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Saponins of Dioscorea Nipponicae Inhibits IL-17A-Induced Changes in Biomechanical Behaviors of In Vitro Cultured Human Airway Smooth Muscle Cells

Airway hyperresponsiveness (AHR) is one of the main pathologic features of bronchial asthma, which is largely attributable to enhanced contractile response of asthmatic airway smooth muscle. Although β2 adrenergic receptor agonists are commonly used to relax airway smooth muscle for treating AHR, there are side effects such as desensitization of long-term use. Therefore, it is desirable to develop alternative relaxant for airway smooth muscle, preferably based on natural products. One potential candidate is the inexpensive and widely available natural herb saponins of Dioscorea nipponicae (SDN), which has recently been reported to suppress the level of inflammatory factor IL-17A in ovalbumin-induced mice, thereby alleviating the inflammation symptoms of asthma. Here, we evaluated the biomechanical effect of SDN on IL-17A-mediated changes of cultured human airway smooth muscle cells (HASMCs) in vitro. The stiffness and traction force of the cells were measured by optical magnetic twisting cytometry (OMTC), and Fourier transform traction microscopy (FTTM), respectively. The cell proliferation was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetry, the cell migration was measured by cell scratch test, and the changes of cell cytoskeleton were assessed by laser confocal microscopy. We found that the stiffness and traction force of HASMCs were enhanced along with the increases of IL-17A concentration and exposure time, and SDN treatment dose-dependently reduced these IL-17A-induced changes in cell mechanical properties. Furthermore, SDN alleviated IL-17A-mediated effects on HASMCs proliferation, migration, and cytoskeleton remodeling. These results demonstrate that SDN could potentially be a novel drug candidate as bronchodilator for treating asthma-associated AHR.
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Keywords: saponins of Dioscorea nipponicae, human airway smooth muscle cells, cell biomechanics, IL-17A

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1 Introduction

Respiratory function can be understood as a series of interactive biomechanical processes involving the structural elements of the lung, particularly the airway smooth muscle cells (ASMCs) playing a decisive role in regulation of pulmonary airway impedance. From the microscopic viewpoint, the cytoskeletal structure of ASMCs is a highly complex and dynamic system with specific micro/nanodynamic characteristics. In addition, the morphology and phenotypic transformation of ASMCs are affected by mechanical factors in the external microenvironment [1–5]. This inspires researchers to focus on the cellular biomechanics of ASMCs in the study of obstructive airway disease such as asthma. For example, previous study has shown that the biomechanical behavior of ASMCs can be altered by localized external stress stimulation, which may directly contribute to excessive ASMC contraction and thus airway stenosis associated with asthma [6]. As ASM dynamics is considered as the common final pathway of airway hyperresponsiveness (AHR) that is characteristic of asthma, ASMC biomechanics may be a key to the understanding of the underlying mechanism of AHR and the pathogenesis of asthma.

Although the pathogenesis of asthma has yet to be fully elucidated, recent evidences suggest that interleukin 17 A (IL-17A), a pro-inflammatory cytokine produced by T helper 17 cells, plays an important role in the development of asthma. For example, it has been shown that the expression of IL-17A is elevated in the bronchoalveolar lavage fluid and lung tissues from both allergen-induced asthmatic mice and patients with asthma in correlation with severity of the disease [7–9]. In addition, it is known that IL-17A can directly bind to the IL-17RA/RC receptor expressed on ASMCs to induce AHR at the tissue level [10], but the effect of IL-17A on ASMC contractility has not been evaluated at the cellular level.

For clinical treatment of asthma, the current therapy mainly uses glucocorticoids and β2 adrenergic receptor agonists to inhibit airway inflammation and dilate bronchial airways, respectively. However, these drugs are known for adverse side effects, in particular about half asthmatic patients are not responsive to them, and many become desensitized after long-term use of β2 agonist treatment [11]. Therefore, there is a need to develop new bronchodilator drugs for the treatment of asthma. An approach is to screen potential drug agent from natural materials such as herbs used in traditional medicine. Accordingly, pharmacopeia records suggest that natural plant rhizome Dioscorea nipponicae has antitussive, expectorant, and anti-inflammatory effects, and its active ingredient is saponins of Dioscorea nipponicae (SDN). Previous study has demonstrated that SDN can reduce airway tissue remodeling by inhibiting IL-17A in murine model of chronic asthma [12]. Since airway remodeling mainly manifests as structural changes of ASMCs including hyperplasia and hypertrophy that contribute to irreversible airflow obstruction and persistent AHR, SDN appears to be an effective agent to alleviate AHR through regulation of IL-17A-mediated human airway smooth muscle cells (HASMCs) structural changes [13]. It is not clear, however, whether SDN could affect HASMCs' biomechanical behaviors through mediation of IL-17A as a therapeutic agent for treating AHR.

Therefore, we hypothesize that in addition to its effect on the structural changes, SDN may also influence the functional changes of ASMCs due to IL-17A. To test this hypothesis, we aimed to evaluate the changes of biomechanical behaviors including stiffness, contraction force, and mobility of ASMCs cultured in vitro in the absence or presence of IL-17A followed by SDN treatment. Such investigation may not only reveal how IL-17A mediates airway contractile function at the cell level but also provide first evidence to demonstrate the potential of SDN as a novel bronchodilator drug agent for treating AHR in asthma.

2 Materials and Methods

2.1 Chemicals and Reagents. Saponins of Dioscorea nipponicae was purchased from Hangzhou Dawen Biotec Co., Ltd. (Hangzhou, Zhejiang, China), IL-17A was purchased from R&D Systems (Minneapolis, MN). Collagen type I, RGD peptide (amino acid sequence Arg-Gly-Asp) and 3-aminopropyltrimemethoxysilane were obtained from Sigma-Aldrich (St. Louis, MO). Sulfo-SANPAH was purchased from Pierce Biotechnology, Inc. (Rockford, IL). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). Insulin and transferrin were purchased from Solarbio (Beijing, China). Fluorescent beads of 0.2 μm diameter (F8811) were purchased from Invitrogen Life Technologies (Carlsbad, CA).

2.2 Culture and Drug Treatment of Human Airway Smooth Muscle Cells. All the HASMCs and cell culture reagents including the double antibody were purchased from ScienCell Research Laboratories, Inc. (Carlsbad, CA) unless noted otherwise. The culture medium of smooth muscle cell was supplemented with 2% fetus bovine serum and 1% smooth muscle cell growth supplement in the equilibrated, poly-L-lysine-coated culture vessel at 37 °C under 5% humidified CO_2. Culture medium was replaced every three days until the cells grew to approximately 70% confluence, and then every other day until the cells grew to approximately 90% confluence. Subsequently, cells were passaged with 0.25% trypsin and 1 mM ethylenediamine tetraacetic acid every 3 to 5 days, and cells from passage 2 to 8 were used for experiments.

Cells were seeded in a dedicated 96-well plate (1×10^4 cells per well) or 6-well plate (1×10^3 cells per well) for assessment of cell stiffness and traction force, or viability and migration, respectively. In each experiment, the cells were first maintained in culture to allow cell adherence to the culture dish for 24 h before drug treatment of IL-17A followed by SDN at different concentrations. The experimental condition for drug treatment of HASMCs with SDN and IL-17A was optimized by initial testing of the dose response of the cells to SDN or IL-17A with time and concentration of drug treatment varying from 6 to 48 h and 0.001 to 10 μg/mL, respectively. The dose response was determined by either MTT assay for cytotoxicity or assessment of cell stiffness.

To determine cytotoxicity by MTT assay, HASMCs were seeded in 96-well plate at 1×10^4 cells each well and cultured for 24 h, then treated with the drug at different concentrations (e.g., SDN at 0.001∼10 μg/mL) for 24 h. Afterward, HASMCs were cultured in MTT (0.5%) containing medium for another 4 h, and then incubated in 150 μL dimethyl sulfoxide each well with gentle oscillation. The absorbance (OD) at 490 nm was measured using a microplate reader, which reflected the number of live cells under the experimental conditions.

2.3 Assessment of Cell Stiffness. Cell stiffness was detected by optical magnetic twisting cytometry (OMTC) [14,15]. HASMCs were plated into a detachable 96-well plate coated with collagen type I at 1×10^4 cells per well. After 24 h, IL-17A was added at different concentrations (0, 0.001, 0.01, 0.1, 1, 10, 1000, 1000 ng/L), and then continued to culture for 24 h in insulin and transferrin medium (containing 0.285% of 2 mg/mL insulin, 0.1% of 5 mg/mL transferrin, 1% double antibody). Afterward, cells were treated with different concentrations (0, 0.001, 0.01, 0.1, 1 μg/mL) of SDN for 24 h. Ferrimagnetic beads specifically designed for OMTC experiments (gifts from the Professor Jeffrey Fredberg's laboratory at Harvard University School of Public Health) were incubated with cells for 20–40 min prior to testing.

Magnetic beads need to be coated with RGD peptide (Sigma-Aldrich) in advance. Briefly, the original stock solution of magnetic beads was resuspended, and the beads were washed once with carbonate buffer, then centrifuged at $200 \times g$ for 5 min, and diluted 1:10 with carbonate buffer. Then, RGD peptide was added

at the ratio of 1:20 to magnetic beads solution, followed by rotating on a HS-3 vertical mixer at 4 °C overnight. After washing in phosphate buffer saline by centrifugation, the beads were suspended into insulin and transferrin medium and stored at 4 °C until use.

The cell sample to be measured was placed in an OMTC culture chamber and magnetized 2–3 times at a voltage of 1000 V so that the magnetic beads were oriented in the same angle and direction. After the OMTC system was stabilized, the torsion (T) detection was continued for 5 min, while the instrument automatically recorded the time and magnetic beads displacement (D), followed by high-throughput analysis with the proprietary MATLAB software to obtain the cell stiffness, Gp as a ratio of T over D.

2.4 Assessment of Cell Traction Force. We adapted the Fourier transform traction microscopy (FTTM) technique for cell traction force measurement based on polyacrylamide substrate described previously [16–19]. Briefly, glass-bottom cell culture dishes were first treated with 0.1 M NaOH, then with 3-aminopropyltrimemethoxysilane for 5 min, followed by 0.5% glutaraldehyde in phosphate buffer saline for 30 min. The pretreated dishes were added with the acrylamide/bis-acrylamide mixture, containing 2% acrylamide and 0.1% or 0.25% bis, mixed with 0.2 μm-diameter fluorescent beads (F8811). The gel was covered with a piece of 12 mm diameter cover glass and turned upside down to let the microbeads move to the surface of the cover glass by gravity. The gel was about 70 μm thick, as determined by confocal microscopy. The free surface of the gel was activated with 1000 μL of 1 mM Sulfo-SANPAH and exposed to ultraviolet light for 90 min. The gel was then coated with collagen type I on the surface and stored at 4 °C before use.

Cells were seeded onto the polyacrylamide gel substrate at a density of 1×10^3 cells per well for 12 h, then exposed to recombinant human IL-17A protein for 24 h in serum-free medium, followed by addition of different concentrations of SDN (0, 0.001, 0.01, 0.1, 1 μg/mL) and incubation for another 24 h. Single HASMCs were imaged with phase contrast by an inverted optical microscope; then fluorescent images were recorded when the cell was attached. Then, the cell was treated with NaOH at 0.1 M and the cell-free bead positions were recorded as a reference point for bead displacements.

2.5 Assessment of Cell Proliferation, Migration, and Cytoskeletal Structure. The effect of IL-17A and SDN on the cell proliferation was detected by MTT assay and the change of cell migration rate was detected by a cell scratch assay. MTT assay was similar as described before for cytotoxicity test except that the HASMCs were seeded in a special 6-well plate at 3×10^5 cells per well, and exposed to IL-17A (0.001∼1000 ng/mL) for 24 h after the cell density reached about 80% followed by further exposure to SDN (0.001∼1 μg/mL) for 24 h. Then cell scratches were performed, and the cell migration on the scratched area was recorded using a live cell workstation. After cellular immunofluorescence staining, the HASMCs cytoskeleton remodeling was visualized by laser scanning confocal microscopy.

2.6 Statistical Analysis. The quantified experimental data were presented as mean ± standard deviation. Statistical analysis was performed using Origin 8.5.1 SR2 (OriginLab Corporation, Northampton, MA). One-way analysis of variance was applied for statistical comparisons between groups. Student's t-test is used for two independent samples. P value less than 0.05 was considered as statistically significant.

3 Results

3.1 Toxicity of Saponins of Dioscorea Nipponicae on Cultured Human Airway Smooth Muscle Cells. The MTT colorimetric method was used to detect the toxicity of SDN on HASMCs as to determine the appropriate range of drug concentrations. From initial testing results, it has been shown that HASMCs always responded to drug treatment time dependently, but appeared to reach maximum at ∼24 h (data not shown). Thus, we exposed cells with drug treatment for 24 h in the following experiments unless noted otherwise.

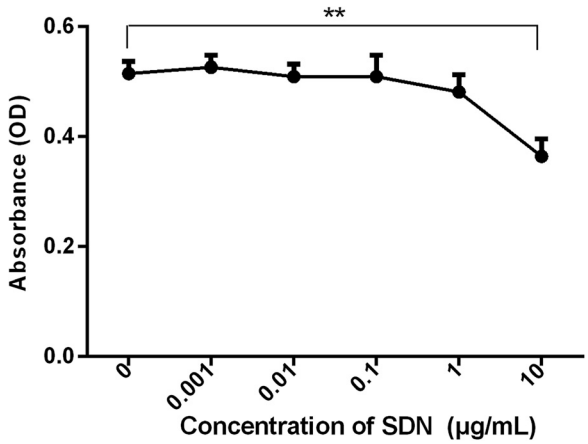

Fig. 1 The evaluation of toxicity of SDN on HASMCs using MTT colorimetric method. **$P < 0.01$ ($n = 3$).

When HASMCs were treated with SDN for 24 h at concentration of 0.001, 0.01, 0.1, 1, and 10 μg/mL, respectively, the growth activity (OD value by MTT assay) of HASMCs appeared to be not affected by SDN as the concentration increased from 0.001 to 1 μg/mL, but significantly inhibited when the concentration reached 10 μg/mL (Fig. 1). Therefore, the concentration of SDN in the range of 0.001∼1 μg/mL was used in the following experiments of our study.

3.2 Effect of Saponins of Dioscorea Nipponicae on IL-17A-Mediated Human Airway Smooth Muscle Cells Stiffness. We used OMTC to detect the change of HASMCs stiffness in response to treatment of IL-17A at different concentrations and treating durations, so to determine the optimal exposure condition for experiment. As shown in Fig. 2, IL-17A treatment did result in a dose- and time-dependent change in cell stiffness of HASMCs ($Gp/Gp_{(0)}$, where $Gp_{(0)}$ was the baseline cell stiffness prior to drug treatment). There was a statistically significant increase in cell stiffness when the IL-17A concentration reached 0.1 ng/mL (*$P < 0.05$) or 10 to 1000 ng/mL (**$P < 0.01$) compared with the control group (0 ng/mL), and the cell stiffness increased more than 50% at 10 ng/mL (Fig. 2(*a*)). Therefore, in the following experiments, we treated HASMCs with IL-17A only at 10 ng/mL. When treated at this concentration over time, the cell stiffness increased significantly at 12 h (*$P < 0.05$), then further increased by more than 50% at 24 h (**$P < 0.01$), and almost reached plateau afterwards (Fig. 2(*b*)). According to these results, we determined drug treatment of SDN and IL-17A at 0.001∼1 μg/mL and 10 ng/mL, respectively, for 24 h as the optimal conditions for the following experiments.

We further looked at whether SDN treatment could alter the cell stiffness after exposure to IL-17A (10 ng/mL). As shown in Fig. 2(*c*), the solvent vehicle (4 mM HCl) of IL-17A appeared to have no significant effect on the cell stiffness regardless of SDN treatment, as the ratio of cell stiffness ($Gp/Gp_{(0)}$) after treatment with HCl alone (vehicle control at 0 μg/mL SDN) or HCl followed by SDN (vehicle control at 0.001, 0.01, 0.1, 1 μg/mL SDN) over that of the drug free control groups remained approximately 1. In contrast, the cell stiffness was significantly increased after exposure to IL-17A at a concentration of 10 ng/mL ($Gp/Gp_{(0)}$ of SDN at 0 μg/mL, **$P < 0.01$). However, SDN reduced IL-17A-induced cell stiffness in a dose-dependent manner when the SDN concentration increased from 0.001 to 1 μg/mL, and the effect of IL-17A on cell stiffness became insignificant or completely abolished

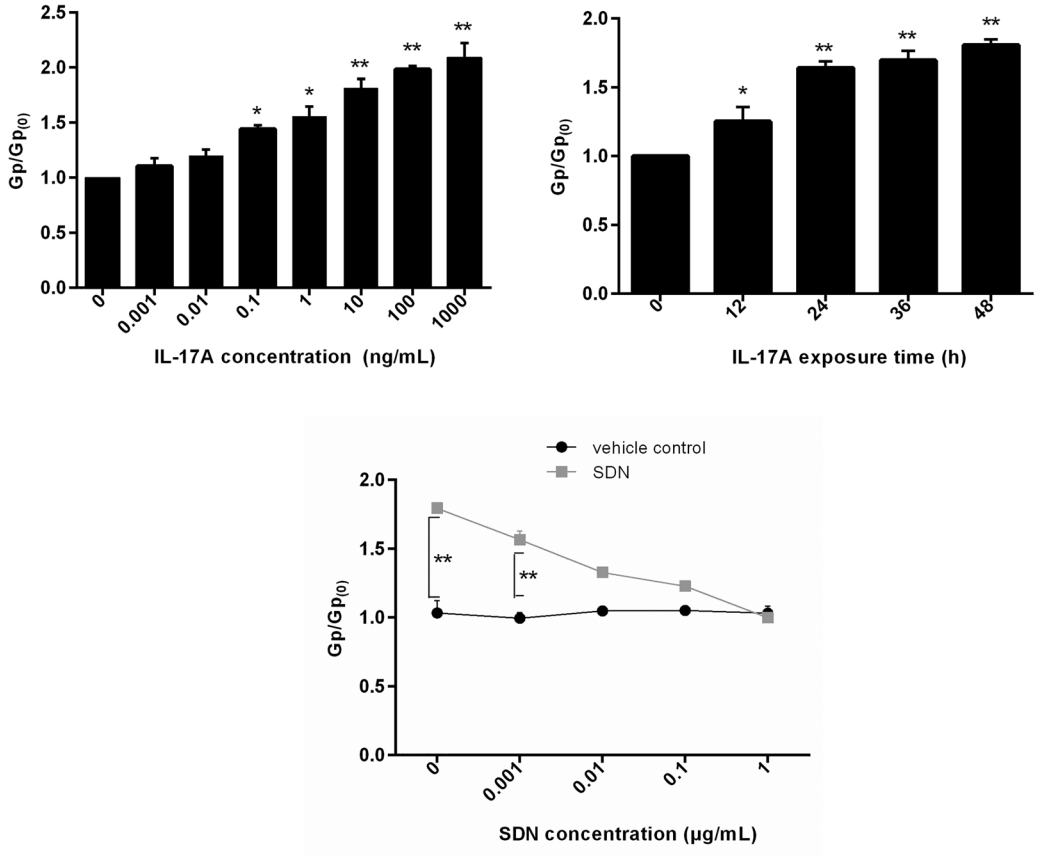

Fig. 2 The effects of SDN on IL-17A-induced change of HASMCs stiffness. (*a*) Cell stiffness changes under different IL-17A exposure concentrations. (*b*) Cell stiffness changes with IL-17A (10 ng/mL) at different time points. (*c*) Cell stiffness change with IL-17A (10 ng/mL) followed by SDN treatment (*$P < 0.05$, **$P < 0.01$ versus 0 group, $n = 3$).

when SDN concentration reached 0.01 μg/mL ($P > 0.05$) or 1 μg/mL ($P > 0.05$), respectively.

3.3 Effect of Saponins of Dioscorea Nipponicae on IL-17A-Mediated Human Airway Smooth Muscle Cells Traction Force. Figure 3 shows the effect of SDN on IL-17A-mediated HASMCs traction force measured by FTTM. When the cell grew on the base gel (Fig. 3(*a*)), the distribution map of fluorescent beads (0.2 mm diameter) differed from that at the base gel due to intracellular tension (Fig. 3(*b*)). The instrument software recorded the distributions of these two states and calculated the cell traction force based on the elasticity theory (Fig. 3(*c*)). After exposure to IL-17A (10 ng/mL) for 24 h, the cell traction force was increased significantly as compared to the vehicle control (379.32±94.97 Pa versus 281.84±89.82 Pa, **$P < 0.01$). However, when the cells were treated with SDN at concentration from 0.001 to 1 μg/mL following the IL-17A treatment, the IL-17A-induced HASMCs' traction force gradually decreased to be either significantly lower than that of IL-17A alone at 0.1 and 1 μg/mL SDN (IL-17A + 0.1 μg/mL SDN versus IL-17A, *$P < 0.05$, IL-17A + 1 μg/mL SDN versus IL-17A, **$P < 0.01$), or comparable with that of vehicle control (276.60±79.80 Pa versus 281.84±89.82 Pa for IL-17A + 1 μg/mL SDN versus vehicle, $P > 0.05$).

3.4 Effect of Saponins of Dioscorea Nipponicae on IL-17A-Mediated Human Airway Smooth Muscle Cells Proliferation, Migration, and Cytoskeletal Organization. Figure 4 displays the effect of IL-17A alone or together with SDN on HASMCs proliferation, migration, and cytoskeletal organization. The proliferation of HASMCs was measured by MTT assay. As the concentration of IL-17A increased from 0.001 to 1000 ng/mL, the cell absorbance (OD) also increased consistently and became significantly greater as the IL-17A concentration reached 0.1 ng/mL (*$P < 0.05$) and beyond (0.1∼100 ng/mL, *$P < 0.01$), indicating that IL-17A could enhance the proliferation of HASMCs in a dose-dependent manner (Fig. 4(*a*)). However, when the cells were treated with SDN after being stimulated with 10 ng/mL IL-17A, the cells responded with decreasing absorbance (OD) as SDN concentration increased from 0.001 ng/mL (*$P < 0.05$) to 0.01∼1 ng/mL (*$P < 0.01$), suggesting that SDN could alleviate the IL-17A-mediated proliferation of HASMCs in a dose-dependent manner (Fig. 4(*b*)).

The cell scratch test is a simple method to detect cell movement, for the coverage of HASMCs on the scratched area reflects the cell migration speed under different experimental conditions. As shown in Fig. 4(*c*), the scratch widths were similar at 0 h with 722.58 μm, 764.73 μm, and 724.59 μm in control group, IL-17A treatment group, and IL-17A + SDN treatment group, respectively. The cell images after 24 h growth indicate that IL-17A caused faster migration of HASMCs compared to the control. After treatment with SDN (1 μg/mL) following IL-17A, however, the scratched area not covered by HASMCs was almost the same as compared to the control group (IL-17A+SDN versus control), indicating that SDN inhibited the migration of HASMCs induced by IL-17A.

The cytoskeletal F-actin staining was observed by laser confocal microscopy, as shown in Figs. 4(*d*)–4(*f*). In normal HASMCs, the microfilaments were evenly distributed and regularly arranged along the long axis of the cells (Fig. 4(*d*)). This stereoscopic arrangement ensures that the contractile tension is evenly distributed from the cell to the extracellular matrix. After treatment with

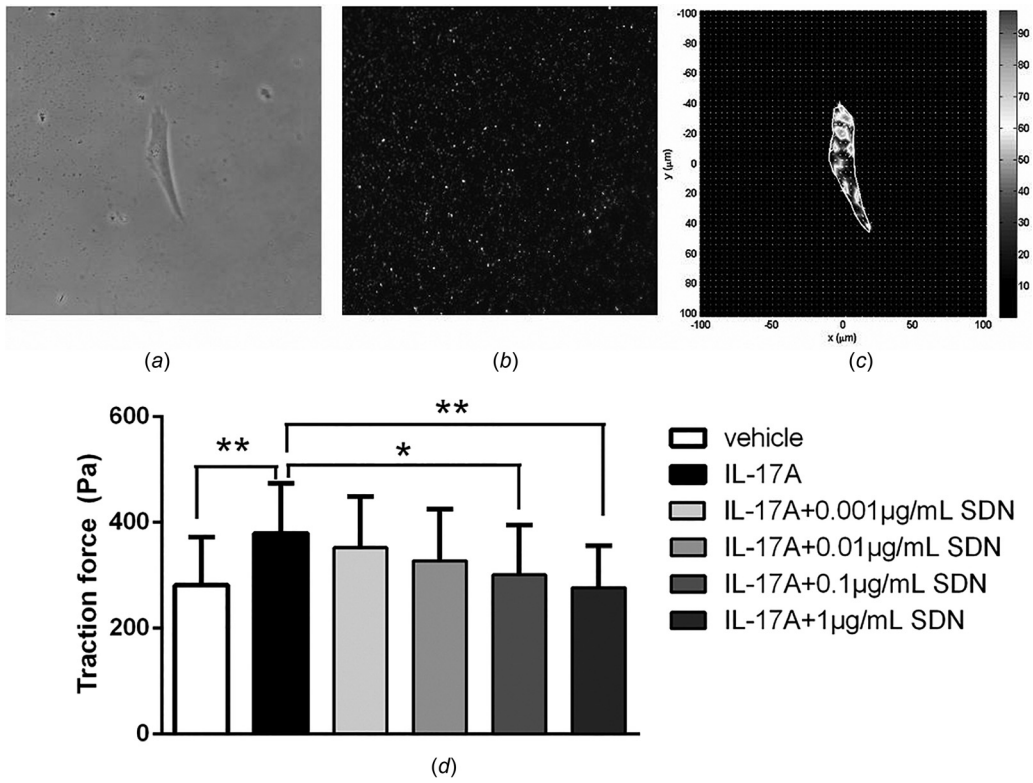

Fig. 3 The effects of SDN on IL-17A-induced HASMCs traction force. (*a*)–(*c*) Representative images of a single cell cultured on polyacrylamide gel (phase-contrast microscopy), fluorescent beads embedded in the base gel and the map of traction force computed from displacement of the fluorescent beads according to FTTM. (*d*) Traction force of HASMCs after treatment with IL-17A followed by SDN (*$P<0.05$, **$P<0.01$, $n=14$, mag. 400×).

IL-17A, the intensity of stress fibers increased significantly (Fig. 4(*e*)), which could in turn cause change in cell contractility. After treatment with SDN, IL-17A-induced increase of intracellular stress fibers was alleviated and diffusely distributed in the cytoplasm, and the geometric arrangement of cells was changed back (Fig. 4(*f*)). These results suggest that IL-17A can lead to remodeling of the cytoskeleton of bronchial smooth muscle, and SDN may inhibit the effect of IL-17A on cytoskeletal remodeling to some extent.

4 Discussion

Studies have shown that there is big difference in the cell mechanical properties between normal and asthmatic (abnormal) ASMCs [20]. Therefore, it is important to evaluate changes in mechanical properties of ASMCs in relation to pathogenesis as well as treatment of AHR airway diseases such as asthma. The two important indexes of cell mechanical properties are the stiffness and the traction force, which were used to quantify the changes of mechanical properties of ASMCs under the action of IL-17A and SDN in this study. According to the results of cell stiffness measured by OMTC and the cell traction force measured by FTTM, IL-17A treatment could increase the stiffness and traction force of the HASMCs, but subsequent SDN treatment could reverse these changes in a dose-dependent manner. It has been shown before that the IL-17RA/RC receptor subtypes are expressed on bronchial airway smooth muscle, and IL-17A binding can induce the enhancement of the contraction response of smooth muscle ring tissue, which plays an important role in the underlying mechanism of AHR [10,21]. The key finding of this study is that SDN could inhibit the effect of IL-17A on the mechanical properties of ASMCs, suggesting that SDN may be a potential drug agent for treatment of IL-17A-mediated AHR. Although IL-17A is a well-known pro-inflammatory cytokine and SDN is a natural product that has long been widely used in traditional medicine for anti-inflammatory purposes, it is the first evidence, to our best knowledge, to show that SDN could counteract IL-17A in regulation of the mechanical behaviors of ASMCs. Such a novel function of SDN to relax contracted HASMCs and its well-known function in inhibition of airway inflammation may be complementary to each other in asthma therapy to result in greater efficacy.

In addition to ASMC mechanical behaviors that ultimately determine airway contraction and relaxation, our results also demonstrated that SDN could counteract IL-17A in modulation of the proliferation, migration and cytoskeleton remodeling of HASMCs. The observation that IL-17A promoted proliferation of the HASMCs is consistent with the role of pro-inflammatory cytokines in inducting ASMC proliferation that leads to greater ASM mass and thus stronger bronchial response to stimuli and exacerbated AHR [22]. As specific to asthma pathogenesis, studies have shown that ASMCs from asthmatic patients proliferate much faster than their nonasthmatic counterparts [23,24]. On the other hand, it has been shown that enhanced migration of ASMCs, particularly the directional migration toward the airway intima induced by various cytokines, inflammatory mediators and growth factors, is equally important in contributing to greater ASM mass and AHR in asthma [25].

In regards the mechanism of SDN in reducing IL-17A-mediated changes in HASMCs behaviors, previous study has shown in mouse model of allergic asthma that SDN can reduce smooth muscle thickening and attenuate airway remodeling by inhibiting MMP-9 and increasing TIMP-1 protein expression [26]. This explains the therapeutic effect of SDN from the perspective

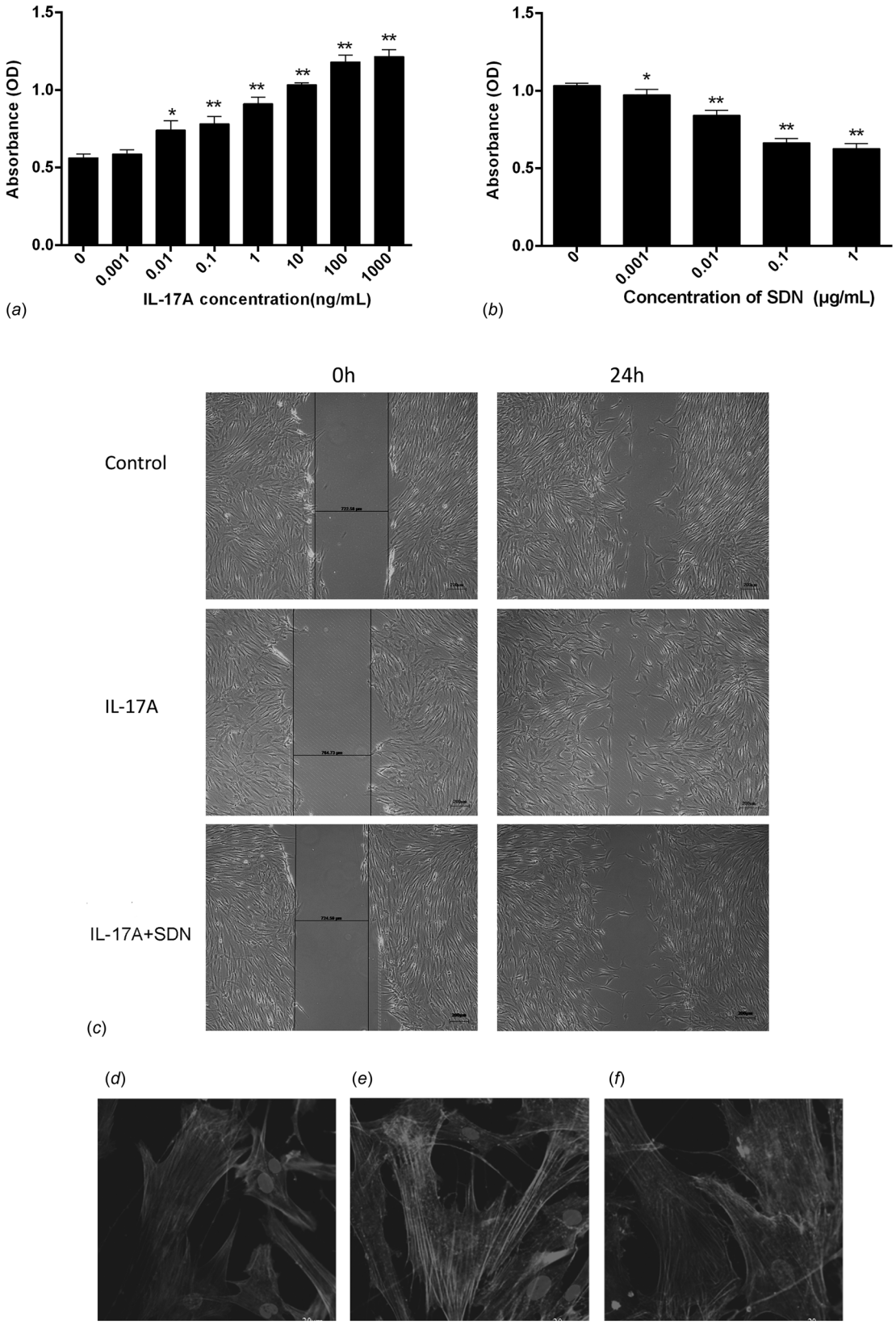

Fig. 4 The effects of IL-17A and SDN treatments on HASMCs proliferation, migration, and cytoskeletal remodeling. ((*a*) and (*b*)) Proliferative capacity of HASMCs as determined by cell absorbance (OD) in MTT assay after treatment with different concentrations of IL-17A (0.01∼10 ng/mL) alone, or with different concentrations of SDN (0.001∼1 μg/mL, 24 h) after 10 ng/mL IL-17A pretreatment for 24 h (*$P < 0.05$, **$P < 0.01$ versus 0 group, $n = 3$). (*c*) Cell migration measured by the scratched cell-free area under different conditions (scale bar = 200 μm). ((*d*)–(*f*)) Images of cytoskeleton F-actin staining by laser confocal microscopy in the absence or presence of drug treatment ((*d*)—control, (*e*)—IL-17A, and (*f*)—IL-17A+SDN, respectively, scale bar = 20 μm).

of airway remodeling at the animal level. Our study instead evaluated the therapeutic effect of SDN from the perspective of biomechanics at the cellular level.

Cell mechanics is thought to be largely determined by the structure and function of the cytoskeleton consisting of various protein filamentous structures including microfilaments, microtubules, and intermediate filaments. Among them, the microfilaments (filamentous actin protein structure also known as F-actin) are the most important for facilitating mechanical behaviors of the cell including maintenance of cell morphology, control of cell deformation, and movement as well as regulation of cell signaling. In particular, the reorganization of cytoskeletal microfilaments has been confirmed as the basis of contractile responses in airway smooth muscle in AHR [27–29]. Our results showed that IL-17A had an obvious effect on the formation and remodeling of F-actin in HASMCs, showing increased F-actin intensity in the presence of the cytokine. However, all the IL-17A-induced changes of F-actin structures in the HASMCs were found to be alleviated by SDN under the given conditions of drug treatment. These results further demonstrate that SDN indeed reduced cell stiffness and traction force of the HASMCs by inhibiting the reorganization of F-actin structures induced by IL-17A, but it remains to be confirmed whether SDN actually acted on HASMCs by specifically antagonizing the IL-17A receptor.

In summary, HASMCs were shown to undergo cytoskeletal reorganizations in response to stimulation with IL-17A, which in turn led to increased stiffness and traction force generation of the cells. These changes may all contribute to the excessive contractile function of ASMCs manifested as the characteristic AHR in asthma. However, we found that SDN could alleviate all the IL-17A-mediated changes in HASMCs, particularly the changes of biomechanical behaviors such as cell stiffness and traction force generation, as well as the cytoskeletal organization such as F-actin stress fiber formation, suggesting that SDN may be a potential drug candidate as bronchodilator for treating IL-17A-associated AHR.

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