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Synergistic cooperation of MDM2 and E2F1 contributes to TAp73 transcriptional activity

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ABSTRACT

TAp73, a structural homologue of p53, plays an important role in tumorigenesis. E2F1 had been reported as a transcriptional regulator of TAp73, however, the detailed mechanism remains to be elucidated. Here we reported that MDM2-silencing reduced the activities of the TAp73 promoters and the endogenous TAp73 expression level significantly; while MDM2 overexpression upregulated them. We further revealed that the regulation of TAp73 transcriptional activity occurs as a synergistic effect of MDM2 and E2F1, most probably through their physical interaction in the nuclei. Furthermore, we also suggested that MDM2 might be involved in DNA damage-induced TAp73 transcriptional activity. Finally, we elucidated that MDM2-silencing reduced the proliferation rate of colon carcinoma cells regardless of the p53 status. Our data show a synergistic effect of MDM2 and E2F1 on TAp73 transcriptional activity, suggesting a novel regulation pathway of TAp73.

1. Introduction

p73 is one of the member of p53 family, which shares relatively high sequence homology with p53, including an N-terminal transactivating (TA) domain and a central DNA binding domain [1–4]. p73 had been reported to activate some p53 responsive genes, such as bax, cyclin G, IGF-BP3 and 14-3-3s [2,5]. Previous studies showed that p73 is expressed in two main isoform classes: TAp73 and DNp73. TAp73 can activate p53-target genes and as the results, induces apoptosis and cell cycle arrest in some carcinoma cells; while DNp73 can act as a dominant negative of p53 family members and thus promotes tumorigenesis [2,3,6,7]. Despite of the above-mentioned similarities, p73 differs from p53 in their activations, regulation mechanisms in responding DNA damage and roles in tumorigenesis. Although p73 had been previously reported to induce cell growth arrest and apoptosis in some cell lines [2–4], a recent report by Vikhanskaya et al. showed that p73 could promote cellular growth by activating AP-1 via c-Jun-dependent pathway [8]. Furthermore, unlike p53, TAp73 mutation is rarely found in tumors; and indeed, TAp73 overexpression could be found in a wide range of cancers [2,6,7]. Moreover, recently Du et al. elucidated that TAp73 could shift the cellular metabolism to meet the tumor cells demands of robust biosynthesis and antioxidant defense by enhancing the pentose phosphate pathway (PPP), and subsequently, promotes cellular growth [9].

E2F1 had been identified as a potent facilitator of DNA synthesis that drives quiescent cells into S phase. During the G1 phase, the activity of E2F1 is inhibited by the binding of tumor suppressor Rb, while in late G1 phase, Rb phosphorylation releases E2F1 and drives cell cycle progression into the S phase through the activations of multiple S phase genes [10,11], *i.e.*, cyclin E, dihydrofolate reductase, thymidine kinase [12] and DNA Pol α [13]. On the other hand, Pediconi et al. had reported that E2F1 could act as the transcriptional regulator of TAp73 by recruiting and activating the TAp73 promoter [14]. Our previous study showed that E2F1 could synergistically act with transcription factor yin yang 1 (YY1) and promotes p73 transcriptional activity [15]. However, whether other factors could interact with E2F1 and participate in regulating TAp73 activation remains to be elucidated.

MDM2 had been known as a negative regulator of p53. MDM2 not only inhibits p53 transcriptional activity [16], but also promotes its ubiquitination [17] and proteolytic degradation [18]. However,

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the roles of MDM2 in the regulations of other p53 family members have not been fully elucidated. MDM2 seemed to have distinct roles in different p53 family members. Calabro et al. had reported that in contrast to p53, MDM2 upregulates the protein level of p63 [19]. Previous studies had also reported that instead of promoting p73 degradation, MDM2 might promote the stability of p73 protein [20,21].

In this study, we elucidated the role of MDM2 in regulating TAp73 transcriptional activity, and investigated the cooperative effect of MDM2 and E2F1 in this regulation. We revealed a novel TAp73 regulatory pathway involving a synergistic cooperation between MDM2 and E2F1.

2. Materials and methods

2.1. Cell lines and culture

U2OS were obtained from the American Type Culture Collection; HEK293T cells were from Riken Cell Bank (Tsukuba, Japan); p53 wild-type human colon carcinoma HCT116 cells (HCT116 $p53^{+/+}$) and p53-null human colon carcinoma HCT116 cells (HCT116 $p53^{-/-}$) were kindly provided by Dr. B. Vogelstein at The Johns Hopkins University Medicine School [22,23]. U2OS and HEK293T cells were maintained in Dulbecco's modified Eagle's medium (Gibco, Life Technologies, Grand Island, NY) containing 10% fetal bovine serum (FBS); HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells were maintained in McCoy's 5A medium (Gibco, Life Technologies) containing 10% FBS. Cells were grown at 37 °C in a humidified incubator with 5% CO_2.

2.2. Construction of vectors

The long human TAp73-luciferase reporter, which contains the TAp73 gene fragment from −4052 to +438, was generously provided by Prof. Levrero (University of Rome 'La Sapienza', Rome, Italy) [14]. The short human TAp73-luciferase reporter (from −857 to +71), human p21-luciferase reporter and pcEF9-Flag-E2F1 vectors were constructed as described previously [15]. For the MDM2 expression vector (pcMDM2), the coding region of human MDM2 was inserted into the pcDNA3 vector (Invitrogen, Grand Island, NY).

2.3. Construction of shRNA expression vectors

Based on the results of applying the design algorithm [24], we identified the target sequences for constructing shRNA expression vectors against MDM2, namely shMDM2-1 and shMDM2-2, and E2F1, namely shE2F1 as below: GACTAAACGATTATATGAT (for shMDM2-1), AGGCAAATGTGCAATACCA (for shMDM2-2), and GGCTGGACCTGGAAACTGA (for shE2F1). The shRNA expression vectors were constructed as described previously [15], and an shRNA expression vector containing a stretch of 7 thymines terminator sequences exactly downstream of the U6 promoter, namely shCont, was used as a control.

2.4. Transient transfection and luciferase reporter assays

For knockdown experiments, cells were transfected with indicated shRNA expression vectors, and 24 h later, transfected cells were selected by using 1 μg/mL puromycin for 36 h. Cells were further transfected with TAp73-luciferase reporters and *Renilla* luciferase expression vector (pRL-SV40, Promega, Madison, WI), then 24 h later, luciferase reporter activities were analyzed using dual luciferase reporter assay as described previously [15]. For overexpression experiments, cells were transfected with indicated overexpression vectors. Twenty-four hours later, cells were further cotransfected with TAp73 reporters or p21 reporter and pRL-SV40 vector, and luciferase activities were analyzed as described above.

2.5. Real-time RT-PCR analysis

Cells were transfected with shRNA expression vectors and selected using puromycin as described above. Thirty-six hours later, total RNA was extracted with RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The first-strand cDNA was synthesized using the QuantiTect Reverse Transcription Kit (Qiagen). Quantitative real-time PCR was performed using QuantiTect® SYBR Green PCR Master Mix (Qiagen) as described previously [23]. The sequences of primers used in this study are listed in Supplemental Table S1. All reactions were run in triplicate and expressional results were normalized to actin.

2.6. Western blotting analysis

For knockdown experiments, cells were transfected with shRNA expression vectors, and puromycin selection was performed as described above. For overexpression experiments, cells were transfected with the indicated overexpression vectors, and 36 h later, the cells were lysed. The lysate samples were electrophorated and transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, MA) as described previously [15]. After blocking, the membrane was incubated with the indicated primary antibodies, and then with horseradish peroxidase (HRP)-conjugated secondary antibody IgG (Amersham Biosciences, Piscataway, NJ). Detections were performed with ECL Plus reagent (Amersham Biosciences). Antibodies used for western blotting were a rabbit polyclonal anti-TAp73 antibody (A300-126A, Bethyl Laboratories, Montgomery, TX), a mouse monoclonal anti-MDM2 antibody (SMP14, Santa Cruz Biotechnology, Santa Cruz, CA), and a rabbit polyclonal anti-actin antibody (#4967: Cell Signaling Technology, Beverly, MA).

2.7. Cell viability assays

HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells were transfected with indicated shRNA expression vectors by using X-tremeGENE HP reagent (Roche Applied Science, Mannheim, Germany), and the transfected cells were selected using puromycin selection as described above. The transfected cells were then seeded into 96-well culture plates at a density of 1×10^4 cells/well. At 24 and 48 h later, cell viabilities were determined using colorimetric assays with 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H-tetrazolioum monosodium salt (Cell Counting Kit-8; Dojindo, Japan) in accordance with the manufacturer's instructions.

2.8. Immunofluorescence staining

The U2OS cells were seeded on cover-slips in 6-well plates and transfected with pcEF9-Flag-E2F1 and pcMDM2. Forty-eight hours after transfection, immunofluorescence staining was performed using with the methods described previously [15]. First antibodies used were rat anti-Flag (Sigma–Aldrich, St. Louis, MO) and rabbit anti-MDM2 antibodies (C-18, Santa Cruz Biotechnology); and secondary antibodies used were Alexa Fluor 488 goat anti-rat IgG (H + L) and Alexa Fluor 568 goat anti-rabbit IgG (H + L) (both from Molecular Probes, Eugene, OR). Nuclei were stained with DAPI.

2.9. Co-immunoprecipitation

HEK293T cells were cotransfected with 10 μg of pcEF9-Flag-E2F1 and pcMDM2 using Lipofectamine 2000 (Invitrogen),

and harvested 48 h later. Cell lysates were solved in lysis buffer (10 mM HEPES, pH7.5, 100 mM KCl, 0.1% NP-40) with protease inhibitors on ice for 30 min and then cleared by centrifugation at 15,000 rpm. The supernatants were incubated at 4 °C for 1 h with protein G-beads in presence of 3 μg of anti-Flag monoclonal antibody (Sigma–Aldrich). Immunoprecipitated proteins were subjected to western blotting analysis using anti-MDM2 antibody as described above.

2.10. Statistical analysis

All values of experimental results were expressed as mean ± SD of triplicates. The Student's *t*-test (two-tail comparisons) was performed to compare the statistical significance of the differences between the two groups. One-way ANOVA was employed for comparisons among multiple groups. A value of $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. MDM2-silencing suppresses the transcriptional activity of TAp73

To elucidate the role of MDM2 in the regulation of TAp73 pathway, we first investigated the effect of MDM2-silencing on the transcriptional activity of TAp73 promoter. We predicted, using the design algorithm [24], target sites for inducing RNAi against MDM2. Based on these, we constructed two shRNA expression vectors, *i.e.*, shMDM2-1 and shMDM2-2. Two shRNA expression

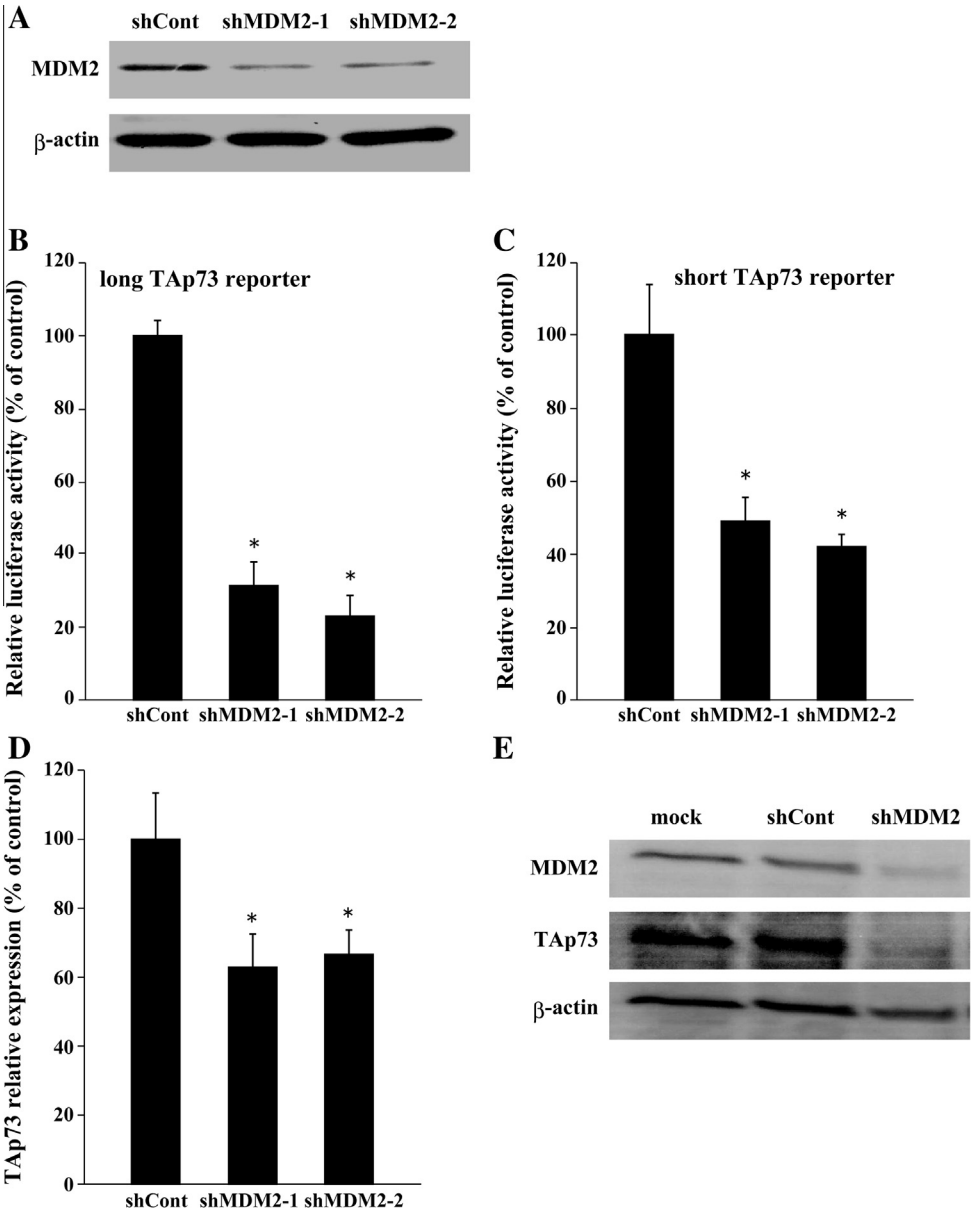

Fig. 1. MDM2-silencing suppresses the transcriptional activity of TAp73. (A) The effects of shRNA vectors on the expression levels of MDM2 in HCT116 $p53^{+/+}$ cells transfected with shMDM2-1, shMDM2-2 or shCont vector. The protein levels of MDM2 were examined by western blots analysis. (B and C) The effect of MDM2-silencing on TAp73 transcriptional activity. HCT116 $p53^{+/+}$ cells were transfected with the indicated shRNA expression vectors, and further cotransfected with pRL-SV40 and either long TAp73 reporter (−4052/+438) (B) or short TAp73 reporter (−857/+71) (C). The activities of TAp73 reporters were determined using dual luciferase reporter assay. The experiments were performed in triplicates. *Indicates $P < 0.05$. (D) The effect of MDM2-silencing on the expression levels of TAp73. HCT116 $p53^{+/+}$ cells were transfected with the indicated vectors, then, the mRNA levels of MDM2 were examined by using quantitative RT-PCR. The experiments were performed in triplicates. *Indicates $P < 0.05$. (E) Western blotting of MDM2 and TAp73 in untransfected HCT116 $p53^{+/+}$ cells and HCT116 $p53^{+/+}$ cells transiently transfected with either shCont or shMDM2-2.

vectors were used to further ensure the effect of MDM2-silencing and to eliminate the off-target effect that might be induced. Western blotting analysis confirmed that both shMDM2-1 and shMDM2-2 could effectively suppress the endogenous protein levels of MDM2 in HCT116 $p53^{+/+}$ cells (Fig. 1A).

Next, we investigated the effect of MDM2-silencing on the transcriptional activity of TAp73 by using either long TAp73 promoter (located at −4052/+438) or short TAp73 promoter (located at −857/+71). We found that in HCT116 $p53^{+/+}$ cells, MDM2-silencing induced a significant reduction of the luciferase activities of both long and short promoters (Fig. 1B and C, respectively), indicating that MDM2-silencing could functionally suppressed the transcriptional activities of TAp73 promoters.

To further confirm the role of MDM2 in TAp73 regulation pathway, we analyzed the effect of MDM2-silencing on the expression levels of endogenous TAp73. As shown in Fig. 1D, the quantitative RT-PCR analysis showed that MDM2-silencing significantly reduced the level of TAp73 mRNA. Consistent with these results, western blotting analysis also showed that the endogenous level of TAp73 protein was grossly suppressed by MDM2-silencing (Fig. 1E).

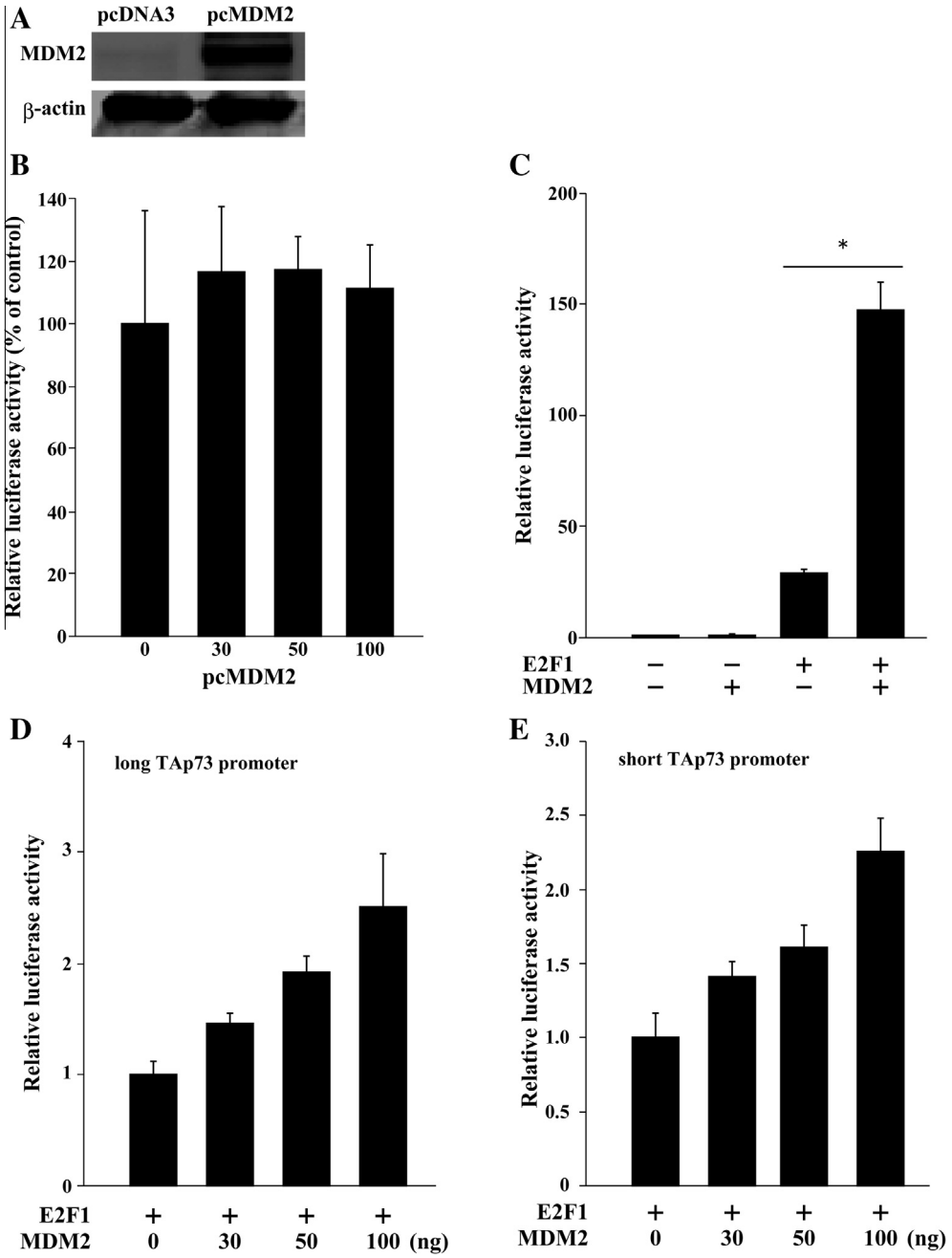

Fig. 2. MDM2 overexpression promotes E2F1-induced TAp73 transcriptional activity. (A) Western blotting of MDM2 and β-actin in HCT116 $p53^{+/+}$ cells transiently transfected with pcDNA3 or pcMDM2 vectors. (B) The effect of MDM2 overexpression on the transcriptional activity of TAp73. U2OS cells were cotransfected with indicated amounts of pcMDM2, long TAp73 reporter and pRL-SV40 gene, and the activities of TAp73 reporter were determined using dual luciferase reporter assay. (C) Overexpressions of MDM2 and E2F1 enhances TAp73 transcriptional activities. U2OS cells seeded in 96 well plate were cotransfected with short TAp73 reporter gene, pRL-SV40 and either pcMDM2 (50 ng) alone, pcEF9-Flag-E2F1 (1 ng) alone or both of them. The activities of TAp73 reporter were determined using dual luciferase reporter assay. (D and E) The dose-dependent effect of MDM2 on TAp73 transcriptional activity in presence of E2F1. U2OS cells were cotransfected with E2F1 (1 ng), the indicated amounts of pcMDM2 vectors, and either long TAp73 reporter (D) or short TAp73 reporter (E). The activities of TAp73 reporters were determined using dual luciferase reporter assay. All experiments were performed in triplicate. *Indicates $P < 0.05$.

MDM2 had been reported to stabilize the expression of TAp73 in its protein level [21]. Our results here showed that MDM2 might be involved in the activation of TAp73 promoter, suggesting that MDM2 regulation of TAp73 might also occur in its translational level. Thus, the robust suppression of the TAp73 protein level as shown in Fig. 1E might be an accumulative effect of MDM2-silencing in both TAp73 transcriptional and translational levels.

3.2. Overexpression of MDM2 induced TAp73 transcriptional activity in presence of E2F1

To further reveal the mechanism of MDM2 regulation on TAp73 activation, we examined the effect of MDM2 overexpression on the transcriptional activity of TAp73 promoter. We generated MDM2 overexpression vector (pcMDM2) and confirmed its expression by performing western blotting analysis using antibody against MDM2 (Fig. 2A). Using a p21 reporter vector expressing firefly luciferase under the p21 promoter, we confirmed that MDM2 overexpression significantly suppressed the transcriptional activity of p21, a well-known p53 downstream target gene, indicating that pcMDM2 vector expressed a functional MDM2 (Supplemental Fig. 1). However, as shown in Fig. 2B, MDM2 overexpression failed to affect the activity of TAp73 promoter. Thus, it seemed that merely overexpressing MDM2 alone was not sufficient to enhance the transcriptional activity of TAp73.

On the other hand, E2F1 had been reported to play a crucial role in the regulation of TAp73 transcriptional activity [14]. Furthermore, our previous results indicated that the regulation of E2F1 on TAp73 is a synergistic cooperation of E2F1 and other transcription factor [15]. Thus, we questioned whether the regulation of MDM2 on TAp73 transcriptional activity also occurs in an E2F1 dependent manner. As shown in Supplemental Fig. 2, we first confirmed that E2F1 overexpression could significantly upregulate TAp73 transcriptional activity dose-dependently. Next, we examined the

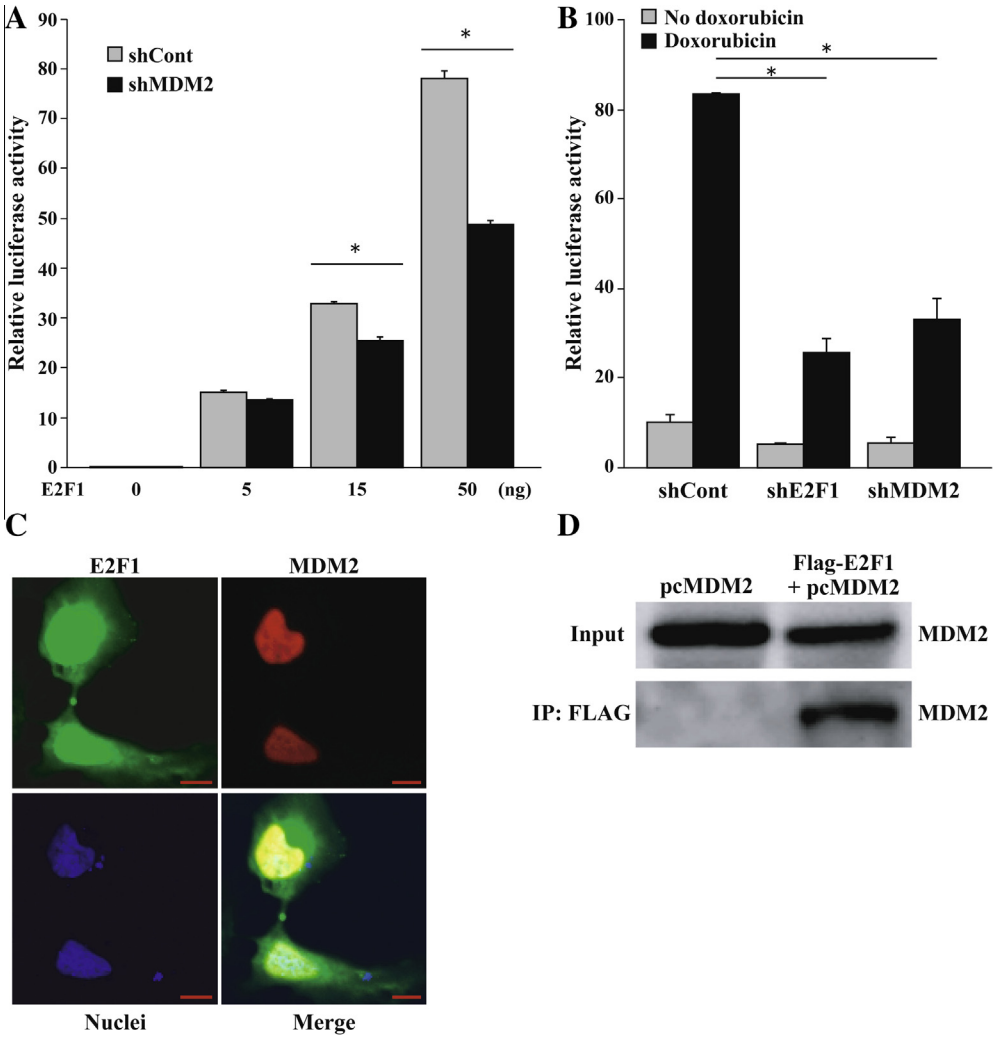

Fig. 3. MDM2 and E2F1 act synergistically in the regulation of TAp73 transcriptional activity. (A) The effect of MDM2-silencing on E2F1-induced TAp73 transcriptional activity. U2OS cells were cotransfected with shMDM2 vectors, long TAp73 reporter, and the indicated amounts of pcEF9-Flag-E2F1. The activities of TAp73 reporter were determined by using dual luciferase reporter assay. Experiments were performed in triplicate. *Indicates $P < 0.05$. Gray bars: shCont; Black bars: shMDM2. (B) The effect of MDM2-silencing on the doxorubicin-induced TAp73 transcriptional activity. U2OS cells were cotransfected with long TAp73 reporter, pRL-SV40 vector, and either shCont, shE2F1 or shMDM2-2. Twenty-four hours after transfection, doxorubicin (2 μM) was added to culture medium, and 12 h later, the activities of TAp73 reporter were measured using dual luciferase reporter assay. Experiments were performed in triplicate. *Indicates $P < 0.05$. Gray bars: no doxorubicin; Black bars: doxorubicin (2 μM). (C) Immunofluorescence staining of MDM2 and E2F1 in U2OS cells cotransfected with pcMDM2 and pcEF9-Flag-E2F1. Immunofluorescence staining was performed by using anti-Flag and anti-MDM2 antibodies. Green: E2F1; red: MDM2; blue: nuclei. Scale bars: 20 μm. (D) Co-immunoprecipitation of MDM2 and E2F1. Cellular lysates from HEK293 cells cotransfected with pcMDM2 and pcEF9-Flag-E2F1 were immunoprecipitated with anti-Flag antibody and blotted with anti-MDM2 antibody.

possibility of synergistic cooperation between MDM2 and E2F1 in this regulatory pathway. We observed that overexpression of MDM2 and E2F1 together robustly enhanced TAp73 transcriptional activity to about 5 times compared to the overexpression of E2F1 alone (Fig. 2C). Furthermore, we found that in presence of exogenous E2F1, the firefly luciferase activities of both long and short TAp73 reporters increased in a MDM2 dose-dependent manner (Fig. 2D and E, respectively). These results indicated a synergistic effect of MDM2 and E2F1 on the regulation of TAp73 transcriptional activity.

3.3. MDM2-silencing decreased E2F1-induced TAp73 transactivation

Next, we investigated whether MDM2-silencing could cancel the E2F1-induced TAp73 transcriptional upregulation. Firstly, we confirmed that overexpression of E2F1 in the U2OS cells significantly promoted the activities of TAp73 reporter (gray bars, Fig. 3A), while MDM2-silencing clearly canceled, at least partly, this upregulation effect (black bars, Fig. 3A). The same tendency could also be confirmed in HCT116 $p53^{+/+}$ cells (Supplemental Fig. 3). Then, we further confirmed the involvement of MDM2 in the upregulation of TAp73 activity induced by endogenous E2F1. As reported previously, doxorubicin, a DNA damaging agent, could induced the activation of the TAp73 promoter in an E2F1 dependent manner [14]. We found that in consistent with E2F1 overexpression, MDM2-silencing significantly suppressed the doxorubicin-induced TAp73 reporter activity to a level similar to that of E2F1-silencing (black bars, Fig. 3B). Although overexpression of MDM2 alone was not sufficient to induce TAp73 transcriptional activity, our findings indicated, for the first time, the crucial role of MDM2 in the

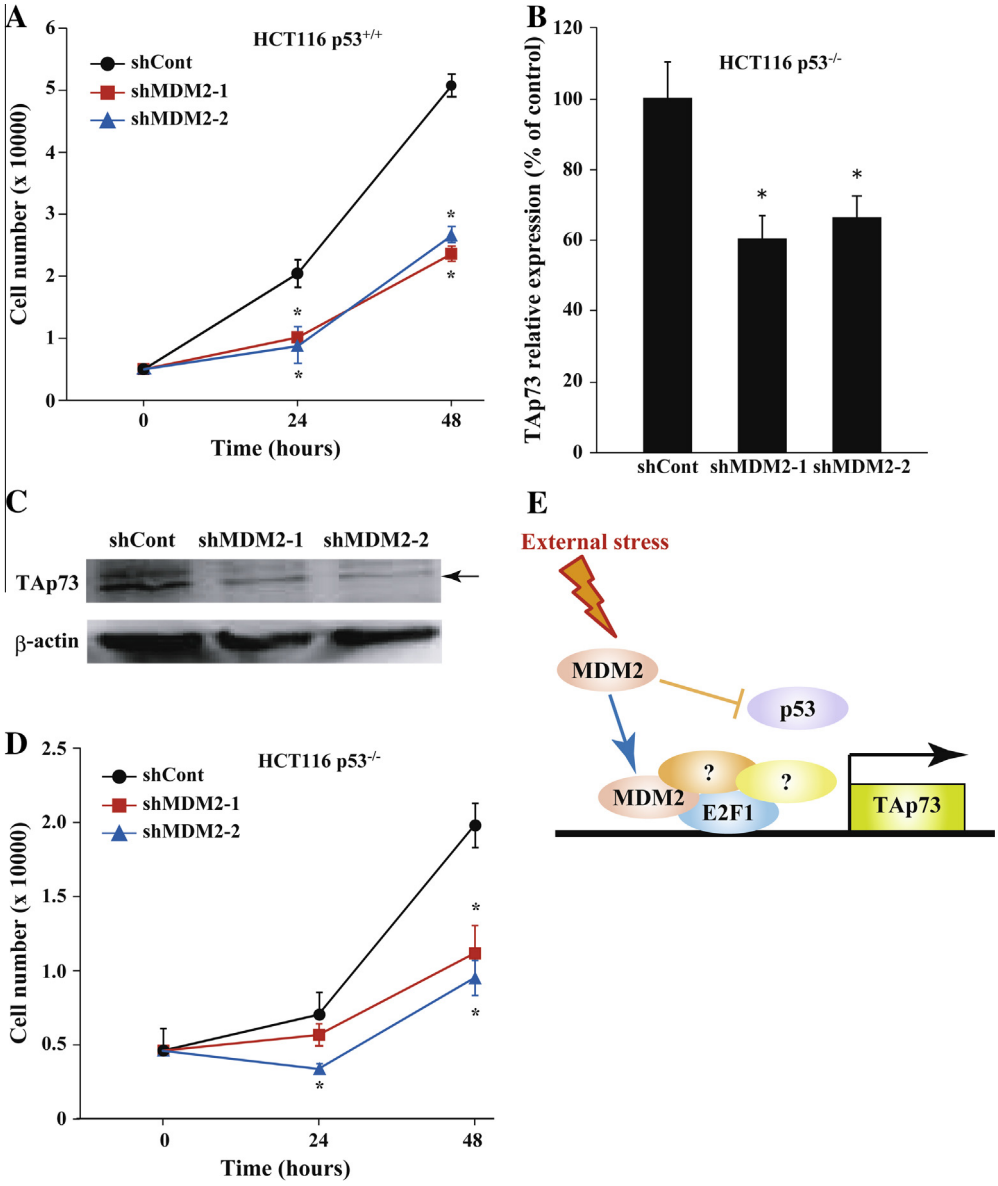

Fig. 4. The effect of MDM2-silecing on cell proliferation in HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells. (A) The effects of MDM2-silencing on the proliferation rate of HCT116 $p53^{+/+}$ cells. HCT116 $p53^{+/+}$ cells were transfected with the indicated shRNA vectors, and the proliferation rates were analyzed at 24 and 48 h after transfection by using MTT assay. Experiments were performed in triplicate. *Indicates $P < 0.05$. (B and C) The effect of MDM2-silencing on the expression levels of TAp73 in HCT116 $p53^{-/-}$ cells. HCT116 $p53^{-/-}$ cells were transfected with the indicated shRNA vectors. (B) The mRNA levels of TAp73 were analyzed using quantitative RT-PCR 48 h after transfection. Experiments were performed in triplicate. *Indicates $P < 0.05$. (C) Western blotting of TAp73 in MDM2-silenced and control HCT116 $p53^{-/-}$ cells. (D) The effects of MDM2-silencing on the proliferation rate of HCT116 $p53^{-/-}$ cells. HCT116 $p53^{-/-}$ were transfected with the indicated shRNA vectors, and the proliferation rates of HCT116 $p53^{+/+}$ cells were analyzed at 24 and 48 h after transfection by using MTT assay. Experiments were performed in triplicate. *Indicates $P < 0.05$. (E) A model of the synergistic role of MDM2 and E2F1 in the regulation of TAp73 transcriptional activity.

E2F1-induced TAp73 transcriptional regulation, indicating that MDM2 might be involved in the DNA damage-induced TAp73 activation. Moreover, in contrast to previously reported E2F1/YY1 regulatory pathway which is specific to TAp73 transcriptional regulation [15], E2F1/MDM2 regulatory pathway might be more general, as it is also involved in the transcriptional activity of DHFR, another well-defined E2F1-dependent gene (Supplemental Fig. 4).

3.4. MDM2 colocalized and interacted physically with E2F1

Next, we tried to reveal more detailed mechanism of this novel regulatory pathway. As E2F1 often binds to other factors in regulating the expression of its target genes [10,11,15], we questioned whether the synergistic regulation of MDM2 and E2F1 on TAp73 transcriptional activity occurs also through their physical interaction. Thus, we first examined the localization of MDM2 and E2F1 in the subcellular compartments. We simultaneously overexpressed MDM2 and Flag-E2F1, and, as shown in Fig. 3C, immunofluorescence staining against anti-Flag and anti-MDM2 confirmed that both E2F1 (upper left image) and MDM2 (upper right image) localized in the nuclei (lower left image). Furthermore, as shown in the merge image (lower right image), MDM2 was colocalized with E2F1 in the nuclei. Moreover, we showed that MDM2 could be detected in the protein lysate fraction immunoprecipitated using anti-Flag antibody (Fig. 3D), indicating the presence of physical interaction between them. Collectively, these results indicated that MDM2 and E2F1 colocalized in the nuclei and interact physically, and thus, further confirm that MDM2 and E2F1 might act synergistically in promoting transcriptional activity of TAp73.

3.5. MDM2-silencing reduced cell proliferation rate partially in a p53-independent manner

Although MDM2 had been reported as a negative regulator of tumor suppressor gene p53, several groups had reported that it acts as a positive regulator of other p53 family members, as it promotes the protein level of p63 and the stability of p73 protein [19–21]. On the other hand, E2F1 had been known to drive cell cycle progression by activating S phase genes. Furthermore, recently, some studies showed the oncogenic roles of TAp73, including its overexpression in a wide range of cancers [2,6,7] and its roles in promoting cellular growth and shifting cellular metabolism to the pentose phosphate pathway, which enable robust biosynthesis and antioxidant defense required by tumor cells [8,9]. Thus, we hypothesized that the regulation of E2F1/MDM2 on TAp73 activity might affect cell proliferation rate. Indeed, we found that in HCT116 $p53^{+/+}$ cells, MDM2-silencing could grossly suppressed cell proliferation rate (Fig. 4A). Our data also demonstrated that shMDM2 vectors used in this study could also significantly suppressed endogenous MDM2 expression in HCT116 $p53^{-/-}$ cells (Supplemental Fig. 5), and that MDM2-silencing also grossly suppressed TAp73 expression in HCT116 $p53^{-/-}$ cells in its transcriptional and translational levels (Fig. 4B and C, respectively). Furthermore, our results showed that MDM2-silencing robustly reduced the proliferation rate of HCT116 $p53^{-/-}$ cells (Fig. 4D). Together, these data revealed that MDM2 might also be involved in cell proliferation in a p53-independent manner, probably due to its regulation on other p53 family members, such as TAp73. Thus, our findings suggested a distinct role of MDM2 in regulating p53 family members, as it could, indeed, in addition to regulate TAp73 protein stability, regulate the transcriptional activity of TAp73.

Taken together, as summarized in the model shown in Fig. 4E, our results demonstrated a novel TAp73 regulatory pathway involving the physical interaction and synergistic cooperation between MDM2 and E2F1. Furthermore, these findings uncovered the distinct regulation mechanisms of MDM2 and E2F1 on different p53 family members, revealing that their roles, especially in tumorigenesis and cancer progression, might be differ according to their targets in carcinoma cells.

Competing financial interests

The authors declare no competing financial interests.

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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.2014.05.026.

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