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Identification of E1AF as a Target Gene of E2F1-induced Apoptosis in Response to DNA Damage

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Transcription factor E1AF plays critical roles in neuronal development and tumour metastasis and is regulated by a number of signalling cascades, including the mitogen-activated protein kinase pathways. Accumulated evidence indicted that E1AF might contribute to cell survival in response to environment factors. Here, we provided evidence the cell cycle and apoptosis regulator E2F1 induces E1AF expression at the transcriptional level. DNA damage by etoposide causes E2F1-dependent induction of E1AF expression at transcriptional level. Furthermore, disruption of E1AF expression by E1AF RNAi decreased E2F1-induced apoptosis in response to etoposide. Thus, we conclude that activation of E1AF provides a means for E2F1 to induce cell apoptosis in response to DNA damage.

Key words: apoptosis, DNA damage, E1AF, E2F1, H1299 cells.

Abbreviations: EMSA, electrophoretic mobility shift assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SDS–PAGE, SDS–poly-acrylamide gel; oligo, oligonucleotide.

E1AF, a member of a subfamily of Ets domain transcription factors, was first identified as a transcription factor that binds to enhancer motifs of the adenovirus E1A gene (*1*). It is capable of regulating transcription by binding to the Ets binding site in the promoter of its target genes, and is involved in a number of processes including neuronal path finding (*2*), mammary gland development and male sexual function (*3–5*).

Pathologically, E1AF plays an important role in mammary oncogenesis and cancer invasiveness and metastasis via directly binding to the promoters of genes involved tumour growth, migration and invasion (*6–18*), indicating the contribution of E1AF in various malignant phenotypes of cancers cells. Recent study revealed that E1AF was up-regulated by DNA-damaging agent cisplatin and could activate the promoter of cell cycle inhibitory factor p21waf1/cip1 and Bax (*5*, *19*). In addition, the expression of E1AF was induced by anti-tumour agent orlistat and inhibitor of fatty acid synthase (*20*, *21*). Consistently, we have reported that E1AF was induced by anti-tumour agent Mithramycin A and contributed to Mithramycin A-induced Huh-7 cell apoptosis (*22*). These data suggested that E1AF might contribute to cell survival in response to environment factors.

DNA damaging agents, such as γ-irradiation or chemotherapeutic drugs, could cause cell cycle arrest as well as apoptosis (*23*, *24*). Accumulated reports have described an increase in E2F1 protein, resulting from stabilization of the E2F1 protein, following the treatment of a variety of tumour cells with DNA damaging agents (*25*). The E2F1 transcription factor plays a major role in regulating a diverse array of cellular functions including proliferation, differentiation and apoptosis (*26–30*). E2F1 stimulates the transcription of several genes in the apoptotic pathway, including p53, p73, p27 and Apaf-1 (*31–34*). Overexpression of E2F1 induces premature S-phase entry and often results in apoptosis (*26*). In addition, E2F1-deficient mice exhibit a defect in thymocyte apoptosis as well as in negative election demonstrating the role of E2F1 during the physiological apoptotic processes (*35*, *36*). The induction of apoptosis by E2F1 may involve the activation of target genes, such as arf, and subsequent signalling of apoptosis through the p53 pathway (*37*). Alternatively, the transcriptional activation of p53 family members, such as the p73 gene, and the Apaf1 gene may contribute to E2F1-mediated apoptosis (*32*). However, the mechanisms of E2F1-mediated apoptosis needed further investigation.

Here, we reported for the first time that the cell cycle and apoptosis regulator E2F1 induces E1AF expression at the transcriptional level. DNA damage by etoposide causes E2F1-dependent induction of E1AF expression and disruption of E1AF expression decreased E2F1-induced apoptosis. Collectively, these data suggested that activation of E1AF provides a means for E2F1 to induce cell apoptosis in response to DNA damage.

MATERIALS AND METHODS

Cell Lines, Plasmids and Antibodies—H1299 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 50 μg/ml streptomycin at 37°C in a humidified CO_2 incubator (5% CO_2, 95% air). Cell transfection was

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performed with Lipofectamine (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Expression constructs for pSilencer-2.0, myc-tagged E1AF, pcDNA3.1-myc, E1AF RNAi and pGL3Basic have been described previously (*15*, *16*, *38*). E1AF promoter plasmid was constructed as previously described (*15*, *39*). Human Bax promoter plasmid containing the 370 bp fragment from the Bax promoter was constructed as previously constructed (*40*). Expression constructs for E2F1 and SiE2F1 were generous gifts from Prof. Cress Doug. Expression constructs for E2F2, E2F3, E2F4, E2F5 and E2F6 were generous gifts from Prof. Deling Lu. Anti-human-E1AF Ab, anti-human-E2F1 Ab, anti-mouse-HRP secondary Ab and anti-rabbit-HRP secondary Ab were purchased from Santa Cruz Biotechnology, California, USA. Anti-myc and anti-GAPDH antibodies were purchased from Oncogene. Anti-human-cleavage PARP cleavage Ab, anti-human-Bcl-2 Ab, anti-human-Bcl-xL Ab, anti-human-Bax Ab, anti-human-Bid Ab, anti-human-Bad Ab, anti-human-Caspase 3 Ab and anti-human-Caspase-9 Ab was purchased from Cell Signaling, Danvers, MA, USA.

Analysis of Nuclear Morphology by Fluorescence Staining—Cells grown on the glass coverslips were fixed with 4% paraformaldehyde/phosphate-buffered saline (PBS) for 30 min, washed for 15 min in 0.1% Triton X-100/PBS and incubated in dark with Hoechst 33258 (10 μg/ml) for 15 min. After the coverslips were washed in PBS, positive nuclei were counted. Normal nuclei and apoptotic nuclei (condensed new moon-type or fragmented chromatin) were easily distinguished.

Analysis of Apoptosis by Flow Cytometry—Adherent and non-adherent cells were collected, washed twice in PBS, and fixed with ice-cold 70% ethanol for at least 1 h. The fixed cells were washed and stained with propidium iodide mixture (50 μg/ml propidium iodide, 0.05% Triton X-100, 37 μg/ml EDTA and 100 U/ml ribonuclease in PBS). After incubation for 45 min at 37°C, the DNA content was determined by quantitative flow cytometry with standard optics of FACScan flow cytometer (Becton–Dickinson FACStar). The percentage of apoptosis was quantitated from sub-G1 events.

Western Blot Analysis—Equal amounts of protein were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and then transferred onto a PVDF membrane. The membrane was blocked for 2 h in a non-fat dried milk solution (5% in Tris–buffered saline) containing 0.5% Tween-20. The membrane was then incubated for 2 h at room temperature with primary antibody, followed by incubation with a HRP-coupled secondary antibody diluted 1:2000 for 1–2 h. Membrane-bound horseradish peroxidase-labelled protein bands were monitored with enhanced chemiluminescent reagents and chemiluminescent signals were detected using X-ray film.

Gel Shift Assay—Gel mobility shift assay was carried out using Gel Shift Assay System (Promega, Wisconsin, USA) as follows. The double-stranded oligonucleotides were annealed, end-labelled with ^{32}P using T4 polynucleotide kinase, and purified using Sephadex G-25 quick spin columns (Roche Molecular Biochemicals, Indianapolis, Ind., USA). Nuclear proteins were pre-incubated for 10 min with 9 μl of electrophoretic mobility shift assay (EMSA) buffer. Then the ^{32}P-end-labelled duplex oligonucleotide (1 μl, 10 fmol) was added, and the reaction was incubated for 20 min on ice. For competition experiments, unlabelled DNA probes were included at 50- to 100-fold molar excess over the ^{32}P-labelled DNA probe. For supershift experiments, 2 μg of rabbit anti-E2F1 polyclonal antibody or control IgG was added to the reaction mixtures and incubated for 30 min prior to addition of the ^{32}P-labelled DNA probe. DNA–protein complexes were separated on 5% non-denaturing polyacrylamide gels in 0.5 × Tris borate/EDTA (pH 8.4) at 4°C and 35 mA. The gels were dried, and the DNA–protein complexes were visualized by autoradiography.

Reverse Transcriptase–Polymerase Chain Reaction—Total RNA (1 μg) were used as a template for cDNA synthesis. cDNA was prepared by use of a TaKaRa RNA PCR Kit. Primers used for PCR were as follows: E1AF forward 5′-ATGGAGCGGAGGATG-3′, reverse 5′-CTGGG GGCTAGTAAGAG-3′; Bax forward, 5′-CTGCAGAGGAT GATTGCCG-3′, reverse 5′-TGCCACTCGGAAAAAGACC T-3′. Amplification was carried out for 22–27 cycles under saturation, each at 94°C, 45 s; 60°C, 45 s; 72°C, 1 min in a 50 μl reaction mixture containing 2 μl each cDNA, 0.2 μM each primer, 0.2 mM dNTP and 2.5 U of Taq DNA polymerase. After amplification, 10 μl of each reaction mixture was analysed by 1% agarose gel electrophoresis, and the bands were then visualized by ethidium bromide staining.

RESULTS

Identification of E1AF as a Target Gene of E2F1 Transcription Factor—When H1299 cells were transiently transfected with E2F1 expression construct, increased E1AF expression was observed. Other known E2F1 targets (Apaf-1, p27Kip1 and DHFR) were also induced (Fig. 1A). Consistent with this, E1AF mRNA expression was also induced by E2F1 in a dose-dependent manner as measured by semi-quantitative reverse transcriptase–polymerase chain reaction (RT–PCR) (Fig. 1B), suggesting that E2F1 induces E1AF expression at transcriptional level. Transfection of a 2.1 kb human E1AF promoter with E2F1 expression construct in H1299 cells resulted in 25-fold activation (Fig. 1C). To test whether the E1AF promoter could be regulated by other E2F proteins in addition to E2F1, E1AF promoter luciferase construct was transiently co-transfected into H1299 cells with expression constructs for E2F1, E2F2, E2F3, E2F4, E2F5, E2F6 or E2F7. As shown in Fig. 1D, the highest activation of the E1AF promoter was obtained by E2F1. These data suggested that E2F1 regulates E1AF expression at transcriptional level.

Deletion analysis was then performed to define functionally important *cis*-elements in this 2100 nt region of E1AF promoter. Luciferase assays showed that a deletion from + 40 to + 83 relative to transcription start resulted in a drastic decrease in the promoter activity and loss of E2F1 activation as compared with that of control construct (data not shown). Inspection of this 44 nt region revealed one potential E2F1 protein binding site (Fig. 1E). EMSA was performed to determine whether E2F1 recognized this region of E1AF promoter. Incubation of the

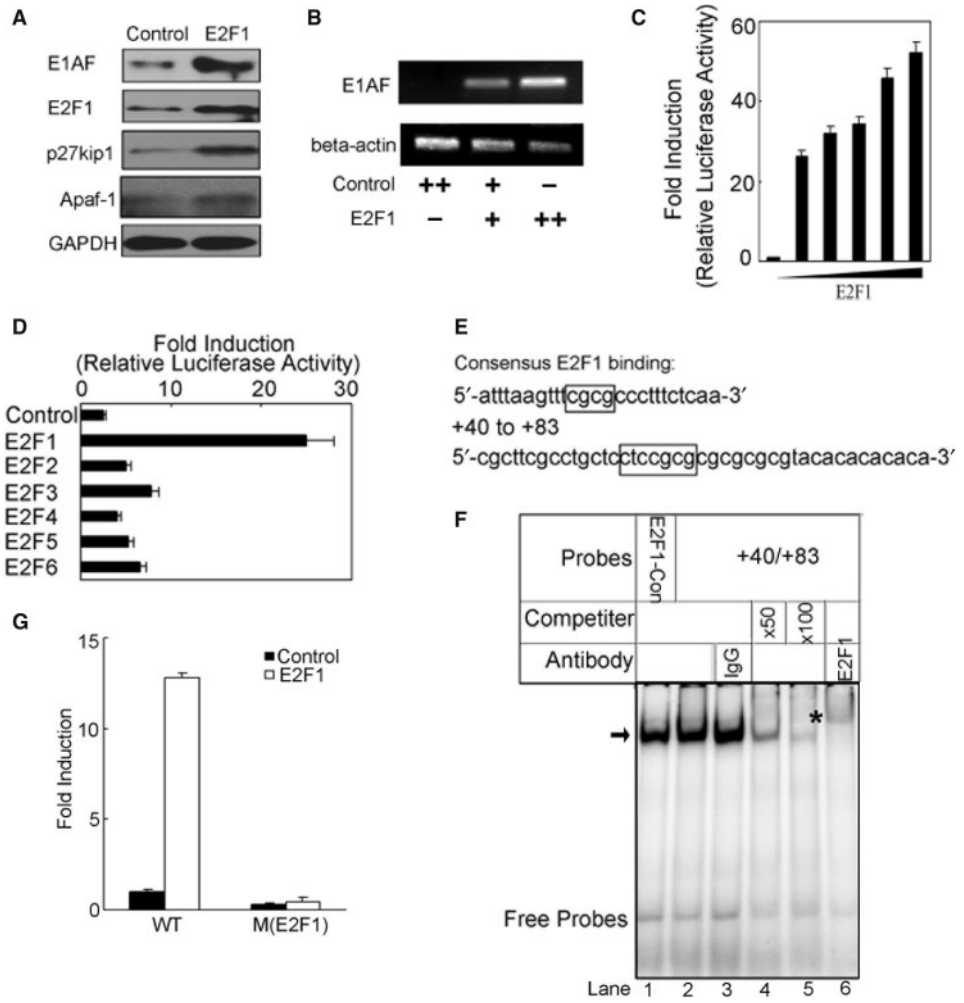

Fig. 1. **Induction of E1AF expression by E2F1.** (A) Western blot analysis of E1AF expression in H1299 cells transiently transfected with E2F1 plasmid or empty control using anti-E1AF antibody. GAPDH expression served as loading controls. (B) RT–PCR analysis of E1AF expression in H1299 cells transfected with control or E2F1 plasmid. The expression of β-actin served as loading controls. (C) Increasing amounts of E2F1 expression plasmids were transiently co-transfected into H1299 cells with E1AF promoter construct pGL3(−2000/+100). Normalized luciferase activity was standardized to pGL3(−2000/+100) with vector alone. Each value is the mean ± SD of at least three independent experiments. (D) E1AF promoter construct pGL3(−2000/+100) was transiently co-transfected into H1299 cells with control or E2F family expression construct. Luciferase activities were measured as described above. (E) Two oligo sequences corresponding to E2F1 binding consensus and the probes containing human E1AF promoter sequence +40 to +83 used in the following EMSA studies were listed and the potential E2F1 binding site was shown in black frame. (F) Nuclear extracts from H1299 cells were incubated with ^{32}P-labelled probes of E2F1 binding consensus sequence or E1AF promoter sequence +40 to +83 untreated or treated with the indicated cold competitors stranded oligonucleotides or an antibody specific to E2F1 or control IgG. (G) Site-directed mutation analyses of E1AF promoter. E1AF promoter construct pGL3(−2000/+100) (WT) and mutated promoter construct MUT were transfected into H1299 cells, together with or without E2F1 expression vector. Normalized luciferase activity was standardized to pGL3(−2000/+100) with vector alone. Each value is the mean ± SD of at least three independent experiments

double-stranded oligo probe between nucleotides + 40 and + 83 with H1299 nuclear extracts formed specific protein–DNA complex (Fig. 1F, lane 2), which was markedly disrupted by incubation with the unlabelled E2F1-consensus oligonucleotides in a dose-dependent manner (Fig. 1F, lanes 4 and 5). To identify specific proteins that bind to this region, we used antibody against E2F1. It was found that antibody against E2F1 supershifted protein–DNA complexes (Fig. 1F, lane 7), indicating the binding of E2F1 to E1AF promoter. To further determine whether this potential E2F1 binding site was necessary for E1AF transcription, we introduced site-directed mutagenesis into this E2F1 binding site (+55 to +60). Mutation of the E2F1 binding site deprived E2F1 responsiveness (Fig. 1G). Correctively, these data identified E1AF as a direct target gene of E2F1 transcription factor.

E2F1 Contributed to Etoposide-induced E1AF Expression—DNA damage induces E2F1 stabilization and activation of a subset of E2F1 targets (*25*, *37*). We examined the effect of DNA damage agents on E1AF expression. Consistent with pro-apoptotic protein E2F1, the expression of E1AF protein was markedly up-regulated in H1299 cells treated with cisplatin, etoposide

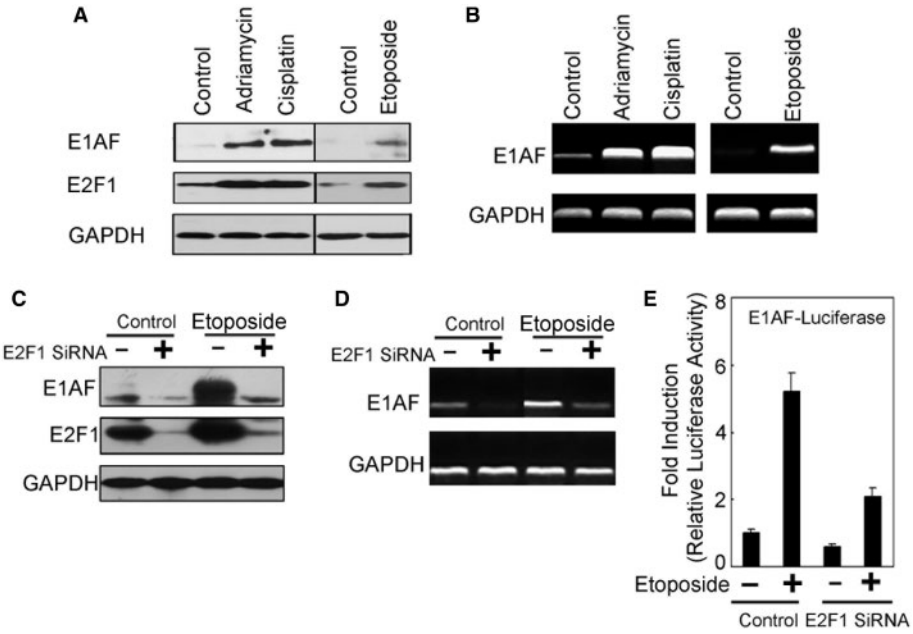

Fig. 2. **E2F1 contributes to etoposide-induced E1AF expression.** (A) H1299 cells were treated with control, adriamycin, cisplatin (5 μg/ml) or etoposide (10 μM) for 24 h and cell extracts were analysed by western blot with anti-E1AF, anti-E2F1 antibodies. GAPDH served as a loading control. (B) RT–PCR analysis of E1AF mRNA expression in H1299 cells treated with control, adriamycin, cisplatin (5 μg/ml) or etoposide (10 μM) for 24 h. GAPDH mRNA expression served as a loading control. (C) H1299 cells were transiently transfected with control or E2F1 RNAi. Twenty-four hours after transfection, cells were treated with control or etoposide (10 μM) for 24 hours and cell extracts were analysed by western blot using anti-E1AF or anti-E2F1 antibody. GAPDH served as a loading control. (D) RT–PCR analysis of E1AF mRNA expression in H1299 cells transiently transfected with control or E2F1 RNAi were treated with or without etoposide (10 μM) for 24 h. GAPDH mRNA expression served as a loading control. (E) E1AF promoter construct pGL3(−2000/+100) was transiently co-transfected into H1299 cells with control or E2F1 RNAi. Twenty-four hours after transfection, cells were treated with control or etoposide (10 μM) for 24 h and luciferase activities were measured as described above.

or adriamycin (Fig. 2A). Consistent with this, E1AF mRNA expression was significantly induced in response to the treatment with cisplatin, etoposide or adriamycin (Fig. 2B). To determine whether E2F1 was necessary for DNA-damage induction of E1AF, H1299 cells treated with etoposide were prior transiently transfected with E2F1 RNAi. Interference of E2F1 expression reduced the protein and mRNA expression of E1AF and inhibited the induction of E1AF after etoposide treatment (Fig. 2C and D). Transfection of E1AF promoter construct into H1299 cells, followed by treatment with etoposide, resulted in 5-fold increase in promoter activity (Fig. 2E). Interference of E2F1 expression reduced the activity of E1AF promoter and inhibited the induction of E1AF promoter activity after etoposide treatment (Fig. 2E). These results suggested that E2F1 mediates etoposide-induced E1AF expression.

E1AF Regulates Etoposide-induced Cell Apoptosis—The effect of etoposide on E1AF expression motivated us to investigate the contribution of E1AF in etoposide-induced apoptosis. Myc-tagged E1AF expression vector was constructed and transiently transfected into H1299 cells. And, we investigated the effect of E1AF over-expression on apoptosis after etoposide treatment for 24 h. As shown in Fig. 3A, the percentage of apoptotic cells in E1AF-overexpressed H1299 cells was markedly increased, compared to that of the controls by fragmented and condensed nuclei, indicating the pro-apoptotic role of E1AF in H1299 cells. Consistent with this, the percentage of apoptotic cells in E1AF overexpression cells was markedly increased compared to that of the controls by FACS assay (Fig. 3B). Furthermore, E1AF over-expression markedly increased etoposide-induced cleavage of PARP, Caspase-3 and Caspase-9 (Fig. 3C), which have been implicated in the apoptosis process in numerous cells induced by DNA-damaging agents (*41*). Furthermore, E1AF overexpression obviously induced the protein and mRNA expression of pro-apoptotic protein Bax (Fig. 3C and D), and induced the activity of Bax promoter in a dose-dependent manner (Fig. 3E). These data suggested that E1AF functions as pro-apoptotic role in etoposide-induced cell apoptosis.

Interference of E1AF Expression could Protect Cells from E2F1-induced Apoptosis in Response to Etoposide—The E2F1 transcription factor plays a major role in cell apoptosis in response to DNA damage (*26*). To clarify the role of E1AF in E2F1-mediated apoptosis, siRNA targeted to E1AF was constructed as reported in our previous study (*15*), and transiently transfected into H1299 cells alone or with E2F1 expression vector, following etoposide treatment. Hochest 33258 staining and flow cytometry analysis showed that the interference of E1AF expression by transfection with E1AF RNAi could protect cells from E2F1-induced apoptosis (Fig. 4A and B), indicating the contribution of E1AF in E2F1-mediated apoptosis. The interference of E1AF expression by transfection with E1AF RNAi could inhibit E2F1-meidated cell apoptosis by MTT assay, and reduced the

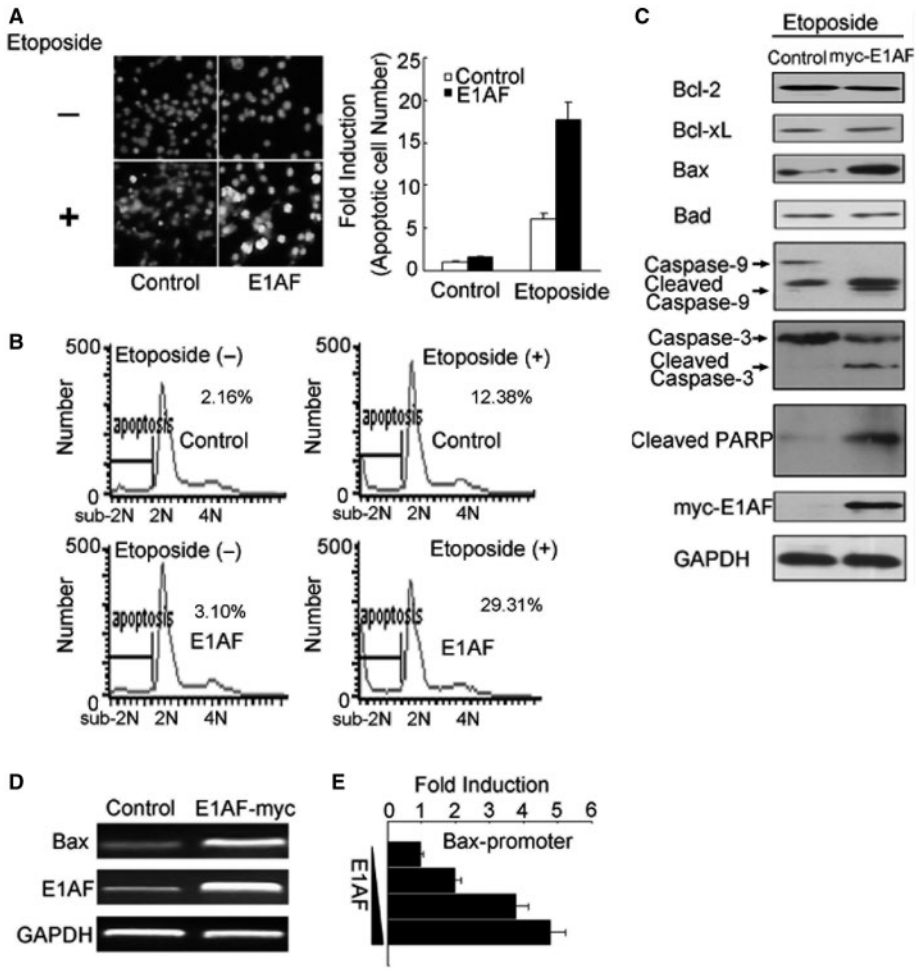

Fig. 3. **The contribution of E1AF in etoposide-induced cell apoptosis.** (A) Hoechst 33258 staining of nuclei from cells transiently transfected with the empty vector or E1AF in the absence or presence of etoposide (10 μM) for 24 h (left panel). Quantification of apoptotic cells. At least 300 cells were counted from three different microscope fields and each value was the mean ± SD of three independent experiments (right panel). (B) Cells transiently transfected with the empty vector or E1AF were harvested after the treatment of etoposide (10 μM) for 24 h, fixed in ethanol, and stained with propidium iodide. The apoptotic rates were counted by flow cytometry analysis. (C) Cells transfected with the empty vector or E1AF in the presence of etoposide (10 μM) for 24 h were harvested. The cell extracts were analysed by western blot using the indicated antibodies. GAPDH served as a loading control. (D) RT–PCR analysis of Bax and E1AF mRNA expression in H1299 cells transiently transfected with control or E1AF-myc plasmid. (E) Increasing amounts of E1AF expression plasmids were transiently co-transfected into H1299 cells with Bax promoter construct. Normalized luciferase activity was standardized to that of Bax promoter construct with vector alone. Each value is the mean ± SD of at least three independent experiments.

cleavage of PARP induced by E2F1 overexpression in etoposide-induced apoptosis (Fig. 4C and D). Taken together, these data suggested that E2F1 increases etoposide-induced apoptosis, at least, partly through up-regulation of E1AF transcription.

DISCUSSION

Apoptosis, or programmed cell death, is a mechanism by which cells undergo death or control cell proliferation in response to DNA damage (*23*). The understanding of apoptosis has provided the basis for novel targeted therapies that can induce death in cancer cells or sensitize them to established cytotoxic agents and radiation therapy. Results from previous studies have demonstrated that E1AF plays an important role in tumour biology, including growth, invasiveness and migration (*1*, *9*, *10*, *42*, *43*). In this study, we explored the relationship between E1AF and the sensitivity of cancer cells to etoposide-induced apoptosis. As shown here, morphological and nuclear changes were inhibited, the loss of cell viability was prevented and the cells in sub-G1 were reduced when E1AF was down-regulated in etoposide-treated H1299 cells, indicating that E1AF might serve as a positive regulator in the apoptotic response of cancer cell to etoposide.

The activation of caspases plays a critical role in apoptosis (*44*), and two main pathways have been identified to lead to it: one is the extrinsic pathway initiated by death receptor activation and the other is the

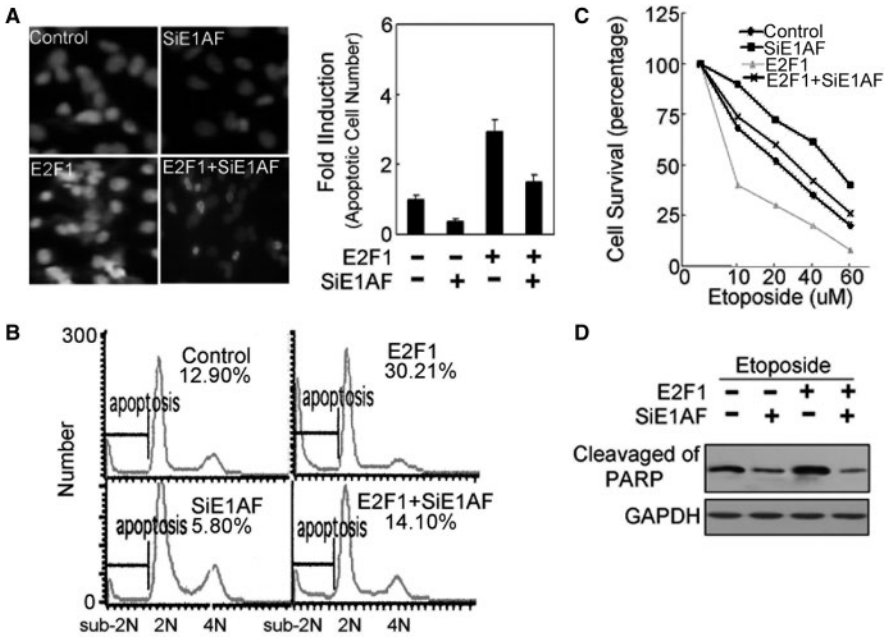

Fig. 4. **Interference of E1AF reduces E2F1-induced apoptosis.** (A) Hoechst 33258 staining of nuclei from H1299 cells transiently transfected with control, E1AF RNAi or/and E2F1 expression vector in the presence of etoposide (10 μM) for 24 h (left panel). Quantification of apoptotic cells. At least 300 cells were counted from three different microscope fields and each value was the mean ±SD of three independent experiments (right panel). (B) Cells transiently transfected with the empty vector or E1AF control, E1AF RNAi or/and E2F1 expression vector were harvested after the treatment of etoposide (10 μM) for 24 h, fixed in ethanol and stained with propidium iodide. The apoptotic rates were counted by flow cytometry analysis. (C) Cells transiently transfected with the empty vector or E1AF control, E1AF RNAi or/and E2F1 expression vector were treated with the indicated dose of etoposide for 24 h and analysed for cell viability by MTT assay. (D) H1299 cells transiently transfected with the empty vector or E1AF control, E1AF RNAi or/and E2F1 expression vector were treated with etoposide (10 μM) for 24 h and analysed for PARP cleavage. GAPDH expression served as loading controls.

intrinsic pathway triggered by various stress signals including DNA damage and acting through mitochondria (*45*). Cross-talk can occur between these two pathways, resulting in the amplification of mitochondrial release of cytochrome c, which in turn leads to enhanced activation of Caspase-9 and downstream executioner caspases that are responsible for the cleavage of specific cellular substrates (*45*). To investigate the mechanism by which E1AF affected etoposide-induced apoptosis, we examined the potential influence of E1AF on the caspases activity. As expected, H1299 cells transfected with E1AF displayed an enhanced activation of Caspase-9, indicating that E1AF overexpression might sensitize H1299 cells to etoposide-induced apoptosis via augmenting the transduction of signals leading to the sequential activation of Caspase-9. This hypothesis was supported by our observations that the expression and promoter activity of Bax, a pro-apoptotic Bcl-2-like protein reported to be essential for mitochondrial dysfunction in response to diverse apoptotic stimuli (*46*, *47*), was significantly increased in E1AF-transfected H1299 cells. In this background, our observations suggest that E1AF overexpression may complement and reinforce the apoptotic effect of etoposide on cancer cells via augmenting the signal transduction in intrinsic pathways, leading to enhanced activation of caspases. The mechanism(s) of E1AF-induced Bax transcription need further investigation.

Another important finding is that E1AF is a target gene of cell cycle and apoptosis regulator E2F1. This is based on the following observations: (i) E2F1 overexpression induced E1AF expression at transcriptional level; (ii) EMSA showed that the E2F1 protein interacted directly with the E1AF promoter; (iii) interference of endogenous E2F1 expression reduced E1AF expression at protein level and transcriptional level; and (iv) interference of E2F1 expression significantly reduced etoposide-induced E1AF expression. E2F1 is an important mediator of apoptotic response to DNA damage. Here, we identified E1AF as a target gene of E2F1-induced cell apoptosis. Interference of E1AF expression significantly reduced E2F1-induced cell apoptosis by Hochest33258 staining, FACS assay and MTT assay. These data suggested E2F1-induced apoptosis in response to DNA damage was, at least partly via up-regulation of E1AF transcription factor.

In conclusion, these data demonstrated a novel mechanism of apoptosis in which an increase in E1AF levels induced by E2F1 results in activation of Caspase-9. Because both of E2F1 and E1AF are frequently deregulated in various pathologic conditions, this finding may contribute to a better understanding of the pathophysiology of many diseases.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Shindoh, M., Higashino, F., and Kohgo, T. (2004) E1AF, an Ets-oncogene family transcription factor. *Cancer Lett.* **216**, 1–8
2. Ladle, R. and Frank, E. (2002) The role of the Ets gene PEA3 in the development of motor and sensory neurons. *Physiol. Behav.* **77**, 571–576
3. Laing, M.A., Coonrod, S., Hinton, B.T., Downie, J.W., Tozer, R., Rudnicki, M.A., and Hassell, J.A. (2000) Male sexual dysfunction in mice bearing targeted mutant alleles of the PEA3 ets gene. *Mol. Cell. Biol.* **20**, 9337–9345
4. Kurpios, N.A., Sabolic, N.A., Shepherd, T.G., Fidalgo, G.M., and Hassell, J.A. (2003) Function of PEA3 Ets transcription factors in mammary gland development and oncogenesis. *J. Mamm. Gland Biol. Neoplasia* **8**, 177–190
5. Firlej, V., Bocquet, B., Desbiens, X., de Launoit, Y., and Chotteau-Lelievre, A. (2005) Pea3 transcription factor cooperates with USF-1 in regulation of the murine Bax transcription without binding to an Ets-binding site. *J. Biol. Chem.* **280**, 887–898
6. Shepherd, T.G., Kockeritz, L., Szrajber, M.R., Muller, W.J., and Hassell, J.A. (2001) The pea3 subfamily Ets genes are required for HER2/Neu-mediated mammary oncogenesis. *Curr. Biol.* **11**, 1739–1748
7. O'Hagan, R.C. and Hassell, J.A. (1998) The PEA3 Ets transcription factor is a downstream target of the HER2/Neu receptor tyrosine kinase. *Oncogene* **16**, 301–310
8. Upadhyay, S., Liu, C., Chatterjee, A., Hoque, M.O., Kim, M.S., Engles, J., Westra, W., Trink, B., Ratovitski, E., and Sidransky, D. (2006) LKB1/STK11 suppresses cyclooxygenase-2 induction and cellular invasion through PEA3 in lung cancer. *Cancer Res.* **66**, 7870–7879
9. Hakuma, N., Kinoshita, I., Shimizu, Y., Yamazaki, K., Yoshida, K., Nishimura, M., and Dosaka-Akita, H. (2005) E1AF/PEA3 activates the Rho/Rho-associated kinase pathway to increase the malignancy potential of non-small-cell lung cancer cells. *Cancer Res.* **65**, 10776–10782
10. Izumiyama, Y., Ohiro, Y., Higashino, F., Yoshida, K., Taguchi, K., Todo, S., Kohgo, T., Totsuka, Y., and Shindoh, M. (2005) E1AF expression is closely correlated with malignant phenotype of tongue squamous cell carcinoma through activation of MT1-MMP gene promoters. *Oncology Reports* **13**, 715–720
11. Davidson, B., Goldberg, I., Tell, L., Vigdorchik, S., Baekelandt, M., Berner, A., Kristensen, G.B., Reich, R., and Kopolovic, J. (2004) The clinical role of the PEA3 transcription factor in ovarian and breast carcinoma in effusions. *Clin. Exp. Metastasis* **21**, 191–199
12. Oikawa, T. and Yamada, T. (2003) Molecular biology of the Ets family of transcription factors. *Gene* **303**, 11–34
13. Tahara, E. (2000) Molecular aspects of invasion and metastasis of stomach cancer. *Verh. Dtsch. Ges. Pathol.* **84**, 43–49
14. Taguchi, K., Yoshida, K., Sasaki, F., and Fujinaga, K. (1997) Two transcription factors, E1AF and N-myc, correlate with the invasiveness of neuroblastoma cell lines. *Jpn J. Cancer Res.* **88**, 394–400
15. Jiang, J., Wei, Y., Liu, D., Zhou, J., Shen, J., Chen, X., Zhang, S., Kong, X., and Gu, J. (2007) E1AF promotes breast cancer cell cycle progression via upregulation of Cyclin D3 transcription. *Biochem. Biophys. Res. Commun.* **358**, 53–58
16. Jiang, J., Wei, Y., Shen, J., Liu, D., Chen, X., Zhou, J., Zong, H., Yun, X., Kong, X., Zhang, S., Yang, Y., and Gu, J. (2007) Functional interaction of E1AF and Sp1 in glioma invasion. *Mol. Cell. Biol.* **27**, 8770–8782
17. Hata, H., Kitamura, T., Higashino, F., Hida, K., Yoshida, K., Ohiro, Y., Totsuka, Y., Kitagawa, Y., and Shindoh, M. (2008) Expression of E1AF, an Ets-oncogene transcription factor, highly correlates with malignant phenotype of malignant melanoma through up-regulation of the membrane-type-1 matrix metalloproteinase gene. *Oncology Reports* **19**, 1093–1098
18. Liu, H.Y., Zhou, B., Wang, L., Li, Y., Zhou, Z.G., Sun, X.F., Xu, B., Zeng, Y.J., Song, J.M., Luo, H.Z., and Yang, L. (2007) Association of E1AF mRNA expression with tumor progression and matrilysin in human rectal cancer. *Oncology* **73**, 384–388
19. Funaoka, K., Shindoh, M., Yoshida, K., Hanzawa, M., Hida, K., Nishikata, S., Totsuka, Y., and Fujinaga, K. (1997) Activation of the p21(Waf1/Cip1) promoter by the Ets oncogene family transcription factor E1AF. *Biochem. Biophys. Res. Commun.* **236**, 79–82
20. Menendez, J.A., Vellon, L., and Lupu, R. (2005) Antitumoral actions of the anti-obesity drug orlistat (XenicalTM) in breast cancer cells: blockade of cell cycle progression, promotion of apoptotic cell death and PEA3-mediated transcriptional repression of Her2/neu (erbB-2) oncogene. *Ann. Oncol.* **16**, 1253–1267
21. Menendez, J.A., Vellon, L., Mehmi, I., Oza, B.P., Ropero, S., Colomer, R., and Lupu, R. (2004) Inhibition of fatty acid synthase (FAS) suppresses HER2/neu (erbB-2) oncogene overexpression in cancer cells. *Proc. Natl Acad. Sci. USA* **101**, 10715–10720
22. Liu, D., Wei, Y., Zhou, F., Ge, Y., Xu, J., Chen, H., Zhang, W., Yun, X., and Jiang, J. (2008) E1AF promotes mithramycin A-induced Huh-7 cell apoptosis depending on its DNA-binding domain. *Arch. Biochem. Biophys.* **477**, 20–26
23. Norbury, C.J. and Zhivotovsky, B. (2004) DNA damage-induced apoptosis. *Oncogene* **23**, 2797–2808
24. Melino, G. (2002) The meaning of death. *Cell Death Differ.* **9**, 347–348
25. Lin, W.C., Lin, F.T., and Nevins, J.R. (2001) Selective induction of E2F1 in response to DNA damage, mediated by ATM-dependent phosphorylation. *Genes Dev.* **15**, 1833–1844
26. Stanelle, J. and Putzer, B.M. (2006) E2F1-induced apoptosis: turning killers into therapeutics. *Trends Mol. Med.* **12**, 177–185
27. Bell, L.A. and Ryan, K.M. (2004) Life and death decisions by E2F-1. *Cell Death Differ.* **11**, 137–142
28. Salon, C., Eymin, B., Micheau, O., Chaperot, L., Plumas, J., Brambilla, C., Brambilla, E., and Gazzeri, S. (2006) E2F1 induces apoptosis and sensitizes human lung adenocarcinoma cells to death-receptor-mediated apoptosis through specific downregulation of c-FLIP(short). *Cell Death Differ.* **13**, 260–272
29. Roos, W.P., Christmann, M., Fraser, S.T., and Kaina, B. (2007) Mouse embryonic stem cells are hypersensitive to apoptosis triggered by the DNA damage O(6)-methylguanine due to high E2F1 regulated mismatch repair. *Cell Death Differ.* **14**, 1422–1432
30. Hershko, T., Chaussepied, M., Oren, M., and Ginsberg, D. (2005) Novel link between E2F and p53: proapoptotic cofactors of p53 are transcriptionally upregulated by E2F. *Cell Death Differ.* **12**, 377–383
31. Kowalik, T.F., DeGregori, J., Leone, G., Jakoi, L., and Nevins, J.R. (1998) E2F1-specific induction of apoptosis and p53 accumulation, which is blocked by Mdm2. *Cell Growth Differ.* **9**, 113–118

32. Stiewe, T. and Putzer, B.M. (2000) Role of the p53-homologue p73 in E2F1-induced apoptosis. *Nat. Genet.* **26**, 464–469
33. Wang, C., Hou, X., Mohapatra, S., Ma, Y., Cress, W.D., Pledger, W.J., and Chen, J. (2005) Activation of p27Kip1 Expression by E2F1. A negative feedback mechanism. *J. Biol. Chem.* **280**, 12339–12343
34. Moroni, M.C., Hickman, E.S., Lazzerini Denchi, E., Caprara, G., Colli, E., Cecconi, F., Muller, H., and Helin, K. (2001) Apaf-1 is a transcriptional target for E2F and p53. *Nat. Cell Biol.* **3**, 552–558
35. Garcia, I., Murga, M., Vicario, A., Field, S.J., and Zubiaga, A.M. (2000) A role for E2F1 in the induction of apoptosis during thymic negative selection. *Cell Growth Differ.* **11**, 91–98
36. Field, S.J., Tsai, F.Y., Kuo, F., Zubiaga, A.M., Kaelin, W.G., Jr., Livingston, D.M., Orkin, S.H., and Greenberg, M.E. (1996) E2F-1 functions in mice to promote apoptosis and suppress proliferation. *Cell* **85**, 549–561
37. Hong, S., Pusapati, R.V., Powers, J.T., and Johnson, D.G. (2006) Oncogenes and the DNA damage response: Myc and E2F1 engage the ATM signaling pathway to activate p53 and induce apoptosis. *Cell Cycle* **5**, 801–803
38. Jiang, J., Chen, X., Shen, J., Wei, Y., Wu, T., Yang, Y., Wang, H., Zong, H., Yang, J., Zhang, S., Xie, J., Kong, X., Liu, W., and Gu, J. (2006) Beta1,4-galactosyltransferase V functions as a positive growth regulator in glioma. *J. Biol. Chem.* **281**, 9482–9489
39. Ishida, S., Higashino, F., Aoyagi, M., Takahashi, A., Suzuki, T., Shindoh, M., Fujinaga, K., and Yoshida, K. (2006) Genomic structure and promoter activity of the E1AF gene, a member of the Ets oncogene family. *Biochem. Biophys. Res. Commun.* **339**, 325–330
40. Thornborrow, E.C. and Manfredi, J.J. (1999) One mechanism for cell type-specific regulation of the Bax promoter by the tumor suppressor p53 is dictated by the p53 response element. *J. Biol. Chem.* **274**, 33747–33756
41. D'Sa-Eipper, C., Leonard, J.R., Putcha, G., Zheng, T.S., Flavell, R.A., Rakic, P., Kuida, K., and Roth, K.A. (2001) DNA damage-induced neural precursor cell apoptosis requires p53 and caspase 9 but neither Bax nor caspase 3. *Development* **128**, 137–146
42. Zhu, X., Jiang, J., Shen, H., Wang, H., Zong, H., Li, Z., Yang, Y., Niu, Z., Liu, W., Chen, X., Hu, Y., and Gu, J. (2005) Elevated beta1,4-galactosyltransferase I in highly metastatic human lung cancer cells. Identification of E1AF as important transcription activator. *J. Biol. Chem.* **280**, 12503–12516
43. Hida, K., Shindoh, M., Yasuda, M., Hanzawa, M., Funaoka, K., Kohgo, T., Amemiya, A., Totsuka, Y., Yoshida, K., and Fujinaga, K. (1997) Antisense E1AF transfection restrains oral cancer invasion by reducing matrix metalloproteinase activities. *Am. J. Pathol.* **150**, 2125–2132
44. Budihardjo, I., Oliver, H., Lutter, M., Luo, X., and Wang, X. (1999) Biochemical pathways of caspase activation during apoptosis. *Ann. Rev. Cell Dev. Biol.* **15**, 269–290
45. Zimmermann, K.C., Bonzon, C., and Green, D.R. (2001) The machinery of programmed cell death. *Pharmacol. Ther.* **92**, 57–70
46. Lalier, L., Cartron, P.F., Juin, P., Nedelkina, S., Manon, S., Bechinger, B., and Vallette, F.M. (2007) Bax activation and mitochondrial insertion during apoptosis. *Apoptosis* **12**, 887–896
47. Reed, J.C. (2006) Proapoptotic multidomain Bcl-2/Bax-family proteins: mechanisms, physiological roles, and therapeutic opportunities. *Cell Death Differ.* **13**, 1378–1386