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A Major Constituent of Green Tea, EGCG, Inhibits the Growth of a Human Cervical Cancer Cell Line, CaSki Cells, through Apoptosis, G_1 Arrest, and Regulation of Gene Expression

WOONG SHICK AHN,[1] SEUNG WON HUH,[2] SU-MI BAE,[2] INSU P. LEE,[2] JUN MO LEE,[1] SUNG EUN NAMKOONG,[1] CHONG KOOK KIM,[3] and JEONG-IM SIN[2]

ABSTRACT

A constituent of green tea, (−)-epigallocatechin-3-gallate (EGCG) has been known to possess antiproliferative properties. In this study, we investigated the anticancer effects of EGCG in human papillomavirus (HPV)-16 associated cervical cancer cell line, CaSki cells. The growth inhibitory mechanism(s) and regulation of gene expression by EGCG were also evaluated. EGCG showed growth inhibitory effects in CaSki cells in a dose-dependent fashion, with an inhibitory dose $(ID)_{50}$ of approximately 35 μM. When CaSki cells were further tested for EGCG-induced apoptosis, apoptotic cells were significantly observed after 24 h at 100 μM EGCG. In contrast, an insignificant induction of apoptotic cells was observed at 35 μM EGCG. However, cell cycles at the G1 phase were arrested at 35 μM EGCG, suggesting that cell cycle arrests might precede apoptosis. When CaSki cells were tested for their gene expression using 384 cDNA microarray, an alteration in the gene expression was observed by EGCG treatment. EGCG downregulated the expression of 16 genes over time more than twofold. In contrast, EGCG upregulated the expression of four genes more than twofold, suggesting a possible gene regulatory role of EGCG. This data supports that EGCG can inhibit cervical cancer cell growth through induction of apoptosis and cell cycle arrest as well as regulation of gene expression *in vitro*. Furthermore, *in vivo* antitumor effects of EGCG were also observed. Thus, EGCG likely provides an additional option for a new and potential drug approach for cervical cancer patients.

INTRODUCTION

CERVICAL CANCER IS AN IMPORTANT CAUSE of death in women worldwide. It is caused mostly by infection with human papillomavirus (HPV) (Yu *et al.*, 1995; Ji *et al.*, 1997). After high-risk HPV infection, two viral oncogenic proteins, E6 and E7, play a critical role in inducing cervical cancers by interacting with p53 and pRb for inactivation of these cellular regulatory proteins, respectively (Scheffner *et al.*, 1990; Werness *et al.*, 1990). Presently, the surgical and radiation therapies have been approached with limited success. Furthermore, an early detection of cervical cancer using the Pap smear has contributed to decreased incidence of cervical cancers. Despite the great progress in treating cervical cancers in the last 3 decades, recurrent or persistent cervical cancers have been problematic, adding the importance of developing anticervical cancer drugs.

Tea is one of the most widely consumed beverages. Its possible beneficial properties have received increased attention. Consumption of green tea has been reported to lower the risk of developing gastric, pancreatic, and colorectal cancers in human populations (Yu *et al.*, 1995; Ji *et al.*, 1997). Furthermore, antiproliferative effects of green tea preparations have been demonstrated in many cancer cell lines, including those involving liver, forestomach, skin, lung, and esophagus (Chen, 1992; Graham, 1992; Yamane *et al.*, 1996; Khafif *et al.*, 1998). The anticarcinogenic and antiproliferative effects of tea have

[1]Department of Obstetrics and Gynecology, [2]Catholic Research Institutes of Medical Science, College of Medicine, The Catholic University of Korea, Seoul, Korea.
[3]College of Pharmacy, Seoul National University, Seoul, Korea.

been attributed to the biological properties of green tea polyphenolic compounds. The polyphenols account for up to 30% of the dry weight of green tea and include flavanols, flavandiols, flavonoids, and phenolic acids (Graham, 1992). In particular, flavanols, known as catechins, are a major component of most green tea. The catechins are composed of (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)epicatechin-3-gallate (ECG), (−)-epicatechin (EC), (+)-gallocatechin (GC), and (+)-catechin (C). Among these, EGCG, a major constituent of catechins, has been shown to be the most effective for inhibiting tumor cell growth as well as inducing apoptosis (Ahmad *et al.*, 1997; Asano *et al.*, 1997). In animal model systems, EGCG reduces the size of human breast and prostate tumors in nude mice (Liao *et al.*, 1995). EGCG has also demonstrated anticarcinogenic activities in animal models including those for skin, lung, gastrointestinal tract cancers (Katiyar *et al.*, 1992; Yang and Wang, 1993). Inhibition of lung metastasis with mouse melanoma cells was also demonstrated by EGCG treatment in mice (Taniguchi *et al.*, 1992). *In vitro* studies have also shown that EGCG can inhibit the growth of human mammary and lung cancer cells (Komori *et al.*, 1993), prostate cancer cells (Paschka *et al.*, 1998), lymphoma cells (Ahmad *et al.*, 1997), leukemic cells (Otsuka *et al.*, 1998), and lung and colon cancer cell lines (Yang *et al.*, 1998). However, no studies on cervical cancer cells have been reported.

In this study, we investigate whether a major green tea constituent, EGCG possesses growth inhibitory properties in an HPV 16-associated human cervical carcinoma cell line, CaSki cells. We observed that EGCG possesses antigrowth effects in CaSki cells in a dose-dependent fashion. This growth inhibition appears to be mediated by apoptosis, cell cycle arrests at the G1 phase, and regulation of gene expression, as determined by FACS and cDNA microarray. Furthermore, *in vivo* antitumor effects of EGCG were also observed, suggesting that a green tea component, EGCG, might be beneficial for controlling cervical cancer clinically.

MATERIALS AND METHODS

Cells

CaSki (HPV-16 positive, wild-type p53) cells were obtained from the Korean Cell Line Bank, Seoul National University, Seoul, Korea. All cells were cultured in DMEM supplemented with 5% heat-inactivated FBS, 50 IU/ml penicillin, and 50 IU/ml streptomycin (cDMEM) at 37°C in the 5% CO_2 incubator.

Cell growth inhibition assay

The growth inhibitory effects of EGCG (a kind gift from Dr. Yukihiko Hara of Mutsui Norin Co., Fujied, Japan) in CaSki cells were measured by direct cell counting. EGCG was diluted in 50% DMSO solution in water. For viable cell counting, 5×10^5 cells per well were treated with EGCG ranging from 10 to 100 μM for various times. At the indicated times, cells were counted using a hemacytometer under a microscope after trypsinization. Cell viability was determined by trypan blue dye exclusion assay.

FACS analysis

CaSki cells were treated with 35, 50, and 100 μM of EGCG for 1 to 2 days. The cells were then trypsinized and washed twice with phosphate-buffered saline (PBS). Cells were stained with both Annexin V-FITC conjugate and propidium iodide. Samples were then analyzed using FACS (Becton Dickinson, San Jose, CA). For DNA contents, cell debris and fixation artifacts were gated out and G_0/G_1, S, and G_2/M populations were quantified using the CellQuest program.

RNA and probe DNA preparation

Total RNA was prepared using Trizol reagent (MRC, Cincinnati, OH) according the manufacturer's protocol. For reverse transcription reaction, 50 μg of total RNA were mixed with 1 μl of control mRNA (λ bactriophage mRNA, 0.5 μg/μl) and 1.5 μg of oligo dT primer (1.5 μg) to a final volume of 20.5 μl. The reaction mixture was heated for 5 min at 70°C and the denatured RNA was reacted for 1 h at 42°C in avian myeloblastosis virus (AMV) buffer (dNTP, Cy3 or Cy5-dUTP, RNase inhibitor, AMV reverse transcriptase). The reaction mixture was added with 10 μl of 1 N NaOH for 10 min at 37°C, followed by addition of 25 μl of 1 M Tris-HCl (pH 7.5). Probes were purified using sephacryl S-100 column. Ethanol-precipitated DNA probes were solubilized in 15 μl of hybridization buffer (6 × SSC, 0.2% SDS, 5 × Denhardt solution, 1 mg/ml salmon sperm DNA).

Probe hybridization

The probes were denatured for 2 min at 95°C and then kept on ice. This was centrifuged for 10 min at 14,000 rpm. Subsequently, the supernatant containing probes was added to a 384 cDNA chip (Macrogen, Seoul, Korea) drop wise. Three hundred eighty-four cDNAs (genes and ESTs) were selected from genes showing a significant change in their expression in cancer cells. These include 352 cancer-related genes, one housekeeping gene, and one positive control gene. After putting a cover glass, the hybridization reaction was performed for 12–16 h at 62°C. After hybridization, the slide was washed twice for 30 min in the 60°C washing buffer (2 × SSC, 0.2% SDS). The slide was then dried at the room temperature.

Scanning and data analyses

DNA chips were scanned using Generation III Array Scanner (Amersharm Pharmacia Biotech, Sunnyvale, CA) and then analyzed via ImaGeneTM Version 3.0 (TaKaRa, Kyoto, Japan) according to the manufacturer's protocols.

Tumor challenge assay

CaSki cells (1×10^6) were injected subcutaneously (s.c.) into the right flank of nude mice. The tumor cells were washed two times with PBS and then injected into mice in a final volume of 100 μl. Two days after tumor challenge, animals started to get 35 μM of EGCG in drinking water orally every day. Tumor growth was measured in millimeters using a caliper, and was recorded as mean diameter [longest surface length (a) and width (b), $(a + b)/2$].

RESULTS

EGCG inhibits the growth of CaSki cells in a dose-dependent manner

To determine antitumor effects of EGCG in an HPV 16-associated cervical cancer cell line, CaSki cells, we treated cells with EGCG ranging from 10 to 100 μM. As shown in Figure 1, EGCG showed a significant growth inhibitory effect in the cancer cell line over the incubation times. The inhibition of cell growth was dose dependent. Furthermore, ID_{50} appeared to be 35 μM in CaSki cells.

Induction of apoptotic cell death by EGCG

We were next interested in testing if at increasing doses EGCG can induce apoptosis in CaSki cells. It has been reported that EGCG can induce apoptosis in certain tumor cells (Otsuka *et al.*, 1998; Paschka *et al.*, 1998; Yang *et al.*, 1998; Okabe *et al.*, 1999; Suganuma *et al.*, 1999). As shown in Figure 2, treating cells with EGCG at 100 μM for 24 and 48 h resulted in a significant induction of both apoptotic (PI negative/annexin V positive) and necrotic cells (PI positive/annexin V positive). Furthermore, treatment of cells with EGCG at 50 μM for 48 h resulted in a significant induction of apoptotic cells, but not necrotic cells. At 35 μM for 24 and 48 h, however, EGCG resulted in an insignificant induction (approximately twofold) of apoptotic cells, compared to negative control. The data suggests that EGCG can suppress the growth of CaSki cells through induction of apoptosis.

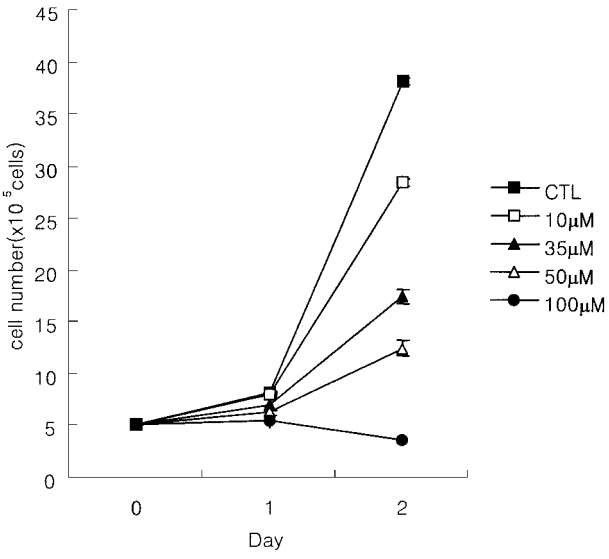

FIG. 1. Dose effects of EGCG on growth inhibition in a cervical cancer cell line, CaSki cells *in vitro*. Cells (5×10^5 cells per well in a 96-well plate) were treated with EGCG ranging in doses from 10 to 100 μM for 1 to 2 days. Cells were then trypsinized for direct cell counting. Values and bars represent mean and SD, respectively. This was repeated twice with similar results.

EGCG-induced cell cycle arrests at the G_1 phase in CaSki cells

We also tested for cell cycle arrests in CaSki cells. To determine if EGCG treatment had any effects on cell cycle perturbations, cell cycle analysis was performed by propidium iodide staining. When treated with 35 μM of EGCG, there was a significant alteration in cell cycle progression in CaSki cells (Fig. 3). In particular, the G_1 phase of cell cycle progression was arrested by EGCG treatment at both 35 and 50 μM. This arrest was detected early at 24 h treatment with EGCG. In contrast, untreated cells showed a normal cell cycle progression. The arrest profiles of EGCG were consistent over time. These collective data suggest that EGCG induces cell cycle arrests at the G_1 phase in CaSki cells.

cDNA microarray analysis of gene regulation by EGCG in CaSki cells

We used 384 cDNA cancer microarray system to determine whether the gene expression of a cervical cancer cell line, CaSki cells could be influenced by the treatment with an ID_{50} of EGCG (35 μM). At least twofold up- or downregulated genes were listed in Table 1. The expression of 16 genes was downregulated more than twofold by both 12- and 24-h treatment with EGCG. These included genes coding for cyclin G-associated kinase, ATPase, NADH dehydrogenase, CD83 antigen, HSPC135 protein, *ras* homolog gene family, dual specificity phosphatase 1, and others. In particular, the expression of dual specificity phosphatase 1 was the most sensitive to EGCG treatment. In contrast, the expression of four genes was upregulated more than twofold by 12, 24, and 48 h treatment with EGCG. The genes were those coding for vimentin, ribosomal proteins L19, mitogen-activated protein kinase type 3, and mitochondrial carrier. Furthermore, some of these genes were regulated commonly by treatment with EGCG at both 10 and 35 μM (Table 2). This suggests that EGCG can influence a gene expression pattern in CaSki cells. Furthermore, it is likely that these regulated genes might possibly be associated with resistance/sensitivity to EGCG in this cell line.

EGCG suppresses tumor growth in nude mice

To determine antitumor effects of EGCG *in vivo*, nude mice were challenged s.c. with CaSki cells and then treated orally with EGCG at 35 μM in drinking water. As shown in Figure 4, EGCG-treated animal groups showed a significant suppression of tumor growth, compared to untreated control groups. Furthermore, in EGCG-treated animals a tumor mass was observed far later than in an untreated control animal group. This suggests that EGCG plays an important role in inhibiting the growth of an HPV 16-associated cervical cancer cell line, CaSki cells *in vivo*.

DISCUSSION

A variety of the chemotherapeutical agents, currently in use for therapy against cervical cancers are known to cause cytotoxicity to normal cells. In this regard, it has been regarded of significance to develop drugs that provide specific cell death to

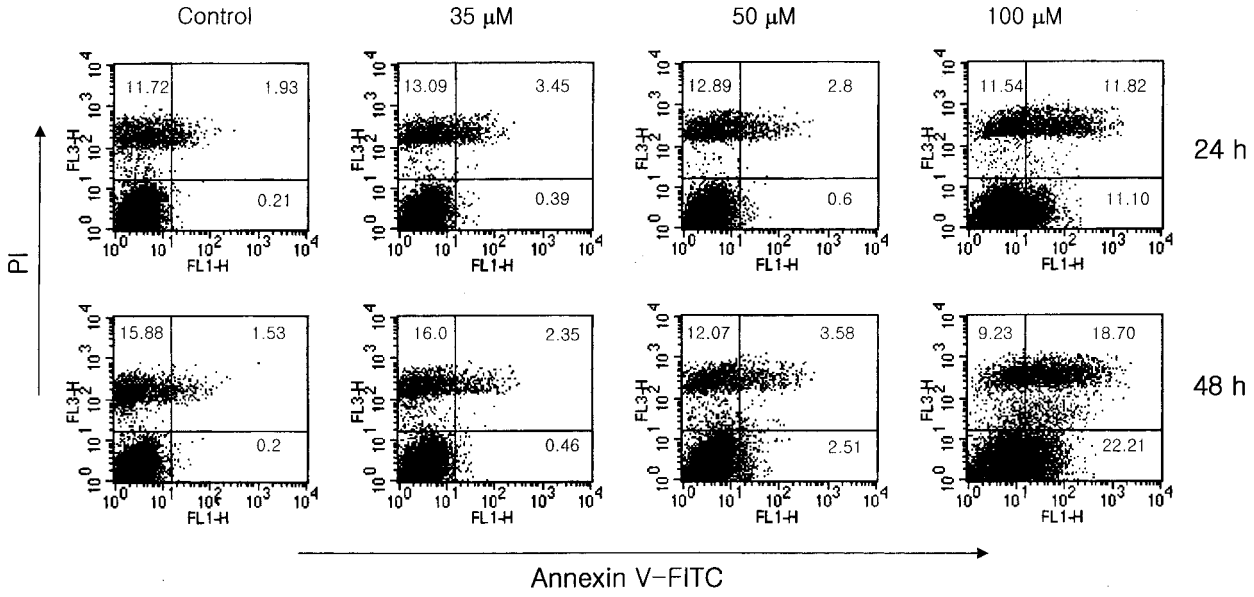

FIG. 2. Effects of EGCG on apoptosis in CaSki cells. Cells (5×10^5 cells per well in a 96-well plate) were treated with 35, 50, and 100 μM of EGCG for 1 and 2 days. For apoptosis and necrosis analyses, cells were trypsinized and washed twice with PBS, followed by staining with Annexin V-FITC and propidium iodide. Stained cells were analyzed via FACS analysis. This was repeated twice with similar results.

cancer cells. The growth inhibitory effects of green tea have been reported in many tumor cell lines (Stoner and Mukhtar, 1995; Otsuka *et al.*, 1998; Paschka *et al.*, 1998; Yang *et al.*, 1998; Okabe *et al.*, 1999; Suganuma *et al.*, 1999). Our observation also demonstrated that a major green tea component, EGCG possesses growth inhibitory activities against a cervical cancer cell line, CaSki cells. This is consistent with the previous reports in other cancer cell lines (Otsuka *et al.*, 1998; Paschka *et al.*, 1998; Yang *et al.*, 1998; Okabe *et al.*, 1999; Suganuma *et al.*, 1999). We also observed that an ID_{50} dose of

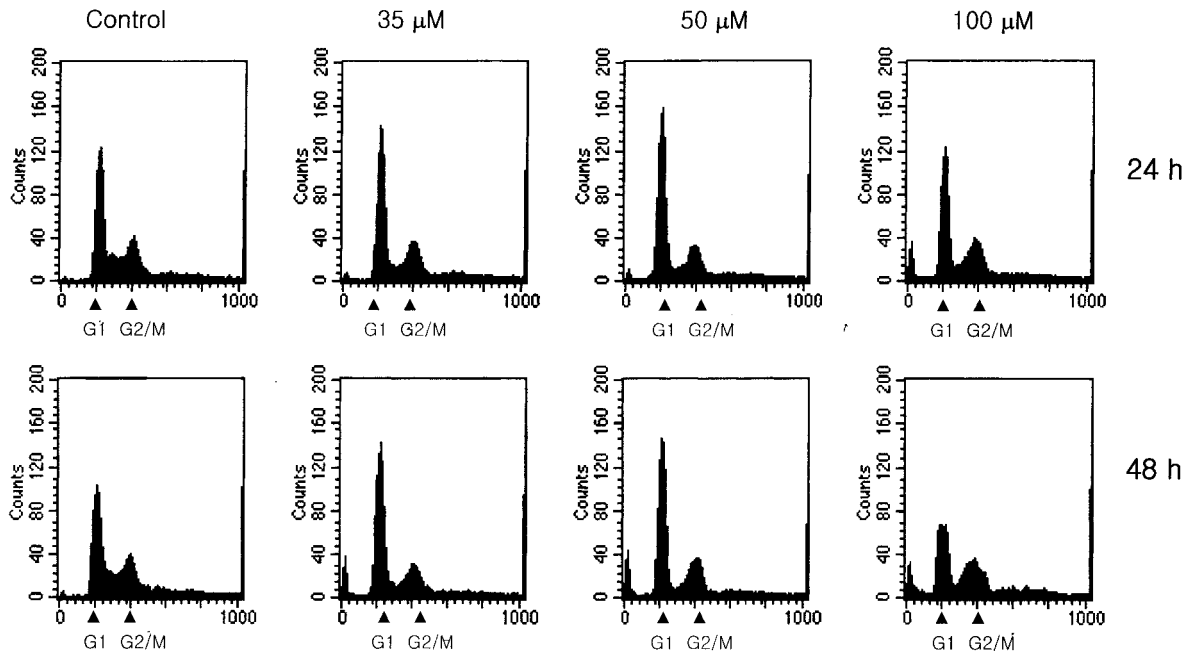

FIG. 3. Cell cycle arrests at the G_1 phase in CaSki cells by EGCG. Cells (5×10^5 cells per well in a 96-well plate) were treated with 35, 50, and 100 μM of EGCG for 1 and 2 days. Cells were trypsinized and washed twice with PBS. Cell cycle distribution was determined by propidium iodide staining. This was repeated twice with similar results.

TABLE 1. LISTS OF GENES/ESTS AT LEAST TWOFOLD REGULATED IN COMMON BY EGCG EXPOSURE TIMES IN CASKI CELLS

	Fold decrease		
Downregulated genes/proteins	*12 h*	*24 h*	*48 h*
1 Hs. 153227 cyclin G-associated kinase	2.0	2.1	<2
2 Hs. 183434 ATPase	2.3	2.1	<2
3 Hs. 19236 NADH dehydrogenase (ubiquinone) 1 beta	2.4	2.1	2.2
4 Hs. 4766 DKFZP586O0120 protein	2.6	3.6	2.0
5 Hs. 79197 CD83 antigen (activated B lymphocytes)	2.6	3.3	2.2
6 Hs. 127496 HSPC135 protein	2.6	2.1	2.0
7 Hs. 15087 KIAA0250 gene product	2.7	2.0	<2
8 Hs. 98693 DKFZP586J0917 protein	2.8	3.4	2.8
9 Hs. 1861 membrane protein, palmitoylated 1 (55 kDa)	2.9	3.4	<2
10 Hs. 75082 *ras* homolog gene family, member G (rho G)	2.9	3.4	2.2
11 Hs. 115740 KIAA0210 gene product	3.0	4.1	2.7
12 Hs. 118978 KIAA0256 gene product	3.1	2.4	<2
13 Hs. 278441 KIAA0015 gene product	3.1	2.1	<2
14 Hs. 72157 DKFZP564I1922 protein	3.2	3.1	
15 Hs. 16165 expressed in activated T/LAK lymphocytes	3.2	4.1	<2
16 Hs. 171695 dual specificity phosphatase 1	4.3	5.9	2.2
	Fold increase		
Upregulated genes/protein	*12 h*	*24 h*	*48 h*
1 Hs. 78713 solute carrier family 25 (mitochondrial carrier)	2.0	2.0	2.2
2 Hs. 2064 vimentin	2.1	2.5	2.7
3 Hs. 75879 ribosomal protein L19	2.3	2.2	2.0
4 Hs. 227400 mitogen-activated protein kinase kinase kinase kinase 3	2.7	2.0	2.1

Cells were treated with 35 μM of EGCG for 12, 24, and 48 h. Probes were synthesized from mRNAs of drug-treated cells (cy5) or nontreated cells (cy3) for hybridization. The genes or ESTs showing more than twofold difference in their expression in common over time were selected from a total of 384 genes. Values represent the number of fold regulation.

EGCG was approximately 35 μM. This dose is similar to that of EGCG in other cancer cells (Otsuka *et al.*, 1998; Yang *et al.*, 1998; Okabe *et al.*, 1999). The ability of EGCG to exert its effect at such a low concentration might be of importance as we consider EGCG to be a chemotherapeutic agent for cervical cancers. Furthermore, it has also been reported that there exists a threshhold level of EGCG that induces apoptosis and growth inhibition in cancer cell lines, but not in normal cells (Ahmad *et al.*, 1997; Chen *et al.*, 1998).

From the epidemiological point of view, the development of

TABLE 2 LISTS OF GENES/ESTS AT LEAST TWOFOLD REGULATED UPON EGCG TREATMENT AT BOTH 10 AND 35 μM IN CASKI CELLS

	EGCG	
Genes/proteins	*10 μM*	*35 μM*
Downregulated genes/ESTs		
1 Hs. 171695 dual specificity phosphatase 1	3.29	2.45
2 Hs. 15087 KIAA0250 gene product	3.23	2.14
3 Hs. 4766 DKFZP586O0120 protein	3.11	3.12
4 Hs. 79197 CD83 antigen (activated B lymphocytes)	3.06	3.11
5 Hs. 75082 *ras* homolog gene family, member G (rho G)	2.82	2.78
6 Hs. 98693 DKFZP586J0917 protein	2.69	2.74
7 Hs. 16165 expressed in activated T/LAK lymphocytes	2.38	3.45
Upregulated genes/ESTs		
1 Hs. 75879 ribosomal protein L19	2.62	2.85
2 Hs. 227400 mitogen-activated protein kinase kinase kinase kinase 3	2.27	2.45
3 Hs. 2064 vimentin	2.12	3.60

Cells were treated with 10 and 35 μM of EGCG for 24 h. Probes were synthesized from mRNAs of drug-treated cells (cy5) or non-treated cells (cy3) for hybridization. The genes or ESTs showing more than 2 fold difference in their expression in common at both doses were selected from a total of 384 genes. Values represent the number of fold regulation.

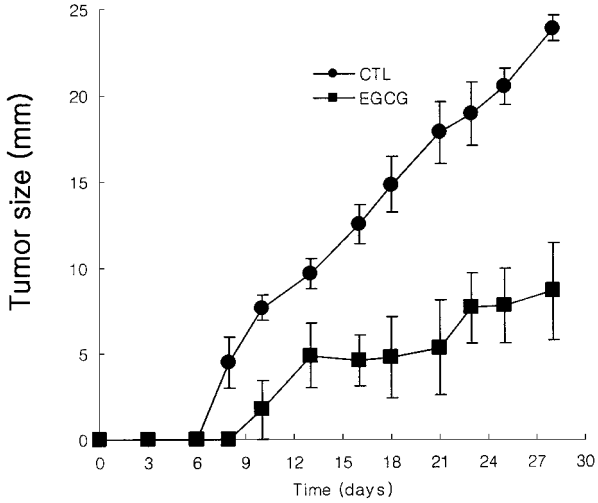

FIG. 4. Suppression of tumor growth by oral delivery with EGCG. Nude mice ($n = 4$) per group were challenged s.c. with CaSki cells in a final volume of 100 μl PBS. Two days after the tumor challenge, mice were orally fed with 35 μM EGCG in drinking water. Values represent mean and SD, respectively. This was repeated once with similar results.

invasive cervical cancer from cervical intraepithelial neoplasm takes at least 10–15 years, during which administration of EGCG or high consumption of green tea could either reduce the incidence of cervical cancer or delay the onset of cancer. It has been reported that the concentration of EGCG in the blood after drinking 1.2 g of green tea reaches a maximum of 0.6 μM in humans (Lee *et al.*, 1995; Unno *et al.*, 1996). However, the average dose of EGCG required for inhibiting tumorigenesis is in the range of 0.2 to 0.3 μM in animals (Yang and Wang, 1993). Yamane *et al.* (1996) reported that a high dose of green tea consumption did not show any harmful side effects in humans, suggesting that optimal effect can be achieved by increasing the dosage of EGCG. We also observed that oral consumption of 35 μM EGCG leads to a significant reduction of tumor mass as well as a delay in tumor formation by CaSki cells in nude mice. Furthermore, no phenotypic side effects were observed. This supports the idea that EGCG might be applicable for chemotherapy against cervical cancer. This is further supported by studies of Japanese groups showing that green tea is a cancer-preventive agent that is effective both before cancer onset and after cancer treatment in breast cancer populations (Nakachi *et al.*, 1998; Suganuma *et al.*, 1999). Moreover, oral delivery with EGCG resulted in decreased growth of cervical cancer cells in animals, suggesting its importance role in suppression of cervical cancer cell growth *in vivo*. Liang and his group also reported using a melanoma animal model that the lung metastasis of melanoma is reduced by EGCG treatment (Liu *et al.*, 2001).

Antitumor effects of EGCG have been reported to be mediated by apoptosis (Ahmad *et al.*, 1997; Paschka *et al.*, 1998; Yang *et al.*, 1998; Kennedy *et al.*, 2001), inactivation of transcription factors (Okabe *et al.*, 1999), inhibition of urokinase and mitogen-activated protein kinase activities (Jankun *et al.*, 1997), suppression of lipooxygenase and cyclooxygenase activities (Stoner and Mukhtar, 1995), and G_1 arrest of the cell cycle (Ahmad *et al.*, 1997, 2002). EGCG has also been reported to be incorporated into the phospholipid bilayer membrane, leading to blocking the tumor promoters from binding to their receptors within the cell membrane as a possible mechanism of EGCG for tumor growth inhibition (Otsuka *et al.*, 1998; Okabe *et al.*, 1999). Kennedy *et al.* recently reported that green tea extract causes cytochrome *c* release and caspase activation for induction of apoptosis (Kennedy *et al.*, 2001). More recently, it has been reported that EGCG inhibits the activity of topoisomerase I, which plays a critical role in DNA replication, transcription, and chromosome condensation (Berger *et al.*, 2001). EGCG also inhibits tumor cell growth by suppressing vascular endothelial growth factor (VEGF) induction in human colon cancer cells (Jung *et al.*, 2001). Taken together, it is likely that EGCG inhibits cancer cell growth through many different regulatory pathways, along with apoptosis and cell cycle arrests. Our observation also confirmed that EGCG induced apoptosis and cell cycle arrests in the G_1 phase in CaSki cells. This is consistent with previous findings in other cancer cell types (Ahmad et al., 1997, 2000, 2002; Paschka *et al.*, 1998; Yang *et al.*, 1998; Kennedy *et al.*, 2001).

Recent development of cDNA microarray technology has allowed us to monitor expression profiles of thousands of genes at once. A generated fingerprint of gene expression profiles could be useful for elucidating cellular changes upon any anticancer drug treatments. This likely sheds light on the determining effects of chemotherapeutic regimen on tumor suppression. We used a 384-cDNA cancer chip to further investigate gene expression patterns of CaSki cells by 35-μM EGCG treatment. Upon EGCG treatment, 16 genes were downregulated, but four genes were upregulated more than twofold over time, compared to nontreated controls. This suggests that EGCG regulates the specific gene expression that might be associated with resistance/sensitivity to EGCG in this CaSki cell line. In particular, cyclin G-associated kinase gene expression was significantly downregulated by EGCG treatment, supporting the arrests of cell cycle progression at the G_1 phase. Cyclins, known as a cell cycle regulator, bind to and activate cyclin-dependent kinases (CDKs), which are also regulators of the cell cycle in eukaryotic cells. Activation and subsequent inactivation of cyclins and CDKs are important for control of the cell cycle (Jeffrey *et al.*, 1995; Sherr, 1996; Dynlacht, 1997). It is likely that inhibition of cyclin G-associated kinase gene expression might play a part in arresting cells in the G_1 phase by interrupting transition to the S phase by activated cyclin G. Furthermore, we observed cell cycle arrests at the G_1 phase after 1-day treatment with 35 μM of EGCG. At this condition, however, apoptosis was insignificantly induced, suggesting that cell cycle arrests might precede apoptosis. The genes encoding proteins involved in cellular metabolism, such as ATPase and NADH dehydrogenase, were also downregulated by EGCG (35 μM), suggesting that EGCG-treated cells might lose some cellular enzyme functions required for cell growth. At 10 μM, however, such a downregulation was not observed, suggesting that the expression of these genes is EGCG dose-dependent. RNA polymerase II gene was also downregulated twofold only at 24-h treatment with 35 μM EGCG (data not shown), suggesting its reduced function

in early EGCG treatment stages. In particular, a gene encoding the regulatory protein, dual specificity phosphatase 1 was more significantly downregulated by 35 μM and even by 10 μM EGCG treatment. This suggests that EGCG might be involved in deregulation of cellular signal pathways. In contrast, four genes were upregulated by EGCG treatment. In particular, three out of four genes were upregulated more than twofold upon both 10 and 35-μM EGCG treatment, suggesting a possible importance of these gene products in cellular metabolisms.

In sum, we observe that a major green tea component, EGCG, suppresses the cervical cancer cell growth *in vitro* through induction of apoptosis and cell cycle arrests at the G_1 phase. EGCG also has an ability to influence gene expression in cervical cancer cells, as determined by cDNA microarray, and furthermore *in vivo* antitumor effects of EGCG were observed. Taken together, this data supports the idea that EGCG likely provides an additional option for a new and potential drug approach for cervical cancer patients.

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Address reprint requests to:
Jeong-Im Sin, Ph.D.
#6004-2, Cancer Research Center
Catholic Research Institutes of Medical Science
The Catholic University of Korea
505 Banpo-Dong, Seocho-Ku
Seoul, 137-040 South Korea

E-mail: jsin1964@hanmail.net

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