Biochemical and Biophysical Research Communications 434 (2013) 185–190

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

A novel role for Gadd45α in base excision repair: Modulation of APE1 activity by the direct interaction of Gadd45α with PCNA

Hye Lim Kim [a,b,c], Sang Uk Kim [d,e], Young Rok Seo [a,b,c,*]

[a] Department of Life Science, Dongguk University, 30 Pildong-ro 1-gil, Jung-gu, Seoul 100-715, Republic of Korea
[b] Institute of Environmental Medicine for Green Chemistry, Dongguk University, 30 Pildong-ro 1-gil, Jung-gu, Seoul 100-715, Republic of Korea
[c] Department of Pharmacology, Institute for Biomedical Science (IBMS), School of Medicine, Kyung Hee University, Seoul 130-701, Republic of Korea
[d] School of Interdisciplinary Bioscience and Bioengineering, Pohang University of Science and Technology, Pohang 790-784, Republic of Korea
[e] Division of Molecular and Life Science, Pohang University of Science and Technology, Pohang 790-784, Republic of Korea

ARTICLE INFO

Article history:
Received 13 February 2013
Available online 26 February 2013

Keywords:
Gadd45α
PCNA
APE1
Base excision repair (BER)

ABSTRACT

The growth arrest and DNA damage inducible, alpha (Gadd45α) protein regulates DNA repair by interacting with proliferating cell nuclear antigen (PCNA). Our previous study suggested a potential role for Gadd45α in the base excision repair (BER) pathway by affecting apurinic/apyrimidinic endonuclease 1 (APE1) protein in addition to its accepted role in nucleotide excision repair (NER). Here, we investigated whether the interaction of Gadd45α with PCNA affects APE1 activity. To address this issue, we used a siRNA directed to Gadd45α and a form of Gadd45α with a mutation to the predicted site of PCNA binding. There was a reduction of APE1 activity in cells transfected with the Gadd45α siRNA. Furthermore, the interaction of Gadd45α with PCNA and APE1 was lower in cells transfected with mutant Gadd45α compared with cells transfected with wild-type Gadd45α. Indeed, we observed that the APE1 activity in the Gadd45α-interacting complex was significantly lower in cells that overexpress mutant Gadd45α compared with cells that overexpress wild-type Gadd45α. We conclude that the PCNA binding site on Gadd45α plays a critical role in modulating the interaction with PCNA and APE1, affecting BER activity. These results provide novel insights into the mechanisms by which BER activity is modulated, although the interaction of Gadd45α with APE1 needs to be clarified.

1. Introduction

Genomic stability is a crucial mechanism for maintaining normal cellular proliferation and cancer avoidance. Such genomic stability is dependent on the ability of the cell to recognize and repair damaged DNA [1]. If alterations in DNA structure are left unrepaired, they cause mutations that increase the risk of cancer [2]. The DNA excision repair systems protect cells from mutations that arise endogenously or as a consequence of environmental exposure. These systems include nucleotide excision repair (NER), base excision repair (BER), and mismatch repair (MMR). In particular, BER is a critical protective mechanism that maintains genomic integrity by correcting DNA base modifications caused by alkylating agents such as methyl methanesulfonate (MMS) [3]. These DNA repair processes involve complexes of several repair proteins that remove and repair damaged DNA.

The growth arrest and DNA damage inducible, alpha (Gadd45α) gene is a downstream target of p53 [4], and is induced by a number of stresses associated with growth arrest [5]. The protein has also been reported to contribute to the regulation of DNA repair. Smith et al. [6] showed that mouse embryo fibroblasts (MEFs) deficient in Gadd45α did not achieve normal levels of UV 6–4 photoproduct removal. There is also evidence that Gadd45α enhances NER by negatively regulating basal p21 expression in keratinocytes [7]. Furthermore, Gadd45α associates with proteins such as proliferating cell nuclear antigen (PCNA), core histones, p21, MAP three kinase 1 (MTK1), and Cdc2. Of these, PCNA participates in the DNA repair pathway via its interaction with Gadd45α [8].

PCNA operates as a communication point and a signal processing center for a variety of cellular processes [9,10]. In particular, PCNA plays a crucial role as a molecular adaptor for recruiting factors to a DNA repair site in a sequence-independent manner [11]. Additionally, PCNA is involved in the DNA repair pathway through recruitment to the sites of DNA damage and interaction with Gadd45α. Smith et al. [6] reported that PCNA foci were reduced in Gadd45α-deficient cells when they were immunostained after UV irradiation. This finding suggests that PCNA recruitment was affected by the presence or absence of Gadd45α. Our previous study showed that Gadd45α strongly interacts with PCNA in nuclear extracts of Gadd45α +/+ MEF cells. Furthermore, through PCNA

* Corresponding author at: Department of Life Science, Dongguk University, 30 Pildong-ro 1-gil, Jung-gu, Seoul 100-715, Republic of Korea. Fax: +82 2 2290 1392.
E-mail addresses: seoyr1@naver.com, seoyr@dongguk.edu (Y.R. Seo).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.02.066

immunostaining, we observed that PCNA was localized within the nucleus of Gadd45α +/+ MEF cells in response to MMS [12].

Several studies, as well as our own, have reported that PCNA was involved in the BER process through co-immunoprecipitation of PCNA and APE1 [12–14]. Apurinic/apyrimidinic (AP) endonuclease 1 (APE1) has been recognized as an essential enzyme responsible for the recognition and excision of AP sites in the BER pathway [15,16]. Some polymorphisms in APE1 reduce BER efficacy by interfering with its ability to communicate with other BER proteins, leading to the risk of lung, colon, breast, and prostate cancer [17,18]. APE1 binds directly to p53, suggesting that p53 may also regulate BER [1,19]. Our earlier studies suggested for the first time the possibility that the p53 target protein, Gadd45α, affects the ability of the APE1 enzyme to function in the BER pathway, as manifested by the slow repair of AP sites in Gadd45α −/− cells and by the disrupted localization of APE1/Ref1 in the nuclei of Gadd45α −/− cells [12,20].

In this study, we predicted the PCNA binding site on the Gadd45α gene sequence using bioinformatic analyses to elucidate the DNA repair mechanism related to Gadd45α. We provide evidence that the interaction of Gadd45α with PCNA plays a vital role in the modulation of APE1 activity via the interaction of Gadd45α with PCNA and APE1.

2. Material and methods

2.1. Cell culture

Human HepG2 liver cancer cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, USA) containing 10% fetal bovine serum (FBS) (Gibco, USA), 10,000 units/ml penicillin, 10,000 μg/ml streptomycin (Gibco, USA) under an atmosphere of 5% CO_2 at 37 °C. The cells were then washed in phosphate-buffered saline (pH 7.4) (Gibco, USA) and removed. After trypsinization for 3 min, the cells were collected by centrifugation at 1500 rpm for 5 min. The cell pellet was resuspended in DMEM and plated on a cell culture dish (SPL Life Sciences, Korea).

2.2. Site-directed mutagenesis and expression

The PCNA binding site on Gadd45α predicted by the PHD software package was mutated by site-directed mutagenesis [21,22]. The Gadd45α expression vector pFlagCMV4-Gadd45α was constructed by inserting the Gadd45α cDNA into pFlagCMV4. Oligonucleotide-mediated mutagenesis of the Gadd45α cDNA was performed by a PCR-based procedure using pFlagCMV4-Gadd45α as the template. Mutation-specific PCR analysis for the Gadd45α mutation was carried out using the following primers. Wild-type Gadd45α sense: 5′-CCCAAGCTTTGCAATATGACTTTGGA-3′, anti-sense: 5′-CTTGGTACCATGCCATCACCGTTCAG-3′, mutant Gadd45α (WVP466VAV) sense: 5′-TTTTGCCGGGAAAGTCGCTACATGGATCAAGTGGCTGTAGTG-3′, anti-sense: 5′-GTTCACCGACATCACTAATTAGAGGGACTTGCCACAGGGACT-3′. *Escherichia coli* strain DH5α was transformed with wild-type or mutant pFlagCMV4-Gadd45α and grown aerobically at 37 °C in LB medium supplemented with ampicillin (100 μg/ml).

2.3. Transfection of Gadd45α plasmid DNA

One day before transfection, HepG2 cells were plated at 1×10^6 cells/100 mm dish in DMEM containing only 10% FBS. The HepG2 cells were then transfected with wild-type or mutant pFlagCMV4-Gadd45α using Fugene 6 transfection reagent according to the manufacturer's instructions (Roche, Germany). For each dish, 18 μl of Fugene 6 was added to 164 μl of free-serum DMEM and incubated for 10 min. After further addition of 6 μg of Gadd45α plasmid DNA, the solutions were incubated at room temperature for 15 min and then added drop-wise to the plated cells. The cells were harvested after 48 h and prepared for immunoprecipitation and APE1 endonuclease activity assays.

2.4. Gadd45α siRNA preparation and transfection

ON-TARGETplus SMARTpool Gadd45α siRNA and Non-targeting siRNA were purchased from Dharmacon, Inc. (Thermo Scientific, USA). Transfections were optimized using different concentrations of siRNA and different cell harvest times. Briefly, one day before transfection, HepG2 cells were plated at 1×10^5 cells/well in 6-well plates. 4 μl of oligofectamine (Invitrogen, USA) was added to 11 μl of serum-free DMEM (Gibco, USA), mixed gently and incubated for 10 min at room temperature. Then 5 μl of stock 20 nmol Gadd45α, siRNA was added to 180 μl of serum-free DMEM and mixed gently. Both siRNA and oligofectamine complexes were mixed and incubated at room temperature for 20 min. While complexes were forming, the growth medium was removed from the cells and 800 μl of serum-free DMEM was added to each well. 200 μl of siRNA and oligofectamine complexes were added to each well, and the 6-well plates were incubated under 5% CO_2 at 37 °C. After 4 h, 500 μl of growth medium containing 3× the normal concentration of serum was added.

2.5. Immunoprecipitation and immunoblotting

In preparation for immunoprecipitation, cell pellets were collected by trypsinization and washed in phosphate-buffered saline (pH 7.4). Harvested cells were homogenized in 500 μl of RIPA buffer containing 50 mM Tris–HCl, pH 7.8, 150 mM NaCl, 0.5 mM EDTA, 0.1% SDS, 1% Triton X-100, 1 mM DTT, protease inhibitors (Roche, Germany) per four 100 mm dishes. The homogenate was incubated for 30 min on ice, sonicated for 5 s and centrifuged at 13,000 rpm for 30 min. The samples were incubated with 2 μg mouse anti-Flag antibody for 12 h and then with 40 μl ExactaCruz™C (Santa Cruz, USA) overnight. After a series of washes, the immunoprecipitated samples were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis [10% (w/v) polyacrylamide].

All immunoblotting was performed as described in Smith et al. [23]. The antibodies were as follows: anti-Flag M2 monoclonal antibody for Gadd45α (F3165, Sigma); anti-PCNA (sc-7907, Santa Cruz); anti-APE1 (NB 100-101, Novus Biologicals). Immunoreactive proteins were detected with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz) and enhanced chemiluminescence (Amersham™ ECL Plus Western Blotting Detection System, GE Healthcare).

2.6. APE1 endonuclease activity assays

Assays were performed as previously described [24]. Briefly, the cells were suspended in 35 μl harvesting buffer containing 20 mM HEPES-KOH (pH 8.0), 10% glycerol, 125 mM NaCl, 0.5 mM EDTA, 1% Triton X-100, 1 mM DTT, and protease inhibitor per well (6-well plate) and kept on ice for 30 min. Debris was removed by centrifugation at 13,000 rpm at 4 °C for 30 min. Oligonucleotides designed for DNA substrates were 17mers containing the abasic site analogue, tetrahydrofuran (THF), at position 9. The complementary oligo with a T opposite THF was also supplied. The sequences were as follows: 5′-AGCATTCGXGACTGGGT-3′, in which X indicates THF; 5′-ACCCAGTCTCGAATGCT-3′ for the complementary strand. The 5′ end-radiolabelling of the oligonucleotides was performed using [γ-^{32}P]ATP and T4 polynucleotide kinase. The radiolabeled strand was then annealed to the complementary strand in 10 mM Tris–HCl (pH 8.0), 100 mM NaCl, and 1 mM EDTA in 90 °C water and

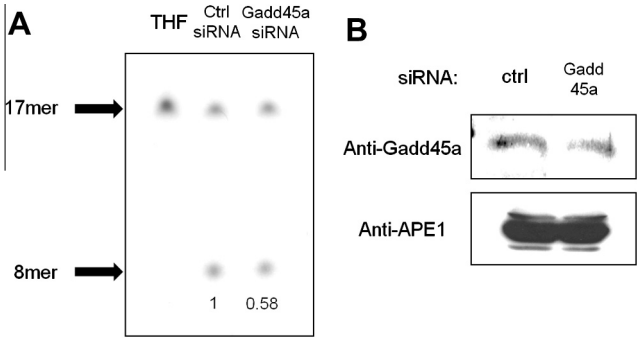

Fig. 1. Reduction of APE1 activity when Gadd45α is silenced. (A) HepG2 cells were transfected with the control or Gadd45α siRNA. APE1 endonucleolytic activity was assessed using the abasic site substrate, THF oligo nucleotide, 24 h after transfection. The APE1 activity of Gadd45α siRNA-treated cells was lower than that of control siRNA-treated cells. (B) Western blots showing relative endogenous levels of Gadd45α and APE1 protein.

cooled to room temperature. APE1 cleavage assays were conducted using 500 ng (Fig. 1) or 2 mg (Fig. 3D) of proteins and 10 pmol of annealed oligo at 37 °C for 15 min in reaction buffer containing 10 mM Tris–HCl (pH 7.5), 50 mM NaCl, and 1 mM EDTA. The substrates were separated from the products in 20% polyacrylamide/7 M urea denaturing gels, and the gels were dried and exposed to X-ray film.

3. Results

3.1. Reduction of APE1 activity in cells treated with Gadd45α siRNA

To investigate whether Gadd45α can affect BER activity based on its interaction with APE1, we performed APE1 endonuclease activity assays after manipulating the levels of Gadd45α. The THF-containing substrate, 17 nucleotides long with the abasic site analogue at position 9, was used as the test lesion for assessing APE1 activity. Cleavage at the abasic site by APE1 results in a 8mer comprised of the 5′ labeled substrate. We found that the level of cleaved product in cells transfected with Gadd45α siRNA was lower than in cells transfected with control siRNA (Fig. 1A). While the APE1 activity was lower in cells transfected with Gadd45α siRNA, the level of APE1 protein was not (Fig. 1B). This result implies that endogenous Gadd45α might be involved in the regulation of BER activity by affecting APE1 function.

3.2. Identification and empirical validation of the PCNA binding site on Gadd45α

To identify the PCNA binding site on Gadd45α, sequence comparisons and secondary structure predictions were employed. It has been suggested that the C-terminal region of Gadd45α (amino acids 137–165) has the capacity to bind PCNA [25]. We therefore analyzed the potential PCNA binding motif on Gadd45α, assuming that the interaction site is accessible to PCNA and that it is conserved within the Gadd45α family of sequences. The assumption is that a highly relevant interaction should be evolutionarily conserved (Fig. 2A). By *in silico* analysis we identified a conserved, surface-exposed sequence comprised of the WVP tripeptide (amino acids 156–158) in the C-terminal PCNA interacting domain of Gadd45α (Fig. 2B). To confirm whether PCNA binds to the identified motif of Gadd45α protein, WVP was mutated to a VAV sequence.

To determine whether PCNA protein binds to the identified PCNA binding site on Gadd45α, we investigated the interaction of Gadd45α with PCNA in flag-tagged wild-type or mutant Gadd45α-transfected cells. As shown in Fig. 3A, Gadd45α interacted effectively with PCNA in cells that overexpress wild-type Gadd45α, but was significantly lower in cells that overexpress mutant Gadd45α. Furthermore, interaction of Gadd45α with APE1 was significantly lower in cells transfected with mutant Gadd45α, compared to cells transfected with wild-type Gadd45α (Fig. 3B). The PCNA, APE1, and GAPDH proteins were expressed equally in cells transfected with wild-type or mutant Gadd45α, indicating that the protein–protein interactions were not affected by respective their overall levels. These data suggest that the PCNA binding site on the Gadd45α protein is a critical region regulating the interaction of Gadd45α with APE1.

3.3. Reduction of APE1 activity in mutant Gadd45α-transfected cells

To validate the functional relevance of the Gadd45α WVP sequence and demonstrate the effects by decreased interaction of mutant Gadd45α with PCNA on APE1 activity, we compared APE1 endonuclease activity in the Gadd45α-interacting complex after immunoprecipitation with flag-tagged wild-type or mutant Gadd45α (Fig. 3C). Our data showed a decreased level of cleaved THF-containing oligonucleotide with mutant Gadd45α compared to that with the wild-type (Fig. 3D), indicating that the decreased interaction of Gadd45α with PCNA might affect BER activity.

4. Discussion

Gadd45α was initially identified as a p53 regulated protein that interacts with PCNA with the suggestion that it might play a role in NER [8]. Subsequently, the domain of Gadd45α that physically interacts with PCNA was identified [25,26]. The specific binding sites within Gadd45α, however, have yet to be identified. To provide further insight into the mechanism of Gadd45α-mediated repair, we have now defined a PCNA binding site on Gadd45α and investigated whether the interaction of Gadd45α with PCNA affects APE1 activity.

Recently, we showed that Gadd45α −/− MEF cells exhibit decreased interaction of Gadd45α with APE1, suggesting that Gadd45α could largely contribute to the BER mechanism [12]. In this study, we have shown a physical interaction between Gadd45α and APE1 and have observed altered APE1 activity in Gadd45α siRNA-treated cells. Together, these data suggest a role for Gadd45α in APE1 activity and consequently in BER. APE1 activity is critical for BER as the predominant activity for excision of AP sites induced by DNA damage [27]. The reduction of APE1 activity in cells treated with Gadd45α siRNA suggests that endogenous Gadd45α might modulate BER activity, although the mechanisms whereby APE1 activity is affected by Gadd45α have not yet been clarified.

In silico analysis of Gadd45α identified a potential PCNA binding motif that was validated by site directed mutagenesis and subsequent immunoprecipitation studies implicating the importance of this site for interaction with PCNA. It is possible that such a binding site may play a physiological role in modulating PCNA interactions with Gadd45α in response to damaged DNA. This proposition is supported by the observation that the C-terminal domain of Gadd45α containing amino acids 137–165 is involved in the interaction with PCNA. Significantly, this domain includes the PCNA binding site identified *in silico* and validated experimentally. Furthermore, some clinicopathological research related to mutations of the gadd45α gene has been reported [28]. These were primarily within exon 4 in 13.6% of tumors from 59 patients with invasive ductal carcinomas of the pancreas, and one of the eight point mutations fell within the PCNA binding domain of Gadd45α. Although it is not yet known whether the identified mutations compromise Gadd45α-mediated interaction and function, our results suggest that the mutations at and around the predicted PCNA

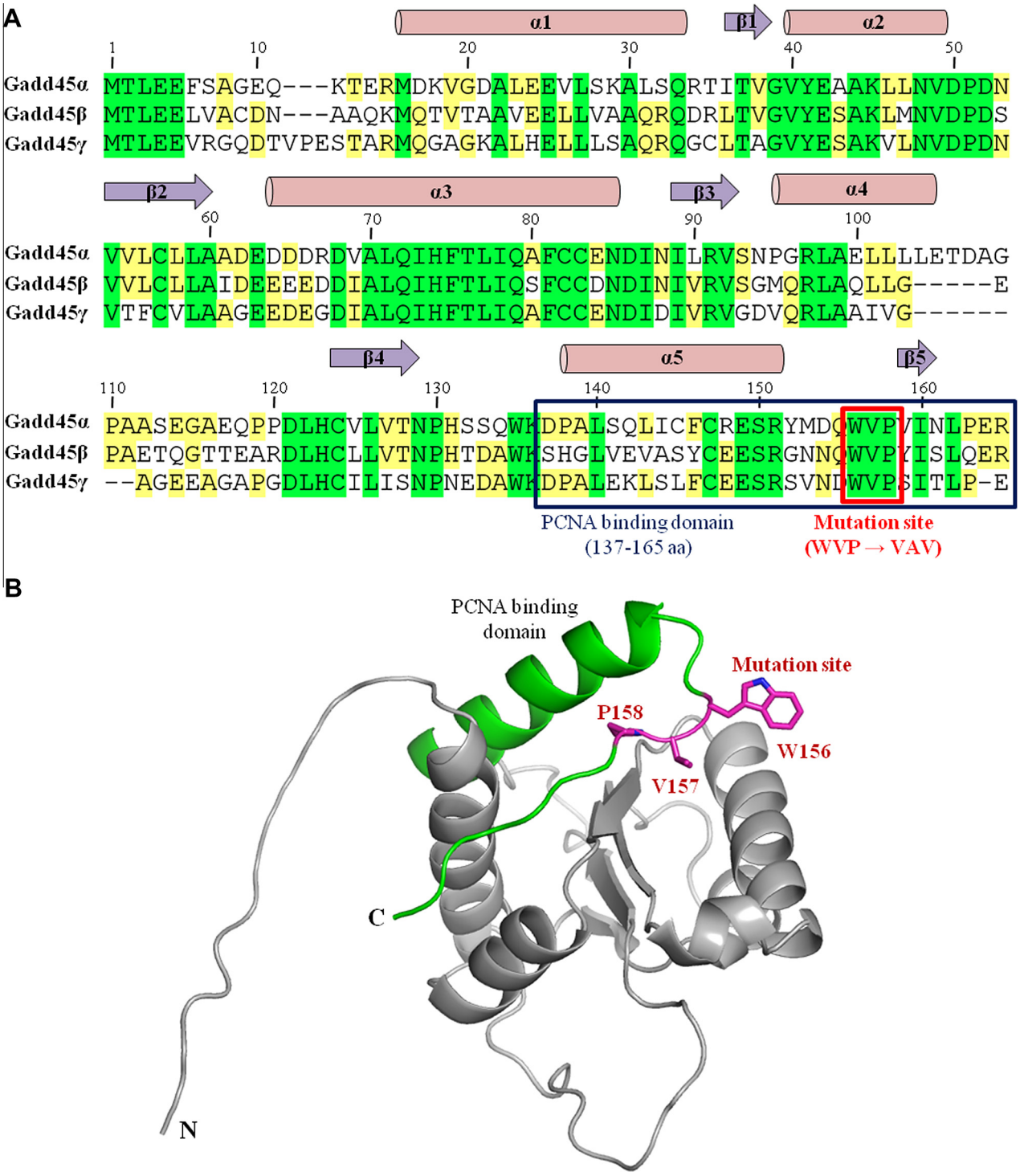

Fig. 2. Sequence alignment and structure of Gadd45α. (A) A sequence alignment of Gadd45α (SWISS-PROT accession code: P24533), Gadd45β (O75923), and Gadd45γ (O95257). Highly conserved residues and partially conserved residues are shaded in green and yellow, respectively. PCNA binding site residues are indicated by the open red box. To demonstrate whether PCNA binds to the identified site of Gadd45α, the predicted binding site was mutated by site-directed mutagenesis and designated WVP466VAV. This figure was drawn with ClustalX [32] and GeneDoc (http://www.nrbsc.org/downloads/). (B) Ribbon diagram of Gadd45α (PED ID code: 2KG4). The PCNA binding sites including Trp156, Val157, and Pro158 are displayed as purple sticks. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

binding site on Gadd45α may contribute to the induction of pancreatic cancer.

The interaction of Gadd45α with multiple repair proteins appears to be an important component in DNA repair. Our group has previously reported the importance of a complex composed of Gadd45α, PCNA, and APE1, providing a potential mechanism for Gadd45α-mediated regulation of BER [12]. Our data now further support the regulation of APE1 function by the binding of Gadd45α to PCNA as well as to APE1, whose interaction with mutant Gadd45α is reduced when compared with wild-type Gadd45α. In aggregate, the data suggest that the interaction of Gadd45α with PCNA is related to both the BER and NER pathways. Although the

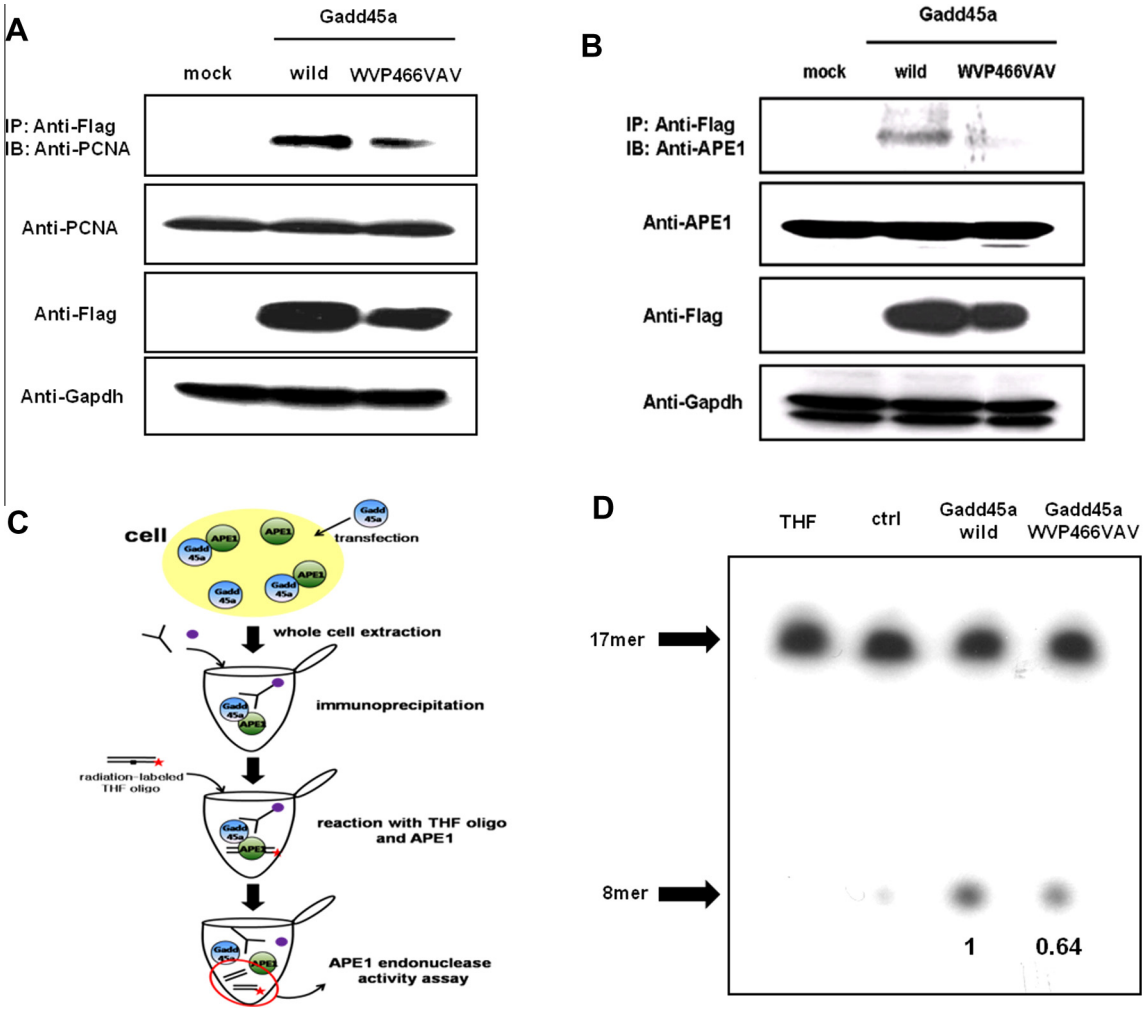

Fig. 3. Altered interaction of Gadd45α with PCNA and APE1 as well as APE1 activity in mutant Gadd45α-transfected cells. (A) Flag-tagged wild-type and mutant Gadd45α plasmids were transfected into HepG2 cells and 48 h later were harvested with RIPA buffer, and subjected to immunoprecipitation using an anti-flag antibody. PCNA antibody was used as a primary antibody for Western blot analysis. In mutant Gadd45α-transfected cells, the interaction of Gadd45α with PCNA was lower. (B) After anti-flag immunoprecipitation, APE1 antibody was used for Western blot analysis. The interaction of Gadd45α with APE1 was likewise lower in cells overexpressing Gadd45α mutant. (C) The APE1 activity, using the THF oligonucleotide substrate, was compared 48 h following transfection of cells with wild-type and mutant Gadd45α vectors. Cells were harvested using APE1 lysis buffer. (D) APE1 activity in the Gadd45α-interacting complex was lower in cells that overexpress mutant Gadd45α compared with those that overexpress wild-type Gadd45α.

data indicate that Gadd45α may modulate APE1 function, it remains unclear whether Gadd45α interacts directly with APE1 or indirectly as part of a complex with other repair proteins.

The modulation of APE1 activity either by direct binding of Gadd45α to APE1 or indirectly by association of Gadd45α with PCNA, or by some other mechanism, is of functional importance since in any of these cases reduction of APE1 activity should result in compromised BER. The absence of or significant reduction of APE1 activity is associated with human disease. The APE1 enzyme is redox sensitive, and several studies have reported an altered redox-associated function of APE1 that may accompany redox-induced neurotoxicity and cancer development [29,30]. Our results, therefore, suggest that the interaction of Gadd45α with PCNA can be considered a general mechanism that contributes to these genetic based diseases. The reduced APE1 activity that accompanies the decrease of APE1 interaction with Gadd45α also indicates that the activity of repair proteins is affected by protein–protein interactions. This is not a new notion for DNA repair processes since He et al. [31] proposed that direct contact between XPG and RPA-XPA bound to DNA lesions as a complex in the NER process could be important for targeting the endonuclease activity of XPG to its sub-

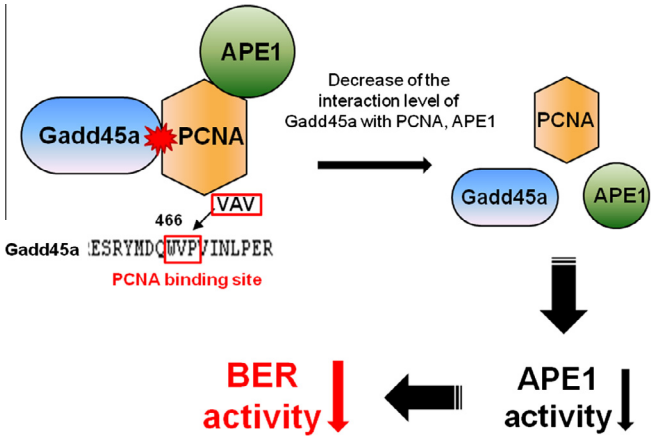

Fig. 4. Model depicting the decrease in interaction between Gadd45α and PCNA and the reduction of APE1 activity as a consequence of mutating the PCNA-interacting site on Gadd45α. The data and model emphasize the importance of Gadd45α-mediated protein–protein interaction in regulation of the base excision repair (BER) activity.

strate. Thus, our results serve as additional evidence showing a protein–protein interaction that affects endonuclease activity *in vivo*.

In conclusion, our data suggest a critical role for Gadd45α in modulating BER activity via interaction with PCNA. We showed for the first time that the interaction site between Gadd45α and PCNA, which we have identified, might ultimately affect the activity of APE1, an enzyme that is important for AP site removal in the BER pathway (Fig. 4). Our results provide novel insights for understanding mechanisms of BER activity modulation although the physical interaction of Gadd45α with APE1 still needs to be elucidated.

Acknowledgments

We thank Dr.Jae Young Lee for helping with the sequence alignment and structure of Gadd45α. This work was supported by the Dongguk University Research Fund of 2010 (S-2010-001037).

References

[1] H. Offer, M. Milyavsky, N. Erez, D. Matas, I. Zurer, C.C. Harris, V. Rotter, Structural and functional involvement of p53 in BER in vitro and in vivo, Oncogene 20 (2001) 581–589.
[2] Y.R. Seo, H.J. Jung, The potential roles of p53 tumor suppressor in nucleotide excision repair (NER) and base excision repair (BER), Exp. Mol. Med. 6 (2004) 505–509.
[3] M. Savio, L.A. Stivala, L. Bianchi, V. Vannini, E. Prosperi, Involvement of the proliferating cell nuclear antigen (PCNA) in DNA repair induced by alkylating agents and oxidative damage in human fibroblasts, Carcinogenesis 19 (1998) 591–596.
[4] F. Carrier, M. Smith, I. Bae, K.E. Kilpatrick, T.J. Lansing, C.Y. Chen, M. Engelstein, S.H. Friend, W.D. Henner, T.M. Gilmer, M.B. Kastan, A.J. Fornace Jr., Characterization of human Gadd45, a p53-regulated protein, J. Biol. Chem. 269 (1994) 32672–32677.
[5] R. Kettenhofen, J. Hoppe, G. Eberhard, C. Seul, Y. Ko, A. Sachinidis, Regulation of Gadd45α mRNA expression in vascular smooth muscle under growth and stress conditions, Cell. Signal. 13 (2001) 787–799.
[6] M.L. Smith, J.M. Ford, M.C. Hollander, R.A. Bortnick, S.A. Amundson, Y.R. Seo, C.X. Deng, P.C. Hanawalt, A.J. Fornace Jr., P53-mediated DNA repair responses to UV-radiation: Studies of mouse cells lacking p53, p21, and/or gadd45 gene, Mol. Cell. Biol. 20 (2000) 3705–3714.
[7] T. Maeda, R.A. Espino, E.G. Chomey, L. Luong, A. Bano, D. Meakins, V.A. Tron, Loss of p21WAF1/Cip1 in Gadd45-deficient keratinocytes restores DNA repair capacity, Carcinogenesis 26 (2005) 1804–1810.
[8] M.L. Smith, I.T. Chen, Q. Zhan, I. Bae, C.Y. Chen, T.M. Gilmer, M.B. Kastan, P.M. O'Connor, A.J. Fornace Jr., Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen, Science 266 (1994) 1376–1380.
[9] D.J. Hosfield, C.D. Mol, B. Shen, J.A. Tainer, Structure of the DNA repair and replication endonuclease and exonuclease FEN-1: coupling DNA and PCNA binding to FEN-1 activity, Cell 95 (1998) 135–146.
[10] Z. Kelman, J. Hurwitz, Protein-PCNA interactions: a DNA-scanning mechanism?, Trends Biochem Sci. 23 (1998) 236–238.
[11] E. Warbrick, The puzzle of PCNA's many partners, Bioessays 22 (2000) 997–1006.
[12] H.J. Jung, E.H. Kim, J.Y. Mun, S. Park, M.L. Smith, S.S. Han, Y.R. Seo, Base excision DNA repair defect in Gadd45α-deficient cells, Oncogene 26 (2007) 7517–7525.
[13] I.I. Dianova, V.A. Bohr, G.L. Dianov, Interaction of human AP endonuclease 1 with flap endonuclease 1 and proliferating cell nuclear antigen involved in long-patch base excision repair, Biochemistry 40 (2001) 12639–12644.
[14] L. Xia, L. Zheng, H.W. Lee, S.E. Bates, L. Federico, B. Shen, T.R. O'Connor, Human 3-methyladenine-DNA glycosylase: effect of sequence context on excision, association with PCNA, and stimulation by AP endonuclease, J. Mol. Biol. 346 (2005) 1259–1274.
[15] L.A. Loeb, B.D. Preston, Mutagenesis by apurinic/apyrimidinic sites, Annu. Rev. Genet. 20 (1986) 201–230.
[16] J. Nakamura, J.A. Swenberg, Endogenous apurinic/apyrimidinic sites in genomic DNA of mammalian tissues, Cancer Res. 59 (1999) 2522–2526.
[17] E.L. Goode, C.M. Ulrich, J.D. Potter, Polymorphisms in DNA repair genes and associations with cancer risk, Cancer Epidemiol. Biomarkers Prev. 11 (2002) 1513–1530.
[18] L. Chen, C.B. Ambrosone, J. Lee, T.A. Sellers, J. Pow-Sang, J.Y. Park, Association between polymorphisms in the DNA repair genes XRCC1 and APE1, and the risk of prostate cancer in white and black americans, J. Urol. 175 (2006) 108–112.
[19] J. Zhou, J. Ahn, S.H. Wilson, C. Prives, A role for p53 in base excision repair, EMBO J. 20 (2001) 914–923.
[20] H.J. Jung, J.H. Lee, Y.R. Seo, Enhancement of methyl methanesulfonate (MMS)-induced base excision repair (BER) in the presence of selenomethionine (SeMet) on p53-dependent Pathway, J. Med. Food 12 (2009) 340–344.
[21] B. Rost, C. Sander, Prediction of protein secondary structure at better than 70% accuracy, J. Mol. Biol. 232 (1993) 584–599.
[22] B. Rost, C. Sander, R. Schneider, PHD–an automatic mail server for protein secondary structure prediction, Comput. Appl. Biosci. 10 (1994) 53–60.
[23] M.L. Smith, I.T. Chen, Q. Zhan, P.M. O'Connor, A.J. Fornace Jr., Involvement of the p53 tumor suppressor in repair of u.v.-type DNA damage, Oncogene 10 (1995) 1053–1059.
[24] K. Hahn, R.C. Faustoferri, R.G. Quivey Jr., Induction of an AP endonuclease activity in streptococcus mutans during growth at low pH, Mol. Microbiol. 31 (1999) 1489–1498.
[25] M. Vairapandi, N. Azam, A.G. Balliet, B. Hoffman, D.A. Liebermann, Characterization of MyD118, Gadd45, and proliferating cell nuclear antigen (PCNA) interacting domains, J. Biol. Chem. 275 (2000) 16810–16819.
[26] P.A. Hall, J.M. Kearsey, P.J. Coates, D.G. Norman, E. Warbrick, L.S. Cox, Characterisation of the interaction between PCNA and Gadd45, Oncogene 10 (1995) 2427–2433.
[27] B. Demple, L. Harrison, Repair of oxidative damage to DNA: enzymology and biology, Annu. Rev. Biochem. 63 (1994) 915–948.
[28] K. Yamasawa, Y. Nio, M. Dong, K. Yamaguchi, M. Itakura, Clinicopathological significance of abnormalities in Gadd45 expression and its relationship to p53 in human pancreatic cancer, Clin. Cancer Res. 8 (2002) 2563–2569.
[29] M.R. Vasko, C. Guo, M.R. Kelley, The multifunctional DNA repair/redox enzyme Ape1/Ref-1 promotes survival of neurons after oxidative stress, DNA Repair (Amst) 4 (2005) 367–379.
[30] Y.L. Lo, Y.S. Jou, C.F. Hsiao, G.C. Chang, Y.H. Tsai, W.C. Su, K.Y. Chen, Y.M. Chen, M.S. Huang, C.Y. Hu, C.J. Chen, C.A. Hsiung, A polymorphism in the APE1 gene promoter is associated with lung cancer risk, Cancer Epidemiol. Biomarkers Prev. 18 (2009) 223–229.
[31] 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.
[32] J.D. Thompson, T.J. Gibson, F. Plewniak, F. Jeanmougin, D.G. Higgins, The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools, Nucleic Acids Res. 25 (1997) 4876–4882.