Effect of green tea catechins on breast carcinogenesis: a systematic review of in-vitro and in-vivo experimental studies

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Catechins (flavan-3-oils) are the main flavonoids present in green tea. The potential cancer chemopreventive and therapeutic properties of green tea catechins have been the focus of research efforts in the last two decades. This systematic review aims to generate in vitro and in vivo data on the effect of green tea catechins on breast carcinogenesis. Electronic databases were searched with the appropriate search terms. Existing evidence suggests that green tea catechins modulate breast cell carcinogenesis. The effect of green tea catechins on breast cell carcinogenesis has been investigated in different experimental models and under different experimental conditions, that is, carcinogen investigated, green tea catechin dosage regimen, treatment with green tea extract versus pure synthetic EGCG, and time point of treatment with green tea catechins in relation to the exposure to the carcinogen. Although the effect of green tea catechins was not always statistically significant, the protective effect of green tea catechins was demonstrated in all the trials, suggesting that treatment with green tea catechins should be further investigated in the clinical setting of chemoprevention of high-risk women. However, it should be emphasized that the reported actions of green tea catechins are observed in high concentrations that are difficult to achieve in the clinical setting. This drawback could be overcome by designing green tea catechins with better bioavailability and/or by cotreatment combining breast cancer endocrine treatment with green tea catechins. *European Journal of Cancer Prevention* 23:84–89 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Polyphenols, particularly flavonoids, constitute the most interesting component of green tea leaves. Catechins (flavan-3-oils) are the main flavonoids present in green tea. The four major catechins are (–)-epigallocatechin-3-gallate (EGCG), which represents ~59% of the total catechins, (–)-epigallocatechin (EGC) (~19%), (–)-epicatechin-3-gallate (ECG) (~13.6%), and (–)-epicatechin (EC) (~6.4%) (Cabrera *et al.*, 2006). The potential cancer chemopreventive and therapeutic properties of teas and tea polyphenols have been the focus of research efforts for the last two decades. Recent data have shown strong chemopreventive and possibly cancer chemotherapeutic effects of green tea polyphenols and EGCG against cancers of the skin (UV radiation and chemically induced), lung, breast, colon, liver, stomach, and prostate (Mann *et al.*, 2009; Fon Sing *et al.*, 2011; Yang *et al.*, 2011; Yuan *et al.*, 2011). Most of the data available are on EGCG, the most abundant catechin in green tea. It is estimated that 1 200 000 new breast cancer cases are diagnosed annually worldwide. Despite advances in breast cancer treatment, mortality from breast cancer is still high. Undoubtedly, novel treatment strategies are needed for chemoprevention in high-risk women. Chemoprevention is a means of cancer control by the use of specific natural or synthetic chemical substances that can suppress, retard, or reverse the process of carcinogenesis. This systematic review aims to synthesize in-vitro and in-vivo data on the effect of green tea catechins on breast carcinogenesis.

Methods

Pubmed, Scopus, Google Scholar, and Science Citation Index were searched using the search terms 'EGCG', 'green tea', 'catechins', 'breast cancer', 'breast carcinogenesis', and 'mammary carcinogenesis'. The search covered the period from 1966 up to and including February 2013. In-vitro or in-vivo experimental trials that investigated the effect of green tea catechins on mammary carcinogenesis fulfilled the inclusion criteria. Only full publications were considered. There was no language restriction. The reference list of all identified trials was checked for additional relevant articles. Double publications were identified. The English publication was included for articles published in non-European languages, whereas the most complete report was included for articles published in European languages.

Results

Initially, 94 possibly relevant trials were identified and after reviewing the title and abstract, 13 experimental

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trials fulfilled the inclusion criteria and were obtained in full text (Table 1).

In-vitro studies

Green tea catechins have been found to block certain steps in carcinogenesis. In-vitro studies provide insights into the mechanisms of modulation of mammary carcinogenesis by green tea catechins. Integration of in-vitro studies with future human trials could evaluate the applicability of these mechanisms in humans.

Rathore and Wang (2012, 2013) and Rathore *et al.* (2012) have repeatedly treated immortalized, noncancerous, human breast epithelial MCF10A cells with picomolar concentrations of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and benzo[α]pyrene (B[α]P) in culture. The authors showed that cumulative exposures to low doses of NNK and B[α]P resulted in cellular acquisition of stem-like cell-associated and epithelial mesenchymal transition-associated properties and markers in addition to cancer-associated properties. The stem-like cell-associated properties and markers included increases in mammosphere formation, and in aldehyde dehydrogenase-positive and $CD44^+/CD24^-$ cell populations. The epithelial mesenchymal transition-associated properties and markers included mesenchymal cell morphology; increased cell migration, invasion, and mobility; and changed expression of E-cadherin, EpCAM, vimentin, and MMP-9.

Table 1 In-vitro and in-vivo studies on the effect of green tea catechins on breast carcinogenesis

References	Experimental model – intervention	Main findings
Rathore and Wang (2013)	Immortalized, noncancerous, human breast epithelial MCF10A cells treated with picomolar concentrations of environmental carcinogens in the presence and absence of green tea catechins	Stem-like cells and EMT-associated properties and markers should be seriously considered as new cancer-associated indicators for detecting breast cell carcinogenesis and as endpoints for intervention of carcinogenesis
Rathore and Wang (2012)	Immortalized, noncancerous, human breast epithelial MCF10A cells treated with picomolar concentrations of environmental carcinogens	Green tea catechins at noncytotoxic levels could suppress chronically induced cellular carcinogenesis by blocking carcinogen-induced ROS elevation, ERK activation, cell proliferation, and DNA damage in each exposure cycle
Rathore *et al.* (2012)	Immortalized, noncancerous, human breast epithelial MCF10A cells treated with picomolar concentrations of environmental carcinogens	Green tea catechins at a noncytotoxic, physiologically achievable concentration of 2.5 μg/ml were effective in suppressing cellular carcinogenesis, as measured by a reduction in the acquired cancer-associated properties of reduced dependence on growth factors, anchorage-independent growth, increased cell mobility, and acinar-conformational disruption
Bigelow and Cardelli (2006)	The immortalized, nontumorigenic breast cell line, MCF10A	Pretreatment with EGCG inhibited hepatocyte growth factor-induced Met phosphorylation and downstream activation of AKT and ERK. Treatment with EGCG blocked the ability of hepatocyte growth factor to induce cell motility
Sakata *et al.* (2011)	C3H/OuJ mice carrying preneoplastic lesions treated with EGCG and tamoxifen alone or in combination	The tumor incidences were decreased in the green tea extract, tamoxifen, and green tea extract and tamoxifen groups. Importantly, in the group treated with green tea extract and tamoxifen, no tumors developed
Kaur *et al.* (2007)	C3(1) SV40 T,t antigen transgenic multiple mammary adenocarcinoma (TAg) mice treated with green tea catechins	0.05% green tea catechins as the sole source of drinking fluid for 25 weeks delayed carcinogenesis as evidenced by a significant decrease in the volume and size of tumors in the mice exposed to green tea extract
Whitsett *et al.* (2006)	Female Sprague–Dawley CD rats treated with DMBA to induce breast cancer after previous exposure to green tea catechins or control diet throughout life	Animals exposed throughout life to EGCG in the drinking water showed a decrease in the latency to first tumor development, although there was no significant difference as compared with the control group with respect to second and third tumor latency. Furthermore, the number of tumors per rat in EGCG-exposed rats was not significantly different from the controls
Hirose *et al.* (1994)	Female Sprague–Dawley rats pretreated with DMBA and exposed to green tea catechins or control diet	The final incidence and multiplicities of mammary tumors were not significantly different between DMBA-treated groups. However, the survival rate of the green tea catechin-treated group was 93.8 vs. 33.3% for rats on the basal diet. At the end of week 18, when all the animals were still alive, the average size of palpable mammary tumors was significantly smaller in the green tea catechin group
Tanaka *et al.* (1997)	Female Sprague–Dawley rats treated with green tea catechins concurrently with exposure to DMBA or after previous exposure to DMBA	No statistically significant differences were found between the groups treated with green tea catechins at the same time as DMBA, compared with the DMBA-alone control group. Thus, green tea catechins had no effect on carcinogenesis when coadministered with DMBA. In contrast, the final number of mammary tumors in groups treated with 1% green tea catechins ($P<0.05$) or 0.01% green tea catechins ($P<0.01$), but not 0.1% green tea catechins, after DMBA treatment were significantly decreased as compared with the control value
Hirose *et al.* (1997)	Female Sprague–Dawley rats treated with green tea catechins after pretreatment with DMBA	No significant differences were observed between EGCG-treated and control groups in the mean diameter of mammary tumors apart from a tendency for a decrease in the group treated with EGCG versus the control group
Hirose *et al.* (1995)	Female Sprague–Dawley rats treated with a diet containing 0.02% PhIP alone, PhIP together with 1% green tea catechins, green tea catechins alone or diet alone for 52 weeks	The survival rate of the PhIP plus green catechin group at the end of the experiment was higher than that of the PhIP-alone group. The incidence of adenocarcinomas was lower in the PhIP plus green tea catechin-treated group compared with the PhIP alone-treated group, although the difference was not statistically significant
Hirose *et al.* (2002)	Female Sprague–Dawley rats treated with 0.02% PhIP alone or PhIP plus 1% green tea catechins for 52 weeks	1% green tea catechins were associated only with reduced mean size of mammary tumors without affecting the total number of mammary tumors
Kavanagh *et al.* (2001)	Female Sprague–Dawley rats treated with green tea catechins after exposure to DMBA	Green tea extract given after initiation significantly increases mammary tumor latency and decreases tumor weight and metastases in DMBA-treated rats

DMBA, dimethylbenz[α]anthracene; EGCG, (–)-epigallocatechin-3-gallate; EMT, epithelial mesenchymal transition; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; ROS, reactive oxygen species.

Green tea catechins at a noncytotoxic, physiologically achievable concentration of 2.5 μg/ml suppressed the NNK-carcinogen-induced and B[α]P-carcinogen-induced cellular carcinogenesis as measured by reduced dependence on growth factors, anchorage-independent growth, increased cell mobility, and acinar-conformational disruption. The effect of green tea catechins was dose dependent. To investigate the mechanisms of action of green tea catechins, the authors used cDNA microarrays to detect differentially regulated genes that were changed in carcinogen-treated cells but whose changes were suppressed by green tea catechins. Initially, the investigators identified genes whose expression was changed in carcinogen-treated cells compared with their counterpart expression levels in untreated cells. After gene expression data analysis, the authors identified 479 differentially expressed genes in carcinogen-treated cells, versus expression in untreated, counterpart cells. Subsequently, the authors identified genes associated with reactive oxygen species (ROS) elevation, cell proliferation, the ERK pathway activation, and DNA damage, which were not induced in green tea catechin and carcinogen-treated cells, and concluded that BAX, COX17, and MRPL41 associated with ROS elevation, B4GALT1, BARHL1, BOLA3, and MT1E associated with cell proliferation, S100P and SPRR1B associated with ERK pathway activation, and ATM and PER1 associated with DNA damage were upregulated in carcinogen-treated cells, but were suppressed in green tea catechin-treated and carcinogen-treated cells. In addition, *TNFRSF8*, a gene associated with negative regulation of cell proliferation, was downregulated in carcinogen-treated cells but not downregulated in GTC-treated and carcinogen-treated cells. In addition, the PCR method was applied to validate cDNA data by arbitrarily choosing a gene from each category. PCR data were in agreement with cDNA data. In an effort to further investigate the mechanism of the protective effect of green tea catechins, the authors showed that short-term exposure of human breast epithelial MCF10A to NNK and B[α]P induced transient ROS elevation leading to ERK pathway activation, cell proliferation, and chromosomal DNA damage, effects that were blocked by nontoxic concentrations of green tea catechins (Rathore and Wang, 2012, 2013; Rathore *et al.*, 2012).

In the above studies, the investigators have attempted to elucidate the mechanisms of modulation of mammary carcinogenesis by green tea catechins taking into account that green tea catechins are multitarget agents. Although they have not entirely delineated the molecular mechanisms underlying the action of green tea catechins, the findings of these studies suggest that green tea catechins modulate breast carcinogenesis through mediation of multiple signaling pathways.

In contrast, Bigelow and Cardelli (2006) have investigated the effect of EGCG on inhibition of hepatocyte growth factor (HGF) signaling in the immortalized, nontumorigenic breast cell line MCF10A. MCF10A is an immortalized, nontumorigenic cell line that represents a preneoplastic cell. HGF treatment induced rapid, sustained activation of Met (HGF receptor), ERK1/2, and AKT pathways. Pretreatment of cells with concentrations of EGCG as low as 0.3 μmol/l inhibited HGF-induced Met phosphorylation and downstream activation of AKT and ERK. Treatment with 5.0 μmol/l EGCG blocked the ability of HGF to induce cell motility. To determine whether EGCG can block HGF signaling at the level of Met receptor activation, MCF10A cells were pretreated for half an hour with increasing concentrations of EGCG (0.07–20 μmol/l). HGF (30 ng/ml) was added in the presence of EGCG for 15 min and protein lysates were prepared. Western blot analysis showed that concentrations of EGCG as low as 0.07 μmol/l EGCG partially blocked Met activation, whereas concentrations of 0.3 μmol/l and above completely blocked phosphorylation of Met, Erk, and Akt. However, (–)-EC could not inhibit HGF-induced events at any concentration tested. In contrast, EGC completely repressed HGF-induced AKT and ERK phosphorylation at concentrations of 10 and 20 μmol/l, but could not block Met activation. Despite these observations, EGC did inhibit HGF-induced motility in MCF10A cells at 10 μmol/l (Bigelow and Cardelli, 2006). These findings suggest that the R1 galloyl group is necessary for Met inhibition as both EGCG and ECG contain this functional group. The R2 hydroxyl group may also contribute toward the inhibition of downstream signaling proteins as it is present in EGCG and EGC. In contrast, epicatechin, which contains neither the R1 galloyl nor the R2 hydroxyl group, could not inhibit HGF-induced events (Bigelow and Cardelli, 2006).

In-vivo studies

Sakata *et al.* (2011) investigated the growth-inhibitory effect of EGCG and tamoxifen alone or in combination in preneoplastic lesions in C3H/OuJ mice. The C3H mouse is a popular animal model of mammary tumor formation carrying the C3H mammary tumor virus type S. Breeding females typically show a high incidence of mammary tumors by 11 months of age. Ten-week-old mice (seven per group) were randomly assigned to treatment groups consisting of green tea extract (0.1 or 1% in drinking water), tamoxifen (10 mg pellet), the combination of 1% green tea extract and 10 mg tamoxifen, and control. The number and size of mammary tumors were measured weekly during a 48-week treatment. The incidence of tumor was decreased in the green tea extract, tamoxifen, and green tea extract and tamoxifen groups. Importantly, in the group treated with green tea extract and tamoxifen, no tumors developed (Sakata *et al.*, 2011), possibly suggesting a synergistic interaction between green tea extract and tamoxifen.

Kaur *et al.* (2007) investigated the effect of green tea catechins on C3(1) SV40 T,t antigen transgenic multiple mammary adenocarcinoma (TAg) mice. In TAg mice, expression of the SV40 transforming sequences is targeted to the mammary epithelium by a fragment of the rat prostatic steroid binding protein promoter C3(1). The T antigen binds and functionally inactivates p53 and Rb tumor suppressor genes, leading to perturbation of cell homeostasis and subsequent mammary carcinogenesis. One hundred percent of female mice carrying the same transgene develop palpable mammary carcinomas with some histologic similarities to human breast cancer, that is, the murine mammary tumors progress from estrogen receptor-positive to estrogen receptor-negative status, and in terms of histology, the intraduct carcinomas resemble human ductal carcinoma in situ. This model has been very useful for the study of various aspects of mammary tumor progression, including changes in genomic organization, cell cycle regulation, apoptosis, and the influence of hormones on the natural history of tumor development. In the trial by Kaur *et al.* (2007), treatment of TAg mice with 0.05% green tea catechins as the sole source of drinking fluid for 25 weeks delayed carcinogenesis as evidenced by a significant decrease in the volume and size of tumors in the mice exposed to green tea extract. The authors suggested that green tea catechins affected carcinogenesis through redox modulation and induction of apoptosis. The hypothesis of redox modulation was supported by experimental data indicating that changes in cancer parameters correlated with tea-polyphenol mediated decreases in the levels of a malondialdehyde DNA adduct in the tumors. The hypothesis of apoptosis induction was supported by the increased tumor levels of cleaved caspase 3 protein that were identified in the tumors (Kaur *et al.* 2007).

Whitsett *et al.* (2006) investigated the potential effect of chemically synthesized EGCG throughout life in the diet on suppression of chemically induced mammary cancer initiated with concentrations of dimethylbenz[α]anthracene (DMBA) resulting in adenocarcinomas. Female Sprague–Dawley CD rats were exposed to either EGCG (0.065% in the drinking water) or control diet for the entire life starting at birth. At 50 days postpartum, rats were treated with 60 mg/kg body weight DMBA to induce mammary cancer. The number of tumors per rat and the time latency to tumor development were estimated. Animals exposed throughout life to EGCG in the drinking water showed a decrease in the latency to first tumor development, although there was no significant difference compared with the control group with respect to second and third tumor latency. Furthermore, the number of tumors per rat in EGCG-exposed rats was not significantly different from the controls. The authors suggested that the lack of effect of EGCG was because of the low bioavailability of pure EGCG (Whitsett *et al.*, 2006). In contrast, it could be suggested that the study did not have the power to detect the effect of EGCG. However, low power does not seem to be a plausible explanation, as the investigators had included 30 animals per group and the dose of DMBA administered resulted in 100% incidence of mammary adenocarcinomas in the control group over the course of the experiment, with an average of more than eight tumors per rat in the control group (Whitsett *et al.*, 2006).

Hirose *et al.* (1994) have investigated the effect of green tea catechins on mammary carcinogenesis in a series of studies without providing any data on the mechanism of modulation of carcinogenesis by green tea catechins. At first, they investigated the effect of green tea catechins on mammary gland carcinogenesis in female Sprague–Dawley rats pretreated with DMBA (Hirose *et al.*, 1994). Groups of 7-week-old rats had been exposed to an intragastric dose of 50 mg/kg DMBA. One week later, rats were treated with a diet containing 1.0% green tea catechins or a basal diet alone for 35 weeks. The final incidence and multiplicities of mammary tumors were not significantly different between DMBA-treated groups. However, the survival rate of the green tea catechin-treated group was 93.8 versus 33.3% for rats on the basal diet. At the end of week 18, when all the animals were still alive, the average size of palpable mammary tumors was significantly smaller in the green tea catechin group. The authors concluded that green tea catechins inhibit rat mammary gland carcinogenesis after DMBA initiation (Hirose *et al.*, 1994). In another trial, Tanaka *et al.* (1997) investigated whether the effect of green tea catechin on DMBA-induced mammary gland carcinogenesis was dose dependent. Six-week-old female Sprague–Dawley rats were treated with dietary 1, 0.1, or 0.01% green tea catechins for 2 weeks and then with diet alone for 35 weeks. At the end of the first week, DMBA was administered intragastrically at a dose of 25 mg/kg. Other groups of 20 7-week-old rats each were administered an intragastric dose of 25 mg/kg DMBA, and 1 week after DMBA treatment they were treated with diet containing 1, 0.1, or 0.01% green tea catechins or basal diet alone for 35 weeks. Control rats were administered 1% green tea catechins or basal diet alone. Parameters evaluated were the final incidence and number of mammary tumors. No statistically significant differences were found between the groups treated with green tea catechins at the same time as DMBA, compared with the DMBA-alone control group. Thus, green tea catechins had no effect on carcinogenesis when coadministered with DMBA. In contrast, the final number of mammary tumors in groups treated with 1% green tea catechins ($P < 0.05$) or 0.01% green tea catechins ($P < 0.01$), but not 0.1% green tea catechins, after DMBA treatment was significantly decreased as compared with the control value. The authors concluded that green tea catechins inhibit mammary carcinogenesis in the postinitiation stage, with the effect being weak and not dose dependent (Tanaka *et al.*, 1997).

In addition, Hirose *et al.* (1997) investigated the effects of green tea catechins on the late promotion or progression stage of mammary gland carcinogenesis. In that study, 84 7-week-old female Sprague–Dawley rats were pretreated with 50 mg/kg body weight intragastric dose of DMBA. Treatment with green tea catechins started at the 13th week when the tumor incidence had reached 50%. Three groups of 28 animals each were placed on a diet containing 0.5% Polyphenon E (Mitsui Norin Co. Ltd, Japan) (58.4% content EGCG), 0.5% EGCG-80 (81% content of EGCG), or basal diet alone for 23 weeks. The experiment was terminated at week 36. The main parameter evaluated was the change in the mean diameter of mammary tumors at the end of the experiment versus the week 13. No significant differences were observed between EGCG-treated and control groups apart from a tendency for a decrease in the group treated with EGCG versus the control group. The authors concluded that green tea catechins were not effective in inhibiting progression of rat mammary carcinogenesis. However, it was suggested that Polyphenon E might exert a weak inhibitory effect on the early promotion stage (Hirose *et al.*, 1997). Again, the results should be interpreted with caution, taking into account the low bioavailability of EGCG. It has been reported that rats receiving decaffeinated green tea showed a higher plasma concentration of EGCG than rats receiving pure EGCG, even though the dose of pure EGCG was five times higher (Chen *et al.*, 1997).

The same group of investigators investigated the effect of green tea catechins on 2-amino-1-methyl-6-phenylimidazo[4,5-β]pyridine (PhIP)-induced mammary carcinogenesis in female F344 rats. Groups of 20–21 6-week-old rats were treated with a diet containing 0.02% PhIP alone, PhIP together with 1% green tea catechins, green tea catechins alone, or diet alone for 52 weeks. The survival rate of the PhIP plus green catechin group at the end of the experiment was higher than that of the PhIP-alone group. The incidence of adenocarcinomas was lower in the PhIP plus green tea catechin-treated group compared with the PhIP alone-treated group, although the difference was not statistically significant (Hirose *et al.*, 1995).

In another trial, Hirose *et al.* (2002) investigated the effect of green tea catechins against heterocyclic amino-induced carcionogenesis in rats. Groups of 20–21 female F344 rats each were treated with 0.02% PhIP alone or PhIP plus 1% green tea catechins for 52 weeks. From the 18th week after the onset of the experiment, rats were carefully checked for the presence of mammary tumors once a week and data on location, size, and number of tumors were recorded. One percent green tea catechins were associated only with reduced mean size of mammary tumors without affecting the total number of mammary tumors (Hirose *et al.*, 2002).

Kavanagh *et al.* (2001) reported that green tea extract administered after initiation significantly increases mammary tumor latency and decreases tumor weight and metastases in DMBA-treated rats.

Discussion

Existing evidence suggests that green tea catechins modulate breast cell carcinogenesis. The effect of green tea catechins on breast cell carcinogenesis has been investigated in different experimental models and under different experimental conditions, that is, carcinogen investigated, green tea catechin dosage regimen, treatment with green tea extract versus pure synthetic EGCG, and time point of treatment with green tea catechins in relation to the exposure to the carcinogen. Although the effect of green tea catechins was not always statistically significant, the protective effect of green tea catechins was shown in all the trials, suggesting that treatment with green tea catechins should be further investigated in the clinical setting of chemoprevention in high-risk women.

The mechanism of inhibition of carcinogenesis by green tea catechins has not been delineated. It has been shown that green tea catechins inhibit the formation of PhIP-induced DNA adduct formation, which leads to reduced tumorigenicity in animal chemoprevention studies. EGCG induces apoptosis of cancer cells through different pathways involving both pro-oxidant and epigenetic modulation of apoptosis-related genes such as human telomerase reverse transcriptase (Min *et al.*, 2012). Furthermore, green tea catechins have also been shown to reduce cell proliferation through modulation of cell cycle progression (Huang *et al.*, 2008).

In addition, green tea catechins modulate multiple signaling pathways including inhibition of the nuclear factor-κB-signaling pathway, inhibition of MAPKs and activator protein 1, inhibition of the synthesis of nitric oxide, inhibition of the epidermal growth factor-mediated signal transduction pathway through suppression of binding of epidermal growth factor to its receptor, inhibition of the insulin-like growth factor-1-mediated signaling pathway, inhibition of overexpression of cyclo-oxygenase-2, and inhibition of proteasome activity (Beltz *et al.*, 2006). Upregulation of tumor suppressor genes by EGCG has been reported (Beltz *et al.*, 2006). Thus, it seems that green tea catechins inhibit breast cell carcinogenesis through modulation of multiple signaling pathways. The effect of green tea catechins on carcinogenesis has been also shown in other tissues. Mantena *et al.* (2005) have shown that oral administration of green tea polyphenols in drinking water of mice leads to significant protection against the development of nonmelanoma skin cancer in terms of tumor incidence (percentage of mice with tumors), tumor multiplicity, and tumor size compared with non-GTPs-treated UVB-irradiated mice (Mantena *et al.*, 2005). Wang *et al.* (1991) reported that a water extract of green tea leaves, containing a mixture of polyphenolic ingredients, when administered as the sole source of drinking water to mice, provided protection against UVB radiation-induced skin tumorigenesis.

However, it should be emphasized that the reported actions of green tea catechins are observed in high concentrations, which are difficult to achieve in the clinical setting (Chow and Hakim, 2011; Visioli, 2011). In fact, human metabolism and bioavailability of green tea catechins have not been fully investigated. In a recently published study that investigated the human bioavailability of green catechins after drinking three doses of green tea, it was shown that irrespective of the dose, green tea catechins appeared rapidly in plasma as monophasic curves, suggesting absorption in the small intestine and minimal enterohepatic circulation (Renouf *et al.*, 2013). This drawback could be overcome by designing green tea catechins with better bioavailability and/or by cotreatment combining breast cancer endocrine treatment with green tea catechins (Landis-Piwowar *et al.*, 2013).

In conclusion, on the basis of experimental evidence, green tea catechins protect breast cells against carcinogenesis through modulation of multiple targets. This protective effect has been shown in different experimental models and under different experimental conditions, suggesting clinical implications of green tea catechins in the chemoprevention of breast cancer in high-risk women. Further investigation in the clinical setting would seem appropriate.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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