PM2.5 Induced Airway Remodeling via Wnt5a/ β -catenin Pathway in Chronic Obstructive Pulmonary Diseases

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Research

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Abstract

Background: PM2.5-associated airway remodeling has recently been recognized as a central feature of COPD. The activation of Wnt/ β -catenin pathway is closely related to the occurrence of airway remodeling. Accordingly, the goal of this study was to determine whether Wnt5a/ β -Catenin is involved in PM2.5-induced smooth muscle proliferation in vivo and vitro, which promoted the development of airway remodeling in COPD.

Methods: The effect of Wnt5a on β -Catenin-mediated airway remodeling was assessed by using an in vivo model of PM2.5-induced COPD and PM2.5-exposed human bronchial smooth muscle cell (HBSMC) in vitro. Small animal spirometry to measure lung function in mice. H&E staining and immunohistological inspection of emphysema and airway remodeling indexes. qPCR to detect Wnt5a, β -Catenin, TGF- β 1, CyclinD1 and c-myc mRNA expression. CCK8 assay for cellular activity. Western blotting for PCNA, α -SMA, Wnt5a, β -Catenin, PDGFR β and TenascinC protein expression. Detection of β -Catenin expression by cellular immunofluorescence.

Results: The exposure to PM2.5 led to emphysema, airway wall thickening, increased smooth muscle layer thickness, decreased lung function and induced the expression of Wnt5a, β -Catenin, PDGFR β and Tenascin C protein expression in lung tissue of mice. BOX5 alleviated PM2.5-induced these outcomes in mice. Moreover, PM2.5 induced the mRNA expression of Wnt5a, β -Catenin, TGF- β 1, CyclinD1 and c-myc in HBSMC. BOX5 also inhibited PM2.5-induced the increase of PCNA, α -SMA, Wnt5a, β -Catenin, PDGFR β and Tenascin C protein expression in HBSMC.

Conclusions: Our findings suggest that PM2.5 exposure induce HBSMC proliferation, contributing to airway remodeling via Wnt5a/ β -Catenin signaling pathway in vivo and in vitro, which could be a target of treatment of COPD.

Background

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 the significantly exposure to harmful particles or gases[1-3]. Airway remodeling is a distinctive pathological feature of COPD, and 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 correlated negatively with the severity of the disease in patients with COPD[4-6]. Although there are advances in the understanding of the inflammatory features of COPD, the mechanisms underlying the remodeling process are still poorly understood.

PM2.5 is an ambient air pollutant with a diameter of $\leq 2.5 \mu\text{m}$, characterized by small particle size, large surface area, and the ability to absorb toxins[7]. Recently, PM2.5 exposure is associated not only with an enhanced risk of developing COPD, but also with the exacerbated deterioration of lung function and

symptoms experienced by COPD patients[8, 9]. We previously showed that PM2.5 induced the expression of Wnt5a in human bronchial epithelial cells[10]. Wnt5a belongs to the Wnt gluco-protein 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 activated non-classical Wnt signaling contributes to the pathogenesis of COPD[12]. Specifically, Wnt5a increased TGF- β 1-induced upregulation of α -SMA expression to enhance smooth muscle cells contractility [13]. TGF- β 1 is involved in β -catenin-independent non-classical Wnt5a signaling, which induces ECM expression in airway smooth muscle cells and promotes tissue fibrosis[14]. However, Wnt5a regulates the proliferation of smooth muscle cells and its role in regulation of airway remodeling in response to PM2.5 remains unknown.

Platelet-derived growth factor receptor (PDGFR β) acts as a cell surface tyrosine kinase receptor and is involved in cell proliferation, survival and migration[15]. Emerging evidence suggests that Wnt signaling can contribute to smooth muscle cell precursor proliferation and adult lung disease development by directly regulating TenascinC transcription participating in the regulation of PDGFR β , which defined the Wnt/Tnc/PDGFR signaling axis[16]. However, it remains unclear whether this axis promotes airway remodeling and accelerates disease progression in COPD.

On this basis, we hypothesized that the Wnt5a/ β -Catenin signaling pathway is participated in PM2.5-induced airway smooth muscle cell proliferation through upregulation of PDGFR β expression, and led to airway remodeling. Hence, using HBSMC and an experimental model of COPD, this study was designed to elucidate the effects of Wnt/Tnc / PDGFR β axis in the airway remodeling of COPD caused by PM2.5.

Methods

Animal Experiments

C57BL/6 mice were purchased from the Experimental Animal Center of Guangzhou University of Chinese Medicine. Animal experiments were performed following the Declaration of Helsinki Convention on the Use and Care of Animals and were approved by the Animal Research Ethics Committee of Guangzhou Medical University.

Collection and Extraction of PM2.5

Traffic ambient PM2.5 was collected by aerodynamic impactors equipped with a glass fiber filter, quartz filter, or teflon membrane, DMSO was used to extract soluble parts of PM2.5. Sufficient specific samples for biological experiments were collected by high volume impactors equipped with glass fiber filter from arterial traffic road. Gravimetric analysis was used to analyze specific physicochemical characterizations of PM2.5. The procedure of PM2.5 samples and analysis methods were obtained as our previously described [10].

PM2.5 exposure in mice

Female C57BL mice(6-8w) were randomly divided into 4 groups of 12 mice each. PBS (20 μ l), PM2.5 (100 μ g/20 μ l), BOX5 (0.5 μ g/ml 10 μ l), and PM2.5+BOX5 (100 μ g/20 μ l+ 0.5 μ g/ml 10 μ l) were injected via tracheal drip, respectively, twice a week for 4 weeks of continuous exposure. Collection of lung tissue for follow-up experiments.

Lung function tests

Four weeks later, mice were anaesthetized intraperitoneally with 1.25 % avertin (2,2,2-tribromoethanol) 24 hours after the last exposure to PM2.5. 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) by Boyle's law maneuvers.

Histology and Immunohistochemistry

Mice lung tissues were collected and analyzed for small airways by histochemistry and immunohistochemistry according to our previous descriptions[17, 18]. The histology was performed on 4.0 μ m thick paraffin sections of lung tissues. Sections were incubated with Anti- α -SMA antibody. (ab5694, Abcam). Immunohistochemistry of α -SMA positive stained area was evaluated with the confocal microscope at 200 $\times$ magnification.

Analysis method of airway remodeling

The thickness of the small airway wall was the total area of the small airway wall (WAt) after normalization of the perimeter of basement membrane(Pbm), i.e., WAt/Pbm ($\mu m^2/\mu m$), which was used as an index of small airway remodeling. The requirement of airway was selected as follows: 1. basement membrane perimeter < 2000 μ m; 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) were 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, i.e. $Area/(Ao-Abm)\%$ (WA%), was used as an indicator of smooth muscle layer in airway remodeling.

Analysis method of emphysema

The mean linear intercept (MLI) was used to evaluate the size of the alveolar cavity, which was used as an index of emphysema. At the same field of view, "⊥" intersecting lines were drawn, and the length of each line (L) and the number of alveoli passing through each line (NS) were calculated, and MLI was expressed as $MLI=L/NS$.

Cell culture and treatment

The human bronchial smooth muscle cell line (HBSMC; American Type Culture Collection, Manassas, VA, USA) was cultured in SMCM (sciencell, USA). Cells were cultured at 37°C with 5% CO₂ with humidification

and then exposed (or not) to PM2.5 (3 µg/ml). HBSMC were pretreated with the Wnt5a antagonist BOX5 (Merck Millipore, Burlington, MA, USA) for 1 h prior to the addition of 3 µg/ml PM2.5.

Cell-viability assay

The viability of HBSMC was detected using the WST8 assay kit (CCK8; Promoter Biotechnology, Wuhan, China) according to the manufacturer's instructions. Cells were exposed to BOX5 (100, 200,300 µM) in SMCM 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

HBSMCs were cultured for 48 h to reach 60% density. Then immunofluorescence was performed following standard procedures. Cells were incubated with Anti-β-catenin antibody (ab32572, Abcam, 1:500) for 12 h at 4°C, followed by 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 examined using an inverted fluorescence microscope (Olympus-FL 500; Olympus Corporation, Tokyo, Japan).

Isolation of mRNA and real-time PCR analysis

Total mRNA was extracted using the Universal RNA Extraction Kit (B0004D, EZB, Shanghai, China) according to the manufacturer's instructions. mRNA 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 a SYBR Premix Ex Taq II (Novozymes) and a Bio-rad instrument II (Roche Diagnostics, Basel, Switzerland). GAPDH served as the housekeeping control.

Table 1

Primer	Primer sequence
Human Wnt5a	F:5'-ATTCTTGGTGGTCGCTAGGTA-3' R: 5'-CGCCTTCTCCGATGTACTGC-3'
Human β -catenin	F:5'-GGAATCCGAGCTGGACCTAAA-3' R: 5'-CCTGAAGCAAATCGACCACAG-3'
Human TGF- β 1	F:5'-GGGATTGGCTGTATGAGCACC-3' R: 5'-GGCGGGAAATTGTGAACTGA-3'
Human c-myc	F:5'-CACCTTGTAGCACGTCCTG -3' R: 5'-GACTCCCCAAGATGTGGTGG-3'
Human CyclinD1	F:5'-CAATGACCCCGCACGATTTC -3' R: 5'-CATGGAGGGCGGATTGGAA-3'
Human GAPDH	F:5'- TGTGGGCATCAATGGATTTGG-3' R: 5'-ACACCATGTATTCCGGGTCAAT-3'

Protein extraction and Western blot analysis

Protein extraction and Western blotting was conducted following 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, 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 hours at room temperature. The proteins of interest were detected by enhanced chemiluminescence reagents (FDbio science), and the band intensities were quantified by using the ImageJ software (National Institute of Health, Bethesda, MD, USA). The expression of the target proteins was normalized against the loading control, β -actin or α -tubulin.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 7.04 for windows (GraphPad Software, San Diego, USA) and are presented as median and IQR. Data was analyzed with a one-way ANOVA with Bonferroni post-hoc test or Kruskal–Wallis with Dunn's test for multiple comparison depending on respectively parametric or non-parametric datasets. $p < 0.05$ was considered statistically significant.

Result

PM2.5 stimulation induced airway remodeling and emphysema in mice.

We used H & E staining to observe the changes of lung histology and immunohistochemistry to detect the expression of α -SMA. Compared with the control group, the lung tissue destruction in different concentration of PM2.5-exposed mice was obvious, showing obvious emphysematous features such as enlarged and fused alveolar cavities and thinning, breaking and widening of alveolar septa (Fig 1A and 1B), indicating the occurrence of emphysema-like lesions. Lung function tests showed that different concentration of PM2.5 exposure downregulated the level of Peak inspiratory flow (PIF) and Peak inspiratory flow (PEF) in mice (Fig 1C and 1D). Based on the structural changes of alveoli, lung function and survival rate of mice, the mice in the medium concentration of PM2.5 exposure group were selected for follow-up experiments with reference to relevant literature. Compared with the control group, the airway wall thickness of mice exposed to PM2.5 for 1 month was significantly thickened (Fig 1E and 1F). Furthermore, the PM2.5 group showed increased α -SMA expression, thickening of the smooth muscle layer, and the percentage of the smooth muscle layer in the airway wall was significantly increased (Fig 1G-1I), suggesting that PM2.5 induced small airway remodeling in vivo.

PM2.5 induced HBSMC proliferation

To investigate the effect of PM2.5 on airway smooth muscle, we detected the expression of α -SMA and Proliferating cell nuclear antigen (PCNA) in airway smooth muscle cells after PM2.5 stimulation. PCNA is an intranuclear protein and involved in DNA replication that is mainly produced in proliferating and transformed cells[19]. After PM2.5 stimulation of airway smooth muscle cells, PCNA and α -SMA expression were upregulated (Fig 2A-2C), indicating that PM2.5 promoted airway smooth muscle proliferation.

PM2.5 increased Wnt5a/ β -Catenin pathway activation in lung tissue of mice

To investigate the effect of PM2.5 on the expression of Wnt5a/ β -Catenin signaling pathway and its potential role in airway remodeling, we measured the expression of Wnt5a/ β -Catenin signaling pathway in PM2.5-exposed lung tissues of mice by Western Blot. Compared with the control group, increased expression of Wnt5a and β -Catenin protein was detected in PM2.5 exposed group (Fig 3A-3C), and PM2.5 exposure also significantly upregulated PDGFR β and Tenascin C protein expression in lung tissue of mice (Fig 3D-3F), indicating that PM2.5 exposure induced the expression of Wnt5a/ β -Catenin signaling pathway in vivo.

PM2.5 increased Wnt5a/ β -Catenin pathway activation in HBSMC

To further investigate the effect of PM2.5 on the expression of Wnt5a/ β -Catenin signaling pathway in vitro, we measured the expression of Wnt5a/ β -Catenin signaling pathway in PM2.5-exposed HBSMC by Western Blot. Compared with the control group, increased expression of Wnt5a and β -Catenin protein was detected in PM2.5 exposed HBSMC (Fig 4A- 4C), and PM2.5 exposure significantly upregulated PDGFR β and Tenascin C protein expression in HBSMC (Fig 4D-4F). We also found increased expression of Wnt5a

and β -Catenin at the transcriptional level, as well as downstream target genes TGF- β 1, CyclinD1, and c-myc (Fig 4H). Cellular immunofluorescence staining of HBSMC revealed that the expression of β -Catenin was increased after PM2.5 stimulation and intranuclear staining was increased compared to the control group (Fig 4G), which suggested that PM2.5 induced the expression of Wnt5a and activated β -Catenin signaling in HBSMC.

Wnt5a-specific antagonist BOX5 alleviated PM2.5-induced airway remodeling in mice

To investigate the role of Wnt5a in PM2.5 exposure-induced airway remodeling in mice. BOX5, a Wnt5a-derived antagonistic peptide was used to block endogenous Wnt5a signaling, then the morphological changes of lung tissues were observed by H & E staining and the expression of α -SMA protein was detected by immunohistochemistry. As depicted in Figure 5A-5E, compared with the PM2.5 group, the airway wall thickness of PM2.5+BOX5-exposed mice thinned, the expression of α -SMA protein in smooth muscle layer decreased, and the ratio of smooth muscle layer to airway thickness (WA%) decreased (Fig 5A-5E), indicated that BOX5 inhibited PM2.5-induced airway smooth muscle layer thickening and airway remodeling. Moreover, the expression of Wnt5a/ β -Catenin signaling pathway in lung tissues of mice were observed by Western Blot. Compared with the PM2.5 group, the expression of Wnt5a, β -Catenin, PDGFR β and Tenascin C protein was downregulated in the BOX5 + PM2.5 group (Fig 5F-5K), indicated that BOX5 inhibited PM2.5-induced the expression of Wnt5a/ β -Catenin signaling pathway during the process of airway remodeling in lung tissues of mice.

Wnt5a-specific antagonist BOX5 alleviated PM2.5-induced HBSMC proliferation

First of all, the cytotoxicity of BOX5 in HBSMC was examined. HBSMC were stimulated with different doses of BOX5 (100-300 μ M) for 48 hours. No significantly changes in cell activity were observed at 100 μ M (Fig 6D), therefore, 100 μ M of BOX5 was subsequently selected as the experimental dose in combination with literature review. As expected, compared with the PM2.5 group, the levels of PCNA and α -SMA showed down-regulation in the BOX5 + PM2.5 group (Fig 6A-6C), indicating that BOX5 inhibited the PM2.5-induced HBSMC proliferation. In addition, BOX5 treatment down-regulated the PM2.5-induced increase of Wnt5a, β -Catenin PDGFR β and Tenascin C compared to PM2.5 group (Fig 6E-6J). These results suggest that 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[20]. Recent epidemiological studies both in China and abroad have found that PM2.5 in air pollution can increase the incidence, risk of acute exacerbation, lung function decline and mortality of COPD[21]. It was demonstrated that the incidence of acute exacerbations of chronic obstructive pulmonary disease (AECOPD) and acute respiratory infections (ARI) is significantly increased when PM2.5 pollution is severe[22].

Our findings demonstrated that PM2.5 exposure caused aberrant upregulation of Wnt5a/ β -Catenin signaling pathway both in HBSMC and lung tissue of mice.

Upregulated Wnt5a led to activation of β -Catenin, then increased the expression of TenascinC and PDGFR β , induced HBSMC proliferation and airway remodeling, targeting the Wnt5a/ β -Catenin signaling pathway may serve as an effective therapeutic strategy for PM2.5- induced airway remodeling in 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[23–25]. We constructed a mice model by tracheal drip injection of PM2.5. It has been found that COPD-like models can be successfully constructed by tracheal drip injection of PM2.5, which mainly exhibits small bronchial mucosal damage, wall thickening, cell squamous hyperplasia, fibrous tissue hyperplasia, and fibrous tissue hyperplasia. Differently from CSE model, PM2.5 mainly causes small airway lesions, such as bronchial dilatation and wall thickening, while CSE mainly shows severe emphysema lesions and poor diffusion function[26–28]. Our study also found that PM2.5 exposure led to thickening of the airway wall in mice, especially the smooth muscle layer, and in vitro PM2.5 stimulation of HBSMC led to proliferation, hypertrophy, and upregulation of intracellular proliferation-related protein PCNA expression. Our results suggest that PM2.5 exposure induced emphysema and airway remodeling in mice and induce airway smooth muscle cell proliferation leading to airway remodeling in vitro, which contributes to the development of COPD.

Furthermore, we found that the expression of Wnt5a and β -Catenin increased in HBSMC after PM2.5 exposure, and the expression of TGF- β 1, CyclinD1 and C-myc mRNA was upregulated in HBSMC. TGF- β 1 is secreted by airway epithelial cells, airway smooth muscle cells and eosinophils, which promotes matrix protein production, contractile protein expression and proliferation of airway smooth muscle cells and airway fibroblasts[29]. Cyclin D1 and c-myc are core transcription factors and target genes of the Wnt/ β -catenin signaling pathway[30]. C-myc and CyclinD1 are widely involved in cell and tissue development and are key downstream effectors of cell proliferation[31, 32]. In addition, Wnt5a and β -catenin expression were upregulated in 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 has been found to be increased in expression in COPD models and COPD patient specimens[14]. Combined with previous reports, the relationship between 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, transduces extracellular signals to intercellular regions, which promotes cell proliferation, survival and migration[15]. Tenascin C is a large hexameric, multimodal extracellular matrix protein and binds to cell surface receptors, ECM proteins, soluble factors or pathogens to regulate cell adhesion, migration, proliferation and differentiation[33, 34]. In a chronic asthma model, blocking the Wnt/ β -catenin signaling pathway inhibits airway remodeling, such as subepithelial fibrosis and smooth muscle proliferation, by downregulating TGF- β and tenascin C/PDGFR β expression[35]. In accordance with previous studies, we also detected that PM2.5 induced the expression

of PDGFR β and Tenascin C protein in the lung tissue and in HBSMC. Therefore, we speculate that Wnt5a/ β -Catenin signaling pathway is probably involved in PM2.5-induced airway remodeling through regulating tenascin C/PDGFR β pathway.

To further investigate the mechanisms by which Wnt5a/ β -Catenin signaling pathway may involve in PM2.5-associated airway remodeling in COPD, we applied Wnt5a-specific antagonist BOX5 to block endogenous Wnt5a signaling in mice and HBSMC. Our current data showed that BOX5 do not change the basal expression of Wnt5a, while BOX5 alleviated PM2.5-induced airway wall thickening, increased smooth muscle layer thickness of mice and attenuated PM2.5-induced HBSMC proliferation. It was found that Wnt, TenascinC and PDGFR expression was increased in adult smooth muscle-related lung disease[36]. β -Catenin siRNA led to a significant decrease in PDGFR β expression. Moreover, study showed that Wnt signaling regulated 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, as well as finding that this signaling axis is upregulated in both mouse 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 expression both in vivo and in vitro, demonstrating that Wnt5a/ β -Catenin signaling pathway is predominantly participated in airway remodeling caused by PM2.5 in COPD.

Conclusions

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

Abbreviations

HBSMC: human bronchial smooth muscle cell; COPD: chronic obstructive pulmonary disease; PIF: Peak inspiratory flow; PEF: Peak inspiratory flow; OD: optical density; ARI: acute respiratory infections; PM2.5: fine particulate matter ≤ 2.5 μm in diameter; MLI: mean linear intercept; Pbm: perimeter of basement membrane; Abm: area of basement membrane; IPF: idiopathic pulmonary fibrosis; PCNA: Proliferating cell nuclear antigen; PDGFR β : Platelet-derived growth factor receptor.

Declarations

Ethics approval and consent to participate

C57BL/6 mice were purchased from the Experimental Animal Center of Guangzhou University of Chinese Medicine. Animal experiments were performed following the Declaration of Helsinki Convention on the Use and Care of Animals and were approved by the Animal Research Ethics Committee of Guangzhou Medical University.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors contributions

Conceptualization: Pixin Ran and Weifeng Zou drafted the manuscript; Weifeng Zou, Ruiting Sun and Jinxing Hu performed the experiments. Xiaoqian Wang and Sha Liu interpreted the data. Meihua Guo and Wei Hong performed the soft analysis. Meihua Guo and Dong Ye review and editing the manuscript; All authors critically reviewed, read and approved the final manuscript.

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Figures

Figure 1

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

Figure 2

PM2.5 induced HBSMC proliferation. HBSMC was stimulated with PM2.5 at a concentration of 3 $\mu\text{g}/\text{ml}$ for 24 h and 48 h. (A,B,C) Western blot analysis showed that PM2.5 induced the expression of PCNA and α -SMA. *P<0.05, **P<0.01, compared to control group, n=3.

Figure 3

PM2.5 increased Wnt5a/β-Catenin pathway activation in lung tissue of mice. (A,B,C)Western blot analysis showed that PM2.5 increased the expression of Wnt5a and β-Catenin in lung tissue of mice. (D,E,F)

Western blot analysis showed that PM2.5 increased the expression of PDGFRβ and TenascinC in lung tissue of mice. *P<0.05, **P<0.01, compared to control group, n=5.

Figure 4

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

Figure 5

BOX5 alleviated PM2.5-induced airway remodeling in mice. Mice were randomly divided into PBS, PM2.5, BOX5, and PM2.5+BOX5. (A) H&E staining showed that BOX5 alleviated PM2.5-induced airway wall thickness. (B) Immunohistochemistry showed that BOX5 decreased PM2.5-induced α-SMA protein expression. (C) BOX5 decreased PM2.5-induced WAt/Pbm ($\mu\text{m}^2/\mu\text{m}$). (D) BOX5 decreased PM2.5-induced WAt%. (E) BOX5 decreased PM2.5-induced α-SMA positive stained area. (F, G, H) Western blot analysis showed that BOX5 downregulated PM2.5-induced Wnt5a and β-Catenin expression in lung tissue of mice. (I, J, K) Western blot analysis showed that BOX5 downregulated PM2.5-induced PDGFRβ and TenascinC expression in lung tissue of mice. * $P < 0.05$, ** $P < 0.01$, $n = 5$.

Figure 6

BOX5 alleviated PM2.5-induced HBSMC proliferation. HBSMC were divided into PBS, PM2.5, BOX5, and PM2.5+BOX5. (A,B,C)Western blot analysis showed that BOX5 downregulated PM2.5-induced PCNA and α-SMA expression in HBSMC.(D)CCK8 assay showed that the cell viability did not significantly change after 48 h of BOX5 (100μM) treatment, but the cell viability were declined at BOX5 treatment of 200μM and 300μM. (E,F,G)Western blot analysis showed that BOX5 downregulated PM2.5-induced Wnt5a and β-Catenin expression in HBSMC. (H,I,J)Western blot analysis showed that BOX5 downregulated PM2.5-induced PDGFRβ and TenascinC expression in HBSMC.*P<0.05, **P<0.01, n=3.