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Antiproliferative effects of the major tea polyphenol, (−)-epigallocatechin gallate and retinoic acid in cervical adenocarcinoma

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Abstract

Objective. To investigate the combined effect of the major tea polyphenol, (−)-epigallocatechin gallate (EGCG) and retinoic acid (RA) on cervical adenocarcinoma.

Methods. Cell growth rate was examined after treatment for 4, 7 and 10 days with 0–100 μM EGCG and/or 1 μM RA in two cervical adenocarcinoma cell lines, HeLa and TMCC-1. The effect of EGCG treatment was examined for the induction of apoptosis by DNA ladder assay and caspase-3-related protease activity in cell lysate. Telomerase activity was detected by stretch PCR telomere extension assay. hTERT expression levels were quantified by a real-time PCR system.

Results. Combining EGCG and RA increased the antiproliferative effect in adenocarcinoma cell lines, whereas EGCG or RA treatment alone caused a less sensitive response in these cells. Neither EGCG nor RA treatment alone affected apoptosis and telomerase activity. The combination treatment of EGCG and RA induced apoptosis and inhibited telomerase activity in adenocarcinoma cell lines. These results were consistent with those of an antiproliferative effect of EGCG and/or RA in cervical adenocarcinoma cells.

Conclusion. Our data suggest that EGCG and RA combined to prevent the carcinogenesis of cervical adenocarcinoma, induce apoptosis and inhibit telomerase activity. The treatments of combining EGCG and RA may be effective in preventing or treating cervical adenocarcinoma.

Keywords: EGCG; Retinoic Acid; Cervical adenocarcinoma

Introduction

Cervical cancer is the second most common cancer in the world. Adenocarcinoma, including adenosquamous carcinoma, represents only 15–20% of all primary carcinoma of the cervix, but this percentage is increasing greatly [1,2]. It has been suggested that adenocarcinoma of the uterine cervix tends to metastasize earlier to lymph nodes and is less sensitive to radiation therapy and to chemotherapy than is squamous cell carcinoma [3,4]. Radical surgery seems to be the treatment of choice for this tumor; however, treatments for advanced or recurring cases have been most often unsuccessful.

Green tea is one of the most common beverages consumed worldwide, and its possible beneficial health effects have received much attention. A number of epidemiological and rodent carcinogenesis studies have provided evidence that green tea has chemopreventive effects for a wide range of malignancies [5–7]. Green tea contains a variety of polyphenols known as catechins. (−)-Epigalocatechin gallate (EGCG) is a major component of polyphenols in green tea [8]. The antitumor effect of EGCG has been demonstrated [5,6,8–11]. Previously, we reported that the treatment with EGCG prevented the carcinogenesis of cervical cancer, induced apoptosis and inhibited telomerase activity [12,13]. It is also reported that EGCG inhibits the growth of human squamous cervical cancer cell lines through apoptosis [12]. EGCG was effective in HPV-18 immortalized endocervical cell lines (premalignant cell) but less effective in cervical adenocarcinoma cell lines. These results suggest that the sensitivity to

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Table 1
Origin and human papillomavirus status of cervical cells and cell lines

Cell line	Origin	HPV DNA type	Tumorigenicity
HEN-18	Endocervix	HPV-18	(−)
HEN-18-S [a]	Endocervix	HPV-18	(−)
HeLa	Cx, adenocarcinoma	HPV-18	(+)
TMCC-1	Cx, adenocarcinoma	HPV-18	(+)

HEN=human endocervical cells; −=negative; +=positive.
[a] Non-tumorigenic, adapted to growth in serum.

EGCG decreases with the progression of the carcinogenic process [12]. The inhibitory effect in cell lines derived from cervical adenocarcinoma cells was less than that from cervical squamous carcinoma cells [12], although several studies have shown that EGCG possesses anticarcinogenic effects in adenocarcinoma, including stomach, colon and breast cancer [5–10]. Recently, Tachibana et al. [14] reported that the combination of EGCG and vitamin A increased expression of the receptor that mediates the anticancer activity of EGCG and enhances the anticancer activity of EGCG.

In the present study, we investigated whether the combined use of EGCG and retinoic acid (RA), the direct acting vitamin A metabolite, possesses growth inhibitory properties in human cervical adenocarcinoma cell lines. Furthermore, we investigated whether the mechanism involves the induction of apoptosis and inhibition of telomerase activity.

Materials and methods

Cells, cell culture and cell growth assay

Primary human end cervical cells (HEN) were prepared as described previously [15,16]. Cervical cell lines are summarized in Table 1. Keratinocyte serum-free medium (K-SFM, GIBCO, Grand Island, NY) was used for growth of HEN; HPV-18-immortalizes HEN. Other cell lines were cultured in Dulbecco's modified Eagle's medium plus 10% fetal calf serum (GIBCO). The uterine cervix adenocarcinoma cell line, TMCC-1, was kindly provided by M. Sakamoto (Tokyo Medical College, Tokyo, Japan). For growth assay, EGCG (Wako Pure Chemical Industries, Osaka, Japan) and RA (Sigma, St. Louis, NY) were diluted in 100% ethanol and stored at −20 °C before use. Cells seeded at 2×10^5 cells per 5 cm plate were incubated with or without 0–100 μM EGCG and/or 1 μM all-trans RA, which was changed every other day. Cell growth rate was determined by counting the number of cells with a Coulter counter and expressed with the following formula:

$$\text{Growth rate (\%)} = \frac{\text{cell number (experiment)}}{\text{cell number (control)}}$$

DNA ladder apoptosis assay

Cells were cultured in medium with 0 μM (control) EGCG and 50–100 μM EGCG and/or 1 μM all-trans RA for 4 days. High molecular weight DNA was extracted, resolved by 1.5% agarose gel electrophoresis and stained using an apoptosis ladder detection kit (Wako Pure Chemicals Industries, Osaka, Japan).

Caspase-3 activity

Caspase-3-related protease activity in cell lysate was determined using a caspase-3 assay kit, fluorometric (Sigma, St. Louis, MO). Briefly, cell lysate was mixed with assay buffer and the caspase-3 substrate, Ac-DEVD-AMC, followed by incubation at room temperature for 1.5 h. Absorbance was then read with a plate fluorometer. The excitation and emission wavelengths were 360 nm and 460 nm, respectively. Caspase-3 activity was expressed as the fluorescence value relative to that for untreated cells (control).

Telomerase assay

Telomerase activity was quantified with the overlap extension PCR assay method using TeloChaser (Toyobo Co., Osaka, Japan). Briefly, 2×10^4 cells grown in medium with 0 μM (control) EGCG and 50–100 μM EGCG and/or 1 μM all-trans RA for 4 days were obtained and suspended in Lysis solution. Cell extracts were assayed in extension mixture. After 30 min incubation at 30 °C for telomerase extension, the telomerase products were purified by Clean-Up solution, followed by isopropyl alcohol precipitation. Recovered pellets were mixed with 30 μl of PCR mixture, heated at 95 °C for 150 s and then subjected to 30 PCR cycles of 95 °C for 30 s, 68 °C for 30 s and 72 °C for 45 s. PCR products were resolved by electrophoresis in a 7% polyacrylamide gel and visualized with SYBR Green I nucleic acid stain (Molecular Probes, Inc., Eugene, USA).

Quantitation of hTERT mRNA

hTERT expression levels were quantified by a real-time PCR system, Light Cycler Telo TAGGG hTERT Quantification kit (Roche Molecular Biochemicals, Indianapolis, IN) according to the manufacture's instructions. Briefly, cells grown in medium with 0 μM (control) EGCG and 50–100 μM EGCG and/or 1 μM all-trans RA for 4 days were obtained. Two hundred nanograms total RNA of each sample was analyzed. hTERT encoding mRNA was reversely transcribed and amplified in a one-step RT-PCR protocol using the LightCycler instrument. Relative expression levels were calculated by dividing the amount of the housekeeping gene PBGD. The resulting ratio is an hTERT value normalized to the expression of PBDG. Duplicates for each sample were performed. PCR products were resolved by electrophoresis in a 7% polyacrylamide gel and visualized with SYBR Green I nucleic acid stain (Molecular Probes, Inc., Eugene, USA).

Table 2
Effect of epigallocatechin gallate (EGCG) and/or retinoic acid on cell growth rate of endocervical and cervical adenocarcinoma cell lines

Cell line	Treatment	Growth rate (% of untreated control)		
		Day 4	Day 7	Day 10
HEN-18	EGCG 100 μM	15.4±4.1*	9.5±0.8*	9.0±0.8*
	EGCG 50 μM	35.3±1.8*	10.9±0.3*	8.2±1.4*
	EGCG 10 μM	51.4±4.1*	9.2±4.8*	7.8±1.3*
HEN-18S	EGCG 100 μM	42.1±0.8*	14.6±0.8*	8.6±0.9*
	EGCG 50 μM	94.2±1.8***	68.2±6.9***	66.6±3.1**
	EGCG 10 μM	120±12.6****	89.3±1.5***	91.1±4.3****
TMCC-1	EGCG 100 μM	72.8±4.5***	92.5±1.1**	108±2.1***
	EGCG 50 μM	89.8±4.5****	106.2±2.3**	99.5±3.1****
	RA	79±5.1**	57.7±2.3*	62.2±4.2*
	EGCG 50 μM+ RA	53.0±00**	22.4±00*	25.4±00*
HeLa	EGCG 100 μM	78.3±5.6***	93.2±1.6***	63.1±1.2*
	IEGCG 50 μM	89.5±3.8***	103±1.5****	101.4±3.8****
	RA	66.4±5.8**	80.1±3.8**	78±4.3**
	EGCG 50 μM+ RA	62.1±7.2**	17.5±5.4*	20.2±6.3*

HEN-18=human papillomavirus type 18-immoratalized human endocervical cell; HEN-18S=non-tumorigenic serum adapted HEC-18; RA=retinoic acid; EGCG=epigallocatechin gallate. The results represent the mean±standard deviation of percent. *P* is the statistical significance of difference in cell growth rate between each day treated and untreated control cells. *$P<0.001$, **$P<0.01$, ***$P<0.05$, ****not significant.

Results

To investigate the effect of EGCG and/or RA on premalignant and malignant cervical adeno cell lines (Table 1), we treated cells for 4, 7 and 10 days with EGCG and/or RA. Cell growth inhibition assays demonstrated that the combination of EGCG and RA treatment inhibited 75–80% growth in cervical adenocarcinoma cell lines, HeLa and TMCC-1, whereas EGCG or RA treatment alone caused a less sensitive response in these cells (Table 2). TMCC-1 was less sensitive than HeLa in EGCG treatment, but the inhibitory effect of RA in TMCC-1 was more than that in HeLa.

The effect of EGCG or RA treatment on apoptosis was examined by DNA ladder apoptosis assay. Cells treated with 0 and 100 μM EGCG or 1 μM RA for 4 days showed that neither EGCG nor RA treatment resulted in the formation of DNA fragments in HeLa and TMCC-1 (Fig. 1, lanes 2, 3, 6 and 7). In comparison with these treatments, the combination of EGCG and RA treatment induced the formation of DNA fragments (Fig. 1, lanes 4 and 8). These results are consistent with those of an antiproliferative effect of EGCG and/or RA in these cell lines.

As caspase-3 plays an important role in apoptotic events, the influence of EGCG and RA on the caspase-3 activity was examined by using a specific flurogenic substrate for caspase-3. The TMCC-1 and HeLa treated with the combination of EGCG and RA had significantly higher activity of caspase-3 than that of no treatment (Fig. 2). These results showed that the activation of caspase-3 was associated with the induction of apoptosis in the combination treatment of EGCG and RA.

Telomerase has been proposed to represent a novel and potentially selective target for cancer therapy. It is also reported that the inhibition of telomerase is one of the major mechanisms of the anticancer effects of EGCG. We studied whether EGCG and RA inhibit telomerase activity by the stretch PCR assay methods. Telomerase was dramatically decreased in HeLa with the combination of EGCG and RA treatment (Fig. 3, lanes 4). Neither EGCG nor RA treatment inhibited the telomerase activity.

A strong correlation between telomerase activity and the expression level of hTERT mRNA has been described in cancer cells. We investigate hTERT expression level by a real-time PCR system. EGCG and RA treatment showed that hTERT expression was decreased in HeLa and TMCC-1 (Fig. 4, lanes 2 and 4). These

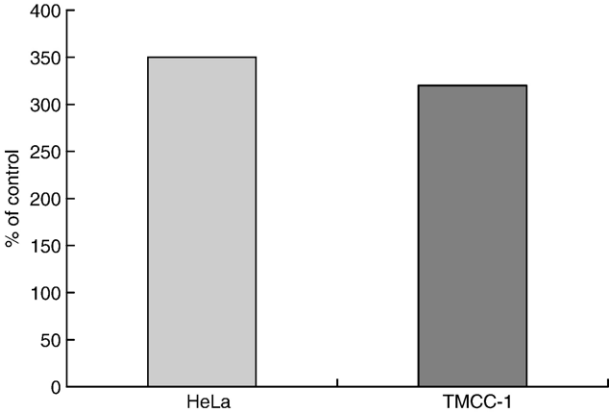

Fig. 2. Effect of epigallocatechin gallate (EGCG) and/or retinoic acid (RA) on caspase-3 activity in HeLa and TMCC-1.

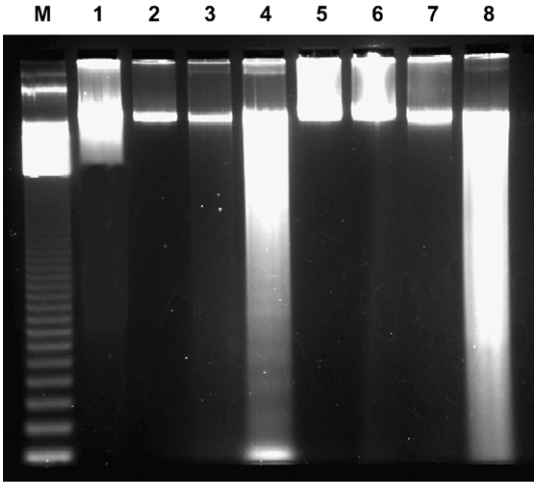

Fig. 1. Effect of epigallocatechin gallate (EGCG) and/or retinoic acid (RA) on apoptosis in TMCC-1 and HeLa. Apoptosis was assayed by DNA ladder formation analysis. TMCC-1 (lanes 1, 2, 3 and 4) and HeLa (lanes 5, 6, 7 and 8) were cultured in medium with EGCG and/or RA. M: 123 bp ladder marker; lanes 1 and 5: control medium; lanes 2 and 6: 1 μM of RA; lanes 3 and 7: 100 μM of EGCG; lanes 4 and 8: 1 μM of RA and 100 μM of EGCG. DNA by ladder formation obtained from cells after EGCG and RA treatment confirmed apoptosis.

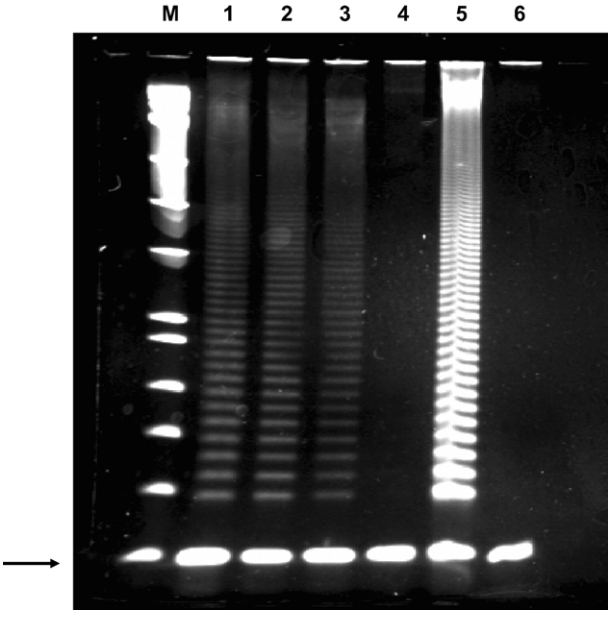

Fig. 3. Effect of epigallocatechin gallate (EGCG) and/or retinoic acid (RA) on telomerase activity in HeLa. Semiquantitative telomerase activity was assayed by stretch PCR assay method. HeLa was cultured in medium with 1 μM of RA and/or 100 μM of EGCG M:φX174/Hinf I marker; lane 1: control medium; lane 2: 1 μM of RA; lane 3: 100 μM of EGCG; lane 4: 1 μM of RA and 100 μM of EGCG; lane 5: positive control (HeLa cell); lane 6: negative control. The arrow indicates the internal standard DNA (65 bp).

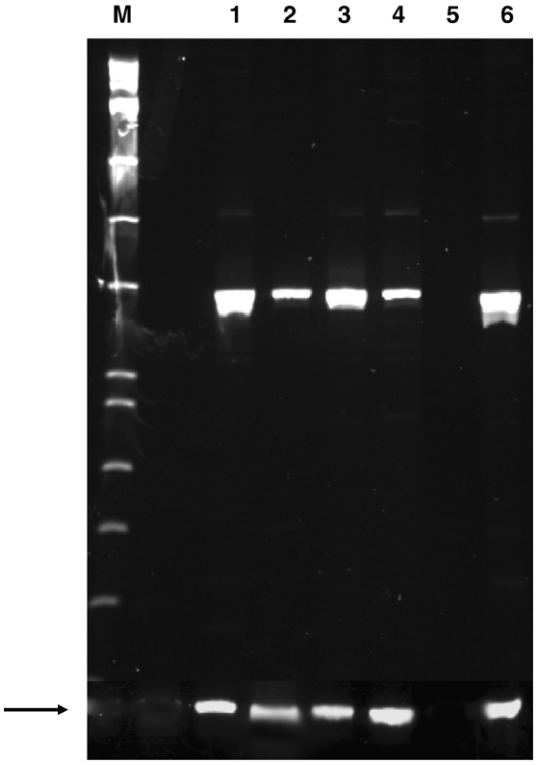

Fig. 4. Effect of epigallocatechin gallate (EGCG) and/or retinoic acid (RA) on the expression of hTERT mRNA in HeLa (lanes 1 and 2) and TMCC-1 (lanes 3 and 4). hTERT expression levels were quantified by a real-time PCR system. Lanes 1 and 3: no treatment; lanes 2 and 4: 1 μM of RA and 100 μM of EGCG; lane 5: negative control; lane 6: positive control (HeLa). The arrow indicates the PBDG.

results are consistent with those of telomerase activity by stretch PCR assay.

Discussion

It has been suggested that cervical adenocarcinoma has relatively more aggressive biological activity, poor prognosis, earlier metastasis and less sensitivity to radiation and chemotherapy, compared to squamous cell carcinoma [3,4]. It is necessary to develop a new anticancer agent for treatment of cervical adenocarcinoma. Polyphenols derived from green tea, particularly EGCG, have been reported to control the proliferation of various cancers and possess the anticarcinogenic and chemopreventive effects in various cancers both in vitro and in vivo [5–7,9–11,17–19]. Previously, we reported that EGCG prevented the carcinogenesis of cervical cancer [12]. It is also reported that the inhibitory effect was less in cervical adenocarcinoma cell lines than squamous cell carcinoma cell lines [12]. EGCG may be effective for treatment of cervical cancer, but, alone, it is inadequate for cervical adenocarcinoma.

Recently, Tachibana et al. [14] reported that all-trans-retinoic acid (RA) enhanced the binding of EGCG to the cell surface of cancer cells. It is also revealed that the cell surface 67-kDa laminin receptor (67 LR) is the target for EGCG and acts as the receptor for antitumor action of EGCG. The growth inhibitory activity of EGCG correlated with the binding strength of EGCG to the cell surface. Our previous study showed that RA EGCG inhibited cell growth for cell lines derived from the endocervix and cervical adenocarcinoma generally less effectively than those derived from their squamous cell counterparts [12]. In the present study, we investigated the efficacy of EGCG and/or RA in cervical adenocarcinoma cells. Our study showed that the combination treatment of EGCG and RA increased the antiproliferative effect in cervical adenocarcinoma cell lines. It is reported that the expression of 67 LR was enhanced by RA treatment [14]. The enhancement of 67 LR by RA treatment may result in the combination effect of EGCG and RA.

The mechanism of cancer inhibition of EGCG is not clear, but several hypotheses have been proposed [20]. It has been reported that EGCG induced apoptosis, and G1 or G2-M arrest of the cell cycle [13,17,21–25]. We previously reported that EGCG treatment resulted in DNA ladder formation in the cell lines from squamous cell, but not the adenocarcinoma counterparts [12]. EGCG treatment induced DNA ladder formation in HPV-18 immortalized endocervical cells, but not in serum adapted HPV-18 immortalized endocervical cells, which confers greater cervical cell growth potential and higher grade cervical lesion [12]. These results supported the hypothesis that the sensitivity to EGCG and induction of apoptosis by EGCG in the carcinogenesis of cervical adenocarcinoma decreases according to the progression to the carcinogenic process. The present study demonstrated that the combination treatment of EGCG and RA induced apoptosis in adenocarcinoma cells. EGCG or RA treatment alone, which was less effective in growth inhibition, did not induce apoptosis. We previously found that RA did not induce apoptosis [26]. These results suggest that the induction of apoptosis by the combination treatment of EGCG and RA is one of the important mechanisms for the EGCG-mediated anticancer effects in cervical adenocarcinoma. The induction of 67 LR by RA may be associated with the induction of apoptosis thorough the enhancement of the binding strength of EGCG to the cell surface. Although the mechanism of EGCG-induced apoptosis is not clear, the activation of caspases has been shown in cancer cells treated with EGCG [27–29]. In the present study, caspase-3 activity was induced by combination treatment of EGCG and RA. These results were consistent with those of an antiproliferative effect of EGCG and/or RA in adenocarcinoma cells.

The activation of telomerase has been proposed to be a critical event in the immortalization of human cells and is characteristic of most human cancer cell lines and tumors, including cervical cancer carcinogenesis [30–32]. It is reported that telomerase inhibition could be one of the major mechanisms in the anticancer effects of EGCG [20,33–36]. Previously, we reported that EGCG treatment inhibited telomerase activity in immortalized cervical cell lines, as well as non-transformed, serum-adapted HPV-18 immortalized endocervical cell lines and transformed HPV-18 immortalized ectocervical cell lines [12]. In this study, we demonstrated that EGCG treatment did not inhibit telomerase activity in cervical adenocarcinoma cells, but the combination treatment of EGCG and RA did. The effect was associated with a decrease of the hTERT expression.

With regard to in vivo studies, EGCG has been reported to prevent the formation of various solid tumors. The effective EGCG

levels were lower than those of in vitro models. The concentration of EGCG shown to have an effect in these previous in vitro studies (10–200 μM) is much higher than those observed in the blood or tissue after drinking tea [37], although the concentration of RA, 1 μM, was a peak plasma level of oral retinoid therapy. There is the disparity between the concentrations needed to achieve the various effects observed in vitro and the plasma levels at which significant anticancer and chemopreventive effects were observed in animal and epidemiological studies. Several mechanisms of cancer inhibition by EGCG in vivo have been proposed [38–40]. It is reported that EGCG inhibited angiogenesis and matrix metalloproteinase in vivo [38,40]. Recently, Tachibana et al. [14] reported that the 67-kDa laminin receptor (67 LR) was associated with EGCG responsiveness to cancer cells at physiologically relevant concentrations. It is also reported that the combination of EGCG and vitamin A increased the expression of the 67 LR that mediates the anticancer activity of EGCG and enhances the anticancer activity of EGCG. Vitamin A in plasma may result in lower levels of effective EGCG in animal and epidemiological studies. Considering our results and these reports, the combination treatment of EGCG and RA could therefore be a promising strategy in treatment for cancer, including cervical adenocarcinoma.

References

[1] Wang SS, Sherman ME, Hildesheim A, Lacey Jr JV, Devesa S. Cervical adenocarcinoma and squamous cell carcinoma incidence trends among white women and black women in the United States for 1976–2000. Cancer 2004;100:1035–44.

[2] Leung TW, Xue WC, Cheung AN, Khoo US, Ngan HY. Proliferation to apoptosis ratio as a prognostic marker in adenocarcinoma of uterine cervix. Gynecol Oncol 2004;92:866–72.

[3] Baalbergen A, Ewing-Graham PC, Hop WC, Struijk P, Helmerhorst TJ. Prognostic factors in adenocarcinoma of the uterine cervix. Gynecol Oncol 2004;92:262–7.

[4] Balega J, Michael H, Hurteau J, Moore DH, Santiesteban J, Sutton GP, et al. The risk of nodal metastasis in early adenocarcinoma of the uterine cervix. Int J Gynecol Cancer 2004;14:104–9.

[5] Suganuma M, Okabe S, Sueoka N, Sueoka E, Matsuyama S, Imai K, et al. Green tea and cancer chemoprevention. Mutat Res 1999;428:339–44.

[6] Mukhtar H, Ahmad N. Green tea in chemoprevention of cancer. Toxicol Sci 1999;52:111–7.

[7] Fujiki H, Suganuma M, Okabe S, Sueoka N, Komori A, Sueoka E, et al. Cancer inhibition by green tea. Mutat Res 1998;402:307–10.

[8] Fujiki H, Suganuma M, Okabe S, Komori A, Sueoka E, Sueoka N, et al. Japanese green tea as a cancer preventive in humans. Nutr Rev 1996;54: S67–70.

[9] Fujiki H, Yoshizawa S, Horiuchi T, Suganuma M, Yatsunami J, Nishiwaki S, et al. Anticarcinogenic effects of (−)-epigallocatechin gallate. Prev Med 1992;21:503–9.

[10] Hirose M, Mizoguchi Y, Yaono M, Tanaka H, Yamaguchi T, Shirai T. Effects of green tea catechins on the progression or late promotion stage of mammary gland carcinogenesis in female Sprague–Dawley rats pretreated with 7,12-dimethylbenz(a)anthracene. Cancer Lett 1997;112:141–7.

[11] Chen ZP, Schell JB, Ho CT, Chen KY. Green tea epigallocatechin gallate shows a pronounced growth inhibitory effect on cancerous cells but not on their normal counterparts. Cancer Lett 1998;129:173–9.

[12] Yokoyama M, Noguchi M, Nakao Y, Pater A, Iwasaka T. The tea polyphenol, (−)-epigallocatechin gallate effects on growth, apoptosis, and telomerase activity in cervical cell lines. Gynecol Oncol 2004;92:197–204.

[13] Noguchi M, Yokoyama M, Watanabe S, Uchiyama M, Nakao Y, Hara K, et al. Inhibitory effect of the tea polyphenol, (−)-epigallocatechin gallate, on growth of cervical adenocarcinoma cell lines. Cancer Lett 2006;234:135–42.

[14] Tachibana H, Koga K, Fujimura Y, Yamada K. A receptor for green tea polyphenol EGCG. Nat Struct Mol Biol 2004;11:380–1.

[15] Yokoyama M, Tsutsumi K, Pater A, Pater MM. Human papillomavirus 18-immortalized endocervical cells with in vitro cytokeratin expression characteristics of adenocarcinoma. Obstet Gynecol 1994;83:197–204.

[16] Yokoyama M, Nakao Y, Yang X, Sun Q, Tsutsumi K, Pater A, et al. Alterations in physical state and expression of human papillomavirus type 18 DNA following crisis and establishment of immortalized ectocervical cells. Virus Res 1995;37:139–51.

[17] Gupta S, Ahmad N, Nieminen AL, Mukhtar H. Growth inhibition, cell-cycle dysregulation, and induction of apoptosis by green tea constituent (−)-epigallocatechin-3-gallate in androgen-sensitive and androgen-insensitive human prostate carcinoma cells. Toxicol Appl Pharmacol 2000;164:82–90.

[18] Thangapazham RL, Singh AK, Sharma A, Warren J, Gaddipati JP, Maheshwari RK. Green tea polyphenols and its constituent epigallocatechin gallate inhibits proliferation of human breast cancer cells in vitro and in vivo. Cancer Lett 2007;245:232–41.

[19] Ahn WS, Yoo J, Huh SW, Kim CK, Lee JM, Namkoong SE, et al. Protective effects of green tea extracts (polyphenon E and EGCG) on human cervical lesions. Eur J Cancer Prev 2003;12:383–90.

[20] Yang CS, Sang S, Lambert JD, Hou Z, Ju J, Lu G. Possible mechanisms of the cancer-preventive activities of green tea. Mol Nutr Food Res 2006;50:170–5.

[21] Ahmad N, Feyes DK, Nieminen AL, Agarwal R, Mukhtar H. Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. J Natl Cancer Inst 1997;89:1881–6.

[22] Yang GY, Liao J, Kim K, Yurkow EJ, Yang CS. Inhibition of growth and induction of apoptosis in human cancer cell lines by tea polyphenols. Carcinogenesis 1998;19:611–6.

[23] Ahmad N, Cheng P, Mukhtar H. Cell cycle dysregulation by green tea polyphenol epigallocatechin-3-gallate. Biochem Biophys Res Commun 2000;275:328–34.

[24] Ahn WS, Huh SW, Bae SM, Lee IP, Lee JM, Namkoong SE, et al. 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. DNA Cell Biol 2003;22:217–24.

[25] Gupta S, Hussain T, Mukhtar H. Molecular pathway for (−)-epigallocatechin-3-gallate-induced cell cycle arrest and apoptosis of human prostate carcinoma cells. Arch Biochem Biophys 2003;410:177–85.

[26] Yokoyama M, Nakao Y, Iwasaka T, Pater A, Sugimori H. Retinoic acid and interferon-alpha effects on cell growth and differentiation in cervical carcinoma cell lines. Obstet Gynecol 2001;98:332–40.

[27] Islam S, Islam N, Kermode T, Johnstone B, Mukhtar H, Moskowitz RW, et al. Involvement of caspase-3 in epigallocatechin-3-gallate-mediated apoptosis of human chondrosarcoma cells. Biochem Biophys Res Commun 2000;270:793–7.

[28] Chen C, Shen G, Hebbar V, Hu R, Owuor ED, Kong AN. Epigallocatechin-3-gallate-induced stress signals in HT-29 human colon adenocarcinoma cells. Carcinogenesis 2003;24:1369–78.

[29] Zhao X, Tian H, Ma X, Li L. Epigallocatechin gallate, the main ingredient of green tea induces apoptosis in breast cancer cells. Front Biosci 2006;11:2428–33.

[30] Iwasaka T, Zheng PS, Yokoyama M, Fukuda K, Nakao Y, Sugimori H. Telomerase activation in cervical neoplasia. Obstet Gynecol 1998;91:260–2.

[31] Zheng PS, Iwasaka T, Yamasaki F, Ouchida M, Yokoyama M, Nakao Y, et al. Telomerase activity in gynecologic tumors. Gynecol Oncol 1997;64:171–5.

[32] Zheng PS, Iwasaka T, Yokoyama M, Nakao Y, Pater A, Sugimori H. Telomerase activation in in vitro and in vivo cervical carcinogenesis. Gynecol Oncol 1997;66:222–6.

[33] Naasani I, Oh-Hashi F, Oh-Hara T, Feng WY, Johnston J, Chan K, et al. Blocking telomerase by dietary polyphenols is a major mechanism for limiting the growth of human cancer cells in vitro and in vivo. Cancer Res 2003;63:824–30.

[34] Naasani I, Seimiya H, Tsuruo T. Telomerase inhibition, telomere shortening, and senescence of cancer cells by tea catechins. Biochem Biophys Res Commun 1998;249:391–6.

[35] Lin SC, Li WC, Shih JW, Hong KF, Pan YR, Lin JJ. The tea polyphenols EGCG and EGC repress mRNA expression of human telomerase reverse transcriptase (hTERT) in carcinoma cells. Cancer Lett 2006;236:80–8.

[36] Mittal A, Pate MS, Wylie RC, Tollefsbol TO, Katiyar SK. EGCG down-regulates telomerase in human breast carcinoma MCF-7 cells, leading to suppression of cell viability and induction of apoptosis. Int J Oncol 2004;24: 703–10.
[37] Webb T. Green tea experiments in lab, clinic yield mixed results. J Natl Cancer Inst 2000;92:1038–9.
[38] Cao Y, Cao R. Angiogenesis inhibited by drinking tea. Nature 1999;398: 381.
[39] Yang CS. Inhibition of carcinogenesis by tea. Nature 1997;389:134–5.
[40] Garbisa S, Biggin S, Cavallarin N, Sartor L, Benelli R, Albini A. Tumor invasion: molecular shears blunted by green tea. Nat Med 1999;5: 1216.