Published OnlineFirst June 8, 2010; DOI: 10.1158/1541-7786.MCR-09-0467

Molecular Cancer Research

XPB Induces C1D Expression to Counteract UV-Induced Apoptosis

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Abstract

Although C1D has been shown to be involved in DNA double-strand break repair, how C1D expression was induced and the mechanism(s) by which C1D facilitates DNA repair in mammalian cells remain poorly understood. We and others have previously shown that expression of xeroderma pigmentosum B (XPB) protein efficiently compensated the UV irradiation–sensitive phenotype of 27-1 cells, which lack functional XPB. To further explore XPB-regulated genes that could be involved in UV-induced DNA repair, differential display analysis of mRNA levels from CHO-9, 27-1, and 27-1 complemented with wild-type XPB was done and *C1D* gene was identified as one of the major genes whose expression was significantly upregulated by restoring XPB function. We found that XPB is essential to induce C1D transcription after UV irradiation. The increase in C1D expression effectively compensates for the UV-induced proteolysis of C1D and thus maintains cellular C1D level to cope with DNA damage inflicted by UV irradiation. We further showed that although insufficient to rescue 27-1 cells from UV-induced apoptosis by itself, C1D facilitates XPB DNA repair through direct interaction with XPB. Our findings provided direct evidence that C1D is associated with DNA repair complex and may promote repair of UV-induced DNA damage. *Mol Cancer Res; 8(6); 885–95. ©2010 AACR.*

Introduction

UV sunlight–induced DNA damage is a common cause of mutagenesis of the human genome. The accumulation of inappropriately repaired DNA damages eventually leads to tumorigenesis. Effective repair of UV-induced DNA damage through a highly conserved DNA repair pathway, nucleotide excision repair (NER), is critical for prevention of genomic instability. Cells with NER defects tend to accumulate DNA damages, which are perpetuated through proliferation and eventually result in carcinogenesis. Several autosomal recessive hereditary diseases, including the classic form of xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD), are characterized by sensitivity to sunlight due to deficiencies in NER. Mutations in xeroderma pigmentosum B gene (*XPB*), one of the major components in NER, have been identified in patients with different clinical phenotypes of XP, CS, TTD, and XP/CS (1–3). For example, missense mutation (c.296T>C, p.F99S) has been connected with both milder XP and milder XP/CS complex patients from two different families (3). The *XPB* gene was initially identified as the gene responsible for NER deficiency in xeroderma pigmentosum group B (XPB) patients (4). It is subsequently identified as a subunit of the general transcription factor TFIIH required for transcriptional regulation of class II genes. The XPB protein contains seven helicase motifs and one ATP binding site essential for its activity in both NER and transcription (4). XPB unwinds double-stranded DNA (dsDNA) along the 3′ to 5′ orientation in the presence of ATP during NER (5). During transcription initiation, its ATPase activity is required for promoter opening, and its helicase activity facilitates promoter clearance and elongation (6, 7). Recently, study has identified a new XPB-unique RED motif, which plays a key role in DNA unwinding (8). Mutation in this motif dramatically reduces the helicase activity of XPB. The dual function of XPB has suggested a connection between the roles of XPB in NER and transcription; for example, in transcription-coupled NER, actively transcribed genes may be preferentially repaired to cope with the urgent need for proteins required for efficient DNA repair (9).

We and others have previously shown that complementing XPB mutation rescued the UV-sensitive phenotype of XPB-defective Chinese hamster ovarian (CHO) 27-1 cells to an extent close to that of the parental

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doi: 10.1158/1541-7786.MCR-09-0467

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CHO-9 cells (10, 11). In this study, we further investigated XPB-regulated candidate genes that could potentially facilitate XPB-mediated NER using these cell lines as models. Through differential display analysis of cells carrying wild-type and mutant *XPB* genes, we identified *C1D,* a recently described nuclear matrix protein-coding gene involved in dsDNA break repair (12), as a target gene of XPB. Interestingly, C1D has been reported to interact with and to activate DNA-dependent protein kinase (DNA-PK; ref. 13). Moreover, C1D interacts with Translin-associated protein X (TRAX) and may regulate Translin/TRAX complex formation at dsDNA break point (14). These findings suggested that C1D may be involved in dsDNA break repair to maintain genomic integrity. Here, we report that functional TFIIH is essential to upregulate C1D transcription in response to UV irradiation and, thus, is important to maintain cellular C1D level after UV irradiation. In human cells, mutations in XPB or XPD attenuate UV-induced C1D transcription. We further show that C1D associates with TFIIH on UV irradiation, and such interaction may be necessary for effective DNA repair.

Materials and Methods

Differential display analysis

Total RNAs of CHO-9, 27-1, and 27-1 stably complemented with human *XPB* gene (27-1/XPB) cells were extracted by Isogen (WAKO). Differential display (DD) analysis was carried out on a Beckman Coulter Fluorescence Differential Display System (genomyxLRS) according to the manufacturer's instructions. Fluoro-DD-PCR was done with the HIEROGLAYPH and Fluoro-DD Kits (Genomyx). Briefly, total RNA was converted to cDNA by Superscript II reverse transcriptase (GIBCO BRL Life Technologies, Inc.) and special anchored primers provided by the manufacturer. Then, aliquots of cDNAs were used for amplification with special sets of fluoro-DD-anchored primers and arbitrary primers provided by the manufacturer. After denaturation, PCR products were loaded onto a genomyxLR Programmable DNA Sequencer and separated under 3,000 V, 100 W at 55°C for 5 hours. Bands of PCR amplicons were visualized through the fluorescence image operating system of genomyxSC. Differentially expressed PCR products were recovered from the gel and eluted into Tris/EDTA buffer for TOPO TA cloning (Invitrogen) followed by sequencing analysis.

Northern hybridization

Equal amounts of total RNA were separated on 1.1% agarose gel in 1× MOPS buffer containing 2.2 mol/L formaldehyde at 60 V for 5 hours and transferred onto Nytran membranes (Schleicher & Schuell) overnight in 20× SSC buffer (1× SSC is 0.15 mol/L NaCl, 0.015 mol/L Na-citrate) and then prehybridized for at least 5 hours at 37°C. Probes were ^{32}P labeled by Klenow (TAKARA) at 37°C for 30 minutes and free ^{32}P was removed by miniquick spin columns (Roche). The probe was hybridized to the blots in 1× SSC buffer for 48 hours at 37°C, washed, and visualized by autoradiography. Two rounds of hybridization were done to confirm expression of genes of interest after DD. For the first round, fragments recovered from DD gels were cloned and used as probe. Gene-specific probes were used for the second round. Genes were amplified by PCR using the following primer sets and cloned into TA-cloning vector (Invitrogen): C1D, 5′-TTGGTCAAAATGGCAGGTGGAGAAATGAAT-3′, 5′-TTAATGTTTACTTTTTCCTTTATTAGCAAC-3′; S3, 5′-CAGAAGATGGCGGTGCAGATT-3′, 5′-TTATGCTGTAGGCACTGGCT-3′; S8, 5′-CCAGCGCCGAGCGATGGGCATCTCTCGGGA-3′, 5′-TTATTTGCCTTTCCGGGCTTTGATCTTCCG-3′; P0, 5′-GTGATGCCCAGGGAAGACA-3′, 5′-TTAGTCGAAGAGACCGAATC-3′.

Cell culture and gene expression

CHO and 27-1 cells were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS). Primary fibroblasts from XPB patients (GM13025 and GM13026 that carry a c.296T>C p.F99S missense mutation on one allele and a c.471+1G>A intron 3 donor site splicing mutation on the other allele), patients' father (GM13028, heterozygous for c.296T>C p.F99S missense mutation), and patients' mother (GM13027, heterozygous for c.471+1G>A splicing mutation) were cultured in DMEM supplemented with 15% (v/v) FBS. For reference of these cell lines, see ref. 3. Telomerase-immortalized GM13026 fibroblasts and its XPB complemented cells were cultured in DMEM supplemented with 10% FBS as described previously (15). XPD [GM08207, R683W on one allele and deletion of amino acids 36-61 (p.G36_R61Del) on the second allele; ref. 16] and its XPD complemented cell line were cultured in DMEM with 10% FBS (15). To generate stable transfectants, full-length Flag-tagged C1D, S3, S8, and PO were amplified by PCR and inserted into the *Bam*HI/*Sna*BI sites of pBabe-IRES (puro^r) vector. DNA constructs including empty vector were then transfected into 27-1 cells at 60% to 80% confluence with Effectene transfection reagent (QIAGEN) following the manufacturer's instruction. Twenty-four hours after transfection, medium was changed to fresh DMEM/FBS (10%) containing 30 μg/mL puromycin (Sigma). After 24 hours of puromycin selection, survived cells were split into 96-well plates. Stable transfected cells were maintained in DMEM/FBS (10%) containing 10 μg/mL puromycin. 27-1/XPB cells were cultured as previously described (10). For transient human XPB transfection, 27-1 cells at 60% to 80% confluence were transfected with 5 to 10 μg of DNA by Superfect Transfection Reagent (QIAGEN), incubated for 48 hours, and harvested for whole-cell extract or RNA preparation.

Antibodies

Antibodies used in Western blotting were monoclonal anti-Flag (M2) (Sigma); anti-ubiquitin (P4D1), anti-C1D (C-17), and polyclonal anti-XPB (S-19) (Santa Cruz Biotechnology; epitope mapped at the COOH terminus);

Published OnlineFirst June 8, 2010; DOI: 10.1158/1541-7786.MCR-09-0467

anti-GTF2H1 (Abnova); and monoclonal anti-actin (Chemicon International). Proteasome inhibitor MG132 was purchased from Sigma.

Reverse transcription-PCR and quantitative real-time PCR

Total RNA from cells was reverse transcribed with Moloney murine leukemia virus reverse transcriptase (Life Technologies) using random hexamers according to the manufacturer's instructions. Aliquots of the cDNA were used for PCR amplification by Platinum PCR Supermix (Invitrogen) with specific primers of C1D as described above and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 5′-AGTTCAAAGGCACAGTCAAG-3′ and 5′-TCCACCACTCTGTTGCTGTA-3′). Real-time PCR was done with IQ SYBR Green Supermix (Bio-Rad) using the same primers.

UV irradiation and survival assay

Cells were plated into six-well plates at a density of 3×10^2 per well and incubated for overnight. Exposure to UV was done as previously described (10). Briefly, cells were gently rinsed twice with PBS, covered with 1 mL of PBS, and then exposed to germicidal UVP light (Blak-Ray Lamp, Ultraviolet Products). After irradiation, cells were incubated in culture medium for 7 days and colonies derived from survived cells of each tested line were counted after being visualized by brief staining.

RNA interference

For C1D silencing, single-strand small interfering RNAs [siRNA; 5′-GGAGCAUCCAGUGAAGCAAtt-3′ (sense) and 5′-UUGCUUCACUGGAUGCUCCtt-3′ (antisense)] were synthesized by Japan Bio Service and resuspended in

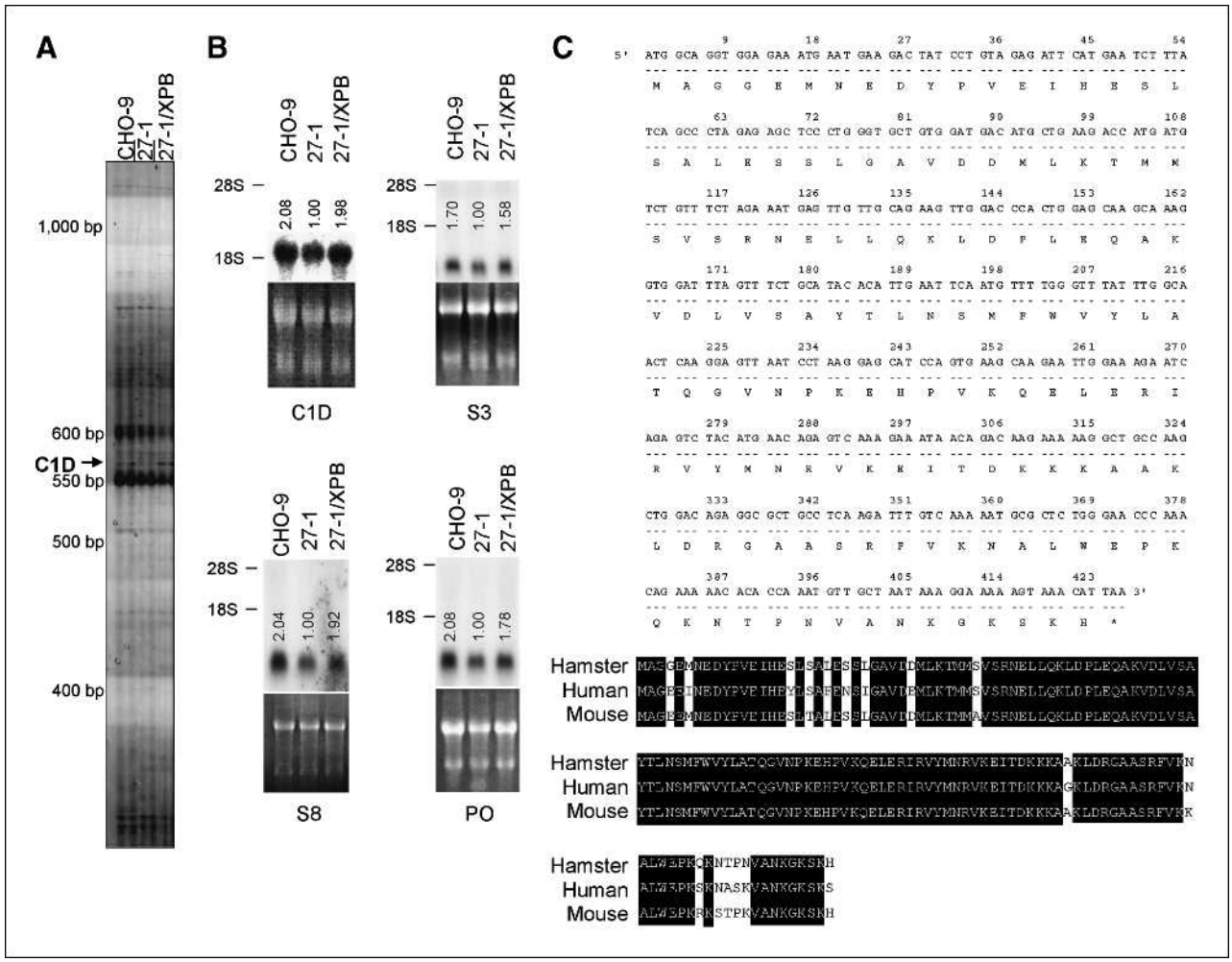

FIGURE 1. Identifying potential XPB-regulated genes by differential display analysis. A, DD gel from which we isolated *C1D* gene fragment (arrow). Each sample was run in duplicate. The positions of size markers were indicated on the left. B, Northern hybridization (top) to examine gene expression of C1D S3, S8, and PO in CHO-9, 27-1, and stable XPB-transfected 27-1 cells. The density of each band was analyzed by densitometry and the density of band from 27-1 cells was designated as 1.00. Total RNA gels (bottom) showing 28S and 18S RNA bands were used as loading control. C, schematic alignment of hamster *C1D* gene and its deduced amino acid sequence (top) and schematic alignment of hamster, mouse, and human C1D proteins showing high identity shared among the three species (bottom).

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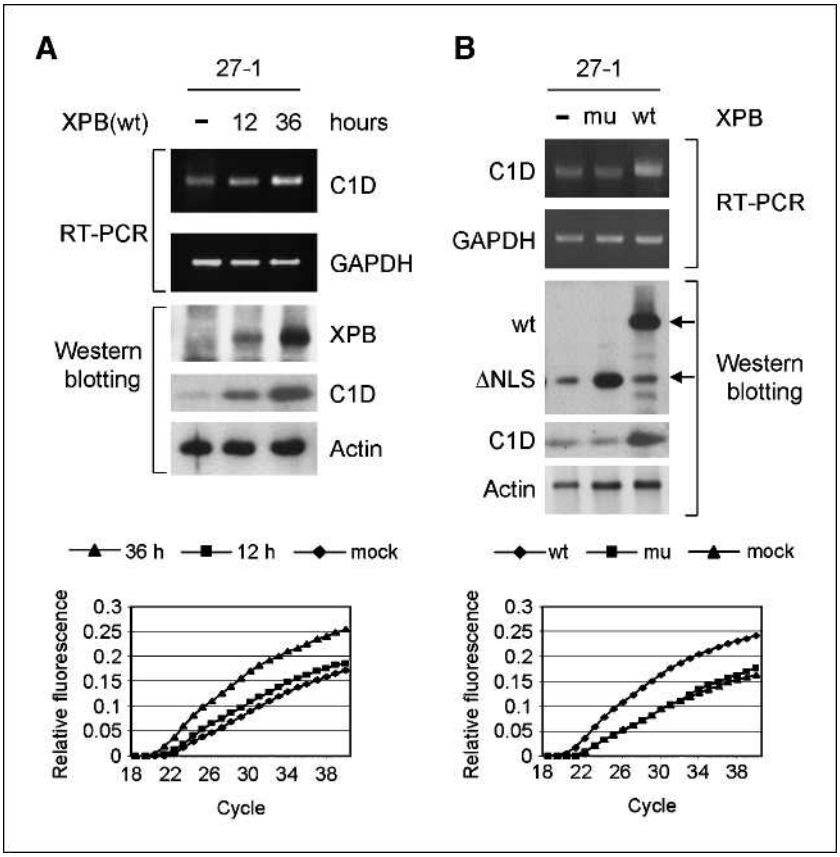

FIGURE 2. XPB transcriptionally induces *C1D* gene expression. A, 27-1 cells were transiently transfected with wild-type human *XPB* gene. Cells were collected at 12 and 36 h after transfection for preparation of total RNA and whole-cell extracts. For PCR and real-time qPCR analyses, signals were normalized to GAPDH. Protein levels of XPB, C1D, and actin were shown. B, 27-1 cells were transiently transfected with wild-type or mutant *XPB* (ΔNLS). Cells were collected 48 h after transfection for protein analysis by immunoblotting and RNA analysis by RT-PCR and real-time PCR, as in A.

RNase-free water at a final concentration of 50 μmol/L. Equal amount of each single-strand siRNA suspension was annealed in annealing buffer provided by Japan Bio Service. The reaction was programmed as 90°C for 1 minute and then 37°C for 1 hour. Successful annealing was confirmed by nondenaturing PAGE. siRNA was then labeled and transfected into target cells by the Label IT siRNA Tracker Cy3 kit (Mirus Corporation). The efficiency of siRNA delivery was monitored with an immunofluorescent microscope.

Ubiquitination assay

Cells were lysed in lysis buffer [50 mmol/L HEPES (pH 7.4), 150 mmol/L NaCl, 0.1% SDS, 1% Triton, 2 mmol/L Na-vanadate, 10 mmol/L NaF, 10 mmol/L $Na_4P_2O_7$, 1 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 20 mg/mL leupeptin] supplemented with 10 mmol/L *N*-ethylmaleimide and 50 μmol/L LL_nL at the indicated time points. Lysates were clarified by centrifugation at 4°C for 10 minutes and incubated with anti-Flag antibody and anti-C1D antibody prebound to protein G-Sepharose beads for 6 hours at 4°C. The immunoprecipitates were washed with lysis buffer, run on 8% to 15% gradient SDS-PAGE and 4% to 12% NuPAGE, and transferred onto Nytran membranes. The membranes were immunoblotted with anti-ubiquitin, anti-Flag, or anti-C1D antibody. MG132-treatment was carried out 2 hours before UV irradiation and cultures were continued in the presence of MG132.

Immunoprecipitation

UV-irradiated HEK293 cells were lysed in lysis buffer [50 mmol/L HEPES (pH 7.4), 150 mmol/L NaCl, 0.1% Triton, 1 mmol/L EDTA, supplemented with protease inhibitor cocktail (Sigma)]. Lysates were then clarified by centrifugation at 4°C for 10 minutes and incubated overnight with anti-XPB antibody prebound to protein A-Sepharose beads. After sequential washes, the immunoprecipitates were separated on 4% to 12% NuPAGE gel, transferred onto a Nytran membrane, and then blotted with anti-XPB, anti-p62, and anti-C1D antibodies.

Results

C1D expression requires functional XPB

27-1 cell line is a subclone of the cell line CHO-9 carrying *A1075G* transition (K359E) located in the helicase motif in the *XPB* gene. The mutation leads to severe defects in NER, and therefore, 27-1 cells are extremely sensitive to UV irradiation compared to its parental CHO-9 cell line (17). However, such defect can be rescued by complementing loss of *XPB* gene with the human *XPB* gene, which shares 96% identity with hamster *XPB* gene

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(10, 11). These fundamental findings established a unique model system for identifying and analyzing genes that are regulated by XPB and their potential roles in facilitating UV-triggered DNA damage repair. We first performed differential display (DD) analysis on CHO-9, 27-1, and 27-1 complemented with human *XPB* gene (27-1/XPB). More than 1,000 amplicons showing significant difference of intensity from CHO-9, 27-1, and 27-1/XPB cells were sequenced and nonspecific background fragments were excluded. Among the potential targets, mRNA levels of nuclear DNA-binding protein C1D (NM_020558, Fig. 1A) and ribosomal proteins S3, S8, and P0 (X51536, NM_031706, and NM_022402 respectively; data not shown) were significantly elevated on stable expression of wild-type XPB. The increases in expression of these four genes were further confirmed by Northern blot analysis using gene-specific probes. Consistent with the differential display results, mRNA levels of C1D, S3, S8, and P0 are all ~2-fold lower in 27-1 cells, as compared with those in CHO-9 and 27-1/XPB under steady-state growth conditions (Fig. 1B). It seemed that under normal growth conditions, complementing XPB only modestly affected the basal level of transcription of these genes. However, XPB may play a more prominent role in activation of these

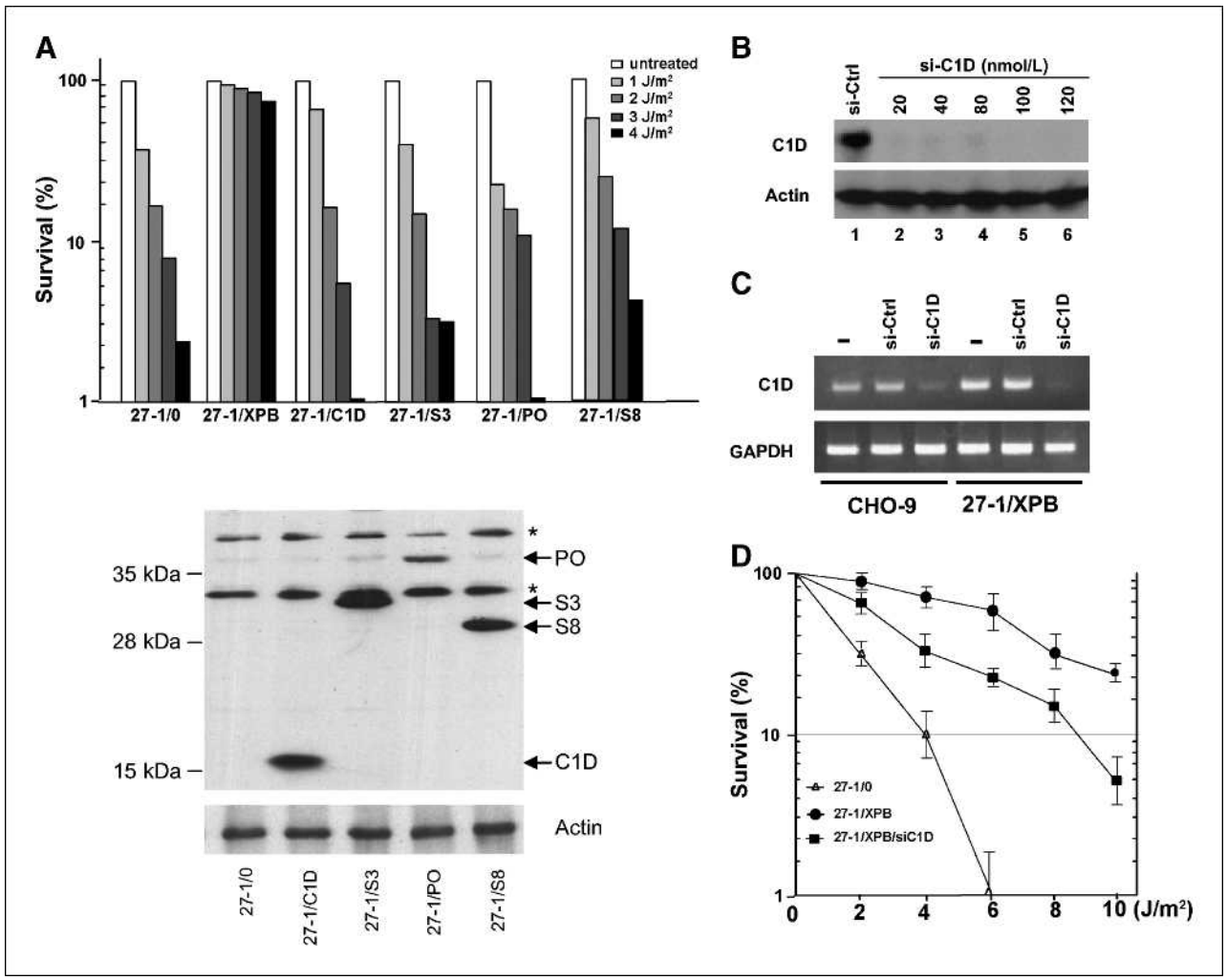

FIGURE 3. C1D is necessary but not sufficient to rescue 27-1 cells from UV-induced apoptosis. A, comparison of survival of 27-1 stably transfected with empty vector (27-1/0), XPB, C1D, S3, S8, and PO after different doses of UV irradiation. Expression of C1D, S3, S8, and PO were confirmed by Western blotting on the bottom. *, nonspecific bands. For cell survival assay, cells were UV irradiated at the indicated doses and cultured for 7 d. Cell colonies are counted and normalized to untreated samples. B, siRNA to C1D blocks C1D expression. 27-1 cells stably expressing Flag-tagged C1D were transfected with the indicated concentrations of si-C1D (lanes 2-6) or 120 nmol/L of single-strand sense RNA (lane 1). Cells were harvested 48 h after transfection and subjected to Western blot. C1D was detected by anti-Flag antibody. Actin was shown as a loading control. C, mRNA level of C1D was also monitored by RT-PCR in CHO-9 and stable XPB-transfected 27-1 cells after si-C1D transfection. si-C1D was transfected at 40 nmol/L. GAPDH was also amplified as input control. D, 27-1/0 cells were transfected with control siRNA (si-Ctrl) and 27-1/XPB cells were transfected with control siRNA or si-C1D and incubated for 48 h. After UV irradiation at the indicated doses, the cells were cultured and survival assays were doneas previously described. Points, mean of three independent experiments; bars, SD.

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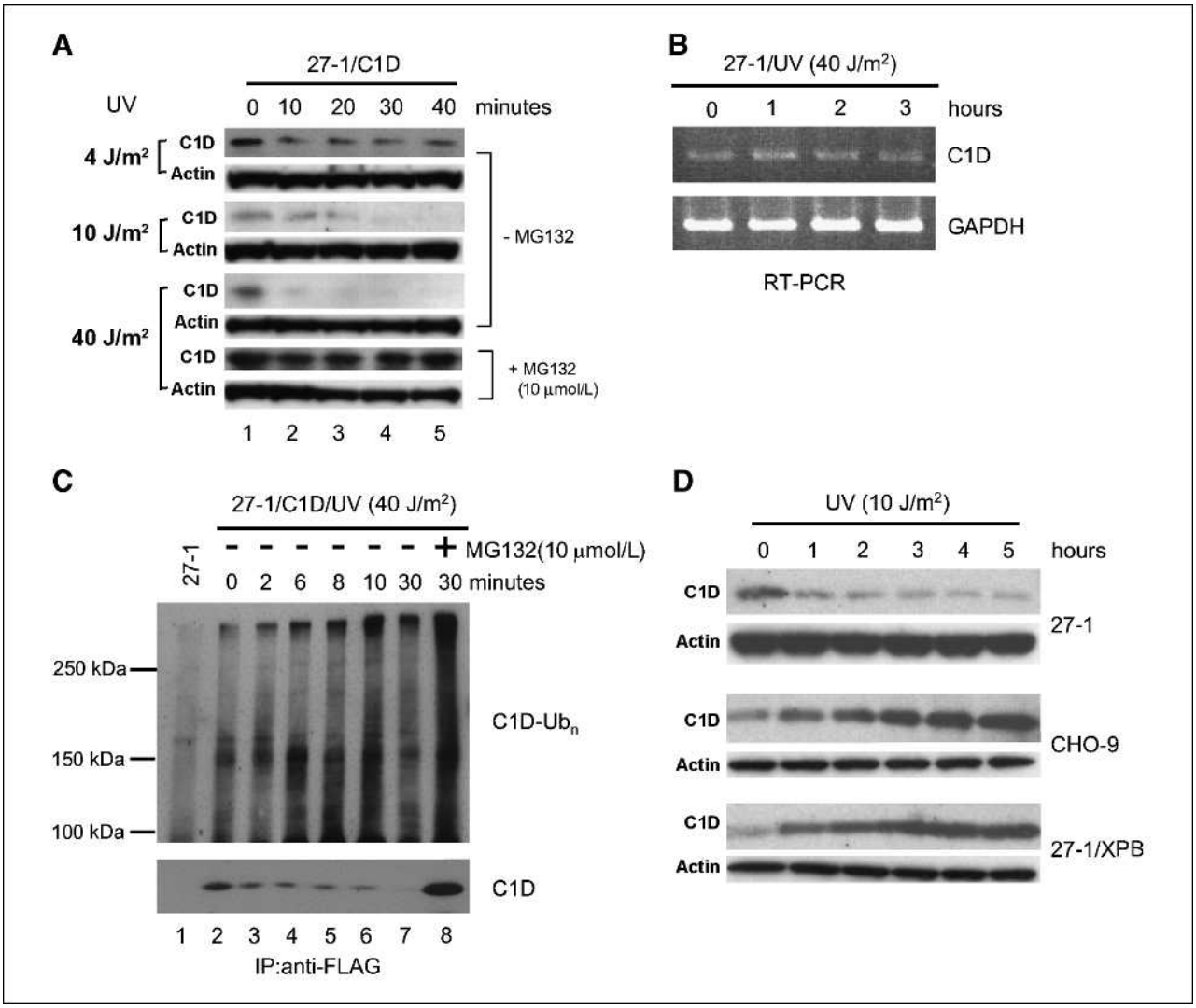

FIGURE 4. XPB is essential to maintain proper cellular C1D protein level in hamster cells after UV irradiation in CHO cells. A, 27-1 cells stably expressing Flag-tagged C1D were irradiated at 4, 10, or 40 J/m^2 in the absence or presence of 10 μmol/L of the proteasome inhibitor MG132. Cells were collected at the indicated time points after irradiation and prepared for anti-Flag Western blotting. B, 27-1 cells were exposed to 40 J/m^2 UV irradiation, which caused a significant decrease in C1D protein level as shown in A. Total RNAs were extracted at the indicated time points for RT-PCR of C1D and GAPDH. C, stable C1D-expressing 27-1 cells were irradiated with 40 J/m^2 UV and collected at the indicated time points. In the case of the sample in lane 8, cells were preincubated in cultural medium containing 10 μmol/L MG132 for 2 h. After irradiation, cells were refed with medium supplemented with 10 μmol/L MG132 and cultured for another 30 min. The cell lysates were immunoprecipitated with anti-Flag antibody and blotted with anti-Flag or anti-ubiquitin (lanes 2-8). Lysates from 27-1 cells not expressing Flag-C1D were also immunoprecipitated with anti-Flag as control (lane 1). C1D-Ubn, polyubiquitinated C1D. D, CHO-9, 27-1, and 27-1/XPB cells were irradiated with 10 J/m^2 UV. Cells were collected at the indicated time points and subjected to anti-C1D immunoblotting.

genes when the cells are subjected to stress or stimulus conditions because it has been shown to be crucial in activated transcription (15, 18).

Among the four XPB target genes, ribosomal proteins S3 and P0 have been shown to contain DNase activity and may therefore participate in other activities beyond their canonical role in protein translation (19–22). C1D was initially identified as a nuclear matrix–associated protein (12) and has been reported to interact with the leucine zipper region of DNA-PKs. C1D can be phosphorylated by DNA-PK and can also activate DNA-PK in the presence of supercoiled plasmid DNA (13). Its expression is induced by γ-irradiation (13). In addition, C1D overexpression can induce p53-dependent apoptosis (23). Thus, C1D is of particular interest considering these established links to the molecular machinery participating in DNA repair. Full-length hamster C1D mRNA sequence was generated by 5′-rapid amplification of cDNA ends; the coding sequence and corresponding amino acid sequence of hamster C1D are shown in Fig. 1C. The cDNA sequence was

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deposited in GenBank under accession no. AY302220. The amino acid sequence of C1D is highly conserved through evolution. Human, mouse, and hamster C1D proteins share ~89% identity (Fig. 1C), indicating that C1D protein is likely to be involved in similar cellular activities across these species. Thus, we decided to further investigate the potential role of C1D in repair of UV-induced DNA damage.

XPB induces C1D transcription

XPB is a subunit of the general transcriptional factor TFIIH. The DNA helicase activity of XPB is essential for activating transcription and NER (6). Our data that C1D mRNA level is higher in 27-1/XPB cells than in 27-1 cells by both differential display and Northern blot analyses indicated that restoring XPB may contribute to enhanced *C1D* gene expression at transcription level. To confirm this, we transiently transfected 27-1 cells with vector expressing human wild-type XPB or a XPB deletion mutant unable to stimulate transcription (10) and monitored C1D mRNA level by reverse transcription-PCR (RT-PCR) and real-time qPCR. The deletion mutant lacks the nuclear localization signal (NLS) and the DNA-binding domain at the NH_2 terminus (2-202 AA deletion, designated as ΔNLS) and is exclusively localized in the cytoplasm (10). C1D mRNA level gradually increased over a time course of 36 hours after transfection. The increase in C1D expression correlated closely with the level of XPB expression (Fig. 2A), suggesting that restoring XPB/TFIIH activity upregulated C1D transcription without UV irradiation. The mutant form (ΔNLS), on the contrary, was unable to promote C1D expression (Fig. 2B). The data further confirm that the nuclear localization and the DNA-binding functions of XPB, the two integral elements for TFIIH to function as a general transcription factor, are essential for maintaining normal level of C1D expression.

C1D is necessary but not sufficient to rescue 27-1 cells from UV-induced DNA damages

Our data from differential display assays indicated that expression levels of C1D and of the other three genes may be correlated to the ability of the cells to recover from damages caused by UV irradiation. It is important to test whether these genes are sufficient to rescue 27-1 cells from damages caused by UV irradiation independently. To address this question, we introduced each individual gene into 27-1 cells and established stable transfectants. A stable line from transfection using the same expression vector without any coding sequence insert was also established as a control cell line (27-1/0). The expression levels of transfected genes were confirmed by Western blotting (Fig. 3A, bottom). The survival of these cell lines after UV irradiation was then evaluated. Unlike complementing

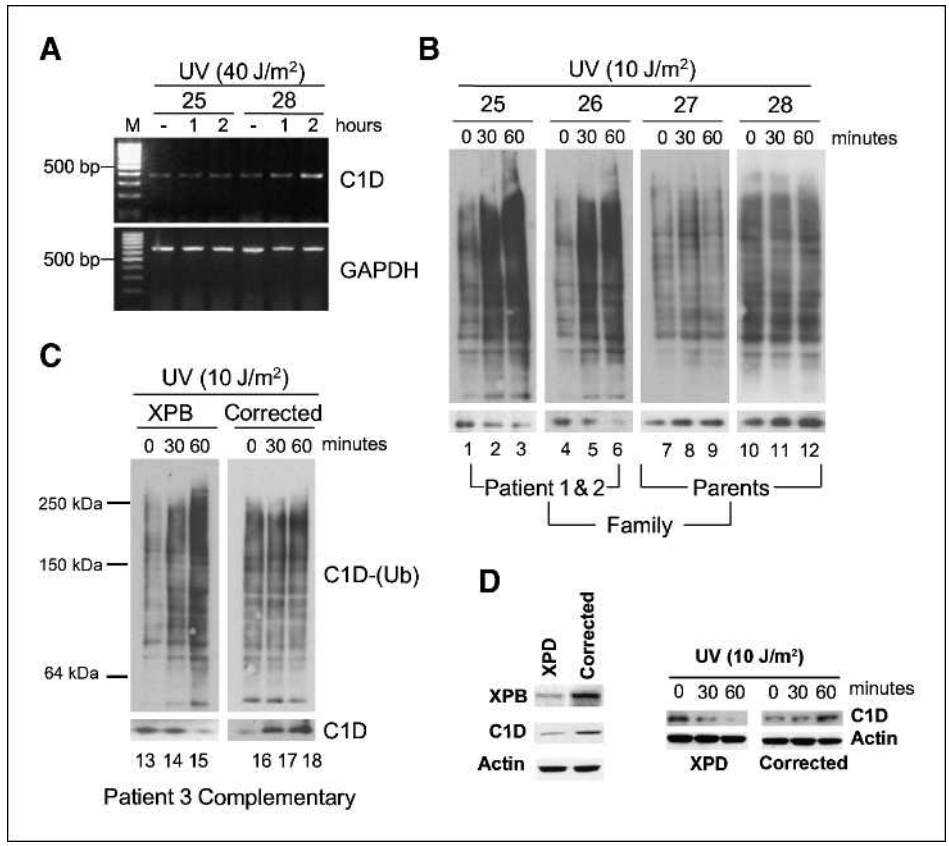

FIGURE 5. XPB is essential to maintain proper cellular C1D protein level in human cells after UV irradiation. A, human primary fibroblasts from XPB patient (GM13025) and father (GM13028) were exposed to 40 J/m^2 UV. Cells were harvested 1 and 2 h after exposure. Total RNA was isolated for RT-PCR of C1D and GAPDH. B, human primary fibroblasts from XPB patients (GM13025 and GM13026) and their parents (GM13027 and GM13028) were exposed to 10 J/m^2 UV and collected 30 min and 1 h after UV irradiation. Cell lysates were immunoprecipitated with anti-C1D and blotted with anti-ubiquitin or anti-C1D. C, GM13026 and GM13026 complemented with XPB were subjected to assay as described in B. D, C1D levels in XPD (GM02807, R683W/DEL36_61) and its XPD complemented derivative line with or without UV irradiation were analyzed by Western blot analysis.

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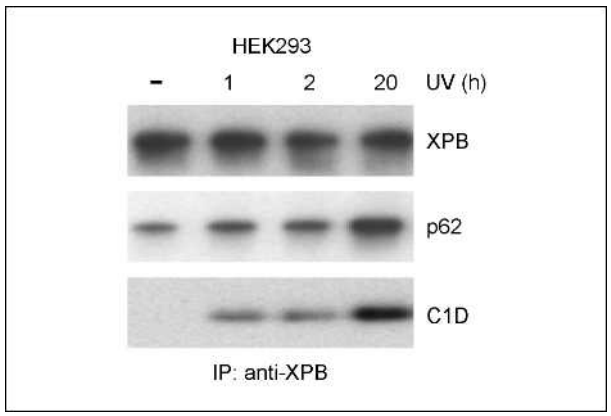

FIGURE 6. XPB forms a complex with C1D after UV irradiation. HEK293 cells irradiated with 40 J/m^2 UV were harvested at the designated time points. Anti-XPB immunopricipitates of these cell lysates were separated on 4% to 12% NuPAGE gel and then subjected to anti-XPB, anti-p62, and anti-C1D immunoblotting.

the *XPB* gene, overexpression of these genes individually failed to rescue the UV-sensitive phenotype of 27-1 cells, suggesting that none of these genes is sufficient to rescue 27-1 cells from defective DNA damage repair following UV irradiation. However, at low UV dose (1 J/m^2), overexpression of C1D seems to be able to provide some protection to the cells (Fig. 3A). However, due to the lack of large sample number, we currently cannot confirm whether the difference is statistically significant.

On the other hand, because the expression and function of C1D are closely related to γ irradiation (23), we hypothesized that elevated C1D levels may facilitate XPB to fulfill its DNA repair activity on UV irradiation. To further verify the involvement of C1D in DNA repair in CHO-9 and 27-1/XPB cells, we transiently silenced C1D expression in these cells by transfecting siRNA. C1D protein and mRNA levels were monitored by Western blot (Fig. 3B) and RT-PCR (Fig. 3C). C1D siRNA (si-C1D) treatment effectively blocked expression of endogenous as well as exogenous C1D. The effects of C1D knockdown on DNA repair of CHO-9 and 27-1/XPB cells were then investigated. After si-C1D treatment, these cells were subjected to UV irradiation and cell survival rates were measured 6 days after irradiation. Consistent with our previous finding, restoration of XPB function rescued 27-1 UV sensitivity. More importantly, under the conditions that C1D expression is blocked, even 27-1/XPB cells, whose TFIIH activity was restored, were up to 50% less resistant to UV irradiation when compared with cells treated with control siRNA (Fig. 3D). A similar phenomenon was observed with CHO cells treated with si-C1D or control siRNA (data not shown). These data suggest that neither C1D nor XPB alone is sufficient to fully support cell resistance to UV-induced DNA damage. Some cooperative efforts from the two factors may be necessary to fully restore effective DNA repair.

XPB is essential to maintain proper cellular C1D protein level after UV irradiation

Besides its critical role in NER, the XPB ATP-dependent DNA helicase activity also plays a major role in promoter opening in transcription (24–27). Because XPB-induced C1D expression is necessary to fully rescue 27-1 cells from UV-induced DNA damages, we suspected that the UV-sensitive phenotype of the 27-1 cell line may be a combined result of loss of XPB repair activity and insufficient level of C1D in these cells. To monitor change in C1D protein level after UV irradiation, 27-1/C1D cells were first subjected to UV irradiation at different doses and then harvested for Western blot analysis. C1D protein diminished quickly. A sharp decline of C1D protein was observed 10 minutes after irradiation even at low-dose UV irradiation (4 J/m^2; Fig. 4A). The rate of C1D protein decline was accelerated at 10 and 40 J/m^2; C1D protein became almost undetectable after 10 minutes of treatment (Fig. 4A). The rapid decline of C1D protein on irradiation was not likely due to shutdown of C1D transcription because no change of C1D mRNA was observed in 27-1 cells even after high-dose UV irradiation (Fig. 4B). Rather, the rapid disappearance of C1D protein may due to rapid degradation through ubiquitin-mediated proteolysis. To test this hypothesis, 27-1/C1D cells were UV irradiated in the absence (Fig. 4C, lanes 2-7) or presence of the proteasome inhibitor MG132 (lane 8). Cells were harvested at the indicated time points after irradiation and the cell lysates were subjected to immunoprecipitation with anti-Flag antibody. Immunoblot of precipitated protein indicated that without MG132, within minutes of irradiation, C1D became polyubiquitinated and monomeric C1D level gradually receded and became undetectable 30 minutes after irradiation. The polyubiquitinated form of C1D gradually accumulated over the same period of time. Pretreatment with MG132 preserved both ubiquitinated and monomeric C1D (Fig. 4C, lane 8), indicating that proteasome-mediated proteolysis plays a major role in UV-induced C1D degradation. To test whether maintenance of C1D protein level after UV irradiation requires functional XPB, CHO-9, 27-1/XPB, and 27-1 cells were irradiated at 10 J/m^2 and harvested at different time points for Western blot analysis. Similar to the previous results, C1D level dropped quickly in 27-1 cells after initial irradiation (Fig. 4D). These data indicate that a fully functional XPB/TFIIH may be necessary to stimulate transcription of *C1D* gene to compensate for the rapid degradation of C1D protein induced by UV irradiation. Indeed, in cells carrying functional XPB/TFIIH (27-1/XPB and CHO-9), C1D levels increased on irradiation (Fig. 4D). Together, these data indicate that maintenance of the level of C1D protein is necessary for cells to counter damages inflicted by UV irradiation. Besides direct involvement in DNA repair activities after initial irradiation, XPB/TFIIH may also directly activate the transcription of genes that facilitate the repair process.

To confirm that functional XPB/TFIIH is required for maintaining C1D protein level on irradiation in human,

Published OnlineFirst June 8, 2010; DOI: 10.1158/1541-7786.MCR-09-0467

we compared C1D mRNA and protein levels in primary human fibroblast cells carrying wild-type or mutant *XPB* gene from patients and their parents of a XPB family. Cell lines GM13027 and GM13028 are derived from clinically unaffected parents and are heterozygous for a c.296T>C, F99S mutation of *XPB* gene (father) and a c.471+1G>A intron 3 donor site splicing mutation of *XPB* gene (mother). Cell lines GM13025 and GM13026 are derived from the two sons of the parents who carry a F99S mutation on one allele and a c.471+1G>A intron 3 donor site splicing mutation on the other allele. Two brothers suffer milder XP/CS complex symptoms (3). GM13025 and GM13026 have been shown to be defective in DNA repair activities and activated transcription in response to stimulations (3, 18, 28). The cells were first irradiated with UV, recovered, and harvested for RNA and total protein preparation. Consistent with our previous findings, C1D RNA level remained unchanged in GM13025 XPB patient cells but gradually increased in GM13028 cells 2 hours after UV irradiation (Fig. 5A). The data indicate that the XPB-dependent induction of C1D transcription in response to UV irradiation may be a common phenomenon across different species. Consistently, for GM13025 and 13026 cells, UV irradiation caused a rapid decline of unubiquitinated C1D protein. The majority of C1D became polyubiquitinated (Fig. 5B, lanes 1-6). In GM13027 and GM13028 cells from the clinically unaffected parents, unubiquitinated C1D gradually increased whereas the polyubiquitinated C1D remained relatively unchanged (Fig. 5B, lanes 7-12). These results suggest that XPB induces C1D transcription on UV irradiation to compensate for the rapid UV-induced degradation of C1D. Hence, XPB plays a critical role in maintaining proper level of C1D protein after UV exposure to cope with urgent need for NER.

To confirm that XPB is the major factor contributing to UV-induced upregulation of *C1D* gene transcription, C1D levels before and after UV irradiation in GM13026 and GM13026 complemented with wild-type XPB were compared. Similar to previously reported that complementing XPB restores *c-myc* induction (18, 29) in these cells, induction of C1D on UV irradiation was observed (Fig. 5C).

If fully functional TFIIH is necessary and sufficient to maintain proper C1D levels in steady state as well as on UV irradiation, mutations in other TFIIH subunits are also expected to influence C1D expression because several components of TFIIH also seem to be coordinately regulated in XPB or XPD patients (30). To test this, we compared C1D protein levels in XPD (XP6BE, R683W/DEL 26_61) and its derivative XPD complemented line before and after UV irradiation. This XPD cell line has been shown to be sensitive to UV irradiation and defective in removal of photoproducts (16). Similar to the results from XPB cells, C1D protein level was lower in mutant cells under normal growth conditions and diminished rapidly on UV irradiation. In the complemented cells, C1D level is higher at steady state, but C1D is clearly induced within 2 hours after UV irradiation (Fig. 5D).

All these data indicate that fully functional TFIIH is necessary and sufficient to maintain proper levels of C1D protein after UV exposure.

XPB forms a complex with C1D after UV irradiation

Our data indicated that XBP/TFIIH and C1D may collaborate to support full UV resistance. If this is true, then some physical association of the two factors would be expected. Indeed, whereas coimmunoprecipitation using anti-XPB antibody did not detect any C1D protein associated with TFIIH complex under normal growth conditions, 1 hour after UV irradiation, C1D copurified with XPB/TFIIH complex. This interaction became stronger and sustained over a 20-hour time course after UV exposure (Fig. 6), possibly due to continuous accumulation of C1D protein during the recovery period in the cell. This suggested that C1D could potentially serve as a loading dock to recruit TFIIH to the site of DNA damage to enhance repair efficiency.

Discussion

The dual role of XBP/TFIIH in transcription regulation and NER implicated that the two processes may be closely implicated. Besides facilitating basal-level transcription of genes, TFIIH has also been shown to mediate activator-dependent transcriptional activation in response to stimuli (18, 31) or physiologic stresses (32). It is conceivable that in response to a particular stress, TFIIH may mediate activation of transcription of a certain gene or a certain group of genes through direct interaction with stress-specific transcription activators. The increase in cellular levels of these gene products would ultimately accommodate the immediate needs that enable cells to cope with the stress condition. In the case of UV irradiation, XPB/TFIIH could serve as a bridge to convey input from transcription activator(s) activated by UV irradiation, such as p53 (33, 34), onto the C1D promoter, although which activator(s) is responsible for activating C1D transcription remains unknown at this time. As nuclear C1D level increases, we hypothesize that C1D protein may enrich at the foci of damaged DNA, and our data indicate that C1D, in turn, recruits XPB/TFIIH to these sites for it to execute its DNA repair activity. Such a scenario presents a typical feed-forward mechanism to accomplish efficient DNA repair so that genomic stability is maintained.

C1D was originally identified as gene encoding a nuclear matrix protein (12) and is ubiquitously expressed in more than 50 human tissues (23). Recent publications have suggested that C1D protein could be involved in the maintenance of genomic integrity by regulating the activity of dsDNA break repair proteins, such as DNA-PK, TRAX/Translin complex, and condensin (13, 14, 35). However, possible involvement of C1D in other DNA repair pathways, such as NER, has not yet been addressed. Our data clearly indicate that although C1D may not play a major role as XPB/TFIIH in NER, its contribution is indispensable for cells to achieve full repair efficiency. Knocking

Published OnlineFirst June 8, 2010; DOI: 10.1158/1541-7786.MCR-09-0467

down C1D expression in a XPB/TFIIH wild-type background undermined the ability of XPB/TFIIH to aid the recovery of cells following UV irradiation. C1D can promote XBP/TFIIH repair by either further activating its 3′-5′ helicase activity or recruiting XPB/TFIIH to the target sites, or both. Whereas our current data cannot distinguish between the two mechanisms, the physical interaction between C1D and XPB/TFIIH induced by UV irradiation suggests that C1D is an integral part of NER.

Interestingly, based on our data, C1D is consistently degraded by ubiquitin-mediated proteolysis both under normal growth conditions and after UV irradiation. To compensate for such loss of C1D and to respond to the urgent need for repairing DNA, cellular C1D protein level needs to be maintained if not further augmented, either by activating its transcription or by blocking proteolysis of C1D protein. Consistent with previous data that C1D protein level increases after UV irradiation, our data indicated that activated transcription is the major step of regulation to compensate for the loss C1D protein and to keep appropriate C1D levels under UV irradiation. Such activation is XPB/TFIIH dependent because XPB mutation or XPB DNA binding region deletion renders *C1D* gene unresponsive to UV induction.

A similar phenomenon was also observed when we compared human primary fibroblast cells from two XPB patients and from their clinically unaffected parents. In cells from two patients, C1D expression could not be induced by UV irradiation; as a result, C1D protein levels dwindled after UV irradiation. It is noteworthy that a higher degree of polyubiquitination of C1D in the patient cells was observed when compared with the cells from the parent. Considering that XPB physically interacts with the 26S proteasome (36), XBP may also somehow modulate C1D protein stability through a yet unknown mechanism. Overall, XPB patients suffer from two layers of defects in NER. First, lack of fully functional XPB/TFIIH undoubtedly compromised the ability of the cells to repair DNA lesions. Second, diminished transcription activation due to XBP/TFIIH mutation also resulted in depletion of C1D protein under UV irradiation. The patient cells were unable to direct the remnant repair activity to sites of DNA damage. The combined effects may be a key cause of the 1,000-fold higher susceptibility to malignancies of XP patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Dr. Yusuke Nakamura (Institute of Medical Science, University of Tokyo) for help in differential display analysis, Dr. Shiro Ikegawa (SNP Research Center, RIKEN, Japan) for advice on differential display analysis, and Dr. Hideo Iba (Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo, Japan) for kindly providing pBabe-IRES (puro^r) vector. Human primary fibroblasts from a XPB family are kind gifts from Dr. David Levens (National Cancer Institute, Bethesda, MD). Human XPD cell line and XPD-complemented cells are kind gift from Dr. Ken Kraemer (National Cancer Institute, Bethesda, MD).

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Received 10/21/2009; revised 04/20/2010; accepted 04/28/2010; published OnlineFirst 06/08/2010.

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Published OnlineFirst June 8, 2010; DOI: 10.1158/1541-7786.MCR-09-0467

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Mol Cancer Res 2010;8:885-895. Published OnlineFirst June 8, 2010.