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Inhibitory role of E2F-1 in the regulation of tumor suppressor p53 during DNA damage response

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ABSTRACT

Appropriate regulation of DNA damage response is pivotal for maintaining genome stability. p53 as well as E2F-1 plays a critical role during DNA damage response, however, the physiological significance of their interaction has been elusive. In the present study, we found that E2F-1 has an inhibitory effect on p53 during adriamycin (ADR)-mediated DNA damage response. Upon ADR exposure, p53 and E2F-1 were markedly induced at protein and mRNA levels in *p53*-procifient U2OS and HCT116 cells, and formed a stable complex as examined by co-immunoprecipitation experiments. Of note, chromatin immunoprecipitation (ChIP) experiments revealed that ADR-mediated induction coincides with the efficient recruitment of p53 and E2F-1 onto the promoters of p53-target genes, such as $p21^{WAF1}$ and *BAX*. Subsequent RT-PCR and luciferase reporter assays demonstrated that E2F-1 strongly attenuates p53-dependent transactivation of p53-target genes. Importantly, siRNA-mediated knockdown of E2F-1 stimulated apoptosis in response to ADR, which was associated with an accelerated response of $p21^{WAF1}$ and *BAX*. Collectively, our present findings suggest that E2F-1 participates in p53-mediated DNA damage response and might have a checkpoint function to limit overactive p53.

1. Introduction

The proper DNA damage response, which monitors and ensures the genomic integrity, has been considered to be a critical barrier to tumorigenesis [1]. p53 is a representative tumor suppressor which plays an important role in the regulation of DNA damage response. Under normal physiological conditions, p53 is expressed at an extremely low level. In response to DNA damage, p53 is quickly induced to accumulate in cell nucleus through the sequential post-translational modifications, such as phosphorylation and acetylation [2,3]. DNA damage-dependent accumulation of p53 is largely mediated by its enhanced protein stability. MDM2, which has an E3 ubiquitin ligase activity for p53 [4], contributes to this stability control of p53. NH_2-terminal phosphorylation of p53 promotes the dissociation of MDM2 from p53, and COOH-terminal acetylation of p53 suppresses MDM2-mediated p53 ubiquitination. Thus, these chemical modifications repress MDM2-dependent proteasomal degradation of p53 and thereby p53 becomes stable.

p53 acts as a nuclear transcription factor. Extensive mutation search demonstrated that *p53* is highly mutated in various primary human tumors, and the majority of loss-of-function mutations is detected within the region encoding its central DNA-binding domain, indicating that the transcriptional activity is critical for its tumor suppressor function [5]. Consistent with these observations, it has been shown that the sequence-specific transactivation ability of p53 is tightly linked to its tumor suppressor function [6]. Mutant forms of p53 lack the sequence-specific DNA-binding activity and exhibit a dominant-negative behavior toward wild-type p53 [7]. Indeed, *p53*-deficient mice developed spontaneous tumors [8].

Upon the severe DNA damage, p53 induces apoptosis to eliminate cells with damaged DNA through transactivating its direct target genes implicated in the induction of apoptosis, such as *BAX*, *PUMA* and *NOXA* [9]. When cells receive repairable DNA damage, p53 instead promotes cell cycle arrest by transactivating $p21^{WAF1}$ and *14-3-3σ* to save time to repair damaged DNA, and then cells with repaired DNA re-enter into the normal cell cycle [9]. Therefore, p53 stands at the crossroad between cell survival and cell death in response to DNA damage.

E2F-1 which belongs to a small family of nuclear transcription factors, acts as a driving force of G1/S transition and DNA replication through the transcriptional regulation of its target gene products involved in cell cycle progression, such as thymidine kinase (TK), DNA polymerase-α, PCNA, and CDK2 [10–12]. Deregulated expression of E2F-1 resulted in the unscheduled entry of cells into *S* phase [13]. In addition to its cell cycle regulatory role, E2F-1 has a pro-apoptotic potential. As described previously [14], forced expression of E2F-1 efficiently induced apoptosis in a wide spectrum of cancerous cells. Consistent with this notion, the early genetic studies demonstrated that mice lacking E2F-1 develop

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spontaneous tumors [15,16]. Intriguingly, Lissy et al. described that E2F-1 has an ability to transactivate p53 homolog p73 [17]. Thus, it is likely that pro-apoptotic p73 participates in E2F-1-mediated apoptosis.

Subsequent studies revealed that E2F-1 is stabilized during DNA damage response [18], indicating that E2F-1 has a role in the regulation of cellular response to DNA damage. Previously, it has been shown that E2F-1 and p53 cooperate to mediate apoptosis [19]. Hsieh et al. described that E2F-1 stimulates p53-mediated apoptosis in response to UV irradiation [20]. In contrast, Nip et al. reported that E2F-1 inhibits the transcriptional activity of p53 [21]. Timmers et al. found that the targeted disruption of E2F-1 results in the induction of p53-target genes [22]. To date, the functional significance of E2F-1 action in p53-mediated DNA damage response has not been completely elucidated.

2. Materials and methods

2.1. Cell lines, cell culture and transfection

Human osteosarcoma-derived U2OS, 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

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. TUNEL staining

Apoptotic cells with fragmented nuclei were detected by *In Situ* Cell Death Detection Kit, TMR red (Roche Molecular Biochemicals). In brief, cells were grown overnight on glass cover slips at 37 °C. At the indicated time points after ADR treatment, cells were washed in phosphate-buffered saline (PBS), fixed in 4% paraformaldehyde for 1 h at room temperature, and then permeabilized with 0.1% Triton X-100 for 2 min on ice. The cells were subsequently incubated with TUNEL reaction mixture for 1 h at 37 °C in a humidified atmosphere in the dark. The cover slips were mounted onto microscope slides using the VECTASHIELD containing DAPI (Vector Laboratories), and examined under a laser scanning confocal microscope (GE Health Science).

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. Indirect immunofluorescence

Cells on cover slips were fixed in 3.7% formaldehyde for 30 min at room temperature and permeabilized with 0.2% Triton X-100 for 5 min at room temperature. After blocking with 3% bovine serum albumin (BSA) in PBS, cover slips were simultaneously incubated with monoclonal anti-p53 (DO-1: Santa Cruz Biotechnologies) and polyclonal anti-E2F-1 (C-20: Santa Cruz Biotechnologies) antibodies in PBS for 1 h at room temperature followed by incubation with FITC-conjugated anti-mouse IgG and rhodamine-conjugated anti-rabbit IgG (Invitrogen) in PBS for 1 h at room temperature. Cell nuclei were stained with DAPI.

2.6. 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-γH2AX (2F3: BioLegend), monoclonal anti-NOXA (114C307: abcam), 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-PUMA (abcam), polyclonal anti-E2F-1 (C-20: Santa Cruz Biotechnologies), 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.

2.7. Immunoprecipitation

Cell lysates (1 mg of proteins) prepared from untreated HCT116 cells were immunoprecipitated with normal mouse serum (NMS) or with monoclonal anti-p53 antibody followed by incubation with 30 μl of 50% slurry of protein G-Sepharose beads. The immunoprecipitates were extensively washed with the ice-cold lysis buffer and analyzed by immunoblotting with polyclonal anti-E2F-1 antibody.

2.8. 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 $p21^{WAF1}$ or *BAX* promoter (100 ng), *Renilla* luciferase expression plasmid (10 ng) together with or without the increasing amounts of the expression plasmid encoding E2F-1 (50, 100 and 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).

2.9. Chromatin immunoprecipitation (ChIP) assay

HCT116 cells were treated with 0.5 μM of ADR or left untreated. Twenty-four hours after ADR treatment, cells were cross-linked with 1% formaldehyde in medium for 15 min at 37 °C. Cells were then washed with ice-cold PBS and resuspended in 200 μl of SDS lysis buffer containing protease inhibitor cocktail. Cell suspension was sonicated to an average length of 200–600 nucleotides in length and pre-cleared with protein A-agarose beads for 30 min at 4 °C. The beads were removed by a brief centrifugation and the chromatin solution was immunoprecipitated with normal rabbit serum (NRS) or with anti-E2F1 antibody overnight at 4 °C, followed by incubation with protein A-agarose beads for additional 1 h at 4 °C. The immune complex was eluted with 100 μl of elution buffer (1% SDS and 0.1 M $NaHCO_3$) and formaldehyde cross-link was reversed by heating at 65 °C for 4 h. Genomic DNA was purified from the immunoprecipitates and analyzed by standard PCR. Specific primers used in this study were as described: *p21*^{WAF1}, 5′-GGTCTGCTACTGTGTCCTCC-3′ (sense) and 5′-CATCTG AACAGAAATCCCAC-3′ (antisense); *BAX*, 5′-AGGCTGAGACGGGGTTA TCT-3 (sense) and 5′-GCGCAGAAGGAATTAGCAAG-3′ (antisense).

3. Results

3.1. Induction of p53 and E2F-1 during ADR-mediated apoptosis

Human osteosarcoma-derived U2OS cells carrying wild-type *p53* were exposed to the various concentrations of anti-cancer drug adriamycin (ADR). Twenty-four hours after ADR treatment, cells were subjected to MTT cell survival assay and TUNEL staining experiments. As shown in Fig. 1A and B, ADR treatment led to a significant reduction of cell viability and a remarkable increase in number of TUNEL-positive cells in a dose-dependent manner, indicating that U2OS cells undergo apoptosis in response to ADR.

Under these experimental conditions, cell lysates and total RNA were prepared and analyzed for the expression levels of p53 and E2F-1 by immunoblotting and RT-PCR, respectively. As seen in Fig. 1C, a dose-dependent accumulation of γH2AX, which serves as a DNA damage marker, and also a proteolytic cleavage of PARP, which is one of substrates of the activated caspase 3 [23], were detected. In accordance with the previous observations [24], ADR-mediated accumulation as well as phosphorylation of p53 at Ser-15 was detectable in association with a remarkable induction of its target gene products such as p21^{WAF1}, BAX, PUMA and NOXA. These results strongly suggest that U2OS cells undergo apoptosis following ADR treatment in a p53-dependent manner. Like p53, E2F-1 was significantly induced in response to ADR. ADR-mediated accumulation of E2F-1 and p53 was further confirmed by the indirect immunofluorescence staining experiments (Supplementary Fig. S1). In addition, RT-PCR experiments revealed that ADR-dependent induction of E2F-1 is regulated at mRNA level (Fig. 1D). The similar response was also apparent in *p53*-proficient human colon carcinoma-derived HCT116 cells, as a consequence of treating with ADR (Supplementary Fig. S2).

3.2. ADR-mediated recruitment of p53 together with E2F-1 onto p53-target gene promoters

As described previously [20], E2F-1 has an ability to form a complex with p53. To confirm these observations under our experimental conditions, we performed co-immunoprecipitation experiments. Cell lysates were prepared from U2OS cells exposed to ADR and immunoprecipitated with normal mouse serum (NMS) or with monoclonal anti-p53 antibody. The immunoprecipitates were analyzed by immunoblotting with polyclonal anti-E2F1 antibody. As shown in Fig. 2A, the anti-p53 immunoprecipitates contained the endogenous E2F-1. The reciprocal experiments demonstrated that the endogenous p53 is co-precipitated with E2F-1 (Fig. 2B), suggesting that E2F1 forms a stable complex with p53 in ADR-treated U2OS cells.

To gain a clue to clarify the functional significance of E2F-1/p53 complex during ADR-mediated apoptosis, we carried out the chromatin immunoprecipitation (ChIP) assays. To this end, U2OS cells were treated with ADR or left untreated. Twenty-four hours after ADR treatment, chromatin DNA was immunoprecipitated with anti-p53 or with anti-E2F-1 antibody and subjected to PCR-based amplification using a primer set specific for *p21*^{WAF1} or *BAX* promoter. As expected, ADR treatment led to a remarkable increase in the amounts of p53 bound to *p21*^{WAF1} and *BAX* promoters (Fig. 2C). Intriguingly, the amounts of E2F-1 recruited onto *p21*^{WAF1} and *BAX* promoters clearly elevated in cells exposed to ADR as compared with those in untreated cells (Fig. 2D). Thus, it is likely that E2F-1 might regulate the transcriptional activity of p53 through the complex formation with p53.

3.3. E2F-1 inhibits the transcriptional activity of p53

To examine a possible effect of E2F-1 on the transcriptional activity of p53, we performed the luciferase reporter assay. *p53*-deficient H1299 cells were transfected with the constant amount of the expression plasmid for p53 and luciferase reporter plasmid carrying p53-responsive *p21*^{WAF1}, *BAX* or *MDM2* promoter, along with or without the increasing amounts of the expression plasmid for E2F-1. As shown in Fig. 3A, p53 had an ability to enhance the luciferase activity driven by *p21*^{WAF1}, *BAX* and *MDM2* promoter, and E2F-1 strongly reduced p53-dependent luciferase activity in a dose-dependent manner.

To further confirm this issue, we carried out RT-PCR. H1299 cells were transfected with the constant amount of p53 expression plasmid, together with or without the increasing amounts of the expression plasmid for E2F-1. Forty-eight hours after transfection, total RNA was prepared and processed for RT-PCR. Consistent with the results obtained from the luciferase reporter assay, p53-dependent induction of the various p53-target genes including *p21*^{WAF1}, *BAX*, *MDM2*, *PUMA* and *NOXA*, was significantly suppressed in cells expressing the exogenous E2F-1 (Fig. 3B). Taken together, our results indicate that E2F1 has an inhibitory effect on the transcriptional activity of p53.

3.4. Knockdown of E2F-1 promotes ADR-mediated apoptosis

To address the functional significance of E2F-1 on DNA damage-induced apoptosis, siRNA-mediated knockdown of the endogenous E2F-1 was carried out. E2F-1 knockdown HCT116 cells were then exposed to ADR. At the indicated time points after ADR treatment, total RNA and cell lysates were prepared and subjected to RT-PCR and immunoblotting, respectively. As clearly shown in Fig. 4A and B, E2F-1 was significantly suppressed in the presence of E2F-1 siRNA. Intriguingly, RT-PCR experiments revealed that a much more rapid response of *p21*^{WAF1} and *BAX* is visible in E2F-1 knockdown cells as compared with that in control cells, as a consequence of ADR treatment. Immunoblot analysis demonstrated that knockdown of E2F-1 causes an enhancement of ADR-dependent phosphorylation of p53 at Ser-15 as well as the proteolytic cleavage of PARP. However, knockdown of E2F-1 had an undetectable effect on ADR-mediated accumulation of p53. In a good agreement with the expression studies, microscopic examination and trypan blue exclusion assay showed that the knockdown of E2F-1 results in a marked decrease in number of the attached cells (Fig. 4C) and also a significant increase in number of trypan blue-positive cells in re-

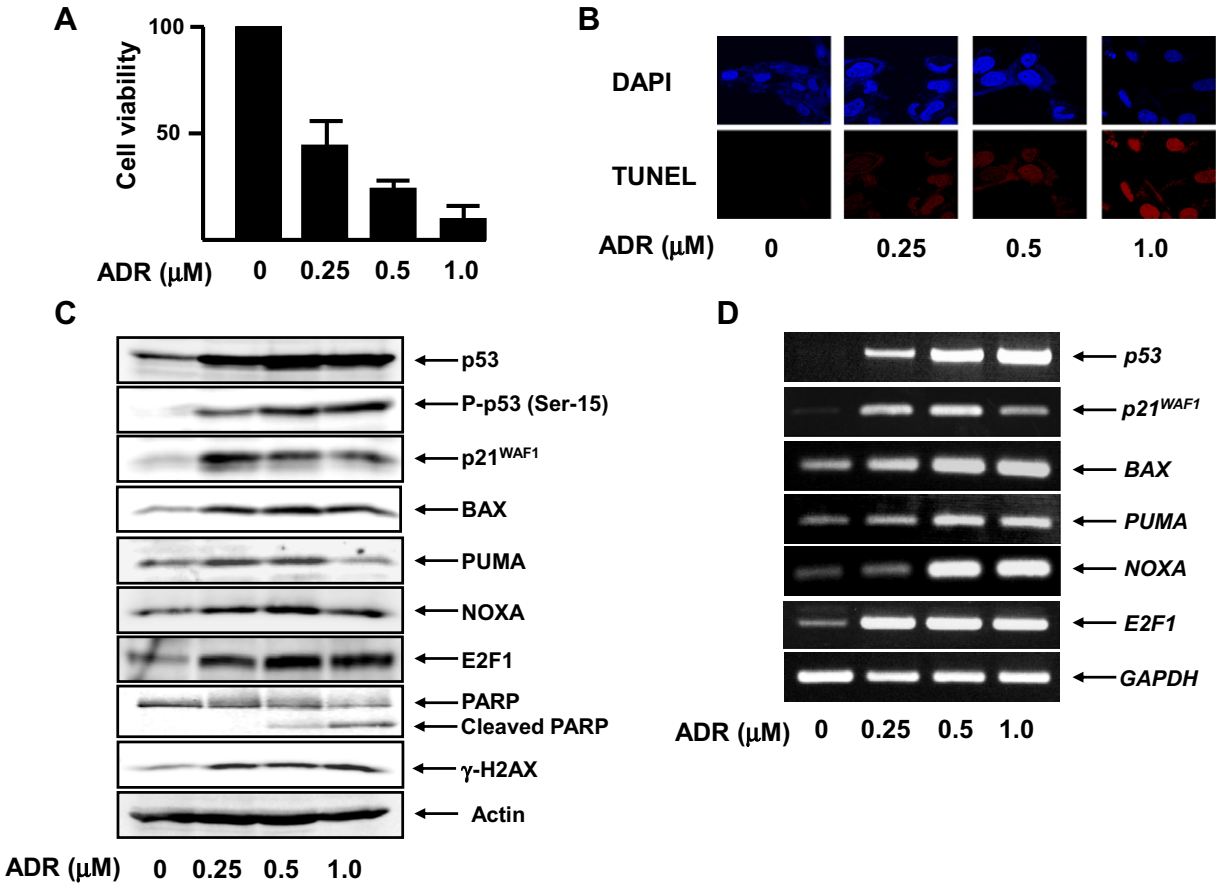

Fig. 1. ADR-mediated induction of E2F-1 and p53. (A) MTT assay. U2OS cells were treated with the indicated concentrations of ADR. Twenty-four hours after ADR treatment, cells were subjected to MTT cell survival assay. (B) TUNEL staining. U2OS cells were treated with ADR as in (A). Twenty-four hours after ADR treatment, cells were processed for TUNEL staining (lower panels). Cell nuclei were stained with DAPI (upper panels). (C) Immunoblotting. Cell lysates were prepared from U2OS cells exposed to the indicated concentrations of ADR for 24 h, and analyzed by immunoblotting with the indicated antibodies. Actin was used as a loading control. (D) RT-PCR. U2OS cells were treated with the indicated concentrations of ADR as in (C). Twenty-four hours after ADR treatment, total RNA was extracted and subjected to RT-PCR. *GAPDH* was used as an internal control.

sponse to ADR (Fig. 4D). Collectively, these findings suggest that E2F-1 has an inhibitory effect on p53 in response to DNA damage.

4. Discussion

In the present study, we have found that E2F-1 has an inhibitory role in the regulation of p53-mediated apoptosis in response to ADR. Considering that ADR treatment leads to the up-regulation of p53 with similar kinetics to its inhibitor E2F-1, E2F-1 might monitor the activity of p53 to avoid an inappropriate apoptosis caused by overactive p53.

Based on our present findings, E2F-1 was up-regulated in response to ADR exposure at mRNA and protein levels. This ADR-mediated transcriptional elevation of E2F-1 was detectable not only in U2OS cells but also in HCT116 cells. Previous studies demonstrated that E2F-1 is stabilized in following X-ray and UV irradiation [25]. Similar induction of E2F-1 was also observed in response to cisplatin and etoposide, which might be caused by DNA damage-mediated prevention of the interaction between E2F-1 and E3 ubiquitin ligase Skip2 [26]. In addition to stability control, the expression of E2F-1 is regulated at transcriptional level in cells exposed to ADR.

siRNA-mediated knockdown of E2F-1 led to a remarkable increase in ADR-dependent phosphorylation of p53 at Ser-15 in association with the induction of apoptosis as well as the proteolytic cleavage of PARP. In accordance with these observations, knocking down of E2F-1 resulted in a rapid response of p53-target genes such as *p21*^{WAF1} and *BAX*. Since ADR-mediated phosphorylation of p53 at Ser-20 and Ser-46 was undetectable in U2OS and HCT116 cells (data not shown), it is likely that Ser-15 phosphorylation is important for ADR-mediated activation of p53 under our experimental conditions. Hershko et al. described that E2F-1 induces phosphorylation of p53 at Ser-46 through up-regulation of TP53INP1 and this phosphorylation is important for E2F-1/p53 cooperation in apoptosis [27]. However, forced expression of E2F-1 failed to induce phosphorylation of p53 at Ser-46 (data not shown), which might be due to the different experimental systems. Intriguingly, knockdown of E2F-1 had a negligible effect on ADR-mediated accumulation of p53, suggesting that DNA damage-induced stabilization of p53 might be a distinct event from DNA damage-mediated phosphorylation at Ser-15 and activation of p53. In support of this notion, it has been shown that DNA damage-induced stabilization of p53 is detectable irrespective of its phosphorylation status [28].

Luciferase reporter assay and RT-PCR analysis demonstrated that E2F-1 has an ability to inhibit the transcriptional activity of p53. Our present observations were consistent with the previous findings as described by Nip et al. [21]. Although the precise molecular mechanisms behind E2F-1-mediated inhibition of the transcriptional activity of p53 remain unclear, Lee et al. reported that both E2F-1 and p53 utilize p300 as a transcriptional

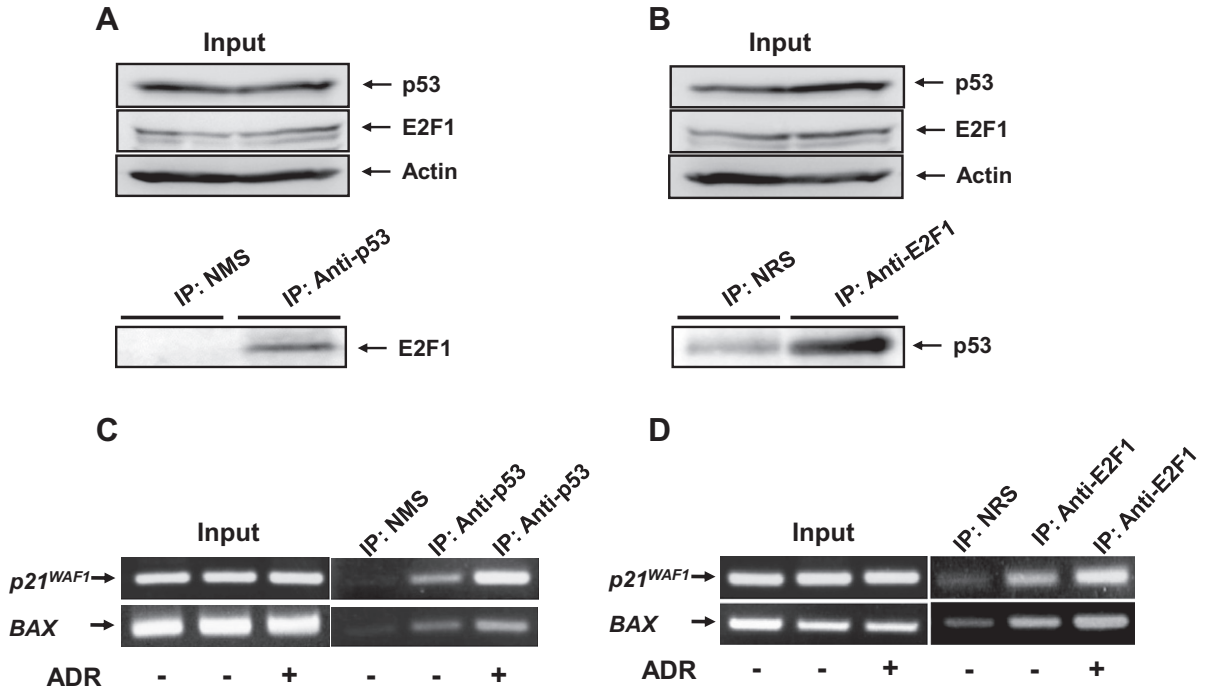

Fig. 2. Complex formation between E2F1 and p53. (A and B) Immunoprecipitation. Cell lysates prepared from U2OS cells exposed to 0.5 μM of ADR were immunoprecipitated with normal mouse serum (NMS) or with monoclonal anti-p53 antibody. The immunoprecipitates were analyzed by immunoblotting with polyclonal anti-E2F-1 antibody. (A). The reciprocal experiments were performed by using normal rabbit serum (NRS) and polyclonal anti-E2F-1 antibody followed by immunoblotting with monoclonal anti-p53 antibody (B). 1/20 of inputs were also shown. (C and D) ChIP assay. U2OS cells were treated with 0.5 μM of ADR or left untreated. Twenty-four hours after ADR exposure, cells were treated with 1% formaldehyde and lysed in SDS-lysis buffer followed by a brief sonication. Chromatin DNA was immunoprecipitated with anti-p53 (C) or anti-E2F1 antibody (D), purified by ethanol precipitation, and then analyzed by standard PCR employing *p21*^{WAF1} (upper panels) or *BAX* (lower panels) promoter-specific primers spanning its p53-responsive site.

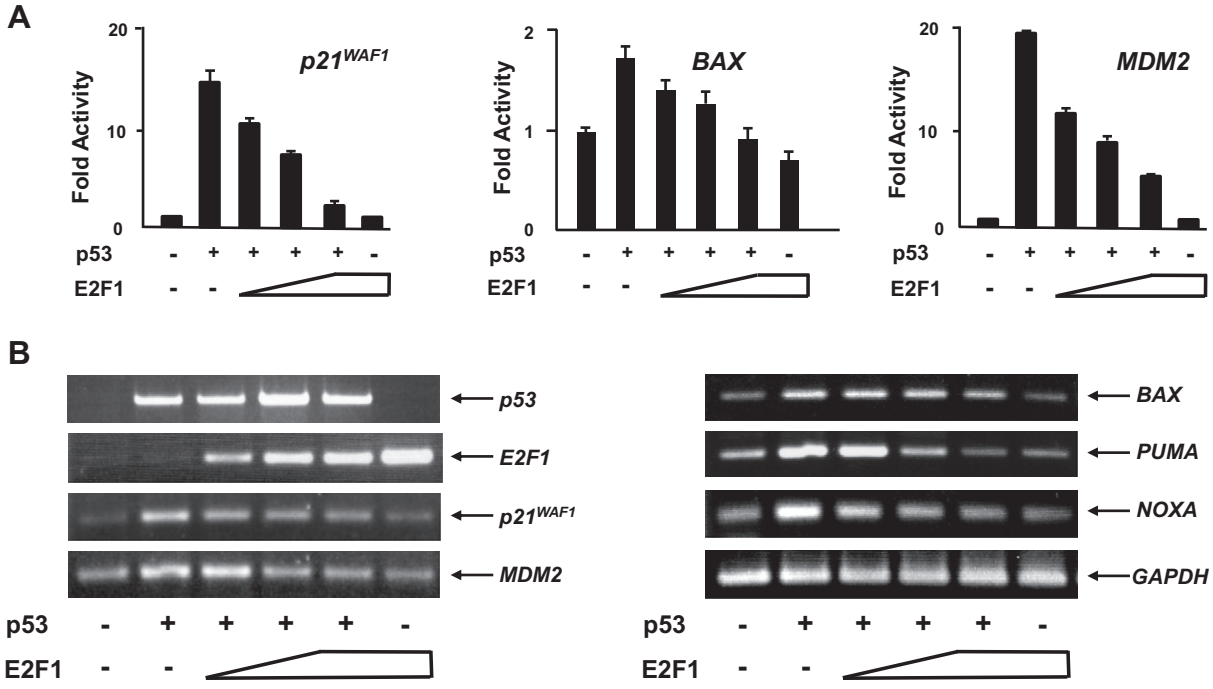

Fig. 3. E2F-1 inhibits the transcriptional activity of p53. (A) Luciferase reporter assay. H1299 cells were transfected with the constant amount of the expression plasmid for p53 (25 ng), luciferase reporter plasmid harboring *p21*^{WAF1}, *BAX* or *MDM2* promoter (100 ng) and *Renilla* luciferase expression plasmid (10 ng), along with or without the increasing amounts of the expression plasmid for E2F-1 (50, 100 and 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. (B) RT-PCR. H1299 cells were transfected with the constant amount of the expression plasmid for p53 (0.5 μg), together with or without the increasing amounts of the expression plasmid for E2F-1 (0.5, 1.0 and 1.5 μg). Forty-eight hours after transfection, total RNA was extracted and subjected to RT-PCR.

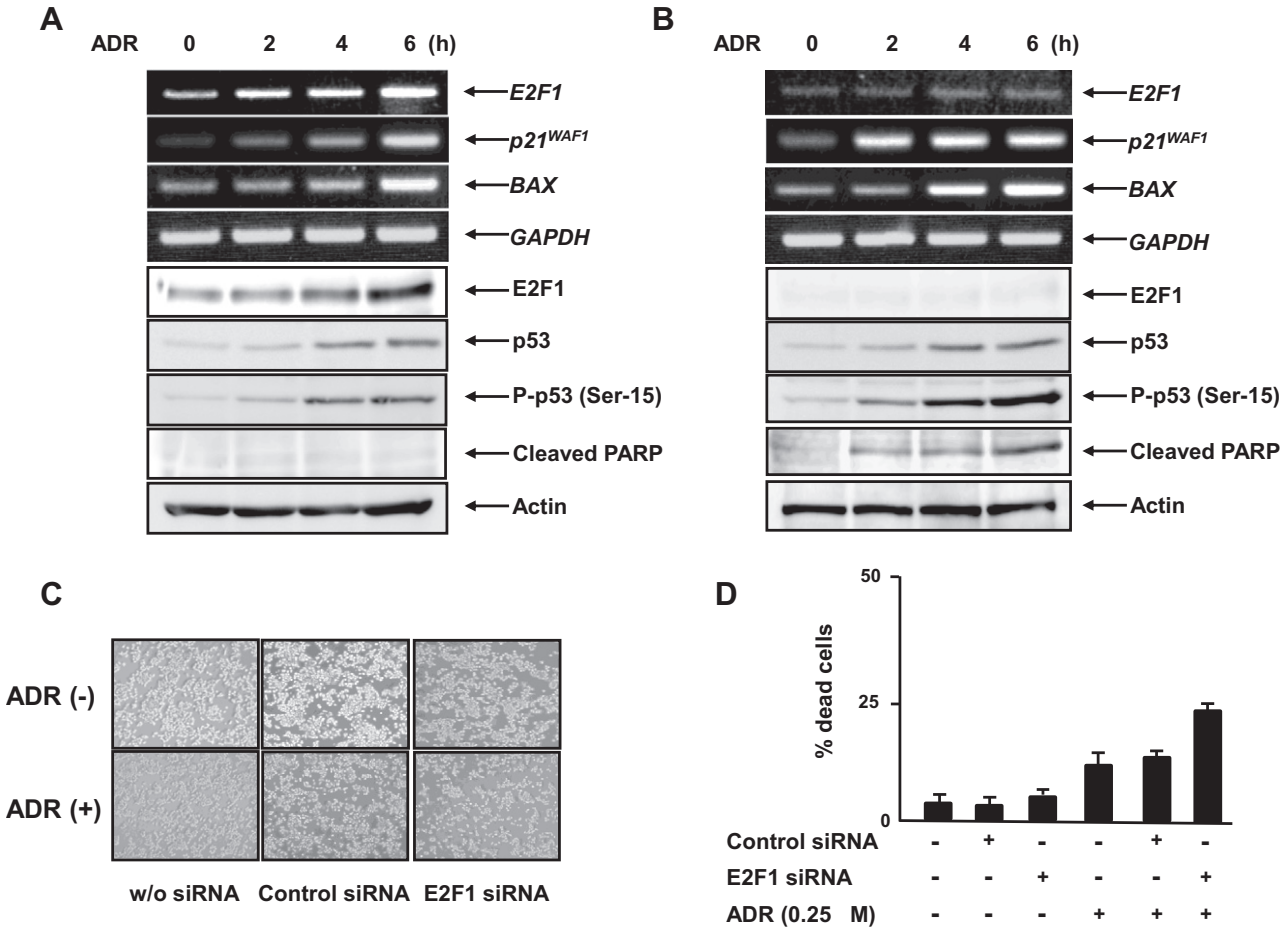

Fig. 4. Knockdown of E2F-1 promotes ADR-mediated apoptosis. (A and B) Expression pattern of p53-target genes in E2F-1-depleted HCT116 cells in response to ADR. HCT116 cells were transfected with control siRNA (A) or with siRNA against E2F-1 (B). Forty-eight hours after transfection, cells were exposed to 0.25 μM of ADR. At the indicated time points after ADR treatment, total RNA and cell lysates were prepared and analyzed by RT-PCR (upper panels) and immunoblotting (lower panels), respectively. (C) Phase-contrast micrograph. E2F-1 knockdown HCT116 cells were exposed to 0.25 μM of ADR. Six hours after ADR treatment, cells were examined by phase-contrast microscope. (D) Trypan blue exclusion assay. E2F-1 knockdown HCT116 cells were treated with 0.25 μM of ADR. Six hours after ADR exposure, adherent and floating cells were collected and subjected to trypan blue exclusion assay. Number of trypan blue-positive cells was scored.

co-activator [29]. Our ChIP assay revealed that p53 as well as E2F-1 efficiently binds to p53-target gene promoters in response to ADR. Thus, it is possible that, upon DNA damage, E2F-1 is recruited onto p53-target gene promoters through complex formation with p53, and then suppresses the interaction between p53 and p300. Since the expression level of p300 remained unchanged regardless of ADR treatment in U2OS and HCT116 cells (data not shown), the intracellular balance between the expression levels of p53 and E2F-1 might be a critical determinant for p53-mediated transcriptional activation and apoptosis in response to DNA damage.

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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.2012.03.108.

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