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Molecular pathway for (−)-epigallocatechin-3-gallate-induced cell cycle arrest and apoptosis of human prostate carcinoma cells

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Abstract

Epigallocatechin-3-gallate (EGCG), the major polyphenolic constituent present in green tea, is a promising chemopreventive agent. We recently showed that green tea polyphenols exert remarkable preventive effects against prostate cancer in a mouse model and many of these effects are mediated by the ability of polyphenols to induce apoptosis in cancer cells [Proc. Natl. Acad. Sci. USA 98 (2001) 10350]. Earlier, we showed that EGCG causes a G0/G1 phase cell cycle arrest and apoptosis of both androgen-sensitive LNCaP and androgen-insensitive DU145 human prostate carcinoma cells, irrespective of p53 status [Toxicol. Appl. Pharmacol. 164 (2000) 82]. Here, we provide molecular understanding of this effect. We tested a hypothesis that EGCG-mediated cell cycle dysregulation and apoptosis is mediated via modulation of cyclin kinase inhibitor (cki)–cyclin–cyclin-dependent kinase (cdk) machinery. As shown by immunoblot analysis, EGCG treatment of LNCaP and DU145 cells resulted in significant dose- and time-dependent (i) upregulation of the protein expression of WAF1/p21, KIP1/p27, INK4a/p16, and INK4c/p18, (ii) down-modulation of the protein expression of cyclin D1, cyclin E, cdk2, cdk4, and cdk6, but not of cyclin D2, (iii) increase in the binding of cyclin D1 toward WAF1/p21 and KIP1/p27, and (iv) decrease in the binding of cyclin E toward cdk2. Taken together, our results suggest that EGCG causes an induction of G1 phase ckis, which inhibits the cyclin–cdk complexes operative in the G0/G1 phase of the cell cycle, thereby causing an arrest, which may be an irreversible process ultimately leading to apoptotic cell death. This is the first systematic study showing the involvement of each component of cdk inhibitor–cyclin–cdk machinery during cell cycle arrest and apoptosis of human prostate carcinoma cells by EGCG.

Keywords: Green tea; Epigallocatechin-3-gallate; Apoptosis; Prostate cancer; Cell cycle

In recent years, (−)-epigallocatechin-3-gallate (EGCG),[1] the major polyphenolic constituent present in green tea, has shown remarkable effects in inhibiting cancer cell growth both in cell culture system and in in vivo tumor models [1–10]. Earlier studies in our laboratory have shown that treatment of androgen-sensitive and androgen-insensitive human prostate carcinoma cells with EGCG resulted in G0/G1 phase cell cycle arrest and apoptosis, irrespective of the p53 status of these cells [11]. This effect was mediated by upregulation in the protein expression of WAF1/p21. Many other laboratories have verified similar antiproliferative responses of EGCG in several other human carcinoma cells [12–14]. More recently, we have demonstrated that consumption of human-achievable doses of green tea polyphenols by TRAMP mice significantly inhibited prostate carcinogenesis and increased cancer-free and overall survival of these mice [15].

Several studies have demonstrated an association between cell cycle regulation and cancer and, in recent years, inhibition of the cell cycle has been appreciated as a target for the management of cancer [16,17]. Studies have shown that cellular proliferation is controlled by

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[1] *Abbreviations used:* EGCG, (−)-epigallocatechin-3-gallate; cdk, cyclin-dependent kinase; cki, cyclin kinase inhibitor; TRAMP, transgenic adenocarcinoma of the mouse prostate; PBS, phosphate-buffered saline.

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the cell cycle and a dysregulated cell cycle is a hallmark of cancer [18,19]. All cancers allow the existence of cellular overload linked in a complex but well-choreographed process of cell cycle regulation with reduced sensitivity of signals to adhere, differentiate, or die. The regulation of the cell cycle is controlled in part by a family of protein kinase complex minimally of a catalytic subunit, the cyclin-dependent kinase (cdk) and its essential activating partner, the cyclin [20]. These cyclin–cdk complexes phosphorylate substrates required for progression through the cell cycle. Furthermore, the cyclin–cdk complexes are associated with various inhibitory molecules including a class of proteins known as cyclin kinase inhibitor (cki) that achieve further regulation of their kinase activity [20]. In the present study, we demonstrate that EGCG, via modulation in the cki–cyclin–cdk machinery, results in a G1 phase arrest of the cell cycle followed by apoptosis of human prostate carcinoma cells. To our knowledge, this is the first systematic study showing the involvement of each component of the cki–cyclin–cdk machinery during cell cycle arrest and apoptosis of prostate carcinoma cells by EGCG.

Materials and methods

Materials

A purified preparation of EGCG (>98% pure) was kindly provided as a gift by Dr. Yukihiko Hara of Mitsui Norin Ltd., Shizuoka, Japan. The mono- and polyclonal antibodies (human reactive anticdk2, 4, and 6, WAF1/p21, KIP1/p27, INK4a/p16, and INK4c/p18) were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). The human reactive monoclonal and polyclonal antibodies for anticyclin D1, D2, and E were obtained from Neomarker (Fremont, CA).

Cell culture

The human prostate carcinoma cells, viz., androgen-sensitive LNCaP and androgen-insensitive DU145 cells, were obtained from American Type Culture Collection (Rockville, MD) and were cultured in RPMI 1640 cell culture medium (Mediatech, Inc., VA) supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin. The cells were maintained at 37 °C and 5% CO_2 in a humid environment.

Treatment of cells

EGCG dissolved in PBS (50 mM, pH 7.4) was employed for the treatment of cells. The cells (70–80% confluent) were treated with 5, 10, 20, and 40 μg/ml concentrations of EGCG for 24 h in complete cell culture medium, whereas cells treated with only PBS served as control. For time-dependent studies the cells were treated with a 20 μg/ml dose of EGCG for desired time intervals.

DNA cell cycle analysis

The growing cells (70% confluent) were treated with EGCG (5, 10, 20, and 40 μg/ml doses) in complete medium for 24 h. The cells were trypsinized thereafter, washed twice with cold PBS, and centrifuged. The pellet was resuspended in 50 μl cold PBS and 450 μl cold methanol for 1 h at 4 °C. The cells were centrifuged at 1100 rpm for 5 min; the pellet was washed twice with cold PBS, suspended in 500 μl PBS, and incubated with 5 μl RNAse (20 μg/ml final concentration) at 37 °C for 30 min. The cells were chilled over ice for 10 min and stained with propidium iodide (50 μg/ml final concentration) for 1 h and analyzed by flow cytometry.

Quantification of apoptosis

For quantification of apoptosis, the cells were grown at a density of 1×10^6 cells in 100-mm culture dishes and were treated with EGCG (10, 20, and 40 μg/ml concentrations) for 24 h. The cells were trypsinized, washed with PBS, and processed for labeling with fluorescein-tagged deoxyuridine triphosphate nucleotide and propidium iodide by use of an APO-DIRECT apoptosis kit obtained from Phoenix Flow Systems (San Diego, CA) as per manufacturer's protocol. The labeled cells were then analyzed by flow cytometry.

Morphological analysis

For assessing morphological changes, 50–60% confluent cells were treated with PBS alone or with 10, 20, and 40 μg/ml concentrations of EGCG. After 24 h of treatment, photographs were taken using a phase-contrast microscope at 200× magnification.

Protein extraction, Western blotting, and immunoprecipitation

The cells (70% confluent) were treated with EGCG (5, 10, 20, and 40 μg/ml doses) in complete media for 24 h. For the time-dependent assay, the cells (50–60% confluent) were treated for 24, 48, and 72 h with 20 μg/ml EGCG, after which the media were aspirated and the cells were washed with cold PBS (50 mM, pH 7.4) and ice-cold lysis buffer (50 mM Tris–HCl, 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 20 mM NaF, 100 mM Na_3VO_4, 0.5% Nonidet-P40, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride (pH 7.4)) with freshly added protease inhibitor cocktail (Protease Inhibitor Cocktail Set III; Calbiochem, La Jolla, CA) over ice for 30 min. The cells were scraped; the lysate was collected

in a microfuge tube and passed through a $21\frac{1}{2}$G needle to break up the cell aggregates. The lysate was cleared by centrifugation at 14,000*g* for 15 min at 4 °C and the supernatant (total cell lysate) was used or immediately stored at −80 °C. The protein concentration was determined by DC Bio-Rad assay using the manufacturer's protocol (Bio Rad Laboratories, Hercules, CA).

For Western blotting, 25–50 μg protein was resolved over 8–12% polyacrylamide gels and transferred to a nitrocellulose membrane. The blot was incubated in blocking buffer (5% nonfat dry milk/1% Tween 20; in 20 mM Tris-buffered saline, pH 7.6) for 1 h at room temperature, incubated with the appropriate monoclonal or polyclonal primary antibody in blocking buffer for from 1 h to overnight at 4 °C followed by incubation with antimouse or antirabbit secondary antibody horseradish peroxidase conjugate obtained from Amersham Life Science Inc. (Arlington Height, IL), and detected by chemiluminescence and autoradiography using XAR-5 film obtained from Eastman Kodak Co. (Rochester, NY). Densitometric measurements of the bands in Western blot analysis were performed using the digitalized scientific software program UN-SCAN-IT purchased from Silk Scientific Corp. (Orem, UT).

For immunoprecipitation, the cell lysates containing 100–200 μg of protein were taken in 1.0 ml RIPA buffer and were precleared by incubating with normal mouse or rabbit IgG (1 μg) and protein A–Sepharose 4B fast flow (20 μl) (Sigma Chemical Co., St. Louis, MO) for 1 h at 4 °C. The supernatant was collected and incubated with 1.0 μg of appropriate primary antibody and 20 μl of protein A–Sepharose overnight at 4 °C. The beads were collected by centrifugation at 3000 rpm for 10 min at 4 °C and washed with RIPA buffer three times after the centrifugation steps. The immunocomplexes were collected, heated at 100 °C over boiling water in 2× denaturing sample buffer for 5 min, and resolved over a SDS 4–12% polyacrylamide gel, followed by Western blot analysis and chemiluminescent detection.

Results and discussion

In recent years, green tea, specifically its major constituent EGCG, has gained much attention for its cancer-chemopreventive properties both in cell culture systems and in several animal tumor model systems [1–14]. Recent studies are suggesting that EGCG may also possess therapeutic effects [21–25]. Much interest in EGCG is due to its differential effects on normal versus cancer cells [4,26,27]. Importantly, at physiologically attainable concentrations EGCG kills cancer cells through apoptosis but has no effect on normal cells [4,26,27]. Earlier studies in our laboratory and elsewhere have shown that green tea-derived polyphenols including EGCG are capable of imparting dose-dependent (i) inhibition of cell growth, (ii) G0/G1 phase arrest of the cell cycle, and (iii) induction of apoptosis in several types of human carcinoma cells [4,11]. Deciphering the molecular mechanism(s) by which green tea or EGCG imparts its antiproliferative effects could be important because it may result in improved opportunities for the management of cancer.

In the present study, we have tested the hypothesis that EGCG-mediated cell cycle dysregulation and apoptosis is mediated via modulation in cki–cyclin–cdk machinery. Therefore, we first evaluated the effect of EGCG on the distribution of human prostate carcinoma cells (androgen-sensitive LNCaP and androgen-insensitive DU145) in the cell cycle. The choice of these cell lines is due to the fact that prostate cancer is known to be intrinsically heterogeneous and represents a mixture of androgen-responsive and -unresponsive cells at the time of clinical diagnosis [28]. Therefore, strategies aimed toward the selective elimination of both types of cells through mechanism-based preventive/therapeutic approaches could be important in the management of prostate cancer. Since green tea and EGCG have been shown to possess growth-inhibitory properties against prostate cancer cells, we first performed DNA cell cycle analysis employing growing LNCaP and DU145 cells. Compared to vehicle-treated controls, EGCG treatment resulted in an appreciable arrest of LNCaP and DU145 cells in the G0/G1 phase of the cell cycle after 24 h of treatment. EGCG treatment of cells caused a dose-dependent increase in arrest of cells in G0/G1 phase of the cell cycle (Fig. 1). This increase in the G0/G1 phase cell population was accompanied by a concomitant decrease in the S phase and G2/M phase cell populations.

Since the cell cycle arrest may lead to induction of apoptosis, in the next series of experiments we determined the extent of apoptosis caused by EGCG in human prostate carcinoma cells. Apoptosis is a physiological process by which cells are removed when an agent damages their DNA [29]. Apoptosis represents a discrete manner of cell death that differs from necrotic cell death and is regarded as an efficient way to eliminate damaged cells [30]. Agents that can modulate apoptosis may be able to affect the steady state cell population, which may be useful in the management and therapy of cancer [31,32]. The LNCaP and DU145 cells were treated with 10, 20, and 40 μg/ml concentrations of EGCG for 24 h, labeled with deoxyuridine triphosphate and propidium iodide, and analyzed for apoptosis by TUNEL assay. As shown by data in Fig. 2A, EGCG treatment of LNCaP cells resulted in 8.2, 14.8, and 28.5% increases (compared to 2.0% in control) in TUNEL-positive cells at 10, 20, and 40 μg/ml EGCG, respectively. In DU145 cells, EGCG treatment resulted in 10.2, 15.0, and 32.6% in TUNEL-positive cells compared to 1.5% in control cells. These results were further

LNCaP Cells

Dose μg/ml	% Cell Population		
	G0/G1	S	G2/M
EGCG 0	56.2	30.0	13.8
EGCG 5	58.3	29.4	12.3
EGCG 10	63.6	25.7	10.7
EGCG 20	67.4	23.3	9.3
EGCG 40	69.5	20.8	9.7

DU145 Cells

Dose μg/ml	% Cell Population		
	G0/G1	S	G2/M
EGCG 0	52.4	32.2	15.4
EGCG 5	53.0	33.6	13.4
EGCG 10	55.3	28.5	16.2
EGCG 20	58.6	25.7	15.7
EGCG 40	65.8	22.4	11.8

Fig. 1. Effect of EGCG on DNA cell cycle analysis in LNCaP and DU145 cells. The cells were treated with vehicle or EGCG (5, 10, 20, and 40 μg/ml, for 24 h) and analyzed by flow cytometry. The percentages of cells in the G0/G1, S, and G2/M phases were calculated using cellfit computer software. Other details are described under Materials and methods. The data shown here are from a representative experiment repeated three times with similar results.

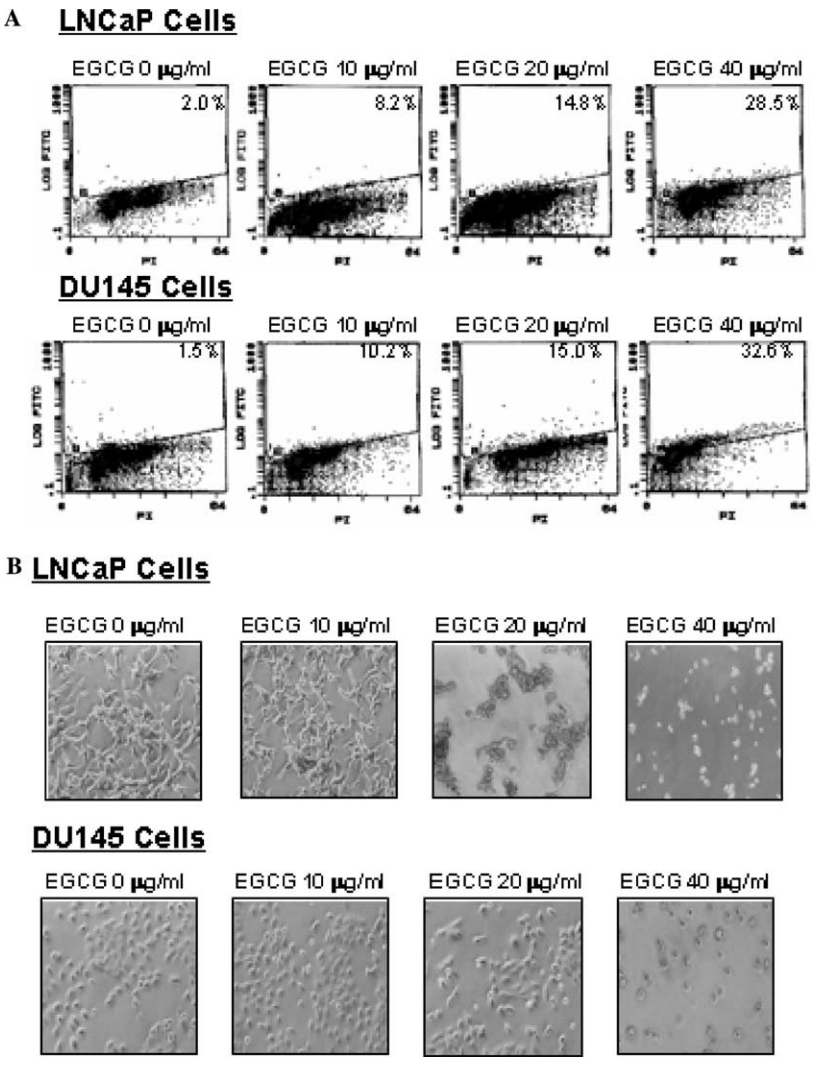

Fig. 2. Effect of EGCG on apoptosis and cell morphology in LNCaP and DU145 cells. (A) Quantification of apoptosis. The cells were treated with vehicle or EGCG (10, 20, and 40 μg/ml for 24 h) and labeled with deoxyuridine triphosphate using terminal deoxynucleotide transferase and PI by using an apoptosis kit followed by flow cytometry. Cells showing deoxyuridine triphosphate fluorescence above that of the control population, as indicated by the line in each graph, are considered apoptotic cells and their percentages of the population are shown in each box. (B) Morphological analysis. Cells were treated with EGCG as indicated above and after 24 h pictures were taken using a phase-contrast microscope at 200× magnification. Other details are described under Materials and methods. The data shown here are from a representative experiment repeated three times with similar results.

verified by microscopic analysis of cells (Fig. 2B). EGCG treatment to both cell lines resulted in inhibition of cell contact and development of early apoptotic morphology such as rounding off and chromatin condensation of cells, a phenomenon consistent with apoptosis.

Several studies have demonstrated an association between cell cycle regulation and cancer as molecular analyses of human cancers have shown that cell cycle regulators are frequently mutated in most common malignancies [18–20,33]. Also, in recent years, inhibition of the cell cycle has been appreciated as a target for the management of cancer [16,17]. The passage through the cell cycle in eukaryotes is orchestrated by the function of a family of protein kinase complexes. Each complex is composed minimally of cyclins (regulatory subunit) that bind to cdks (catalytic subunit) to form active cyclin–cdk complexes. These complexes are activated at various checkpoints after specific intervals during the cell cycle and can also be regulated by a number of exogenous factors [20,34]. However, in transformed cells, cell cycle progression could be a mitogenic signal-dependent or -independent process [35,36]. Cdk activity is further regulated by activating or inhibitory phosphorylation and by small proteins known as cyclin kinase inhibitors. The cki include the CIP/KIP and INK4 family of proteins. We next studied the modulation in cell cycle regulatory events during EGCG-mediated cell cycle dysregulation and apoptosis in both cell lines. The two cell lines, viz., LNCaP and DU145, employed in this study differ in their p53 status as the LNCaP cells harbor wild-type p53, and the DU145 cells carry mutant p53. The choice of these cells is due to the following facts. The tumor suppressor gene p53 is regarded as a key element in maintaining a balance between cell growth and cell death in the living system [37]. p53, in response to DNA damage, triggers a variety of cell cycle regulatory events to limit the proliferation of damaged cells. This "gate keeper gene," in a number of human tumors, is inactivated by a mutation resulting in uncontrolled cellular proliferation [38]. In our earlier studies we have shown that EGCG treatment results in an upregulation and/or stabilization of p53 in LNCaP cells (with wild-type p53) but not in DU145 cells (mutant p53) [11]. We next examined the effect of EGCG on cell cycle regulatory molecules operative in G0/G1 phase of the cell cycle in both cell lines. As shown by Western blot analysis (Fig. 3), EGCG treatment (5, 10, 20, and 40 μg/ml for 24 h) of LNCaP cells resulted in significant dose-dependent upregulation of cyclin kinase inhibitors, viz., WAF1/p21, KIP1/p27, INK4a/p16, and INK4c/p18. To investigate the time-dependent effect of EGCG on these cell cycle regulatory molecules, we selected the 20 μg/ml dose of EGCG. At this dose, EGCG treatment was also found to result in a time-dependent upregulation of these molecules. Similar results were obtained with DU145 cells where treatment of EGCG resulted in dose- and time-dependent increases in protein expression of WAF1/p21, KIP1/p27, INK4a/p16, and INK4c/p18. Many studies have shown that these ckis regulate the progression of cells in G0/G1 phase arrest of the cell cycle and an induction of these molecules causes a blockade of the G1 to S transition, thereby resulting in a G0/G1 phase arrest of the cell cycle [39]. Further, studies have shown a loss of functional cki in different human cancers and derived cell lines that leads to uncontrolled

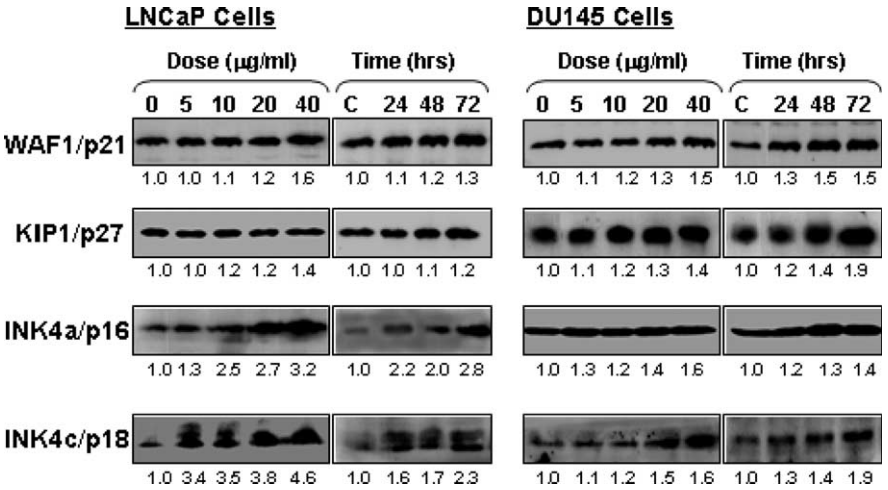

Fig. 3. Effect of EGCG on protein expression of WAF1/p21, KIP1/p27, INK4a/p16, and INK4c/p18 in LNCaP and DU145 cells. The cells were treated with vehicle or EGCG (5, 10, 20, and 40 μg/ml for 24 h and 20 μg/ml for 24, 48, and 72 h) and then harvested. Total cell lysates were prepared and 25–50 μg protein was subjected to SDS–PAGE followed by Western blot analysis and chemiluminescent detection. Data shown below the blots represent fold modulation in the protein expression normalized to β-actin. The details are described under Materials and methods. The data shown here are from a representative experiment repeated three times with similar results.

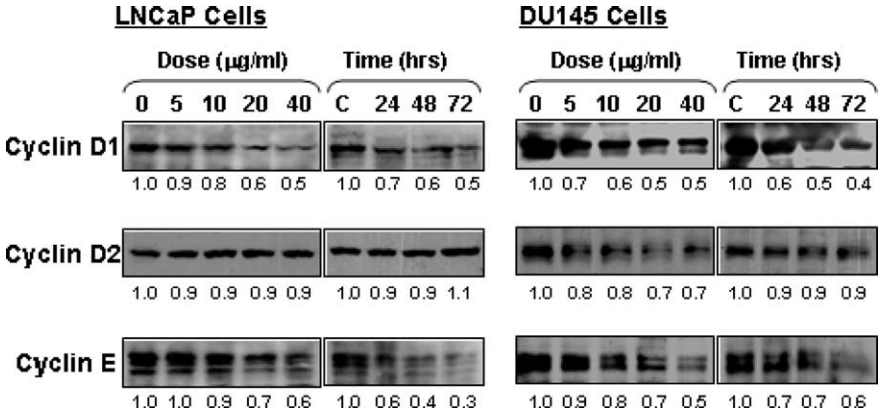

Fig. 4. Effect of EGCG on protein expression of cyclin D1, cyclin D2, and cyclin E in LNCaP and DU145 cells. The cells were treated with vehicle or EGCG (5,10, 20, and 40 μg/ml for 24 h and 20 μg/ml for 24, 48, and 72 h) and then harvested. Total cell lysates were prepared and 25–50 μg protein was subjected to SDS–PAGE followed by Western blot analysis and chemiluminescent detection. Data shown below the blots represent fold modulation in the protein expression normalized to β-actin. The details are described under Materials and methods. The data shown here are from a representative experiment repeated three times with similar results.

cell proliferation due to an increase in the levels of cdk–cyclin complex [40].

The most important member of the CIP/KIP family is WAF1/CIP1/p21 which is regarded as an almost universal inhibitor of cdks [41,42]. Many studies have shown that certain exogenous stimuli may result in a p53-dependent and p53-independent induction of WAF1/p21, which in turn, may trigger a series of events ultimately resulting in a cell cycle arrest and/or apoptosis [39–42]. Our data showing an induction of WAF1/p21 by EGCG appears to be p53 dependent in LNCaP cells (with wild-type p53) and p53 independent in DU145 cells (with mutant p53). Therefore, EGCG seems to be capable of imposing its responses in prostate carcinoma cells with or without mutations in p53. Similarly, recent studies [43,44] have also shown that KIP1/p27 is an important regulator of cell cycle progression through G0/G1 phase and induction of apoptosis. The INK4 cki family members, especially INK4a/p16 and INK4c/p18, have also been implicated in the regulation of cell cycle progression at the G1 to S transition via inhibiting cdk4 and/or cdk6 [39,40]. Our studies have shown that EGCG may impart cell cycle dysregulation in both androgen-sensitive and androgen-insensitive human prostate carcinoma cells via an up-regulation of almost all major ckis involved in G0/G1 phase.

In the next series of experiments, we assessed the effect of EGCG treatment on modulations in the levels of the major cyclins operative in G0/G1 phase of the cell cycle, viz., cyclin D1, cyclin D2, and cyclin E. Recent studies have shown that overexpression of cyclin D1 and cdk4 is commonly associated with several human malignancies and leads to the shortening of G1 phase, causing uncontrolled cellular proliferation [40,45]. In the present study, treatment of LNCaP and DU145 cells with EGCG resulted in a dose- and time-dependent decrease in the protein expression of cyclin D1 and cyclin E; however, the protein levels of cyclin D2 remained unchanged (Fig. 4).

Progression of cells to G1 and entry into the S phase of the cell cycle is regulated by two distinct cdks, cdk4 (or cdk6) and cdk2 [40,46]. Inactivation of either kinase leads to cell cycle arrest and withdrawal of cells from the cell cycle [46]. Genetic alterations usually affect cdk4 and cdk6 and the regulator cyclin D1 or cyclin D2, whereas deregulation of cdk2 activity frequently results from the alterations in the expression levels of their regulators cyclin E [39,40,45,46]. In this study, treatment of LNCaP cells with EGCG resulted in a dose-dependent decrease in cdk2, cdk4, and cdk6 (Fig. 5). The decrease in cdk2 protein expression was more pronounced than that of cdk4 and cdk6. Similar time-dependent inhibition was observed in cdk2, cdk4, and cdk6 after treatment of cells with 20 μg/ml concentration of EGCG for 24, 48, and 72 h. Next, we assessed the effect of EGCG treatment on the protein expression of cdks in DU145 cells. As shown by Western blot analysis, EGCG treatment resulted in significant dose- and time-dependent inhibition of cdk2, cdk4, and cdk6. These results lead to the conclusion that EGCG is capable of restoring proper checkpoint control to both types of human prostate carcinoma cells.

For a normal progression of cells through the cell cycle, a balance between each component of the cki–cyclin–cdk complex plays an important role. We therefore, investigated the effect of EGCG on the binding between WAF1/CIP1/p21–cyclin D1, KIP1/p27–cyclin D1, and cyclin–cdk. For this, one of the two proteins was immunoprecipitated using appropriate antibody and the effect on the binding was assessed by probing the immunoblot using antibody directed against the other

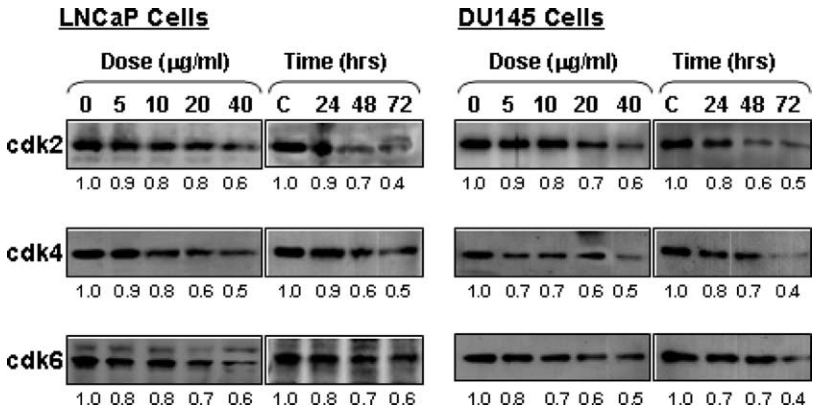

Fig. 5. Effect of EGCG on protein expression of cdk2, cdk4, and cdk6 in LNCaP and DU145 cells. The cells were treated with vehicle or EGCG (5, 10, 20, and 40 μg/ml for 24 h and 20 μg/ml for 24, 48, and 72 h) and then harvested. Total cell lysates were prepared and 25–50 μg protein was subjected to SDS–PAGE followed by Western blot analysis and chemiluminescent detection. Data shown below the blots represent fold modulation in the protein expression normalized to β-actin. The details are described under Materials and methods. The data shown here are from a representative experiment repeated three times with similar results.

protein. As shown in Fig. 6, EGCG treatment of LNCaP and DU145 cells resulted in a dose- and time-dependent increase in the binding of cyclin D1 toward WAF1/CIP1/p21 and KIP1/p27. EGCG treatment of both cell lines also resulted in a dose- and time-dependent decrease in the binding of cyclin E toward cdk2.

Cell cycle regulatory molecules are believed to be critical regulatory elements which control the progression of cells in early and late G1 phases of the cell cycle [39–46]. Our data, showing a decrease in the protein levels of the cyclins (D1 and E) and the cdks (2, 4, and 6) by EGCG treatment in both cell lines, are in agreement with the fact that the cdks and cyclins operate in association with each other by forming complexes which may bind to and are inhibited by ckis. This series of events imposes a blockade of G1 to S transition, causing a G0/G1 phase arrest of the cell cycle. The effect of EGCG on the interbinding between the different components of the cki–cyclin–cdk network, however, needs further investigation.

Based on the outcome of this study and available knowledge on the mechanism of cell cycle regulation, in a composite diagram (Fig. 7), we suggest the series of events, that ultimately results in an imposition of an artificial checkpoint at the G1 to S transition of prostate carcinoma cells by EGCG, thereby resulting in an arrest of cells in G0/G1 phase of the cell cycle. This arrest in cell cycle is an irreversible process and the cells, unable to repair these damages, ultimately undergo apoptosis. The present study provides further evidence for a role of a naturally occurring agent such as green tea polyphenols and EGCG as an anticancer agent for the management of prostate cancer.

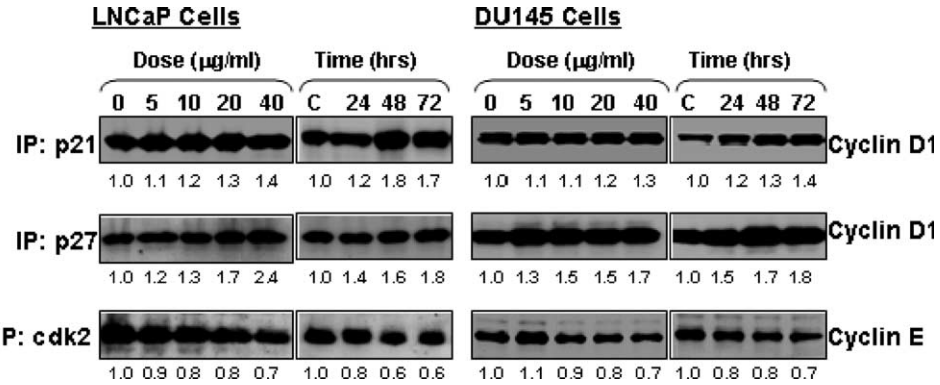

Fig. 6. Effect of EGCG on interbinding between WAF1/p21, KIP1/p21, and cdk2 with cyclins in LNCaP and DU145 cells. The cells were treated with vehicle or EGCG (5, 10, 20, and 40 μg/ml for 24 h, 20 μg/ml for 24, 48, and 72 h) and then harvested. Total cell lysates were prepared, 100–200 μg protein was immunoprecipitated and subjected to SDS–PAGE, and the binding with the other protein was measured by Western blot analysis directed against the other protein. Data shown below the blots represent fold modulationin the protein expression normalized to β-actin. The details are described under Materials and methods. The data shown here are from a representative experiment repeated three times with similar results.

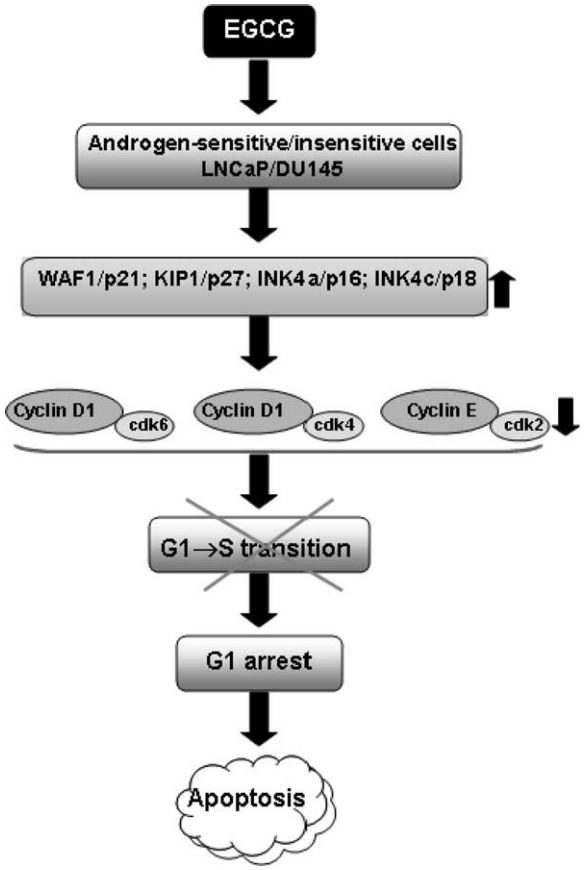

Fig. 7. Proposed model for EGCG-mediated cell cycle dysregulation and apoptosis of human prostate carcinoma cells. The ↑ indicate increases and ↓ indicate decreases in protein expression.

Acknowledgments

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