

PM2.5 Induces Airway Remodeling in Chronic Obstructive Pulmonary Diseases via the Wnt5a/ β -Catenin Pathway

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Background: Fine-particulate matter ≤ 2.5 μm in diameter (PM2.5)-associated airway remodeling has recently been recognized as a central feature of COPD. Activation of the Wnt/ β -catenin pathway is closely related to the occurrence of airway remodeling. Accordingly, the goal of this study was to determine whether the Wnt5a/ β -Catenin pathway is involved in PM2.5-induced smooth muscle proliferation in vivo and in vitro, which promotes the development of airway remodeling in subjects with COPD.

Methods: The effect of Wnt5a on β -Catenin-mediated airway remodeling was assessed using an in vivo model of PM2.5-induced COPD and PM2.5-exposed human bronchial smooth muscle cells (HBSMCs) in vitro. Small animal spirometry was used to measure lung function in mice. H&E staining and immunohistochemistry were performed to inspect emphysema and airway remodeling indices. Real-time PCR was used to detect Wnt5a, β -Catenin, TGF- β 1, CyclinD1 and c-myc mRNA expression. The CCK8 assay was performed to detect cellular activity. Western blotting was performed to assess PCNA, α -SMA, Wnt5a, β -Catenin, PDGFR β and TenascinC protein expression. β -Catenin expression was detected using cellular immunofluorescence.

Results: Exposure to PM2.5 led to emphysema, airway wall thickening, an increased smooth muscle layer thickness, decreased lung function and increased expression of the Wnt5a, β -Catenin, PDGFR β and Tenascin C proteins in the mouse lung tissue. BOX5 (a Wnt5a antagonist) alleviated these PM2.5-induced outcomes in mice. Moreover, PM2.5 induced the expression of the Wnt5a, β -Catenin, TGF- β 1, CyclinD1 and c-myc mRNAs in HBSMCs. BOX5 also inhibited the PM2.5-induced increases in PCNA, α -SMA, Wnt5a, β -Catenin, PDGFR β and Tenascin C protein expression in HBSMCs.

Conclusion: Our findings suggest that PM2.5 exposure induces HBSMC proliferation, contributing to airway remodeling via the Wnt5a/ β -Catenin signaling pathway in vivo and in vitro, which might be a target for COPD treatment.

Keywords: COPD, airway remodeling, PM2.5, Wnt5a/ β -Catenin

Introduction

Chronic obstructive pulmonary disease (COPD) is a common chronic disease that can be prevented and treated. It is accompanied by persistent respiratory symptoms and airflow limitation, generally with airway and alveolar abnormalities related to significant exposure to harmful particles or gases.^{1–3} Airway remodeling is a distinctive pathological feature of COPD, and the airway wall thickness correlates with the severity of COPD. Smooth muscle cell proliferation plays an important role in airway remodeling, and the smooth muscle layer of the small airways is thickened and negatively correlated with the severity of the disease in patients with

COPD.⁴⁻⁶ Although advances in our understanding of the inflammatory features of COPD have been achieved, the mechanisms underlying the remodeling process are still poorly understood.

Fine-particulate matter ≤ 2.5 μm in diameter (PM_{2.5}) is characterized by a small particle size, large surface area, and the ability to absorb toxins.⁷ Recently, PM_{2.5} exposure was reported to be associated not only with an increased risk of developing COPD but also with the exacerbated deterioration of lung function and symptoms experienced by patients with COPD.^{8,9} As shown in our previous study, PM_{2.5} induced Wnt5a expression in human bronchial epithelial cells.¹⁰ Wnt5a is a member of the Wnt glucoprotein family, which activates a variety of downstream signaling pathways to regulate cell migration, polarity, proliferation and survival.¹¹ Previous studies have shown that increased expression of Wnt5a activates non-classical Wnt signaling and contributes to the pathogenesis of COPD.¹² Specifically, Wnt5a increases the TGF- β 1-induced upregulation of α -Smooth muscle actin (α -SMA) expression to enhance smooth muscle cell contractility.¹³ TGF- β 1 is involved in β -catenin-independent nonclassical Wnt5a signaling, which induces extracellular matrix (ECM) expression in airway smooth muscle cells and promotes tissue fibrosis.¹⁴ Wnt5a also regulates the proliferation of smooth muscle cells, but its role in the regulation of airway remodeling in response to PM_{2.5} remains unknown.

Platelet-derived growth factor receptor (PDGFR β) is a cell surface tyrosine kinase receptor that is involved in cell proliferation, survival and migration.¹⁵ Emerging evidence suggests that Wnt signaling contributes to smooth muscle cell precursor proliferation and adult lung disease development by directly regulating Tenascin C transcription and participating in the regulation of PDGFR β , which defines the Wnt/Tnc/PDGFR signaling axis.¹⁶ However, researchers have not clearly determined whether this axis promotes airway remodeling and accelerates disease progression in subjects with COPD.

Based on this information, we hypothesized that the Wnt5a/ β -Catenin signaling pathway participates in PM_{2.5}-induced airway smooth muscle cell proliferation through the upregulation of PDGFR β expression and leads to airway remodeling. Hence, using HBSMCs and an experimental model of COPD, this study was designed to elucidate the effects of the Wnt/Tnc/PDGFR β axis on PM_{2.5}-induced airway remodeling in a COPD model.

Methods

Animal Experiments

C57BL/6 mice were purchased from the Experimental Animal Center of Guangzhou University of Chinese Medicine. Animal experiments were performed according to the Chinese Association for Laboratory Animal Science Policy, and were approved by the Institutional Animal Care and Use Committee of Guangzhou Medical University.

Collection and Extraction of PM_{2.5}

Traffic ambient PM_{2.5} was collected by aerodynamic impactors equipped with a glass fiber filter, quartz filter, or Teflon membrane, and DMSO was used to extract soluble parts of PM_{2.5}. Sufficiently specific samples for biological experiments were collected with high-volume impactors equipped with glass fiber filters from arterial traffic roads. Gravimetric analysis was used to analyze specific physicochemical characteristics of PM_{2.5}. The procedure of PM_{2.5} sampling and analysis methods were described in our previous study.¹⁰

PM_{2.5} Exposure in Mice

C57BL/6 mice (6–8w) were randomly divided into 4 groups of 12 mice each. PBS (20 μL), PM_{2.5} (100 $\mu\text{g}/20$ μL), BOX5 (0.5 $\mu\text{g}/\text{mL}$ 10 μL), and PM_{2.5}+BOX5 (100 $\mu\text{g}/20$ μL +0.5 $\mu\text{g}/\text{mL}$ 10 μL) were injected via tracheal drip twice a week for 4 weeks of continuous exposure. Lung tissue was collected for subsequent experiments.

Lung Function Tests

After four weeks of treatment, mice were anaesthetized intraperitoneally with 1.25% avertin (2, 2, 2-tribromoethanol) 24 h after the last exposure to PM_{2.5}. Tracheotomized mice were placed in a whole-body manometer (Buxco-Force Pulmonary Maneuvers) to determine the peak expiratory flow rate (PEF) and peak inspiratory flow rate (PIF) using Boyle's law maneuvers.

Histology and Immunohistochemistry

Mouse lung tissues were collected, and small airways were analyzed using histochemistry and immunohistochemistry as described in our previous studies.^{17,18} Histology was performed on 4.0 μm thick paraffin sections of lung tissues. Sections were incubated with an anti- α -SMA antibody (ab5694, Abcam). Immunohistochemical staining to determine the α -SMA-positive stained area was evaluated with a confocal microscope at 200 \times magnification.

Method for Analyzing Airway Remodeling

The thickness of the small airway wall was calculated as the total area of the small airway wall (WAt) after normalization of the perimeter of the basement membrane (Pbm), ie, WAt/Pbm ($\mu\text{m}^2/\mu\text{m}$), which was used as an index for small airway remodeling. The airways were selected based on the following criteria: 1. basement membrane perimeter <2000 μm ; 2. minimum internal diameter of airway/maximum internal diameter of airway >0.5; Pbm, area of basement membrane (Abm) and airway wall outer membrane area (Ao) measured using Image-Pro Plus 6.0 (IPP6.0) software. The percentage of smooth muscle layer area (smooth muscle-specific marker protein α -SMA positive area) to airway wall area, ie, Area/(Ao-Abm)% (WA%), was used as an indicator of the smooth muscle layer in airway remodeling.

Method for Analyzing Emphysema

The mean linear intercept (MLI) was used to evaluate the size of the alveolar cavity, which was used as an index of emphysema. In the same field of view, “+” intersecting lines were drawn, the length of each line (L) and the number of alveoli passing through each line (NS) were calculated, and the MLI was calculated as $\text{MLI}=\text{L}/\text{NS}$.

Cell Culture and Treatment

The human bronchial smooth muscle cell line (HBSMC; American Type Culture Collection, Manassas, VA, USA) was cultured in SMC (ScienCell, USA). Cells were cultured at 37°C with 5% CO₂ in a humidified chamber and then exposed (or not) to PM2.5 (3 $\mu\text{g}/\text{mL}$). HBSMCs were pretreated with the Wnt5a antagonist BOX5 (Merck Millipore, Burlington, MA, USA) for 1 h prior to the addition of 3 $\mu\text{g}/\text{mL}$ PM2.5.

Cell Viability Assay

The viability of HBSMCs was detected using a WST8 assay kit (CCK8; Promoter Biotechnology, Wuhan, China), according to the manufacturer's instructions. Cells were

exposed to BOX5 (100, 200, or 300 μM) in SMC culture medium (100 μL). The optical density (OD) at 450 nm was detected with a microplate reader (Multiskan MK3; Thermo Fisher Scientific, Waltham, MA, USA).

Immunofluorescence Staining

HBSMCs were cultured for 48 h to reach a 60% density. Then, immunofluorescence staining was performed using standard procedures. Cells were incubated with an anti- β -catenin antibody (ab32572, Abcam, 1:500) for 12 h at 4°C, followed by a goat anti-rabbit IgG antibody (ab150077, Abcam, 1:400) for 1 h at room temperature. Finally, cell nuclei were stained with 4'-6-diamidino-2-phenylindole (DAPI; Beyotime, Shanghai, China) for 5 min. Images were captured using an inverted fluorescence microscope (Olympus-FL 500; Olympus Corporation, Tokyo, Japan).

Isolation of mRNA and Real-Time PCR Analysis

Total RNA was extracted using the Universal RNA Extraction Kit (B0004D, EZB, Shanghai, China) according to the manufacturer's instructions. RNA was quantified using a NanoDrop spectrophotometer (NanoDrop Tech, Rockland, DE, USA). Gene-specific primers (listed in Table 1) were provided by Sangon Biotech (Shanghai, China). Quantitative real-time PCR was performed using SYBR Premix Ex Taq II (Novozymes) and a Bio-Rad instrument II (Roche Diagnostics, Basel, Switzerland). GAPDH served as the housekeeping control.

Protein Extraction and Western Blot Analysis

Protein extraction and Western blotting were conducted according to the manufacturer's instructions. The membranes were blocked with 5% BSA (G5001, Servicebio) and then incubated with antibodies (anti-PCNA antibody, ab18197, Abcam; anti- α -SMA antibody, ab5694; anti-Wnt5a antibody, ab179824; anti- β -catenin antibody,

Table 1 Primer Sequence

Genes	Forward Primer	Reverse Primer
Human Wnt5a	ATTCTTGGTGGTCGCTAGGTA	CGCCTTCTCCGATGTACTGC
Human β -catenin	AGCTTCCAGACACGCTATCAT	CGGTACAACGAGCTGTTTCTAC
Human TGF- β 1	TATTGAGCACCTTGGGCACTG	GGGTGTATCAGAGTCCCTGC
Human c-myc	CCCTCCACTCGGAAGGACTA	GCTGGTGCATTTTCGGTTGT
Human CyclinD1	CAATGACCCCGCACGATTTTC	CATGGAGGGCGGATTGGAA
Human GAPDH	TGTGGGCATCAATGGATTGG	ACACCATGTATCCGGGTCAAT

ab32572; anti-PDGFR β antibody, ab69506; anti-TenascinC antibody, ab108930; anti- α -tubulin, ab7291; anti- β -actin, ab8226, Abcam) at 4°C for 12 h. Subsequently, the blots were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 2 h at room temperature. The proteins of interest were detected using enhanced chemiluminescence reagents (FDBio science), and the band intensities were quantified using ImageJ software (National Institute of Health, Bethesda, MD, USA). The expression of the target proteins was normalized to the loading control β -actin or α -tubulin.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism software version 7.04 for Windows (GraphPad Software, San Diego, USA) and are presented as the medians and IQRs. Data were analyzed with one-way ANOVA followed by the Bonferroni post hoc test or Kruskal-Wallis with Dunn's test for multiple comparisons, depending on whether datasets had a parametric or non-parametric distribution. $P < 0.05$ was considered statistically significant.

Result

PM2.5 Stimulation Induced Airway Remodeling and Emphysema in Mice

We performed H&E staining to observe the changes in lung histology and immunohistochemistry to detect the expression of α -SMA. Compared with the control group, the lung tissue destruction in mice exposed to different concentrations of PM2.5 was obvious and characterized by emphysematous features, such as enlarged and fused alveolar cavities and thinning, breaking and widening of alveolar septa (Figure 1A and B), indicating the occurrence of emphysema-like lesions. Lung function tests showed that exposure to different concentrations of PM2.5 (50 μ g/20 μ L and 100 μ g/20 μ L) reduced PIF and PEF in mice (Figure 1C and D). Based on the structural changes in alveoli, lung function and survival rate of mouse, the mouse in the group exposed to PM2.5 (100 μ g/20 μ L) were selected for follow-up experiments with reference to the relevant literature.¹⁹ Compared with the control group, the airway wall thickness of mice exposed to PM2.5 for 1 month was significantly thickened (Figure 1E and F). Furthermore, the PM2.5 group showed increased α -SMA expression, thickening of the smooth

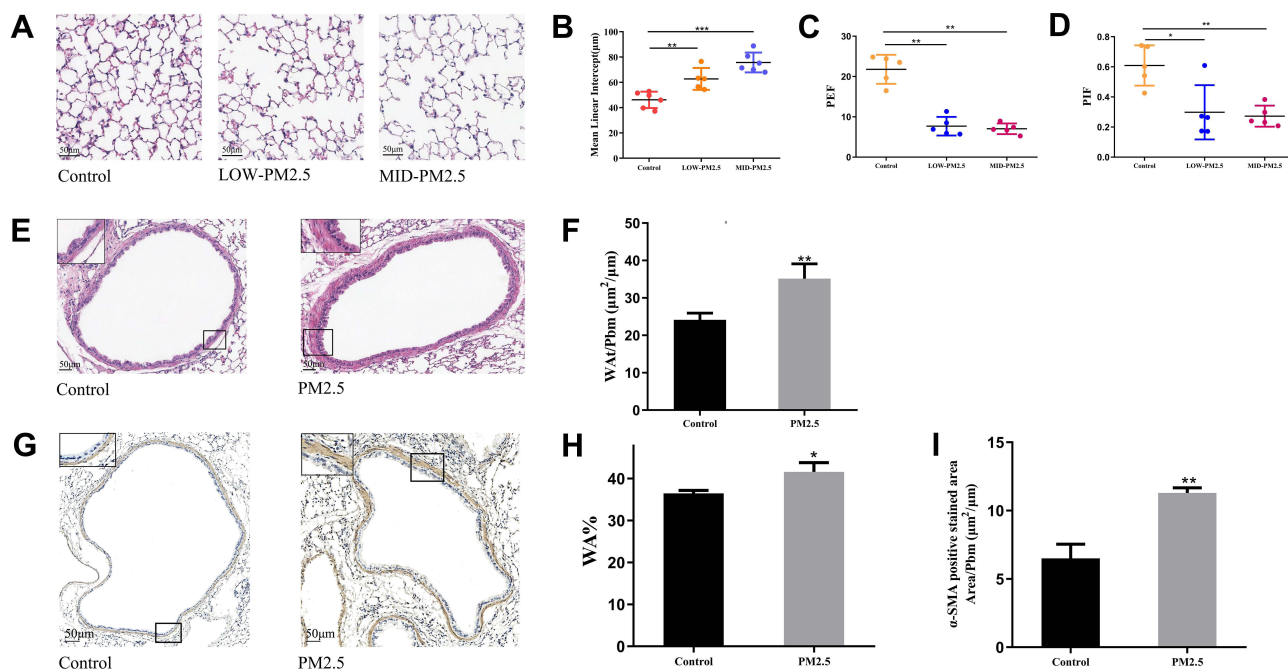


Figure 1 PM2.5 stimulation induced airway remodeling and emphysema in mice. Mice were exposed to LOW-PM2.5 (50 μ g/20 μ L) and MID-PM2.5 (100 μ g/20 μ L) via a tracheal drip twice a week for 4 weeks. (A) H&E staining showed that different concentrations of PM2.5 induced emphysema-like lesions in mice. (B) Different concentrations of PM2.5 increased the MLI in mice. (C) Lung function tests showed that different concentrations of PM2.5 decreased the PEF. (D) The lung function test showed that different concentrations of PM2.5 decreased the PIF. (E) H&E staining showed that PM2.5 induced airway remodeling. (F) PM2.5 increased the level of WA/Pbm ($\mu\text{m}^2/\mu\text{m}$). (G) Immunohistochemistry showed that PM2.5 induced α -SMA expression in the mouse lung tissue. (H) PM2.5 increased the ratio of WA%. (I) PM2.5 increased the area of α -SMA-positive staining. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to the control group, $n = 5$.

Abbreviations: PM2.5, particulate matter $\leq 2.5 \mu\text{m}$; MLI, mean linear intercept; PEF, peak expiratory flow rate; PIF, peak inspiratory flow rate.

muscle layer, and a significantly increased percentage of the smooth muscle layer in the airway wall (Figure 1G–I), suggesting that PM2.5 induced small airway remodeling in vivo.

PM2.5 Induced HBSMC Proliferation

We detected the expression of α -SMA and proliferating cell nuclear antigen (PCNA) in airway smooth muscle cells after PM2.5 stimulation to investigate its effect on the airway smooth muscle. PCNA is an intranuclear protein that is involved in DNA replication and is mainly produced in proliferating and transformed cells.²⁰ After PM2.5 stimulation of airway smooth muscle cells, PCNA and α -SMA expression were upregulated (Figure 2A–C), indicating that PM2.5 promoted airway smooth muscle cell proliferation.

PM2.5 Increased Wnt5a/ β -Catenin Pathway Activation in the Mice Lung Tissue

We measured the expression of proteins in the Wnt5a/ β -Catenin signaling pathway in PM2.5-exposed lung tissues of mice using Western blotting to investigate the effect of PM2.5 on the expression of proteins in the Wnt5a/ β -Catenin signaling pathway and their potential roles in airway remodeling. Compared with the control group, increased expression of the Wnt5a and β -Catenin proteins was detected in the PM2.5-exposed group (Figure 3A–C), and PM2.5 exposure also significantly upregulated PDGFR β and Tenascin C protein expression in mouse lung tissue (Figure 3D–F), indicating that PM2.5 exposure induced the expression of proteins involved in the Wnt5a/ β -Catenin signaling pathway in vivo.

PM2.5 Increased Wnt5a/ β -Catenin Pathway Activation in HBSMCs

We measured the expression of proteins in the Wnt5a/ β -Catenin signaling pathway in PM2.5-exposed HBSMCs using Western blotting to further investigate the effect of

PM2.5 on the expression of proteins in the Wnt5a/ β -Catenin signaling pathway in vitro. Compared with the control group, increased expression of the Wnt5a and β -Catenin proteins was detected in PM2.5-exposed HBSMCs (Figure 4A–C), and PM2.5 exposure significantly upregulated PDGFR β and Tenascin C protein expression in HBSMCs (Figure 4D–F). We also detected increased expression of Wnt5a and β -Catenin, as well as their downstream target genes TGF- β 1, CyclinD1, and c-myc, at the transcript level (Figure 4H). Cellular immunofluorescence staining of HBSMCs revealed increased expression of β -Catenin after PM2.5 stimulation and increased intranuclear staining compared to the control group (Figure 4G), suggesting that PM2.5 induced the expression of Wnt5a and activated β -Catenin signaling in HBSMCs.

The Wnt5a-Specific Antagonist BOX5 Alleviated PM2.5-Induced Airway Remodeling in Mice

BOX5, a Wnt5a-derived antagonistic peptide, was used to block endogenous Wnt5a signaling and investigate the role of Wnt5a in PM2.5 exposure-induced airway remodeling in mice. Then, morphological changes in lung tissues were observed using H&E staining, and the expression of the α -SMA protein was detected using immunohistochemistry. As depicted in Figure 5A–E, compared with the PM2.5 group, the airway wall thickness of PM2.5+BOX5-exposed mice was thinner, the expression of α -SMA protein in the smooth muscle layer decreased, and the ratio of smooth muscle layer to airway thickness (WA%) decreased (Figure 5A–E), indicating that BOX5 inhibited PM2.5-induced airway smooth muscle layer thickening and airway remodeling. Moreover, the expression of proteins in the Wnt5a/ β -Catenin signaling pathway in the mice lung tissues was observed using Western blotting. Compared with the PM2.5 group, the expression of Wnt5a, β -Catenin, PDGFR β and Tenascin

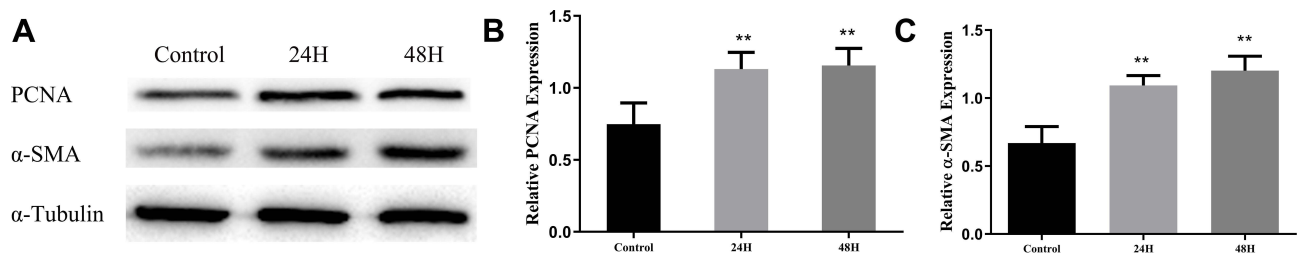


Figure 2 PM2.5 induced HBSMC proliferation. HBSMCs were stimulated with 3 μ g/mL PM2.5 for 24 h or 48 h. (A–C) Western blot analysis showed that PM2.5 induced the expression of PCNA and α -SMA. ** P <0.01, compared to the control group, n =3.

Abbreviations: PM2.5, particulate matter \leq 2.5 μ m; HBSMC, human bronchial smooth muscle cell; PCNA, proliferating cell nuclear antigen; α -SMA, α -Smooth muscle actin.

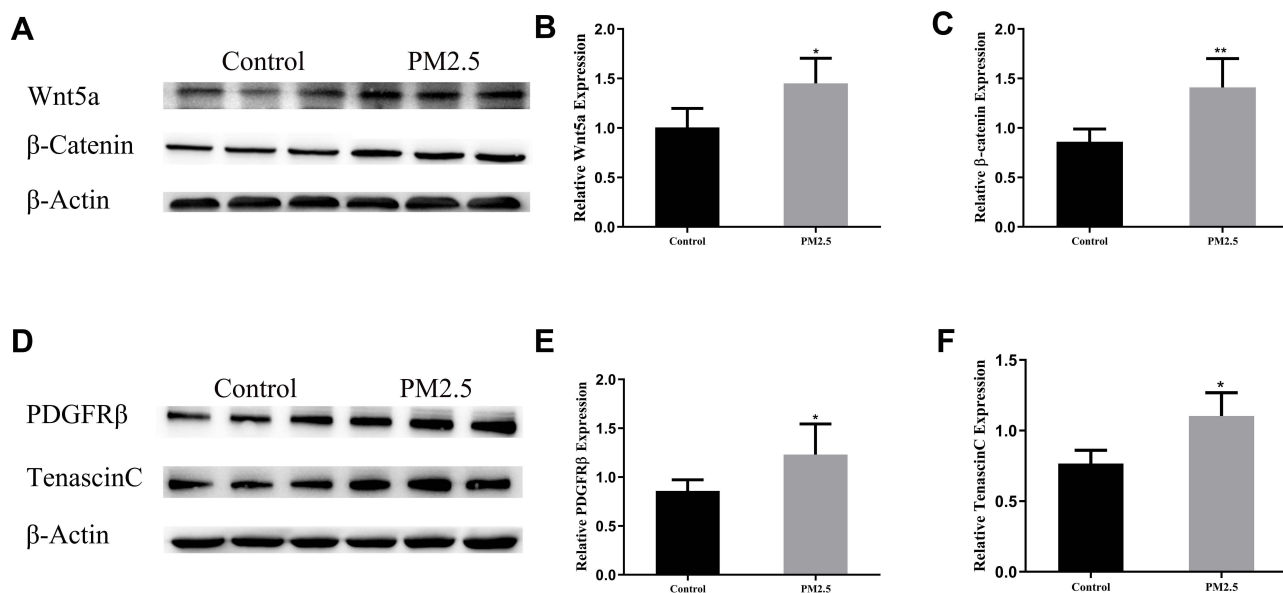


Figure 3 PM2.5 increased Wnt5a/β-Catenin pathway activation in the mice lung tissue. (A–C) Western blot analysis showed that PM2.5 increased Wnt5a and β-Catenin expression in the mice lung tissue. (D–F) Western blot analysis showed that PM2.5 increased PDGFRβ and tenascin C expression in the mice lung tissue. *P<0.05, **P<0.01, compared to the control group, n=5.

Abbreviations: PM2.5, particulate matter ≤ 2.5 μm; PDGFRβ, Platelet-derived growth factor receptor.

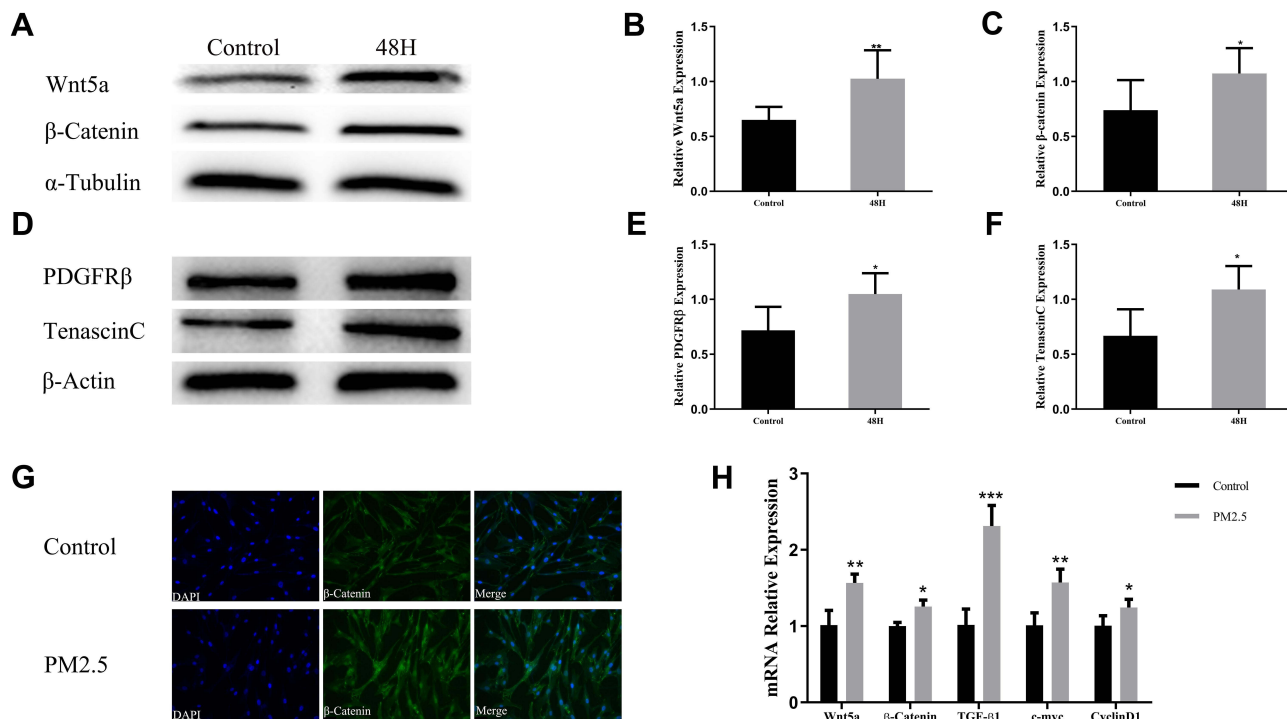


Figure 4 PM2.5 increased Wnt5a/β-Catenin pathway activation in HBSMCs. HBSMC were exposed to PM2.5 (3 μg/mL) for 48 h. (A–C) Western blot analysis showed that PM2.5 induced Wnt5a and β-Catenin expression in HBSMCs. (D–F) Western blot analysis showed that PM2.5 induced PDGFRβ and Tenascin C expression in HBSMC. (G) Immunofluorescence staining showed that PM2.5 increased the expression of β-Catenin in HBSMCs. (H) Real-time PCR results showed that PM2.5 increased the expression of the Wnt5a, β-Catenin, TGF-β1, c-myc and CyclinD1 mRNAs in HBSMCs. *P<0.05, **P<0.01, ***P<0.001, compared to the control group, n=3.

Abbreviations: PM2.5, particulate matter≤2.5 μm; HBSMC, human bronchial smooth muscle cell; PDGFRβ, Platelet-derived growth factor receptor.

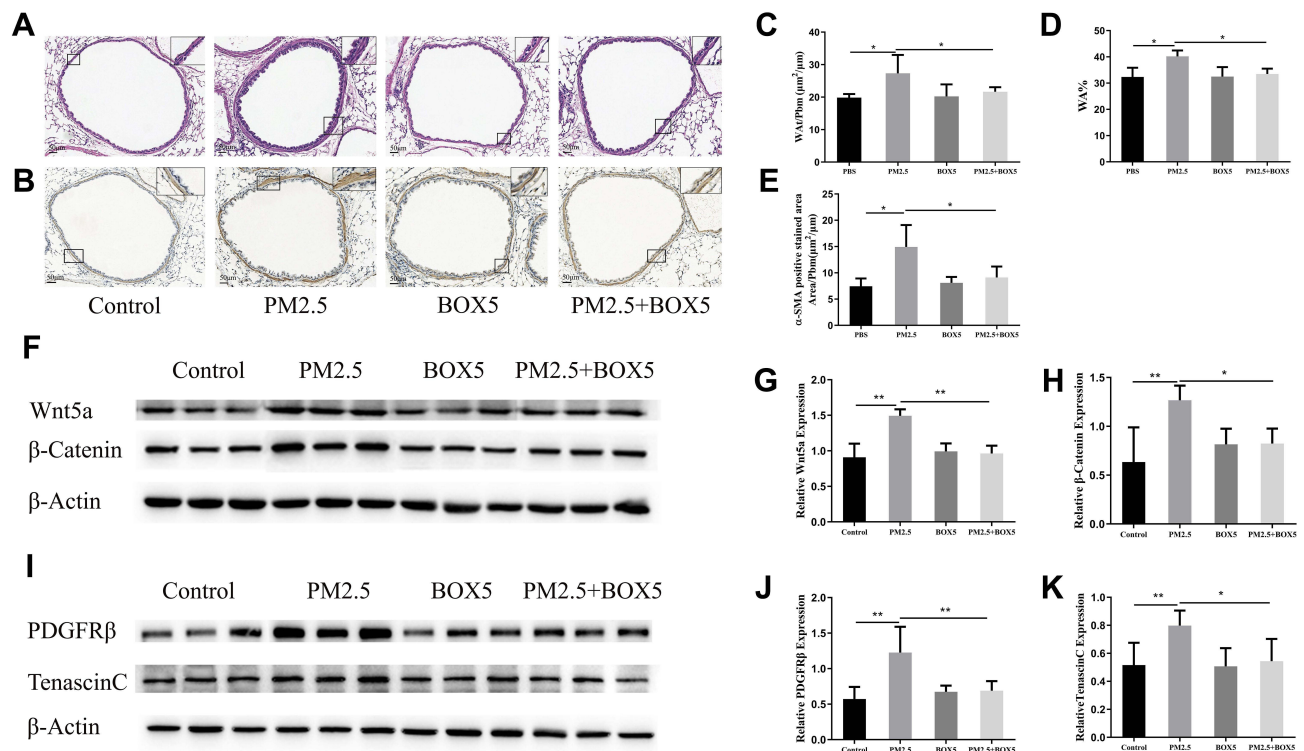


Figure 5 BOX5 alleviated PM2.5-induced airway remodeling in mice. Mice were randomly divided into PBS, PM2.5, BOX5, and PM2.5+BOX5 groups. (A) H&E staining showed that BOX5 alleviated the PM2.5-induced airway wall thickening. (B) Immunohistochemistry showed that BOX5 decreased PM2.5-induced increase in α -SMA protein expression. (C) BOX5 decreased the PM2.5-induced increase in WAI/Pbm ($\mu\text{m}^2/\mu\text{m}$). (D) BOX5 decreased the PM2.5-induced increase in WA%. (E) BOX5 decreased the PM2.5-induced increase in the α -SMA-positive area. (F–H) Western blot analysis showed that BOX5 reduced PM2.5-induced increases in Wnt5a and β -Catenin expression in the mouse lung tissue. (I–K) Western blot analysis showed that BOX5 reduced the PM2.5-induced increases in PDGFR β and tenascin C expression in the mice lung tissue. * $P < 0.05$, ** $P < 0.01$, $n = 5$.

Abbreviations: PM2.5, particulate matter $\leq 2.5 \mu\text{m}$; PDGFR β , Platelet-derived growth factor receptor; α -SMA, α -Smooth muscle actin.

C proteins was downregulated in the BOX5 + PM2.5 group (Figure 5F–K), indicating that BOX5 inhibited PM2.5-induced expression of proteins in the Wnt5a/ β -Catenin signaling pathway during the process of airway remodeling in the mice lung tissues.

The Wnt5a-Specific Antagonist BOX5 Alleviated PM2.5-Induced HBSMC Proliferation

First, the cytotoxicity of BOX5 in HBSMCs was examined. HBSMCs were stimulated with different doses of BOX5 (100–300 μM) for 48 h with reference to the relevant literature.²¹ No significant changes in cell activity were observed after the treatment with 100 μM BOX5 (Figure 6D); therefore, 100 μM BOX5 was subsequently selected as the experimental dose in combination with a literature review. As expected, compared with the PM2.5 group, the levels of PCNA and α -SMA were downregulated in the BOX5 + PM2.5 group (Figure 6A–C), indicating that BOX5 inhibited PM2.5-induced HBSMC proliferation. In

addition, BOX5 treatment reduced the PM2.5-induced increase in Wnt5a, β -Catenin, PDGFR β and Tenascin C levels compared to the PM2.5 group (Figure 6E–J). These results suggest that the Wnt5a/ β -Catenin signaling pathway may participate in the regulation of PM2.5-induced HBSMC proliferation in vitro.

Discussion

With the continuous advancement of industrialization and urbanization, PM2.5 is playing an increasingly important role in the development of COPD.²² Recent epidemiological studies conducted both in China and abroad have found that PM2.5 in air pollution increases the incidence, risk of acute exacerbation, lung function decline and mortality of patients with COPD.²³ The incidence of acute exacerbations of chronic obstructive pulmonary disease (AECOPD) and acute respiratory infections (ARIs) is significantly increased when PM2.5 pollution is severe.²⁴

Our findings revealed that PM2.5 exposure caused aberrant upregulation of components of the Wnt5a/ β -Catenin signaling pathway in both HBSMCs and mice

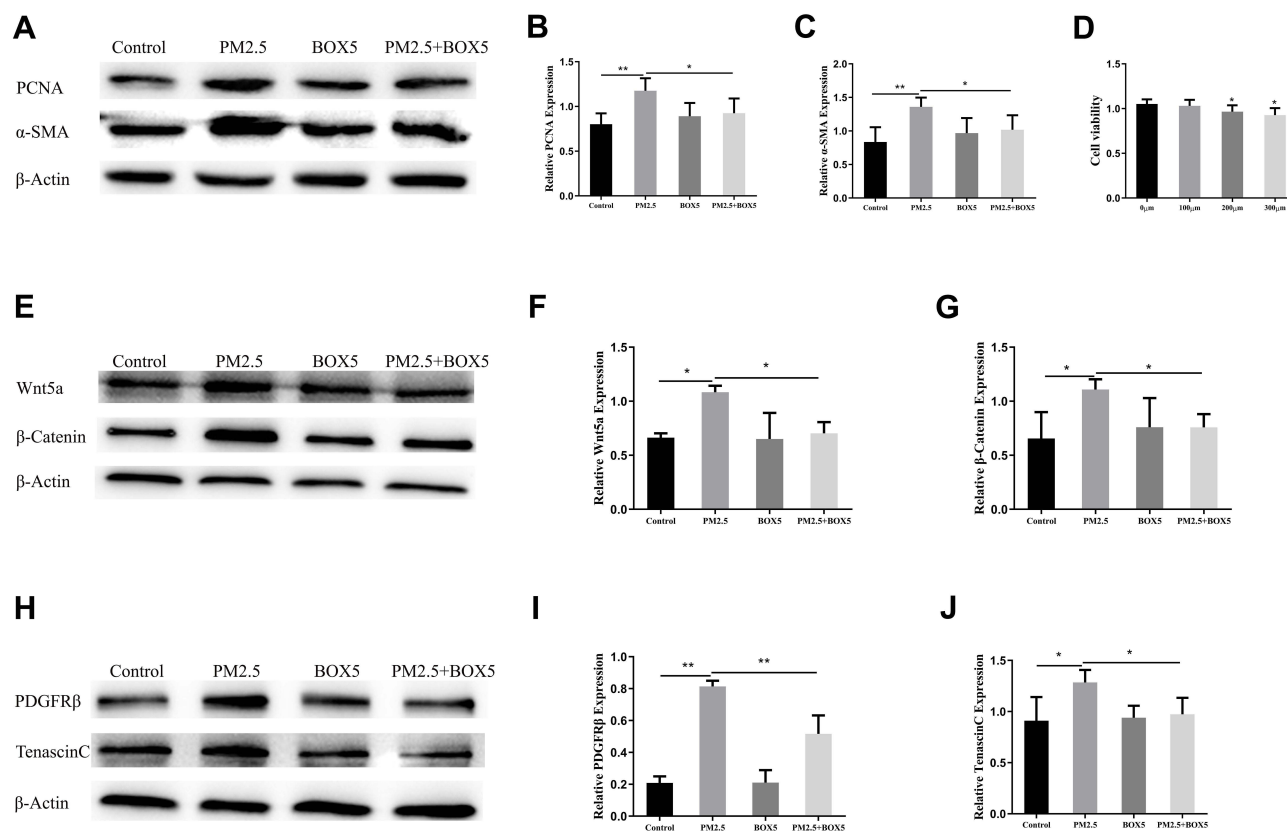


Figure 6 BOX5 alleviated PM2.5-induced HBSMC proliferation. HBSMCs were divided into PBS, PM2.5, BOX5, and PM2.5+BOX5 groups. (A–C) Western blot analysis showed that BOX5 downregulated PM2.5-induced increases in PCNA and α -SMA expression in HBSMCs. (D) The CCK8 assay showed that cell viability did not significantly change after 48 h of BOX5 (100 μ M) treatment, but cell viability decreased after 200 μ M and 300 μ M BOX5 treatment. (E–G) Western blot analysis showed that BOX5 downregulated the PM2.5-induced increases in Wnt5a and β -Catenin expression in HBSMCs. (H–J) Western blot analysis showed that BOX5 downregulated the PM2.5-induced increases in PDGFR β and tenascin C expression in HBSMCs. * $P < 0.05$, ** $P < 0.01$, $n = 3$.

Abbreviations: PM2.5, particulate matter $\leq 2.5 \mu$ m; HBSMC, human bronchial smooth muscle cell; PDGFR β , Platelet-derived growth factor receptor; PCNA, proliferating cell nuclear antigen; α -SMA, α -Smooth muscle actin.

lung tissue. Upregulated Wnt5a led to the activation of β -Catenin, increased expression of TenascinC and PDGFR β , and the induction of HBSMC proliferation and airway remodeling. Approaches targeting the Wnt5a/ β -Catenin signaling pathway may serve as an effective therapeutic strategy for PM2.5-induced airway remodeling in patients with COPD.

Epidemiological studies have shown that exposure to PM2.5 poses a serious threat and risk to human health and is significantly associated with increased mortality from respiratory disease, lung cancer and cardiovascular disease.^{25–27} We constructed a mice model by administering a tracheal drip injection of PM2.5. COPD-like models have been successfully constructed by a tracheal drip injection of PM2.5, which are mainly characterized by small bronchial mucosal damage, wall thickening, squamous cell hyperplasia, and fibrous tissue hyperplasia. In contrast to the cigarette smoke extract (CSE) model, PM2.5 mainly causes small airway lesions, such as

bronchial dilatation and wall thickening, while CSE mainly induces severe emphysema lesions and poor diffusion function.^{28–30} Our study also found that PM2.5 exposure led to thickening of the airway wall in mice, especially the smooth muscle layer, and PM2.5 stimulation of HBSMCs in vitro led to proliferation, hypertrophy, and upregulation of intracellular proliferation-related protein PCNA expression. Based on our results, PM2.5 exposure induced emphysema and airway remodeling in mice and induced airway smooth muscle cell proliferation, leading to airway remodeling in vitro, which contributes to the development of COPD.

Furthermore, the expression of Wnt5a and β -Catenin increased in HBSMCs after PM2.5 exposure, and the expression of the TGF- β 1, CyclinD1 and C-myc mRNAs was upregulated in HBSMCs. TGF- β 1 is secreted by airway epithelial cells, airway smooth muscle cells and eosinophils and promotes matrix protein production, contractile protein expression and proliferation of airway

smooth muscle cells and airway fibroblasts.³¹ Cyclin D1 and c-myc are the core transcription factors and target genes of the Wnt/ β -catenin signaling pathway.³² C-myc and CyclinD1 are widely involved in cell and tissue development and are key downstream effectors of cell proliferation.^{33,34} In addition, Wnt5a and β -catenin expression were upregulated in the lung tissues of mice exposed to PM2.5. Dysregulation of Wnt5a signaling has been observed in many lung diseases, including COPD, idiopathic pulmonary fibrosis (IPF) and asthma. Fibroblast-derived Wnt5a levels are increased in COPD models and specimens from patients with COPD.¹⁴ Very recently, a study indicated that smooth muscle-derived Wnt5a induced inflammatory cytokines and the α -SMA expression in the airway smooth muscle in asthma, resulting in increased airway wall inflammation and remodeling.³⁵ Combined with previous reports, the relationship between the Wnt5a/ β -catenin signaling pathway and PM2.5-associated airway remodeling was also established in our study.

PDGFR β is a cell surface tyrosine kinase receptor that transduces extracellular signals into intercellular regions and promotes cell proliferation, survival and migration.¹⁵ Tenascin C is a large hexameric, multimodal extracellular matrix protein that binds to cell surface receptors, ECM proteins, soluble factors or pathogens to regulate cell adhesion, migration, proliferation and differentiation.^{36,37} In a chronic asthma model, blockade of the Wnt/ β -catenin signaling pathway inhibited airway remodeling, such as subepithelial fibrosis and smooth muscle proliferation, by downregulating TGF- β 1 and tenascin C/PDGFR β expression.³⁸ Consistent with previous studies, we also detected that PM2.5 induced the expression of the PDGFR β and tenascin C proteins in the lung tissue and in HBSMCs. Therefore, we speculate that the Wnt5a/ β -Catenin signaling pathway is probably involved in PM2.5-induced airway remodeling by regulating the tenascin C/PDGFR β pathway.

We applied the Wnt5a-specific antagonist BOX5 to block endogenous Wnt5a signaling in mice and HBSMCs as a method to further investigate the mechanisms by which the Wnt5a/ β -Catenin signaling pathway may be involved in PM2.5-associated airway remodeling in COPD. Our current data showed that BOX5 did not change basal Wnt5a expression, while BOX5 alleviated PM2.5-induced airway wall thickening and the smooth muscle layer thickness of mice, attenuated PM2.5-induced HBSMC proliferation. Wnt, tenascin

C and PDGFR expression are increased in adults with smooth muscle-related lung disease.³⁹ Administration of a β -Catenin siRNA led to a significant decrease in PDGFR β expression. Moreover, Wnt signaling regulates PDGFR β in part by directly regulating Tnc transcription to promote SMC precursor proliferation and adult lung disease development.¹⁶ This study defines the Wnt/Tnc/PDGFR β signaling axis, which is essential for lung smooth muscle development and disease progression, and finds that this signaling axis is upregulated in both mice and human models of asthma and pulmonary arterial hypertension. Consistently, we also found that BOX5 decreased β -Catenin activation and downregulated Tenascin C and PDGFR β protein expressions in both *in vivo* and *in vitro*, indicating that the Wnt5a/ β -Catenin signaling pathway predominantly participates in airway remodeling caused by PM2.5 in COPD models.

Limitations

The limitations of our study could not be ignored, primary human bronchial smooth muscle cells should ideally be used for experimental *in vitro* studies; however, because of technical limitations for culturing primary cells, we used the human bronchial smooth muscle cell line, and cannot exclude the possibility of differences due to altered sensitivity.

Conclusions

In summary, we investigated the possible role of PM2.5 in COPD by focusing on the airway remodeling mechanism and showed that PM2.5 induced the upregulation of Wnt5a, which in turn promoted β -Catenin activation and increased the expression of Tenascin C and PDGFR β to promote HBSMC proliferation, contributing to airway remodeling *in vivo* and *in vitro*. These results provide evidence that treatments targeting Wnt5a/ β -Catenin signaling may be a new therapeutic approach for PM2.5-associated airway remodeling in individuals with COPD.

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Disclosure

The authors report no conflicts of interest in this work.

References

- Barnes PJ, Burney PG, Silverman EK, et al. Chronic obstructive pulmonary disease. *Nat Rev Dis Primers*. 2015;1:15076. doi:10.1038/nrdp.2015.76
- Lange P, Celli B, Agustí A, et al. Lung-function trajectories leading to chronic obstructive pulmonary disease. *N Engl J Med*. 2015;373(2):111–122. doi:10.1056/NEJMoa1411532
- Rabe KF, Watz H. Chronic obstructive pulmonary disease. *Lancet*. 2017;389(10082):1931–1940. doi:10.1016/S0140-6736(17)31222-9
- Barnes PJ. Small airway fibrosis in COPD. *Int J Biochem Cell Biol*. 2019;116:105598. doi:10.1016/j.biocel.2019.105598
- Black PN, Ching PS, Beaumont B, Ranasinghe S, Taylor G, Merrilees MJ. Changes in elastic fibres in the small airways and alveoli in COPD. *Eur Respir J*. 2008;31(5):998–1004. doi:10.1183/09031936.00017207
- Hollins F, Sutcliffe A, Gomez E, et al. Airway smooth muscle NOX4 is upregulated and modulates ROS generation in COPD. *Respir Res*. 2016;17(1):84. doi:10.1186/s12931-016-0403-y
- Kim Y, Seo J, Kim JY, Lee JY, Kim H, Kim BM. Characterization of PM(2.5) and identification of transported secondary and biomass burning contribution in Seoul, Korea. *Environ Sci Pollut Res Int*. 2018;25(5):4330–4343. doi:10.1007/s11356-017-0772-x
- Schultz ES, Litonjua AA, Melen E. Effects of long-term exposure to traffic-related air pollution on lung function in children. *Curr Allergy Asthma Rep*. 2017;17(6):41. doi:10.1007/s11882-017-0709-y
- Zhao B, Zheng H, Wang S, et al. Change in household fuels dominates the decrease in PM2.5 exposure and premature mortality in China in 2005–2015. *Proc Natl Acad Sci U S A*. 2018;115(49):12401–12406. doi:10.1073/pnas.1812955115
- Zou W, Wang X, Hong W, et al. PM2.5 induces the expression of inflammatory cytokines via the Wnt5a/Ror2 pathway in human bronchial epithelial cells. *Int J Chron Obstruct Pulmon Dis*. 2020;15:2653–2662. doi:10.2147/COPD.S270762
- Oishi I, Suzuki H, Onishi N, et al. The receptor tyrosine kinase Ror2 is involved in non-canonical Wnt5a/JNK signalling pathway. *Genes Cells*. 2003;8(7):645–654. doi:10.1046/j.1365-2443.2003.00662.x
- Mikels AJ, Nusse R, Arias AM. Purified Wnt5a protein activates or inhibits beta-catenin-TCF signaling depending on receptor context. *PLoS Biol*. 2006;4(4):e115. doi:10.1371/journal.pbio.0040115
- Koopmans T, Kumawat K, Halayko AJ, Gosens R. Regulation of actin dynamics by WNT-5A: implications for human airway smooth muscle contraction. *Sci Rep*. 2016;6:30676. doi:10.1038/srep30676
- Baarsma HA, Skronska-Wasek W, Mutze K, et al. Noncanonical WNT-5A signaling impairs endogenous lung repair in COPD. *J Exp Med*. 2017;214(1):143–163. doi:10.1084/jem.20160675
- Mellgren AM, Smith CL, Olsen GS, et al. Platelet-derived growth factor receptor beta signaling is required for efficient epicardial cell migration and development of two distinct coronary vascular smooth muscle cell populations. *Circ Res*. 2008;103(12):1393–1401. doi:10.1161/CIRCRESAHA.108.176768
- Cohen ED, Ihida-Stansbury K, Lu MM, Panettieri RA, Jones PL, Morrissey EE. Wnt signaling regulates smooth muscle precursor development in the mouse lung via a tenascin C/PDGFR pathway. *J Clin Invest*. 2009;119(9):2538–2549. doi:10.1172/JCI38079
- He F, Liao B, Pu J, et al. Exposure to ambient particulate matter induced COPD in a rat model and a description of the underlying mechanism. *Sci Rep*. 2017;7:45666. doi:10.1038/srep45666
- Bracke KR, D'Hulst AI, Maes T, et al. Cigarette smoke-induced pulmonary inflammation, but not airway remodelling, is attenuated in chemokine receptor 5-deficient mice. *Clin Exp Allergy*. 2007;37(10):1467–1479. doi:10.1111/j.1365-2222.2007.02808.x
- Xu Z, Li Z, Liao Z, et al. PM2.5 induced pulmonary fibrosis in vivo and in vitro. *Ecotoxicol Environ Saf*. 2019;171:112–121. doi:10.1016/j.ecoenv.2018.12.061
- He S, Chen M, Lin X, Lv Z, Liang R, Huang L. Triptolide inhibits PDGF-induced proliferation of ASMCs through G0/G1 cell cycle arrest and suppression of the AKT/NF-κB/cyclinD1 signaling pathway. *Eur J Pharmacol*. 2020;867:172811. doi:10.1016/j.ejphar.2019.172811
- Wang Z, Zhao J, Wang T, Du X, Xie J. Fine-particulate matter aggravates cigarette smoke extract-induced airway inflammation via Wnt5a-ERK pathway in COPD. *Int J Chron Obstruct Pulmon Dis*. 2019;14:979–994. doi:10.2147/COPD.S195794
- Osornio-Vargas AR, Bonner JC, Alfaro-Moreno E, et al. Proinflammatory and cytotoxic effects of Mexico City air pollution particulate matter in vitro are dependent on particle size and composition. *Environ Health Perspect*. 2003;111(10):1289–1293. doi:10.1289/ehp.5913
- Churg A, Brauer M, Del Carmen Avila-Casado M, Fortoul TI, Wright JL. Chronic exposure to high levels of particulate air pollution and small airway remodeling. *Environ Health Perspect*. 2003;111(5):714–718. doi:10.1289/ehp.6042
- Horne BD, Joy EA, Hofmann MG, et al. Short-term elevation of fine particulate matter air pollution and acute lower respiratory infection. *Am J Respir Crit Care Med*. 2018;198(6):759–766. doi:10.1164/rccm.201709-1883OC
- Guo W, Tan Y, Yin X, Sun Z. Impact of PM(2.5) on second birth intentions of China's floating population in a low fertility context. *Int J Environ Res Public Health*. 2019;16(21):4293. doi:10.3390/ijerph16214293
- Mirabelli MC, Boehmer TK, Damon SA, et al. Air quality awareness among U.S. adults with respiratory and heart disease. *Am J Prev Med*. 2018;54(5):679–687. doi:10.1016/j.amepre.2018.01.037
- Li P, Xin J, Wang Y, et al. The acute effects of fine particles on respiratory mortality and morbidity in Beijing, 2004–2009. *Environ Sci Pollut Res Int*. 2013;20(9):6433–6444. doi:10.1007/s11356-013-1688-8
- Zhao D, Zhou Y, Jiang C, Zhao Z, He F, Ran P. Small airway disease: a different phenotype of early stage COPD associated with biomass smoke exposure. *Respirology (Carlton, Vic)*. 2018;23(2):198–205. doi:10.1111/resp.13176
- Rivera RM, Cosio MG, Ghezzi H, Salazar M, Pérez-Padilla R. Comparison of lung morphology in COPD secondary to cigarette and biomass smoke. *Int J Tuberc Lung Dis*. 2008;12(8):972–977.
- Camp PG, Ramirez-Venegas A, Sansores RH, et al. COPD phenotypes in biomass smoke- versus tobacco smoke-exposed Mexican women. *Eur Respir J*. 2014;43(3):725–734. doi:10.1183/09031936.00206112
- Januskevicius A, Vaitkiene S, Gosens R, et al. Eosinophils enhance WNT-5a and TGF-β1 genes expression in airway smooth muscle cells and promote their proliferation by increased extracellular matrix proteins production in asthma. *BMC Pulm Med*. 2016;16(1):94. doi:10.1186/s12890-016-0254-9
- Jia XX, Zhu TT, Huang Y, Zeng XX, Zhang H, Zhang WX. Wnt/β-catenin signaling pathway regulates asthma airway remodeling by influencing the expression of c-Myc and cyclin D1 via the p38 MAPK-dependent pathway. *Exp Ther Med*. 2019;18(5):3431–3438. doi:10.3892/etm.2019.7991
- Roy PG, Thompson AM. Cyclin D1 and breast cancer. *Breast (Edinburgh, Scotland)*. 2006;15(6):718–727. doi:10.1016/j.breast.2006.02.005
- Poli V, Fagnocchi L, Fasciani A, et al. MYC-driven epigenetic reprogramming favors the onset of tumorigenesis by inducing a stem cell-like state. *Nat Commun*. 2018;9(1):1024. doi:10.1038/s41467-018-03264-2

35. Koopmans T, Hesse L, Nawijn MC, et al. Smooth-muscle-derived WNT5A augments allergen-induced airway remodelling and Th2 type inflammation. *Sci Rep.* 2020;10(1):6754. doi:10.1038/s41598-020-63741-x
36. Chiquet-Ehrismann R. Tenascins. *Int J Biochem Cell Biol.* 2004;36(6):986–990. doi:10.1016/j.biocel.2003.12.002
37. Midwood KS, Chiquet M, Tucker RP, Orend G. Tenascin-C at a glance. *J Cell Sci.* 2016;129(23):4321–4327. doi:10.1242/jcs.190546
38. Kwak HJ, Park DW, Seo JY, et al. The Wnt/ β -catenin signaling pathway regulates the development of airway remodeling in patients with asthma. *Exp Mol Med.* 2015;47(12):e198. doi:10.1038/emm.2015.91
39. Haczku A, Atochina EN, Tomer Y, et al. *Aspergillus fumigatus*-induced allergic airway inflammation alters surfactant homeostasis and lung function in BALB/c mice. *Am J Respir Cell Mol Biol.* 2001;25(1):45–50. doi:10.1165/ajrcmb.25.1.4391

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