Cellular Consequences of Overproduction of DNA Topoisomerase II in an Ataxia-Telangiectasia Cell Line

P. J. Smith and T. A. Makinson

INTRODUCTION

Active regulation of the superhelical density of eukaryotic cellular DNA is thought to be under the control of DNA topoisomerases (for review see Refs. 1 and 2). These enzymes can relax supercoiled DNA by virtue of their single (type I topoisomerase) or double (type II topoisomerase)-strand-passing activities which lead to reductions in linking number. The major type II DNA topoisomerase (topoisomerase II) also has the unique ability to unknot and decatenate DNA molecules, giving the cell an enzymatic pathway for the resolution of topologically compromised products of replication and recombination (1-3). In view of its activity and strategic location (4) within the axial domains at the base of chromatin loops (5), topoisomerase II has been implicated in the function [e.g., transcription, replication and recombination (1)] of genomic DNA and in the structural organization of chromatin [e.g., chromosome condensation and separation (6)].

Recently it has been recognized (for review see Ref. 7) that topoisomerase II is an important intracellular target for certain DNA intercalating drugs (e.g., mAMSA) and the epipodophyllotoxins (e.g., VP16-213). Such drugs induce covalent trapping of topoisomerase II molecules as “cleavable complexes” on cellular DNA. Although the exact nature of the drug-protein-DNA interaction is not clear, it is important to establish the potentially cytotoxic nature of such topoisomerase II-dependent lesions since the drugs which stimulate complex formation are useful in the chemotherapeutic management of cancer (8-11). Recent evidence suggests that variations in nuclear topoisomerase II levels as a consequence of cell cycle commitment (12, 13) or cell type (14) can dictate cellular responses to topoisomerase II-active drugs. There is no information in the literature on the effect of overproduction of topoisomerase II in human cells, on cellular phenotype and responsiveness to topoisomerase II-active drugs. We address this question in an A-T cell line, permitting additional observations to be made on the involvement of topoisomerase II in the in vitro A-T phenotype.

The human genetic disorder A-T has been widely studied in attempts to elucidate the genetic determinants of cellular responses to DNA damage (reviews: Refs. 15, 16). A-T cells show enhanced sensitivity to ionizing radiation both in vitro and in vivo (15). A-T cells also demonstrate an unusual resistance to the inhibition of de novo DNA synthesis by ionizing radiation (17-21). Several lines of evidence have suggested that A-T cells may have some defect in the expression of DNA topoisomerase II, namely: some A-T cell lines (22) have been found to be sensitive to novobiocin [a coumarin which blocks the ATPase activity of the B subunit of bacterial gyrase—the eukaryotic representative of which is topoisomerase II (11)]; unusually low levels of DNA unknotting activity have been reported in some, but not all, A-T cell lines (23); the observations that A-T derived cell lines are unusually sensitive to VP16-213 (24, 25) but not to mAMSA (26). Current dogma (7) would predict that low levels of DNA topoisomerase II in A-T cells should impart resistance to the toxic effects of topoisomerase II interactive drugs if the main route of cell kill by a given agent is predominantly dependent upon the frequency of cleavable complex formation. Clearly, there are contradictions in the literature given the drug sensitivity profiles of A-T cells.

In an attempt to resolve these contradictions we report here on the relationship between the cellular responses of various normal and A-T cell lines [together with a Xp cell line included for comparative purposes (26)] to the topoisomerase II-active drug mAMSA and the nuclear availability of the target enzyme. In particular we define the in vitro characteristics of an SV40-transformed fibroblast A-T cell line with unusually high levels of topoisomerase II expressed in all phases of the cell cycle.

MATERIALS AND METHODS

Cell Culture

The SV40-transformed human fibroblast cells lines MRC5CVI (normal donor), AT5B1VA (A-T homozygote donor), and XP12ROSV40 (XP donor; Group A) were kindly supplied by Dr. C. Arlett (MRC Cell Mutation Unit, Palmer, UK). GM0637 is an SV40-transformed fibroblast from a normal donor (Human Genetic Cell Mutant Cell Reposi-
Drug Preparation and Cell Survival Assay

The aminocaridine drug mAMSA (amsacrin; Park-Davis, Eastleigh, UK) was stored at −20°C as a 10 mM stock solution in dimethyl sulfoxide. Monolayer cultures in exponential growth phase were detached using trypsin/versene and plated at low density (0.25–5 × 10^5 cells/9 cm dish) and allowed to attach for a period of 16 h. mAMSA was added directly to each plate and cultures were incubated for 1 h before drug removal by washing plates with PBS and assaying for clonogenic potential by continued incubation (14 days) in fresh growth medium.

Cell Cycle Phase Analysis

A rapid, one-step ethidium bromide DNA-staining technique for RNase digested cells was used for the measurement of cellular DNA content, with subsequent computer evaluation of cell cycle phase distributions. Details have been described previously (27, 28). Monolayer cultures were grown in six-well plates (8 × 10^5 cells/well) and incubated for 48 h prior to drug treatment. A 24-h delay-expression period was permitted before versene/trypsin detachment of cells for cell-cycle analysis. Suspension cultures were treated in midphase 6–8 × 10^6 cell/ml for continuous drug exposures.

DNA Damage Measurement

Lesions detectable as DNA strand breaks under alkaline conditions (including protein-associated strand-breaks (13, 29)) were measured by microscale adaptation of the method described by Kanter and Schwartz (30). The method relies upon the strand-break-dependent enhancement of DNA unwinding in alkaline solutions as detected by fluorimetry. Cells were plated in 96-well (flat-bottom, cell culture quality) microtiter plates at 2.5 × 10^4 cells/well and allowed to attach for a 16-h incubation period. Columns of eight wells received a range of drug doses by direct addition of concentrated drug solutions. After a 1-h exposure period, plates were washed twice with PBS and each well overlaid with 50 μl of a trypsin/versene solution to aid cell detachment. The assay employed all of the reagents described previously (30) except that the fluorochrome buffer contained Hoechst 33342 at 1.25 mM. The assay employed all of the reagents described previously (30) except that the fluorochrome buffer contained Hoechst 33342 at 1.25 mM. The assay required the addition of 50 μl of fluorochrome buffer (column T; no denaturation) and each well briefly sonicated. A second column received 50 μl 0.1 N NaOH (column B; full denaturation) and each well briefly sonicated. A series of other columns representing one untreated control and several treated with drug received 50 μl 0.1 N NaOH/well (column P; partial denaturation). After a 30-min period at room temperature B and P columns received 50 μl 0.1 N HCl/well and 50 μl fluorochrome buffer/well. Column T received 100 μl/well of a 1:1 mixture of 0.1 N HCl and 0.1 N NaOH. The contents of all wells were then homogenized by brief sonication. The fluorescence of Hoechst 33342-DNA in each well was measured using a Fluoroskan II 96-well microplate fluorometer (Flow Laboratories; Rickmansworth, UK) employing excitation at 355 nm and emission monitored at 480 nm. The method relies upon the strand-break-dependent enhancement of DNA unwinding (F, due to strand breaks of alkali-labile damage) was determined by the expression F = -log(Dx/Dc), where Dx and Dc represent the percentage of double-stranded DNA in experimental or control samples, respectively [percentage of double-stranded DNA = 100 (P–B/T–B) / (31)]. Absolute strand-break frequencies can be calculated with reference to X-irradiated standards (32, 33) which gave 5.5 F units/Gy for each fibroblast line.

Filter Binding Assay of DNA-Protein Cross-Linking

The assay is essentially that described by Minford et al. (34) except that linear pBR322 plasmid DNA (a gift from Dr. P. Rabbitts; Ludwig Institute, Cambridge, UK) was used as a substrate as follows. Preparation of Radiolabeled DNA. pBR322 supercoiled plasmid DNA was linearized using EcoRI restriction endonuclease and Klenow enzyme (large fragment DNA polymerase I; Boehringer Mannheim GmbH) was used to fill in 3′-recessed ends in the presence of dTTP (Pharmacia) and [α-32P]dATP (100 μCi/10 μg DNA; Amersham). DNA was separated using a G-50 course Sephadex column eluted in buffer (10 mM Tris, 0.1 mM EDTA, 50 mM NaCl, pH 8.0) under gravity. DNA containing fractions were pooled according to their specific activity.

Preparation of Crude Nuclear Protein Extracts. Cells were grown in 850-cm² roller cultures and harvested in exponential growth phase by versene detachment. Cells were resuspended in growth medium (total 5–8 × 10^6 cells), sedimented and washed twice with cold nucleus buffer (0.15 M NaCl, 5 mM MgCl₂, 1 mM KH₂PO₄, 1 ethylene glycol bis (β-aminoethoxy ether)-N,N′,N′,N′-tetraacetic acid, 0.1 mM diithiothreitol and 10% glycerol, pH 6.5) and resuspended in 1 ml of ice cold buffer. Cells were then permeabilized by the addition of 9 ml nucleus buffer containing 0.35% Triton X-100 (NEN Products) and 0.1 mM phenylmethylsulfonyl fluoride (Sigma Chemical Co, St Louis, MO) and mixed gently for 20 min at 4°C. Nuclei were pelleted and washed in Triton-free buffer and resuspended prior to protein extraction in 0.4–1 ml of ice-cold nucleus buffer containing 350 mM NaCl for 20 min. The suspension was centrifuged twice to remove nuclei and the protein concentration of the supernatant determined.

Filter Binding Assay. The assay was performed exactly as described previously (34). Briefly, 200-μl reaction volumes containing 15 ng radiolabeled pBR322 DNA, crude protein extract and 20 μg mAMSA in nucleus buffer were prepared and incubated at 37°C for 25 min. Reactions were stopped by dilution in 20 mM EDTA (pH 10.0; i.e., stop buffer) and solutions allowed to pass through polyvinyl chloride filters (Millipore type BS, 25-mm diameter, 2-μm pore size) followed by a wash with a high salt/detergent solution and finally stop buffer. DNA retained on the filter was measured by scintillation counting.

Topoisomerase II Decatenation Assay

Activity was assayed according to the method of Sahai and Kaplan (35) using the ability of crude nuclear extracts (see filter binding assay above) to catalyse the decatenation of a catenated network of tritium-labeled kinetoplast DNA obtained from Crithidia fasciculata (culture generously provided by Dr. J. Thacker, MRC Radiobiology Unit, Harwell, UK). Crithidia cultures were grown in radiolabeling medium (containing [3H]thymidine) as described and kinetoplast DNA purified by CsCl banding to yield a preparation with a specific activity of 2.2 × 10^6 cpm/μg DNA. Assay conditions were as described previously (35) except that the reaction mixture contained 2 mM ATP, 1.2 μg DNA, and 32 ng protein. Decatenation reactions were stopped using sodium dodecyl sulfate and the products (single DNA circles and small catenanes) separated by centrifugation at 13,000 × g for 7.5 min prior to quantification by scintillation counting.

Flow Cytometric Assay for DNA Topoisomerase II

Nucleic preparations from exponentially growing cultures were obtained as described for the filter binding assay (see above). Nuclei were resuspended in PBS and fixed by slow addition of methanol (final 50% v/v). Samples were washed twice in PBS and 10 μl rabbit antisera (1:10 diluted in PBS; against recombinant human topoisomerase II polypeptide; a generous gift from Dr. L. Liu, The Johns Hopkins University, MD) was added to nuclei pellets for 60 min at room temperature; samples were then washed with PBS and resuspended in fluorescein isothiocyanate-conjugated swine anti-rabbit IgG (1:20 in PBS) for 60 min. DNA was stained by addition of 500 μl propidium iodide.
iodide (50 μg/ml) solution containing 100 μg/ml ribonuclease A prior to dual parameter (DNA/antibody) analysis by flow cytometry (36).

**Topoisomerase II Immunoblotting**

Western blotting was performed by separating proteins on a discontinuous 10% sodium dodecyl sulfate-polyacrylamide gel using the method of Laemmli (37). Nuclear extracts were prepared as described above. Transfer of resolved proteins from gels to nitrocellulose filter paper was performed as described by Towbin et al. (38). After transfer, additional protein binding sites on nitrocellulose were blocked by incubation of the paper for 16 h in NGA buffer (5 mM EDTA, 150 mM NaCl, 50 mM Tris-base, 10 mM NaN₃, 0.25% gelatin, and 0.05% Nonidet P40). The blots were then rinsed in saline and incubated at 4°C for a further 16 h with polyclonal rabbit anti-human topoisomerase II IgG antibody (diluted 1:1000 in NGA buffer). The nitrocellulose sheet was then washed in NGA and incubated with ¹²⁵I-labeled protein A for 16 h at 4°C. Autoradiography was then used to visualize antibody bound to proteins.

**RESULTS**

**Enzyme Expression.** Nuclear extracts of transformed normal (MRC5CVI) and A-T (AT5BIVA) fibroblasts were assayed for their capacity to decatenate kDNA in the presence of ATP. Fig. 1a shows that the AT5BIVA nuclear extract showed high type II topoisomerase activity with the reaction running to completion with 16 ng protein (1-h incubation; Fig. 1a), whereas only 10% of DNA was released into the supernatant in the presence of 16 ng MRC5CVI nuclear extract. Fig. 1b shows the impact of such high topoisomerase II activity in the AT5BIVA extract on the kinetics of decatenation. Western blotting (Fig. 2) of nuclear extracts using an anti-human topoisomerase II polyclonal antibody showed the overexpression of a protein with a molecular weight of 170,000 (indicated as P in Fig. 2) and an array of smaller proteins within the molecular weight range expected of proteolytic fragments of DNA topoisomerase II (39). This overexpression of DNA topoisomerase II in the AT5BIVA was not a consistent feature of all A-T derived cells since two independent A-T lymphoblastoid cell lines showed reduced expression at the p170 position compared to lymphoblastoid cell lines derived from normal donors (Fig. 2).

To visualize the pattern of cell cycle expression of DNA topoisomerase II in MRC5CVI and AT5BIVA, a flow cytometric immunofluorescence assay was established and the patterns of second antibody (fluorescein isothiocyanate labeled) binding versus DNA content are shown in Fig. 3. The results suggest that there is heterogeneous immunoreactivity in all cell cycle phases in MRC5CVI with some cells showing high levels of fluorescence. In the case of AT5BIVA many more cells show significant levels of anti-topoisomerase II-dependent fluorescence. Multiparametric analysis of gated populations of cells (Table 1) shows that anti-topoisomerase II antibody binding increases through the cell cycle in both cell types, with
AT5BIVA cells showing 2.2-, 1.5-, and 1.7-fold greater binding than normal cells for G₁, S, and G₂ phases, respectively. Interestingly the 90° light scatter values for AT5BIVA nuclei were significantly greater than those for MRC5CVI nuclei for each cell cycle phase despite equivalent DNA contents (and similar size as determined by microscopy). For example, G₁ phase AT5BIVA nuclei were similar to late S phase/G₂ MRC5CVI nuclei in terms of the 90° light scatter signal. Thus, the permeabilized nuclei derived from the AT5BIVA cell culture appear to have a different internal organization of nuclear material as determined by light scatter characteristics.

DNA Intercalator-dependent Protein-DNA Cross-Linking Activity in Nuclear Extracts. It is a property of DNA topoisomerase II that certain intercalating drugs (i.e., mAMSA) can trap enzyme molecules as covalent complexes with double-stranded DNA. We have used an in vitro assay to examine mAMSA induced DNA-protein cross-linking activities in nuclear extracts of various cell lines. Fig. 4a shows the effect of increasing protein concentration on the filter retention of protein-cross-linked DNA for extracts from normal, A-T and XP Group A cell lines. The two normal cell lines (MRC5CVI and GM0637) and the XP cell line (XP12ROSV40) showed significant levels of cross-linking activity with the reaction becoming limited at protein concentrations of greater than 0.25 μg/reaction. The A-T-derived cell line (AT5BIVA) showed much greater levels of mAMSA-dependent cross-linking in keeping with an overexpression of DNA topoisomerase II per unit protein extracted. However, the relationship between topoisomerase II content and filter binding activity is clearly complex since all extracts (including A-T) should achieve the same maximal level of filter binding. Such a lack of stoichiometry has been noted previously (34).

Fig. 4b shows comparative results for similar assays on extracts from two normal (SC and JR) and two A-T (GM717 and GM1526) lymphoblastoid cell lines. Clearly overexpression of mAMSA-dependent cross-linking activity is not a general feature of A-T-derived cell lines. Indeed the relatively low levels (at least two-fold lower than fibroblasts) of cross-linking (particularly in GM717) are consistent with the enzyme levels indicated by immunoblotting (Fig. 2). Extraction rates were 9–12 μg protein/10⁶ cells, for lymphoblastoid cells compared with 28–47 μg protein/10⁶ cells for the fibroblast lines. Thus, compared with normal transformed fibroblasts, lymphoblastoid cells appear to have 6–8-fold less cross-linking activity per nucleus extracted.

Intercalator-induced Strand Scission in Genomic DNA. The above results predict that the AT5BIVA cell line, in particular, should have an enhanced capacity for cleavable-complex formation in intact cells if the overproduced topoisomerase II is not limited in its accessibility to genomic DNA. We have used a sensitive DNA unwinding technique to show that AT5BIVA has a greatly enhanced rate of DNA strand breakage upon challenge with mAMSA (Fig. 5). Equivalent levels of DNA damage [approximately three breaks/Mₙ, 10⁶; assuming 2.7 breaks/Mₙ, 10⁶ DNA/Gy X-radiation (33)] were induced in one XP and two normal cell lines exposed to 2.5 μM mAMSA (for 1 h) and in the AT5BIVA cell line exposed to 0.5 μM mAMSA.

Cytotoxic Effects of mAMSA. Fig. 6 shows the greatly enhanced sensitivity of AT5BIVA cells to cell killing by mAMSA. Computer analyses of the survival responses yield D₀ values of

![Graph](image)

**Fig. 4.** mAMSA-dependent DNA-protein cross-linking activities in nuclear extracts of transformed fibroblasts (a) and lymphoblastoid (b) cell lines. Reactions were run in the presence (solid lines) or absence (dotted lines) of 20 μM mAMSA. a: O, MRC5CVI; △, GM0637; ▲, XP12ROSV40; •, AT5BIVA. b: O, △, SC; △, ▲, JR; •, GM717; ▲, GM1526. Data points are arithmetic means (standard error approximately 1.5%) of four to nine determinations.

![Graph](image)

**Fig. 5.** mAMSA-induced DNA strand breakage in transformed fibroblast cell lines. O, MRC5CVI; △, GM0637; ▲, XP12ROSV40; •, AT5BIVA. Data points are arithmetic means of two independent determinations.

![Graph](image)

**Fig. 6.** Survival responses of MRC5CVI (O) and AT5BIVA (•) cells to mAMSA. Collected data points (standard error approximately 5%) from two independent experiments.
2.92 and 0.21 μM mAMSA (1-h exposure) for the normal (MRC5CVI) and A-T (AT5BIVA) cell lines, respectively. This greater than 10-fold increase in A-T cell sensitivity to a DNA damaging agent is greater than the "A-T like" enhancement observed for X-radiation (27).

The capacity of DNA damaging agents (27) and DNA topoisomerase interactive drugs (24) to induce G<sub>2</sub> delay is closely related to toxicity measured by clonogenic survival assays. Fig. 7, a–h, shows the effect of 0.5 μM mAMSA on the cell cycle distribution of exponentially growing fibroblasts. The AT5BIVA cell line shows a marked propensity to enter a G<sub>2</sub>-delayed state. Fig. 8a shows the DNA dose dependency of the G<sub>2</sub> block where even the highest dose (2 μM) of mAMSA administered to either two normal (MRC5CVI and GM0637) or an XP (XP12ROSV40) cell line cannot induce a level of G<sub>2</sub> block equivalent to that achieved by the lowest dose (0.25 μM) of mAMSA given to the A-T (AT5BIVA) cell line, indicating a dose modification effect of >8-fold. For comparative purposes Fig. 8b shows the G<sub>2</sub> block responses for normal and A-T lymphoblastoid cell lines. The results indicate that the two A-T lymphoblastoid cell lines are not abnormally sensitive to mAMSA, consistent with the concept that A-T cells are not intrinsically sensitive to this drug by virtue of their A-T genotype (26).

DISCUSSION

The present findings relate to three distinct problems. First, the cytotoxic nature of intercalator (mAMSA)-dependent protein-DNA complex formation and its dependence on the nuclear availability of topoisomerase II. Second, the involvement of topoisomerase II expression in the in vitro phenotype of A-T-derived cells. Third, whether overexpression of topoisomerase II has any impact on the in vitro characteristics of a human cell line. We present evidence that overproduction of topoisomerase II can occur in all parts of the cell cycle of an A-T cell line with no apparent effect on the A-T phenotype or significant changes in cell cycle phase distribution. However, unusually high levels of topoisomerase II result in marked hypersensitivity to mAMSA-induced cell cycle delay and cell kill, in a manner which is directly related to the excess capacity for the production of DNA damage.

Comparing the results for AT5BIVA and MRC5CVI cells shows a close correlation between the generation of DNA damage and the overproduction of topoisomerase II. Nuclear extracts of AT5BIVA reveal that an approximately 10-fold elevation of decatenation activity is accompanied by overproduction of the protein. In attempting to relate the level of topoisomerase II with the frequency of DNA strand breakage it is important to note that complex formation does not require ATP hydrolysis by the enzyme molecule (34). Furthermore, the DNA damage detection assay (DNA unwinding) employed is relatively insensitive to multiple strand scissions in a localized area. Presumably the extensive levels of mAMSA-induced damage in AT5BIVA reflects increased complex formation throughout the genome rather than increased interaction within pre-existing "hot spots." It would be appropriate to determine the distribution of lesions within a defined gene and its flanking sequences as reported for the c-myc locus and the drug VP16-213 (40).

In attempting to evaluate the cytotoxic potential of a given class of DNA damage (in this case DNA-protein cross-linking), regard must be given to the cell type involved and other modes of action of the damaging agent (7). In human breast cancer cells it has been shown (13, 29) that stimulation of the capacity for cleavable complex formation increases sensitivity to some (e.g., VP16-213 and mAMSA) but not all (e.g., doxorubicin and mitoxantrone) topoisomerase II-interactive drugs. Clearly, some agents could effect cell killing by other means such as; membrane damage (41) and radical generation (42). In this paper we have concentrated on responses to the intercalating drug mAMSA given the body of literature (7) which relates low levels of cleavable complex formation in plateau phase/quiescent cultures or modified topoisomerase II enzyme with drug resistance. The study provides complementary evidence, when compared with reports on the effects of low levels of topoisomerase II on drug sensitivity, which supports the notion that cleavable complex formation is potentially the primary route for mAMSA toxicity in human cells.

Our findings with EBV-transformed lymphoblastoid cell lines are consistent with the recent report of low or normal levels of topoisomerase II activity in A-T lymphoblastoid (23). Both the immunoblotting and filter-binding studies indicate that lymphoblastoid cells have lower levels of topoisomerase II and extractable cross-linking activity than the SV40-transformed fibroblast lines. The hypersensitivity of A-T cells to chromo-

Fig. 7. DNA content versus frequency histograms showing cell cycle phase distributions for mAMSA (0.5 μM X 1 h)-treated transformed fibroblasts assayed at 24 h after drug treatment (b, d, f, and h) compared with untreated controls (a, c, e, and g). Cell lines: MRC5CVI (a and b); GM0637 (c and d); XP12ROSV40 (e and f) and AT5BIVA (g and h).

Fig. 8. mAMSA-induced G<sub>2</sub> + M delay in transformed fibroblasts (a) and lymphoblastoid cells (b) determined at 24 h after a 1-h drug exposure or at the end of a 24-h continuous exposure. Symbols (and control values for % G<sub>i</sub>, % S, and % G<sub>2</sub> + M) in a: O, MRC5CVI (46.5, 38.2, 15.3); △, GM0637 (30.5, 39.9, 29.6); •¿, XP12ROSV40 (36.3, 38.8, 24.9); ⊙, AT5BIVA (26.8, 46.8, 26.7). Symbols (and control values for % G<sub>i</sub>, % S, and % G<sub>2</sub> + M) in b: O, SC (59.8, 31.1, 9.1); △, JR (61.6, 27.8, 10.6); ⊙, GM717 (54.5, 32.6, 12.9); △, GM1526 (58.1, 27.5 and 14.4). Data points are arithmetic means of two determinations (range, ±3%).
some damage has been demonstrated for noncycling G2 phase cultures (43) in which topoisomerase II levels should be reduced (7, 39). A preliminary study using the filter-binding assay to measure mAMSA-dependent DNA-protein cross-linking activities in A-T cells recruited into a G2-delayed state following X-irradiation (27) reveals putative levels of topoisomerase II which are merely commensurate with cell cycle position (data not shown). Taken together with the current findings of high levels of topoisomerase II in an A-T-derived cell line, which shows the expected level of X-ray sensitivity for cell kill (27) and resistance to the inhibition of de novo DNA synthesis (44), we conclude that there is no clear involvement of topoisomerase II in the in vitro A-T phenotype. We may also deduce from the current study that modified topoisomerase II expression does not contribute to the expression of UV sensitivity in the XP (Group A) cell line studied.

A recent report on the expression of topoisomerase I and II throughout the cell cycle in chicken lymphoblastoid cells (39) suggests that topoisomerase II is actively degraded following the expected level of X-ray sensitivity for cell kill (27) and resistance to the inhibition of de novo DNA synthesis (44), we conclude that there is no clear involvement of topoisomerase II in the in vitro A-T phenotype. We may also deduce from the current study that modified topoisomerase II expression does not contribute to the expression of UV sensitivity in the XP (Group A) cell line studied.

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