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Brca1 is involved in tolerance to adefovir dipivoxil-induced DNA damage

HAO LIU[1*], YANG WU[2*], FANG HE[3*], ZIYUAN CHENG[1], ZILU ZHAO[1], CUIFANG XIANG[1], XIAOYU FENG[1], XIN BAI[1], SHUNICHI TAKEDA[4], XIAOHUA WU[2] and YONG QING[1]

[1]Department of Pharmacology, West China School of Pharmacy, Sichuan University; [2]State Key Laboratory of Biotherapy and Cancer Center, [3]Center of Infectious Diseases, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, P.R. China; [4]Department of Radiation Genetics, Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan

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Abstract. Nucleos(t)ide analogues (NAs) are currently the most important anti-viral treatment option for patients with chronic hepatitis B (CHB). Adefovir dipivoxil (ADV), a diester pro-drug of adefovir, has been widely used for the clinical therapy of hepatitis B virus infection. It has been previously reported that adefovir induced chromosomal aberrations (CAs) in the *in vitro* human peripheral blood lymphocyte assay, while the genotoxic mechanism remains elusive. To evaluate the possible mechanisms, the genotoxic effects of ADV on the TK6 and DT40 cell lines, as well as DNA repair-deficient variants of DT40 cells, were assessed in the present study. A karyotype assay revealed ADV-induced CAs, particularly chromosomal breaks, in wild-type DT40 and TK6 cells. A γ-H2AX foci formation assay confirmed the presence of DNA damage following treatment with ADV. Furthermore, *Brca1*$^{-/-}$ DT40 cells exhibited an increased sensitivity to ADV, while the knockdown of various other DNA damage-associated genes did not markedly affect the sensitivity. These comprehensive genetic studies identified the genotoxic capacity of ADV and suggested that Brca1 may be involved in the tolerance of ADV-induced DNA damage. These results may contribute to the development of novel drugs against CHB with higher therapeutic efficacy and less genotoxicity.

Correspondence to: Dr Yong Qing, Department of Pharmacology, West China School of Pharmacy, Sichuan University, No. 17, Section 3, People's South Road, Chengdu, Sichuan 610041, P.R. China
E-mail: qingyong@scu.edu.cn

Dr Xiaohua Wu, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, No. 17, Section 3, People's South Road, Chengdu, Sichuan 610041, P.R. China
E-mail: wuxh@scu.edu.cn

[*]Contributed equally

Abbreviations: HBV, hepatitis B virus; cccDNA, covalently closed circular DNA; NAs, nucleos(t)ide analogues; CHB, chronic hepatitis B; ETV, entecavir; ADV, adefovir dipivoxil; DSBs, double-strand DNA breaks; CPT, camptothecin; DMSO, dimethyl sulfoxide; IC_{50}, 50% inhibiting concentration; ISCN, International System for Human Cytogenetic Nomenclature; *WT*, wild-type; CAs, chromosomal aberrations; BER, base excision repair; HR, homologous recombination; NHEJ, non-homologous end joining; TLS, translesion DNA synthesis; NER, nucleotide excision repair

Key words: adefovir dipivoxil, DNA damage, Brca1, DT40 cells, TK6 cells, homologous recombination

Introduction

Chronic hepatitis B (CHB) is a major health problem worldwide (1). It is estimated that at least one-third of the world population have been infected with hepatitis B virus (HBV) (2) and 240 million individuals are chronic carriers; however, a curative therapy remains unavailable (3). HBV, a hepadnaviridae, stabilizes in hepatocytes by forming covalently closed circular DNA (cccDNA) (4,5). At present, nucleos(t)ide analogues (NAs) are one of the two major options for CHB treatment (6,7). The NAs approved for HBV treatment include tenofovir alafenamide, entecavir (ETV) and adefovir dipivoxil (ADV). Although ADV has not been recommended as the first-line therapy, it is commonly used in numerous Asian countries due to the relatively lower resistance rate and lower cost compared with those of other therapies (6-10).

NAs markedly inhibit reverse transcriptase to reduce the DNA levels of HBV (11,12). However, an increasing body of evidence has indicated that cccDNA stably attaches to the host hepatocyte genome in order to avoid elimination by NA (4,11,13,14). Thus, the majority of patients require long-term NA therapy, even if HBV DNA has decreased to undetectable levels for a short time. Therefore, it is important to study the safety of NA therapy and the re-treatment efficacy following a relapse (15). By using specific assays, studies have determined that the nucleoside analogue ETV has genotoxic (16) and carcinogenic effects (17). Various types of DNA lesions, including single-strand DNA breaks, double-strand DNA breaks (DSBs), alkylation of DNA bases and covalent links between bases (intrastrand and inter-strand crosslinks), may be caused by genotoxic chemicals (18). Unrepaired or incorrectly repaired lesions result in mutations and/or genetic instability, which may then be risk factors of carcinogenesis. In addition, the US prescription information sheet (19) states that ADV was indicated to be mutagenic in an

in vitro mouse lymphoma cell assay. However, the underlying mechanisms of the genotoxicity of ADV remain elusive. Further studies are required to gain a better understanding of the genetic toxicity mechanisms of ADV. The DT40 cell line originates from a chicken B-lymphocyte line (20) and TK6 lymphoblastoid cells are a human-derived cell line (21-24). By using the wild-type (*WT*) or specific gene knockout variants of these cell lines, the toxicity of ADV was evaluated and the underlying mechanisms were investigated in the present study.

Materials and methods

Chemicals. ADV (purity, ≥99%) and camptothecin (CPT; purity, ≥99%) were purchased from MedChemExpress (Monmouth Junction, NJ, USA). These chemicals were dissolved in dimethyl sulfoxide (DMSO). Stock solutions of ADV (10 mM) and CPT (100 μM) were stored at -20˚C in aliquots. In each experiment, the final concentration of DMSO never exceeded 0.1%.

Cell lines and cell culture. All of the cell lines used in the present study were provided by Professor Shunichi Takeda (Kyoto University, Kyoto, Japan). The DT40 cell lines (25-30) with different phenotypes used in the present study are summarized in Table I. The DT40 cells were incubated in RPMI-1640 medium containing 10% newborn calf serum, 1% chicken serum, 1% penicillin streptomycin (all Wisent, Inc., St. Bruno, QC, Canada), 200 mM L-glutamine and 50 μM β-mercaptoethanol (both Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 5% CO_2 at 39.5˚C. The TK6 cells were routinely maintained in RPMI-1640 medium (Wisent, Inc.) including 10% horse serum (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (Wisent Inc.) at 37˚C in the presence of 5% CO_2. These sera (except chicken serum) were heat-deactivated at 56˚C for 30 min prior to use.

Cell viability assay. The anti-proliferative effects of ADV and CPT on cells was determined with MTT assay (31-33). Briefly, the cells ($1x10^4$ cells/ml) were seeded into 96-well plates in complete medium, followed by incubation in the presence of various concentrations of ADV or CPT for 72 h. The concentrations of ADV were 0, 0.06, 0.12, 0.18, 0.24 and 0.3 μM for DT40 cells or 0, 1, 2, 3, 4 and 5 μM for TK6 cells. The CPT concentrations were 0, 10, 20, 30, 40 and 50 nM for DT40 cells, or 0, 2, 4, 6 and 8 nM for TK6 cells. The concentrations of CPT were 0, 1, 2, 3, 4 and 5 nM, or 0, 10, 20, 30, 40 and 50 nM for DT40 cells specifically for sensitivity experiments. DMSO (<0.1%) was applied as a solvent and vehicle control with 3 replicates for each concentration of the drugs. Following 69 h treatment at 39.5˚C for DT40 and at 37˚C for TK6 cells, 20 μl of 5 mg/ml MTT (Amresco, LLC, Solon, OH, USA) was added to the cells for 3 h at 39.5˚C for DT40 and at 37˚C for TK6 cells, and subsequently, the formazan dye crystals were dissolved by 50 μl of 20% sodium dodecyl sulfate overnight at 39.5˚C for DT40 and at 37˚C for TK6 cells. The absorbance was detected at a wavelength of 570 nm by a microplate reader. The wells without cells served as a blank control. All the experiments were repeated at least 3 times. The 50% inhibitory concentration (IC_{50}) of the drugs on the cells was calculated with SPSS 25.0 software (IBM Corp, Armonk, NY, USA).

Chromosome aberration (CA) analysis. Preparation of chromosome samples was performed as described previously (34-37), with certain modifications. Briefly, cells ($2x10^5$ cells/ml) were seeded in 6-well plates and were treated with ADV or CPT in complete medium. DT40 cells were treated with 0.2 or 0.4 μM ADV for 9-30 h and 5 or 25 nM CPT for 6-24 h at 39.5˚C. TK6 cells were performed with 5 μM ADV for 12-36 h and 2.5 μM ADV for 12 h at 37˚C. Colcemid (0.2 μg/ml; Gibco; Thermo Fisher Scientific, Inc.) was added 3 h prior to harvesting in order to enrich mitotic cells. The cells were then incubated in 1 ml hypotonic KCl solution (75 mM) for 25 min at room temperature. Subsequently, the cells were fixed with 3 ml Carnoy's solution [methanol/acetic acid, 3:1, (v/v)] for 35 min. A drop of this suspension was placed onto ethanol-washed glass slides and immediately dried by a flame. Finally, the dried slides were dyed with 5% Giemsa solution for 5 min at room temperature and carefully rinsed with water prior to air-drying. In the present study, 50 metaphase cells per each experiment were analyzed under a light microscope (magnification, x1,000). All assays were performed 3 times. A break or gap in a chromosome was evaluated and defined according to The International System for Human Cytogenetic Nomenclature (ISCN) (37).

Immunofluorescence analysis. γ-H2A histone family member X (H2AX) formation is a rapid and sensitive cellular response to DSBs (38). Immunofluorescent staining was performed as reported previously (39). Briefly, cells ($2x10^5$ cells/ml) cultured on 12-well-plates. DT40 cells were treated with 0.1 μM ADV or 0.1 μM CPT for 3, 6 and 9 h at 39.5˚C, and TK6 cells were treated with 1 μM ADV or 20 nM CPT for 3, 6 and 9 h at 37˚C and then harvested on glass slides. The cells were fixed with 3% paraformaldehyde for 10 min at room temperature followed by washing with PBS 3 times. The fixed cells were permeabilized with 0.1% Nonidet P-40 for 20 min at room temperature and washed with PBS again. Following blocking with 3% bovine serum albumin (cat. no. A8020; Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) for 30 min at room temperature, the cells were probed with anti-γ-H2AX mouse monoclonal antibody (cat. no. 05-636; 1:1,000 dilution; EMD Millipore, Billerica, MA, USA) in a humidified environment at 4˚C overnight. Following washing with PBS, the cells were incubated for 1 h at 37˚C with Alexa Fluor 488-labeled goat anti-mouse IgG (H+L) (cat. no. A0428; 1:1,000 dilution; Beyotime Institute of Biotechnology, Wuhan, China). The nuclei were stained with 4',6-diamidino-2-phenylindole for 10 min at room temperature. Finally, a fluorescence microscope (IX81; Olympus Corporation, Tokyo, Japan) was used to visualize the γ-H2AX foci (magnification, x1,000). The foci in 100 nuclei were counted. The visible foci as described in previous reports were counted by eye using Photoshop (version 12.0.3; Adobe Systems, Inc., San Jose, CA, USA) (40). The experiments were performed 3 times.

Statistical analysis. Statistical analysis was performed with SPSS 25.0 software (IBM Corp.). The statistically significant differences were determined with a Student's t-test or two-way

Table I. DNA repair genes mutated in the analyzed DT40 clones.

Author, year	Gene	Function	(Refs.)
Sonoda *et al*, 2003	*Rev3*	TLS, HR (catalytic subunit of Polζ)	(25)
Okada *et al*, 2002	*Xpa*	Initial step of NER	(26)
Masson *et al*, 1998	*Parp1*	Poly (adenosine diphosphate) ribosylation, associated with single-strand break and BER	(27)
Qing *et al*, 2011; Rosen, 2013	*Brca1*	HR, NHEJ	(28,30)
Takata *et al*, 1998	*Ku70*	Initial step for NHEJ-dependent DSB repair	(29)

TLS, translesion DNA synthesis; HR, homologous recombination; NER, nucleotide excision repair; BER, base excision repair; NHEJ, nonhomologous end joining; DSB, double-strand break; *Parp1*, poly (adenosine diphosphate-ribose) polymerase; *Rev3*, REV3-like DNA directed polymerase ζ catalytic subunit; *Ku70*, Ku autoantigen 70 kDa; *Xpa*, xeroderma pigmentosum group A-complementing protein.

analysis of variance with Tukey's post hoc test. Values are expressed as the mean ± standard deviation. P<0.05 was considered to indicate a statistically significant difference.

Results

ADV induces DSBs in WT DT40 and TK6 cells. Adefovir was previously demonstrated to induce CAs by a human peripheral blood lymphocyte assay *in vitro* without metabolic activation (19). As a diester prodrug of adefovir (19), it cannot be excluded that ADV may also has mutagenic effects. DSBs, one type of DNA damage, may be reliably identified by γ-H2AX foci detection (41). In the present study, DT40 and TK6 cells were continuously exposed to various concentrations of ADV for 72 h and CPT was used as a positive control. The results of the MTT assay indicated that ADV exerts a notable cytotoxic effect in *WT* DT40 and TK6 cells (Fig. 1). Furthermore, DT40 cells were treated with 0.1 μM ADV for 3, 6 or 9 h to dynamically investigate the changes in the number of γ-H2AX foci. In addition, TK6 cells were also exposed to 1 μM ADV for the same durations. As presented in Figs. 2 and S1, the number of γ-H2AX foci was significantly increased following treatment with ADV and exhibited a peak at 6 h. According to the quantitative distribution of γ-H2AX foci in *WT* DT40 and TK6 cells (Fig. S1), ADV induced a greater percentage of γ-H2AX-positive cells.

In addition, DNA damage was analyzed by measuring cytologically detectable CAs in mitotic cells. *WT* DT40 cells were exposed to 0.2 or 0.4 μM ADV, and CAs were determined at 9, 12, 15, 24 and 30 h. The maximum number of CAs was observed at 12 h of ADV treatment (Fig. 3A and B). Similarly, TK6 cells were exposed to 5 μM ADV for 12-36 h and with 2.5 μM ADV for 12 h. CAs were determined at 12, 24 and 36 h. The maximum number of chromosomal breaks was observed at 12 h (Fig. 3C-E). These results confirmed that ADV was able to generate DNA DSBs in DT40 and TK6 cells.

Brca1$^{-/-}$ cells defective in DNA repair pathways are sensitive to ADV. To study the genotoxic mechanisms of ADV, an MTT assay using *WT* and mutant DT40 cells was performed. The sensitivity of ADV was assessed in a panel of DT40 clones, each of which was deficient in a different DNA repair pathway (Table I) (25-30). DT40 cells were treated with ADV at various concentrations for 72 h. It was revealed that ADV inhibited cell growth in a dose-dependent manner (Fig. 4). As presented in Fig. 4A-C, only *Brca1$^{-/-}$* (28,30) cells exhibited significant sensitivity to ADV. The present study also exposed *WT* and *Brca1$^{-/-}$* DT40 cells to ADV and evaluated their cellular response using a colony survival assay (Data S1). Compared with *WT* DT40 cells, the colony survival of *Brca1$^{-/-}$* cells was significantly reduced at 14 days (Fig. S2). The results of the clonogenic assays also confirmed this sensitivity (Fig. S2). The cell variants deficient in other DNA repair genes, including poly (adenosine diphosphate-ribose) polymerase 1 (*Parp1$^{-/-}$*) (27), REV3-like DNA directed polymerase ζ catalytic subunit (*Rev3$^{-/-}$*) (25), Ku autoantigen 70 kDa (*Ku70$^{-/-}$*) (29) and xeroderma pigmentosum group A-complementing protein (*Xpa$^{-/-}$*) (26), were not more sensitive to ADV than *WT* cells (Fig. 4). In the present experiments, CPT was selected as a positive control (Fig. 4D-F).

Brca1 deficiency sensitizes DT40 cells to ADV-induced CAs. In the present study, CAs were classified as chromosome or chromatid gaps, breaks and exchanges according to The ISCN (37). *WT* or *Brca1$^{-/-}$* DT40 cells were exposed to 0.2 or 0.4 μM ADV for 9, 12, 15, 24 or 30 h. Compared with *WT* DT40 cells, *Brca1$^{-/-}$* cells exhibited significantly increased CAs at 15, 24 and 30 h, and the higher the dose the greater the increase in CAs observed (Fig. 5A and B). Notably, a monophasic pattern of CAs was induced in *WT* and *Brca1$^{-/-}$* cells. In these 2 cell lines, a peak was detectable at 12 h for *WT* and 24 h for *Brca1$^{-/-}$* cells, respectively, and it was much higher in *Brca1$^{-/-}$* cells than in *WT* cells (Fig. 5A and B). As a positive control, *WT* and *Brca1$^{-/-}$* DT40 cells were treated with 5 nM CPT for 6, 12, 18 and 24 h (Fig. 5C). At the same time, *WT* DT40 cells were treated with 25 nM CPT (Fig. 5D). The results indicated that CPT induced more DNA damage in *Brca1$^{-/-}$* cells when compared with *WT* DT40 cells. These results further confirmed that *Brca1* participates in repairing ADV-induced DNA damage.

Discussion

NAs are effective inhibitors against reverse transcriptase to inhibit HBV DNA replication; however, NAs, including ETV, adefovir and ADV, have been reported to have genotoxic effects by various

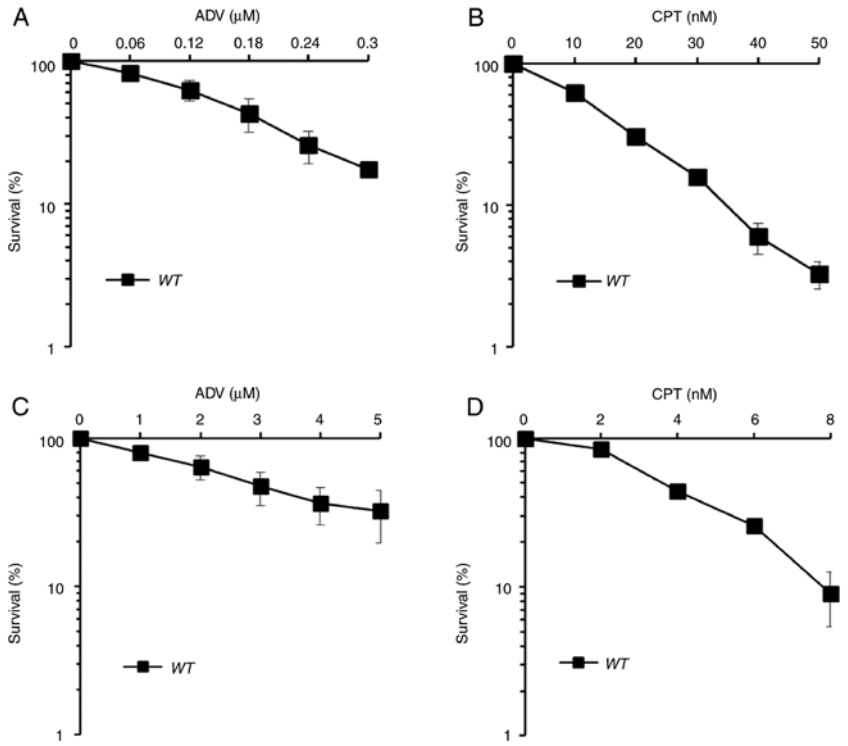

Figure 1. ADV reduces the survival of *WT* DT40 and TK6 cells. Sensitivity of *WT* DT40 cells to (A) ADV and (B) CPT. Sensitivity of *WT* TK6 cells to (C) ADV and (D) CPT. The x-axis represents the concentration of ADV or CPT and the y-axis represents the relative percentage of surviving cells at 72 h. Survival data were log-transformed to approximate normality. ADV, adefovir dipivoxil; *WT*, wild-type; CPT, camptothecin.

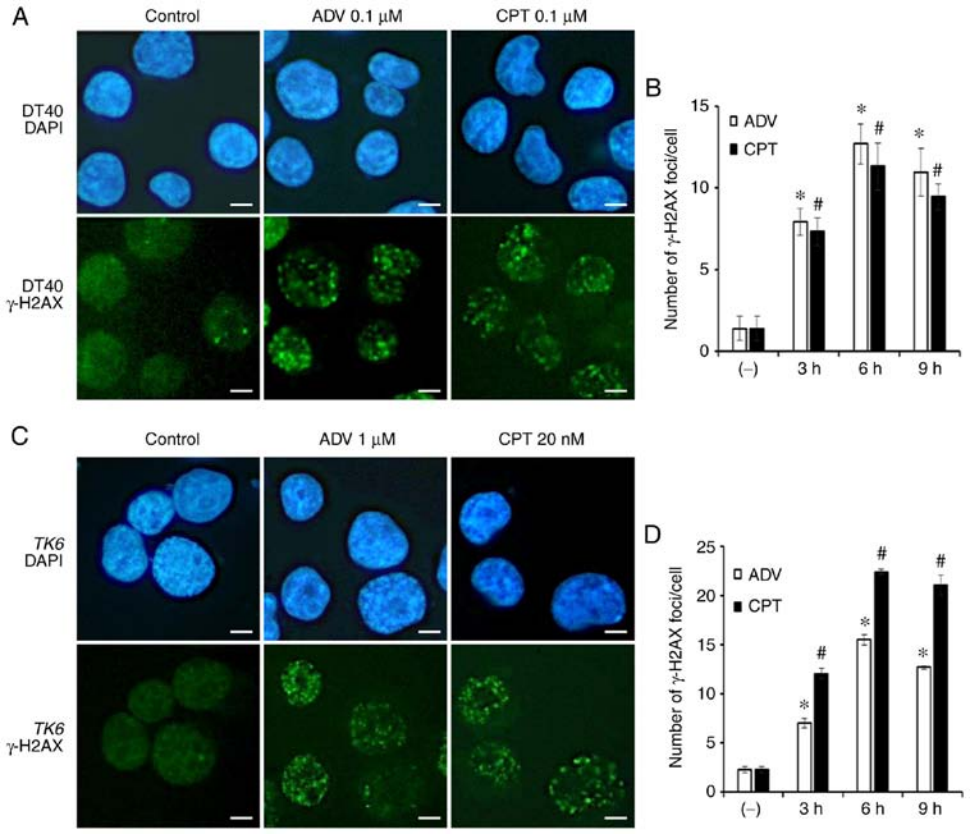

Figure 2. ADV induces the accumulation of γ-H2AX in the nuclei of *WT* DT40 and TK6 cells. (A) Immunostaining of *WT* DT40 cells using an anti-γ-H2AX antibody and DAPI. Cells were treated with 0.1 μM ADV or 0.1 μM CPT for 6 h (scale bar, 10 μM; magnification, x1,000). (B) Quantification of γ-H2AX foci in the nuclei of *WT* DT40 cells treated with 0.1 μM ADV or 0.1 μM CPT for different durations. (C) Immunostaining of *WT* TK6 cells using an anti-γ-H2AX antibody and DAPI. Cells were exposed to 1 μM ADV or 20 nM CPT for 6 h (scale bar, 10 μM; magnification, x1,000). (D) Quantification of γ-H2AX foci in the nuclei of *WT* TK6 cells treated with 1 μM ADV or 20 nM CPT for different durations. Values are expressed as the mean ± standard deviation. *P<0.05 vs. ADV control; $^{\#}$P<0.05 vs. CPT control. ADV, adefovir dipivoxil; γ-H2AX, γ-H2A histone family member X; *WT*, wild-type; CPT, camptothecin; DAPI, 4',6-diamidino-2-phenylindole.

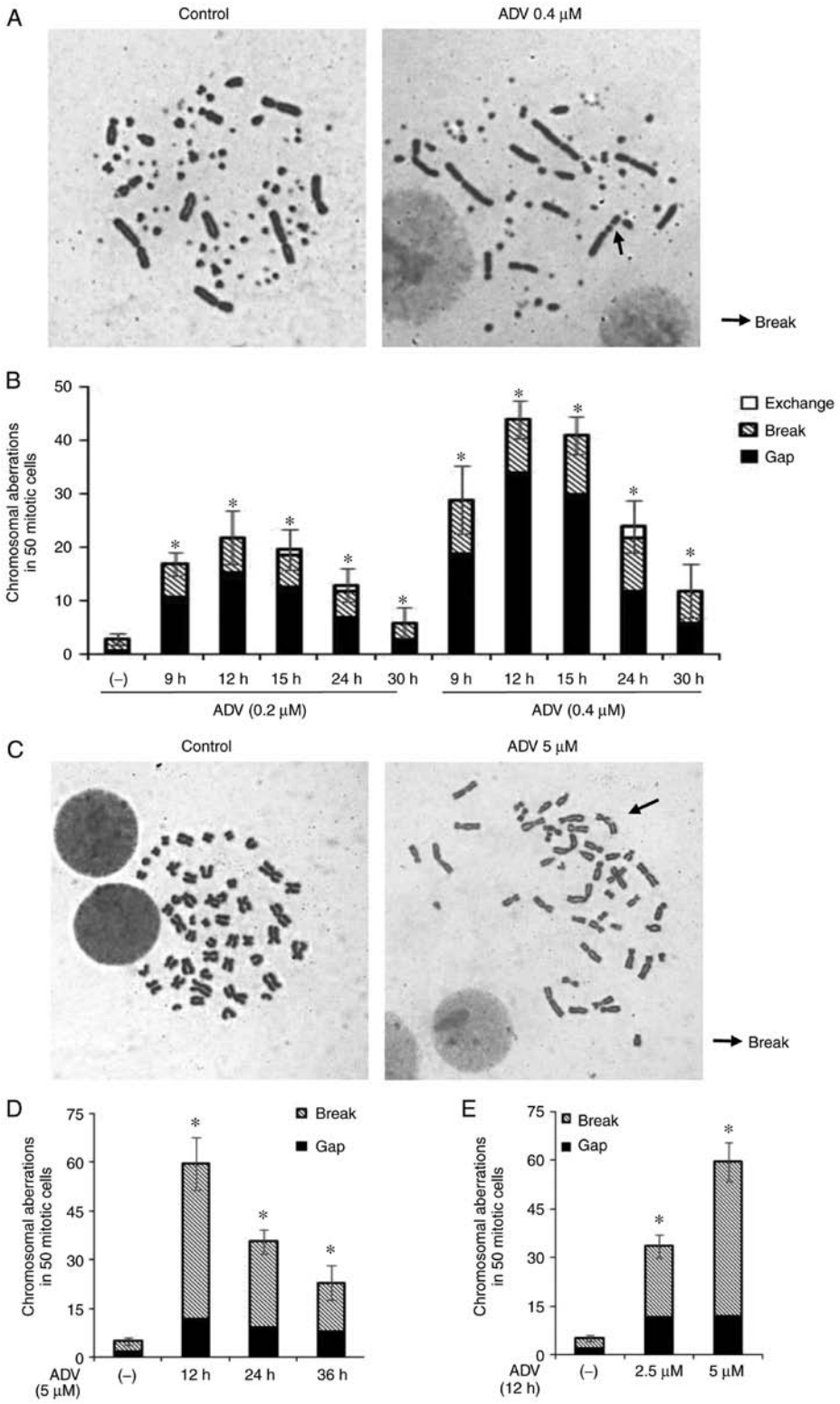

Figure 3. ADV induces DNA double-strand breaks in *WT* DT40 and TK6 cells. (A) Representative karyotype analysis of *WT* DT40 cells left untreated or exposed to 0.4 μM ADV for 12 h (magnification, x1,000). (B) ADV induced CAs in *WT* DT40 cells depending on the concentration. Cells were exposed to 0.2 or 0.4 μM ADV for 9-30 h. (C) Representative karyotype analysis of *WT* TK6 cells left untreated or exposed to 5 μM ADV for 12 h (magnification, x1,000). (D) An increased frequency of CAs in *WT* TK6 cells was detected following treatment with 5 μM ADV for 12-36 h. (E) Cells were exposed to 2.5 or 5 μM ADV for 12 h. ADV induced CAs in *WT* TK6 cells in a dose-dependent manner. Arrows indicate double-strand breaks. Values are expressed as the mean ± standard deviation. *P<0.05 vs. control. ADV, adefovir dipivoxil; *WT*, wild-type; CAs, chromosomal aberrations.

studies (16,19). In the present study, the possible mechanisms underlying the genotoxic effects of ADV were assessed, and it was indicated that DSBs were generated following ADV treatment in *WT* DT40 and TK6 cells. Furthermore, the increase in γ-H2AX foci and CAs confirmed that DNA damage was induced by ADV. By determining the quantitative distribution of γ-H2AX foci in *WT* DT40 and TK6 cells, the DNA damage induced by ADV was described in detail.

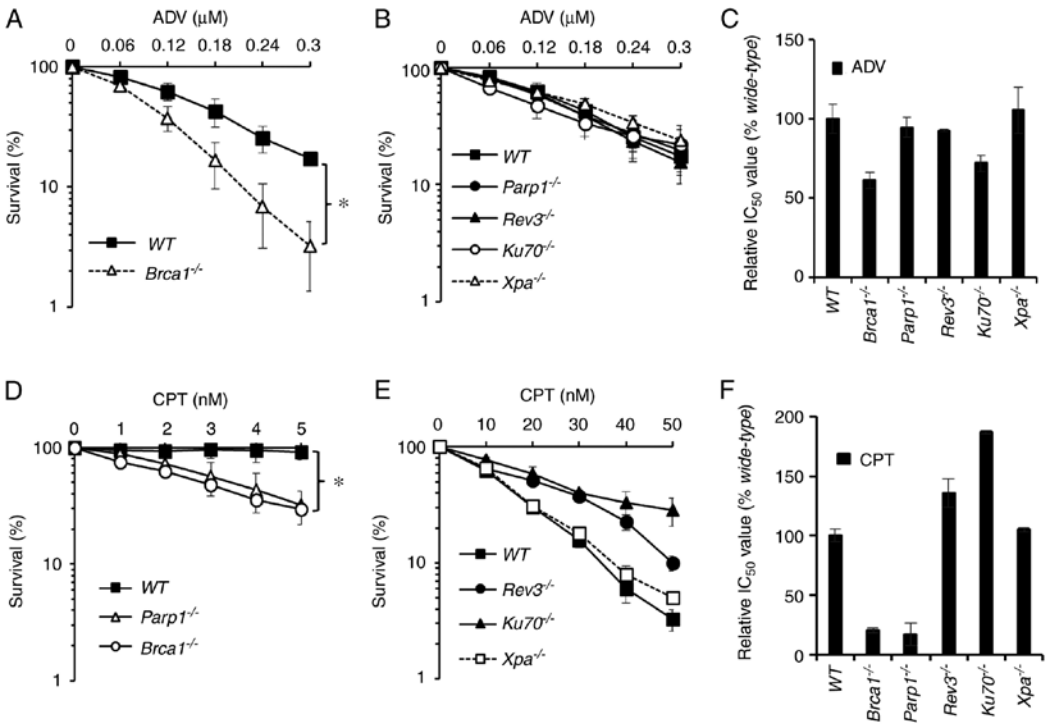

Figure 4. *Brca1*$^{-/-}$ cells were sensitive to ADV. (A and B) Sensitivity of *Parp1*$^{-/-}$, *Xpa*$^{-/-}$, *Rev3*$^{-/-}$, *Brca1*$^{-/-}$ and *Ku70*$^{-/-}$ cells to ADV. The x-axis represents the concentration of ADV and the y-axis represents the relative percentage of surviving cells at 72 h. (C) Relative IC_{50} values of ADV in DT40 cells. (D and E) Sensitivity of *Parp1*$^{-/-}$, *Xpa*$^{-/-}$, *Rev3*$^{-/-}$, *Brca1*$^{-/-}$ and *Ku70*$^{-/-}$ cells to CPT. The x-axis represents the concentration of CPT and the y-axis represents the relative percentage of surviving cells at 72 h. (F) Relative IC_{50} values of CPT in DT40 cells. Survival data were log-transformed to approximate normality. Two-way analysis of variance was used to test for differences in the linear dose-response curves between *WT* cells and mutant cells. *P<0.05, as indicated. ADV, adefovir dipivoxil; CPT, camptothecin; *WT*, wild-type; IC_{50}, 50% inhibitory concentration; *Parp1*$^{-/-}$, poly (adenosine diphosphate-ribose) polymerase deficient cells; *Rev3*$^{-/-}$, REV3-like DNA directed polymerase ζ catalytic subunit deficient cells; *Ku70*$^{-/-}$, Ku autoantigen 70 kDa deficient cells; *Xpa*$^{-/-}$, xeroderma pigmentosum group A-complementing protein deficient cells.

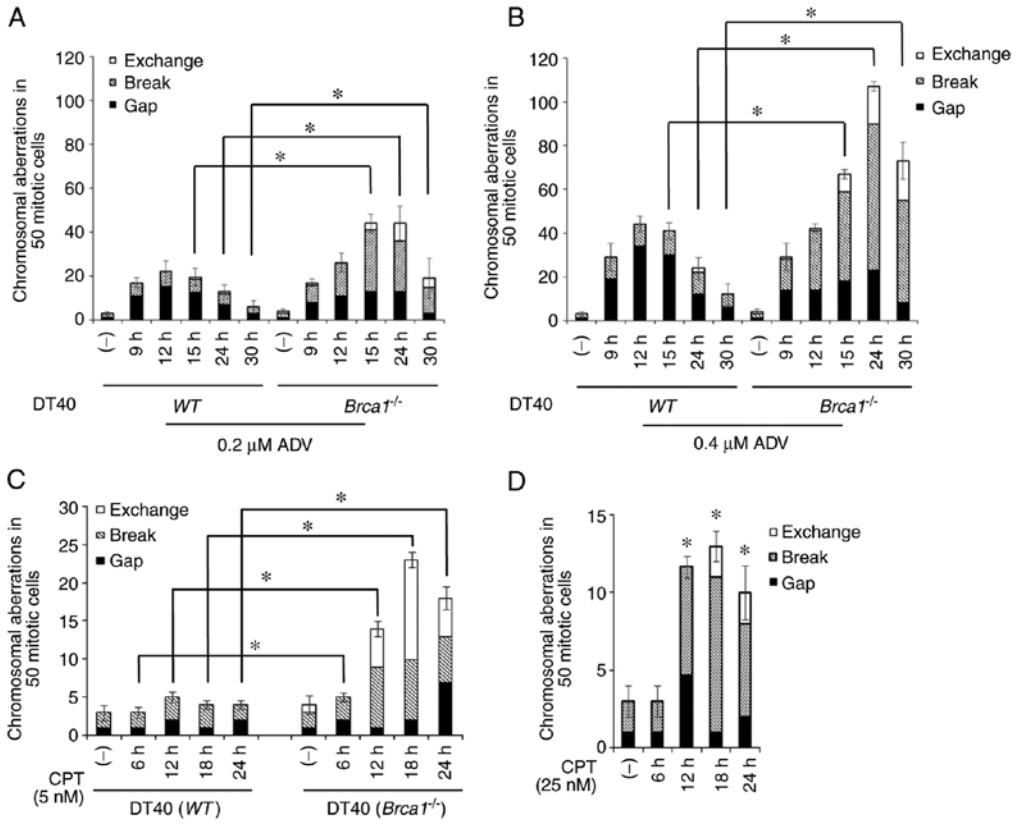

Figure 5. *Brca1*$^{-/-}$ cells exhibit increased DNA double-strand breaks in response to ADV and CPT. (A and B) CAs in *Brca1*$^{-/-}$ and *WT* cells following treatment with ADV (0.2 or 0.4 μM) for 9-30 h. (C) CAs in *Brca1*$^{-/-}$ and *WT* cells following treatment with CPT (5 nM) for 6-24 h. *P<0.05, as indicated. (D) *WT* DT40 cells were exposed to 25 nM CPT for 6-24 h. *P<0.05 vs. control (-). ADV, adefovir dipivoxil; CPT, camptothecin; CAs, chromosomal aberrations; *WT*, wild-type.

To the best of our knowledge, the present study was the first to screen the sensitivity of a series of isogenic DNA repair-deficient cell lines to ADV in a quantitative manner. These cell lines include a base excision repair-mutant (*Parp1*$^{-/-}$), a homologous recombination (HR) repair mutant (*Brca1*$^{-/-}$), a non-homologous end-joining (NHEJ) repair mutant (*Ku70*$^{-/-}$), a translesion DNA synthesis repair-mutant (*Rev3*$^{-/-}$) and a nucleotide excision repair-mutant (*Xpa*$^{-/-}$). Notably, the sensitivity profile (IC_{50}) evaluation indicated that only *Brca1*$^{-/-}$ cells displayed a higher sensitivity to ADV compared with any other DNA repair-deficient cell lines. The increased CAs in *Brca1*$^{-/-}$ cells compared with the *WT* cell line reflected that Brca1 may have a critical role in preventing ADV-induced CAs. These results suggested that ADV may be genotoxic with Brca1 dependence. The results of the clonogenic assays also confirmed that *Brca1*$^{-/-}$ cells were significantly more sensitive to ADV when compared with *WT* DT40 cells.

Brca1 protein is generally considered as not only a tumor suppressor but a DNA repair factor involved in multiple DNA repair and genome stability processes (42-44). Much of the research on DNA repair associated with Brca1 has focused on HR repair as well as NHEJ (30,45,46). Firstly, Brca1 has an important role in the HR of DSBs, which is comprised of the dynamic removal of NHEJ proteins from DSBs to prevent inappropriate end-ligation and promote reliable sister chromatid repair (47-50). Secondly, Brca1 is also associated with NHEJ (51). DNA breaks in *Brca1*$^{-/-}$ cells are abnormally connected to complex chromosome rearrangements via NHEJ factor tumor protein p53 binding protein 1 (53BP1). 53BP1 is able to block the resection of DNA breaks and inhibit HR in *Brca1*$^{-/-}$ cells. The capacity of *Brca1*$^{-/-}$ cells to accurately repair DSBs is limited by the presence of 53BP1. HR and NHEJ compete to deal with DNA breaks (51). Brca1 and 53BP1 adjust the balance between HR and NHEJ, which may be used to selectively protect or kill *Brca1*$^{-/-}$ cells (51).

The present results illustrated that *Ku70*$^{-/-}$ cells did not have an increased sensitivity to ADV compared with *WT* DT40 cells. Furthermore, the results also demonstrated that 53BP1-deficient cells had a similar viability following treatment with ADV compared with *WT* TK6 cells (data not shown). Based on all of these results, it was concluded that Brca1 took part in the repair of ADV-induced DNA damage, and the molecular mechanisms may mainly be associated with HR.

In conclusion, ADV-induced cellular genotoxicity in DT40 and TK6 cells, and Brca1 are involved in tolerance to DNA damage induced by ADV. The present results suggest that it is necessary to monitor the genotoxicity of ADV and to restrict the usage or limit the treatment period. A better understanding of the mechanisms underlying ADV-induced genotoxicity may contribute to the development of novel drugs against CHB with higher therapeutic efficacy and less genotoxicity. To date, at least 6 NAs have been approved for HBV treatment, including telbivudine, lamivudine, ETV, ADV, tenofovir alafenamide and tenofovir disoproxil fumarate (6-8). However, further research on their molecular mechanisms is worthwhile.

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Availability of data and materials

All data generated or analyzed during this study are included in this article and in the supplementary materials.

Authors' contributions

YQ, XW and HL conceived and designed the experiments. HL and ZC performed the experiments. HL, YQ, XW, YW and FH analyzed the data. ZZ, CX, XF and XB performed part of chromosome aberration analysis work for TK6 cells and analyzed the data. HL, XW, YQ, YW and FH wrote the paper. ST generated the gene knockout cells. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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