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Suppression of androgen receptor signaling and prostate specific antigen expression by (−)-epigallocatechin-3-gallate in different progression stages of LNCaP prostate cancer cells

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ABSTRACT

The green tea polyphenol, (−)-epigallocatechin-3-gallate (EGCG), inhibits the development and progression of prostate cancer in TRAMP mice and in men. We examined the effects of EGCG on LNCaP human prostate cancer sublines 104-S, 104-R1 and R1Ad representing different progression stages of prostate cancer. EGCG suppressed cell proliferation, prostate specific antigen (PSA) expression, and AR transcriptional activity in the different LNCaP sublines. Intraperitoneal administration of EGCG also suppressed the growth of relapsing R1Ad tumors and decreased tumor-derived serum PSA. Effects of EGCG on tumor PSA expression have the potential to affect accurate monitoring of patient tumor burden by serum PSA measurements.

1. Introduction

Green tea has many health benefits, including anticancer effects, which are attributed to the high catechin content of tea especially (−)-epigallocatechin-3-gallate (EGCG) [1,2]. Catechins account for 30–40% of green tea's dry weight [3]. The major catechins contained in green tea are (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epicatechin (EC) and catechin. EGCG is known to regulate a variety of signaling pathways, including pathways involving insulin-like growth factor-1 (IGF-1) [2,4,5]. Green tea is a common beverage consumed in China, Japan, Korea, and Taiwan. These East Asian countries have a much lower prostate cancer incidence compared to Western countries. Prostate cancer, a very common male-specific malignancy, is the third leading cause of cancer death among males in the United States and the leading cause of cancer death in men over 65 years old. Oral infusion of green tea polyphenols inhibits IGF-1-induced signaling and suppresses prostate carcinogenesis in Transgenic Adenocarcinoma Mouse Prostate (TRAMP) mice, a mouse model that spontaneously develops metastatic prostate cancer [5–7]. Oral administration of green tea catechins (600 mg/day) also prevents the progression of prostate cancer in men with high-grade prostate intra-epithelial neoplasia [8].

PSA, a 33 kDa protein in semen, is a kallikrein-related serine protease secreted by the epithelia cells of the prostate gland. PSA cleaves two abundant proteins in semen, semenogelin and fibronectin, and triggers liquefaction of the seminal plasma coagulum facilitating spermatozoa release. Serum PSA levels increase dramatically in men with prostate cancer and PSA measurement is widely used for early detection and monitoring of prostate cancer [9]. PSA expression is androgen-dependent and the promoter of the PSA gene is regulated by the androgen receptor (AR) [10].

Previously, we have used LNCaP human prostate cancer cells to establish a prostate cancer progression model

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both *in vitro* and *in vivo* [11–16]. Proliferation of the androgen-dependent LNCaP 104-S clonal subline is stimulated by androgen. 104-S cells progress to androgen-independent 104-R1 cells following androgen deprivation. The proliferation of LNCaP 104-R1 cells is androgen-independent [12]. Androgens paradoxically inhibit the proliferation of 104-R1 cells partially by down-regulating c-myc and inducing the cyclin-dependent kinase inhibitor $p27^{Kip1}$, which causes G1 cell cycle arrest in these cells. 104-R1 cells adapt to androgen-induced growth suppression and eventually R1Ad cells emerge. Proliferation of R1Ad cells is stimulated by androgen. AR protein levels increase during the progression from androgen-dependent LNCaP 104-S cells to androgen-independent LNCaP 104-R1 cells, but AR levels decrease during the transition from 104-R1 to R1Ad cells [12]. Androgens stimulate PSA expression in 104-S and 104-R1 cells. Implantation of testosterone pellets causes regression of 104-R1 xenografts in castrated mice, although the tumors relapse after a few weeks and emerge as R1Ad tumors. R1Ad tumors express lower levels of AR protein compared to parental 104-R1 tumors and the growth of R1Ad tumors is stimulated by androgen, however, R1Ad tumors do not regress after androgen withdrawal [15]. Recently, testosterone was reported to effectively control the progression of castration-resistant metastatic prostate cancer in one patient with androgen-independent prostate cancer [17], suggesting that our prostate cancer progression model may, in certain circumstances, mimic the clinical progression of prostate tumors from androgen-dependence to androgen-independence.

EGCG inhibits cell proliferation, PSA and Human tissue kallikreins 2 (hK2) secretion, expression of AR mRNA, and transcriptional activity of AR in androgen-dependent LNCaP cells treated with androgen [18,19]. EGCG, but not EGC, ECG, or EC, suppresses tumor growth of androgen receptor (AR)-negative androgen-insensitive PC-3 prostate xenografts, AR-positive androgen-independent LNCaP 104-R1 prostate xenografts, and MCF breast xenografts in athymic mice [1]. Since EGCG is a potential therapeutic agent to treat prostate cancer and since PSA is an important marker to monitor growth and progression of prostate tumors in patients, we have investigated if different LNCaP sublines representing different progression stages of prostate cancer exhibit different sensitivities to EGCG. In addition we have determined if the inhibitory effects of EGCG on cell proliferation are modulated by androgen in the different LNCaP sublines and whether EGCG affects AR and PSA protein and mRNA expression and AR transcriptional activity in LNCaP 104-S and 104-R1 cells. Finally, using R1Ad tumors as a model of advanced prostate cancer we have examined if EGCG affects tumor growth in castrated mice and if EGCG affects PSA secretion by these tumors.

2. Materials and methods

2.1. Cell culture

Androgen-dependent LNCaP 104-S and androgen-adapted LNCaP R1Ad cells were maintained and cultured in Dulbecco's modified Eagle's medium (DMEM) (Mediatech, Herndon, VA) supplemented with 1 nM dihydrotestosterone (DHT), 8% fetal bovine serum (FBS) (Atlas, Fort Collins, CO), 50 I.U. penicillin, and 50 μg/ml streptomycin (Cellgro, Mediatech). Androgen-independent LNCaP 104-R1 cells were maintained and cultured in DMEM supplemented with 8% dextran-coated charcoal stripped fetal bovine serum (CS-FBS), 50 I.U. penicillin, and 50 μg/ml streptomycin. Trypsin (Mediatech) was used for cell passage [11–16].

2.2. Cell proliferation assay

Cell number after 4 days of growth was analyzed by measuring DNA content with the fluorescent dye Hoechst 33258 (Sigma, St. Louis, MO) as described previously [13,20,21].

2.3. Luciferase-reporter assay

LNCaP 104-S or 104-R1 cells were seeded in at 3×10^4 cells in each well of a 48-well plate in DMEM containing 8% CS-FBS. LNCaP cells were transiently transfected with phRL-CMV-Renilla luciferase plasmid (normalization vector; 1 ng/well), PSA-luc (reporter gene vector; 50 ng/well), Bluescript SKII + (carrier; 750 ng/well) using the calcium phosphate co-precipitation method [21]. The PSA-luc reporter vector was prepared by isolating a 5.8 kb PSA (kallikrein 3) promoter sequence by PCR using a human chromosome 19 BAC (clone RP11-795B6; BACPAC Resources Center http://www.bacpac.chori.org) as template. First round PCR was performed with 5′-TGCTCATCTGCTGC GTGGG and 5′-GGAGGTGGACCAGTGATACGG as forward and reverse primers using a combination of Taq DNA polymerase (Advantage II Taq; Clontech) and Pfu DNA polymerase (Stratagene) according to Barnes [22]. After agarose gel electrophoresis, a 5 to 8 kb region was eluted and used as template for a second round of PCR using nested primers 5′-AAGACAAGGAGAACTCAAAGTGCG and 5′-AGCCCCACAAAGAGAAACACG. The first round PCR reaction consisted of 20 cycles of 94 °C for 20 s, 58 °C for 30 s, 72 °C for 7 min; the second round was 25 cycles of 94 °C for 20 s, 58 °C for 30 s, 72 °C for 7 min. An approximately 6.2 kb band was eluted and digested with Hind III. A 5.8 kb Hind III fragment was inserted into pBluescript II (Stratagene) for sequence verification. This fragment was then inserted into Hind III-digested pGL3-basic (Promega) to drive expression of a luciferase reporter gene. The 3′ Hind III site is 12 bp past the PSA transcription start site [23].

2.4. Western blotting analysis

Cells were lysed in Laemmli buffer without bromophenol blue dye. AR and PSA protein expression was determined by immunoblotting using anti-AR (AN-21) and anti-PSA (DAKO, Elostrup, Denmark) antibodies as described previously [11–16]. Anti-rabbit and anti-mouse IgG secondary antibodies were from Invitrogen (Carlsbad, California) and blots were scanned on an Odyssey infrared imaging system (LI-COR, Lincoln, NE).

2.5. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated with the TRIZOL Reagent (Invitrogen, Carlsbad, CA) and contaminating DNA was removed using DNase I (DNA-free, Ambion, Austin, TX). cDNA was synthesized from total RNA using the Omniscript RT synthesis kit (Qiagen, Valencia, CA). TaqMan primer/probes were designed using Primer Express (Applied Biosystems, Foster City, CA). A QuantiTect Probe PCR kit (Qiagen, Valencia, CA) and ABI Prism 7700 cycler (Applied Biosystems) were used in a dual-labeled probe protocol. Primers and probes used for androgen receptor (AR) and prostate specific antigen (PSA) mRNA detection were as described [15,16]. All transcript levels were normalized to human glyceraldehyde-3-phosphate dehydrogenase levels as described [15,16]. The 5′-end and 3′-end of probes were labeled with the reporter-fluorescent dye carboxyfluorescein and the quencher dye 6-carboxytetramethylrhodamine, respectively.

2.6. Animals

Experiments involving mice were approved by the University of Chicago Institutional Animal Care and Use Committee. Intact male BALB/c nu/nu mice (National Cancer Institute, Frederick, MD) were castrated and 14 days after castration mice were injected subcutaneously in both flanks with 1×10^6 LNCaP 104-R1 cells suspended in 0.5 mL Matrigel (BD Biosciences, Franklin Lakes, NJ). Mice with tumors were treated by implantation of testosterone propionate pellets as described [15]. Testosterone caused a transient regression of tumors. Volume of relapsed tumors were measured weekly using calipers, and volume was calculated using the formula: volume = length × width × height × 0.52 [15,16]. Testosterone propionate was purchased from Sigma (St. Louis, MO).

2.7. PSA assay

Blood was collected from the orbital sinus of mice and serum was prepared from blood and stored at −80 °C. PSA in mice sera was measured using an ACTIVE PSA EIA kit (Diagnostic System Laboratories, Inc., Webster, TX).

2.8. Data analysis

Data are presented as the mean plus standard deviation (SD) or standard error (SE) of at least three experiments or are representative of experiments repeated at least three times. Statistical significance was assessed using Student's *T*-test.

3. Results

3.1. EGCG suppresses proliferation of LNCaP sublines

In order to determine if different progression stages of LNCaP prostate cancer cells exhibit different sensitivities to EGCG treatment, we treated androgen-dependent LNCaP 104-S, androgen-independent LNCaP 104-R1, and androgen-adapted LNCaP R1Ad cells with increasing concentrations of EGCG in the absence or presence of 0.1 nM of the synthetic androgen R1881 (Fig. 1). As observed previously [11–16], proliferation of 104-S cells was stimulated by 0.1 nM R1881, proliferation of 104-R1 was suppressed by 0.1 nM R1881 and proliferation of R1Ad cells was slightly stimulated by androgen. EGCG suppressed the proliferation of 104-S, 104-R1, and R1Ad cells in the presence or absence of androgen. There was no significant differences in the sensitivities to EGCG among 104-S, 104-R1, and R1Ad cells when androgen was absent. When 0.1 nM R1881 was present, 80 μM EGCG caused a 50% reduction of cell number in 104-S and R1Ad cells and a 30% reduction of cell number in 104-R1, indicating that androgen-independent 104-R1 cells may be more resistant to EGCG treatment when androgen is present.

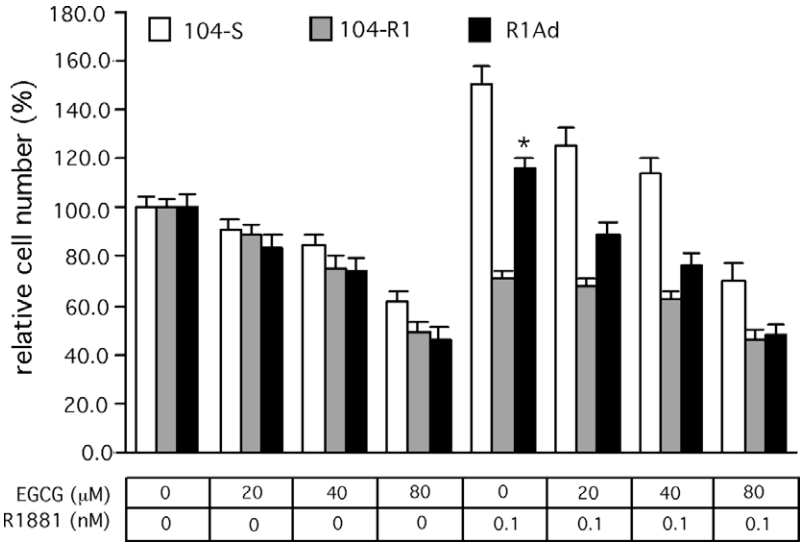

Fig. 1. The effect of EGCG on proliferation of androgen-dependent LNCaP 104-S cells, androgen-independent LNCaP 104-R1 cells, and androgen-adapted LNCaP R1Ad cells. LNCaP 104-S, 104-R1, and R1Ad cells were treated with increasing concentrations (0, 20, 40, 80 μM) of EGCG in the presence or absence of 0.1 nM R1881 for 96 h. Relative cell number was determined using a fluorometric DNA assay described in Section 2. Relative number of 104-S, 104-R1, and R1Ad cells were normalized to the number of cells at 0 nM R1881 and 0 μM EGCG. Asterisk (*) indicates that cell number difference of R1Ad cells between 0 nM R1881 and 0.1 nM R1881 in the absence of EGCG is statistically significant ($P < 0.05$).

3.2. EGCG suppresses AR and PSA protein and mRNA expression

We next determined the effect of EGCG on protein and mRNA expression of AR and PSA in our LNCaP progression model. Since R1Ad cells express very low amounts of AR in cell culture [12], we did not include R1Ad cells in this experiment. Similar to previous observations [12], androgen-independent 104-R1 cells expressed a higher level of AR protein (2-fold) and mRNA (6-fold) compared to androgen-dependent 104-S cells. EGCG dose-dependently decreased the expression of AR protein in cells treated with androgen and had little effect in the absence of androgen (Fig. 2A). EGCG also decreased AR mRNA in both 104-S and 104-R1 cells; the effect was most readily seen only at the highest dose and was greatest in 104-S cells in the absence of androgen and in 104-R1 cells in the presence of androgen (Fig. 2B). PSA protein in 104-S cells was not detectable with or without treatment with 0.1 nM R1881, while 0.1 nM R1881 clearly stimulated detectable PSA protein expression in 104-R1 (Fig. 2C), perhaps due to a higher level of AR in 104-R1 cells. The basal level of PSA mRNA expression in androgen-independent 104-R1 cells was much higher then that in 104-S cells. Androgen dramatically stimulated expression of PSA mRNA in both 104-S and 104-R1 cells. EGCG at higher doses decreased PSA protein and mRNA expression in 104-R1 and 104-S cells, an effect that was greatest in the presence of 0.1 nM R1881. This effect was more dose-dependent and of greater magnitude in 104-R1 than 104-S cells (Fig. 2D).

3.3. EGCG suppresses AR transcriptional activity

Since EGCG suppressed PSA protein and mRNA expression, we determined if EGCG suppresses AR transcription activity in our LNCaP progression model. A luciferase reporter gene construct containing the PSA promoter region was transfected into both androgen-dependent 104-S and androgen-independent 104-R1 cells. R1881 stimulated transcription of the reporter gene, while EGCG dose-dependently suppressed reporter gene expression in both 104-S cells and 104-R1 cells (Fig. 3), suggesting that EGCG suppressed PSA protein and mRNA expression, at least in part, by regulation of AR transcriptional activity. The effect of EGCG on reporter gene expression was greatest in the presence of androgen, although a 40–50% reduction in basal reporter gene expression was observed in the absence of androgen with 80 μM EGCG

3.4. EGCG suppresses tumor growth and serum PSA level of relapsed tumors

Previously, we showed that androgen ablation by castration causes shrinkage of androgen-dependent LNCaP 104-S tumors [16], while androgen treatment suppresses growth of androgen-independent 104-R1 tumors [15]. 104-R1 tumors adapt to androgenic suppression and relapse as R1Ad tumors [15]. Androgen ablation does not cause regression of R1Ad tumor growth, but androgen stimulates the growth of R1Ad tumors [15]. Although R1Ad cells in culture express very low levels of AR [12], R1Ad tumors express AR at levels comparable to that of 104-S tumors [15]. Androgen treatment increases the serum PSA level in castrated mice bearing R1Ad tumors, and removal of androgen decreases the serum PSA level comparable to that in intact mice bearing 104-S tumors [15]. Since R1Ad tumors may mimic clinically relapsing hormone-refractory prostate tumors after androgen treatment [17], we determined if EGCG could suppress tumor growth as well as serum PSA level in mice bearing R1Ad tumors. Mice in the treatment group received daily intraperitoneal injections of 1 mg EGCG, while mice in the control group received vehicle (water) only. Similar to what we have observed previously [15], R1Ad tumors in castrated mice without a testosterone supply did not grow over 6 weeks, while 11 weeks of EGCG treatment caused a 40% reduction in average tumor volume (Fig. 4A). Although the serum PSA level and PSA density (serum PSA level divided by tumor volume) in the control group did not change significantly during treatment with vehicle, EGCG

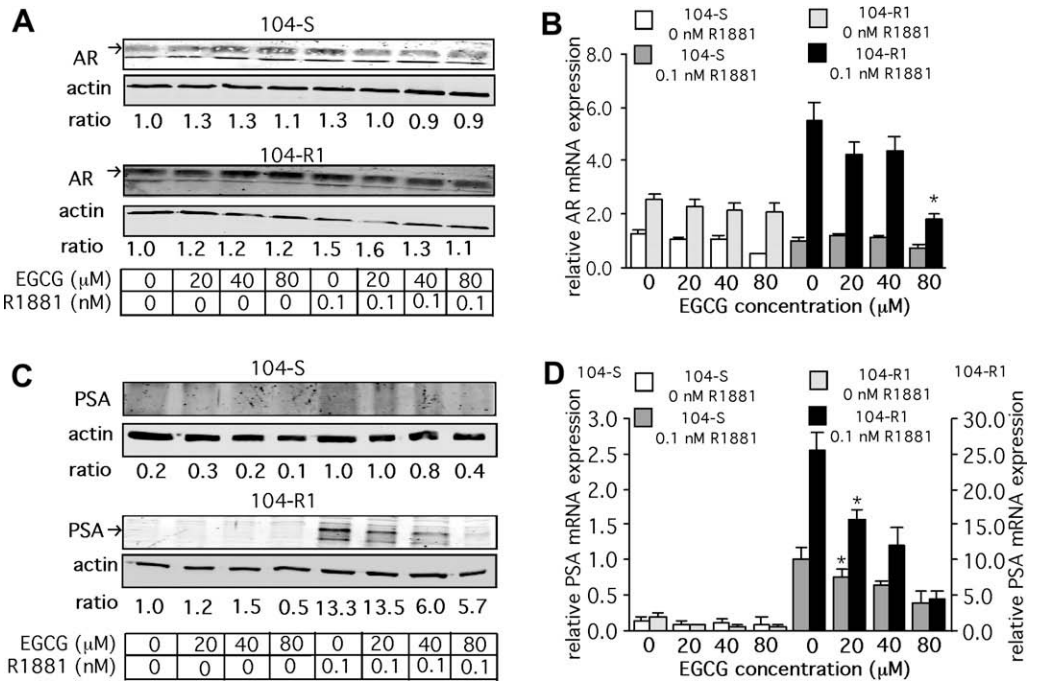

Fig. 2. EGCG suppression of AR and PSA protein and mRNA expression in LNCaP sublines. AR protein levels in 104-S and 104-R1 (A) cells as well as PSA protein levels in 104-S and 104-R1 (C) cells treated with increasing concentrations (0, 20, 40, 80 μM) of EGCG in the presence or absence of 0.1 nM R1881 for 96 h were assayed by Western blotting. Expression level of AR mRNA in 104-S and 104-R1 cells (B) and PSA mRNA in 104-S and 104-R1 cells (C) treated with increasing concentrations (0, 20, 40, 80 μM) of EGCG in the presence or absence of 0.1 nM R1881 for 96 h were assayed by real-time quantitative PCR. AR and PSA protein levels were normalized to that of 104-S or 104-R1 cells being treated with no EGCG and no androgen. AR and PSA mRNA levels in both 104-S and 104-R1 cells were normalized to that of 104-S cells not treated with EGCG but treated with 0.1 nM R1881. Since the PSA mRNA level in 104-R1 cells is much higher than that in 104-S cells, we used a different scale (right ordinate) for PSA mRNA in 104-R1 cells. Asterisk (*) indicates that the expression level of AR mRNA of 104-R1 cells after treatment with 80 μM EGCG and 0.1 nM R1881 compared to treatment with 0 μM EGCG and 0.1 nM R1881 is statistically significant ($P < 0.05$). Differences in the level of PSA mRNA between 104-S and 104-R1 cells after treatment with 20 μM EGCG and 0.1 nM R1881 and after treatment with 0 μM EGCG and 0.1 nM R1881 is also statistically significant ($P < 0.05$).

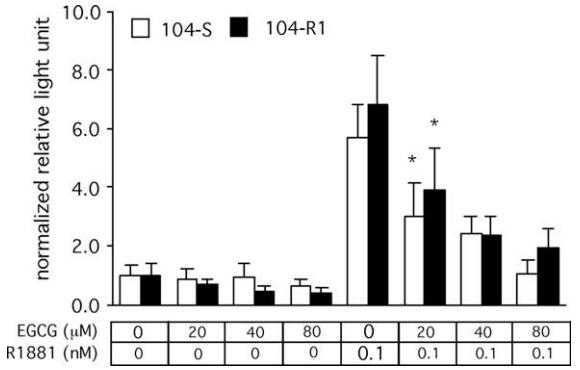

Fig. 3. Inhibition of androgen-regulated reporter gene expression by EGCG. LNCaP 104-S and 104-R1 cells were transiently transfected with a PSA promoter luciferase reporter gene construct. A CMV-*Renilla* luciferase reporter vector was used for normalization. After transfection, the cells were treated with various concentrations of EGCG in the absence or presence of 0.1 nM R1881 for 24 h. Luciferase expression was measured as described in Section 2. Normalized reporter gene expression is presented relative to activity in 104-S or 104-R1 cells not treated with EGCG or androgen. Asterisks (*) indicate that the difference in the relative light units of 104-S and 104-R1 cells treated with 0 and 20 μM EGCG in the presence of 0.1 nM R1881 are statistically significant ($P < 0.05$).

decreased both the serum PSA level and PSA density in the treatment group (Fig. 4B). Decreases in serum PSA did not totally correlate with the decreases in tumor volume. In a subgroup of 3 mice with five tumors treated with EGCG, serum PSA and PSA density dropped by 50%, while the average tumor volume reduction was less than 15% (Fig. 4C).

4. Discussion

The proliferation of androgen-dependent 104-S, androgen-independent 104-R1, and androgen-adapted R1Ad cells was affected similarly by EGCG in the absence of androgen (Fig. 1). EGCG, therefore, may be useful for suppressing prostate tumors at different progression stages, especially after androgen ablation. The fact that proliferation of 104-R1 cells in the presence of androgen was more resistant to EGCG treatment compared to 104-S and androgen-adapted R1Ad cells may be due to androgen inhibition of cell proliferation in this cell type. If androgen and EGCG suppress 104-R1 cell proliferation by modulating the same signaling processes, their combined effect would not be additive and the effect of EGCG would diminish in the presence of androgen. EGCG at concentrations above 80 μM is reported to induce G1 cell cycle arrest, apoptosis, and accumulation of cell cycle inhibitors $p21^{CIP/waf1}$, $p27^{Kip1}$, $p16^{INK4a}$, and $p18^{INK4c}$, to decrease expression of cyclin D1, cyclin E, cdk2, cdk4, and cdk6, as well as decrease binding of cyclin E and cdk2 [24,25]. Although withdrawal of androgen from androgen-dependent LNCaP 104-S cells or androgen treatment of androgen-independent LNCaP 104-R1 cells does not induce apoptosis, these treatments do cause G1 cell cycle arrest by up-regulating $p27^{Kip1}$, down-regulating c-myc, and suppressing cdk2 activity [11–13].

Because prostate cancer cell proliferation is regulated by androgens, we examined the effect of EGCG on AR expression and function. EGCG had little or no effect on AR protein levels in 104-S and 104-R1 cells in the absence

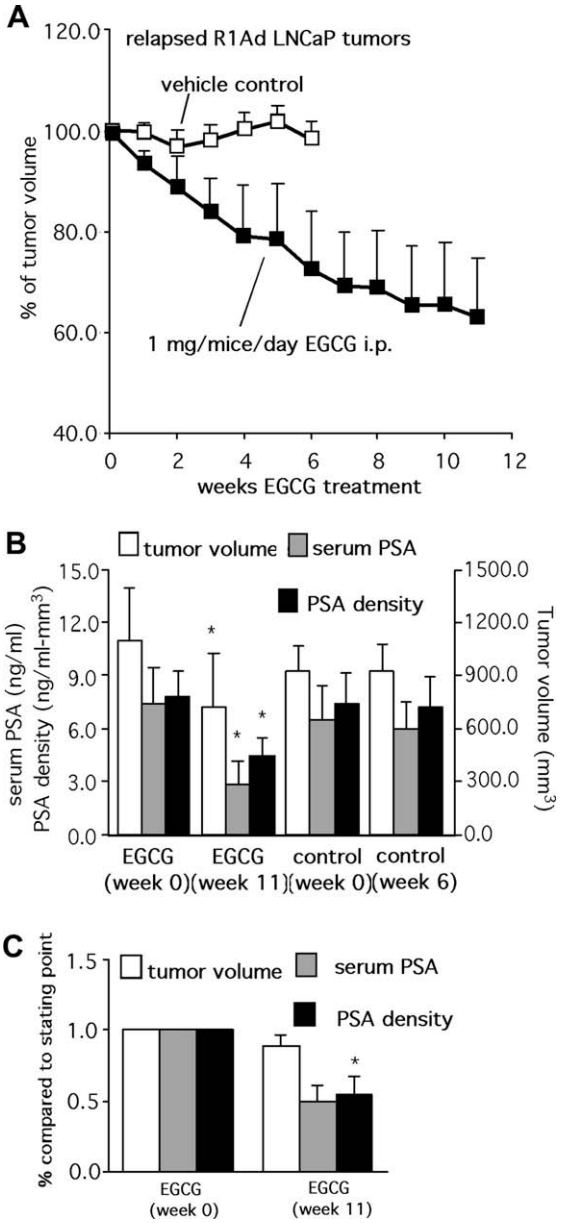

Fig. 4. Suppression of relapsed LNCaP tumor growth and serum PSA level by EGCG. Balb/c athymice mice were inoculated with androgen-independent LNCaP 104-R1 cells. Mice were implanted with 20 mg testosterone propionate pellets when the average volume of 104-R1 tumors reached 400 mm^3. Tumors initially regressed but relapsed after 4 weeks. Mice with relapsed tumors were separated into two groups, control (7 mice, 9 tumors) and treatment (7 mice, 10 tumors). Testosterone propionate pellets were removed after 8 weeks. At this point (assigned as week 0), the average tumor volume of control and treatment group was 679 mm^3 and 763 mm^3, respectively. Mice in the treatment group were then given EGCG intraperitoneally (1 mg/mouse/day) while mice in control group received water and tumor volume was monitored every week (A). Tumor volume, serum PSA, and serum PSA density (PSA level divided by tumor volume) before and after the treatment was determined (B). Asterisk (*) indicates that Volume, PSA, and PSA density difference among groups before and after EGCG treatment is statistically significant with $P < 0.05$ (B). Five tumors (from three mice) in the EGCG treatment group with a tumor volume reduction less than 15% were assayed for PSA and PSA density change (C).

of androgen, while in the presence of androgen, EGCG reduced AR protein levels in these cells by 30% (Fig. 2). The effect of EGCG on AR protein levels in the presence of androgen may be due, in part, to effects of EGCG on AR mRNA expression, since EGCG decreased AR mRNA by 20% and 68% in 104-S and 104-R1 cells, respectively. EGCG at lower concentrations had little effect on AR mRNA expression in the absence of androgen, mirroring effects of EGCG on AR protein levels. However, EGCG did significantly lower AR mRNA levels at the highest dose in contrast to the lack of an effect on AR protein levels. Since AR protein levels are maintained by a combination of synthesis and degradation, it is possible that EGCG affects AR degradation pathways, which alters AR levels independent of AR mRNA levels. Previously, it was reported that EGCG down-regulates the expression of AR and inhibits cell proliferation in LNCaP cells in part by modulating expression and activity of the transcriptional activator Sp1 that has a role in transcription of the AR gene [19].

EGCG also decreased expression of both PSA protein and mRNA in 104-S and 104-R1 cells in the presence and absence of androgen (Fig. 2C and D). PSA protein levels were decreased by 40–50% and mRNA levels by 30–80% by 80 μM EGCG. This effect may be related in part to the effect of EGCG on AR levels or perhaps due to direct effects on AR-induced PSA gene transcription. EGCG decreased basal and androgen-induced expression of a luciferase reporter gene linked to the PSA promoter/enhancer in both 104-S and 104-R1 cells (Fig. 3). Since PSA is used to screen patients and monitor for recurrence of prostate cancer after various therapies, the effect of EGCG has the potential to confound PSA monitoring and screening in patients consuming large amounts of green tea beverages. Limited oral bioavailability of EGCG may, however, limit such an effect. In this regard, a clinical trial using green tea polyphenols to treat prostate intra-epithelial neoplasia did not observe a significant decrease in plasma PSA in men consuming green tea polyphenols [8].

EGCG has been shown to inhibit the growth of prostate tumor xenografts in mice, the development of prostate cancer in TRAMP mice and the progression of prostatic intra-epithelial neoplasia in men [1,5–8]. EGCG also suppresses the growth of androgen-dependent and androgen-independent CWR22 human prostate cancer xenografts and tumor-derived serum PSA in nude mice [26,27], indicating that suppression of PSA by EGCG is not restricted to the LNCaP cell model. In this study we show that EGCG inhibits the growth of R1Ad tumors in castrated male mice (Fig. 4). EGCG decreased tumor volume by nearly 40% after 11 weeks of daily treatment. Consistent with the effect of EGCG on the PSA promoter (Fig. 3) and on expression of PSA in cells in culture (Fig. 2C and D), secretion of PSA by tumor xenografts decreased 60% after 11 weeks of EGCG treatment. Part of this decrease is undoubtedly due to decreased tumor volume but even PSA density decreased after treatment indicating effects on PSA expression independent of that related to tumor burden. The effect of EGCG on PSA expression in castrated mice may appear to be independent of an effect on AR signaling. However, recently it was shown that all enzymes necessary for androgen synthesis are expressed in LNCaP xenografts and some enzymes are up-regulated during the progression towards androgen-independence after withdrawal of androgen [28]. In addition, adrenal androgens may be present in prostate cancer tissue and activate the PSA promoter and stimulate PSA expression in LNCaP cells via AR [29], although circulating adrenal androgen levels in mice are much lower compared to that in humans. Since R1Ad tumors express AR, *de novo* androgen generated from LNCaP R1Ad tumors and murine adrenal androgens may activate AR and the PSA promoter in R1Ad tumors, which express AR at levels comparable to LNCaP 104-S tumors, and stimulate expression of PSA in castrated athymic mice. Our observation that EGCG can further suppress the serum PSA level in castrated mice suggests that EGCG can inhibit AR signaling and PSA promoter activity perhaps induced by *de novo* tumor-derived androgen and/or adrenal androgen. This observation indicates that EGCG may be beneficial for advanced prostate cancer patients with high serum PSA after androgen deprivation therapy.

Conflict of interest

There is no conflict of interest for any of the authors.

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References

[1] S. Liao, Y. Umekita, J. Guo, J.M. Kokontis, R.A. Hiipakka, Growth inhibition and regression of human prostate and breast tumors in athymic mice by tea epigallocatechin gallate, Cancer Lett. 96 (1995) 239–243.
[2] S. Liao, Y.H. Kao, R.A. Hiipakka, Green tea: biochemical and biological basis for health benefits, Vitam. Horm. 62 (2001) 1–94.
[3] E.C. Stuart, M.J. Scandlyn, R.J. Rosengren, Role of epigallocatechin gallate (EGCG) in the treatment of breast and prostate cancer, Life Sci. 79 (2006) 2329–2336.
[4] Y.H. Kao, R.A. Hiipakka, S. Liao, Modulation of endocrine systems and food intake by green tea epigallocatechin gallate, Endocrinology 141 (2000) 980–987.
[5] V.M. Adhami, I.A. Siddiqui, N. Ahmad, S. Gupta, H. Mukhtar, Oral consumption of green tea polyphenols inhibits insulin-like growth factor-I-induced signaling in an autochthonous mouse model of prostate cancer, Cancer Res. 64 (2004) 8715–8722.
[6] S. Gupta, K. Hastak, N. Ahmad, J.S. Lewin, H. Mukhtar, Inhibition of prostate carcinogenesis in TRAMP mice by oral infusion of green tea polyphenols, Proc. Natl. Acad. Sci. USA 98 (2001) 10350–10355.
[7] C.E. Harper, B.B. Patel, J. Wang, I.A. Eltoum, C.A. Lamartiniere, Epigallocatechin-3-gallate suppresses early stage, but not late stage prostate cancer in TRAMP mice: mechanisms of action, Prostate 67 (2007) 1576–1589.
[8] S. Bettuzzi, M. Brausi, F. Rizzi, G. Castagnetti, G. Peracchia, A. Corti, Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study, Cancer Res. 66 (2006) 1234–1240.
[9] H. Lilja, D. Ulmert, A.J. Vickers, Prostate-specific antigen and prostate cancer: prediction, detection and monitoring, Nat. Rev. Cancer 8 (2008) 268–278.
[10] K.B. Cleutjens, C.C. van Eekelen, H.A. van der Korput, A.O. Brinkmann, J. Trapman, Two androgen response regions cooperate in steroid hormone regulated activity of the prostate-specific antigen promoter, J. Biol. Chem. 271 (1996) 6379–6388.

[11] J. Kokontis, K. Takakura, N. Hay, S. Liao, Increased androgen receptor activity and altered c-myc expression in prostate cancer cells after long-term androgen deprivation, Cancer Res. 54 (1994) 1566–1573.
[12] J.M. Kokontis, N. Hay, S. Liao, Progression of LNCaP prostate tumor cells during androgen deprivation: hormone-independent growth, repression of proliferation by androgen, and role for 27Kip1 in androgen-induced cell cycle arrest, Mol. Endocrinol. 12 (1998) 941–953.
[13] J.M. Kokontis, S. Hsu, C.-P. Chuu, M. Dang, J. Fukuchi, R.A. Hiipakka, S. Liao, Role of androgen receptor in the progression of human prostate tumor cells to androgen independence and insensitivity, Prostate 65 (2005) 287–298.
[14] Y. Umekita, R.A. Hiipakka, J.M. Kokontis, S. Liao, Human prostate tumor growth in athymic mice: inhibition by androgens and stimulation by finasteride, Proc. Natl. Acad. Sci. USA 93 (1996) 11802–11807.
[15] C.-P. Chuu, R.A. Hiipakka, J. Fukuchi, J.M. Kokontis, S. Liao, Androgen causes growth suppression and reversion of androgen independent prostate cancer xenografts to an androgen-stimulated phenotype in athymic mice, Cancer Res. 65 (2005) 2082–2084.
[16] C.-P. Chuu, R.A. Hiipakka, J.M. Kokontis, J. Fukuchi, R.-Y. Chen, S. Liao, Inhibition of tumor growth and progression of LNCaP prostate cancer cells in athymic mice by androgen and liver X receptor agonist, Cancer Res. 66 (2006) 6482–6486.
[17] P. Mathew, Prolonged control of progressive castration-resistant metastatic prostate cancer with testosterone replacement therapy: the case for a prospective trial, Ann. Oncol. 19 (2008) 395–396.
[18] E. Pezzato, L. Sartor, I. Dell'Aica, R. Dittadi, M. Gion, C. Belluco, M. Lise, S. Garbisa, Prostate carcinoma and green tea: PSA-triggered basement membrane degradation and MMP-2 activation are inhibited by (−)epigallocatechin-3-gallate, Int. J. Cancer 112 (2004) 787–792.
[19] F. Ren, S. Zhang, S.H. Mitchell, R. Butler, C.Y. Young, Tea polyphenols down-regulate the expression of the androgen receptor in LNCaP prostate cancer cells, Oncogene 19 (2000) 1924–1932.
[20] R. Rago, J. Mitchen, G. Wilding, DNA fluorometric assay in 96-well tissue culture plates using Hoechst 33258 after cell lysis by freezing in distilled water, Anal. Biochem. 191 (1990) 31–34.
[21] C.-P. Chuu, R.-Y. Chen, R.A. Hiipakka, J.M. Kokontis, K.V. Warner, J. Xiang, S. Liao, The liver X receptor agonist T0901317 acts as androgen receptor antagonist in human prostate cancer cells, Biochem. Biophys. Res. Commun. 357 (2007) 341–346.
[22] W.M. Barnes, PCR amplification of up to 35-kb DNA with high fidelity and high yield from l bacteriophage templates, Proc. Natl. Acad. Sci. USA 91 (1994) 2216–2220.
[23] K.B. Cleutjens, H.A. van der Korput, C.C. van Eekelen, H.C. van Rooij, P.W. Faber, J. Trapman, An androgen response element in a far upstream enhancer region is essential for high, androgen-regulated activity of the prostate-specific antigen promoter, Mol. Endocrinol. 11 (1997) 148–161.
[24] S. Gupta, N. Ahmad, A.L. Nieminen, H. Mukhtar, 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. 64 (2000) 82–90.
[25] S. Gupta, T. Hussain, H. Mukhtar, Molecular pathway for (−)-epigallocatechin-3-gallate-induced cell cycle arrest and apoptosis of human prostate carcinoma cells, Arch. Biochem. Biophys. 410 (2003) 177–185.
[26] S.C. Lee, W.K. Chan, T.W. Lee, W.H. Lam, X. Wang, T.H. Chan, Y.C. Wong, Effect of a prodrug of the green tea polyphenol (−)-epigallocatechin-3-gallate on the growth of androgen-independent prostate cancer in vivo, Nutr. Cancer 60 (2008) 483–491.
[27] I.A. Siddiqui, N. Zaman, M.H. Aziz, S.R. Reagan-Shaw, S. Sarfaraz, V.M. Adhami, N. Ahmad, S. Raisuddin, H. Mukhtar, Inhibition of CWR22Rnu1 tumor growth and PSA secretion in athymic nude mice by green and black teas, Carcinogenesis 27 (2006) 833–839.
[28] J.A. Locke, E.S. Guns, A.A. Lubik, H.H. Adomat, S.C. Hendy, C.A. Wood, S.L. Ettinger, M.E. Gleave, C.C. Nelson, Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer, Cancer Res. 68 (2008) 6407–6415.
[29] A. Mizokami, E. Koh, H. Fujita, Y. Maeda, M. Egawa, K. Koshida, S. Honma, E.T. Keller, M. Namiki, The adrenal androgen androstenediol is present in prostate cancer tissue after androgen deprivation therapy and activates mutated androgen receptor, Cancer Res. 64 (2004) 765–771.