Ku86 Modulates DNA Topoisomerase I–Mediated Radiosensitization, but not Cytotoxicity, in Mammalian Cells

Shyh-Jen Shih, Tara Erbele, and Allan Y. Chen

Department of Radiation Oncology, University of California Davis Medical Center, Sacramento, California

Abstract

Ku86 is an integral component of the nonhomologous end-joining (NHEJ) pathway of cellular double-strand break repair. In the current study, we investigated the role of Ku86 in DNA topoisomerase I–mediated radiosensitization induced by camptothecin in mammalian cells. Interestingly, as examined by clonogenic survival assay, a 30-minute camptothecin treatment induced significantly higher levels of radiosensitization in the Ku86-deficient Chinese hamster ovary xrs-6 cells than in the hamster Ku86-complemented xrs-6+hamKu86 cells, albeit exhibiting similar drug toxicity in these two cell lines. To confirm these findings, similar studies were conducted in two pairs of transfectant sublines established from the Ku86-deficient Chinese hamster lung fibroblast XR-V15B cells. Compared with the vector-alone sublines, radiation resistance was restored in the human Ku86-complemented sublines without alteration of cell cycle distributions. Again, significantly higher levels of camptothecin-induced radiosensitization were observed in the vector-alone sublines than in the Ku86-complemented XR-V15B sublines. In contrast, camptothecin treatments, ranging from 0.5 to 24 hours, induced similar cytotoxicities in both vector-alone and Ku86-complemented sublines. Because neither the DNA-damaging etoposide and cisplatin nor the tubulin-binder vinblastine induced enhanced levels of radiosensitization in the Ku86-deficient cells, Ku86 seems to uniquely affect topoisomerase I–mediated radiosensitization induced by camptothecin. Furthermore, cotreatment with DNA replication inhibitor aphidicolin abolished both camptothecin-induced cytotoxicity and radiosensitization in the vector-alone, as well as the Ku86-complemented subline cells, indicating both events are initiated by replication-dependent topoisomerase I–mediated DNA damages. Taken together, our data show a novel role of Ku86 in modulating topoisomerase I–mediated radiosensitization, but not cytotoxicity, in mammalian cells. (Cancer Res 2005; 65(20): 9194-9)

Introduction

Human DNA topoisomerase I is the cytotoxic target of a unique class of anticancer drugs including camptothecin derivatives (1–3). The up-regulated levels of topoisomerase I expression in cancer cells provide the molecular basis for selective targeting of cancer cells by using topoisomerase I drugs (1–3). Camptothecin derivatives exert their biological effects by trapping the topoisomerase I–cleavable complex, a key reaction intermediate of topoisomerase I formed during its catalytic cycle (1–3). The drug-trapped topoisomerase I–cleavable complexes can serve as DNA-breaking poisons and damage DNA through interactions with cellular processes such as replication of DNA (4, 5). The nature and the repair pathways involved of the various replication-dependent topoisomerase I–mediated DNA damages (T1DD) have been the subject of intense study and remain unsolved (1–3). Topoisomerase I also mediates radiosensitization induced by camptothecin derivatives (6, 7). Camptothecin derivatives were shown to induce radiosensitization in a schedule-dependent manner, which requires camptothecin treatment to be before or concurrently with but not following radiation (7). Our previous findings indicate that compounds capable of trapping topoisomerase I–cleavable complexes may possess radiosensitization activity (7, 8). Indeed, certain topoisomerase I–targeted indolocarbazoles have recently been shown to induce radiosensitization in mammalian cells (9). The mechanism of topoisomerase I–mediated radiosensitization remains largely unknown. Eukaryotic cells have evolved two major repair pathways for DNA double-strand breaks (DSB) including the homologous recombination and the nonhomologous end-joining (NHEJ) pathways (10, 11). Whereas homologous recombination ensures accurate repair by using an undamaged sister chromatid or homologous chromosome as a template, NHEJ uses either no or limited sequence homology to rejoin ends in a manner that is often error prone. Ku is a heterodimeric complex composed of Ku70 and Ku86 subunits that serve as a DNA-binding subunit of the DNA-dependent protein kinase (DNA-PK) complex of the NHEJ pathway (12, 13). Ku is believed to bind to broken DNA ends and recruits and activates DNA-PK$_{cs}$, DNA ligase IV, XRCC4 (a DNA ligase IV accessory factor) and Artemis, which are required for the rejoining of DNA DSBs (12, 13). In the present study, we investigated the role of Ku86 in topoisomerase I–mediated cytotoxicity and radiosensitization in mammalian cells. Furthermore, we studied the role of active DNA synthesis in camptothecin-induced radiosensitization in both Ku86-deficient and Ku86-complemented cells. Based on our data, a model of how mammalian Ku86 selectively modulates topoisomerase I–mediated radiosensitization, but not cytotoxicity, is proposed.

Materials and Methods

Drugs and materials. All drugs and chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Except for fetal bovine serum (FBS) that was from Gemini Bio-Products (Woodland, CA), media and reagents for tissue culture work and transfection were purchased from Life Technologies/Bethesda Research Laboratories (Grand Island, NY). DNase-free RNase and propidium iodide were purchased from Roche Molecular Biochemicals (Indianapolis, IN).

Cell cultures. The Chinese hamster ovary (CHO) xrs-6 and hamster Ku86 cDNA-complemented xrs-6+hamKu86 cells (14) were purchased from the European Collection of Cell Cultures (Wiltshire, United Kingdom). The Chinese hamster lung fibroblast XR-V15B cells (15) were purchased from the American Type Culture Collection (Manassas, VA). All cell lines were

Requests for reprints: Allan Y. Chen, Department of Radiation Oncology, University of California Davis Medical Center, 4501 X Street, G-150, Sacramento, CA 95817. Phone: 916-734-8252; Fax: 916-454-4614; E-mail: allan.chen@ucdmc.ucdavis.edu.

doi:10.1158/0008-5472.CAN-05-2387

grown in HAM's F12 medium supplemented with 10% FBS, glutamate, penicillin, and streptomycin, in a humidified 37°C incubator at 5% CO_2. The media for the xrs-6+hamKu86 and the four XR-V15B-transfectant sublines (V#1, V#11, K#4, and K#8) were supplemented with 400 μg/mL of G418.

Clonogenic survival assay. Clonogenic survival assays were conducted as described previously (7). Briefly, log-phased cells were plated overnight before being treated with various protocols, trypsinized, counted, and plated for colony formation. Following 7 to 10 days of incubation, colonies were fixed with methanol/acetic acid (3:1), stained with crystal violet, and counted. All survival points were done in triplicate, and experiments were conducted a minimum of twice. Error bars shown in the figures represent SDs.

Irradiation of cells. Cells were irradiated using a radiosensitization 2000 Biological Irradiator (Rad Source Technologies, Inc., Boca Raton, FL) at a dose rate of 105 cGy/min. The irradiator was calibrated periodically by using thermoluminescence dosimetry.

Analysis of radiation survival curve. Chemoradiation survival curves were corrected for cytotoxicity induced by drug alone. Radiation enhancement ratio (RER) was calculated by dividing the survival fraction of radiation ($SF_{radiation}$) in the absence of drug over the survival fraction of treatment ($SF_{drug+radiation}$) in the presence of drug at the designated radiation dose (RER = $SF_{radiation}$ / $SF_{drug+radiation}$).

Cell cycle analysis. Log-phased cells were fixed in 70% ethanol, treated with DNase-free RNase at 37°C for 40 minutes, stained with 5 μg/mL of propidium iodide at room temperature for 15 minutes, and analyzed for DNA content by using a Coulter Epics XL flow cytometer (Beckman Coulter, Miami, FL) as described (16). Analysis of cell cycle position was done using the Phoenix Multicycle software (Phoenix Flow Systems, San Diego, CA).

Generation of the Chinese hamster XR-V15B sublines by transfection. pMHLacZ (LacZ, neo resistance expression vector), pBJ5 (as a control), and pBJ5+Ku86 (containing cDNA encoding human Ku86; ref. 15), were kindly provided by Dr. Gilbert Chu (Stanford University). Transfections were done as described by Smider et al. (15), with minor modifications. Briefly, 2×10^5 of cells were incubated overnight with 2.0 μg of plasmid DNA (0.5 μg pMHLacZ plus 1.5 μg of either pBJ5 or pBJ5+Ku86) and 12.5 μL of LipofectAMINE 2000 reagent (250 μL each). Clones were selected by using 400 μg/mL of G418.

Immunoblotting analysis. Cell lysates were separated by 8% SDS-polyacrylamide gel and electrotransferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA). The membranes were probed with human scleroderma antiserum for topoisomerase I (TopoGEN, Columbus, OH), monoclonal mouse Ku15 antibody for Ku86 (Sigma, St. Louis, MO), and 6C5 antibody for glyceraldehyde-3-phosphate dehydrogenase (BioDesign International, Saco, ME) to verify equal loading. The membranes were incubated with peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) before the proteins were detected by enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ).

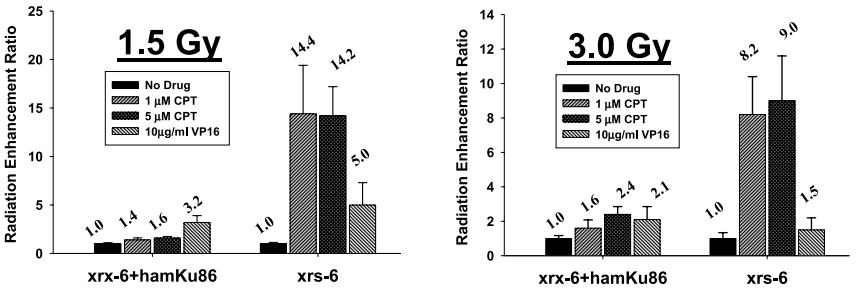
Results and Discussion

Camptothecin induced significantly higher levels of radiosensitization in the Ku86-deficient xrs-6 than in the hamster Ku86-complemented xrs-6+hamKu86 cells. The role of Ku86 in camptothecin-induced cytotoxicity and radiosensitization was investigated in the CHO Ku86-deficient xrs-6 and its hamster Ku86-complemented xrs-6+Ku86 cells by clonogenic survival assay. As reported previously (14), the xrs-6 cells are more sensitive than the xrs-6+hamKu86 cells to radiation and the DNA topoisomerase II–targeted etoposide (VP-16; Fig. 1*A*). In comparison, both the xrs-6 and the xrs-6+hamKu86 cells exhibited similar sensitivity to the cytotoxicity of camptothecin (Fig. 1*A*). Interestingly, 30 minutes of camptothecin treatment at either 1 or 5 μmol/L induced a significantly higher level of radiosensitization in the xrs-6 cells than in the xrs-6+hamKu86 cells (Fig. 1*B*). As measured by RER (RER = $SF_{radiation}$ / $SF_{drug+radiation}$), camptothecin induced 10-fold higher RER values in the xrs-6 cells than in the xrs-6+hamKu86 cells at 1.5 Gy (14.4 and 14.2 comparing with 1.4 and 1.6). At 3 Gy, camptothecin induced 5-fold higher RER values in the xrs-6 cells than in the

A Chemoradiation sensitivity (% Survival)

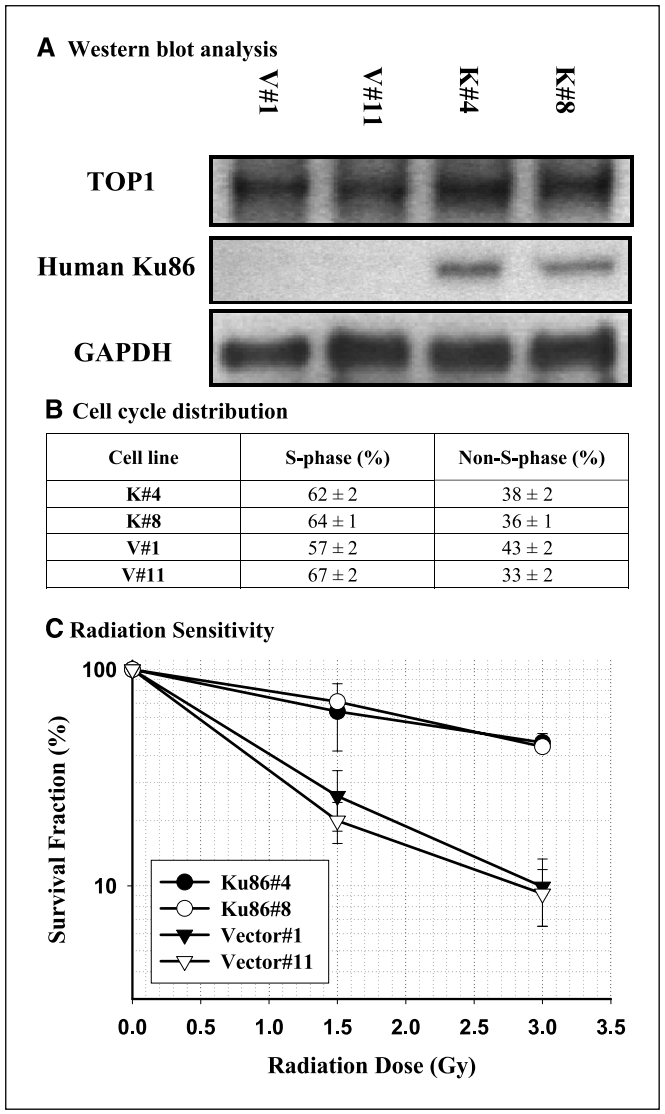
Treatments		xrs-6	xrs-6+hamKu86
No treatment control		100 ± 10	100 ± 7
Radiation (Gy)	1.5	6.2 ± 0.9	76 ± 8
	3.0	0.6 ± 0.2	49 ± 8
	6.0	0.04 ± 0.04	6 ± 2
CPT (μM)	0.2	53 ± 3	51 ± 4
	1.0	40 ± 10	51 ± 7
	5.0	40 ± 6	49 ± 4
VP16 (μg/ml)	2.0	26 ± 5	103 ± 2
	10.0	4 ± 3	60 ± 20

B RERs at 1.5 Gy and 3.0 Gy.



*RER = $SF_{radiation}$/ $SF_{drug+radiation}$.

Figure 1. Cytotoxicity and radiosensitization induced by camptothecin (*CPT*) and VP-16 in the CHO Xrs6 and its hamster Ku86-complemented Xrs6+hamKu86 cell lines. Clonogenic survival assays of exponentially growing cells were conducted as described in Materials and Methods. DMSO (0.1%) was used for no drug control. All points were done in triplicate, and experiments were conducted a minimum of two times. *A,* cytotoxicity for ionizing radiation, 30 minutes of camptothecin, and 30 minutes of VP-16 treatments. *B,* RERs induced by camptothecin and VP-16 at 1.5 and 3.0 Gy. RER was calculated by dividing the survival fraction of radiation ($SF_{radiation}$) in the absence of drug over the survival fraction of treatment ($SF_{drug+radiation}$) in the presence of drug at the designated radiation dose (RER = $SF_{radiation}$ / $SF_{drug+radiation}$). Cells were preincubated for 30 minutes with 1 or 5 μmol/L of camptothecin or 10 μg/mL of VP-16 followed by treatment with 0, 1.5, or 3.0 Gy of radiation. The number above each column denotes the RER induced by the indicated drug treatment of the individual cell line.



Cell line	S-phase (%)	Non-S-phase (%)
K#4	62 ± 2	38 ± 2
K#8	64 ± 1	36 ± 1
V#1	57 ± 2	43 ± 2
V#11	67 ± 2	33 ± 2

Figure 2. Ku86 expressions, cell cycle distributions, and radiation sensitivities of the Ku86-deficient (V#1 and V#11) and human Ku86-complemented (K#4 and K#8) XR-V15B sublines. *A,* Western blot analysis. Equivalent amounts of whole cell extract derived from the four sublines were separated in 8% SDS-polyacrylamide gels and subjected to Western blotting as described in Materials and Methods by using antibodies against human topoisomerase I (*TOP1*), Ku86, and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as indicated. *B,* cell cycle distributions of the four sublines were determined by flow cytometric analysis of exponentially growing cells as described in Materials and Methods. *C,* radiation sensitivities of the four XR-V15B sublines were determined by clonogenic survival assay of exponentially growing cells as described in Materials and Methods. *Bars,* SD (from at least three independent experiments).

xrs-6+hamKu86 cells (8.2 and 9.0 comparing with 1.6 and 2.4). In comparison, VP-16 induced no such radiosensitization enhancement in the Ku86-deficient xrs-6 cells at both 2 and 10 μg/mL (Fig. 1*B*).

Human Ku86 complementation restored radiation resistance in the Ku86-deficient XR-V15B cells without altering cell cycle distribution. To confirm the above findings, two pairs of isogenic sublines were established from the Ku86-deficient Chinese hamster lung fibroblast XR-V15B cells by transfection with either vector alone or vector-containing human Ku86 cDNA as described in Materials and Methods (15). As shown by Western blot, all four XR-V15B sublines express similar levels of topoisomerase I; however, only the Ku86-complemented K#4 and K#8 sublines, but not the vector-alone transfectant V#1 and V#11 sublines, express Ku86 (Fig. 2*A*). Cell cycle distribution, particularly the S-phase proportion, is known to be a major determinant for cytotoxicity of topoisomerase I–targeted drugs (1–3). As examined by flow cytometry, the four XR-V15B sublines exhibit similar cell cycle distributions with ~60% of cells residing in the S phase (Fig. 2*B*), indicating that Ku86 complementation confers no alteration on cell cycle distribution. However, compared with the V#1 and V#11 sublines, Ku86 complementation restores radiation resistance in the K#4 and K#8 sublines (Fig. 2*C*). These results show that the Ku86 expressed in the K#4 and K#8 sublines is functional and involved in repairing radiation–induced damage.

Ku86 does not affect cytotoxicity of camptothecin in the XR-V15B cells. Chemoradiation-clonogenic survival assays were conducted in the four XR-V15B transfectant sublines. As shown in Fig. 3*A*, compared with the vector-alone V#1 and V#11 sublines, cellular resistance to VP-16 was restored in the Ku86-complemented K#4 and K#8 sublines. In contrast, all four XR-V15B sublines exhibited similar sensitivity to the cytotoxicity of a 30-minute treatment of either 1 or 5 μmol/L of camptothecin (Fig. 3*A*). Because S-phase cells are most sensitive to camptothecin-induced cytotoxicity (4, 5), the observed similar cytotoxicity to camptothecin of the four sublines may simply reflect their parallel proportion of S-phase distribution (Fig. 2*B*). To ensure an accurate assessment of cytotoxicity in all cell cycle phases, extended duration of camptothecin treatment was examined in the K#4 and V#11 subline cells. As shown in Fig. 3*A* and *B*, both K#4 and V#11 cells exhibited similar sensitivity to the cytotoxicity of 4 hours of 1 μmol/L, as well as 4, 12, and 24 hours of 0.1 μmol/L of camptothecin treatments. Our findings seem to disagree with previous reports showing altered sensitivity to camptothecin in eukaryotic cells with genetic alterations in the NHEJ pathways (17, 18). In mammalian cells, mutations in DNA-PK_{cs} were reported to be associated with camptothecin hypersensitivity (17). In contrast, surprisingly, mutant chicken embryo DT40 cells lacking NHEJ molecules, including Ku70, DNA-PK_{cs}, and DNA ligase IV, were shown to be resistant to camptothecin (18). These reported conflicting results of camptothecin-induced cytotoxicity in cells with alterations in the NHEJ pathway remain unclear at present. Possible explanation may include variations in cytotoxicity assay methodologies with differences between growth inhibition assays and clonogenic survival assay as well as the difference in DNA damage response pathways between chicken embryo cells and mammalian cells. Nevertheless, based on stringent clonogenic survival assay in three pairs of independently isolated cell lines, our results clearly show that Ku86 is not involved in camptothecin-induced cytotoxicity in mammalian cells.

Camptothecin-induced radiosensitization is significantly affected by Ku86 in the XR-V15B cells. Camptothecin-induced radiosensitization was investigated in the two pairs of XR-V15B sublines. As shown in Fig. 3*C*, camptothecin induced high levels of radiosensitization with RER values of 13.5 and 15.8 at 1.5 Gy and 16.0 and 23.0 at 3 Gy in the vector-alone V#1 and V#11 sublines. In contrast, significantly lower levels of radiosensitization with RER values of 1.0 and 1.5 at 1.5 Gy and 1.1 to 1.3 at 3 Gy were induced by camptothecin in the Ku86-complemented K#4 and K#8 sublines (Fig. 3*C*). In comparison, the topoisomerase II–targeted VP-16 induced similar levels of radiosensitization in all four XR-V15B sublines (Fig. 3*C*). In addition, neither the DNA-damaging cisplatin, nor the tubulin binder vinblastine induced enhanced levels of radiosensitization in the Ku86-deficient cells

A Cytotoxicity for 0.5 hour drug treatment with CPT and VP16 (% survival)

Cell line / Treatments (0.5 h)	K#4	K#8	V#1	V#11
No treatment	100 ± 7	100 ± 12	100 ± 10	100 ± 7
1.0 μM CPT	31 ± 3	28 ± 5	37 ± 3	37 ± 6
5.0 μM CPT	33 ± 10	32 ± 12	37 ± 4	32 ± 6
10 μg/ml VP16	64 ± 13	70 ± 14	6 ± 4	12 ± 4

B Cytotoxicity for extended durations of drug treatment (% survival)

CPT Treatment / Cell line		K#4	V#11
No treatment control		100 ± 5	100 ± 8
1 μM	4 hr	27 ± 1.2	24 ± 3.0
0.1 μM	4 hr	28 ± 1.4	29 ± 1.3
0.1 μM	12 hr	2.2 ± 0.3	1.9 ± 0.1
0.1 μM	24 hr	0.2 ± 0.06	0.14 ± 0.02

C Radiation enhancement ratio (RER) induced by CPT or VP-16

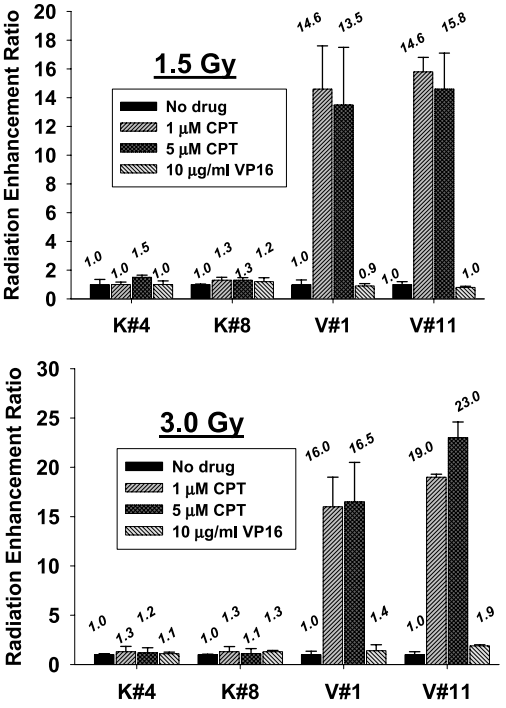


Figure 3. Cytotoxicity and radiosensitization induced by camptothecin (*CPT*) and VP-16 in the Ku86-deficient (V#1 and V#11) and human Ku86-complemented (K#4 and K#8) XR-V15B sublines. Clonogenic survival assays of exponentially growing cells were conducted as described in Materials and Methods. DMSO (0.1%) was used for no drug control. All points were done in triplicate, and experiments were conducted a minimum of two times. *A,* cytotoxicity for a 30-minute drug treatment with camptothecin and VP-16. *B,* cytotoxicity for extended durations of camptothecin treatment at 1 and 0.1 μmol/L. *C,* RERs induced by camptothecin and VP-16 at 1.5 and 3.0 Gy. RER = $SF_{radiation}$ / $SF_{drug+radiation}$ as described in Fig. 1. Cells were preincubated for 30 minutes with 1 or 5 μmol/L of camptothecin or 10 μg/mL of VP-16 followed by treatment with 0, 1.5, or 3.0 Gy of radiation. The number above each column denotes the RER induced by the indicated drug treatment of the individual cell line.

(data not shown). These results confirm the observation in the xrs-6 and xrs-6+hamKu86 cells (Fig. 1*B*) and indicate that Ku86 uniquely affect radiosensitization induced by camptothecin but not by other cytotoxic agents. Consistently, our recent results showed that the indolocarbazole derivatives F1 and F7, two non-camptothecin topoisomerase I–targeted drugs (9), also induced significantly higher levels of radiosensitization in the Ku86-deficient V#11 cells than in the Ku86-complemented K#4 XR-V15B cells.[1] Our findings are the first to show a novel role of Ku86 in modulating topoisomerase I–mediated radiosensitization in mammalian cells.

Replication inhibitor aphidicolin cotreatment abolished camptothecin-induced radiosensitization in both Ku86-deficient and Ku86-proficient XR-V15B cells. The S phase–specific cytotoxicity of camptothecin can be abolished by cotreatment with replication inhibitors such as aphidicolin (4, 5), presumably by stalling replication fork to prevent "collision" between camptothecin-trapped topoisomerase I–cleavable complexes and DNA replication machinery (1–5). Our previous work indicates that camptothecin-induced radiosensitization can be partially reversed by cotreatment with DNA replication inhibitor aphidicolin but not inhibitors for transcription and protein synthesis in CHO cells (19). The effect of replication inhibition by aphidicolin cotreatment in camptothecin-induced radiosensitization was investigated in the Ku86-deficient V#11 and the Ku86-complemented K#4 cells. As measured by ^{3}H-thymidine incorporation, a 30-minute treatment of 2.5 μmol/L of aphidicolin inhibited 90% of DNA synthesis in the CHO cells. As shown in Fig. 4*A*, a 30-minute cotreatment of 2.5 μmol/L of aphidicolin abolished both cytotoxicity and radiosensitization induced by 1 μmol/L of camptothecin in the Ku86-complemented K#4 cells. More impressively, in the Ku86-deficient V#11 cells, the high RER values of 17 at 1.5 Gy and 25 at 3 Gy induced by camptothecin were near completely reversed to 1.7 and 1.4, respectively (Fig. 4*A*). These results indicate that the initiation of camptothecin-induced radiosensitization, similar to camptothecin-induced cytotoxicity, is a replication-dependent event that is not affected by Ku86.

Ku86 modulates topoisomerase I–mediated radiosensitization, but not cytotoxicity, in mammalian cells. Ku86 complexes with Ku70 and DNA-PK_{cs} to form the DNA-PK complex of the NHEJ pathway (12, 13). Because camptothecin is known to induce unique T1DDs, Ku86 may modulate topoisomerase I–mediated radiosensitization through activation of DNA-PK. Indeed, our recent data showed that camptothecin also induced significantly enhanced levels of radiosensitization in mammalian cells deficient in DNA-PK_{cs}.[2] Additional studies using other NHEJ mutants, including DNA ligase IV, XRCC4, and Artemis, may further define the role of NHEJ

[1] A.Y. Chen et al., unpublished result.

[2] A.Y. Chen and S-J. Shih, unpublished result.

pathway for topoisomerase I–mediated radiosensitization. How does Ku86 affect camptothecin-induced radiosensitization without altering cytotoxicity of camptothecin? The collision between the drug-trapped topoisomerase I–cleavable complex and the DNA replication machinery is responsible for the S phase–specific T1DDs induced by camptothecin (1–5). As observed in a cell-free SV40 DNA replication system, the collision leads to at least three major T1DDs, including DNA DSB, arrest of replication fork, and an aborted "cleaved" topoisomerase I-DNA complex (20). The biochemical consequences of each of these T1DDs remain largely undefined (2, 3). It is plausible that different T1DDs, as well as distinct repair mechanisms, are involved for the induction of topoisomerase I–mediated cytotoxicity and topoisomerase I–mediated radiosensitization. In supporting such theory, our recent results show that topoisomerase I–mediated radiosensitization, but not cytotoxicity, induced by 30-minute camptothecin treatment is completely reversed during a time course of eight hours in CHO cells.[2] These results suggest that camptothecin may induce topoisomerase I–mediated radiosensitization through a "repairable" T1DD, which leads to cell death when it is combined with radiation-induced damage. The exact nature of the different T1DDs leading to cytotoxicity and radiosensitization remains to be determined. Taken together, as shown in Fig. 4*B*, our data suggest a model that the collision between the drug-trapped topoisomerase I–cleavable complexes and DNA replication machinery leads to two separate pathways: the cytotoxicity pathway and the radiosensitization pathway. Whereas the topoisomerase I–mediated radiosensitization pathway is affected by Ku86, the topoisomerase I–mediated cytotoxicity pathway is not.

Acknowledgments

Received 7/8/2005; revised 8/5/2005; accepted 8/16/2005.

Grant support: University of California Cancer Research Coordinating Committee fund and University of California Davis Health System Award (A.Y. Chen).

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

We thank Dr. Andrew Vaughan for critical reading of the article.

A Effect of co-treatment with DNA replication inhibitor aphidicolin (APH) on cytotoxicity and radiosensitization(RS) induced by camptothecin (CPT).

XR-V15B Sublines	Drug Treatment	% Survival without radiation	$SF_{1.5}$[a] (%)	$RER_{1.5}$[b]	SF_3 (%)	RER_3
K#4	No Drug	100 ± 4	73± 2	1.0	42 ± 1	1.0
	APH	97 ± 4	70 ± 2	1.0	45 ± 2	0.9
	CPT	34 ± 4	67 ± 4	1.1	37 ± 1	1.2
	APH + CPT	89 ± 2	80 ± 1	0.9	46 ± 2	0.9
V#11	No Drug	100 ± 5	20 ± 3	1.0	5.8 ± 0.5	1.0
	APH	98 ± 6	23 ± 4	0.9	6.2 ± 0.8	0.9
	CPT	35 ± 1	1.2 ± 0.2	17.0	0.20 ± 0.02	25.0
	APH + CPT	94 ± 4	12 ± 1	1.7	4.1 ± 0.4	1.4

[a] $SF_{1.5}$ and SF_3 : Survival Fraction at 1.5 Gy and 3 Gy.
[b] RER, radiation enhancement ratio = $SF_{radiation}$ / $SF_{drug+radiation}$.

B A proposed model for the induction of TOP1-mediated cytotoxicity and RS.

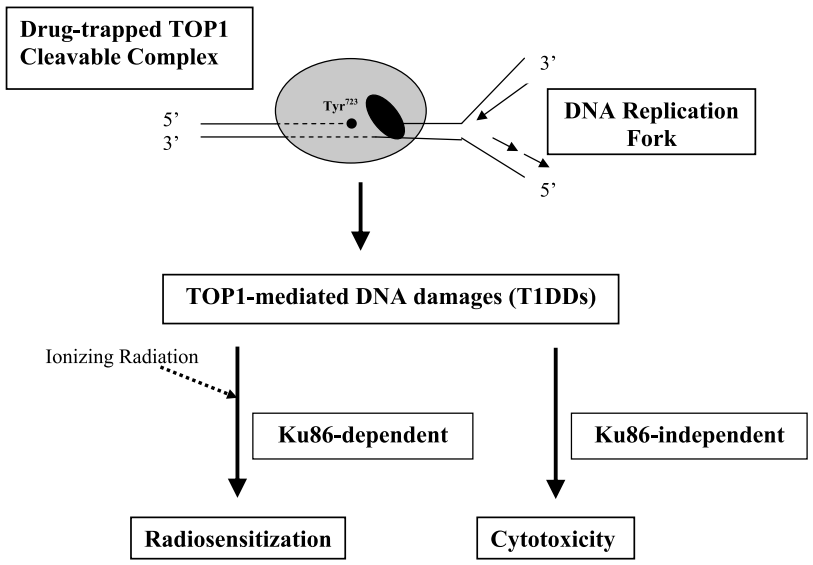


Figure 4. The role of DNA replication in the induction of topoisomerase I (*TOP1*)–mediated cytotoxicity and radiosensitization (*RS*). *A,* effect of cotreatment with DNA replication inhibitor aphidicolin (*APH*) on cytotoxicity and radiosensitization induced by camptothecin (*CPT*). The Ku86-proficient (K#4) and Ku86-deficient (V#11) XRV-15B cells were preincubated for 30 minutes with either no drug, 2.5 μmol/L of aphidicolin, 1 μmol/L of camptothecin, or 2.5 μmol/L of aphidicolin plus 1 μmol/L of camptothecin. Cells were then irradiated with graded doses of radiation and plated for colony formation as described in Materials and Methods. DMSO (0.1%) was used for no drug control. All points were done in triplicate, and the results of one of two experiments are shown. *B,* proposed model for two separate pathways leading to the induction of topoisomerase I–mediated cytotoxicity and radiosensitization in mammalian cells. According to this model, the collision between drug-trapped topoisomerase I–cleavable complexes and replication machinery may generate different T1DDs that lead to two distinct pathways: the topoisomerase I–mediated cytotoxicity pathway and topoisomerase I–mediated radiosensitization pathway. Whereas the topoisomerase I–mediated radiosensitization pathway is modulated by Ku86, the topoisomerase I–mediated cytotoxicity pathway is not.

References

1. Chen AY, Liu LF. DNA topoisomerases: essential enzymes and lethal targets. Annu Rev Pharmacol Toxicol 1994;34:191–218.
2. Li TK, Liu LF. Tumor cell death induced by topoisomerase-targeting drugs. Annu Rev Pharmacol Toxicol 2001;41:53–77.
3. Pommier Y, Redon C, Rao VA, et al. Repair of and checkpoint response to topoisomerase I-mediated DNA damage. Mutat Res 2003;532:173–203.
4. Holm C, Covey JM, Kerrigan D, Pommier Y. Differential requirement of DNA replication for the cytotoxicity of DNA topoisomerase I and II inhibitors in Chinese hamster DC3F cells. Cancer Res 1989;49:6365–8.
5. D'Arpa P, Beardmore C, Liu LF. Involvement of nucleic acid synthesis in cell killing mechanisms of topoisomerase poisons. Cancer Res 1990;50:6919–24.
6. Mattern MR, Hofmann GA, McCabe FL, Johnson RK. Synergistic cell killing by ionizing radiation and DNA topoisomerase I inhibitor topotecan (SK&F 104864). Cancer Res 1991;51:5813–6.
7. Chen AY, Okunieff P, Pommier Y, Mitchell J. Mammalian DNA topoisomerase I mediates the enhancement of radiation cytotoxicity by camptothecin derivatives. Cancer Res 1997;57:1529–36.
8. Chen AY, Chou R, Shih S-J, Lau D, Gandara D. Enhancement of radiotherapy with DNA topoisomerase I-targeted drugs. Crit Rev Oncol Hematol 2004;50:111–9.
9. Chen AY, Shih S-J, Hsiao M, Rothenberg ML, Prudhomme M. Induction of radiosensitization by indolocarbazole derivatives: the role of DNA topoisomerase I. Mol Pharmacol 2004;66:553–60.
10. Jeggo PA. DNA breakage and repair. Adv Genet 1998;38:186–218.
11. Khanna KK, Jackson SP. DNA double strand breaks: signaling, repair and the cancer connection. Nat Genet 2001;27:247–54.
12. Featherstone C, Jackson SP. Ku, a DNA repair protein with multiple cellular functions. Mutat Res 1999;434:3–15.
13. Tuteja R, Tuteja N. Ku autoantigen: a multifunctional DNA-binding protein. Crit Rev Biochem Mol Biol 2000;35:1–33.
14. Singleton BK, Priestley A, Steingrimsdottir H, et al. Molecular and biochemical characterization of xrs mutants defective in Ku80. Mol Cell Biol 1997;17:1264–73.
15. Smider V, Rathmell WK, Lieber MR, Chu G. Restoration of X-ray resistance and V(D)J recombination in mutant cells by Ku cDNA. Science 1994;266:288–91.
16. Mack PC, Gandara DR, Lau AH, Lara PN, Jr., Edelman MJ, Gumerlock PH. Cell cycle-dependent potentiation of cisplatin by UCN-01 in non-small-cell lung carcinoma. Cancer Chemother Pharmacol 2003;51:337–48.
17. Culmsee C, Bondada S, Mattson MP. Hippocampal neurons of mice deficient in DNA-dependent protein kinase exhibit increased vulnerability to DNA damage, oxidative stress and excitotoxicity. Brain Res Mol Brain Res 2001;87:257–62.
18. Adachi N, So S, Koyama H. Loss of nonhomologous end joining confers camptothecin resistance in DT40 cells. Implications for the repair of topoisomerase I-mediated DNA damage. J Biol Chem 2004;279:37343–8.
19. Chen AY, Scruggs PB, Geng L, Rothenberg ML, Hallahan DE. p53 and p21 are major cellular determinants for DNA topoisomerase I-mediated radiation sensitization in mammalian cells. Ann N Y Acad Sci 2000;922:298–300.
20. Tsao YP, Russo A, Nyamuswa G, Silber R, Liu LF. Interaction between replication forks and topoisomerase I-DNA cleavable complexes: studies in a cell-free SV40 DNA replication system. Cancer Res 1993;53:5908–14.

Ku86 Modulates DNA Topoisomerase I–Mediated Radiosensitization, but not Cytotoxicity, in Mammalian Cells

Shyh-Jen Shih, Tara Erbele and Allan Y. Chen

Cancer Res 2005;65:9194-9199.