Protein kinase CK2 modulates apoptosis induced by resveratrol and epigallocatechin-3-gallate in prostate cancer cells

Kashif A. Ahmad,[1,2] Nathan H. Harris,[1] Andrew D. Johnson,[1] Hans C.N. Lindvall,[1,2] Guixia Wang,[1,2] and Khalil Ahmed[1,2,3]

[1]Cellular and Molecular Biochemistry Research Laboratory, Veterans Affairs Medical Center and [2]Department of Laboratory Medicine and Pathology and [3]Cancer Center, University of Minnesota, Minneapolis, Minnesota

Abstract

Resveratrol and epigallocatechin-3-gallate (EGCG) are important candidates as chemopreventive agents by virtue of their ability to induce apoptosis in cancer cells. Casein kinase 2 (CK2) is a ubiquitous protein ser/thr kinase that plays diverse roles in cell proliferation and apoptosis. We have previously shown that overexpression of CK2 suppresses apoptosis induced by a variety of agents, whereas down-regulation of CK2 sensitizes cells to induction of apoptosis. We therefore investigated whether or not CK2 played a role in resveratrol and EGCG signaling in androgen-sensitive (ALVA-41) and androgen-insensitive (PC-3) prostate cancer cells. Resveratrol- and EGCG-induced apoptosis is associated with a significant down-regulation of CK2 activity and protein expression in both the ALVA-41 and PC-3 cells. Overexpression of CK2α protected prostatic cancer cells against resveratrol- and EGCG-induced apoptosis. Relatively low doses (10 μmol/L) of resveratrol and EGCG induced a modest proliferative response in cancer cells that could be switched to cell death by moderate inhibition of CK2. These findings characterize, for the first time, the effects of polyphenolic compounds on CK2 signaling in androgen-sensitive and androgen-insensitive prostatic carcinoma cells and suggest that resveratrol and EGCG may mediate their cellular activity, at least in part, via their targeting of CK2. Further, the data hint at the potential of using these polyphenols alongside CK2 inhibitors in combination chemotherapy. [Mol Cancer Ther 2007;6(3):1006–12]

Received 8/14/06; revised 10/20/06; accepted 1/22/07.

Grant support: U.S. Department of Veterans Affairs Medical Research Fund and National Cancer Institute, Department of Health and Human Services USPHS research grant CA-15062.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Khalil Ahmed, Cellular and Molecular Biochemistry Research Laboratory (151), Veterans Affairs Medical Center, One Veterans Drive, Minneapolis, MN 55417. Phone: 612-467-2594; Fax: 612-725-2093. E-mail: ahmedk@umn.edu

doi:10.1158/1535-7163.MCT-06-0491

Introduction

In recent years, many bioactive polyphenolic compounds have gained recognition as candidate agents in chemoprevention and/or in cancer chemotherapy. Of this class, resveratrol and epigallocatechin-3-gallate (EGCG) are two of the most widely studied compounds (1–3). Resveratrol is a naturally occurring *trans*-3,5,4′-trihydroxystilbene that is a constituent of various plants, such as grapes, berries, and peanuts. Of note is its consumption in red wine that has been suggested to prevent cardiovascular diseases (commonly called the French Paradox; ref. 4). In addition to its cardioprotective effects, resveratrol has been shown to suppress proliferation in a wide variety of tumor cells, including those of the lymphoid, breast, colon, stomach, and prostate. Resveratrol signaling is complex, and its action modulates a wide range of cellular activities and pathways (1, 5). In this context, it is noteworthy that resveratrol has been used as a sensitizer in drug-induced apoptosis (6). Likewise, EGCG is a polyphenolic compound present in green tea. It induces apoptosis in a variety of tumor cells and has been proposed as a chemopreventive agent for prostate cancer (2, 3, 7). The functional role of resveratrol and EGCG has generated a considerable interest in identifying their cellular targets.

Casein kinase 2 (CK2) is a ubiquitous protein serine/threonine kinase consisting of a heterotetrameric complex of α, α′, and β subunits, such that the catalytic α subunits are linked via two regulatory β subunits to form configurations, such as $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, or $\alpha'_2\beta_2$, depending upon the cell type. CK2 is a multifunctional protein kinase that is involved in cell growth, proliferation, and survival (8–12). CK2 has also been shown to act as a potent suppressor of apoptosis in cancer cells (13–15). The significance of these functions of CK2 relates to the observations that CK2 is consistently dysregulated in various cancer, including prostate cancer (8, 11, 16, 17). Recently, CK2 has also been proposed as a target for cancer therapy (13, 18–20). We decided to examine the role of CK2 as a possible target for the effects of resveratrol and EGCG in prostate cancer cells. Thus far, with the exception of one report on the effect of resveratrol on COP9 signalosome–associated CK2 (21), no detailed investigations have been undertaken to relate resveratrol and EGCG signaling to CK2.

Our findings suggest that CK2 is an upstream target wherein down-regulation leads to apoptosis, whereas its overexpression impedes cell death signal (15, 22). In the present study, we hypothesized that resveratrol and EGCG signaling, at least in part, may occur via an effect on CK2 in prostate cancer cells. Prostate cancer is the second most common cancer in males, and initially the disease starts as an androgen-responsive tumor that eventually progresses

to the androgen-resistant phenotype (23). Thus, in this report we have used both androgen-sensitive (ALVA-41), androgen-insensitive (PC-3), and BPH-1 cell lines to determine whether they show a differential response to resveratrol and EGCG with regard to CK2 signaling. Our results indicate that resveratrol- and EGCG-induced apoptosis is associated with down-regulation of CK2 in prostate cells, and that the level of cellular CK2 exerts a significant effect on resveratrol and EGCG signaling. Both ALVA-41 and PC-3 cells showed similar qualitative response to the action of resveratrol and EGCG with respect to CK2 signal. This study is the first to delineate the role of CK2 in resveratrol and EGCG apoptotic signaling in the androgen-sensitive and androgen-insensitive prostate carcinoma cell lines.

Materials and Methods

Cell Lines and Reagents

Prostate cancer cells (ALVA-41 and PC-3) were grown in RPMI 1640 (Invitrogen/Life Technologies, Carlsbad, CA) supplemented with 2 mmol/L L-glutamine and 6% or 10% fetal bovine serum for ALVA-41 and PC-3 cells, respectively. BPH-1 cells were obtained form Dr. Simon Hayward (Vanderbilt University, Nashville, TN) and were grown in RPMI 1640 supplemented with 10% bovine growth serum and 2 mmol/L L-glutamine. Resveratrol and EGCG were purchased from Sigma (St. Louis, MO). 4,5,6,7-Tetrabromobenzotriazole (TBB), a chemical inhibitor of CK2, was purchased from Calbiochem (San Diego, CA).

Cell Treatment and Transfection

For cell viability studies, ALVA-41, PC-3, and BPH-1 cells were treated for 24 h with 10 μmol/L and 100 μmol/L concentrations of resveratrol or EGCG. Down-regulation of CK2 by resveratrol and EGCG was studied in parallel experiments. For sensitization of cells to low levels of resveratrol and EGCG, PC-3 and ALVA-41 cells (0.25×10^6) were first treated with 40 μmol/L TBB for 6 h or small interfering RNA–CK2α (0.25 μg/mL) using *N*-[1-(2,3-dioleoyloxyl)propyl]-*N*,*N*,*N*-trimethylammoniummethyl sulfate (DOTAP) for 12 h and then maintained with or without 5 to 10 μmol/L resveratrol or EGCG for a total of 24 h. To accomplish transient overexpression of CK2 in PC-3 and ALVA-41 cells, 0.25×10^6 cells were plated in six-well plates overnight followed by transfection with pcDNA6 (empty vector) or pcDNA6-CK2α at a dose of 2.0 μg/mL using DOTAP for a period of 24 h, as described previously (15). Transfected PC-3 and ALVA-41 cells were treated overnight with 100 μmol/L resveratrol or EGCG to evaluate the effect of overexpression of CK2 on resveratrol- and EGCG-induced apoptosis.

Cell Viability and Proliferation Assay

WST-1 reagent (Roche, Indianapolis, IN) was used to determine viability and proliferation in cells after various treatments. Aliquots (200 μL) of treated or untreated cells ($5–10 \times 10^3$) were seeded in each well of a 96-well plate. Cells were allowed to reattach overnight. After various treatments of cells, media in each well was replaced with 100 μL of fresh media containing 100 μL/mL WST-1, and incubation was carried out at 37°C for an additional 60 min. An automated plate reader (Molecular Devices, Sunnyvale, CA) was used to measure absorbance at 450 nm. The results were confirmed in at least three independent experiments.

Determination of Caspase-3 Activity

Caspase-3 activity was determined using the fluorescent caspase substrate from BioMol (Plymouth, PA). PC-3 and ALVA-41 cells (0.25×10^6) were plated in six-well plates and treated with 100 μmol/L resveratrol or EGCG for 18 h. Cells were washed with 1 × PBS and suspended in chilled cell lysis buffer (50–200 μL per sample) on ice for 15 min. A 50-μL aliquot of 2× reaction buffer (10 mmol/L HEPES, 2 mmol/L EDTA, 10 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 10 mmol/L DTT) with 5 μL of the conjugate substrate (DEVD-AFC) was added to cell lysates. Caspase activity was determined by the relative fluorescence intensity at 505 nm after excitation at 400 nm using a spectrofluorometer (Molecular Devices).

Preparation of Whole-Cell Lysates

After the desired treatment, ALVA-41, PC-3, and BPH-1 cells ($0.25–2.5 \times 10^6$) were collected and washed twice with 1 × PBS by centrifuging at 100 × *g* for 5 min. Cell pellets were resuspended in ice-cold 1× radioimmunoprecipitation assay buffer lysis and lysates were used for immunoblot analysis.

CK2 Activity Assay

CK2 activity in cell lysates was determined by using the CK2 assay kit (MBL International, Woburn, MA).

Immunoblot Analysis

PC-3, ALVA-41, and BPH-1 cells were plated in six-well plates at a concentration of 0.25×10^6 and subjected to desired treatments. Whole-cell lysates were prepared as described previously (15). Membranes were probed with mouse anti-CK2α primary antibody (1:1,000; BD Biosciences, San Diego, CA). The membranes were then probed with goat antimouse IgG (1:50,000; Pierce, Rockford, IL) and analyzed for chemiluminescence (Western Pico). Membranes were stripped with Restore Stripping buffer (Pierce) and immunoblotted with rabbit antiactin (1:10,000; Calbiochem).

Results

Effect of Resveratrol and EGCG on Prostate Cancer Cell Viability

It is generally believed that chemopreventive effects of resveratrol and EGCG are related to their ability to induce apoptosis in cancer cells (1–4). Because CK2 seems to regulate apoptotic activity in cells (8, 9), we decided to examine whether or not resveratrol and EGCG exerted any effect on CK2 activity. By using purified CK2, we observed that both resveratrol and EGCG caused a dose-dependent inhibition of the enzyme activity *in vitro*. The inhibitory effect was moderate (~25% at 25 μmol/L, ~60% at 50 μmol/L, and 60–80% at 100 μmol/L concentration of these agents) considering that TBB, a chemical inhibitor of

CK2, produced over 90% inhibition of the purified enzyme at 25 μmol/L concentration. We then treated ALVA-41, PC-3, and BPH-1 cells with 10 μmol/L or 100 μmol/L concentrations of resveratrol or EGCG for 24 h to investigate the effect of resveratrol and EGCG on prostate cancer cell viability (Fig. 1). The results show that resveratrol at low doses (10 μmol/L) induced a proliferative response in ALVA-41 and BPH-1 cells at 24 h, which was not evident in PC-3 cells (Fig. 1A). However, at a higher dose (100 μmol/L), resveratrol produced a significant toxicity in prostate cancer cells, although the effect on ALVA-41 cells was relatively greater than on PC-3 cells (Fig. 1A). Analogous results were observed with EGCG tested at the same concentrations (Fig. 1B), except that the effect on BPH-1 cells was somewhat lower. To confirm that cell death that was observed was apoptotic in nature, we determined caspase-3 activity in ALVA-41 and PC-3 cells treated with 100 μmol/L of resveratrol or EGCG for 18 h. Both resveratrol and EGCG at 100 μmol/L produced a significant caspase-3 activity in both ALVA-41 and PC-3 cells (Fig. 1C) correlating with the data on cell viability. Next, we showed that activity of CK2 in ALVA-41 and PC-3 cells treated with resveratrol and EGCG (at a concentration of 100 μmol/L for 6 h) was inhibited by 25% to 60% (Fig. 1D). These results suggest that the down-regulation of CK2 activity caused by resveratrol and EGCG is upstream of the induction of apoptosis by these agents in both ALVA-41 and PC-3 prostate cancer cells.

Modulation of CK2 by Resveratrol and EGCG Is Independent of Prostate Cancer Cell Phenotype

Because CK2 is elevated in cancer cells, we investigated whether the nature of the response of CK2 depended on the concentrations of resveratrol and EGCG, which induced a proliferative or apoptotic response. Immunoreactive CK2 was analyzed in lysates from cells treated with proliferation-inducing dose (10 μmol/L) or apoptosis-inducing dose (100 μmol/L) of resveratrol or EGCG. The results in Fig. 2 show a reduction in the catalytic subunit of CK2 in ALVA-41 and PC-3 cells treated with 100 μmol/L resveratrol for 24 h (Fig. 2A and B). It may be noted that the predominant form of the catalytic subunit in ALVA-41 and PC-3 cells is α, with α′ being relatively minor. Similar responses were observed for ALVA-41 and PC-3 cells treated with 100 μmol/L of EGCG (Fig. 2C). Interestingly, low doses of the two agents seem to elicit a modest increase in the immunoreactive catalytic α and α′ subunits of CK2 in ALVA-41 cells (Fig. 2D) but seemed to have no effect on the α subunit in PC-3 cells (Fig. 2B). We also tested the effect of low and high doses of resveratrol and EGCG on BPH-1 cells which showed analogous results for cancer cells (Fig. 2E). Together, it seems that the response of CK2 signal to resveratrol and EGCG action in prostate cancer cells is not dependent on the nature of their phenotype, which accords with our previous observations demonstrating the similar functionality of the CK2 signal in these prostate cancer cells (15).

Sensitization of Prostate Cancer Cells to Low Concentrations of Resveratrol and EGCG via Modest Down-regulation of CK2

Because treatment of cells with resveratrol and EGCG at low doses for 24 h seemed to induce some proliferation accompanied by a subtle increase in CK2 protein level, we decided to examine the cell response evoked by low doses of resveratrol and EGCG subsequent to chemical and

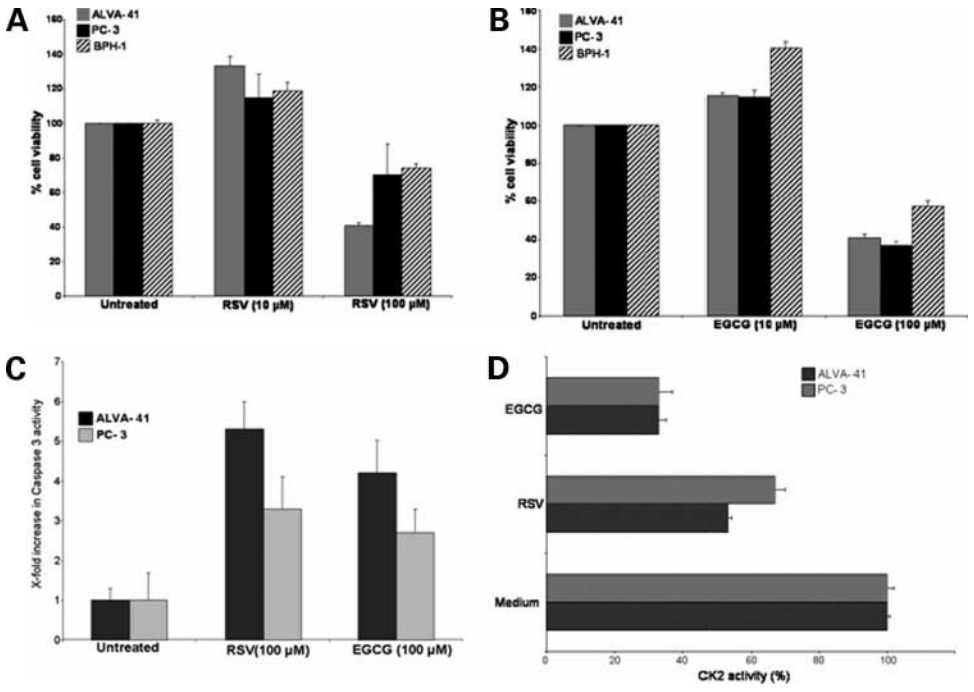

Figure 1. Down-regulation of CK2 induced by resveratrol (*RSV*) and EGCG leads to apoptosis in prostate cancer cells. ALVA-41, PC-3, and BPH-1 cells (0.25×10^6) were treated with 10 or 100 μmol/L resveratrol for 24 h (**A**) and 10 or 100 μmol/L EGCG for 24 h (**B**), and cell viability was assessed by WST-1 assay. Data based on three different experiments, each carried out in triplicate. **C,** ALVA-41 and PC-3 cells ($0.25 - 2.5 \times 10^6$) were treated with 100 μmol/L of resveratrol or EGCG, and caspase-3 activity was determined by spectrofluorimetric analysis. **D,** ALVA-41 and PC-3 cells ($0.25 - 2.5 \times 10^6$) were treated with 100 μmol/L resveratrol and EGCG for 6 h, and CK2 activity was determined using Cyclex CK2 activity kit (MBL).

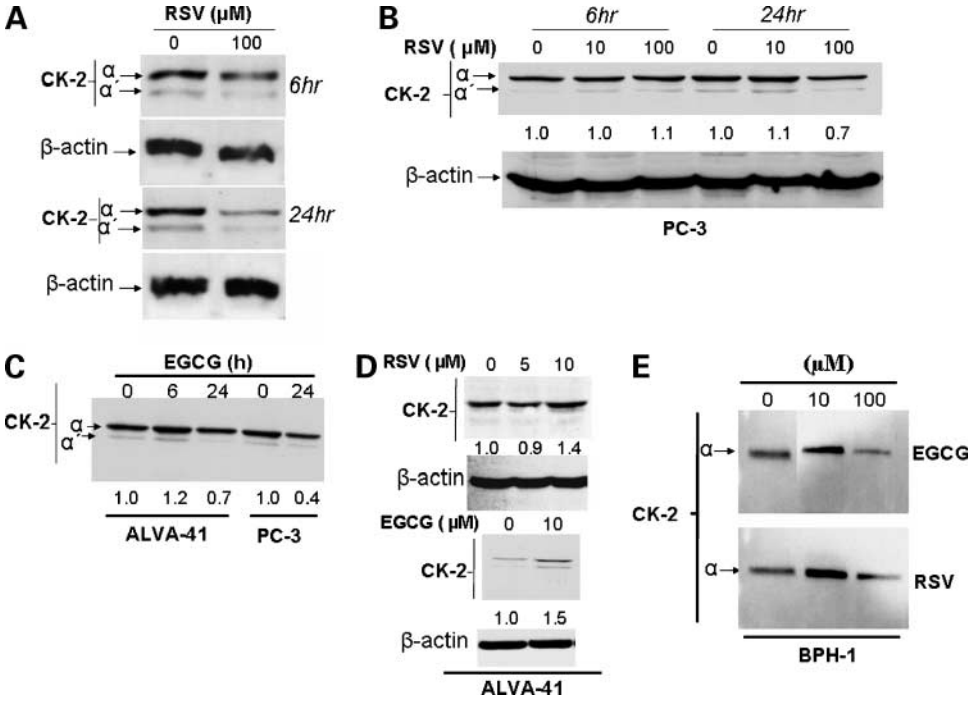

Figure 2. Cell death and cell proliferation induced by resveratrol and EGCG corresponds to CK2 protein expression in cells. ALVA-41 (**A**) and PC-3 (**B**) cells (0.5×10^6) were plated in T25 flasks and treated with 10 or 100 μmol/L resveratrol for 6 and 24 h. **C,** ALVA-41 and PC-3 cells were treated with 100 μmol/L EGCG for 6 and 24 h. **D,** ALVA-41 cells (0.5×10^6) were treated with resveratrol and EGCG (at 5, 10, or 100 μmol/L) for 24 h. **E,** BPH-1 cells (0.5×10^6) were plated in T25 flasks and treated with 10 or 100 μmol/L resveratrol or EGCG for 24 h. Expression of the CK2α and CK2α′ subunits was determined by Western blotting and relative densitometric values for the bands.

molecular down-regulation of CK2. We treated ALVA-1 and PC-3 cells with the CK2 inhibitor TBB at a suboptimal concentration (40 μmol/L for 6 h) or transfected the cells with 0.25 μg/mL of small interfering RNA–CK2α for 12 h. The results in Fig. 3A show that these treatments elicit only a modest down-regulation of CK2 activity (~25%), which is insufficient to induce apoptosis in 24 h. Subsequent to the treatment of cells with these suboptimal levels of inhibitors, either resveratrol or EGCG, was added at concentrations that promote cell proliferation (i.e., 5–10 μmol/L resveratrol or 10–20 μmol/L EGCG) for a total period of 24 h. The cell viability data indicated that, in ALVA-41 and PC-3 cells with prior exposure to TBB, there was not only a lack of proliferative response to the polyphenols, but rather these cells were sensitized so as to show a significant reduction in viability compared with treatments of either TBB or resveratrol or EGCG alone (Fig. 3B). Similar results were observed when CK2 was down-regulated by small interfering RNA treatment before exposure of cells to resveratrol (Fig. 3C) or to EGCG (Fig. 3D). Together, these observations support the notion that with moderate down-regulation of CK2 activity, the polyphenols evoke an even greater reduction in cell viability. These results accord with the previous data demonstrating that moderate down-regulation of CK2 causes a sensitization of prostate cancer cells to agents, such as TRAIL (15).

Effect of Overexpression of CK2 on Resveratrol- and EGCG-Induced Cell Death

We next investigated the effects of forced overexpression of CK2 on apoptosis-inducing activity of resveratrol and EGCG by transfecting ALVA-41 cells with 2 μg/mL pcDNA6 (empty vector) or pcDNA6-CK2α for 24 h and then followed by treatment with 100 μmol/L resveratrol or EGCG for 24 h. The results in Fig. 4 show that overexpression of CK2α increased CK2 activity and protein expression (Fig. 4A and B). Cell death induced by resveratrol and EGCG was blocked by overexpression of CK2α, further suggesting the involvement of CK2 as a target in apoptotic signaling of resveratrol and EGCG.

Discussion

Antioxidants, such as naturally occurring polyphenolic compounds have gained considerable attention as important chemopreventive agents and as modulators of tumor cell response to chemotherapy (2). In this context, several reports have described apoptotic signaling of the polyphenolic compounds resveratrol (1, 24) and EGCG (3, 25) in various cancers, and numerous downstream targets of resveratrol and EGCG have been suggested for the induction of apoptosis (e.g., refs. 1–3). Among these, nuclear factor-κB, Akt, and Bcl-2 systems have attracted the most attention, especially with regard to their relation to cell cycle progression and induction of apoptosis (e.g., refs. 1–3). Considering that CK2 has emerged as a key protein involved in cell growth, proliferation, and apoptosis (8–12), we investigated its role in resveratrol and EGCG signaling in prostate cancer cell lines by using the androgen-dependent (ALVA-41) and androgen-independent (PC-3) cells as models mimicking the general pathophysiology of prostate cancer. We have shown that concentrations of resveratrol and EGCG that induce cell death are associated with CK2 down-regulation in both the androgen-dependent and androgen-independent prostate cancer cells. Further,

blocking the death signaling of resveratrol and EGCG by overexpression of CK2 suggests that CK2 levels in cancer cells influence the effectiveness of these polyphenols. With respect to the relation of prostate cancer cell phenotype to the action of the polyphenols resveratrol and EGCG, we have found the androgen-insensitive PC-3 cells to behave in a manner similar to the androgen-sensitive and ALVA-41 cells. Whereas we noted that PC-3 cells were slightly more resistant to the action of resveratrol and EGCG than ALVA-41 cells, the targeting of CK2 signal seemed to be analogous in both cell lines, suggesting that mechanisms linking the apoptotic signaling to CK2 would be accessible in both forms of prostate cancer. It has previously been documented that CK2 signal in prostate cancer cells is responsive to both the androgenic and growth-factor stimuli in responsive cells (26).

To address the involvement of CK2 in resveratrol and EGCG signaling, we need to consider the mounting knowledge that links CK2 to several of the proposed targets for these agents, which could conceivably relate to an upstream engagement of the CK2 signal. Examples of these observations in the context of certain aforementioned targets are considered as follows. A significant role of CK2 in the activity/function of nuclear factor-κB is indicated in studies demonstrating an involvement of CK2 in the aberrant activation of nuclear factor-κB by direct phosphorylation of RelA/p65 and/or via action on IκB (17, 27–29). Likewise, several reports have documented the role of CK2 in p53 and MDM2 activity (30–32). Further, many growth-related molecules have been proposed as potential substrates of CK2, and its role in cell cycle progression and DNA repair has also been documented (8–14, 33–36). A number of proteins that become resistant to caspase degradation upon phosphorylation by CK2 have been identified; these include Bid, Max, ARC, c-myc, and procaspase-2 (37–40). With respect to the proposed targeting of Akt by resveratrol and EGCG (1, 3, 41), it may be noted that a considerable amount of information on the regulation of PTEN by CK2 exists (42, 43), and, indeed, it has been reported that CK2 can directly affect the phosphorylation of Akt (44). Overexpression of CK2 has been shown to block apoptosis mediated by DNA-damaging agents as well as via death receptors, whereas the reverse seems to be the case on down-regulation of CK2 by diverse means (8, 14, 15, 45, 46). Accordingly, our observations on the actions of resveratrol and EGCG on

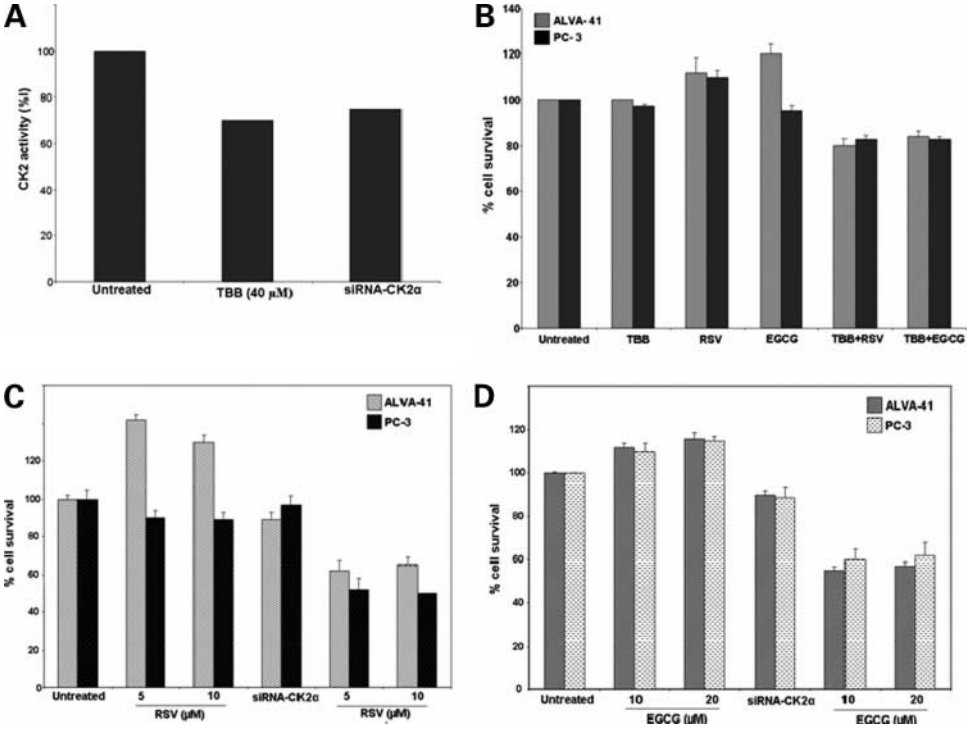

Figure 3. Chemical and molecular down-regulation of CK2 sensitizes prostate cancer cells to induction of apoptosis by low doses of resveratrol and EGCG. **A,** ALVA-41 cells ($0.25 - 2.5 \times 10^6$) were treated with 40 μmol/L TBB for 6 h or transfected with small interfering RNA – CK2α (0.25 μg/mL, using DOTAP) for 12 h. CK2 activity was determined using CK2 assay kit (MBL). **B,** ALVA-41 and PC-3 cells (0.25×10^6) were treated with 10 μmol/L resveratrol or EGCG for a total time of 24 h with or without prior 6 h treatment with TBB (40 μmol/L). Cells treated with TBB (40 μmol/L) for 24 h were also included as control. **C,** ALVA-41 and PC-3 cells (0.25×10^6) were transfected with small interfering RNA – CK2α (0.25 μg/mL, using DOTAP) alone for 24 h, or for 12 h followed by treatment with 5 μmol/L or 10 μmol/L resveratrol for a total of 24 h. Cells treated with resveratrol (5 – 10 μmol/L) for a total of 24 h were also included as control. **D,** ALVA-41 and PC-3 cells (0.25×10^6) were transfected with small interfering RNA – CK2α (0.25 μg/mL; using DOTAP) alone for 24 h, or for 12 h followed by treatment with 10 μmol/L or 20 μmol/L EGCG for a total of 24 h. Cells treated with EGCG (10 – 20 μmol/L) for 24 h were also included as control. Cell viability was determined by WST-1 assay, and data are based on three different experiments carried out in triplicate.

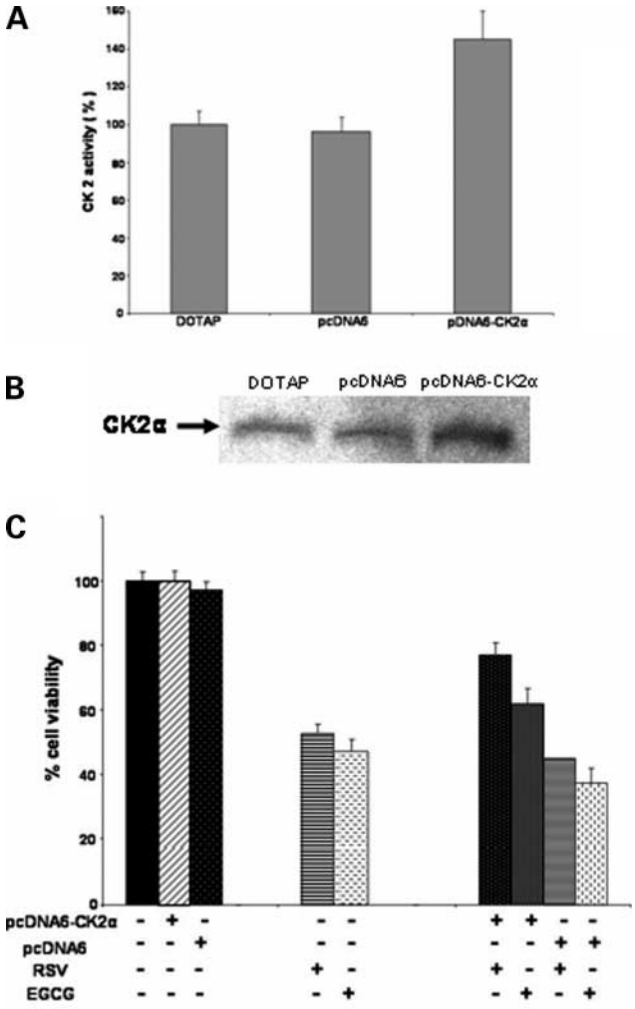

Figure 4. Overexpression of CK2 protects prostate cancer cells from resveratrol- and EGCG-induced cell death. **A,** ALVA-41 cells (0.5×10^6) were transfected with pcDNA6 or pcDNA6-CK2α (2.0 μg/mL) for 24 h using DOTAP, and CK2 activity was determined using the CK2 assay kit (MBL). **B,** analysis of CK2 protein expression as detailed in **A. C,** ALVA-41 cells (0.5×10^6) were transfected with pcDNA6 or pcDNA6-CK2α (2.0 μg/mL) for 24 h using DOTAP, and then followed by treatment with 100 μmol/L resveratrol or EGCG for 24 h. Cell viability was determined by WST-1 assay.

CK2 may have strong implications regarding their cell biological effects. Taken together, it is reasonable to propose that CK2 can be regarded as a target of resveratrol and EGCG, and that the other aforementioned proposed targets may be, at least in part, downstream of this signal.

It is noteworthy that moderate down-regulation of CK2, to the extent that is insufficient to induce apoptosis, causes sensitization of cells to low doses of resveratrol and EGCG. This accords with our previous observations on sensitization of prostate cancer cells to induction of apoptosis by suboptimal doses of TRAIL in the presence of a small reduction in CK2 activity (15, 22, 47). It may be reiterated that levels of CK2 are tightly controlled in the cell as discussed previously (8). Thus, whereas a modest up-regulation of CK2 in cells enhances the oncogenic potential (48), a modest down-regulation is equally effective in promoting sensitivity to induction of death in the cells (15, 47). This is an important consideration because CK2 levels can affect the activity of resveratrol and EGCG in the cells. Accordingly, the suboptimal doses of resveratrol and EGCG could be switched to a cytotoxic effect in the presence of a slight down-regulation of CK2. We also observed that proliferation of cells at low doses of resveratrol and EGCG was associated with an up-regulation of CK2 expression in tumor cells. It may be noted that proliferation of cells in the presence of low doses of resveratrol has been well documented previously (49, 50). This accords with our result that overexpression of CK2 can block the apoptotic signaling of resveratrol and EGCG. Based on these observations, it is also conceivable that other polyphenolic and bioactive compounds could be used to target CK2 in cancer chemotherapy.

In summary, the present work is the first to implicate CK2 as a target in resveratrol- and EGCG-induced apoptosis in both the androgen-sensitive and androgen-insensitive prostatic carcinoma cells. A direct effect of resveratrol and EGCG in modulating the activity of CK2 signal implicates CK2 as a target of these agents in mediating an apoptotic response. Further, based on various considerations, we propose that engagement of CK2 signal is upstream of several other previously proposed targets, such as nuclear factor-κB, Akt, and Bcl-2. The observations on CK2 activity and protein level in both ALVA-41 and PC-3 cells suggest that resveratrol and EGCG targeting of CK2 is similar in both types of prostate cancer cells. The conversion of the proliferative or nonapoptotic response of cancer cells at low doses of resveratrol and EGCG to induction of apoptosis by modest inhibition of cellular CK2 activity hints at a possible strategy for a combination chemotherapy.

References

1. Aggarwal BB, Bhardwaj A, Aggarwal RS, et al. Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. Anticancer Res 2004;5:2783 – 840.
2. Manson MM. Inhibition of survival signalling by dietary polyphenols and indole-3-carbinol. Eur J Cancer 2005;41:1842 – 53.
3. Khan N, Afaq F, Saleem M, et al. Targeting multiple signaling pathways by green tea polyphenol (−)epigallocatechin-3-gallate. Cancer Res 2006; 66:2500 – 5.
4. Pervaiz S. Resveratrol: from grapevines to mammalian biology. FASEB J 2003;17:1975 – 85.
5. Clement MV, Hirpara JL, Chawdhury SH, et al. Chemopreventive agent resveratrol, a natural product derived from grapes, triggers CD95 signaling-dependent apoptosis in human tumor cells. Blood 1998;92: 996 – 1002.
6. Fulda S, Debatin SM. Sensitization for anticancer drug-induced apoptosis by the chemopreventive agent resveratrol. Oncogene 2004; 23:6702 – 11.
7. Gupta S, Ahmad N, Nieminen AL, et al. 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.
8. Tawfic S, Yu S, Wang H, et al. Protein kinase CK2 signal in neoplasia. Histol Histopathol 2001;16:573 – 82.

9. Ahmed K, Gerber DA, Cochet C. Joining the cell survival squad: an emerging role for protein kinase CK2. Trends in Cell Biol 2002;12:226 – 9.

10. Litchfield DW. Protein kinase CK2: Structure, regulation and role in cellular decisions of life and death. Biochem J 2003;369:1 – 15.

11. Guerra B, Issinger O-G. Protein kinase CK2 and its role in cellular proliferation, development, and pathology. Electrophoresis 1999;20: 391 – 408.

12. Pinna LA. Protein kinase CK2: a challenge to canons. J Cell Sci 2002; 115:3873 – 8.

13. Wang G, Ahmad KA, Unger G, et al. CK2 signaling in androgen dependent and independent prostate cancer. J Cell Biochem 2006;99: 382 – 91.

14. Guo C, Yu S, Davis AT, Green JE, Ahmed K. A potential role of nuclear matrix-associated protein kinase CK2 in protection against drug-induced apoptosis in cancer cells. J Biol Chem 2001;276:5992 – 9.

15. Wang G, Ahmad KA, Ahmed K. Role of Protein kinase CK2 in regulation of TRAIL induced apoptosis in prostate cancer cells. Cancer Res 2006;66:2242 – 9.

16. Ahmed K. Significance of casein kinase system in cell growth and proliferation with emphasis on studies of the androgenic regulation of the prostate. Cell Mol Biol Res 1994;40:1 – 11.

17. Romieu-Mourez R, Landesman-Bollag E, Seldin DC, et al. Protein kinase CK2 promotes aberrant activation of nuclear factor κB, transformed phenotype, and survival of breast cancer cells. Cancer Res 2002;62: 6670 – 80.

18. Ahmad KA, Wang G, Slaton JW, et al. Targeting CK2 for cancer therapy. Anticancer Drugs 2005;16:1037 – 43.

19. Unger GM, Davis AT, Slaton JW, et al. Protein kinase CK2 as regulator of cell survival: Implications for cancer therapy. Curr Cancer Drug Targets 2004;4:77 – 84.

20. Wang G, Unger G, Ahmad KA, et al. Downregulation of CK2 induces apoptosis in cancer cells—a potential approach to cancer therapy. Mol Cell Biochem 2005;274:77 – 84.

21. Uhle S, Medalia O, Waldron R, et al. Protein kinase CK2 and protein kinase D are associated with the COP9 signalosome. EMBO J 2003;6: 1302 – 12.

22. Ahmad KA, Wang G, Ahmed K. Intracellular hydrogen peroxide production is an upstream event in apoptosis induced by down-regulation of CK2 in prostate cancer cells. Mol Cancer Res 2006;4:331 – 8.

23. Tang DG, Porter AT. Target to apoptosis: a hopeful weapon for prostate cancer. Prostate 1997;32:284 – 93.

24. Kuwajerwala N, Cifuentes E, Gautam S, et al. Resveratrol induces prostate cancer cell entry into S phase and inhibits DNA synthesis. Canc Res 2002;62:2488 – 92.

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. Guo C, Yu S, Davis AT, et al. Nuclear matrix targeting of the protein kinase CK2 signal as common downstream response to androgen or growth factor stimulation of prostate cancer cells. Cancer Res 1999;59: 1146 – 51.

27. Wang D, Westerheide SD, Hanson JL, et al. Tumor necrosis factor α-induced phosphorylation of RelA/p65 is controlled by casein kinase II. J Biol Chem 2000;275:32592 – 7.

28. Yu M, Yeh J, Van Waes C. Protein kinase casein kinase 2 mediates inhibitor κB kinase and aberrant nuclear factor κB activation by serum factor(s) in head and neck squamous carcinoma cell. Canc Res 2006;66: 6722 – 31.

29. Kato T, Jr., Delhase M, Hoffmann A, et al. CK2 is a C-terminal IκB kinase responsible for the NF-κB activation during the UV response. Cell 2003;12:829 – 39.

30. Keller DM, Zeng X, Wang Y, et al. A DNA damage-induced p53 serine 392 kinase complex contains CK2, hSpt16, and SSRP1. Mol Cell 2001;7: 283 – 92.

31. Götz C, Kartarius S, Scholtes P, et al. Identification of a CK2 phosphorylation site in mdm2. Eur J Biochem 1999;266:493 – 501.

32. Prowald A, Schuster N, Montenarh M. Regulation of DNA binding of p53 by its interaction with protein kinase CK2. FEBS Lett 1997;408: 99 – 104.

33. Barz T, Ackermann K, Dubois G, et al. Genome-wide expression screens indicate a global role of protein kinase CK2 in chromatin remodeling. J Cell Sci 2003;116:1563 – 77.

34. Xu X, Landesman-Bollag E, Channavajhala PL, et al. Murine protein kinase CK2: gene and oncogene. Mol Cell Biochem 1999;191:65 – 74.

35. Loizou JI, El-Khamisy SF, Zlatanou A, et al. The protein kinase CK2 facilitates repair of chromosomal DNA single-strand breaks. Cell 2004; 117:17 – 28.

36. Channavajhala P, Seldin DC. Functional interaction of protein kinase CK2 and c-Myc in lymphomagenesis. Oncogene 2002;21:5280 – 8.

37. Li PF, Li J, Muller EC, et al. Phosphorylation by protein kinase CK2: a signaling switch for the caspase-inhibiting protein ARC. Mol Cell 2002;10: 247 – 58.

38. Shin S, Lee Y, Kim W, et al. Caspase-2 primes cancer cells for TRAIL-mediated apoptosis by processing procaspase-8. EMBO J 2005;24: 3532 – 42.

39. Krippner-Heidenreich A, Talanian RV, Sekul R, et al. Targeting of the transcription factor Max during apoptosis: phosphorylation-regulated cleavage by caspase-5 at an unusual glutamic acid residue in position P1. Biochem J 2001;358:705 – 15.

40. Desagher S, Osen-Sand A, Montessuit S, et al. Phosphorylation of Bid by casein kinase I and II regulates its cleavage by caspase 8. Mol Cell 2002;8:601 – 11.

41. Siddiqui IA, Adhami VA, Afaq F, et al. Modulation of phosphatidylinositol-3-kinase/protein kinase B- and mitogen-activated protein kinase-pathways by tea polyphenols in human prostate cancer cells. J Cell Biochem 2004;91:232 – 42.

42. Torres J, Pulido R. The tumor suppressor PTEN is phosphorylated by the protein kinase CK2 at its C terminus: Implications for the PTEN stability to the proteasome-mediated degradation. J Biol Chem 2001; 276:933 – 8.

43. Vazquez F, Grossman SR, Takahashi Y, et al. Phosphorylation of the PTEN tail acts as an inhibitory switch by preventing its recruitment into a protein complex. J Biol Chem 2001;276:48627 – 30.

44. Maira DG, Salvi M, Arrigoni G, et al. Protein kinase CK2 phosphorylates and upregulates Akt/PKB. Cell Death Differ 2005;12:668 – 77.

45. Slaton JW, Sloper DT, Unger G, et al. Induction of apoptosis by antisense CK2 in human prostate cancer xenograft model. Mol Cancer Res 2004;2:712 – 21.

46. Yamane K, Kinsella TJ. Casein kinase 2 regulates both apoptosis and the cell cycle following DNA damage induced by 6-thioguanine. Clin Cancer Res 2005;11:2355 – 63.

47. Wang G, Ahmad KA, Ahmed K. Modulation of receptor mediated apoptosis by CK2. Mol Cell Biochem 2005;274:201 – 5.

48. Kelliher MA, Seldin DC, Leder P. Tal-1 induces T cell acute lymphoblastic leukemia accelerated by casein kinase IIα. EMBO J 1996; 15:5160 – 6.

49. Ahmad KA, Clement MV, Hanif IM, Pervaiz S. Resveratrol inhibits drug-induced apoptosis in human leukemia cells by creating an intracellular milieu non-permissive for death execution. Cancer Res 2004;64:1452 – 9.

50. Ahmad KA, Clement MV, Pervaiz S. Pro-oxidant activity of low doses of resveratrol inhibits hydrogen peroxide induced apoptosis. Ann N Y Acad Sci 2003;1010:365 – 73.