Biochemical and Biophysical Research Communications 430 (2013) 1034–1039

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Co-chaperon DnaJC7/TPR2 enhances p53 stability and activity through blocking the complex formation between p53 and MDM2

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ARTICLE INFO

Article history:
Received 16 November 2012
Available online 21 December 2012

Keywords:
DnaJC7
MDM2
p53
Protein stability
TPR2
Two-hybrid

ABSTRACT

Tumor suppressor p53 plays a critical role in the regulation of DNA damage response. Upon severe DNA damage, p53 promotes apoptosis to eliminate cells with seriously damaged DNA to maintain genomic integrity. Pro-apoptotic function of p53 is tightly linked to its sequence-specific transactivation ability. In the present study, we have identified co-chaperon DnaJC7/TPR2 as a novel binding partner of p53 by yeast-based two-hybrid screening. In the two-hybrid screening, we used the central DNA-binding domain of p53 as a bait. Co-immunoprecipitation experiments demonstrated that DnaJC7 is associated with p53 in mammalian cells. Luciferase reporter and colony formation assays revealed that DnaJC7 enhances p53-dependent transcriptional as well as growth-suppressive activity. Forced expression of DnaJC7 induced to extend a half-life of p53, indicating that DnaJC7-mediated activation of p53 might be at least in part due to its prolonged half-life. Consistent with these observations, the amount of p53/MDM2 complex was markedly reduced in the presence of DnaJC7, suggesting that DnaJC7 dissociates MDM2 from p53. Taken together, our present findings strongly suggest that DnaJC7 participates in p53/MDM2 negative feedback regulatory pathway, and thereby enhancing the stability and activity of p53.

1. Introduction

The representative tumor suppressor p53 plays a pivotal role in the regulation of DNA damage response [1]. p53 acts as a nuclear sequence-specific transcription factor, and transactivates numerous target genes implicated in cell cycle arrest ($p21^{WAF1}$) and apoptosis (*BAX*, *NOXA* and *PUMA*) following DNA damage [2,3]. Initial mutation searches demonstrated that *p53* is highly mutated in various human primary tumors and over 90% of *p53* mutations are detectable within its genomic region encoding the central DNA-binding domain [4]. Mutant forms of p53 lack the sequence-specific transcriptional activity and acquire oncogenic potential [2]. Mutation-dependent dysfunction of p53 results in tumor development and progression. Therefore, the proper conformation of p53 DNA-binding domain is required for its sequence-specific transcriptional as well as pro-apoptotic activity [5].

Under normal conditions, p53 is expressed at a barely detectable level. Upon DNA damage, p53 is quickly stabilized and activated dramatically through post-translational modifications such as phosphorylation and acetylation [6,7]. The stability of p53 is primarily regulated by MDM2 [8]. MDM2 binds to NH_2-terminal region of p53, and acts as a specific E3 ubiquitin protein ligase for p53. The ubiquitin-conjugated p53 is subsequently degraded through ubiquitin/proteasome system. Since MDM2 is a direct transcriptional target gene of p53, MDM2 and p53 form a negative feedback loop, in which p53 induces the expression of MDM2, which in turn down-regulates p53 [9,10]. DNA damage-induced NH_2-terminal phosphorylation of p53 promotes a dissociation of MDM2 from p53, and COOH-terminal acetylation blocks MDM2-mediated ubiquitination of p53. These chemical modifications suppress the ubiquitin/proteasome-dependent degradation of p53 and thereby p53 becomes stable.

In addition to chemical modifications, p53 is regulated by protein–protein interaction. It has been shown that ASPP1/ASPP2 interact with DNA-binding domain of p53 to allow induction of its target genes [11]. Yang et al. demonstrated that 14-3-3σ forms a complex with p53 in response to DNA damage, and enhances the transcriptional activity of p53 [12]. Recently, Lew et al. found that hexamethylene bisacetamide-inducible protein 1 (HEXIM1) is associated with COOH-terminal region of p53 and prevents MDM2-mediated degradation of p53 [13]. According to their results, HEXIM1 had an ability to increase p53-mediated transcriptional activation. Additionally, Lopez-Mateo et al. described that CREBZF which is a member of ATF/CREB transcription factor, interacts with p53 and promotes the stability as well as transcriptional activity of p53 [14].

In the present study, we have identified co-chaperon DnaJC7/TPR2 (DnaJ heat shock protein 40 family) as a novel binding partner of p53, and found that DnaJC7 extends a half-life of p53

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http://dx.doi.org/10.1016/j.bbrc.2012.11.121

by inducing a dissociation of MDM2 from p53, and enhances its activity.

2. Materials and methods

For yeast two-hybrid screening, we used the Matchmaker Gold Yeast Two-Hybrid System (Clontech). The cDNA encoding the DNA-binding domain of p53 (amino acid residues 113–290) was amplified by PCR-based strategy using the full-length p53 cDNA as a template. The PCR product, which was produced by an additional *Eco*RI site in 5′-upstream and *Bam*HI in 3′-downstream, was digested completely with *Eco*RI and *Bam*HI, and subcloned in-frame into the identical restriction sites of pGBKT7 to generate a "bait" plasmid, pGBKT7-p53DB. The bait plasmid was introduced into yeast strain Y2HGold using the lithium acetate/heat-shock procedure. The transformed yeast cells were plated on SD medium lacking tryptophan and incubated at 30 °C for 1 week. Viable colonies were mated with yeast strain Y187 containing cDNA library derived from human fetal brain (Clontech), and diploid cells were plated on SD medium lacking tryptophan, leucine, histidine, and adenine in the presence of X-α-Gal and aureobasidin A. Positive colonies showing blue color were picked up and library plasmids were isolated by using Yeastmaker yeast plasmid isolation kit (Cloctech). The nucleotide sequences of the positive cDNA clones were determined by the dideoxy terminator cycle sequencing method.

2.1. Cell lines, cell culture and transfection

Monkey embryonic kidney-derived COS7, Human osteosarcoma-derived U2OS, human colon carcinoma-derived HCT116 and human lung carcinoma-derived H1299 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Invitrogen), penicillin (50 U/ml) and streptomycin (50 μg/ml). Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in the air. For transfection, cells were transfected using LipofectAMINE 2000 (Invitrogen) according to the manufacturer's instructions.

2.2. Cell survival assay

U2OS cells were seeded at a density of 5×10^3 cells/96-well cell culture plates and allowed to attach overnight. Cells were then treated with the indicated concentrations of ADR. At the indicated time points after ADR treatment, 10 μl of a modified 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl-tetrazolium bromide solution (Dojindo) were added to the culture and reaction mixtures were incubated at 37 °C for 2 h. The absorbance readings for each well were carried out at 570 nm using the microplate reader (Model 450, Bio-Rad Laboratories).

2.3. DNA fragmentation assay

U2OS cells were exposed to the indicated concentrations of ADR. Twenty-four hours after ADR treatment, floating and adherent cells were collected and incubated with lysis buffer (5 mM Tris–HCl, pH 7.5, 20 mM EDTA, pH 8.0, and 0.5% Triton X-100) at 4 °C for 30 min. The reaction mixture was centrifuged at 15,000 rpm at 4 °C for 20 min, and the supernatant was incubated with proteinase K (at a final concentration of 50 μg/ml) at 50 °C for 1 h followed by the additional incubation with RNase A (at a final concentration of 40 μg/ml) at 37 °C for 1 h. Genomic DNA was purified by phenol/chloroform extraction and ethanol precipitation. DNA concentration was measured at 260 nm in a spectrophotometer. DNA ladder was visualized in 1.5% agarose gel.

2.4. RT-PCR

Total RNA was prepared from U2OS cells by using RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions and reverse transcribed in the presence of random primers and SuperScript II reverse transcriptase (Invitrogen). The resultant first-strand cDNA was amplified by PCR to examine expression levels of genes of interest. The list of primer sets used will be provided upon request. *GAPDH* was used as an internal control. PCR products were analyzed by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining. The expression of *GAPDH* was measured as an internal control.

2.5. Immunoblotting

Cells were washed in ice-cold PBS and lysed in a lysis buffer containing 25 mM Tris–HCl pH 7.5, 137 mM NaCl, 2.7 mM KCl, 1% Triton X-100 and protease inhibitor cocktail (Sigma). The protein concentration of cell lysates was determined by using Bio-Rad protein assay dye reagent (Bio-Rad Laboratories) according to the manufacturer's instructions. Equal amounts of cell lysates were separated by 10% SDS–polyacrylamide gel electrophoresis, electro-transferred onto Immobilon-P membrane filters (Millipore) and blocked with 0.5% non-fat milk in Tris-buffered saline (TBS) containing 0.1% Tween 20 at 4 °C. The membranes were probed with monoclonal anti-p53 (DO-1: Santa Cruz Biotechnologies), monoclonal anti-FLAG (M2: Sigma), monoclonal anti-MDM2 (SMP-14: Santa Cruz Biotechnologies), monoclonal anti-γH2AX (2F3: BioLegend), monoclonal anti-NOXA (114C307: abcam), polyclonal anti-DnaJC7 (Proteintech), polyclonal anti-phospho-p53 at Ser-15 (Cell Signaling Technology), polyclonal anti-$p21^{WAF1}$ (H-164: Santa Cruz Biotechnology), polyclonal anti-BAX (Cell Signaling Technology), polyclonal anti-PARP (Cell Signaling Technology), or with polyclonal anti-Actin (20–33: Sigma) antibody at room temperature for 1 h followed by incubation with appropriate horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology) at room temperature for 1 h. Immunoreactive bands were visualized by using ECL system (Amersham Biosciences) according to the manufacturer's instructions.

2.6. Immunoprecipitation

COS7 cells were transfected with the expression plasmid for FLAG-DnaJC7. Forty-eight hours after transfection, cell lysates were prepared and precleared with protein G-Sepharose beads at 4 °C for 1 h. The precleared cell lysates (1 mg of proteins) were immunoprecipitated with normal mouse serum (NMS) or with monoclonal anti-p53 antibody at 4 °C for 12 h. The reaction mixtures were incubated with 30 μl of 50% slurry of protein G-Sepharose beads at 4 °C for 2 h. The immunoprecipiates were extensively washed with the ice-cold lysis buffer and analyzed by immunoblotting with monoclonal anti-FLAG or with monoclonal anti-p53 antibody.

2.7. Luciferase reporter assay

H1299 cells were seeded at a density of 5×10^4/12-well cell culture plates and transfected with the constant amount of the expression plasmid for p53 (25 ng), luciferase reporter plasmid carrying p53-responsive element derived from $p21^{WAF1}$, *MDM2* or *BAX* promoter (100 ng), *Renilla* luciferase expression plasmid (10 ng) together with or without the increasing amounts of the expression plasmid encoding FLAG-DnaJC7 (100 or 200 ng). Forty-eight hours after transfection, cell lysates were prepared and their luciferase activities (firefly and *Renilla*) were measured with Dual-luciferase reporter assay system according to the manufacturer's instructions (Promega).

3. Results

3.1. Identification of DnaJC7 as a p53-binding protein in a two-hybrid screening

To identify cellular protein(s) which could interact with the central DNA-binding domain of p53 and regulate its activity, we used an yeast two-hybrid system to screen a human fetal brain cDNA library with a "bait" plasmid (pGBKT7-p53DB) encoding the central DNA-binding domain of p53 (amino acid residues 113–290). After extensive screening, we have finally obtained 21 independent positive colonies showing blue color. Plasmids carrying these 21 positive candidates were rescued into *Escherichia coli*, and their nucleotide sequences were determined. One clone, termed NK-17, contained a partial human cDNA for DnaJC7/TPR2, which is a member of DnaJ heat shock protein 40 (Hsp40) family [15].

To ask whether DnaJC7 could interact with p53 in mammalian cells, we generated a full-length FLAG-DnaJC7 expression plasmid. COS7 cells which express a large amount of endogenous p53 [16], were transfected with FLAG-DnaJC7 expression plasmid. Forty-eight hours after transfection, cell lysates were prepared and subjected to immunoprecipitation experiments. As shown in Fig. 1, the anti-FLAG immunoprecipitates contained the endogenous p53. Reciprocal experiments demonstrated that FLAG-DnaJC7 co-precipitates with the endogenous p53, suggesting that DnaJC7 is associated with p53 in mammalian cells.

3.2. DnaJC7 has an ability to enhance the transcriptional and growth-suppressive activities of p53

To address whether DnaJC7 could affect the transcriptional activity of p53, we performed luciferase reporter assay. To this end, *p53*-deficient H1299 cells were transfected with the constant amount of the expression plasmid for p53, luciferase reporter plasmid carrying p53-target promoters ($p21^{WAF1}$, *MDM2* and *BAX*) and *Renilla* luciferase reporter plasmid together with or without the increasing amounts of FLAG-DnaJC7 expression plasmid. Forty-eight hours after transfection, cells were lysed and their luciferase activities were measured. Consistent with the previous observations [17], ectopic expression of p53 alone led to an increase in the luciferase activities driven by those p53-target gene promoters (Fig. 2A–C). Of note, co-expression of p53 with FLAG-DnaJC7 resulted in a strong increase in the luciferase activities as compared with those in cells transfected with p53 expression plasmid alone, suggesting that DnaJC7 has an ability to enhance p53-dependent transcriptional activation. Similar results were also obtained in *p53*-proficient U2OS cells (Supplemental Fig. S1).

Next, we sought to examine a possible effect of DnaJC7 on p53-mediated growth suppression. For this purpose, H1299 cells were transfected with the empty plasmid, p53 expression plasmid, p53 expression plasmid plus FLAG-DnaJC7 expression plasmid or with FLAG-DnaJC7 expression plasmid. Forty-eight hours after transfection, cells were maintained in the presence of G418 for 2 weeks. In accordance with the previous observations [17], the exogenous expression of p53 resulted in a decrease in number of G418-resistant colonies (Fig. 2D). Intriguingly, co-expression of p53 with FLAG-DnaJC7 led to a much more decrease in number of drug-resistant colonies relative to that of p53 alone, whereas FLAG-DnaJC7 alone had an undetectable effect on colony formation. Collectively, these results indicate that DnaJC7 enhances the transcriptional and growth-suppressive activities of p53 through the complex formation with p53.

3.3. DnaJC7 prolongs a half-life of p53

To examine whether DnaJC7 could regulate the endogenous p53, U2OS cells were transfected with the empty plasmid or with the increasing amounts of the expression plasmid for FLAG-DnaJC7. Forty-eight hours after transfection, cell lysates and total RNA were isolated and subjected to immunoblotting and RT-PCR, respectively. As shown in Fig. 3A, forced expression of FLAG-DnaJC7 elevated the expression level of p53 in association with the up-regulation of p53-target gene products such as $p21^{WAF1}$ and BAX. RT-PCR analysis revealed that DnaJC7 promotes the expression of $p21^{WAF1}$ and *BAX*, whereas the expression level of

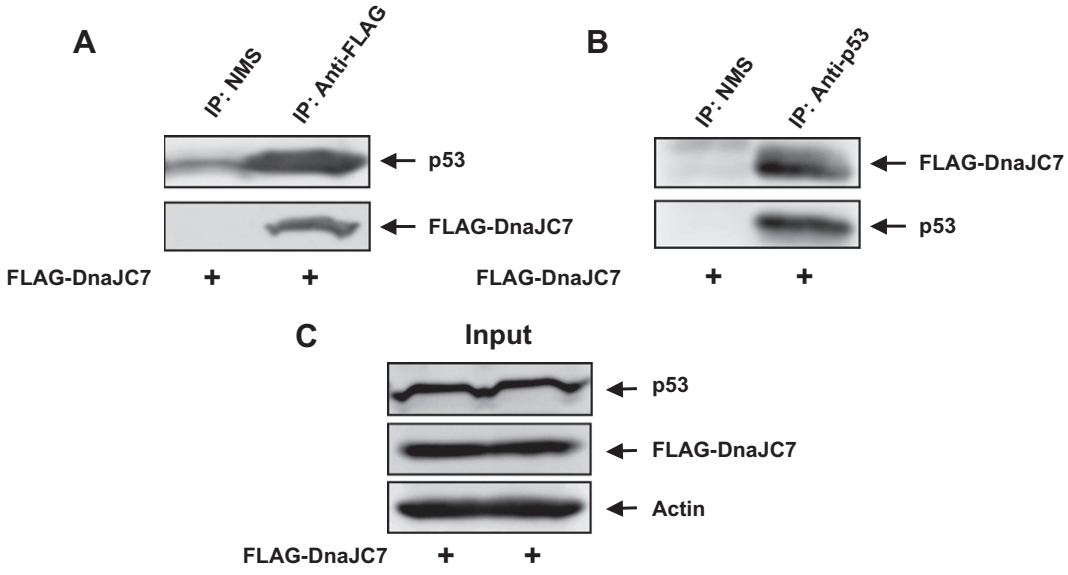

Fig. 1. Complex formation between DnaJC7 and p53 in cells. COS7 cells were transfected with the expression plasmid for FLAG-DnaJC7. Forty-eight hours after transfection cell lysates were prepared and immunoprecipitated with normal mouse serum (NMS) or with anti-FLAG antibody. The immunoprecipitates were analyzed by immunoblotting with anti-p53 or with anti-FLAG antibody (A). The reciprocal experiments were performed by using NMS and anti-p53 antibody followed by immunoblotting with anti-FLAG or with anti-p53 antibody (B). The input contains 1/20 of the lysates used in the experiments (C). The expression levels of actin were examined as a loading control.

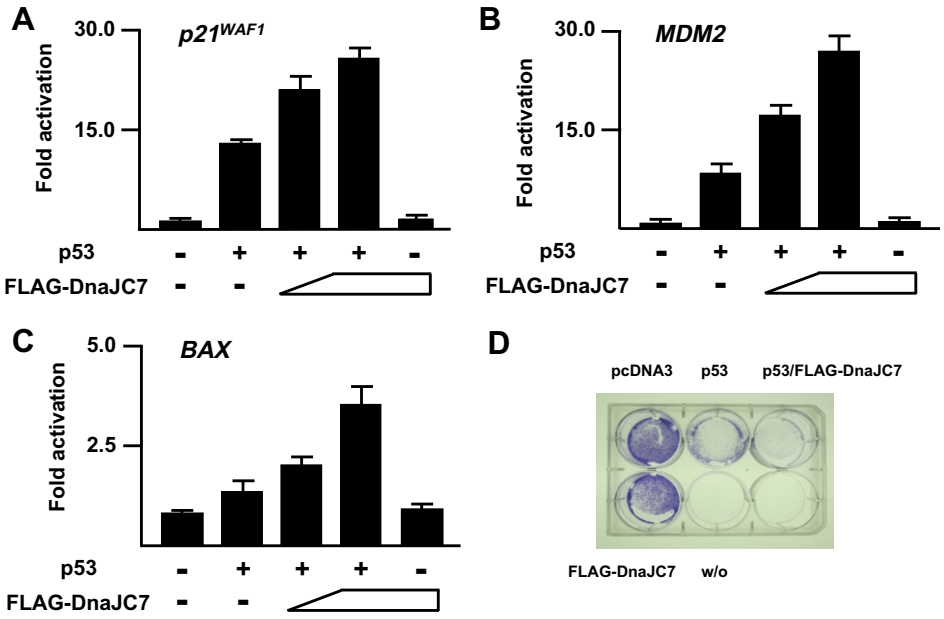

Fig. 2. DnaJC7 enhances p53-mediated transcriptional activation and growth suppression. (A–C) Luciferase reporter assay. *p53*-deficient H1299 cells were transfected with constant amount of the expression plasmid for p53 (25 ng), luciferase reporter plasmid carrying p53-responsive element derived from $p21^{WAF1}$ (A), *MDM2* (B) or *BAX* (C) promoter (100 ng) and *Renilla* luciferase reporter plasmid (10 ng) together with or without the increasing amounts of the expression plasmid for FLAG-DnaJC7 (100 or 200 ng). Total amount of plasmid DNA was kept constant (510 ng) with pcDNA3. Forty-eight hours post-transfection, cell lysates were prepared and their luciferase activities were measured by Dual-Luciferase reporter system. The firefly luminescence signal was normalized based on the *Renilla* luminescence signal. Results represented averages of from three independent transfections. (D) Colony formation assay. H12299 cells were transfected with the indicated combinations of the expression plasmids. Forty-eight hours after transfection, cells were transferred into fresh medium containing G418 (at a final concentration of 800 μg/ml). Two weeks after the selection, G418-resistant colonies were fixed in methanol and stained with Giemsa's solution.

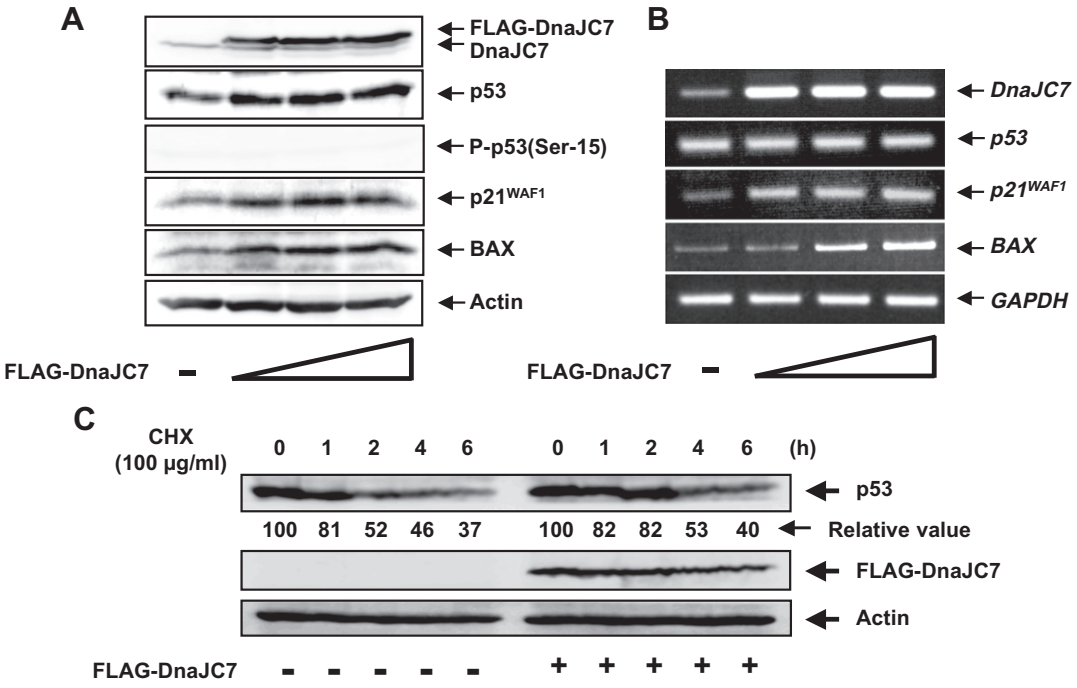

Fig. 3. DnaJC7 enhances p53-dependent transcriptional activity through increasing its stability. (A) Immunoblotting. *p53*-proficient U2OS cells were transfected with the empty plasmid (pcDNA3) or with the increasing amounts of the expression plasmid for FLAG-DnaJC7 (0.5, 1.0 or 1.5 μg). Total amount of plasmid DNA was kept constant (2 μg) with pcDNA3. Forty-hours after transfection, cell lysates were prepared and subjected to immunoblotting with the indicated antibodies. The expression levels of actin were examined as a loading control. (B) RT-PCR. U2OS cells were transfected as in (A). Forty-eight hours post transfection, total RNA were extracted and processed for RT-PCR. The expression levels of *GAPDH* were examined as an internal control. (C) DnaJC7 prolongs a half-life of p53. U2OS cells were transfected with the empty plasmid or with the expression plasmid for FLAG-DnaJC7 (0.5 μg). Twenty-four hours after transfection, cells were transferred into fresh medium containing 100 μg/ml of cycloheximide (CHX). At the indicated time points after the treatment with CHX, cell lysates were prepared and analyzed by immunoblotting with the indicated antibodies. Actin was used for equal protein loading. Densitometry was used to quantify the amounts of p53 which normalized to actin.

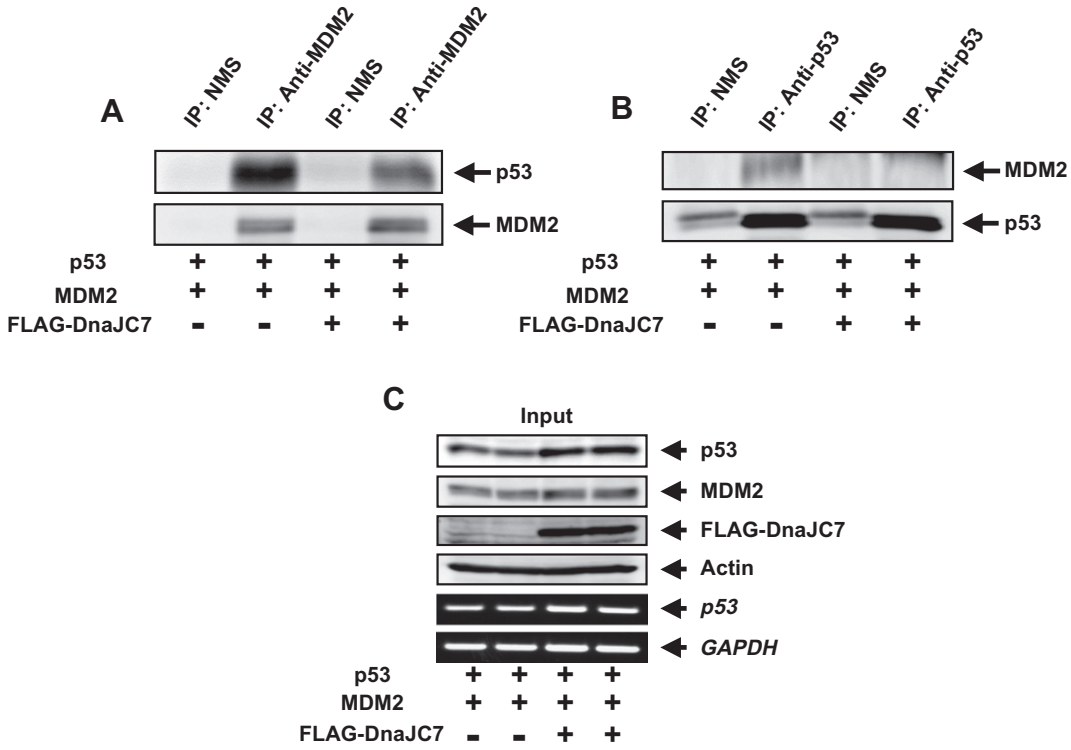

Fig. 4. DnaJC7 promotes the dissociation of MDM2 from p53. (A–C) Immunoprecipitation. H1299 cells were transfected with the constant amount of the expression plasmids for p53 and MDM2 together with or without the expression plasmid for FLAG-DnaJC7. Forty-eight hours after transfection, cells were exposed to 10 μM of MG-132. Six hours after the treatment, cell lysates were immunoprecipitated with NMS or with anti-MDM2 antibody. The immunoprecipitates were analyzed by immnoblotting with anti-p53 or with anti-MDM2 antibody (A). The reciprocal experiments were performed by using NMS and anti-p53 antibody followed by immunoblotting with anti-MDM2 or with anti-p53 antibody (B). The input contains 1/20 of the lysates used in the experiments (C). The expression levels of actin were examined as a loading control. Under the same experimental conditions, RT-PCR was carried out to examine the expression levels of *p53*. *GAPDH* was used as an internal control.

p53 remains unchanged in the presence of FLAG-DnaJC7 (Fig. 3B), suggesting that DnaJC7 might stabilize p53.

To confirm this issue, we performed cycloheximide blockage. To this end, U2OS cells were transfected with the empty plasmid or with the expression plasmid for FLAG-DnaJC7. Twenty-four hours after transfection, cells were exposed to the translation inhibitor cycloheximide. At the indicated time periods after cycloheximide treatment, cell lysates were prepared and analyzed by immunoblotting. As clearly shown in Fig. 3C, a half-life of the endogenous p53 was significantly extended in the presence of FLAG-DnaJC7.

3.4. DnaJC7 induces the dissociation of MDM2 from p53

Since the stability of p53 is regulated largely by MDM2-mediated ubiquitin/proteasome system [8], we sought to check whether DnaJC7 could affect the complex formation between p53 and MDM2. H1299 cells were transfected with the constant amount of the expression plasmids for p53 and MDM2 together with or without FLAG-DnaJC7 expression plasmid. Forty-eight hours after transfection, cells were exposed to proteasome inhibitor MG-132 for 6 h, and cell lysates were then prepared. Equal amount of cell lysates were immunoprecipitated with NMS or with anti-MDM2 antibody followed by immunoblotting with anti-p53 or with anti-MDM2 antibody. As shown in Fig. 4A, the amount of MDM2 detected in the anti-MDM2 immunoprecipitates in the presence of FLAG-DnaJC7 was less than that in the absence of FLAG-DnaJC7. The similar results were also obtained in the reciprocal experiments (Fig. 4B). Under our experimental conditions, the exogenously expressed p53 was increased in the presence of FLAG-DnaJC7 as compared with that in the absence of FLAG-DnaJC7, whereas *p53* mRNA levels remained unchanged regardless of FLAG-DnaJC7 (Fig. 4C). Taken together, our results suggest that DnaJC7 enhances the stability and function of p53 by inhibiting the complex formation between p53 and MDM2.

4. Discussion

In the present study, we found that DnaJC7 interacts with p53 and acts as a positive regulator for p53. Considering that DnaJC7 has an ability to dissociate MDM2 from p53, it is likely that DnaJC7 might participate in p53/MDM2 negative feedback regulatory pathway. To our knowledge, functional interaction between DnaJC7 and p53 has not been described.

According to our present results, forced expression of DnaJC7 prolonged a half-life of p53 without DNA damage. Upon adriamycin (ADR)-mediated DNA damage, p53 was significantly induced to accumulate in association with the up-regulation of its target gene products such as $p21^{WAF1}$, MDM2, BAX and NOXA in U2OS cells, whereas the expression level of DnaJC7 remained unchanged in the presence or absence of ADR (Supplemental Fig. S2). Similar results were also obtained in *p53*-proficient colon carcinoma-derived HCT116 cells (data not shown). During the proper DNA damage response, MDM2-mediated degradation of p53 is required to avoid the inappropriate apoptosis caused by a large amount of over-active p53. On the other hand, p53 escaped from MDM2-mediated degradation to eliminate cells with seriously damaged DNA in response to DNA damage. Thus, it is likely that the regulation of p53/MDM2 negative feedback loop might be one of the critical determinants of cell fate (cell death or cell survival). It has been described that DNA damage-mediated NH_2-terminal phosphorylation

of p53 such as Ser-15 prevents the interaction between p53 and MDM2, and thereby increasing the stability of p53 [18]. In addition to DNA damage-dependent chemical modifications of p53, Xu et al. described that PDCD5 disrupts the interaction between p53 and MDM2 and thus enhances the stability of p53 by antagonizing MDM2-mediated protesomal degradation [19]. Based on our present observations, like PDCD5, DnaJC7 accelerated the dissociation of MDM2 from p53, and inhibited the proteolytic degradation of p53. Since the expression level of DnaJC7 remained unchanged regardless of ADR treatment, DnaJC7 might contribute to maintain p53 at an appropriate level in the presence or absence of DNA damage.

In addition to the stability control of p53, DnaJC7 enhanced p53-dependent transcriptional and growth-suppressive activities. As shown in Supplemental Fig. S3, DnaJC7 induced ADR-mediated proteolytic cleavage of PARP in *p53*-proficient HCT116 cells following ADR exposure in a dose-dependent manner, whereas DnaJC7 had undetectable effect on the amounts of cleaved PARP in *p53*-deficient HCT116 cells exposed to ADR, suggesting that DnaJC7 also enhances the pro-apoptotic activity of p53 in response to ADR. Since we have employed DNA-binding domain of p53 as a bait in the two-hybrid screening, DnaJC7 bound to p53 through at least in part its DNA-binding domain. As described previously [11], like DnaJC7, ASPP1/ASPP2 bound to DNA-binding domain of p53, and significantly enhanced its transactivation as well as pro-apoptotic activity. Initial structural analysis demonstrated that the COOH-terminal region of the latent form of p53 exists in close proximity to the DNA-binding domain [20], whereas DNA damage-mediated chemical modifications lead to expose this domain and thereby exerting its transactivation function [2]. Mutant forms of p53 bearing nonfunctional conformation of their DNA-binding domains lack the sequence-specific transcriptional ability [2], suggesting that the proper conformation of its DNA-binding domain is responsible for its sequence-specific transactivation function. Intriguingly, it has been shown that the large T antigen of simian virus 40 is associated with DNA-binding domain of p53 and strongly inhibits its transcriptional activity by inducing the dramatic conformational changes of its DNA-binding domain [21], indicating that protein–protein interaction might affect the conformation of p53 DNA-binding domain. In contrast to the large T antigen, Hsp90 and Hsp70 molecular chaperones maintained the native conformation of p53 under heat-shock conditions, and assisted the refolding of p53 under normal conditions [22]. In addition, Hsp90 was involved in the stabilization of p53 [23]. Moffatt et al. found that DnaJC7 forms a complex with Hsp90 and Hsp70 [24]. Although the precise molecular mechanisms by which DnaJC7 activates p53, it is likely that the interaction of DnaJC7 with p53 might unlock its DNA-binding domain and also induce its conformation change from the latent form to the functional one in collaboration with Hsp90 and/or Hsp70. Further studies should be required to address this issue.

Acknowledgments

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and a Hamaguchi Foundation.

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.2012.11.121.

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