Overexpression of Damaged-DNA-Binding Protein 2 (DDB2) Potentiates UV Resistance in Hamster V79 Cells

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Background: Ultraviolet radiation (UV) damaged-DNA binding (DDB) activity comprises two major components: damaged-DNA binding protein 1 (DDB1) and 2 (DDB2). Although the function of DDB is unclear, mutation on DDB2 is associated with cellular sensitivity to a variety of genotoxic agents including UV. It has been suggested that DDB2 may play a role in UV-induced DNA repair. However, evidence that DDB2 involves in DNA repair and UV sensitivity is lacking.

Methods: To examine the role of DDB2, we established DDB2-overexpressing hamster V79 cell lines, V79ddb2, by stable transfection with full-length open reading frame of human ddb2 cDNA. Cells were irradiated with UV and determined its DNA repair activity by testing the remaining photoproducts on the chromatin and measuring the plasmid reactivation, respectively. UV induced cytotoxicity was determined by the colorimetric assay (MTT assay), and apoptotic cells exhibiting morphological features of chromatin condensation and nuclear fragmentation were counted after 4-diamidino-2-phenylindole (DAPI) staining.

Results: DDB activity was increased in DDB2-overexpressing cell lines. Analysis on DNA repair indicated that UV photoproducts were removed in a time-dependent manner and there was greater than 50% of damage removed within 12 h in DDB2-overexpressing cells. In contrast, nearly all the damage remained unrepaired in V79 cells. However, using bacterial CAT gene as a reporter, both V79 and V79ddb2 cells demonstrated no difference in the reactivation of plasmid DNA carrying UV damage. These results suggest that DDB2 may involve in repair of bulky genomic DNA damage. Although a maximum of only 30% of apoptosis was induced, UV irradiation caused a dose-dependent apoptosis and cytotoxicity in these cell lines. V79ddb2 cells displayed resistance to UV-induced apoptosis and cytotoxicity.

Conclusion: Our findings indicate that overexpression of DDB2 in V79 cell potentiates DNA repair and protects cells from UV-induced cytotoxicity. These results also suggest that DDB2 may be involved in the development of UV resistance.

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The recognition and incision of damaged DNA are crucial in nucleotide excision repair and cell sensitivity to genotoxic agents including ultraviolet radiation (UV). Nucleotide excision repair is a versatile strategy for defending against genotoxic insults to chromosome DNA in organisms ranging from microplasma to mammals, and represents a major mechanism of DNA repair in mammalian cells. Cells from the heritable human disorder xeroderma pigmentosum (XP) complementation groups A through G have reduced nucleotide excision repair.[1] Defects in DNA repair of XP cells often occur in the recognition and incision of damaged DNA. Indeed, there is as yet only indirect evidence which suggests that damaged-DNA-binding (DDB) activity may be involved in DNA repair in mammalian cells.[2-5] UV-DDB activity has been purified to apparent homogeneity and characterized from human placenta and HeLa cells,[6-8] and is identical to the activity originally identified from human placenta.[9] In vitro reconstitution studies indicate that UV-DDB proteins, although not essential, can stimulate nucleotide excision repair.[10] UV-DDB activity appears to be enhanced in UV-resistant human cells[2-4,11] and to be lost or reduced in several XP-E cell lines.[5,12-14] Microinjection of purified UV-DDB into XP-E cells restores the repair defect.[15,16]

UV-DDB was originally identified as a complex with 2 subunits of ~127 kDa (i.e., DDB1) and ~48 kDa (i.e., DDB2).[7,17,18] DDB1 cDNA was isolated from both monkeys[19] and humans.[20,21] Although DDB1 was proposed to be involved in nucleotide excision repair,[22] several studies have shown that DDB1 is not essential for this step.[23] Human DDB2 cDNA was isolated,[20] and together with DDB1 is required for the recognition of UV damage.[24] Since a subset of XP-E cells, which lack DDB activity, retains mutations in DDB2,[18] DDB2 may contribute to nucleotide excision repair and cell sensitivity. UV irradiation has been shown to induce apoptosis, genetically programmed cell death, in a variety of cell types. Although UV-induced cytotoxicity and resistance have been extensively reported, the mechanism remains unclear. In the present study, we found that overexpression of DDB2 in cells can potentiate DNA repair and apoptotic resistance in response to UV.

METHODS

Reagents and cell lines

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin were obtained from GIBCO (Gaithersburg, MD). [^{32}P]dCTP (3000 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). Cisplatin, vincristine, DAPI, G418, and other were purchased from Sigma (St. Louis, MO). Chinese hamster V79 cells were obtained originally from American Type Culture Collection.

To generate DDB2-overexpressing cell variants, V79 cells were transfected with pcDNA3ddb2, a pcDNA3 plasmid (Invitrogen, Carlsbad, CA) that contains a full-length ORF of human DDB2 cDNA and a neomycin-resistant gene. Potential cell candidates were selected after being cultured in media containing G418 (for selecting resistance to geneticin) for 2 weeks. The G418 concentration (400 μg/ml), which killed all cells without plasmid transfection, was empirically determined in the cell line.

DDB2 cDNA and antibody

An 1820-nucleotide segment of human DDB2 cDNA was isolated from a placenta cDNA library (Quick-Clone cDNA, Clontech) by polymerase chain reaction (PCR)(25) according to the human DDB2 cDNA sequence (GenBank database accession no. U18300).[20] PCR primer pairs were designed according to the reported DDB2 sequence to cover the full-length open reading frame. For convenience of plasmid construction, the recognition sequences of Sma I and Hind III were added to each primer at the 5' and 3' ends, respectively. The PCR products were cloned into a plasmid pGEM-T Easy vector (Promega), designated as pGTddb2. Plasmid DNA was sequenced by the dideoxynucleotide method[26] using a T7 or SP6 primer complementary to a vector region immediately outside the cDNA insert. To test for its continuity in the predicted ORF, proteins were made using the TNT reticulocyte lysate system (Promega) from pGTddb2 as described.[27]

To produce DDB2 antibodies, pGTddb2 was digested with the restriction enzymes Sma I and Hind III, and inserted in frame into pET15bDH, a modified form of pET15b (Invitrogen), to produce

pETddb2. Proteins were made in bacteria and purified with a nickel column according the specifications of the supplier (Qiagen). Polyclonal DDB2 antibodies were generated in New Zealand rabbits according to the described methods.[28]

Cell extracts and immunoblot analysis

For whole-cell extracts, cells were washed with phosphate-buffered saline (PBS) twice and lysed in 1 ml of modified radio immunoprecipitation buffer (RIPA; 50 mM Tris HCl, pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM PMSF, 1 μg/ml each of aprotinin, leupeptin, and pepstatin, 1 mM Na_3VO_4, and 1 mM NaF) on ice for 30 min. The insoluble material was removed by centrifugation at 14,000 rpm for 10 min at 4 °C. The protein concentration was measured with the Bradford assay using the BioRad dye reagent,[29] and separated by SDS-polyacrylamide gel electrophoresis.[30] Equivalent amounts of protein from each sample were separated by SDS-polyacrylamide electrophoresis (PAGE), transferred onto PVDF membranes and incubated with antibodies. Antigen-antibody complexes were visualized by the standard enhanced chemiluminescence reaction according to the specifications of the supplier (Pierce, Rockford, IL).

Nuclear extracts and gel mobility shift assay

Crude nuclear extracts were prepared according to Dignam et al.[31] Protein-DNA binding was performed in 15 ml of buffer containing 12% glycerol, 12 mM Hepes (pH 7.9), 100 mM KCl, 5 mM $MgCl_2$, 4 mM Tris HCl, 1 mM EDTA, 1 mM dithiothreitol, and 300 mg/ml BSA at 25 °C for 30 min.[32] DNA probes were prepared as described previously.[33] Hind III- and EcoR I-generated f103 fragments were [^{32}P]dCTP-labeled (3 ¡ 10^4 cpm/ng DNA) using Klenow DNA polymerase and purified using column chromatography by standard methods.[27] DNA at a concentration of 100 μg/ml was exposed to a fluence rate of 25 J/m²/s from a VL-100C UV irradiation unit (Vilbert Lourmat, France). UV exposures were measured with a VLX-254 radiometer (Vilbert Lourmat, France). The reaction mixtures were then subjected to 5% polyacrylamide gel electrophoresis under low ionic strength (6.7 mM Tris HCl, pH 7.9, 3.3 mM sodium acetate, 1 mM EDTA) at 30 °C and a constant current of 15 mA. The resolved gel was dried and exposed to Kodak XAR-5 X-ray film at -70 °C with an intensifying screen.

DNA repair assays

To measure DNA repair, cells were irradiated with 10 J/m² of UV and incubated to allow repair of the photoproducts as described.[34] The relative number of UV-induced DNA photoproducts was determined by a radioimmunoassay as previously described.[35] Antiserum was raised in rabbits by injection with UV-irradiated, denatured calf thymus DNA coupled to methylated bovine serum albumin.[36] Two grams of heat-denatured sample was allowed to compete against 32P-labeled poly(dA): poly(dT) irradiated with 40 kJ/m² UV for antibody-binding sites. Using a standard curve, inhibition in the repaired samples was converted to dose equivalents, and the removal of antibody-binding sites was determined.

To measure plasmid reactivation,[33] cells were seeded at 3 ¡ 10^6 cells per 100-mm plate 1 day before electroporation. One milliliter of cell suspensions, in Hepes buffer, was added to a sterile cuvette containing 20 μg pRSVcat and 10 μg pSVβ (Clontech) plasmids. This was gently mixed and subjected to electroporation[27] as described in the manufacturer's instructions (GenePulser, BioRad). The following day, fresh medium was provided to the cells, and they were incubated for another 48 h. Cells were then harvested for CAT assay as previously described.[37] The β-gal activity of the same preparation for CAT assay was also analyzed as an internal control.[27] After autoradiography, the density on the X-ray film corresponding to the modified chloramphenicol was quantitated with a scanning densitometer (Personal Densitometer SI, Molecular Dynamics). Being normalized to β-gal activity, the CAT activity was calculated as the percent of chloramphenicol converted into acetylated derivatives.

Analysis of cytotoxicity and apoptosis

Cytotoxicity was determined by MTT assay.[38] Cells were plated in 96-well dishes in a total volume of 100 μl. Cells were exposed to UV for 12 hours after plating, and the culture was incubated for 72 hours at 37 °C. The percentage of cells surviving UV was determined by the ability of the cells to convert

the tetrazolium MTT salt into a formazan product solubilized in acid-propanol.

For analysis of apoptosis, cells growing in 6-well plates were exposed to UV for 24 hours at 37 °C. Cells were fixed with methanol and incubated with a DAPI (4-diamidino-2-phenylindole) solution for 30 min in the dark. Floating cells from each well were also fixed and then placed back into the respective wells and analyzed using a microscope at 420 nm. The apoptotic cells exhibiting morphological features of chromatin condensation and nuclear fragmentation[39] were counted in 6-8 randomly selected fields. Exactly 500 nuclei were examined from each sample, and the results were calculated as the number of apoptotic nuclei over the total number of nuclei counted from 3 independent experiments.

RESULTS

Overexpression of DDB2 potentiates UV-damaged DNA binding

To examine the function of DDB2, DDB2-overexpressing cell lines were established from a Chinese hamster V79 cell line, which displays either none or only trace amounts of the protein. Two typical candidate clones, V79ddb2#1 and #2, were isolated for detection of the DDB2 protein (Fig. 1). Using an antibody for DDB2, both DDB2-overexpressing cell lines displayed several times the specific DDB2 proteins (around 47 kDa) compared to parental V79 cells. In contrast, no protein was detected for the preimmune serum on the same protein membrane blot. The same membrane blot, after being stained with Amido black, revealed equal amounts of proteins for each cell extract. The DDB activities of the selected DDB2-overexpressing cell lines were compared (Fig. 2A). On an electrophoretic mobility shift assay (EMSA), V79 nuclear extracts displayed no DDB activity, whereas both V79ddb2 cell lines displayed dose-dependent DDB activity (indicated by "bound"). However, the damage-binding activity was much higher in V79ddb2#2 than in V79ddb2#1. It should be noted that both lines overexpressed similar amounts of DDB2 (see Fig. 1). The data suggest that DDB activity is not solely determined by the concentration of DDB2 in these cells. To ensure that the DDB activity was specific to UV damage, competitive EMSA was carried out (Fig. 2B). An

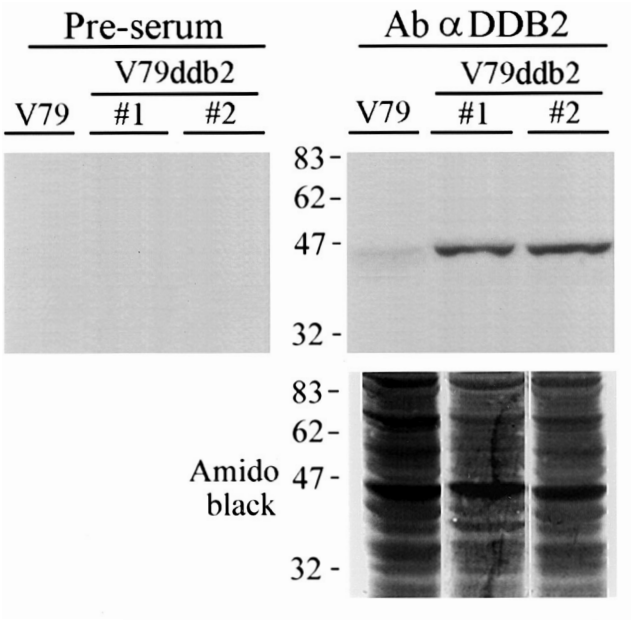

Fig. 1 Establishment of DDB2-overexpressing cell lines. (A) Immunoblot analysis of DDB2 in V79, and DDB2-overexpressing V79 [V79ddb2] cells. Fifty micrograms of whole-cell extracts was used for the immunoblot analysis. Amido black staining is shown below. Immunodetection using preimmune serum is shown to the left. Molecular weight markers (kDa) are also indicated.

increasing molar excess of a specific competitor (f103/UV) inhibited the DDB activity. In contrast, there was no measurable inhibition of DDB activity by a 100-fold increase in untreated f103 or single-stranded f103 (ssf103). However, a majority of the DDB activity was inhibited by a 100-fold increase in UV-irradiated ssf103 (ssf103/UV). Hence, the DDB2-overexpressing cell lines displayed enhanced DDB activity preferential for UV damage.

Overexpression of DDB2 potentiates DNA repair

To examine the role of DDB2 in DNA repair, the DDB2-overexpressing cell lines were irradiated with UV and assayed for DNA repair. UV induces mainly cyclobutane pyrimidine dimers and (6-4) photoproducts on cellular DNA.[1] Using an antibody against UV-induced photoproducts, we measured the majority of DNA remaining in V79 cells (Fig. 3). In

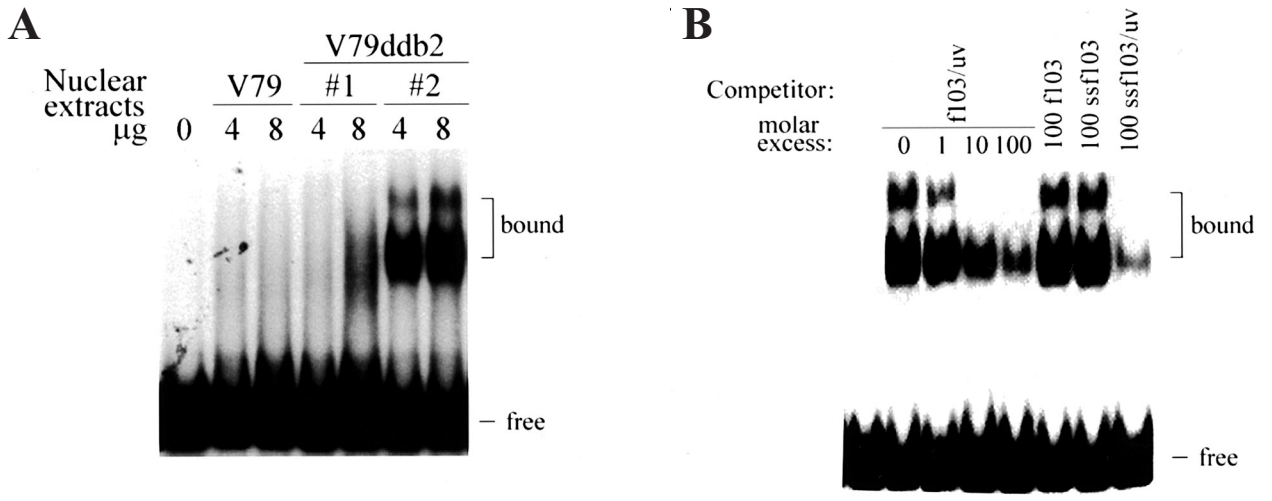

Fig. 2 UV-DDB activity in DDB2-overexpressing V79 cells. (A) Increased DDB activity in DDB2-overexpressing V79 cells. Four or eight microliters of nuclear extracts of indicated cells was incubated with a DNA probe and f103, and irradiated with 6000 J/m^2 UV. Free and bound probes are indicated. (B) Specificity of DDB activity. Eight microliters of nuclear extracts was used to detect UV-DDB activity in the presence of the indicated molar excess of competitors. Symbols: f103/UV and f103, double-stranded DNA f103 with or without UV irradiation (6000 J/m^2); ssf103/UV and ssf103, single-stranded DNA f103 with or without UV irradiation.

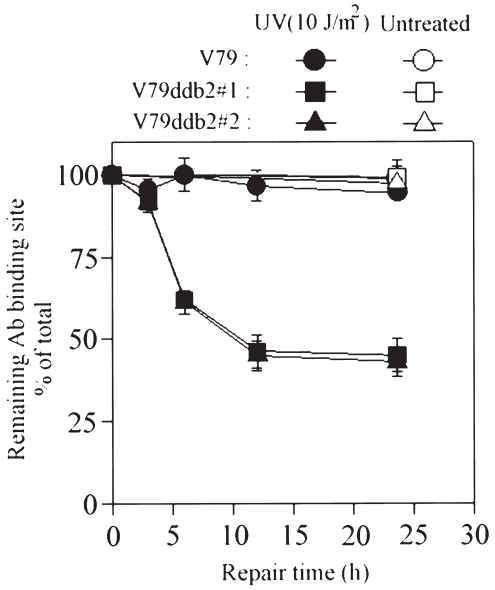

Fig. 3 Chromosomal DNA repair in cells. V79, V79ddb2#1, or V79ddb2#2 cells were irradiated with 10 J/m^2 UV, and after incubation, the UV photoproducts remaining on the chromosome DNA were measured at 25 or 4 °C with a damage-specific antibody. The plotted values represent the mean ¡ S.D. of 3 independent experiments.

contrast, 50% or more of the photoproducts were removed within 12 hours in a time-dependent manner in the DDB2-overexpressing cell lines. Repair became saturated at 12 hours since no more repairs occurred with a longer incubation. There was essentially no detectable repair at 4 °C in these cells, suggesting that an enzymatic reaction may be required. These results indicate that overexpression of DDB2 potentiates the repair of UV damage in cells. Since V79 cells lack photoreactivating enzyme activity, which removes the major dimer photoproducts,[1] enhanced DNA repair detected in DDB2-overexpressing cells is likely preferential for cyclobutane pyrimidine dimers.

Overexpression of DDB2 did not potentiate plasmid reactivation

Plasmid reactivation using CAT enzymatic activity as a reporter can be used in certain conditions to quantify DNA repair.[37] Using this assay, the reporter plasmids carrying UV damage were transfected into V79 cells, and the relative CAT activity was measured (Fig. 4). There was dose-dependent inhibition of CAT activity in each cell line. By assuming that plasmid reactivation or CAT activity is expressed by intact DNA, DNA damage on plasmid

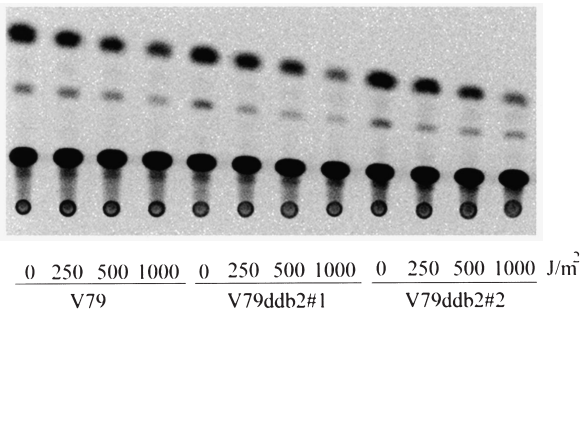

Fig. 4 Naked DNA repair in cells. DNA repair was determined using plasmid reactivation in cells, and the CAT activity was isolated and measured in vitro. Typical CAT activity of transfected cells is shown in the top panel. Relative CAT activity, normalized to β-galactosidase activity (see "Materials and Methods"), was calculated. The plotted values represent the mean ¡ S.D. of 3 independent experiments.

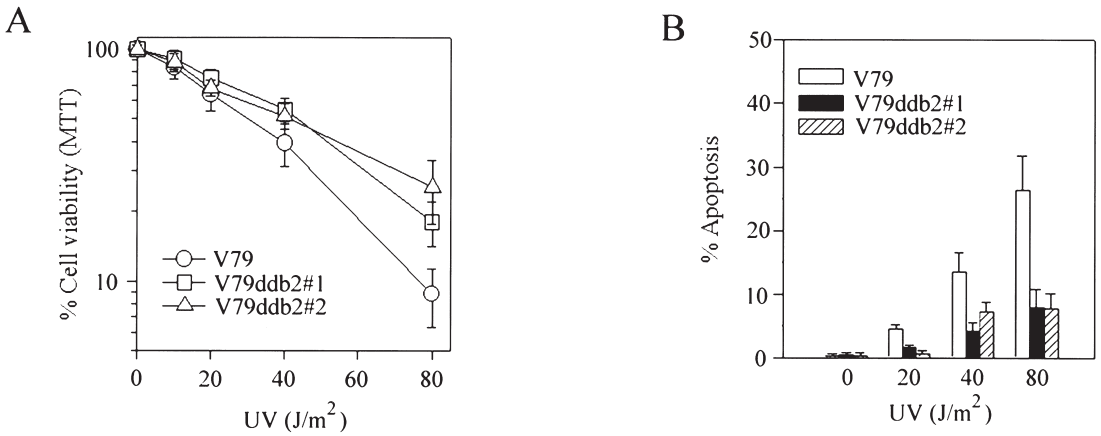

Fig. 5 Overexpression of DDB2 inhibition of UV-induced cytotoxicity and apoptosis. (A) UV-induced cytotoxicity. Cytotoxicity was analyzed after 72 hours using the MTT assay, and is represented as the percent (%) survival by setting untreated cells as 100%. (B) UV-induced apoptosis. The percent of cells undergoing apoptosis was determined after 24 hours. The plotted values represent the mean ¡ S.D. of 3 independent experiments.

DNA was apparently repaired in the cells. However, there was no difference between parental and DDB2-overexpressing cells. A typical CAT activity pattern is shown in the top panel of Fig. 4. These results indicate that overexpression of DDB2 did not potentiate plasmid reactivation in V79 cells.

Overexpression of DDB2 protects cells from UV-induced apoptosis

To examine the role of DDB2 in UV sensitivity,

we further investigated the effects of DDB2 overexpression in UV-treated hamster V79 cells, which express no of only trace amounts of DDB2. Cytotoxicity measured by the MTT assay indicated dose dependence. The cytotoxicity was partially reduced in V79ddb2 cells (Fig. 5A). Apoptotic cells were induced, to a maximum of 30%, in a dose-dependent manner in both V79 and V79ddb2 cells. V79ddb2 cells displayed reduced UV-induced apoptosis compared to V79 cells (Fig. 5B). Overexpression of DDB2 inhibited UV-induced apoptosis by more than 50%. These results indicate that overexpressing DDB2 might protect cells from UV-induced apoptosis and cytotoxicity.

DISCUSSION

In the present study, we found that DDB2 is expressed only in trace amounts, if any, in hamster V79 cells, and that overexpression of DDB2 potentiates cell resistance to UV. Independently selected DDB2-overexpressing cell lines displayed increased, but different, levels of UV-DDB activity. In addition, V79 cells overexpressing DDB2 repaired UV damage more efficiently than did parental cells. Other investigators have also shown that damaged-DNA-binding activity is potentiated by transient transfection of DDB2 in V79 and human 293T cells, but not by mutated DDB2 from several XP-E cell lines.[24] The involvement of DDB2 in the response to UV is also supported by the findings that a subset of XP-E cells, although containing DDB1, did not exhibit damaged-DNA-binding activity due to a mutation in DDB2.[18] Further, overexpression of DDB1 by transient transfection in human 293T cells failed to induce damaged-DNA-binding activity.[24] In addition, DDB activity is associated with and probably responsible for enhanced DNA repair in a HeLa cell model.[3] It is likely that increased DDB activity in DDB2-overexpressing V79 cells may be attributed to enhanced DNA repair. However, different DDB2 activities occur in DDB2-overexpressing V79 cell lines, whereas the levels of DNA repair capacity were the same in these DDB2-overexpressing cell lines. The efficacy of DNA repair in this cell system, therefore, cannot be fully explained by enhanced DDB activity. In fact, 3 DDB proteins have now been implicated in nucleotide DNA repair: the XPA protein,[40] the XPC/HR23B heterodimer,[41] and UV-DDB. Although there may be cell-type dependence, UV-DDB is probably not the only factor affecting DNA repair in V79 cells. Since insertion of exogenous DNA into cellular chromosomes occurs randomly during establishment of DDB2-overexpressing cell lines, other genes involved in DDB activity may also be affected. Recently, DNA repair detected with damage-specific antibodies has been demonstrated, and the results revealed that DDB2 enhances global genomic repair of cyclobutane pyrimidine dimers, but not (6-4) photoproducts.[42] In addition, microinjection of purified DDBs into XP-E cells, but not in an in vitro system, restored repair synthesis. These results suggest that the DDB protein in its DDB1 or DDB1-DDB2 form may play a specific role in the repair of chromosomal DNA in the nuclear environment, which is not easily revealed in vitro.[15,16] Since V79 cells contain minimal photoreactivating enzyme activity,[43] which removes the major cyclobutane dimer photoproducts, enhanced DNA repair detected in DDB2-overexpressing cells is likely preferential for cyclobutane dimer photoproducts. Taken together, DDB2 is involved in cyclobutane dimer repair that may regulate cell responses to UV. Interestingly, overexpression of DDB2 in cells did not improve repair of naked DNA damage. DDB2 contains a WD motif with some identity to the WD motifs in a subfamily of WD repeat proteins involved in the reorganization of chromatin.[24] The results strongly suggest that the regulation of DNA repair, and maybe cytotoxicity, is affected by other chromosome-binding proteins. This may explain why DDB2 alone cannot potentiate DNA excision repair in vitro and naked DNA repair in cells.

In this study, we also found that overexpression of DDB2 protected cells from UV-induced cytotoxicity and apoptosis. This is expected because DNA repair is enhanced in DDB2-overexpressing cells. It has been well demonstrated that cells with improved DNA repair are often associated with resistance to DNA damage.[22,44,45] Our results suggest that apoptotic resistance may represent acquired UV resistance, and that apoptotic resistance may be due to overexpression of DDB2. Overexpression of DDB2 thereby establishes an opportunity for cells to develop resistance to UV. Indeed, resistant HeLa cells, which

exhibit enhanced DNA repair,[37,46] became sensitive to UV-induced apoptosis by inhibiting DDB2 expression.[47] It is likely that the role of DDB2 in modulating UV-induced sensitivity in hamster cells may also occur in other mammalian cells. However, inhibition of DDB2 did not affect cisplatin- and mitomycin c-induced cytotoxicity or apoptosis (data not shown), suggesting that modulation of apoptosis by DDB2 may depend on the stimulus involved.

Activation of caspases and their regulators is required for apoptosis, and this plays a predominant role in cell sensitivity or resistance to a variety of DNA-damaging agents.[45,48] Using commercial antibodies, we had difficulty detecting apoptotic molecules in V79 cells. However, 80 J/m^2 of UV induced a maximum of 30% apoptosis in V79 cells. In contrast, 40 J/m^2 of UV induced more than 80% apoptosis in human cells.[39] Although the induction of apoptosis in V79 cells was less effective, the apoptotic phenotype (chromosome fragmentation and chromatin condensation) was the same as that in human cells. Incubation of V79 cells for longer than 24 hours to allow repair showed no greater elimination of DNA damage. In addition, components of apoptosis are conserved between Caenorhabditis elegans, Drosophila melanogaster, and humans.[19] There may be a different epitope on hamster apoptotic proteins. A recent report by other investigators revealed that overexpression of DDB2 by stable transfection in hamster V79 cells also potentiated damaged-DNA-binding activity.[42] However, they detected no protection against UV toxicity (by colony-forming assay) by DDB2 overexpression.[42] In contrast to that report, we found that overexpression of DDB2 protected V79 cells from apoptosis and thus enhanced cell survival. The contradiction is probably due to different cell clones and/or assays employed. Therefore, the association of DDB2 with DDB1 and DDB2's effect on DNA repair and apoptosis deserve further investigation.

Acknowledgments

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受損DNA結合蛋白DDB2的表現可增強倉鼠V79細胞抗紫外輻射

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背 景： 細胞中具有對紫外輻射損傷DNA的結合活性主要包含兩種蛋白：受損DNA結合蛋白1 (DDB1) 和2 (DDB2)，雖然還不清楚損傷DNA結合活性在細胞中之功能，但是已知DDB2發生突變會導致細胞對多種基因毒性物質的敏感性增加；其中包括紫外輻射。因此可推測DDB2在紫外輻射引起的DNA修補機制中扮演一定的角色。但是實際上有關DDB2參與DNA修補與對紫外輻射敏感性的證據仍舊缺乏。

方 法： 爲了進一步研究DDB2的功能，我們建立一株可表現DDB2蛋白的倉鼠細胞株V79ddb2。在細胞經紫外輻射處理後，利用抗體檢測基因體上留存的光化合物；以及將帶有報導基因 (CAT) 的質體DNA，在細胞外經紫外輻射處理後送入細胞內，藉由其報導基因的表現，得以分析細胞修補受損DNA的效率。利用呈色分析 (MTT) 的方法檢測紫外輻射對細胞毒殺的敏感性，以及在顯微鏡下用螢光染色法 (DAPI) 計算因紫外輻射引起染色體發生變化的凋亡細胞。

結 果： 我們發現該細胞株在12小時內可移除近50%的紫外輻射損傷的DNA；但是缺乏DDB2表現的母細胞株卻不具有這樣的修補能力。然而利用CAT基因當作報導基因的修補分析，顯示兩株細胞對紫外輻射損傷DNA的修補並無差異，此結果可推測DDB2可能只參與基因體之修補。另外在紫外輻射引起細胞凋亡與細胞毒性的分析方面，V79ddb2細胞也顯示比母細胞株有抗紫外輻射的能力。

結 論： 實驗結果顯示當V79細胞表現DDB2蛋白可提升細胞DNA修補能力以及降低紫外輻射對細胞的毒殺。因此推測DDB2可參與細胞抗紫外輻射的作用。

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關鍵字： 細胞凋亡，受損DNA結合蛋白2，DNA修補，抵抗性，紫外輻射。

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