Abstract. Telomerase is elevated in >90% of breast carcinomas and therefore has received much attention as a target for breast cancer therapy and cancer diagnostic research. Dietary components that are capable of inhibiting the growth of cancer cells without affecting the growth of normal cells are receiving considerable attention in developing novel cancer-preventive approaches. Studies have shown that (-)-epigallocatechin-3-gallate (EGCG) from green tea imparts a growth inhibitory effect on cancer cells. Here, we show that treatment of EGCG dose-dependently inhibited (20-100%) the reproductive or colony forming potential, and also decreased cell viability at different time points studied (~80% inhibition) in human breast carcinoma MCF-7 cells but had no adverse effect on the growth of normal mammary cells. Treatment of EGCG for 48 and 72 h markedly increased the percentage of apoptotic cells (32-51%) in MCF-7 cells compared to that of non-EGCG treated cells (8-14%). In order to identify the possible mechanism of decreased cell viability and induction of apoptosis in breast carcinoma cells by EGCG, we found that treatment of MCF-7 cells with EGCG dose-dependently inhibited telomerase activity (40-55%), and also inhibited the mRNA expression (40-55%) of hTERT, a catalytic subunit of telomerase. Additional studies demonstrated that EGCG also inhibited the protein expression of hTERT, which indicated that inhibition of telomerase was associated with down-regulation of hTERT.

Together, our results indicate that EGCG down-regulates telomerase in human breast carcinoma MCF-7 cells, leading to the suppression of cell viability and induction of apoptosis, thus providing the molecular basis for the development of EGCG as a novel chemopreventive and pharmacologically safe agent against breast cancer.

Introduction

Breast cancer is the most commonly diagnosed invasive malignancy in women affecting as many as 1 in every 8 females. Current therapies for the treatment of breast cancer may result in drug resistance or toxicity (1). Since telomerase is elevated in over 90% of the breast carcinomas, this enzyme that maintains the ends of chromosomes during the replicative process has received attention as a target for breast cancer therapy and cancer diagnostic research. Telomerase may also be a potential target for breast cancer prevention or therapy because its activity is specific to neoplastic tissues but does not activate in normal breast cells (2). In most cancers, telomerase activity is detectable in the early stages, and higher levels of telomerase correlate with a poor prognosis (3). Ablation of telomerase expression leads to telomeric attrition and growth inhibition of cultured neoplastic cells (4,5). The upregulation of telomerase allows tumor cells to escape cellular senescence and to proliferate indefinitely (6). Telomerase activation is thought to be required for cells to continuously divide beyond replicative senescence and may, therefore, be a critical step in cellular immortality and carcinogenesis (7,8). Three major subunits comprising the human telomerase complex have been identified. First, the RNA component of human telomerase (hTR) provides the template for telomere repeat synthesis (4). Second, telomerase-associated protein (TP1) is a cloned telomerase component, the function of which remains unclear (9,10) and third, the most important component responsible for the enzyme activity of telomerase is human telomerase reverse transcriptase (hTERT) (11,12). Studies have shown that hTERT is expressed in most of the malignant tumors but not in most normal tissues (much lower levels in certain normal cells like lymphocytes, intestinal crypt cells, germinal cells etc.) and that hTERT expression is closely associated with telomerase activity,
whereas 2 other subunits (hTR and TP1) are constitutively expressed in both tumors and normal tissues (13). These observations demonstrate that disruption of telomere maintenance limits cellular lifespan in human cancer cells, thus further validating hTERT as an important target for the development of anti-neoplastic therapies. As telomerase activity seems to play a crucial role in tumor cell immortality, it has importance both as a molecular prognostic parameter and as a target molecule for anti-cancer therapy. High telomerase activity correlates with the degree of malignancy and the likelihood of tumor progression (14,15). Together, these observations validate the view that inhibition of telomerase function may constitute a new strategy of chemoprevention and antineoplastic therapy.

The treatment of cancer patients with chemotherapy is empirical and often produces unsatisfactory and adverse toxic effects. Even the most commonly used drugs produce meaningful responses in <50% patients. As a result, too many patients are needlessly exposed to highly toxic drugs and suffer the side effects without reaping the benefits. Therefore the search for non-toxic or less cytotoxic and more effective anticancer drugs is warranted. Herbs and medicinal plants have been used for centuries to treat many diseases throughout Asia and Europe. Chemoprevention through the consumption of nutraceuticals or dietary supplements like polyphenols from green tea may reduce both morbidity and mortality in cancer. However, a clear understanding of the mechanisms was observed only with specific targets, or different experimental models (16), however, since many of the mechanisms were observed only with specific targets, or were examined under exceptional and nonphysiological conditions, it is still lacking. In recent years, several lines of evidence from epidemiological and animal studies have emerged, showing chemopreventive and anticancer potential of green tea polyphenols, which have suggested a positive association between epidemiological and animal studies have emerged, showing chemopreventive and anticancer potential of green tea polyphenols, which have suggested a positive association between green tea and a lower incidence of gastric, esophageal, ovarian, pancreatic, colorectal and skin cancers (reviewed in ref. 16). Further, treatment with (−)-epigallocatechin-3-gallate (EGCG), the major and most potent chemopreventive agent found in green tea, effectively inhibits carcinogenesis in various animal organs (16). Various mechanisms were suggested to explain the anticancer effects of green tea polyphenols in different experimental models (16), however, since many of these mechanisms were observed only with specific targets, were examined under exceptional and nonphysiological conditions, it is still not clear how green tea polyphenols uniquely affect cancer in a universal, selective and nontoxic manner. Moreover, EGCG has been shown to have a profound growth inhibitory effect on cancer cells but not on their normal counterparts such as normal breast cells (17). It has been shown that EGCG and other related polyphenols inhibited telomerase activity in some tumor cell lines (18,19), but the anti-carcinogenic effect of EGCG in human mammary carcinoma cells is not well studied or understood. Therefore, we determined the anti-carcinogenic or chemopreventive effect of EGCG on the cellular viability, proliferation potential, induction of apoptosis, and telomerase activity and hTERT gene regulation in human breast carcinoma MCF-7 cells.

Materials and methods

Chemicals, antibodies and cell line. EGCG was procured as a gift from Dr Y. Hara (Tokyo Food Techno Co. Ltd, Shizuoka, Japan). Human breast adenocarcinoma MCF-7 cells were purchased from American Tissue Culture Collection (ATCC, Manassas, VA). Annexin V-conjugated Alexafluor 488 Apoptosis Detection kit was purchased from Molecular Probes Inc. (Eugene, OR). A TRAP assay kit was purchased from Serologicals Corporation (Norcross, GA). Enhanced chemiluminescence Western blotting kit was purchased from Amersham Pharmacia Biotech (Piscataway, NJ). MITT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) and all other chemicals employed in this study were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO). Cells were cultured in monolayers in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone, Logan, UT), 100 µg/ml penicillin, and 100 µg/ml streptomycin, both from Invitrogen (Carlsbad, CA) and maintained at 37˚C in a humidified atmosphere of 5% CO₂.

Colony formation assay. The anti-proliferative effect of EGCG on MCF-7 cells was measured by a colony formation assay. Approximately 200 cells were seeded into 35 mm petriplates in triplicate for each group and allowed to adhere for 24 h. Thereafter cell culture medium was removed, and EGCG, pre-dissolved in the medium at concentrations of 5-50 µg/ml, was added to the cells. The cells were allowed to incubate at 37°C in the incubator undisturbed for 14 days. During this time each individual surviving cell would proliferate and form colonies. On day 15, the colonies were then washed with cold phosphate buffer saline (PBS), fixed with 70% ethanol and stained with 0.02% aqueous trypan blue solution. The colonies which had ≥50 cells per colony were counted. Data were represented as number of colonies formed per 200 cells plated and converted to percentage to determine the plating efficiency for the control group. The percent surviving fraction of each treatment group, compared with cells treated with vehicle alone (non-EGCG), was then calculated and expressed as the colony forming potential.

Cell viability assay. The MTT assay was performed to determine the cell viability of MCF-7 cells under the influence of EGCG and other epicatechins from green tea as described previously with some modification (20). Approximately 5,000 MCF-7 cells/well were plated in 96-well plates and kept in the incubator at 37°C using previously described cell culture conditions. After overnight incubation to allow them to adhere, the cells were fed with normal medium or without varying concentrations of EGCG (10-100 µg/ml), and also with equal doses of different epicatechin derivatives (60 µg/ml) such as (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC) and EGCG for 24, 48 and 72 h. Each treatment and time point was assayed in at least 8 replicates. Cell viability was assessed by the MTT assay at the different time intervals. Briefly, at the stipulated time following the treatment of epicatechin derivatives, medium was aspirated, MTT (50 µl of a 5 mg/ml stock solution in PBS) was added in each well of the 96-well culture plate and incubation continued at 37°C for 2 h. The plates were spun, supernatants were discarded and purple colored precipitates of formazan were dissolved in 150 µl of dimethyl sulfoxide. The color absorbance was recorded at 540 nm of
Each aliquot with a BioRad 3350 microplate reader with a reference at 650 nm serving as a blank. The reduction in viability of MCF-7 cells in each well was expressed as the percentage compared to control cells (non-epicatechin treatment).

Measurement of apoptotic cells by flow cytometry. Apoptotic cell death caused by EGCG treatment was determined using the Annexin V-Alexafluor488 Apoptosis Detection kit following the manufacturer's instructions. Briefly, the MCF-7 cells were treated with and without EGCG for 48 and 72 h. Cells were harvested, washed and subjected to Annexin V and propidium iodide staining in binding buffer. Stained cells were analyzed by fluorescence activated cell sorting (FACSCalibur, BD Biosciences, San Jose, CA) using CellQuest 3.3 Software. The kit contains recombinant Annexin V conjugated to Alexafluor 488 fluorescent dye, providing maximum sensitivity. The apoptotic cells stained with Annexin V showed green fluorescence and were present in the lower right (LR) quadrant of the histogram. Cells stained with both Annexin V and propidium iodide, showed red and green fluorescence, and occupied the upper right (UR) quadrant of the histogram.

Preparation of samples for the telomerase activity detection assay and RT-PCR. In preliminary experiments of cell viability and proliferation experiments, EGCG was found to be the most effective inhibitor of MCF-7 cell proliferation as compared to other epicatechin derivatives (EC, ECG and EGC); therefore, in further experiments only EGCG was tested to determine its effect on telomerase activity. The cells were seeded into 24-well culture plates and allowed to adhere overnight. The next day, cells were treated with various concentrations of EGCG for 48 and 72 h. At the stipulated time, the cells were washed with cold PBS buffer, trypsinized, centrifuged and the cell pellet was obtained and washed in PBS buffer. After re-suspending the cells in PBS, cells were counted and 1x10^6 cells for the telomeric repeat amplification protocol (TRAP assay) and 3x10^6 cells for the RT-PCR were isolated from each treatment group and pelleted down. The PBS was discarded completely and the pellets were frozen and stored at -80°C for further estimations.

Detection of telomerase by TRAP assay. The cell pellets obtained from different treatment groups for the Telomere Repeat Amplification Protocol (TRAP) assay was assayed by the TRAPEze XL Telomerase Detection kit (Serologicals Corporation) according to the manufacturer's instructions. Briefly, the cell pellet was thawed on ice and lysed with CHAPS lysis buffer and the supernatant containing the active telomerase enzyme was isolated and the telomerase activity was determined by the PCR-based TRAP assay. Equal volumes of samples obtained from an equal number of cells with active telomerase were analysed in the TRAP reaction along with their respective heat-inactivated counterparts. The basic principle behind the TRAP assay is underlined as follows: After extension of the substrate TS (5'-AATTCGG TCGAGGAGAGTT-3') oligonucleotide by telomerase, the telomerase products were amplified by PCR in the presence of Ampilfluor™ primers which fluoresce only upon incorporation into the TRAP products or the internal control. The PCR conditions constituted 36 cycles of the 3 steps of PCR at 94°C/30 sec, 59°C/30 sec, 72°C/1 min followed by a 55°C/25 min extension step. Therefore, the net increase of fluorescence in the reaction vessel directly correlated to the amount of amplified DNA. Each reaction product was amplified in the presence of a 56-bp internal TRAP assay standard. A TSR8 quantitation standard (which serves as a standard to estimate the amount of product extended by telomerase in a given extract) was included for each set of TRAP assays. The telomerase products were resolved on 10% non-denaturing polyacrylamide gels and stained with Sybr-Green dye. A fluorescent ladder of products with 6 base pair increments starting at 61 nucleotides (61, 67, 73, 79 etc.) was observed. The fluorescence emission produced was directly proportional to the amount of TRAP products generated.

Determination of telomerase by RT-PCR. Total cellular RNA was extracted from frozen cell pellets from different treatment groups using the RNeasy mini kit (Qiagen Molecular Diagnostics, Valencia, CA) according to the manufacturer's instructions. Total RNA (2 µg) from each sample was used for cDNA production using the Superscript 1st strand synthesis system for RT-PCR (Invitrogen Co., Carlsbad, CA) with random hexamers. The cDNA samples were then amplified with the PCR Master mix from Promega Corporation. Specifically, hTERT cDNA was amplified using sense 5'-TGA ACTTGGCGGAGAGAGTTT and antisense 5'-ATG CGTCAAACCTGTA CGCCT-3' oligonucleotides with an initial heating at 94°C for 5 min, followed by 34 cycles of 94°C for 45 sec, 62°C for 30 sec and 72°C for 45 sec (21). Amplified products were electrophoresed on a 2% agarose gel in 1X Tris-borate EDTA buffer (90 mM Tris-borate, pH 8.3; 1 mM EDTA) with the standard of a 100 bp ladder. The gel was stained with ethidium bromide. Images were made and the intensity of bands was measured with Kodak EDAS (Kodak, New York, NY) gel documentation system. A real-time PCR for detecting the hTERT subunit of telomerase was also performed but was found to be terminated in the exponential phase (data not shown).

Western blot analysis. Western blot analysis was performed to determine the expression of telomerase at the protein level. For this purpose, cells were harvested at desired time points with or without treatments of EGCG, washed with ice cold PBS and lysed with buffer containing 50 mM Tris HCl (pH 7.4), 150 mM NaCl, 1% NP-40, 1 mM EDTA, 1 mM sodium ortho-vanadate and other protease inhibitors as described previously (22). An equal amount of the protein samples (50 µg) was separated on a 12% SDS-polyacrylamide gel and transferred onto nitrocellulose membranes (Amersham Pharmacia Biotech). The membranes were immunoblotted with an antibody against hTERT (SantaCruz Biotechnology, Inc., SantaCruz, CA), and protein bands were visualized using the ECL detection system (Amersham) according to the manufacturer's instructions. To compare the relative intensity of each protein band from different treatment groups in the Western blot, computerized densitometry was performed using Optimas 6.2 software program.
Statistical analysis. Statistical analysis was performed through use of the Student's t-test to determine the statistical significance of difference in the absolute values of the clonogenic potential, cell viability and apoptotic cell death between the EGCG treated and non-EGCG treated groups. The chemopreventive effect of EGCG or other epicatechins was considered significant when $p<0.05$.

Results and Discussion

Treatment with EGCG inhibits cellular proliferation and viability of MCF-7 cells. To assess the cytotoxic or anti-carcinogenic effect of EGCG on MCF-7 cells, dose response curves were generated using data from an anchorage dependent colony-forming assay and MTT assay. The colony-forming assay is based on the fact that single, isolated cells in medium proliferate independently and ultimately form individual colonies. Thus, it measures the ability of the cells to maintain their reproductive capacity under various treatments. We found that EGCG treatment results in strong cytotoxic effects on the human breast carcinoma MCF-7 cells (Fig. 1, upper panel), and significantly inhibited the colony forming potential of MCF-7 cells. There was a drastic reduction in the ability of the breast carcinoma cells to form colonies with increasing doses of EGCG. The doses of EGCG treatment from 30-50 $\mu$g/ml have shown almost complete cessation in the colony forming potential or proliferation ability with no colonies being observed at the end of the experimental time period of 14 days.

Further, to determine the effect of EGCG on the cell viability and proliferating potential of MCF-7 cells, the MTT assay was carried out. The MCF-7 cells were treated with varying doses of EGCG (10-100 $\mu$g/ml) for 24, 48 and 72 h time points (Fig. 1, middle panel). Treatment of EGCG with 40 $\mu$g/ml concentration significantly reduced the cell viability (23-52% reduction, $p<0.05$ to $p<0.001$) of MCF-7 cells from 24 h onwards to 72 h whereas 55-77% inhibition ($p<0.0001$) of MCF-7 cells from 40 $\mu$g/ml concentration were found to be in between 40-52% and 58-76% by EGC and EGCG treatment, respectively in comparison to that of non-epicatechin treated control group; ** $p<0.0001$ vs control (non-treated). Data are presented in terms of the mean ± SD of 8 replicates. * $p<0.001$ vs control (non-treated) group.

We also determined and compared the effect of other epicatechins from green tea at the same dose level, and at different time points (Fig. 1, lower panel). In a previous experiment (Fig. 1, middle panel) we found that treatment of MCF-7 cells with a 60 $\mu$g/ml dose of EGCG very significantly inhibited (55-77%, $p<0.001$) cell viability; therefore, this dose was used for each epicatechin derivative to determine their effects on the cell viability or proliferating potential of MCF-7 cells. Treatment of EC and ECG were found to be ineffective in inhibiting the proliferating potential of MCF-7 cells at each time point studied (Fig. 1, lower panel). However, treatment of EGC and EGCG inhibited cell viability by 40-52% and 58-76% respectively at all the time points studied. These observations also indicated that inhibitory effect of EGCG was significantly greater ($p<0.001$) than that of EGC treatment. Further, amongst all the epicatechins present in green tea
EGCG, EGC, ECG and EC), EGCG has been shown to be the most potent anti-carcinogenic agent in several in vivo as well as in vitro systems (16,18).

**Treatment of EGCG induces apoptosis in MCF-7 cells.** Since, treatment of EGCG at the doses of 40 and 60 µg/ml for 48 and 72 h resulted in a significant inhibition of cell viability in MCF-7 cells, we selected these doses and time points to determine the effect of EGCG on the induction of apoptosis. As shown in Fig. 2, treatment with EGCG (40 and 60 µg/ml) resulted in induction of apoptosis in MCF-7 cells both after 48 h (panel A) and 72 h (panel B) of its treatment compared to control (non-EGCG) cells. The number of apoptotic cells was divided as late apoptotic cells shown in upper right (UR) quadrant and early apoptotic cells as shown in lower quadrant (LR) of the histograms (23). We found that treatment with EGCG at the dose of 40 and 60 µg/ml for 48 h (Fig. 2, panel A) increased the number of early apoptotic cells (LR), respectively, from 4.5 to 25.3% and 31.4% while the number of late apoptotic cells (UR) was increased from 4.0 to 7.4% and 6.1%. Thus, total percentage of apoptotic cells (UR+LR) increased from 8.5 to 32.7% following the treatment of 40 µg/ml of EGCG and 8.5 to 37.5% following the treatment of 60 µg/ml of EGCG. The induction of apoptosis in MCF-7 cells was found higher when cells were treated for 72 h (Fig. 2, panel B). Treatment with EGCG at the dose of 40 and 60 µg/ml to MCF-7 cells for 72 h resulted in marked increase in LR cells, respectively, from 8.5 to 29.5% and 8.5 to 42.5%. Thus, total percentage of apoptotic cells (UR+LR) increased from 14.8 to 39.1% following the treatment of 40 µg/ml of EGCG and 14.8 to 51.4% following the treatment of 60 µg/ml EGCG (Fig. 2, panel B). Marked induction of apoptosis by EGCG indicates its anti-carcinogenic effect and its chemopreventive potential against mammary carcinogenesis. EGCG-induced apoptosis after 24 h of treatment was not very significant compared to non-EGCG treated MCF-cells (data not shown).

**Treatment with EGCG inhibits telomerase activity, hTERT mRNA and protein expressions.** To explore the possible mechanism of EGCG in suppression of cell viability and induction of apoptosis, we determined the effect of EGCG on telomerase in MCF-7 cells. Telomerase, a polymerase with reverse transcriptional activity that leads to repair of the telomeric attrition in the genome, has been shown to be up-regulated in most of the breast carcinomas (2). Therefore, we were interested in determining the mechanism of the anti-carcinogenic effect of EGCG on the telomerase activity in MCF-7 cells using PCR-based TRAPeze XL Telomerase Detection kit. Since treatment of EGCG significantly inhibited cell viability, cellular proliferation and induction of apoptosis in MCF-7 cells at 48 h after its treatment, we specifically selected this time point to determine dose-dependent effect of EGCG on telomerase activity. Treatment of MCF-7 cells with EGCG (40-80 µg/ml) resulted in a dose-dependent decrease in the telomerase activity (40-55%, p<0.01) following its treatment for 48 h as evident by reduced intensity of the 6-bp...
of chemoprevention strategies that specifically target telomerase is of great interest. A definitive knowledge of the mechanisms by which human telomerase is regulated might be important for a better understanding of the pathogenesis of breast carcinoma, and chemopreventive strategies for breast cancer. As telomerase activity seems to be a key player in tumor cell immortality, it has importance both as a molecular prognostic parameter and as a target molecule for anti-cancer therapy. Telomerase has been shown to correlate with poor clinical outcome in neuroblastoma, gastric cancer etc. (24,25), indicating that tumors expressing this enzyme may be more aggressive. For breast cancer, however, telomerase activity is considered as a controversial prognostic marker; whereas some studies suggest that telomerase, clinicopathological parameters and disease outcome are linked, others do not find this association (14,26-29). However, telomerase is emerging as a molecular marker which, when activated or upregulated, allows tumor cells to escape from cellular senescence and to proliferate indefinitely (6). Recent studies indicated that telomerase activity inhibition may lead directly to telomere shortening and cellular damage, or it may indirectly, by blocking telomerase-mediated cell survival, enhance cell damage elicited by other factors (30-33). In context with this information, the treatment of EGCG may therefore be an effective chemopreventive agent for the prevention of human breast cancer incidence, which not only decreases cell viability and induction of apoptosis but also inhibits telomerase activity of human breast carcinoma cells in vitro.

To gain insight into the mechanisms controlling telomerase activity in MCF-7 cells, we examined the effect of EGCG on the protein expression of catalytic subunit of the enzyme as well as mRNA expression. The hTERT subunit of the telomerase enzyme is the catalytic subunit which is known to be primarily correlated with the telomerase activity as it is shown to be the rate limiting step in telomerase activation. The data from RT-PCR showed that treatment of 40 and 60 µg/ml of EGCG to MCF-7 cells markedly reduced the mRNA expression of hTERT by 40 and 55% respectively after 48 h of treatment as observed by the reduced intensity of the fluorescent bands (Fig. 3, panel B).

Further, we were interested in looking at the effect of EGCG on telomerase at the protein level. Therefore, we performed the Western blot analysis. Here, we conducted an experiment to determine the dose-dependent effect of EGCG (20-60 µg/ml) on the expression of hTERT protein at 48 and 72 h after its treatment in MCF-7 cells (Fig. 4). The treatment of EGCG at the dose of 20 µg/ml was not effective in the inhibition of hTERT protein expression. This dose of EGCG was also not effective to inhibit cell viability (Fig. 1, middle panel) and to induce apoptosis in MCF-7 cells (data not shown). However, the treatment of EGCG at the dose of 60 µg/ml resulted in marked inhibition of hTERT protein expression at 48 and 72 h time points. Densitometric analysis of the protein bands on the autoradiograph indicated about 60% reduction in hTERT protein expression following EGCG treatment at 48 and 72 h compared to that of non-EGCG treated control samples. The marked inhibition of telomerase activity and hTERT protein expression by EGCG treatment seems to be associated with the marked induction of apoptosis in MCF-7 cells.
Since ~90% of breast cancers exhibit telomerase activity and activation of telomerase is an early event in breast cancer progression, the development of chemoprevention strategies that specifically target telomerase is of great interest. Chemoprevention is considered as a promising strategy to control cancer (34), and many natural or dietary substances have been shown to inhibit experimental carcinogenesis (35). EGCG is one of the dietary chemopreventive phytochemicals that has attracted great interest because of its remarkable multifunctional inhibitory effects on in vitro and in vivo models of carcinogenesis (16,36,37). EGCG is one of the dietary chemopreventive phytochemicals that has attracted great interest because of its remarkable multifunctional inhibitory effects on in vitro and in vivo models of carcinogenesis (16,36,37). EGCG has recently been demonstrated to inhibit breast cancer cell proliferation by estrogen receptor mediated effects (38). MCF-7 cells are estrogen receptor positive and thus, there is a strong possibility that the manifestation of EGCG as an anticancer agent and telomerase regulator could be estrogen-receptor mediated and interdependent. Importantly, EGCG treatment has no adverse effect on normal mammary gland cells (17).

The data obtained in this study provide evidence that EGCG inhibits telomerase activity at the transcriptional as well as at the post-transcriptional levels, thereby leading to its anti-carcinogenic effect in human breast carcinoma MCF-7 cells. We also observed that the telomerase activity and the telomerase protein were decreased more than the mRNA levels. Possibly, this effect may be corroborated to the fact that the activity of the catalytic subunit of the telomerase, hTERT, is regulated by various post-transcriptional mechanisms like binding to heat shock proteins (hsp90) and involvement of PI3/Akt protein (39). The results of this study suggest that down regulation of telomerase by EGCG treatment leads to the suppression of cell viability and induction of apoptosis in MCF-7 cells, thus providing the molecular basis for the development of EGCG as a pharmacologically safe agent for breast cancer prevention among high-risk population.

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References


