Role of Protein Kinase CK2 in the Regulation of Tumor Necrosis Factor–Related Apoptosis Inducing Ligand–Induced Apoptosis in Prostate Cancer Cells

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Abstract

Protein kinase CK2 (formerly casein kinase 2 or II) is a ubiquitous and highly conserved protein Ser/Thr kinase that plays diverse roles such as in cell proliferation and apoptosis. With respect to the latter, we originally showed that elevated CK2 could suppress various types of apoptosis in prostate cancer cells; however, the downstream pathways that respond to CK2 for mediating the suppression of apoptosis have not been fully elucidated. Here, we report studies on the role of CK2 in influencing activities associated with tumor necrosis factor–related ligand (TRAIL/Apo2-L)–mediated apoptosis in prostate carcinoma cells. To that end, we show that both androgen-insensitive (PC-3) and androgen-sensitive (ALVA-41) prostate cancer cells are sensitized to TRAIL by chemical inhibition of CK2 using its specific inhibitor 4,5,6,7-tetrabromobenzotriazole (TBB). Furthermore, we have shown that overexpression of CK2α using pcDNA6-CK2α protected prostatic cancer cells from TRAIL-mediated apoptosis by affecting various activities associated with this process. Thus, overexpression of CK2 resulted in the suppression of TRAIL-induced apoptosis via its effects on the activation of caspases, DNA fragmentation, and downstream cleavage of lamin A. In addition, the overexpression of CK2 blocked the mitochondrial apoptosis machinery engaged by TRAIL. These findings define the important role of CK2 in TRAIL signaling in androgen-sensitive and -insensitive prostatic carcinoma cells. Our data support the potential usefulness of anticancer strategies that may involve the combination of TRAIL and down-regulation of CK2. (Cancer Res 2006; 66(4): 2242-9)

Introduction

Casein kinase 2 (CK2) is a highly conserved and ubiquitous protein Ser/Thr kinase localized in the nuclear and cytoplasmic compartments in the cell. It consists of a heterotetrameric structure comprised of α, α′, and β subunits such that the catalytic α subunits are linked via the regulatory β subunit to form configurations such as $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, or $\alpha'_2\beta_2$ in different cells. Many signals affect cellular activities that control growth, proliferation, and death in cells. Protein kinase CK2 is one such signal for which much evidence has emerged, implicating it as a key player in many cellular processes especially including those related to cell growth and cell death. It has long been recognized that CK2 plays a role in cell growth and proliferation, and as such, numerous growth-related proteins seem to be substrates of CK2. An important aspect of CK2 biology is its consistent dysregulation in various cancers that have been examined (1–8).

A more recently identified aspect of CK2 functional activity is that besides promoting cell growth and proliferation, CK2 could also act as a suppressor of apoptosis (3, 4, 9, 10). The role of CK2 as a suppressor of apoptosis may be particularly important in the context of cancer cell phenotype where CK2 is up-regulated. It is well recognized that cancer cells show a dysregulation not only of growth and proliferation but also of apoptosis (programmed cell death). Thus, it is possible that elevated levels of CK2, besides having a role in promoting cell growth, would also be involved in the suppression of cell death in cancer cells. The latter aspect may be particularly important for the prostate cancer where it seems that a dysregulation of apoptosis compared with proliferation may be even more important to its pathogenesis (11, 12).

A key property of the kinase is that it can undergo dynamic shuttling to different loci in the cell in response to diverse stimuli (1–3, 13). This is of particular interest in view of enhanced localization of the kinase to the nuclear structures such as chromatin and nuclear matrix in cancer cells as compared with normal cells (1–3). We previously documented that CK2 shuttles out of the nucleus in response to removal of growth or survival factors (such as hormones and growth factors) whereas the administration of these factors results in its shuttling to the nuclear structures. These observations have hinted that nuclear shuttling of CK2 may serve to protect against cell death and promote cell growth whereas the opposite may promote cell death and suppress growth (1–3, 10). More direct evidence of the role of CK2 as a suppressor of apoptosis was obtained when it was shown that overexpression of the α subunit of CK2 prior to treatment of cells with chemical mediators of apoptosis such as etoposide or diethylstilbestrol resulted in protection against cell death (9). More recently, we have observed that CK2 could also exert a potent suppression of death receptor–mediated apoptosis in prostate cancer cells (14, 15). Together, these observations suggest that CK2 has a broad effect on the apoptosis machinery.

TRAIL, a member of the tumor necrosis factor family, is one of the ligands that promotes cell death via the death receptors TRAIL-R1 (DR4) and TRAIL-R2 (DR5). TRAIL has been found to be effective in inducing apoptosis in certain transformed cells whereas nontransformed cells are generally resistant to TRAIL (see ref. 16), thus prompting the notion that TRAIL may be useful in cancer therapy especially in combination with other modalities (see ref. 17). Binding of TRAIL to cognate receptors activates the apoptosis machinery by recruiting the death-inducing signaling complex and leading to activation of effector caspases (18). Considerable evidence has implicated the involvement of mitochondrial proteins

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in the regulation of TRAIL-induced apoptosis (for a review, see ref. 19).

Our initial observations suggested that CK2 could modulate TRAIL-mediated apoptosis in prostate cancer cells (14). To further investigate the effects of CK2 on pathways associated with TRAIL-mediated apoptosis, here we show that manipulation of the CK2 levels by overexpression of CK2 or its down-regulation by a specific inhibitor results in the respective inhibition or augmentation of TRAIL activity in the cells. CK2-mediated modulations of TRAIL-induced apoptosis involves effects on caspase-3, caspase-8, and caspase-9, and mitochondrial apoptotic proteins such as Bax, Bcl-2, and Bcl-X_L. Importantly, suboptimal concentrations at which TRAIL and CK2 inhibitor are individually insufficient to induce apoptosis result in induction of apoptosis when added together suggesting that down-regulation of CK2 sensitizes the prostate cancer cells to TRAIL-mediated apoptosis. The present data are the first to detail the mechanism of CK2-mediated modulation of TRAIL function in the androgen-sensitive and -insensitive prostate carcinoma cell lines.

Materials and Methods

Cell lines and reagents. Prostate cancer cell line PC-3 was maintained in RPMI 1640 (Invitrogen/Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum and 2 mmol/L L-glutamine in T-75 flasks. ALVA-41 cells were grown in RPMI 1640 supplemented with 6% fetal bovine serum and 2 mmol/L L-glutamine. TBB was purchased from Calbiochem (San Diego, CA) and TRAIL from R&D Systems (Minneapolis, MN).

Cell treatment and transfection. To achieve 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-CK2α at a dose of 2.0 μg/mL using *N*-[1(2,3dioleoyloxyl)propyl]-*N,N,N*-trimethylammonium-methylsulfate (DOTAP) for a period of 24 hours (14, 15). Subsequently, PC-3 cells were treated with 25.0 ng/mL of TRAIL for 24 hours, and ALVA-41 cells were treated with 10.0 ng/mL for 24 hours; these concentrations of TRAIL induced apoptosis in the two types of prostate cancer cells. To promote down-regulation of CK2, PC-3 cells and ALVA-41 cells were treated with 60 μmol/L of TBB and 40 μmol/L TBB, respectively, for 3 hours, and then maintained for 21 hours with or without the desired amount of TRAIL.

Cell viability and proliferation assay. The cell proliferation assay reagent WST-1 (Roche, Indianapolis, IN), a tetrazolium salt that is cleaved by mitochondrial dehydrogenases in viable cells, was employed to determine cell viability and proliferation with or without TRAIL in cells treated with TBB to inhibit CK2 activity, or transiently transfected with pcDNA6-CK2α to achieve forced overexpression of CK2. An aliquot of 200 μL containing a suspension of treated or untreated cells ($2\text{-}5 \times 10^3$) was placed in each well of a 96-well plate. Cells were allowed to re-attach over a period of 24 hours. Following various treatments of cells as above, the medium in each well was replaced with 100 μL of fresh medium containing 100 μL/mL WST-1, and incubation was carried out at 37°C for an additional 60 minutes. An automated plate reader was employed to measure OD_{450}. The results were confirmed in at least three independent experiments.

Determination of caspase-3, caspase-8 and caspase-9 activity. Caspase-3, caspase-8, and caspase-9 activity was determined using the fluorescent assay caspase substrates from BioMol (Plymouth, PA). PC-3 and ALVA-41 cells (0.25×10^6) were plated in six-well plates, and treated with TBB or pcDNA6-CK2α in the presence or absence of TRAIL. Cells were washed with 1× PBS and suspended in chilled cell lysis buffer (50-200 μL/sample) and incubated on ice for 15 minutes. 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 for caspase-3, IETD for caspase-8 and LEHD-AFC for caspase-9) was added to cell lysates. Caspase activity was determined by the relative fluorescence intensity at 505 nm following excitation at 400 nm using a spectrofluorometer (Spectramax Gemini, Switzerland).

Determination of DNA fragmentation. Propidium iodide staining was done for analyzing DNA fragmentation. Cells (0.25×10^6) were plated in six-well format. After desired treatment(s) with TBB ± TRAIL or pcDNA6-CK2α ± TRAIL, as described above, cells were collected and washed twice with 1× PBS. Supernatant was removed, and the residual pellet was gently suspended in 2.5 mL of 70% ethanol in 1× PBS (1.0 mL). Samples were incubated for 1 hour at 4°C, and then washed twice with 1× PBS. The pellet was resuspended in propidium iodide (2.5 μg/mL) solution + 12.5 μL of RNase A (10 mg/mL) + 500 μL of 38 mmol/L sodium citrate buffer. After incubation at 37°C for 45 minutes, the stained cells were analyzed by flow cytometry (FACSVANTAGE SE; Becton Dickinson, San Jose, CA) with the excitation and emission wavelengths set at 488 and 610 nm, respectively. At least 10,000 events were analyzed by Cell Quest software (BD Biosciences, San Jose, CA).

Preparation of whole cell lysates and cytosolic extracts. Following the desired treatment, PC-3 and ALVA-41 cells (2.5×10^6) were collected and washed twice with 1× PBS by centrifuging at 100 × *g* for 5 minutes. The pellet was resuspended in ice-cold 1× radioimmunoprecipitation assay (RIPA) lysis buffer. Lysates were incubated on ice for 10 to 15 minutes and then stored at −20°C. The lysate was centrifuged at 10,000 × *g* to discard the DNA, and the supernatant fraction (lysate) was employed for immunoblot analysis. For detection of cytochrome *c* release, 5×10^6 cells were subjected to the desired treatment, followed by two washes with 1× PBS by centrifugation at 100 × *g* for 5 minutes. The pellet was suspended in extraction buffer (9) and then subjected to differential centrifugation at 600 × *g* for 5 minutes. The supernatant fraction was further centrifuged at 14,000 × *g* for 15 minutes to isolate the mitochondrial fraction. The resulting supernatant fraction was designated as the final cytosolic extract, and was employed to measure the released cytochrome *c* by Western blot analysis.

Immunoblot analysis of caspase-3, caspase-8, c-FLIP$_L$, lamin A, Bax, Bcl-2, Bcl-X$_L$, and cytochrome *c*. PC-3 and ALVA-41 cells were plated in six-well plates at a concentration of 0.5×10^6 and subjected to desired treatments. Whole cell lysates were prepared after washing the cells twice with 1× PBS and then suspending them in 1× RIPA lysis buffer. Western blot analysis was done, and membranes were probed with rabbit anti-lamin A (1:1,000), rabbit anti-caspase-3 (1:500), mouse anti-caspase-8 (1:500), mouse anti-Bax, mouse anti-Bcl-2, rabbit anti-Bcl-X_L (1:1,000; Cell Signaling, Beverly, MA), rabbit anti-FLIP (1:500; Calbiochem), mouse anti-cytochrome *c* (1:1,000; R&D Systems) primary antibodies. After three washes with TBS in 0.1% Tween 20 (TBS-T) the membranes were reprobed with either goat anti-mouse IgG (1:50,000) or mouse anti-rabbit IgG (1:20,000; Pierce Biotechnology, Inc., Rockford, IL) depending on the primary antibody type. Membranes were washed again with TBS-T and allowed to chemiluminesce (Western Pico). They were then developed on Kodak X-ray films. Membranes were also stripped with Restore Stripping buffer (Pierce Biotechnology), and immunoblotted with rabbit anti-lamin A (1:200; Sigma-Aldrich, St. Louis, MO).

Results

Effect of chemical inhibition of CK2 on TRAIL-induced apoptosis. We have recently documented that down-regulation of CK2 in prostate carcinoma cells can induce apoptosis by itself, and likewise can influence apoptosis mediated via death receptors (14, 15). To further delineate the effects of modulation of CK2 on TRAIL function, we initially employed the CK2-specific chemical inhibitor TBB. Both TBB and TRAIL can induce cell death in different cell types in a concentration-dependent manner. Based on this consideration, we determined the concentrations of TBB and TRAIL at which they induced minimal apoptosis when added individually to ALVA-41 or PC-3 cells as a prerequisite to examining whether the addition of these agents together at the suboptimal concentrations would result in induction of apoptosis, i.e., that even partial inhibition of CK2 would result in sensitization of these cells to TRAIL. We found that 60 and 40 μmol/L of TBB inhibited

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CK2 activity by 25% in PC-3 and ALVA-41 cells; however, these concentrations of TBB were insufficient to induce apoptosis in the two cell lines. Similarly, TRAIL at concentrations of 2 and 10 ng/mL in ALVA-41 and PC-3 cells, respectively, did not induce significant apoptosis. Accordingly, PC-3 cells were treated with 60 μmol/L of TBB for 3 hours, and then maintained for 21 hours with or without 10 ng/mL of TRAIL, whereas ALVA-41 cells were treated with 40 μmol/L TBB for 3 hours followed by a period of 21 hours with or without 2.0 ng/mL of TRAIL at the end of which cell lysates were prepared and assessed for caspase-3, caspase-8, and caspase-9 activity. The results in Fig. 1 show that treatment of

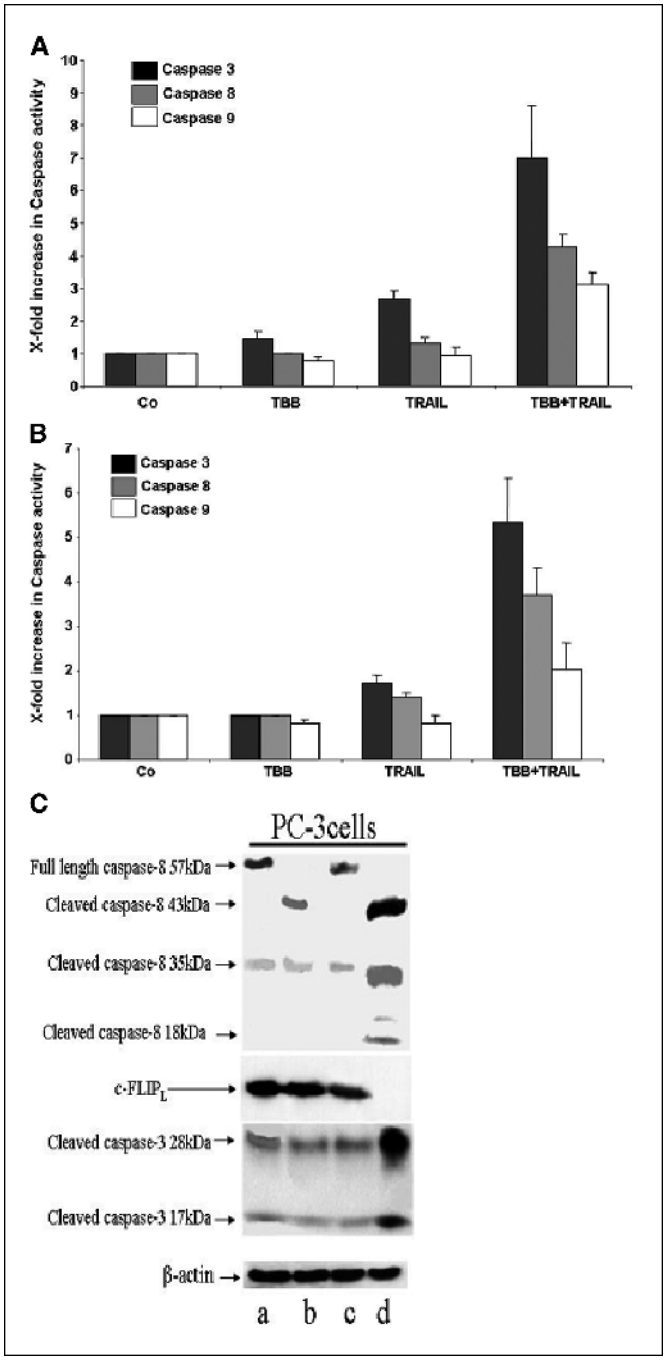

Figure 1. Augmentation of TRAIL-mediated caspase activity and c-FLIP$_L$ down-regulation by chemical inhibition of CK2 in prostate cancer cells. Caspase activity was assessed by spectrofluorimetric assay, as described in Materials and Methods. *A,* ALVA-41 cells (0.25×10^6) treated with TBB (40 μmol/L) or TRAIL alone (2.0 ng/mL) for 24 hours and after pretreatment with 40 μmol/L TBB for 3 hours and subsequent addition of TRAIL for 21 hours. *B,* PC-3 cells (0.25×10^6) treated with TBB (60 μmol/L) or TRAIL alone (10 ng/mL) for 24 hours and after pretreatment with 60 μmol/L TBB for 3 hours and subsequent addition of TRAIL for 21 hours. *C,* processing of caspase-3 and caspase-8, and c-FLIP$_L$ expression in PC-3 cells was determined by immunoblot analysis. *Lane a*, untreated control; *lane b*, TRAIL (10 ng/mL); *lane c*, TBB (60 μmol/L); *lane d*, TBB (60 μmol/L) plus TRAIL (10 ng/mL).

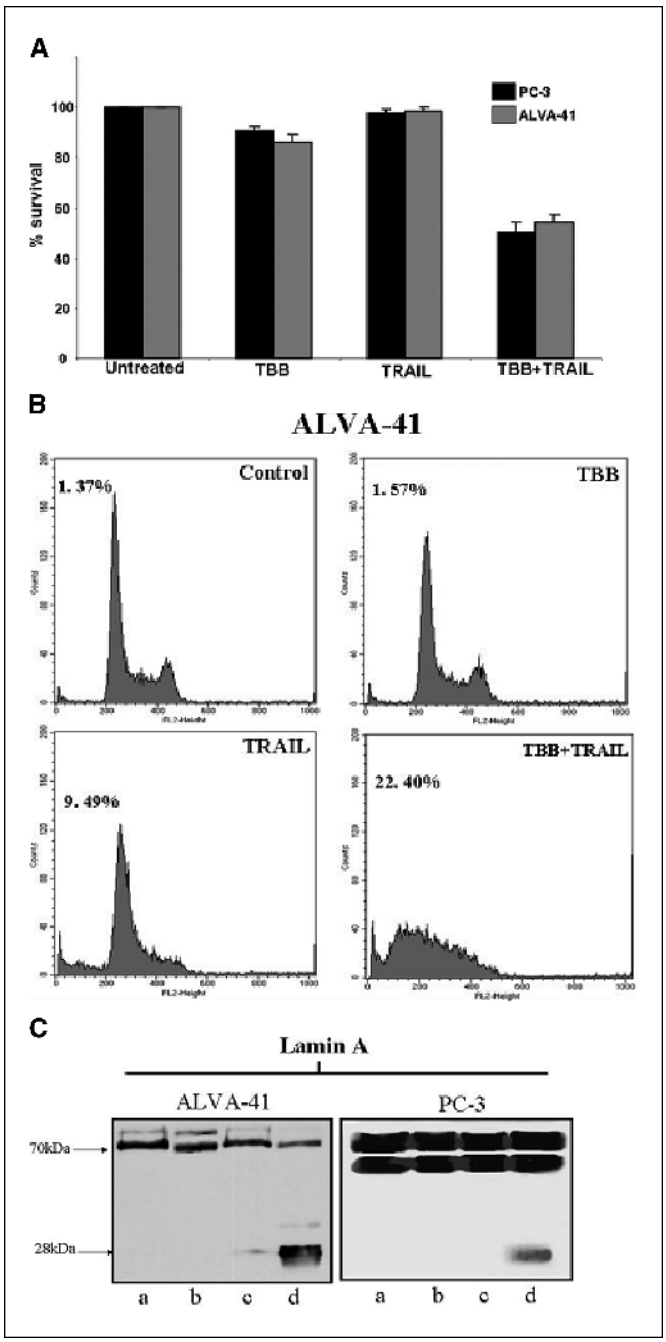

Figure 2. Sensitization of TRAIL-induced apoptosis in prostate cancer cells by treatment with TBB. ALVA-41 and PC-3 cells (0.25×10^6) were treated with TRAIL in the presence or absence of TBB as in Fig. 1. *A,* cell death in ALVA-41 and PC-3 cells was assessed by WST-1 assay. *B,* DNA fragmentation in ALVA-41 cells was measured by propidium iodide staining. *C,* lysates from PC-3 and ALVA-41 cells treated under the same conditions as above were subjected to immunoblot analysis using anti-lamin A as described in Materials and Methods. *Lane a*, untreated control; *lane b*, TRAIL; *lane c*, TBB; *lane d*, TBB plus TRAIL.

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androgen-sensitive ALVA-41 cells (Fig. 1*A*) and androgen-insensitive PC-3 cells (Fig. 1*B*) with TBB followed by TRAIL compared with TBB or TRAIL alone resulted in an enhancement in caspase-3, caspase-8, and caspase-9 activity. Representative immunoblot analysis of the processing of caspase-3 and caspase-8 in PC-3 cell lysates provided further evidence for activation of these caspases. As shown in Fig. 1*C*, caspase-3 and caspase-8 cleavage was augmented when PC-3 cells were subjected to TBB prior to treatment with TRAIL. In addition, c-FLIP$_L$ expression was down-regulated, which also accorded with sensitization of cells to TRAIL in the presence of TBB. Analogous results were observed for ALVA-41 cells under similar conditions (data not shown). Thus, it seems that both the androgen-insensitive (PC-3) and androgen-sensitive (ALVA-41) phenotypes of prostate cancer were sensitized to TRAIL by down-regulation of CK2 activity in the presence of TBB. We next did assays to confirm that cell death in both ALVA-41 and PC-3 cells treated with TBB and TRAIL was due to apoptosis. The results in Fig. 2*A* show that when TBB and TRAIL were added to cells individually at the concentrations employed for the above described experiments, there was no significant reduction in cell survival. However, the two agents added together at these suboptimal concentrations significantly reduced cell survival. The decrease in cell survival observed in response to the down-regulation of CK2 combined with TRAIL was via apoptosis as confirmed by analysis of the population of cells present in different phases of the cell cycle. The results in Fig. 2*B* show that the presence of TBB and TRAIL together as compared with the corresponding controls evoked a marked increase in sub-G$_1$ fraction (an indicator of DNA fragmentation) in ALVA-41 cells. These suboptimal concentrations of TBB and TRAIL alone had minimal effects on DNA fragmentation. Furthermore, we analyzed lamin A cleavage in lysates prepared from ALVA-41 and PC-3 cells treated with TBB, TRAIL, or TBB plus TRAIL. The results in Fig. 2*C* show that there was a marked enhancement in lamin A cleavage in cells treated with TBB plus TRAIL compared with TRAIL or TBB treatment alone. Because lamin A is a downstream target for caspases, its cleavage indicates that cells had undergone efficient apoptosis. These results further substantiated that chemical inhibition of CK2 by TBB sensitized prostate cancer cells to TRAIL-induced apoptosis, thereby affecting several of the TRAIL-mediated activities.

Restoration of mitochondrial circuitry engaged by TRAIL on chemical inhibition of CK2. Depending on the dose employed, TRAIL has been shown to induce both type I and type II apoptotic response in tumor cells. This suggests that besides type I response, mitochondria play an important role in the function of TRAIL (20). We therefore examined the response of mitochondrial pathways to the induction of cell death by TRAIL on down-regulation of CK2 by TBB. Again, for this purpose, we employed suboptimal concentrations of TRAIL and TBB at which they individually did not engage any of the mitochondrial proteins. Cell lysates of treated PC-3 and ALVA-41 cells were subjected to Western blotting for the detection of Bax, Bcl-2, Bcl-X$_L$, and cytochrome *c*. The results in Fig. 3 show that TBB treatment prior to the addition of TRAIL up-regulated the proapoptotic protein Bax with simultaneous down-regulation of mitochondrial antiapoptotic proteins Bcl-2 and Bcl-X$_L$. Furthermore, the increase in expression of cytochrome *c* in the cytosol coincided with the increase in Bax and decrease in Bcl-2 and Bcl-X$_L$ expression (Fig. 3). These results implicated the restoration of the mitochondrial circuitry upon sensitization of cells to TRAIL as a result of CK2 inhibition in both the androgen-sensitive and -insensitive prostate cancer cells.

Blocking of TRAIL-induced caspase activity by overexpression of CK2. We next investigated the effects of CK2 overexpression on TRAIL-induced apoptotic activities in prostate cancer cells. PC-3 and ALVA-41 cells were transfected with pcDNA6-CK2α to achieve transient overexpression of CK2α prior to treatment with TRAIL at apoptosis-inducing concentrations, i.e., 10 and 25 ng/mL of TRAIL for ALVA-41 and PC-3 cells, respectively. Determination of caspase-3, caspase-8, and caspase-9 activity in treated cells showed that the activity of these caspases peaked at 12 hours with TRAIL alone, whereas prior overexpression of CK2 significantly impeded the activity of caspase-3, caspase-8, and caspase-9 induced by TRAIL in ALVA-41 and PC-3 cells (Fig. 4*A* and *B*). Furthermore, Western blot analysis of caspase-8 and caspase-3 in PC-3 cells showed that the TRAIL-induced cleavage of caspases was inhibited by overexpression of CK2. Because it has been previously shown that c-FLIP$_L$ expression is necessary and sufficient to maintain resistance to TRAIL-induced apoptosis in certain prostate cancer cells (21), we examined c-FLIP$_L$ activity in response to overexpression of CK2 in PC-3 cells subjected to TRAIL-mediated apoptosis. The results in Fig. 4*C* show that forced overexpression of CK2 resulted in a significant

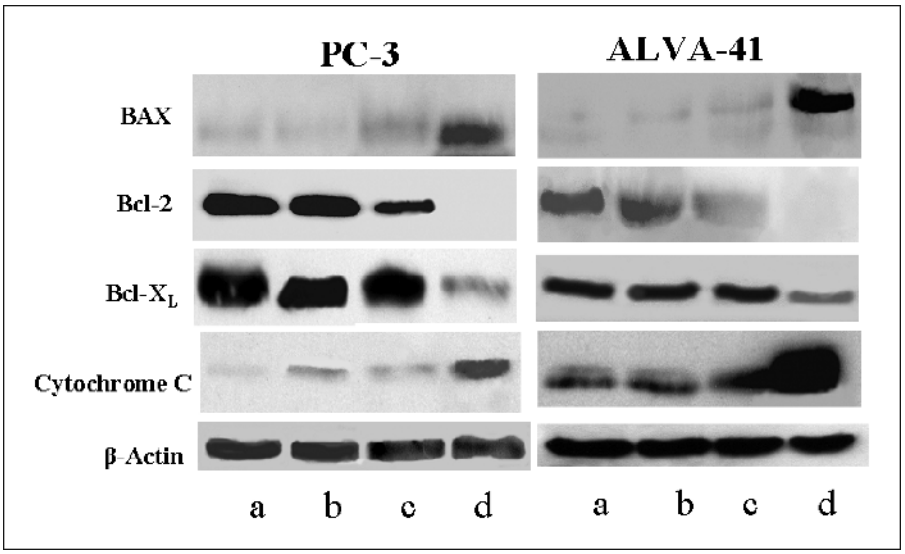

Figure 3. Effect of TBB on TRAIL-mediated expression of Bax, Bcl-2, Bcl-X$_L$, and release of cytochrome *c* in prostate cancer cells. Lysates prepared from ALVA-41 and PC-3 (2.5×10^6) cells treated with desired concentrations of TBB with or without TRAIL as in Fig. 1 were subjected to immunoblot analysis for anti-Bax, anti-Bcl-2, anti-Bcl-X$_L$, anti-cytochrome *c*, and anti-actin. *Lane a*, untreated control; *lane b*, TRAIL; *lane c*, TBB; *lane d*, TBB plus TRAIL.

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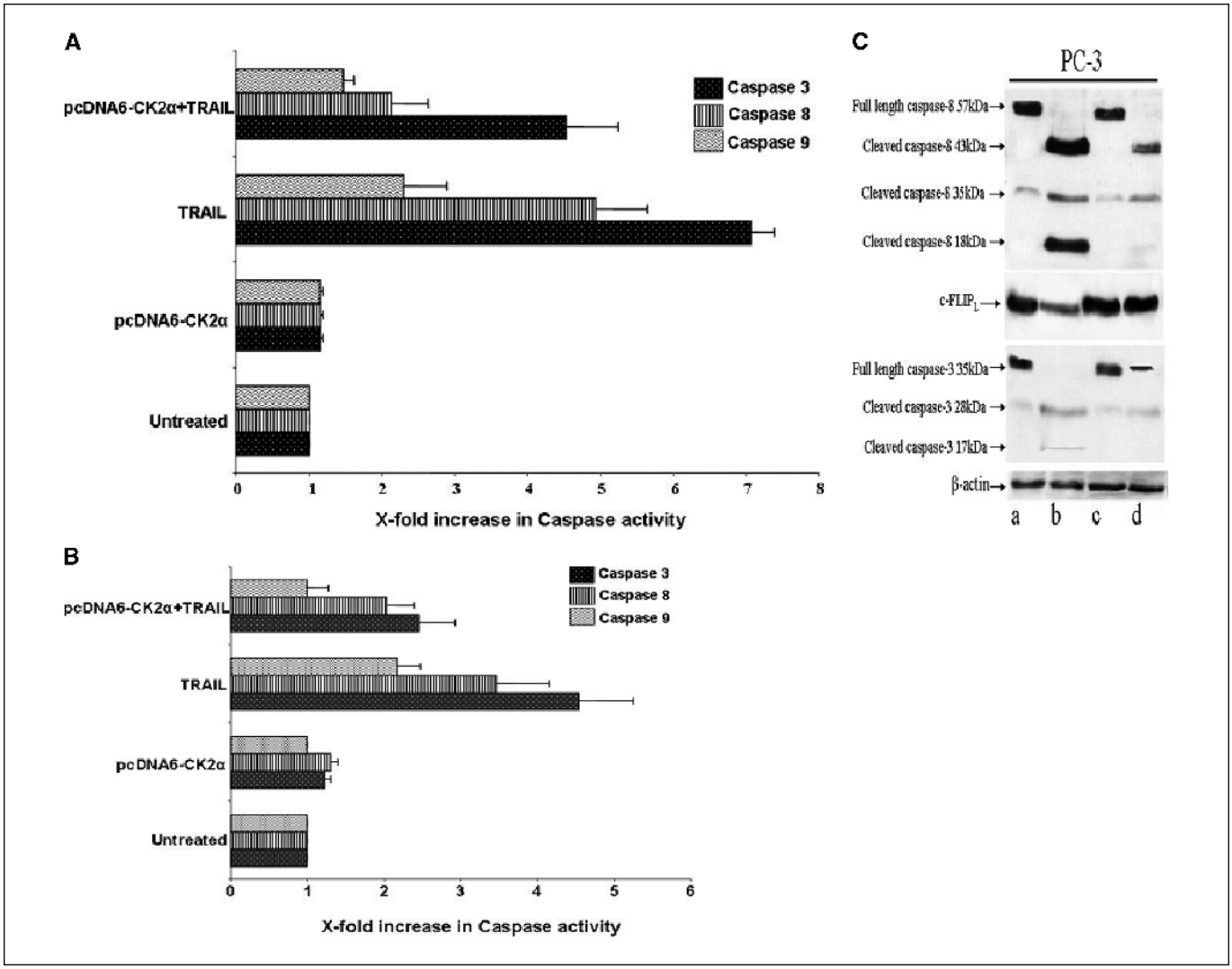

Figure 4. Effect of overexpression of CK2 on TRAIL-induced caspase activity in prostatic cancer cells. Caspase activity was determined by spectrofluorimetric assay as described in Materials and Methods. *A,* ALVA-41 cells (0.25×10^6) were transfected with pcDNA6-CK2α (2 μg/mL) using DOTAP transfection reagent for 24 hours followed by treatment with 10 ng/mL of TRAIL for 24 hours. *B,* PC-3 cells (0.25×10^6) were transfected with pcDNA6-CK2α (2 μg/mL) for 24 hours, followed by 25 ng/mL of TRAIL for 24 hours. *C,* representative immunoblot analysis of processing of caspase-3, caspase-8, and c-FLIP$_L$ is shown for PC-3 cells. *Lane a*, untreated control; *lane b*, TRAIL (25 ng/mL); *lane c*, pcDNA6-CK2α (2 μg/mL), and *lane d*, pcDNA6-CK2α (2 μg/mL) plus TRAIL (25 ng/mL).

reversal of TRAIL-induced c-FLIP$_L$ down-regulation. The response of ALVA-41 cells was also similar to that of PC-3 cells shown in Fig. 4*C* (data not shown). Together, the results on the function of TRAIL in the presence of CK2 overexpression were opposite to those observed when CK2 was down-regulated by TBB.

Effect of overexpression of CK2 on TRAIL-mediated DNA fragmentation and lamin A cleavage. Having shown that overexpression of CK2 blocked TRAIL-induced caspase activation in prostatic cancer cells, we further analyzed TRAIL-treated cells for evidence of apoptosis by employing WST-1 assay and by analysis of DNA fragmentation using propidium iodide staining. The results in Fig. 5 show that TRAIL alone (at appropriate concentrations) induces cells to undergo cell death or apoptosis (~60%) in ALVA-41 and PC-3 cells (as indicated by the percentage of cells in sub-G_1 phase of cell cycle). However, prior transfection of cells with pcDNA6-CK2α resulted in a significant reduction in TRAIL-mediated cell death or apoptosis (~20% of cells undergoing apoptosis; Fig. 5*A* and *B*). In contrast to the above experiments showing that CK2 down-regulation resulted in the enhancement of TRAIL-induced caspase activation and breakdown of the downstream target lamin A, the present results showed that breakdown of lamin A was inhibited under conditions of CK2α overexpression in both ALVA-41 and PC-3 cells suggesting the suppression of TRAIL-induced apoptosis (Fig. 5*C*).

Engagement of the mitochondrial apoptotic proteins activated by TRAIL is blocked by CK2 overexpression. Considering that mitochondrial death–related activities are engaged by TRAIL, and as shown above, the effect of TRAIL is enhanced in the presence of TBB (i.e., down-regulation of CK2), we examined if TRAIL-responsive mitochondrial signals were blocked upon overexpression of CK2. PC-3 and ALVA-41 cells were transfected with pcDNA6-CK2α prior to treatment with TRAIL at apoptosis-inducing concentrations, and cell lysates were subjected to Western blot analysis for Bax, Bcl-X$_L$, Bcl-2, and cytochrome *c* expression (Fig. 6). We observed that TRAIL-induced up-regulation of Bax was inhibited when cells were transfected with pcDNA6-CK2α to

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promote overexpression of CK2α. In addition, TRAIL-induced down-regulation of Bcl-2 and Bcl-X_L expression was reversed by overexpression of CK2. Likewise, TRAIL-mediated release of cytochrome *c* in the cytoplasm was blocked under these conditions of elevated CK2 in both the PC-3 and ALVA-41 cells, thus indicating that the response of mitochondrial death circuitry to TRAIL was impeded under these conditions. Overall, these results were opposite to those observed above when TBB was employed to down-regulate CK2 with consequent sensitization of cells to TRAIL activity, and further confirm that modulations in CK2 could exert a significant effect on activity of TRAIL in both the androgen-sensitive and androgen-insensitive prostate cancer cells.

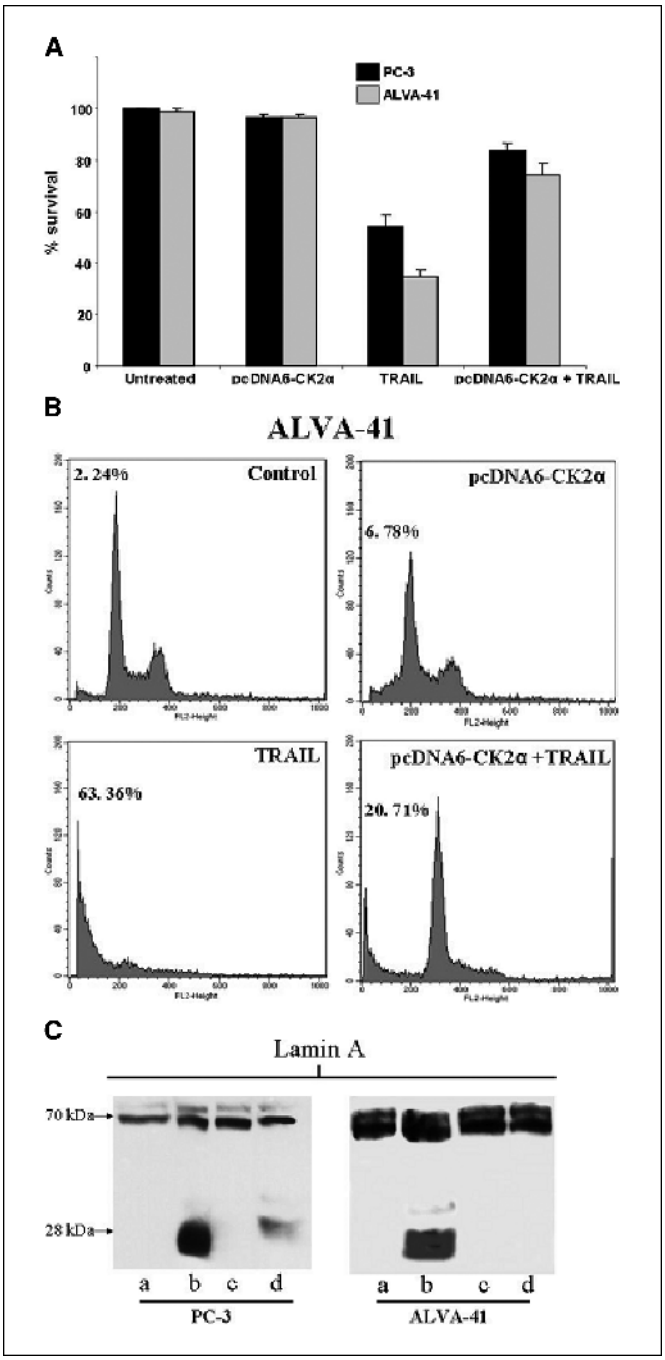

Figure 5. Effect of overexpression of CK2 on TRAIL-induced apoptosis in prostate cancer cells. *A,* ALVA-41 and PC-3 (0.25×10^6) cells were transfected with pcDNA6-CK2α (2 μg/mL) using DOTAP for 24 hours followed by treatment with 10 ng/mL TRAIL for ALVA-41 and 25 ng/mL TRAIL for PC-3 cells. Cell death was assessed by WST-1 assay. *B,* PC-3 (0.25×10^6) cells were transfected with pcDNA6-CK2α (2 μg/mL) using DOTAP for 24 hours followed by TRAIL (25 ng/mL) for 24 hours. DNA fragmentation was assayed by propidium iodide staining. *C,* lysates from treated cells were subjected to immunoblot analysis for lamin A cleavage. *Lane a*, untreated control; *lane b*, TRAIL; *lane c*, pcDNA6-CK2α; and *lane d*, pcDNA6-CK2α plus TRAIL.

Discussion

Much previous evidence has underscored the function of protein kinase CK2 in cell growth and proliferation (e.g., refs. 1–9). However, a new paradigm for its function in cancer biology emerged with our demonstration that CK2 is also capable of suppressing chemical-mediated and survival factor deprivation–mediated apoptosis (2, 3, 9, 10), along with the subsequent recent recognition that CK2 can also affect death receptor–mediated apoptosis in prostate cancer, colon cancer, and rhabdomyosarcoma cells (14, 22–24). Together, these observations have prompted the notion that analogous to its role in cell growth (25), CK2 may have a global role in affecting the apoptosis machinery (14).

Previous studies investigating the role of CK2 down-regulation on sensitization of cells to TRAIL employed agents such as 5,6-dichlorobenzimide, apigenin, and emodin (22–24). Although these studies supported the role of CK2 in the regulation of TRAIL activity in colon carcinoma and rhabdomyosarcoma cells, the issue of using a highly specific inhibitor of CK2 warrants further consideration. In the present work, we have employed TBB which is currently regarded as the most specific CK2 inhibitor available (26), and additionally, we have also examined the effects of overexpression of CK2 on TRAIL function. Thus far, studies have not been undertaken along these lines on prostate cancer cells, or dealing with the effects of overexpression of CK2 on TRAIL-mediated activities in any cell. To that end, we have attempted to delineate the regulation of TRAIL function in both the androgen-sensitive ALVA-41 and androgen-insensitive PC-3 prostatic carcinoma cell lines by modulating cellular CK2 by its down-regulation (using the chemical inhibitor TBB) as well as its up-regulation by forced overexpression of CK2α. We hypothesized that the results of TRAIL function under the two experimental conditions of CK2 modulation would be complementary.

At appropriate concentrations (which vary with the cell type) both TBB and TRAIL can individually induce apoptosis in prostate cancer cells (14, 15). In the present work, we tested the hypothesis that the combined effect of TBB plus TRAIL at suboptimal concentrations would be considerably greater than that observed when they are tested individually in the two types of prostatic carcinoma cells (PC-3 and ALVA-41). Indeed, the results supported this notion, thus providing evidence for a synergy between the action of TBB and TRAIL. A noteworthy observation resulting from these studies is that the effects of CK2 on the regulation of TRAIL are analogous in the androgen-sensitive and androgen-insensitive phenotypes of prostate cancer cells. This observation corresponds with our previous results documenting that CK2 was a downstream nuclear signal for growth in both the androgen-sensitive (LNCaP) and androgen-insensitive (PC-3) cells, suggesting that regardless of the prostate cancer cell phenotype, the CK2 signal was functionally active in cells responsive to the corresponding growth stimuli (27). Germane to these considerations are the observations that both the androgen-sensitive and androgen-insensitive prostate carcinoma cell lines also respond to antisense CK2α in a similar manner (28). To further examine the role of CK2 in affecting TRAIL activity, we studied the effects of transient overexpression of CK2α in the prostatic

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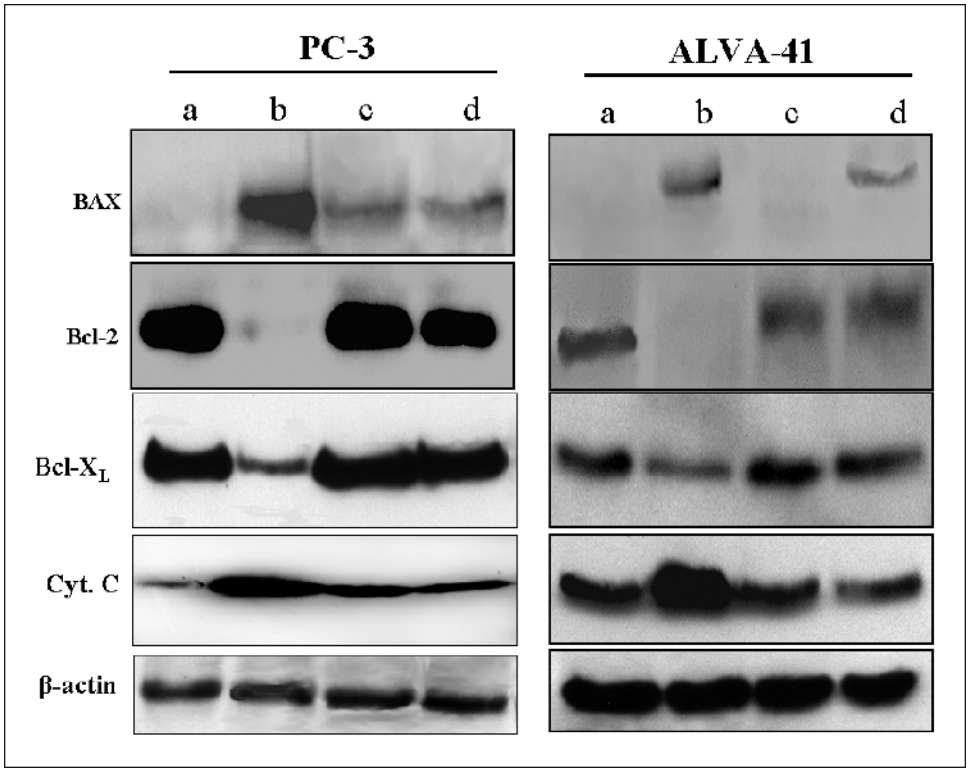

Figure 6. Effect of overexpression of CK2 on mitochondrial engagement by TRAIL in prostate cancer cells. ALVA-41 and PC-3 cells (2.5×10^6) were transfected in T25 flasks with pcDNA6-CK2α (2.0 μg/mL) for 24 hours using DOTAP, followed by treatment with 10 ng/mL TRAIL for ALVA-41 cells and 25 ng/mL for PC-3 cells for 24 hours. Lysates of the treated cells were subjected to immunoblot analysis using anti-Bax, anti-Bcl-2, anti-Bcl-X_L, anti-cytochrome *c*, and anti-actin antibodies. *Lane a*, untreated control; *lane b*, TRAIL; *lane c*, pcDNA6-CK2α; and *lane d*, pcDNA6-CK2α plus TRAIL.

carcinoma cells (ALVA-41 and PC-3). These experiments also bore out the role of CK2 as a suppressor of apoptosis because the TRAIL-mediated activities under these conditions were markedly attenuated, and the effects of CK2 modulation were apparent at several reactions that are affected by TRAIL-mediated apoptosis.

It is well documented that TRAIL signaling involves caspases and mitochondrial apoptotic proteins for transduction of its effects (20). Our results clearly indicate that CK2 affects TRAIL-mediated activities in regulating apoptosis in target cells such as the prostatic carcinoma cells examined here. The effects of down-regulation or overexpression of CK2 on TRAIL-mediated changes in cell viability and DNA fragmentation are apparent at several levels in the apoptosis cascade. These include, for example, effects on caspase-3, caspase-8, and caspase-9, expression of c-FLIP$_L$, and downstream cleavage of lamin A. Recent studies have documented that c-FLIP$_L$ expression is necessary and sufficient to maintain resistance to TRAIL-induced apoptosis in prostate cancer cells (21). Our results coincide with this observation and further show that CK2 up-regulation or down-regulation markedly affects TRAIL-mediated regulation of c-FLIP$_L$. The effect of CK2 on the involvement of the mitochondrial pathway is further underscored by its dramatic effects on changes in TRAIL-mediated response of Bax, Bcl-2, Bcl-X_L, and cytochrome *c* release. To reiterate, all the results observed on the function of TRAIL and its modulation by CK2 were analogous in both the androgen-sensitive ALVA-41 and androgen-insensitive PC-3 prostate carcinoma cell lines indicating full functionality of the CK2 signal in prostate cancer cells regardless of their phenotype.

In summary, the present work is the first to provide a detailed analysis of the mechanism of CK2-mediated regulation of activities associated with TRAIL-induced apoptosis in prostatic carcinoma cells. The observations may be of particular importance in the context of prostate cancer as the CK2 signal is equally accessible in the androgen-sensitive and -insensitive phenotypes (27, 28). The present results also provide further support for our recent proposal on the usefulness of CK2 as a target for cancer therapy (15, 29–31). A potential outcome of these studies is the possibility of a significant improvement in the chemotherapeutic potential of TRAIL by down-regulation of CK2, and as such, consideration of combined therapy with TRAIL and CK2 inhibition may be an attractive strategy to enhance prostate tumor cell death.

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Role of Protein Kinase CK2 in the Regulation of Tumor Necrosis Factor–Related Apoptosis Inducing Ligand–Induced Apoptosis in Prostate Cancer Cells

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