Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 361 (2007) 20–25
BBRC
www.elsevier.com/locate/ybbrc

TFDP3 inhibits E2F1-induced, p53-mediated apoptosis

Chan Tian [a], Dan Lv [a], Huan Qiao [a], Jun Zhang [a], Yan-hui Yin [a], Xiao-ping Qian [a], Yu-ping Wang [b], Yu Zhang [a,*], Wei-feng Chen [a,*]

[a] Department of Immunology, Peking University Health Science Center, 38 Xue Yuan Road, Beijing 100083, China
[b] Department of Pathology, Peking University Health Science Center, Beijing, China

Received 16 June 2007
Available online 5 July 2007

Abstract

By dimerizing with E2F proteins, TFDP has profound influence on cellular E2F activities. While TFDP1 and 2 enhance the DNA binding and the transcriptional activity of E2F, the newly identified member of the DP family, TFDP3 primarily functions as a negative regulator. To further characterize the inhibitory property of TFDP3, the present study specifically examined the modulatory role of TFDP3 on E2F1-induced cell death. HEK-293 cells underwent apoptosis following ectopic expression of E2F1. This effect was virtually abolished by co-transfection with TFDP3. In the meantime, the accumulation of p53 proteins and the increased expression of the pro-apoptotic molecules, including Bax, Puma, Noxa, and Bid were found to be suppressed. These data suggest a new mechanism for the regulation of E2F1-induced apoptosis and provide further evidence for the general involvement of TFDP3 in the regulation of E2F functions.

Keywords: TFDP3; E2F1; DP; Apoptosis; p53

The E2F family of transcription factors is group of structurally related DNA binding proteins. In mammals, this family consists of eight members [1,2]. All together, they constitute a complicated regulatory network with contrasting roles in transcriptional activation and repression: E2F1–3 are potent transactivators, whereas the rest of family primarily acts as transcriptional repressors.

E2F factors are involved in the regulation of a number of important cellular events. The best studied is its role in the control of cell cycle progression [1,3–5]. Much of our understanding of the molecular mechanism by which E2F regulates gene expression comes from these studies. In resting cells, the activator E2Fs (E2F1–3) are expressed at low levels and are sequestered by the retinoblastoma (Rb) protein. Meanwhile, the repressor E2Fs (E2F4 and 5), which are prevalent, form complexes with Rb-related pocket proteins, p107 and p130 at most of the E2F-regulated promoters, where they actively repress the transcription by association with various chromatin–remodeling complexes. In response to mitogenic signals, pocket proteins are phosphorylated by cyclinD/cdk4, 6 and cyclinE/cdk2 and dissociate from E2Fs. The repressor E2Fs are relocated to the cytoplasm, whereas the activator E2Fs, which are induced to high levels, bind the vacated promoters and drive the expression of genes encoding DNA replication proteins and cell cycle regulators.

In addition to cell proliferation, E2Fs have important roles in the induction of apoptosis [6,7]. Ectopic expression of E2F1 leads to apoptosis in tissue culture under a number of conditions, including serum deprivation, growth factor withdrawal, γ-radiation, and even normal culture conditions [8–12]. Increased cell death is also observed in several E2F1 transgenic mouse models [13–17]. On the other hand, mice deficient in E2F1 have an excess of mature T cells due to an apoptotic defect in developing thymocytes [18]. Moreover, loss of E2F1 can rescue the apoptosis in

* Corresponding authors. Fax: +86 10 82801436.
E-mail addresses: zhangyu007@bjmu.edu.cn (Y. Zhang), wfchen@public.bta.net.cn (W. Chen).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.06.128

Rb-deficient mouse embryos [19]. Several studies indicate a similar activity for E2F3: overexpression of E2F3 triggers apoptosis both *in vitro* and *in vivo* [20,21], and loss of E2F3 suppresses apoptosis that occurs in the absence of Rb [22]. Notably, the E2F3-induced apoptosis is associated with increased E2F1 levels and is dependent on E2F1 [21], supporting the notion that induction of apoptosis is a function specific for E2F1 [23,24].

Multiple pathways link E2F1 to apoptosis, which are either dependent or independent of the tumor suppressor p53. The first p53-dependent mechanism to be recognized is the transactivation of p14ARF by E2F1. The p14ARF protein binds to the p53 regulator Mdm2 and inhibits its ability to target p53 to ubiquitination and subsequent degradation. The p53 protein is therefore found to be accumulated in E2F1-transfected cells [25,26]. In addition to regulating the level of p53, E2F1 induces the phosphorylation of p53 via an Atm/Nbs1/Chk2 pathway. The disturbance of this pathway impairs E2F1-induced apoptosis [27,28]. Still another p53-dependent mechanism involves the enhanced transcription of the pro-apoptotic cofactors of p53, such as p53-ASPP1, ASpp2, JMY, and TP53INP1 [29]. E2F1-induced apoptosis may also occur independently of p53. In this situation, E2F1 directly activates transcription of the p53 homolog p73 [30,31], Apaf-1 [32,33], caspases [34], and BH3-only proteins [35].

With the exception of E2F7 and E2F8 which contain a duplicated DNA-binding domain and are organized to mimic an E2F-DP heterodimer [36,37], E2F factors exist and function *in vivo* as heterodimeric complexes formed with members of the DP family of transcription factors [38]. TFDP1 and TFDP2 are two well-characterized members of this family. Although they themselves demonstrate little or no DNA binding activity, their association with E2F enhances both the DNA binding and the transcriptional activity of E2F.

We and others have recently reported the identification of the third member of the DP family, TFDP3 [39,40]. In contrast to the enhancing effect of the two known DP proteins, TFDP3 inhibits the DNA binding and transcriptional activities of E2F factors. Moreover, cell proliferation induced by ectopic expression of E2F is subverted by coexpression of TFDP3. In the present study, we explored the potential influence of TFDP3 on another important aspect of E2F function, namely the induction of apoptosis.

Materials and methods

Expression constructs. The expression plasmid pcDNA3-TFDP3-flag, pCMV-E2F1-HA, and pEGFP-N1 have been described previously [40].

Cell culture, transfection, and γ-irradiation. HEK-293 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (vol/vol) new-born calf serum (NCS). For transient transfection, cells were plated at a density of 50,000 cells per cm^2, and the expression constructs were delivered into the cells on the next day using the standard calcium phosphate precipitation method. Twenty-four hours after transfection, cells were switched to cultures in DMEM containing 0.1% NCS, or directly exposed to γ-radiation at a dose of 12.5 Gy.

Analysis of cell apoptosis. At various time points after serum starvation and γ-irradiation, HEK-293 transfectants were subjected to analysis for apoptosis either by staining with Annexin-V and propidium (PI) or by measuring the DNA content. Annexin-V/PI staining was performed according to manufacturer's protocol (Baosai Biotechnology, Beijing). For the measurement of DNA content, cells were fixed in 75% cold alcohol for at least 1 h at 4 °C. After washing twice with PBS, cells were resuspended in 0.5 ml PBS containing RNaseA (50 μg/ml), and incubated at 37 °C for 30 min. Subsequently, they were stained with PI, and analyzed on a FACScan cytometer (Becton–Dickinson).

Western blot analysis. HEK-293 transfectants were collected and counted. For each of 10^6 cells, 200 μl of lysis buffer [20 mM Tris (pH7.5), 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 5 μg/ml aprotinin, 5 μg/ml leupeptin, and 2 mM PMSF] was added. After incubation for 30 min on ice, the cell lysate was clarified by centrifugation. The protein concentration in the supernatant was determined using the Bio-Rad protein assay kit. Samples were fractionated by electrophoresis in 12.5% sodium dodecyl sulfate–polyacrylamide gels and then blotted onto nitrocellulose membranes (Amersham Bioscience). Immunoblotting was performed with monoclonal antibodies specific for E2F1 (CST), the FLAG tag, p53 (DO-1; Santa Cruze Biotechnology), or β-actin.

Reverse transcription (RT)-PCR. Total RNA was isolated from HEK-293 transfectants using Trizol reagent (Invitrogen). The mRNA expression was determined by RT-PCR using the Promega RT-PCR kit. The primers used for PCR were as follows: 5′-TGTAGAGGAGACAG GAATC CACGG-3′ and 5′-AGGCACCTAATTGGGCTCCATCTC-3′ for *Puma*; 5′-GCAGGCCTACCCTAGAGACA-3′ and 5′-GTCCATCCCATTTCT GGCTA-3′ for *Bid*; 5′-AGATGCCTGGGAAGAAG-3′ and 5′-AGTCC CCTCATGCAAGT-3′ for *Noxa*; 5′-ATCCAGGATCGAGCAGGGCG-3′ and 5′-AC TCGCTCAGCTTCTTGGTG-3′ for *Bax*; and 5′-ACCACA GTCC ATGCCATCAC-3′ and 5′-TCCACCACCCTGTTGCTGTA-3′ for *G3PDH*.

Statistical analysis. Statistical analysis was performed using *t* test.

Results and discussion

Inhibition of E2F1-induced apoptosis by TFDP-3

Our previous experiments have demonstrated that unlike TFDP1, TFDP3 negatively regulates E2F-driven cell cycle progression [40]. Similar results were also obtained in a separate study [39]. To examine the effect of TFDP3 on E2F1-induced apoptosis, HEK-293 cells were transiently transfected with E2F1, E2F1 + TFDP3 or empty vectors. The expression of the exogenous proteins was monitored by Western blot. As shown in Fig. 1A, E2F1 and TFDP3 had a similar pattern of expression, reaching their highest level at 24 h post-transfection but decreasing remarkably afterwards. As cells growing in normal concentrations of serum (5–10%) were relatively insensitive to E2F1-induced cell death (9, and data not shown), the cultures were switched to low-serum media (0.1%) 24 h after transfection to fully reveal the apoptotic effect. At the time when the cells were about to be transferred to low-serum cultures, cells were overall healthy and no obvious difference in morphology was noted among the three groups. Twenty-four hours after serum starvation (i.e., 48 h post-transfection), a large number of refractile-looking cells arose in E2F1-transfected cultures. Hoechst staining showed condensed chromatin and fragmented nuclei, which was typical of apoptotic cells

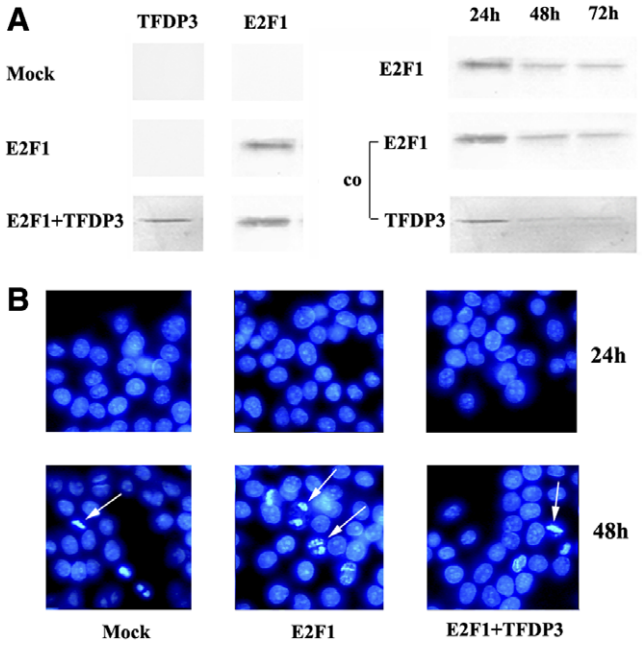

Fig. 1. Overexpression of E2F1/TFDP3 and cell death. HEK-293 cells were transfected with pCMV-E2F1-HA (2 μg) (E2F1), pCMV-E2F1-HA (2 μg) plus pcDNA3-DP3-Flag (2 μg) (E2F1 + DP3), or pcDNA3 empty vector (4 μg) (Mock). In E2F1 transfection, 2 μg of pcDNA3 was added to compensate for a total of 4 μg plasmid DNA. In addition, 1 μg of pEGFP-N1 was included in each transfection to monitor transfection efficiency, which was usually in the range of 50–60%. (A) Protein expression. HEK293 transfectants were examined for E2F1 and TFDP3 expression at 24 h post-transfection (left panel). A time course of E2F1 and TFDP3 expression was determined in E2F1 and E2F1 + TFDP3 transfectants (right panel). Each lane was loaded with an equal amount of total cellular proteins. E2F1 and TFDP3 were detected with antibodies specific for E2F1 and the Flag tag, respectively. (B) Morphological change of transfected cells. HEK-293 transfectants were stained with Hoechst 33342 at 24 and 48 h after transfection and examined under fluorescence microscope. Arrows indicate cells with condensed chromatin and/or fragmented nuclei.

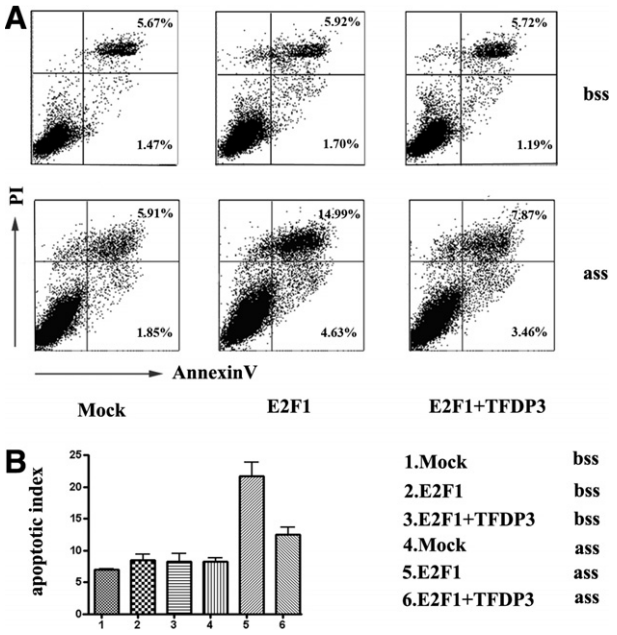

Fig. 2. Flow cytometry analysis of cell death in HEK-293 transfectants. HEK-293 cells were transfected as described in Fig. 1, and switched to low-serum culture 24 h after transfection. Cells before (bss) and 24 h after serum starvatios (ass) were stained with Annexin-V/PI and analyzed by flow cytometry. (A) A typical staining profile of the various transfectants before and after serum starvation. The number in each quadrant indicates the percentage of Annexin-V single positive or Annexin-V/PI double positive cells. (B) The percentage of apoptotic cells (defined as apoptotic index) in various transfectants before and after serum starvation. The apoptotic cells included all Annexin-V positive cells. The data were presented as means ± SD from three independent experiments.

(Fig. 1B). In contrast, such cells remained limited both in mock-transfected and TFDP3-cotransfected cultures.

To more closely follow cell apoptosis under these conditions, we stained the cells with Annexin-V and PI and analyzed them by flow cytometry. Fig. 2A showed a typical result of such analysis, whereas the summary of three independent experiments is presented in Fig. 2B. The percentage of positively stained cells was relatively low and consistent in all three groups until 24 h post-transfection. In the first 24 h in low-serum culture, the staining profile of the mock-transfected cells remained largely unaltered. In contrast, the Annexin-V/PI double positive cells as well as the Annexin-V single positive cells were almost tripled in E2F1-transfected cultures. Interestingly, co-transfection with TFDP3 significantly reduced the percentage of apoptotic cells in the culture ($P = 0.03$), leaving it essentially indistinguishable from the mock control. By 72 h after transfection, more than half of the cells were found to be dead in all three cultures probably due to serum deprivation. The difference between these groups was largely obscured because of the massive cell death (data not shown).

Although the E2F1-induced apoptosis has been extensively investigated, the function of DP proteins in this process has seldom been addressed partly because of the general belief that the endogenous TFDP1 is always present in excess. However, a few studies did reveal a modulatory role of TFDP1 in apoptosis. In one study, simultaneous expression of TFDP1 transgene was shown to cooperate with the E2F1 transgene in the induction of testicular atrophy [14]. In another study with an IL-3-dependent hematopoietic cells line, it was found that co-transfection of E2F1 and TFDP1 led to rapid cell death even in the presence of IL-3, whereas E2F1 alone induced cell death only in the absence of IL-3 [11]. Our study shows for the first time that the E2F1-induced apoptosis can be inhibited by a unique member of the DP members, adding further complexity to the mechanisms regulating this important function of E2F1.

Several studies using E2F1 mutants have demonstrated that the transactivation domain is dispensable for E2F1-induced apoptosis [41,42]. This raises the possibility that the action of E2F1 in apoptosis may be transcription independent. However, our finding that E2F1-induced apoptosis can be largely suppressed by TFDP3 argues against the general importance of transcription-independent mechanisms.

Blockage of E2F1-mediated sensitization of cells to radiation-induced apoptosis by TFDP3

Previous studies have indicated that E2F1 has the property to potentiate cell death in response to DNA damage caused by γ-radiation or chemotherapeutic drugs [43,44]. In consideration of its potential implication in chemo- and radiotherapy of tumors, we sought to determine whether such a function may be modulated by TFDP3 as well. To this end, HEK-293 transfectants were exposed to γ-radiation at a dose of 12.5 Gy 24 h after transfection, and the cell death was analyzed by flow-cytometry after an additional 24 h of culture. As shown in Fig. 3A, E2F1 transfection resulted in a significant increase in the sensitivity of HEK-293 cells to γ-radiation ($P = 0.04$). On average, the percentage of apoptotic cells (defined as apoptotic index) increased by nearly 50% over that in the mock control. On the other hand, coexpression of TFDP3 almost abolished the enhancing effect.

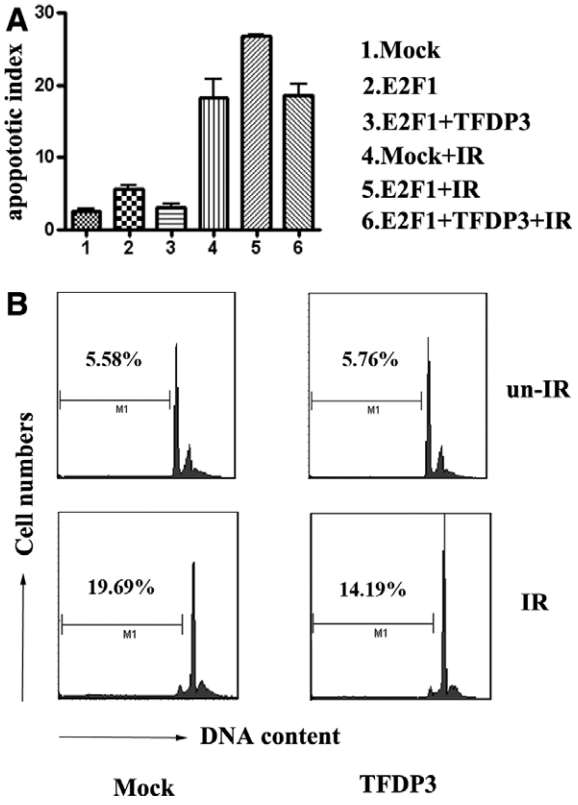

Fig. 3. Inhibition of E2F1 effect on γ-radiation-induced apoptosis by TFDP3. (A) TFDP3 inhibition of E2F1 potentiation to γ-radiation-induced apoptosis. HEK-293 transfectants as described in Fig. 1 were γ-irradiation (IR) or sham treated 24 h after transfection. Cells were then analyzed for DNA content using PI staining after an additional 24 h of culture. The percentage of cells with sub-diploid DNA content was calculated, and the data from three independent experiments were presented as means ± SD. (B) Inhibition of endogenous E2F1 by TFDP3 and γ-radiation-induced apoptosis. HEK-293 cells were transfected with pcDNA3-DP3-Flag (TFDP3) or pCDNA3 empty vector (Mock). The transfectants were γ-irradiation 24 h after transfection. Cells before (un-IR) and 24 h after irradiation (IR) were analyzed for DNA content. A typical result was shown. The number in the histogram indicates the percentage of cells with sub-diploid DNA content.

Interestingly, DNA damage often results in increased levels of E2F1 protein or activity, presumably due to protein stabilization following E2F1 phosphorylation by ATM and Chk2 kinases [7]. This provides a good model to test the effect of TFDP3 on the function of "endogenous" E2F1. HEK-293 cells were transfected with TFDP3 or empty vectors. The transfectants were then analyzed for radiation-induced apoptosis. The apoptotic cells, as indicated by their sub-diploid DNA content, reached nearly 20% in the mock control 24 h after irradiation, which was in comparison to 14% in the TFDP3 transfectant (Fig. 3B). This result indicates that E2F1 may contribute to radiation-induced apoptosis through a mechanism that can be blocked by TFDP3.

Role of p53 in the inhibition of E2F1-induced apoptosis by TFDP3

E2F1-induced apoptosis occurs via both p53-dependent and p53-independent pathways. The relative importance of these two pathways presumably varies from one cell type to another. As p53 is functionally intact in HEK-293 cells, we focused on the p53-dependent pathway in our analysis of the molecular mechanisms underlying TFDP3-mediated inhibition of E2F1-induced cell death. First, the protein level of p53 was determined in different transfectants using Western blot analysis (Fig. 4A). Consistent with many other studies, E2F1 transfection resulted in p53 accumulation in HEK-293 cells. This effect, however, diminished following co-transfection with TFDP3. In fact, the p53/β-actin ratio indicated an even lower level in E2F1/TFDP3 transfectants than in mock control.

The role p53 in apoptosis has been well established. Primarily, it functions as a transcriptional regulator, providing fine control over the expression of a variety of apoptosis-related genes. On one hand, it suppresses the expression of anti-apoptotic genes, such as Bcl-2, Bcl-xL, and survivin. On the other hand, it directly activates a number of pro-apoptotic genes, including Bax and several BH3-only members of the Bcl-2 family [45]. In view of the elevated p53 levels in E2F1-transfected cells, we next examined the mRNA expression of a select subset of p53 target genes and observed for any changes resulting from TFDP3 co-transfection. Bax, Puma, Noxa, and Bid were all found to be up-regulated in cells overexpressing E2F1. Again, co-transfection with TFDP3 reduced the expression to basic levels (Fig. 4B).

Increased expression of Puma and Noxa, as well as Bim and Hrk in E2F1-transfected cells has been previously reported by Ginsberg's group [35]. They proposed that Puma and Noxa were two critical mediators for E2F1-induced apoptosis on the basis that inhibition of either Puma or Noxa resulted in significant reduction in cell death. Our study revealed two additional potential target molecules, Bax and Bid. Given that the potential targets

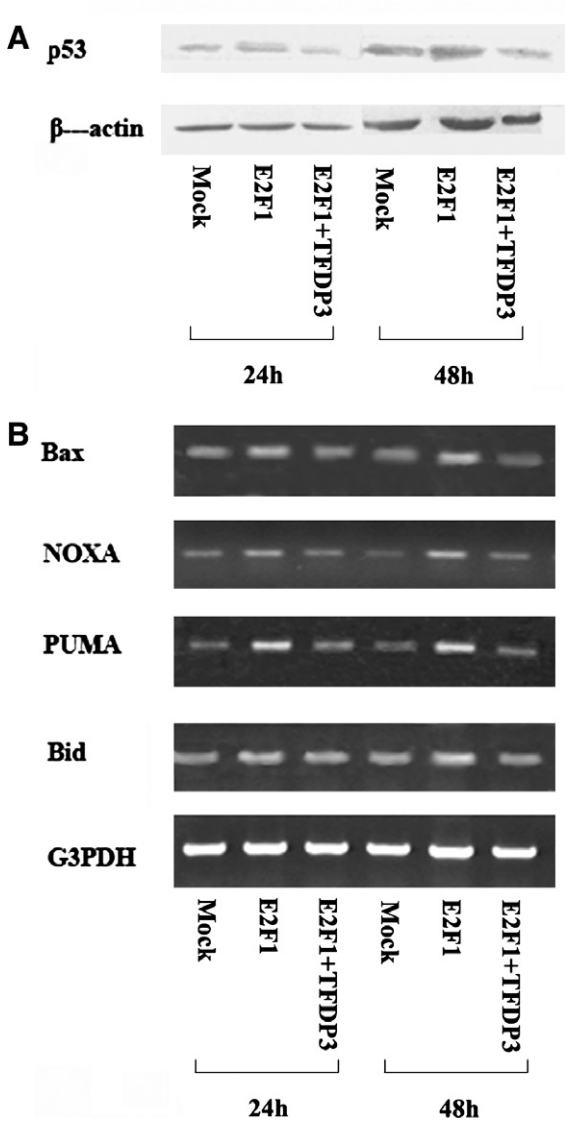

Fig. 4. Expression of apoptosis-related molecules in HEK-293 transfectants. (A) Western blot analysis of p53 expression. Total cellular protein was prepared from HEK-293 transfectants 24 and 48 h after transfection. The p53 protein was detected by Western blot with specific antibodies. β-Actin was used as a loading control. (B) RT-PCR analysis of Bax, Noxa, Puma, and Bid expression. Total RNA was prepared from HEK-293 transfectants 24 and 48 h after transfection. Expression of Bax, Noxa, Puma, and Bid mRNA was determined using RT-PCR. G3PDH was used a control for the amount of RNA initially added into the reactions.

of E2F1 may overlap with that of p53, it remains to be determined whether their increased transcription is induced by E2F1 or indirectly by p53.

Bak and Bax are at the center of the intrinsic apoptotic pathway by permeabilizing the mitochondrial membrane. Their activity is fine tuned by the BH3-only proteins, which include Bim, Bad, Bid, Bik, Bmf, Puma, Noxa, and Hrk. Two basic models have been proposed for how BH3-only proteins activate Bax and Bak. In the direct binding model, BH3-only proteins are thought to trigger activation of Bax and Bak directly. The displacement model, on the other hand, proposes that BH3-only proteins activate Bax and Bak by displacing them from the anti-apoptotic proteins [46]. The critical role of Bax, Bid, Puma, and Noxa in apoptosis suggests that suppression of their transcription could represent an important mechanism for TFDP3-mediated inhibition of E2F1-induced apoptosis.

In conclusion, we have demonstrated that TFDP3 is a negative regulator of E2F1-induced cell death. Furthermore, we have shown that suppression of the p53 pathway is at least partly responsible for the inhibitory effect of TFDP3. These findings should allow better understanding of inhibitory properties of TFDP3 and the regulation of E2F1-induced apoptosis. In addition, this study provides a simple explanation for the differential expression of TFDP3 in tumor versus normal tissues.

Acknowledgments

This work was supported by National 863 Program in China Grant 2003AA215110, National Natural Science Foundation of China Grant 30525044, and Beijing Natural Science Foundation Grant 7061003.

References

[1] D.K. Dimova, N.J. Dyson, The E2F transcriptional network: old acquaintances with new faces, Oncogene 24 (2005) 2810–2826.
[2] P.K. Tsantoulis, V.G. Gorgoulis, Involvement of E2F transcription factor family in cancer, Eur. J. Cancer 41 (2005) 2403–2414.
[3] D. Cobrinik, Pocket proteins and cell cycle control, Oncogene 24 (2005) 2796–2809.
[4] J.M. Trimarchi, J.A. Lees, Sibling rivalry in the E2F family, Nat. Rev. Mol. Cell. Biol. 3 (2002) 11–20.
[5] J. DeGregori, The genetics and the E2F family of transcription factors: shared function and unique roles, Biochim. Biophys. Acta 1602 (2002) 131–150.
[6] L.A. Bell, K.M. Ryan, Life and death decision by E2F-1, Cell Death Differ. 11 (2004) 137–142.
[7] D. Ginsberg, E2F1 pathways to apoptosis, FEBS Lett. 529 (2002) 122–125.
[8] X.Q. Qin, D.M. Livingston, W.G. Kaelin Jr., P.D. Adams, Deregulated E2F1 expression leads to S-phase entry and p53-mediated apoptosis, Proc. Natl. Acad. Sci. USA 91 (1994) 10918–10922.
[9] B. Shan, W.H. Lee, Deregulated expression of E2F-1 induces S-phase entry and leads to apoptosis, Mol. Cell. Biol. 14 (1994) 8166–8173.
[10] X. Wu, A.J. Levine, p53 and E2F-1 cooperate to mediate apoptosis, Proc. Natl. Acad. Sci. USA 91 (1994) 3602–3606.
[11] S.W. Hiebert, G. Packham, A.K. Strom, R. Haffner, M. Oren, G. Zambetti, J.L. Cleveland, E2F-1: DP-1 induces p53 and overrides survival factors to trigger apoptosis, Mol. Cell. Biol. 15 (1995) 6864–6874.
[12] T.F. Kowalik, J. DeGregori, J.K. Schwarz, J.R. Nevins, E2F1 overexpression in quiescent fibroblasts leads to induction of cellular DNA synthesis and apoptosis, J. Virol. 69 (1995) 2491–2500.
[13] C.T. Guy, W. Zhou, S. Kaufman, M.O. Robinson, E2F-1 blocks terminal differentiation and causes proliferation in transgenic megakaryocytes, Mol. Cell. Biol. 16 (1996) 685–693.
[14] C. Holmberg, K. Helin, M. Sehested, O. Karlstrom, E2F-1 induced p53-independent apoptosis in transgenic mice, Oncogene 17 (1998) 143–155.
[15] A.M. Pierce, S.M. Fisher, C.J. Conti, D.G. Johnson, Deregulated expression of E2F1 induces hyperplasia and cooperates with ras in skin tumor development, Oncogene 16 (1998) 1267–1276.

[16] A.M. Pierce, I.B. Gimenez-Conti, R. Schneider-Broussard, L.A. Martinez, C.J. Conti, D.G. Jognson, Increased E2F1 activity induces skin tumors in mice heterozygous and nullizygous for p53, Proc. Natl. Acad. Sci. USA 95 (1998) 8858–8863.
[17] A.M. Pierce, R. Schneider-Broussard, I.B. Gimenez-Conti, J.L. Russell, C.J. Conti, D.G. Johnson, E2F1 has both oncogenic and tumor-suppressive properties in a transgenic model, Mol. Cell. Biol. 19 (1999) 6408–6414.
[18] S.J. Field, F.Y. Tsai, F. Kuo, A.M. Zubiaga, W.G. Kaelin Jr., D.M. Livingston, S.H. Orkin, M.E. Greenberg, E2F-1 functions in mice to promote apoptosis and suppress proliferation, Cell 85 (1996) 549–561.
[19] K.Y. Tsai, Y. Hu, K.F. Macleod, Mutation of E2f-1 suppresses apoptosis and inappropriate S phase entry and extends survival of Rb-deficient mouse embryos, Mol. Cell 2 (1998) 293–304.
[20] E. Vigo, H. Muller, E. Prosperini, G. Hateboer, P. Cartwright, M.C. Moroni, K. Helin, CDC25A phosphatase is a target of E2F and is required for efficient E2F-induced S phase, Mol. Cell. Biol. 19 (1999) 6379–6395.
[21] E.L. Denchi, K. Helin, E2F1 is crucial for E2F-dependent apoptosis, EMBO Rep. 6 (2005) 661–667.
[22] U. Ziebold, T. Reza, A. Caron, J.A. Lees, E2F3 contributes both to the inappropriate proliferation and to the apoptosis arising in Rb mutant embryos, Genes Dev. 15 (2001) 386–391.
[23] J. DeGregori, G. Leone, A. Miron, L. Jakoi, J.R. Nevins, Distinct roles for E2F proteins in cell growth control and apoptosis, Proc. Natl. Acad. Sci. USA 94 (1997) 7245–7250.
[24] T.F. Kowalik, J. DeGregori, G. Leone, L. Jakoi, J.R. Nevins, E2F1-specific induction of apoptosis and p53 accumulation, which is blocked by Mdm2, Cell Growth Differ. 9 (1998) 113–118.
[25] 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.
[26] K.D. Robertson, P.A. Jones, The human ARF cell cycle regulatory gene promoter is a CpG island which can be silenced by DNA methylation and down-regulated by wild-type p53, Mol. Cell. Biol. 18 (1998) 6457–6473.
[27] H.A. Rogoff, M.T. Pickering, F.M. Frame, M.E. Debatis, Y. Sanchez, S. Jones, T.F. Kowalik, Apoptosis associated with deregulated E2F activity is dependent on E2F1 and Atm/Nbs1/Chk2, Mol. Cell. Biol. 24 (2004) 2968–2977.
[28] J.T. Powers, S. Hong, C.N. Mayhew, P.M. Rogers, E.S. Knudsen, D.G. Johnson, E2F1 uses the ATM signaling pathway to induce p53 and chk2 phosphorylation and apoptosis, Mol. Cancer Res. 2 (2004) 203–214.
[29] T. Hershko, M. Chaussepied, M. Oren, D. Ginsberg, Novel link between E2F and p53: proapoptotic cofactors of p53 are transcriptionally upregulated by E2F, Cell Death Differ. 12 (2005) 377–383.
[30] 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 Jr., Role for the p53 homologue p73 in E2F-1-induced apoptosis, Nature 407 (2000) 645–648.
[31] T. Stiewe, B.M. Putzer, Role of the p53-homologue p73 in E2F1-induced apoptosis, Nat. Genet. 26 (2000) 464–469.
[32] M.C. Moroni, E.S. Hickman, E.L. Denchi, G. Caprara, E. Colli, F. Cecconi, H. Muller, K. Helin, Apaf-1 is a transcriptional target for E2F and p53, Nat. Cell. Biol. 3 (2001) 552–558.
[33] Y. Furukawa, N. Nishimura, Y. Furukawa, M. Satoh, H. Endo, S. Iwase, H. Yamada, M. Matsuda, Y. Kano, M. Nakamura, Apaf-1 is a mediator of E2F-1-induced apoptosis, J. Biol. Chem. 277 (2002) 39760–39868.
[34] 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.
[35] T. Hershko, D. Ginsberg, Up-regulation of Bcl-2 homology 3 (BH3)-only proteins by E2F1 mediates apoptosis, J. Biol. Chem. 279 (2004) 8627–8634.
[36] L. Di Stefano, M.R. Jensen, K. Helin, E2F7, a novel E2F featuring DP-independent repression of a subset of E2F-regulated genes, EMBO J. 22 (2003) 6289–6298.
[37] B. Maiti, J. Li, A. de Bruin, F. Gordon, C. Trimmers, R. Opavsky, K. Patil, J. Tuttle, W. Cleghorn, G. Leone, Cloning and characterization of mouse E2F8, a novel mammalian E2F family member capable of blocking cellular proliferation, J. Biol. Chem. 280 (2005) 18211–18220.
[38] M.R. Hitchens, P.D. Robbins, The role of the transcription factor DP in apoptosis, Apoptosis 8 (2003) 461–468.
[39] A. Milton, K. Luoto, L. Ingram, S. Munro, N. Logan, A.L. Graham, T.R. Brummelkamp, E.M. Hijmans, R. Bernards, N.B. La Thangue, A functionally distinct member of the DP family of E2F subunits, Oncogene 25 (2006) 3212–3218.
[40] H. Qiao, L. Di Stefano, C. Tian, Y.Y. Li, Y.H. Yin, X.P. Qian, X.W. Pang, Y. Li, M.A. McNutt, K. Helin, Y. Zhang, W.F. Chen, Human TFDP3, a novel DP protein, inhibits DNA binding and transactivation by E2F, J. Biol. Chem. 282 (2007) 454–466.
[41] J.K. Hsieh, S. Fredersdorf, T. Kouzarides, K. Martin, X. Lu, E2F1-induced apoptosis requires DNA binding but not transactivation and is inhibited by the retinoblastoma protein through direct interaction, Genes Dev. 11 (1997) 1840–1852.
[42] A.C. Phillips, S. Bates, K.M. Ryan, K. Helin, K.H. Vousden, Induction of DNA synthesis and apoptosis are separable functions of E2F-1, Genes Dev. 11 (1997) 1853–1863.
[43] M. Pruschy, C. Wirbelauer, C. Glanzmann, S. Bodis, W. Krek, E2F-1 has properties of a radiosensitizer and its regulation by cyclin A kinase is required for cell survival of fibrosarcoma cells lacking p53, Cell Growth Differ. 10 (1999) 141–146.
[44] J. Nip, D.K. Strom, B.E. Fee, G. Zambetti, J.L. Cleveland, S.W. Hiebert, E2F-1 cooperates with topoisomerase II inhibition and DNA damage to selectively augment p53-independent apoptosis, Mol. Cell. Biol. 17 (1997) 1049–1056.
[45] J. Yu, L. Zhang, The transcriptional atargets of p53 in apoptosis control, Biochem. Biophys. Res. Commun. 331 (2005) 851–858.
[46] S.N. Willis, J.M. Adams, Life in the balance: how BH3-only proteins induce apoptosis, Curr. Opin. Cell. Biol. 17 (2005) 617–625.