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14-3-3 σ is a new target up-regulated by transforming growth factor-β1 through a Smad3-dependent mechanism

Hye-Young Hong [a], Woo-Kwang Jeon [a], Seong-Jin Kim [b], Byung-Chul Kim [a,*]

[a] Department of Biochemistry, College of Natural Sciences, Kangwon National University, Chuncheon 200-701, Republic of Korea
[b] Cha Cancer Institute, Cha University, Seoul 135-081, Republic of Korea

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ABSTRACT

The seven members of the human 14-3-3 family play crucial roles in a diverse range of cellular responses including cell cycle progression, DNA damage checkpoint, and apoptosis. One particular isoform, 14-3-3 σ, the p53 target gene, is a unique tumor suppressor. We here report 14-3-3 σ as a transforming growth factor-beta (TGF-β) target gene. In mammary epithelial cells, TGF-β selectively induced expression of 14-3-3 σ at both mRNA and protein levels, and this induction was dependent on Smad3 not on p53. In addition, blockade of non-canonical Smad-independent pathways, including MAP kinases and Rho GTPases, did not affect the TGF-β1-induced 14-3-3 σ expression. Our data provides the first evidence that 14-3-3 σ is a Smad3-dependent target gene of TGF-β1.

1. Introduction

Transforming growth factor-β (TGF-β) is a multifunctional cytokine that modulate a diverse of cellular functions such as cell proliferation, differentiation, cell adhesion, apoptosis, and inflammatory responses [1,2]. TGF-β initiates intracellular signaling through two types of transmembrane serine and threonine kinase receptors, TβRI and TβRII. The activated receptor signaling complex propagates intracellular signal transduction via two effector pathways, the canonical Smad-dependent or non-canonical Smad-independent pathway. In the canonical pathway, receptor-activated Smad proteins (R-Smads; Smad2 and Smad3) are phosphorylated and translocated into nucleus to elicit TGF-β-specific transcriptional responses [3,4]. An inhibitory Smad, Smad7 acts as a negative feedback molecule induced by TGF-β1 [5]. The non-canonical TGF-β pathway utilizes various signaling components, including extracellular signal-regulated kinase 1/2 (ERK1/2), p38 kinase, c-Jun NH2-terminal kinase 1/2 (JNK1/2), RhoA, Rac1, Cdc42, and phosphatidylinositol 3-kinase (PI3K), to induce Smad-independent responses, yet also regulates Smad-mediated responses [6–8].

The 14-3-3 family of proteins contains seven isotypes, β, ε, η, γ, τ, ζ, and σ. They are highly conserved and ubiquitously expressed in a wide range of mammalian species [9,10]. The 14-3-3 family proteins participate in diverse range of cell signaling pathways with adaptor function and regulate fundamental cellular functions such as cell proliferation, apoptosis, and cytoskeleton rearrangement [11–13]. Up- or down-regulation of 14-3-3 proteins has been founded in human cancers; for example, expression of 14-3-3 σ, a transcriptional target of p53, is lost in breast cancer [14] whereas 14-3-3 β, γ, and τ expression levels are increased in lung cancer [15].

Although TGF-β1 is well known to be inhibitory to cell proliferation, relatively limited progress has been made in the identification of target genes that are ultimately responsible for the anti-proliferative function of TGF-β1. Previously, we showed that 14-3-3 σ may be crucial for TGF-β1-induced growth inhibition [16]. In this study, we demonstrate that 14-3-3 σ is a new target up-regulated by TGF-β1 in a Smad3-dependent manner in mammary epithelial cells, suggesting a possibility that loss of 14-3-3 σ in breast cancer significantly contributes to insensitivity of cancer cells to the growth inhibitory effects of TGF-β.

2. Materials and methods

2.1. Cell culture

The HepG2 human hepatoblastoma cell lines were purchased from the American Type Culture Collection (Rockville, MD). Smad3$^{+/+}$ mouse embryonic fibroblasts (MEFs), Smad3$^{-/-}$ MEFs, and Eph4 mouse mammary epithelial cells were obtained from Dr. Anita B. Roberts of the National Cancer Institute (USA). Cells were grown in Dulbecco's modified Eagle medium (Invitrogen, Grand Island, NY) supplemented with 10% heat-inactivated fetal

* Corresponding author. Address: Department of Biochemistry, College of Natural Sciences, Kangwon National University, 192-1 Hyoja-2-dong, Chuncheon 200-701, Republic of Korea. Fax: +82 33 242 0459.
E-mail address: bckim@kangwon.ac.kr (B.-C. Kim).

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bovine serum (FBS) (Invitrogen, Grand Island, NY), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C under a humidified 95/5% (v/v) mixture of air and CO_2. HaCaT cells were maintained in Dulbecco's modified Eagle medium (Invitrogen, Grand Island, NY) supplemented with 10% heat-inactivated FBS.

2.2. Plasmid DNAs, DNA transfection and reporter assay

The pBSD2-wt-Luc and pBSD2-mt-Luc plasmid DNAs were a gift from Dr. Bert Vogelstein (Johns Hopkins University School of Medicine). All plasmids were transiently transfected using polyethylenimine (Roche, Mannheim). To control variations in cell numbers and transfection efficiency, all clones were cotransfected with 0.2 μg of CMV-β-GAL, a eukaryotic expression vector in which *Escherichia coli* β-galactosidase (Lac Z) structural gene is under the transcriptional control of the CMV promoter. Luciferase reporter activity was assessed on a luminometer with a luciferase assay system (Promega, Madison, WI) according to the manufactures' protocol. The data for luciferase assays represent the mean ± SD of three independent experiments.

2.3. Reverse transcription polymerase chain reaction (RT-PCR) and quantitative real-time RT-PCR

Total RNA was extracted using the phenol-guanidinium isothiocyanate method [17]. RT-PCR was performed by Access RT-PCR system (Promega, Madison, WI) according to the manufacturer' instructions using the following mouse 14-3-3 σ primers; forward 5′-CCT GCT GGA CTC GCA CCT CA-3′ and reverse, 5′-TGT CGG CTG TCC ACA GCG TC-3′, mouse 14-3-3 β primers; forward 5′-GAG CGC TAC GAC GAC ATG GCC-3′ and reverse, 5′-AAT TCC AGG ACC GTG GTG CAG ATG-3′, mouse 14-3-3 ε primers; forward 5′-GAG CGA TAC GAC GAA ATG GTG-3′ and reverse 5′-CCT TGG ACT CGC CAG TGT TAG-3′, mouse 14-3-3 η primers; forward 5′-CTG GCG GAG CAG GCG GAG CGC-3′ and reverse 5′-CTG TCT CCA GCT CCT TTT CAA TCT TCT CCC-3′, mouse γ 14-3-3 primers; forward 5′-CCG GGA GAA GAT CGA GAA GGA GT-3′ and revere 5′-CTG CAT GAT CAG AGT GGA GTC CTT G-3′, mouse 14-3-3 τ primers; forward 5′-GCA GCT GAT CAA GGA CTA TCG GG-3′ and reverse 5′-GCC TCT TGG TAG GCT CCT TGG G-3′, mouse 14-3-3 ζ primers; forward 5′-CCC ACT CCG GAC ACA GAA TAT CAG-3′ and reverse 5′-CTC TGT ATT CTC GAG CCA TCT GCT G-3′, and mouse β-actin was amplified as a control, using the following primers: forward, 5′-ACG TTG CTA TCC AGG CTG TG-3′, and reverse, 5′-GCG ACG TAG CAC AGC TTC TC-3′. PCR amplifications were performed under the following conditions: 25 cycles of 94 °C for 30 s, 55 °C for 1 min, and 72 °C for 1 min; for β-actin, 25 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min. For quantitative RT-PCR, a Prism 7000 real-time PCR machine (Applied Biosystems, Poster City, CA) and QantiTec SYBR PCR kit (Quiagen, Valencia, CA) were used to measure the expression of 14-3-3 σ under the following conditions: 35 cycles of 94 °C for 15 s, 60 °C for 20 s, and 72 °C for 30 s. Quantitative real-time RT-PCR experiments were independently repeated at least three times to ensure reproducibility.

2.4. Adenoviral infections

Adenoviruses expressing LacZ and N-terminally Flag-tagged Smad2 Smad3, Smad4, and Smad7 were provided by Dr. Kohei Miyazono (The University of Tokyo, Japan). High-titered stocks of recombinant viruses were amplified and titrated in HEK293T cells as described by Fujii et al. [18]. Adenoviral infections of cells were used at a multiplicity of infection (m.o.i.) of less than 8×10^2 pfu/cells.

2.5. Immunoblot analysis

Cytosolic extracts were obtained in mammalian cell lysis buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 0.5% Nonidet P-40, 5 mM EDTA, 2 mM DTT, 1 mM sodium orthovanadate, 10 mM β-glycerophosphate, 1 mM NaF, 1 μM microcystin, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 10 μg/ml aprotinin). Western blotting was performed using anti-p53 and anti-14-3-3 σ (Santa Cruz Biotechnology, Santa Cruz, CA), anti-Smad2 and anti-Smad3 (Upstate, Chicago, IL), anti-phospho-Smad3 (Cell Signaling Technology, Danvers, MA), and anti-Flag M2 and anti-β-actin (Sigma–Aldrich, St. Louis, MO) antibodies. Protein samples were heated at 95 °C for 5 min and analyzed by SDS–PAGE. Immunoblot signals were developed using Super Signal Ultra chemiluminescence detection reagents (Pierce, Rockford, IL).

2.6. Statistical analysis

Statistical analyses were performed using SigmaPlot 2001 (Systat Software, Inc., Richmond, CA). Statistical significance was assessed by comparing the means values (±SD) using a Student's *t*-test for paired data.

3. Results and discussion

3.1. Identification of 14-3-3 σ as a TGF-β1-inducible gene in mammary epithelial cells

To examine whether TGF-β1 affects 14-3-3 σ gene expression, we treated the mouse mammary epithelial cell line, Eph4, with TGF-β1 (5 ng/ml) for various time periods. Western-blot analysis revealed time-dependent induction of 14-3-3 σ by TGF-β1 (Fig. 1A). Induction was evident as early as 1.0 h, and reached a maximum within 12 h. Concentrations of TGF-β1 as low as 1.0 ng/ml were capable of inducing maximal expression of 14-3-3 σ (Fig. 1B). Consistent with these results, three independent experiments of quantitative real time PCR analysis showed that the 14-3-3 σ mRNA increased in dose (Fig. 1C)-, and time (Fig. 1D)-dependent manner under TGF-β1 stimulation. Notably, the TGF-β1-induced increases of 14-3-3 σ mRNA was not observed in other 14-3-3 isoforms (Fig. 2). To determine whether TGF-β1 increases 14-3-3 σ mRNA at transcriptional level, we treated Eph4 cells with TGF-β1 in the absence or presence of actinomycin D, an inhibitor of RNA polymerase II-dependent gene transcription. Actinomycin D strongly blocked the TGF-β1-induced increases of 14-3-3 σ mRNA (Fig. 1E) and 14-3-3 σ protein (Fig. 1F), suggesting that the increase is at the transcriptional level. We next determined if the TGF-β1-induced increase in transcriptional activity is mediated by post-transcriptional regulation of a pre-existing protein. Our results showed that both cyclohexamide and puromycin, inhibitors of *de novo* protein synthesis, significantly blocked the TGF-β1-induced increase of 14-3-3 σ protein (Fig. 1F). However, the TGF-β1-induced increase of 14-3-3 σ mRNA did not affected by cyclohexamide (Fig. 1E), indicating that new protein synthesis is not required for transducing the TGF-β1 signal to the increase in 14-3-3 σ mRNA.

3.2. TGF-β1-induced 14-3-3 σ gene expression is dependent on Smad3 but not on p53

14-3-3 σ has been identified as a p53-responsive gene in DNA damage checkpoints [19]. Because p53 has also been implicated in TGF-β1-mediated signaling [20], we determined whether TGF-β1-induced 14-3-3 σ gene expression is dependent on p53 or not. We firstly addressed this by using reporter constructs specific

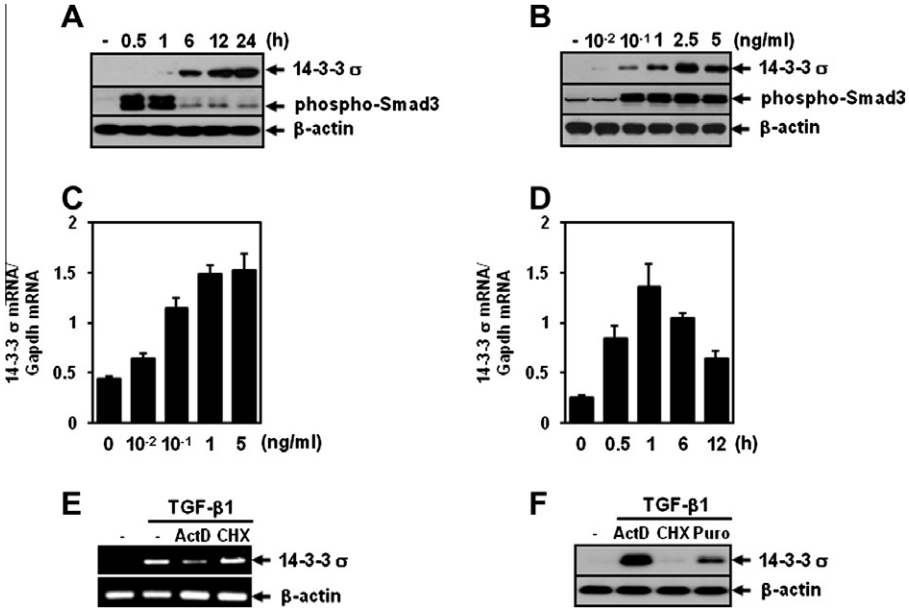

Fig. 1. TGF-β1 induces 14-3-3 σ gene expression in mammary epithelial cells. Western blots showing the induction of 14-3-3 σ and the extent of phosphorylation of Smad3 in Eph4 cells incubated (A) with TGF-β1 (5 ng/ml) for the indicated times and (B) with the indicated concentrations of TGF-β1. Quantitative real time RT-PCR showing the mRNA expression of 14-3-3 σ in Eph4 cells incubated (C) with the indicated concentrations of TGF-β1 and (D) with TGF-β1 (5 ng/ml) for the indicated times. RT-PCR (E) and western blot (F) showing 14-3-3 σ expression form Eph4 cells incubated with or without TGF-β1 (5 ng/ml) in the absence or presence of 10 μg/ml actinomycin D (Act D) or 100 ng/ml cycloheximide (CHX). The effect of puromycin (Puro) on the expression of 14-3-3 σ protein induced by TGF-β1 (5 ng/ml) was also examined (F). All data are representative results of at least three independent experiments.

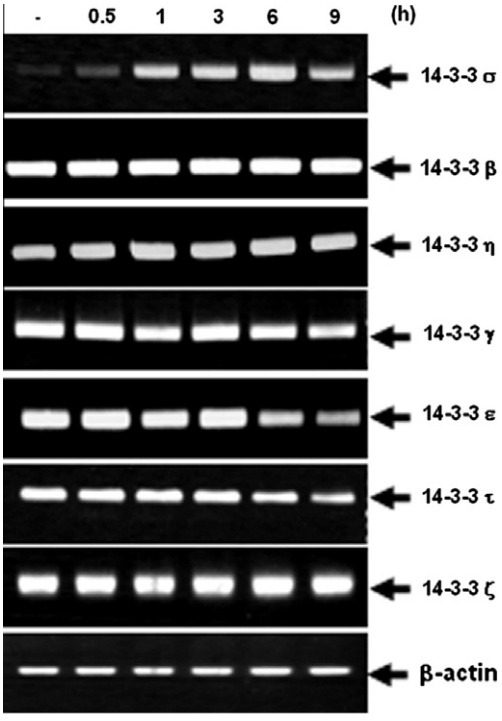

Fig. 2. Isoform-selective induction of 14-3-3 σ expression by TGF-β1. RT-PCR showing 14-3-3 isoform mRNAs from Eph4 cells incubated with TGF-β1 (5 ng/ml) for the indicated times. β-Actin expression was used as a loading control and for normalization. Data are representative results of at least three independent experiments.

for p53. As shown in Fig. 3A, pBSD2-wt-Luc plasmid contains functionally active p53 binding region of 14-3-3 σ promoter inserted upstream of adenovirus E1B minimal promoter fused to luciferase coding sequences. The oligonucleotide in pBSD2-mt-Luc plasmid contains point mutations that abolish p53 binding (Fig. 3A). As expected, co-transfection with plasmid expressing p53 cDNA (Fig. 3B) or treatment of adriamycin (Fig. 3C) increased the activity of pBSD2-wt-Luc efficiently, but failed to induce the Smad-binding element-containing Luc (pSBE-Luc) reporter activity. In contrast, co-transfection with a plasmid encoding a constitutively active form of TGF-β type I receptor, namely TβRI (T204D) (Fig. 3B), or treatment of TGF-β1 (Fig. 3C) strongly increased pSBE-Luc reporter activity, but failed to induce the pBSD2-wt-Luc reporter activity. The reporter activity of pBSD2-mt-Luc was not increased by p53, TβRI (T204D), TGF-β1, or adriamycin (Fig. 3B and C). To ensure that p53 does not involve in TGF-β1-induced 14-3-3 σ gene expression, we transfected cells with small interference RNA (siRNA) against p53. Western-blot analysis showed that p53 siRNA effectively reduced the levels of p53 but did not prevent the stimulatory effect of TGF-β1 on 14-3-3 σ expression (Fig. 3D). These results suggest that p53 is not involved in the induction of 14-3-3 σ gene expression by TGF-β1.

TGF-β1 transduces signal into cells through two main pathways, the canonical Smad-dependent pathway and non-canonical Smad-independent pathway [3]. Non-canonical Smad-independent pathways, including mitogen-activated protein kinase (MAPK) and Rho pathway, are important for optimal transmission of TGF-β signal. In order to investigate the role of Smad-independent pathways in the TGF-β1-mediated up-regulation of 14-3-3 σ gene expression, we employed specific inhibitors for each of these pathways together with TGF-β1. As shown in Fig. 4A, TGF-β1-induced 14-3-3 σ gene expression was not significantly inhibited by U0126, an inhibitor of MAPK kinase (MEK1), SB203580, an inhibitor of p38 MAPK, and SP600125, an inhibitor of c-Jun NH2-terminal kinase 1/2 (JNK1/2). Furthermore, overexpression of dominant negative mutants of Rho GTPases such as Rac1N17, Cdc25N17, and RhoAN19, had no effects on 14-3-3 σ gene expression induced by TGF-β1 (Fig. 4B). These results suggest that Non-canonical Smad-independent pathway may not be crucial for induction of 14-3-3 σ gene expression by TGF-β1.

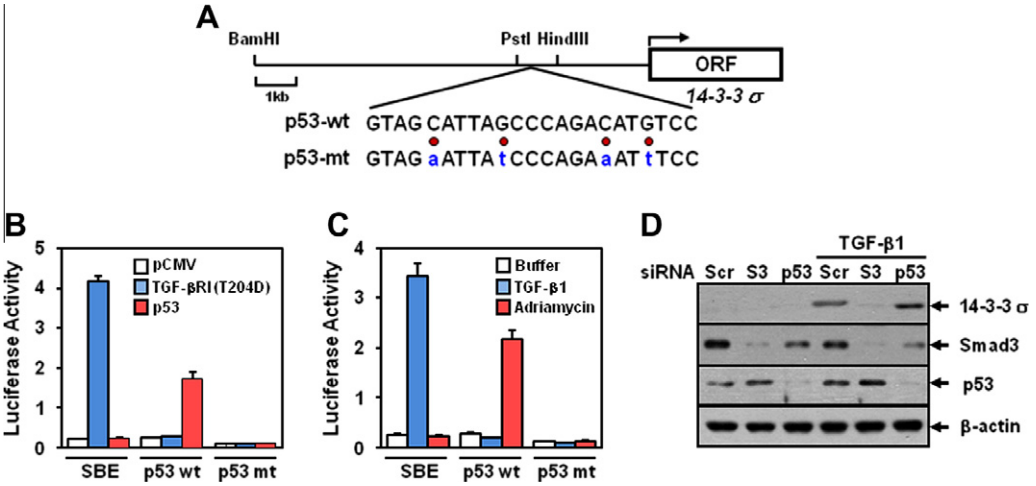

Fig. 3. TGF-β1 induces 14-3-3 σ expression in a p53-independent manner. (A) Schematic representation of 14-3-3 σ promoter-fused reporter plasmids carrying a wild type (pBSD2-wt) or mutant (pBSD2-mt) p53 binding site. Small letters indicating the base changes within the p53 binding site. The Smad binding element (SBE)-Luc, 14-3-3 σ (p53 wt)-Luc and 14-3-3 σ (p53 mt)-Luc reporter activities in Eph4 cells (B) transiently transfected with TGF-βRI(T204D), a constitutively active form of TGF-β type I receptor, or p53 or (C) treated with TGF-β1 (5 ng/ml) or adriamycin (10 μM) for 24 h. (D) Western blot showing 14-3-3 σ protein expression in Eph4 cells transfected with scrambled siRNA, Smad3 siRNA, or p53 siRNA, followed by TGF-β1(5 ng/ml) treatment for 24 h. All data are representative results of at least three independent experiments.

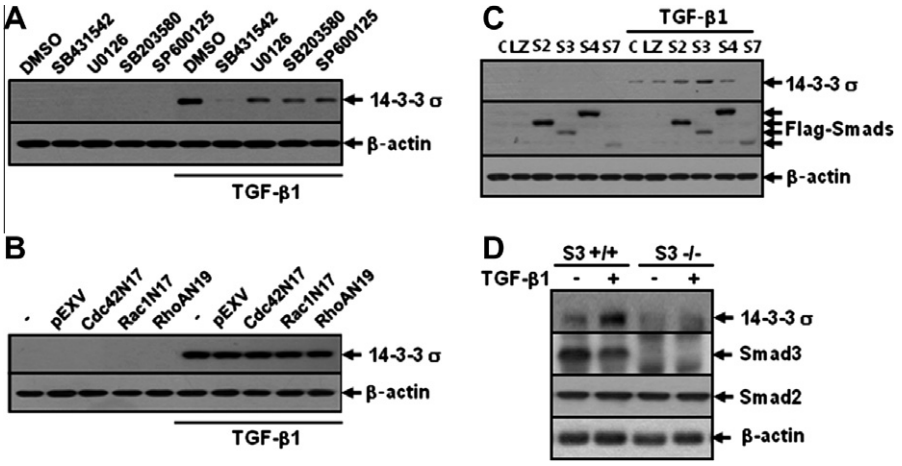

Fig. 4. TGF-β1 induces 14-3-3 σ expression in a Smad3-dependent manner. (A) Eph4 cell were preincubated for 30 min in the absence or presence of SB431542 (5 μM), U0126 (10 μM), SB203580 (5 μM), or SP600125 (15 μM), followed by treatment without or with TGF-β1 (5 ng/ml) for additional 24 h. (B) Cells transfected with pEXV, Cdc42N17, RacN17, or RhoAN19 were treated with TGF-β1 (5 ng/ml) for 24 h. (C) Cells infected with adenovirus carrying the LacZ, Flag-tagged Smad2, Flag-tagged Smad3, Flag-tagged Smad4, or Flag-tagged Smad7 were incubated in the absence or presence of TGF-β1(5 ng/ml) for 24 h. (D) Smad3 wild type (+/+) and Smad3 knockout (−/−) mouse embryonic fibroblasts (MEFs) were treated with TGF-β1(5 ng/ml) for 24 h. Changes in expression of 14-3-3 σ, Flag-tagged Smads, Smad2, and Smad3 were examined by western blot. β-actin was used as a loading in western blot analysis of cell extracts. All data are representative results of at least three independent experiments.

To determine the involvement of Smad pathway in TGF-β1-induced 14-3-3 σ gene expression, we infected FLAG-tagged Smad cDNAs in Eph4 cells using a recombinant adenovirus system. As shown in Fig. 4C, infection of the inhibitory Smad7 adenovirus clearly diminished the induction of 14-3-3 σ gene expression by TGF-β1. Notably, the induction of 14-3-3 σ gene expression was strongly enhanced in the Eph4 cells infected with the Smad3 adenovirus but not in the cells infected with the Smad2 or Smad4 adenovirus (Fig. 4C). Consistently, TGF-β1-induced 14-3-3 σ gene expression was substantially reduced in cells transfected with siRNA Smad3 (Fig. 3D) and in Smad3 knockout mouse embryo fibroblasts (MEFs) (Fig. 4D). Taken together, these results reveal that 14-3-3 σ gene expression is induced by TGF-β1 through a Smad3-dependent pathway in mammary epithelial cells.

14-3-3 σ was originally characterized as a target gene induced by p53 in cell cycle checkpoint signaling [19]. A growing body of evidences suggests a putative role of 14-3-3 σ as a tumor suppressor. For example, hypermethylation of CpG islands and loss of expression of the 14-3-3 σ gene has been reported in human breast and hepatocellular carcinoma [21,22]. In addition, 14-3-3 σ has been reported to suppress protein kinase B/Akt-activated cancer [23]. In spite of the important role of 14-3-3 σ gene in cancer, the molecular mechanism underlying the regulation of 14-3-3 σ gene expression has not been almost addressed. In this study, we demonstrate that TGF-β1 is an important key modulator of 14-3-3 σ gene expression through a Smad3-dependent mechanism in mammary epithelial cells. Our present results, together with previous report that 14-3-3 σ has an important role for TGF-β1-induced growth inhibition [16], strongly suggest that TGF-β1-mediated Smad3-dependent activation of 14-3-3 σ may be a new regulatory axis in anti-proliferative TGF-β signaling. Therefore, elucidation of the molecular mechanism underlying role of 14-3-3 σ in TGF-β1-induced growth inhibition might be provide important insights into developing therapeutic approaches for cancers that are associated with perturbations of TGF-β signaling.

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References

[1] M. Dong, G.C. Blobe, Role of transforming growth factor-beta in hematologic malignancies, Blood 107 (2006) 4589–4596.
[2] B.C. Kim, M. Mamura, K.S. Choi, B. Carabretta, S.J. Kim, Transforming growth factor beta 1 induces apoptosis through cleavage of BAD in a Smad3-dependent mechanism in FaO hepatoma cells, Mol. Cell. Biol. 22 (2002) 1369–1378.
[3] R. Derynk, Y.E. Zhang, Smad-dependent and Smad-independent pathways in TGF-beta family signaling, Nature 425 (2003) 577–584.
[4] J. Massague, D. Wotton, Transcriptional control by the TGF-beta/Smad signaling system, EMBO J. 19 (2000) 1745–1754.
[5] H. Hayashi, S. Abdollah, Y. Qiu, J. Cai, Y.Y. Xu, B.W. Grinnell, et al., The MAD-related protein Smad7 associates with the TGF-beta receptor and functions as an antagonist of TGF-beta signaling, Cell 89 (1997) 1165–1173.
[6] K. Horiguchi, T. Shirakihara, A. Nakano, T. Imamura, K. Miyazono, M. Saitoh, Role of Ras signaling in the induction of snail by transforming growth factor-beta, J. Biol. Chem. 284 (2009) 245–253.
[7] F. Blanchette, N. Rivard, P. Rudd, F. Grodin, L. Attisano, C.M. Dubois, Cross-talk between the p42/p44 MAP kinase and Smad pathways in transform growth factor β1-induced furin gene transactivation, J. Biol. Chem. 276 (2001) 33986–33994.
[8] A.K. Kamaraju, A.B. Roberts, Role of Rho/ROCK and p38 MAP kinase pathways in transforming growth factor-beta-mediated Smad-dependent growth inhibition of human breast carcinoma cells in vivo, J. Biol. Chem. 280 (2005) 1024–1036.
[9] D.K. Morrison, The 14-3-3 proteins: integrators of diverse signaling cues that impact cell fate and cancer development, Trends Cell Biol. 19 (2009) 1–23.
[10] Y. Takahashi, The 14-3-3 proteins: gene, gene expression, and function, Neurochem. Res. 28 (2003) 1265–1273.
[11] T.A. Chan, H. Hermeking, C. Lengauer, K. Kinzler, B. Vogelstein, 14-3-3 Sigma is required to prevent mitotic catastrophe after DNA damage, Science 401 (1999) 616–620.
[12] R.R. Subramanian, S.C. Maters, H. Zhang, H. Fu, Functional conservation of 14-3-3 isoforms in inhibiting bad-induced apoptosis, Exp. Cell Res. 271 (2001) 142–151.
[13] N. Kakinuma, B.C. Roy, Y. Zhu, Y. Wang, R. Kiyama, Kank regulates RhoA-dependent actin stress fibers and cell migration via 14-3-3 in PI3K-Akt signaling, J. Cell Biol. 181 (2008) 537–549.
[14] J.M. Moreira, G. Ohlsson, F.E. Rank, J.E. Cells, Down-regulation of the tumor suppressor protein 14-3-3 sigma is a sporadic event in cancer of the breast, Mol. Cell. Proteomics 4 (2005) 555–569.
[15] S.A. Maxwell, Z. Li, D. Jaya, S. Ballard, J. Ferrell, H. Fu, 14-3-3 zeta mediates resistance of diffuse large B cell lymphoma to an anhracycline-based chemotherapeutic regimen, J. Biol. Chem. 284 (2009) 22379–22389.
[16] H.Y. Hong, W.K. Jeon, E.J. Bae, S.T. Kim, H.J. Lee, S.J. Kim, B.C. Kim, 14-3-3 sigma and 14-3-3 zeta plays an opposite role in cell growth inhibition mediated by transforming growth factor-beta1, Mol. Cells 29 (2010) 305–309.
[17] P. Chomczynski, N. Sacchi, Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction, Anal. Biochem. 162 (1987) 575156–575159.
[18] M. Fujii, K. Takeda, T. Imamura, H. Aoki, T.K. Sampath, S. Enomoto, et al., Roles of bone morphogenetic protein type I receptors and Smad proteins in osteoblast and chondroblasts differentiation, Mol. Biol. Cell 10 (1999) 3801–3813.
[19] H. Hermeking, C. Lengauer, K. Polyak, T.C. He, L. Zhang, S. Thiagaliangam, K.W. Kinzler, B. Vogelstein, 14-3-3 sigma is a p53-regulated inhibitor of G2/M progression, Mol. Cell 1 (1997) 3–11.
[20] R. Samarakoon, J.M. Overstreet, P.J. Higgins, TGF-β signaling in tissue fibrosis: redox controls, target genes and therapeutic opportunities, Cell Signal 25 (2013) 264–268.
[21] A.T. Ferguson, E. Evron, C.B. Umbricht, T.K. Pandita, T.A. Chan, H. Hemeking, et al., High frequency of hypermethylation at the 14-3-3 sigma locus leads to gene silencing in breast cancer, Proc. Natl. Acad. Sci. USA 97 (2000) 6049–6054.
[22] N. Iwata, H. Yamamoto, S. Sasaki, F. Itoh, H. Suzuki, T. Chikuchi, et al., Frequent hypermethylation of CpG islands and loss of expression of the 14-3-3 sigma gene in human hepatocellular carcinoma, Oncogene 19 (2000) 5298–5302.
[23] H. Yang, Y.Y. Wen, R. Zhao, Y.L. Lin, K. Fournier, et al., DNA damage-induced protein 14-3-3 sigma inhibits protein kinase B/Akt activation and suppresses Akt-activated cancer, Cancer Res. 66 (2006) 3096–3105.