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Topoisomerase II beta levels are a determinant of melphalan-induced DNA crosslinks and sensitivity to cell death

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ABSTRACT

The role of topoisomerase (topo) II in DNA repair has yet to be fully elucidated. Current evidence suggesting a role for topo II in the repair of DNA damage has been obtained by using in vitro model systems or inferred from correlative data in drug resistant cell lines. In this study we directly examined the role of topo IIα and β in mediating the repair of melphalan-induced crosslinks in cellular DNA. To accomplish this, we used siRNA technology to knock down either topo IIα or β in human chronic myelogenous leukemia K562 and histiocytic lymphoma U937 cell line. Our data demonstrate that topo IIβ levels, (but not α), are a determinant of melphalan-induced crosslinks and sensitivity to melphalan. Furthermore, we show that knocking down topo IIβ inhibits the repair of melphalan-induced crosslinks in K562 cells. These studies represent the first direct evidence that topo IIβ participates in the repair of DNA damage induced by an alkylating agent in cellular DNA. Finally, these results suggest non-redundant roles for these two isoforms in mediating repair of DNA crosslinks.

1. Introduction

Topoisomerases (topo) are nuclear enzymes critical for maintaining the topology of DNA and contribute to regulating transcription, replication and segregation of chromosomes [1]. It is attractive to speculate that topo II may play a critical role in localized unwinding of DNA, allowing for recruitment of DNA repair complexes to damaged DNA. Indeed, several reports have shown a correlation between topo II activity and sensitivity to crosslinking agents in drug resistant cell lines. For example, Tan et al. showed that selection of a human Burkitt lymphoma cell line with the nitrogen mustard mechlorethamine resulted in increased activity of topo II and increased sensitivity to topo II inhibitors [2]. In addition, Barret et al. demonstrated that selection of L1210 murine leukemia cells with cisplatinum resulted in a three-fold increase in topo II activity [3]. However, to date, studies correlating topo II levels with sensitivity to crosslinking agents have been limited to drug resistant cell line models. Although these models are valuable, they can be difficult to interpret, as multiple changes occur during drug selection. To simplify the interpretation of the role of topo II, and delineate whether a functional overlap exists between the two isoforms of topo II, we used siRNA technology to selectively reduce either topo IIα or β levels. In this study we provide evidence that topo IIβ is a determinant of the levels of melphalan-induced crosslinks

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and sensitivity to apoptosis. In addition, we show that this result is not due to alterations in cell cycle kinetics, as cells with reduced topo IIα or β levels are not growth arrested.

2. Materials and methods

2.1. Cell culture

The U937 human histiocytic lymphoma cell line and the K562 chronic myelogenous leukemia cell line were obtained from the American Type Culture Collection (Rockville, MD). Both cell lines were maintained in RPMI media supplemented with 10% FBS. Cells were maintained at 37 °C in 5% CO_2/95% air atmosphere.

2.2. SiRNA transfection

2 million U937 or K562 cells were transfected with either 1 μm of non-silencing siRNA (Qiagen, Valencia Ca), topo IIβ siRNA (Ambion Foster City, CA) or topo IIα siRNA (Dharmacon, Chicago IL) using solution V and Nucleofactor technology per manufacturer's instructions (Amaxa, Gaithersburg, MD). For U937 cells, assays were performed 24 h following transfection. For K562 cells, a repeat transfection was performed at 24 h and cells were assayed an additional 24 h later.

2.3. Western blot analysis of topo II levels

Cells were lysed in a 0.1% NP40 buffer containing 350 mM NaCl, 5 mM EDTA, 50 mM NaF, 50 mM Tris–HCl (pH 7.5), 1 mM phenylmethylsulfonyl fluoride, 20 μg/ml leupeptin, 20 μg/ml aprotonin, 20 μg/ml antipaoen, 20 μg/ml chymostatin, 20 μg/ml pepstatin A, 20 μg/ml soybean trypsin inhibitor and 1 mM benzamidine. Lysates were sonicated and quantified using the BCA protein assay (Pierce Biochemicals, Rockland, IL). Fifty microgram of extract were loaded and separated on a 5–15% gradient SDS-PAGE gel and transferred to a polyvinylidene difluoride (PVDF) membrane. The upper portion of the blot was probed with either a topo IIα or topo IIβ monoclonal antibody (BD Biosciences α cat # 611327, β Cat # 611493) and the lower portion of the blot was probed with a β-actin monoclonal antibody (Sigma, St. Loius MO cat # 5316). The band of interest was detected by chemiluminscence (Pierce Biochemical) and quantified using imagequant software (Molecular Dynamics, Sunnyvale CA).

2.4. Detection of melphalan induced crosslinks

The alkaline comet assay was used to detect melphalan induced DNA crosslinks as previously described [4]. Briefly, following transfection of siRNA (24 h for U937 and 48 h for K562 cells), cells were plated at a density of 500,000 cells per ml in a 35 mm culture dish. Following a 2 h drug treatment, cells were processed immediately for melphalan-induced crosslinks. For assessing the repair of melphalan-induced crosslinks, cells were treated for 2 h with melphalan and then washed once and placed in drug free media. Crosslinks were then measured at the indicated time points. Control samples consisted of cells treated with vehicle control (0.86 nM HCl in 0.15% ethanol). Following drug treatment, single strand breaks were induced by irradiating appropriate samples with 900 rad (MARK I model 68A Irradiator, Sheperd and Associates, San Fernado, CA). After drug treatment and irradiation, 5000 cells were placed in a microcentrifuge tube containing 1 ml cold PBS, and the alkaline comet assay was performed as previously described [4]. Fifty-one images were randomly captured per slide, and images from fluorescence microscopy were quantified using Loats Associates comet analysis software (Loats Associates, Westminster, MD).

The percent crosslinking was calculated as follows:

$$\text{relative crosslinking} = 1 - \left(\frac{(\text{comet moment drug treated} + 900\,\text{rad} - \text{comet moment vehicle control})}{(\text{comet moment 900 rad only} - \text{comet moment vehicle control})} \right) \times 100.$$

2.5. Apoptosis

Annexin V staining of apoptotic cells was used to determine whether reducing the levels of either topo IIα or β increased the sensitivity of K562 cells to melphalan induced cell death. Forty-eight hours after transfection with appropriate siRNA constructs, cells were treated with 100 μM melphalan for 2 h and then placed in drug free media for an additional 24 h. Twenty-four hours after drug exposure, melphalan induced apoptosis was measured by annexin V staining and FACS analysis.

2.6. Clonagenic assay

K562 cells were transfected with non-silencing siRNA, topoisomerase IIα, or topoisomerase IIβ using the nucleofactor (AMAXA) per manufacturer's instructions. Cells were counted and resuspended in RPMI containing 10% FBS at a concentration of 10,000 cells/ml. Cells were treated with varying concentrations of drug for 2 h. The cells were washed twice with 1 ml RPMI containing 15% FBS and resuspended in 1.6 ml RPMI containing 15% FBS. 2.4 ml of 0.5% agar (Gibco Select Agar) was added to each sample and 1 ml was transferred to three separate 17 mm × 100 mm sterile tubes. The tubes were incubated at 37 °C for 8 days. On the 8th day the colonies were counted on 2 mm grid culture dishes. All colonies over 50 cells were counted in each sample.

2.7. Cell cycle

The effects of reducing topo IIβ and α levels on cell cycle progression were measured by a two-color flow cytometry method, where S-phase was detected by FITC conjugated anti-BrdU antibody and G1 and G2-M populations were identified by PI staining of DNA content as previously described [5]. Following 48 h of culture, 30 μg/ml bromodeoxyuridine (BrdU, Sigma) was added for 1 h. Following incubation with BrdU, cells were fixed overnight with 66% cold ethanol. After fixation, cells were digested with 4 ml of a 0.04% pepsin solution containing 0.1% HCl for 1 h at 37 °C. Nuclei were centrifuged and then incubated in a 2N HCl solution at 37 °C for an additional 30 min. Following acid (HCl) denaturation of DNA, samples were washed once in

0.1M sodium borate and once in PBTB (PBS containing 0.0025% Tween 20 and 0.5% BSA). Cell pellets were resuspended in 200 μl PBTB, and then incubated with 5 μl of anti-BrdU FITC-conjugated antibody (Dako, Carpinteria, CA) for 60 min in the dark. Samples were subsequently washed once in PBTB and resuspended in 500 μl of PBTB containing 10 μg/ml propidium iodide and 25 μl of 10 mg/ml RNase A (Boehringer Mannheim, IN). Following a 30 min incubation at 37 °C, samples were analyzed for BrdU incorporation and propidium iodide staining by using flow cytometry.

2.8. Statistical analysis

In order to generate 95% confidence intervals, we fit the data to an analysis of variance (ANOVA). Least squares estimates of the means and pooled standard errors were then calculated, and the corresponding 95% confidence intervals generated. SAS© software was used in the calculations, using Proc GLM. Student's *t*-tests were used to test for differences in the means of non-silencing and topo IIα siRNA and non-silencing and topo IIβ siRNA samples.

3. Results

3.1. Reducing topo II levels using siRNA constructs

As shown in Fig. 1A and B, siRNA targeting of topo IIβ selectively reduced the expression of topo IIβ protein levels in K562 and U937 cells by 95 and 69%, respectively (average of three independent experiments). Cells transfected with topo IIβ siRNA showed no significant changes in topo IIα protein levels. Furthermore, as shown in Fig. 1C and D, transfection of topo IIα siRNA reduced topo IIα protein levels by 83% and 66% (mean of three independent experiments) in K562 and U937 cells, respectively. In order to control for effects due to the presence of high concentrations of short RNA sequences, a siRNA construct that does not hybridize to any known genes was used for all experiments and is referred to as non-silencing siRNA.

3.2. Melphalan-induced crosslinks

Once optimization of reducing topo IIα and β protein levels was achieved, the alkaline comet assay was used to quantify melphalan-induced crosslinks. As shown in Fig. 2A and B, reducing topo IIβ levels was sufficient to significantly enhance melphalan-induced crosslinks in both K562 and U937 cells compared to the non-silencing control sample ($p < 0.025$ for both, Student's *t*-test). In contrast as shown in Fig. 2C and D, reducing the levels of topo IIα did not significantly alter melphalan-induced crosslinks ($p > 0.025$, pooled *t*-test). The results were very similar in both U937 and K562 cells, suggesting that the observation is not cell line specific. In addition, these data indicate that topo IIβ levels are a determinant of the levels of melphalan-induced crosslinks.

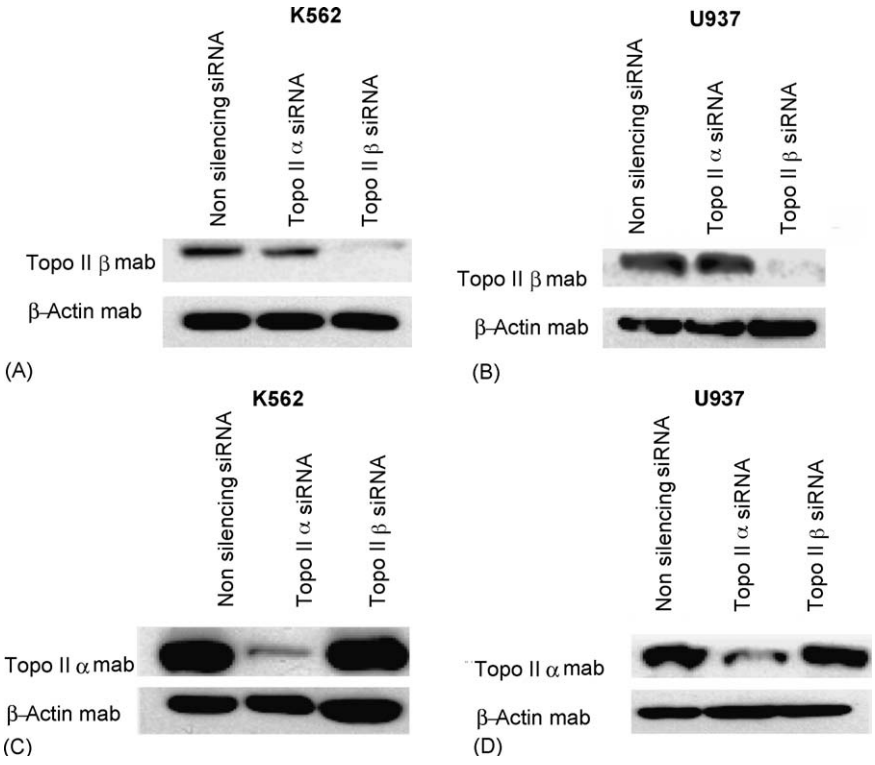

Fig. 1 – SiRNA constructs reduced topo II levels in both K562 and U937 cells as determined by Western blot analysis. (A and B): Targeting topo IIβ reduced topo IIβ levels in K562 and U937 cells by 95 and 69%, respectively compared to the non-silenced transfected control cells ($n = 3$ independent experiments). (C and D): Targeting topo IIα with siRNA constructs reduced topo IIα levels in K562 and U937 cells by 83% and 66%, respectively ($n = 3$ independent experiments).

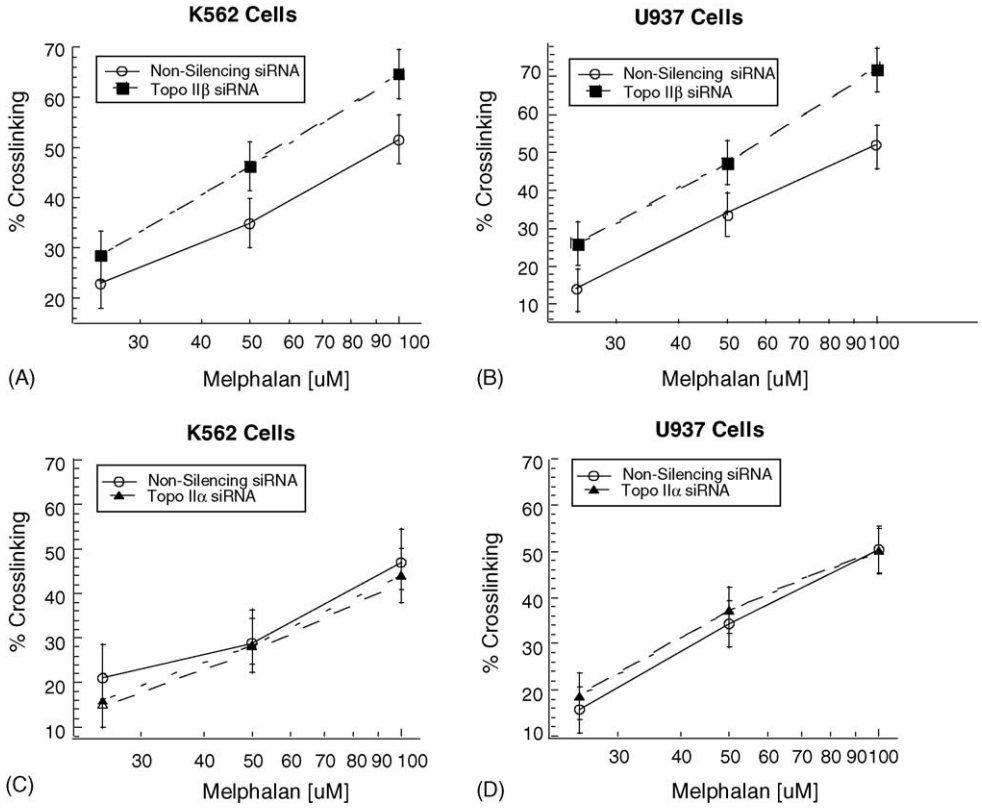

Fig. 2 – Reducing topo IIβ (but not topo IIα) levels increases melphalan-induced crosslinks. K562 cells and U937 cells were silenced for either topo IIα or β as previously described. Following siRNA treatment, cell populations were either exposed to vehicle control or varying dose of melphalan for 2 h. After drug exposure the alkaline comet assay was used to detect melphalan induced-crosslinks. (A and B): K562 and U937 cells with reduced topo IIβ levels show significantly increased levels of melphalan induced crosslinks ($p < 0.025$ $n = 3$ independent experiments, 51 images were analyzed per experiment, Student's *t*-test), compared to cell transfected with non-silencing siRNA. Shown are the estimated mean, and the upper and lower 95% confidence interval as determined by ANOVA. (C and D): Reducing topo IIα levels in K562 or U937 cells did not change the levels of melphalan-induced crosslinks ($p > 0.025$, $n = 3$ independent experiments and 51 images were analyzed per experiment, Student's *t*-test) compared to non-silenced control cells. Shown are the estimated mean, and the upper and lower 95% confidence interval as determined by ANOVA.

3.3. *Repair of melpahlan induced crosslinks*

In order to address the concern of DNA damage due to apoptosis, we chose to measure the levels of melphalan-induced crosslinks at an early time point of 2 h-post melphalan treatment. However, these studies do not clearly define whether the difference in crosslinks is the result of increased crosslink formation or the inhibition of the repair of melpahlan-induced DNA crosslinks. Thus, to assess repair of melphalan induced crosslinks, we performed a time course study. K562 cells were chosen for this assay as BCR-ABL positive cells are relatively resistant to apoptosis, which limits the concerns of measuring DNA damage induced by activation of endonucleases [6]. Previous studies have indicated that maximal levels of melphalan-induced crosslinks are typically observed between 5 and 8 h post drug treatment [7]. In order to assess equivalent crosslink repair, the non-silencing and topo IIα siRNA transfected K562 cells were treated with 50 μM melphalan, while topo IIβ siRNA transfected K562 cells were treated with 25 μM melphalan. Following the 2 h drug treatment, cells were washed, drug-free media added and crosslinks were measured at the indicated time points (see Fig. 3). As shown in Fig. 3, this dosing schedule resulted in the expected near equal numbers of crosslinks in all test groups at the 5 h time point. However, as predicted, the topo IIβ silenced cells demonstrated an increase in the level of melphalan-induced crosslinks at the 24 h time point ($p < 0.025$ Student's *t*-test) compared to non-silenced control cells. Decreased repair of melphalan-induced crosslinks in topo IIβ depleted cells is most pronounced at the early time points. These data suggest that repair of melphalan induced-crosslinks may be biphasic with respect to the dependency of topo IIβ and that topo IIβ is only involved in the initial phase of repair of melphalan-induced crosslinks. Consistent with our previous data, reducing topo IIα levels did not alter the level of melphalan-induced crosslinks at the 24 h time point compared to the non-silenced controls ($p > 0.025$, ANOVA).

3.4. *Melphalan induced apoptosis*

To assess whether results from the comet assay correlated with cell survival, we measured melphalan-induced apoptosis

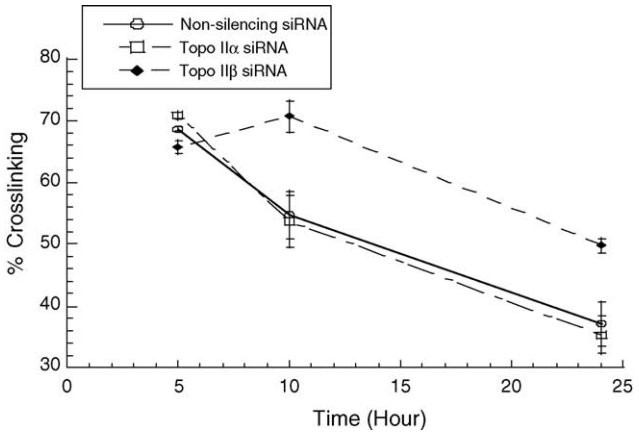

Fig. 3 – K562 cells with reduced topo IIβ levels show a decrease in the rate of repair of melphalan-induced crosslinks. In order to start with an equivalent number of crosslinks, topo IIβ silenced cells were treated with 25 μM melphalan and topo IIα and non-silenced cells were treated with 50 μM melphalan for 2 h. Following 2 h of drug treatment, cells were centrifuged and re-suspended in drug-free media. Crosslinks were measured at the indicated times by the alkaline comet assay. Shown are the estimated mean, and the upper and lower 95% confidence interval as determined by ANOVA ($n = 3$ independent experiments, 51 images analyzed per experiment).

in cells with reduced topo IIα and β levels. As shown in Fig. 4A, reducing either topo IIβ or topo IIα levels did not alter the amount of background cell death. This suggests that depleting topo II levels does not induce cell death. In addition, cells with reduced topo IIβ levels showed a two-fold increase in melphalan-induced apoptosis compared to the non-silenced control cells (see Fig. 4B). In contrast, reducing topo IIα levels did not sensitize cells to melphalan-induced apoptosis. Shown in Fig. 4C is the drug specific apoptosis (drug-treated-vehicle control) of a representative experiment performed in triplicate. The level of increased sensitivity to apoptosis is relatively small. However, this two-fold difference is similar to what we recently reported for silencing of FANCF [8]. FANCF is a known component of the FA/BRCA DNA repair pathway, and mutation in the FA pathway results in chromosome fragility, hypersensitivity to crosslinking agents and increased susceptibility to cancer [9]. Together, these data indicate that increased levels of melphalan induced crosslinks, as detected by the alkaline comet assay, correlate with sensitivity to melphalan-induced apoptosis. Furthermore, agents regulating topo IIβ levels and/or activity may represent a viable strategy for increasing the efficacy of alkylating agents.

3.5. *Clonogenic assay*

A clonogenic assay was performed to determine whether reducing topo IIβ levels increased cell survival as measured by the ability of cells to divide and form colonies following drug treatment. As shown in Fig. 5, cells with reduced topo IIβ levels showed a trend towards increased sensitivity of melphalan induced cell death. This difference in survival between cells with reduced topo IIβ and either control or cells with reduced topo IIα is smaller compared to differences observed when apoptosis was used an endpoint. This observation may be due to (a) differences in sensitivities of the assays (b) knocking down topo IIβ with siRNA is transient and thus siRNA strategies may not be appropriate for clonogenic assays requiring 9 days to measure the endpoint or (c) topo IIβ is a determinant of apoptosis but plays a smaller role in cell death and survival mediated by other pathways such as necrosis. Further studies are warranted to determine whether topo IIβ is a determinant of only apoptotic cell death induced by alkylating agents.

3.6. *Cell cycle progression*

Topo II plays an important role in DNA replication and mitosis. In fact, Akimitsu et al., demonstrated that topo IIα−/− mice are embryonic lethal at the four or eight-cell stage, suggesting that topo IIα is critical for cell division [10]. Because cell cycle kinetics are intricately linked to DNA repair processes, we wanted to determine what effect reducing the levels of topo IIα or β had on cell cycle progression. We used BrdU labeling to determine the percentage of cells actively incorporating nucleotides in S-phase. PI staining was used to determine the percentage of cells residing in G1 and G2-M. As shown in Fig. 6A–D, reducing either topo IIα or β levels did not alter the cell cycle kinetics of K562 cells compared to non-silencing control. These data suggest that differences in the repair of melphalan-induced crosslinks in topo IIβ depleted cells are not caused by alterations in cell cycle kinetics prior to melphalan treatment.

Although topo IIα is required for embryonic cell division, Sakaguchi et al. recently demonstrated that the dependency on topo IIα for cell division is not required for HeLa cells [11]. These investigators, using siRNA strategies demonstrated that knocking down the expression of either topo IIα or β was not sufficient for inhibiting condensation or segregation of chromosomes. However, reducing both topo IIα and β levels by siRNA did inhibit segregation of chromosomes [12]. We made a similar observation, as reducing topo II levels with siRNA did not result in accumulation of cells in a specific phase of the cell cycle. The observed differences in the requirement of topo IIα for cell division may reflect either differences in malignant versus embryonic cells or the fact that siRNA transfection does not completely silence the expression of topo II.

4. Discussion

Although in vitro studies have shown similar biochemical properties for topo II, in vivo studies have revealed important functional differences between topo II isoforms. For example, as mentioned earlier, Akimitsu et al. demonstrated that topo IIα−/− mice are embryonic lethal at the four or eight-cell stage suggesting that topo IIα is critical for cell division [10]. In contrast, topo IIβ −/− mice die at birth due to neural and neuromuscular defects [12,13]. These data suggest that in

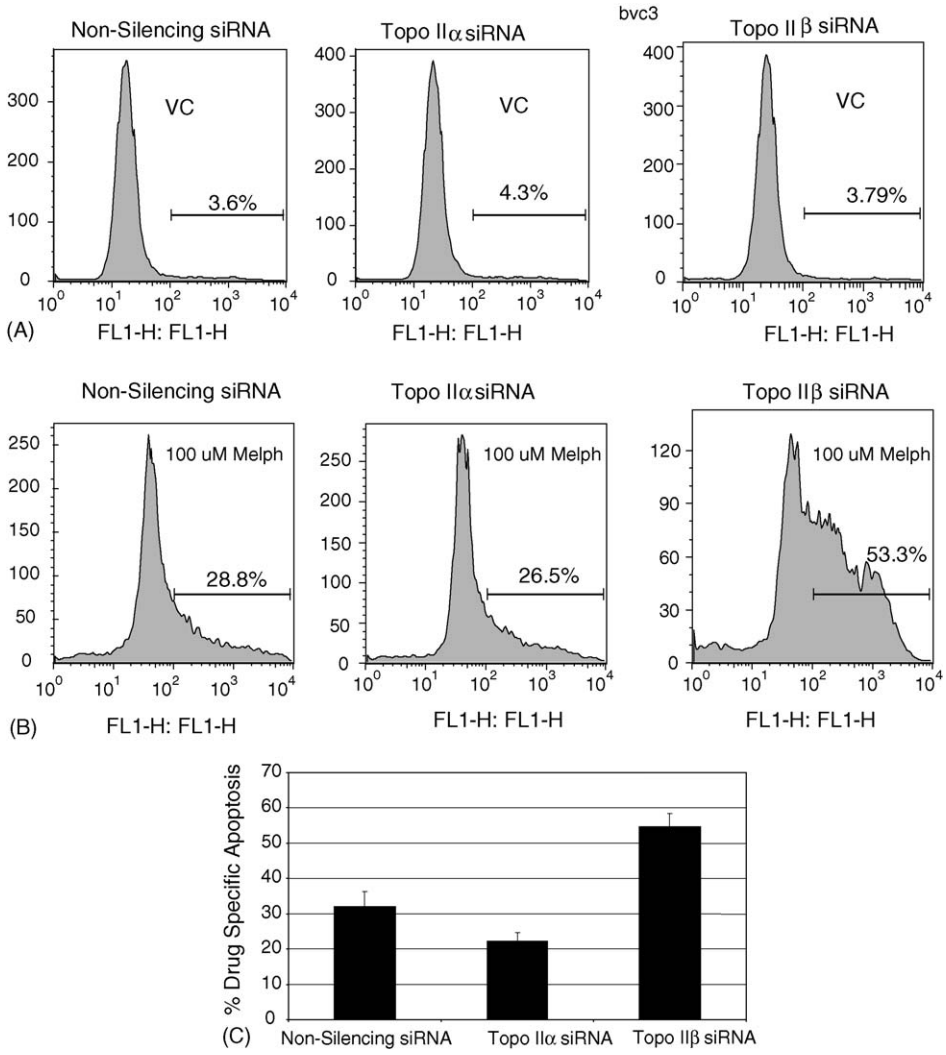

Fig. 4 – K562 cells with reduced topo IIβ levels are more sensitive to melphalan induced cell death compared to topo IIα silenced cells or cells transfected with non-silencing siRNA ($p < 0.05$ Student's *t*-test). (A and B): Following siRNA treatment, cells were treated with either vehicle control (vc) or 100 μM melphalan for 2 h and then placed in drug free media for an additional 24 h. Twenty-four hours after drug exposure, melphalan induced apoptosis was measured by annexin V staining and FACS analysis. (C): Shown is a representative figure performed in triplicate. The experiment was repeated in three independent experiments and similar results were obtained. Percent drug specific apoptosis = drug treated − vehicle control.

contrast to topo IIα, topo IIβ is not required for cell division but rather is essential for neuronal development. The exact mechanism whereby topo IIβ contributes to neuronal differentiation is currently poorly understood. However, there is evidence that deficiency in DNA repair pathways can lead to a similar neurological phenotype in mice [14,15].

It is attractive to speculate that topo II may be required for multi-protein repair complexes to gain access to localized damaged lesions of DNA. Circumstantial evidence in drug resistant lines has indicated that a correlation exists between topo II activity and sensitivity to crosslinking agents. However, no direct evidence for the participation of topo II in DNA repair in intact cells has been reported. In vitro data indicate that the catalytic activity of both topo IIα and β can be stimulated by abasic, oxidized and mono-alkylated DNA [16]. However, these studies were performed with either plasmids or short double stranded oligonucleotides, and thus it is difficult to extrapolate the significance of these findings for highly structured cellular DNA. Prior to this report, only correlative data were available to suggest that topo II may play a role in the repair of DNA damage in mammalian cell based systems.

Several DNA repair pathways can contribute to the repair of crosslinks induced by alkylating agents. Further studies are warranted to determine what DNA repair pathway(s) involve topo IIβ. These repair pathways include non-homologous end joining (NHEJ), and homologous recombination (HR) pathways. Recent experimental evidence suggests that homo-

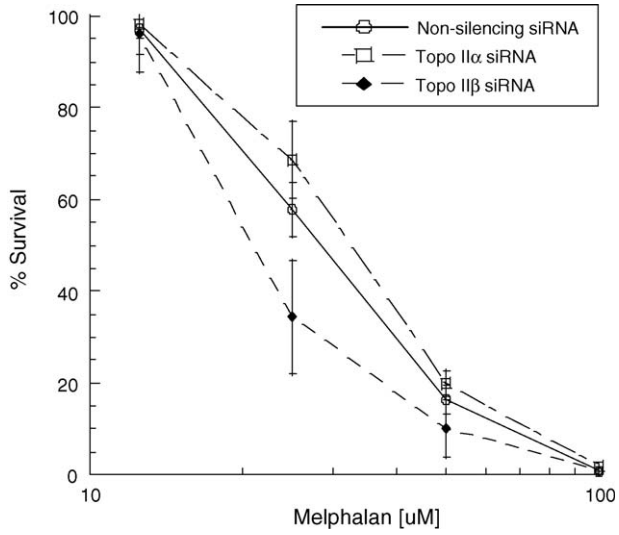

Fig. 5 – K562 cells with reduced topo IIβ show increased melphalan induced cell death as measured by a clonogenic assay. Two independent experiments were performed and shown is a representative figure.

logous recombination and specifically the Fanconi anemia (FA/BRCA pathway) is a key determinant of repair of interstrand DNA crosslinks [4,8,17,18]. NHEJ is predominately mediated by the PI-3 kinase family member DNA-PK. Mutations in DNA-PK result in hypersensitivity to both gamma radiation and crosslinking agent-mediated cell death [19]. Moreover, Ku80 knockouts show increased sensitivity to alkylating agents [20]. In summary, experimental evidence indicates that both the FA/BRCA and DNA-PK pathway can contribute to the repair of DNA damage induced by an alkylating agent. We are currently pursuing whether topo IIβ contributes to either of these two repair pathways.

In conclusion, our data show that topo IIβ levels are a determinant of the magnitude of DNA crosslinks and cell survival following treatment with melphalan. These studies suggest non-redundant roles for the two isoforms of topo II in DNA repair. Furthermore, the observed results could not be attributed to differences in cell cycle kinetics in topo II depleted cells. Additional studies are warranted to determine which DNA repair pathway(s) topo IIβ facilitates and whether topo IIβ contributes to genome surveillance and tumorigenesis.

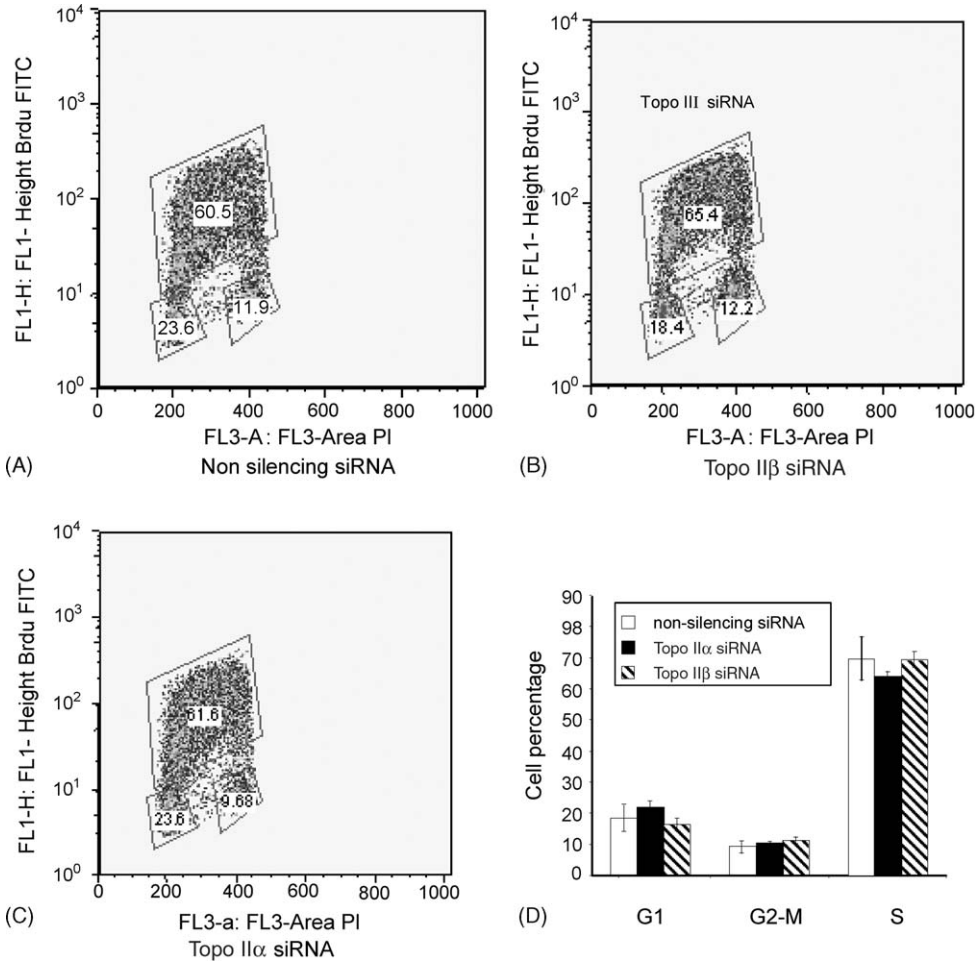

Fig. 6 – K562 cells with reduced topo IIα or β are not growth arrested. (A–C): Fitc-anti-BrdU was used to detect cells in S-phase, while PI staining of DNA content was used to detect the percentage of cells residing in G1 and G2-M phase of the cell cycle. (D): Composite figure of mean and standard deviation of three independent experiments.

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REFERENCES

[1] Wang JC. Cellular roles of DNA topoisomerases: a molecular perspective. Nat Rev Mol Cell Biol 2002;3:430–40.
[2] Tan KB, Mattern MR, Boyce RA, Schein PS. Elevated DNA topoisomerase II activity in nitrogen mustard-resistant human cells. Proc Natl Acad Sci USA 1987;84:7668–71.
[3] Barret JM, Calsou P, Larsen AK, Salles B. A cisplatin-resistant murine leukemia cell line exhibits increased topoisomerase II activity. Mol Pharmacol 1994;46:431–6.
[4] Hazlehurst LA, Enkemann SA, Beam CA, Argilagos RF, Painter J, Shain KH, et al. Genotypic and phenotypic comparisons of de novo and acquired melphalan resistance in an isogenic multiple myeloma cell line model. Cancer Res 2003;63:7900–6.
[5] Hazlehurst LA, Damiano JS, Buyuksal I, Pledger WJ, Dalton WS. Adhesion to fibronectin via beta1 integrins regulates p27kip1 levels and contributes to cell adhesion mediated drug resistance (CAM-DR). Oncogene 2000;19:4319–27.
[6] Bedi A, Barber JP, Bedi GC, el-Deiry WS, Sidransky D, Vala MS, et al. BCR-ABL-mediated inhibition of apoptosis with delay of G2/M transition after DNA damage: a mechanism of resistance to multiple anticancer agents. Blood 1995;86:1148–58.
[7] O'Connor PM, Kohn KW. Comparative pharmacokinetics of DNA lesion formation and removal following treatment of L1210 cells with nitrogen mustards. Cancer Commun 1990;2:387–94.
[8] Chen Q, Van der Sluis PC, Boulware D, Hazlehurst LA, Dalton WS. The FA/BRCA pathway is involved in melphalan-induced DNA interstrand cross-link repair and accounts for melphalan resistance in multiple myeloma cells. Blood 2005;106:698–705.
[9] Wong JC, Buchwald M. Disease model: Fanconi anemia. Trends Mol Med 2002;8:39–42.
[10] Akimitsu N, Adachi N, Hirai H, Hossain MS, Hamamoto H, Kobayashi M, et al. Enforced cytokinesis without complete nuclear division in embryonic cells depleting the activity of DNA topoisomerase IIalpha. Genes Cells 2003;8:393–402.
[11] Sakaguchi A, Kikuchi A. Functional compatibility between isoform alpha and beta of type II DNA topoisomerase. J Cell Sci 2004;117(Pt 7):1047–54.
[12] Lyu YL, Wang JC. Aberrant lamination in the cerebral cortex of mouse embryos lacking DNA topoisomerase IIbeta. Proc Natl Acad Sci USA 2003;100:7123–8.
[13] Yang X, Li W, Prescott ED, Burden SJ, Wang JC. DNA topoisomerase IIbeta and neural development. Science 2000;287:131–4.
[14] Frappart PO, Tong WM, Demuth I, Radovanovic I, Herceg Z, Aguzzi A, et al. An essential function for NBS1 in the prevention of ataxia and cerebellar defects. Nat Med 2005;11:538–44.
[15] Lee Y, Barnes DE, Lindahl T, McKinnon PJ. Defective neurogenesis resulting from DNA ligase IV deficiency requires Atm. Genes Dev 2000;14:2576–80.
[16] Sabourin M, Osheroff N. Sensitivity of human type II topoisomerases to DNA damage: stimulation of enzyme-mediated DNA cleavage by abasic, oxidized and alkylated lesions. Nucleic Acids Res 2000;28:1947–54.
[17] D'Andrea AD. The Fanconi Anemia/BRCA signaling pathway: disruption in cisplatin-sensitive ovarian cancers. Cell Cycle 2003;2:290–2.
[18] Rothfuss A, Grompe M. Repair kinetics of genomic interstrand DNA cross-links: evidence for DNA double-strand break-dependent activation of the Fanconi anemia/BRCA pathway. Mol Cell Biol 2004;24:123–34.
[19] Tanaka T, Yamagami T, Oka Y, Nomura T, Sugiyama H. The scid mutation in mice causes defects in the repair system for both double-strand DNA breaks and DNA cross-links. Mutat Res 1993;288:277–80.
[20] Muller C, Calsou P, Salles B. The activity of the DNA-dependent protein kinase (DNA-PK) complex is determinant in the cellular response to nitrogen mustards. Biochimie 2000;82:25–8.