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Schisandrin B suppresses TGFβ1 signaling by inhibiting Smad2/3 and MAPK pathways

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ABSTRACT

TGFβ1 plays a crucial role in the pathogenesis of vascular fibrotic diseases. *Schisandra chinensis* (*S. chinensis*), which is used as an oriental herbal medicine, is effective in the treatment of vascular injuries that cause aberrant TGFβ1 signaling. In this study, we investigated whether *S. chinensis* extract and its active ingredients inhibit TGFβ1 signaling in A7r5 vascular smooth muscle cells. We found that *S. chinensis* extract suppressed TGFβ1 signaling via inhibition of Smad2/3 phosphorylation and nuclear translocation. Among the active ingredients of *S. chinensis* extract, schisandrin B (SchB) most potently inhibited TGFβ1 signaling. SchB inhibited sustained phosphorylation and nuclear translocation of Smad2/3. Moreover, SchB suppressed TGFβ1-induced phosphorylation of p38 and JNK, which contributed to Smad2/3 inactivation. The present study is the first to demonstrate that *S. chinensis* extract and SchB inhibit TGFβ1 signaling. Our results may help future investigations to understand vascular fibrosis pathogenesis and to develop novel therapeutic strategies for treatment of vascular fibrotic diseases.

1. Introduction

Transforming growth factor β1 (TGFβ1) regulates tissue homeostasis via multiple cellular processes, including cell proliferation, migration, apoptosis, and extracellular matrix remodeling [1,2]. Aberrant or inappropriate TGFβ1 signaling is associated with a wide range of human diseases, such as cardiovascular, autoimmune and fibrotic diseases, and cancers [3–6]. Particularly, TGFβ1 participates in the pathogenesis of vascular fibrotic diseases, such as hypertension, restenosis, and atherosclerosis. In vascular fibrotic diseases, TGFβ1 acts on vascular smooth muscle cells at sites of vascular injury to induce cell switching from a quiescent contractile phenotype to a proliferative synthetic phenotype [7,8]. Therefore, inhibitors of TGFβ1 signaling have emerged as potential therapies to treat vascular fibrotic diseases [9–11].

Upon TGFβ1 engagement of the TGFβ type II receptor kinase (TβRII) at the plasma membrane, TβRII recruits and phosphorylates TβRI/ALK5 [12,13]. In turn, ALK5 phosphorylates Smad2 and Smad3 to promote their binding to Smad4 to form a heteromeric Smad complex [12,13]. The Smad complex enters the nucleus to initiate the transcription of extracellular matrix proteins, such as fibronectin and collagens [14,15]. Conversely, TGFβ1 activates mitogen-activated protein kinase (MAPK) signaling in a Smad2/3-independent manner [16,17]. Although this activity is not well characterized, TGFβ1-induced MAPK activation contributes to the transcriptional activity of the Smad complex [16,17].

Schisandra chinensis (*S. chinensis*) has been used as a traditional oriental medicine and possesses diverse biological activities [18]. *S. chinensis* is effective in the treatment of various diseases, including hepatitis [19], cancers [20,21] and vascular injury [22,23], where deregulation of TGFβ1 signaling is commonly observed [7,24]. However, the underlying pharmacologic mechanisms of *S. chinensis* extract are largely unknown. Furthermore, the effect of *S. chinensis* extract on TGFβ1 signaling has not been previously studied.

In this study, we discovered that *S. chinensis* extract inhibited TGFβ1 signaling in A7r5 vascular smooth muscle cells. Among the active ingredients of *S. chinensis* extract that were examined,

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schisandrin B (SchB) was most potently suppressed TGFβ1 signaling and inhibited Smad 2/3 and MAPK activities. Our results provide a basis for future investigations that are aimed at treating vascular fibrotic diseases.

2. Materials and methods

2.1. Cell culture and reagents

The A7r5 rat aortic smooth muscle cell line was obtained from ATCC. The cells were cultured according to the supplier's recommendations. Prior to treatment with TGFβ1 (R&D Systems, Minneapolis, MN), A7r5 cells were maintained in DMEM containing 0.2% FBS for 12 h. All cell culture reagents were purchased from Invitrogen (Carlsbad, CA). All other reagents not specified were supplied by Sigma (St. Louis, MO).

2.2. Preparation of the extract and ingredients from S. chinensis

S. chinensis and its active ingredients (schisandrol A, schisandrol B, gomisin N, schisandrin A, schisandrin B, and schisandrin C) were prepared as previously described [25]. Briefly, *S. chinensis* extract and its active ingredients were purified using open column chromatography and semi-preparative HPLC. The chemical structures of the ingredients were confirmed based on ^{1}H NMR and ^{13}C NMR data. The purity (greater than 95%) of the isolated active ingredients was determined using HPLC–DAD and ^{1}H NMR spectra.

2.3. Luciferase assay

A7r5 cells were transfected with pGL2-3TP-Luc [26] or pGL3-SBE-4-Luc [27] reporter gene plasmids [28]. At 24 h after transfection, the cells were incubated with TGFβ1 and/or *S. chinensis* extract or its active ingredients for 24 h. The cells were harvested and assayed for luciferase activity using a commercial kit (Promega, Madison, WI). The luciferase activity was normalized to β-galactosidase activity.

2.4. Western blot analysis

The crude extracts were prepared by incubation with RIPA buffer containing protease and phosphatase inhibitor cocktails

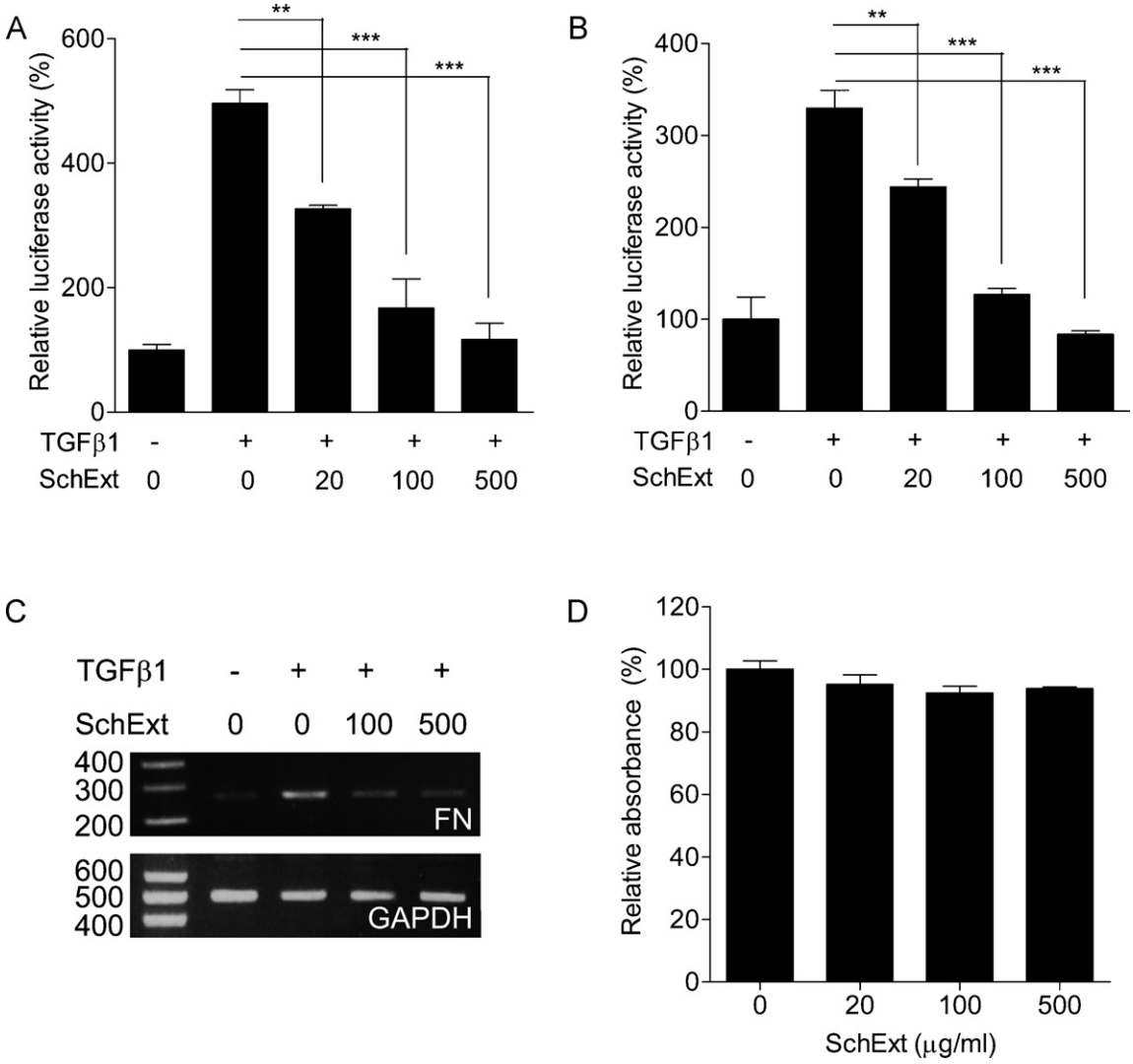

Fig. 1. Inhibition of TGFβ1 signaling by *S. chinensis* extract in A7r5 cells. The cells were transfected with 3TP-Luc (A) or SBE4-Luc (B) reporter constructs, and treated with TGFβ1 (1 ng/ml) and/or *S. chinensis* extract (SchExt) (20, 100, or 500 μg/ml) for 24 h. The luciferase activity was expressed as a relative value compared to that of the untreated cells which was set to 100%. The data were expressed as the mean ± SEM ($n = 4$). $^{**}P < 0.01$ and $^{***}P < 0.005$. (C) The cells were treated with TGFβ1 (1 ng/ml) and/or *S. chinensis* extract (100 or 500 μg/ml) for 48 h prior to RT-PCR analysis. GAPDH was used as a loading control. The numbers on the left denote sizes of DNA marker bands. (D) The cells were treated with *S. chinensis* extract at the indicated concentrations for 48 h prior to MTT assays. Cell viability was expressed as a relative absorbance value compared to that of the untreated cells which was set to 100%. The data were expressed as the mean ± SEM ($n = 4$).

(Calbiochem, La Jolla, CA). The proteins were resolved using 8% or 10% SDS-PAGE and probed with the indicated antibodies. Antibodies against pSmad2, pSmad3, pp38, pJNK, pERK, Smad2, Smad3, p38, and ERK were purchased from Cell Signaling Technology (Danvers, MA). Antibodies against JNK and GAPDH were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The data were representative of at least three independent experiments.

2.5. Confocal microscopy

Cells were grown on glass coverslips in 24-well plates. After treatment with TGFβ1 and/or *S. chinensis* extract or SchB for 1 h, the cells were fixed with 4% formaldehyde in PBS for 10 min, permeabilized with 0.1% Triton X-100 for 5 min, and blocked with 5% normal goat serum in PBS for 5 min. The cells were labeled with anti-Smad3 antibody overnight and probed with FITC-conjugated anti-rabbit IgG antibody (Invitrogen) and DAPI (Roche, Mannheim, Germany). The cells were photographed using the FluoView 1000 confocal microscope (Olympus, Tokyo, Japan).

2.6. RT-PCR

Using RNeasy mini kit (Qiagen, Valencia, CA), total RNA was isolated from cells that were treated with TGFβ1 and/or *S. chinensis* extract or SchB for 48 h. Reverse transcription was performed using a commercial kit according to the manufacturer's instructions (Invitrogen). PCR analysis using fibronectin or GAPDH primers was performed as previously described [29,30].

2.7. Statistical analysis

The data were expressed as the mean ± SEM. Comparison of mean values among experimental groups was performed using one-way ANOVA followed by a post hoc test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. S. chinensis extract inhibits TGFβ1/Smad signaling in A7r5 cells

To assess the effect of *S. chinensis* extract on TGFβ1 signaling, we first performed luciferase assays using two different reporter gene constructs containing Smad-binding elements. *S. chinensis* extract suppressed TGFβ1-mediated luciferase activity in a dose-dependent manner (Fig. 1A and B), indicating that *S. chinensis* extract inhibits TGFβ1 signaling. These reporter assay results were confirmed by RT-PCR analysis. *S. chinensis* extract suppressed TGFβ1-induced fibronectin transcription (Fig. 1C). Under the experimental conditions, *S. chinensis* extract did not exhibit cytotoxicity (Fig. 1D).

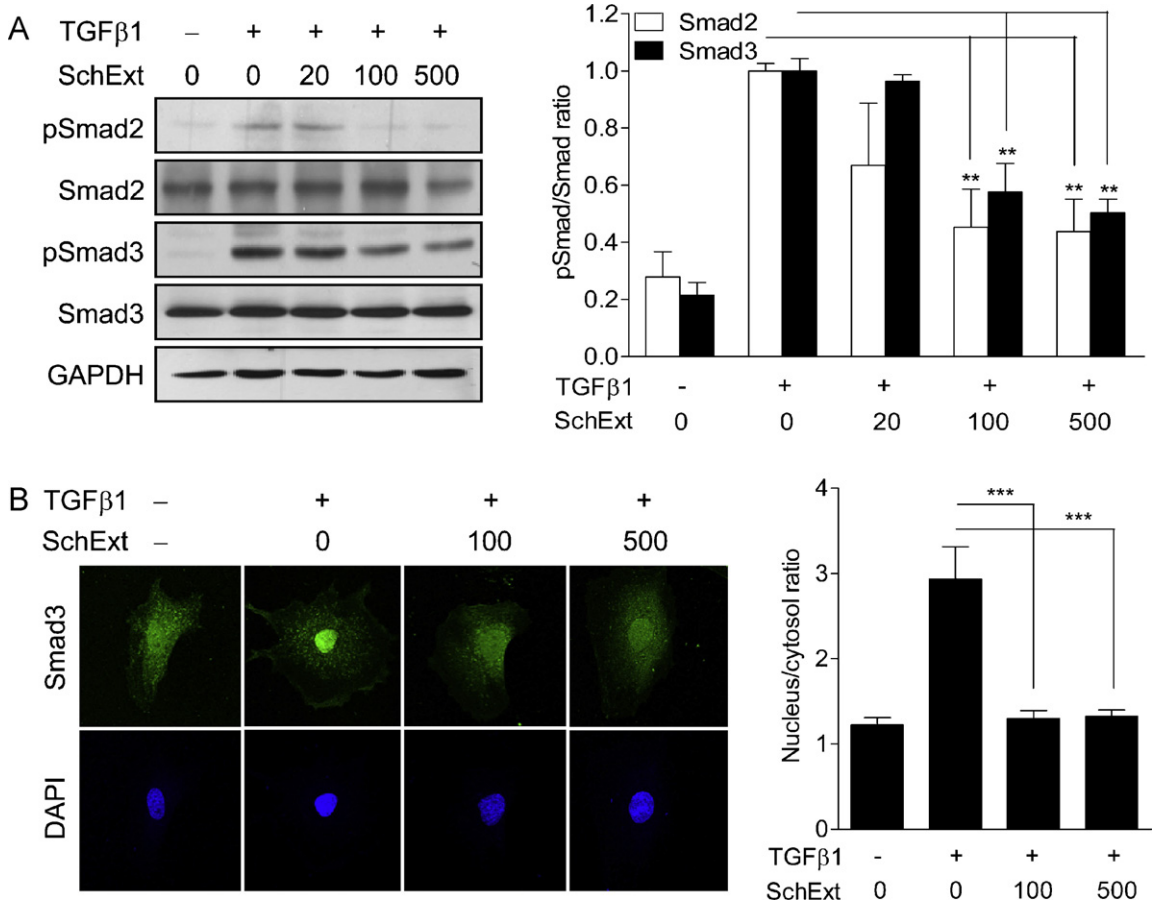

Fig. 2. Inhibition of TGFβ1-induced Smad activation and nuclear translocation by *S. chinensis* extract. (A) The cells were treated with TGFβ1 (1 ng/ml) and/or *S. chinensis* extract (20, 100, or 500 μg/ml) for 2 h prior to Western blot analysis. The phosphorylated/total ratio of Smad2/3 was plotted based on the quantification of the band intensities. The data were expressed as the mean ± SEM ($n = 4$). $^{**}P < 0.01$. (B) The cells were treated with TGFβ1 (1 ng/ml) and/or *S. chinensis* extract (100 or 500 μg/ml) for 2 h prior to confocal microscopy. Localization of Smad3 was assessed using anti-Smad3 antibody and FITC-conjugated IgG antibody. DAPI was used to visualize the nucleus. The nuclear/cytosolic ratio of Smad3 staining was measured in at least 20 independent fields that were derived from three separate experiments. The data were expressed as the mean ± SEM. $^{***}P < 0.005$.

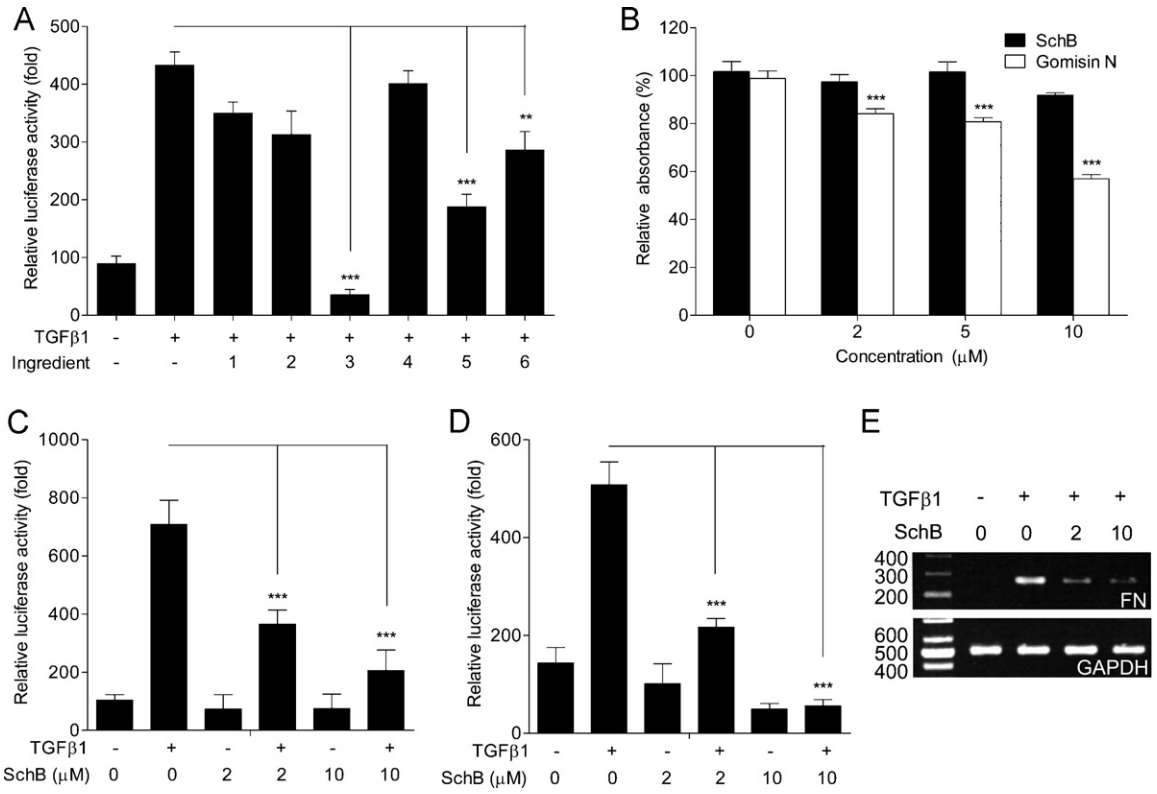

Fig. 3. SchB-mediated inhibition of TGFβ1 signaling in A7r5 cells. The cells were transfected with 3TP-Luc (A and C) or SBE4-Luc (D) reporter constructs, and treated with TGFβ1 (1 ng/ml) and/or the indicated ingredients (10 μM each) or SchB (2 or 10 μM) for 24 h. The luciferase activity was expressed as a relative value compared to that of the untreated cells which was set to 100%. The data were expressed as the mean ± SEM (n = 4–6). $^{**}P < 0.01$; $^{***}P < 0.005$. Ingredient 1, schisandrol A; 2, schisandrol B; 3, gomisin N; 4, schisandrin A; 5, schisandrin B; and 6, schisandrin C. (B) The cells were treated with SchB or Gomisin N at the indicated concentrations for 48 h prior to MTT assays. Cell viability was expressed as a relative absorbance value compared to that of the untreated cells which was set to 100%. The data were expressed as the mean ± SEM (n = 4). $^{***}P < 0.005$. (E) The cells were treated with TGFβ1 (1 ng/ml) and/or SchB (2 or 10 μM) for 48 h prior to RT-PCR analysis. GAPDH was used as a loading control. Numbers on the left denote sizes of DNA marker bands.

To further confirm these results, we examined whether *S. chinensis* extract reduces Smad activity. Western blot analysis showed that *S. chinensis* extract reduced Smad2/3 phosphorylation (Fig. 2A). In addition, confocal microscopic analysis revealed that *S. chinensis* extract inhibited TGFβ1-induced nuclear translocation of Smad3 (Fig. 2B and Fig. S3A). These data demonstrated that *S. chinensis* extract (at concentrations greater than 100 μg/ml) suppressed TGFβ1/Smad signaling via inhibition of Smad2/3 phosphorylation and nuclear translocation.

3.2. SchB inhibits TGFβ1/Smad signaling in A7r5 cells

To identify the effective components of *S. chinensis* extract against TGFβ1 signaling, we screened six ingredients (Figs. S1 and S2) of *S. chinensis* extract using luciferase assays. Among these compounds, gomisin N and SchB inhibited TGFβ1 signaling (Fig. 3A). However, gomisin N induced cell death (Fig. 3B), which prevented the evaluation of its effects on TGFβ1 signaling. Therefore, we chose SchB as an effective ingredient for the following studies.

The inhibitory effect of SchB on TGFβ1 signaling was confirmed using luciferase assays with two different reporter constructs. SchB suppressed TGFβ1-mediated luciferase activity in a dose-dependent manner (Fig. 3C and D). These reporter assay results were further verified by RT-PCR analysis. SchB inhibited TGFβ1-induced transcription of fibronectin (Fig. 3E). These data demonstrated that SchB (at concentrations greater than 2 μM) suppressed TGFβ1 signaling.

3.3. SchB inhibits sustained phosphorylation and nuclear translocation of Smad2/3 in A7r5 cells

To determine the mechanisms of SchB action on TGFβ1 signaling, we examined the phosphorylation level of Smad2/3. Regardless of the presence of SchB, TGFβ1 induced Smad2/3 phosphorylation (Fig. 4A) until 1 h of treatment. Following 2 h of treatment with TGFβ1, SchB markedly decreased the level of phosphorylated Smad2/3 (Fig. 4A), suggesting that SchB stimulated Smad2/3 dephosphorylation rather than inhibited ALK5 activity. Because PPM1A is a Smad2/3 phosphatase [31], we questioned whether SchB-induced decrease in Smad2/3 phosphorylation is mediated by PPM1A. Knockdown of PPM1A with siRNA markedly restored Smad2/3 phosphorylation in the presence of SchB (Figs. S4A and S4B), indicating that SchB stimulated Smad2/3 dephosphorylation via PPM1A pathways.

To further confirm these results, we examined whether SchB inhibits TGFβ1-induced nuclear translocation of Smad3. As presented in Fig. 4 and S3B, SchB reduced nuclear Smad3 in TGFβ1-treated cells to the levels that were comparable to that in untreated cells, which were further corroborated by subcellular fraction experiments (Fig. S5). Our results demonstrated that SchB suppresses TGFβ1 signaling by inhibiting sustained phosphorylation and nuclear translocation of Smad2/3.

3.4. SchB inhibits TGFβ1-induced MAPK activation in A7r5 cells

Because TGFβ1 activates MAPK signaling [16,17], we first confirmed TGFβ1-induced activation of MAPK in A7r5 cells. As

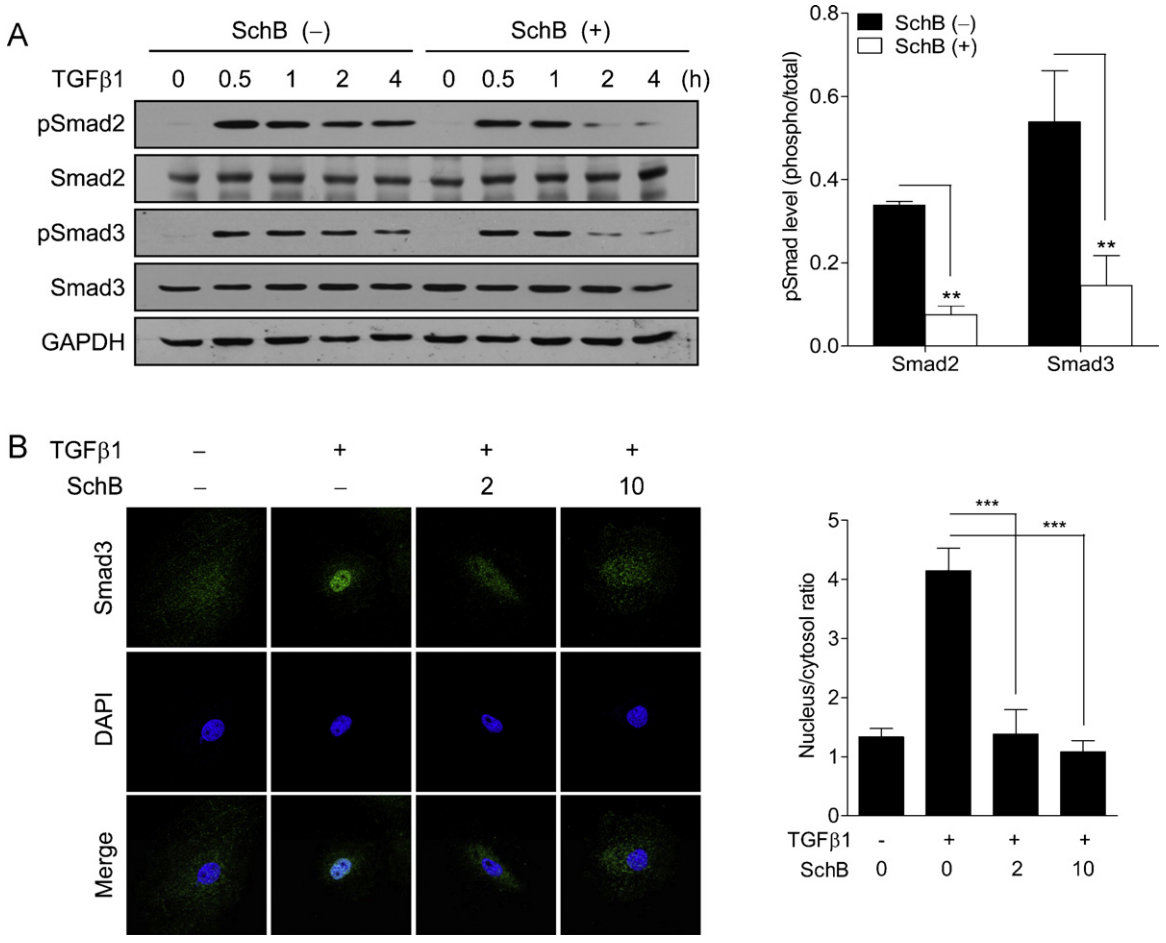

Fig. 4. SchB-mediated inhibition of Smad2/3 in A7r5 cells. (A) The cells were treated with TGFβ1 (1 ng/ml) and/or SchB (2 μM) for the indicated times prior to Western blot analysis. The phosphorylated/total ratio of Smad2/3 was plotted based on the quantification of the band intensities that were obtained after 2 h of treatment. The data were expressed as the mean ± SEM ($n = 4$). $^{**}P < 0.01$. (B) The cells were treated with TGFβ1 (1 ng/ml) and/or SchB (2 or 10 μM) for 2 h prior to confocal microscopy. The localization of Smad3 was assessed using anti-Smad3 antibody and FITC-conjugated IgG antibody. DAPI was used to visualize the nucleus. The nuclear/cytosolic ratio of Smad 3 staining was measured in at least 20 independent fields that were derived from three separate experiments. The data were expressed as the mean ± SEM. $^{***}P < 0.005$.

shown in Fig. 5 A, TGFβ1 notably increased phosphorylation levels of p38 and JNK, but not ERK. TGFβ1-induced p38 and JNK activation reached the maximum at the 1 h mark after treatment with SchB and decreased thereafter. Because the role of MAPK signaling in TGFβ1 signaling in vascular smooth muscle cells has been little characterized, we performed reporter gene analyses using ALK5 (as a positive control) and MAPK inhibitors to assess the effect of MAPK signaling on TGFβ1 signaling, Luciferase assays showed that ALK5, p38, and JNK inhibitors but not the ERK inhibitor suppressed TGFβ1 signaling (Fig. 5B). We ascertained that these inhibitors were not toxic at the concentrations that were used in this study (Fig. 5C). Nonetheless, p38 and JNK inhibitors did not affect the phosphorylation level of Smad2/3 (Fig. 5D). Moreover, p38 and JNK inhibitors did not block the nuclear translocation of Smad3 (Fig. 5E). These results suggest that p38 and JNK inhibitors affect Smad2/3 action inside the nucleus.

We then examined whether SchB inhibits TGFβ1-mediated p38 and JNK activation. Fig. 6 shows that SchB suppressed TGFβ1-induced phosphorylation of p38 and JNK. Our results demonstrate that SchB suppresses TGFβ1 signaling via inhibition of Smad2/3 and MAPK (particularly, p38 and JNK) signaling pathways. The specificity of SchB action on kinase signaling pathways was assessed by responses to EGF. SchB did not reduce EGF-induced Akt phosphorylation (Fig. S6).

4. Discussion

TGFβ1 plays a crucial role in tissue remodeling in normal or injured tissues, regulating cell growth, fibrosis, and inflammation [8]. However, aberrant regulation of TGFβ1 leads to pathologic fibrosis by increased cell proliferation and excessive accumulation of extracellular matrix proteins [32,33]. Here, our results show that *S. chinensis* extract and its active ingredient SchB inhibit TGFβ1 signaling via two different mechanisms in A7r5 cells: (1) SchB inhibits sustained phosphorylation and nuclear translocation of Smad2/3; and (2) SchB suppresses TGFβ1-induced phosphorylation of p38 and JNK. Therefore, our results suggest that SchB is a promising compound for developing therapies to treat vascular fibrotic diseases.

In this study, we provided SchB-mediated mechanisms that regulate inhibition of TGFβ1 signaling in A7r5 cells. Fig. 4A shows that SchB rapidly terminated TGFβ1 responses by inhibiting sustained phosphorylation of Smad2/3. Moreover, SchB stimulated Smad2/3 dephosphorylation via PPM1A pathways (Fig. S4). However, there are still other possibilities: SchB may need time to penetrate into cells, or may act indirectly via a mechanism that needs protein synthesis. It also remains to be determined how SchB regulates PPM1A activity. Further studies with SchB as a useful chemical tool may assist understanding of the molecular mechanisms underlying termination of TGFβ1 responses.

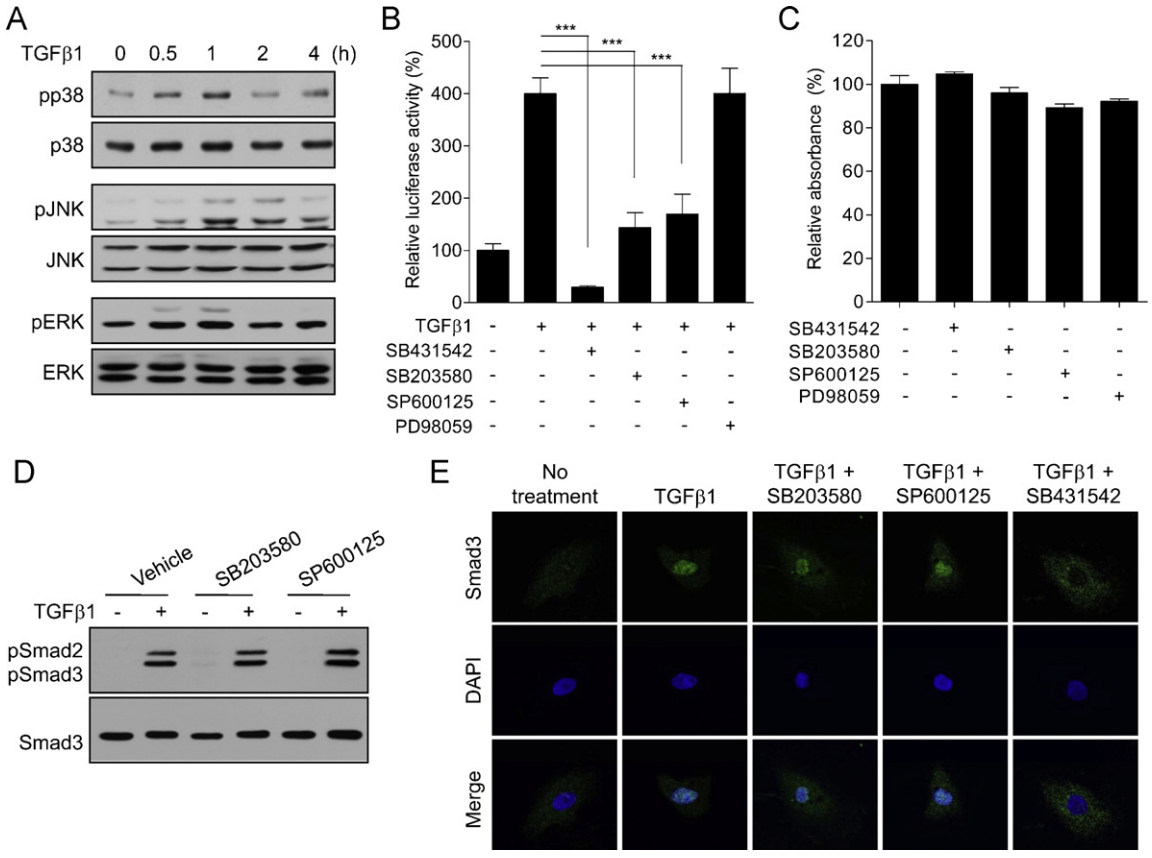

Fig. 5. The effect of MAPK activation on TGFβ1 signaling in A7r5 cells. (A) The cells were treated with TGFβ1 (1 ng/ml) for the indicated times prior to Western blot analysis. (B) The cells were transfected with 3TP-Luc reporter construct and treated with TGFβ1 (1 ng/ml) and/or the indicated inhibitors. The luciferase activity is expressed as a relative value compared to that of the untreated cells which is set to 100%. The data were expressed as the mean ± SEM ($n = 4$). ***$P < 0.005$. SB431542, ALK5 inhibitor; SB203580, p38 kinase inhibitor; SP600125, JNK inhibitor; and PD98059, ERK inhibitor. (C) The cells were treated with the indicated inhibitors (10 μM) for 48 h prior to MTT assays. Cell viability was expressed as a relative absorbance value compared to that of the untreated cells which was set to 100%. The data were expressed as the mean ± SEM ($n = 4$). (D) The cells were treated with TGFβ1 (1 ng/ml) and/or the indicated inhibitors for 1 h prior to Western blot analysis. (E) The cells were treated with TGFβ1 (1 ng/ml) and/or the indicated inhibitors for 1 h prior to confocal microscopy.

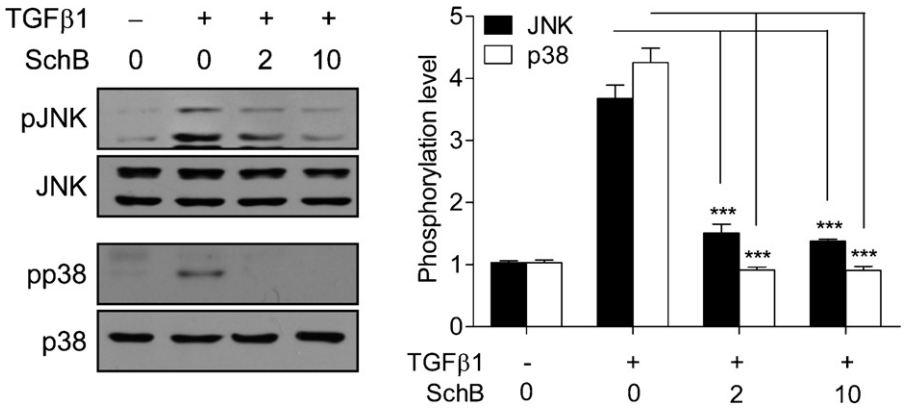

Fig. 6. SchB-mediated inhibition of TGFβ1-induced MAPK activation in A7r5 cells. The cells were treated with TGFβ1 (1 ng/ml) and/or SchB (2 or 10 μM) for 1 h prior to Western blot analysis. The phosphorylated/total ratio of JNK and p38 was plotted based on the quantification of the band intensities that were obtained after 1 h of treatment with TGFβ1 (1 ng/ml) and/or SchB (2 μM). The data were expressed as the mean ± SEM ($n = 4$). ***$P < 0.005$.

Our study may provide insight into elucidating the mechanisms of cross-talk between Smad2/3 and MAPK signaling. Reporter assays with Smad-binding elements showed that p38 and JNK was crucial for Smad2/3 transcriptional activity. However, inhibition of p38 and JNK did not affect the phosphorylation level and nuclear translocation of Smad2/3 in TGFβ1-treated cells (Fig. 5D and E). These results suggest that p38 and JNK regulates Smad2/3 action inside the nucleus. Taken together, our data imply that the inhibitory actions of SchB on TGFβ1 signaling are achieved in the cytoplasm and the nucleus.

TGFβ1 induces oxidative stress, which can participate in fibrotic changes in vascular tissues [8,34]. Conversely, oxidative stress elevates TGFβ1 production [35]. Indeed, fibrosis is closely associated with chronic inflammation leading to sustained

production of reactive oxygen species [36,37]. Many studies have shown that antioxidant activity of *S. chinensis* extract and SchB [38–40]. Therefore, SchB-mediated antioxidant activity may be a possible mechanism of the inhibitory action of SchB on TGFβ1 signaling. In summary, the present study demonstrated that SchB suppresses TGFβ1 signaling by inhibiting Smad2/3 and MAPK pathways. Because SchB is a naturally occurring compound, it may be advantageous for attaining a balance between therapeutic outcome and undesirable side effects. Our results provide a basis for future investigation aiming at treating vascular fibrotic diseases.

Conflict of interest

None declared.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bcp.2011.11.002.

References

[1] Massague J, Blain SW, Lo RS. TGFbeta signaling in growth control, cancer, and heritable disorders. Cell 2000;103:295–309.
[2] Derynck R, Akhurst RJ. Differentiation plasticity regulated by TGF-beta family proteins in development and disease. Nat Cell Biol 2007;9:1000–4.
[3] Cain K, Freathy C. Liver toxicity and apoptosis: role of TGF-beta1, cytochrome c and the apoptosome. Toxicol Lett 2001;120:307–15.
[4] Sookoian S, Gianotti TF, Rosselli MS, Burgueno AL, Castano GO, Pirola CJ. Liver transcriptional profile of atherosclerosis-related genes in human nonalcoholic fatty liver disease. Atherosclerosis 2011.
[5] Doyle KP, Cekanaviciute E, Mamer LE, Buckwalter MS. TGFbeta signaling in the brain increases with aging and signals to astrocytes and innate immune cells in the weeks after stroke. J Neuroinflamm 2010;7:62.
[6] Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor beta in human disease. N Engl J Med 2000;342:1350–8.
[7] Ryan ST, Koteliansky VE, Gotwals PJ, Lindner V. Transforming growth factor-beta-dependent events in vascular remodeling following arterial injury. J Vasc Res 2003;40:37–46.
[8] Ruiz-Ortega M, Rodriguez-Vita J, Sanchez-Lopez E, Carvajal G, Egido J. TGF-beta signaling in vascular fibrosis. Cardiovasc Res 2007;74:196–206.
[9] Khanna A, Li B, Li P, Suthanthiran M. Transforming growth factor-beta 1: regulation with a TGF-beta 1 antisense oligomer. Kidney Int Suppl 1996;53:S2–6.
[10] Dhanani S, Huang M, Wang J, Dubinett SM. Interferon-alpha inhibits murine macrophage transforming growth factor-beta mRNA expression. Inflammation 1994;18:301–9.
[11] Chan W, Krieg Jr RJ, Norkus EP, Chan JC. alpha-Tocopherol reduces proteinuria, oxidative stress, and expression of transforming growth factor beta 1 in IgA nephropathy in the rat. Mol Genet Metab 1998;63:224–9.
[12] Heldin CH, Miyazono K, ten Dijke P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. Nature 1997;390:465–71.
[13] Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell 2003;113:685–700.
[14] Schiller M, Javelaud D, Mauviel A. TGF-beta-induced SMAD signaling and gene regulation: consequences for extracellular matrix remodeling and wound healing. J Dermatol Sci 2004;35:83–92.
[15] Ryer EJ, Hom RP, Sakakibara K, Nakayama KI, Nakayama K, Faries PL, et al. PKCdelta is necessary for Smad3 expression and transforming growth factor beta-induced fibronectin synthesis in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 2006;26:780–6.
[16] Engel ME, McDonnell MA, Law BK, Moses HL. Interdependent SMAD and JNK signaling in transforming growth factor-beta-mediated transcription. J Biol Chem 1999;274:37413–20.
[17] Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature 2003;425:577–84.
[18] Panossian A, Wikman G. Pharmacology of *Schisandra chinensis* Bail.: an overview of Russian research and uses in medicine. J Ethnopharmacol 2008;118:183–212.
[19] Melhem A, Stern M, Shibolet O, Israeli E, Ackerman Z, Pappo O, et al. Treatment of chronic hepatitis C virus infection via antioxidants: results of a phase I clinical trial. J Clin Gastroenterol 2005;39:737–42.
[20] Yasukawa K, Ikeya Y, Mitsuhashi H, Iwasaki M, Aburada M, Nakagawa S, et al. Gomisin A inhibits tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mouse skin. Oncology 1992;49:68–71.
[21] Min HY, Park EJ, Hong JY, Kang YJ, Kim SJ, Chung HJ, et al. Antiproliferative effects of dibenzocyclooctadiene lignans isolated from *Schisandra chinensis* in human cancer cells. Bioorg Med Chem Lett 2008;18:523–6.
[22] Chiu PY, Ko KM. Schisandrin B protects myocardial ischemia-reperfusion injury partly by inducing Hsp25 and Hsp70 expression in rats. Mol Cell Biochem 2004;266:139–44.
[23] Kim EY, Baek IH, Rhyu MR. Cardioprotective effects of aqueous *Schizandra chinensis* fruit extract on ovariectomized and balloon-induced carotid artery injury rat models: effects on serum lipid profiles and blood pressure. J Ethnopharmacol 2011;134:668–75.
[24] Shimizu N, Yoshiyama M, Omura T, Hanatani A, Kim S, Takeuchi K, et al. Activation of mitogen-activated protein kinases and activator protein-1 in myocardial infarction in rats. Cardiovasc Res 1998;38:116–24.
[25] Kim HK, Bak YO, Choi BR, Zhao C, Lee HJ, Kim CY, et al. The role of the lignan constituents in the effect of *Schisandra chinensis* fruit extract on penile erection. Phytother Res 2011.
[26] Wrana JL, Carcamo J, Attisano L, Cheifetz S, Zentella A, Lopez-Casillas F, et al. The type II TGF-beta receptor signals diverse responses in cooperation with the type I receptor. Cold Spring Harb Symp Quant Biol 1992;57:81–6.
[27] Zawel L, Dai JL, Buckhaults P, Zhou S, Kinzler KW, Vogelstein B, et al. Human Smad3 and Smad4 are sequence-specific transcription activators. Mol Cell 1998;1:611–7.
[28] Shin DM, Jeon JH, Kim CW, Cho SY, Lee HJ, Jang GY, et al. TGFbeta mediates activation of transglutaminase 2 in response to oxidative stress that leads to protein aggregation. FASEB J 2008;22:2498–507.
[29] Gao DF, Niu XL, Hao GH, Peng N, Wei J, Ning N, et al. Rosiglitazone inhibits angiotensin II-induced CTGF expression in vascular smooth muscle cells—role of PPAR-gamma in vascular fibrosis. Biochem Pharmacol 2007;73:185–97.
[30] Kim SH, Bae HC, Park EJ, Lee CR, Kim BJ, Lee S, et al. Geraniol inhibits prostate cancer growth by targeting cell cycle and apoptosis pathways. Biochem Biophys Res Commun 2011;407:129–34.
[31] Lin X, Duan X, Liang YY, Su Y, Wrighton KH, Long J, et al. PPM1A functions as a Smad phosphatase to terminate TGFbeta signaling. Cell 2006;125:915–28.
[32] Branton MH, Kopp JB. TGF-beta and fibrosis. Microbes Infect 1999;1:1349–65.
[33] Brazova J, Sismova K, Vavrova V, Bartosova J, Macek Jr M, Lauschman H, et al. Polymorphisms of TGF-beta1 in cystic fibrosis patients. Clin Immunol 2006;121:350–7.
[34] Li S, Li X, Zheng H, Xie B, Bidasee KR, Rozanski GJ. Pro-oxidant effect of transforming growth factor-beta1 mediates contractile dysfunction in rat ventricular myocytes. Cardiovasc Res 2008;77:107–17.
[35] Zhao W, Zhao T, Chen Y, Ahokas RA, Sun Y. Oxidative stress mediates cardiac fibrosis by enhancing transforming growth factor-beta1 in hypertensive rats. Mol Cell Biochem 2008;317:43–50.
[36] Sakurai T, Kudo M, Fukuta N, Nakatani T, Kimura M, Park AM, et al. Involvement of angiotensin II and reactive oxygen species in pancreatic fibrosis. Pancreatology 2011;11(Suppl. 2):7–13.
[37] Kamp DW, Shacter E, Weitzman SA. Chronic inflammation and cancer: the role of the mitochondria. Oncology (Williston Park) 2011;25(13):400–10.
[38] Ip SP, Ko KM. The crucial antioxidant action of schisandrin B in protecting against carbon tetrachloride hepatotoxicity in mice: a comparative study with butylated hydroxytoluene. Biochem Pharmacol 1996;52:1687–93.
[39] Chiu PY, Leung HY, Ko KM. Schisandrin B enhances renal mitochondrial antioxidant status, functional and structural integrity, and protects against gentamicin-induced nephrotoxicity in rats. Biol Pharm Bull 2008;31:602–5.
[40] Lu H, Liu GT. Anti-oxidant activity of dibenzocyclooctene lignans isolated from Schisandraceae. Planta Med 1992;58:311–3.