Smad2/3 signaling involved in urotensin II-induced phenotypic differentiation, collagen synthesis and migration of rat aortic adventitial fibroblasts

Weizhao Lin,[1,2] Xu Yang,[1,2] Fan Zheng,[1,2] Jianshe Yang,[3] Yonggang Zhang,[1,2]

[1]Department of Cardiology, Second Affiliated Hospital, Shantou University Medical College, Shantou; [2]Cardiovascular Laboratory, Centre for Translational Medicine, Shantou University Medical College, Shantou; [3]Department of Nuclear Medicine, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China

Correspondence: Dr. Yonggang Zhang. Department of Cardiology, Second Affiliated Hospital, Shantou University Medical College, No. 69, Dongxia North Road, Shantou, Guangdong 515041, China.
Tel.: +86.75483141143.
Fax: +86.75488346543.
E-mail: zhangyg8686@hotmail.com

Key words: urotensin II; adventitial fibroblasts; Smad; signal transduction; vascular fibrosis.

Acknowledgments: this project was supported by the National Natural Science Foundation of China (No. 30971273 & 81270223) and the Natural Science Foundation of Guangdong Province of China (No. 9151051501000016 & S2011010000450) Health Sciences & Technology Program of Shantou, Guangdong, China (210623156491437).

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: none.

Ethical approval and consent to participate: animal care and experimental protocols complied with the Animal Management Rules of China and the Guide for Care and Use of Laboratory Animals, Shantou University Medical College. Experimentations were conducted in agreement with the standards of animal care (National Institutes of Health Guide for the Care and Use of Laboratory Animals).

Availability of data and material: data and materials are available by the authors.

Received: 3 August 2023.
Accepted: 22 August 2023.

Licensee PAGEPress, Italy
Italian Journal of Medicine 2023; 17:1637
doi:10.4081/itjm.2023.1637

ABSTRACT

Objective. To investigate whether Smad2/3 signaling is involved in urotensin II (UII) induced activation of aortic adventitial fibroblasts. Materials and Methods. Growth-arrested adventitial fibroblasts were stimulated with UII in the presence or absence of urotensin II receptor (UT) antagonist SB710411 or transfected with Smad2/3 small inhibitory RNA (siRNA). UII-stimulated Smad2/3 phosphorylation, α-smooth muscle actin (α-SMA), and collagen I expression and migration of adventitial fibroblasts were evaluated by western blot analysis, real-time reverse transcription polymerase chain reaction, immunofluorescence, ELISA, and transwell migration assay, respectively. Results. In cultured adventitial fibroblasts, UII time- and dose-dependently stimulated Smad2/3 protein phosphorylation, with maximal effect at 10^{-8} mol/l (increased by 147.2%, P<0.001). UII stimulated Smad2/3 upregulation and nuclear translocation. SB710411 significantly inhibited these effects. In addition, UII potently induced α-SMA and procollagen 1 protein or mRNA expression (P<0.01), which were completely blocked by Smad2 (decreased by 75.1%, 54.2% in protein, and by 73.3% and 38.2% in mRNA, respectively, P<0.01) or Smad3 siRNA (decreased by 80.3% and 47.0% in protein, and by 72.3% and 47.7% in mRNA, respectively, P<0.01). Meanwhile, Smad2 or smad3 siRNA significantly inhibited the UII-induced collagen 1 secretion and cell migration. Conclusions. UII may stimulate adventitial-fibroblast phenotype conversion, migration, and collagen I synthesis via phosphorylated-Smad2/3 signal transduction pathways.

Introduction

Vascular fibrosis is a critical pathophysiological procedure of many diseases such as hypertension, diabetes, and atherosclerosis,[1-3] characterized by excessive deposition and extracellular matrix remodeling in the vessel wall. Previous studies of vascular dysfunction focused on the vascular intima and media. However, recent studies revealed that vascular adventitia also plays an important role in vascular regulation and dysfunction.[4,5] Importantly, the phenotype differentiation from adventitial fibroblasts into myofibroblasts plays a central role in vascular fibrosis, with α-smooth muscle actin (α-SMA) upregulation.[6,7] The adventitia is a

source of several vasoactive factors such as endothelin 1,[8] angiotensin II,[9] and transforming growth factor β (TGF-β),[10] which may regulate vascular structure and function via autocrine and/or paracrine mechanisms.[11]

We previously found that urotensin II (UII), a potent vasoconstrictive peptide, is an autocrine/paracrine vasoactive factor for aortic adventitia.[12] UII could stimulate proliferation,[13,14] migration of adventitial fibroblasts,[15] as well as vascular smooth muscle cells (VSMCs), and promote foam cell formation and collagen production,[16-18] involved in the process of vascular fibrosis and remodeling. However, the mechanisms have not been completely clarified.

Smad signaling participates in the process of vascular fibrosis.[1,19,20] The receptor-regulated Smads, including Smad2/3, are closely associated with vascular fibrosis. Phosphorylated Smad2/3 forms a heterotrimeric complex with Smad4 and moves into the nucleus to modulate transcription. The cytokine TGF-β promotes fibrosis via phosphorylation of Smad2/3.[21] In addition, other vasoactive factors such as angiotensin II and advanced glycosylation end products can promote vascular fibrosis by activating Smad signaling.[2,22,23]

In this study, we investigated whether Smad2/3 signaling is involved in UII-induced adventitial-fibroblast phenotype differentiation, migration, and collagen production.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing 180-200 g were supplied by the Animal Center, Shantou University Medical College, Shantou, China. Animal care and experimental protocols complied with the Animal Management Rules of China and the Guide for Care and Use of Laboratory Animals, Shantou University Medical College. Experimentations were conducted in agreement with the standards of animal care (National Institutes of Health Guide for the Care and Use of Laboratory Animals).

Cell culture

The isolation and culture of adventitial fibroblasts from rat aorta were essentially as described.[12] Cells were cultured in serum-free DMEM (Gibco, Invitrogen-Gibco, Carlsbad, CA, USA) in the presence or absence of UII (Phoenix Pharmaceuticals, Belmont, CA, USA) (10^{-10}, 10^{-9}, 10^{-8} or 10^{-7} mol/l). To examine the molecular basis of the action of UII, urotensin II receptor (UT) antagonist SB710411 (Phoenix Pharmaceuticals) (10^{-6} mol/l) and, smad2 and smad3 small inhibitory RNA (siRNA) were used. Cells from passages 3 to 5 were used for all experiments.

Small inhibitory RNA knockdown

The primer sequences for siRNA targeting for Smad2 were 5'-GAGCCACAGAGUAAUUACATT -3', and 5'-UGUAAUUACUCUGUGGCUCTT -3', and for Smad3, 5'-GCUGCUGCCUGAUUGAAAUTT- 3' and 5'-UAAGACACGCUGGAACAGCGG-3'. All siRNAs were chemically synthesized by Shanghai GenePharma Co. Adventitial fibroblasts were seeded at 70-80% for transfection, then treated with Lipofectamine RNAiMAX Reagent (Invitrogen, Carlsbad, CA, USA) and 100 pmol/ml siRNA mixed in Opti-MEM. At 24 h after transfection, cells were incubated with UII (10^{-8}mol/l) for another 24 h. Smad2/3 phosphorylation, α-SMA, and collagen I expression were determined.

Western blot analysis

After incubation, the culture medium was removed, and adventitial fibroblasts were lysed directly in the dish for 30 min with lysis buffer RIPA supplemented with 1 mol/l phenylmethylsulphonyl fluoride. Lysates were cleared by centrifugation at 12 000 g for 5 min at 4°C. Protein content in the lysate was analyzed by use of bicinchonininc acid (BCA). Equal amounts of protein were resolved by electrophoresis on 10% SDS-PAGE (10% running gel, 5% stacking gel) and transferred onto polyvinylidene fluoride membrane with a 0.45 mm pore size. The membrane was incubated in blocking buffer for 1 h at room temperature, then incubated with primary monoclonal antibody for α-SMA (Sigma, St. Louis, MO, USA; 1:2000), procollagen1 (1:200), Phospho-Smad2 and 3 (Cell Signaling Technology, Boston, USA; 1:1000; 1:1000), phosphorylated Smad2/3 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Proteintech Group, USA; 1: 3000) 4°C overnight. Then, the membrane was rinsed 3 times with TBS/Tween 20 and incubated in goat anti-mouse IgG-peroxidase conjugate (1:5000) for 1 h at room temperature. Immunocomplexes were visualized by renaissance chemiluminescence reagents and exposed to radiographic film. The optical density of immunoblots was determined by scanning densitometry.

Immunofluorescence

Cells were seeded on coverslips, then fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) (pH 7.4) for 15 min at room temperature, washed twice with ice-cold PBS, then incubated for 10 min in PBS containing 0.25% Triton X-100 (PBST). Then cells were incubated with 1% bovine serum albumin in PBST for 30 min to block unspecific binding of antibodies and incubated with primary antibodies, followed by antibodies.

Cell migration

Cell migration was measured by the use of a transwell chamber apparatus with 8-mm pore size and apolycarbonate membrane (Corning). Adventitial fibroblasts (5×10^{-5} cells/ml) were seeded in the upper chamber in serum-free DMEM. After 6-h incubation, the filters were removed, membranes were washed with PBS, and cells beneath the membrane were fixed with cold methanol for 15 min and stained with hematoxylin and eosin. Migrated adventitial fibroblasts were quantified by counting 5 random fields under a light microscope at 200× magnification in each membrane.

Enzyme-linked immunosorbent assay

The type I collagen secretion from adventitial fibroblasts was measured by ELISA (Uscnlife, Wuhan, China) according to the manufacturer's instructions. After incubation, the culture medium was collected, centrifuged immediately, and added to 96-well plastic plates. Absorbance of colored products was determined with a microplate reader set to 450 nm. Experiments were performed in triplicate, repeated 6 times.

Real-time reverse transcription polymerase chain reaction

Total RNA was isolated directly from cells by use of Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA concentration and purity were analyzed by use of the NanoDrop ND-1000 system with 260 nm absorbance and 260/280 nm absorbance, respectively. Real-time reverse transcription polymerase chain reaction (RT-PCR) reactions involved the TaKaRa SYBR PrimeScriptTM RT-PCR Kit (TaKaRa Biotechnology, Dalian, China). Reverse transcription to cDNA was accomplished at 37°C for 15 min, then 85°C for 5 sec. The primer sequences are in Table 1. PCR cycles were run at 95°C for 3 sec and then 95°C for 5 sec, and 60°C for 30 sec, for 40 cycles. The fluorescence emitted by SYBR Green I dye was measured at the end of each cycle. For each PCR product, a single narrow peak was obtained by melting curve analysis at the specific melting curve temperature, indicating specific amplifications. Experiments were performed in triplicate. The relative mRNA expression of the genes was determined by the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Results are shown as mean±SEM. Comparisons involved one-way ANOVA, followed by the Student-Newman-Keuls test. All data were analyzed by use of GraphPad Prism 4.0. Two-tailed $P<0.05$ was considered statistically significant.

Results

Urotensin II stimulated smad2/3 phosphorylation in a urotensin II receptor-dependent manner in adventitial fibroblasts

UII time-dependently stimulated Smad2/3 protein phosphorylation. Smad2/3 phosphorylation was increased by 21.6% ($P>0.05$), 68.1% ($P>0.05$), 117.8% ($P<0.01$), and 147.2% ($P<0.001$), respectively, after 3-, 6-, 12- and 24-h incubation with UII (10^{-8} mol/l) (Figure 1A). UII (10^{-10}-10^{-8} mol/l) also dose-dependently stimulated Smad 2/3 phosphorylation, with maximal effect at 10^{-8} mol/l (increased by 82.8%, $P<0.05$, Figure 1B), which was inhibited by SB710411, the UT antagonist (decreased by 67.5%, $P<0.01$, Figure 1C). Smad2/3 proteins were mainly located in the cytosol, with weak expression in control cells. UII induced Smad2/3 nuclear translocation and upregulation, which was also significantly inhibited by SB710411 (Figure 1D). These findings suggest that UII stimulated smad2/3 phosphorylation in a UT-dependent manner.

Smad2/3 small inhibitory RNA inhibited the urotensin II-induced α-smooth muscle actin expression and collagen 1 synthesis and migration in adventitial fibroblasts

To evaluate whether Smad2/3 is involved in UII-induced upregulation of α-SMA (a marker of adventitial fibroblast differentiation into myofibroblast) and procol-

Table 1. Primers for real-time reverse transcription polymerase chain reaction.

Gene	Primers	
Smad2	Sense	5'-GAGCCACAGAGUAAUUACATT-3'
	Anti- sense	5'-UGUAAUUACUCUGUGGCUCTT-3'
Smad3	Sense	5'-GCUGCUGCCUGAUUGAAAUTT-3'
	Anti- sense	5'-AUUUCAAUCAGGCAGCAGCTT-3'
Negative control	Sense	5'-UUCUCCGAACGUGUCACGUTT-3'
	Anti- sense	5'-ACGUGACACGUUCGGAGAATT-3'

lagen 1 expression, cells were treated with 100 pmol/ml siRNA to knock down smad2 or smad3. UII-induced α-SMA and procollagen 1 protein expression were impaired significantly with siRNA knockdown of Smad2 (decreased by 75.1% and 54.2%, respectively, P<0.001, Figure 2) or Smad3 (decreased by 80.3% and 47.0%, respectively, P<0.01 or P<0.001, Figure 3). In addition, the effect of UII on promoting the phenotypic differentiation of adventitial fibroblasts into myofibroblasts was inhibited with siRNA knockdown of Smad2 or Smad3 (Figure 4A). Meanwhile, UII induced collagen 1 secretion from cells (P<0.01), which was also inhibited by Smad2 or smad3 siRNA (decreased by 33.3% and 39.0%, respectively, P<0.01, Figure 4B). Similarly, the UII-induced mRNA expression of α-SMA and procollagen 1 were significantly impaired with smad2 (decreased by 73.3% and 38.2%, respectively, P<0.01) or smad3 knockdown (decreased by 72.3% and 47.7%, respectively, P<0.01, Figure 4C-D). Smad2 or smad3 knockdown also obviously decreased the UII induced migration (Figure 5). All of these findings suggest that Smad2/3 mediated the UII-induced α-SMA expression and collagen 1 synthesis and migration.

Discussion

In the present study, we examined whether Smad2/3 signaling is involved in UII-induced activation of adventitial fibroblasts. UII time- and dose-dependently stimulated Smad2/3 phosphorylation and induced Smad2/3 nuclear translocation. The effects were inhibited by UT antagonism, which suggests that they were accomplished via UT. In addition, the UII promotion of a-SMA and collagen I mRNA and protein expression and cell migration were inhibited by siRNA knockdown of Smad2/3, so Smad2/3 mediated the UII-induced phenotypic differentiation, collagen synthesis, and migration of adventitial fibroblasts.

UII is involved in the development of cardiovascular diseases via cellular phenotypic differentiation, migration, proliferation, and collagen production. In neonatal cardiac fibroblasts, UII significantly induces collagen synthesis, which is modulated by UT and TGF-β1.[24] UII also simulates collagen synthesis in cardiac fibroblasts and migration of endothelial progenitor cells and human aortic VSMCs via a RhoA/Rho-associated protein kinase- or extracellular signal-regulated kinase-dependent pathway.[25-27] *In vivo*, chronic UII infusion induced diastolic dysfunction and enhanced collagen production in rats.[18] We previously demonstrated that UII may stimulate adventitial fibroblast differentiation, migration, and collagen synthesis through protein kinase C, mitogen-activated protein kinase (MAPK), calcineurin, Rho kinase, and/or Ca2+ signal transduction pathways,[15] and osteopontin may mediate the UII-induced migration of adventitial fibroblasts.[28] Others

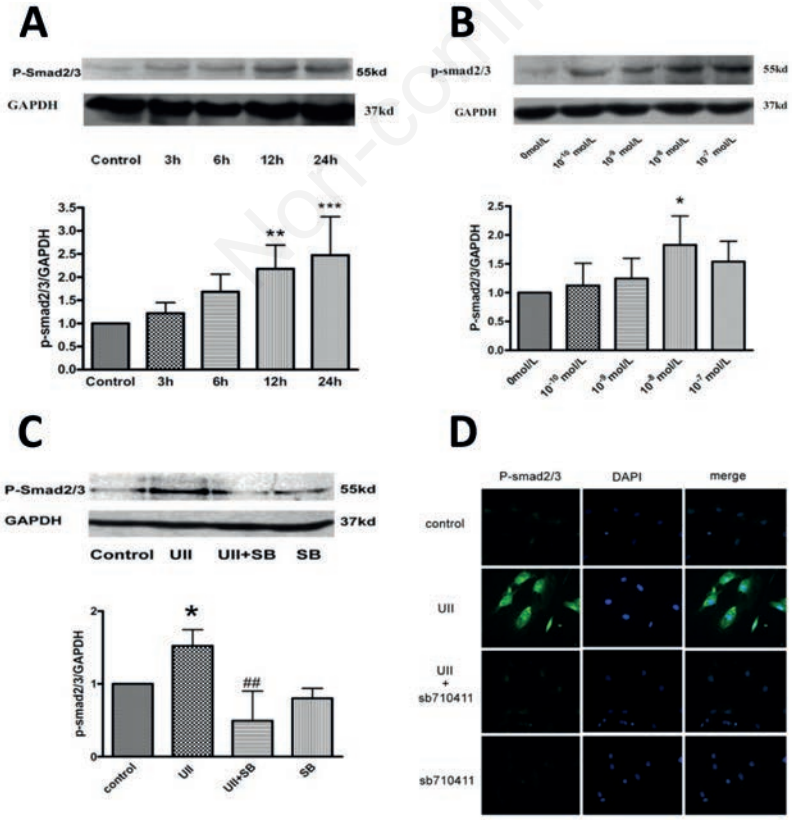

Figure 1. Urotensin II (UII) activates Smad2/3 phosphorylation in an UT-dependent way. Adventitial fibroblasts were treated with UII (10^{-8} mol/l) for the indicated times (A) or with concentrations of UII (10^{-10}-10^{-8} mol/l) for 24 h (B), or preincubated with SB710411, the UT antagonist, for 30 min, then with UII (10^{-8} mol/l) and SB710411 for 24 h (C and D). Smad2/3 phosphorylation and nuclear translocation were determined by western blot analysis and immunofluorescence staining, respectively, and quantification. Glyceraldehyde-3-phosphate dehydrogenase was used to verify equal loading of cell lysates. Data are mean±SEM ratio of Smad2/3 phosphorylation to glyceraldehyde-3-phosphate dehydrogenase from 5 (A and B) or 3 (C) independent experiments. *P<0.05, **P<0.01, ***P<0.001 *vs.* Control; ##P<0.01 *vs.* UII. D, immunofluorescence of UII promoting p-Smad2/3 expression and translocation via UT, Magnification×400, n=3.

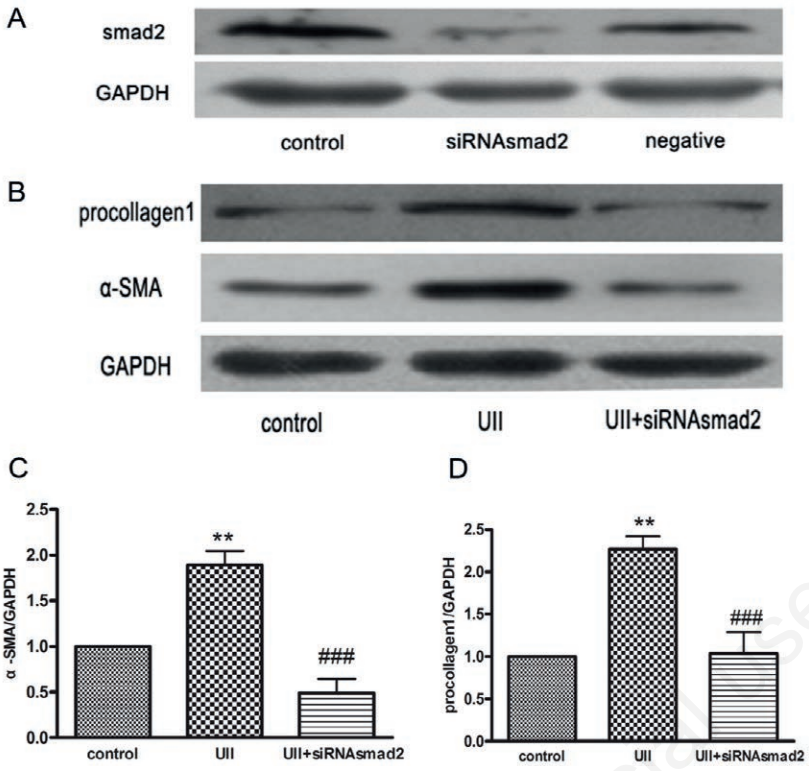

Figure 2. Smad2 small inhibitory RNA inhibited the urotentin II-induced procollagen 1 and α-smooth muscle actin protein expression in rat adventitial fibroblasts. Cells were transfected with (A) Smad2 small inhibitory RNA, then with urotensin II (10^{-8} mol/l) for 24 h. Cell lysates were assayed for procollagen 1 and α-smooth muscle actin protein expression. Western blot analysis of knockdown efficiency of Smad2 with quantification. Data are mean±SEM ratio of procollagen 1 and α-smooth muscle actin to glyceraldehyde-3-phosphate dehydrogenase from 4 independent experiments. **P<0.01 *vs.* control; ###P<0.001 *vs.* UII.

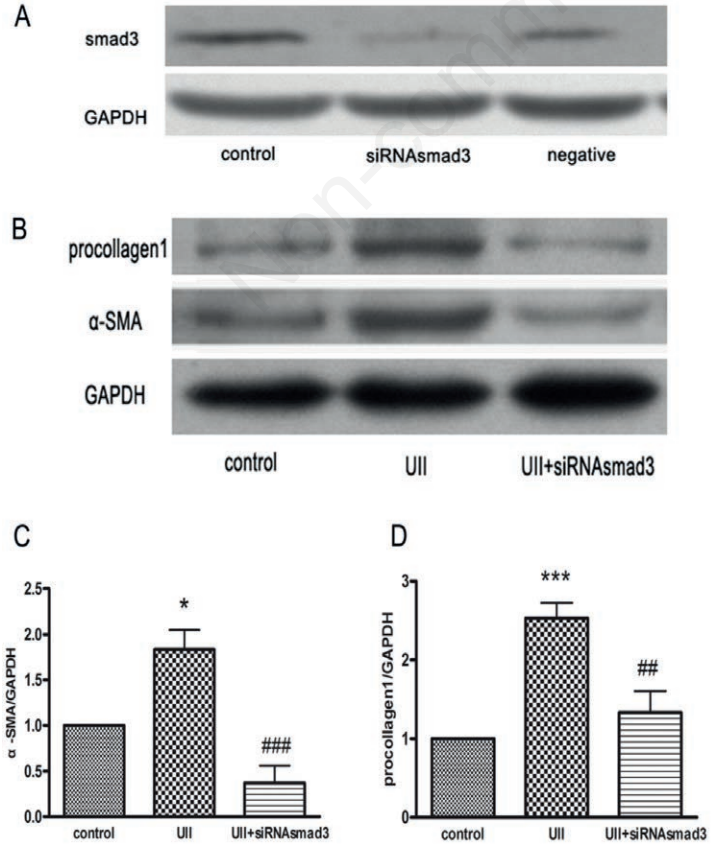

Figure 3. Smad3 small inhibitory RNA inhibited the urotentin II-induced procollagen 1 and α- smooth muscle actin protein expression in rat adventitial fibroblasts. Cells were transfected with Smad3 small inhibitory RNA, then with urotensin II (10^{-8} mol/l) for 24 h. Cell lysates were assayed for procollagen 1 and α- smooth muscle actin protein expression. Western blot analysis of knockdown efficiency of Smad3 with quantification. Data are mean±SEM ratio of procollagen 1 and α-smooth muscle actin to glyceraldehyde- 3-phosphate dehydrogenase from 4 independent experiments. *P<0.05,*P<0.001 *vs.* control; ##P<0.01, ###P<0.001 *vs.* UII.**

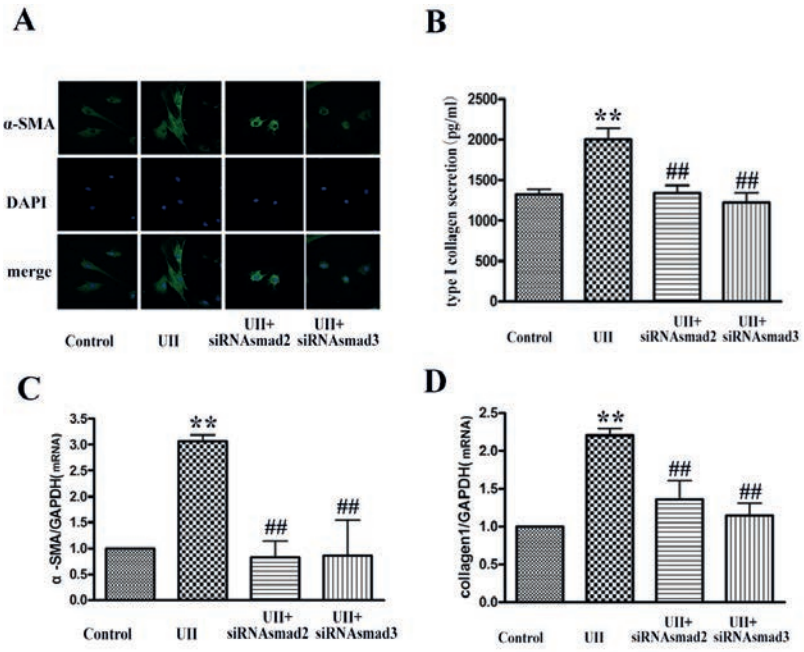

Figure 4. Smad2 or Smad3 knockdown inhibited urotensin II-induced differentiation, collagen 1 secretion, α-smooth muscle actin and procollagen 1 mRNA expression in adventitial fibroblasts. Cells were transfected with Smad2 or Smad3 small inhibitory RNA, then incubated with urotensin II (10^{-8} mol/l) for 24 h. Myofibroblast formation was determined by immunofluorescence staining (A, Magnification×400, n=3). ELISA of secretion of collagen 1 (B, n=6). qPCR analysis of α-smooth muscle actin (C) and procollagen 1 (D) mRNA expression (n=3). Data are mean±SEM. **P<0.01 *vs.* control; ## P<0.01 *vs.* urotensin II.

have reported that UII promotes collagen synthesis with activation of TGF-β1/Smad2/3 signaling in VSMCs.[17] Here, we found that knockdown of Smad2/3 significantly inhibited the UII effects, for direct evidence that the activation of Smad2/3 signaling is a new mechanism mediating the UII-induced phenotypic differentiation, collagen synthesis, and migration in adventitial fibroblasts.

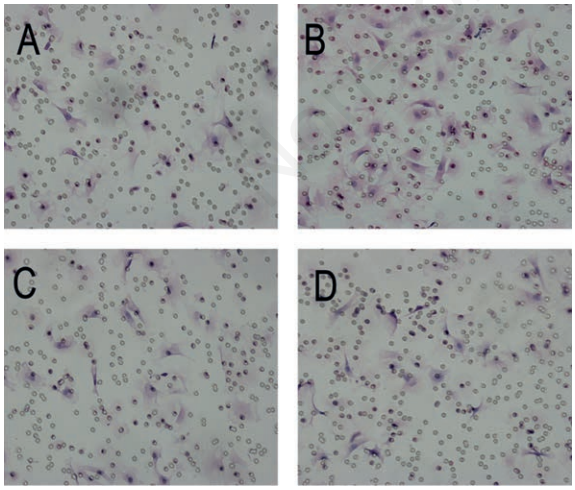

Figure 5. Smad2/Smad3 small inhibitory RNA inhibited the urotensin II-induced migration of adventitial fibroblasts. Cells were transfected with Smad2 or Smad3 small inhibitory RNA for 24 h, then stimulated with urotensin II (10^{-8} mol/l) and incubated for another 24 h. Cell migration was measured in transwell chambers. (A) Control; (B) urotensin II; (C) urotensin II +siRNAsmad2; (D) urotensin II +siRNAsmad3; (n=6 experiments).

The activation of Smad2/3 is a key signal involved in the differentiation of fibroblasts into myofibroblasts, which play an important role in tissue fibrosis. Smad2/3 mediates TGF-β1-induced proliferation, migration, and SMA and collagen expression in adventitial fibroblasts. Treating Smad3-expressing VSMCs with TGF-β1 promoted collagen 3 secretion from fibroblasts.[29,30] The use of 15-lipoxygenase/15-hydroxyeicosatetraenoic acid also depended on TGF-β1/Smad2/3 activation to promote vascular fibrosis. Angiotensin II-induced cardiac fibrosis and inflammation were prevented in Smad3-knockout cardiac fibroblasts. The present study shows that UII may induce Smad2/3 signaling activation via UT, then promote adventitial fibroblast phenotypic differentiation via Smad2/3 signal transduction pathways, so simultaneous inhibition of UII and Smad2/3 signaling might be a promising measure to control the development of fibrosis.

Many studies have demonstrated that TGF-β1 is an essential cytokine to activate Smad. UII could induce TGF-β1 secretion in adventitial fibroblasts,15 neonatal cardiac fibroblasts, and VSMCs.[17] In addition to TGF-β, other factors are involved in Smad2/3 activation. Therefore, the cross-talk between Smad and TGF-β and the other signal transduction pathways, such as protein kinase C and MAPK pathways, in UII-involved pathogenesis needs further investigation.

In summary, we revealed that in adventitial fibroblasts, UII stimulated Smad2/3 protein phosphorylation and nuclear translocation in a UT-dependent manner. The UII-promoted α-SMA and collagen 1 expression and cell migration were blocked completely by siRNA knockdown of Smad2/3.

Smad2/3 signaling may mediate the UII-induced phenotypic differentiation, collagen synthesis, and migration of adventitial fibroblasts, which provides new insights into vascular fibrosis promoted by UII.

References

1. Rodriguez-Vita J, Sanchez-Lopez E, Esteban V, et al. Angiotensin II activates the Smad pathway in vascular smooth muscle cells by a transforming growth factor-beta-independent mechanism. Circulation 2005;111: 2509-17.
2. Liu Y, Liang C, Liu X, et al. AGEs increased migration and inflammatory responses of adventitial fibroblasts via RAGE, MAPK and NF-kappaB pathways. Atherosclerosis 2010;208:34-42.
3. Lan TH, Huang XQ, Tan HM. Vascular fibrosis in atherosclerosis. Cardiovasc Pathol 2013;22:401-7.
4. Stenmark KR, Yeager ME, El KK, et al. The adventitia: essential regulator of vascular wall structure and function. Annu Rev Physiol 2013;75:23-47.
5. Wu X, Lu Q. Expression and significance of alpha-SMA and PCNA in the vascular adventitia of balloon-injured rat aorta. Exp Ther Med 2013;5:1671-6.
6. Evans RA, Tian YC, Steadman R, Phillips AO. TGF-beta1-mediated fibroblast-myofibroblast terminal differentiation-the role of Smad proteins. Exp Cell Res 2003;282:90-100.
7. Forte A, Della CA, De Feo M, et al. Role of myofibroblasts in vascular remodelling: focus on restenosis and aneurysm. Cardiovasc Res 2010;88:395-405.
8. Boyd R, Ratsep MT, Ding LL, Wang HD. ETA and ETB receptors are expressed in vascular adventitial fibroblasts. Am J Physiol Heart Circ Physiol 2011;301:H2271-H2278.
9. Song N, Ding W, Chu S, et al. Urotensin II stimulates vascular endothelial growth factor secretion from adventitial fibroblasts in synergy with angiotensin II. Circ J 2012;76:1267-73.
10. Zhang YG, Hu YC, Mao YY, et al. Transforming growth factor-beta1 involved in urotensin II-induced phenotypic differentiation of adventitial fibroblasts from rat aorta. Chin Med J (Engl) 2010;123:3634-9.
11. Di Wang H, Ratsep MT, Chapman A, Boyd R. Adventitial fibroblasts in vascular structure and function. the role of oxidative stress and beyond. Can J Physiol Pharmacol 2010;88:177-86.
12. Zhang YG, Li YG, Wei RH, et al. Urotensin II is an autocrine/paracrine growth factor for aortic adventitia of rat. Regul Pept 2008;151:88-94.
13. Shi L, Ding W, Li D, et al. Proliferation and anti-apoptotic effects of human urotensin II on human endothelial cells. Atherosclerosis 2006;188:260-4.
14. Vaudry H, Leprince J, Chatenet D, et al. International Union of Basic and Clinical Pharmacology. XCII. Urotensin II, urotensin II-related peptide, and their receptor: from structure to function. Pharmacol Rev 2015;67:214-58.
15. Zhang YG, Li J, Li YG, Wei RH. Urotensin II induces phenotypic differentiation, migration, and collagen synthesis of adventitial fibroblasts from rat aorta. J Hypertens 2008;26:1119-26.
16. Shiraishi Y, Watanabe T, Suguro T, et al. Chronic urotensin II infusion enhances macrophage foam cell formation and atherosclerosis in apolipoprotein E-knockout mice. J Hypertens 2008;26:1955-65.
17. Zhao J, Ding W, Song N, et al. Urotensin II-induced collagen synthesis in cultured smooth muscle cells from rat aortic media and a possible involvement of transforming growth factor-beta1/Smad2/3 signaling pathway. Regul Pept 2013;182:53-8.
18. Tzanidis A, Hannan RD, Thomas WG, et al. Direct actions of urotensin II on the heart: implications for cardiac fibrosis and hypertrophy. Circ Res 2003;93: 246-53.
19. Wang W, Huang XR, Canlas E, et al. Essential role of Smad3 in angiotensin II-induced vascular fibrosis. Circ Res 2006;98:1032-9.
20. Rodrigues DR, Rodrigues-Diez R, Lavoz C, et al. Statins inhibit angiotensin II/Smad pathway and related vascular fibrosis, by a TGF-beta-independent process. PLoS One 2010;5:e14145.
21. Javelaud D, Mauviel A. Mammalian transforming growth factor-betas: Smad signaling and physio-pathological roles. Int J Biochem Cell Biol 2004;36:1161-5.
22. Yang F, Chung AC, Huang XR, Lan HY. Angiotensin II induces connective tissue growth factor and collagen I expression via transforming growth factor-beta-dependent and -independent Smad pathways: the role of Smad3. Hypertension 2009;54:877-84.
23. Li JH, Huang XR, Zhu HJ, et al. Advanced glycation end products activate Smad signaling via TGF-beta-dependent and independent mechanisms: implications for diabetic renal and vascular disease. FASEB J 2004;18: 176-8.
24. Dai HY, Kang WQ, Wang X, et al. The involvement of transforming growth factor-beta1 secretion in urotensin II-induced collagen synthesis in neonatal cardiac fibroblasts. Regul Pept 2007;140:88-93.
25. Tran L, Kompa AR, Kemp W, et al. Chronic urotensin-II infusion induces diastolic dysfunction and enhances collagen production in rats. Am J Physiol Heart Circ Physiol 2010;298:H608-H613.
26. Matsusaka S, Wakabayashi I. Enhancement of vascular smooth muscle cell migration by urotensin II. Naunyn Schmiedebergs Arch Pharmacol 2006;373:381-6.
27. Xu S, Jiang H, Wu B, et al. Urotensin II induces migration of endothelial progenitor cells via activation of the RhoA/Rho kinase pathway. Tohoku J Exp Med 2009;219: 283-388.
28. Zhang YG, Kuang ZJ, Mao YY, et al. Osteopontin is involved in urotensin II-induced migration of rat aortic adventitial fibroblasts. Peptides 2011;32:2452-8.
29. Hinz B. Formation and function of the myofibroblast during tissue repair. J Invest Dermatol 2007;127:526-37.
30. Desmouliere A, Darby IA, Gabbiani G. Normal and pathologic soft tissue remodeling. role of the myofibroblast, with special emphasis on liver and kidney fibrosis. Lab Invest 2003;83:1689-707.