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Expression of Tumor Suppressor p53 Facilitates DNA Repair But Not UV-Induced G2/M Arrest or Apoptosis in Chinese Hamster Ovary CHO-K1 Cells

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Abstract Tumor suppressor p53 is an essential regulator in mammalian cellular responses to DNA damage including cell cycle arrest and apoptosis. Our study with Chinese hamster ovary CHO-K1 cells indicates that when p53 expression and its transactivation capacity was inhibited by siRNA, UVC-induced G2/M arrest or apoptosis were unaffected as revealed by flow cyotmetric analyses and other measurements. However, inhibition of p53 rendered the cells slower to repair UV-induced damages upon a plasmid as shown in host cell reactivation assay. Furthermore, the nuclear extract (NE) of p53 siRNA-treated cells was inactive to excise the UV-induced DNA adducts as analyzed by comet assay. Consistently, the immunodepletion of p53 also deprived the excision activity of the NE in the similar experiment. Thus, tumor suppressor p53 of CHO-K1 cells may facilitate removal of UV-induced DNA damages partly via its involvement in the repair mechanism. J. Cell. Biochem. 103: 528–537, 2008.

Key words: Chinese hamster ovary CHO-K1 cells; UVC; apoptosis; G2/M arrest; caffeine; p53; comet assay; host cell reactivation assay; DNA repair

Tumor suppressor p53 plays an important role in cellular responses to DNA damage. Signaling of DNA damage stabilizes p53 protein, which can act as transcription factor to induce many genes' expression that may lead to cell cycle arrest or apoptosis [Bargonetti and Manfredi, 2002; Heinrichs and Deppert, 2003]. Although the DNA-damage induced cell cycle arrest at G1 is p53-dependent, the influence of p53 on G2/M arrest is controversial [Yonish-Rouach, 1996; Taylor and Stark, 2001]. It is considered that p53 may cause G2/M arrest via induction of $p21^{(Waf1/Cip1)}$, the general cyclin dependent kinase (CDK) inhibitor. Or, p53 may induce G2/M arrest through its induction of the expression of 14-3-3σ protein that can sequestrate CDK1 and cyclin B [Hermeking et al., 1997; Innocente et al., 1999]. In addition, p53 may modulate DNA repair via transcription dependent and independent fashions [Seo and Jung, 2004; Sengupta and Harris, 2005]. Expression of DNA repair-specific genes such as XPC and p48-XPE [Hwang et al., 1999; Adimoolam and Ford, 2002] and MSH2 is p53-inducible [Scherer et al., 2000]; p53 may enhance base excision repair via interacting with APE1/REF1 and DNA polymerase β [Zhou et al., 2001; Achanta and Huang, 2004]. The loss of p53 in human cells may impair the excision of UV-induced DNA adducts [Smith et al., 1995; Ford and Hanawalt, 1997]. In addition, recruitment of nucleotide excision repair (NER) factors XPC and TFIIH to DNA damage is p53-dependent [Wang et al., 2003]. Previously, we have reported [Tzang et al., 1999] that the p53 of Chinese hamster CHO-K1 cells has transactivational activity despite the missense mutation at codon 211. Furthermore, we have shown that while the p53 is accumulated in CHO-K1 cells following UV treatment, the $p21^{(Waf1/Cip1)}$ protein remains absent regardless of UV treatment. The deficiency of $p21^{(Waf1/Cip1)}$ protein

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is correlated with the absence of G1 arrest and the deregulation of Cdk2 activity, which is critical to UV-induced apoptosis [Liao et al., 2006].

In this study, we describe our study on the role of p53 in UV-induced G2/M arrest and apoptosis of the CHO-K1 cells. We find that inhibition of p53 expression by siRNA had little effect on UV-induced G2/M arrest and apoptosis. However, the p53 is essential to the cellular repair capacity of UV-induced DNA damage.

MATERIALS AND METHODS

Cell Cultures, UV Irradiation and Caffeine Treatment

CHO-K1 cells were originally obtained from the American Type Culture Collection (Manassas, VA, USA). Human lung H1299 cells and their derivatives were provided by Dr. Hai-Mei Huang (National Tsing-Hua University). The CHO-K1 and H1299 cells were maintained in 1× McCoy's 5A medium (Sigma, St. Louis, MO) and 1× RPMI-1640 medium (Gibco, Grand Island, NY), respectively. Both media were supplemented with 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and 0.03% glutamine, and were cultured at 37°C in a water-saturated atmosphere containing 5% CO_2.

UV treatment was done as previously described [Liao et al., 2006]. Briefly, cells were exposed, without cover, to UV using a germicidal lamp (254 nm, Sankyo Denki Co., Tokyo, Japan) in a UV box at the indicated dose. The dose of UV-irradiation was calibrated with a UV radiometer (UVP Inc., San Gabriel, CA).

For caffeine treatment, stock solution (125 mM) of caffeine (Sigma) was prepared in 1 × PBS and stored at room temperature before use. Cells were treated with caffeine at 2.5 mM for 24 h.

Flow Cytometric Analyses

Flow cytometric analysis of cellular DNA contents was done as previously described [Liao et al., 2006]. In brief, cells were fixed in 70% alcohol for 12–16 h at −20°C. The fixed cells were then incubated with RNase (10 μg/ml) and propidium iodide (50 μg/ml) at room temperature for 30 min before analysis with a flow cytometer (FACScan, Becton Dickinson, Franklin Lakes, NJ).

DNA Fragmentation Assay

The DNA fragmentation assay was performed as described previously [Kondo et al., 1995]. In brief, cells were lysed in buffer (10 mM Tris-HCl, 10 mM EDTA and 0.2% Triton X-100, pH 7.5) for 10 min on ice. The lysate was centrifuged (12,000*g* at 4°C for 30 min) and the DNA in the supernatant was purified with standard procedures. The DNA in the samples was separated by electrophoresis on 1.5% agarose gels for analysis.

Western Blot Analysis

Western blot analysis was performed according to a standard protocol. Proteins in the whole cell lysate after being transferred to PVDF membranes (Amersham Pharmacia Biotech, Amersham, UK) were probed with antibodies, such as Bcl-2 (sc-7382; Santa Cruz Biotechnology, Santa Cruz, CA), actin (sc-1616; Santa Cruz), and p53 (sc-6248; Santa Cruz). To detect the Ab:Ag complexes, an ECL detection kit (Pierce Chemical Co., Chester, UK) was used according to the manufacturer's instruction.

Small Interference RNA (siRNA) of p53 and Transfection

The oligonucleotides for p53 siRNA and nonspecific siRNA were synthesized by Qiagen (Hilden, Germany) with standard purification. The p53 siRNA antisense sequence is AAG GUG GAC AGA ACA UUG U dTdT; the sense sequence is ACA AUG UUC UGU CCA CCU U dTdT. The sequence corresponds to the p53 mRNA (nt 84–104) in addition to a two dT 3′-overhang. The p53 specific siRNA was transfected into CHO-K1 cells with the GenePORTER™ 2 transfection reagent (Gene therapy systems, Inc., GTS) and the transfection was done according to the manufacturer's instruction. The assay for the targeted gene was performed at 24 or 48 h after transfection. The exact time sequence of transfection, UV irradiation, and assay was indicated below in each specific assay.

RNA Isolation and RT-PCR

Total RNA was isolated with REzol reagent (PROtech Technologies, Taipei, Taiwan) and according to the manufacturer's instruction. The reverse transcription (RT) was done with RAV-2 RTase (TaKaRa Bio Inc., Japan) and

according to the manufacturer's instruction. The polymerase chain reaction (PCR) was performed in a mixture containing the template (generated from the RT reaction), the specific primers, and Taq DNA polymerase (TaKaRa) and according to the standard procedure modified with the following conditions. The amplification started with denaturation step at 95°C for 10 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at suitable temperature (GAPDH 55°C, Gadd45 50°C) for 30 s and extension at 72°C for 30 s. The PCR products were separated on 1.5% agarose gel and analyzed by a computer software Gel-Pro Analyzer (Media Cybernetics, Sliver Spring, MD). The primers for GAPDH were: forward primer 5′-ACCACAGTCCATGCCATCAC-3′, and reverse primer, 5′-TCCACCACCCTGTTGCTGTA-3′; for Gadd45 were: forward primer 5′-GCAGAAGACCGAAAGGATGGAC-3′, and reverse primer 5′-CCATGTAGCGACTTTCCCGAC-3′.

Assay for p53 Transactivation Activity

Plasmid WWP-Luc, a luciferase reporter driven by a derivative of the promoter of $p21^{(Waf1/Cip1)}$ (gift of Dr. B. Vogelstein of Johns Hopkins University, Baltimore, MD) was used for assaying the transactivation activity of p53. The EGFP-N3 (Clontech Co., Germany) was used to control the transfection efficiency. Exponentially growing cells were co-transfected with WWP-Luc and EGFP-N3 plasmids with or without p53 siRNA by using GenePORTER^{TM} 2 transfection reagent. Two days after transfection, the cells were lysed and the luciferase activity in the lysate was assayed with a commercial kit (Promega Co., Madison, WI) and according to the protocols provided by the manufacturer. Luciferase activity was measured with a luminescence reader (Wallac Victor 1420 Multilabel Counter, Perkin-Elmer, Monza, Italy). To see the effect of UV, the cells, 24 h after transfection, were irradiated with UV and harvested for luciferase activity assay at 24 h after UV treatment. Data were expressed as relative levels to that of cells without both siRNA and UV treatments after the normalization with EGFP expression.

Host Cell Reactivation

Plasmid CMV-Luc, a luciferase reporter containing CMV promoter which can stably express luciferase in the cells. To induce damage on the plasmid, the CMV-Luc plasmid was UV-irradiated at dose of 100 J/m^2. To see the effect of p53 siRNA, cells were first transfected with p53 siRNA or non-specific siRNA (1 μg per 4×10^5 cells in a 35 mm dish), and 24 h later, the siRNA transfected cells were transfected with CMV-Luc (damaged or undamaged) by using GenePORTER^{TM} 2 transfection reagent and according to the protocols provided by the manufacturer. The cells were harvested at the indicated time (1–3 days after CMV-Luc transfection) for luciferase activity assay as described above. Similarly, cells were also transfected with the plasmid EGFP-N3 for transfection-efficiency control. Results were expressed as the ratio between the level of cells transfected with damaged plasmid and that of cells transfected with undamaged plasmid after the normalization with EGFP expression.

Cell Viability Assay

CHO-K1 cells were transfected with p53 siRNA or nonspecific siRNA, 24 h after transfection, the cells were UV-irradiated. Cells after UV irradiation were plated in 96-well tissue culture plates (2×10^3 cells per well) and were cultured for 1–2 day before viability assay with [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] (MTS) kit (Promega). Viability is expressed as the ratio relative to the unirradiated cells.

Nuclear Extract (NE) Preparation

The NE of CHO-K1 cells was prepared by the method described by [Bergstein et al., 1979]. Briefly, cells were first treated with 2.5 mM hydroxyurea and 25 μM cytosine-β-D-arabinofuranoside for 16 h for depleting the cellular deoxyribonucleotide triphosphate (dNTP) pool. The cells were washed with hypotonic buffer (20 mM HEPES, pH 7.5, 5 mM KCl, 0.5 mM $MgCl_2$, 0.5 mM dithiothreitol, and containing 0.2 M sucrose) and swollen in hypotonic buffer without sucrose for 10 min on ice. The swollen cells were ruptured with 10 strokes of Dounce homogenizer and the homogenate was forced to pass through 22G needle for 10 times. The mixture was centrifuged at 2,000*g* for 5 min to isolate the nuclei. The nuclei were resuspended in the buffer (20 mM Hepes, pH 7.5, 5 mM KCl, 0.5 mM $MgCl_2$, and 0.5 mM dithiothreitol containing 10% sucrose), and stored in −70°C.

The nuclei were thawed on ice and allowed to swell in 100 mM NaCl on ice for 1 h. The ruptured nuclei were centrifuged at 15,000*g* for 20 min at 4°C to isolate the supernatant, and the supernatant was filtered through a YM-10 Microcon filter (Millipore, Bedford, MA) to reduce the residual dNTP. Protein concentration was determined by the BCA Protein Assay Kit (Pierce, USA) using bovine serum albumin as a standard.

Immunodepletion

To test if a specific protein in NEs is essential for excision of DNA adducts, immunodepletion of the specific protein from the NE was performed as the following. NE was added with an antibody (1:10, w/w), and the mixture was gently shaken in a rotator for more than 12 h at 4°C. The mixture was centrifuged at 8,000*g* for 10 min to remove the precipitate and the supernatant was used as immunodepleted NE in comet-NE assay. Antibodies used for the experiment were p53 (sc-6248; Santa Cruz), XPB (s-19, Santa Cruz), and actin (sc-1616; Santa Cruz) and PCNA (Ab-1, Oncogen, Darmstadt, Germany).

Comet-NE Assay

The assay was performed as described previously [Wang et al., 2005; Li et al., 2007]. For preparing the gels on each slide, 100 μl of 1.4% agarose in phosphate-buffered saline (PBS) at 65°C was placed onto a glass microscope slide pre-warmed at 60°C. The gel was covered with coverslip immediately and the slide was chilled on ice. The coverslip was removed and an aliquot of 100 μl of cells containing agarose was then added. This was done by mixing equal volume of 1.2% low-melting agarose and cell suspension (1×10^6 cells/ml in PBS); the mixture was kept at 40°C. The coverslip was added and removed as described above. Then, 100 μl of 1.2% low-melting agarose was applied as the third layer of agarose. After the third layer of gel was made, the slide was immersed in ice-cold cell lysis solution and stored at 4°C for at least 2 h. Cell lysis solution contained 2.5 M NaCl, 100 mM EDTA, and 10 mM Tris (pH adjusted to 10 with NaOH), and 1% N-laurylsarcosine, 1% Triton X-100, and 10% DMSO were added immediately before use.

After cell lysis, the slides were washed three times with deionized water. The NE digestion was done by adding a total of 20 μl of excision mixture containing 0.6 μg of NE, 50 mM Hepes-KOH (pH 7.9), 70 mM KCl, 5 mM $MgCl_2$, and 0.4 mM EDTA, 2 mM ATP, 40 mM phosphocreatine, and 2.5 mM creatine phosphokinase onto each slide. A coverslip was applied and the slides were incubated at 37°C for 2 h in a sealed box containing a piece of wet tissue paper. After the incubation, the slides were denatured in 0.3 N NaOH, 1 mM EDTA for 20 min. Electrophoresis was carried out in the same denaturation solution at 25 V, 300 mA for 25 min. The slide was washed briefly in deionized water, blotted, and then transferred to 0.4 M Tris-HCl, pH 7.5. DNA was stained by adding 40 μl of 50 μg/ml propidium iodide onto the slide. A coverslip was applied and the slide was examined under a fluorescence microscope (Axioplan 2, Zeiss Co., Thornwood, NY). The image of 50 cells per treatment was recorded with close-circuit display camera (CoolSNAP). The migration of DNA from the nucleus of each cell was measured with a computer program (http://tritekcorp.com) using the parameter of tail moment. The tail moment is defined as the product of tail length and fraction of total DNA in the tail.

Statistics

Data are expressed as mean ± standard derivation throughout this article. All experiments were performed independently at least twice. Statistical analyses were performed with Student's *t*-test.

RESULTS

UV-Induced G2/M Arrest and Apoptosis in CHO-K1 Cells

Cells at 24 h following UV-irradiation of various doses (0–25 J/m^2) were harvested for DNA content analysis by flow cytometry. UV-induced apoptosis, as indicated by the appearance of sub-G1 population, gradually increased as dose of UV radiation increased from 10 J/m^2 to 25 J/m^2 (↓ marked and the numerals at the upper right corners of Fig. 1). The UV treatment (at the doses of 10 and 15 J/m^2) induced G2/M arrest as indicated by increase of the peak with double DNA content in the cells. No apparent G2/M arrest or apoptosis could be detected at lower UV doses (i.e., 5 J/m^2 or less; data not shown). Caffeine, an inhibitor of G2/M arrest, sensitized the cells to UV radiation as indicated

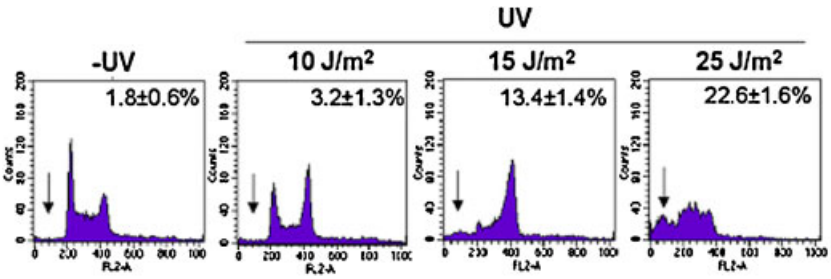

Fig. 1. UV induced G2/M arrest and apoptosis in CHO-K1 cells. Flow cytometric analysis of cellular DNA contents. Cells were harvested for flow cytometric analysis at 24 h after UV irradiation at the indicated doses (0–25 J/m^2). The sub-G1 fraction as a measurement of apoptosis is marked with arrows. Quantitative analyses of sub-G1 fractions are summarized on the upper right corners as the means ± SE of at least three independent experiments. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

by the increase of sub G1(Fig. 2A). The increase of sub G1 due to the presence of caffeine is consistent with the increase of DNA fragmentation and the decrease of a pro-apoptotic protein Bcl-2 shown in Figure 2B,C.

Knock-Down of p53 Expression Did Not Affect the UV-Induced G2/M Arrest and Apoptosis

The level of tumor suppressor p53 protein was elevated in cells following UV-irradiation (Fig. 3). To investigate if p53 is involved in the UV-induced G2/M arrest and apoptosis, we knocked down the expression of p53 by using siRNA (Fig. 4A). The inhibition of p53 expression due to siRNA suppressed the UV-induction of Gadd45 gene expression as examined by RT-PCR (Fig. 4B); Gadd45 is one of the targets of p53, as a transcription activator. The reduction of p53 due to siRNA was further confirmed in the experiment using p21$^{(Waf1/Cip1)}$ promoter as p21$^{(Waf1/Cip1)}$ gene expression is also transactivated by p53. The p21 promoter activity was activated by UV irradiation (due to p53), whereas such activation was greatly reduced in the p53 siRNA treated cells (Fig. 4C). The UV-induced G2/M arrest and apoptosis were apparently unaffected by the inhibition of p53 expression as examined by flow cytometry (Fig. 5A). This is verified with the viability assay shown in Figure 5B. The cells treated with p53 siRNA or with non-specific siRNA showed similar sensitivity to UV irradiation at various doses.

Involvement of p53 in DNA Repair

The effect of p53 expression knocked down on DNA repair was examined by the recently developed comet-nuclear extract assay [Wang et al., 2005]. The comet-nuclear extract assay allowed us to examine the activity in NEs to excise the UV-induced DNA adducts. Figure 6A shows clearly that the NEs prepared from cells with low p53 due to siRNA had much reduced excision activity as compared with those from control cells. The importance of p53 on excision activity was validated with the nuclear extract immunodepleted of p53 (Fig. 6B). Moreover, the conclusion of the above in vitro studies was confirmed by the host reactivation assay done in CHO-K1 cells. As shown in Figure 7, the recovery of the promoter activity in cells treated with p53 siRNA (curve with close circles) was apparently slower than that in cells with non-specific siRNA (open circles).

Involvement of p53 in UV-Induced Apoptosis and DNA Repair in Human H1299 Cells

As a comparative study, the involvement of p53 in the UV-induced apoptosis and DNA repair was examined with p53 null, human H1299 cells and the cells stably transfected with human wild-type p53. The flow cytometric analysis and host cell reactivation assay were used for the study, and the results are shown in Figure 8. First, the absence of p53 in H1299 cells and the expression of p53 in the transfected cells were confirmed by Western blotting analysis (see Fig. 8A). The expression of p53 in the H1299 cells markedly reduced the sub-G1 fraction as revealed by the flow cytometry (from 23.5 to 7.2%; Fig. 8B). Also, the presence of p53 greatly enhanced the repair of UV-induced DNA damages upon the plasmids in the host reactivation assay (Fig. 8C). Thus, p53 in the human H1299 cells, distinct from its counterpart in CHO-K1 cells, may play active role in both UV-induced apoptosis and repair.

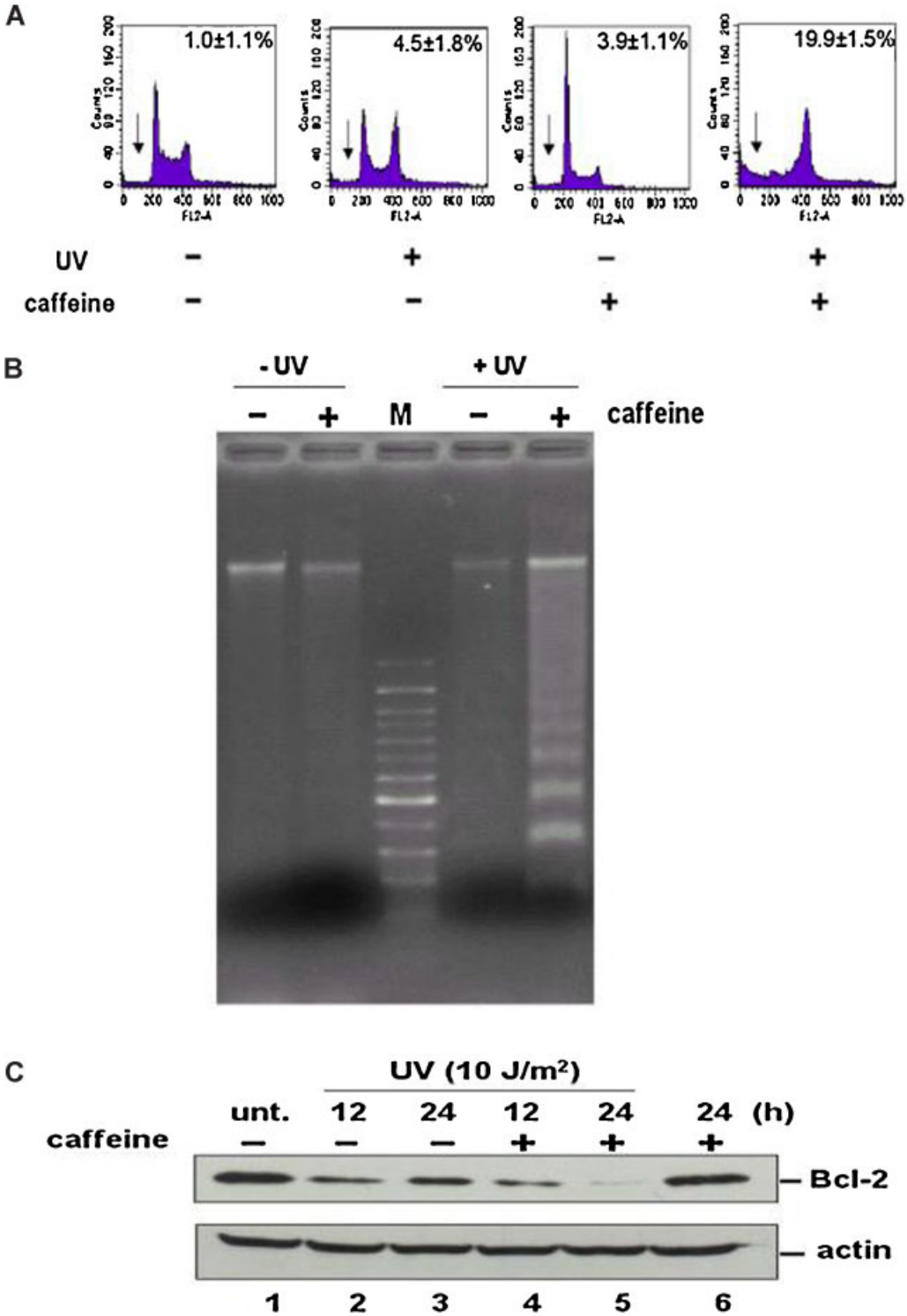

Fig. 2. Caffeine increased the UV-induced apoptosis with reduction of the Bcl-2 level in CHO-K1 cells. Immediately after UV irradiation (10 J/m^2 or mock treatment), cells were treated with or without caffeine (2.5 mM) for 24 h before harvest for (**A**) flow cytometric analysis and (**B**) DNA fragmentation analysis. In panel A, the sub-G1 fraction is marked with arrows. Quantitative analyses of sub-G1 fractions are summarized on the upper right corners; in panel B, M, molecular size marker. **C**: Bcl-2 was analyzed by Western blotting analysis in cells treated with or without caffeine (2.5 mM) for 12 or 24 h after exposure to UV. Actin was used as a loading control. Unt, untreated cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

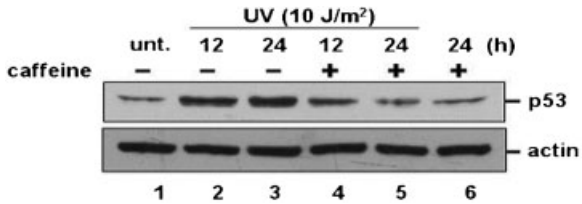

Fig. 3. UV induced p53 level and caffeine attenuated the effect in CHO-K1 cells. Western blotting analysis of p53 in cells treated with or without caffeine (2.5 mM) for 12 or 24 h after exposure to UV. Actin was used as a loading control. Unt, untreated cells.

DISCUSSION

Our previous study [Tzang et al., 1999] has suggested that the tumor suppressor p53 of Chinese hamster CHO-K1 cells, despite a missense mutation at its codon 211, is active to induce the endogenous Gadd45 expression and stimulate the exogenous p21 promoter's activity. In this study, we show that the p53 may play role in DNA repair. In addition, we also showed that UV could induce G2/M arrest and/or apoptosis depending on the dose of UV. The G2/M arrest could be released by caffeine. Release of G2/M arrest by caffeine was accompanied with apoptosis, suggesting the role of G2/M arrest in protecting CHO-K1 cells from UV-induced apoptosis. According to the current knowledge, UV-induced G2/M arrest may result from the down-regulation of Cdk1 activity via ATR-Chk1-Cdc25 signaling pathway. Thus, Cdk1 may play role in the UV-induced apoptosis of CHO-K1 cells. Previously, we have found that deregulation of Cdk2 activity, because of the absence of $p21^{(Waf1/Cip1)}$, is correlated with

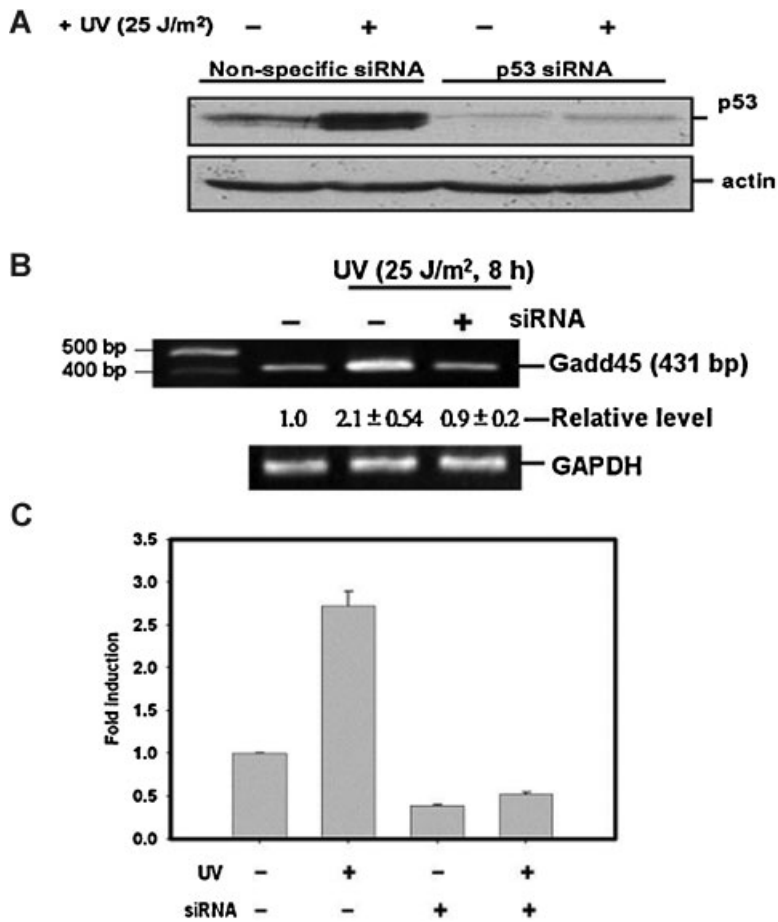

Fig. 4. Specific siRNA to inhibit p53 level in CHO-K1 cells. **A**: Western blotting analysis of p53 in cells pre-treated with non-specific or p53 siRNA at 24 h after exposure to UV. Actin was used as a loading control. **B**: RT-PCR of Gadd45 gene expression in cells pre-treated with or without p53 siRNA at 8 h after exposure to UV. The effects on Gadd45 expression were summarized as relative levels with GAPDH as the control. **C**: Transactivational activity of p53 on p21 promoter-reporter in cells pre-treated with or without p53 siRNA at 24 h after exposure to UV.

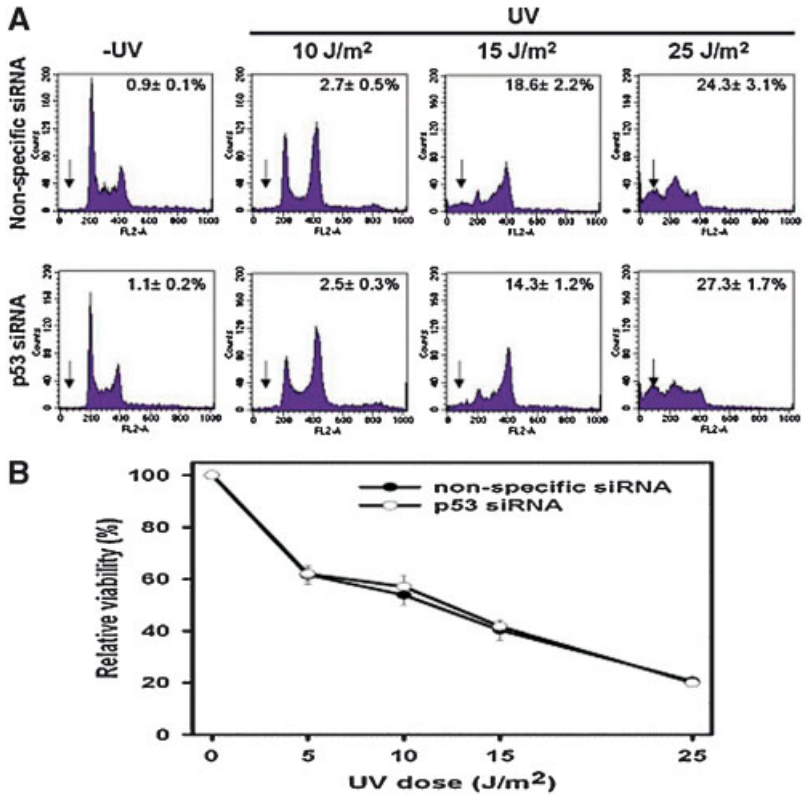

Fig. 5. Inhibition of p53 level did not affect the UV-induced apoptosis. **A**: Cells were pre-treated with p53 siRNA or non-specific siRNA for 24 h and then irradiated with UV at the indicated doses (0–25 J/m^2). Cells were harvested at 24 h after irradiation for the flow cytometric analysis of cellular DNA contents. The sub-G1 fraction is marked with arrow. Quantitative analyses of sub-G1 fractions are shown on the upper right corners. **B**: Cells were pre-treated with p53 siRNA or non-specific siRNA for 24 h and then irradiated with UV at the indicated doses (0–25 J/m^2). Cells were continuously grown for 1–2 days before viability assay.

UV-induced apoptosis in CHO-K1 cells. Cdk1 activity of CHO-K1 cells, unlike Cdk2, appeared to be less affected due to the absence of p21. Cdk1 activity may become deregulated due to the overload of DNA damages at high doses of UV ($\geq$25 J/m^2).

Although p53 protein accumulated in UV-irradiated cells (Fig. 3), the accumulation is not correlated with UV-induced G2/M arrest or apoptosis. Inhibition of p53 expression by siRNA, verified by Western analysis of p53 and by p53 functional analyses, did not affect the UV-induced G2/M arrest or apoptosis (Figs 4 and 5). Furthermore, caffeine, the inhibitor of G2/M arrest, enhanced UV-induced apoptosis despite its reducing the accumulation of p53 (Figs 2A and 3). In contrast, the accumulation of p53 may be linked to DNA repair. First, immunodepletion of p53, as that of XPB, rendered the NE inactive to excise UV-induced DNA adducts in comet-NE assay (Fig. 6B). Second, NE of p53-siRNA-treated cells was also inactive in the similar assay. Third, cells treated with p53 siRNA had lower repair rate than the control cells in the host reactivation assay (Fig. 7). In a similar study, wild-type p53 also facilitated DNA repair as the p53-null human H1299 cells and the isogenic cells expressing the wild-type p53 were compared [Chang and Liu, unpublished results]. The immunodepletion experiment of the comet-NE assay suggests that p53 may participate DNA repair process. NER mechanism is responsible for repairing the UVC-induced DNA lesions including cyclobutane pyrimidine dimers (CPD) and 6,4 photoproducts [Mitchell, 1988; Sancar, 1994]. NER consists of four steps: damage-site recognition and verification, dual incision, DNA synthesis, and ligation (reviewed by [Costa et al., 2003]). To investigate the involvement of p53 in NER, we conducted the immunofluorescence study and found that p53 was essential for recruiting XPB [Chang et al., unpublished results]. XPB and XPD, the two helicases, are the components

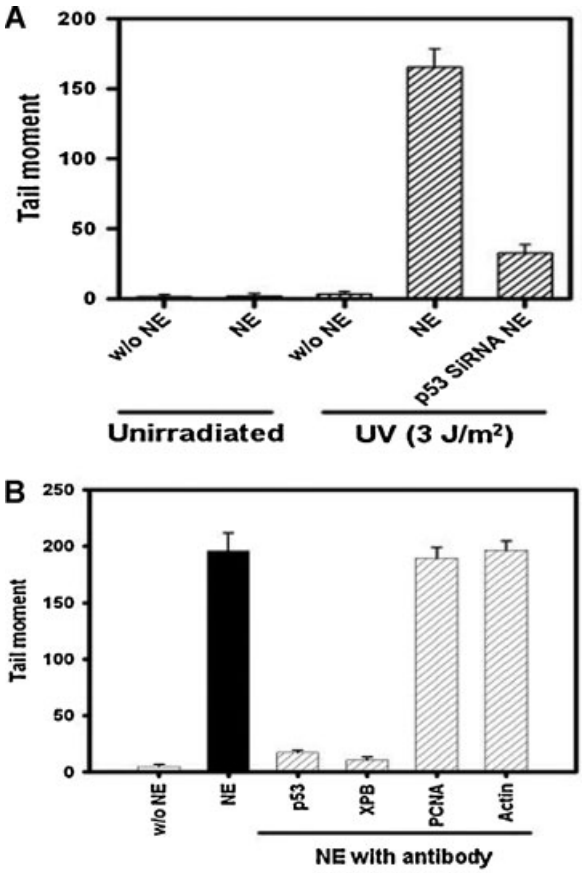

Fig. 6. p53 of CHO-K1 is essential to the activity of the nuclear extract to excise UV-induced DNA adducts. Comet-NE assay in (**A**) and (**B**). A: The cells irradiated with UV or not were used in the assay as the substrates. Nuclear extracts (NEs) used in the assay were prepared from the cells pre-treated with or without p53 siRNA. B: The cells irradiated with UV were used as the substrates. The NEs prepared from the exponentially growing cells were either immunodepleted of p53, XPB, PCNA or actin or without immunodepletion.

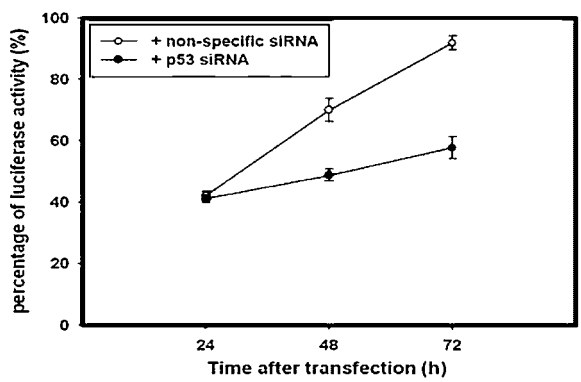

Fig. 7. p53 of CHO-K1 facilitated the DNA repair in CHO-K1 cells. Host cell reactivation assay of UV-irradiated CMV-Luc plasmid in CHO-K1 cells pre-treated with specific p53 siRNA or non-specific siRNA.

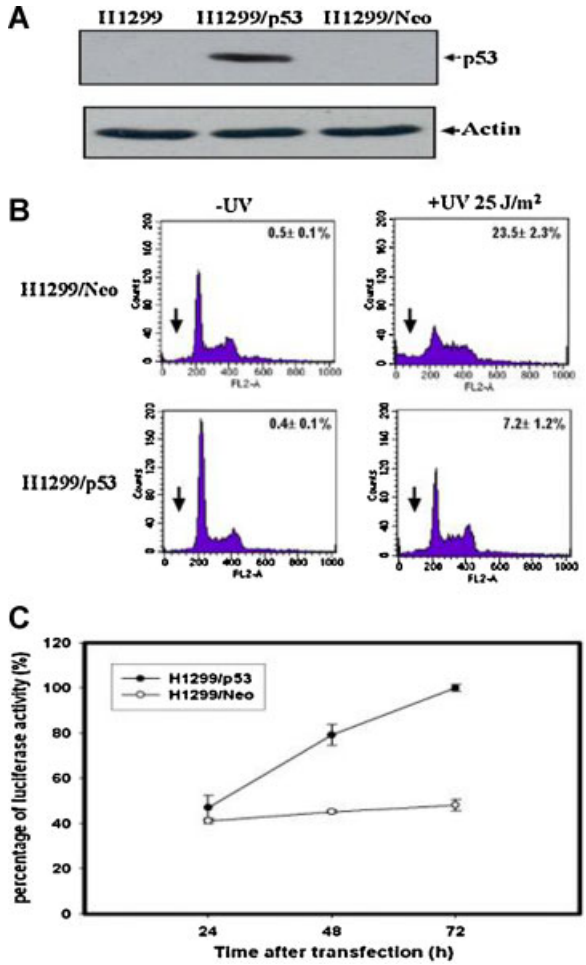

Fig. 8. p53 inhibited the UV-induced apoptosis and enhanced the DNA repair in H1299 cells. H1299/p53:H1299 cells stably transfected with human p53 expression vector; H1299/Neo:H1299 cells stably transfected with empty vector. **A**: The Western blotting analysis of p53 protein in H1299, H1299/p53 or H1299/Neo cells. Actin was used as a loading control. **B**: The flow cytometric analysis of cellular DNA contents in cells at 36 h after 25 J/m^2 of UV irradiation. The sub-G1 fractions are marked with arrows. Quantitative analyses of sub-G1 fractions are shown on the upper right corners. **C**: Host reactivation of UV-irradiated CMV-Luc plasmid in H1299/p53 or H1299/Neo cells. Cells were transfected with CMV-Luc (damaged or undamaged) and harvested at the indicated time (1–3 days after CMV-Luc transfection) for luciferase activity assay as described above. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

of TFIIH complex, which is involved in the initial step of NER.

In the comparative study, we found that p53 in the human H1299 cells may play active role in both UV-induced apoptosis and repair (Fig. 8). This is apparently different from that of the p53 in CHO-K1. The hamster p53 enhanced repair but did not affect UV-induced G2/M arrest or apoptosis. The difference, in our interpretation,

is partly due to the missing of $p21^{(Waf1/Cip1)}$ in CHO-K1 cells. The absence of p21 may fail the G1 arrest in the hamster cells following UV irradiation, and G2/M arrest becomes prominent. On the other hand, H1299 cells in which p21 is inducible if p53 is present has G1 arrest. Apparently, G2/M arrest was absent in p53-null H1299 cells (Fig. 8B). Previous study [Rohaly et al., 2005] with lower dose of UV ($10\ J/m^2$) also failed to detect G2/M arrest. Thus, G2/M arrest in H1299 cells, unlike that in CHO-K1 cells, may be p53-dependent.

In summary, p53 of the CHO-K1 cells is not involved in UV-induced G2/M arrest, and fails to regulate UV-induced apoptosis plausibly due to the absence of p21 in the cells. Furthermore, p53 of the CHO-K1 facilitates repairing the DNA damages induced by UV. This facilitation may be in part due to its direct involvement in NER repair process.

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