

Protein Kinase CK2 Is a Central Regulator of Topoisomerase I Hyperphosphorylation and Camptothecin Sensitivity in Cancer Cell Lines[†]

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ABSTRACT: Topoisomerase I (topo I) is required to unwind DNA during synthesis and provides the unique target for camptothecin-derived chemotherapeutic agents, including Irinotecan and Topotecan. While these agents are highly effective anticancer agents, some tumors do not respond due to intrinsic or acquired resistance, a process that remains poorly understood. Because of treatment toxicity, there is interest in identifying cellular factors that regulate tumor sensitivity and might serve as predictive biomarkers of therapy sensitivity. Here we identify the serine kinase, protein kinase CK2, as a central regulator of topo I hyperphosphorylation and activity and cellular sensitivity to camptothecin. In nine cancer cell lines and three normal tissue-derived cell lines we observe a consistent correlation between CK2 levels and camptothecin responsiveness. Two other topo I-targeted serine kinases, protein kinase C and cyclin-dependent kinase 1, do not show this correlation. Camptothecin-sensitive cancer cell lines display high CK2 activity, hyperphosphorylation of topo I, elevated topo I activity, and elevated phosphorylation-dependent complex formation between topo I and p14ARF, a topo I activator. Camptothecin-resistant cancer cell lines and normal cell lines display lower CK2 activity, lower topo I phosphorylation, lower topo I activity, and undetectable topo I/p14ARF complex formation. Experimental inhibition or activation of CK2 demonstrates that CK2 is necessary and sufficient for regulating these topo I properties and altering cellular responses to camptothecin. The results establish a cause and effect relationship between CK2 activity and camptothecin sensitivity and suggest that CK2, topo I phosphorylation, or topo I/p14ARF complex formation could provide biomarkers of therapy-responsive tumors.

Topoisomerase I (topo I)¹ catalyzes DNA unwinding during DNA synthesis and transcription (1, 2) and plays a central role in cancer as the unique cellular target for an increasingly important class of chemotherapeutic drugs derived from the plant alkaloid, camptothecin, that includes Irinotecan (Camptosar, CPT-11) and Topotecan (Hycamtin) (3). Although complete absence of topo I is lethal to mammalian cells, the level of topo I can be highly variable among tumor specimens and cell lines (4–8), and this can lead to variable cellular responses to camptothecin and related drugs (6). Low level expression of topo I in cultured cells can be selected by long-term exposure to camptothecin (9) and correlates with camptothecin resistance (reviewed in refs 10–12). In addition, it is also apparent that cancer cells have mechanisms to regulate topo I activity in the absence of changes in topo I protein expression (6, 13). These mechanisms have not been well delineated, although they may play an equal or greater role in the clinical response to therapy than do expression changes. A better understanding of how topo I activity is regulated is therefore critical not only to our understanding of the biology of this essential enzyme but also to the clinical application of topo I-targeted drugs.

There is considerable evidence that phosphorylation is critical to the regulation of topo I activity. Topo I purifies as a phosphoprotein, and its activity and ability to associate with DNA is inhibited by treatment with alkaline phosphatase (14–16). Topo I activity is stimulated *in vitro* by treatment with the serine kinases, protein kinase C (PKC) or protein kinase CK2 (CK2, formerly casein kinase II) (14, 16–20). Phosphorylation also correlates with increased topo I activity *in vivo* (6, 21, 22), where it occurs primarily on serine residues in most systems examined (15, 16, 20, 23–25). Specific *in vivo* serine phosphorylation sites have now been identified at positions 10, 21, 112, and 394, targeted by CK2 (serine 10), PKC (serine 21), and cyclin-dependent protein kinase-1 (cdk-1, serines 112 and 394) (22). Furthermore, topo I mutants lacking a serine site identified as a PKC target are less active when expressed ectopically in cells and when assayed *in vitro* following ectopic expression in cells (22).

The phosphorylation status of topo I correlates with cellular sensitivity to camptothecin. In OVCAR3 ovarian cancer cells, for example, the failure of ectopic overexpression of topo I to increase overall topo I activity or cellular sensitivity to camptothecin can be attributed to a reduced ability of that cell line to phosphorylate the enzyme (13). In sublines of murine L5178 lymphoma cells, cellular sensitivity to camptothecin has been linked to the phosphorylation status of topo I and to CK2 (26–30). We have previously found that two non-small cell lung cancer cell lines, H358 and H23, express similar levels of topo I protein but have high and low sensitivity to camptothecin, respectively, that correlates with high or low levels of topo I serine phosphorylation and topo I activity (6). Furthermore, the underphosphorylated and less active form of topo I in H23 cells can be activated by

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Abbreviations: topo I, topoisomerase I; CPT, camptothecin; PKC, protein kinase C; cdk1, cyclin-dependent kinase 1; CK2, protein kinase CK2; TBB, 4,5,6,7-tetrabromobenzotriazole; EDTA, ethylenediamine-tetraacetic acid; EGTA, ethylene glycol tetraacetic acid; DTT, dithiothreitol; SDS, sodium dodecyl sulfate; DSB, double strand break.

CK2 treatment *in vitro*, consistent with a possible role for CK2 in regulating cellular sensitivity to camptothecin (6). Phosphorylation of serine 21 by PKC has been found to promote increased sensitivity of topo I to camptothecin (22), consistent with a previous study showing that PKC enhances the sensitivity of the enzyme to camptothecin (16). Taken together, these observations suggest that one or more topo I serine phosphorylating activities could have a general role in a variety of cancers to regulate topo I activity *in vivo* in ways that affect the cellular response to camptothecin-related drugs. However, this possibility has not been fully explored, nor has the critical kinase been identified.

In this study we have carried out a more extensive correlative analysis across a panel of normal and cancer-derived cell lines of camptothecin sensitivity, topo I phosphorylation status and activity, and endogenous levels of three topo I phosphorylating enzyme activities. We find that CK2 is frequently upregulated in cancer cell lines relative to normal cell lines and that levels of CK2, unlike PKC and cdk-1, display consistent correlation with the appearance of hyperphosphorylated topo I and with increased cellular sensitivity to camptothecin. As in our earlier study (6), we find that hyperphosphorylation of topo I also correlates with its ability to form a complex with the p14 alternate reading frame (p14ARF) tumor suppressor, a protein overexpressed in many cancer cell lines and cancers that has previously been shown to bind to and activate topo I (31, 32). Furthermore, through experimental modulation of cellular CK2 activity, we demonstrate a functional relationship between CK2 overexpression, topo I hyperphosphorylation, and cellular sensitivity to camptothecin. These results identify CK2 as a frequent and central regulator of topo I properties and cellular sensitivity to camptothecin in cancer cell lines and provide a rationale to further investigate the clinical potential of CK2-mediated phosphorylation of topo I as a biomarker of tumor responsiveness to therapy.

MATERIALS AND METHODS

Cell Lines. The following cell lines were obtained from the American Type Culture Collection (Rockville, MD): the non small cell lung cancer cell lines H358 and H23, the prostate cancer cell lines PC-3, DU145, and LnCAP, the breast cancer cell line MDA-MB-435 [which expresses genes associated with both breast cancer and melanoma (33)], the colon cancer cell lines SW480 and HT29, the HET1A normal human epithelial cell line immortalized with SV40 T-antigen, and the BJ-1 normal human fibroblast cell line immortalized with human telomerase. The OC3 esophageal adenocarcinoma cell line was provided by Dr. Rebecca Fitzgerald, MRC Cancer Cell Unit, Hutchison-MRC Research Center, Cambridge. The GT41F cell line was established from a skin punch biopsy at the Sidney Kimmel Cancer Center, San Diego, CA. Cell lines were maintained at 37 °C in 10% CO₂ in DMEM supplemented with nonessential amino acids, pyruvate, L-glutamine, gentamycin, and 10% FBS.

Drugs and Drug Treatments. Camptothecin was purchased from Sigma, St. Louis, MO. A stock solution of 1 mM was prepared in DMSO. The CK2 inhibitor 4,5,6,7-tetrabromobenzotriazole (TBB) was purchased from Calbiochem/EMD, Gibbstown, NJ. A stock solution of 1 mM was prepared in DMSO. The CK2 activator 1-ethyl-4,5-dicarbamoylimidazole (34) was synthesized according to the published methods (35, 36). A 1 M stock solution was prepared in PBS. Cells were treated with camptothecin (5–80 nM) for 18 h. Cells were treated with TBB (10 μ M) for 1 h, following a modified protocol taken from ref 37. At this dose,

TBB has no effect on 3 day cell viability but reduced cellular CK2 activity, 3 days later, to about 25% of control. Cells were treated with 10 nM CK2 activator, 1-ethyl-4,5-dicarbamoylimidazole (34), without changing medium over the 3 day course of the assays. This was the lowest dose (assayed over the range of 5–100 nM) that could activate CK2 in H23 cells while having no effect on 3 day cell viability. Further details on the properties of the CK2 activator are provided in Supporting Information Figure S2.

Cell Viability Assays. Cell viability assays were carried out in 96-well plates, starting as 2000 cells per well. Following overnight attachment, triplicate wells of cells were treated with increasing doses of camptothecin (Sigma, St. Louis, MO) for 18 h. Viability was scored 3 days after the start of treatment by the MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium inner salt (Promega, Madison, WI)] bio-conversion assay as previously described (6). Under these conditions, control, untreated cells remained in exponential growth. In some assays, cells were first exposed for 1 h to TBB (10 μ M) and then to camptothecin for 18 h. In other assays, cells were exposed concurrently to the CK2 activator and camptothecin for 18 h and then to the CK2 activator alone for the remainder of the assay.

Serine Kinase Activity Assays. Protein kinase C (PKC), protein kinase CK2 (CK2), and cyclin-dependent kinase I (cdk1) assay kits were purchased from Upstate Biotechnology/Millipore (Temecula, CA) and used according to the instructions supplied in the kit. [γ -³³P]ATP, 3000 Ci/mmol, was purchased from MP Biomedicals (Solon, OH). Assays were performed for 10 min at 30 °C in a total reaction volume of 50 μ L using the respective peptide substrates supplied in each kit. PKC activity was assayed using 10 μ L (10 μ g) of cell lysate prepared by extraction of whole cells in a PKC extraction buffer described in ref 38: 50 mM HEPES, pH 7.5, 150 mM NaCl, 0.1% Tween 20, 1 mM EDTA, 2.5 mM EGTA, 10% glycerol, protease inhibitors (10 μ g/mL aprotinin, 10 μ g/mL leupeptin, 1 mM phenylmethanesulfonyl fluoride (PMSF), and phosphatase inhibitors (1 mM NaF, 0.1 mM Na₃VO₄, 10 mM β -glycerophosphate). For cdk1 assays, cdk1 protein was first immunoprecipitated from 50 μ g of cell lysate prepared in RIPA buffer [10 mM sodium phosphate, pH 7, 0.15 M NaCl, 0.1% SDS, 1% NP40, 1% sodium deoxycholate, 1 mM phenylmethanesulfonyl fluoride (PMSF), and complete protease inhibitors (Roche, Nutley, NJ)] followed by resuspension in 50 μ L of cdk1 assay buffer supplied in the kit. CK2 activity was assayed using 10 μ L (10 μ g) of high salt nuclear extracts, prepared as for the topo I assays (see below). Further details of reaction conditions for CK2 are provided in Supporting Information Figure S2. Purified cdk1 was purchased from Upstate Biotechnology/Millipore (Temecula, CA). Purified PKC was purchased from Sigma (St. Louis, MO). CK2 holoenzyme was purchased from Promega (Madison, WI). CK2 α 1 catalytic subunit was purchased from Active Motif (Carlsbad, CA).

CK2 siRNA Treatment. An siRNA oligonucleotide mixture specific for the α 1 and α 2 catalytic subunits of CK2 and a negative control siRNA scrambled sequence were purchased from Upstate/Millipore (Temecula, CA) and transfected into cells using Lipofectamine (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.

Antibodies. For detection of topo I in all experiments except the Western analysis in Figure 4A, we used a goat polyclonal anti-topo I recognizing a topo I C-terminal epitope (Santa Cruz Biotechnology, Santa Cruz, CA). In Figure 4A (lower panel) we used a mouse monoclonal antibody recognizing a topo I N-terminal

epitope (LifeSpan Biosciences, Seattle, WA). Other antibodies used were rabbit polyclonal anti-full-length p14ARF (Zymed Laboratories, South San Francisco, CA), mouse monoclonal anti-phosphoserine (Sigma, St. Louis, MO), mouse monoclonal anti-actin (Santa Cruz Biotechnology, Santa Cruz, CA), rabbit monoclonal anti-CK2 α subunit (C-terminus) antibody (Upstate/Millipore, Temecula, CA), rabbit polyclonal anti-protein kinase C (Sigma, St. Louis, MO), rabbit polyclonal anti-histone H2A.X (Bethyl Laboratories, Montgomery, TX), rabbit polyclonal anti- γ -H2A.X (Ser¹³⁹) (Novus Biologicals, Littleton, CO), and mouse monoclonal anti-cdk1 (Millipore, Temecula, CA). All primary antibodies were used at 1:100 for Western blots. Secondary antibodies for Western blots were goat anti-rabbit HRP, goat anti-mouse HRP, and donkey anti-goat HRP (all purchased from Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and were used at 1:1000.

Western Analyses. SDS-PAGE Western was carried out as previously described (39) using 60 μ g of protein. Protein concentrations were determined by using the fluorescence-based Quant-iT assay kit and Qubit fluorometer (Invitrogen, Carlsbad, CA). Lysates were prepared by one of two methods: For Figures 1–3, whole cell lysates were prepared by direct cell lysis on culture plates following a procedure adapted from ref 22. Briefly, culture plates of adherent cells were washed twice with cold PBS followed by addition of cold lysis buffer [1% Triton X-100, 400 mM NaCl, 50 mM HEPES, pH 7.5, 10% glycerol, 5 mM MgSO₄, 1 mM EDTA, complete protease and phosphatase inhibitors (Roche, Nutley, NJ)] directly to the plates. Following centrifugation, supernatants were concentrated by acetone precipitation and resuspended in electrophoresis sample buffer. For Figure 4, we used our previously described procedure (6), adapted from ref 40, involving first the preparation of nuclei in 10 mM HEPES (pH 7.5), 100 mM NaCl, 300 mM sucrose, 3 mM MgCl₂, 1 mM EDTA, 1 mM DTT, 0.5% Triton X-100, 1 mM PMSF, and complete protease and phosphatase inhibitors (Roche, Nutley, NJ), followed by a 15 min incubation with DNase I at 37 °C to solubilize nuclear proteins. Blots were developed using Amersham/GE Healthcare (Buckinghamshire, U.K.) ECL reagent, and in some cases band intensities were quantitated digitally using an Alpha imager.

Coimmunoprecipitation (IP)/Western Blot (Immunoblot). For detecting phosphorylated topo I, or p14ARF-topo I complex formation, whole cell lysates (Figures 1–3) or nuclear extracts (Figure 4) were prepared as described above (see Western Analyses). Whole cell lysates were adjusted to 150 mM NaCl to allow for complex formation. Immunoprecipitations were carried out with an anti-topo I antibody (recognizing topo I C-terminus), according to our previously described procedure (41), followed by SDS-PAGE/Western (immunoblot) analysis for p14ARF or for phosphoserine in the immunoprecipitated material.

Topo I Enzymatic Assays. High-salt nuclear extracts (20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, 10% glycerol, and complete protease inhibitors) were prepared as described (42) and assayed using the Topo I assay kit (TopoGEN, Port Orange, FL), according to the manufacturer's instructions. Briefly, 0.125 μ g of supercoiled plasmid DNA was incubated with 0.75 μ g of nuclear extract (in some assays of camptothecin-resistant cell lines, 7.5 μ g was used, as indicated) for 30 min at 37 °C. The reaction was stopped by adding stop loading dye supplied in the kit and electrophoresed on a 1% agarose/TAE (10 mM Tris-acetate/1 mM EDTA) gel, and the reaction products were visualized by ethidium bromide staining (0.5 μ g/mL). In some

cases band intensities were quantitated digitally using an Alpha imager.

Cleavage Complexes. H358 or H23 cells (2×10^5) (before or 3 days after treatments to alter CK2 levels) were treated with 1.2 μ Ci of [³H]thymidine (Perkin-Elmer, Waltham, MA) overnight, followed by a 1 h incubation in nonradioactive medium and a 25 min incubation in 0.08 μ M camptothecin to cross-link topo I to DNA. Cells were washed in cold PBS, and cross-linked topo I/DNA complexes were recovered using the K⁺/SDS method (42), resuspended in water, and subjected to liquid scintillation counting.

Camptothecin-Mediated DNA Damage. H358 or H23 cells (2×10^5) (before or 3 days after treatments to alter CK2 levels) were exposed to 10 μ M camptothecin for 1 h to induce double strand DNA breaks, and histones were acid-extracted from whole cells in 0.2 M H₂SO₄ as previously described (43). Extracts were then subjected to Western analysis of the histone variant, γ -H2A.X, the phosphorylated form of the histone variant H2A.X, which serves as a marker for DNA double strand breaks. Unphosphorylated H2A.X served as a control.

RESULTS

Cellular Responses to Camptothecin Correlate with Topo I Serine Hyperphosphorylation and Activity. We have screened nine cancer cell lines and three normal cell lines for sensitivity to increasing concentrations of camptothecin. The results of the 3 day, 96-well viability assays, where cell viability is expressed as a percentage of control (untreated) cell viability, are shown in Figure 1A. Six of the cancer cell lines (PC3, DU145, and LnCAP prostate cancer cells, H358 lung cancer cells, MDA-MB-435 breast cancer cells, and OC3 esophageal cancer cells) are camptothecin-sensitive and retain only 15–25% viability relative to untreated cells at the highest dose of camptothecin (Figure 1A, left panel). All of the normal cell lines (GT41F human skin fibroblasts, BJ-1 immortalized human skin fibroblasts, and HET1A immortalized human epithelial cells) and three of the cancer cell lines (H23 lung cancer cells and HT29 and SW480 colon cancer cells) are relatively resistant to camptothecin, retaining over 50–70% viability relative to untreated cells at the highest dose of camptothecin (Figure 1A, right panel). The camptothecin resistance of the H23, HT29, and SW480 cell lines is consistent with previously published studies (6, 44).

All of the cell lines proliferate with doubling times of about 24 h. In the absence of treatment, 3 day cell viabilities of representative camptothecin-sensitive and -insensitive cell lines (H358, H23, BJ-1) do not vary by more than 10% (Supporting Information Figure S1) indicating that the observed differences in camptothecin sensitivities are independent of growth rates. Furthermore, all of the cancer cell lines, irrespective of their sensitivity to camptothecin, express topo I protein at roughly equivalent levels as normal cells, as shown by the topo I immunoprecipitation (IP)/Western analysis of nuclear extracts in Figure 1B, top row. However, we observe marked differences among the cell lines with respect to topo I serine phosphorylation that correlate with cellular responses to camptothecin. When nuclear extracts are immunoprecipitated with an anti-topo I antibody followed by Western analysis of phosphoserine in the immunoprecipitated material, we observe that the three camptothecin-resistant cancer cell lines (H23, HT29, SW40) and the three normal cell lines (GT41F, BJ-1, HET1A) have only some 20–30% of the serine phosphorylated topo I as do the six camptothecin-sensitive cell

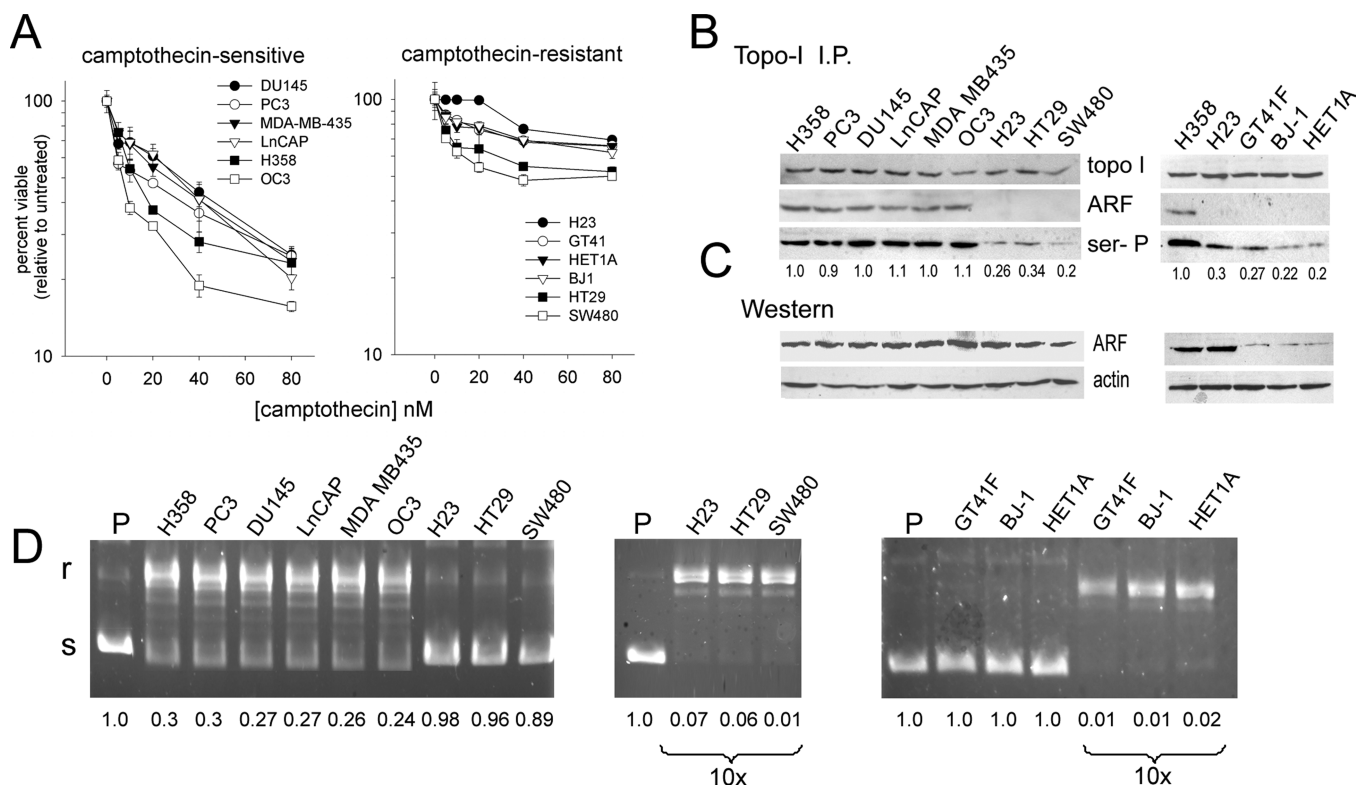


FIGURE 1: Cellular responses to camptothecin and their relationship to topo I serine phosphorylation and activity. (A) 96-well viability assays of camptothecin-sensitive cell lines (left panel) and camptothecin-resistant cell lines (right panel) carried out with increasing doses of camptothecin (18 h incubation). Cell viability, expressed as a percent of untreated cell viability) was measured 3 days post start of camptothecin treatment. Each point represents the average of triplicate wells, with standard deviations shown. (B) Immunoprecipitations of cell extracts with anti-topo I antibody, followed by Western analysis of topo I (top row), p14ARF (middle row), or phosphoserine (bottom row) in the immunoprecipitated material. Numbers below lanes represent digital analyses of phosphoserine band intensities relative to H358. (C) Western analysis of p14ARF (top row) or actin (bottom row) in the same series of cell lines as in part B. (D) Enzymatic assays of topoisomerase I-mediated conversion of supercoiled plasmid (s) to relaxed form (r) by nuclear extracts of the indicated cell lines using 0.75 μ g of lysates per reaction (left panel) or 7.5 μ g of lysate protein per reaction (10 \times , middle panel). Right panel shows results for the three normal cell lines, GT41F, BJ-1, and HET1A, at 0.75 μ g of lysates per reaction or 7.5 μ g of lysate protein per reaction (10 \times). P = plasmid only. Digital quantitations of band intensities relative to plasmid only are shown below each lane.

lines (Figure 1B, bottom row, digital quantitation of band intensities relative to H358 shown below lanes).

To determine how topo I serine phosphorylation in our panel of cell lines correlates with endogenous levels of topo I activity, we carried out in vitro topo I activity assays on nuclear extracts from these cell lines. The assays measure the ability of topo I to convert a supercoiled plasmid "s" to a slower migrating relaxed form "r" as revealed by agarose gel electrophoresis and ethidium bromide staining. Using quantities of nuclear extracts (0.75 μ g/reaction) that convert 70% or more of the supercoiled plasmid form to relaxed form in the case of the six camptothecin-sensitive cell lines, we observe virtually no conversion of supercoiled form in the case of the three camptothecin-resistant cell lines, H23, HT29, and SW480 (Figure 1D, left panel), although 10-fold higher amounts of these latter three extracts (7.5 μ g/reaction) are able to carry out efficient conversion (Figure 1D, middle panel). These results are consistent with our previous study showing that topo I activity in H358 cells is some 10-fold higher than in H23 cells (6). Nuclear extracts from normal cells (GT41F, BJ-1, and HET1A) display low topo I activity profiles similar to profiles of the camptothecin-resistant cancer cell lines at both lower and 10-fold higher amounts of extract (Figure 1D, right panel). The numbers below each lane represent the digital quantitation of supercoiled band intensities, expressed as a fraction of the intensity of control, untreated supercoiled plasmid "P".

Topo I is known to participate in a variety of protein-protein interactions that can affect its activity, among them an interaction with the topo I activator, p14ARF (32). We have previously shown that the p14ARF/topo I complex formation is topo I serine phosphorylation-dependent and is observed in H358 cells, with hyperphosphorylated topo I, but not in H23 cells (6). As a further demonstration for increased topo I phosphorylation and activity in camptothecin-sensitive cell lines, we carried out a Western analysis of p14ARF in the anti-topo immunoprecipitated material from the same nuclear extracts used for phosphoserine analysis in Figure 1B, bottom panel. As shown in Figure 1B, middle panel, the presence of the topo I/p14ARF complex parallels the presence of serine phosphorylated topo I across the panel of cell lines, as well as the pattern of topo I activity in Figure 1D. All of the cancer cell lines we used express similar levels of endogenous p14ARF, as shown by a direct Western analysis of nuclear extracts in Figure 1C (top panel), while barely detectable levels of p14ARF are expressed in GT41F, BJ-1, and HET1A normal cells (Figure 1C), consistent with other reports of low p14ARF expression in normal tissues (45). Thus, the camptothecin responses of this panel of cancer cell lines and normal cells correlate with topo I phosphorylation, topo I activity, and topo I/p14ARF complex formation.

Elevated CK2, but Not PKC or cdk1, Correlates with Cellular Sensitivity to Camptothecin. We assayed extracts of our panel of cancer cell lines for endogenous PKC, cdk-1, and

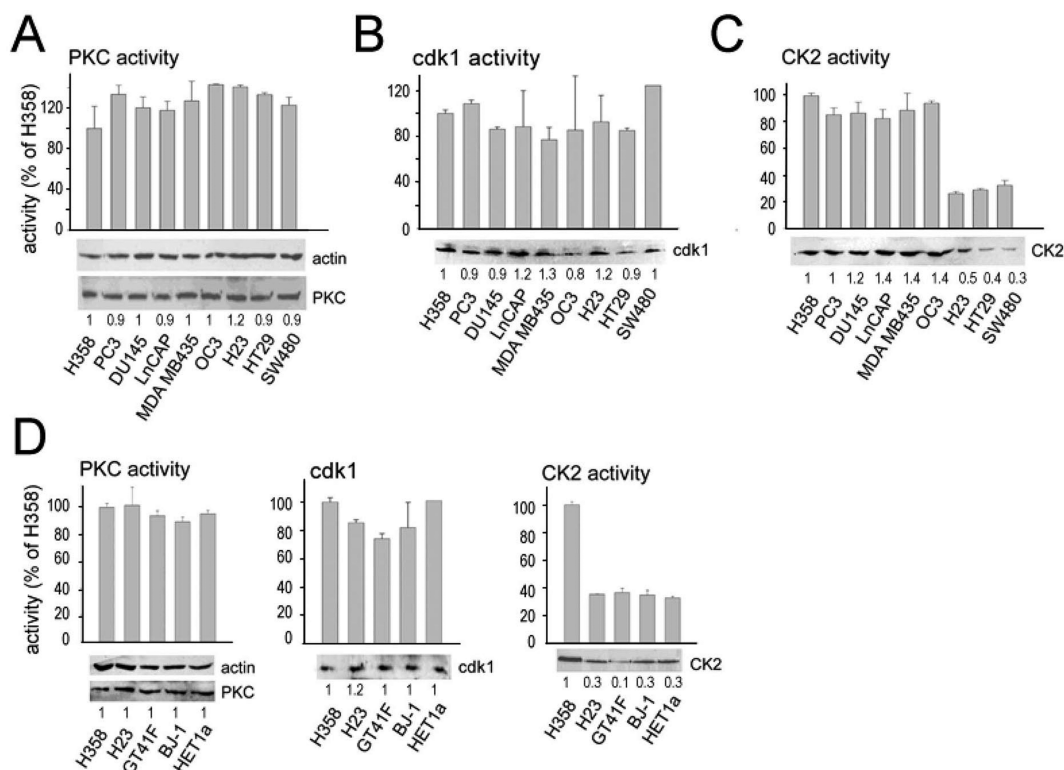


FIGURE 2: Correlation of elevated CK2, but not PKC or cdk1, with cellular sensitivity to camptothecin. Extracts were prepared from the indicated cancer cell lines and assayed for enzymatic activities (bar graphs) and protein levels (Western blots) of (A) PKC, (B) cdk1, or (C) CK2. Actin control in part A, top Western panel, is the same for all Westerns. (D) Cell lysates of H358 and H23 cells and normal cells GT41F, BJ-1, and HET1A were assayed for PKC, cdk1, and CK2 enzymatic activities (bar graphs) and protein levels (Western blots) as in parts A–C. Actin control is the same for all. Assays were repeated on one additional occasion with similar results. Numbers below Western blots indicate digital reading of band intensities of PKC, cdk1, or CK2 Western blots, relative to H358.

CK2 protein levels and enzymatic activity to determine possible correlations between levels of these enzymes and cellular responses to camptothecin. As shown by the bar graphs and Western blots in Figure 2A, the levels of PKC enzymatic activity and protein, respectively, are roughly equivalent in all the cell lines and do not correlate with cellular responsiveness to camptothecin. Similarly, as shown by the bar graphs and Western blots in Figure 2B, the levels of cdk1 enzymatic activity and protein, respectively, are roughly equivalent in all the cell lines and do not correlate with cellular responses to camptothecin. In contrast, levels of CK2 activity (Figure 2C, bar graph) are reduced in the camptothecin-resistant cancer cell lines to some 20–30% of that observed in camptothecin-sensitive H358 cells, and this corresponds to a parallel drop in CK2 protein to about one-half to one-third the level observed in H358 cells (Figure 2C, Western blot). Levels of CK2 enzymatic activity and protein in the camptothecin-sensitive cell lines are roughly equivalent to higher levels observed in H358 cells (Figure 2C, bar graph and Western blot). Actin controls, applicable to panels A–C in Figure 2, are shown in panel A. Digital quantitations of band intensities relative to H358 are indicated below the Western blots.

The three normal cell lines, GT41F, BJ-1, and HET1A, display PKC and cdk-1 enzymatic activity and protein levels that are similar to levels observed in the cancer cell lines H23 and H358, with no correlation with the degree of camptothecin sensitivity of the cells (Figure 2D, left and middle panels, bar graph and Western blot). In contrast, levels of CK2 enzymatic activity and protein in the normal cell lines are similar to the lower levels found in camptothecin-resistant H23 cells and correlate with the camptothecin resistance of these normal cell lines (Figure 2D,

right panel, bar graph and Western blot). The absolute level of CK2 activity in H23 cells, in picomoles of phosphate incorporated per minute per microgram of protein as calculated based on the specific activity of the ATP, is about $9.2 \text{ pmol min}^{-1} (\mu\text{g of protein})^{-1}$, a level comparable to a previously reported level of $8 \text{ pmol min}^{-1} (\mu\text{g of protein})^{-1}$ in human dermal fibroblasts (46).

Levels of CK2 enzymatic activity in the normal cell lines are about one-third the level observed in camptothecin-sensitive H358 cells (Figure 2D, right panel, bar graph). Similarly, levels of CK2 protein in normal cell lines are about 10–30% of the level observed in H358 cells (Figure 2D, right panel, Western blot). Actin controls, applicable to all panels in Figure 2D, are shown below the PKC bar graph. Digital quantitations of band intensities relative to H358 are indicated below the Western blots. Taken together, these results suggest a functional relationship between CK2, topo I phosphorylation, and cellular sensitivity to camptothecin.

CK2 Is Necessary and Sufficient To Maintain Topo I Phosphorylation and Phosphorylation-Dependent Properties of Topo I in Vivo. To determine whether cellular levels of CK2 have functional significance with regard to topo I properties, we examined how experimental modulation of CK2 activity in two representative cell lines, namely, camptothecin-sensitive H358 cells and camptothecin-resistant H23 cells, affects topo I phosphorylation, topo I complex formation with p14ARF, topo I activity, and camptothecin-induced DNA damage.

We downregulated CK2 activity in H358 cells either by treating them with the highly selective CK2 inhibitor TBB (4,5,6,7-tetrabromobenzotriazole), which has minimal effects on PKC or cdk1 (47), or by downregulating CK2 expression using an siRNA

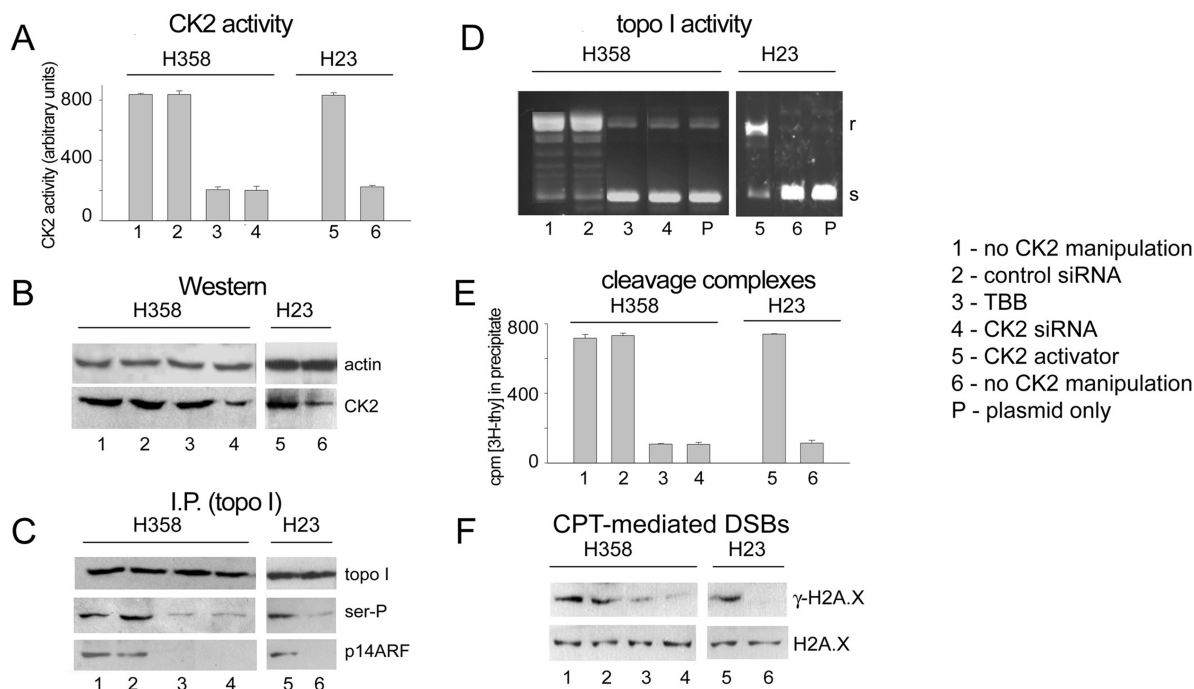


FIGURE 3: Experimental modulation of CK2 activity and consequences for topo I properties. (A) Assays of CK2 activity in nuclear extracts prepared from H358 cells (lanes 1–4) and H23 cells (lanes 5 and 6) 3 days after the following treatments: 1, no CK2 manipulation (H358); 2, transfection with control, scrambled siRNA (H358); 3, treatment 1 h with 10 μ M TBB (H358); 4, transfection with CK2 siRNA (H358); 5, treatment (for duration of assay) with CK2 activator (H23); 6, no CK2 manipulation (H23). Each assay represents the average of duplicate samples, with standard deviations shown. (B) Western analysis of CK2 or actin (control) protein in lysates of H358 or H23 cells 3 days after treatments in part A. (C) Immunoprecipitations with anti-topo I of cell lysates of H358 or H23 cells 3 days after treatments in part A, followed by Western analysis of topo I (top row), phosphoserine (middle row), or p14ARF (bottom row) in the immunoprecipitated material. (D) Enzymatic assays of topoisomerase I-mediated conversion of supercoiled plasmid (s) to relaxed form (r) by nuclear extracts prepared from H358 or H23 cells 3 days after treatments in part A. P = supercoiled plasmid only. (E) K^+ /SDS precipitation of covalent, camptothecin-stabilized cleavage complexes between topo I and cellular DNA of H358 or H23 cells. Cells were treated as in part A, followed 3 days later by overnight labeling with [3 H]thymidine, followed by a 25 min incubation in 0.08 μ M camptothecin. (F) Western analysis of γ -H2A.X and total H2A.X (control) in acid extracts of H358 and H23 cells 3 days after the treatments in part A.

mixture with specificity for the $\alpha 1$ and $\alpha 2$ isoforms of the CK2 catalytic subunit. Conversely, we upregulated CK2 activity in H23 cells by treating them with a CK2 activator, 1-ethyl-4,5-dicarbamoylimidazole (34). The activator has a specific effect on purified CK2 activity, stimulating the activity of purified CK2 holoenzyme or the $\alpha 1$ catalytic subunit of CK2 some 6-fold *in vitro* when used at the concentration used to treat cells (10 nM), while having no effect on either purified PKC and cdk1 activity or on endogenous PKC or cdk1 activity in treated H23 cells (Supporting Information Figure S2). As shown by the bar graph in Figure 3A, TBB or CK2 siRNA treatment of H358 cells reduces CK2 activity 3 days later to some 25% of levels in untreated cells (Figure 3A, bars 3 and 4). A control scrambled sequence siRNA has no effect (Figure 3A, bar 2). CK2 activator treatment of H23 cells results in a 4-fold increase in CK2 activity relative to untreated H23 cells (Figure 3A, bar 5). A similar treatment of H358 cells results in only a 10% increase in CK2 activity (bar not shown), suggesting that topo I in untreated H358 is nearly maximally phosphorylated at potential CK2-targeted sites.

We carried out a Western analysis of CK2 protein (α subunit) in lysates of H358 and H23 cells 3 days following the start of various treatments described in Figure 3A. As shown in the Western blot in Figure 3B, TBB treatment of H358 cells has no effect on CK2 protein levels, as expected (Figure 3B, lane 3), but CK2 siRNA treatment of H358 cells reduces CK2 protein levels to about 47% of control levels (Figure 3B, lane 4, as determined by digital analyses of band intensities). CK2 activator treatment

of H23 cells enhances the accumulation of CK2 protein about 2-fold (Figure 3B, lane 5, as determined by digital analyses of band intensities) but not CK2 α transcription (Supporting Information Figure S3). Thus the increased activity of CK2 observed in CK2 activator-treated H23 cells appears to involve both direct activation of the enzyme and increased enzyme accumulation, possibly through increased translation or increased protein stability.

We then examined levels of total topo I protein, topo I serine phosphorylation, and topo I complex formation with p14ARF in H358 or H23 cells, 3 days following the start of various treatments described in Figure 3A, by carrying out anti-topo I immunoprecipitations of nuclear extracts of treated cells, followed by Western analyses of topo I, phosphoserine, or p14ARF in the immunoprecipitated material (Figure 3C). We found that total topo I protein levels are not affected by these treatments and remain similar in H358 and H23 cells (Figure 3C, top panel), while the level of topo I phosphorylation and complex formation with p14ARF parallels the pattern of CK2 activity (Figure 3C, middle and lower panels, respectively, compared to Figure 3A). TBB or CK2-siRNA treatment of H358 cells reduces topo I serine phosphorylation to about 10% that of untreated H358 cells (Figure 3C, middle panel, lanes 3 and 4, as determined by digital quantitation of band intensities), indicating that the majority of topo I hyperphosphorylation is under CK2 control in these cells. Conversely, CK2 activator treatment of H23 cells increases topo I phosphorylation by some 4.7-fold relative to untreated H23 cells (Figure 3C, middle panel, lane 5, as determined by digital quantitation of band intensities), making it

roughly equivalent to endogenous topo I phosphorylation levels in untreated H358 cells. Finally, a Western analysis of p14ARF in the topo I-immunoprecipitated material (Figure 3C, lower panel) shows that p14ARF/topo I complex formation occurs only in the presence of hyperphosphorylated topo I in both untreated H358 and CK2 activator-treated H23 cells (Figure 3C, lower panel, lanes 1, 2, and 5). The results indicate that CK2-mediated phosphorylation has functional significance *in vivo* in whole cells, consistent with our previous results *in vitro* (6).

We confirmed that the changes in topo I phosphorylation status correspond to the predicted changes in topo I activity by assaying nuclear extracts of H358 and H23 cells, 3 days following the start of various treatments described in Figure 3A, for their ability to convert a supercoiled plasmid “s” to a relaxed form “r”. Figure 3D shows an ethidium bromide stained agarose gel following electrophoresis of the reaction products obtained from these assays. Under conditions where untreated H358 cell nuclear extract converts virtually all supercoiled plasmid form to relaxed form (Figure 3D, lane 1, 0.75 μ g of lysate per reaction), essentially none is converted using nuclear extracts from H358 cells treated with TBB or CK2 siRNA (Figure 3D, lanes 3 and 4), indicating that topo I activity is effectively inhibited *in vivo* by treatments that inhibit CK2 activity. Conversely, under conditions where nuclear extract from untreated H23 cells is essentially inactive (Figure 3D, lane 6, 0.75 μ g of lysate protein per reaction), nuclear extract from CK2 activator-treated H23 cells converts virtually all of the supercoiled form to relaxed form (Figure 3D lane 5), indicating that CK2 activation is sufficient to activate topo I activity to levels observed in H358 cells. Taken together, these results indicate that CK2 is necessary and sufficient to maintain topo I activity and function in these cancer cells.

The activation and suppression of topo I activity is predicted to produce a corresponding increase and decrease in camptothecin-induced DNA damage. Human topo I acts by introducing a single strand break in the DNA double helix via an intermediate covalent complex between the enzyme and DNA termed a “cleavable complex”, in which an active tyrosyl residue at position 723 in the C-terminal domain of topo I becomes linked to the 3'-end of the DNA break, leaving a 5'-OH on the other side of the break. The passage of the noncleaved strand unwinds the DNA by one linkage number and is followed by a resealing of the single strand break and release of the enzyme (see ref 1 for a review). Camptothecin and related topo I inhibitors bind at the enzyme–DNA interface and trap cleavage complexes so that DNA unwinding, resealing, and enzyme release are prevented (48–54). The single strand break can become a lethal double strand break upon passage of the replication fork (55). This mechanism, which converts topoisomerase I into a cellular poison, has been proposed to account for the cytotoxicity of camptothecin (56) and explains why low levels of topoisomerase I, by limiting the frequency of cleavage complex formation, favor cell survival in the presence of camptothecin.

To determine levels of covalent cleavage complex formation, cells treated as described in Figure 3A were DNA-labeled 3 days later by overnight incubation in [³H]thymidine, followed by exposure to camptothecin to stabilize cleavage complexes and K⁺/SDS precipitation of covalent topo I–DNA cleavage complexes as described in Materials and Methods. Under these conditions of precipitation, only DNA covalently linked to topo I will coprecipitate with it. As shown by the bar graph of coprecipitated [³H]thymidine-labeled DNA in Figure 3E, camptothecin-stabilized cleavage complexes are some 5–7-fold more frequent in cells

expressing the highest levels of CK2 and phosphorylated topo I (Figure 3E, bars 1, 2, and 5), indicating that more topo I molecules become associated with cellular DNA under these conditions.

Finally, since camptothecin treatment ultimately leads to the production of double strand DNA breaks (DSBs) in growing cells, we examined how the various treatments in Figure 3A affect camptothecin-mediated induction of the phosphorylated form of the histone variant, H2A.X (denoted γ -H2A.X), which accumulates at sites of DSBs (57). Total H2A.X served as a control. Camptothecin exposure was for 1 h, initiated 3 days after treatment of cells as described in Figure 3A. As shown in Figure 3F, γ -H2A.X, and hence DNA double strand breaks, accumulates in camptothecin-treated cells expressing high levels of CK2 and phosphorylated topo I (Figure 3F, lanes 1, 2, and 5), confirming that the increased cellular sensitivity to camptothecin correlates with increased DNA damage.

Topo I Hyperphosphorylation Involves Serine Site(s) Outside of the N-Terminal Domain of Topo I. A CK2-mediated phosphorylation site has been recently identified at serine 10 in the N-terminal region of topo I (22). To determine whether the hyperphosphorylation of topo I observed in H358 cells or CK2 activator-treated H23 cells occurs in the N-terminal domain of the protein or in other regions of the protein, we took advantage of the fact that two molecular mass species of topo I are formed when extracts are prepared by DNase I digestion of nuclei (see Materials and Methods), namely, the 90 kDa full-length protein (765 amino acid residues) and a 67 kDa truncated protein lacking about 200 amino acid residues of the N-terminal region, arising most likely through proteolytic cleavage (58). The cleavage event is likely to be favored during the 15 min DNase I digestion step at 37 °C. Both bands can be visualized by SDS–PAGE/Western using a C-terminal-specific antibody to topo I, while only the 90 kDa band is recognized by an N-terminal-specific antibody. As expected, we find that the C-terminal-specific antibody recognizes both species of topo I in DNase I-solubilized nuclear extracts from H23 and H358 cell nuclei and that CK2 activator treatment of H23 cells or TBB treatment of H358 cells (treatments as described in Figure 3A) have no effect on the levels of these two species (Figure 4A, top panel). Also as expected, we find that the N-terminal antibody recognizes only one band corresponding to the full-length species (Figure 4A, bottom panel). A topo I immunoprecipitation with the C-terminal-specific antibody, followed by Western analysis of phosphoserine in the immunoprecipitated material (Figure 4B), reveals phosphorylation in both the 90 and 67 kDa bands of untreated H358 cells (Figure 4B, lane 2). Phosphorylation of both bands is inhibited by treatment of H358 cells with the CK2 inhibitor, TBB (Figure 4B, lane 1). Conversely, untreated H23 cells show low levels of phosphorylation in both bands (Figure 4B, lane 4), while treatment with the CK2 activator results in enhanced phosphorylation of both bands (Figure 4B, lane 3). It therefore appears that the abnormal hyperphosphorylation of topo I that results from high levels of CK2 activity involves at least one CK2-targeted site outside of the N-terminal region, in addition to possible sites within the N-terminal region.

Experimental Modulation of CK2 Activity Alters Cellular Responses to Camptothecin. We then examined how the camptothecin responses of two representative cancer cell lines, H358 and H23, are affected by TBB or CK2 siRNA pretreatment to inhibit CK2 or by the CK2 activator to activate CK2, respectively (treatments as described in Figure 3A). Camptothecin treatments were initiated immediately after siRNA transfection

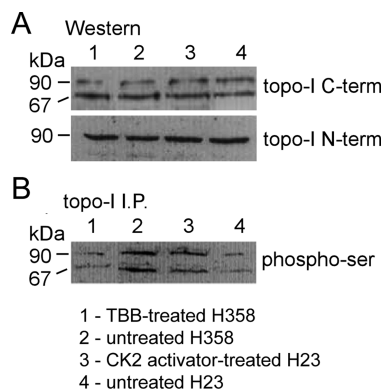


FIGURE 4: Location of topo I hyperphosphorylation outside of the N-terminal region. (A) Western analysis of DNase I-generated nuclear extracts prepared from TBB-treated H358 cells (lane 1), untreated H358 cells (lane 2), CK2 activator treated H23 cells (lane 3), or untreated H23 cells (lane 4) using a topo I C-terminal-specific antibody (top panel) or a topo I N-terminal-specific antibody (lower panel). (B) Topo I immunoprecipitation (C-terminal-specific antibody) followed by phosphoserine Western of the same samples as in part A.

or TBB treatment or concurrently with CK2 activator treatment, as described in Materials and Methods, and cell viabilities were determined 3 days post start of camptothecin treatment. As shown by the viability assay in Figure 5A, H358 cells without pretreatment are sensitive to camptothecin but become more resistant following TBB or siRNA treatment to inhibit CK2 activity. Conversely, as shown by the viability assay in Figure 5B, H23 cells grown without CK2 activator are resistant to camptothecin but become more sensitive during exposure to the CK2 activator. H358 cells are not further sensitized to camptothecin by treatment with the CK2 activator (data not shown), consistent with observations that the activator had minimal effect on H358 topo I phosphorylation. Taken together, the results argue that elevated CK2 enzymatic activity and protein in certain cancer cells, resulting in abnormal hyperphosphorylation of topo I, has a direct effect on the cellular response to topo I-targeted drugs, such as camptothecin, and is a hallmark of camptothecin-sensitive cancer cells.

DISCUSSION

This study demonstrates a cause and effect relationship in human cancer cell lines between high CK2 activity and increased cellular sensitivity to camptothecin, a topo I-targeted drug. The results establish CK2 as a physiological regulator of cancer cell topo I in ways that enhance camptothecin-mediated cleavage complex formation and cellular vulnerability to camptothecin-related drugs. Among the three serine kinases known to phosphorylate topo I in vivo, namely, PKC, cdk1, and CK2 (22), only CK2 shows this relationship, suggesting that CK2 plays a unique role in the camptothecin response of cancer cells. Camptothecin sensitivity correlates with abnormally elevated levels of phosphorylation of topo I compared to normal cell lines, with abnormally elevated topo I activity and with the presence of the serine phosphorylation-dependent complex between topo I and p14ARF, a topo I activator that is overexpressed in certain cancers (59–62). In contrast, camptothecin-resistant cancer cell lines resemble normal cell lines with regard to their lower level of CK2, a lower level of serine phosphorylated topo I, a lower level of topo I activity, and undetectable levels of topo I-p14ARF complexes. Importantly, we observe no differences among the cell lines with

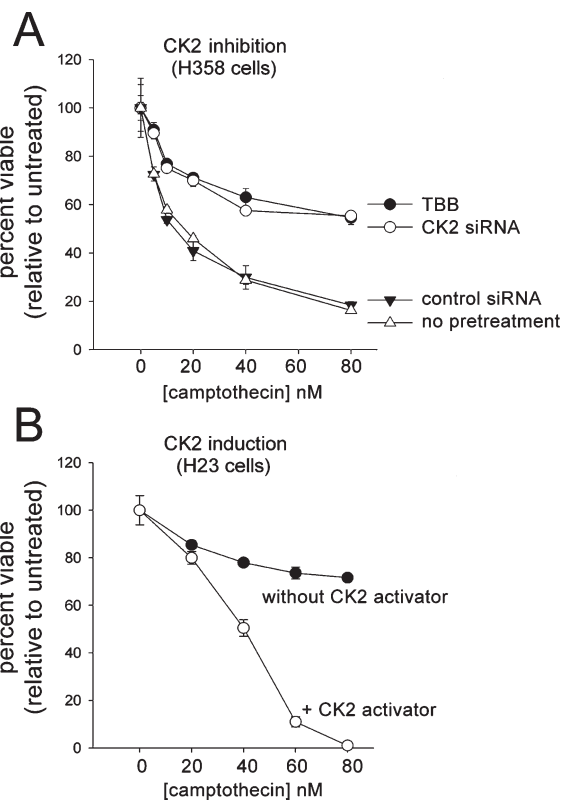


FIGURE 5: Cellular responses to experimental CK2 modulation. (A) 96-well viability assay of camptothecin-sensitive H358 lung cancer cells pretreated with TBB (10 μ M, 1 h, ●) or transfected with CK2 siRNA (○) or with control, scrambled sequence siRNA (▼), followed immediately by treatment with increasing doses of camptothecin for 18 h. Control cells (△) received no pretreatment. (B) 96-well viability assay of camptothecin-resistant H23 cells treated for 18 h with increasing doses of camptothecin either without the CK2 activator, 1-ethyl-4,5-dicarbamoylimidazole (●), or in the presence of CK2 activator for the duration of the assay (○). Cell viability, expressed as a percent of untreated cell viability, was measured 3 days post start of camptothecin treatment. Each point represents the average of triplicate wells, with standard deviations shown.

respect to overall serine phosphatase activity levels (Supporting Information Figure S4), further supporting a role for increased kinase activity rather than decreased phosphatase activity in mediating topo I hyperphosphorylation. The elevated levels of CK2, topo I phosphorylation, topo I activity, and topo I complex formation with p14ARF therefore appear to reflect an abnormal state associated with certain forms of cellular transformation.

Based on our data in Figure 4, we conclude that the hyperphosphorylation event in camptothecin-sensitive cancer cell lines must involve at least one CK2-targeted site outside of the N-terminal domain of topo I, where a CK2-targeted site at serine 10 has previously been identified (22). Four domains of topo I have been defined through a combination of approaches, including sequence alignments, analysis of proteolytic fragments, and crystallography (see ref 1 for a review and references therein). These domains include a poorly conserved N-terminal domain, a highly conserved DNA-binding core domain containing most of the catalytic residues, a poorly conserved linker domain, and a conserved C-terminal domain that contains the active site tyrosine. The additional CK2-targeted phosphorylation site(s) suggested by our data could therefore occur within the core or C-terminal domains so as to enhance the DNA binding or catalytic activity of topo I, thereby promoting an increased cellular sensitivity to camptothecin.

The previously identified CK2-targeted site at serine 10, together with a PKC-targeted site at serine 21 and two cdk-1-targeted sites at serines 112 and 394, are preferentially phosphorylated in mitotic cells, suggesting a possible role for these sites during mitosis (22). Of these four sites, only the PKC-targeted site at serine 21 has been linked to increased topo I activity (22). It is noteworthy that we find that the human cancer cell lines used to identify these serine phosphorylation sites, A549 human lung cancer cells and K562 human leukemia cells, display overall levels of topo I phosphorylation, similar to the lower levels we report here in the normal cell line, BJ-1 (Supporting Information Figure S5). Because no CK2-targeted site outside of the N-terminal domain has been reported in topo I from A549 or K562 cells and because the cdk-1-targeted site at serine 394 (in the central core domain) does not affect topo I activity (22), we speculate that the hyperphosphorylation of topo I in camptothecin-sensitive cancer cell lines is an aberrant event, involving either a novel site(s) targeted only under conditions of high CK2 activity or a site(s) targeted so infrequently in normal cells or camptothecin-resistant cancer cells that it is undetectable. While further analysis is required to determine whether this hyperphosphorylation event is cell cycle dependent and whether a single serine site or multiple serine sites are involved, the fact that it is associated with a greatly enhanced sensitivity to the S-phase specific drug, camptothecin, suggests a possible S-phase-specific effect.

The identification of the p14ARF tumor suppressor as a topo I-activating protein (31, 32) has raised the possibility that this protein could play a role in cancer through activation of topo I, a role distinct from its well-described p53-dependent tumor suppressor activity (63–67). We have previously reported that the interaction of p14ARF with topo I requires topo I serine phosphorylation and is only detectable in cancer cell lines with hyperphosphorylated topo I (6), an observation corroborated by the present study. Furthermore, in cell lines expressing a hyperphosphorylated topo I and p14ARF, we previously reported that camptothecin sensitivity is increased or decreased, respectively, by increasing or decreasing the expression level of p14ARF (6). Thus, it is likely that p14ARF contributes to topo I activity and camptothecin sensitivity in the topo I hyperphosphorylated cell lines in this study, all of which express p14ARF, making the presence of the p14ARF/topo I complex a possible biomarker of camptothecin sensitivity. Nevertheless, because purified topo I is regulated by phosphorylation in the absence of ARF (14–18), the primary driver of topo I activity and camptothecin sensitivity appears to be the phosphorylation status of topo I.

The role of CK2 in activating topo I *in vivo* is consistent with its well-established involvement in a broad array of cellular processes generally related to cell proliferation, cell viability, and oncogenesis (68). For example, coexpression of CK2 with the c-myc oncogene transforms murine lymphocytes (69), and CK2 alpha transgenic mice that are p53 null show increased propensity for tumor formation compared to p53 null mice lacking the CK2 alpha transgene (70). In humans, elevated levels of CK2 relative to normal cells or adjacent normal tissue have been observed in leukemias (71, 72) and solid tumors of the colon and breast (73), lung (74), head and neck (75), and kidney (76). A high level of CK2 expression correlates with poor prognosis in acute myeloid leukemia (77). Furthermore, downregulation of CK2 can reduce cancer cell viability (78). Of note, constitutive activation of CK2 has been reported in the HT29 cell line (79), a cell line found in the present study to express lower levels of CK2 associated with

camptothecin resistance. The overall level of CK2 relative to other cancer cell lines was not investigated in the earlier study, so there is no discrepancy with our present findings. However, the earlier finding that downregulation of CK2 promotes apoptosis on HT29 cells (79), taken together with the present data, suggests that CK2 may offer dual opportunities for cancer treatment, on the one hand as a therapeutic target (see ref 80 for a review) irrespective of endogenous expression level and on the other hand as a relevant biomarker for chemosensitivity.

Camptothecin-based therapeutics such as Irinotecan and Topotecan, whose therapeutic effect is achieved via the same mechanism as camptothecin, are being used for a growing array of cancer types (81), but their efficacy is often compromised by intrinsic or acquired therapy resistance. A variety of possible tumor resistance mechanisms have been identified using cultured cancer cell lines and by evaluation of tumor tissues (reviewed in refs 12, 82, and 83), but clinical resistance remains a multifaceted and poorly understood phenomenon. Because the side effects of treatment can be severe, there is intense interest in defining biomarkers of tumor sensitivity that could identify patients most likely to benefit from camptothecin-based agents. Our results suggest that a common, underlying feature of camptothecin sensitivity is the presence of CK2-mediated topo I hyperphosphorylation that increases topo I enzymatic activity. Assays to detect elevated CK2 protein or hyperphosphorylated topo I protein in biopsy specimens could therefore be used to identify tumors most likely to respond to camptothecin-derived chemotherapeutic drugs and to guide treatment choices for cancer patients.

Hyperphosphorylated topo I leading to abnormally elevated levels of topo I activity could also contribute to the toxicity of other DNA damaging chemotherapies in addition to those derived from camptothecin and, as such, could serve as a biomarker of therapy response to these agents as well. Covalent topo I trapping to DNA is observed in response to DNA cross-links, nearby nicks or gaps, abasic sites, and mismatches (84–86), all of which can be consequences of exposure to DNA damaging chemotherapeutic drugs. We have found that treatment of cancer cells with the chemotherapeutic agent, 5-fluorouracil, which can be aberrantly incorporated into DNA in place of thymidine, or cisplatin (Platinol), which produces DNA adducts, mimics the effects of camptothecin with regard to the generation of DNA double strand breaks (87), consistent with the formation of irreversible cleavage complexes. If further studies confirm that sensitivity to these agents also correlates with topo I phosphorylation, they may provide a rationale for an even broader application of CK2 or phospho topo I-based diagnostics.

SUPPORTING INFORMATION AVAILABLE

(1) Growth rates of camptothecin-sensitive and -insensitive cell lines (Figure S1), (2) effect of CK2 activator on purified PKC, cdk1, and CK2 activities and on endogenous PKC and cdk1 activity in H23 cells (Figure S2), (3) effect of CK2 activator on CK2 α transcription (Figure S3), (4) levels of serine/threonine phosphatase activity in cell lines (Figure S4), and (5) topo I phosphorylation levels in A549 and K562 cell lines (Figure S5). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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