Effects of Arkadia on airway remodeling through enhancing TGF- β signaling in allergic rats

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Upregulation of transforming growth factor- β (TGF- β) signaling is interrelated with the development of airway remodeling. In this study, we examined the role of two E3 ubiquitin ligases, Arkadia and Smurf2, which are critically required for TGF- β signaling in airway remodeling. Rats were immunized with ovalbumin (OVA) and then challenged with an OVA aerosol. In *in vitro* experiments, normal human bronchial epithelial cells were stimulated with TGF- β_1 with or without the preincubation of Arkadia/Smurf2 small interfering RNA (siRNA) or lactacystin (an inhibitor of proteasomal degradation). In the lungs of OVA-treated rats, a large number of inflammatory cells were present near the airways. An increased subepithelial collagen deposition was associated with high expression levels of Smad7, SnoN and Ski mRNAs, Arkadia, Smurf2, and TGF- β type I receptor (T β RI), but low expression levels of Smad7, SnoN and Ski proteins. Smad7, SnoN and Ski interacted with both Arkadia and Smurf2 while T β RI only interacted with Smurf2 but not with Arkadia. In *in vitro* experiments, the inhibitory effect of TGF- β_1 on the expression of Smad7, SnoN and Ski was reversed by Arkadia siRNA and lactacystin, whereas the stimulatory effect of TGF- β_1 on the expression of T β RI protein and Smad7/SnoN/Ski mRNAs was not affected. In contrast, Smurf2 siRNA did not influence the effects of TGF- β_1 on the expression of the above proteins. Our results suggest that Arkadia may contribute to the pathogenesis of airway remodeling through enhancing TGF- β signaling by inducing the reduction of Smad7, SnoN and Ski proteins in OVA-sensitized and -challenged rats.

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Asthma is a chronic inflammatory disease of the airways, and recurrent inflammation results in airway remodeling characterized by subepithelial fibrosis, myofibroblast and myocyte hyperplasia, together with epithelial damage, goblet cell metaplasia, edema and increased vascularity.¹ Transforming growth factor β (TGF- β), a fibrogenic cytokine, has been implicated in the development of airway remodeling in asthma on the basis of its strong capacity to induce the production of extracellular matrix factors including type I collagen (COL-I).² It is, therefore, possible that regulators of TGF- β activity might also have important role in the modulation of the airway remodeling process in asthma.

The process of TGF- β signaling pathway activation is known as follows. On TGF- β stimulation and activation of the TGF- β type I receptor (T β RI), R-Smads (Smad2 and Smad3) are phosphorylated. Then, phosphorylated R-Smads

bind to Smad4, and are translocated into the nucleus, in which such a complex, together with additional DNA-binding co-factors, controls the transcription of TGF- β responsive genes.³ In the process, negative regulators such as Smad7, SnoN and Ski can downregulate TGF- β signaling through multiple mechanisms.^{3,4} Accumulative evidence indicates that the expression of Smad7, SnoN and Ski proteins in some fibrotic models is obviously lower than that in the normal controls,^{5–13} and also shows that upregulation of the expression of Smad7, SnoN or Ski proteins reverses or blocks fibrogenesis.^{14–17} Nakao *et al*¹⁸ reported that Smad7 immunoreactivity, which was present mainly in bronchial epithelial cells, was markedly decreased in asthmatic patients, compared with that of control subjects. However, the expression levels of SnoN and Ski in asthmatic patients are unclear.

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The expression of Smad7, SnoN and Ski is modulated by the ubiquitin-proteasome degradation pathway,^{19–21} and in the process of regulation, E3 ubiquitin ligases, such as Arkadia and Smurf2, induce ubiquitin-dependent degradation of Smad7, SnoN and Ski.^{11–13,19–23} Whereas Fukasawa *et al*^{6,13} reported that Smurf2 mediated the degradation of Smad7, SnoN and Ski in a mouse model of renal tubulointerstitial fibrosis, Liu *et al*^{7,24} have shown that the reduction of Smad7 in a rat model of renal tubulointerstitial fibrosis and normal human renal tubular epithelial cells may be mainly attributed to Arkadia, but not to Smurf2. Thus, how Arkadia and Smurf2 affect Smad7, SnoN and Ski in organ fibrosis remains to be clarified. Moreover, the interactions between Arkadia/Smurf2 and Smad7/SnoN/Ski in allergic airway remodeling are unknown. Therefore, this study was designed to clarify the effects of Arkadia and Smurf2 on the expression of Smad7, SnoN, Ski as well as T β RI in pathogenesis of airway remodeling in ovalbumin (OVA)-sensitized and -challenged rats *in vivo* and TGF- β ₁-treated bronchial epithelial cells *in vitro*.

MATERIALS AND METHODS

Animals

All animals used in this study were 6- to 8-week-old male Sprague–Dawley rats obtained from the Experimental Animal Center of Central South University (Changsha, China). Animal study protocols were approved by the Animal Care Committee of Central South University and the experiments were carried out according to the guidelines of the committee.

Chronic Airway Remodeling Model

Eight rats were sensitized and challenged with OVA (OVA-treated group) to induce chronic airway remodeling. Briefly, the rats were immunized with an intraperitoneal injection of 100 mg of OVA (Sigma, St Louis, MO, USA), 200 mg of aluminum hydroxide (Sigma), and 6×10^9 heat-killed *Bordetella pertussis* (Wuhan Institute of Biological Products, Wuhan, China) in 1 ml normal saline on days 0 and 14. From day 21, the rats were challenged for 30 min by nebulization of 1% (wt/vol) OVA in saline solution on 3 day/week for 6 weeks. As a control group, eight rats were sensitized with 1 ml normal saline and challenged with nebulized saline solution. All animals were killed 48 h after the final antigen or saline challenge.

Cell Culture and Treatment

Normal human bronchial epithelial cells (NHBEs) (Cell Center of Central South University, Changsha, China) were grown in a 5% CO₂ atmosphere at 37 °C in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum in six-well plastic plates for 24 h, and then a recombinant human TGF- β ₁ (R&D Systems, Minneapolis, MN, USA) at a final concentration of 5 ng/ml was added into the cell culture. The cells were collected 72 h later, and the expression of

Arkadia, Smurf2, T β RI, Smad7, SnoN and Ski proteins and mRNAs in TGF- β ₁-stimulated NHBEs were detected by western blot and real-time RT-PCR, respectively. In an additional experiment, NHBEs were cultured with or without Arkadia, Smurf2 or control small interfering RNA (siRNA) (2 μ g/ml, Sofarbio Technology, Hangzhou, China) for 24 h or lactacystin (10 μ M, Sigma) for 3 h. Then, TGF- β ₁ was added into the cell culture as described above. The cells were collected 72 h later, and the expression of Arkadia, Smurf2, T β RI, Smad7, SnoN and Ski proteins and mRNAs in TGF- β ₁-stimulated NHBEs were detected as described above.

Histopathological Staining

Lung tissues were fixed in 4% paraformaldehyde in phosphate-buffered saline and embedded in paraffin. Tissue sections (4 μ m thick) were stained with hematoxylin and eosin and Masson's trichrome for histopathological examination.

Western Blotting

The lung tissues and NHBEs were lysed with Nonidet P-40 (NP-40) lysis buffer as previously described.^{7,24} Proteins (20 μ g) were loaded and separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PolyScreen polyvinylidene difluoride transfer membranes (Pharmacia Corporation, Piscataway, NJ, USA). Immunoblotting was performed with different antibodies and visualized by the enhanced chemiluminescence (Amersham, Piscataway, NJ, USA) method. The primary antibodies purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) were goat anti-Arkadia, goat anti-Smurf2, goat anti-SnoN, rabbit anti-Smad7, rabbit anti-Ski and rabbit anti-T β RI. Mouse monoclonal anti- β -actin (Sigma) was used as an internal control.

Immunoprecipitation

Each extract from lung tissues was incubated with 4 μ g of specific anti-Smad7, anti-SnoN, anti-Ski or anti-T β RI antibody and 50 μ l of protein G-Sepharose beads for 3 h at 4 °C. The immunoprecipitates were washed three times with cold lysis buffer and then separated by SDS-PAGE for immunoblotting of associated proteins in the complex. The lysates incubated with anti-rabbit IgG or anti-goat IgG, instead of the specific antibodies were used as negative controls.

Real-Time RT-PCR

Total RNA was extracted from the lung tissues and NHBEs by using Trizol (Invitrogen, Carlsbad, CA, USA). Reverse transcription of the RNA was performed by using a first-strand complementary DNA synthesis kit (Promega, Madison, MI, USA). The PCR with SYBR Green PCR Master Mix (Takara, Shiga, Japan) was used to analyze the mRNA expression of TGF- β ₁, COL-I, Smad7, SnoN and Ski. Real-time PCR was performed by using the ABI PRISM 7900-HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The primer sequences used were as follows: rat TGF- β ₁: forward 5'-CCTGAGTGGCTGTCTTTTGACG-3',

reverse 5'-AGTGAGCGCTGAATCGAAAGC-3'; rat COL-I: forward 5'-AGAGCATGACCGATGGATTCC-3', reverse 5'-TTGCCAGTCTGCTGGTCCATG-3'; rat Smad7: forward 5'-TCGGACAGCTCAATTCGGAC-3', reverse 5'-GGTAACTGCTGCGTTGTAA-3'; rat SnoN: forward 5'-GAGGAGCAGGAGAAAATGG-3', reverse 5'-GAATCACGCTTGCTTGTG-3'; rat Ski: forward 5'-TGA CTCTGGACACAGCAGGA-3', reverse 5'-GAGAGGACAGCGAGGACAAG-3'; rat β -actin: forward 5'-TGGGTCAGAAGGACTCCTATG-3', reverse: 5'-CAGGCAGCTCATAGCTCTTCT-3'; human Smad7: forward 5'-CCTTAGCCGACTCTGCGAACTA-3', reverse 5'-CCAGATAATTTCGTTCCCCCTGT-3'; human SnoN: forward 5'-AAGGCTCACAGTAGTGGTAA-3', reverse 5'-GTGGCAGACTCAGATTTC-3'; human Ski: forward 5'-CTGCTGAGCCAGGATT-3', reverse 5'-TTGTACTTGTGCCATAGTC-3'; and human β -actin: forward 5'-TGGGTCAGAAGGACTCCTATG-3', reverse 5'-CAGGCAGCTCATAGCTCTTCT-3'. Real-time PCR data were analyzed according to the manufacturer's instructions.

Statistical Analysis

Data were presented as means $\pm$ s.d. The differences in the expression levels of proteins and mRNAs between control rats and OVA-treated rats were determined by the independent samples *t*-test. The differences in the expression levels of proteins and mRNAs among control NHBEs and NHBEs treated with TGF- β_1 , Arkadia siRNA, Smurf2 siRNA, control siRNA and/or lactacystin were determined by one-way ANOVA with least significant difference as an adjusted *post hoc* test. All statistical analyses were performed using SPSS 11.0 (SPSS, Chicago, IL, USA). A *P*-value of <0.05 was considered statistically significant.

RESULTS

Histopathological Changes and the Expression of TGF- β_1 and COL-I in the Lungs of OVA-Treated Rats

A large numbers of inflammatory cells were present near the airways and there was a significant increase in the sub-epithelial collagen deposition in the lungs of OVA-treated rats, compared with those of control rats (Figure 1a). These pathological changes were associated with a significant increase in TGF- β_1 and COL-I mRNAs expression in the lungs of OVA-treated rats, compared with those of control rats (Figure 1b).

Reduction of Smad7, SnoN and Ski Proteins, but not mRNAs, in the Lungs of OVA-Treated Rats

Western blotting showed that the expression of Smad7, SnoN and Ski proteins was significantly decreased in OVA-treated lungs, compared with saline-treated lungs (Figure 2a). To analyze whether the reduction of Smad7, SnoN and Ski proteins resulting from the downregulation of Smad7, SnoN and Ski mRNAs, we analyzed the mRNA expression of Smad7, SnoN and Ski by real-time RT-PCR. In contrast to the significant decrease of the three proteins, the expression of Smad7, SnoN and Ski mRNAs was increased in OVA-treated lungs, compared with saline-treated lungs (Figure 2b).

Increased Expression of Arkadia, Smurf2 and T β RI in the Lungs of OVA-Treated Rats

Western blotting showed that the expression of Arkadia, Smurf2 and T β RI was significantly increased in OVA-treated lungs, compared with saline-treated lungs (Figure 3).

Figure 1 Histopathological changes and expression of TGF- β_1 and COL-1 mRNAs in the lungs of allergic rats. (a) H&E staining and Masson's trichrome staining in OVA-treated (OVA) and saline-treated (saline) lungs (original magnification $\times 200$). Note: the progressive development of inflammatory infiltration and collagen deposition in OVA-treated lungs. (b) The relative ratios of TGF- β_1 and COL-1 mRNAs to β -actin mRNA in OVA-treated lungs and saline-treated lungs. Real-time RT-PCR shows a significant increase of TGF- β_1 and COL-1 mRNAs in the lungs of OVA-treated rats, compared with those of saline-treated rats. Data are expressed as mean $\pm$ s.d. ($n=8$). * $P<0.01$, compared with saline-treated rats.

Figure 2 Expression levels of Smad7, SnoN, and Ski proteins and mRNAs in the lungs of allergic rats. **(a)** Western blotting shows a significant reduction in the expression of Smad7, SnoN and Ski proteins in OVA-treated lungs, compared with saline-treated lungs. Data are relative ratios of expression intensities of Smad7, SnoN and Ski to β -actin in OVA-treated lungs and saline-treated lungs. **(b)** The relative ratios of Smad7, SnoN and Ski mRNAs to β -actin mRNA in OVA-treated and saline-treated lungs. Real-time RT-PCR shows a significant increase in the expression of Smad7, SnoN and Ski mRNAs in OVA-treated lungs, compared with saline-treated lungs. Data are expressed as mean $\pm$ s.d. ($n = 8$). * $P < 0.01$, compared with saline-treated lungs.

Figure 3 Expression levels of Arkadia, Smurf2 and T β RI in the lungs of allergic rats. Western blotting shows that the expression of Arkadia, Smurf2 and T β RI is increased in OVA-treated and saline-treated lungs. Data are relative ratios of expression intensities of Arkadia, Smurf2 and T β RI to β -actin in OVA-treated lungs and saline-treated lungs. Data are expressed as mean $\pm$ s.d. ($n = 8$). * $P < 0.01$, compared with saline-treated lungs.

The Interactions of Arkadia/Smurf2 with Smad7/SnoN/Ski/T β RI in the Lungs of OVA-Treated Rats

To determine the effects of Arkadia and Smurf2 on TGF- β signaling and airway remodeling, we analyzed the interactions of Arkadia/Smurf2 with Smad7, SnoN, Ski and T β RI in the lungs of OVA-treated rats using co-immunoprecipitation. In OVA-treated lungs, Arkadia-Smad7, Smurf2-Smad7, Arkadia-SnoN, Smurf2-SnoN, Arkadia-Ski and Smurf2-Ski complexes were all observed (Figures 4a–c). In addition, the Smurf2-T β RI complex was observed; however, there was no complex composed of Arkadia and T β RI (Figure 4d). These findings indicate that both Smurf2 and Arkadia interact with Smad7, SnoN and Ski, respectively, and Smurf2, but not Arkadia, interacts with T β RI in OVA-treated lungs.

Effects of Preincubation of Arkadia siRNA on the Expression of Arkadia, T β RI, Smad7, SnoN and Ski in TGF- β_1 -Stimulated NHBEs

Western blotting showed that the expression of Arkadia and T β RI were increased, while that of Smad7, SnoN and Ski were decreased in TGF- β_1 -stimulated NHBEs, compared with control NHBEs. The expression of Arkadia in TGF- β_1 -stimulated NHBEs was inhibited by preincubation of Ar-

kadia siRNA or lactacystin, while control siRNA did not affect the expression of Arkadia. Similar to the control siRNA, neither Arkadia siRNA nor lactacystin affected the expression of T β RI in TGF- β_1 -stimulated NHBEs. The low expression of Smad7, SnoN and Ski in TGF- β_1 -stimulated NHBEs was reversed by Arkadia siRNA or lactacystin, but not by the control siRNA (Figures 5a–c). However, real-time RT-PCR showed that Arkadia siRNA or lactacystin did not significantly affect the mRNA expression of Smad7, SnoN and Ski in TGF- β_1 -stimulated NHBEs (Figure 5d).

Effects of Preincubation of Smurf2 siRNA on the Expression of Smurf2, T β RI, Smad7, SnoN and Ski in TGF- β_1 -Stimulated NHBEs

Similarly, western blotting showed that the expression of Smurf2 and T β RI was increased, while Smad7, SnoN and Ski were decreased in NHBEs stimulated with TGF- β_1 , compared with control NHBEs. In addition, the expression of Smurf2 in TGF- β_1 -stimulated NHBEs was inhibited by preincubation of Smurf2 siRNA or lactacystin, but not by the control siRNA. Similar to the control siRNA and lactacystin, preincubation of Smurf2 siRNA did not affect the expression

Figure 4 The interactions of Arkadia/Smurf2 with Smad7/SnoN/Ski/TβRI in the lungs of allergic rats. Lung extracts collected from OVA-treated and saline-treated rats were subjected to Smad7, SnoN, Ski or TβRI immunoprecipitation (IP), followed by Arkadia or Smurf2 immunoblotting (IB). In OVA-treated lungs, Smad7-Arkadia and Smad7-Smurf2 (**a**), SnoN-Arkadia and SnoN-Smurf2 (**b**), Ski-Arkadia and Ski-Smurf2 (**c**) and TβRI-Smurf2 (**d**) complexes are noted. However, there is no TβRI and Arkadia complex. Representative data of three independent experiments are shown.

Figure 5 Effects of Arkadia siRNA on the expression of Arkadia, TβRI, Smad7, SnoN and Ski in TGF-β₁-stimulated NHBEs. (**a**) Western blotting showing the effects of Arkadia siRNA, control siRNA and lactacystin on the protein expression of Arkadia, TβRI, Smad7, SnoN and Ski in TGF-β₁-stimulated NHBEs. (**b**, **c**) The relative ratios of expression intensities of Arkadia, TβRI, Smad7, SnoN and Ski to that of β-actin in TGF-β₁-stimulated NHBEs. (**d**) Real-time RT-PCR showing effects of Arkadia siRNA, control siRNA and lactacystin on the mRNA expression of Smad7, SnoN and Ski in TGF-β₁-stimulated NHBEs. Data are relative ratios of Smad7, SnoN and Ski mRNAs to β-actin mRNA. NHBEs, control NHBEs; TGF-β₁, NHBEs treated with TGF-β₁; Arkadia siRNA, TGF-β₁-stimulated NHBEs in the preincubation of Arkadia siRNA; control siRNA, TGF-β₁-stimulated NHBEs in the preincubation of control siRNA; and lactacystin, TGF-β₁-stimulated NHBEs in the preincubation of lactacystin. Experiments were repeated three times with similar results, and the data are expressed as the mean ratio ± s.d. **P* < 0.01, compared with the control NHBEs; ▲*P* < 0.01, compared with the TGF-β₁-stimulated NHBEs.

of TβRI in TGF-β₁-stimulated NHBEs. In contrast to lactacystin, preincubation of Smurf2 siRNA or control siRNA did not reverse the low expression of Smad7, SnoN and Ski proteins in TGF-β₁-stimulated NHBEs (Figure 6).

DISCUSSION

In this study, we showed that the lung of rats treated with OVA showed the progressive development of inflammatory infiltration and collagen deposition, which were associated

Figure 6 Effects of Smurf2 siRNA on the expression of Smurf2, T β RI, Smad7, SnoN and Ski in NHBEs. (a) Western blotting showing effects of Smurf2 siRNA, control siRNA and lactacystin on the expression of Arkadia, T β RI, Smad7, SnoN and Ski in TGF- β_1 -stimulated NHBEs. (b, c) Relative ratios of expression intensities of Smurf2, T β RI, Smad7, SnoN and Ski to that of β -actin in TGF- β_1 -stimulated NHBEs. NHBEs, control NHBEs; TGF- β_1 , TGF- β_1 -stimulated NHBEs; Smurf2 siRNA, TGF- β_1 -stimulated NHBEs in the preincubation of Smurf2 siRNA; control siRNA, TGF- β_1 -stimulated NHBEs in the preincubation of control siRNA; and lactacystin, TGF- β_1 -stimulated NHBEs in the preincubation of lactacystin. Experiments were repeated three times with similar results, and the data are expressed as the mean ratio $\pm$ s.d. * P < 0.01, compared with the control NHBEs; ΔP < 0.01, compared with the TGF- β_1 -stimulated NHBEs.

with increased expression levels of TGF- β_1 , COL-I, Smad7, SnoN and Ski mRNAs, but simultaneous diminished levels of Smad7, SnoN and Ski proteins. We then found that the expression of Arkadia and Smurf2, which are known to induce ubiquitin-dependent degradation of Smad7, SnoN and Ski, was increased in OVA-treated lungs. Finally, we used co-immunoprecipitation, which showed that Smurf2 interacted with Smad7, SnoN, Ski and T β RI, and Arkadia only interacted with Smad7, SnoN and Ski, but not with T β RI. These findings suggest that there is little possibility of decreased translation of Smad7, SnoN and Ski proteins, and that Arkadia and Smurf2 may contribute to the reduction of these three proteins in the pathogenesis of allergic airway remodeling.

Arkadia and Smurf2, two E3 ubiquitin ligases, have a pivotal role in regulating TGF- β signaling through selectively targeting key components of the Smad pathway for degradation.^{11,12,20–23} Smurf2 is known to induce the degradation of TGF- β receptors through interaction with Smad7, which enhances the inhibitory effect of Smad7 on TGF- β signaling.^{19,22} In addition, Lin *et al*²⁵ reported that Smurf2-regulated TGF- β signaling by potentially reducing the transcriptional activity of Smad2. However, Bonni *et al*²³ have shown that Smurf2 may partner with phosphorylated Smad2 to enhance TGF- β signaling by degrading inhibitors such as SnoN. When these findings are considered together, it seems that Smurf2 may exert bi-functional effects (ie, either amplify or inhibit) on the TGF- β signaling through partnership with different classes of signal proteins and regulatory factors. As shown by immunoprecipitation, Smurf2 interacted with T β RI in OVA-treated lungs. Previous studies have shown that Smurf2 induces the degradation of the T β RI protein and enhances the inhibitory effect of negative reg-

ulators on TGF- β signaling.^{19,22} These results and our findings seem to indicate that Smurf2 could result in the inhibition of TGF- β signaling in airway remodeling pathogenesis, which is contradictory to the notion that the upregulation of TGF- β signaling is crucial for the pathogenesis of airway remodeling. There, we showed that T β RI was increased in OVA-treated lungs. When these findings are considered together, it seems that Smurf2 is not competent to degrade the Smad7, SnoN and Ski in airway remodeling pathogenesis. Unlike Smurf2, Arkadia, which is widely expressed in embryonic and adult tissues, especially in lungs, is of relative specificity in modulation of TGF- β signaling. Arkadia interacts strongly with Smad6/7, SnoN and Ski, degrades these proteins,^{20,21} and specifically activates the transcription of TGF- β through Smad3/Smad4-binding sites and induces degradation of SnoN.²⁶ In addition, it is very important that Arkadia is not recruited to TGF- β receptors and does not degrade the receptors.²⁰ In other words, Arkadia regulates the transcriptional activity of TGF- β through degradation of Smad7, SnoN and Ski, but not TGF- β receptors. Therefore, Arkadia amplifies TGF- β signaling through degradation of Smad7, SnoN and Ski.^{20,21} Taken together, we presume that the reduction of Smad7, SnoN, and Ski could be mainly attributed to the high expression of Arkadia in allergic airway remodeling.

To confirm this presumption, we knocked down the expression of endogenous Arkadia and Smurf2 using siRNA in NHBEs. It was observed that there was a high level of T β RI expression with low levels of Smad7, SnoN and Ski expression in TGF- β_1 -stimulated NHBEs compared with control NHBEs. When NHBEs were preincubated with Arkadia siRNA and lactacystin, the reduction in the expression of Smad7, SnoN and Ski proteins induced by TGF- β_1 was partly

reversed; however, the expression of T β RI protein and Smad7/SnoN/Ski mRNA were not affected. In contrast to Arkadia siRNA, Smurf2 siRNA had no influence on the expression of Smad7, SnoN and Ski proteins. Similar to Arkadia siRNA and lactacystin, Smurf2 siRNA also did not affect the expression of T β RI. In a previous study, Asano *et al*²⁷ have shown that the overexpression of Smurf2 and coexpression of Smad7 along with Smurf2 all did not affect the levels of T β RI protein in scleroderma fibroblasts. These findings, in agreement with ours, also suggest that Arkadia-mediated positive regulation of TGF- β signaling may have a promoting role in allergic airway remodeling.

In conclusion, Arkadia may contribute to the pathogenesis of airway remodeling through enhancing TGF- β signaling by inducing the reduction of the expression of Smad7, SnoN and Ski proteins in OVA-sensitized and -challenged rats. Therefore, the identification of key molecular players such as Arkadia would be useful for therapeutic intervention in airway remodeling, and further investigation is required to determine the exact mechanisms.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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