Tyrosyl-DNA Phosphodiesterase 1 (TDP1) Repairs DNA Damage Induced by Topoisomerases I and II and Base Alkylation in Vertebrate Cells

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Background: Tdp1 is a DNA repair enzyme conserved across eukaryotes.

Results: Tdp1 repairs not only 3’-tyrosyl-DNA bonds and 3’-phosphoglycolates but also 5’-tyrosyl-DNA bonds and 3’-deoxyribose phosphates.

Conclusion: The end processing functions of Tdp1 extend to the repair of Top2-DNA adducts and DNA breaks from base alkylation.

Significance: Tdp1 has a broad range of DNA repair activities and is a potential drug target in anticancer therapy.

Tyrosyl-DNA phosphodiesterase 1 (Tdp1) removes Topoisomerase I (Top1)3 is an essential eukaryotic enzyme that regulates DNA topology by relaxing both positive and negative DNA supercoiling generated during replication and transcription (1–3). To untwist the DNA, Top1 nicks one DNA strand by covalently linking its catalytic tyrosine residue to a 3’-phosphate, which allows the controlled rotation of the broken strand around the intact strand (4, 5). Once supercoiling is removed, Top1 is released by religation of the DNA 3’-phosphate with the 5’-hydroxyl end (2, 6). Camptothecin (CPT) derivatives, such as topotecan and irinotecan, are Top1 inhibitors widely used in cancer chemotherapy (7). The indenoisoquinolines, another class of non-CPT Top1 inhibitors, are in clinical development (8). These agents kill cancer cells by stabilizing Top1 cleavage complex (Top1cc) (9) and inducing the formation of DNA double strand breaks (DSBs) upon replication fork collisions with Top1cc (3, 10–13). Transcription has also been shown to contribute to the formation of DSBs (14–16).

Tyrosyl-DNA phosphodiesterase 1 (Tdp1) removes Top1cc (17–23), while Top1cc-induced DSBs resulting from replication fork collisions are primarily repaired by homologous recombination (24–28). Mutation and genetic inactivation of Tdp1 cause the neurodegenerative disease spinocerebellar ataxia neuropathy 1 (29). Spinocerebellar ataxia neuropathy 1 lymphoblastoid cells, Tdp1 knock-out mice, and murine cells derived from these mice all exhibit hypersensitivity to CPT (20, 21, 30–36). The recent finding that Tdp1 localizes to mitochondria and contributes to efficient repair of oxidative damage in mitochondrial DNA revealed a novel role for Tdp1 in mitochondrial DNA repair (6).

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The abbreviations used are: Top1, topoisomerase I; Top2, topoisomerase II; CPT, camptothecin; Top1cc, topoisomerase I DNA cleavage complex(es); Top2cc, topoisomerase II DNA cleavage complex(es); MMS, methyl methanesulfonate; 3’-dRP, 3’-deoxyribose phosphate; TDP1, tyrosyl-DNA phosphodiesterase 1; DSF, double strand break; PG, phosphorylcollate; AP, apurinic/apyrimidinic; IR, ionizing radiation; nt, nucleotide(s); FAM, fluorescein.
In addition to 3'-phosphotyrosyl bonds, Tdp1 hydrolyzes other adducts that block DNA ends, including 3'-phosphoglycolates (3'-PGs) (37) and AP sites (19, 38) albeit less effectively (18, 39). Endogenous oxidative damage or anticancer treatments (i.e., chemotherapy or ionizing radiations) can lead to such 3'-DNA blocking lesions. Ionizing radiation (IR) induces a variety of DNA lesions, including a small number of lethal DSBs and many more single strand breaks with 3'-PG ends (40, 41). Bleomycin induces DSBs with 3'-PG ends (42). The involvement of Tdp1 in the repair of such lesions is significant given that several of the agents listed above are clinically used for cancer treatment.

To better understand the role of Tdp1 in vertebrate cells, we took advantage of the relative ease to delete specific genes in chicken DT40 cells (43), which have well characterized repair pathways (44). We generated Tdp1-deficient chicken DT40 (Tdp1−/−) cell lines and Tdp1+/− cells complemented with human Tdp1. Notably, there is a high degree of amino acid sequence identity and similarity between human and chicken Tdp1 (69 and 11%, respectively) with all catalytic residues conserved (45) (Fig. 1). We then examined the role of Tdp1 in repairing a broad range of DNA damages using the Tdp1+/− cells in cell survival assays and biochemical assays.

The role of Tdp1 in topoisomerase II cleavage complex (Top2cc) removal has been controversial. Etoposide, a clinically used Top2 inhibitor, stabilizes Top2cc, while the Top2 homodimers are covalently linked to extruding 4-nucleotide overhangs on the 5'-end of DNA (3, 9, 46). Some reports have suggested the involvement of Tdp1 in Top2cc repair (47, 48), whereas others have provided contradictory data (18, 30, 32, 49). The results of the present study clarify the involvement of Tdp1 in resolving Top2cc-mediated DNA damage. Finally, because CtIP (RBBP8) has recently emerged as a critical factor for the repair of topoisomerase-DNA complexes (26, 27, 50), we generated double mutant DT40 cells for Tdp1 and CtIP and investigated the relative contribution of the Tdp1- and CtIP-dependent pathways in the cellular responses to Top1- and Top2-targeting drugs as well as methyl methanesulfonate (MMS).

EXPERIMENTAL PROCEDURES

Cell Culture—DT40 cells were cultured at 37 °C with 5% CO₂ in RPMI 1640 medium supplemented with 1% chicken serum (Invitrogen), 10−5 M β-mercaptoethanol, penicillin, streptomycin, and 10% fetal calf serum.
**Tdp1-mediated DNA Repair**

**Generation of Tdp1−/− DT40 Cells—** Tdp1 gene disruption construct 1 (Tdp1-1-puro) was generated from genomic PCR products combined with puromycin-resistant selection cassettes flanked by loxP sites using MultiSite Gateway technology (Invitrogen). All procedures were performed according to the manufacturer’s instructions. Genomic DNA sequences of wild type cells were amplified using primers 5′-tttgggacctgttgctctgtcc-3′ and 5′-gctcaaatctacactgcggc-3′ for the 5′-arm and 5′-gaagaacctgttgctc-3′ and 5′-cttgcaaggctgctc-3′ for the 3′-arm. To generate the 5′- and 3′-arm entry clones, 2.7 kb from the 5′- and 3.7 kb from the 3′-arm were subcloned by BP recombination into the donor vectors pDONR™ P4-P1R and pDONR P2R-P3, respectively. To generate the targeting vector by LR recombination, we used the primers 5′-ctgcttt-3′ and 5′-acagctgtttgc-3′ for the 5′-arm. The amplified 3′-arm PCR product (2.2 kb) was subcloned into pCR2.1-TOPO vector (Invitrogen). All procedures were performed according to the manufacturer’s instructions. Genomic DNA sequences of wild type cells, Tdp1-2-hyg was linearized using the SacI restriction enzyme and transfected by electroporation (Bio-Rad).

**To generate the targeting vector by LR recombination,** we used the primers 5′-ctgcttt-3′ and 5′-acagctgtttgc-3′ for the 5′-arm. The amplified 3′-arm PCR product (2.2 kb) was subcloned into pCR2.1-TOPO vector (Invitrogen). All procedures were performed according to the manufacturer’s instructions. Genomic DNA sequences of wild type cells, Tdp1-1-puro was linearized by the AscI restriction enzyme and transfected by electroporation (Bio-Rad).

**Generation of CtIP S332A/+; Tdp1−/− DT40 Cells—** To generate the double knock-out CtIP S332A/+; Tdp1−/− DT40 cells, the linearized Tdp1 disruption constructs Tdp1-1-puro and Tdp1-2-hyg were transfected sequentially by electroporation. Gene disruption was confirmed by Southern blot and RT-PCR (see above).

**Immunoblotting and Antibodies—** To prepare whole cell lysates, cells were lysed by Celllytic™ M lysis reagent (C2978, Sigma-Aldrich). After thorough mixing and incubation at 4 °C for 30 min, lysates were then centrifuged at 12,000 × g at 4 °C for 20 min. Supernatants were collected, aliquoted, and stored at −80 °C. Preparation of mitochondrial and nuclear extracts was performed as described (6). Lysates were prepared in the same manner as whole cell lysates. Immunoblotting was carried out using standard procedures. Rabbit polyclonal anti-Tdp1 antibody was obtained from Abcam (Ab4166; Cambridge, MA). Mouse monoclonal anti-γH2AX antibody was purchased from Upstate Biotechnology (Lake Placid, NY). Actin antibodies were purchased from Sigma. Mouse monoclonal anti-Top1 antibody was purchased from BD Biosciences (556597). Rabbit polyclonal anti-Porin (AB-5; voltage-dependent anion channel) antibody was purchased from EMD Millipore (PC548T-5UG). Secondary antibodies were horseradish peroxidase (HRP)-conjugated antibodies to mouse or rabbit Ig (GE Healthcare).

**Preparation of Radiolabeled Oligonucleotides and Substrates—** Oligonucleotides with 5′- and 3′-phosphotyrosine linkages were synthesized by Midland Certified Reagent Co., Inc. (Midland, TX). All other oligonucleotides were synthesized by Integrated DNA Technologies (Corvala, IA). T4 polynucleotide kinase (New England Biolabs, Cambridge, MA) and [α-32P]ATP (PerkinElmer Life Sciences) were used for 5′-end labeling, and terminal deoxynucleotidyl transferase (Invitrogen) and [α-32P]dCTP (PerkinElmer Life Sciences) were used for 3′-end labeling. For the preparation of internally labeled DNA oligonucleotides, a 22-nt DNA (5′-gcgcagctagcggcggatggca-3′) and a 3′-phosphate was labeled with [32P] at the 5′-end. An 18-nt DNA (5′-tcctgtagcctgcttt-3′) harboring the phosphotyrosine, hydroxyl, or phosphate at the 5′-end was mixed with 5′-labeled 22-nt DNA before annealing to a 36-nt DNA with complementary sequence. The nicks were sealed with T4 DNA ligase (New England Biolabs). The resulting internally labeled 40-nt product harboring 5′-phosphotyrosine, -hydroxyl, or -phosphate was then gel-purified and eluted for use (named Y40, OH40, and P40, respectively). Y40 was then annealed to a complementary 40-nt DNA (5′-tgcagctgcggcggatggca-3′) or to shorter complementary DNA strands (missing 2, 4, or 6 nt from the 3′-end) to generate Y40/40, Y40/38, Y40/36, or Y40/34, respectively (see Fig. 4). Double-stranded OH40/36 and P40/36 were generated in the same manner. For the 3′-deoxyribose phosphate (3′-dRP) substrate, 5′-labeled 25-nt DNA carrying uracil at the 15th nt from the 5′-end was annealed to a complementary 25-nt DNA harboring adenine opposite the uracil (supplemental Fig. S4). Annealed DNA was incubated with uracil-DNA glycosylase for 1 h at 37 °C, and then Endonuclease III (New England BioLabs) was added for 1 h at 37 °C to generate the 3′-dRP at a nicked DNA site. Unincorporated radioactive nucleotides were removed using a mini Quick Spin Oligo column (Roche Diagnostics).

Complementary oligodeoxynucleotides were annealed in equimolar amounts by heating a solution to 95 °C for 3 min followed by slow cooling to room temperature. The other sequences of the oligonucleotides used in the experiments are as follows: 14Y, 5′-gatcagaaaaagcct-3′; 14P, 5′-gatcagaaaaagcct-3′; 14OH, 5′-gatcagaaaaagcctOH-3′; Y19, 5′-Yp-tcctgtagccgcaagctgcttt-3′; Y19/19, 5′-Yp-tcctgtagcgcggcggatggca-3′ and 3′-agcagctgcggcggatggca-5′; P19, 5′-p-tcctgtagcgcggcggatggca-3′; OH19, 5′-OH-tcctgtagcgcggcggatggca-3′; and 6-FAM19, 5′-FAM-
tccgtgagctgtgcttt-3’. The bold “a” indicates the added adenosine through the 3’-end labeling using [α-32P]cordycepin 5’-triphosphate (Yp, tyrosylphospho linkage; p, phosphate linkage; pY, phosphotyrosyl linkage).

DNA Reactions and Gel Analyses—Preparation of cell lysate was carried out as same as for immunoblotting described above. One nanomolar labeled DNA substrates in a 10-μl reaction volume were incubated with the indicated concentration of recombinant human TDP1 (52) or cell lysate for the indicated time at 25 °C in a buffer containing 80 mM KCl, 2 mM EDTA, 1 mM dithiothreitol (DTT), 40 μg/ml bovine serum albumin, 50 mM Tris-HCl, pH 7.5, and 0.01% Tween 20. Reactions were terminated by adding 1 volume of gel loading buffer (96% (v/v) formamide, 10 mM EDTA, 1% (w/v) xylene cyanol, and 1% (w/v) bromphenol blue). Double-stranded substrates were heated at 95 °C for 3 min before loading. Samples were subjected to 16% denaturing PAGE. Gels were dried and exposed on PhosphorImager screens. Imaging and quantification were done using a Typhoon 8600 and ImageQuant software (GE Healthcare).

Measurement of Cellular Sensitivity to DNA-damaging Agents—To assess IR sensitivity, 3 × 10^6 cells in 10 ml of medium were irradiated with a 137Cs source. To measure the sensitivity of cells to CPT, etoposide, MMS, bleomycin, and cisplatin, cells were continuously exposed to various concentrations of the drugs. For exposure of cells to hydrogen peroxide (H2O2), 3 × 10^5 cells were treated in 1 ml of medium containing H2O2 for 30 min and then washed with PBS. Two hundred cells were seeded into a 384-well white plate (6007680, PerkinElmer Life Sciences) with 40 μl of medium/well. Plates were incubated at 37 °C for 72 h. Cell survival was determined using the AtPlite 1step kit (PerkinElmer Life Sciences). Briefly, 40 μl of AtPlite solution was added to each well. After 5 min, luminescence was measured by an Envision 2104 Multilabel Reader (PerkinElmer Life Sciences).

Immunostaining—Cells were treated with or without 10 nM CPT for 2 h. After cytospin, cells were fixed with 4% paraformaldehyde for 10 min at room temperature. Primary antibody against γH2AX was detected using anti-mouse IgG secondary antibodies labeled with Alexa Fluor 488/568 (Invitrogen). Cells were mounted in antifade solution with DAPI (Vector Laboratories, Burlingame, CA) and examined using a laser-scanning confocal microscope (Zeiss LSM510) with a ×63 oil objective. Images were collected and processed using the Zeiss AIM software and sized in Adobe Photoshop 7.0.

RESULTS

Generation of Tdp1+/− DT40 Cells—We disrupted one of two Tdp1 alleles using targeting construct 1 (Tdp1-1-puro) carrying a puromycin resistance gene (Fig. 2A) to generate Tdp1+/− cells. Gene targeting was confirmed by Southern blotting (Fig. 2B). However, disruption of the other allele was not successful using targeting construct 1 carrying a hygromycin resistance gene. This indicated the possibility of an allele-specific mutation(s) between the two Tdp1 gene alleles. Therefore, we generated targeting construct 2 (Tdp1-2-hyg; Fig. 2A) in which the homology arms were amplified using the genomic DNA from the Tdp1+/− cells as template. Targeting construct 2 successfully disrupted the other Tdp1 allele (Fig. 2B). We confirmed Tdp1 gene disruption by RT-PCR using paired primers a/b that were designed to flank both resistance genes containing stop codons. Other paired primers c/d that were designed from the 5’-side of the targeted sites were used as a control. As expected, primers a/b amplified the cDNA of wild type and Tdp1+/− cells, whereas none of three independent Tdp1+/− cell clones yielded detectable product (Fig. 2C).

To complement the Tdp1+/− cells, we transfected the FLAG-tagged human TDP1 cDNA in Tdp1+/− cells. The chiken Tdp1+/− cells complemented with human TDP1 (hereafter referred to as Tdp1+/−/hTDP1) showed a similar amount of TDP1 protein expression compared with human 293T cells (Fig. 2D). Several available antibodies raised against human TDP1 did not react with the chicken Tdp1 (Fig. 2D and data not shown). The proliferative properties of generated cells were indistinguishable from those of wild type cells as monitored by growth curves (Fig. 2E) and by cell cycle analysis (data not shown).

To examine the tyrosyl-DNA phosphodiesterase activity of the mutant cells, we performed gel-based assays using a 14-nt single-stranded DNA oligonucleotide bearing a 3’-phosphotyrosine (14Y) labeled at the 5’ terminus with 32P (19, 23, 49). Tdp1 activity was measured by the extent of substrate conversion into a 3’-phosphate DNA product (14P). The 14P product can be readily converted further to a 3’-hydroxyl DNA product (14OH) by polynucleotide kinase 3’-phosphatase (Fig. 2F, upper panel) (53). The 32P-labeled 14Y substrate was incubated with serially diluted whole cell lysates from wild type, Tdp1+/−, and Tdp1+/−/hTDP1 cells in buffer without magnesium. Wild type and Tdp1+/−/hTDP1 cells showed similar processing activity that was detectable with as little as 0.1 ng/μl whole cell lysate. By contrast, no processing activity was observed in Tdp1+/− cells extract even at 8,800-fold excess (880 ng/μl) (Fig. 2F, lower panel). These results show that Tdp1 is the only factor able to process the 3’-phosphotyrosyl-DNA bond under divergent cation-free conditions and that human TDP1 can complement chicken Tdp1 for the 3’-phosphotyrosyl-DNA bond processing activity.

To determine whether Tdp1 was also functioning in DT40 cell mitochondria, we examined the Tdp1 activity of mitochondrial extracts by fractionating wild type and Tdp1+/− cells into nuclear and mitochondrial fractions and then performed the gel-based assays using 14Y substrates (supplemental Fig. S1). The mitochondrial fraction from wild type cells showed robust 3’-phosphotyrosyl diesterase activity, whereas no detectable processing activities were observed for the mitochondrial fraction of Tdp1+/− cells. These data demonstrate that the nuclear Tdp1 gene provides 3’-phosphotyrosyl-diesterase activity to both nuclear and mitochondrial DNA.

Broad Involvement of Tdp1 for DNA Repair—We examined the sensitivity of Tdp1+/− cells to various types of DNA-damaging agent (Fig. 3). As expected (31, 32), Tdp1+/− cells showed hypersensitivity to CPT. Low dose CPT (10 nM) also induced high γH2AX signal (12) in the Tdp1+/− cells as detected by immunostaining and Western blotting (supplemental Fig. S2, A and B) and induced the accumulation of cells at the G2 phase in Tdp1+/− cells (supplemental Fig. S2C).
Tdp1 cells showed consistent hypersensitivity to etoposide, a selective Top2 inhibitor (3, 46), marginal sensitivity to IR, and strong sensitivity to bleomycin in agreement with the fact that both IR and bleomycin induce DSBs and/or single strand breaks with 3'-PG ends that can be processed by Tdp1 (6, 37, 39, 54).

Tdp1 cells also showed significant sensitivity to MMS and mild sensitivity to H2O2. Complementation of the Tdp1 cells with human TDP1 reduced the sensitivities to these treatments. These results suggest that Tdp1 has broad involvement in the repair of lesions induced by a variety of DNA-damaging agents.

Cleavage of Top2cc by Tdp1—Our cell survival results showing that Tdp1-/- cells are hypersensitive to etoposide prompted us to examine the involvement of Tdp1 for the repair of Top2cc in greater detail. To test the activity of Tdp1 for Top2cc removal, we performed gel-based cleavage assays using DNA substrates with 5'-phosphotyrosyl ends. In addition to a blunt-ended double strand substrate (Y40/40), we tested substrates with 5'-overhangs of 2, 4, or 6 bases (Y40/38, Y40/36, and Y40/34, respectively; Fig. 4). The internally labeled substrates contained phosphate groups on the 3'-end to prevent the 3'-nucleosidase activity of Tdp1 from removing the last..
revealed that TDP1 did not process 5'-fluorescein (6-FAM)-labeled oligonucleotide. Additional experiments (Fig. 5) we conclude that TDP1 cannot process a 5'-nucleosidase activity of TDP1.

Involvement of Tdp1 for Repair of Abasic Sites—Because the hypersensitivity of Tdp1−/− cells to MMS was strong and almost fully reverted by human TDP1 (Fig. 3), we investigated the biochemical basis of this hypersensitivity. MMS is a strong electrophile that attacks the most nucleophilic centers in DNA, resulting in DNA base methylation (55). Methylated DNA bases can be converted into abasic (AP) sites by DNA N-glycosylase. H2O2 also generates modified bases such as 8-oxoguanine, which can lead to AP sites (56) (Fig. 6A). AP sites are then repaired by various pathways, including base excision repair, nucleotide excision repair, translesion synthesis, or homologous recombination. In the process of base excision repair, a 3′-dRP can form as a result of β-elimination by OGG1 and NTH1 that inhibits the gap filling by DNA polymerase β (57).

Although 3′-dRP is known to be removed by AP endonuclease 1, a recent study revealed that both an AP site and a 3′-dRP are also cleaved by Tdp1 (38). Consistent with these results, we also demonstrated that recombinant human TDP1 can process a 3′-dRP substrate. The processing efficiency of 3′-dRP by TDP1 was 17-fold less than that of 3′-phosphotyrosine in terms of EC50 (the half-maximal effective concentration) (Fig. 6, B and C, and supplemental Fig. S4). Therefore, the sensitivity of Tdp1−/− cells to MMS and H2O2 is consistent with impaired repair activity of AP sites and 3′-dRP in these cells.

**DISCUSSION**

The role of Tdp1 is well established for the repair of Top1-induced DNA damage (20, 21, 31, 32, 35, 58, 59). Because inactivation of Tdp1 augments the antiproliferative effect of Top1 inhibitors, Tdp1 inhibitors are being explored as anticancer drugs in cancer cells with preexisting Top1cc repair defects (45, 60).
Our results expand the spectrum of repair pathways that involve Tdp1, including the repair of DNA lesions produced by etoposide, IR, bleomycin, MMS, and H2O2. Biochemical assays corroborate this view given that human TDP1 can process 3'-PGs (6, 37, 39, 54), 3'-dRP (present study), 5'-phosphotyrosine (present study), and AP sites (19, 38). 3'-PG ends represent up to half of the breaks induced by IR (40) and are present at a large fraction of the DSB ends produced by bleomycin (37, 54). Accordingly, cells from Tdp1 knock-out mice are defective in the repair of IR- and H2O2-induced breaks and hypersensitive to bleomycin (31, 32). Spinocerebellar ataxia neuropathy 1 cells are also radiosensitive and unable to rapidly repair H2O2-induced single strand breaks (20). Consistent with these results, our finding that Tdp1-mediated DNA Repair

FIGURE 4. Processing activity of recombinant human TDP1 on double-stranded substrate harboring 5'-phosphotyrosyl linkage with blunt end (Y40/40) or 2'- (Y40/38), 4'- (Y40/36), or 6-base (Y40/34) 5'-overhangs. A, the substrates were incubated with 1 μM TDP1 for the indicated time. P40 was used as marker. A gel representative of consistent results in independent experiments is shown. B, quantification of TDP1 processing activity from A (left). The error bars represent the S.E. (n = 3). A scheme of the DNA substrates used in A is shown (right). An asterisk indicates the internal radiolabeled site.

FIGURE 5. Differential activity of recombinant human TDP1 for single-stranded DNA substrates harboring 5'-phosphotyrosine (Y19) and 5'-fluorescein (6-FAM19). A, scheme for the processing pathways of Y19 (left) and 6-FAM19 (right) by TDP1. Because of the nucleosidase activity of TDP1 (19), which removes the last 3'-end base, the Y19 substrate can be processed in two ways. One is that the 5'-tyrosine is removed first (P19) before the 3'-terminal adenine (P18) (clockwise). The other is that the 3'-adenine is removed first (Y18) before the 5'-tyrosine (P18) (counterclockwise). An asterisk indicates the radiolabeled site. B, each substrate was incubated with serial dilutions (1:3) of TDP1 at 25 °C for 30 min with the highest TDP1 concentration starting at 1 μM. Processing of 6-FAM19 by TDP1 yielded one product (right), whereas processing of Y19 yielded two distinct products (left), indicating that TDP1 processes 5'-phosphotyrosine but not 5'-fluorescein.
duced by IR and H₂O₂ are known to lead to the formation of Top1cc (61), which would also require Tdp1 for their removal.

Phosphodiesterase activity has been reported for yeast Tdp1. Moreover, deletion of the Tdp1 gene in yeast confers hypersensitivity to etoposide (47), and overexpression of human TDP1 in human cells counteracts the DNA damage mediated by etoposide (48). On the other hand, Tdp1 knock-out mice and embryonic fibroblasts as well as spinocerebellar ataxia neuropathy 1 cells are not noticeably hypersensitive to etoposide (21, 32). Thus, the potential role of Tdp1 in the repair of Top2-mediated DNA damage has been a long standing controversy (18, 47). Our cell survival data with etoposide clearly indicate that Tdp1 can be involved in Top2cc repair in metazoans (Fig. 3). Why the sensitivity to etoposide is detectable in chicken Tdp1 cells but not in mouse and human Tdp1 mutant cells is unclear. One possibility is that chicken DT40 cells might be limited in their alternative repair pathways for Top2cc, whereas redundant pathways are present in human and murine cells. The repair protein complexes involved in the repair of Top2cc are not well understood in vertebrate cells except for the recent discovery of Tdp2 (62, 63). It is also possible that DT40 cells show a differential response to etoposide because of the rapid cell cycle of DT40 cells (8 h for one cycle) with most of the cells in S phase. This may also contribute to the hypersensitivity of DT40 cells to CPT. The complementation by exogenous expression of human TDP1 in Tdp1 cells only partially restored the resistance of Tdp1 cells to etoposide, whereas it almost fully restored normal responses to CPT and MMS (Fig. 3). This difference might be due to species-specific protein-protein interactions whereby human TDP1 fails to interact fully with other chicken-specific repair complexes that are critical for Top2cc and alkylation damage removal. Despite these differences, our biochemical results using recombinant human TDP1 (Figs. 4 and 5) strongly support that Tdp1 plays a role in the removal of Top2cc and contributes to the response to Top2 inhibitors.

To our knowledge, the involvement of Tdp1 in the repair of the DNA lesions induced by the classical alkylating agent MMS has not been reported. MMS produces N7-guanine methyl
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adducts that are readily converted to AP sites by glycosylases or/and spontaneous base elimination. Such lesions are known to trap Top1cc (64–66), which could explain the participation of Tdp1 in their repair. However, our present study also suggests a more direct involvement of Tdp1 in the repair of abasic sites by direct cleavage of the 3’-blocking dRP lesions (19, 38).

The repair of Top1- and Top2-induced DNA lesions involves redundant pathways (46, 66). A recent study revealed that CtIP, together with BRCA1, acts in the nuclease-mediated elimination of Top1cc and Top2cc (26). Deletion of Ctp1, the ortholog of CtIP in Schizosaccharomyces pombe, markedly sensitizes cells to CPT (50), and deletion of Sae2, the Saccharomyces cerevisiae ortholog of CtIP, produces a mild sensitization to CPT (36, 67). Our data show at least an additive sensitization of Tdp1−/−;CtIP S322A−/− double mutant cells compared with the single mutants, indicating parallel (redundant) activities of CtIP and Tdp1 for Top1cc repair in DT40 cells. By contrast, we found that Tdp1 and CtIP are epistatic for the repair of Top2cc, suggesting that Tdp1 and CtIP work together for the removal of Top2cc. Further investigations are warranted to elucidate the possible interactions of Tdp1 and CtIP for the repair of Top2-induced DNA lesions.

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