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Original Article

Epicatechins Purified from Green Tea (*Camellia sinensis*) Differentially Suppress Growth of Gender-Dependent Human Cancer Cell Lines

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The anticancer potential of catechins derived from green tea is not well understood, in part because catechin-related growth suppression and/or apoptosis appears to vary with the type and stage of malignancy as well as with the type of catechin. This *in vitro* study examined the biological effects of epicatechin (EC), epigallocatechin (EGC), EC 3-gallate (ECG) and EGC 3-gallate (EGCG) in cell lines from human gender-specific cancers. Cell lines developed from organ-confined (HH870) and metastatic (DU145) prostate cancer, and from moderately (HH450) and poorly differentiated (HH639) epithelial ovarian cancer were grown with or without EC, EGC, ECG or EGCG. When untreated cells reached confluency, viability and doubling time were measured for treated and untreated cells. Whereas EC treatment reduced proliferation of HH639 cells by 50%, EGCG suppressed proliferation of all cell lines by 50%. ECG was even more potent: it inhibited DU145, HH870, HH450 and HH639 cells at concentrations of 24, 27, 29 and 30 μM, whereas EGCG inhibited DU145, HH870, HH450 and HH639 cells at concentrations 89, 45, 62 and 42 μM. When compared with EGCG, ECG more effectively suppresses the growth of prostate cancer and epithelial ovarian cancer cell lines derived from tumors of patients with different stages of disease.

Keywords: Green tea – epicatechin (EC) – epigallocatechin (EGC) – EC 3-gallate (ECG) – EGC 3-gallate (EGCG) – organ-confined – metastatic – prostate cancer – epithelial ovarian cancer – viability – doubling time – 50% inhibitory concentration (IC50)

Introduction

There is accruing evidence that green tea may have anticancer activity (1), but the mechanisms for this action are poorly understood. Green tea is produced from the shrub *Camellia sinensis* (Fig. 1); leaves are dried but not fermented so that the green coloration attributed to polyphenols is retained. Commercially prepared green tea extracts contain ∼60% polyphenols (1). These polyphenols are the source of bioflavonoids, which have strong antioxidant activity.

The major bioflavonoids in green tea are epicatechins. Like all bioflavonoids, the tea catechins have three hydrocarbon rings; hydroxyl molecules are found at the 3, 5, and 7 positions (Fig. 2). The four major tea catechins are epicatechin (EC), EC 3-gallate (ECG), epigallocatechin (EGC) and EGC 3-gallate (EGCG). The relative proportions of EC, ECG, EGC and EGCG in non-decaffeinated green tea are 792 ± 3, 1702 ± 16, 1695 ± 1 and 8295 ± 92 mg 100 g^{-1} dry wt, respectively; corresponding proportions in non-decaffeinated black tea are 240 ± 1, 761 ± 4, 1116 ± 24 and 1199 ± 0.12 mg 100 g^{-1} dry wt (1).

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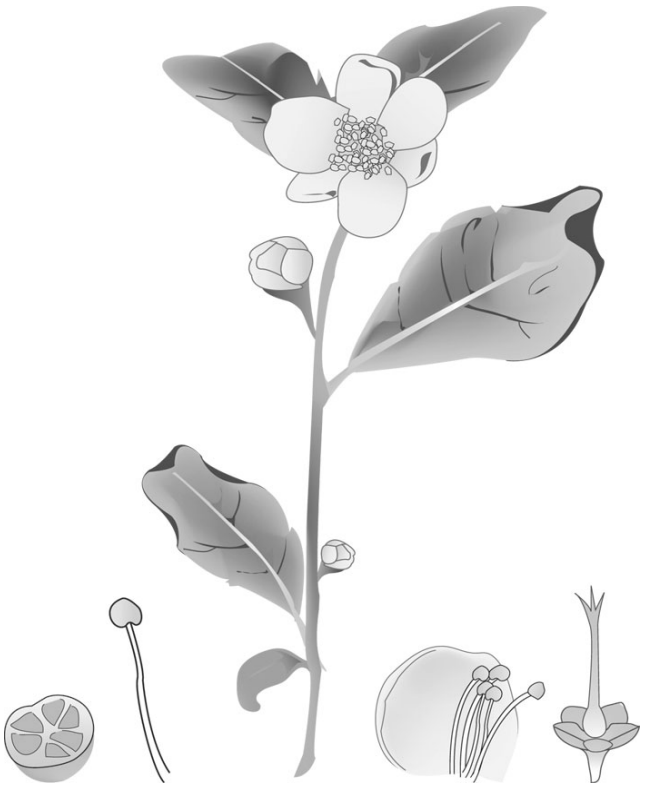


Figure 1. Morphology of Green Tea, *Camellia sinensis.*

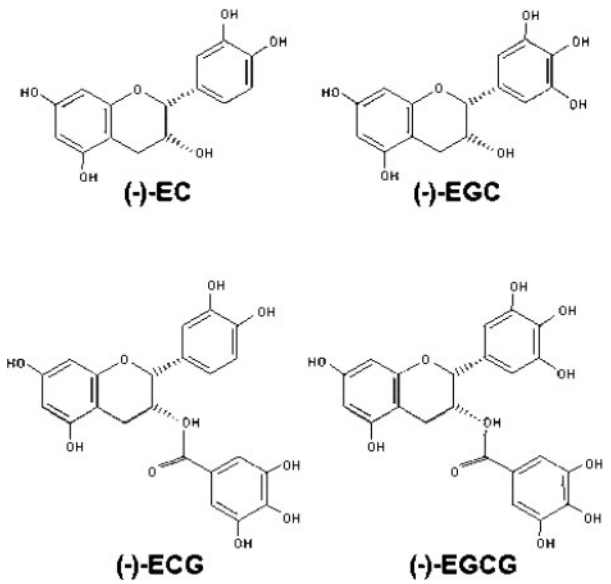


Figure 2. Structure of epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) used in this investigation. All bioflavanoids have three rings; tea catechins are flavan-3-ols with an hydroxyl group at the 3, 5 and 7 positions.

Epicatechins have apparent activity against human cancer: they reportedly may promote apoptosis (2–6), arrest metastasis by inhibiting metalloproteinases (7,8), impair angiogenesis (9,10) and reverse multidrug resistance (11,12). Although all epicatechins except EC can potentially suppress cell proliferation (13–18), EGCG appears the most promising and is therefore under clinical investigation in chemoprevention trials (19). However, given the wide range in physiologic potency of the different catechins, an exclusive focus on EGCG is probably short-sighted. EGCG is reportedly more effective than EGC in decreasing the intestinal absorption of cholesterol (20) and it is the most potent catechin inhibitor of HIV-1 reverse transcriptase (21), but ECG has the strongest collagenase inhibitory effect (22) and the highest antioxidant potential (23). By contrast, only EGC is a potent mediator of oxidative modification and an inhibitor of xanthine oxidase during hepatic catabolism of purines (24).

We hypothesized that the *in vitro* anticancer action of the various catechins varies with the type and stage of malignancy. We tested this hypothesis by examining proliferation of catechin-treated cell lines derived from organ-confined or metastatic prostate cancer (CaP) and from moderately or poorly differentiated epithelial ovarian cancer (EOC). The goal was to obtain data that would be useful for developing chemopreventive and therapeutic clinical trials in patients with gender-specific and non-specific solid tumors.

Materials and Methods

Human Cancer Cell Lines

Four gender-specific human cancer cell lines were used. The HH870 androgen-receptor-negative CaP cell line was developed at Hoag Cancer Center, Newport Beach, CA, from an organ-confined primary tumor that had been resected from a 56-year-old, previously untreated Caucasian (25). This tumor was Gleason Grade 3/4, with no evidence of vascular or perineural invasion or extracapsular extension (stage T2b). The DU145 metastatic CaP cell line (American Type Culture Collection line HTB-81) was derived from a brain lesion of 69-year-old male Caucasian. It is androgen insensitive and does not express prostate-specific antigen. Two EOC cell lines developed at Hoag Cancer Center were also used: HH639 was from a poorly differentiated clear cell, Grade 3 carcinoma in the omentum and left ovary of a 56-year-old Caucasian female; HH450 was from moderately differentiated metastatic cells recovered from the abdominal fluid of a 52-year-old Asian female.

All four cell lines were cryopreserved in liquid nitrogen freezer at -70^{o}C. For recovery of cryopreserved cells, the vials were transferred to a 37^{o}C water bath for 15–30 s, further thawed at room temperature and then transferred to a 15 ml polypropylene tube with a Pasteur pipette. An aliquot of 9 ml of RPMI-9% fetal bovine serum (FBS) was added in drops. The cells were allowed to settle for 5 min and then centrifuged at 4^{o}C for 10 min at 300 g. Supernatant was removed, and cells were suspended in fresh RPMI, gently tapped and vortexed. Cell viability was monitored by 0.2% trypan blue dye exclusion, and cell count was determined using a hemocytometer.

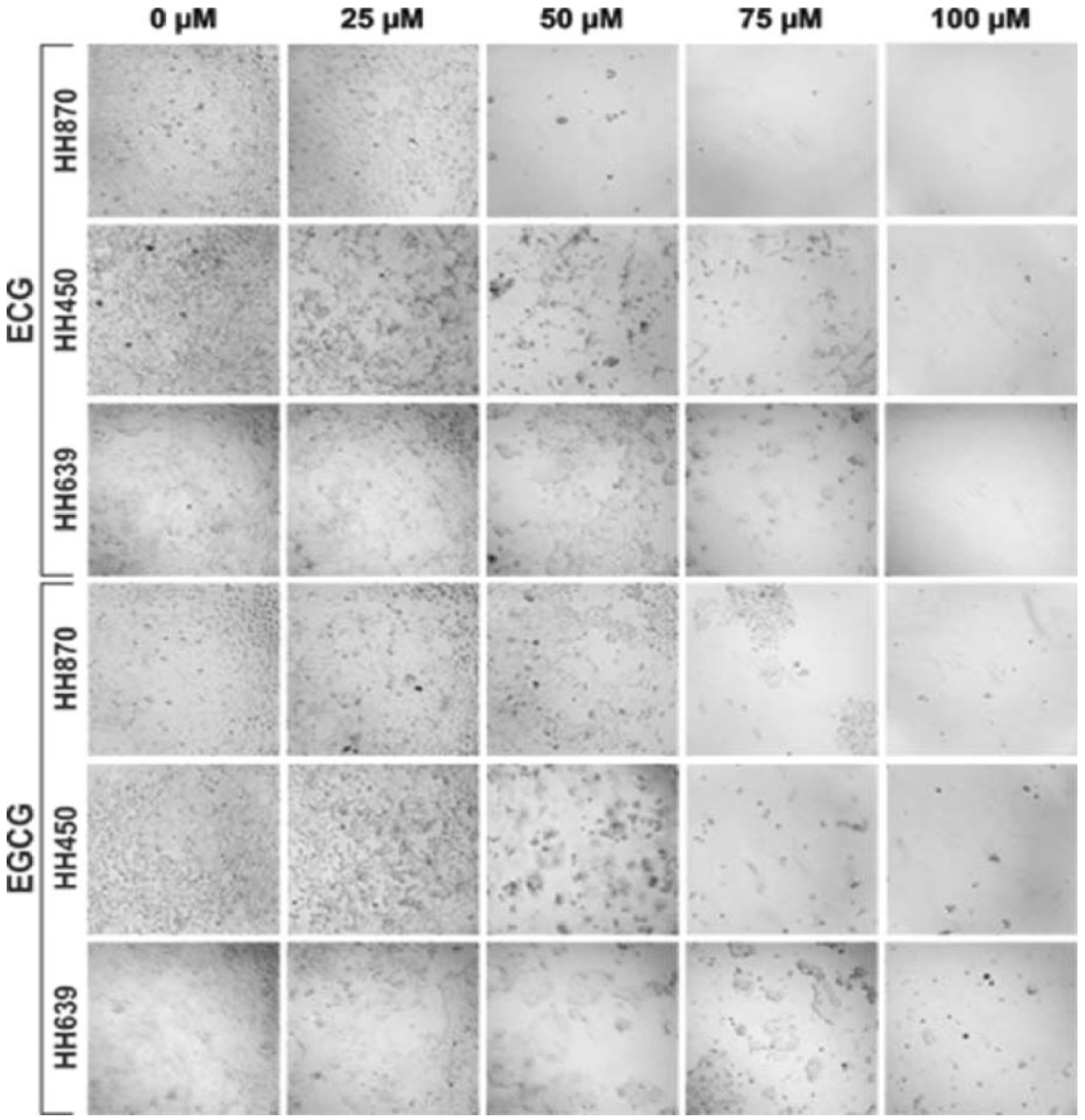


Figure 3. Photomicrographs of cells grown in culture with 0, 25, 50, 75 or 100 μM of ECG or EGCG. Organ-confined prostate cancer cell line HH870 and primary and metastatic epithelial ovarian cancer cell lines (HH450 and HH639) were seeded (2.5×10^5 cells per line) into flasks containing culture medium (RPMI-1640 with 9% FBS-antibiotics) with or without ECG or EGCG. When growth of untreated (control) cells reached confluency, cell monolayers in each flask were photographed under a light microscope. Both ECG and EGCG significantly affected the density of each cell line. Decrease in cell density was observed at higher concentrations of ECG and EGCG. Magnification: ×100.

Cells recovered from cryovials were grown in RPMI-1640 with glutamine (Invitrogen, Carlsbad, CA) supplemented with 9% FBS, HEPES buffer, gentamycin (5 mg%) and fungizone (0.5 mg%), at 37°C in a humidified atmosphere of 5% CO_2. Upon confluency, cells were detached with sterile EDTA-dextrose (137 mM sodium chloride, 5.4 mM potassium chloride, 5.6 mM dextrose, 0.54 mM ethylene diamine tetra acetate (EDTA), 7.1 mM sodium bicarbonate) at 37°C for 5–15 min (or ~45 min for HH639), recovered with cold RPMI-1640-9% FBS and resuspended in the same medium. Use of trypsin was avoided for harvesting the cells. Cell viability and cell count were reassessed before cells were seeded in culture flasks.

Tea Epicatechins

All epicatechins used in this study (Fig. 2) were obtained from Sigma (EC, Sigma E4018; FW 290.3; ECG , Sigma E3892, FW 442.4; EGC, Sigma E3768, FW 306.3; EGC, Sigma E4143, FW 458.4) and were 98% pure as assessed by high-performance liquid chromatography (by the commercial source). Stock solutions were prepared under sterile conditions

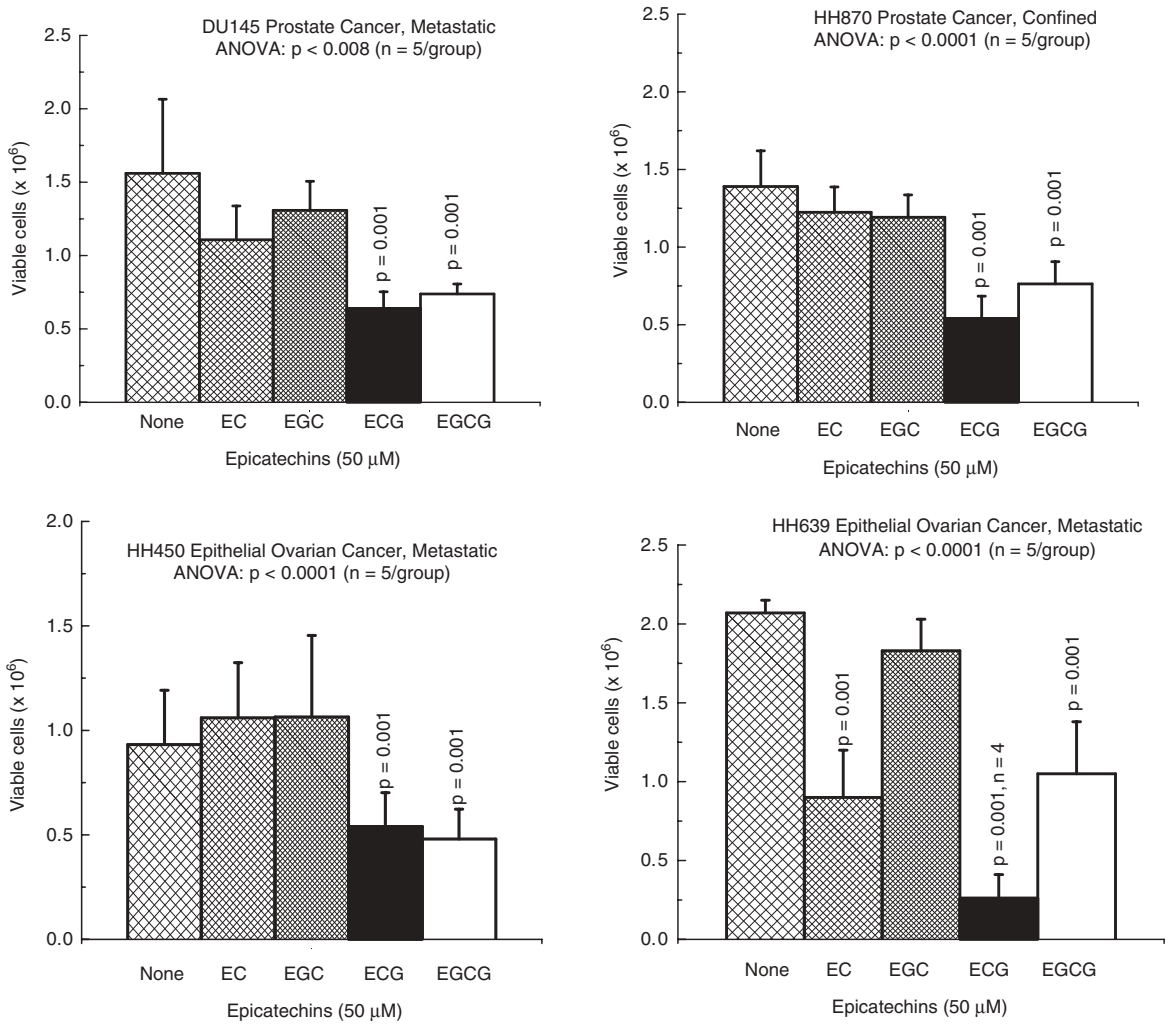


Figure 4. Density of cancer cells seeded (2.5×10^5 cells per line) into flasks containing culture medium (RPMI-1640 with 9% FBS-antibiotics) with or without catechins (50 μM) ($n = 5$ per treatment). When growth of untreated cells reached confluency, cells from each flask were harvested and viable/dead cells were counted. Mean and standard deviation are represented. *P*-values obtained with pairwise comparison and ANOVA are shown.

with 50, 60 or 100 μM of each epicatechin or with no epicatechin (control) in RPMI-1640 with glutamine (Invitrogen), 9% FBS, 0.54% HEPES buffer, gentamycin (5 mg%) and fungizone (0.5 mg%).

Growth Conditions

All experiments used 25 ml sterile polystyrene tissue culture flasks with a vented blue plug seal cap (Beckton Dickinson, Franklin Lakes, NJ, Cat. No. 353107). Each flask contained stock solution with or without epicatechin in concentrations of 50 μM (five flasks for each epicatechin and five flasks for control) and 25, 75 and 100 μM (three flasks for each epicatechin and three flasks for control). Cells (0.25×10^6) suspended in 10 ml of the RPMI-1640-FBS solution described above were transferred to each flask and allowed to grow until control cells reached confluency. The cells were detached with sterile EDTA-dextrose at 37°C for 5 min, recovered with cold RPMI-1640-FBS medium and resuspended in the same medium.

Cells were counted using a hemocytometer; trypan blue dye exclusion was used to determine the number of viable versus dead cells. The interval between seeding and confluent growth of control cells was used to calculate the doubling time and the number of cell cycles. The 50% inhibitory concentration (IC50) of each catechin in each cell line was calculated using a software program (Microcal Origin Corp, OriginLab Corporation, Northampton, MA). The cells were photographed directly from the flask using light microscopy (Olympus IX-70, Japan).

Statistics

Analyses of variance and Fisher's least significant difference (LSD) method were used for pairwise comparisons of values significant at the 0.05 level.

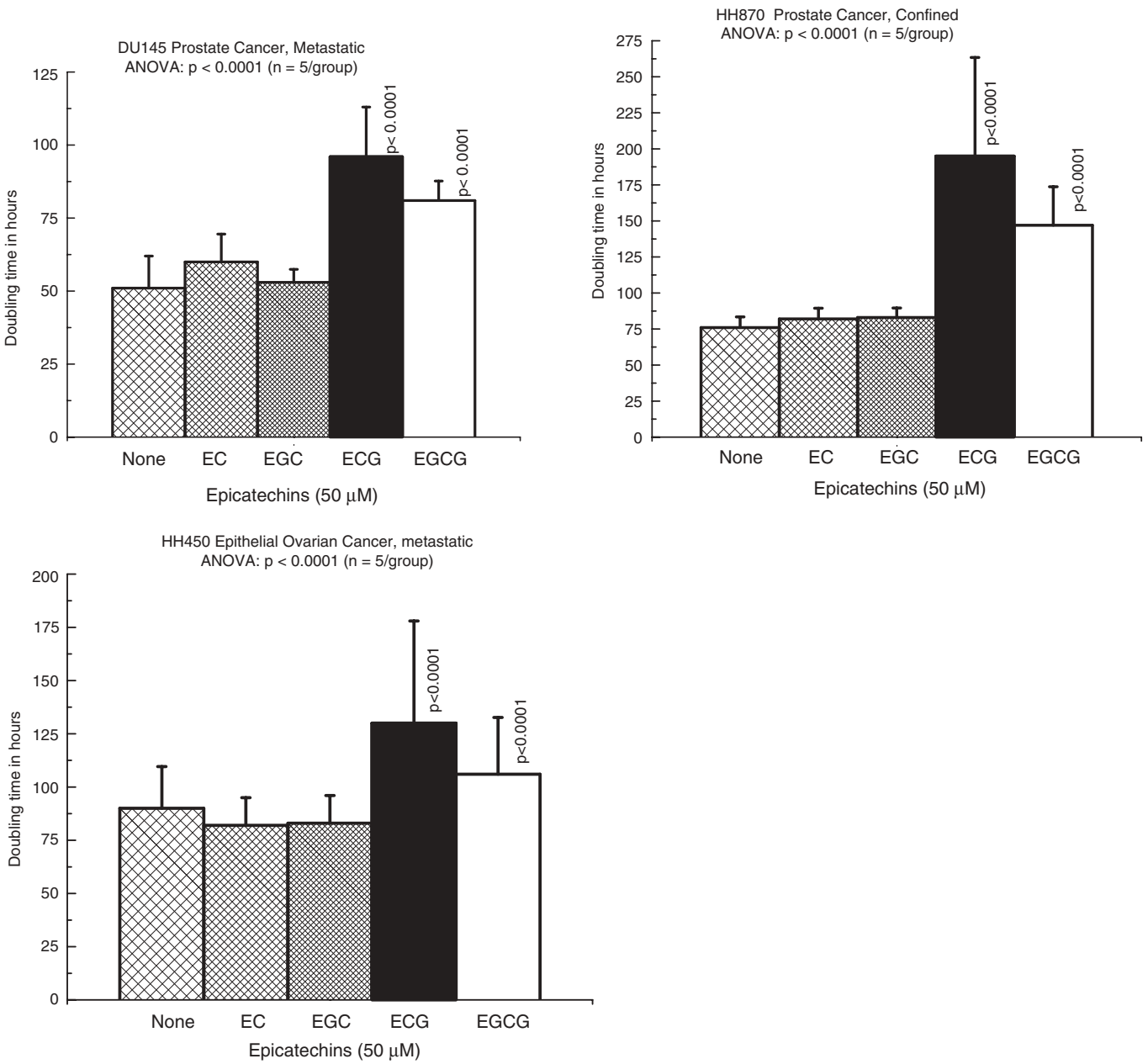


Figure 5. Doubling time of cancer cells seeded (0.25×10^6 per line) into flasks containing culture medium (RPMI-1640 with 9% FBS-antibiotics) with or without catechins (50 μM) ($n = 5$ per treatment). When untreated cells reached confluency, cells from each flask were harvested and viable/dead cells were counted. Vertical bars refer to standard deviation. The mean doubling time was calculated from the mean of five viable cell counts. *P*-values were obtained with pairwise comparison and ANOVA.

Results

ECG as a Better Growth Suppressor Than EGCG: Microscopic Observations

Organ-confined prostate cancer cell line HH870 and primary and metastatic epithelial ovarian cancer cell lines (HH450 and HH639) seeded (2.5×10^5 cells) in flasks with or without various concentrations (25, 50, 75 or 100 μM) of ECG or EGCG were photographed under a light microscope after the untreated control cells reached confluency (Fig. 3). Both ECG and EGCG significantly affected the density of each cell line at or above 75 μM. The decrease in cell density at higher concentrations is much pronounced for ECG than for EGCG, a finding significant considering recommendations of clinical trials with EGCG (19).

ECG Suppresses Viable Cell Density Better Than EGCG

The mean density or viable cell number (in millions) ($n = 5$ per treatment) of different cell lines was examined with or without

Table 1. Four different epicatechins (50 μM) on cell number, cell cycles and mean doubling time of prostate and epithelial ovarian cancer cell lines

Parameters	Control	EC	ECG	EGC	EGCG
Organ-confined prostate cancer (HH870), 186 h* for confluent growth of untreated cells					
Number of flasks	5	5	5	5	5
Initial seeding	0.25×10^6	0.25×10^6	0.25×10^6	0.25×10^6	0.25×10^6
Cell number†	1.39×10^6	1.22×10^6	**0.54×10^6**	1.19×10^6	0.76×10^6
Fold increase (approx.)	>2	>2	**1**	>2	>1
Number of cell cycles	2.5	2	**1**	2	1.5
Dead cell count	0.22×10^6	0.12×10^6	**0.1×10^6**	0.2×10^6	0.16×10^6
Mean doubling time	76 h	82 h	**195 h**	83 h	147 h
Metastatic prostate cancer (DU145), 125 h* for confluent growth of untreated cells					
Number of flasks	5	5	5	5	5
Initial seeding	0.25×10^6	0.25×10^6	0.25×10^6	0.25×10^6	0.25×10^6
Cell number†	1.56×10^6	1.11×10^6	**0.64×10^6**	1.31×10^6	0.74×10^6
Fold increase (approx.)	>2	~2	**>1**	>2	>1
Number of cell cycles	2.5	2	**1.3**	2.4	1.6
Dead cell count	0.45×10^6	0.3×10^6	0.24×10^6	0.34×10^6	**0**
Mean doubling time	51 h	60 h	**96 h**	53 h	81 h
Epithelial ovarian cancer (HH450), 219 h* for confluent growth of untreated cells					
Number of flasks	5	5	5	5	5
Initial seeding	0.16×10^6	0.16×10^6	0.16×10^6	0.16×10^6	0.16×10^6
Cell number†	0.93×10^6	1.06×10^6	0.66×10^6	1.06×10^6	**0.48×10^6**
Fold increase (approx.)	>3	>3	3	>3	**>2**
Number of cell cycles	2.5	2.7	1.9	2.7	**1.5**
Dead cell count	0.66×10^6	0.40×10^6	0.29×10^6	0.46×10^6	**0.14×10^6**
Mean doubling time	90 h	82 h	**130 h**	83 h	106 h
Epithelial ovarian cancer (HH639), 170 h‡ for confluent growth of untreated cells					
Number of flasks	5	5	5	5	5
Initial seeding	0.25×10^6	0.25×10^6	**0.25×10^6**	0.25×10^6	0.25×10^6
Cell number†	2.1×10^6	0.90×10^6	**0.20×10^6***	$1.83 \times 10^{6\dagger}$	1.10×10^6
Fold increase (approx.)	3	~3	**0**	3	2
Number of cell cycles	3	~2	**0**	3	2
Dead cell count	0.27×10^6	0.16×10^6	**0.11×10^6**	0.014×10^6	0.30×10^6
Mean doubling time	56 h	124 h	**0**	51 h	88 h

*Four flasks were counted; †mean viable cell count; ‡three flasks were counted. Significant values are shown in bold.

catechins (50 μM) (Fig. 4). The cell density was measured when growth of untreated cells reached confluency. Statistical analysis by ANOVA as well as by pairwise comparison showed that both ECG and EGCG significantly affected the cell density. ECG decreased the cell density of prostate cancer cells DU145, HH870 and ovarian cancer cell line HH639 more potently than EGCG. But EGCG inhibited the growth of ovarian cancer cell line HH450 better than ECG, suggesting the need to determine relative efficacy of ECG and EGCG in clinical trials for different cancers.

Tumor Cell Doubling Time: ECG versus EGCG

Figure 5 shows the influence of the four epicatechins on cell doubling time. ECG and/or EGCG prolonged the doubling

Table 2. Relative inhibitory potency (IC50) of epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) on organ-confined (HH870) and metastatic (DU145) prostate cancer and ovarian cancer (HH450 and HH639)

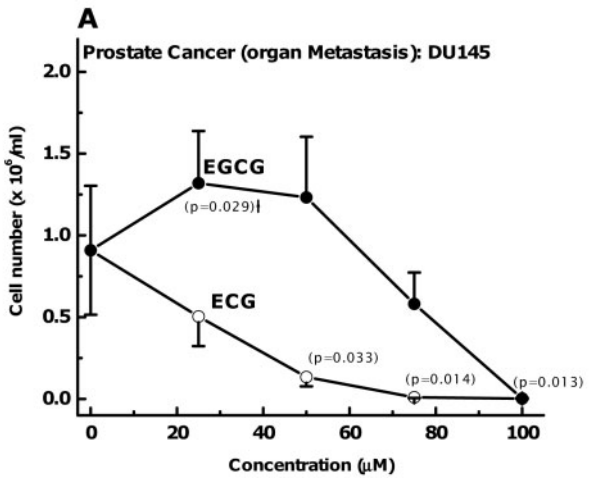
Tumor cell line	IC50 in μM	
	ECG	EGCG
Prostate cancer		
HH870	27.44	45.43
DU145	24.09	88.66
Epithelial ovarian cancer		
HH450	28.95	62.25
HH639	29.59	42.21

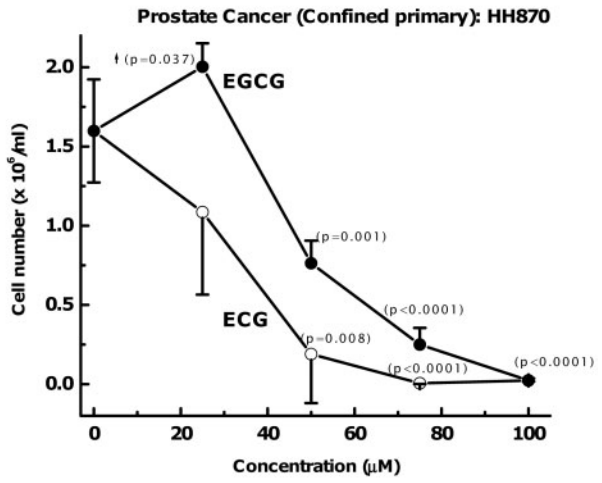
A

	0 μM	25 μM	50 μM	75 μM	100 μM
ECG					
Mean		0.504	0.134	0.010	0.002
SD		0.181	0.057	0.004	0.003
p (two-tail)		0.701	0.033	0.014	0.013
EGCG					
Mean	0.909	1.319	1.232	0.581	0.003
SD	0.394	0.319	0.371	0.192	0.004
p (two-tail)		0.029	0.066	0.990	0.013

B

	0 μM	25 μM	50 μM	75 μM	100 μM
ECG					
Mean		1.0858	0.1900	0.0058	0.0233
SD		0.5214	0.3097	0.0052	0.0058
p (two-tail)		0.223	0.008	0.0001	0.0001
EGCG					
Mean	1.5979	2.0025	0.7633	0.2500	0.0242
SD	0.3249	0.1475	0.1432	0.1047	0.0126
p (two-tail)		0.037	0.0011	0.0001	0.0001



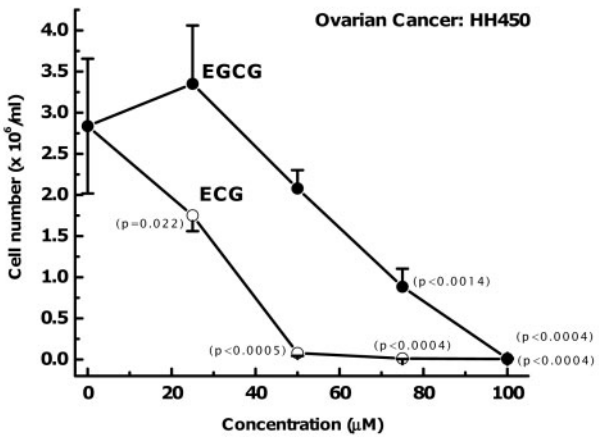


C

	0 μM	25 μM	50 μM	75 μM	100 μM
ECG					
Mean		*1.7508*	*0.0783*	*0.0125*	*0.0058*
SD		0.1913	0.0341	0.0066	0.0014
p (two-tail)		0.0217	0.0005	0.0004	0.0004
EGCG					
Mean	2.837	3.3508	2.0792	0.8842	0.0108
SD	0.819	0.709	0.222	0.220	0.008
p (two-tail)		0.378	0.077	0.0014	0.0004

D

	0 μM	25 μM	50 μM	75 μM	100 μM
ECG					
Mean		1.6242	0.0967	0.0267	0.0100
SD		0.7082	0.0104	0.0138	0.0043
p (two-tail)		0.0964	0.0005	0.0004	0.0004
EGCG					
Mean	2.7088	1.4925	1.2650	0.8242	0.0067
SD	0.7699	0.5802	0.6817	0.1254	0.0014
p (two-tail)		0.0422	0.0386	0.0015	0.0004



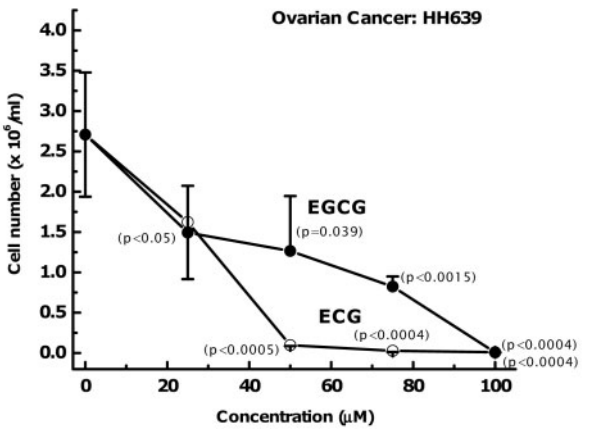


Figure 6. Suppression of cell growth by ECG and EGCG. (**A**) DU145; (**B**) HH870; (**C**) HH450; (**D**) HH639. Cells (0.25×10^6 per line) were seeded in flasks containing culture medium (RPMI-1640 with 9% FBS-antibiotics) with or without ECG or EGCG at concentrations of 0, 25, 50, 75 and 100 μM (three flasks for each dose). Mean (circles) and standard deviation (vertical lines) are represented. When untreated cells reached confluency, cell monolayers in each flask were photographed under a light microscope, harvested and counted. The suppressive effect on cell density was striking at higher concentrations of ECG and EGCG. At 25 μM of EGCG, cell counts for HH870 and DU145 were significantly higher than control values. *P*-values indicate significant differences between mean values of treated and untreated cells.

Table 3. The effects of tea catechins on human cancer cell lines

Human tumor	Cell line	Catechin	Source	Observed effects
Breast	MCF-7	EGCG	Valcic *et al.* (13)	EC, EGC, ECG, EGCG, GC and C were tested against MCF-7 breast carcinoma cell line. Of all the green tea components, EGCG was the most potent inhibitor of growth
Breast	MCF-7 T47D MDA-MB-231 HS578T	EGCG	Chisholm *et al.* (17)	The aim to determine if low concentrations of EGCG, EGC and ECG inhibit the proliferation of many different cancer cell lines with and without 4-hydroxytamoxifen (4-OHT), which would cause significant cytotoxicity (CTX) in estrogen-receptor-positive (ERα+) and receptor-negative (ERα−) human breast cancer cells. Therefore, MCF-7, T47D, MDA-MB-231 and HS578T cells were incubated with EGCG, EGC or ECG (5–25 μM) individually and in combination with 4-OHT for 7 days. Cell number was determined by the sulforhodamine B cell proliferation assay. As single agents, none of the catechins was CTX to T47D cells, while only EGCG (20 μM) elicited CTX in MCF-7 cells. No benefit was gained by combination treatment with 4-OHT. ERα− human breast cancer cells were more susceptible as all three catechins were significantly CTX to HS578T cells at concentrations of 10 μM. In this cell line, combination with 4-OHT did not increase CTX. However, the most striking results were produced in MDA-MB-231 cells. In this cell line, EGCG (25 μM) produced a greater CTX effect than 4-OHT (1 μM). The combination of the two resulted in synergistic CTX
Colon	HT-29	EGCG	Valcic *et al.* (13)	GC, EC, EGC, ECG and EGCG extracted from green tea leaves and catechin (C) were tested against HT-29 colon cancer cell line. Of all the catechins, EGCG was the most potent inhibitor of growth
Colon	HT-29	EGCG	Jung *et al.* (9)	EGCG, the most abundant catechin in green tea extract, inhibited Erk-1 and Erk-2 activation in serum-deprived HT-29 human colon cancer cells *in vitro* in a dose-dependent manner. EGC, ECG and EC did not affect Erk-1 or 2 activation at a concentration of 30 μM. EGCG also inhibited the increase of VEGF expression and promoter activity induced by serum starvation. *In vivo*, in athymic BALB/c nude mice inoculated subcutaneously with HT-29 cells were treated with daily intraperitoneal injections of EC (negative control) or EGCG at 1.5 mg day(-1) mouse(-1) starting 2 days after tumor cell inoculation. Treatment with EGCG inhibited tumor growth (58%), microvessel density (30%), and tumor cell proliferation (27%) and increased tumor cell apoptosis (1.9-fold) and endothelial cell apoptosis (3-fold) relative to the control condition ($P < 0.05$ for all comparisons)
Gastric	MK-1	EGCG	Kinjo *et al.* (6)	Among the six active flavan-3-ols, EC, EGC, EGCG, GC, ECG, GCG, EGCG and GCG showed the highest antiproliferative activity against human stomach cancer (MK-1) cells. These data suggest that the presence of the three adjacent hydroxyl groups (pyrogallol or galloyl group) in the molecule would be a key factor for enhancing the activity. Since reactive oxygen species play an important role in cell death induction, radical scavenging activity was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. The order of scavenging activity was ECG $\geq$ EGCG $\geq$ EGC $\geq$ GC $\geq$ EC. The compounds having a galloyl moiety showed more potent activity. The contribution of the pyrogallol moiety in the B-ring to the scavenging activity seemed to be less than that of the galloyl moiety
Melanoma	UACC-375	EGCG	Valcic *et al.* (13)	GC, EC, EGC, ECG and EGCG extracted from green tea leaves and catechin (C) were tested against UACC-375 melanoma cell line. Of all the green tea components, EGCG was the most potent inhibitor of growth
Glioblastoma	A172	EGCG= ECG	Sachinides *et al.* (28)	The effect of C, EC, EGCG, ECG and CG on the tyrosine phosphorylation of PDGF beta-receptor (PDGF-Rβ) and on the anchorage-independent growth of A172 glioblastoma cells was investigated. Treatment of A172 glioblastoma with 50 μM CG, ECG, EGCG and 25 μM Tyrphostin 1296 resulted in an 82 ± 17%, 77 ± 21%, 75 ± 8% and 55 ± 11%, respectively (mean ± SD, $n = 3$) inhibition of the PDGF-BB-induced tyrosine phosphorylation of PDGF-Rb. The PDGF-Rb downstream intracellular transduction pathway including tyrosine phosphorylation of phospholipase C-γ1 (PLC-γ1) and phosphatidylinositol 3′-kinase (PI3′-K) was also inhibited. Spheroid formation was completely inhibited by 50 μM ECG, CG, EGCG and by 25 μM Tyrphostin 1296. The catechins possessing the gallate group act as anticancer agents probably partly via their ability to suppress the tyrosine kinase activity of the PDGF-Rβ
Lung	PC-9	EGCG = ECG/ EGC	Okabe *et al.* (14)	EGC and ECG inhibited the growth of a human lung cancer cell line, PC-9 cells as potently as did EGCG, but EC did not show significant growth inhibition. The mechanism of growth inhibition by EGCG was studied in relation to cell-cycle regulation. EGCG (50 and 100 μM) increased the percentages of cells in the G_2-M-phase from 13.8 to 15%. [^{3}H]EGCG was incorporated into the cytosol, as well as the nuclei
Lung	A549	EGCG = ECG	Fujimoto *et al.* (16)	EGCG or ECG and genistein as a control dose dependently inhibited the growth of human lung cancer cell line, A549 cells, strongly elevated hnRNP B1 protein and increased G_2/M-phase cells associated with induction of apoptotic cells. Treatment of A549 cells with EGCG, ECG or genistein significantly inhibited the expression levels of hnRNP B1 mRNA and the elevated levels of hnRNP B1 protein, both of which are constitutively elevated in cancer cells. Furthermore, both EGCG and genistein inhibited the promoter activity of hnRNP A2/B1 gene expression, with IC50 values 29 mM for EGCG and 66 mM for genistein, suggesting the interaction of EGCG or genistein with the transcriptional complex
Pancreatic	HPAC	EGCG = ECG	Lyn-Cook *et al.* (36)	The effects of ECG and EGCG on the growth of human pancreatic adenocarcinoma (HPAC) were determined. ECG and EGCG inhibited growth as well (∼95%). Black and green tea extracts, EGCG decreased the expression of the K-ras gene and the multidrug-resistant gene (mdr-1)

Table 3. *Continued*

Human tumor	Cell line	Catechin	Source	Observed effects
Prostate	LnCaP	EGCG = ECG	Lyn-Cook *et al.* (36)	ECG and EGCG significantly inhibited growth of prostate tumor (LNCaP) and increased expression of the mdr-1 gene in LNCaP
Oral squamous	SCC-25	EGCG = ECG	Elattar *et al.* (37)	The effect of EGCG, ECG and EGC (at concentrations of 50, 80, 100 and 200 mM) on the growth and DNA synthesis of human oral squamous carcinoma cell line SCC-25 was determined. At the four dose levels used, the three compounds induced significant dose-dependent inhibition in cell growth. In DNA study, the three compounds exhibited stimulatory effect at 50 μM followed by significant dose-dependent inhibitory effect (10–100%) at 80, 100 and 200 μM dose levels. Dose-dependent changes in cell morphology were also observed with phase-contrast microscopy after cell treatment with EGCG
Hepatoma	HepG2-ARE-C8	EGCG/ ECG	Chen *et al.* (27)	Tea catechin treatment significantly increased cell viability, decreased lipid peroxidation levels and protected cell membrane fluidity in lead-exposed HepG2 cells in a concentration-dependent manner. The galloylated catechins showed a stronger effect than non-galloylated catechins. Co-treatment with EGC, EC, ECG and the tea catechins may have a role to play in modulating oxidative stress in lead-exposed $HepG_2$ cells
Colon	LoVo	EGCG = **EGC**	Tan *et al.* (38)	Treatment of LoVo colon cancer cells with EGCG and EGC resulted in the growth suppression and induction of apoptosis in a time- and concentration-dependent manner. ECG and EC, however, did not have the same effects. In addition, treatment with EGCG, EGC and ECG caused LoVo cells arrest at G_1-phase in the cell-cycle progression, whereas EC resulted in an arrest at S-phase
Oral squamous	HSC-2	ECG	Babich *et al.* (18)	The relative cytotoxicity (CTX) of ECG to carcinoma HSC-2 cells and normal HGF-2 fibroblasts cells from the human oral cavity, as compared with other polyphenols in tea, was evaluated. For the HSC-2 carcinoma cells, ECG, CG and EGCG grouped as highly toxic, EGC as moderately toxic, and C and EC as least toxic. For the HGF-2 fibroblasts, ECG and CG grouped as highly toxic, EGCG as moderately toxic, and EGC, C and EC as least toxic. The CTX effects of the polyphenols were more pronounced to the carcinoma, than to the normal, cells. The addition of ECG to cell culture medium led to the generation of hydrogen peroxide (H_2O_2). But ECG, as compared with EGCG, was a poor generator of H_2O_2 and, hence, the CTX of ECG was unaffected by the presence of the antioxidants, N-acetyl cysteine and glutathione, and catalase. The CTX of ECG was unaffected by a metabolic activating system, i.e. a hepatic microsomal S-9 mix. ECG induced apoptosis in the carcinoma HSC-2 cells, but not in the normal HGF-2 fibroblasts
Prostate	DU145	ECG	Chung *et al.* (4)	EGCG, EGC and ECG but not EC suppress the growth and induce apoptosis in human prostate cancer DU145 cells largely through an increase in reactive oxygen species (ROS) formation and mitochondrial depolarization. The growth suppression, apoptosis induction, ROS formation and mitochondrial depolarization are in a similar order, i.e. ECG > EGCG > EGC > EC. EGCG did not alter the expression of BCL-2, BCL-X(L) and BAD in DU145 cells
Fibrosarcoma	HT1080	ECG	Maeda-Yamamoto *et al.* (8)	EGCG, EGC and theaflavin strongly suppressed the invasion of HT1080 cells into the monolayer of HUVECs/gelatin membrane, whereas EC, EGC, tea flavonols, tea flavones and gallate derivatives had no effect. Both theaflavin-digallate and theasinensin D showed a weak invasion inhibitory effect. ECG significantly inhibited the invasion without cytotoxicity (CTX) against cancer cells and HUVECs. Ester-type catechins (ECG and EGCG) and theaflavin strongly suppressed matrix metalloproteinase (MMP) 2 and MMP-9, which were secreted into the conditioned medium of HT1080 cells. ECG showed the most potential antimetastasis activity because it inhibited invasion in the absence of CTX
Stomach	KATO III	ECG	Okabe *et al.* (2)	Various tea polyphenols induced growth inhibition and apoptosis of human stomach cancer cell line KATO III, and inhibition of tumor necrosis factor-alpha (TNF-α) release from the cells, in the order of ECG > EGCG > EGC > theaflavins (TF) > EC. EGCG inhibited TNF-α gene expression in KATO III cells, as well as okadaic acid-induced AP-1 and NF-κB activation. The inhibitory potencies of EGCG for AP-1 and NF-κB binding to DNA were different between KATO III cells and mouse fibroblast cell line BALB/3T3
Prostate, metastatic	DU145	ECG	Present study	ECG suppressed cell proliferation of DU145 prostate cancer cells at a concentration 24 μM, whereas EGCG suppressed at the same level at 89 μM
Prostate, confined	HH870	ECG	Present study	ECG suppressed cell proliferation of HH870, a cell line developed from confined prostate cancer at a concentration 27 μM, whereas EGCG suppressed at the same level at 45 μM
Ovarian	HH450	ECG	Present study	ECG suppressed cell proliferation of HH450, an ovarian cancer cell line at a concentration 29 μM, whereas EGCG suppressed at the same level at 63 μM
Ovarian	HH639	ECG	Present study	ECG suppressed cell proliferation of HH639, an ovarian cancer cell line at a concentration 30 μM, whereas EGCG suppressed at the same level at 42 μM

EC, epicatechin; EGC, epigallocatechin; ECG, epicatechin gallate; EGCG, epigallocatechin gallate and epimers of EGC [GC], ECG [CG] and EGCG [GCG]. EGC potency indicated in bold.

time of CaP cell lines DU145 and HH870 and EOC line HH450. No doubling was observed for HH639 cells treated with ECG; instead cell number decreased, indicating cell death. Table 1 summarizes the effects of EC, ECG, EGC and EGCG on viability, doubling time and cycling of the four cell lines. Untreated cells from each line reached confluency in about 2.5 cell cycles. EC did not affect the proliferation of DU145, HH870 or HH450 cells but it reduced the proliferation of HH639 cells by half ($P < 0.05$) and prevented their confluent growth (Table 1). EGC did not affect the proliferation of any cell line (Table 1), whereas EGCG arrested proliferation of all four lines. ECG, followed by EGCG, was the most potent inhibitor of cell growth and cycling.

Dosimetric Analysis of Growth to Tumor cells: IC50 of ECG is Superior to EGCG

Proliferation of each cell line ($n = 3$ per treatment) was monitored with or without ECG or EGCG at concentrations of 0, 25, 50, 75 and 100 μM. The dosimetric results plotted in Fig. 6 shows concentration-dependent suppression of cell growth by ECG and EGCG. The suppressive effect on cell density was striking at higher concentrations of ECG and EGCG. ECG was a more potent inhibitor of cell growth than EGCG. At 25 μM of EGCG, cell numbers for HH870 and DU145 were significantly higher than control values. Based on the results plotted in Fig. 6, IC50 values were calculated. The IC50 values are 24–30 μM for ECG, versus 42–89 μM for EGCG (Table 2). ECG suppressed growth at all higher concentrations tested (Fig. 6), whereas EGCG significantly ($P < 0.05$) enhanced proliferation of CaP cells at 25 μM, a finding relevant to chemoprevention trials with EGCG only.

Discussion

Green tea is widely consumed in Japan and China and its polyphenolic components have a chemopreventive effect against cancer *in vitro* and *in vivo* (39). A cup of green tea contains 100–150 mg catechins, of which 8% are EC, 15% are EGC, 15% are ECG and 50% are EGCG (40). Although numerous investigations have shown the role of EGCG in cancer chemoprevention, only a few studies have attempted to compare the relative antitumor efficacy of all four catechins (Table 3). When we used a systematic approach to assess the effect of various catechins on cell lines derived from gender-based cancers, we found that each catechin's antitumor activity depended on the type of tumor. EGCG was not always the most potent chemopreventive agent.

Most of the earlier literature (Table 3) indicates that EGCG is the most potent growth inhibitor of cell lines from glioblastoma, melanoma and cancers of the breast, colon, lung, prostate (androgen-receptor-positive), pancreas, liver and mouth. EGCG prevents proliferation of DU145 cells by arresting the cell cycle at G_0/G_1-phase (19). Gupta and others (26) have documented that G_0/G_1-phase arrest is independent of p53 mutation, and EGCG treatment of DU145 induces the cyclin kinase inhibitor WAF1/p21. These observations suggest that EGCG imposes a cell-cycle checkpoint (19). However, our results showed that ECG may be more potent than EGCG for inhibition of primary and metastatic CaP and EOC cells (Fig. 4, Tables 1 and 2). ECG significantly reduced cell proliferation (Table 1, Figs 2 and 3) and increased mean doubling time (Table 1, Fig. 4).

The *in vitro* effect of chemopreventive agents can be studied when tumor cells are in a matrix (1,4,27) or in a suspension (28,29). We used the suspension method because it exposes the entire cell surface to the chemotherapeutic agent. Our findings confirm an earlier report that used the matrix method to show that ECG is more potent than EGCG in suppressing the proliferation of DU145 CaP cells (4). Thus reported differences in the relative efficacy of different catechins may not be due to differences in methodology.

Not all tumor cells are killed by catechins. In our study, ECG (50 μM) induced death of most but not all HH639 cells. Doubling ECG's IC50 concentration might increase the tumor kill rate if ECG does not epimerize to CG. Our *in vitro* dose of 100 μM is equivalent to 29 mg (EC/EGC) to 45 mg (EGCG/ECG), far less than the 100–150 mg (50% of which is EGCG) in one cup of green tea. However, Lee *et al*. (41) reported that plasma levels of EGCG and EGC in healthy volunteers increased to 78 and 223 ng ml^{-1}, respectively, 20 min after drinking brewed green tea (1.2 g of tea solids in 200 ml hot water). This suggests that drinking more than 10 cups of green tea may be necessary to maintain a plasma concentration of EGCG equivalent to that used *in vitro* by a dose of 50 μM or 22.5 mg. Kaegi (42) suggested a daily intake of 13 cups of green tea as a chemopreventive measure. Because this level of tea consumption is impractically high, chemoprevention of cancer with catechins may require administration of the appropriate catechin in a purified form.

In conclusion it may be stated that both green and black tea polyphenols are important components of antitumor aspect of complementary and alternative medicine (CAM), which play a significant role in the American health care system and in patients who suffer from chronic problems (43). While green tea catechin gallates such as EGCG and ECG possess potent antitumor activities, their epimers, commonly found in black tea, act as potent inhibitor of proteases involved in replication of viruses, including coronoviruses (44). There is a need to understand preventive and therapeutic potential of catechin gallates from both green and black teas. We are currently designing a phase I chemopreventive study to examine the effects of purified EGCG and ECG in patients who have been chosen observational management of organ-confined prostate cancer.

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