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Transcription factor Sox4 is required for PUMA-mediated apoptosis induced by histone deacetylase inhibitor, TSA

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ABSTRACT

PUMA is a crucial regulator of apoptotic cell death mediated by p53-dependent and p53-independent mechanisms. In many cancer cells, PUMA expression is induced in response to DNA-damaging reagent in a p53-dependent manner. However, few studies have investigated transcription factors that lead to the induction of PUMA expression via p53-independent apoptotic signaling. In this study, we found that the transcription factor Sox4 increased PUMA expression in response to trichostatin A (TSA), a histone deacetylase inhibitor in the p53-null human lung cancer cell line H1299. Ectopic expression of Sox4 led to the induction of PUMA expression at the mRNA and protein levels, and TSA-mediated up-regulation of *PUMA* transcription was repressed by the knockdown of Sox4. Using luciferase assays and chromatin immunoprecipitation, we also determined that Sox4 recruits p300 on the *PUMA* promoter region and increases *PUMA* gene expression in response to TSA treatment. Taken together, these results suggest that Sox4 is required for p53-independent apoptotic cell death mediated by PUMA induction via TSA treatment.

1. Introduction

PUMA is a member of the BH3-only Bcl-2 family, and mediates apoptotic cell death through mitochondria dysfunction-mediated activation of caspases [1–3]. In many human cancer cell types, expression of PUMA is barely detected. However, it is rapidly induced in response to a variety of stress leading to apoptotic cell death [1,2]. Especially, DNA-damaging agents can induce PUMA expression via the function of tumor suppressor p53. Genotoxic stress leads to p53 recruitment to the consensus sequences at the *PUMA* promoter [4,5]. p53 and the p53 binding elements in the *PUMA* promoter are necessary for the induction of PUMA by DNA damaging stimuli [5]. In addition to genotoxic stress, histone deacetylase (HDAC) inhibitor induces apoptotic cell death in gastric cancer cells by enhancing p53 binding to the *PUMA* promoter, whereas HDAC3 is dissociated from the *PUMA* promoter [6]. These reports supported the contention that PUMA induction by various cellular stimuli occurs in a p53-dependent fashion. Recent studies have revealed that PUMA expression is also induced by p53-independent mechanisms in response to several stimuli. Transcription factor p73, which is a p53 homologue, can regulate PUMA expression in p53-deficient cancer cells by binding to p53 consensus sites in the *PUMA* promoter in a serum starved condition [7,8]. The transcriptional level of *PUMA* is controlled by the forkhead family member FOXO3a in $p53^{-/-}$ mouse embryonic fibroblast cell lines in response to cytokine withdrawal [9]. In this regard, the finding of a stimulus-mediated novel molecular mechanism that can induce PUMA expression might be important in the understanding of p53-independent apoptotic cell death. However, these correlations between PUMA-inducible stimuli and transcriptional modulators remain to be elucidated.

Sox4, a transcription factor of the sex-determining gene on the Y chromosome (SRY), is characterized by a highly conserved sequence in the high-mobility group (HMG) DNA-binding domain (DBD) [10]. Sox4 plays important roles in many developmental processes, including embryonic cardiac, thymocyte and nervous system development [11–13]. In addition, Sox4 is also involved in tumorigenesis. Especially, Sox4 has dual-functions – oncogenic or tumor-suppressive – in a number of tumors. For example, the expression of Sox4 is highly increased in carcinomas of the lung, breast and colon [14–16]. In contrast, Sox4 functions as a tumor suppressor in the bladder and hepatocarcinoma [17,18]. Moreover, Sox4 displays pro-apoptotic effects when Sox4 is overexpressed in HeLa and HEK293 cells [18–20]. Furthermore, many genes related to the inhibition of cancer cell viability have been identified as putative Sox4 target genes by microarray analysis [21]. In addition,

Abbreviations: TSA, trichostatin A; DBD, DNA binding domain; TAD, transactivation domain.

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a recent report described that increased Sox4 expression by DNA-damage acts as a co-activator of p53 in colon and lung cancer, implicating Sox4 as a tumor suppressor [22]. However, the precise roles of Sox4-mediated inhibition of cancer progression remain unclear.

In this report, we demonstrate that trichostatin A (TSA), a HDAC inhibitor, induces PUMA expression in the p53-deficient human lung cell line, H1299, by recruitment of Sox4 and Sox4-dependent p300 co-activator in the *PUMA* promoter. These findings suggest that Sox4 is required for TSA-mediated apoptotic cell death in p53-deficient cancer cells.

2. Materials and methods

2.1. Cell culture and transfection

H1299 cells were obtained from the ATCC (American type culture collection; Manassas, VA). H1299 cells were maintained RPMI 1640 medium. This medium was supplemented with 10% fetal bovine serum (Invitrogen) and penicillin–streptomycin (50 units/ml). Transient transfection was performed by Lipofectamine 2000 (Invitrogen) with different plasmid DNA according to the manufacturer's instructions.

2.2. Plasmid constructs

Details for plasmid constructions are described in the Supplemental material.

2.3. Western blotting

Western blot analysis was performed as described previously [24]. The monoclonal antibody against GFP (11814460001) was purchased from Roche Diagnostics (Indianapolis, IN, USA) and PUMA (1652-S) was purchased from Epitomics Inc. (Burlingame, CA). PARP1 (51-6639GR) was purchased from BD Biosciences (Franklin. 162. Lakes, NJ). Polyclonal antibodies against β-tubulin (sc-9104) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Western blotting was visualized by chemiluminescence using an ECL system (Santa Cruz Biotechnology Inc.).

2.4. RNA preparation and quantitative real-time PCR

Total RNA extraction and quantitative real-time PCR was performed as described previously [25]. The expression levels of human *PUMA* and *18SrRNA* in H1299 cells were measured by qRT-PCR with the following specific primers: *PUMA*, forward, 5′-GAC GAC CTC AAC GCA CAG TA-3′; reverse, 5′-CAC CTA ATT GGG CTC CAT CT-3′; *18SrRNA*, forward, 5′-GAT TAA GCC ATG CAT GTC TA-3′; reverse, 5′-GTC GGG CGC CGG CGG CTT TG-3′.

2.5. TUNEL assay

H1299 cells were incubated for 24 h with or without 1 μM TSA and the TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling) assay was performed with the use of the DeadEnd™ Fluorometric TUNEL System (Promega), according to the manufacturer's instructions.

2.6. Cell viability assays

Cell viability was measured by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay as described previously [24]. For investigation of apoptosis by nuclear staining, transfected H1299 cells were washed with phosphate-buffered saline (PBS) and fixed for 10 min in 4% paraformaldehyde. The cells were permeabilized with 0.3% Triton X-100 for 15 min. After incubation in blocking solution for 30 min, cells were incubated in 300 nM DAPI in PBS for 2 min at room temperature. Chromatin condensation in cells was visualized using a Carl Zeiss LSM-510 META laser scanning microscope (Oberkochen, Germany). To evaluate cell death rate, TSA-treated H1299 cells were stained by trypan blue solution (0.4%) and the number of apoptotic cells was counted and presented as a percentage versus the total population of control cells.

2.7. Luciferase assay

H1299 cells were cultured in 60 mm dishes and were transfected using Lipofectamine 2000, with the luciferase reporter constructs (0.1 μg), pCMV-β-galactosidase and GFP-Sox4 or siRNA-Sox4. The cells were lysed in reporter lysis buffer 48 h after transfection (Promega). Cell lysates were then analyzed with the luciferase reporter assay system, using a glomax luminometer (Promega). Luciferase activities were normalized on the basis of the β-galactosidase activity of the cotransfected vector. All transfection experiments were repeated independently at least three times.

2.8. Polyclonal antibody production of Sox4

Details of Sox4 antibody production are described in the Supplemental material.

2.9. Chromatin immunoprecipitation (ChIP)

A ChIP assay was conducted following the protocol provide by Upstate Biotechnology (Lake Placid, NY, USA), and as described previously [25]. The chromatin fragments from H1299 cells were immunoprecipitated with antibodies against Sox4, p300, acetylated histone H3 and H4, and HDAC1. DNA of the immunoprecipitates and control input DNA was analyzed by quantitative real-time PCR with the *PUMA* promoter-specific primers: forward, 5′-TCT CCA AAC CCC GCG AGG GAC-3′; reverse, 5′-ACC CCT GGG GTC GAC CCT CTT-3′.

2.10. Statistical analysis

Statistical analysis of variances between two different experimental groups was conducted with Tukey's post hoc comparison test using SPSS, version 12 (SPPS Inc., Chicago, IL, USA). All experiments were repeated at least three times. The levels were considered significant at $p < 0.05$ (*) and very significant at $p < 0.01$ (**), obviously significant at $p < 0.001$ (***), or not significant (*n.s.*).

3. Results

3.1. TSA increases H1299 lung carcinoma cell death through the enhancement of PUMA mRNA and protein expression

Although the HDAC inhibitor TSA modulates the transcriptional activity of p53, leading to cell cycle arrest or apoptotic cell death, the p53-independent molecular mechanisms by HDAC inhibitors are currently unclear. To test the TSA-mediated cell death through the p53-independent pathway, H1299 cells were treated for three days either in 1 μM TSA or in the same volume of ethanol, as a control, and cell viability and death were determined by MTT assay and trypan blue staining. As shown in Fig. 1A, the viability of H1299 cells was decreased (upper panel) and cell death was significantly increased (lower panel) twenty-four hours after TSA treatment. Furthermore, TUNEL positive cells, apoptotic cell death markers, were detected in TSA-treated H1299 cells (Fig. 1B). It

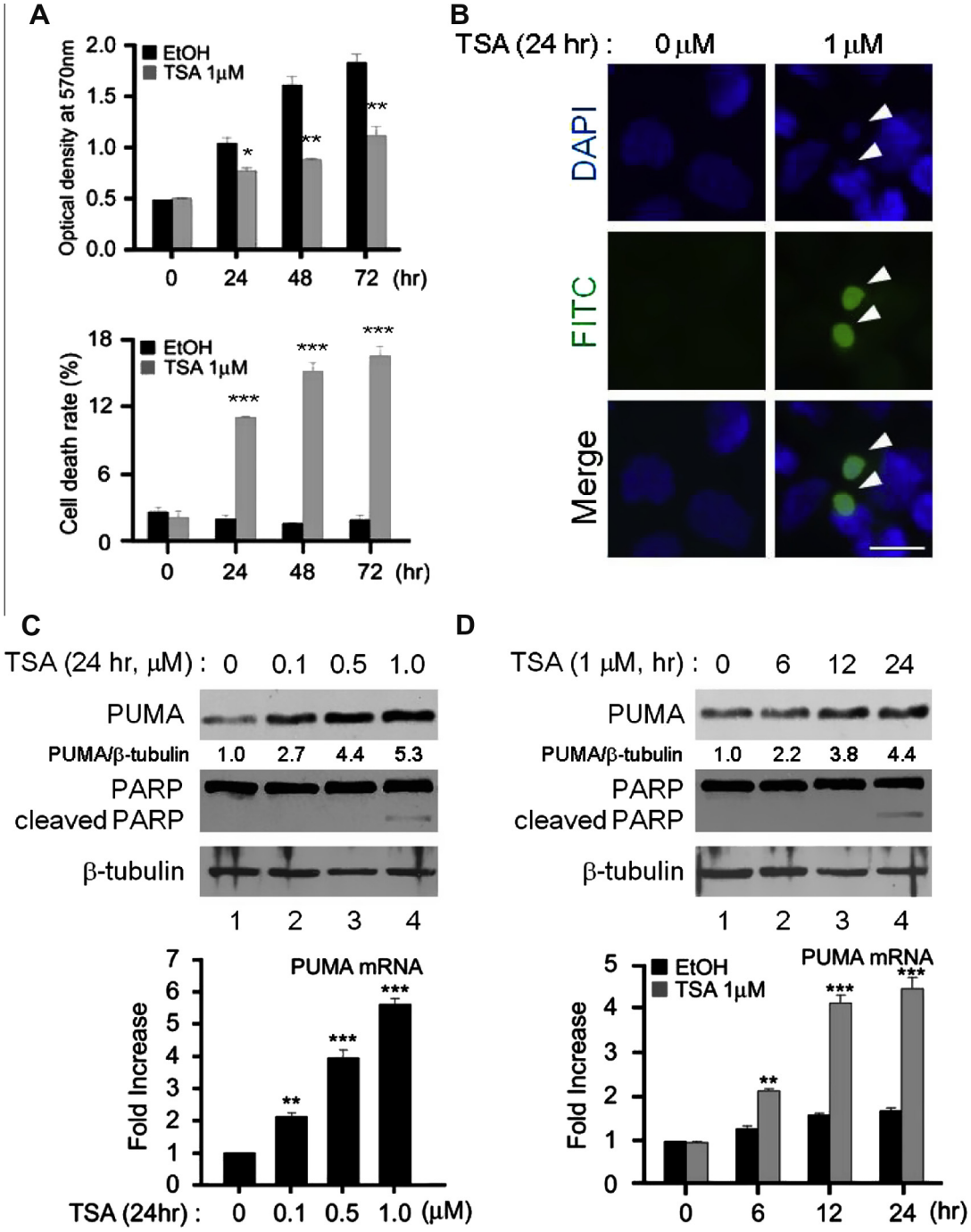

Fig. 1. Expressions of PUMA protein and mRNA are induced by TSA treatment, which results in apoptotic cell death. (A) H1299 cells were treated with TSA for the indicated times and cell viability was examined by MTT assay (upper panel) and trypan blue staining (bottom panel). (B) TUNEL assay was performed for H1299 cells in response to TSA. White arrows indicate DNA-fragmented apoptotic cells. Scale bar, 25 μm. (C and D) The level of PUMA protein and transcripts in H1299 cells treated with TSA, as indicated, were analyzed using Western blotting and qRT-PCR. PUMA protein or mRNA was normalized by β-tubulin or *18SrRNA* gene, respectively. All data are representative of three independent experiments and statistical significance is represented by Tukey's post hoc test ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

was recently reported that TSA treatment induces gastric cancer cell death through the induction of PUMA expression [6]. To test whether the apoptotic death of H1299 cells by TSA treatment was mediated by PUMA induction, H1299 cells were treated with TSA of various concentrations (Fig. 1C) for different time points (Fig. 1D). Then, PUMA protein and mRNA expressions were examined by Western-blotting and quantitative real time PCR (qRT-PCR). As shown in Fig. 1C and D, both PUMA protein and transcript were markedly increased by TSA treatment in dose- and time-dependent manners. Moreover, PARP cleavage shows the apoptotic cell death in 1 μM of TSA treated H1299 cells for 24 h. Taken together, these results indicate that TSA induces apoptosis through the enhancement of PUMA expression in p53-null H1299 cells.

3.2. Transcription factor Sox4 regulates PUMA transcription in H1299 cells in response to TSA

To reveal the novel transcriptional regulator of *PUMA*, its promoter region (−500 ~ +500 from transcriptional start site) was used for a transcription factor binding motif search program.

Because the *PUMA* promoter has two Sox4 binding sites (Fig. 2A), we tested the possibility of the involvement of Sox4 during *PUMA* transcription in a p53-independent manner. H1299 cells were transfected with GFP-fused Sox4 expression plasmid for 24 h, and the PUMA mRNA expression level was then analyzed by qRT-PCR. As shown in Fig. 2B, overexpression of Sox4 significantly increased *PUMA* transcription levels in a Sox4 dose-dependent manner (lane 2 and 3). However, transfection of Sox4 small interfering RNA (siRNA) that effectively repressed the level of overexpressed GFP-Sox4 proteins reduced the *PUMA* mRNA level (lane 4). To examine whether Sox4 directly bound to its responsive elements (SB1 and SB2) of *PUMA* gene promoter, H1299 cells were transiently transfected with Sox4 expression vector, together with luciferase reporter constructs which are regulated by *PUMA* intact promoter (WT) and mutant promoters (mutant 1 ~ 3) on Sox4 binding sites (SB1 and SB2) (Fig. 2C, upper panel). As shown in Fig. 2C, the promoter activity of reporter constructs containing two intact Sox4 binding sites (*PUMA*p WT) was significantly increased in a Sox4 dose-dependent manner. In contrast, reporter constructs including point mutations of each SB site (*PUMA*p Mut1-3) did not show increased promoter activity. We also tested whether the induction of *PUMA* transcripts by TSA treatment was mediated by the transcriptional activity of Sox4. H1299 cells were transfected with Sox4 expression plasmids or siRNA-Sox4 constructs, and the *PUMA* mRNA level was then analyzed after TSA treatment. As shown in Fig. 2D, exogenous Sox4 induced *PUMA* transcripts, and levels of *PUMA* mRNA were further increased by TSA treatment in the presence of exogenous Sox4 (lane 1 and 2). In contrast, H1299 cells transfected with Sox4-siRNA did not show the induction of *PUMA* mRNA in spite of TSA treatment (Fig. 2D, lane 3 and 4). To further confirm the function of Sox4 as a transcription factor, various truncated constructs for Sox4 were transiently transfected in H1299 cells, and *PUMA* transcript levels were then analyzed by qRT-PCR. The Sox4 wild-type construct, containing both DBD and TAD increased *PUMA* mRNA expression (Fig. 3A, lane 3), whereas Sox4 truncated mutants deficient in DBD and/or TAD failed to induce the transcription of *PUMA* (Fig. 3A, lane 4–6). Consistent with the induction of *PUMA* transcripts (Fig. 3A, lane 3), the expression level of PUMA protein was also increased by Sox4 overexpression (Fig. 3B, lane 3). The induction of PUMA protein resulted in apoptotic cell death with the appearance of PARP cleavage and compacted chromatins in the nucleus (Fig. 3B). All together, these results suggest that Sox4 significantly induces *PUMA* gene transcription in a p53-independent manner in response to TSA.

3.3. Sox4 is recruited to PUMA gene promoter and is required for chromatin opening through histone acetylation by p300

To determine whether the enhancement of *PUMA* transcription was mediated by the binding of transcription factor Sox4 on its promoter region, H1299 cells were transfected with Sox4

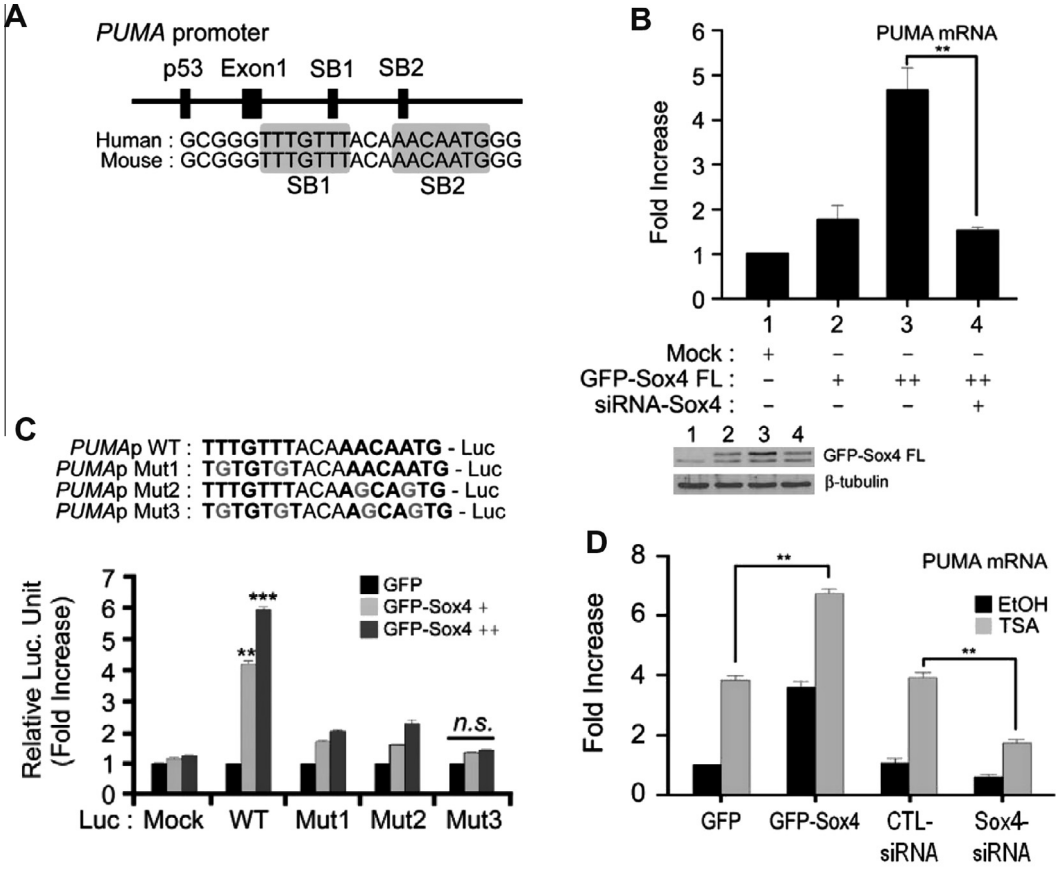

Fig. 2. Sox4 regulates *PUMA* transcription in TSA-treated H1299 cells. (A) The conserved Sox4 binding sites (SB) in the human and mouse *PUMA* promoters were identified. (B) Total RNA from H1299 cells transfected with increasing amounts of plasmids encoding Sox4 DNA or Sox4 siRNA vectors were analyzed for *PUMA* mRNA levels by qRT-PCR. *PUMA* transcripts were normalized by *18SrRNA*. Protein levels were verified by Western blotting using antibodies against GFP and β-tubulin (as a loading control). (C) Substituted nucleotide in the Sox4-response elements was indicated as a gray bold. Various mutants of human *PUMA* promoters were used, and are indicated as mut1, mut2 and mut3. Each of the constructs and pCMV-β-galactosidase were transiently transfected into H1299 cells, together with increasing amounts of plasmids encoding Sox4 cDNA. Forty-eight hours after transfection, luciferase activity was measured. Data were normalized against β-galactosidase activity and are expressed as relative luciferase units compared to the control. (D) H1299 cells were transfected with either Sox4 expression plasmids or Sox4-siRNA constructs. The amount of *PUMA* transcript was analyzed by qRT-PCR after TSA treatments in transfected cells. All data are representative of three independent experiments, and statistical significance was determined using Tukey's post hoc test (**$p < 0.01$, ***$p < 0.001$, *n.s.*, not significant).

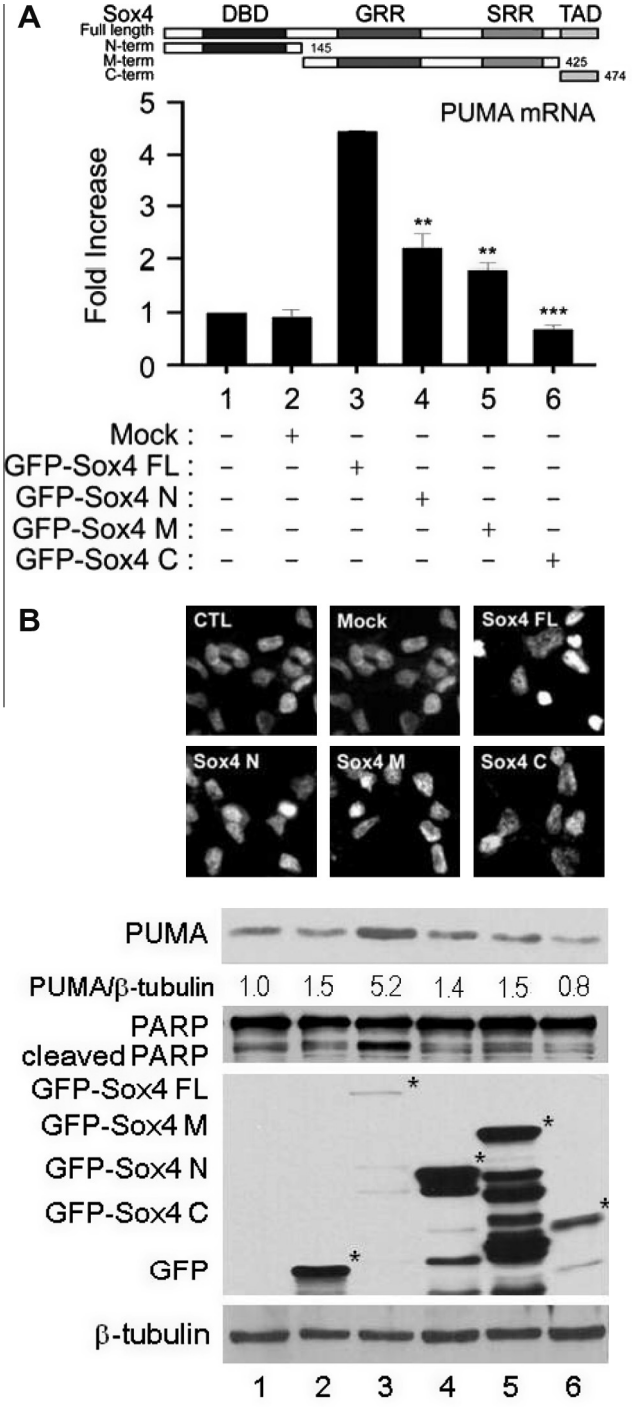

Fig. 3. Sox4 increases *PUMA* transcription as a transcription factor function. (A) H1299 cells were transiently transfected with various Sox4 truncation mutants (upper panel). Total RNA was analyzed for *PUMA* mRNA levels by qRT-PCR. (B) After transfection, as in (A), the nucleus was stained using 4′,6-diamidino-2-phenylindole for detection of chromosomal condensation (upper panel). The expression levels of proteins were assessed by immunoblotting (bottom panels). All data are representative of three independent experiments, and statistical significance was determined using Tukey's post hoc test ($^{**}p < 0.01$, $^{***}p < 0.001$).

expression plasmid followed by TSA treatment, and chromatin immunoprecipitation was then performed. As shown in Fig. 4A, overexpressed Sox4 markedly precipitated the *PUMA* promoter. Moreover, Sox4 binding to the *PUMA* promoter was increased under TSA-treated conditions. In a time course study, endogenous Sox4 specifically precipitated *PUMA* promoter in response to TSA treatment (data not shown). p300, which is a known co-activator for histone acetylation of the *PUMA* promoter [5], was also recruited to the *PUMA* promoter in the presence of TSA (data not shown). To further investigate whether p300 recruitment can occur in a Sox4-dependent manner, TSA treatment was carried out in Sox4-silencing H1299 cells and the ChIP assay was performed using p300 specific antibodies. Consistently, the *PUMA* promoter was precipitated by p300 when TSA was treated in endogenous Sox4 expressed cells, whereas TSA treatment in Sox4-silenced H1299 cells led to decreased amounts of fragments of *PUMA* promoter in the ChIP assay. According to p300 recruitment, acetylated histones H3 and H4 were also increased by TSA in Sox4-existent H1299 cells. However, when we treated TSA in H1299 cells transfected with Sox4-siRNA, acetylated histones were decreased on the *PUMA* promoter (Fig. 4B). Interestingly, HDAC1, which is known as a repressor of *PUMA* promoter was dissociated in response to TSA not correlated with Sox4 expression (Fig. 4C). Taken together, these results indicate that TSA-mediated histone acetylation in *PUMA* promoter is regulated by Sox4-dependent p300 recruitment.

4. Discussion

PUMA is known as the inducer of apoptotic cell death, and is down-regulated in many cancer cells, including colorectal, breast and lung cancer [1,23]. Although the induction of PUMA expression in response to signals including DNA-damaging reagents or HDAC inhibitors in a p53-dependent manner is well known, the signals or factors underlying this induction remain to be elucidated in p53-null conditions. In the present study, we demonstrated that *PUMA* transcription is induced by TSA treatment in p53-null H1299 cells. The protein and mRNA levels of PUMA were increased by TSA, resulting in apoptotic cell death (Fig. 1). Moreover, we demonstrated that Sox4 is a crucial transcription factor for TSA-mediated induction of PUMA expression. Ectopic expression or knockdown of Sox4 can modulate the *PUMA* transcription and TSA-mediated *PUMA* transcription, and promoter activity was abolished by Sox4 silencing (Figs. 2 and 3). Furthermore, TSA treatment in H1299 cells recruits Sox4 and Sox4-dependent p300 on the *PUMA* promoter (Fig. 4).

To identify the transcription factors that can modulate the transcription of *PUMA*, we analyzed putative transcription factor binding elements within *PUMA* promoter, and found Sox4 to be a putative transcription modulator. Recent reports have shown that Sox4 indirectly induces the p53 target gene expression in response to DNA-damage signals, including doxorubicin, UV irradiation and ionizing radiation thorough contribution of the enhancement of p53 acetylation by interacting with p53 and modulating a more stable interaction between p53 and p300 [22]. However, our unbiased binding motif search result suggests that Sox4 may regulate the PUMA expression directly through binding on its target gene promoter. Indeed, PUMA expression was increased by the ectopic expression of Sox4, whereas overexpression of truncated Sox4 constructs could not induce PUMA expression. As shown in Figs. 2–4, qRT-PCR, luciferase assays and ChIP assays clearly showed that Sox4 modulates *PUMA* transcription via direct binding to the Sox4 binding sites in the *PUMA* promoter region. Furthermore, Sox4 gene silencing using its siRNA abolished gene expression and promoter activity of PUMA. These findings clearly show that Sox4 regulated PUMA gene expression in response to TSA treatment.

There are two possible reasons for the modulation of PUMA expression by Sox4 in response to TSA. First, it was possible that TSA-mediated an enhancement of Sox4 transcriptional activity by maintaining Sox4 acetylation. In our observation, however, Sox4 acetylation was not detected by TSA treatment (data not shown).

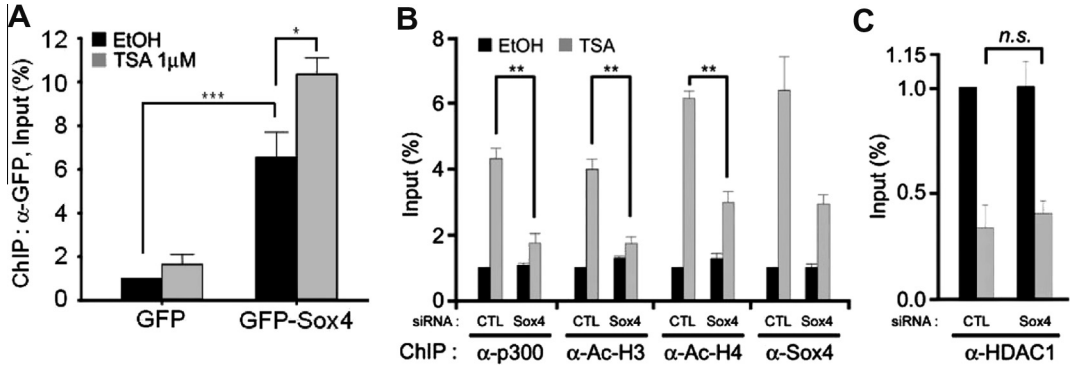

Fig. 4. Histone acetylation of *PUMA* promoter is mediated by Sox4-dependent p300 recruitment in TSA-treated H1299 cells. (A) Sox4-overexpressing H1299 cells were incubated with TSA, and ChIP analysis was conducted using GFP-antibodies. The precipitated chromatin fragments were analyzed using primers specific to the *PUMA* promoter. (B and C) H1299 cells transfected with pBabe-dual siRNA empty vector or Sox4-siRNA constructs were incubated with TSA, and ChIP assays were performed using antibodies against p300, acetylated histones H3 and H4 and HDAC1 (C). The precipitated *PUMA* promoter fragments were analyzed as in (A). All data are the mean ± SEM of three independent experiments performed in triplicate, and statistical significance between different groups was determined by Tukey's post hoc test ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$,*n.s.*, not significant).

Another possibility is the requirement of Sox4 for TSA-mediated histone acetylation in the Sox4 target gene promoter. Quantitative ChIP assays in TSA-treated H1299 cells revealed that Sox4 recruitment on the *PUMA* promoter correlated with changing patterns of histone acetylation in the *PUMA* promoter (Fig. 4). These results suggest that the other co-regulators with acetyltransferase or deacetylase activity were involved in histone acetylation in the *PUMA* promoter in a Sox4-dependent manner. Indeed, the present observation of the recruitment of Sox4-dependent histone acetyltransferase p300 is supported by the prior report of a Sox4-p300 interaction [22]. In contrast to p300, HDAC1 recruitment on the *PUMA* promoter was Sox4-independent, suggesting that HDAC1 is already recruited at the *PUMA* promoter by other transcription factors, prior to TSA treatment.

Despite intensive studies, the mechanisms in response to cancer therapeutic agents capable of inducing cell death in abnormal p53 cancer cells still remain to be fully elucidated. In this respect, we demonstrated that TSA-mediated apoptotic cell death is caused by PUMA expression through Sox4 transcriptional activity. These results indicate a novel molecular mechanism that may further the understanding of p53-independent apoptotic cell death, and suggests a novel possibility for cancer treatment.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.07.099.

References

[1] J. Yu, L. Zhang, P.M. Hwang, K.W. Kinzler, B. Vogelstein, PUMA induces the rapid apoptosis of colorectal cancer cells, Mol. Cell 7 (2001) 673–682.
[2] K. Nakano, K.H. Vousden, PUMA, a novel proapoptotic gene, is induced by p53, Mol. Cell 7 (2001) 683–694.
[3] J. Yu, Z. Wang, K.W. Kinzler, B. Vogelstein, L. Zhang, PUMA mediates the apoptotic response to p53 in colorectal cancer cells, Proc. Natl. Acad. Sci. USA 100 (2003) 1931–1936.
[4] M.D. Kaeser, S. Pebernard, R.D. Iggo, Regulation of p53 stability and function in HCT116 colon cancer cells, J. Biol. Chem. 279 (2004) 7598–7605.
[5] P. Wang, J. Yu, L. Zhang, The nuclear function of p53 is required for PUMA-mediated apoptosis induced by DNA damage, Proc. Natl. Acad. Sci. USA 104 (2007) 4054–4059.
[6] L. Feng, M. Pan, J. Sun, H. Lu, Q. Shen, S. Zhang, T. Jiang, L. Liu, W. Jin, Y. Chen, X. Wang, H. Jin, Histonedeacetylase 3 inhibits expression of PUMA in gastric cancer cells, J. Mol. Med. (Berl) 91 (2012) 49–58.
[7] G. Melino, F. Bernassola, M. Ranalli, K. Yee, W.X. Zong, M. Corazzari, R.A. Knight, D.R. Green, C. Thompson, K.H. Vousden, P73 Induces apoptosis via PUMA transactivation and Bax mitochondrial translocation, J. Biol. Chem. 279 (2004) 8076–8083.
[8] L. Ming, T. Sakaida, W. Yue, A. Jha, L. Zhang, J. Yu, Sp1 and p73 activate PUMA following serum starvation, Carcinogenesis 29 (2008) 1878–1884.
[9] H. You, M. Pellegrini, K. Tsuchihara, K. Yamamoto, G. Hacker, M. Erlacher, A. Villunger, T.W. Mak, FOXO3a-dependent regulation of Puma in response to cytokine/growth factor withdrawal, J. Exp. Med. 203 (2006) 1657–1663.
[10] A.I. Penzo-Mendez, Critical roles for SoxC transcription factors in development and cancer, Int. J. Biochem. Cell Biol. 42 (2010) 425–428.
[11] M.W. Schilham, M.A. Oosterwegel, P. Moerer, J. Ya, P.A. de Boer, M. van de Wetering, S. Verbeek, W.H. Lamers, A.M. Kruisbeek, A. Cumano, H. Clevers, Defects in cardiac outflow tract formation and pro-B-lymphocyte expansion in mice lacking Sox-4, Nature 380 (1996) 711–714.
[12] M.W. Schilham, P. Moerer, A. Cumano, H.C. Clevers, Sox-4 facilitates thymocyte differentiation, Eur. J. Immunol. 27 (1997) 1292–1295.
[13] M. Cheung, M. Abu-Elmagd, H. Clevers, P.J. Scotting, Roles of Sox4 in central nervous system development, Brain Res. Mol. Brain Res. 79 (2000) 180–191.
[14] R.S. Friedman, C.S. Bangur, E.J. Zasloff, L. Fan, T. Wang, Y. Watanabe, M. Kalos, Molecular and immunological evaluation of the transcription factor SOX-4 as a lung tumor vaccine antigen, J. Immunol. 172 (2004) 3319–3327.
[15] J.D. Graham, S.M. Hunt, N. Tran, C.L. Clarke, Regulation of the expression and activity by progestins of a member of the SOX gene family of transcriptional modulators, J. Mol. Endocrinol. 22 (1999) 295–304.
[16] T. Reichling, K.H. Goss, D.J. Carson, R.W. Holdcraft, C. Ley-Ebert, D. Witte, B.J. Aronow, J. Groden, Transcriptional profiles of intestinal tumors in Apc(Min) mice are unique from those of embryonic intestine and identify novel gene targets dysregulated in human colorectal tumors, Cancer Res. 65 (2005) 166–176.
[17] M. Aaboe, K. Birkenkamp-Demtroder, C. Wiuf, F.B. Sorensen, T. Thykjaer, G. Sauter, K.M. Jensen, L. Dyrskjot, T. Orntoft, SOX4 expression in bladder carcinoma: clinical aspects and in vitro functional characterization, Cancer Res. 66 (2006) 3434–3442.
[18] S.G. Ahn, H.S. Kim, S.W. Jeong, B.E. Kim, H. Rhim, J.Y. Shim, J.W. Kim, J.H. Lee, I.K. Kim, Sox-4 is a positive regulator of Hep3B and HepG2 cells' apoptosis induced by prostaglandin PG)A(2) and delta(12)-PGJ(2), Exp. Mol. Med. 34 (2002) 243–249.
[19] B.E. Kim, J.H. Lee, H.S. Kim, O.J. Kwon, S.W. Jeong, I.K. Kim, Involvement of Sox-4 in the cytochrome c-dependent AIF-independent apoptotic pathway in HeLa cells induced by Delta12-prostaglandin J2, Exp. Mol. Med. 36 (2004) 444–453.
[20] E.H. Hur, W. Hur, J.Y. Choi, I.K. Kim, H.Y. Kim, S.K. Yoon, H. Rhim, Functional identification of the pro-apoptotic effector domain in human Sox4, Biochem. Biophys. Res. Commun. 325 (2004) 59–67.
[21] P. Liu, S. Ramachandran, M. Ali Seyed, C.D. Scharer, N. Laycock, W.B. Dalton, H. Williams, S. Karanam, M.W. Datta, D.L. Jaye, C.S. Moreno, Sex-determining region Y box 4 is a transforming oncogene in human prostate cancer cells, Cancer Res. 66 (2006) 4011–4019.
[22] X. Pan, J. Zhao, W.N. Zhang, H.Y. Li, R. Mu, T. Zhou, H.Y. Zhang, W.L. Gong, M. Yu, J.H. Man, P.J. Zhang, A.L. Li, X.M. Zhang, Induction of SOX4 by DNA damage is critical for p53 stabilization and function, Proc. Natl. Acad. Sci. USA 106 (2009) 3788–3793.
[23] J. Yu, W. Yue, B. Wu, L. Zhang, PUMA sensitizes lung cancer cells to chemotherapeutic agents and irradiation, Clin. Cancer Res. 12 (2006) 2928–2936.

[24] S.M. Jang, J.W. Kim, C.H. Kim, D. Kim, S. Rhee, K.H. Choi, P19(ras) Represses proliferation of non-small cell lung cancer possibly through interaction with neuron-specific enolase (NSE), Cancer Lett. 289 (2010) 91–98.

[25] J.W. Kim, S.M. Jang, C.H. Kim, J.H. An, E.J. Kang, K.H. Choi, Neural retina leucine-zipper regulates the expression of Ppp 2r5c, the regulatory subunit of protein phosphatase 2A, in photoreceptor development, FEBS J. 277 (2010) 5051–5060.