Published in final edited form as:

Ann N Y Acad Sci. 2009 August ; 1171: 591–599. doi:10.1111/j.1749-6632.2009.04916.x.

Involvement of Reactive Oxygen Species in Apoptosis Induced by Pharmacological Inhibition of Protein Kinase CK2

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Abstract

It has been reported that inhibition of protein kinase CK2 (CK2) with antisense oligodeoxynucleotides (ODN) is a potent inducer of apoptosis in cancer cells but not in normal cells. In this regard, the apoptotic-inducing effect is attributed to the catalytic activity of the enzyme, which phosphorylates proapoptotic proteins to inhibit their functions. In this study we investigate the role of intracellular redox status in the proapoptotic activity of CK2 inhibition in human leukemia CEM cells. We provide evidence that inhibition of CK2 activity induces apoptotic cell death as evident by activation of caspase 3, DNA fragmentation, and phosphatidylserine externalization. Inhibition of CK2 resulted in a significant increase in intracellular hydrogen peroxide production, which we show as a critical mediator of apoptosis. To that end, apoptotic hallmarks, like DNA fragmentation and phosphatidylserine externalization, were blocked with the specific hydrogen peroxide scavenger catalase. We also show that inhibition of CK2 reduces cytosolic intracellular superoxide, a precursor of hydrogen peroxide. In summary, decreasing CK2 activity increases intracellular hydrogen peroxide, creating an intracellular environment conducive for death execution. Taken together, these data provide information on novel pathways involved in CK2 biology with implications for effective tools against drug-resistant tumors.

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Conflicts of Interest

The authors declare no conflicts of interest.

Keywords

human leukemia cells; CK2; apoptosis; ROS

Introduction

Protein kinase CK2 (CK2) is a ubiquitous serine/threonine protein kinase consisting of two catalytic α (GeneID: 1457), α' (GeneID: 1459) subunits and a regulatory β (GeneID: 1460) subunit. The catalytic subunits are linked via the regulatory subunits to form the heterotetrameric holoenzyme with configurations, such as $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, or $\alpha'\alpha_2\beta_2$, in varying amounts in different cells. This multifunctional protein kinase has been shown to impact cell growth and proliferation as numerous growth-related proteins are substrates of CK2. Furthermore, deregulation of CK2 activity in the neoplastic phenotype underscores the relevance of CK2 biology to cancer.¹ Of particular note, recent evidence indicates apoptosis-suppressing activity of CK2,¹ which renders CK2 as a logical target for novel therapeutic strategies, considering that cancer cells invariably harbor defects or deficiencies in death execution machinery.

Apoptosis is an orderly series of intracellular events that may be triggered by various factors, including death receptor ligation and anticancer drugs.^{2,3} In this regard, our recent work has highlighted the role of cellular redox status in the regulation of apoptotic death signaling.^{4–7} Cellular redox state is dependent on the products of intracellular reactive oxygen species (ROS), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), and the inherent ability of the cells to maintain a nontoxic constitutive concentration of these reactive species via their antioxidant defense systems.⁸ While an overwhelming increase in intracellular ROS could be detrimental to cells and tissues by triggering various modes of cell death, a slight pro-oxidant state has also been associated with signaling for growth and proliferation.^{9,10} Indeed, a pro-oxidant milieu is invariably associated with cellular transformation and appears to provide cancer cells with a survival advantage over their normal counterparts.^{11–13} Along these lines, we demonstrated that a slightly elevated intracellular concentration of O_2^- was inhibitory to apoptotic signaling, irrespective of the trigger,^{14,15} whereas an increase in the intracellular ratio of H_2O_2 to O_2^- facilitated death execution.^{16–18}

Intrigued by findings that CK2 is overexpressed in tumor cells, has an inhibitory effect on apoptosis, and that silencing of CK2 selectively induces apoptosis in tumor cells, we investigated the role of intracellular ROS in apoptosis induced upon inhibition of CK2.¹⁹ Here we show that pharmacological inhibition of CK2 triggers apoptosis in human leukemia cells, which is dependent upon intracellular H_2O_2 production.

Materials and Methods

Cell Culture

The human T-lymphoblastic CEM leukemia cells were purchased from American Type Culture Collection (ATCC, Rockville, MD) and maintained in RPMI 1640 (Hyclone, Logan,

UT) supplemented with 1% L-glutamine, 1% penicillin/streptomycin, and 5% fetal bovine serum with 1% Geneticin. The cell line was cultured in an incubator at 37°C with 5% CO₂.

Inhibitor Treatment and Flow Analysis

Typically, 1×10^6 cells were plated in 24-well plates and treated with the CK2 inhibitor 4,5,6,7-tetrabromobenzotriazole (TBB) (Cal-biochem, San Diego, CA) before they were washed with RPMI 1640 and analyzed. In a typical flow cytometry analysis, the cells were incubated with the various intracellular dyes in the culture medium for 20 min at 37°C in the dark before they were washed and resuspended in 500 µL of RPMI 1640; at least 10,000 events were analyzed using the WINMDI software Version 2.8 (Scripps, La Jolla, CA).

Determination of Cell Viability

Cell viability was analyzed by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) (Sigma, St. Louis, MO) as described previously.⁵ Briefly, following treatments as above, 1×10^5 cells were plated in a 96-well plate with 50 µL of MTT and incubated for 4 h at 37°C in the dark. After incubation, the crystals formed were dissolved in 200 µL dimethylsulfoxide + 10 µL Sorenson's glycine buffer and absorbance was measured with an automated ELISA reader (Tecan, Maennedorf, Switzerland), using an absorbance wavelength of 570 nm.

Determination of CK2 Activity

Protein kinase CK2 (CK2) activity was determined with the CK2-sensitive synthetic p53 peptide (RRRDDDSDDD) (Cyclex, Nagano, Japan), which is phosphorylated at serine46 by CK2, as described previously.²⁰ Briefly, 2×10^6 cells were homogenized with 25 strokes in a Denounce homogenizer (B. Braun, Bethlehem, PA) in the extraction buffer [20 mmol/L Tris-HCl (pH 8.0), 150 mmol/L NaCl, 10% glycerol, 0.25% Triton X-100, 1 mmol/L dithiothreitol (DTT), 1 mmol/L EDTA, 1 mmol/L EGTA, 0.2 mmol/L phenylmethanesulfonyl fluoride (PMSF), 1 µg/mL pepstatin, 0.5 µg/mL leupeptin, 5 mmol/L β-glycerophosphate, 5 mmol/L NaF, 1 mmol/L Na₃VO₄] at 4°C and purified with phosphocellulose P11 (Whatman, Maidstone, UK) and a hydroxyapatite column (Bio-Rad, Hercules, CA) to achieve a rich CK2 fraction, as recommended by the manufacturer. The enriched fractions were then incubated with the synthetic substrate p53 peptide coated on a 96-well plate with the kinase buffer (provided) and 125 µmol/L ATP. The wells were then washed and incubated with the horseradish peroxidase-conjugated anti-phospho-p53 serine46 antibody (Cyclex) with an automated ELISA reader (Tecan) at an absorbance wavelength of 540 nm.

Analysis of Caspase 3 Activity

Caspase 3 activity was analyzed with fluorescent-conjugated caspase 3 substrate (Alexis, Lausen, Switzerland), as described previously.¹⁶ Briefly, 2×10^6 cells were washed with plain medium and lysed with 210 µL of caspase lysis buffer (20 mmol/L Tris-HCl, 150 mmol/L NaCl, 1 mmol/L Na₂-EDTA, 1 mmol/L EGTA, 1% Triton, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L β-glycerophosphate, 1 mmol/L Na₃VO₄, 1 µg/mL leupeptin, 1 mmol/L PMSF) for 10 min at 4°C. Fifty microliters of the lysate was then plated on a 96-

well plate with 50 μ L 2 $\times$ reaction buffer, 10 mmol/L DTT, and 2 μ mol/L of the conjugate caspase substrate DEVD-AFC. Kinetic measurements were then made with a spectrofluorometer (Tecan) with excitation wavelength of 400 nm and fluorescence wavelength of 505 nm. Results were then tabulated as fold increase with respect to the lysate from untreated cells.

Analysis of Intracellular Hydrogen Peroxide and Superoxide

Intracellular H₂O₂ was assayed by 5-(and-6)-chloromethyl-2',7'-dichlorofluorescein diacetate (CM-H₂DCFDA) (Molecular Probes, Eugene, OR), while intracellular O₂⁻ was assayed by hydroethidine (HE) (Molecular Probes), as described previously.²¹ Briefly, cells were incubated with 5 μ mol/L DCF-DA or 5 μ mol/L HE and analyzed by flow cytometry at 488 nm and 525 nm wavelengths, respectively.

Analysis of DNA Fragmentation

DNA fragmentation was analyzed with propidium iodide (PI), as previously described.¹⁶ Briefly, following treatment the cells were washed and resuspended in 500 μ L of 1 $\times$ PBS and immediately fixed with 70% ethanol before they were resuspended in 500 μ L of PI:RNase A solution and analyzed by flow cytometry at 488 nm excitation wavelength and 610 nm emission wavelength.

Analysis of Phosphatidylserine Externalization

Externalization of phosphatidylserine (PS) was analyzed by dual staining with fluorescein isothiocyanate (FITC) Annexin V probe (BD Pharmingen, Franklin Lakes, NJ) and PI, as previously described.³² Briefly, cells were incubated with 2 μ L FITC Annexin V and 1 μ L PI in 1 $\times$ binding buffer for 20 min at 37°C in the dark. After incubation, they were immediately kept at 4°C and analyzed by flow cytometry at 525 nm wavelength.

Data Analysis

Data presented in each figure is mean $\pm$ SD of at least three independent experiments performed in triplicate, unless otherwise indicated. Statistical significance was determined by the Student's *t*-test. A representative of the triplicates was presented for the flow cytometry figure.

Results and Discussions

Inhibition of CK2 Decreases Cell Viability in Human Leukemia Cem Cells

It was previously shown that CK2 expression and activity were enhanced in various human tumor cells and this was not a reflection of the proliferative status of the tumors but a pathological feature of cancer cells.¹ It has also been demonstrated that overexpression of the different CK2 subunits prior to chemotherapeutic drug treatment protects cancer cells against apoptotic cell death and increases cell viability.²² Recent findings have also established that inhibition of CK2 with antisense oligodeoxynucleotides (ODN) is a potent inducer of apoptosis in cancer cells but not in normal cells.¹⁹ Here we showed that exposure of human leukemia Cem cells to various concentrations (3.125–100 μ mol/L) of CK2

inhibitor TBB resulted in a significant dose-dependent decrease in cell survival as measured by the MTT assay at 12 h (Fig. 1A). TBB has been shown to act at the posttranslation level as a highly selective ATP/GTP-competitive inhibitor of CK2.^{23,24} In line with the latter, we showed that 1 h treatment of leukemia cells with TBB significantly reduced (80% and 60% reduction at 25 $\mu\text{mol/L}$ and 50 $\mu\text{mol/L}$, respectively) the activity of the CK2 enzyme (Fig. 1B).

Inhibition of CK2 Induces Apoptosis in Human Leukemia Cem Cells

The execution of apoptosis in cells is orchestrated by intricate networking between caspases and apoptogenic factors, thus rendering caspases integral effectors of the apoptotic machinery. The activation of executioner caspase 3 is fundamental to the commitment of cells toward the final phase of apoptosis,²⁵ thus rendering its activation an important hallmark of apoptotic death. In order to elucidate the nature of the cell death induced by CK2 inhibition, we measured the activity of the executioner caspase 3.²⁶ We showed that the inhibition of CK2 in Cem cells induced a dose-dependent increase in the activity of caspase 3 as measured by the fold increase in fluorescence of DEVD-AFC compared to the untreated cells at 4 h (Fig. 2A). In addition, we also performed cell cycle analysis using PI to detect the proportion of sub-G1 cells. We showed that treatment (12 h) of Cem cells with TBB induced an increase in the sub-G1 fraction (Fig. 2B) in a dose-dependent manner. These data corroborate earlier findings that inhibition of CK2 in human tumors triggers apoptotic cell death.^{22,27,28}

Inhibition of CK2 Induces Intracellular H_2O_2 Production and a Decrease in Intracellular O_2^-

We have previously demonstrated that drug exposure of human leukemia cells resulted in H_2O_2 -dependent apoptosis.¹⁶ While a high concentration of H_2O_2 elicits necrotic cell death, a low concentration of H_2O_2 induces classical apoptotic response.¹⁸ Here we showed that inhibition of CK2 with TBB in Cem cells resulted in a significant increase in intracellular H_2O_2 after 3 h of treatment in a dose-dependent manner, as shown by the right shift of the DCF histogram (Fig. 3A). CM- H_2DCFDA is cell permeant and enters the cells where its acetate groups are cleaved by intracellular esterases, yielding the DCF moiety that is oxidized by H_2O_2 to yield green fluorescence. The positive shift by flow cytometry could be inhibited by preincubation of the cells for 1 h with 5000 units of catalase, the specific H_2O_2 scavenger (Fig. 3A), indicating that the ROS detected by the DCF fluorescence was H_2O_2 . The addition of catalase was able to scavenge about 50% of the H_2O_2 produced by 50 $\mu\text{mol/L}$ TBB. These data suggest that apoptotic response of the cells to inhibition of CK2 may be a function of a reducing milieu created by intracellular H_2O_2 , as previously described.^{4-7,16,18,29}

Intrigued by the above findings, we next investigated the source of the intracellular H_2O_2 . Here we showed that CK2 inhibition reduced the intracellular O_2^- levels of the cells. Treatment of Cem cells with CK2 inhibitor TBB resulted in a dose-dependent reduction of intracellular O_2^- concentration after 2 h of treatment, as measured by a left shift of the HE histogram (Fig. 3B). DDC (diethyldithiocarbamate) inhibited Cu/Zn superoxide dismutase (SOD), thereby increasing the intracellular O_2^- , while DPI (diphenyleneiodonium chloride) reduced the intracellular O_2^- level by inhibiting the NADPH oxidase system; thus they are

used as positive and negative controls, respectively, in the HE assay. The reduced pro-oxidant state of the cells may further facilitate the induction of apoptosis in the cells upon CK2 inhibition, as previously described.^{4,18} Interestingly, the reduction in intracellular O_2^- occurred at 2 h prior to the increase in intracellular H_2O_2 at 3 h. Taken together, these data suggest that, via its dismutation, the inherent intracellular O_2^- of the leukemia cells may be the source of the increase in intracellular H_2O_2 produced upon inhibition of CK2 activity in the cells.

The principal intracellular O_2^- scavengers are the SODs, which convert O_2^- to H_2O_2 , thus making it a key antioxidant defense system in the cells. In human cells, there are two isoforms of SOD, namely Cu/Zn SOD, which is located in the cytosol, and Mn SOD, which is located in the mitochondria, using copper and zinc or manganese, respectively, as coenzymes. The coenzymes function to transfer electrons in the dismutation process.³⁰ The above preliminary observations suggest that inhibition of CK2 activity may involve SODs for the production of intracellular H_2O_2 .

Apoptotic Cell Death Induced by Inhibition of CK2 Is Contingent on Intracellular H_2O_2 Production

We have previously shown that an efficient apoptotic death execution is dependent upon a reducing intracellular milieu attained by the production of intracellular H_2O_2 .^{4-7,16,18,29} We have also shown that the production of intracellular H_2O_2 is vital in the induction of drug-induced apoptosis in human leukemia cells.^{16,31} Here we provided evidence that the cytotoxic activity of CK2 inhibition is contingent upon intracellular H_2O_2 produced. We showed by dual staining of Cem cells with Annexin V and PI that the key intracellular H_2O_2 scavenger, catalase, is able to attenuate the externalization of PS without an increase in the uptake of PI (Fig. 4A); preincubation of the cells with 5000 units of catalase for 1 h is able to attenuate 50% of PS externalization. Loss of the plasma membrane is one of the earliest morphological features of apoptosis. In early apoptotic cells, the membrane phospholipid PS is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment and facilitating the removal of cells programmed to die by the external surrounding cells. An early feature of cells undergoing apoptosis is the externalization of PS without the uptake of PI.³² We provided further evidence that the degradation of DNA is inhibited by catalase. Prior incubation of cells with 5000 units of catalase for 1 h rescues about 50% of the sub-G1 DNA fragmentation induced by inhibition of CK2 (Fig. 4B). These data suggest that apoptosis induced by CK2 inhibition is dependent upon intracellular H_2O_2 production and support our earlier findings that H_2O_2 is an important signaling molecule in apoptotic execution.^{4-7,16,18,29,31}

Concluding Remarks

Taken together, the data presented support the prodeath effect of CK2 inhibition in human leukemia cells through an H_2O_2 -mediated mechanism, thereby creating an intracellular reducing milieu, conducive for apoptotic death execution (Fig. 5). The precise mechanisms and source of the intracellular H_2O_2 induced upon CK2 inhibition and the downstream effects of H_2O_2 that induce the apoptotic response are the focus of our current

investigations. Our preliminary observations suggest that a prime redox regulation system, which may manipulate the intracellular H_2O_2 and O_2^- levels upon inhibition of CK2 activity, could be SODs. However, to date no phosphorylation site has been identified on the SOD protein sequence, and thus the activity of SODs in the cells is not known to be directly regulated by known intracellular kinases. More work is needed to elucidate if SODs are responsible for the redox regulation triggered upon CK2 inhibition.³⁰

These findings could potentially be important in light of recent interest for combination chemotherapy regimens of cancer treatment.³³ The use of kinase inhibitors as adjunct in combination with anticancer drugs could have implications as they could synergize the effects of these clinical drugs while reducing the side effects in cancer patients.

Acknowledgments

This work was supported by research grants from the National Medical Research Council and the Biomedical Research Council of Singapore to S.P.; the National Cancer Institute (CA-15062) and the Department of Veterans Affairs of USA to K.A. We also wish to thank Nafis M. Hanif for help in preparation of this manuscript.

References

1. Tawfic S, et al. Protein kinase CK2 signal in neoplasia. *Histol. Histopathol.* 2001; 16:573–582. [PubMed: 11332713]
2. Strasser A, O'Connor L, Dixit VM. Apoptosis signaling. *Annu. Rev. Biochem.* 2000; 69:217–245. [PubMed: 10966458]
3. Hengartner MO. The biochemistry of apoptosis. *Nature.* 2000; 407:770–776. [PubMed: 11048727]
4. Pervaiz S, Clement MV. A permissive apoptotic environment: function of a decrease in intracellular superoxide anion and cytosolic acidification. *Biochem. Biophys. Res. Commun.* 2002; 290:1145–1150. [PubMed: 11811982]
5. Pervaiz S, Clement MV. Hydrogen peroxide-induced apoptosis: oxidative or reductive stress? *Methods Enzymol.* 2002; 352:150–159. [PubMed: 12125343]
6. Clement MV, Pervaiz S. Intracellular superoxide and hydrogen peroxide concentrations: a critical balance that determines survival or death. *Redox. Rep.* 2001; 6:211–214. [PubMed: 11642710]
7. Clement MV, Pervaiz S. Reactive oxygen intermediates regulate cellular response to apoptotic stimuli: an hypothesis. *Free Radic. Res.* 1999; 30:247–252. [PubMed: 10230803]
8. Fridovich I. The biology of oxygen radicals. *Science.* 1978; 201:875–880. [PubMed: 210504]
9. Burdon RH, Gill V, Rice-Evans C. Cell proliferation and oxidative stress. *Free Radic. Res. Commun.* 1989; 7:149–159. [PubMed: 2511085]
10. Burdon RH. Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. *Free Radic. Biol. Med.* 1995; 18:775–794. [PubMed: 7750801]
11. Hampton MB, Orrenius S. Redox regulation of apoptotic cell death in the immune system. *Toxicol. Lett.* 1998; 102–103:355–358.
12. Cerutti PA. Prooxidant states and tumor promotion. *Science.* 1985; 227:375–381. [PubMed: 2981433]
13. Burdon RH, Gill V, Rice-Evans C. Oxidative stress and tumor cell proliferation. *Free Radic. Res. Commun.* 1990; 11:65–76. [PubMed: 1963620]
14. Pervaiz S, et al. Superoxide anion inhibits drug-induced tumor cell death. *FEBS Lett.* 1999; 459:343–348. [PubMed: 10526162]
15. Clement MV, Stamenkovic I. Superoxide anion is a natural inhibitor of FAS-mediated cell death. *EMBO J.* 1996; 15:216–225. [PubMed: 8617197]
16. Hirpara JL, Clement MV, Pervaiz S. Intracellular acidification triggered by mitochondrial-derived hydrogen peroxide is an effector mechanism for drug-induced apoptosis in tumor cells. *J. Biol. Chem.* 2001; 276:514–521. [PubMed: 11016925]

17. Hampton MB, Orrenius S. Dual regulation of caspase activity by hydrogen peroxide: implications for apoptosis. *FEBS Lett.* 1997; 414:552–556. [PubMed: 9323034]
18. Clement MV, Ponton A, Pervaiz S. Apoptosis induced by hydrogen peroxide is mediated by decreased superoxide anion concentration and reduction of intracellular milieu. *FEBS Lett.* 1998; 440:13–18. [PubMed: 9862415]
19. Kim JS, et al. Protein kinase CK2 α as an unfavorable prognostic marker and novel therapeutic target in acute myeloid leukemia. *Clin. Cancer Res.* 2007; 13:1019–1028. [PubMed: 17289898]
20. Litchfield DW. Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. *Biochem. J.* 2003; 369(Pt 1):1–15. [PubMed: 12396231]
21. Walrand S, et al. Flow cytometry study of polymorphonuclear neutrophil oxidative burst: a comparison of three fluorescent probes. *Clin. Chim. Acta.* 2003; 331:103–110. [PubMed: 12691870]
22. Guo C, et al. Apoptotic role of nuclear matrix-associated protein kinase CK2 in protection against drug-induced apoptosis in cancer cells. *J. Biol. Chem.* 2001; 276:5992–5999. [PubMed: 11069898]
23. Pagano MA, et al. 2-Dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole: a novel powerful and selective inhibitor of protein kinase CK2. *Biochem. Biophys. Res. Commun.* 2004; 321:1040–1044. [PubMed: 15358133]
24. Borowski P, et al. Halogenated benzimidazoles and benzotriazoles as inhibitors of the NT-Pase/helicase activities of hepatitis C and related viruses. *Eur. J. Biochem.* 2003; 270:1645–1653. [PubMed: 12694177]
25. Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. *Science.* 2004; 305:626–629. [PubMed: 15286356]
26. Boulares AH, et al. Role of poly(ADP-ribose) polymerase (PARP) cleavage in apoptosis. Caspase 3-resistant PARP mutant increases rates of apoptosis in transfected cells. *J. Biol. Chem.* 1999; 274:22932–22940. [PubMed: 10438458]
27. Wang G, et al. Downregulation of CK2 induces apoptosis in cancer cells—a potential approach to cancer therapy. *Mol. Cell Biochem.* 2005; 274:77–84. [PubMed: 16342410]
28. Wang G, Ahmad KA, Ahmed K. Role of protein kinase CK2 in the regulation of tumor necrosis factor-related apoptosis inducing ligand-induced apoptosis in prostate cancer cells. *Cancer Res.* 2006; 66:2242–2249. [PubMed: 16489027]
29. Ahmad KA, et al. Hydrogen peroxide-mediated cytosolic acidification is a signal for mitochondrial translocation of Bax during drug-induced apoptosis of tumor cells. *Cancer Res.* 2004; 64:7867–7878. [PubMed: 15520193]
30. Zelko IN, Mariani TJ, Folz J. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and ECSOD (SOD3) gene structures, evolution, and expression. *Free Radic. Biol. Med.* 2002; 33:337–349. [PubMed: 12126755]
31. Ahmad KA, et al. Resveratrol inhibits drug-induced apoptosis in human leukemia cells by creating an intracellular milieu nonpermissive for death execution. *Cancer Res.* 2004; 64:1452–1459. [PubMed: 14973069]
32. Vermes I, et al. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. *J. Immunol. Methods.* 1995; 184:39–51. [PubMed: 7622868]
33. Sarkar FH, Li Y. Using chemopreventive agents to enhance the efficacy of cancer therapy. *Cancer Res.* 2006; 66:3347–3350. [PubMed: 16585150]

Figure 1.

Inhibition of protein kinase CK2 (CK2) activity induces cell death. Cem cells were treated with various doses (3.125–100 $\mu\text{mol/L}$) of 4,5,6,7-tetrabromobenzotriazole (TBB) and (A) cell death was detected by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) at 12 h. (B) The inhibition of CK2 activity with TBB was measured with the synthetic p53 peptide (RRRDDSDDD) at 1 h. Inhibition of CK2 activity is reflected by a decrease in absorbance of the horseradish peroxidase-conjugated anti-phospho-p53 serine46 antibody at 540 nm.

Figure 2.

Apoptotic cell death is induced upon inhibition of CK2. Cem cells were treated with 25 $\mu\text{mol/L}$ or 50 $\mu\text{mol/L}$ TBB and apoptosis was detected by (A) the activation of executioner caspase 3 at 4 h and (B) propidium iodide (PI) staining of the sub-G1 DNA fragmentation populations at 12 h.

Figure 3. Inhibition of CK2 induces intracellular hydrogen peroxide (H_2O_2) production and decreases intracellular superoxide (O_2^-). Cem cells were treated with 25 $\mu\text{mol/L}$ or 50 $\mu\text{mol/L}$ TBB and (A) intracellular H_2O_2 production was detected by a right shift of the DCF fluorescence after 3 h of inhibitor treatment with or without preincubation with 5000 units of catalase for 1 h, while (B) the decrease in intracellular O_2^- was detected by the left shift of the hydroethidine (HE) fluorescence after 2 h of inhibitor treatment. Diethyldithiocarbamate (DDC) and diphenyleneiodonium chloride (DPI) were used as positive and negative controls, respectively. Flow cytometry data shown represent three independent experiments.

Figure 4.

Apoptosis induced by CK2 inhibition is dependent upon the intracellular H_2O_2 production. Cem cells were treated with 25 μ mol/L or 50 μ mol/L TBB with or without preincubation with 5000 units of catalase (CAT) for 1 h and apoptosis was detected by (A) PI staining of the sub-G1 DNA fragmentation populations after 12 h of inhibitor treatment and (B) externalization of Annexin V receptors, as observed by a right shift of the fluorescein isothiocyanate (FITC) Annexin V fluorescence after 4 h of inhibitor treatment. Flow cytometry data shown represent three independent experiments.

Figure 5.

Diagrammatic representation of redox regulation in inhibition of CK2-induced apoptosis. Inhibition of CK2 activity altered the redox status of the cells by inducing the production of intracellular H_2O_2 and decreasing the intracellular O_2^- , creating an intracellular milieu permissive for apoptotic death execution.