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*Original Research*

# Excessive Stretch of Airway Smooth Muscle (but Not Epithelial) Cells Activates Paracrine TGF-β1 to Induce Smad2-Mediated G1 Arrest in Airway Epithelial Cells

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**Abstract**

**Background:** Although mechanical ventilation (MV)-associated excessive stretch directly damages airway epithelial cells (AECs) and contributes to ventilator-induced lung injury (VILI), the contribution of paracrine factors from airway cells, particularly airway smooth muscle cells (ASMCs) and AECs, remains poorly understood. As stretch-sensitive cells, ASMCs and AECs may respond to excessive stretch by secreting paracrine factors such as transforming growth factor β1 (TGF-β1), thereby disrupting airway epithelial integrity. This study compared the sensitivity of cultured ASMCs and AECs to MV-associated excessive stretch and their ability to induce paracrine factors, particularly TGF-β1, that inhibit AEC proliferation *in vitro*. **Methods:** Human ASMCs and AECs (16HBE14o-) were cultured under static, physiological stretch (5% strain), or pathological excessive stretch (13% strain) conditions for 72 h. Conditioned media were collected and used to culture 16HBE14o- cells for 48 hours. Active and total TGF-β1 levels in the conditioned media were measured using an enzyme-linked immunosorbent assay. Cell viability, proliferation, migration, stress fiber formation, cell cycle distribution, and Smad2 signaling were assessed using the Cell Counting Kit-8 assay, cell counting, wound healing assay, immunofluorescence, flow cytometry, quantitative reverse transcription polymerase chain reaction, and Western blotting, respectively. **Results:** Conditioned media derived from ASMCs, but not 16HBE14o- cells, cultured under 13% excessive stretch inhibited 16HBE14o- cell proliferation. In addition, excessive stretch enhanced TGF-β1 activation in ASMCs in an integrin αV-dependent manner. Experiments using a TGF-β1-neutralizing antibody and transforming growth factor β receptor I inhibitors demonstrated that inhibition of 16HBE14o- cell proliferation by conditioned media derived from excessively stretched ASMCs was dependent on TGF-β1 activation and Smad2 signaling. **Conclusion:** Conditioned media derived from ASMCs, but not AECs, exposed to MV-associated excessive stretch induced G1 cell cycle arrest in AECs via TGF-β1/Smad2 signaling. These findings suggest that ASMCs are more sensitive than AECs to excessive stretch-induced TGF-β1 activation and may thereby contribute to disruption of airway epithelial integrity. Targeting this paracrine pathway may warrant further investigation as a potential strategy for preventing or treating VILI.

**Keywords:** mechanical ventilation; excessive stretch; crosstalk of airway smooth muscle cells and airway epithelial cells; transforming growth factor-β1; proliferation

## 1. Introduction

Mechanical ventilation (MV) is a life-saving intervention for acute respiratory distress syndrome (ARDS) patients, but it unambiguously induces lung injury known as ventilator-induced lung injury (VILI) [1,2,3]. This remains an unmet clinical challenge, as multiple clinical trials have failed to prevent or effectively treat VILI, and no specific pharmacological therapies are currently available.

Although abundant evidence shows that MV-associated excessive stretch (>10% strain) leads to lung injury, the underlying mechanisms are thus far insufficiently elucidated, thereby hindering the development of clinical treatment [4,5,6,7]. Currently, studies have primarily focused on a single cell type for cellular responses to stretch, e.g., either airway smooth muscle cells (ASMCs) or airway epithelial cells (AECs) [5,7,8,9,10]. These studies largely overlook the fact that ASMCs and AECs do function collectively as a local trophic unit to respond to stretch [6]. Additionally, different types of cells in the airway may crosstalk via paracrine factors to contribute to airway pathogenesis [11,12,13]. For example, human AECs subjected to endoplasmic reticulum (ER) stress can release IL-1 to trigger inflammatory responses in human lung fibroblasts [14]. However, few studies have focused on comparing the sensitivity of ASMCs and AECs to MV-associated excessive stretch in the secretion of paracrine factors to induce AEC dysfunction [15].

Healthy AECs form a protective barrier of the airway against environmental hazards, and their dysfunction may

disrupt airway barrier integrity and thus induce lung injury [8]. On the other hand, ASMCs act not only as a mechanical sensor but also as a secretory effector to produce modulators such as transforming growth factor-β (TGF-β) to induce the dysfunction of AECs [16]. These cell types are directly exposed to excessive stretch during MV, yet their distinct roles (contractile/secretory vs. barrier/secretory) may elicit fundamentally different paracrine responses. The differential sensitivity of these two cell types to stretch-induced TGF-β activation and release has important implications for VILI, suggesting that they may contribute disproportionately to its pathogenesis.

As a pleiotropic growth factor, TGF-β1 induces cell cycle arrest of AECs via activating Smad signaling [17]. Interestingly, TGF-β1 is typically secreted as a latent form that binds to the matrix via latency-associated peptide (LAP) and latent TGF-β binding protein-1 (LTBP1), and its activation is tightly regulated by protease or stretch [18,19,20]. For example, it has been revealed that the stretch-induced activation of TGF-β1 is dependent on integrin αV, and a small fraction of activation of this latent TGF-β generates maximal cellular responses [21,22,23]. However, it remains unclear whether ASMCs and AECs are directly linked in the activation of TGF-β1 under the conditions of MV-associated excessive stretch and the regulation of airway epithelial proliferation. We hypothesize that excessive stretch activates TGF-β1 paracrine signaling differently in ASMCs versus AECs, which subsequently leads to inhibition of AECs proliferation.

In this study, we compared the effect of conditioned medium derived from ASMCs and AECs cultured in static, 5% stretch, and 13% stretch on AEC proliferation, especially focusing on the activation of TGF-β1. We found that only the conditioned media derived from excessively stretched ASMCs but not that from excessively stretched AECs inhibited proliferation of AECs, together with TGF-β1 activation in ASMCs and cell cycle arrest in AECs, which may underlie the pathological pathways involved in mechanically disrupting airway integrity and thus may be targeted for the treatment of VILI.

## 2. Materials and Methods

### *2.1 Chemicals*

α-smooth muscle actin (α-SMA) primary antibody was purchased from BOSTER (#BM0002, BOSTER, Wuhan, China). TGF-β1 (#T7039, Sigma, St. Louis, MO, USA), CWHM-12 (an integrin αV inhibitor, #HY-18644) and Galunisertib (TGF-β receptor 1 (TGFR1) inhibitor, synonyms LY2157299, #HY-13226) were purchased from MedChemExpress (Shanghai, China). Collagen type I was purchased from Merck (#08-115, Merck Ltd, Beijing, China). Trypsin (#25200056), penicillin-streptomycin (#15140122), Dulbecco's modified Eagle's medium (#11965-084, DMEM), and fetal bovine serum (#16000-044, FBS) were purchased from ThermoFisher (Waltham, MA, USA). RNase A (#ST578) and propidium iodide (#ST511) were purchased from Beyotime Biotechnology (Shanghai, China).

### *2.2 Cell Culture and Stretch or Conditioned Medium Treatment*

Human primary ASMCs (#BNCC339826, BNCC, Beijing, China) were cultured in a humidified incubator (5% $CO_2$, 37 °C) with DMEM (10% FBS, 2 mM L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin). All primary ASMCs were validated for their identity by smooth muscle cell marker analysis using the immunofluorescence technique to observe the α-SMA distribution and tested negative for mycoplasma.

Human AECs from the 16HBE14o- cell line (#SCC150, Merck Ltd, Beijing, China), hereafter 16HBE14o- cells, were cultured under the same conditions as ASMCs. The 16HBE14o- cell line is a basal cell type characterized by functional tight junctions and well-developed barrier properties. Therefore, healthy cells with cubic-like morphology were used in this study. All cell lines were validated by short tandem repeats (STR) profiling and tested negative for mycoplasma.

For stretch treatment [24], exponentially proliferating ASMCs ($2 \times 10^4$ cells/cm$^2$) at passages 3–8, and 16HBE14o- cells ($4 \times 10^4$ cells/cm$^2$) were seeded, respectively, on Bioflex 6-well plates (coated with type I collagen, FlexCell International, Hillsborough, NC, USA) for 24 h in FBS-free medium. Subsequently, the cells were subjected to cyclic strain at 0.5 Hz: either 5% strain (simulating the physiological airway strain) or 13% strain (simulating the pathological airway strain during MV) for 72 h using the FlexCell 5000 cell stretching system (FlexCell International) according to the literature reports [7,25]. Cells cultured on Bioflex plates under static conditions were used as negative controls (Static, hereafter). At the end of the 72 h stretch treatment, conditioned media were collected from each well and stored at –70 °C before use in the following experiment.

For conditioned medium treatment, subconfluent, exponentially proliferating 16HBE14o- cells ($1 \times 10^4$ cells/cm$^2$) were plated in 96-well plates (ThermoFisher, Waltham, MA, USA) for 24 h. Subsequently, the media were replaced with the conditioned media collected from the stretch treatment experiments. Then the cells were maintained in the conditioned media for up to 48 h, and at different time points, cell proliferation, cell cycle, and stress fiber distribution were detected, respectively (Fig. 1).

### *2.3 Inhibitor Treatment*

To investigate whether integrin αV is involved in the stretch-induced activation of TGF-β1 in ASMCs and AECs, cells were pretreated with CWHM-12 (a pan integrin αV inhibitor, 5 µM) for 30 min before stretch treatment.

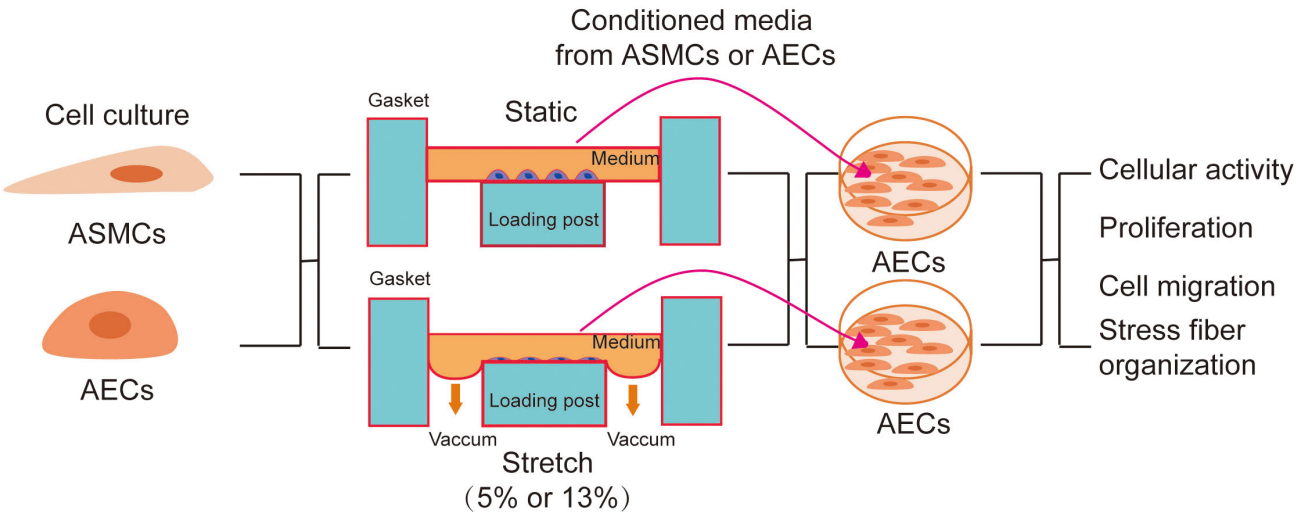


**Fig. 1. Schematic of the experimental methods**. ASMCs, airway smooth muscle cells; AECs, airway epithelial cells.

To investigate whether TGF-β1 signaling is involved in the proliferation of AECs in conditioned media from the stretch-treated ASMCs and AECs, 16HBE14o- cells were pretreated with 7.5 μg/mL TGF-β1 neutralizing antibody (AF8145, Beyotime Biotechnology, Shanghai, China) or Galunisertib (a TGFR1 inhibitor, also known as LY2157299, 10 μM). TGF-β1 treatment (1 ng/mL) was used as a positive control.

### *2.4 Enzyme-Linked Immunosorbent Assay (ELISA)*

Active TGF-β1 was measured from the medium using the TGF-β1 ELISA kit (#EK981, Multisciences Biotech, Hangzhou, China) according to the manufacturer's protocol. The linear detection range is 31.25–2000 pg/mL with a sensitivity of 3.36 pg/mL. The intra-assay coefficient of variation (CV) is 2.0–5.3%, and the inter-assay CV is 4.1–6.0%. Total TGF-β1 was measured from the medium heated at 80 °C for 8 min to activate all latent TGF-β1 as described elsewhere [19].

### *2.5 Cell Proliferation Evaluation*

Cell proliferation was evaluated in terms of the increase in cell activity and cell number, respectively, with time. The increase in cell activity with time was evaluated with the Cell Counting Kit-8 (CCK-8) assay kit (#C0037, Beyotime Biotechnology, Shanghai, China). Briefly, 16HBE14o- cells were inoculated in 96-well plates ($1 \times 10^4$ cells/well) and cultured for 24 h, and then treated with conditioned media derived from the stretched ASMCs or 16HBE14o- cells for up to 48 h. At 0, 12, 24, 36, and 48 h time points, respectively, CCK-8 solution (10 μL) was added to the cells and maintained for 4 h at 37 °C. Absorbance was determined using a microplate reader at 450 nm (Elx100, ThermoFisher). Cell viability was expressed as the fold change of the OD value in the experimental group to that of the control group.

The increase in cell number with time was evaluated similarly by using the trypan blue exclusion cell counting method. Briefly, 16HBE14o- cells were seeded in 6-well plates (ThermoFisher, Waltham, MA, USA) at a density of $2 \times 10^4$ cells/cm$^2$ for 24 h, then treated with conditioned media derived from the stretched ASMCs or 16HBE14o- cells for 48 h, with or without TGF-β1 signaling inhibitor. At 0, 12, 24, 36, and 48 h time points, respectively, the cell number was counted by using a cytometer after staining with 0.4% trypan blue for living cells.

### *2.6 Cell Cycle Analysis*

The cell cycle was analyzed by using flow cytometry. Briefly, 16HBE14o- cells in 6-well plates seeded ($2 \times 10^4$ cells/cm$^2$) for 24 h were treated with conditioned media derived from the excessively stretched ASMCs for 48 h with or without inhibitors. Afterwards, the cells were collected, fixed with 70% ethanol, and incubated on ice for 30 min. Cells were washed with ice-cold phosphate-buffered saline (PBS) and then centrifuged at 1200 rpm for 5 min. The pellets were resuspended in PBS containing RNase A (100 μg/mL) and propidium iodide (40 μg/mL), and incubated at 37 °C for 30 min in the dark. The cell cycle profile was determined with a flow cytometer (BD Accuri C6, BD Biosciences, San Jose, CA, USA) and analyzed with FlowJo software (version 10, FlowJo LLC).

### *2.7 Quantitative Reverse Transcription PCR (qRT-PCR) Assessment of mRNA Expression*

The mRNA expression of *Cyclins* (*Cyclin A*, *B*, *D*, and *E*) and *SMADs* (*SMAD1*, *2*, *3*, *4*, *5*, *6*, and *7*) was measured by qRT-PCR. Briefly, total RNA was extracted using the TRI Reagent RNA Isolation Reagent (#T9424, Sigma, St. Louis, MO, USA). 500 ng RNA was used to generate first-strand cDNA using the Revert Aid First Strand cDNA Synthesis Kit (#K1622, ThermoFisher). Primers for *Cyclins*

and *SMADs* as target genes and *GAPDH* as reference gene (**Supplementary Table 1**) were purchased from General Biosystems (Anhui, China). PowerUp SYBR Green Master Mix (#A25742, Applied Biosystems, CA, USA) was used. The reaction was run in the StepOnePlus qRT-PCR system (Applied Biosystems, Carlsbad, CA, USA) using 1 µL of the cDNA in a 10 µL reaction solution in triplicate. Fold changes in mRNA expression were calculated using $C_T$ values from three independent experiments. Calibration and normalization were done using the $2^{-\Delta\Delta CT}$ method, where $\Delta C_T = C_T$ (target gene) – $C_T$ (reference gene).

### *2.8 Western Blot*

Protein expression levels of phosphorylated and total Smad2 (pSmad2 and Smad2) in 16HBE14o- cells were evaluated by using Western blot. Briefly, 16HBE14o- cells treated with conditioned media from excessively stretched ASMCs were lysed, and 30 µg of protein was added per well. Electrophoresis was performed in 10% polyacrylamide separating gel with a 5% stacking gel. Proteins were transferred to polyvinylidene fluoride (PVDF) membranes (0.45 µm, Millipore, Billerica, MA, USA). After 1 h incubation in blocking solution (5% bovine serum albumin, BSA), membranes were incubated with respective primary antibodies: Smad2 (AF1300, Beyotime Biotechnology, Shanghai, China), pSmad2 (AF2545, Beyotime Biotechnology). Goat anti-mouse secondary antibody (1:1000, DyLight 800, ThermoFisher Scientific, #SA5-10036) or goat anti-rabbit secondary antibody (1:1000, DyLight 680, ThermoFisher Scientific, #35518) were used as a secondary antibody.

### *2.9 Statistical Analysis*

Statistical analysis was performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA). All data that followed a normal distribution, verified using the Shapiro-Wilk test, were therefore reported as means ± standard deviation (S.D.), and group size (n) represents 3 independent experiments with different biological replicates in each group. Comparison of means between two groups was performed by an unpaired Student's *t*-test. Comparison of means among three or more groups was performed using One-way analysis of variance (ANOVA), followed by a Post Hoc test (Tukey HSD method) or Two-way ANOVA, followed by the Tukey HSD method. Statistical significance is represented by asterisks (*$p < 0.05$; **$p < 0.01$).

## 3. Results

### *3.1 Excessive Stretch Induces Soluble Factors From ASMCs but Not AECs to Inhibit AECs Proliferation*

Fig. 2 shows the effects of conditioned media derived from the stretched-ASMCs or AECs on the proliferation of AECs. In all cases, AECs, i.e., 16HBE14o- cells appeared to grow with time for up to 48 h, in terms of fold change of cell activity assayed by CCK-8 (panel A), and cell counting (number of cells/cm$^2$, panel B). However, the increase in cell activity and cell counting of 16HBE14o- cells were both inhibited by ~50% at 48 h when the cells were treated with conditioned media derived from ASMCs cultured in 13% Stretch, as compared to the cells treated with conditioned media derived from ASMCs cultured in Static ($p$ = 0.001). On the contrary, none of the other conditioned media had any effect on the proliferation of 16HBE14o- cells, whether it was derived from ASMCs cultured in Static, 5% Stretch, or derived from 16HBE14o- cells cultured in Static, 5% Stretch, 13% Stretch. We also analyzed the cellular activity and proliferation of ASMCs and AECs under Static, 5% Stretch, and 13% Stretch with CCK-8 assay and cell counting (**Supplementary Fig. 1**). The results show that 13% excessive stretch had no significant effect on the cell activity and proliferation of ASMCs and AECs, thereby excluding the influence of cell number on the secretion of soluble factors. These data indicate that only excessive stretch of ASMCs but not AECs could induce certain soluble factors in the conditioned media, thereby inhibiting the proliferation of AECs.

### *3.2 Excessive Stretch Activates TGF-β1 in the Conditioned Media of Cultured ASMCs Depending on Integrin αV*

Fig. 3 shows the effects of stretch on the activation of TGF-β1 in the conditioned media derived from ASMCs or AECs. We measured active and total TGF-β1 levels, and then calculated the active/total TGF-β1 ratio (%) in the conditioned media using ELISA. Results from the conditioned media derived from ASMCs indicate that 13% Stretch increased the active TGF-β1 level by ~3 fold (panel A left, $p$ = 0.001), the total TGF-β1 level by ~45% (panel A middle, $p$ = 0.029), and the active/total TGF-β1 ratio from 15 ± 2% to 42 ± 2% (Panel A right, $p$ = 0.001), whereas 5% Stretch had no significant effect in any case, as compared to the Static group.

As shown in panel B, 13% Stretch also significantly increased the active ($p$ = 0.014) and total ($p$ = 0.017) TGF-β1 levels in the conditioned media derived from 16HBE14o- cells, as compared to the Static group. However, the extent of increase of active and total TGF-β1 levels in the conditioned media from 16HBE14o- cells appeared lower than that from ASMCs, and the active/total TGF-β1 ratio was not increased by 13% Stretch, as compared to the Static group. 5% Stretch also did not affect the TGF-β1 level in the conditioned media from 16HBE14o- cells. These data show that although 13% excessive stretch enhanced the TGF-β1 activation of AECs, it did not directly activate latent TGF-β1 of AECs.

In addition, we found that the increase in TGF-β1 level in the conditioned media derived from ASMCs cultured in 13% Stretch condition was largely inhibited when the cells were pretreated with CWHM-12 (integrin αV inhibitor), but not with the pan-protease inhibitor consisting of 1 mM p-toluenesulfonyl-l-arginine methyl ester (protease inhibitor) (panel C). These results indicate that excessive stretch can

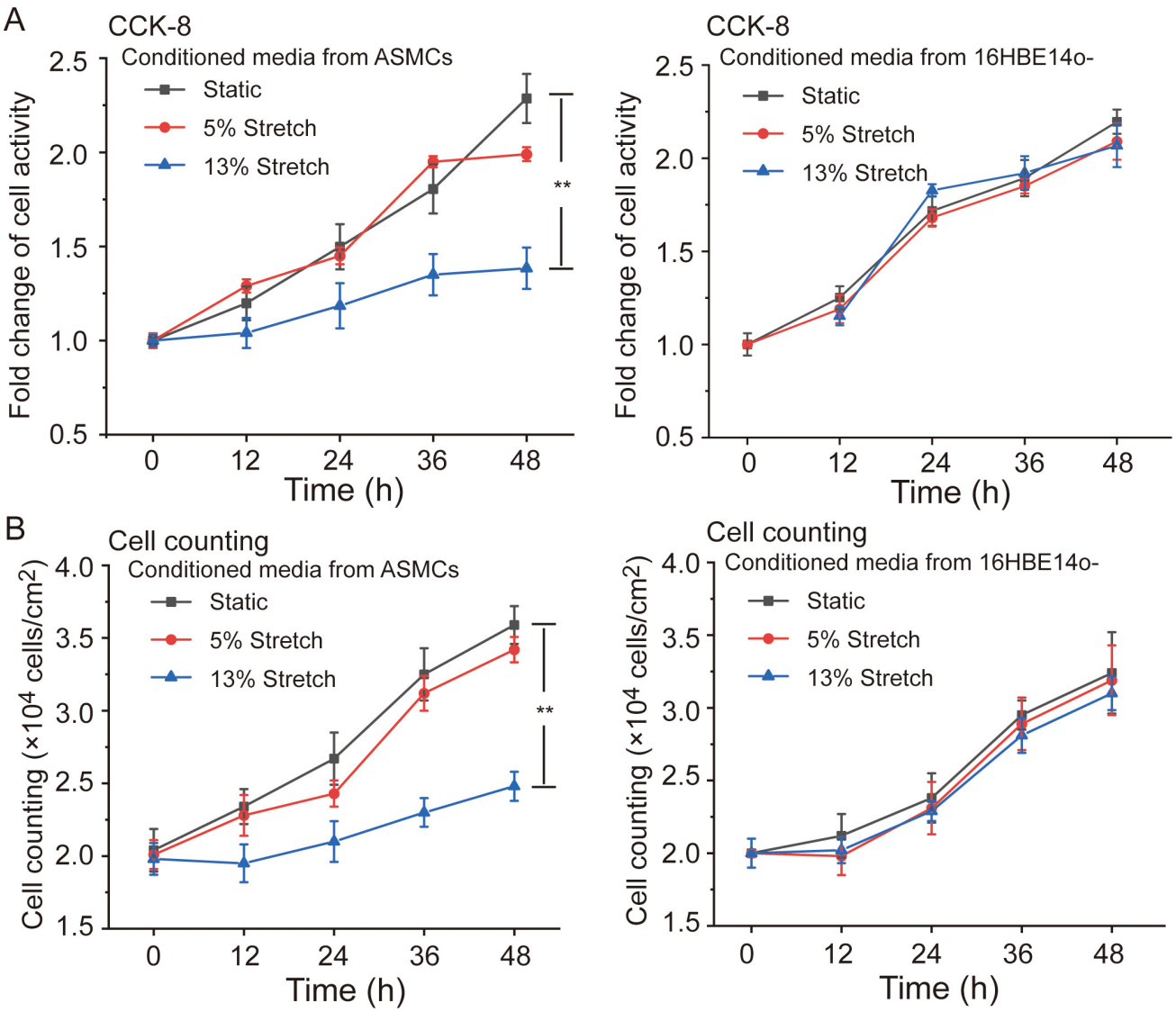


**Fig. 2. Effect of conditioned media derived from stretched-ASMCs or 16HBE14o- cells on the proliferation (cell activity and cell counting) of 16HBE14o- cells**. (A) Cell activity of 16HBE14o- cells detected using CCK-8 assay. (B) Cell counting of 16HBE14o- cells detected using the cell counting assay. 16HBE14o- cells were cultured in conditioned media derived from ASMCs (left panel) or 16HBE14o- cells (right panel) cultured in Static, 5% Stretch, or 13% Stretch conditions. Data were presented as means ± S.D., n = 3 independent experiments in each group (3 biological replicates for each experiment). $^{**}p < 0.01$ compared with the static group. CCK-8, cell counting Kit-8; S.D., standard deviation.

induce and activate TGF-β1 from ASMCs via integrin αV pathway.

### *3.3 Excessive Stretch Activates TGF-β1 From ASMCs to Activate Smad2 Signaling in AECs*

Using qRT-PCR, we compared the mRNA expression levels of *SMAD1*, *2*, *3*, *4*, *5*, *6*, *7* in 16HBE14o- cells cultured in normal medium. The data indicate that *SMADs* mRNA expression levels in 16HBE14o- cells were rank-ordered as: *SMAD2* > *SMAD5* > *SMAD3* > *SMAD4* > *SMAD7* > *SMAD6*, and *SMAD1* was not detected (**Supplementary Fig. 2**). Since *SMAD2* was the most expressed *SMAD* mRNA in 16HBE14o- cells, we further evaluated the protein expression levels of phosphorylated and total Smad2 (pSmad2, Smad2) in 16HBE14o- cells treated with conditioned media derived from ASMCs under a 13% stretch condition, using Western blot. As shown in Fig. 4, unlike the media derived from static ASMCs, the conditioned media derived from excessively stretched ASMCs caused a significant increase in Smad2 phosphorylation, which was similar to the effect of treatment with 1 ng/mL exogenous TGF-β1, reflecting TGF-β1 signaling. In addition, the Smad2 phosphorylation in 16HBE14o- cells cultured in conditioned media from stretched ASMCs was inhibited in the presence of TGF-β1 neutralizing antibody or TGFR1 inhibitor.

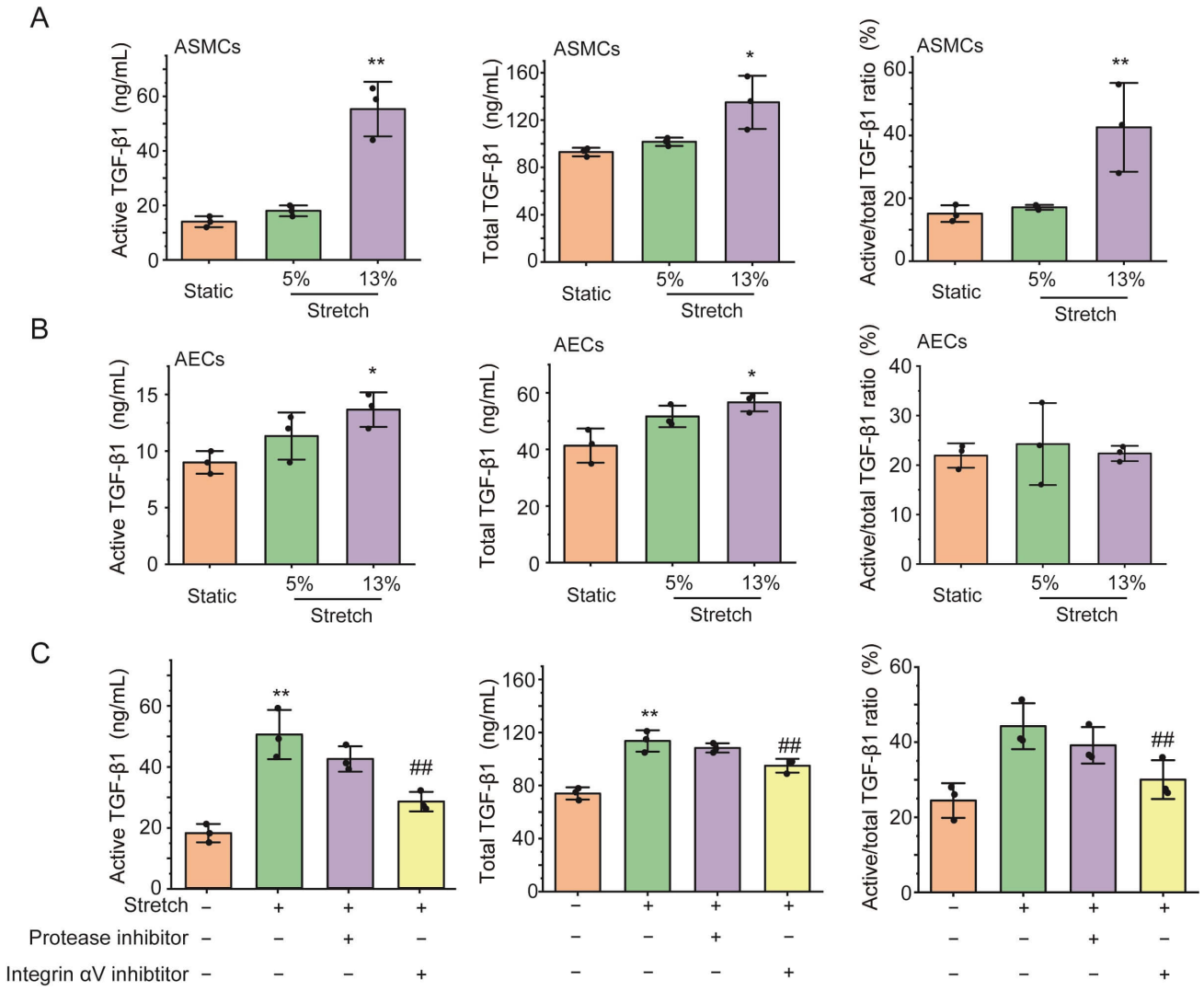


**Fig. 3. TGF-β1 activation in the media of stretched ASMCs or AECs**. (A,B) From left to right panel, Active, Total TGF-β1 level (ng/mL), and Active/total TGF-β1 ratio (%) in the media of ASMCs, AECs (16HBE14o- cells) cultured in Static, 5% Stretch, or 13% Stretch conditions. (C) From left to right panel, Active, Total TGF-β1 level (pg/mL), and Active/total TGF-β1 ratio (%) in the media of ASMCs cultured in Stretch condition (13%, 72 h), in the absence or presence of 30 min pretreatment with serine protease inhibitor (1 mM, p-toluenesulfonyl-l-arginine methyl ester) or integrin αV inhibitor (5 μM, CWHM-12). Data are presented as means ± S.D., n = 3 independent experiments in each group (3 biological replicates for each experiment), $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the Static group, $^{\#\#}p < 0.01$ compared to the Stretch group. TGF-β1, transforming growth factor β1.

### *3.4 Cell Cycle Arrest of AECs in the Conditioned Media Derived From Stretched ASMCs*

Since conditioned media derived from ASMCs cultured in the excessive stretch condition inhibited AEC proliferation, we then investigated the cell cycle progression of AECs in the conditioned media from excessively stretched ASMCs by using flow cytometry. Cells were stained with propidium iodide (PI) and analyzed by flow cytometry. The sequential gating strategy was as follows: debris was excluded on an FSC-A vs. SSC-A dot plot by gating the main cell population; doublets and aggregates were excluded using an FSC-H vs. FSC-A gate to retain singlets; further doublet discrimination was performed using a PI-A vs. PI-W plot; and cell cycle distribution was analyzed on the PI-A histogram, with G0/G1 defined as 2N DNA content, S phase as the population between 2N and 4N, and G2/M as 4N. A minimum of 10,000 events within the singlet gate were collected per sample. Data were analyzed using FlowJo software (version 10, FlowJo LLC). Representative cell cycle profiles (percentage of $G_0/G_1$, S, and $G_2$/M phase) of 16HBE14o- cells treated with conditioned media from ASMCs cultured in static or stretch conditions (Static or 13% Stretch) clearly indicate a blocking effect of the media from stretched ASMCs on the cell cycle progression of 16HBE14o- cells (**Supplementary Fig. 3**). Quantitative analysis of the cell cycle profiles further illustrates the

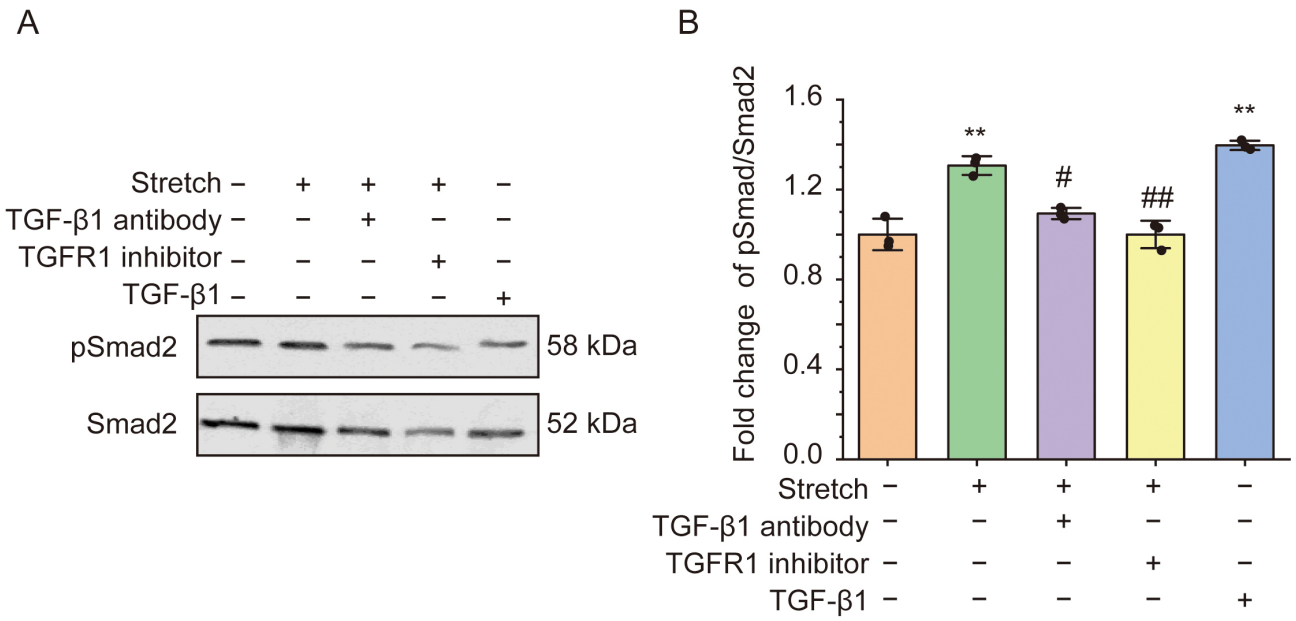


**Fig. 4. Effect of conditioned media derived from stretched ASMCs on the phosphorylation of Smad2 in AECs**. (A) Representative Western blot images showing phosphorylated or total Smad2 (pSmad2, Smad2) in original lanes. (B) Quantified fold change of protein expression of phosphorylated Smad2 normalized to that of total Smad2 (pSmad2/Smad2). AECs (16HBE14o- cells) were treated with conditioned media derived from ASMCs cultured in static or 13% stretch conditions (Stretch – or +), with or without 30 min pretreatment with TGF-β1 neutralizing antibody (7.5 μg/mL), TGFR1 inhibitor (Galunisertib, 10 μM), or exogenous TGF-β1 (1 ng/mL). Cells were then lysed and used for Western blotting. Data are presented as means ± S.D., n = 3 independent experiments in each group, $^{**}p < 0.01$ (compared to Stretch – group), $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ (compared to Stretch + group). TGFR1, TGF-β receptor 1.

cell cycle arrest in 16HBE14o- cells, with an 11% increase in $G_0/G_1$ phase (from 53% to 64%), and a 12% decrease in S phase (from 27% to 15%) in the 13% Stretch group, as compared with the Static group (Fig. 5A).

Furthermore, the qRT-PCR results show that *Cyclin D* mRNA expression was significantly decreased in 16HBE14o- cells cultured in conditioned media derived from stretched ASMCs, but not *Cyclin A*, *B*, and *E* (Fig. 5B). The migration and stress fiber organization of 16HBE14o- cells were also promoted and disorganized markedly, as demonstrated by the representative images of wound healing assay and immunofluorescent microscopy, respectively (Fig. 5C,D).

### *3.5 TGF-β1 Mediates Proliferation Inhibition and Cell Cycle Arrest of AECs in the Conditioned Media Derived From Stretched ASMCs*

To confirm whether TGF-β1 mediated the proliferation inhibition and cell cycle arrest of AECs in the conditioned media derived from stretched ASMCs, we further evaluated cell proliferation (cell activity by CCK-8 assay, and cell counting), cell cycle distribution and *Cyclin D* mRNA expression of 16HBE14o- cells cultured in the conditioned media derived from ASMCs under static (Stretch –) or 13% stretch (Stretch +) condition, with or without pretreatment of TGF-β1 antibody (7.5 μg/mL) or TGFR1 inhibitor (Galunisertib, 10 μM). Fig. 6 shows that the effects of the conditioned media derived from stretched ASMCs on the proliferation inhibition and cell cycle arrest of 16HBE14o- cells were all attenuated to variable extents by TGF-β1 antibody or inhibitor. These together suggest that TGF-β1 mediates the effect of conditioned media of excessively stretched ASMCs on the proliferation and cell cycle of AECs.

## 4. Discussion

In this study, we found that conditioned media from ASMCs but not AECs subjected to MV-associated excessive stretch induced G1-phase cell cycle arrest in AECs, as exemplified with primary human ASMCs and 16HBE14o- cells, respectively. Furthermore, we found that the excessively stretched ASMCs activated TGF-β1 in the culture media to inhibit AECs proliferation by blocking cell cycle G1-to-S phase transition via integrin αV pathway, which could be largely attenuated by inhibiting the TGF-β1 receptors in 16HBE14o- cells. These results indicate that ASMCs are more sensitive and/or more responsive to MV-associated excessive stretch in terms of releasing harmful soluble factors to impair cellular functions such as proliferation and cell cycle progression of AECs through TGF-β1 mediation, which may result in dysfunction or even damage of airway epithelia and ultimately contribute to VILI.

Epithelial–mesenchymal interaction is thought to play a key role in regulating airway injury in VILI. In addition

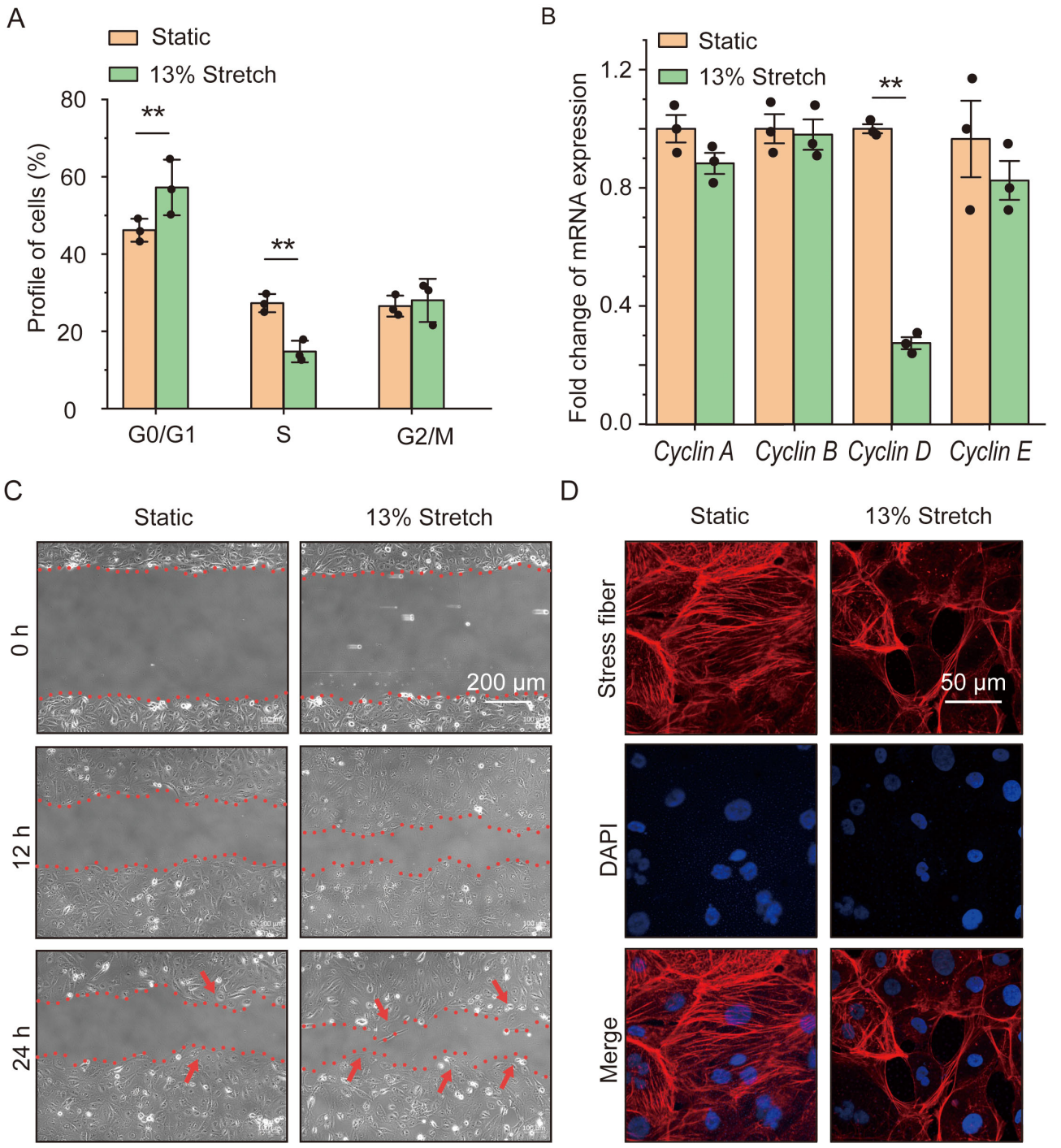


**Fig. 5. Effect of conditioned media derived from excessively stretched ASMCs on cell cycle progression of AECs**. (A) The cell cycle distribution of 16HBE14o- cells assessed by flow cytometry. (B) The mRNA expression of *Cyclin A*, *B*, *D*, *E* of 16HBE14o- cells assessed by qRT-PCR. (C,D) Representative images (scale bar, 200 μm) of wound healing assay for assessment of time-dependent migration, and immunofluorescent microscopy (scale bar, 50 μm) for assessment of stress fiber organization of 16HBE14o- cells treated with conditioned media derived from ASMCs cultured in Static or 13% Stretch condition. Data were presented as means ± S.D., n = 3 independent experiments in each group (3 biological replicates for each experiment), $^{**}p < 0.01$. qRT-PCR, quantitative reverse transcription PCR.

to contractile mechanical functions, ASMCs also possess the capacity to produce numerous proinflammatory factors that modulate epithelial cell behaviors [13]. In this study, we demonstrated that only the mesenchymal ASMCs under excessive stretch indeed influence multiple cellular functions, including cell proliferation, cell cycle progression,

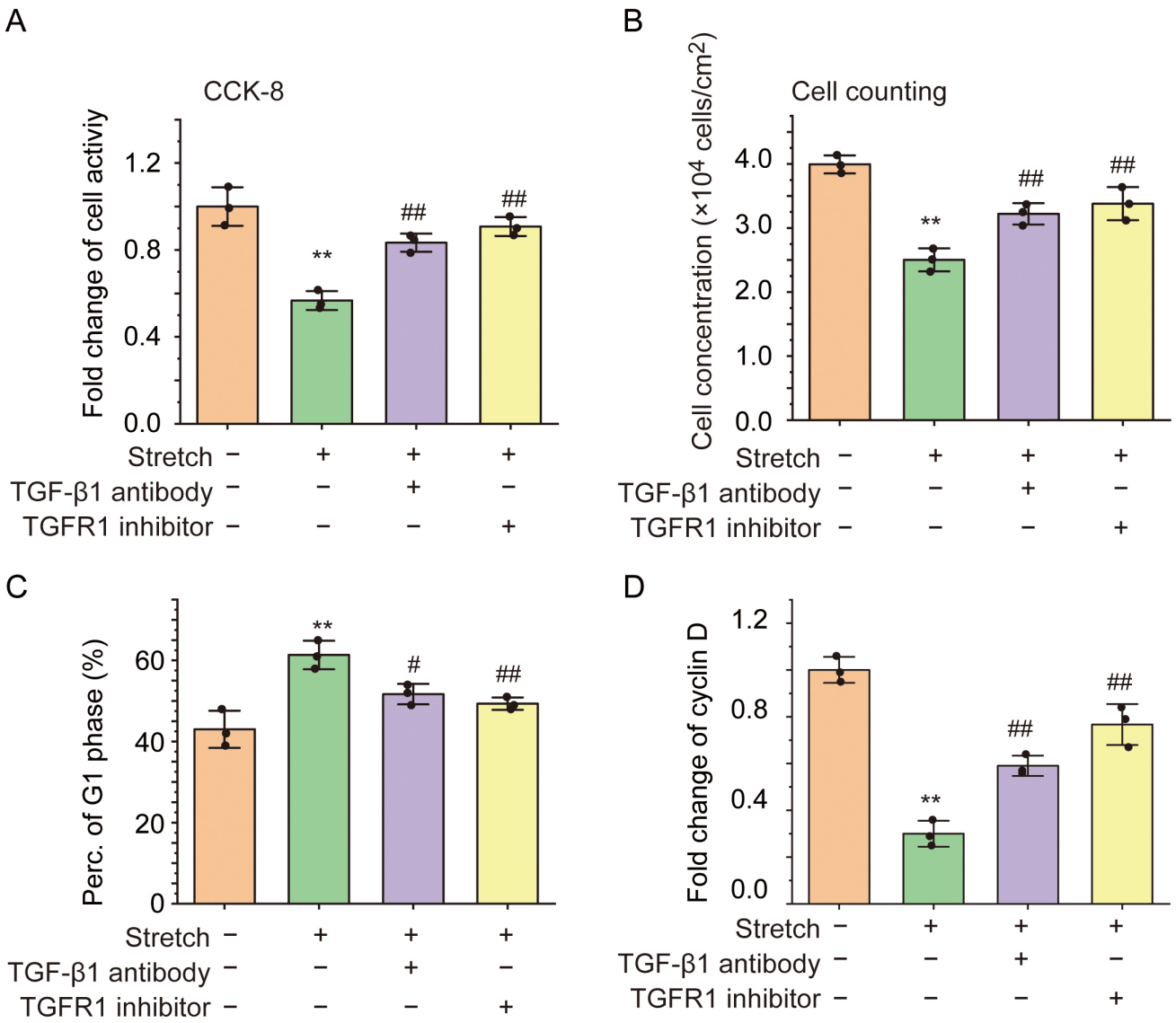


**Fig. 6. TGF-β1-dependent proliferation inhibition and cell cycle arrest of AECs cultured in the conditioned media from excessively stretched ASMCs**. (A–D) Fold change of cell activity (CCK-8 assay), Cell concentration, Percentage of G1 phase, and fold change of *Cyclin D* mRNA expression of 16HBE14o- cells cultured in conditioned media derived from ASMCs under static (Stretch –) or 13% stretch (Stretch +) condition, with or without 30 min pretreatment of TGF-β1 antibody (7.5 μg/mL) or TGFR1 inhibitor (10 μM). Data were presented as means ± S.D., n = 3 independent experiments in each group (3 biological replicates for each experiment), $^{**}p < 0.01$ compared to the Static group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to the Stretch group.

and stress fiber organization of AECs, not through direct interactions with the AECs but through indirect paracrine signaling via the conditioned media. Unlike ASMCs, AECs under excessive stretch could not directly activate latent TGF-β1, which underscores the differential mechanosensitivity between mesenchymal and epithelial cells. This finding suggests that ASMCs play a unique role in VILI pathogenesis through paracrine signaling.

Stretch plays a critical role in the lung tissue under physiological and pathological conditions [26,27,28,29,30,31,32]. Therefore, lung cells are physiologically adapted to the periodic stretch associated with normal breathing. However, during MV, the lung is exposed to excessive stretch, which may cause lung injury [33,34]. In this study, we found that MV-associated 13% excessive stretch, but not physiological 5% stretch, significantly activated TGF-β1 directly, depending on integrin αV. Understanding the relationship between stretch magnitude and severity of VILI and the associated cellular dysfunction may lead to improved MV strategy in clinical practice.

Several signaling molecules, including mechanosensitive ion channels and intracellular signaling molecules, impact high stretch-induced TGF-β1 activation [21]. As a

mechanosensitive ion channel, Piezo1 may mediate stretch-induced $Ca^{2+}$ influx in ASMCs, which could activate downstream signaling cascades leading to integrin αV activation. This is supported by our previous finding that Piezo1 mediates stretch-induced ASMC migration [5], suggesting that Piezo1 may serve as an upstream mechanosensor that couples mechanical stretch to integrin-dependent TGF-β1 activation. Focal adhesion kinase (FAK) is a key signaling intermediate that links integrin engagement to cytoskeletal reorganization and force generation. Stretch-induced integrin αV clustering may activate FAK autophosphorylation, which in turn recruits Src family kinases and activates the RhoA/ROCK pathway to generate the contractile forces necessary for mechanical dissociation of latent TGF-β1 from the LAP-integrin complex [23]. This pathway may represent the final mechanical step linking stretch sensing to TGF-β1 activation. These proposed pathways are testable using pharmacological inhibitors (e.g., GsMTx4 for Piezo1, FAK inhibitor VS-4718, Y-27632 for ROCK) and genetic approaches (siRNA/shRNA), which we plan to pursue in future studies.

On the other hand, TGF-β1 signaling has been a topic of intense investigation, and much has been uncovered regarding its structure and function [35]. TGF-β1 is typically secreted as a latent tripartite complex comprising the mature TGF-β1 dimer, the LAP, and the LTBP1. The mature TGF-β1 forms a complex with LAP noncovalently, which is called the small latent complex, and in this configuration, the mature TGF-β1 dimer is unable to bind to its receptors. The latent tripartite complex serves as an extracellular sensor in which LAP functions as the detector, LTBP1 as the localizer, and the mature TGF-β1 dimer as the effector of TGF-β1 receptor (TGFR1) [36]. The mature TGF-β1 dimer is typically released from this latent tripartite complex during interaction of the Arg-Gly-Asp (RGD) sequence on the LAP with integrins such as $\alpha$V. Only the released mature TGF-β1 dimer can function as active TGF-β1 to bind to its receptors (e.g., TGFR1) on target cells to initiate intracellular signaling, primarily through the phosphorylation of Smad2/3 proteins. The extracellular concentration of active TGF-β1 is primarily regulated by several mechanisms identified *in vitro* and *in vivo*, involving both protease and/or mechanical stimulation [37]. In this study, we revealed that excessive stretch directly activated TGF-β1 via integrin αV, which has also been shown to cause cytoskeletal reorganization [22].

Furthermore, TGF-β1 is known to exert multiple levels of control over Cyclin D/E, cdk4/6, and cdk inhibitors [38]. We found that *Cyclin D* mRNA expression was significantly inhibited in 16HBE14o- cells when cultured in conditioned media derived from excessively stretched ASMCs. Given that the cyclin proteins promote cell cycle transition through checkpoints and TGF-β1 controls the level of *Cyclin D*, together with our finding that TGF-β1 was activated in cultured ASMCs during excessive stretch as well as the conditioned media from excessively stretched ASMCs caused cell cycle arrest with downregulated *Cyclin D* mRNA expression in 16HBE14o- cells, these results suggest that the excessively stretched ASMCs may block the cell cycle progression in AECs by downregulating *Cyclin D* mRNA expression via TGF-β1 paracrine signaling.

TGF-β1 is also a master regulator of epithelial-mesenchymal transition (EMT) through downstream Smad2/3 pathways. Given that our conditioned media from stretched ASMCs activated Smad2 phosphorylation in AECs, it is plausible that TGF-β1 signaling could initiate EMT in AECs, particularly under prolonged exposure conditions that more closely mimic sustained MV. G1 cell cycle arrest may serve as a permissive state for EMT, as cell cycle exit can facilitate phenotypic plasticity by allowing cells to redirect resources from proliferation to transcriptional reprogramming. This is supported by evidence that TGF-β1-induced G1 arrest and EMT share common downstream effectors, including downregulation of Cyclin D and upregulation of EMT transcription factors (e.g., Snail, Slug, Twist), suggesting that conditioned media from excessively stretched ASMCs may induce EMT of AECs.

Although it is well-known that activated TGF-β1 is involved in VILI, therapeutics aim at inhibiting freely diffusible TGF-β have been associated with toxicities [39]. Targeting TGF-β activation thus has the potential to increase specificity while limiting toxicities. From a translational perspective, our findings suggest two complementary therapeutic strategies for reducing VILI during clinical MV. First, integrin αV antagonists (e.g., CWHM-12, cilengitide) could be administered prophylactically to prevent MV-induced TGF-β1 activation in ASMCs, thereby blocking the upstream initiation of the paracrine injury cascade. Second, TGFR1 inhibitors (e.g., Galunisertib/LY2157299) could be used to block paracrine TGF-β1 signaling in AECs, preventing downstream Smad2 activation and cell cycle arrest. The choice between upstream (integrin αV) and downstream (TGFR1) targeting may depend on the clinical context: prophylactic use during anticipated prolonged MV may favor upstream blockade, while therapeutic use after injury onset may require downstream inhibition. However, these strategies require rigorous *in vivo* validation in animal VILI models before clinical translation.

It needs to be noted that there are some limitations in this study. First, the study was limited to *in vitro* models with cultured ASMCs and AECs, and our findings need to be further explored in advanced models such as an *in vivo* animal model. Second, although integrin αV in ASMCs was found to be implicated in excessive stretch-induced activation of TGF-β1, the detailed mechanisms underlying this activation remain to be fully elucidated.

Our *in vitro* model represents a simplified system that does not capture the complexity of the *in vivo* airway microenvironment during VILI. For example, the het-

erogeneous lung stretch *in vivo* produces regional variations in strain magnitude that cannot be fully recapitulated by our uniform biaxial stretch system. In addition, infiltrating immune cells, particularly macrophages and neutrophils, contribute significantly to TGF-β1 activation through protease-dependent mechanisms (e.g., plasmin, MMPs), which could synergize with or compete against the integrin αV-dependent pathway identified in our study. Finally, extracellular matrix remodeling during VILI may alter the mechanical properties of the airway wall, thereby modifying the transmission of mechanical strain to ASMCs and influencing integrin αV-dependent TGF-β1 activation. These represent apparent translational gaps that need to be addressed in future studies.

## 5. Conclusion

Taken together, our findings *in vitro* reveal that MV-associated excessive stretch directly activates pleiotropic TGF-β1 in the mesenchymal ASMCs but not AECs, which in turn induces G1 phase cell cycle arrest in AECs. These results suggest that the ASMCs are highly mechanosensitive to abnormal external mechanical stimulation such as MV-associated excessive stretch in initiating and regulation AEC dysfunction, which warrants further validation *in vivo* models before clinical translation of therapeutically targeting ASMCs for treatment of VILI.

## Availability of Data and Materials

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author Contributions

ML and LD conceived and designed the experiments. JZ, KN, XS, and HL performed the experiments. KN analyzed the data. YP, JL, and LL collected some data for this manuscript. ML wrote the manuscript. ML and LD revised the manuscript. All authors read and approved the final version of the manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

Not applicable.


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## Conflicts of Interest

The authors declare no conflict of interest.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBL55462.

## References


[1] Madahar P, Beitler JR. Emerging concepts in ventilation-induced lung injury. F1000Research. 2020; 9: F1000 Faculty Rev-222. https://doi.org/10.12688/f1000research.20576.1

[2] von Düring S, Parhar KKS, Adhikari NKJ, Urner M, Kim SJ, Munshi L, et al. Understanding ventilator-induced lung injury: The role of mechanical power. Journal of Critical Care. 2025; 85: 154902. https://doi.org/10.1016/j.jcrc.2024.154902

[3] Slutsky AS, Ranieri VM. Ventilator-induced lung injury. The New England Journal of Medicine. 2013; 369: 2126–2136. https://doi.org/10.1056/NEJMra1208707

[4] The Acute Respiratory Distress Syndrome Network, Brower RG, Matthay MA, Morris A, Schoenfeld D, Thompson BT, et al. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The New England Journal of Medicine. 2000; 342: 1301–1308. https://doi.org/10.1056/NEJM200005043421801

[5] Luo M, Gu R, Wang C, Guo J, Zhang X, Ni K, et al. High Stretch Associated with Mechanical Ventilation Promotes Piezo1-Mediated Migration of Airway Smooth Muscle Cells. International Journal of Molecular Sciences. 2024; 25: 1748. https://doi.org/10.3390/ijms25031748

[6] López-Martínez C, Martín-Vicente P, Amado-Rodríguez L, López-Alonso I, Fernández-Rodríguez M, González-López A, et al. Prediction of lung overdistension during mechanical ventilation using micro-RNA and gene expression. Intensive Care Medicine Experimental. 2025; 13: 60. https://doi.org/10.1186/s40635-025-00768-2

[7] Bagchi A, Vidal Melo MF. Follow the Voxel-A New Method for the Analysis of Regional Strain in Lung Injury. Critical Care Medicine. 2018; 46: 1033–1035. https://doi.org/10.1097/CCM.0000000000003109

[8] Li M, Wang Y, Hu Z, Huang S, Chen P, Chen L, et al. PTEN-mediated senescence of lung epithelial cells drives ventilator-induced pulmonary fibrosis. Theranostics. 2025; 15: 8360–8376. https://doi.org/10.7150/thno.117523

[9] Yang C, Guo J, Ni K, Wen K, Qin Y, Gu R, et al. Mechanical Ventilation-Related High Stretch Mainly Induces Endoplasmic Reticulum Stress and Thus Mediates Inflammation Response in Cultured Human Primary Airway Smooth Muscle Cells. International Journal of Molecular Sciences. 2023; 24: 3811. https://doi.org/10.3390/ijms24043811

[10] Bocchi E, Pitozzi V, Pontis S, Caruso PL, Beghi S, Caputi M, et al. Investigation of Aberrant Basaloid Cells in a Rat Model

of Lung Fibrosis. Frontiers in Bioscience (landmark Edition). 2024; 29: 305. https://doi.org/10.31083/j.fbl2908305

[11] Calzetta L, Passeri D, Kanabar V, Rogliani P, Page C, Cazzola M, et al. Brain natriuretic peptide protects against hyperresponsiveness of human asthmatic airway smooth muscle via an epithelial cell-dependent mechanism. American Journal of Respiratory Cell and Molecular Biology. 2014; 50: 493–501. https://doi.org/10.1165/rcmb.2013-0119OC

[12] Holgate ST, Lackie PM, Howarth PH, Roche WR, Puddicombe SM, Richter A, et al. Invited lecture: activation of the epithelial mesenchymal trophic unit in the pathogenesis of asthma. International Archives of Allergy and Immunology. 2001; 124: 253–258. https://doi.org/10.1159/000053726

[13] Abohalaka R. Bronchial epithelial and airway smooth muscle cell interactions in health and disease. Heliyon. 2023; 9: e19976. https://doi.org/10.1016/j.heliyon.2023.e19976

[14] Suwara MI, Green NJ, Borthwick LA, Mann J, Mayer-Barber KD, Barron L, et al. IL-1α released from damaged epithelial cells is sufficient and essential to trigger inflammatory responses in human lung fibroblasts. Mucosal Immunology. 2014; 7: 684–693. https://doi.org/10.1038/mi.2013.87

[15] Luo M, Sun C, Guo J, Zhang X, Zhang J, Shi X, et al. Stretch Enhances Secretion of Extracellular Vehicles from Airway Smooth Muscle Cells via Endoplasmic Reticulum Stress Signaling in Relation to Ventilator-Induced Lung Injury. BIOCELL. 2025; 49: 833–855. https://doi.org/10.32604/biocell.2025.063869

[16] Semlali A, Jacques E, Rouabhia M, Milot J, Laviolette M, Chakir J. Regulation of epithelial cell proliferation by bronchial fibroblasts obtained from mild asthmatic subjects. Allergy. 2010; 65: 1438–1445. https://doi.org/10.1111/j.1398-9995.2010.02376.x

[17] Riemondy KA, Jansing NL, Jiang P, Redente EF, Gillen AE, Fu R, et al. Single cell RNA sequencing identifies TGFβ as a key regenerative cue following LPS-induced lung injury. JCI Insight. 2019; 5: e123637. https://doi.org/10.1172/jci.insight.123637

[18] Humphrey JD, Milewicz DM, Tellides G, Schwartz MA. Cell biology. Dysfunctional mechanosensing in aneurysms. Science (new York, N.Y.). 2014; 344: 477–479. https://doi.org/10.1126/science.1253026

[19] Zhen G, Guo Q, Li Y, Wu C, Zhu S, Wang R, et al. Mechanical stress determines the configuration of TGFβ activation in articular cartilage. Nature Communications. 2021; 12: 1706. https://doi.org/10.1038/s41467-021-21948-0

[20] Froese AR, Shimbori C, Bellaye PS, Inman M, Obex S, Fatima S, et al. Stretch-induced Activation of Transforming Growth Factor-β1 in Pulmonary Fibrosis. American Journal of Respiratory and Critical Care Medicine. 2016; 194: 84–96. https://doi.org/10.1164/rccm.201508-1638OC

[21] Dong X, Zhao B, Iacob RE, Zhu J, Koksal AC, Lu C, et al. Force interacts with macromolecular structure in activation of TGF-β. Nature. 2017; 542: 55–59. https://doi.org/10.1038/nature21035

[22] Noble PB, Pascoe CD, Lan B, Ito S, Kistemaker LEM, Tatler AL, et al. Airway smooth muscle in asthma: linking contraction and mechanotransduction to disease pathogenesis and remodelling. Pulmonary Pharmacology & Therapeutics. 2014; 29: 96–107. https://doi.org/10.1016/j.pupt.2014.07.005

[23] Shi M, Zhu J, Wang R, Chen X, Mi L, Walz T, et al. Latent TGF-β structure and activation. Nature. 2011; 474: 343–349. https://doi.org/10.1038/nature10152

[24] Bossé Y. The Strain on Airway Smooth Muscle During a Deep Inspiration to Total Lung Capacity. Journal of Engineering and Science in Medical Diagnostics and Therapy. 2019; 2: 0108021–1080221. https://doi.org/10.1115/1.4042309

[25] Wen K, Ni K, Guo J, Bu B, Liu L, Pan Y, et al. MircroRNA Let-7a-5p in Airway Smooth Muscle Cells is Most Responsive to High Stretch in Association With Cell Mechanics Modulation. Frontiers in Physiology. 2022; 13: 830406. https://doi.org/10.3389/fphys.2022.830406

[26] Doryab A, Tas S, Taskin MB, Yang L, Hilgendorff A, Groll J, et al. Evolution of Bioengineered Lung Models: Recent Advances and Challenges in Tissue Mimicry for Studying the Role of Mechanical Forces in Cell Biology. Advanced Functional Materials. 2019; 29: 1903114. https://doi.org/10.1002/adfm.201903114

[27] Maksym GN, Deng L, Fairbank NJ, Lall CA, Connolly SC. Beneficial and harmful effects of oscillatory mechanical strain on airway smooth muscle. Canadian Journal of Physiology and Pharmacology. 2005; 83: 913–922. https://doi.org/10.1139/y05-091

[28] Bokka KK, Jesudason EC, Warburton D, Lubkin SR. Quantifying cellular and subcellular stretches in embryonic lung epithelia under peristalsis: where to look for mechanosensing. Interface Focus. 2016; 6: 20160031. https://doi.org/10.1098/rsfs.2016.0031

[29] Liu M, Tanswell AK, Post M. Mechanical force-induced signal transduction in lung cells. The American Journal of Physiology. 1999; 277: L667–L683. https://doi.org/10.1152/ajplung.1999.277.4.L667

[30] Sanchez-Esteban J, Wang Y, Gruppuso PA, Rubin LP. Mechanical stretch induces fetal type II cell differentiation via an epidermal growth factor receptor-extracellular-regulated protein kinase signaling pathway. American Journal of Respiratory Cell and Molecular Biology. 2004; 30: 76–83. https://doi.org/10.1165/rcmb.2003-0121OC

[31] Diem K, Fauler M, Fois G, Hellmann A, Winokurow N, Schumacher S, et al. Mechanical stretch activates piezo1 in caveolae of alveolar type I cells to trigger ATP release and paracrine stimulation of surfactant secretion from alveolar type II cells. FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology. 2020; 34: 12785–12804. https://doi.org/10.1096/fj.202000613RRR

[32] Wirtz HR, Dobbs LG. Calcium mobilization and exocytosis after one mechanical stretch of lung epithelial cells. Science (new York, N.Y.). 1990; 250: 1266–1269. https://doi.org/10.1126/science.2173861

[33] Gattinoni L, Carlesso E, Cadringher P, Valenza F, Vagginelli F, Chiumello D. Physical and biological triggers of ventilator-induced lung injury and its prevention. The European Respiratory Journal. Supplement. 2003; 47: 15s–25s. https://doi.org/10.1183/09031936.03.00021303

[34] Pinhu L, Whitehead T, Evans T, Griffiths M. Ventilator-associated lung injury. Lancet (london, England). 2003; 361: 332–340. https://doi.org/10.1016/S0140-6736(03)12329-X

[35] Anderton MJ, Mellor HR, Bell A, Sadler C, Pass M, Powell S, et al. Induction of heart valve lesions by small-molecule ALK5 inhibitors. Toxicologic Pathology. 2011; 39: 916–924. https://doi.org/10.1177/0192623311416259

[36] Annes JP, Munger JS, Rifkin DB. Making sense of latent TGF-beta activation. Journal of Cell Science. 2003; 116: 217–224. https://doi.org/10.1242/jcs.00229

[37] Hinz B, Suki B. Does Breathing Amplify Fibrosis? American Journal of Respiratory and Critical Care Medicine. 2016; 194: 9–11. https://doi.org/10.1164/rccm.201601-0149ED

[38] Slingerland JM, Hengst L, Pan CH, Alexander D, Stampfer MR, Reed SI. A novel inhibitor of cyclin-Cdk activity detected in transforming growth factor beta-arrested epithelial cells. Molecular and Cellular Biology. 1994; 14: 3683–3694. https://doi.org/10.1128/mcb.14.6.3683-3694.1994

[39] Akhurst RJ. Targeting TGF-β Signaling for Therapeutic Gain. Cold Spring Harbor Perspectives in Biology. 2017; 9: a022301. https://doi.org/10.1101/cshperspect.a022301