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SUMOylation of damaged DNA-binding protein DDB2

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ABSTRACT

Damaged DNA-binding protein (DDB) is a heterodimer composed of two subunits, p127 and p48, which have been designated DDB1 and DDB2, respectively. DDB2 recognizes and binds to UV-damaged DNA during nucleotide excision repair. Here, we demonstrated that DDB2 was SUMOylated in a UV-dependent manner, and its major SUMO E3 ligase was PIASy as determined by RNA interference-mediated knockdown. The UV-induced physical interaction between DDB2 and PIASy supported this notion. PIASy knockdown reduced the removal of cyclobutane pyrimidine dimers (CPDs) from total genomic DNA, but did not affect that of 6-4 pyrimidine pyrimidone photoproducts (6-4PPs). Thus, DDB2 plays an indispensable role in CPD repair, but not in 6-4PP repair, which is consistent with the observation that DDB2 was SUMOylated by PIASy. These results suggest that the SUMOylation of DDB2 facilitates CPD repair.

1. Introduction

The post-translational modification of proteins by the conjugation of small ubiquitin-related modifiers (SUMOs) is an important regulatory mechanism that affects many cellular processes, including transcriptional regulation, nuclear transport, the maintenance of genome integrity, and signal transduction based on the capability of SUMOs to cause rapid changes in the function, distribution, and protein–protein interactions of pre-existing proteins [1–4]. Three SUMO isoforms have been identified in mammals, SUMO1, SUMO2 and SUMO3, and their functions have been shown to partly overlap [5]. SUMO is covalently attached to proteins through the activities of an enzyme cascade similar to that of ubiquitination. There is only one known heterodimeric SUMO-activating E1 enzyme (Aos1/Uba2) and SUMO-conjugation E2 enzyme (Ubc9). Once activated, SUMO is transferred to Ubc9 and then is attached to the ε-amino group of a specific lysine residue of a target protein that contains the consensus sequence ψKXE (ψ, large hydrophobic residue) recognized by Ubc9 [1,3–6]. An E3 ligase enhances the transfer of SUMO molecules from E2 to substrates. To date, several structurally unrelated proteins have been identified as SUMO E3 ligases such as RanBP2, PIASs (protein inhibitor of activated STAT proteins), and the polycomb group protein Pc2 [1,2].

DNA repair is an indispensable process that protects genomic integrity against DNA damage generated by environmental agents or DNA metabolism, and the SUMOylation of proteins implicated in DNA damage repair seems to play a key role in progressing the entire process; however, the mechanisms have not been completely elucidated. For example, the depletion of PIAS1 and PIASy in mammalian cells causes a decrease in double strand break repair by homologous recombination and non-homologous end joining [7]. Siz1 and Siz2, orthologues of mammalian PIAS family SUMO E3 ligases are required for efficient global genome nucleotide excision repair (GG-NER) in *Saccharomyces cerevisiae* [8].

Nucleotide excision repair (NER) is an important DNA repair pathway for UV damage that eliminates a wide variety of base lesions such as UV-induced 6-4 pyrimidine pyrimidone photoproducts (6-4PPs) and cyclobutane pyrimidine dimers (CPDs). In humans, a partial deficiency in the function of one of the GG-NER proteins has been shown to cause autosomal recessive disorders such as xeroderma pigmentosum (XP). Seven GG-NER-deficient XP complementation groups (XPA through XPG) have been identified [9]. Mammalian NER includes two distinct subpathways that remove lesions from the entire genome (GG-NER), and that eliminate DNA damage in the transcribed strand (transcription-coupled NER (TC-NER)). A major difference between the two mechanisms is in the damage recognition step. In TC-NER, lesion recognition is mediated by stalled RNA polymerase II at the damaged sites. In GG-NER, XPC and DDB2 (XPE) proteins play key roles in recognizing DNA lesions [10–16]. Despite this difference, subsequent repair reactions are shared by both systems [17]. Among XP proteins, XPC is conjugated to SUMO in response to UV irradiation [18] and its binding partner centrin-2 is also SUMOylated [19]. However, it remains unclear whether the SUMOylation of these factors has an impact on GG-NER.

In this study, we showed that DDB2 was SUMOylated following UV irradiation. RNA interference studies suggested that PIASy was a major SUMO E3 ligase for DDB2. We also demonstrated that the efficiency of CPD removal, but not that of 6-4PP was reduced in PIASy-knockdown cells, which suggests that the SUMOylation of DDB2 may be required for efficient GG-NER.

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2. Materials and methods

2.1. Cell culture and UV irradiation

HeLa cells and their derivatives that stably expressed FLAG-tagged DDB2 (HeLa/FLAG-DDB2), and both HA-tagged SUMO1 and FLAG-tagged DDB2 (HeLa/FLAG-DDB2 + HA-SUMO1) were maintained in DMEM high glucose medium (SIGMA) containing 10% fetal bovine serum (FBS), penicillin, and streptomycin. To induce photo lesions, exponentially growing cells were washed with phosphate-buffered saline (PBS) and UV irradiated (20 J/m^2) using a germicidal lamp calibrated to deliver a dose of 1 J/m^2/s. Cells were incubated in growth medium following UV exposure to allow the repair of DNA lesions for a defined period. Procedures for constructing expression vectors are shown in the supplemental methods.

2.2. Immunoprecipitation and immunoblotting

Immunoprecipitation was conducted under denaturing conditions to detect UV-induced SUMOylated DDB2. HeLa/FLAG-DDB2 or HeLa/FLAG-DDB2 + HA-SUMO1 cells (1×10^7 cells) were washed twice with PBS, and resuspended in SDS lysis buffer (62.5 mM Tris–HCl, pH 6.8, 10% glycerol, 2% SDS, and 10 mM *N*-ethylmaleimide). Well mixed lysates were boiled to denature proteins, and were then diluted 10-fold with RIPA buffer (50 mM Tris–HCl, pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 1 mM EDTA, 10 mM *N*-ethylmaleimide, and 150 mM NaCl) followed by two rounds of sonication. After separating insoluble materials by centrifugation, ANTI-FLAG M2 affinity gel (SIGMA) or anti-HA-tag Agarose (Medical & Biological Laboratories) was added, and FLAG or HA-associated immunoprecipitates were recovered, followed by denaturation in 1× SDS sample buffer (65 mM Tris–HCl, pH 6.8, 3% SDS, 10% glycerol, and 5% β-mercaptoethanol). Proteins were detected by Western blotting with primary antibodies and horseradish peroxidase (HRP)-conjugated secondary antibodies. Rabbit anti-SUMO1 and rabbit anti-SUMO2 were generated by immunizing rabbits with affinity purified proteins. Rat anti-HA high affinity (Roche Applied Science), rabbit anti-FLAG™ (ROCKLAND) and mouse anti-b-actin (SIGMA) were used as primary antibodies.

2.3. Knockdown of E3 ligases and DDB2

In order to identify the E3 ligase for DDB2, siRNAs for E3 ligases (40 nM) were transfected to a half-confluent HeLa/FLAG-DDB2. Scramble siRNA was also transfected as a control. UV was irradiated (20 J/m^2) at 48 h post-transfection. Cells were recovered 30 min post-irradiation and analyzed. To assess the influence of PIASy knockdown against CPD and 6-4PP removal, 40 nM of siRNAs were transfected to semi-confluent HeLa cells, and UV (5 J/m^2 for CPDs and 10 J/m^2 for 6-4PPs) was irradiated at 48 h post-transfection. The sequences of siRNAs used were shown in Supplementary Table 2.

2.4. ELISA

Genomic DNA was purified from mock-treated or UV irradiated cells using MagExtractor-Genome- (TOYOBO). DNAs (10 ng in PBS) were denatured and distributed to protamine sulfate-precoated 96-well assay plates (Asahi Glass), and plates were dried completely. Plates were then washed with PBS containing 0.05% Tween-20, followed by blocking with 2% FBS. After washing, either anti-CPD or anti-(6-4)PP antibodies (Cosmo Bio) diluted in PBS were distributed to DNA-coated plates, and plates were incubated at 37 °C for 30 min. After washing, plates were incubated with the Biotin-F(ab′)2 fragment of anti-mouse IgG (H+L) (Life Technologies) at 37 °C for 30 min, followed by incubation with Peroxidase-Streptavidin (Life Technologies). Captured CPDs and 6-4PPs were visualized using BD OptEIA™ Substrate Reagent A and B (BD Biosciences).

2.5. Micropore UV irradiation and immunofluorescence

EGFP-PIASy was transfected to HeLa/FLAG-DDB2 grown on a cover glass, and 12 h after transfection, cells were washed twice with PBS, covered by a Cyclopore™ Track Etched Membrane (pore size 5.0 μm in diameter) (Watman), and exposed to UV light (100 J/m^2). Cells were fixed with 4% formaldehyde, and permeabilized and masked with PBS containing 0.3% TritonX-100 and 5% FBS. Cells were incubated with primary antibodies, and then with goat anti-mouse F(ab′)2 Rhodamine Conjugated (MP Biochemicals, Inc.) for CPD, or goat anti-rabbit IgG (H+L) TRITC conjugate (ZYMED) for FLAG-DDB2. Fluorescence images were obtained with BIO ZERO-8000 (KEYENCE) or a confocal microscope.

3. Results

3.1. DDB2 was SUMOylated upon UV irradiation

GG-NER is a multistep process in which many proteins are implicated. The precise interactions and assembly of these proteins are critical for the accuracy of the repair reaction and subsequent cellular responses. DDB2 is known as a DNA repair protein in GG-NER and is subjected to many kinds of post-translational modifications, such as phosphorylation [20], ubiquitination [21–24], and poly(ADP-ribosyl)ation [25,26]. In general, one of the most important consequences of SUMOylation is the modification of protein–protein interactions, and many proteins involved in DNA repair and DNA replication are subjected to this modification, which may affect the accurate progress of the entire process through the formation of precise repair and replication complexes. Thus, it is of interest to know whether DDB2 is SUMOylated in addition to already reported post-translational modifications. For this, FLAG-DDB2 and HA-SUMO1 or HA-SUMO2 were cotransfected to HeLa cells. Transfected cells were exposed to UV light, harvested 30 min after irradiation, and immunoprecipitation was then conducted under denaturing conditions (Fig. 1A left). SUMOylated FLAG-DDB2 was detected by the anti-HA antibody with an estimated molecular mass of 67 kDa. Between the two SUMO paralogues, SUMO1 gave a dense band; however, a certain level of SUMO2 conjugation was also observed. The appearance of the band of SUMO-conjugated DDB2 was UV-dependent, while the faint band was detected in mock-irradiated cells either with SUMO1 or SUMO2. Mouse-derived DDB2 was used in these experiments; however, a similar experiment showed that human DDB2 was also SUMOylated (Fig. 1A right). Thus, we considered that DDB2 was conjugated with both SUMO1 and SUMO2, but preferentially with SUMO1 in a UV irradiation-dependent manner. We then attempted to elucidate whether endogenous DDB2 was SUMO-conjugated. Immunoprecipitation was conducted under denaturing conditions to precipitate just covalently modified DDB2, and to prevent the degradation of SUMO-conjugated protein by SUMO-specific proteases. However, we could not identify the modified endogenous DDB2 and this may be because SUMO conjugation is short-lived and the steady-state level of SUMO-conjugated protein is usually low. To overcome this problem, we used cell lines that stably expressed FLAG-DDB2 for further studies in order to improve the efficiency of immunoprecipitation. Prior to analyses, we studied the physiological behavior of FLAG-DDB2 in HeLa/FLAG-DDB2 cells.

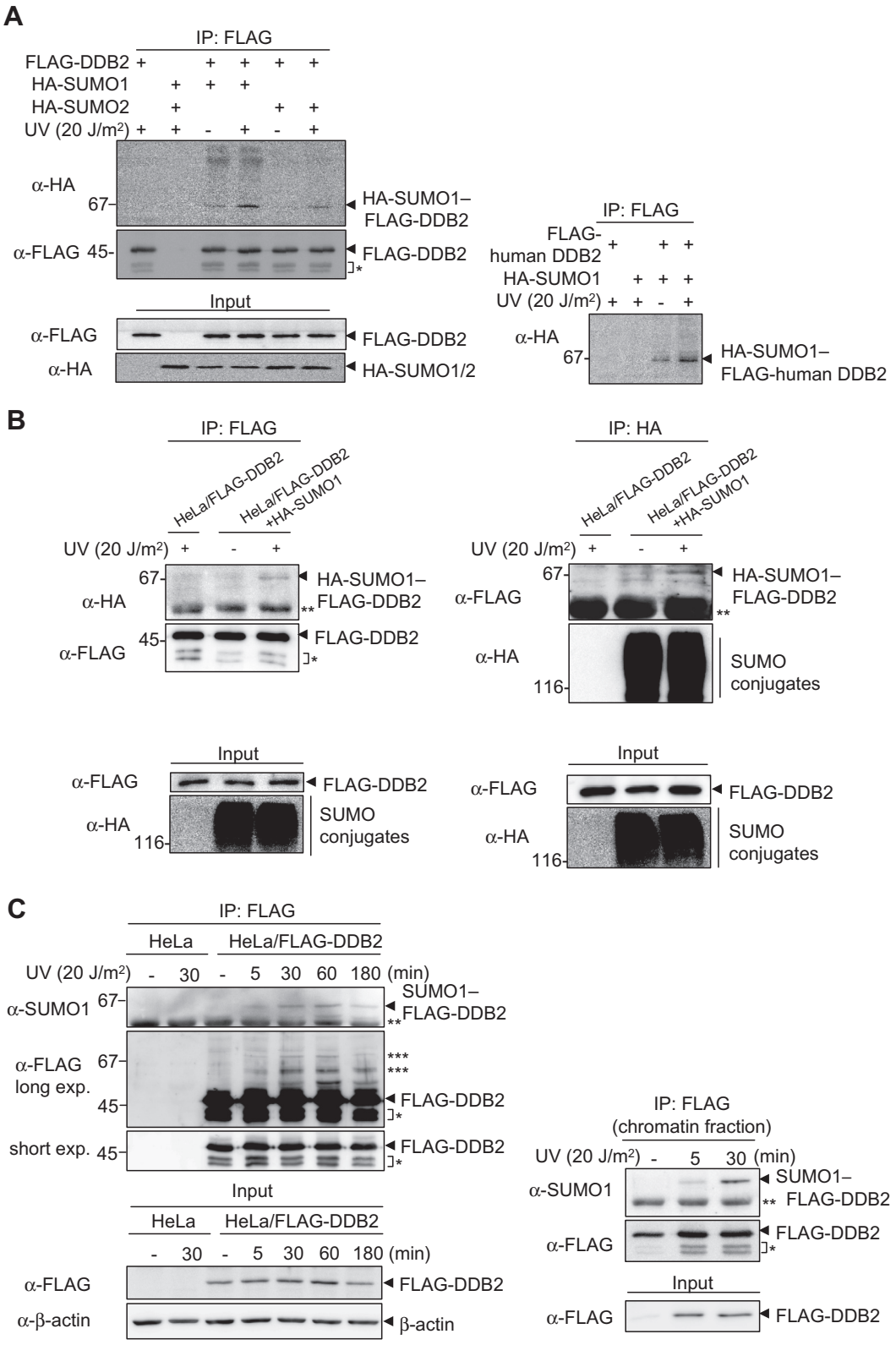

Fig. 1. SUMOylation of DDB2. (A) Left: HA-SUMO1 or HA-SUMO2 was cotransfected with FLAG-DDB2 to HeLa cells. Cells were allowed to repair UV damage for 30 min following irradiation. HA-SUMO and FLAG-DDB2 were detected by Western blotting after immunoprecipitation either with the FLAG or HA antibody. Right: SUMO1 conjugation of human DDB2 was analyzed as mouse DDB2. (B) Immunoprecipitation was conducted under denaturing conditions with HeLa/FLAG-DDB2 + HA-SUMO1 exposed to UV light (20 J/m^2). HeLa/FLAG-DDB2 was used for a negative control of the HA antibody. Left: FLAG immunoprecipitates were detected with anti-HA, right: Inverse immunoprecipitation and Western blotting. (C) Left: HeLa/FLAG-DDB2 cells were harvested 5, 30, 60, and 180 min after irradiation (20 J/m^2). SUMO-conjugated DDB2 was detected using the anti-SUMO1 antibody with FLAG-related immunoprecipitates. Right: A similar experiment with the chromatin fraction. A representative result of a minimum of triplicated experiments is shown. *unidentified bands possibly derived from FLAG-DDB2, **the immunoglobulin heavy chain, and ***these bands were also detected by the anti-Ubiquitin antibody.

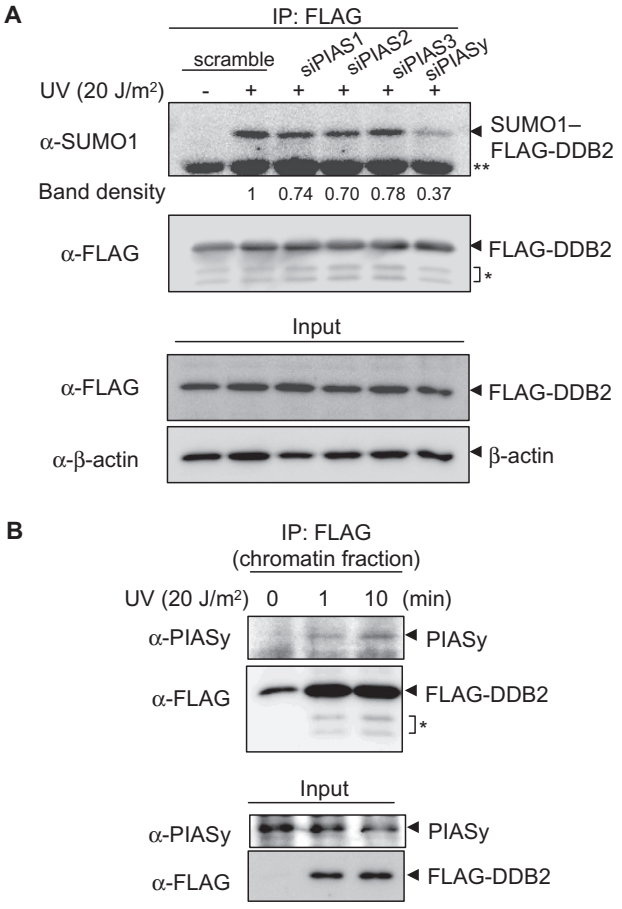

Fig. 2. DDB2 is SUMOylated by PIASy. (A) siRNAs for E3 ligases were transfected to HeLa/FLAG-DDB2, and cells were UV irradiated. Cells were then lysed and immunoprecipitated with the FLAG antibody, followed by Western blotting with anti-SUMO1. (B) After UV irradiation, soluble chromatin fraction of HeLa/FLAG-DDB2 was subjected to co-immunoprecipitation by the FLAG-M2 resin, and PIASy was detected by rabbit anti-PIASy (Cell Signaling Technology). A representative result of a minimum of triplicated experiments is shown. *shows bands possibly derived from FLAG-DDB2, and **indicates the immunoglobulin heavy chain.

The protein level of FLAG-DDB2 in this cell line was about four times higher than that of endogenous DDB2 (Supplemental Fig. S1A). The CUL4–DDB2 complex is well-known to recognize UV-induced DNA damage at the first step of GG-NER, and recruits the XPC complex to the damaged site [15,16]. DDB2 is then poly-ubiquitinated and degraded by proteasome [21–24]. Thus, we confirmed that FLAG-DDB2 levels decreased after UV irradiation in a similar time course to that of endogenous DDB2 (Supplemental Fig. S1B). In addition, mobilization of DDB2 to chromatin upon UV irradiation was also confirmed: almost all FLAG-DDB2 proteins were detected in chromatin, but not in the nuclear extract fractions after UV irradiation (Supplemental Fig. S1C). From these results, we assumed that FLAG-DDB2 exhibited similar behavior to that of endogenous DDB2. We also established cell lines that expressed HA-SUMO1 besides FLAG-DDB2. Using these cell lines, we also conducted similar immunoblot experiments. SUMO-conjugated protein with the molecular mass of the expected HA-SUMO–FLAG-DDB2 was detected in the FLAG-associated immunoprecipitates of UV irradiated HeLa/FLAG-DDB2 + HA-SUMO1 (Fig. 1B left), and HA-SUMO–FLAG-DDB2 was detected at the same position in the inverse immunoprecipitates, which again suggested that DDB2 was SUMOylated (Fig. 1B right).

We next examined whether the amount of DDB2 SUMOylation changed in a time-dependent manner. HeLa/FLAG-DDB2 cells were exposed to UV light followed by incubation for a certain period. As shown in Fig. 1C (left), the Western blotting with FLAG immunoprecipitates detected a band of SUMO-conjugated protein with the molecular mass of the expected SUMO–FLAG-DDB2 protein using the anti-SUMO1 antibody. This band appeared immediately after UV irradiation and the modification peaked around 30 min post-irradiation. In addition, the SUMOylation of DDB2 was clearly observed in the chromatin fraction (Fig. 1C right). The blot was also probed with the anti-ubiquitin, poly(ADP-ribose), and FLAG antibodies. Smear bands with several distinct bands were observed when the blot was detected with the anti-ubiquitin antibody (data not shown). Multiple bands were observed by the FLAG antibody. Among them, the mobility of two bands was coincident with those detected by the anti-ubiquitin antibody. DDB2 has been reported to be poly(ADP-ribosyl)ated soon after UV damage [25,26]. In the present study, a smear pattern was observed with the anti-poly(-ADP-ribose) antibody with the same blot (data not shown), which suggested that the weak and smear bands detected by the anti-FLAG antibody may partly correspond to poly(ADP-ribosyl)ated DDB2. In contrast, we could not detect the distinct FLAG-conjugated protein corresponding to SUMOylated DDB2 by the anti-FLAG antibody, which indicated that SUMO-modified DDB2 was less abundant. A band corresponding to SUMO2–DDB2 could not be detected in this blot.

3.2. SUMO E3 ligase for DDB2

We next attempted to identify the SUMO E3 enzyme of DDB2. There are generally three types of SUMO E3 ligases: RanBP2, PIASs,

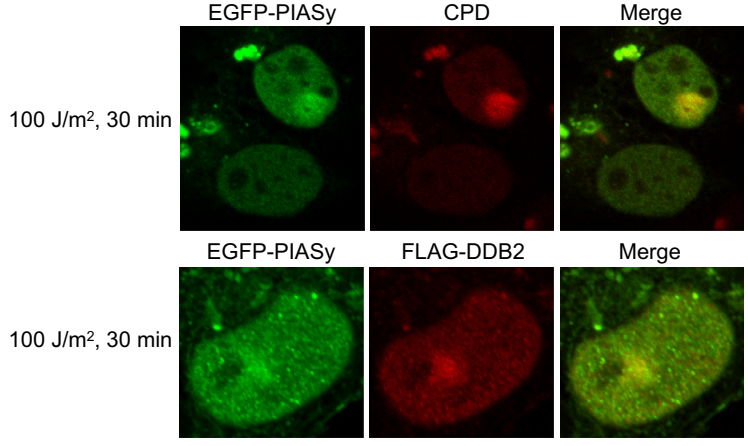

Fig. 3. EGFP-PIASy colocalizes with FLAG-DDB2 and CPDs after UV irradiation in HeLa/FLAG-DDB2. N-terminal EGFP-tagged PIASy was expressed in HeLa/FLAG-DDB2 cells for 12 h. Fixed cells were stained with the anti-CPD and anti-FLAG antibodies.

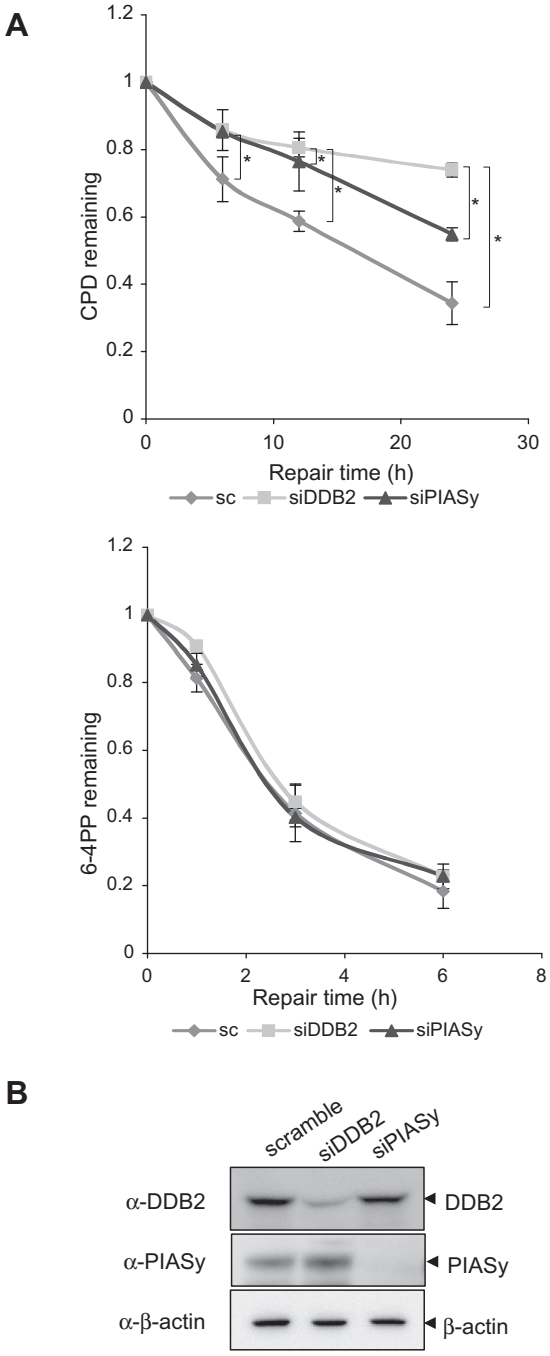

Fig. 4. SUMOylation of DDB2 stimulates GG-NER. (A) siRNA of PIASy and DDB2 were transfected to HeLa cells, and UV irradiated. The remaining CPDs (top) and 6-4PPs (bottom) were detected at the indicated time points after UV irradiation. (B) The knockdown of PIASy and DDB2 was shown by Western blotting. *indicates significant differences by the Student's *t* test ($p < 0.05$). A representative result of a minimum of triplicated experiments is shown.

and Pc2 [1]. The siRNAs of these E3 ligases including PIAS1, 2, 3 and PIASy were transfected into HeLa/FLAG-DDB2 cells. The expression levels of PIAS1 and PIASy were almost zero by Western blotting and those of PIAS2 and 3, RanBP2, and Pc2 were less than 30% of the original levels by RT-PCR. Immunoprecipitation of SUMOylated DDB2 demonstrated that PIASy knockdown decreased the SUMOylation of DDB2 in a UV irradiation-dependent manner, while other E3 enzymes did not show a significant reduction (Fig. 2A and data not shown). Since the detection of SUMO1-conjugated DDB2 gave clear results by our hand, E3 activity was estimated based on SUMO1 modifications. Overall, we assumed that PIASy is a major E3 ligase for DDB2. Since DDB2 does not contain the reported consensus sequence of SUMOylation, we attempted to determine whether PIASy binds to the substrate DDB2 to ensure that PIASy is a major E3 ligase for the SUMOylation of DDB2. Since DDB2 mobilized to chromatin upon UV irradiation, interaction of DDB2 and PIASy in soluble chromatin fraction was assessed. We found that PIASy physically interacted with DDB2 by co-immunoprecipitation experiments (Fig. 2B).

To clear the involvement of PIASy in GG-NER, relocalization of PIASy to damaged sites was assessed with the EGFP-PIASy fusion protein. We confirmed that EGFP-PIASy colocalized in a UV-dependent manner with both FLAG-DDB2 and CPDs by immunocytochemical analyses (Fig. 3), which indicated that PIASy was involved in CPD repair.

3.3. Physiological function of SUMOylation

To know the possible consequence of the SUMOylation of DDB2, we next examined the effect of PIASy knockdown on GG-NER (Fig. 4A). By this knockdown, the remaining DDB2 and PIASy were 20% and almost zero, respectively (Fig. 4B). In scramble siRNA-transfected cells, CPDs remaining in the genome were around 35% after 24 h irradiation (Fig. 4A upper). On the other hand, unrepaired CPDs in cells to which siRNA for DDB2 was introduced were more than 70%. Due to the knockdown of PIASy, the amount of remaining CPDs was between those of these two siRNAs (Fig. 4A upper). With 6-4PPs, three siRNAs gave a similar level of the remaining photoproducts, which suggested that PIASy knockdown affected the repair of CPDs, but not that of 6-4PPs (Fig. 4A lower). In these experiments, growth of siRNA-treated cells was not obviously different from that of control cells. Therefore, PIASy-mediated SUMOylation of the DDB2 protein possibly facilitated CPD repair.

4. Discussion

We showed that DDB2 was SUMOylated using cells that overexpressed DDB2 and SUMOs, and cell lines that stably expressed DDB2. Global analyses of putative SUMO-conjugated protein by mass spectrometry previously suggested that DDB2 may be subjected to both SUMO1 and 2 conjugation, and SUMO2 conjugation seems to be major [5]. Our results showed that both SUMO species may conjugate to DDB2 in a UV-dependent manner. Although we did not clearly detect UV-dependent SUMO2 conjugation of DDB2 in a stable cell line, we could not rule out the possibility that SUMO2 modification affected GG-NER. DDB2 does not have a consensus motif for the SUMO conjugation, ψKXE [5]. However, recent proteomic studies revealed that an increasing number of SUMOylated proteins do not contain the consensus sequence [1,27,28]. To identify the site of SUMO conjugation, we have already studied the lysine mutants of DDB2. Twenty-four lysine residues are common between mice and humans. Each of these lysine residues was replaced by arginine. A considerable reduction in SUMOylation was observed in some of these mutants; however, we have not yet identified unique SUMOylation sites (Masuda et al., unpublished results). In other words, it is possible that DDB2 is SUMOylated at several sites. Further study on this is essential to clarify the physiological meaning of SUMOylation.

As for SUMO E3 ligase, the most possible candidate was PIASy. Although the expression of PIASy was completely repressed, a certain level of SUMO conjugation was observed with DDB2 as shown in Fig. 2A, suggesting that the other E3 ligase weakly contributed to SUMOylation. In fact, SUMOylation of many substrate proteins can be stimulated by several E3 ligases with low specificity. For exam-

ple, PIAS1, PIAS3, and PIASy all facilitate the SUMOylation of p53 [2]. In contrast, E3 ligase RanBP2 physically interacts with the substrate and promotes SUMOylation in the case of RanGAP1 [2]. In this regard, low level SUMO conjugation in the absence of PIASy may be catalyzed by other E3 ligases in a non-specific manner. We observed UV-dependent physical interactions between PIASy and DDB2 *in vivo*. Thus far, the precise molecular mechanism of UV-dependent interactions has remained unclear. However, it is noteworthy that PIASy interacted with the poly(ADP-ribosyl)ated protein [29]. Thus, one possible explanation based on this fact may be that DDB2 is poly(ADP-ribosyl)ated in a UV-dependent manner [25,26], and PIASy interacts with this modified form of DDB2.

The repair of CPDs was largely impaired, whereas that of 6-4PPs was not affected by the knockdown of PIASy. The repair pathway for 6-4PPs and CPDs share the same enzymes including XPA, B, C, D, F and G, except for the protein that senses the lesion: DDB2 is indispensable for the recognition of CPDs [9,10]. Therefore, our result that the repair of CPDs, but not 6-4PPs was impaired by PIASy knockdown suggests that the major target of SUMOylation by PIASy is DDB2. SUMO conjugation of DDB2 may accelerate the repair of CPDs. This result agrees with the finding that yeast Siz1 and Siz2 are indispensable for GG-NER [8]. In addition, we observed that the knockdown of PIASy partly elongated the lifespan of endogenous DDB2 (Masuda et al., unpublished results). We are now trying to elucidate the physiological role of SUMOylation.

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.bbrc.2013.07.013.

References

[1] J.R. Gareau, C.D. Lima, The SUMO pathway: emerging mechanisms that shape specificity, conjugation and recognition, Nat. Rev. Mol. Cell Biol. 11 (2010) 861–871.
[2] E.S. Johnson, Protein modification by SUMO, Annu. Rev. Biochem. 73 (2004) 355–382.
[3] R. Geiss-Friendlander, F. Melchior, Concepts in sumoylation: a decade on, Nat. Rev. Mol. Cell Biol. 8 (2007) 947–956.
[4] A.D. Capili, C.D. Lima, Taking it step by step: mechanistic insights from structural studies of ubiquitin/ubiquitin-like protein modification pathway, Curr. Opin. Struct. Biol. 17 (2007) 726–735.
[5] A.C.O. Vertegaal, J.S. Andersen, S.C. Ogg, R.T. Hay, M. Mann, A.I. Lamond, Distinct and overlapping sets of SUMO-1 and SUMO-2 target proteins revealed by quantitative proteomics, Mol. Cell Proteomics 5 (2006) 2298–2310.
[6] M.S. Rodriguez, C. Dargemont, R.T. Hay, SUMO-1 conjugation in vivo requires both a consensus modification motif and nuclear targeting, J. Biol. Chem. 276 (2001) 12654–12659.
[7] Y. Galanty, R. Belotserkovskaya, J. Coates, S. Polo, K.M. Miller, S.P. Jackson, Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks, Nature 462 (2009) 935–939.
[8] H.R. Silver, J.A. Nissley, S.H. Reed, Y.-M. Hou, E.S. Johnson, A role of SUMO in nucleotide excision repair, DNA Repair 10 (2011) 1243–1251.
[9] R.D. Wood, M. Mitchell, J. Sgouros, T. Lindahl, Human DNA repair genes, Science 291 (2001) 1284–1289.
[10] J. Moser, M. Volker, H. Kool, S. Alekseev, H. Vrieling, A. Yasui, A.A. van Zeeland, L.H.F. 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.
[11] T. Yasuda, K. Sugasawa, Y. Shimizu, S. Iwai, T. Shiomi, F. Hanaoka, Nucleosomal structure of undamaged DNA regions suppresses the non-specific DNA binding of the XPC complex, DNA Repair 4 (2005) 389–395.
[12] A. Scrima, R. Konícková, B.K. Czyzewski, Y. Kawasaki, P.D. Jeffrey, R. Groisman, Y. Nakatani, S. Iwai, N.P. Pavletich, N.H. Thomä, Structural basis of UV DNA-damage recognition by the DDB1–DDB2 complex, Cell 135 (2008) 1213–1223.
[13] M. Araki, C. Masutani, M. Takemura, A. Uchida, K. Sugasawa, J. Kondoh, Y. Ohkuma, F. Hanaoka, Centrosome protein centrin 2/caltractin 1 is part of the xeroderma pigmentosum group C complex that initiates global genome nucleotide excision repair, J. Biol. Chem. 276 (2001) 18665–18672.
[14] R. Nishi, S. Alekseev, C. Dinant, D. Hoogstraten, A.B. Houtsmuller, J.H.J. Hoeijmakers, W. Vermeulen, F. Hanaoka, K. Sugasawa, UV-DDB-dependent regulation of nucleotide excision repair kinetics in living cells, DNA Repair 8 (2009) 767–776.
[15] M.E. Fitch, S. Nakajima, A. Yasui, J.M. Ford, In vivo recruitment of XPC to UV-induced cyclobutane pyrimidine dimmers by the DDB2 gene product, J. Biol. Chem. 278 (2003) 46906–46910.
[16] K. Sugasawa, Regulation of damage recognition in mammalian global genomic nucleotide excision repair, Mutat. Res. 685 (2010) 29–37.
[17] P.C. Hanawalt, Subpathways of nucleotide excision repair and their regulation, Oncogene 21 (2002) 8949–8956.
[18] Q.-E. Wang, Q. Zhu, G. Wani, M.A. El-Mahdy, J. Li, A.A. Wani, DNA repair factor XPC is modified by SUMO-1 and ubiquitin following UV irradiation, Nucleic Acids Res. 33 (2005) 4023–4034.
[19] U.R. Klein, E.A. Nigg, SUMO-dependent regulation of centrin-2, J. Cell Sci. 122 (2009) 3312–3321.
[20] Q. Zhao, B.M. Barakat, S. Qin, A. Ray, M.A. El-Mahdy, G. Wani, E.-S. Arafa, S.N. Mir, Q.-E. Wang, A.A. Wani, The p38 mitogen-activated protein kinase augments nucleotide excision repair by mediating DDB2 degradation and chromatin relaxation, J. Biol. Chem. 283 (2008) 32553–32561.
[21] V. Rapić-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.
[22] K. Sugasawa, Y. Okuda, M. Saijo, R. Nishi, N. Matsuda, G. Chu, T. Mori, S. Iwai, K. Tanaka, K. Tanaka, F. Hanaoka, UV-induced ubiquitylation of XPC protein mediated by UV-DDB-ubiquitin ligase complex, Cell 121 (2005) 387–400.
[23] X. Chen, Y. Zhang, L. Douglas, P. Zhou, UV-damaged DNA-binding proteins are targets of CUL-4A-mediated ubiquitination and degradation, J. Biol. Chem. 276 (2001) 48175–48182.
[24] A. Nag, T. Bondar, S. Shiv, P. Raychaudhuri, The xeroderma pigmentosum group E gene product DDB2 is a specific target of cullin 4A in mammalian cells, Mol. Cell. Biol. 21 (2001) 6738–6747.
[25] A. Pines, M.G. Vrouwe, J.A. Marteijn, D. Typas, M.S. Luijsterburg, M. Cansoy, P. Hensbergen, A. Deelder, A. de Groot, S. Matsumoto, K. Sugasawa, N. Thoma, W. Vermeulen, H. Vrieling, L. Mullenders, PARP1 promotes nucleotide excision repair through DDB2 stabilization and recruitment of ALC1, J. Cell Biol. 199 (2012) 235–249.
[26] M. Robu, R.G. Shah, N. Petitclerc, J. Brind'Amour, F. Kandan-Kulangara, G.M. Shah, Role of poly(ADP-ribose) polymerase-1 in the removal of UV-induced DNA lesions by nucleotide excision repair, Proc. Natl. Acad. Sci. USA 110 (2013) 1658–1663.
[27] H.A. Blomster, S.Y. Imanishi, J. Siimes, J. Kastu, N.A. Morrice, J.E. Eriksson, L. Sistonen, In vivo identification of sumoylation sites by a signature tag and cysteine-targeted affinity purification, J. Biol. Chem. 285 (2010) 19324–19329.
[28] H.-H. Hsiao, E. Meulmeester, B.T.C. Frank, F. Melchior, H. Urlaub, "ChopNSpice", a mass spectrometric approach that allows identification of endogenous small ubiquitin-like modifier-conjugated peptides, Mol. Cell Proteomics 8 (2009) 2664–2675.
[29] M. Stilmann, M. Hinz, S.C. Arslan, A. Zimmer, V. Schreiber, C. Scheidereit, A nuclear poly(ADP-ribose)-dependent signalosome confers DNA damage-induced IkappaB kinase activation, Mol. Cell 36 (2009) 365–378.