Biochemical and Biophysical Research Communications 386 (2009) 207–211

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Acetylation status of E2F-1 has an important role in the regulation of E2F-1-mediated transactivation of tumor suppressor p73

Toshinori Ozaki *, Rintaro Okoshi, Meixiang Sang, Natsumi Kubo, Akira Nakagawara

Division of Biochemistry, Chiba Cancer Center Research Institute, Chiba 260-8717, Japan

ARTICLE INFO

Article history:
Received 3 June 2009
Available online 11 June 2009

Keywords:
Acetylation
Adriamycin
Apoptosis
DNA damage
E2F-1
p73

ABSTRACT

Tumor suppressor p73 plays an important role in the regulation of DNA damage response. E2F-1 acts as a transcriptional regulator for *p73*. In the present study, we have found that acetylation of E2F-1 has a critical role in the E2F-1-mediated transactivation of *p73*. In response to adriamycin (ADR), p73 was stabilized in HeLa cells and the expression levels of its target genes increased in association with an induction of apoptosis. Of note, E2F-1 and several its target genes were transactivated in response to ADR, whereas *p73* mRNA level remained unchanged. Immunoprecipitation analysis revealed that ADR has a marginal effect on acetylation status of E2F-1. Intriguingly, acetylation level of E2F-1 remarkably increased in the presence of trichostatin A (TSA) and thereby inducing the expression level of *p73* mRNA. Taken together, our present findings suggest that acetylation status of E2F-1 contributes to the selective activation of its target genes.

Introduction

p73 is one of the tumor suppressor p53 family members and has an ability to induce apoptosis in certain cancerous cells through transactivating cell cycle-regulating and pro-apoptotic target genes such as $p21^{WAF1}$, *BAX*, *NOXA* and *PUMA* [1]. Although *p73* is mapped at human chromosome 1p36 where the chromosome aberrations are frequently observed in a variety of human tumors, *p73* is rarely mutated in human tumor tissues [2], indicating that p73 is not a classical tumor suppressor. Recently, it has been shown that, in contrast to the previous observations [3], *p73*-deficient mice develop spontaneous tumors [4], suggesting that p73 acts as a tumor suppressor.

Like p53, p73 is also involved in the regulation of DNA damage response [1]. In response to DNA damage, p73 is subjected to the chemical modifications including phosphorylation and acetylation [1] and thereby its protein stability significantly increases. Stabilized p73 exerts its pro-apoptotic function. Accumulating evidence suggests that MDM2, which is an E3 ubiquitin protein ligase for p53, inhibits transcriptional as well as pro-apoptotic function of p73 without affecting its protein stability [5]. Rossi et al. have found that a HECT-type E3 ubiquitin protein ligase termed Itch promotes a rapid proteolytic degradation of p73 through ubiquitin–proteasome system [6].

Previously, Chen et al. described that p73 is transcriptionally up-regulated in response to a subset of DNA damaging drugs such as camptothecin [7]. Subsequent studies demonstrated that the promoter region of *p73* contains several E2F-1-responsive elements and indeed E2F-1 acts as a transcriptional regulator for *p73* [8–10]. E2F-1 belongs to the E2F family of nuclear transcription factors and E2F-1/DP-1 complex recognizes E2F-1-responsive elements within the promoter regions of its target genes [11]. As described previously [12,13], E2F-1 has dual roles in the regulation of cell proliferation and cell fate. For example, enforced expression of E2F-1 resulted in cell cycle progression from G1 phase to S phase through the up-regulation of its target genes implicated in DNA synthesis [14–19]. In addition to its proliferative role, E2F-1 has an ability to induce apoptosis through $p14^{ARF}$-mediated stabilization of p53 and/or transactivation of *p73* [8–10,20]. In response to DNA damage, ATM/ATR-dependent phosphorylation of E2F-1 at Ser-31 leads to stabilization and activation of E2F-1. During DNA damage, 14-3-3 binds to phosphorylated Ser-31 of E2F-1 and inhibits its ubiquitination [21].

In the present study, we have found that acetylation of E2F-1 is involved in the selective transactivation of *p73*.

Materials and methods

Cell line and culture. Human cervical carcinoma HeLa cells were maintained 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 grown at 37 °C in a humidified atmosphere of 5% CO_2 in the air. Where indicated, cells were exposed to the indicated concentration of adriamycin (ADR) or trichostatin A (TSA).

* Corresponding author. Fax: +81 43 265 4459.
E-mail address: tozaki@chiba-cc.jp (T. Ozaki).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.06.035

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 concentration 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 1 h. The absorbance readings for each well were carried out at 570 nm using the microplate reader (Model 450, Bio-Rad Laboratories).

Preparation of genomic DNA. HeLa cells were exposed to the indicated concentration of ADR. Twenty-four hours after ADR treatment, genomic DNA was prepared by using Apoptosis Ladder Detection Kit (Wako) according to the manufacturer's recommendations. Genomic DNA was analyzed by 1.5% agarose gel electrophoresis and stained with ethidium bromide.

RT-PCR. Total RNA was isolated from HeLa 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 measure expression levels of genes of interest. The oligonucleotide primers used in this study were as follows: *p53*, 5′-ATTTGATGCTGTCCCCGGACGATATTGAAC-3′ (sense) and 5′-ACCCTTTTTGGACTTCAGGTGGCTGGAGTG-3′ (antisense); *p73α*, 5′-CCGGGAGAACTTTGAGATCC-3′ (sense) and 5′-AT CTTCAGGGCCCCCAGGTC-3′ (antisense); *BAX*, 5′-TTTGCTTCAGGGT TTCATCC-3′ (sense) and 5′-CAGTTGAAGTTGCCGTCAGA-3′ (antisense); *PUMA*, 5′-GCCCAGACTGTGAATCCTGT-3′ (sense) and 5′-TC CTCCCTCTTCCGAGATTT-3′ (antisense); *NOXA*, 5′-CTGGAAGTCG AGTGTGCTACT-3′ (sense) and 5′-TCAGGTTCCTGAGCAGAAGAG-3′ (antisense); *MDM2*, 5′-ACTTGAGCCGAGGAGTTCAA-3′ (sense) and 5′-TCCCGGCAAAAACAAATAAG-3′ (antisense); *Itch*, 5′-CTGGAAGT CGAGTGTGCTACT-3′ (sense) and 5′-CTTGGATGTGGAGCCATCAT-3′ (antisense); *E2F-1*, 5′-GGTGAGGTCCCCAAAGTCAC-3′ (sense) and 5′-GCCACCATAGTGTCACCACC-3′ (antisense); *Caspase-7*, 5′-CAAAG CCACTGACTGACTGAGATG-3′ (sense) and 5′-CAACCCAATGAATAA ATGAT-3′ (antisense); *Bid*, 5′-GCATGTCAACAGCGTTCCTA-3′ (sense) and 5′-GGAACCTGCACAGTGGAAAT-3′ (antisense); *Bmf*, 5′-ATG GAGCCATCTCAGTGTGTG-3′ (sense) and 5′-CCCCGTTCCTGTTCTCTT CT-3′ (antisense); *GAPDH*, 5′-ACCTGACCTGCCGTCTAGAA-3′ (sense) and 5′-TCCACCACCCTGTTGCTGTA-3′ (antisense). PCR products were separated by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining.

Immunoblotting. Cells were washed in ice-cold phosphate-buffered saline (PBS) and lysed in SDS-sample buffer containing 10% glycerol, 5% β-mercaptoethanol, 2.3% SDS and 62.5 mM Tris–HCl (pH 6.8). 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. Bovine serum albumin (BSA) was used as a standard. Equal amounts of cell lysates were separated by 10% SDS–polyacrylamide gel electrophoresis, electro-transferred onto Immobilon-P membrane filters (Millipore) and blocked with 0.3% 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: Oncogene Research Products), monoclonal anti-Itch (BD Transduction Laboratories), monoclonal anti-p73 (Ab-4: NeoMarkers), monoclonal anti-RB (Ab-1: Oncogene Science), polyclonal anti-E2F-1 (C-20: Santa Cruz Biotechnology), polyclonal anti-phospho-p53 at Ser-15 (Cell Signaling Technology), polyclonal anti-PARP (Cell Signaling Technology), polyclonal anti-DP-1 (K-20: Santa Cruz Biotechnology), or with anti-actin (20–33: Sigma) antibody at room temperature for 1 h followed by incubation with 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.

Immunoprecipitation. Whole cell lysates (1 mg of protein) prepared form HeLa cells were precleared with 30 μl of protein G-Sepharose beads and used for immunoprecipitation with anti-E2F-1 antibody. After the addition of 30 μl of protein G-Sepharose beads, the reaction mixtures were incubated for additional 2 h at 4 °C. The beads were then collected by brief centrifugation and washed extensively with the lysis buffer. The precipitated proteins were subjected to immunoblotting with polyclonal anti-acetyl-Lysine antibody (Upstate Biotechnology).

Results

HeLa cells undergo apoptotic cell death in response to ADR

Since it has been shown that the endogenous p53 is inactivated in human cervical cancer-derived HeLa cells, which might be due to the presence of E6-AP [22], we have examined whether HeLa cells could be sensitive to strong chemotherapeutic drug, adriamycin (ADR) [23]. To this end, HeLa cells were treated with ADR at the indicated concentration or left untreated. Twenty-four or forty-eight hours after ADR treatment, cells were subjected to MTT assays. As clearly shown in Fig. 1A, cell viability significantly reduced in a dose-dependent manner. Consistent with those observations, ADR treatment in HeLa cells resulted in apoptotic cell death as examined by Trypan blue exclusion assays (Fig. 1B). Finally, we have prepared genomic DNA from HeLa cells treated with or without ADR and genomic DNA was analyzed by agarose gel electrophoresis. As shown in Fig. 1C, ADR treatment led to a promotion of DNA fragmentation. Taken together, HeLa cells underwent apoptotic cell death in response to ADR.

Expression patterns of p73 and E2F-1 in response to ADR

To examine the expression patterns of p73 and its related gene products in response to ADR, HeLa cells were exposed to the indicated concentration of ADR and whole cell lysates were subjected to immunoblotting. As shown in Fig. 2A, ADR-mediated proteolytic cleavage of PARP was detected, indicating that HeLa cells underwent apoptotic cell death in response to ADR. Consistent with the previous observations [6,24,25], p73 and its transcriptional activator E2F-1 were induced in the presence of ADR, whereas E3 ubiquitin protein ligase Itch which targets p73 for proteasome-dependent degradation of p73, decreased in response to ADR. This inverse relationship between the expression levels between p73 and Itch in response to DNA damage was consistent with the previous observations [6]. DNA damage-induced down-regulation of Itch might contribute to the increased stability of p73 in response to DNA damage. Intriguingly, p53 and its phosphorylation at Ser-15 were induced in the presence of ADR, suggesting that ADR treatment overcomes the negative effect of E6-AP on p53.

Semiquantitative RT-PCR experiments demonstrated that the expression levels of p53/p73 target genes such as *BAX*, *NOXA* and *MDM2* [1] remarkably increase in response to ADR, which might be attributed to the ADR-mediated up-regulation of p53 and p73 at protein level, whereas *p53* and *Itch* remains unchanged irrespective of ADR treatment. It was worse noting that, upon ADR treatment, the expression level of DNA damage responsive gene *E2F-1* significantly increases, however, its direct transcriptional target *p73* remains unchanged, suggesting that E2F-1 might not act as a transcriptional activator for *p73* in HeLa cells exposed to ADR.

Selective induction of E2F-1 target genes in response to ADR

As described above, E2F-1 might not act as a transcription factor for *p73* during ADR-mediated apoptotic cell death in HeLa cells, we

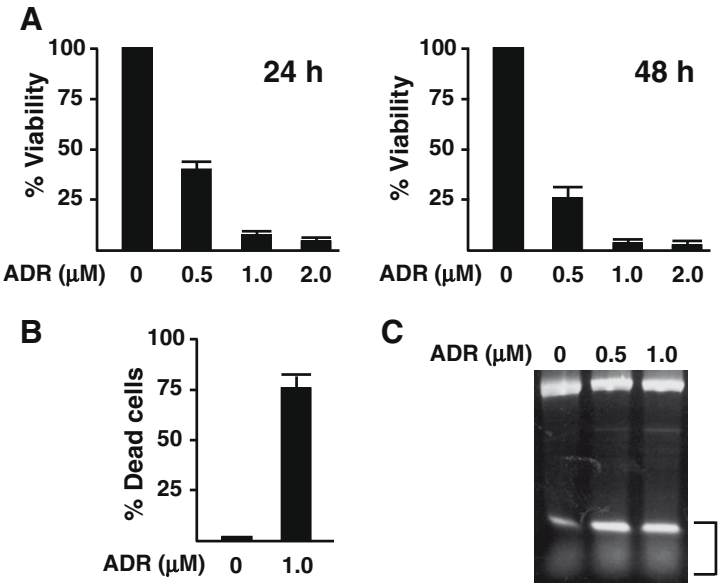

Fig. 1. HeLa cells undergo apoptosis cell death in response to ADR. (A) Cell survival assays. HeLa cells were treated with the indicated concentration of ADR or left untreated. Twenty-four (left panel) or 48 (right panel) hours after the treatment with ADR, cell viability was examined by MTT assay. (B) Trypan blue exclusion assay. HeLa cells were exposed to 1 μM of ADR or left untreated. Twenty-four hours after ADR treatment, cells were stained with Trypan blue and the extent of apoptotic cell death was calculated as the percentage of Trypan blue-positive cells in each cell population. Results are typical of two independent experiments performed in triplicate. (C) DNA fragmentation assay. HeLa cells were treated with the indicated concentration of ADR or left untreated. Twenty-four hours after ADR treatment, genomic DNA was prepared from each cell and analyzed by agarose gel electrophoresis. The position of the fragmented DNA was indicated.

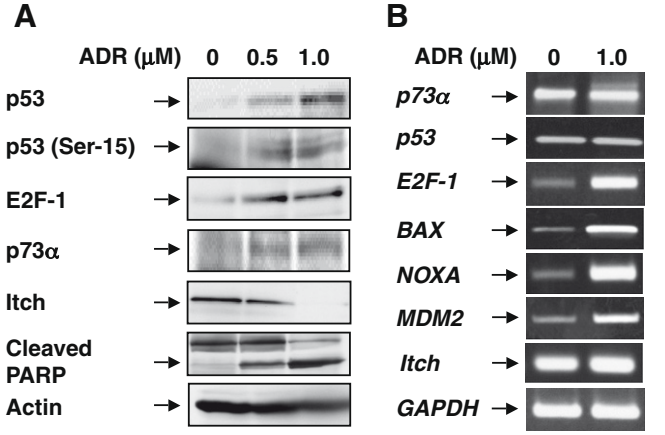

Fig. 2. ADR-mediated induction of p73 without transcriptional activation of *p73*. (A) Immunoblotting. Whole cell lysates prepared from HeLa cells treated with or without ADR were subjected to immunoblotting with the indicated antibodies. Actin was used as a loading control. (B) RT-PCR. HeLa cells were exposed to 1 μM of ADR or left untreated. Twenty-four hours after ADR treatment, total RNA was prepared and processed for RT-PCR. *GAPDH* was used as an internal control.

have examined the expression patterns of the other E2F-1 target genes in response to ADR. HeLa cells were exposed to 1 μM of ADR for 24 h and then total RNA was subjected to RT-PCR. As shown in Fig. 3A, the expression levels of *Caspase-7* [14], *Bid* [26] and *Bmf* [14] were induced in response to ADR, indicating that ADR treatment might enhance the transcriptional potential of E2F-1. Since it has been well established that E2F-1 is regulated by RB and DP-1 [12,13], we then examined the expression patterns of them in HeLa cells exposed to the indicated concentration of ADR. As shown in Fig. 3B, ADR treatment had undetectable effect on the expression levels of RB and DP-1. Next, we asked whether ADR treatment could affect the complex formation between E2F-1 and DP-1. For this purpose, HeLa cells were treated with ADR or left untreated, and then whole cell lysates were immunoprecipitated with normal rabbit serum (NRS) or with polyclonal anti-DP-1 antibody followed by immunoblotting with anti-E2F-1 antibody. As seen in Fig. 3C, the amounts of E2F-1 contained in the anti-DP-1 immunoprecipitates increased in the presence of ADR, which might be due to ADR-mediated up-regulation of E2F-1. Thus, the increased E2F-1/DP-1 complex might contribute to the detectable transcriptional up-regulation of *Caspase-7*, *Bid* and *Bmf*.

As described previously [23], DNA damage-mediated acetylation of E2F-1 is involved in the differential regulation of E2F-1 target gene expression. These observations prompted us to check the acetylation status of E2F-1 in response to ADR. To this end, HeLa cells were treated with 1 μM of ADR or left untreated. Twenty-four hours after ADR treatment, whole cell lysates were immunoprecipitated with NRS or with polyclonal anti-E2F-1 antibody followed by immunoblotting with anti-acetyl-Lysine antibody. As shown in Fig. 3D, ADR treatment had negligible effect on the acetylation level of E2F-1.

Acetylated form of E2F-1 has an ability to transactivates p73

To address whether acetylation status of E2F-1 could be involved in E2F-1-mediated transcriptional activation of *p73*, we have treated HeLa cells with histone deacetylase inhibitor (TSA) for 24 h and examined the expression level of the endogenous *p73* by RT-PCR. As shown in Fig. 4A, the expression level of p73 significantly increased in the presence of TSA as compared with that of cells exposed to ADR. In support with those results, immunoprecipitation experiments revealed that TSA treatment remarkably enhances the acetylation level of E2F-1 as compared with that of cells treated with ADR (Fig. 4B). These results strongly suggest that acetylation status of E2F-1 is involved in the selective induction of *p73*.

Discussion

Based on our present observations, ADR treatment resulted in a massive apoptotic cell death in association with a significant

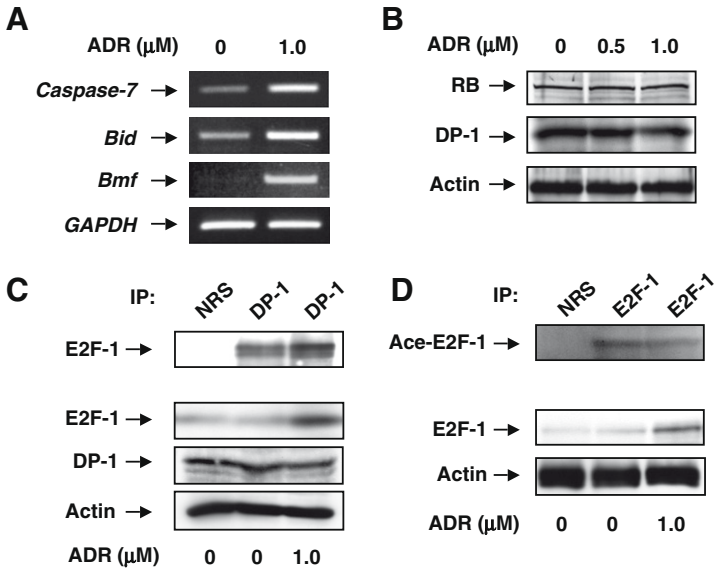

Fig. 3. Acetylation status of E2F-1 remains unchanged irrespective of ADR treatment. (A) Differential transcriptional activation of E2F-1 target genes in response to ADR. Twenty-four hours after ADR treatment, total RNA was prepared from HeLa cells and subjected to RT-PCR. *GAPDH* was used as an internal control. (B) Immunoblotting. HeLa cells were exposed to ADR or left untreated. Twenty-four hours after ADR treatment, whole cell lysates were prepared and processed for immunoblotting with the indicated antibodies. Actin was used as a loading control. (C) Immunoprecipitation. Whole cell lysates were prepared from HeLa cells treated with ADR or left untreated and immunoprecipitated with normal rabbit serum (NRS) or with anti-DP-1 antibody followed by immunoblotting with anti-E2F-1 antibody (upper panel). 1/20 of inputs were shown in lower panels. (D) Acetylation status of E2F-1 in the presence of ADR. Whole cell lysates prepared from HeLa cells treated with or without 1 μM of ADR for 24 h were immunoprecipitated with NRS or with anti-E2F-1 antibody. The anti-E2F-1 immunoprecipitates were analyzed by immunoblotting with polyclonal anti-acetyl-Lysine antibody.

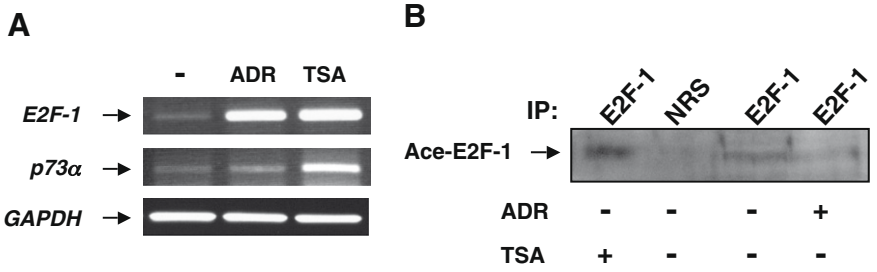

Fig. 4. TSA treatment enhances the acetylation level of E2F-1 and results in the transcriptional activation of *p73*. (A) RT-PCR. HeLa cells were treated with ADR (1 μM), TSA (1 μg/ml) or left untreated. Twenty-four hours after the treatment, total RNA was processed for RT-PCR. *GAPDH* was used as an internal control. (B) E2F-1 is significantly acetylated in the presence of TSA. HeLa cells were treated with ADR (1 μM), TSA (1 μg/ml) or left untreated. Twenty-four hours after the treatment, whole cell lysates were prepared and immunoprecipitated with NRS or with anti-E2F-1 antibody. The anti-E2F-1 immunoprecipitates were subjected to immunoblotting with anti-acetyl-Lysine antibody.

induction of pro-apoptotic p53 as well as p73 at protein level. As described [1], post-translational modifications of p53 as well as p73 contribute to an increase in their protein stability. For example, p73 is phosphorylated at Tyr-99 and Ser-289 by non-receptor tyrosine kinase c-Abl [27–29] and protein kinase Cδ catalytic fragment [30] in response to DNA damage, respectively. Therefore, DNA damage-mediated induction of p73 is largely regulated at protein level. On the other hand, *p73* is transactivated in response to a subset of DNA damaging drugs in certain cancerous cells. Chen et al. demonstrated that *p73* is transcriptionally induced in response to camptothecin in human osteosarcoma-derived SAOS-2 cells [7]. In addition, Marabese et al. described that, following ADR treatment, *p73* is transactivated in human neuroblastoma-derived SH-SY5Y cells [31]. It has been shown that E2F-1 has a critical role in the transactivation of *p73* [8–10].

Under our experimental conditions, DNA damage response gene product E2F-1 was significantly induced in response to ADR, whereas the expression level of its direct transcriptional target *p73* remained unchanged, indicating that certain post-translational modification(s) of E2F-1 might be required for E2F-1-mediated transcriptional induction of *p73*. In support with this notion, Pediconi et al. described that ADR treatment in human glioblastoma-derived T98G cells results in an extensive induction of E2F-1 acetylation in association with an efficient recruitment of E2F-1 onto the *p73* promoter region [32]. In a sharp contrast to T98G cells, ADR-mediated induction of E2F-1 acetylation was not detectable in HeLa cells and we did not detect ADR-dependent transcriptional induction of *p73*. TSA treatment in HeLa cells led to a remarkable increase in the acetylation level of E2F-1 as examined by immunoprecipitation experiments and also induced the transcription of *p73*, suggesting that acetylation status of E2F-1 plays an important role in the selective transcriptional regulation of *p73*. However, it is unknown why ADR treatment induces E2F-1 acetylation in T98G cells but not in HeLa cells. This issue should be addressed.

Another finding of our present study was that *E2F-1* is transcriptionally induced in response to DNA damage. Accumulating evidence suggests that E2F-1 is induced to be stabilized and activated in response to DNA damage through post-translational

modifications such as phosphorylation and acetylation [24,33]. Upon DNA damage, E2F-1 is phosphorylated at Ser-31 in an ATM-dependent manner and thereby stabilized caused by inhibition of Skp2-mediated proteolytic degradation through ubiquitin/proteasome system [24]. In addition to its phosphorylation, E2F-1 is acetylated at several Lysine residues adjacent to its DNA-binding domain, which enhances its DNA-binding activity [33]. To our knowledge, DNA damage-mediated transcriptional up-regulation of *E2F-1* is a novel mechanism behind DNA damage-mediated accumulation of E2F-1. To better understand the precise molecular mechanisms underlying DNA damage response, it is important to identify upstream mediator(s) which has an ability to transactivate *E2F-1* in response to DNA damage.

Acknowledgments

This work was supported in part by a Grant-in-Aid from the Ministry of Health, Labour and Welfare for the Third Term Comprehensive Control Research for Cancer, a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science and Technology, Japan, a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science, Uehara Memorial Foundation and Hisamitsu Pharmaceutical Co.

References

[1] T. Ozaki, A. Nakagawara, p73, a sophisticated p53 family member in the cancer world, Cancer Sci. 96 (2005) 729–737.
[2] S. Ikawa, A. Nakagawara, Y. Ikawa, p53 family genes: structural comparison, expression and mutation, Cell Death Differ. 6 (1999) 1154–1161.
[3] A. Yang, N. Walker, R. Bronson, M. Kaghad, M. Oosterwegel, J. Bonnin, C. Vagner, H. Bonnet, P. Dikkes, A. Sharpe, F. McKeon, D. Caput, p73-deficient mice have neurological, pheromonal and inflammatory defects but lack spontaneous tumours, Nature 404 (2000) 99–103.
[4] E.R. Flores, S. Sengupta, J.B. Miller, J.J. Newman, R. Bronson, D. Crowley, A. Yang, F. McKeon, T. Jacks, Tumor predisposition in mice mutant for p63 and p73: evidence for broader tumor suppressor functions for the p53 family, Cancer Cell 7 (2005) 363–373.
[5] X. Zeng, L. Chen, C.A. Jost, R. Maya, D. Keller, X. Wang, W.G. Kaelin, M. Oren, J. Chen, H. Lu, MDM2 suppresses p73 function without promoting p73 degradation, Mol. Cell. Biol. 19 (1999) 3257–3266.
[6] M. Rossi, V. De Laurenzi, E. Munarriz, D.R. Green, Y.C. Liu, K.H. Vousden, G. Cesareni, G. Melino, The ubiquitin-protein ligase Itch regulates p73 stability, EMBO J. 24 (2005) 836–848.
[7] X. Chen, Y. Zheng, J. Zhu, J. Jiang, J. Wang, p73 is transcriptionally regulated by DNA damage, p53, and p73, Oncogene 20 (2001) 769–774.
[8] N.A. Lissy, P.K. Davis, M. Irwin, W.G. Kaelin, S.F. Dowdy, A common E2F-1 and p73 pathway mediates cell death induced by TCR activation, Nature 407 (2000) 642–645.
[9] M. Irwin, M.C. Marin, A.C. Phillips, R.S. Seelan, D.I. Smith, W. Liu, E.R. Flores, K.Y. Tsai, T. Jacks, K.H. Vousden, W.G. Kaelin, Role for the p53 homologue p73 in E2F-1-induced apoptosis, Nature 407 (2000) 645–648.
[10] T. Stiewe, B.M. Putzer, Role for the p53-homologue p73 in E2F-1-induced apoptosis, Nat. Genet. 26 (2000) 464–469.
[11] J.M. Trimarchi, J.A. Lees, Sibling rivalry in the E2F family, Nat. Rev. Mol. Cell Biol. 3 (2002) 11–20.
[12] C. Stevens, N.B. La Thangue, E2F and cell cycle control: a double-edged sword, Arch. Biochem. Biophys. 412 (2003) 157–169.
[13] N.B. La Thangue, The yin and yang of E2F-1: balancing life and death, Nat. Cell Biol. 5 (2003) 587–589.
[14] H. Müller, A.P. Bracken, R. Vernell, M.C. Moroni, F. Christians, E. Grassilli, E. Prosperini, E. Vigo, J.D. Oliner, K. Helin, E2Fs regulate the expression of genes involved in differentiation, development, proliferation, and apoptosis, Genes Dev. 15 (2001) 267–285.
[15] S. Ishida, E. Huang, H. Zuzan, R. Spang, G. Leone, M. West, J.R. Nevins, Role for E2F in control of both DNA replication and mitotic functions as revealed from DNA microarray analysis, Mol. Cell. Biol. 21 (2001) 4684–4699.
[16] J. Stanelle, T. Stiewe, C.C. Theseling, M. Peter, B.M. Pützer, Gene expression changes in response to E2F1 activation, Nucleic Acids Res. 30 (2002) 1859–1867.
[17] Z. Nahle, J. Polakoff, R.V. Davuluri, M.E. McCurrach, M.D. Jacobson, M. Narita, M.Q. Zhang, Y. Lazebnik, D. Bar-Sagi, S.W. Lowe, Direct coupling of the cell cycle and cell death machinery by E2F, Nat. Cell Biol. 4 (2002) 859–864.
[18] B. Ren, H. Cam, Y. Takahashi, T. Volkert, J. Terragni, R.A. Young, B.D. Dynlacht, E2F integrates cell cycle progression with DNA repair, replication, and G(2)/M checkpoints, Genes Dev. 16 (2002) 245–256.
[19] J. Wells, C.R. Graveel, S.M. Bartley, S.J. Madore, P.J. Farnham, The identification of E2F1-specific target genes, Proc. Natl. Acad. Sci. USA 99 (2002) 3890–3895.
[20] S. Bates, A.C. Phillips, P.A. Clark, F. Stott, G. Peters, R.L. Ludwig, K.H. Vousden, p14ARF links the tumour suppressors RB and p53, Nature 395 (1998) 124–125.
[21] A.H. Milton, N. Khaire, L. Ingram, A.J. O'Donnell, N.B. La Thangue, 14-3-3 proteins integrate E2F activity with the DNA damage response, EMBO J. 25 (2006) 1046–1057.
[22] M. Scheffner, J.M. Huibregtse, R.D. Vierstra, P.M. Howley, The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53, Cell 75 (1993) 495–505.
[23] A. Costanzo, P. Merlo, N. Pediconi, M. Fulco, V. Sartorelli, P.A. Cole, G. Fontemaggi, M. Fanciulli, L. Schiltz, G. Blandino, C. Balsano, M. Levrero, DNA damage-dependent acetylation of p73 dictates the selective activation of apoptotic target genes, Mol. Cell 9 (2002) 175–186.
[24] W.C. Lin, F.T. Lin, J.R. Nevins, Selective induction of E2F1 in response to DNA damage, mediated by ATM-dependent phosphorylation, Genes Dev. 15 (2001) 1833–1845.
[25] M.S. Irwin, K. Kondo, M.C. Marin, L.S. Cheng, W.C. Hahn, W.G. Kaelin, Chemosensitivity linked to p73 function, Cancer Cell 3 (2003) 403–410.
[26] Q. Cao, Y. Xia, M. Azadniv, I.M. Crispe, The E2F-1 transcription factor promotes caspase-8 and bid expression, and enhances Fas signaling in T cells, J. Immunol. 173 (2004) 1111–1117.
[27] J.G. Gong, A. Costanzo, H.Q. Yang, G. Melino, W.G. Kaelin, M. Levrero, J.Y. Wang, The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage, Nature 399 (1999) 806–809.
[28] R. Agami, G. Blandino, M. Oren, Y. Shaul, Interaction of c-Abl and p73alpha and their collaboration to induce apoptosis, Nature 399 (1999) 809–813.
[29] Z.M. Yuan, H. Shioya, T. Ishiko, X. Sun, J. Gu, Y.Y. Huang, H. Lu, S. Kharbanda, R. Weichselbaum, D. Kufe, p73 is regulated by tyrosine kinase c-Abl in the apoptotic response to DNA damage, Nature 399 (1999) 814–817.
[30] J. Ren, R. Datta, H. Shioya, Y. Li, E. Oki, V. Biedermann, A. Bharti, D. Kufe, p73beta is regulated by protein kinase Cdelta catalytic fragment generated in the apoptotic response to DNA damage, J. Biol. Chem. 277 (2002) 33758–33765.
[31] M. Marabese, F. Vikhanskaya, C. Rainelli, T. Sakai, M. Broggini, DNA damage induces transcriptional activation of p73 by removing C-EBPalpha repression on E2F1, Nucleic Acids Res. 31 (2003) 6624–6632.
[32] N. Pediconi, A. Ianari, A. Costanzo, L. Belloni, R. Gallo, L. Cimino, A. Porcellini, I. Screpanti, C. Balsano, E. Alesse, A. Gulino, M. Levrero, Differential regulation of E2F1 apoptotic target genes in response to DNA damage, Nat. Cell. Biol. 5 (2003) 552–558.
[33] C. Wang, F.J. Rauscher, W.D. Cress, J. Chen, Regulation of E2F1 function by the nuclear corepressor KAP1, J. Biol. Chem. 282 (2007) 29902–29909.