Biochemical and Biophysical Research Communications 454 (2014) 518–523

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

p53 negatively regulates Pin1 expression under ER stress

Kwon Jeong [a,1], Seong-Jin Kim [b,1], Yoojung Oh [a], Hunsung Kim [a], Young-Seok Lee [a], Byung-Su Kwon [c], Sanghyun Park [a], Key-Chung Park [d], Kyung-Sik Yoon [a], Sung Soo Kim [a], Joohun Ha [a], Insug Kang [a], Wonchae Choe [a,*]

[a] Department of Biochemistry and Molecular Biology (BK21 Project), Medical Research Center for Bioreaction to Reactive Oxygen Species and Biomedical Science Institute, School of Medicine, Kyung Hee University, Seoul 130-701, Republic of Korea
[b] Neurodegeneration Control Research Center, School of Medicine, Kyung Hee University, Seoul 130-701, Republic of Korea
[c] Department of Obstetrics and Gynecology, Kyung Hee University, Seoul 130-701, Republic of Korea
[d] Department of Neurology, School of Medicine, Kyung Hee University, Seoul 130-701, Republic of Korea

ARTICLE INFO

Article history:
Received 10 October 2014
Available online 27 October 2014

Keywords:
ER stress
Pifithrin-α
Pin1
p53
p53REs

ABSTRACT

Accumulating evidence suggests that endoplasmic reticulum (ER) stress plays a major role in the development of many diseases. A previous study indicated that the apoptotic regulator p53 is significantly increased in response to ER stress and participates in ER stress-induced apoptosis. However, the regulators of p53 expression during ER stress are still not fully understood. Here, we investigated whether p53 contributes to the impairment of Pin1 signaling under ER stress. We found that treatment with thapsigargin, a stimulator of p53 expression and an inducer of ER stress, decreased Pin1 expression in HCT116 cells. Also, we identified functional p53 response elements (p53REs) in the *Pin1* promoter. Overexpression of p53 significantly decreased Pin1 expression in HCT116 cells while abolition of *p53 gene* expression induced Pin1 expression. Pin1 expression was significantly increased by treatment with the p53 inhibitor pifithrin-α or down-regulation of p53 expression. Taken together, ER stress decreased Pin1 expression through p53 activation, and this mechanism may be associated with ER stress-induced cell death. These data reported here support the importance of Pin1 as a potential target molecule mediating tumor development.

1. Introduction

The endoplasmic reticulum (ER) is an organelle that plays an important role in protein folding and post-translational modification. When protein folding fails, misfolded proteins accumulate in the ER, which leads to cell stress called ER stress [1]. The ER senses alterations in intracellular homeostasis provoked by stimuli, subsequently inducing the unfolded protein response (UPR) [2–5]. The UPR decreases protein translation, increases chaperone expression, and accelerates the degradation of misfolded proteins to alleviate cellular stress and restore ER homeostasis via three main signal transducers. These transducers include inositol-requiring enzyme-1α (IRE-1α), PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6) [6–8]. Severe or prolonged ER stress induces the activation of unique pathways that lead to cell death through apoptosis. Recently, several pathways have been directly implicated in ER stress-induced apoptosis, including the caspase-12/caspase-4, CHOP/GADD153, IRE1/PERK/JNK, and p53 signaling pathways [9–12]. Previous studies have indicated that NF-κB signaling is required for ER stress-induced p53 expression [13]. Elevation of p53 expression during ER stress in MEF, MCF-7, and HCT116 cells has been reported [12], and p53 has been shown to play an important role in ER dysregulation [1,14].

Pin1 is the peptidyl-prolyl *cis*/*trans* isomerase (PPIase) that binds and isomerizes specific phosphorylated serine/threonine-proline (pS/pT-P) motifs in a subset of proteins, resulting in protein conformational changes [15,16]. Pin1 has been shown to regulate the function of several key proteins that are involved in various cellular stress responses. Pin1 regulates cell growth and apoptosis by catalyzing the isomerization of various molecules [17,18]. Pin1 is also involved in controlling the accumulation and activation of p53 in cells exposed to DNA damaging agents [19]. Pin1 can also enhance the DNA binding activity and transcriptional activity of both p53 and p73 towards their target genes [19,20]. Functionally, Pin1 is required for maintaining DNA damage checkpoints that protect cells from DNA damage-induced apoptosis [19], and can

* Corresponding author at: Department of Biochemistry and Molecular Biology, School of Medicine, Kyung Hee University, #1 Hoegi-dong, Dongdaemoon-gu, Seoul 130-701, Republic of Korea. Fax: +82 2 959 8168.
E-mail address: wchoe@khu.ac.kr (W. Choe).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.10.101
0006-291X/

also induce apoptosis by enhancing pro-apoptotic gene expression [20]. However, the exact molecular mechanism by which Pin1 mediates apoptotic regulation under ER stress is unknown.

Since ER stress activates p53, we hypothesized that ER stress might regulate Pin1 expression through p53 activation. To test this hypothesis, we investigated the effect of p53 on Pin1 expression in HCT116 colon cancer cells and conducted promoter analysis to characterize the p53-responsive element in the *Pin1* promoter. In this report, we show for the first time that p53 negatively regulates Pin1 expression under ER stress and that Pin1 plays a preventive role in p53-mediated apoptosis under ER stress.

2. Materials and methods

2.1. Cell culture and reagents

The parental HCT116 human colon adenocarcinoma cell line containing the wild-type p53 ($p53^{+/+}$) and a p53-deficient derivative ($p53^{-/-}$) were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) in humidified air containing 5% CO_2 at 37 °C. RPMI 1640 and FBS were obtained from Hyclone Laboratories, Inc. (South Logan, Utah, USA). All antibodies were obtained from Cell Signaling Technology (Danvers, MA, USA) or Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA), and chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Plasmids and transient transfection

A cDNA encoding *Pin1* or *p53* was subcloned into a pcDNA (Invitrogen, Carlsbad, CA, USA) between the HindIII and EcoRI restriction sites for protein expression that tagged the protein with a Flag-tag or HA-tag at the C-terminal end. Regions of the human *Pin1* promoter (−2.7 kb) were amplified by PCR (reverse transcription polymerase chain reaction) from human genomic DNA and inserted into the KpnI/XhoI restriction sites in the pGL3 basic vector (Promega, Madison, WI, USA) using the following primers: forward 5′-CGGGGTACCATTTCCTTGCTCTATCCA-3′ and reverse 5′-AAACTCGAGGCTCCGCCTCAGCTGCGC-3′. A deletion mutant reporter construct lacking putative p53 binding sites was constructed in the *Pin1* promoter (−1.0 kb) using a two-step PCR method. HCT116 cells were transfected with Pin1 expression vectors using the X-tremeGENE™ HP DNA transfection reagent from Roche Diagnostics, (Indianapolis, IN, USA). After a 24 h incubation period, growth media was replaced and cells were grown for an additional 24 h.

2.3. Cell viability assay

Cell viability was measured in 96-well plates using a quantitative colorimetric assay with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), which is an indicator of mitochondrial activity in living cells. Briefly, 30 μl MTT (final concentration, 0.5 mg/mL) was added to the medium at the indicated time after treatment and then incubated at 37 °C for 4 h. The MTT solution was removed, and 100 μl DMSO was added to each well. The absorbance of each aliquot was measured at 540 nm using a microplate reader (Bio–Rad).

2.4. Real-time PCR analysis

Total cellular RNA was extracted from cells using the TRIzol reagent (Invitrogen). A cDNA was synthesized from 1.0 μg total RNA using M-MLV reverse transcriptase (Fermentas, Hanover, MD, USA). The specific primers for RT-PCR included the following:

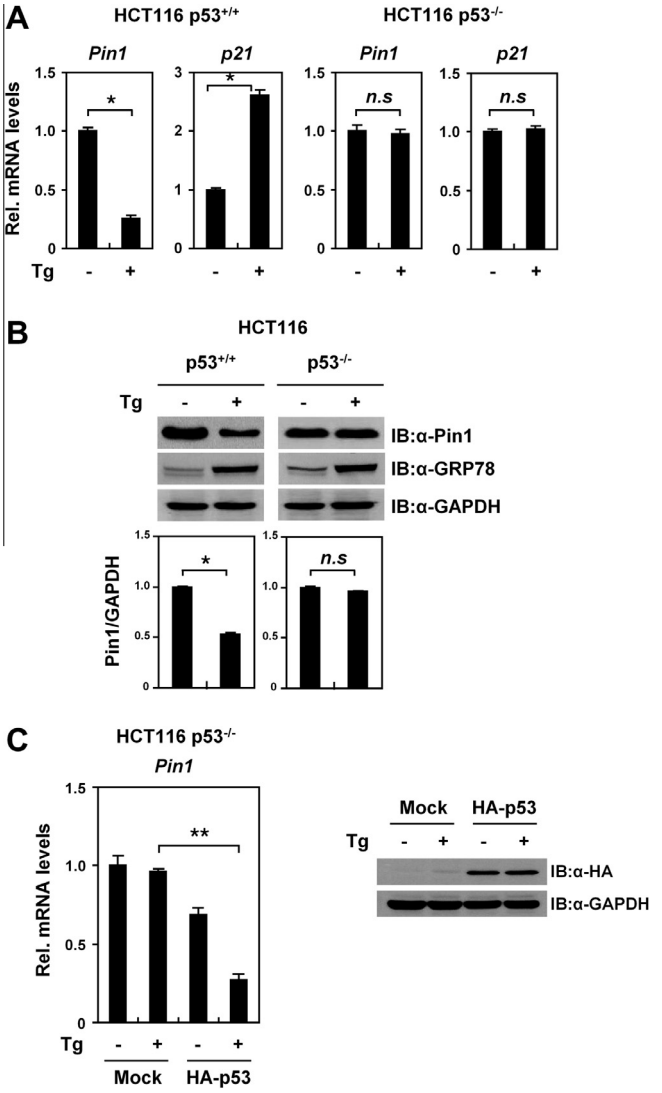

Fig. 1. p53 down-regulates Pin1 expression under ER stress in HCT116cells. (A) HCT116 $p53^{+/+}$ or $p53^{-/-}$ cells were treated with 1.0 μM thapsigargin. After 24 h, cells were harvested and total RNAs were extracted. *Pin1* and *p21* mRNA levels were analyzed by real-time PCR. (B) HCT116 $p53^{+/+}$ or $p53^{-/-}$ cells treated with 1.0 μM thapsigargin for 24 h were analyzed by Western blot for Pin1 and GRP78 protein. GAPDH is shown as an internal standard. (C) p53 reduced human *Pin1* mRNA levels. Both pcDNA and p53 expression plasmids were used to transfect HCT116 $p53^{-/-}$ cells, which were then treated with 1.0 μM thapsigargin for 24 h. After transfection, the cells were assayed by real-time PCR. The data are expressed as fold increase relative to the untreated control. The values are shown as the mean ± SD of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, and n.s., not significant.

Pin1, forward 5′-TGCCACCGTCACACAGTATT-3′ and reverse 5′-CTGACCAGGCCTTCTCTTTG-3′; *p21*, forward 5′-GCAGACCAGCATGACAGATTTC-3′ and reverse 5′-GGATTAGGGCTTCCTCTTGGA-3′; and GAPDH, forward 5′-CAAGGTCATCCATGACAACTTTG-3′ and reverse 5′-GTCCACCACCCTGTTGCTGTAG-3′. Real-time PCR was performed using an ABI prism 7300 Sequence Detection System (Applied Biosystems, Branchburg, NJ, USA) with SYBRGreen PCR Master Mix (Applied Biosystems). The PCR reaction was carried out for 40 thermal cycles. Expression of the target gene was analyzed by an absolute quantification method and normalized using GAPDH levels.

2.5. Western blot analysis

Cells were lysed in NP40 lysis buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1% NP40, 2 mM EDTA, 10 mM NaF, 2 mM Na_3VO_4,

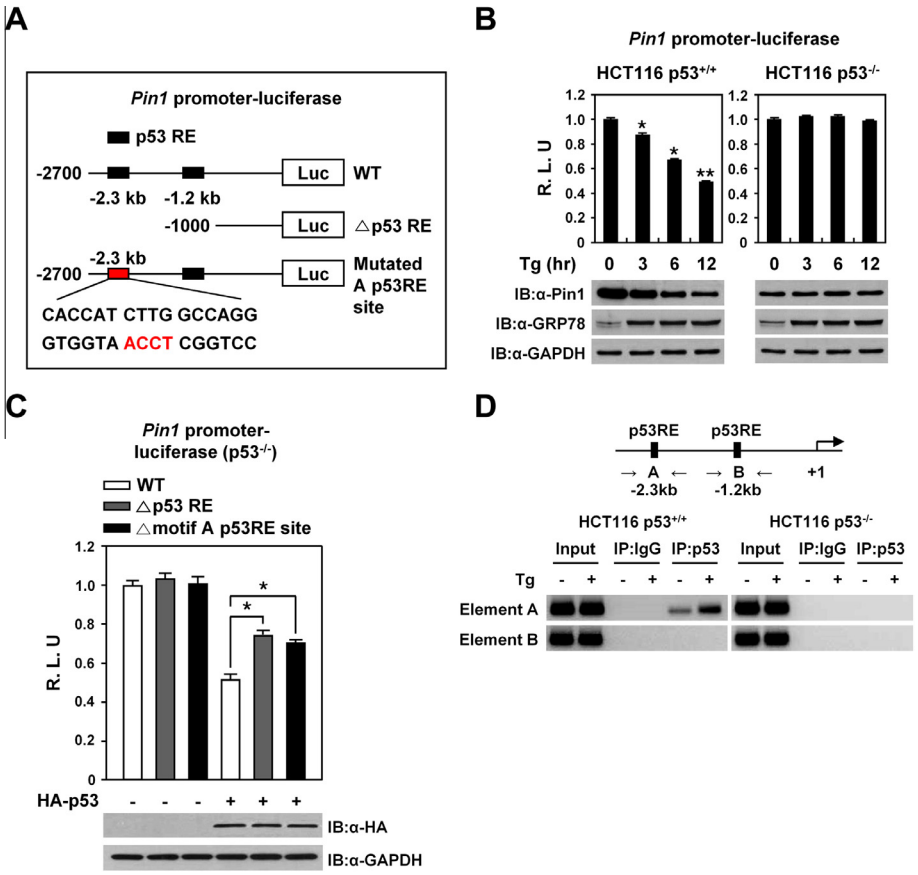

Fig. 2. p53 represses human *Pin1* promoter activity. (A) A schematic representation of the *Pin1* promoter region. The *Pin1* promoter–luciferase fusion (WT; from −2700 to +100), p53REs-deleted *Pin1* promoter–luciferase fusion (Δp53RE; from −1200 to +100) and *Pin1* promoter–luciferase fusion containing the mutated A p53RE site at position −2300 (mutated motif A p53RE; the nucleotide sequence changed from "CTTG" to "ACCT"). The p53REs are shown in bold. (B) *Pin1* promoter–luciferase fusion plasmids were used to transfect HCT116 $p53^{+/+}$ or $p53^{-/-}$ cells. Luciferase activity was measured after treatment with 1.0 μM thapsigargin at the indicated times and normalized to β-galactosidase activity. (C) Introduction of p53 plasmids into HCT116 $p53^{-/-}$ cells decreased the wild-type *Pin1* promoter–luciferase activity, but did not significantly change the p53REs-deleted or mutated motif A p53RE *Pin1* promoter–luciferase activity. Luciferase activity was measured after treatment with 1.0 μM thapsigargin for 24 h and normalized to β-galactosidase activity. (D) HCT116 cells were treated with or without 1.0 μM thapsigargin for 24 h and then p53 binding to the element A and B regions of the *Pin1* promoter was analyzed by ChIP assay. The values are shown as the mean ± SD of three independent experiments. $^{*}p < 0.05$.

and 0.01% protease inhibitor cocktail). Protein concentrations of the total lysates were determined by the Bradford method (Bio–Rad, Hercules, CA, USA). Twenty micrograms of protein from the total lysates were subjected to SDS-PAGE, transferred to Immobilon-P membranes (Millipore, Bedford, MA), and incubated with primary antibodies in blocking solution (5% skim milk). Proteins were visualized using the ECL-plus detection system (Santa Cruz).

2.6. Luciferase assay

HCT116 cells were co-transfected with 0.8 μg of the reporter vector together with 0.4 μg *p53* of expression vector. The empty pcDNA vector was used to adjust the total amount of DNA and 0.8 μg of pCMV-β-galactosidase expression plasmid was used as an internal control. Luciferase activity was determined using a luciferase assay kit (Promega) according to the manufacturer's instructions. The relative luciferase activity was normalized against the β-galactosidase activity.

2.7. Chromatin immunoprecipitation (ChIP) assay

The ChIP assay was conducted using the protocol according to the manufacturer's instructions (Upstate Biotechnology, Lake Placid, NY, USA). Primers used were as follows: element A, forward 5′-CCAGGGGTTCAAGCAATTTTC-3′ and reverse 5′-AAATAAAAG GCTGGGTGCGGT-3′ and element B, forward 5′-ACCCACCTCGGCCT TCCAAAG-3′ and reverse 5′-GAGCCAGACTTGGGTTTAAAG-3′.

2.8. Site-directed mutagenesis

DpnI-mediated site-directed mutagenesis was employed for the generation of mutant p53 binding site. PCR was performed using 50 ng of DNA template and a QuikChange Site-directed mutagenesis kit (Stratagene) was used according to the instruction manual. A mutant reporter construct containing a putative p53 binding sites in the *Pin1* promoter (−2.3 kb) was generated by primers 5′-TTTTC ACCATACCTGCCAGGCTGG-3′ (forward) and 5′-AAAAGTGGTATGGA CGGTCCGACC-3′ (reverse). After PCR amplification, DpnI endonuclease was added and the mixture was incubated at 37 °C for 2 h to allow for digestion of the parental methylated DNA. The DpnI-treated dsDNA was used to transform DH5α competent cells. Colonies were selected and the mutations were confirmed by DNA sequencing.

2.9. TUNEL assay

The TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end-labeling) assay was conducted by using the Apo-BrdU *in situ* DNA Fragmentation Assay Kit following the protocol of the supplier (BioVision, Mountain View, CA). The TUNEL

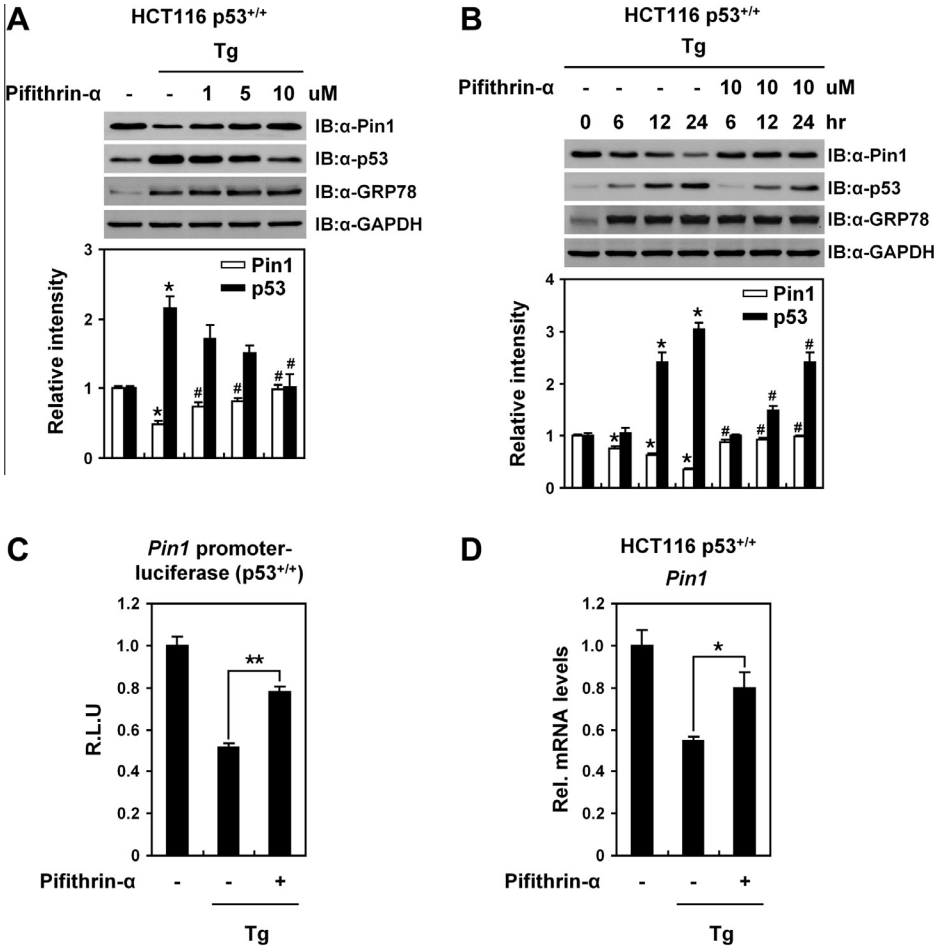

Fig. 3. Regulation of Pin1 expression depends on p53 under ER stress. Effect of the p53 pharmacologic inhibitor pifithrin-α on p53 activity. (A, B) HCT116 $p53^{+/+}$ cells were cultured with 1.0 μM thapsigargin after treatment with or without pifithrin-α at the indicated doses and times. Pin1, p53, and GRP78 protein levels were analyzed by Western blot. (C) HCT116 $p53^{+/+}$ cells transiently transfected with the wild-type *Pin1* promoter construct were cultured with 1.0 μM thapsigargin after treatment with or without pifithrin-α for 24 h. Luciferase activity is expressed relative to that of non-treated cells. (D) The effect of pifithrin-α on *Pin1* mRNA expression in HCT116 $p53^{+/+}$ cells was monitored by to real-time PCR analysis. Total RNA was isolated from HCT116 $p53^{+/+}$ cells and then subjected to RT-PCR analysis. The values shown are the mean ± SD of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{\#}p < 0.05$.

reaction was performed according to the manufacturer's instructions. The number of apoptotic cells is expressed as a percentage of the total cells counted from five random fields for each cover slip.

2.10. Statistical analysis

The results are expressed as the mean ± SD from at least three independent experiments. Statistical analyses were conducted using Student's *t*-tests. By convention, a *p* value of <0.05 was considered statistically significant.

3. Results

3.1. Pin1 expression is p53 dependent under ER stress

To evaluate whether p53 regulates Pin1 expression, we first examined the effect of the ER stress-inducer thapsigargin on *Pin1* transcript level in HCT116 colon cancer cells. As shown in Fig. 1A, treatment with thapsigargin decreased Pin1 mRNA level in the presence of p53, concomitant with an increase in *p21* mRNA level, which is a gene known to be induced by DNA damage [21]. However, in the absence of p53 (HCT116 $p53^{-/-}$), treatment with thapsigargin had no effect on either *Pin1* or *p21* mRNA levels. Next, Pin1 protein production was also observed upon treatment with thapsigargin with GRP78 protein production used as a positive control for ER stress (Fig. 1B). In the presence of p53, treatment with thapsigargin decreased Pin1 protein level, consistent with the mRNA results. In the absence of p53, Pin1 protein level was not changed by treatment with thapsigargin [22]. In order to confirm these results, we compared *Pin1* transcript levels upon thapsigargin treatment in the presence or absence of p53. Real-time PCR analysis revealed that ectopic expression of p53 significantly reduced *Pin1* transcript levels (Fig. 1C). Taken together, these results demonstrate that p53 negatively regulates Pin1 expression under ER stress.

3.2. p53 down-regulates Pin1 promoter activity

To further understand how Pin1 is down-regulated by p53 during ER stress, we examined the effect of p53 on *Pin1* promoter activity. Using a promoter analysis program, two potential p53-responsive elements (REs) were identified in an upstream 2.7 kb noncoding region of the *Pin1* gene (Fig. 2A). We then examined whether the presence of this DNA segment was sufficient to reduce luciferase reporter activity upon thapsigargin treatment in a time-dependent manner (Fig. 2B). *Pin1* promoter activity observed in HCT116 $p53^{+/+}$ cells was reduced in a time-dependent manner in response to thapsigargin treatment compared to that in HCT116 $p53^{-/-}$ cells. To verify that the putative p53REs are crucial to

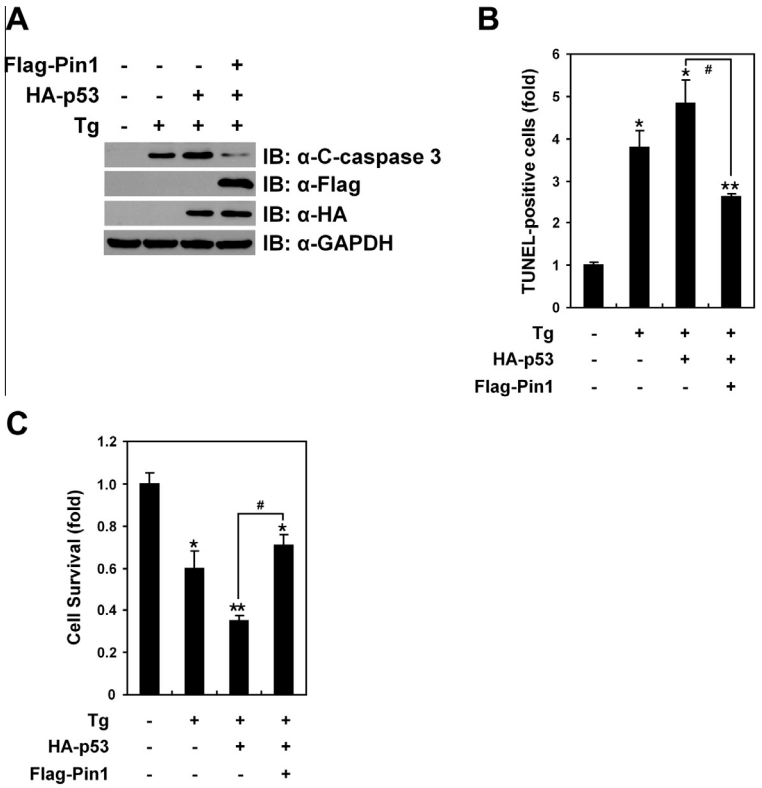

Fig. 4. Pin1 inhibits p53-mediated cell death under ER stress. (A) HCT116 $p53^{+/+}$ cells co-transfected with Flag-Pin1 and HA-p53 were treated with 1.0 μM thapsigargin for 24 h, followed by Western blot analysis for caspase-3 activation. α-GAPDH was used as an internal loading control. (B, C) HCT116 $p53^{+/+}$ cells co-transfected with Flag-Pin1 and HA-53 were exposed to 1.0 μM thapsigargin for 24 h. The cells were fixed, permeabilized, and followed by TUNEL assay (B). TUNEL-positive cells were quantified and plotted. MTT assays were performed to determine cell viability (C). The values shown are the mean ± SD of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{\#}p < 0.05$.

p53-mediated repression of the *Pin1* promoter, we deleted the RE sites and the resulting construct was then used to transfect HCT116 $p53^{-/-}$ cells. As shown in Fig. 2C, transfection with the deletion mutant reporter significantly restored the reduced promoter activity by p53, indicating that the putative p53REs are crucial for p53-mediated repression of the *Pin1* promoter. To confirm that p53 is capable of binding to these putative p53REs, we performed ChIP assays with thapsigargin-treated or untreated HCT116 colon cancer cells. p53 was recruited to the region containing the p53RE binding sites in HCT116 $p53^{+/+}$ cells but not in HCT116 $p53^{-/-}$ cells and it interacted with A p53RE site but not B p53RE site (Fig. 2D). To verify that the motif A p53RE site is crucial to p53-mediated repression of the *Pin1* promoter, we mutated the motif A p53RE site (CTTG to ACCT) and the resulting construct was then used to transfect HCT116 $p53^{-/-}$ cells with or without the p53-expressing vector (Fig. 2C). The luciferase reporter containing the mutated A p53RE site significantly restored the p53-mediated reduced promoter activity as much as the deletion mutant reporter, indicating that the A p53RE site is crucial for p53-mediated repression of the *Pin1* promoter.

3.3. p53 activity is required for the increased expression of Pin1 in response to ER stress

We used the p53 inhibitor pifithrin-α to test whether attenuation of p53 signaling leads to increased Pin1 protein production. To investigate the effect of pifithrin-α on Pin1 expression under ER stress, Pin1 protein expression level was monitored by Western blot after co-treatment of pifithrin-α with thapsigargin. GRP78 was used as an ER stress marker. As shown in Figs. 3A and B, when HCT116 $p53^{+/+}$ cells were incubated with pifithrin-α, p53 protein level was decreased in a dose- and time-dependent manner. In contrast, Pin1 protein level increased significantly. We also demonstrated that pifithrin-α treatment restores p53-mediated repression of *Pin1* by luciferase assays and RT-PCR analysis under ER stress (Figs. 3C and D), thereby suggesting that p53 is a key transcriptional repressor of *Pin1* under ER stress.

3.4. Pin1 protects p53-mediated ER stress-induced apoptosis

In HCT116 human colon adenocarcinoma cells, p53 expression is known to trigger apoptosis since p53 is a key activator for the expression of caspase-3, which induces the intrinsic apoptotic pathway [23]. Thus we hypothesized that Pin1 could play a role in p53-mediated apoptosis under ER stress. To test the hypothesis, we co-transfected Flag-Pin1 and HA-p53 expression plasmids in HCT116 $p53^{+/+}$ cells treated with or without thapsigargin. ER stress activated caspase-3 in HCT116 $p53^{+/+}$ cells. However, overexpressed Pin1 reduced caspase-3 cleavage induced by ER stress (Fig. 4A). These results were verified by TUNEL assays (Fig. 4B) and cell survival analyses (Fig. 4C). Our results clearly demonstrate that Pin1 decreased p53-induced apoptosis under ER stress in HCT116 $p53^{+/+}$ cells.

4. Discussion

In this study, the relationship between Pin1 and p53 under ER stress was investigated in the HCT116 human colon adenocarcinoma cell line. We found that Pin1 expression levels were reduced by ER stress and that this effect was mediated by p53. Furthermore, functional p53 responsive elements were identified in the *Pin1* promoter. Therefore, we concluded that p53 is able to negatively regulate Pin1 expression levels. These results clearly show a

functional relationship between Pin1 and p53, which are two major proteins involved in human diseases such as Alzheimer's disease and cancer.

Several signal transduction pathways have been identified that can explain how cells trigger programmed cell death when faced with unfolded protein accumulation. Prolonged ER stress leads to cell apoptosis. ER stress can induce multiple signaling pathways involved in ER stress-induced apoptosis, such as caspase-12/caspase-9/caspase-3, PERK/ATF4/CHOP, IRE-1/ASK1/JNK, and p53 pathways [9,24–26]. In this study, we first identified Pin1 as a direct p53 target gene that contains functional p53REs in the promoter region, and we demonstrated that Pin1 is repressed by p53 in response to ER stress (Fig. 2). We also found that Pin1 plays a protective role from p53-mediated apoptosis under ER stress in HCT116 colon cancer cells (Fig. 4). It has been suggested that tumor suppressor p53 can induce growth arrest and cell death, and the increase of p53 correlates with the potentiation of its ability to initiate apoptosis through activation of multiple pro-apoptotic genes, such as Bax, Noxa, Puma, and Apaf–1 [27]. Also, Pin1 has been known to regulate cell apoptosis by directly regulating pro-apoptosis proteins such as B-cell lymphomas2 (Bcl-2)-associated X protein (BAX) and death-associated protein 6 (Daxx) or anti-apoptotic proteins such as Bcl-2 and myeloid cell Leukemia-1 [28]. It has been shown that Pin1 is crucial for p53 to take apoptotic response through conformational changes of phosphorylated serine or threonine residues of p53 [29]. Pin1 is also required for dissociation of p53 from relA-associated apoptosis inhibitor iASPP [30]. On the contrary, the protective role of Pin1 against apoptosis has been reported recently. Pin1 was found to be overexpressed in human cancers including prostate, breast, lung, and cervical tumors [31,32]. Consistent with its overexpression in cancers, Pin1 activates a number of oncogenes including Ras signaling, suggesting that Pin1 may act as a novel anti-cancer target [32]. However, the mechanism how Pin1 is amplified in cancer has not been intensively studied. Although our studies strongly suggest a protective role of Pin1 in p53-mediated apoptosis under ER stress, the mechanism by which Pin1 mediates protection against p53-mediated apoptosis is not well understood. More research focusing on the target of Pin1 in p53-mediated cell death would be needed.

It was previously reported that Pin1 levels are down-regulated during ER stress in human neuroblastoma cells [33], which is consistent with our results. However, the mechanism by which Pin1 expression levels are down-regulated by ER stress remained to be elucidated. Here, for the first time, we demonstrate that p53 represses Pin1 expression under ER stress and that Pin1 protects cells from p53-mediated apoptosis under ER stress. These results may provide an important therapeutic strategy for chemotherapeutic drugs through ER stress-associated signaling pathways.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (No. NRF-2013R1A1A2007105).

References

[1] C. Xu, B. Bailly-Maitre, J.C. Reed, Endoplasmic reticulum stress: cell life and death decisions, J. Clin. Invest. 115 (2005) 2656–2664.
[2] R.J. Kaufman, Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls, Genes Dev. 13 (1999) 1211–1233.
[3] C. Patil, P. Walter, Intracellular signaling from the endoplasmic reticulum to the nucleus: the unfolded protein response in yeast and mammals, Curr. Opin. Cell Biol. 13 (2001) 349–355.
[4] K. Mori, Tripartite management of unfolded proteins in the endoplasmic reticulum, Cell 101 (2000) 451–454.
[5] H.P. Harding, M. Calfon, F. Urano, I. Novoa, D. Ron, Transcriptional and translational control in the mammalian unfolded protein response, Annu. Rev. Cell Dev. Biol. 18 (2002) 575–599.
[6] Y. Shi, K.M. Vattem, R. Sood, J. An, J. Liang, L. Stramm, R.C. Wek, Identification and characterization of pancreatic eukaryotic initiation factor 2 alpha-subunit kinase, PEK, involved in translational control, Mol. Cell Biol. 18 (1998) 7499–7509.
[7] H.P. Harding, Y. Zhang, D. Ron, Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase, Nature 397 (1999) 271–274.
[8] C.Y. Liu, M. Schroder, R.J. Kaufman, Ligand-independent dimerization activates the stress response kinases IRE1 and PERK in the lumen of the endoplasmic reticulum, J. Biol. Chem. 275 (2000) 24881–24885.
[9] T. Nakagawa, J. Yuan, Cross-talk between two cysteine protease families. Activation of caspase-12 by calpain in apoptosis, J. Cell. Biol. 150 (2000) 887–894.
[10] S.J. Marciniak, C.Y. Yun, S. Oyadomari, I. Novoa, Y. Zhang, R. Jungreis, K. Nagata, H.P. Harding, D. Ron, CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum, Genes Dev. 18 (2004) 3066–3077.
[11] F. Urano, X. Wang, A. Bertolotti, Y. Zhang, P. Chung, H.P. Harding, D. Ron, Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1, Science 287 (2000) 664–666.
[12] J. Li, B. Lee, A.S. Lee, Endoplasmic reticulum stress-induced apoptosis: multiple pathways and activation of p53-up-regulated modulator of apoptosis (PUMA) and NOXA by p53, J. Biol. Chem. 281 (2006) 7260–7270.
[13] W.C. Lin, Y.C. Chuang, Y.S. Chang, M.D. Lai, Y.N. Teng, I.J. Su, C.C. Wang, K.H. Lee, J.H. Hung, Endoplasmic reticulum stress stimulates p53 expression through NF-κB activation, PLoS One 7 (2012) e39120.
[14] D. Ron, P. Walter, Signal integration in the endoplasmic reticulum unfolded protein response, Nat. Rev. Mol. Cell Biol. 8 (2007) 519–529.
[15] K.P. Lu, X.Z. Zhou, The prolyl isomerase Pin1: a pivotal new twist in phosphorylation signalling and disease, Nat. Rev. Mol. Cell Biol. 8 (2007) 904–916.
[16] K. Nakamura, A. Greenwood, L. Binder, E.H. Bigio, S. Denial, L. Nicholson, X.Z. Zhou, K.P. Lu, Proline isomer-specific antibodies reveal the early pathogenic tau conformation in Alzheimer's disease, Cell 149 (2012) 232–244.
[17] K.P. Lu, S.D. Hanes, T. Hunter, A human peptidyl-prolyl isomerase essential for regulation of mitosis, Nature 380 (1996) 544–547.
[18] P.E. Shaw, Peptidyl-prolyl isomerases: a new twist to transcription, EMBO Rep. 3 (2002) 521–526.
[19] H. Zheng, H. You, X.Z. Zhou, S.A. Murray, T. Uchida, G. Wulf, L. Gu, X. Tang, K.P. Lu, Z.X. Xiao, The prolyl isomerase Pin1 is a regulator of p53 in genotoxic response, Nature 419 (2002) 849–853.
[20] F. Mantovani, S. Piazza, M. Gostissa, S. Strano, P. Zacchi, R. Mantovani, G. Blandino, G. Del Sal, Pin1 links the activities of c-Abl and p300 in regulating p73 function, Mol. Cell 14 (2004) 625–636.
[21] G.M. Wulf, Y.C. Liou, A. Ryo, S.W. Lee, K.P. Lu, Role of Pin1 in the regulation of p53 stability and p21 transactivation, and cell cycle checkpoints in response to DNA damage, J. Biol. Chem. 277 (2002) 47976–47979.
[22] E.S. Stavridi, T.D. Halazonetis, P53 and stress in the ER, Genes Dev. 18 (2004) 241–244.
[23] S.P. Cregan, J.G. MacLaurin, C.G. Craig, G.S. Robertson, D.W. Nicholson, D.S. Park, R.S. Slack, Bax-dependent caspase-3 activation is a key determinant in p53-induced apoptosis in neurons, J. Neurosci. 19 (1999) 7860–7869.
[24] H. Zinszner, M. Kuroda, X. Wang, N. Batchvarova, R.T. Lightfoot, H. Remotti, J.L. Stevens, D. Ron, CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum, Genes Dev. 12 (1998) 982–995.
[25] J.H. Lin, H. Li, Y. Zhang, D. Ron, P. Walter, Divergent effects of PERK and IRE1 signaling on cell viability, PLoS One 4 (2009) e4170.
[26] K.M. Ryan, A.C. Phillips, K.H. Vousden, Regulation and function of the p53 tumor suppressor protein, Curr. Opin. Cell Biol. 13 (2001) 332–337.
[27] T. Shibue, S. Suzuki, H. Okamoto, H. Yoshida, Y. Ohba, A. Takaoka, T. Taniguchi, Differential contribution of Puma and Noxa in dual regulation of p53-mediated apoptotic pathways, EMBO J. 5 (2006) 4952–4962.
[28] Y.C. Liou, X.A. Zhou, K.P. Lu, Prolyl isomerase Pin1 as a molecular switch to determine the fate of phosphoproteins, Trends Biochem. Sci. 36 (2011) 501–514.
[29] G. Sorrentino, M. Mioni, C. Giorgi, N. Ruggeri, P. Pinton, U. Moll, F. Mantovani, G. Del Sal, The prolyl-isomerase Pin1 activates the mitochondrial death program of p53, Cell Death Differ. 20 (2013) 198–208.
[30] F. Mantovani, F. Tocco, J. Girardini, P. Smith, M. Gasco, X. Lu, T. Crook, G. Del Sal, The prolyl isomerase Pin1 orchestrates p53 acetylation and dissociation from the apoptosis inhibitor iASPP, Nat. Struct. Mol. Biol. 14 (2007) 912–920.
[31] L. Bao, A. Kimzey, G. Sauter, J.M. Sowadski, K.P. Lu, D.G. Wang, Prevalent overexpression of prolyl isomerase Pin1 in human cancers, Am. J. Pathol. 164 (2004) 1727–1737.
[32] G.M. Wulf, A. Ryo, G.G. Wulf, S.W. Lee, T. Niu, V. Petkova, K.P. Lu, Pin1 is overexpressed in breast cancer and cooperates with Ras signaling in increasing the transcriptional activity of c-Jun towards cyclin D1, EMBO J. 20 (2001) 3459–3472.
[33] Y.S. Kap, J.J. Hoozemans, A.J. Bodewes, R. Zwart, O.C. Meijer, F. Baas, W. Scheper, Pin1 levels are downregulated during ER stress in human neuroblastoma cells, Neurogenetics 8 (2007) 21–27.