Environmental and Molecular Mutagenesis 53:608–618 (2012)

Research Article

DNA-PKcs-Dependent NHEJ Pathway Supports the Progression of Topoisomerase II Poison-Induced Chromosome Aberrant Cells

Maria Eugenia Elguero, Marcelo de Campos-Nebel, and Marcela González-Cid*

Laboratorio de Mutagenesis, Instituto de Medicina Experimental, IMEX-CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina

The role of DNA double strand break (DSB) repair pathways, non-homologous end joining (NHEJ), and homologous recombination (HR) was evaluated to prevent the chromosome instability induced by the topoisomerase II (Top2) poisons, idarubicin, and etoposide in Chinese hamster cell lines. XR-C1 (DNA-PKcs deficient) and V-C8 (BRCA2 deficient) showed higher sensitivity to increased concentrations of Top2 poisons compared with their normal counterparts, CHO9 and V79. Both proficient and deficient cells exhibited a marked DSB induction in all phases of the cell cycle. Additionally, deficient cells showed persistent DNA damage 24 hr post-treatment. Chromosomal aberrations increased in the first mitosis following Top2 poison-treatments in G1 or G2 in proficient and deficient cells. CHO9 and V79 demonstrated chromosome and chromatid exchanges following treatments in G1 and G2 phases, respectively. Deficient cells showed high frequencies of chromatid exchanges following treatments in G1 and G2. Simultaneously, we analyzed the micronuclei (MN) induction in interphase cells after treatments in G1, S, or G2 of the previous cell cycle. Both Top2 poisons induced an important increase in MN in CHO9, V79, and V-C8 cells. XR-C1 exhibited an increased MN frequency when cells were treated in G1 phase but not in S or G2. This MN reduction was due to a cell accumulation at G2/M and death in G2-treated cells. Our data suggest that NHEJ and HR operate differentially throughout the cell cycle to protect from Top2 poison-induced chromosome instability, and that DNA-PKcs-dependent NHEJ pathway allows the survival of chromosome damaged cells during S/G2 to the next interphase. Environ. Mol. Mutagen. 53:608–618, 2012.

Key words: double strand break repair; chromosome integrity; micronucleus; idarubicin; etoposide; Chinese hamster cell lines

INTRODUCTION

DNA-topoisomerase type II (Top2) is a well-established target in cancer therapy as it plays critical roles in a wide range of cellular processes, such as, replication, transcription, and chromosome segregation [McClendon and Osheroff, 2007]. Top2 causes transient breaks in both strands (DSB) of the DNA involving a covalent enzyme-DNA intermediate, the cleavable complex. Under normal conditions, these cleavable complexes are present at low levels and are well tolerated by the cell [Mosesso et al., 1998]. However, anticancer drugs, such as, the anthracycline idarubicin (IDA) and the epipodophyllotoxin etoposide (ETO) stabilize the cleavable complex preventing the religation of DNA DSB induced by the enzyme [de Campos-Nebel et al., 2010]. As these drugs transform the enzyme into a potent cellular toxin, they are called Top2 poisons [Fortune and Osheroff, 2000].

Despite the importance of these drugs in cancer chemotherapy, patients that are treated with regimens that include Top2-targeted agents eventually develop secondary neoplasms. The risk of therapy-related myeloid neoplasms after epipodophyllotoxins averages from 2% to 12%, with a latency time ranging from 1 to 3 years from the primary treatment [Leone et al., 2010].

DNA DSB is one of the most dangerous DNA lesions experienced in human cells, as a single DSB can poten-

Grant sponsor: National Research Council (CONICET), Grant Number PIP 5896; Grant sponsor: A. J. Roemmers Foundation.

*Correspondence to: Marcela González-Cid, Lab. de Mutagenesis, Instituto de Medicina Experimental IMEX-CONICET, Academia Nacional de Medicina, J. A. Pacheco de Melo 3081, 1425-Buenos Aires, Argentina. E-mail: margoncid@hematologia.anm.edu.ar

Received 6 June 2012; provisionally accepted 18 July 2012; and in final form 30 July 2012

DOI 10.1002/em.21729

Published online 18 September 2012 in Wiley Online Library (wileyonlinelibrary.com).

tially lead to loss of more than 100 million base pairs of genetic information (e.g., loss of an entire chromosome arm) [Helleday et al., 2007]. Moreover, DNA DSB can result in an extensive diversity of genetic alterations including large- or small-scale deletions, loss of heterozygosity, translocations, and chromosome loss [Riches et al., 2008]. This genomic instability at the chromosomal level is a common feature of most human tumors and occurs early in the multistep process of tumorigenesis.

DNA repair is essential for the successful maintenance and propagation of genetic information [Kanaar et al., 1998]. In mammals, non-homologous end joining (NHEJ) and homologous recombination (HR) are the two major pathways that repair DSB. However, NHEJ repairs DSB through an imprecise pathway that joins ends together, HR used a complementary template, usually provided by the sister chromatid, for an accurate repair process.

DSB repair is tightly regulated during the cell cycle. In G1 phase, the absence of a sister chromatid makes NHEJ the prominent pathway for DSB repair. In late S and G2 phases, even though NHEJ remains functional, there is an increase in the repair of DSB by HR [Takata et al., 1998; Rothkamm et al., 2003; Mao et al., 2008; Natarajan et al., 2008; Takashima et al., 2009].

The enzyme DNA-PKcs plays a direct role in DSB repair by acting as a key component of NHEJ and defects in its kinase activity or its expression result in cancer predisposition [Auckley et al., 2001; Fu et al., 2003; Lee et al., 2007; Peddi et al., 2010]. A lack of functional DNA-PKcs leads to increased sensitivity to ionizing radiation, the anthracycline adriamycin and other DNA damaging agents [Shen et al., 1998; Lee and Kim, 2002].

There are many proteins involved in HR including the breast cancer associated gene, BRCA2. This gene is an important component of the DNA damage response pathway, and loss of its function is considered a major factor in cancer predisposition [Abaji et al., 2005]. BRCA2 mutation carriers also have increased risks of developing a variety of cancers including ovarian, pancreatic, prostate, stomach, breast cancer, and melanoma [Antoniou et al., 2003; Turnbull and Hodgson, 2005; Tai et al., 2007]. BRCA2-mutant cells and embryos are hypersensitive to ionizing radiation and other DNA damaging agents and develop numerous spontaneous chromosomal abnormalities [Abbott et al., 1998; Treszezamsky et al., 2007].

Our previous results have demonstrated that DNA DSB induced by IDA and ETO were repaired by both NHEJ and HR in a specific cell cycle manner [de Campos-Nebel et al., 2010].

Therefore, we hypothesized that a defective genetic background in NHEJ or HR could affect differently, the chromosome stability maintenance throughout the cell cycle. To evaluate this hypothesis, we analyzed the contribution of both DSB repair pathways to protect the chromosome integrity and to prevent the propagation of abnormal cells following treatment with Top2 poisons in Chinese hamster cell lines.

MATERIALS AND METHODS

Cells and Culture Conditions

Mutant Chinese hamster cell lines XR-C1, DNA-PKcs deficient cells [Errami et al., 1998], and V-C8, BRCA2 deficient cells [Kraakman-van der Zwet et al., 2002] with their correspondent parental cell lines, CHO9 and V79 were kindly provided by Prof. Dr. M.Z. Zdzienicka (Nicolaus Copernicus University, Bydgoszcz, Poland) and Dr. W. Wiegant (Leiden University Medical Center, Leiden, The Netherlands). CHO9 and XR-C1 were grown in F-12 medium (Gibco, Life Technologies Corporation, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (Natocor, Villa Carlos Paz, Córdoba, Argentina), 2 mM L-glutamine (Gibco, Life Technologies Corporation, Grand Island, NY), penicillin (100 units/ml), and streptomycin (100 μg/ml). V79 and V-C8 were cultured in Mc Coy's 5A medium (without hypoxanthine and thymidine, Gibco, Life Technologies Corporation, Grand Island, NY) supplemented with 10% FBS, L-glutamine, and antibiotics. Cultures were maintained at 37°C under a 5% CO_2 humidified atmosphere.

Chemicals

IDA (CAS no. 58957-92-9, Zavedos®, Pharmacia y Upjohn), thymidine (CAS no. 50-89-5, Sigma-Aldrich, St Louis, MO) and 5-bromo-2′-deoxyuridine (BrdU, CAS no. 59-14-3, Sigma-Aldrich, St Louis) were dissolved in bidistilled water. ETO (CAS no. 33419-42-0, Sigma-Aldrich, St Louis) was dissolved in dimethyl sulfoxide (DMSO) (CAS no. 67-68-5, JT Baker, Mallinckrodt Baker, Phillipsburg, NY). Control cultures were exposed to equivalent volumes of corresponding solvents of the drugs. Unless otherwise specified, the cells were treated with IDA 0.005 μg/ml or ETO 5.0 μg/ml.

MTT Assay

Cell viability was determined by the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich, St Louis) reduction assay. Cells ($1.0–2.5 \times 10^3$ per well) were seeded into 96-well plates and incubated overnight at 37°C. Different doses of IDA (0.0001, 0.00025, 0.0005, 0.00075, and 0.001 μg/ml) or ETO (0.05, 0.1, 0.25, 0.5, and 0.75 μg/ml) were added for 20 hr. After drug treatment, cells were washed twice in phosphate buffered saline (PBS) and incubated in fresh medium. At 72 hr, 100 μl of MTT (1 mg/ml) in PBS was added to each well followed by incubation for 3–4 hr in the dark at 37°C. The supernatant was removed and, after washing, 100 μl DMSO was added. The absorbance of each sample was measured using an Elisa reader at 570 nm and expressed as the percentage of control. Four independent experiments were performed in triplicates.

γH2AX Detection by Flow Cytometry

Exponentially growing cell lines were plated in 60 mm dishes for at least 20 hr before a 1-hr treatment with IDA or ETO. Thereafter, the medium was removed; cells were washed twice in PBS and immediately harvested or incubated in fresh medium for an additional of 3 or 24 hr. The cells were trypsinized, and cellular suspensions were fixed with 1% paraformaldehyde for 10 min at 37°C. After blocking for 30 min in blocking buffer (3% FBS, 0.25% Triton X-100 in PBS), cells were incubated with a mouse anti-γH2AX antibody (Upstate Biotechnology, NY, 1:800) for 18 hr at 4°C. The cells were then resuspended in blocking buffer containing an anti-mouse secondary antibody conjugated with fluorescein isothiocyanate (FITC, Vector Laboratories, CA, 1:100) for 30 min at room temperature in the dark. Samples were counterstained with 10 μg/ml propidium iodide in the presence of 150 μg/ml RNAse A for 15 min at room temperature. A total of 20,000 cells per sample were evaluated using a FACScan flow cytometer (Becton-Dickinson, San Jose, CA). After normalizing for histone content, the mean value of γH2AX

intensity of fluorescence (IF) was calculated as described by Huang et al. [2004]. Two independent experiments were performed for each treatment.

Chromosomal Aberrations and Mitotic Index

Cell lines were plated in 60 mm dishes, enriched in G1 and G2 phases and treated with IDA or ETO for 1 hr. Cultures treated in G1 or G2 were then washed twice with PBS and incubated in fresh medium for 18 or 4 hr, respectively, to allow the cells to arrive to the first metaphase. Colcemid (0.2 μg/ml) was added 2.5–3.0 hr before harvesting, and then, cells were trypsinized and collected by centrifugation. Cells were exposed to hypotonic solution 0.075 M KCl, fixed in methanol:acetic acid (3:1) and stained with 10% Giemsa for 4 min. For each treatment, 50 metaphases were analyzed for the induction of chromosomal aberrations (CA). The gaps were excluded from the analysis of CA frequencies. Aberrations were classified as chromosome and chromatid breaks, and chromosome and chromatid exchanges. Chromatid and chromosome breaks were scored as one break and quadriradial and triradial chromosome configurations, dicentric and rings chromosomes were scored as two breaks. Complete and incomplete chromatid exchanges and intrachanges in the same configuration were considered as complex exchange configurations. Mitotic index (MI) was calculated as the number of metaphases among 1,000 nuclei. Two independent experiments were performed for each end point.

Micronucleus Formation

Cell lines were grown on coverslips in 35 mm dishes and enriched in G1, S, and G2 phases. At each phase, the cells were treated with IDA or ETO for 1 hr. After washing twice in PBS, complete medium was added for 20, 12–14, or 8–10 hr; respectively, to reach the following cell cycle interphase. The cells were fixed with 1% paraformaldehyde and permeabilized with 0.25% Triton X-100 in PBS for 20 min at room temperature. DNA was stained with 4,6-diamidino-2-phenylindole (DAPI, Vector Laboratories, CA) containing mounting medium. MN frequencies were scored in 1,000 nuclei per sample in three independent experiments.

Statistical Analysis

Differences between the mean of cell viability, γH2AX IF, MI, and MN were analyzed using the Students' t-test. The CA were evaluated by the χ^2 test (Primer of Biostatistics, version 3.0 by S.A. Glantz, McGraw-Hill, 1992).

RESULTS

To elucidate whether DNA DSB repair defects may affect the cellular sensitivity to Top2 poisons, we evaluated the response to these agents in NHEJ- and HR-deficient hamster cell lines using the MTT assay. The results from cell viability experiments are shown in Figure 1. The cell lines studied showed a concentration related decrease of cell viability following IDA or ETO treatment. V-C8 cells appear substantially more sensitive than XR-C1. The deficient cells, V-C8 and XR-C1 are hypersensitive to ETO and V-C8 cells to IDA. However, the hypersensitivity of XR-C1 to IDA is only observed at the highest concentration.

The cellular induction of phosphorylated H2AX on Ser 139 (γH2AX) following exposure to genotoxic agents is considered to be a sensitive and specific reporter of DNA damage, in particular, DNA DSB [Huang et al., 2004]. To assess the induction of DSB and the different profiles of remaining DNA damage resulting from poisoning Top2 in NHEJ- and HR-deficient genetic backgrounds, we analyzed the levels of γH2AX at different times in the Chinese hamster cell lines. The time of maximal induction of DSB was determined in CHO9 and V79 cells following 1 hr treatment with IDA 0.005 μg/ml or ETO 5.0 μg/ml and sampling at different times post-treatment (1, 3, and 6 hr, data not shown). Both proficient cells exhibited a marked induction of DSB in all phases of the cell cycle, with values peaking up to 1 and 3 hr after treatment with ETO and IDA, respectively (Fig. 2A). Similarly, DNA-PKcs and BRCA2-deficient cell lines showed a higher increase of γH2AX levels compared with wild type cells at the times assayed (data not shown). In addition, these mutant cell lines accumulated increased levels of persistent DNA damage 24 hr post-treatment with Top2 poisons (Figs. 2B–2D). After IDA or ETO treatment, the residual damage was 11.08% and 13.95% for CHO9 cells, while XR-C1 cells showed percentages of 76.68% and 36.78%, respectively. Likewise, residual levels of damage in V79 cell line were 37.72% and 4.74% for IDA and ETO, respectively, while V-C8 mutant cells showed levels of 47.35% and 37.83%. Thus, the analysis of γH2AX revealed a high number of remaining DNA breaks in either NHEJ- or HR-deficient cells, when compared with their isogenic wild type counterparts.

As DNA DSB are the ultimate lesions that may be converted into CA by misrepair or unrepair, the persistence of increased numbers of DSB will raise the probability of CA formation. To assess the contribution of NHEJ and HR pathways to protect from chromosomal instability at different cell cycle stages, we analyzed the structural CA in the first mitosis following treatments in G1 or G2 phase in the Chinese hamster cell lines. The cell cultures were enriched at the different cell cycle phases by using the protocol described in Figure 3.

The types and frequencies of CA are presented in Tables I and II. The high rate of spontaneous CA in V-C8 cells was previously described [Kraakman-van der Zwet et al., 2002; de Campos-Nebel et al., 2008]. The percentage of abnormal cells following treatment with both IDA and ETO was increased in proficient and deficient cell lines ($P = 0.0001$). The proficient cell lines treated in G1 or G2 showed lower levels of chromatid and chromosome breaks than the mutant cells. A high frequency of spontaneous chromatid and chromosome types of exchanges was observed in V-C8 cells. Though, these CA were rarely found in the untreated V79 cell line.

It is well known that DNA damage occurring in G1 or prereplicative S-phase is manifested at mitosis as chromosome-type aberrations, whereas postreplicative DNA breakage results in chromatid-type aberrations [Wang

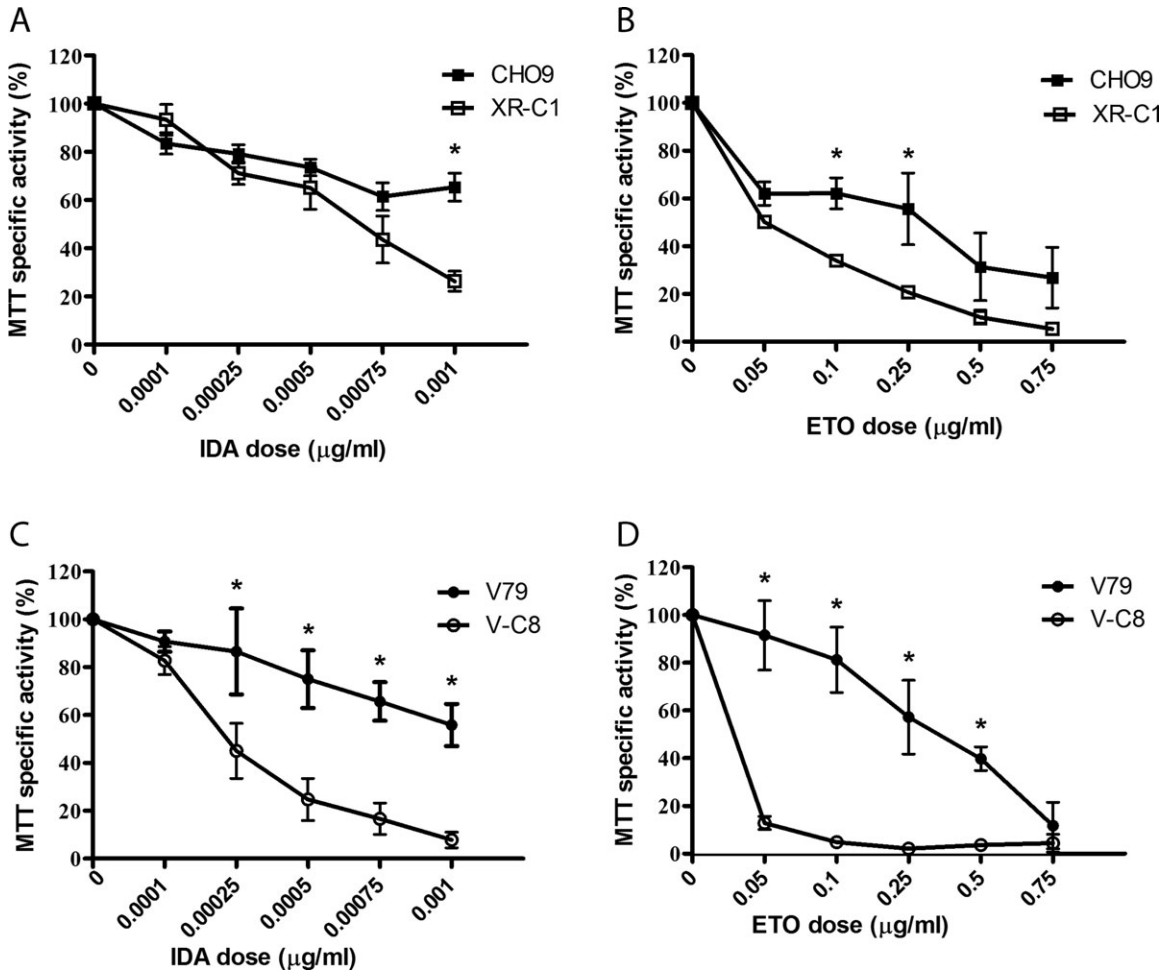

Fig. 1. Cell viability assessed by MTT assay. DNA repair proficient (CHO9 and V79) and deficient (XR-C1 and V-C8) Chinese hamster cell lines were treated with different concentrations of IDA (**A** and **C**) or ETO (**B** and **D**) for 20 hr and evaluated 72 hr later. Data represent the mean ± SD. * $P <$ 0.04, Students' t-test vs. respective deficient cell line.

et al., 2007]. CHO9 and V79 cells showed chromosome and chromatid exchanges after treatments in G1 and G2 phases, respectively. The DNA repair deficient cells showed high frequencies of chromatid exchanges following both G1- and G2-treatments, being the NHEJ mutant cells particularly susceptible. Complex exchange configurations and highly damaged cells were found in the wild-type cells treated with ETO in G2, as well as in the mutant cell lines treated with IDA or ETO in G1 and G2.

MI data are also presented in Tables I and II. A significant reduction of MI was shown after treatments in the proficient and deficient cells, indicating that both IDA and ETO partially blocked the entrance of cells into mitosis. Despite the decrease of the MI, chromosomal damage induced by both Top2 poisons was detected in metaphases.

To evaluate the activities of NHEJ and HR pathways in avoiding the induction of CA by treatments in G1 or G2 with Top2 poisons, a comparison was made by calculating the total break index (TBI) for each cell line (Fig. 4). For the quantification of the total breaks, the complex aberrations and the highly damaged cells were excluded, and a twofold induction over control was considered to be positive.

In the NHEJ-deficient cells, TBI values were 3.8- or 3.4-fold higher than their wild type counterpart CHO9 cells after G1-treatment with IDA or ETO, respectively. Meanwhile, the TBI between V79 and V-C8-treated cells revealed no significant differences.

Following the Top2-poison treatment in G2 phase, TBI in XR-C1 cells increased 5.4-fold after IDA and 2.0-fold after ETO compared with CHO9 cells. On the other hand, TBI calculated for IDA in the HR-deficient VC-8 cells exceeded the values for V79 cells by 5.2-fold. However, for ETO, an index of 1.6 was found. The existence of high levels of ETO-induced chromosomal damage in G2-treated CHO9 and V79 makes it difficult to evaluate the rise of damage in the deficient cells.

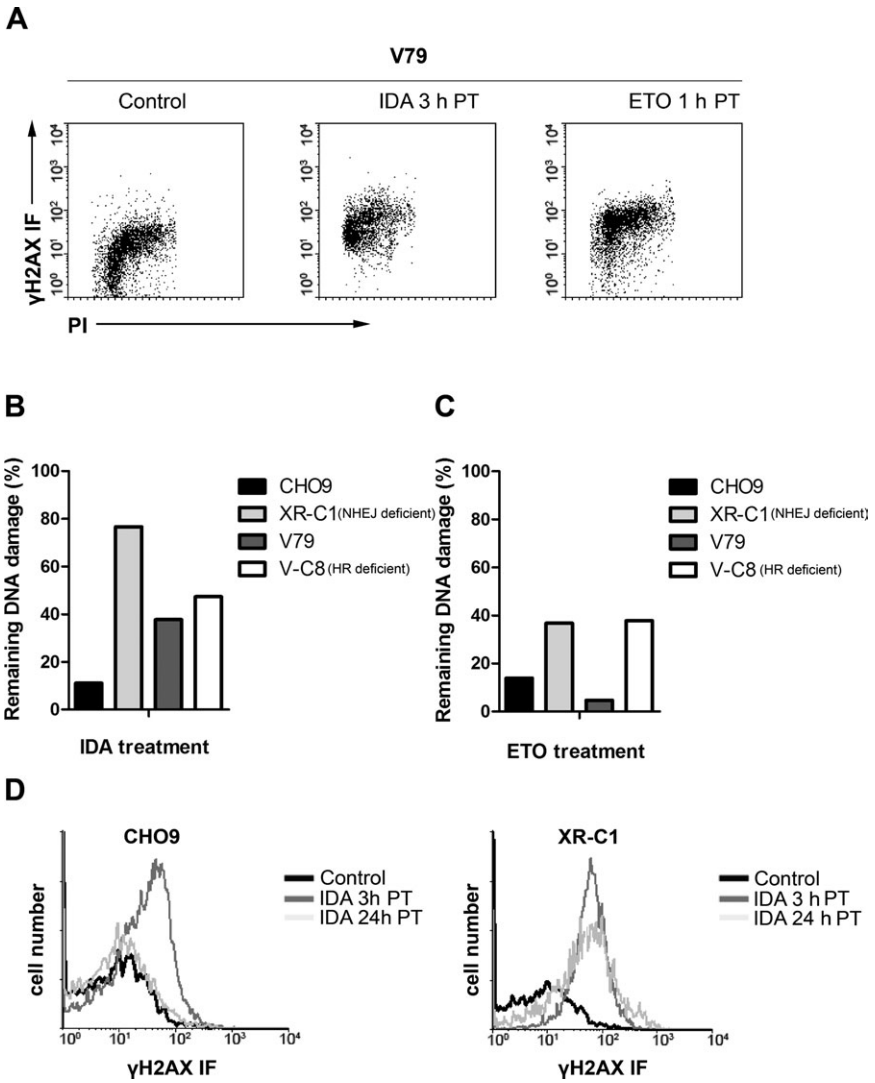

Fig. 2. DNA DSB repair deficient Chinese hamster cells retain increased γH2AX signals at 24 hr post-treatment (PT) with IDA (0.005 μg/ml) or ETO (5.0 μg/ml) by 1 hr. (**A**) Representative dot-plot graphics showing the maximal induction of γH2AX signals throughout the cell cycle in V79 cells by treatments with IDA or ETO. (**B** and **C**) The remaining DNA damage (γH2AX IF in treated cells at 24 hr PT-γH2AX IF in control cells/γH2AX IF in treated cultures at 1 or 3 hr PT-γH2AX IF in control cells × 100) at 24 hr PT with IDA or ETO. (**D**) Representative histograms of the γH2AX signals induced at different times by IDA in the wild-type (CHO9) and the DNA-PKcs-deficient (XR-C1) cell lines.

These data suggest that for the protection of Top2 poison-induced CA formation, NHEJ pathway is important throughout the cell cycle, and HR is required in G2 phase.

To further characterize the role of the DSB repair pathways to avoid the postmitotic persistence of DNA damage, we analyzed the induction of MN in interphase cells after treatments in G1, S, or G2 of the previous cell cycle. The clastogenic origin of MN induced by IDA and ETO was formerly assessed by immunofluorescence analysis of γH2AX signals in MN. We observed the percentage of γH2AX positive MN in V79 cell line treated with IDA 0.005 μg/ml or ETO 5.0 μg/ml for 1 hr at G2 phase. The results demonstrated that most of the micronucleated (MN) cells (86% for IDA and 89% for ETO) showed MN with a positive labeling for γH2AX.

Both IDA and ETO treatment induced a significant increase ($P < 0.02$) in the percentage of MN in all cell lines (Fig. 5). This increase was observed in proficient CHO9 and V79 cells treated at G1, S, and G2 phases, demonstrating that DNA damage occurring at different cell cycle phases is able to reach the next interphase in the DNA repair competent cells.

On the other hand, the NHEJ-mutant cell line exhibited an increased frequency of MN ($P < 0.001$) when cells were treated in G1 phase or in asynchronously growing cultures, where both of them showed similar amounts of cells in G1 (~80%, data not shown). This percentage was

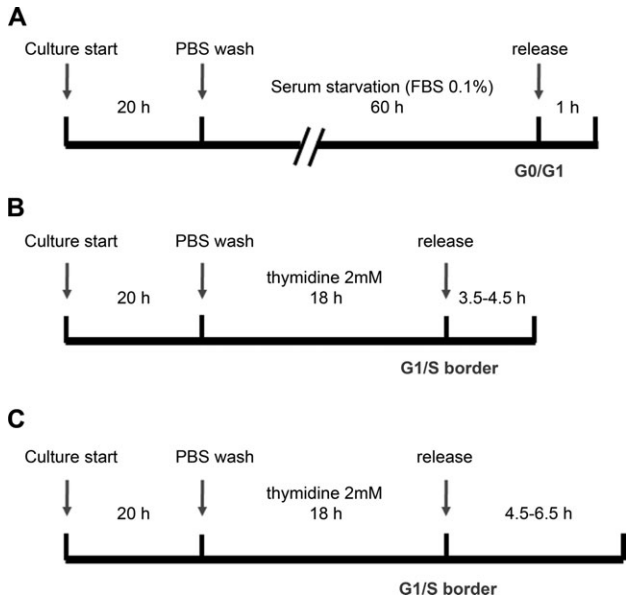

Fig. 3. Experimental schedule for the accumulation of CHO9 and V79 cells in G1, S, and G2 phases of cell cycle. (**A**) Enrichment of cell populations in G1 phase: After 20 hr of seeding, cells were washed in PBS and grown in medium with 0.1% FBS for 60 hr. Then, cells were released for 1 hr. (**B**) Enrichment of cell populations in S phase: After 20 hr of seeding, cells were washed in PBS and grown in complete medium with thymidine 2 mM for 18 hr. Then, cells were released for 3.5–4.5 hr. (**C**) Enrichment of cell populations in G2 phase: After 20 hr of seeding, cells were washed in PBS and grown in complete medium with thymidine 2 mM for 18 hr. Then, cells were released for 4.5–6.5 hr. Cell population distribution was evaluated in cells exposed to BrdU 150 μM during the last 30 min of release. Then cells were fixed in methanol 90%, treated with HCl 2 N and neutralized with borax buffer 0.1 M ($Na_2B_4O_7$, pH 8.5). BrdU incorporation was detected using a mouse anti-BrdU antibody (1:100, Santa Cruz Biotechnology, Santa Cruz, CA) followed by FITC conjugated anti-mouse antibody (Vector Laboratories, CA). The percentage of cells in the different phases of cell cycle obtained with these methodologies was checked by flow cytometry. The percentages were for G1 88.27% in CHO9 and 97.23% in V79, for S 78.79% in CHO9 and 83.30% in V79 and for G2 42.85% in CHO9 and 46.27% in V79. In asynchronously growing cultures, these percentages (mean ± SD) were for G1 62.80 ± 0.58 in CHO9 and 62.65 ± 0.77 in V79, for S 22.00 ± 0.49 in CHO9 and 33.38 ± 0.58 in V79 and for G2 15.20 ± 0.12 in CHO9 and 3.97 ± 0.19 in V79.

higher than that observed in the other cell lines (% of cells at G1 phase in asynchronous cultures was 62.8 ± 1.2 in CHO9, 62.7 ± 0.8 in V79, and 58.5 ± 8.2 in V-C8, data not shown). When the XR-C1 cells were treated in S ($P < 0.005$) or G2 ($P < 0.001$) phases, the MN frequencies diminished. To identify the possible cause of this reduction, we analyzed the effects of IDA and ETO on the cell cycle progression and cell death (Fig. 6). XR-C1 cells were treated in G2 phase and collected 8 hr after removal of Top2 poisons. Cellular DNA content was estimated using flow cytometry. Both IDA and ETO induced an accumulation at G2/M phases in a large proportion of cells. The simultaneous analysis by fluorescence microscopy after treatments showed cell death in XR-C1 cells. These effects could be responsible for the diminished MN formation in NHEJ-mutant cells.

In addition, the MN frequencies induced by IDA and ETO in the HR-mutant cells during different phases of the cell cycle were significantly increased ($P < 0.001$, Fig. 5).

However, V-C8 cells untreated or treated in G1 yield MN in a lower extension than those treated at the other cell cycle phases. We presume the methodology used to enrich the cell population at G1 phase may affect the cell viability of this particular cell line. Despite that, the MN values induced in S- and G2-treated V-C8 cells were higher than those found in G1-treated cells.

Together, these data suggest that NHEJ and HR operate differentially throughout the cell cycle to protect from Top2 poison-induced chromosome instability, and that the DNA-PKcs-dependent NHEJ pathway allows the survival of chromosome damaged cells during S and G2 to the next interphase.

DISCUSSION

Chromosome instability is a common genetic feature following Top2 poisons-based chemotherapy [Ahuja et al., 2000; Degrassi et al., 2004]. In this study, we investigated the contribution of the NHEJ and HR repair pathways to prevent the chromosome instability induced by Top2 poisons in Chinese hamster cell lines. The higher sensitivity displayed by the DNA-PKcs- and BRCA2-deficient cells to Top2 poisons highlighted the requirement of both DSB repair pathways.

The exposure of the Chinese hamster cells to Top2 poisons was previously reported to lead to DNA DSB formation [Jensen et al., 2004; Smart et al., 2008]. In the same way, we show here that IDA- and ETO-induced phosphorylation of H2AX throughout the cell cycle. In addition, we observed that after 24 hr the DSB repair-mutant cells retained higher amounts of γH2AX signals. Similarly, Kuhfittig-Kulle et al. [2007] reported that after X-irradiation the disappearance of γH2AX foci was faster in CHO wild-type cells compared with XR-C1 cells. Iliakis et al. [2004] showed that impaired DSB repair systems leads to increased levels of DSB and the persistence of these lesions over longer periods of time. A more recent study on LIG-4- or XRCC4-deficient cells demonstrated a higher persistence of γH2AX foci on metaphase chromosomes following irradiation on interphase cells [Kato et al., 2009].

Elevated levels of unprocessed DSB have been reported to act as a primary source for CA formation [Ferguson et al., 2000]. The Top2 poisons induced CA independently of the phase of the cell cycle in which the treatment has been performed, acting by an S-independent mechanism.

TABLE I. Structural Chromosome Aberrations (CA) and Mitotic Index (MI) in IDA- and ETO-treated Chinese Hamster Cells in G1 Phase

Cell line	Treatment (μg/ml)	Abnormal cells (%)	CA in 100 cells			Complex exchange configuration	Highly damaged cells (> 15 breaks)	Total breaks	MI (% ± SD)
			ctb+csb	cte	cse				
CHO9	Control	17	16	1	0	0	0	18	4.75 ± 0.35
	IDA 0.005	51*	47	9	11	0	0	87	2.45 ± 0.21
	ETO 5.0	43*	37	4	7	0	0	59	2.40 ± 0.71
XR-C1	Control	12	12	0	0	0	0	12	4.55 ± 0.21
	IDA 0.005	76*	79	37	33	2	5	219	1.55 ± 0.01†
	ETO 5.0	48*	62	17	19	1	6	134	1.80 ± 0.28†
V79	Control	14	15	0	1	0	0	17	8.05 ± 0.78
	IDA 0.005	52*	53	0	4	0	0	61	5.80 ± 0.28
	ETO 5.0	49*	57	2	5	0	0	71	5.00 ± 0.71
V-C8	Control	38	29	10	5	0	0	59	4.15 ± 0.35
	IDA 0.005	89*	179	71	29	11	18	379	1.90 ± 0.42†
	ETO 5.0	75*	83	44	21	4	9	213	1.70 ± 0.28†

Abbreviations: ctb, chromatid-type breaks; csb, chromosome-type breaks; cte, chromatid-type exchanges; cse, chromosome-type exchanges.
*$P = 0.0001$, χ^2 test.
†$P < 0.03$, Students' t-test vs. respective controls.

TABLE II. Structural Chromosome Aberrations (CA) and Mitotic Index (MI) in IDA- and ETO-treated Chinese Hamster Cells in G2 Phase

Cell line	Treatment (μg/ml)	Abnormal cells (%)	CA in 100 cells			Complex exchange configuration	Highly damaged cells (> 15 breaks)	Total breaks	MI (% ± SD)
			ctb+csb	cte	cse				
CHO9	Control	21	20	1	0	0	0	22	3.95 ± 0.07
	IDA 0.005	60*	73	25	2	0	0	127	2.81 ± 0.91
	ETO 5.0	76*	237	70	8	18	35	393	1.10 ± 0.14†
XR-C1	Control	13	14	0	0	0	0	14	6.95 ± 0.21
	IDA 0.005	83*	327	48	7	4	36	437	3.70 ± 0.28†
	ETO 5.0	82*	298	87	12	7	65	496	3.30 ± 0.42†
V79	Control	22	28	2	0	0	0	32	9.17 ± 0.51
	IDA 0.005	52*	51	5	3	0	0	67	6.56 ± 1.05
	ETO 5.0	89*	97	39	8	5	22	191	3.25 ± 0.37†
V-C8	Control	38	42	7	5	0	0	66	7.85 ± 0.35
	IDA 0.005	96*	403	135	25	29	91	723	4.90 ± 0.14†
	ETO 5.0	92*	330	125	15	27	65	610	3.90 ± 0.28†

Abbreviations: ctb, chromatid-type breaks; csb, chromosome-type breaks; cte, chromatid-type exchanges; cse, chromosome-type exchanges.
*$P = 0.0001$, χ^2 test.
†$P < 0.01$, Students' t-test vs. respective controls.

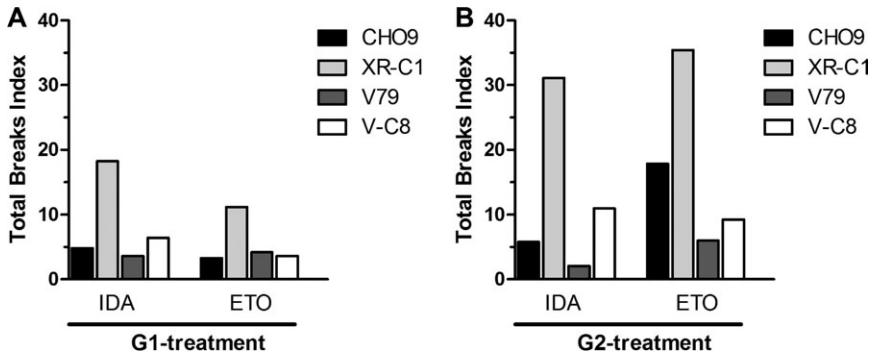

Fig. 4. Total breaks index induced by IDA (0.005 μg/ml) or ETO (5.0 μg/ml) in G1- (**A**) and G2- (**B**) DNA DSB repair proficient and deficient Chinese hamster cell lines. Total breaks index = Total breaks in treated cells/Total breaks in control untreated cells.

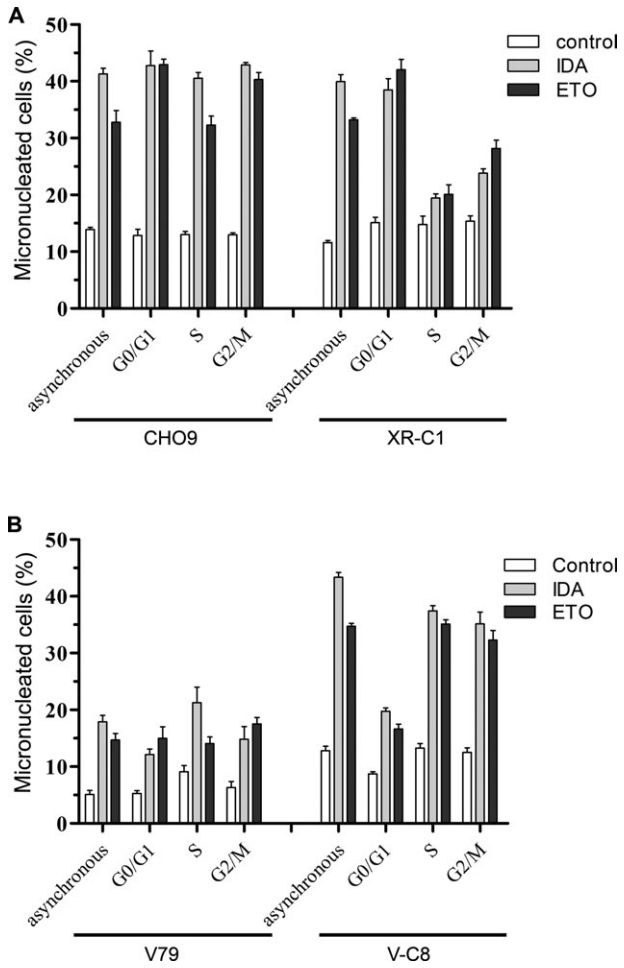

Fig. 5. Percentage of MN cells in DNA DSB repair proficient and deficient Chinese hamster cell lines treated with IDA (0.005 μg/ml) or ETO (5.0 μg/ml) at different phases of the cell cycle. (**A**) MN frequencies (mean ± SD) in CHO9 and XR-C1 cells. (**B**) MN frequencies (mean ± SD) in V79 and V-C8 cells. Significant differences (Students' t-test) in proficient ($P < 0.02$) and deficient ($P < 0.005$) cell lines compared with their respective control cells were found in all cases.

After treatment in G1 with the Top2 poisons, DNA repair proficient cells showed a slight increase of chromosome-type exchanges in comparison with chromatid-type in the first metaphases. On the other hand, XR-C1 and V-C8 cells showed an increase of both chromosome- and chromatid-type aberrations. Similar findings were obtained by Natarajan et al. [2008] in CHO mutant cell lines following irradiation.

It has been previously established that the Chinese hamster cell lines used in this study are p53 deficient [Chaung et al., 1997; Hu et al., 1999; Wilson et al., 2010]. In agreement with those studies, we have determined by flow cytometry, a lack of G1 arrest in asynchronously growing proficient and deficient cells treated with IDA or ETO (data not shown). Therefore, the formation of chromatid exchanges in DNA-PKcs-deficient cells treated in G1 phase is compatible with a checkpoint defect that allows cells with unrepaired DSB to enter S phase. In the S phase, HR is the obvious candidate for repairing those DSB by making use of the sister chromatid as a template through an error-free process [Saleh-Gohari and Helleday, 2004]. Previous reports have demonstrated that in γ-ray or I-SceI endonuclease-treated G1 cells, defects in NHEJ lead to over-stimulation of HR in S/G2 [Delacôte et al., 2002; Saintigny et al., 2007]. However, when DSB are generated before replication, HR cannot use the sister chromatid, as both chromatids contain a DSB at the same position. In this situation, HR will be forced to use homologous sequences dispersed through the genome increasing the risks of chromosomal instability [Delacôte and Lopez, 2008]. Moreover, the absence of DNA-PKcs can recruit a slow, error-prone repair process, which can operate as a backup of NHEJ (B-NHEJ) favoring the misjoining of DSB that remained open for a long time [Iliakis, 2009]. B-NHEJ is also enhanced in G2 phase after the induction of DSB by radiation [Wu et al., 2008].

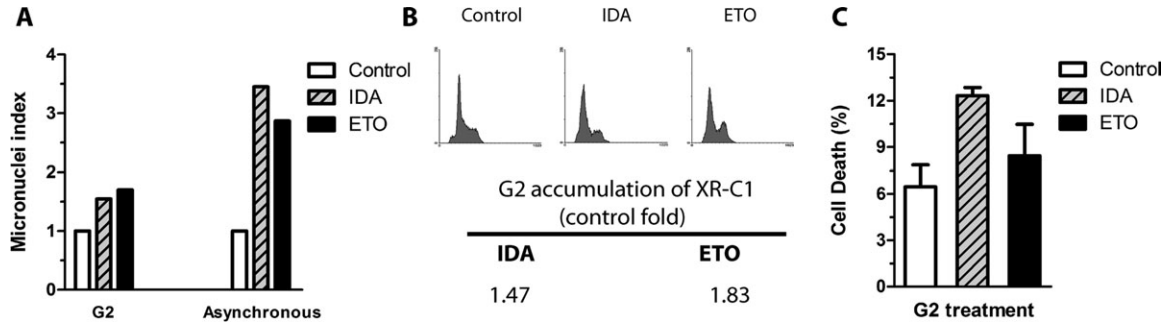

Fig. 6. Cell cycle progression and cell death induced by IDA (0.005 μg/ml) or ETO (5.0 μg/ml) in G2-enriched XR-C1 cells. (**A**) MN index (%MN in treated cells/%MN in control cells) comparison of asynchronous and G2-enriched populations. (**B**) Cell cycle analysis by flow cytometry and (**C**) cell death measurement after staining with acridine orange/ethidium bromide of G2-enriched populations treated with IDA or ETO by 1 hr and then released by 8 hr.

Likewise, G1-treated BRCA2-deficient cell line showed an increase in the frequency of chromatid exchanges in comparison with the wild-type cells, though it was less pronounced than that obtained in DNA-PKcs-deficient cells. In BRCA2-mutant cells, the NHEJ pathway is supposed to repair most of the DSB induced in G1 phase in the course of the cell cycle. In support of this, Wang et al. [2001] showed that the activity of NHEJ was not affected by mutations in BRCA2.

Taking into account the TBI induced by IDA and ETO in the G1-treated cell lines, we observed that DNA-PKcs-deficient cells were highly susceptible to Top2 poisons in comparison with the parental CHO9, while BRCA2-deficient cells showed a slight increase with respect to V79. These results indicate that NHEJ plays a major role in the protection of chromosomal integrity after insults in G1.

The yield of chromatid-type exchanges was increased in all cell lines following treatment with Top2 poisons in G2 with respect to G1. This effect may be explained by the fact that Top2α has a maximal decatenation activity in G2 [Burden et al., 1993] and by the lower efficiency of DSB repair of G2 cells [Wilson et al., 2010]. Moreover, both mutant cell lines showed higher frequencies of chromatid-type exchanges than their proficient counterparts. These findings are in agreement with an early work in CHO-K1 and xrs5 and xrs6 (Ku86-mutant) cells treated with m-amsacrine and ETO in the G2 stage of the cell cycle [Darroudi and Natarajan, 1989]. The TBI induced by IDA after treatment in G2 in DNA-PKcs- or BRCA2-deficient cells showed a substantial increase, though this effect was lower with ETO. This seems to be due to an extensive chromosome damage induced by ETO in the parental cell lines. Thus, both NHEJ and HR pathways are important for the maintenance of chromosomal stability after treatment in G2 with Top2 poisons.

To determine the fate of the CA induced by both IDA and ETO in the subsequent interphase cells, we analyzed MN formation. It is known that the kind of CA contained on MN depends on the inducting agent [Norppa and Falck, 2003]. Top2 poisons promote either structural or numerical CA [Degrassi et al., 2004]. We determined that most of the IDA or ETO-induced MN has DSB-derived CA by the presence of γH2AX foci on them, and therefore, suggesting the presence of acentric chromosomes or chromatid fragments derived from unrepaired and/or misrepaired DSB.

Both Top2 poisons induced an increased frequency of MN in CHO and V79 cells independently of the cell cycle stage in which the cells were treated. In agreement with our results, Wang et al. [2007] reported that ETO caused a significant increase in MN when V79 cells were treated during G1, S, and G2 phases. When we analyzed the DNA-PKcs-defective cells, a higher frequency of MN was found in cells treated in G1 compared with S and G2. However, treatments in G2 resulted in an extensive G2 arrest and cell death. Both phenomena prevented the hazardous proliferation of severely damaged cells. On the contrary, the MN values induced by treatments in S and G2 in BRCA2-mutant cells were higher than those resulting from treatments in G1. It is well known that NHEJ may act imprecisely but restores efficiently the chromosomal structural integrity [Lieber, 2008; Revaud et al., 2011], being the most prominent activity of DSB repair. Although the nonconservative HR subpathway single-strand annealing was shown to be enhanced in BRCA2-mutant cells [Larminat et al., 2002], its activity on DSB is limited by the context of the genomic sequence. Therefore, it is likely that the DNA-PKcs-dependent NHEJ activity on BRCA2-mutant cells promotes the survival of cells with chromosomal abnormalities induced in S and G2 phases.

A large body of evidence has demonstrated the effect of the cell cycle stage on the DSB repair ability in different vertebrate cells following exposure to ionizing radiation [Takata et al., 1998; Rothkamm et al., 2003; Natarajan et al., 2008; Frankenberg-Schwager et al., 2009] and I-SceI endonuclease [Mao et al., 2008; Takashima et al., 2009] and Top2 poisons [de Campos-Nebel et al., 2010]. However, less is known about the influence of both NHEJ and HR repair pathways to support chromosome instability following treatments with chemical agents, such as Top2 poisons. In this sense, our results suggest that although impairments in both DSB repair pathways result in increased frequencies of chromosome aberrations, the DNA-PKcs-dependent NHEJ activity in G2 cells is important to promote the progression of aberrant cells to the next cell cycle. Therefore, as several DNA-PKcs chemical inhibitors have been developed, combined therapies using low doses of Top2 poisons may result in a high antitumoral activity diminishing the risk of promoting chromosome instability in actively proliferating normal cells.

AUTHOR CONTRIBUTIONS

MdCN and MGC conceived and designed the experiments. MEE, MdCN, and MGC performed the experiments. MEE, MdCN, and MGC analyzed the data. MdCN and MGC wrote the paper. All authors approved the final article.

ACKNOWLEDGMENTS

The authors thank Dr. C. D. Pasqualini for her critical revision of the article. They also thank Dres. N. Galassi, N. Riera, and M. Filippo for helping them with flow cytometry experiments.

REFERENCES

Abaji C, Cousineau I, Belmaaza A. 2005. BRCA2 regulates homologous recombination in response to DNA damage: Implications for genome stability and carcinogenesis. Cancer Res 65:4117–4125.

Abbott DW, Freeman ML, Holt JT. 1998. Double-strand break repair deficiency and radiation sensitivity in BRCA2 mutant cancer cells. J Natl Cancer Inst 90:978–985.

Ahuja HG, Felix CA, Aplan PD. 2000. Potential role for DNA topoisomerase II poisons in the generation of t(11;20)(p15;q11) translocations. Genes Chromosomes Cancer 29:96–105.

Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, Loman N, Olsson H, Johannsson O, Borg A, Pasini B, Radice P, Manoukian S, Eccles DM, Tang N, Olah E, Anton-Culver H, Warner E, Lubinski J, Gronwald J, Gorski B, Tulinius H, Thorlacius S, Eerola H, Nevanlinna H, Syrjäkoski K, Kallioniemi OP, Thompson D, Evans C, Peto J, Lalloo F, Evans DG, Easton DF. 2003. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: A combined analysis of 22 studies. Am J Hum Genet 72:1117–1130.

Auckley DH, Crowell RE, Heaphy ER, Stidley CA, Lechner JF, Gilliland FD, Belinsky SA. 2001. Reduced DNA-dependent protein kinase activity is associated with lung cancer. Carcinogenesis 22:723–727.

Burden DA, Goldsmith LJ, Sullivan DM. 1993. Cell-cycle-dependent phosphorylation and activity of Chinese-hamster ovary topoisomerase II. Biochem J 293:297–304.

Chaung W, Mi LJ, Boorstein RJ. 1997. The p53 status of Chinese hamster V79 cells frequently used for studies on DNA damage and DNA repair. Nucleic Acids Res 25:992–994.

Darroudi F, Natarajan AT. 1989. Cytogenetical characterization of Chinese hamster ovary X-ray-sensitive mutant cells, xrs 5 and xrs 6. IV. Study of chromosomal aberrations and sister-chromatid exchanges by restriction endonucleases and inhibitors of DNA topoisomerase II. Mutat Res 212:137–148.

de Campos-Nebel M, Larripa I, González-Cid M. 2008. Non-homologous end joining is the responsible pathway for the repair of fludarabine-induced DNA double strand breaks in mammalian cells. Mutat Res 646:8–16.

de Campos-Nebel M, Larripa I, González-Cid M. 2010. Topoisomerase II-mediated DNA damage is differently repaired during the cell cycle by non-homologous end joining and homologous recombination. PLoS ONE 5:e12541.

Degrassi F, Fiore M, Palitti F. 2004. Chromosomal aberrations and genomic instability induced by topoisomerase-targeted antitumour drugs. Review. Curr Med Chem Anticancer Agents 4:317–325.

Delacôte F, Han M, Stamato TD, Jasin M, Lopez BS. 2002. An xrcc4 defect or Wortmannin stimulates homologous recombination specifically induced by double-strand breaks in mammalian cells. Nucleic Acids Res 30:3454–3463.

Delacôte F, Lopez BS. 2008. Importance of the cell cycle phase for the choice of the appropriate DSB repair pathway, for genome stability maintenance: The trans-S double-strand break repair model. Cell Cycle 7:33–38.

Errami A, He DM, Fried AA, Overkamp WJ, Morolli B, Hendrickson EA, Eckardt-Schupp F, Oshimura M, Lohman PH, Jackson SP, Zdzienicka MZ. 1998. XR-C1, a new CHO cell mutant which is defective in DNA-PKcs, is impaired in both V(D)J coding and signal joint formation. Nucleic Acids Res 26:3146–3153.

Ferguson DO, Sekiguchi JM, Chang S, Frank KM, Gao Y, DePinho RA, Alt FW. 2000. The nonhomologous end-joining pathway of DNA repair is required for genomic stability and the suppression of translocations. Proc Natl Acad Sci USA 97:6630–6633.

Fortune JM, Osheroff N. 2000. Topoisomerase II as a target for anticancer drugs: When enzymes stop being nice. Review. Prog Nucleic Acid Res Mol Biol 64:221–253.

Frankenberg-Schwager M, Gebauer A, Koppe C, Wolf H, Pralle E, Frankenberg D. 2009. Single-strand annealing, conservative homologous recombination, nonhomologous DNA end joining, and the cell cycle-dependent repair of DNA double-strand breaks induced by sparsely or densely ionizing radiation. Radiat Res 171:265–273.

Fu YP, Yu JC, Cheng TC, Lou MA, Hsu GC, Wu CY, Chen ST, Wu HS, Wu PE, Shen CY. 2003. Breast cancer risk associated with genotypic polymorphism of the nonhomologous end-joining genes: A multigenic study on cancer susceptibility. Cancer Res 63:2440–2446.

Helleday T, Lo J, van Gent DC, Engelward BP. 2007. DNA double-strand break repair: From mechanistic understanding to cancer treatment. DNA Repair 6:923–935.

Hu T, Miller CM, Ridder GM, Aardema MJ. 1999. Characterization of p53 in Chinese hamster cell lines CHO-K1, CHO-WBL, and CHL: Implications for genotoxicity testing. Mutat Res 426:51–62.

Huang X, Okafuji M, Traganos F, Luther E, Holden E, Darzynkiewicz Z. 2004. Assessment of histone H2AX phosphorylation induced by DNA topoisomerase I and II inhibitors topotecan and mitoxantrone and by the DNA cross-linking agent cisplatin. Cytometry A 58:99–110.

Iliakis G, Wang H, Perrault AR, Boecker W, Rosidi B, Windhofer F, Wu W, Guan J, Terzoudi G, Pantelias G. 2004. Mechanisms of DNA double strand break repair and chromosome aberration formation. Review. Cytogenet Genome Res 104:14–20.

Iliakis G. 2009. Backup pathways of NHEJ in cells of higher eukaryotes: Cell cycle dependence. Review. Radiother Oncol 92:310–315.

Jensen LH, Dejligbjerg M, Hansen LT, Grauslund M, Jensen PB, Sehested M. 2004. Characterisation of cytotoxicity and DNA damage induced by the topoisomerase II-directed bisdioxopiperazine anti-cancer agent ICRF-187 (dexrazoxane) in yeast and mammalian cells. BMC Pharmacol 4:31.

Kanaar R, Hoeijmakers JH, van Gent DC. 1998. Molecular mechanisms of DNA double strand break repair. Review. Trends Cell Biol 8:483–489.

Kato TA, Okayasu R, Bedford JS. 2009. Signatures of DNA double strand breaks produced in irradiated G1 and G2 cells persist into mitosis. J Cell Physiol 219:760–765.

Kraakman-van der Zwet M, Overkamp WJ, van Lange RE, Essers J, van Duijn-Goedhart A, Wiggers I, Swaminathan S, van Buul PP, Errami A, Tan RT, Jaspers NG, Sharan SK, Kanaar R, Zdzienicka MZ. 2002. Brca2 (XRCC11) deficiency results in radioresistant DNA synthesis and a higher frequency of spontaneous deletions. Mol Cell Biol 22:669–679.

Kuhfittig-Kulle S, Feldmann E, Odersky A, Kuliczkowska A, Goedecke W, Eggert A, Pfeiffer P. 2007. The mutagenic potential of non-homologous end joining in the absence of the NHEJ core factors Ku70/80, DNA-PKcs and XRCC4-LigIV. Mutagenesis 22:217–233.

Larminat F, Germanier M, Papouli E, Defais M. 2002. Deficiency in BRCA2 leads to increase in non-conservative homologous recombination. Oncogene 21:5188–5192.

Lee HS, Choe G, Park KU, Park do J, Yang HK, Lee BL, Kim WH. 2007. Altered expression of DNA-dependent protein kinase catalytic subunit (DNA-PKcs) during gastric carcinogenesis and its clinical implications on gastric cancer. Int J Oncol 31:859–866.

Lee SH, Kim CH. 2002. DNA-dependent protein kinase complex: A multifunctional protein in DNA repair and damage checkpoint. Review. Mol Cells 13:159–166.

Leone G, Fianchi L, Pagano L, Voso MT. 2010. Incidence and susceptibility to therapy-related myeloid neoplasms. Chem Biol Interact 184:39–45.

Lieber MR. 2008. The mechanism of human nonhomologous DNA end joining. Review. J Biol Chem 283:1–5.

Mao Z, Bozzella M, Seluanov A, Gorbunova V. 2008. DNA repair by nonhomologous end joining and homologous recombination during cell cycle in human cells. Cell Cycle 7:2902–2906.

McClendon AK, Osheroff N. 2007. DNA topoisomerase II, genotoxicity, and cancer. Mutat Res 623:83–97.

Mosesso P, Darroudi F, van den Berg M, Vermeulen S, Palitti F, Natarajan AT. 1998. Induction of chromosomal aberrations (unstable

and stable) by inhibitors of topoisomerase II, m-AMSA and VP16, using conventional Giemsa staining and chromosome painting techniques. Mutagenesis 13:39–43.

Natarajan AT, Berni A, Marimuthu KM, Palitti F. 2008. The type and yield of ionising radiation induced chromosomal aberrations depend on the efficiency of different DSB repair pathways in mammalian cells. Mutat Res 642:80–85.

Norppa H, Falck GC. 2003. What do human micronuclei contain? Mutagenesis 18:221–233.

Peddi P, Loftin CW, Dickey JS, Hair JM, Burns KJ, Aziz K, Francisco DC, Panayiotidis MI, Sedelnikova OA, Bonner WM, Winters TA, Georgakilas AG. 2010. DNA-PKcs deficiency leads to persistence of oxidatively induced clustered DNA lesions in human tumor cells. Free Radic Biol Med 48:1435–1443.

Revaud D, Martins LM, Boussin FD, Sabatier L, Desmaze C. 2011. Different DNA-PKcs functions in the repair of radiation-induced and spontaneous DSBs within interstitial telomeric sequences. Chromosoma 120:309–319.

Riches LC, Lynch AM, Gooderham NJ. 2008. Early events in the mammalian response to DNA double-strand breaks. Review. Mutagenesis 23:331–339.

Rothkamm K, Krüger I, Thompson LH, Löbrich M. 2003. Pathways of DNA double-strand break repair during the mammalian cell cycle. Mol Cell Biol 23:5706–5715.

Saintigny Y, Delacôte F, Boucher D, Averbeck D, Lopez BS. 2007. XRCC4 in G1 suppresses homologous recombination in S/G2, in G1 checkpoint-defective cells. Oncogene 26:2769–2780.

Saleh-Gohari N, Helleday T. 2004. Conservative homologous recombination preferentially repairs DNA double-strand breaks in the S phase of the cell cycle in human cells. Nucleic Acids Res 32:3683–3688.

Shen H, Schultz M, Kruh GD, Tew KD. 1998. Increased expression of DNA-dependent protein kinase confers resistance to adriamycin. Biochim Biophys Acta 1381:131–138.

Smart DJ, Halicka HD, Schmuck G, Traganos F, Darzynkiewicz Z, Williams GM. 2008. Assessment of DNA double-strand breaks and gammaH2AX induced by the topoisomerase II poisons etoposide and mitoxantrone. Mutat Res 641:43–47.

Tai YC, Domchek S, Parmigiani G, Chen S. 2007. Breast cancer risk among male BRCA1 and BRCA2 mutation carriers. J Natl Cancer Inst 99:1811–1814.

Takashima Y, Sakuraba M, Koizumi T, Sakamoto H, Hayashi M, Honma M. 2009. Dependence of DNA double strand break repair pathways on cell cycle phase in human lymphoblastoid cells. Environ Mol Mutagen 50:815–822.

Takata M, Sasaki MS, Sonoda E, Morrison C, Hashimoto M, Utsumi H, Yamaguchi-Iwai Y, Shinohara A, Takeda S. 1998. Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. EMBO J 17:5497–5508.

Treszezamsky AD, Kachnic LA, Feng Z, Zhang J, Tokadjian C, Powell SN. 2007. BRCA1- and BRCA2-deficient cells are sensitive to etoposide-induced DNA double-strand breaks via topoisomerase II. Cancer Res 67:7078–7081.

Turnbull C, Hodgson S. 2005. Genetic predisposition to cancer. Clin Med 5:491–498.

Wang H, Zeng ZC, Bui TA, DiBiase SJ, Qin W, Xia F, Powell SN, Iliakis G. 2001. Nonhomologous end-joining of ionizing radiation-induced DNA double-stranded breaks in human tumor cells deficient in BRCA1 or BRCA2. Cancer Res 61:270–277.

Wang L, Roy SK, Eastmond DA. 2007. Differential cell cycle-specificity for chromosomal damage induced by merbarone and etoposide in V79 cells. Mutat Res 616:70–82.

Wilson PF, Hinz JM, Urbin SS, Nham PB, Thompson LH. 2010. Influence of homologous recombinational repair on cell survival and chromosomal aberration induction during the cell cycle in gamma-irradiated CHO cells. DNA Repair 9:737–744.

Wu W, Wang M, Wu W, Singh SK, Mussfeldt T, Iliakis G. 2008. Repair of radiation induced DNA double strand breaks by backup NHEJ is enhanced in G2. DNA Repair 7:329–338.

Accepted by—
K. Vasquez