DNA Repair 12 (2013) 284–292

Contents lists available at SciVerse ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Checkpoint protein Rad9 plays an important role in nucleotide excision repair

Tiepeng Li[a,b], Zhixin Wang[c], Yun Zhao[a], Wei He[a,d], Lili An[a], Shengquan Liu[c], Yuheng Liu[a], Hailin Wang[c], Haiying Hang[a,*]

[a] Key Laboratory of Protein and Peptide Pharmaceuticals, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China
[b] University of Chinese Academy of Sciences, Beijing 100039, China
[c] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[d] University of California Davis, One Shields Ave, Davis, CA 95616, United States

ARTICLE INFO

Article history:
Received 20 July 2012
Received in revised form 11 January 2013
Accepted 17 January 2013
Available online 21 February 2013

Keywords:
Rad9
DNA damage checkpoint
Nucleotide excision repair
Transcription regulation

ABSTRACT

Rad9, an evolutionarily conserved checkpoint gene with multiple functions for preserving genomic integrity, has been shown to play important roles in homologous recombination repair, base excision repair and mismatch repair. However, whether Rad9 has an impact on nucleotide excision repair remains unknown. Here we demonstrated that Rad9 was involved in nucleotide excision repair and loss of Rad9 led to defective removal of the UV-derived photoproduct 6-4PP (6,4 pyrimidine-pyrimidone) and the BPDE (*anti*-benzo(*a*)pyrene-trans-7,8-dihydrodiol-9,10-epoxide)–DNA adducts in mammalian cells. We also demonstrated that Rad9 could co-localize with XPC in response to local UV irradiation. However, our data showed that Rad9 was not required for the photoproducts recognition step of nucleotide excision repair. Further investigation revealed that reduction of Rad9 reduced the UV-induced transcription of the genes of the nucleotide excision repair factors *DDB2*, *XPC*, *DDB1* and *XPB* and DDB2 protein levels in human cells. Interestingly, knockdown of one subunit of DNA damage recognition complex, hHR23B impaired Rad9-loading onto UV-damaged chromatin. Based on these results, we suggest that Rad9 plays an important role in nucleotide excision repair through mechanisms including maintaining DDB2 protein level in human cells.

1. Introduction

Organisms must accurately transmit the genetic information from one cell to its daughters to survive. Aside from great accuracy in replication of DNA and precision in chromosome distribution, eukaryotes have evolved the DNA damage response (DDR) to protect DNA from various damages induced by environmental agents or endogenous factors generated during cellular activities. DNA damage checkpoint is an important aspect of DDR, and it not only arrests or slows down the cell cycle at specific phases in response to DNA damage, allowing adequate time for repair, but also coordinates with the activation of DNA repair pathways, the recruitment of DNA repair proteins to sites of DNA damage, activation of transcriptional programs and apoptosis. Rad9, an evolutionarily conserved gene with multiple functions for preserving genomic integrity, is not only required for the intra-S, and G_2 checkpoint [1–3], but also directly involved in homologous recombination repair (HR), base excision repair (BER) and mismatch repair (MMR) pathways [4–8]. Moreover, it was demonstrated that Rad9 transactivated p53 target genes, including *p21* [9,10], which directly controls the G_1 to S-phase transition. In addition, Rad9 has also been shown to induce apoptosis through its interaction with Bcl-2 and Bcl-xL [11].

Nucleotide excision repair (NER) [12,13] is a versatile DNA repair pathway that processes a wide range of helix-distorting DNA lesions such as chemically induced bulky adducts and UV-derived DNA photoproducts. Mammalian NER consists of two distinct subpathways: global genome NER (GG-NER) removes damage from the entire genome, whereas transcription-coupled NER (TC-NER) preferentially eliminates lesions on the transcribed strand of actively transcribed genes. The major difference between these two subpathways appears to be in the strategies they use to recognize damaged bases. In TC-NER, RNA polymerase II stalls at damage sites and triggers the repair reaction, while in GG-NER DNA is initially surveyed for lesions by UV-DDB and/or XPC-hHR23B complex. Upon the recognition of lesions by a specific sub-pathway, subsequent repair processes are thought to involve a common mechanism. After recruited by either arrested transcription apparatus or XPC complex [14–16], TFIIH unwinds the DNA duplex and

* Corresponding author at: Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China. Tel.: +86 10 6488 8473; fax: +86 10 6488 8473.
E-mail address: hh91@sun5.ibp.ac.cn (H. Hang).

1568-7864/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.dnarep.2013.01.006

then RPA, XPA and XPG are recruited to verify the DNA structure alterations and assemble the pre-incision complex. Next, the dual incision occurs after the join of ERCC1-XPF. Finally, DNA patch is filled by the concerted action of PRC, PCNA and DNA polymerase δ and ε whereas ligase I and ligase III/XRCC1 seal the nick.

As mentioned above, it is unknown if Rad9 has any impact on NER although Rad9 has been shown to be directly involved in BER, MMR and HR pathways [4–8]. Previous studies showed Rad9 played an important role in radioresistance and loss of Rad9 made cells highly sensitive to UV exposure in an evolutionary conserved manner [2,17,18]. In addition, we demonstrated that mice with conditional deletion of *mRad9* in keratinocytes are prone to skin tumor following painting with 7,12-dimethylbenzanthracene (DMBA) which induces DNA lesions processed by NER [19]. These two results hint that Rad9 might play a role in NER. Furthermore, Giannattasio et al. reported that there was a physical interaction between the budding yeast counterparts of human Rad9 and XPA (DDC1 and RAD14 respectively), and binding of Rad9 to UV-damaged DNA required the presence of functional XPA [20]. Thus, it is interesting to examine the relationship between Rad9 and NER. Results presented here identified a novel role of Rad9 in NER. Further investigations showed that the combined effects of Rad9's impact on the gene transactivation of NER factors, Rad9 stabilized DDB2 protein level in human cells and associated with XPC-hHR23B complex in response to UV irradiation, these events together may contribute to efficient NER in human cells.

2. Materials and methods

2.1. Cell culture and antibodies

Human HeLa, HEK293T and mouse ES cells were cultured according to previously published methods [2,7]. Sources of commercial antibodies are as follows: mouse monoclonal anti-BPDE-dG antibody (mAb 8E11) (Trevigen); Qdot 625-conjugated goat anti-Mouse IgG (Invitrogen); anti-flag (Sigma); anti-XPC (Sigma); anti-XPA (Santa Cruz); anti-XPB (p89) (Santa Cruz); anti-ERCC1 (Santa Cruz); anti-XPG (Sigma); anti-DDB2 (Abcam); anti-GAPDH (Kang Chen); anti-Rad9 (BD Biosciences); anti-Rad9 (for IF, Zen Bioscience); anti-HA (Abcam); anti-6-4PP (MBL).

2.2. siRNA transfection

Small interference RNA (siRNA) duplexes against human Rad9 [3] (1#: 5′-AAGUCUUUCCUGUCUGUCUUC-3′) were synthesized by Invitogen Inc. Transfection experiments were carried out using Lipofectamin RNAiMAX Reagent (Invitrogen) following the manufacture's instructions.

2.3. Quantification of 6-4PP by ELISA

Mouse ES cells, HEK293T cells and Rad9-depleted HEK293T cells were cultured to 60% confluence and irradiated with UV (15 J/m^2) and harvested at various time points to allow DNA repair. Genomic DNA was isolated with Wizard genomic DNA purification Kits (Promega), and DNA concentrations were determined using Nanodrop ND2000 (Gene). Details of ELISA have been described previously [17]. In brief, the denatured DNA was immobilized in the protamine sulfate-precoated microtiter, and incubated with 64M-2 and HRP-conjugated goat anti-Mouse IgG respectively. The absorbance of colored products derived from *O*-phenylenediamine dihydrochloride was measured at 490 nm.

2.4. BPDE treatment and CE-LIF immunoassay of BPDE–DNA adducts

Mouse ES cells were cultured to 60% confluence and treated with 100 nM BPDE (*anti*-benzo(*a*)pyrene-trans-7,8-dihydrodiol-9,10-epoxide, a reactive metabolite of the potent carcinogen benzo(*a*)pyrene). After 2 h, the cells were subsequently washed twice with PBS and continued to culture for indicated time prior to the harvest. The genomic DNA was extracted using Wizard genomic DNA purification Kits (Promega) and the concentration of DNA were estimated at UV 260 nm. Details of capillary electrophoresis-laser induced fluorescence immunoassay (CE-LIF) analysis were described previously[21]. Briefly, genomic DNA was denatured by heating at 95 °C for 5 min followed by chilling on ice. The denatured DNA was mixed with 1.5 nM primary antibody mAb 8E11, 5 nM secondary antibody QD-2Ab, and 100 μg/ml BSA in a Tris-acetate buffer (pH = 7.8). The mixture was incubated for 30 min at room temperature and then electrokinetically injected into the capillary by applying an injection voltage of −20 kV for 10 s.

2.5. Local UV irradiation and immunofluorescence

Co-localization of NER factors with 6-4PP was performed as previously described [22]. Briefly, HeLa cells growing on glass coverlips were transfected with siRNA against either Rad9 or control siRNA. Transfected cells were irradiated with UV through a 5-μm isopore polycarbonate filter (Millipore) after 48 h. 30 min later, the cells were fixed with 4% paraformaldehyde in PBS for 10 min at room temperature and permeabilized with 0.5% Triton X-100 in PBS for 5 min on ice. For DNA denaturation, the cells were incubated in 2 N HCl for 30 min at room temperature. Then the cells were blocked with 20% FBS in PBS at 37 °C for 30 min. Primary rabbit anti-XPC, anti-HA (DDB2) and mouse anti-6-4PP as well as fluorescent-conjugated secondary antibodies were all prepared in PBS containing 5% FBS and laid on coverslips for 30 min at 37 °C. Following each antibody incubation step, the cells were washed with PBS for 5 times. Fluorescence images were obtained using a Nikon Fluorescence Microscope TE2000-S (Nikon).

2.6. Quantitative real-time RT-PCR

Total RNA was isolated with RNeasy Mini Kit (Qiagen) following the manufacture's protocol. First strand cDNA was synthesized with 2 μg of total RNA using Superscript III reverse transcriptase (Invitrogen). Real-time PCR was performed using the StepOnePlus system (ABI) with SYBR Green I (Takara) to label amplified DNA. The thermal cycling program was 94 °C for 5 min followed by 50 cycles of denaturing at 94 °C for 15 s, annealing at 60 °C for 20 s and extension at 72 °C for 15 s. The final extension was at 72 °C for 3 min. The primer sequences used in this assay were listed in Supplementary table S1. The relative levels of NER factors were the average ratios of the qRT-PCR results relative to *GAPDH*.

2.7. Dual-luciferase reporter assay

XPCL [23] and REhDDB2L [24] were the two well-studied consensus p53 binding sites in the promoter regions of human XPC and DDB2 genes respectively. We fused XPCL and REhDDB2L into pGL-3-basic vector, generating pGL-3-XPCL and pGL-3-REhDDB2L respectively. The constructs were transfected into HEK293T cells seeded in a 12-well plate. Luciferase activity was measured using dual-luciferase assays (Promega). As the internal control, each sample was co-transfected with pRL-CMV, containing the Renilla luciferase gene under CMV promoter, and the relative luciferase

activity was calculated as the ratio of *Firefly* to *Renilla* to adjust the transfection efficiency.

3. Results

3.1. Loss of Rad9 causes defective nucleotide excision repair of UV-induced 6-4PP in mammalian cells

Loss of Rad9 made cells highly sensitive to UV treatment [2,7,18,25]. To investigate whether Rad9 plays a role in the repair of UV-derived photoproducts, we irradiated wild-type mouse ES cells (*mRad9*$^{+/+}$ cells) and *mRad9*-deleted mouse ES cells (*mRad9*$^{-/-}$ cells) with 15 J/m^2 UVC. The cells were harvested at 1, 2 and 4 h, and the genomic DNA was prepared to assess the repair of 6-4PP using ELISA with a widely used antibody (64M-2, MBL). As shown in Fig. 1A, 6-4PP were gradually removed in wild-type mouse ES cells during 4 h incubation time after UVC treatment, while it remained almost unrepaired in *mRad9*-deleted mouse ES cells. To determine whether this function of Rad9 is conserved in human cells, we knocked down the endogenous Rad9 in HEK293T cells using Rad9-specific siRNA and subjected the genomic DNA from these cells to ELISA. As seen in Fig. 1B, HEK293T cells transfected with control siRNA were able to repair about 18.8% of 6-4PP at 6 h after UV radiation while the cells in which Rad9 was knockdown using Rad9-specific siRNA (Fig. 1C) removed only 7.7% of 6-4PP at the same time point. Taken together, these data suggest that loss and reduction of Rad9 in mouse and in human cells respectively leads to defective NER of UV-induced 6-4PP.

3.2. Rad9 is important for the removal of BPDE–DNA adducts in mouse ES cells

Chemically induced bulky adducts are another type of NER substrates besides UV-induced DNA photoproducts. To determine whether Rad9 has any impact on the NER of bulky adducts, we exposed *mRad9*$^{+/+}$ and *mRad9*$^{-/-}$ mouse ES cells to 100 nM BPDE and an ultrasensitive CE-LIF assay [21] was performed to measure the concentration of BPDE–DNA adducts remaining in genomic DNA. We chose BPDE as the DNA-damaging substrate instead of the other substrates in this experiment because a local two-sided bulge is formed at guanine adducts of BPDE through disruption of base pairing interactions and this feature makes it an excellent NER substrate [26]. As shown in Fig. 2, wild-type mouse ES cells removed significantly more BPDE–DNA adducts than *mRad9*-deleted mouse ES through the whole repairing course of 24 h after BPDE exposure. The wild-type cells repaired 35% of BPDE–DNA adducts at 12 h while *mRad9*-deleted mouse ES cells only removed 5% of the adducts at the same time. This result suggests that Rad9 plays an important role in repairing BPDE-damaged DNA. Compared to those repaired by the wild-type cells, the majority of BPDE–DNA adducts was removed at various time points in the *mRad9*$^{-/-}$ cells ectopically expressing mRad9 at the wild-type levels (Fig. 2; the ectopically expressed mRad9 level was shown in [7]), indicating that defective NER of BPDE–DNA adducts in *mRad9*-deleted mouse ES cells was due to the loss of *mRad9*.

3.3. Rad9 co-localizes with XPC in response to local UV irradiation

According to a generally accepted working model, Rad9, in complex with Rad1 and Hus1, binds to chromatin early in response to the DNA damage [27] in a Rad17-dependent manner [28] and this complex serves as a recruiting platform for the downstream proteins involved in cell cycle arrest [29], DNA repair [8] and apoptosis [11]. To search for proteins associate with Rad9, we pulled down Flag-tagged Rad9 using anti-Flag antibody from the lysate

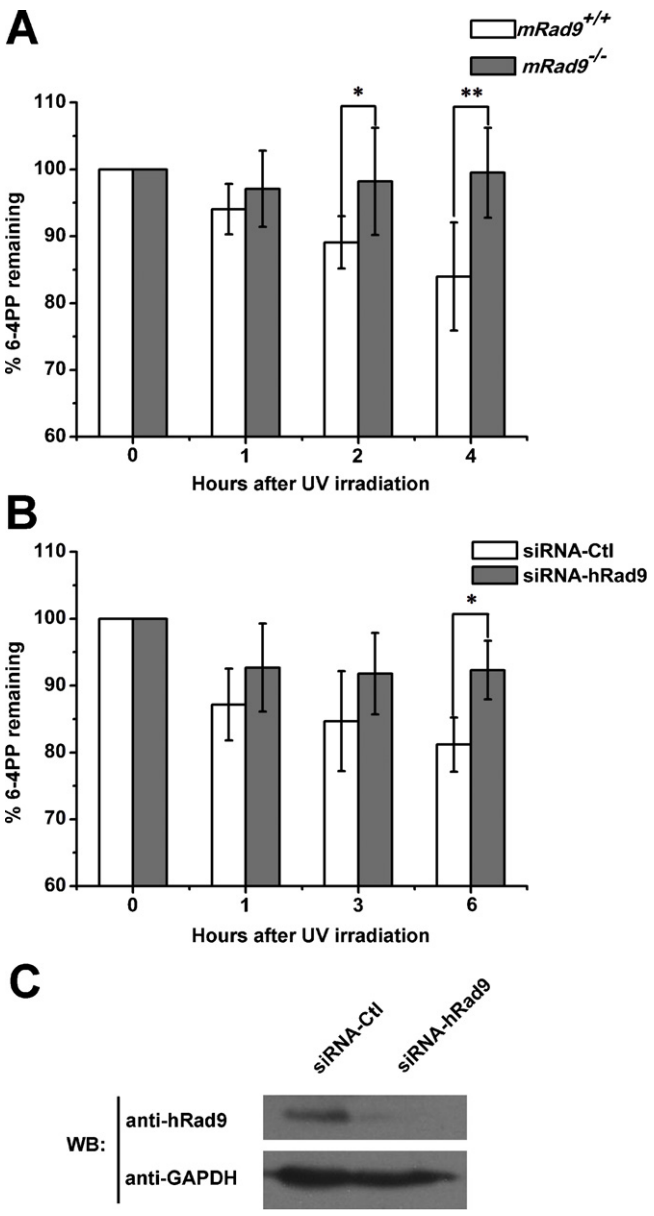

Fig. 1. Loss of Rad9 causes defective nucleotide excision repair of UV-induced 6-4PP in mammalian cells. (A) Mouse ES cells were irradiated with 15 J/m^2 of UVC. Genomic DNA was extracted at indicated time and subjected to ELISA. DNA damage was detected using antibody specific for 6-4PP (*t*-test; $n = 6$, $^*p < 0.05$, $^{**}p < 0.01$). (B) HEK293T cells were transfected with control siRNA and siRNA against Rad9 respectively and 48 h later exposed to 15 J/m^2 of UVC. DNA damage was determined as described in A (*t*-test; $n = 5$, $^*p < 0.05$). (C). Western analysis of HEK293T cells transfected with siRNA against endogenous Rad9.

extracted from HEK293T cells expressing Flag-Rad9 and identified Rad9-associated proteins with mass spectrometry [7]. One of those proteins was hHR23B. This interaction was confirmed by co-immunoprecipitation in HEK293T cells and GST-pulldown assay using GST-hHR23B and His-Rad9 purified from two lines of *Escherichia coli.* cells that overexpressed these two proteins respectively (Fig. S1). Previous studies showed that hHR23B, in complex with XPC and centric 2, plays an important role in the damage sensing step within GG-NER, triggering subsequent NER factors recruitment [14–16]. To investigate whether Rad9 was associated with this complex *in vivo*, we used local UV irradiation-coupled immunofluorescence assay. We observed that Rad9 rapidly accumulated at the sites of XPC-hHR23B complex after localized UV-irradiation (Fig. 3A). These results suggest that the interaction

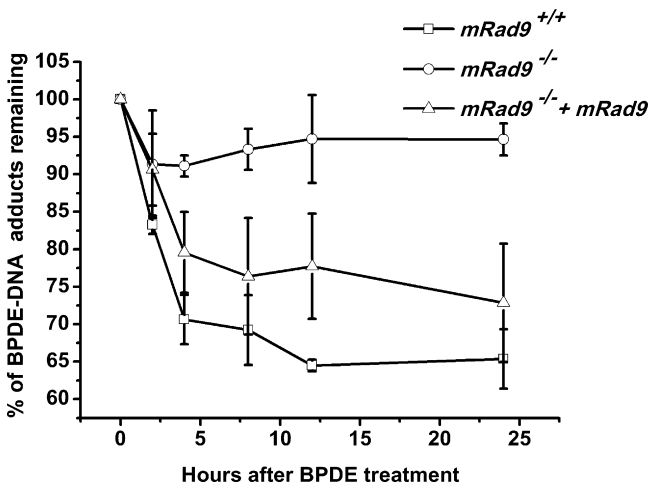

Fig. 2. Deletion of Rad9 causes defective nucleotide excision repair of BPDE–DNA adducts in mouse ES cells. Mouse ES cells were cultured to 60% confluence and exposed to 100 nM BPDE for 2 h. The cells were harvested to extract genomic DNA at indicated time points. The remaining of BPDE–DNA adducts in genomic DNA was determined by CE-LIF assay as described in Section 2. The results were the averages of three replicate experiments.

of Rad9 with XPC-hHR23B complex might participate in NER after UV treatment, possibly in the lesions recognition step.

3.4. Rad9 is dispensable for the recruitments of XPC and DDB2 to DNA damage sites

To address the question that Rad9 might be involved in DNA damage recognition step of NER through the interaction between Rad9 and XPC-hHR23B complex, we examined the impact of Rad9 knockdown on the recruitment of XPC complex to DNA damage sites. Immunofluorescent staining of HeLa cells transfected with Rad9-specific siRNA or negative control siRNA showed that the presence of Rad9 was dispensable for the efficient XPC recruitment to 6-4PP sites (Fig. 3C). Furthermore, we quantified immunofluorescence intensities of foci using two doses at two different times after UV irradiation (15 min and 45 min; 60 J/m^2 and 100 J/m^2). There is no statistically significant difference between Rad9-depleted and control cells at the co-localization levels of XPC to the DNA damage sites (Supplementary Fig. S4A). Therefore, knockdown of Rad9 has no influence on the recruitment of XPC to the damage sites and this interaction does not contribute to the impaired removal of 6-4PP observed in Rad9-knockdown cells.

Besides the XPC-hHR23B complex, UV-DDB complex also plays an important role in the recognition step of GG-NER in mammalian cells [12,13,30]. DDB2, the small subunit of UV-DDB complex, localizes to UV-induced photoproducts ahead of XPC and facilitates recruitment of XPC to DNA damage sites [31–33]. Furthermore, DDB complex has a much higher binding affinity and specificity for damaged DNA than XPC [34], particularly with regard to UV-induced 6-4PP [35]. To further investigate whether knockdown of Rad9 in HeLa cells has any influence on the recruitment of DDB2 to DNA damage sites, the co-localization of DDB2 with 6-4PP was also performed. As seen in Fig. 3D, the recruitment of DDB2 to photoproducts was intact in HeLa cells in which Rad9 was knockdown. Similar results were also derived from immunofluorescence study using two doses at two different times after localized UV-irradiation (15 min and 45 min; 60 J/m^2 and 100 J/m^2) (Supplementary Fig. S4B). Taken together, these results demonstrate that Rad9 is dispensable for the recruitments of XPC and DDB2 to the DNA damage sites, and knockdown of Rad9 has no impact on the damage recognition step of NER in HeLa cells.

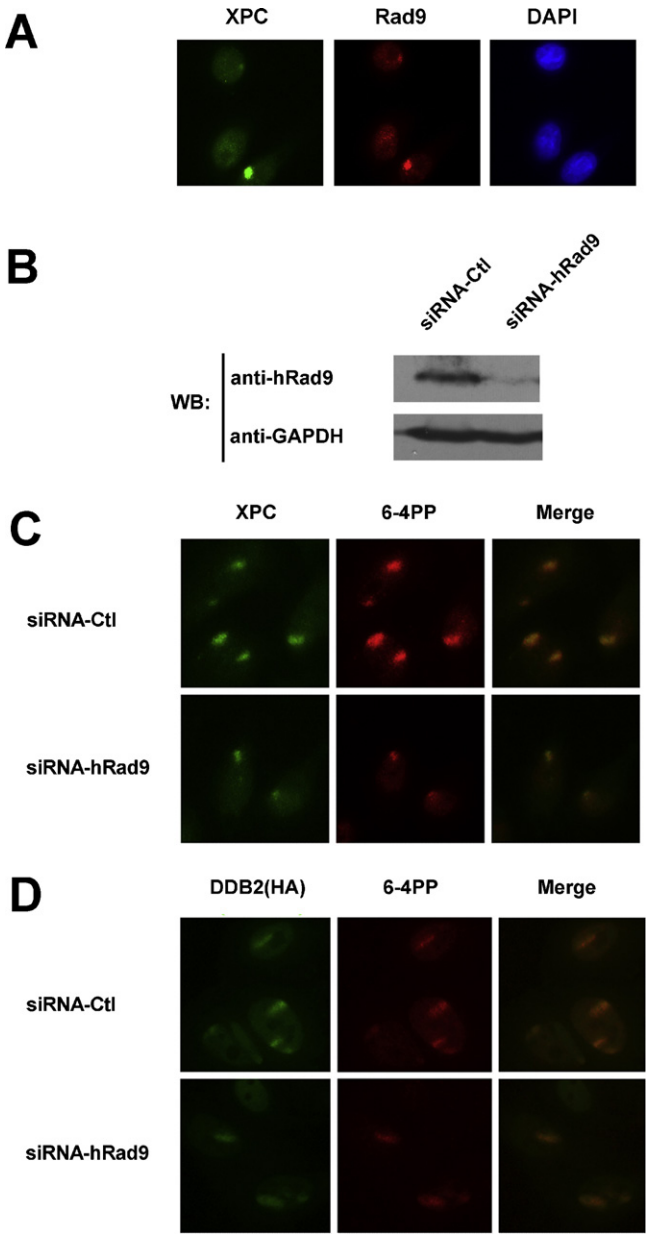

Fig. 3. Rad9 co-localizes with XPC in response to local UV irradiation but it is dispensable for the recruitments of XPC and DDB2 to DNA damage sites. (A) Rad9 co-localized with XPC after local UV irradiation. HeLa cells were locally irradiated with 100 J/m^2 UVC through a 5-μm isopore polycarbonate filter and 30 min later the cells were fluorescently stained for Rad9 (red) and XPC (green). (B). Western analysis of HeLa cells transfected with siRNA against endogenous Rad9. (C) HeLa cells were transfected with control siRNA (upper panels) or Rad9-targeting siRNA (lower panels). Transfected cells were locally irradiated with 100 J/m^2 UVC through a 5-μm isopore polycarbonate filter and 30 min later the cells were incubated in 2 N HCl for DNA denaturation followed by fluorescently staining for 6-4PP (red) and XPC (green). (D) HeLa cells expressing HA-DDB2 and transfected with control siRNA (upper panels) or Rad9-targeting siRNA (lower panels) were locally irradiated with UVC after 48 h. Cells were then double immunostained with anti-6-4PP (red) and anti-HA (green) antibodies.

3.5. Rad9 plays a role in the transcriptional regulation of DDB2 and XPC in response to UV exposure in human cells

Yin et al. [9] demonstrated that Rad9 bound to the p53 DNA-binding consensus sequences in the promoter region of *p21* and enhanced the expression of this gene. Another group further investigated this question and showed that Rad9 association to the

p53-binding sites in the *p21* promoter was increased after UV exposure [10]. Therefore we hypothesized that Rad9 transcriptionally regulated the expression of NER factors after exposed to UV radiation, similar to its regulatory role in the transactivation of *p21* and the reduced expression of these factors might contribute to the defective NER of 6-4PP in *Rad9*-dificient cells. To test this possibility, *XPC* and *DDB2* were chosen as candidates because expression of both genes are induced by UV radiation in a p53-dependent manner [23,24,36] and both genes have consensus p53 binding sites in their promoter region [23,24]. Employing qRT-PCR, the expression of the XPC and DDB2 gene products after UV exposure were examined in HEK293T cells transfected with siRNA toward Rad9 or control siRNA. As shown in Fig. 4A, the level of DDB2 mRNA expression substantially increased after UV radiation followed by a decline after reaching to its maximal level, and this expression pattern was similar to that of *p21* [10]. However, the UV-induced expression of *DDB2* was obviously subdued in the *Rad9*-deficient cells in response to UV radiation. The expression pattern of *XPC* mRNA was also investigated, and a result similar to that of *DDB2* was shown in Fig. 4B. To confirm the transcriptional alterations of NER factors are due to a lack of Rad9 but not an off target effect, we used two more different siRNA duplexes to knockdown the endogenous Rad9 in HEK293T cells. Both the duplexes efficiently reduced endogenous Rad9 protein in HEK293T cells (Fig. S5E). Using those two duplexes, we repeated the qRT-PCR assays on the mRNA levels of XPC and DDB2 (Fig. S5A and S5B) and obtained results similar to those derived from experiments using the first siRNA duplex (Fig. 4). Taken together, these results suggest that Rad9 plays a role in the transcriptional regulation of *XPC* and *DDB2* in response to UV exposure in HEK293T cells.

Next, Western blotting was performed to examine whether this transcriptional alterations of NER factors would have any impact on protein expression levels. As shown in Fig. 4C, UV irradiation evidently reduced DDB2 protein levels in both *Rad9*-knockdown and control cells. Afterwards, the DDB2 level was gradually restored from 3 to 24 h in control cells, and it was unable to be detected in *Rad9*-knockdown cells until 24 h after UV light treatment. Consistently, Rapic-Otrin et al. reported that DDB2 protein levels were significantly reduced soon after UV treatment, and the protein levels restored to normal levels by 24 h post-UV [37]. Surprisingly, *Rad9* knockdown significantly reduced DDB2 protein levels in HEK293T cells even before UV light treatment. This unexpected effect of Rad9 knockdown on BBD2 protein level suggests that Rad9 might maintain DDB2 protein in human cells leading to UV light resistance.

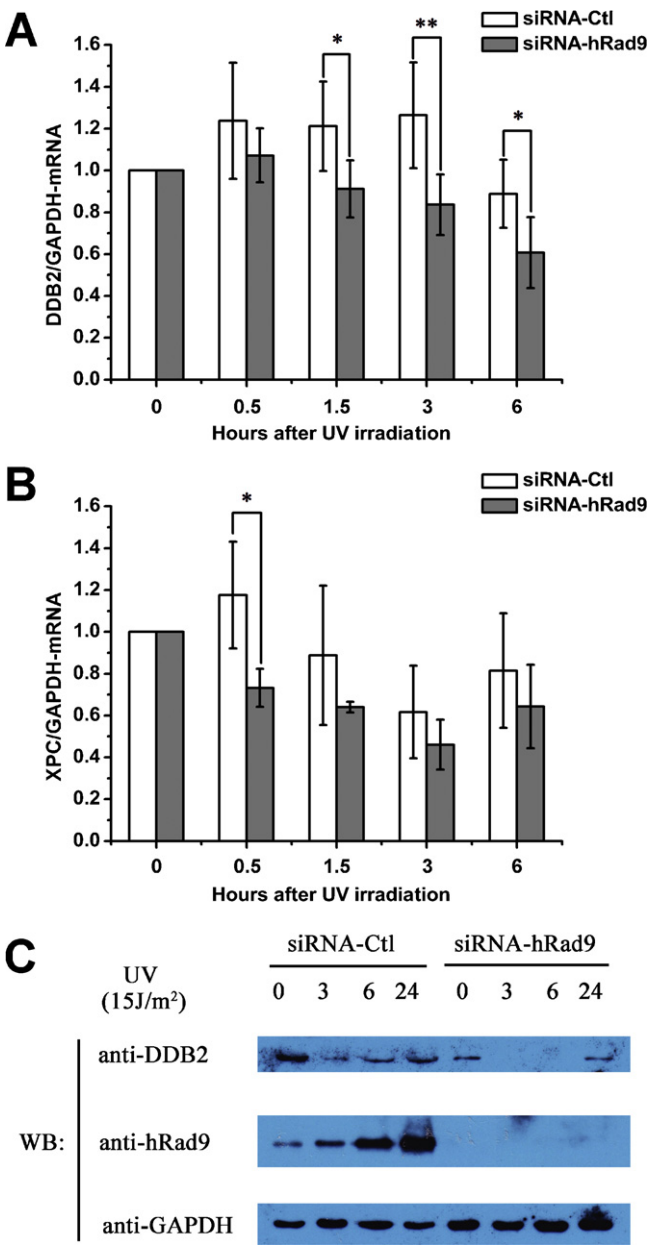

Fig. 4. Rad9 plays a role in the transcriptional regulation of *DDB2* and *XPC* in response to UV exposure in human cells. Endogenous Rad9 of HEK293T cells was depleted as described in Fig. 1B. Then the cells were irradiated with UVC (15 J/m^2) and allowed to repair for indicated times before harvested for RNA extraction and cDNA synthesis. Real-time PCR was performed to determine the relative levels of *DDB2* (A) and *XPC* (B) (*t*-test; $n = 5$, $^*p < 0.05$, $^{**}p < 0.01$). (C) Expression of DDB2 was evaluated in HEK293T cells transfected with control siRNA and siRNA against Rad9. The times indicated are incubation times after treatment with UVC (15 J/m^2).

3.6. Loss of Rad9 also alters expression pattern of additional NER factors in human cells

A previous study to identify p53-targeted genes on the whole-genome level showed that *DDB2* and *XPC* are two of those genes which belong to NER pathway [38]. To further investigate whether Rad9 transactivates other NER factors in addition of p53-regulated *DDB2* and *XPC* in response to UV treatment, qRT-PCR was performed. As seen in Fig. 5 and Fig. S5, UV-induced expression pattern of *XPB* and *DDB1* were altered in the *Rad9*-deficient HEK293T cells. Moreover, there were general trends that Rad9 knockdown reduced the transcription of the genes of the other NER factors *XPG*, *XPF*, *ERCC1*, *hHR23B* and *CETN2* although the differences were not statistically significant in these experimental settings (Fig. S3). Taken together, these results demonstrate that loss of *Rad9* also alters expression pattern of additional NER factors in human cells.

3.7. Rad9 induces DDB2 transcription through its p53 response element

In an effort to verify that regulation of NER factors by Rad9 is at the level of transcription, we tested whether Rad9 could activate the p53 response elements in the promoter region of these genes using Dual-luciferase reporter assay system. As shown in Fig. 6A, co-transfection of Rad9 and pGL3-REhDDB2L (pGL3-basic vectors containing p53 response element, REhDDB2L) significantly induced luciferase activity (Fig. 6A, column 2). p53, used as a positive control, was also shown to induce luciferase activity (Fig. 6A, column 5). Next, we investigated whether this induction depends on the phosphorylation states of the C-terminal of Rad9 which is critical

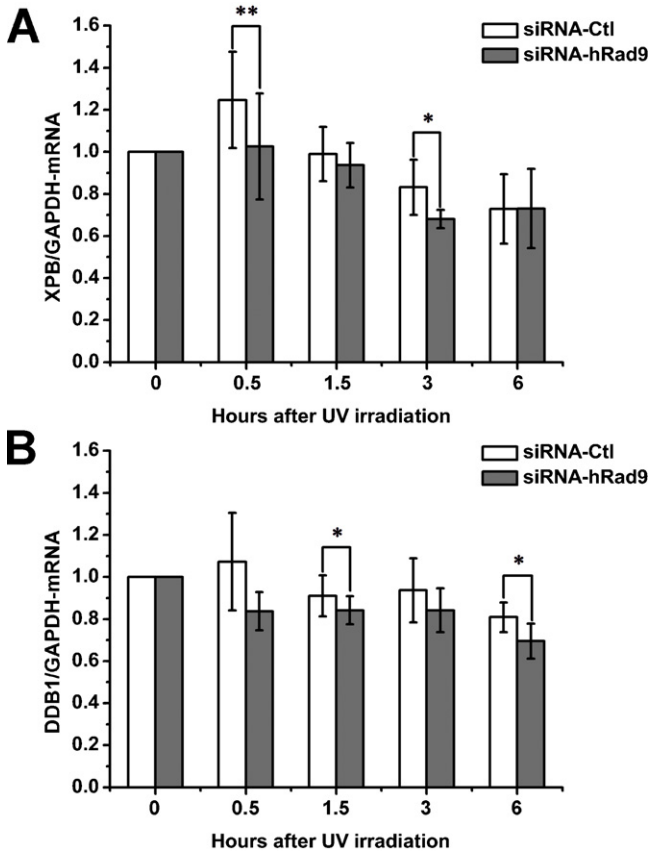

Fig. 5. UV-induced expression pattern of *XPB* and *DDB1* were altered in the *Rad9*-deficient HEK293T cells. The mRNA level of *XPB* and *DDB1* were determined by real-time PCR as described in Fig. 4 (*t*-test; $n = 5$, $^{*}p < 0.05$, $^{**}p < 0.01$).

for Chk1 activation and G_2 checkpoint maintenance [1,29,39]. The phosphorylation-defective Rad9 mutant Rad9-9A (all the 9 phosphorylated sites in C-terminus mutated) were expressed at the similar level of its wide-type counterpart (Fig. 6B), but the luciferase activity was not affected in cells expressing the Rad9-9A, suggesting that phosphorylation states of C-terminal of Rad9 had no significant impact on the Rad9 binding to the promoter region of *DDB2* (Fig. 6A, column 3). We also tested the potential role of arginine methylation of Rad9 in *DDB2* transactivation. In a previous study, we demonstrated that the methylation of the three arginines of a Rad9 arginine-rich sequence RGRR was important for Chk1 activation and G_2 cell cycle checkpoint maintenance [40]. Compared to the wild-type Rad9, the Rad9 mutant Rad9-3RK did not impair the luciferase activity, suggesting that the activation of REhDDB2L by Rad9 does not require the methylation of Rad9 RGRR (Fig. 6A, column 2 for the wild-type Rad9 and column 4 for the mutant Rad9). Dual-luciferase reporter assay was also performed to investigate whether Rad9 could activate p53 response element in the promoter region of *XPC*, pGL3-XPCL. However, Rad9 did not induce luciferase activity above the negative control, while p53 significantly induced luciferase activity (Fig. 6C). Taken together, these results suggest that Rad9 activates the reporter containing REhDDB2L but not the reporter containing XPCL, and this activation is not dependent on the post-translational modifications of Rad9.

4. Discussion

4.1. Rad9 plays an important role in NER in mammalian cells

NER is a versatile DNA repair pathway through which cells remove a variety of DNA lesions following DNA damage. The checkpoint protein Rad9 has been shown to be directly involved in multiple DNA repair pathways, including HR, BER and MMR [4–8]. In an effort to determine the potential connection between Rad9

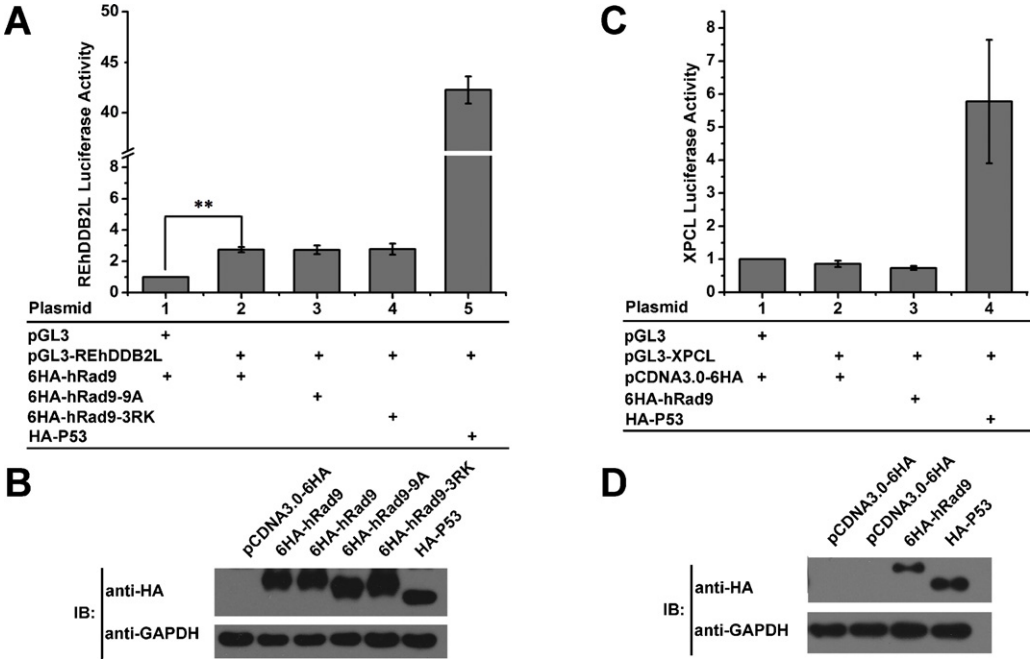

Fig. 6. Rad9 induces luciferase activity through the control of consensus p53 response element in the promoter region of *DDB2* but not *XPC*. (A) Transcriptional activation of the REhDDB2L-containing reporter construct by wild-type or modification-defective Rad9. p53 was used as a positive control. The luciferase activities were shown as the ratios of *Firefly* to *Renilla* (*t*-test; $n = 3$, $^{**}p < 0.01$). 9A and 3RK denote the mutant with all nine sites mutated (Ser272, Ser277, Ser328, Ser336, Ser341, Thr355, Ser375, Ser380 and Ser387) and the mutant with all three sites mutated (Arg172, Arg174 and Arg175) respectively. (B) Lysate of HEK293T cells transfected with HA-tagged wild-type or phosphorylation-defective Rad9 mutants and p53 plasmids in A were immunoblotted with anti-HA (top) and anti-GAPDH (bottom) antibodies respectively. (C) Transcriptional activation of the XPCL-containing reporter construct by Rad9 and P53 was performed as described in (A). (D) Western analysis of lysates of HEK293T cells used in (C).

and NER, we examined the repair kinetics of UV-induced 6-4PP in both mouse and human cells with or without Rad9 gene, and our data showed that knockout or knockdown of Rad9 resulted in low efficiency in NER of 6-4PP (Fig. 1). Thus, we reasoned unrepaired UV-derived damages due to loss of survival-promoting function of Rad9 could make cells susceptible to UV exposure which was consistent with previous observations that *mRad9*$^{-/-}$ cells showed enhanced UV sensitivity and ectopic expression of *mRad9* or *hRad9* in *mRad9*$^{-/-}$ cells at levels of found in *mRad9*$^{+/+}$ cells could restore wild-type radioresistance [2,7].

Chemical carcinogens, such as DMBA and BPDE, can cause the formation of carcinogen-DNA adducts which are another type of NER substrates besides UV-induced DNA photoproducts. Incomplete removal of these adducts can enhanced risk of mutagenesis and tumorigenesis. Previously, we demonstrated that mice with *mRad9* deleted in keratinocytes were prone to skin tumorigenesis following painting with DMBA which induces DNA lesions processed by NER. We also observed there were more DNA lesions induced by DMBA treatment in *mRad9*-deleted keratinocytes than in its wild-type counterpart [19]. One explanation is that deficiency in removal of DMBA-DNA adducts caused by *mRad9*-deletion contributes to the susceptibility of *mRad9*$^{-/-}$ mice to increased DNA damage in keratinocytes and skin tumorigenesis. In consistent with these observations, the result presented here showed that loss of *mRad9* led to defective repair of BPDE induced DNA lesions (Fig. 2) and this strongly supported our hypothesis mentioned above. Additionally, the present finding is also consistent with the result of another previous study, in which cells lacking *Rad9* were reported highly sensitive to the antiproliferative effects of Cisplatin [41], another substrate repaired by NER.

4.2. Rad9 associates with XPC-hHR23B complex in response to local UV irradiation

As mentioned above, Rad9 was shown as DNA damage sensor and recruiting platform for the recruitment of down stream DNA repair factors in response to the DNA damage in BER and MMR pathways [8,42]. Previous studies reported that Rad9 was rapidly recruited to the damage sites after the UV treatment [28,43,44]. The finding that Rad9 was associated with DNA damage recognition complex, XPC-hHR23B, in response to local UV irradiation (Fig. 3A and Fig. S1), suggests that Rad9 might have an impact on the NER through assisting recruitment of NER factors to DNA damage sites. However, our results from immunofluorescence assays using two doses at two different times after localized UV-irradiation showed that the recruitments of DNA damage recognition factors XPC-hHR23B complex as well as UV-DDB complex remained unaffected by the knockdown of *Rad9* in HeLa cells (Fig. 3 and Fig. S4). XPA is believed to verify the damage sites following initial recognition of a lesion and stabilize pre-incision complex for NER [45,46]. While a previous study reported that there was a physical interaction between the budding yeast counterparts of human Rad9 and XPA (DDC1 and RAD14 respectively) [20], the evidence presented here demonstrated that depletion of *Rad9* had no influence on the localization of XPA to the UV-induced photo lesions (Fig. S2A). In addition, Rad9 is also dispensable for the recruitment of the additional downstream factors XPB (p89), ERCC1 and XPG (Fig. S2B, S2C and S2D respectively). Taken together, recruitments of NER factors to DNA damage sites remain intact by the reduction Rad9, indicating involvement of Rad9 in NER, unlike in BER and MMR pathways [8,42], are not through recruiting core NER factors to DNA damage sites.

NER and ATR-dependent cell cycle checkpoint are the major pathways responsible for repair of UV-induced DNA damage. Evidences showed that these pathways were not independent with but coordinated with each other to impact on DNA damage survival. Therefore, as for the biological significance of associations between Rad9 and XPC complex, one possibility is that Rad9 interacts with XPC-hHR23B complex to sense DNA lesions and transmits the damage information to cell cycle checkpoint machineries instead of recruiting NER repair proteins to fix DNA lesions. In consistent with this hypothesis, a previous study showed that triggering of the DNA damage cascade required recognition and processing of the lesions by the GG-NER in the specific phase of the cell cycle [47]. Furthermore, inactivation of NER by the knockdown of XPA or XPC partially affected the UV-induced focus formation of Rad9 [44]. In addition, we observed that hHR23B knockdown disrupts Rad9 accumulation to the chromatin isolated from HEK293T cells irradiated by UV-light (80 J/m^2) (Fig. S6). Furthermore, we also found that loss of *mXPC* in MEF cells leads to defective accumulation of Rad9 onto UV-damaged chromatin (data not shown). These results suggest there might be cross-talk between NER and ATR-dependent cell cycle checkpoint pathway through interaction between Rad9 and XPC-hHR23B complex.

4.3. Knockdown of Rad9 alters gene expression of NER factors and reduces DDB2 protein levels in human cells

To further explore the mechanism(s) by which Rad9 is involved in NER of photoproducts, we turned to its role in regulation of gene expression [9,10]. We showed here, similar to its function in regulation control of *p21*, disruption of Rad9 using specific siRNA altered the UV-induced expression patterns of XPC and DDB2 genes (Fig. 4 and Fig. S5). These data fit with the current view, developed in yeast and extended to mammalian cells that checkpoint responses result in the activation of a variety of cellular pathways, including transcription, to impact on DNA damage survival [48–51]. It has been suggested that checkpoint protein BRCA1 and p53 transcriptionally induced *DDB2* and *XPC* after UV exposure, however, it seemed that the involvement of BRCA1 and p53 in regulation of *DDB2* and *XPC* occurred at late times post-UV (24 h and thereafter) and at that time NER had been completed [23,36,49,50]. By contrast, our data showed reduction of Rad9 altered expression pattern of *DDB2* and *XPC* and affected NER efficiency at the early times after UV radiation (within 6 h), in which NER was happening as shown in Fig. 1. In addition to this, Yin et al. suggested that Rad9 might direct the more global response of cells to DNA damage by transactivating a set of downstream genes [9], which is similar to the situation in p53-regualated gene expression [52]. Therefore, besides p53-regulated *DDB2* and *XPC*, we also screened for additional NER factors potentially regulated by Rad9 under UV treatment. Consistently, we demonstrated that the differences in gene expression of *XPB* and *DDB1* as a function of Rad9 depletion were statistically significant (Fig. 5 and Fig. S5). Our study showed that the Rad9-dependent upregulation in the UV-induced transcription of each NER factor was not as great as the p53-dependent [23,53] or BRCA1-dependent upregulation [49,50]. However, in this study we did observe transcription patterns of multiple NER factors were altered and this was not equivalent to the conditions found in the heterozygous NER knockout cells in which only a single NER factor was changed. The reduced transcription of multiple NER genes likely has a combined effect on NER efficiency not caused by partial reduction of a single NER gene and this might contribute in part to NER in HEK293T cells. More studies are needed to reveal molecular mechanism(s) by which Rad9 transactivates multiple NER genes in response to UV irradiation.

We also subjected *mRad9*$^{+/+}$ and *mRad9*$^{-/-}$ mouse ES cells to qRT-PCR to examine the function of Rad9 for the induction of NER factors genes in the mouse cells, however, we did not observe significant induction of these genes in these two cells (Fig. S7). These results suggest that Rad9 does not play a role in transactivating these genes in the mouse ES cells, which is unlike the situation

in human HEK293T cells. To further investigate whether this difference is due to cell type specific (differentiated cells versus ES cells) or to the difference in human and mouse species, we analyzed the transcription of nine NER genes in control and UV-irradiated wild-type 3T3 cells. As shown in Fig. S8, we did not observe significant induction of these genes in response to UV irradiation in this differentiated mouse cells. This result suggests that the difference mentioned above is due to different cell types but not different species. Similarly, Tan and Chu reported that p53 regulated *DDB2* transcription in human cells but not in rodent cells [24] and this indicated different mechanisms used by human and rodent cells to regulate UV-dependent induction of NER factor genes.

The above result using mouse cells also suggests that at least one other mechanism instead of transactivation of NER factor genes through which Rad9 regulates NER is important. One possible mechanism might be due to unexpected observation that Rad9 knockdown reduced DDB2 protein levels in HEK293T cells even before UV light treatment (Fig. 4C), though we are unable to successfully check this possibility in mouse ES cells because we found that detection using the indicated anti-DDB2 antibody did not show clean band of mouse DDB2 on Western blotting membranes. Another possible mechanism might be through cross-talk between NER and ATR-dependent cell cycle checkpoint pathway mediated by interaction between Rad9 and XPC-hHR23B complex.

One might think that the Rad9-dependent UV-induced transactivation of NER genes conflicts with no influence on recruitments of NER proteins to the sites of damage induced by localized UV-irradiation. To examine the re-localization of NER factors *in vivo*, we employed localized UV-irradiation for immunofluorescence assay which was not equivalent to the conditions and resulted effects of the global UV irradiation. A study by Fitch et al. showed that an UV dose of 400 J/m^2 delivered through the filter induced just little more than 100% of the lesions observed from 20 J/m^2 without a filter [53]. That is, the dosage of the localized UV-irradiation was about 5% of that of global UV-irradiation. In addition, Mone et al. demonstrated that transcription was almost completely inhibited in areas that have been irradiated, while chromatin outside of the damaged areas continues to support normal levels of transcription [22]. Therefore, localized UV-irradiation used here hardly changed the level of NER proteins studied in Figs. 5 and 6, Fig. S5 and the phenomenon that the recruitments of NER proteins were not affected by Rad9 knockdown was not contradictory to that Rad9 knockdown reduced UV-induced the transcription of NRE genes.

4.4. Rad9 can transactivate REhDDB2L element in the promoter of DDB2

To further elucidate the mechanism by which Rad9 transactivates *DDB2* and *XPC*, we determined that Rad9 induced luciferase activity like p53 did for the REhDDB2L element (Fig. 6A) and this suggests that Rad9 transcriptionally regulates the expression of NER factors by binding to the promotor regions of *XPC* and *DDB2* after exposure to UV radiation, similar to its regulatory role in the transactivation of p21 [9,10]. Post-translational modifications like phosphorylation and arginine methylation in Rad9 were suggested to be important for Chk1 activation and G_2 cell cycle checkpoint maintenance [1,29,39,40], but Rad9 mutants Rad9-9A (all the 9 phosphorylated sites in C-terminus mutated) and Rad9-3RK (the three arginine methylation sites mutated) had the same ability to induce luciferase activity as its wide-type counterpart. The irrelevance of the phosphorylation of Rad9 C-terminus to *DDB2* transcription is in contrast to that the phosphorylation of Rad9 C-terminus to *p21* transcription, and this difference indicates different detailed mechanisms used by Rad9 for the transcriptional regulation of *p21* and *DDB2*. The above described finding also suggests that Rad9's function in *DDB2* transactivation is not through cell cycle checkpoint activation. By contrast, we did not observe any inductive effect of Rad9 on the XPCL element controlled luciferase (Fig. 5C); however, we cannot rule out the possibility that Rad9 can bind to other regulatory elements within the promoter region of *XPC* and this also indicates that Rad9 and p53 use different sequences to transactivate *XPC*. To elucidate this question, the robust approach should be introduced to map Rad9 downstream genes in the whole genome, like a previous work did by Wei et al. [38].

5. Conclusions

Herein, we showed that Rad9 played an important role in the repair of UV-derived 6-4PP and BPDE–DNA adducts, which are two main types of substrates processed by NER. Rad9 associates with XPC-hHR23B complex in response to local UV irradiation. However, reduction of Rad9 using specific siRNA did not impair the recruitments of DDB2 and XPC to UV-induced photo lesions, excluding that Rad9 functioned in NER through recruiting the core NER factors to damage sites. Instead, one or all of the following three Rad9-involved mechanisms contributes to NER: (1) the combined effects of Rad9's impact on the UV-induced expression patterns of multiple NER factors, (2) the Rad9-mediated DDB2 protein level stabilization, and (3) the signal flow from XPC-hHR23B complex to Rad9 through the interaction of Rad9 with XPC-hHR23B complex. Other mechanisms such as that Rad9 regulates the functional efficiency of one or more NER factors gathered at DNA damage sites cannot be ruled out.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

We thank Dr. Hongyu Deng for providing pGL-3-basic and pRL-CMV vectors and Dr. Zengqiang Yuan for pcDNA3.0-p53. We also thank Chunchun Liu and Zhenwei Yang for technical advice. This study was funded by National Natural Science Foundation grant 30971604, National Natural Science Foundation grant 11179040 and "Strategic Priority Research Program" of the Chinese Academy of Sciences, grant no. XDA04020000.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.dnarep.2013.01.006.

References

[1] P. Roos-Mattjus, K.M. Hopkins, A.J. Oestreich, B.T. Vroman, K.L. Johnson, S. Naylor, H.B. Lieberman, L.M. Karnitz, Phosphorylation of human Rad9 is required for genotoxin-activated checkpoint signaling, J. Biol. Chem. 278 (2003) 24428–24437.
[2] K.M. Hopkins, W. Auerbach, X.Y. Wang, M.P. Hande, H. Hang, D.J. Wolgemuth, A.L. Joyner, H.B. Lieberman, Deletion of mouse rad9 causes abnormal cellular responses to DNA damage, genomic instability, and embryonic lethality, Mol. Cell. Biol. 24 (2004) 7235–7248.
[3] T. Dang, S. Bao, X.F. Wang, Human Rad9 is required for the activation of S-phase checkpoint and the maintenance of chromosomal stability, Genes Cells 10 (2005) 287–295.
[4] R.K. Pandita, G.G. Sharma, A. Laszlo, K.M. Hopkins, S. Davey, M. Chakhparonian, A. Gupta, R.J. Wellinger, J. Zhang, S.N. Powell, J.L. Roti Roti, H.B. Lieberman, T.K. Pandita, Mammalian Rad9 plays a role in telomere stability, S- and G2-phase-specific cell survival, and homologous recombinational repair, Mol. Cell. Biol. 26 (2006) 1850–1864.
[5] X. Guan, H. Bai, G. Shi, C.A. Theriot, T.K. Hazra, S. Mitra, A.L. Lu, The human checkpoint sensor Rad9-Rad1-Hus1 interacts with and stimulates NEIL1 glycosylase, Nucleic Acids Res. 35 (2007) 2463–2472.

[6] X. Guan, A. Madabushi, D.Y. Chang, M.E. Fitzgerald, G. Shi, A.C. Drohat, A.L. Lu, The human checkpoint sensor Rad9-Rad1-Hus1 interacts with and stimulates DNA repair enzyme TDG glycosylase, Nucleic Acids Res. 35 (2007) 6207–6218.
[7] W. He, Y. Zhao, C. Zhang, L. An, Z. Hu, Y. Liu, L. Han, L. Bi, Z. Xie, P. Xue, F. Yang, H. Hang, Rad9 plays an important role in DNA mismatch repair through physical interaction with MLH1, Nucleic Acids Res. 36 (2008) 6406–6417.
[8] H. Bai, A. Madabushi, X. Guan, A.L. Lu, Interaction between human mismatch repair recognition proteins and checkpoint sensor Rad9-Rad1-Hus1, DNA Repair 9 (2010) 478–487.
[9] Y. Yin, A. Zhu, Y.J. Jin, Y.X. Liu, X. Zhang, K.M. Hopkins, H.B. Lieberman, Human RAD9 checkpoint control/proapoptotic protein can activate transcription of p21, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 8864–8869.
[10] K. Ishikawa, H. Ishii, Y. Murakumo, K. Mimori, M. Kobayashi, K. Yamamoto, M. Mori, H. Nishino, Y. Furukawa, K. Ichimura, Rad9 modulates the P21WAF1 pathway by direct association with p53, BMC Mol. Biol. 8 (2007) 37.
[11] K. Komatsu, T. Miyashita, H. Hang, K.M. Hopkins, W. Zheng, S. Cuddeback, M. Yamada, H.B. Lieberman, H.G. Wang, Human homologue of *S. pombe* Rad9 interacts with BCL-2/BCL-xL and promotes apoptosis, Nat. Cell Biol. 2 (2000) 1–6.
[12] J.T. Reardon, A. Sancar, Nucleotide excision repair, Prog. Nucleic Acid Res. Mol. Biol. 79 (2005) 183–235.
[13] L.C. Gillet, O.D. Scharer, Molecular mechanisms of mammalian global genome nucleotide excision repair, Chem. Rev. 106 (2006) 253–276.
[14] R.Y. Li, P. Calsou, C.J. Jones, B. Salles, Interactions of the transcription/DNA repair factor TFIIH and XP repair proteins with DNA lesions in a cell-free repair assay, J. Mol. Biol. 281 (1998) 211–218.
[15] M. Yokoi, C. Masutani, T. Maekawa, K. Sugasawa, Y. Ohkuma, F. Hanaoka, The xeroderma pigmentosum group C protein complex XPC-HR23B plays an important role in the recruitment of transcription factor IIH to damaged DNA, J. Biol. Chem. 275 (2000) 9870–9875.
[16] S.J. Araujo, E.A. Nigg, R.D. Wood, Strong functional interactions of TFIIH with XPC and XPG in human DNA nucleotide excision repair, without a preassembled repairosome, Mol. Cell. Biol. 21 (2001) 2281–2291.
[17] T. Mori, M. Nakane, T. Hattori, T. Matsunaga, M. Ihara, O. Nikaido, Simultaneous establishment of monoclonal antibodies specific for either cyclobutane pyrimidine dimer or (6-4)photoproduct from the same mouse immunized with ultraviolet-irradiated DNA, Photochem. Photobiol. 54 (1991) 225–232.
[18] H.B. Lieberman, K.M. Hopkins, M. Nass, D. Demetrick, S. Davey, A human homolog of the *Schizosaccharomyces pombe* rad9+ checkpoint control gene, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 13890–13895.
[19] Z. Hu, Y. Liu, C. Zhang, Y. Zhao, W. He, L. Han, L. Yang, K.M. Hopkins, X. Yang, H.B. Lieberman, H. Hang, Targeted deletion of Rad9 in mouse skin keratinocytes enhances genotoxin-induced tumor development, Cancer Res. 68 (2008) 5552–5561.
[20] M. Giannattasio, F. Lazzaro, M.P. Longhese, P. Plevani, M. Muzi-Falconi, Physical and functional interactions between nucleotide excision repair and DNA damage checkpoint, EMBO J. 23 (2004) 429–438.
[21] Z. Wang, M. Lu, X. Wang, R. Yin, Y. Song, X.C. Le, H. Wang, Quantum dots enhanced ultrasensitive detection of DNA adducts, Anal. Chem. 81 (2009) 10285–10289.
[22] M.J. Mone, M. Volker, O. Nikaido, L.H. Mullenders, A.A. van Zeeland, P.J. Verschure, E.M. Manders, R. van Driel, Local UV-induced DNA damage in cell nuclei results in local transcription inhibition, EMBO Rep. 2 (2001) 1013–1017.
[23] S. Adimoolam, J.M. Ford, p53 and DNA damage-inducible expression of the xeroderma pigmentosum group C gene, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 12985–12990.
[24] T. Tan, G. Chu, p53 binds and activates the xeroderma pigmentosum DDB2 gene in humans but not mice, Mol. Cell. Biol. 22 (2002) 3247–3254.
[25] J.M. Murray, A.M. Carr, A.R. Lehmann, F.Z. Watts, Cloning and characterisation of the rad9 DNA repair gene from *Schizosaccharomyces pombe*, Nucleic Acids Res. 19 (1991) 3525–3531.
[26] M. Cosman, C. de los Santos, R. Fiala, B.E. Hingerty, V. Ibanez, E. Luna, R. Harvey, N.E. Geacintov, S. Broyde, D.J. Patel, Solution conformation of the (+)-cis-anti-[BP]dG adduct in a DNA duplex: intercalation of the covalently attached benzo[a]pyrenyl ring into the helix and displacement of the modified deoxyguanosine, Biochemistry 32 (1993) 4145–4155.
[27] P. Roos-Mattjus, B.T. Vroman, M.A. Burtelow, M. Rauen, A.K. Eapen, L.M. Karnitz, Genotoxin-induced Rad9-Hus1-Rad1(9-1-1) chromatin association is an early checkpoint signaling event, J. Biol. Chem. 277 (2002) 43809–43812.
[28] L. Zou, D. Cortez, S.J. Elledge, Regulation of ATR substrate selection by Rad17-dependent loading of Rad9 complexes onto chromatin, Genes Dev. 16 (2002) 198–208.
[29] S. Delacroix, J.M. Wagner, M. Kobayashi, K. Yamamoto, L.M. Karnitz, The Rad9-Hus1-Rad1(9-1-1) clamp activates checkpoint signaling via TopBP1, Genes Dev. 21 (2007) 1472–1477.
[30] K. Sugasawa, Multiple DNA damage recognition factors involved in mammalian nucleotide excision repair, Biochemistry (Moscow) 76 (2011) 16–23.
[31] J. Moser, M. Volker, H. Kool, S. Alekseev, H. Vrieling, A. Yasui, A.A. van Zeeland, L.H. Mullenders, The UV-damaged DNA binding protein mediates efficient targeting of the nucleotide excision repair complex to UV-induced photo lesions, DNA Repair 4 (2005) 571–582.
[32] K. Sugasawa, Y. Okuda, M. Saijo, R. Nishi, N. Matsuda, G. Chu, T. Mori, S. Iwai, K. Tanaka, F. Hanaoka, UV-induced ubiquitylation of XPC protein mediated by UV-DDB-ubiquitin ligase complex, Cell 121 (2005) 387–400.
[33] G. Yasuda, R. Nishi, E. Watanabe, T. Mori, S. Iwai, D. Orioli, M. Stefanini, F. Hanaoka, K. Sugasawa, In vivo destabilization and functional defects of the xeroderma pigmentosum C protein caused by a pathogenic missense mutation, Mol. Cell. Biol. 27 (2007) 6606–6614.
[34] J.T. Reardon, A.F. Nichols, S. Keeney, C.A. Smith, J.S. Taylor, S. Linn, A. Sancar, Comparative analysis of binding of human damaged DNA-binding protein (XPE) and *Escherichia coli* damage recognition protein (UvrA) to the major ultraviolet photoproducts: T[c,s]T, T[t,s]T, T[6–4]T, and T[Dewar]T, J. Biol. Chem. 268 (1993) 21301–21308.
[35] D.K. Treiber, Z. Chen, J.M. Essigmann, An ultraviolet light-damaged DNA recognition protein absent in xeroderma pigmentosum group E cells binds selectively to pyrimidine (6-4) pyrimidone photoproducts, Nucleic Acids Res. 20 (1992) 5805–5810.
[36] B.J. Hwang, J.M. Ford, P.C. Hanawalt, G. Chu, Expression of the p48 xeroderma pigmentosum gene is p53-dependent and is involved in global genomic repair, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 424–428.
[37] V. Rapic-Otrin, M.P. McLenigan, D.C. Bisi, M. Gonzalez, A.S. Levine, Sequential binding of UV DNA damage binding factor and degradation of the p48 subunit as early events after UV irradiation, Nucleic Acids Res. 30 (2002) 2588–2598.
[38] C.L. Wei, Q. Wu, V.B. Vega, K.P. Chiu, P. Ng, T. Zhang, A. Shahab, H.C. Yong, Y. Fu, Z. Weng, J. Liu, X.D. Zhao, J.L. Chew, Y.L. Lee, V.A. Kuznetsov, W.K. Sung, L.D. Miller, B. Lim, E.T. Liu, Q. Yu, H.H. Ng, Y. Ruan, A global map of p53 transcription-factor binding sites in the human genome, Cell 124 (2006) 207–219.
[39] R.P. St Onge, B.D. Besley, J.L. Pelley, S. Davey, A role for the phosphorylation of hRad9 in checkpoint signaling, J. Biol. Chem. 278 (2003) 26620–26628.
[40] W. He, X. Ma, X. Yang, Y. Zhao, J. Qiu, H. Hang, A role for the arginine methylation of Rad9 in checkpoint control and cellular sensitivity to DNA damage, Nucleic Acids Res. 39 (2011) 4719–4727.
[41] J.M. Wagner, L.M. Karnitz, Cisplatin-induced DNA damage activates replication checkpoint signaling components that differentially affect tumor cell survival, Mol. Pharmacol. 76 (2009) 208–214.
[42] M.J. Park, J.H. Park, S.H. Hahm, S.I. Ko, Y.R. Lee, J.H. Chung, S.Y. Sohn, Y. Cho, L.W. Kang, Y.S. Han, Repair activities of human 8-oxoguanine DNA glycosylase are stimulated by the interaction with human checkpoint sensor Rad9-Rad1-Hus1 complex, DNA Repair 8 (2009) 1190–1200.
[43] A.L. Medhurst, D.O. Warmerdam, I. Akerman, E.H. Verwayen, R. Kanaar, V.A. Smits, N.D. Lakin, ATR and Rad17 collaborate in modulating Rad9 localisation at sites of DNA damage, J. Cell Sci. 121 (2008) 3933–3940.
[44] D.O. Warmerdam, R. Freire, R. Kanaar, V.A. Smits, Cell cycle-dependent processing of DNA lesions controls localization of Rad9 to sites of genotoxic stress, Cell Cycle 8 (2009) 1765–1774.
[45] Z. He, L.A. Henricksen, M.S. Wold, C.J. Ingles, RPA involvement in the damage-recognition and incision steps of nucleotide excision repair, Nature 374 (1995) 566–569.
[46] L. Li, X. Lu, C.A. Peterson, R.J. Legerski, An interaction between the DNA repair factor XPA and replication protein A appears essential for nucleotide excision repair, Mol. Cell. Biol. 15 (1995) 5396–5402.
[47] F. Marini, T. Nardo, M. Giannattasio, M. Minuzzo, M. Stefanini, P. Plevani, M. Muzi Falconi, DNA nucleotide excision repair-dependent signaling to checkpoint activation, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 17325–17330.
[48] A.L. Carcagno, M.F. Ogara, S.V. Sonzogni, M.C. Marazita, P.F. Sirkin, J.M. Ceruti, E.T. Canepa, E2F1 transcription is induced by genotoxic stress through ATM/ATR activation, IUBMB Life 61 (2009) 537–543.
[49] R. Takimoto, T.K. MacLachlan, D.T. Dicker, Y. Niitsu, T. Mori, W.S. el-Deiry, BRCA1 transcriptionally regulates damaged DNA binding protein (DDB2) in the DNA repair response following UV-irradiation, Cancer Biol. Ther. 1 (2002) 177–186.
[50] A.R. Hartman, J.M. Ford, BRCA1 induces DNA damage recognition factors and enhances nucleotide excision repair, Nat. Genet. 32 (2002) 180–184.
[51] A.P. Gasch, M. Huang, S. Metzner, D. Botstein, S.J. Elledge, P.O. Brown, Genomic expression responses to DNA-damaging agents and the regulatory role of the yeast ATR homolog Mec1p, Mol. Biol. Cell 12 (2001) 2987–3003.
[52] A.J. Levine, p53, the cellular gatekeeper for growth and division, Cell 88 (1997) 323–331.
[53] M.E. Fitch, I.V. Cross, J.M. Ford, p53 responsive nucleotide excision repair gene products p48 and XPC, but not p53, localize to sites of UV-irradiation-induced DNA damage, in vivo, Carcinogenesis 24 (2003) 843–850.