### Original Article Fibroblast growth factor 10 protects against particulate matter-induced airway inflammatory response through regulating inflammatory signaling and apoptosis

Lingjing Liu<sup>1\*</sup>, Ziqiang Xia<sup>2\*</sup>, Jingli Li<sup>1</sup>, Yiran Hu<sup>1</sup>, Qiang Wang<sup>1</sup>, Junjie Chen<sup>1</sup>, Shiqian Fan<sup>1</sup>, Jinming Wu<sup>2</sup>, Nian Dong<sup>1</sup>, Chengshui Chen<sup>1</sup>

<sup>1</sup>Department of Respiratory and Critical Care Medicine, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325006, China; <sup>2</sup>Department of Gastroenterology, First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325006, China. <sup>\*</sup>Equal contributors.

Received July 23, 2019; Accepted October 25, 2019; Epub November 15, 2019; Published November 30, 2019

Abstract: Chronic respiratory disorders are some of the most frequent and severe chronic diseases in China. Epidemiological research has shown that particulate matter (PM) is a risk factor and is closely correlated to the progression of numerous respiratory diseases. Fibroblast growth factor 10 (FGF10) is a mesenchymal-epithelial signaling messenger essential for the development and environmental stability of several tissues. Nevertheless, its role in PM-induced airway inflammation remains unclear. The present study aimed to explore the mechanisms underlying the FGF10-related slowing of lung injury and inflammation in vivo and in vitro, as well as the therapeutic potential of these phenomena. Mice were intraperitoneally injected with a vehicle (PBS) or FGF10 (0.5 mg/kg) at one hour before intratracheal treatment with vehicle (PBS) or PM (4 mg/kg) for two consecutive days. Human airway epithelial BEAS-2B cells were exposed to a vehicle (PBS) or FGF10 (10 ng/ml) in vitro at one hour prior to incubation with a vehicle or PM (200 ug/ml) for 24 hours. Then, the impact on inflammatory molecules was investigated. In vivo, it was found that FGF10 diminished the inflammatory cell aggregation and reduced the apoptosis. Interestingly, in the PM group, the level of FGF10 increased in the bronchoalveolar lavage fluid (BALF). However, the pre-treatment with FGF10 markedly impaired the PM-induced increase in IL-6, IL-8, TNF-α and PGE2 levels in BALF and the cell supernatant. In conclusion, the present findings indicate that FGF10 attenuates PM-induced airway inflammation by inhibiting apoptosis and inflammation. This may be exploited for the prevention and management of PM-induced airway inflammation.

**Keywords:** Airway inflammation, apoptosis, chronic respiratory disorders (CRD), fibroblast growth factor 10 (FGF10), particulate matter

#### Introduction

Chronic respiratory disorders (CRD), including chronic obstructive pulmonary disease (COPD) and asthma, are some of the most frequent and severe chronic diseases in China. The Chinese Adult Lung Health study has identified approximately 100 million cases of COPD in China [1] and 384 million cases worldwide [2]. Furthermore, 30 million people suffer from asthma in China [3], which corresponds to one of the largest CRD-related burdens worldwide. Moreover, in China, the upturn in industrialization and urbanization has propelled air pollution as one of the key factors behind the high incidence of these disorders [4]. Particulate matter (PM) has been recognized as the principal component of polluted air. Epidemiological research has shown that PM is closely correlated to the progression of numerous respiratory diseases [5]. PM comprises of various toxic substances, including metals, organic carbon and mineral salts, as well as bacteria and viruses. The American Cancer Society has shown that cardiopulmonary mortality increases by 6% for every 10  $\mu$ g/m<sup>3</sup> of PM2.5 concentration [6]. Since air pollution and respiratory disorders have increasingly become severe public health problems, the prevention and treatment of CRD in China has become a priority. The treatment of CRD presently centers on inhaled corticosteroids and bronchodilators. Although these treatments are effective for symptom relief, these do not effectively change the disease

progression. Therefore, elucidating the molecular mechanism that links air pollution and especially PM to the pathogenesis of CRD is of great importance for public health systems, since this is essential for developing novel therapeutic and preventive alternatives.

Epithelial integrity has attracted abundant attention in investigations correlated to CRD, which mainly focus on epithelial structural damage and abnormal immune function. These are both key pathophysiological components involved in the inflammation and remodeling of the airway observed in CRD [7]. Airway epithelium is the primary barrier against air pollution, and is thereby a deposit for PM attracted by respiratory airflow. Loss of epithelial integrity in the airway is one of the initial phenomena observed in the development of CRD [8]. Unlike airway remodeling, which involves incompletely reversible pathophysiological processes such as fibroblast activation and abnormal deposition of the extracellular matrix (ECM), the mechanisms underlying the destruction of airway epithelia remain unclear. At present, research on this aspect has mainly focused on inflammatory injury, and neglected this tissue's selfrepairing capabilities [9]. These aspects display a large potential for the identification of drug targets for promoting airway epithelial repair, in order to achieve early and effective treatments for CRD.

Fibroblast growth factor 10 (FGF10) belongs to the fibroblast growth factor family, which exhibits classic paracrine activity, and mediates stromal and epithelial cell signaling via mesenchymal-epithelial interactions [10]. Previous studies have focused on the role of FGF10 in embryonic lung morphogenesis and development, in which it promotes alveolar-bronchial epithelial mitosis to regulate the formation of the bronchial tree. The role of FGF10 in the repair of bronchial-pulmonary epithelial damage has sparked great scientific attention over the past decade [11]. At present, FGF10 has been considered to be able to regulate the mobilization and differentiation of mesenchymal stem cells, as well as the homeostasis of intrinsic cells of the lung structure, thereby promoting the repair of bronchial-pulmonary epithelial injury [12, 13].

Given the importance of FGF10 to repair functions [14], an animal model of PM-induced airway disease was established to assess the expression of FGF10 in bronchoalveolar lavage fluid (BALF). As expected, an elevated FGF10 expression was found in this setting. Thus, the present study aimed to evaluate the therapeutic potential and mechanisms underlying the attenuating effect of FGF10 on PM, and inflammation-induced lung injury *in vivo* and *in vitro*.

In the present study, the protective effect for FGF10 in PM-induced lung injury was discovered. These findings provide new insights into the pathogeny of this condition, allowing for the development of novel treatment alternatives. The present study may also promote the discovery of the beneficial effects of FGF10 and its therapeutic application in the context of other disorders.

#### Materials and methods

#### Reagents and antibodies

The PM (SRM NIST 1649b) was purchased from the Standard Reference Material Program, and certified by the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). Human recombinant FGF10 was purchased from PeproTech (Shanghai, China). The One Step TUNEL Apoptosis Assay Kit was purchased from Roche (Mannheim, Germany). The anticleaved caspase-3, Anti-Bcl-2, Anti-Bax, Anti-Ki67 and anti-GAPDH antibodies were obtained from Cell Signaling Technology (Beverly, MA, USA). The reagents implemented in the western blot assays were obtained from Beyotime (Shanghai, China).

#### Cells and animals

BEAS-2B cells were obtained from the Chinese Academy of Sciences (Shanghai, China), and cultured in RPMI 1640 medium (Hyclone, Logan, UT, USA) supplemented with 100 U/ml of penicillin, 100 mg/ml of streptomycin, and 10% heat inactivated fetal bovine serum (FBS) at 37°C with 5% carbon dioxide. Male C57BL/6J mice with weeks of age and 20-25 g of weight were provided by Shanghai SLAC Laboratory Animal Co., Ltd., and housed in a specific pathogen-free (SPF) facility in Wenzhou Medical University. All procedures performed on these animals were approved by the Institutional Animal Care and Use Committee.

#### Experiment design

For the in vitro assays, the PM was suspended in PBS at a stock concentration of 4 mg/cm<sup>3</sup>, and BEAS-2B cells before treatment with 200 ug/ml of PM. FGF10 (10 ng/ml) was used to treat the BEAS-2B cells at one hour before stimulation with PM. Regarding the in vivo experiments, a total of 40 male C57BL/6J mice were allocated at random to each of the four groups: (1) Sham group, mice received intraperitoneal PBS at one hour before the intratracheal instillation of PBS at the same dose as the PM group (Figure 1A); (2) FGF10 group, mice received intraperitoneal FGF10 (0.5 mg/kg) at one hour before the intratracheal instillation of PBS (Figure 1B); (3) PM group, mice received intraperitoneal PBS at one hour before intratracheal instillation of 100 µg of PM in 25 µL of PBS per day for two consecutive days (Figure 1C); (4) PM+FGF10 group, mice received intraperitoneal FGF10 (0.5 mg/kg) at one hour before treatment with PM for two consecutive days (Figure 1D).

## Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot

RIPA lysis buffer (Beyotime, Shanghai, China) with protease inhibitors was used to extract the intracellular proteins (Selleck, Shanghai, China). A Pierce BCA Protein Assay Kit (Thermo Fisher Scientific) was used for protein quantification. Samples with equal concentrations were separated with SDS-PAGE before transferring onto a PVDF membrane for the western blot with the previously mentioned antibodies. The immunoreactive bands were assessed on a Bio-Rad Laboratories system with an ECL reagent (Thermo Scientific, Waltham, MA, USA). The ImageJ software was used to assess the protein band intensity.

#### Dysfunction and inflammation of the pulmonary endothelial barrier

The inflammation in lung tissues was evaluated through the assessment of leukocyte migration into the alveolar space, and the local and systemic production of inflammatory mediators. Mice lungs underwent intratracheal lavage with 1-mL PBS injections, and approximately 0.8 mL was obtained for 10-minute centrifugation at 1,000 rpm at 4°C. Then, the protein content in the BALK samples was assessed using BCA assays (Bio-Rad Laboratories). The absorbance was read at 570 nm (Molecular Devices, USA).

## Histopathology and immunohistochemical staining

For the histopathology, the lung was inflated with 20 cm  $H_20$  pressure, and fixed with 4% paraformaldehyde and paraffinized. Lung injury severity was assessed *via* the observation of the morphological changes in the hematoxylin and eosin (H&E)-stained 5 µm sections. For the immunohistochemical staining, the same procedure was performed before incubation with the anti-cleaved Capase-3 and anti-KI67 antibodies, as well as the corresponding secondary antibodies.

#### Cell viability assays

Cell viability was determined *via* Cell Counting Kit-8 (CCK-8) assays. Cells that were seeded into 96 wells were PM treated for 24 hours, and the CCK-8 was added to the wells for two hours. The absorbance was read at 450 nm on a spectrophotometer (Tecan, Männedorf, Switzerland).

#### Cell apoptosis measurement

Apoptosis was quantified through Annexin V/ Propidium iodide (PI) staining and TUNEL apoptosis assays. Briefly, the treated cells were resuspended in 200  $\mu$ l of binding buffer, and treated with 5  $\mu$ l of Annexin V-FITC and PI (Beyotime) for 15 minutes in the dark. Then, the staining was assessed on a FACS flow cytometer (BD Biosciences, CA, USA) to quantify the apoptotic cells.

One Step TUNEL Apoptosis Detection Kits (Roche, Mannheim, Germany) were used to detect the DNA fragmentation following the *in vivo* lung injury. A Nikon ECLPSE Ti microscope was used for the imaging analysis (Nikon, Japan).

#### Wound healing assays

In order to assess the cell migration,  $1 \times 10^{6}$  cells were seeded into 6-well plates and drug treated for 24 hours under standard culture conditions. A wound was scraped into the cells using a p200 pipette tip, and cells were washed and incubated in RPMI-1640 containing 2% fetal bovine serum (FBS).



Figure 1. Schematic diagram of the in vivo experimental design. PM, Particulate matter; FGF10, Fibroblast growth factor.



**Figure 2.** Effect of PM on airway inflammation in mice. A. Histological changes of lung were detected by H&E staining after PM induction. The inflammatory cells were increased in the airway after PM instillation. The hematoxylin staining (blue) indicates the localization of nuclei, while the eosin staining (red) indicates the localization of the cytoplasm. These images were taken using a BX51 microscope DP71 camera at ×40, ×200, or ×400 magnification. B. The inflammation scores for the two groups. C. The level of FGF10 in the BALF was detected by ELISA. The results are presented as mean  $\pm$  standard error of the mean (SEM; \**P*<0.05, \*\**P*<0.01, *n* = 6).

#### Enzyme-linked immunosorbent assay (ELISA)

ELISA kits (R&D Systems) were implemented for the quantification of IL-6, IL-8, TNF- $\alpha$ , PGE2 and FGF10 concentrations in the BALF and cell supernatant, according to manufacturer's protocols.

#### Statistical analysis

Data were expressed as means  $\pm$  SEM from several independent experiments (n $\geq$ 5). Graph-Pad Prism 5.0 (San Diego, CA, USA) was used to perform all statistical analysis. The differences between two groups were assessed using Student *t*-test. Multiple group comparisons were performed by one-way ANOVA, followed by Tukey's post-hoc analysis. Results with P<0.05 were considered statistically significant.

#### Results

## Pulmonary inflammation and endogenous FGF10 elevation induced by PM in mice

The pro-inflammatory effects of PM on mice were evaluated through the intratracheal instillation of a vehicle (PBS) or PM (4 mg/kg) for two consecutive days. The histological changes in lung tissue were examined by H&E staining (**Figure 2A**). No damage was apparent in the samples from the vehicle group, while those from the PM group presented with evident inflammatory cell aggregates surrounding the PM deposits. The histopathology also revealed a significant increase in PM-induced airway inflammation (*P*<0.05, **Figure 2B**). Notably, the BALF FGF10 levels were greater in the PM group (*P*<0.01, **Figure 2C**).

# Pretreatment with FGF10 protects against PM-induced lung damage and inflammatory response

Exogenous recombinant FGF10 was administered for the treatment of PM-induced inflammation to investigate the impact of FGF10 in this context more in depth. First, in order to investigate the effects of PM-induced lung injury and the protective effects of FGF10 during disease development, lung tissue samples were subjected to pathologic analysis (Figure 3A). The H&E staining revealed that PM exposure caused the infiltration of inflammatory cells and alveolar septal thickening, when compared to the vehicle group. In contrast, the pretreatment with FGF10 significantly diminished the lung damage and preserved the normal tissue architecture and integrity. Although the lung injury scores were significantly higher in the PM group than in controls (P<0.01), the pretreatment with FGF10 obviously reduced these scores, when compared to the PM group (P<0.05, Figure 3B). In order to evaluate the PM-induced injury to the lung and tracheal epithelium, the total protein in BALF samples



**Figure 3.** FGF10 protects against lung histological and inflammatory after PM induction. A. The histological changes of lungs detected by H&E staining at 48 hours. The animals were randomly assigned into four groups: vehicle group, FGF10 group, PM group, and PM+FGF10 group. The images were taken using a BX51 microscope DP71 camera at ×40, ×200, or ×400 magnification. B. The inflammation scores for the four groups. C. The total protein level in the BALF was detected using a BSA protein assay kit. D-G. The expression of IL-6, IL-8, TNF- $\alpha$  and PGE2 in the BALF was measured by ELISA assay. The results are presented as mean ± standard error of the mean (SEM; \*\**P* < 0.01 vs Vehicle group; ##*P* < 0.01 vs PM group, *n*=3).



**Figure 4.** FGF10 protects against PM-induced lung injury, reduces the apoptosis level and increases the cell proliferation in lung tissue. (A) The representative sections of nuclear DNA fragmentation staining using TUNEL in the different groups, and the quantitative analysis of the number of TUNEL-positive airway epithelial cells (B) The IHC of cleaved-caspase 3 and (C) Ki67 of lung tissue sections obtained from the indicated groups, and the quantification of cleave-caspase 3 expression and Ki67 in lung tissue are shown. Scale bar = 100  $\mu$ m. The results are presented as mean ± standard error of the mean (SEM; \*\**P* < 0.01 vs Vehicle group; ##*P* < 0.01 vs PM group, *n*=3).

was quantified for each experimental group (**Figure 3C**). The total protein levels in BALF were higher than those in the vehicle groups (P<0.01), but these decreased in response to the FGF10 treatment (P<0.01). FGF10 alone had no effects on basal BALF protein levels, suggesting that this specifically prevents PM-induced alveolar leakage. In addition to these pathological changes, a significant increase in secreted IL-6, IL-8, TNF- $\alpha$  and PGE2 (**Figure 3D-G**) was observed in BALF in the PM groups, when compared to the vehicle groups (P<0.01). The FGF10 pretreatment substantially reduced the pro-inflammatory cytokine secretion (P<0.01).

#### FGF10 reduced apoptosis in lung tissue via the regulation of pro-apoptotic proteins and increasing cell proliferation

TUNEL staining was performed to assess the levels of apoptosis in lung tissue (**Figure 4A**). The number of TUNEL-positive epithelial cells increased in response to PM, but decreased in the presence of FGF10 (*P*<0.05). In order to explore the mechanisms underlying these effects, the cleaved caspase-3 expression was assessed through immunohistochemical analysis (**Figure 4B**), which increased in the PM group, but decreased following the FGF10 treatment. FGF10 alone had no effects on the

cleaved caspase-3 levels. The Ki67 staining revealed that the FGF10 treatment significantly increased the cell proliferation (Figure 4C).

## FGF10 promotes cell migration and protects BEAS-2B cells against PM-induced injury

PM exposure slowed the rates of cell migration, while FGF10 pre-treatment promoted this process (Figure 5A). In addition, FGF10 exhibited robust inhibitory effects on the IL-6, IL-8, TNF- $\alpha$  and PGE2 release from PM-challenged BEAS-2B cells, suggesting that this protects BEAS-2B cells against PM-induced apoptosis (Figure 5B-E).

## FGF10 prevents BEAS-2B cell death through the regulation of pro-apoptotic proteins

It was further determined whether FGF10 could protect against PM-induced apoptosis and increase cell proliferation in BEAS-2B cells. The apoptosis was evaluated in PM-induced lung cell models through Annexin V/PI labeling (**Figure 6A**), which was effectively reduced following FGF-10 treatment (*P*<0.05). The CCK-8 assays (**Figure 6B**) revealed that FGF10 pretreatment increased the BEAS-2B cell proliferation (*P*<0.01). In order to understand the protective mechanisms of FGF10, the expression of pro-apoptotic proteins cleaved caspase-3,



**Figure 5.** Effects of FGF10 on PM-induced cell migration, and the increase in proinflammatory marker in BEAS-2B cells. Cells treated with FGF10 (10 ng/ml) were used to treat BEAS-2B cells at one hour before stimulation with PM, and subsequently stimulated for 24 hours. A. Cell migration growth was analyzed by scratch assay. B-E. The intracellular concentrations of IL-6, IL-8, TNF- $\alpha$  and PGE2 were measured using ELISA. The results are presented as mean  $\pm$  standard error of the mean (SEM; \*\*P < 0.01 vs Vehicle group; ##P < 0.01 vs PM group, n=3).

Bcl-2 and Bax were examined by western blot (Figure 6C). The expression of cleaved caspase-3 and Bax significantly increased after PM injury, while the expression of Bcl-2 decreased. The FGF10 treatment significantly inhibited the expression/activation of all pro-apoptotic genes assessed. Taken together, these results indicate that FGF10 protects BEAS-2B cells from PM-induced apoptosis.

#### Discussion

Epidemiological studies have demonstrated that PM exposure induces acute inflammatory responses and chronic lung inflammation, which in turn promotes CRD, such as asthma and chronic bronchitis [15, 16]. Despite the abundant research, no effective treatment has been found for PM-induced lung injury, which remains an urgent medical necessity. In this scenario, it is essential to explore the mechanism underlying PM-induced airway inflammation. In the present study, the PM instillation model was used for the first time to investigate the protective effects of FGF10 on lung injury. It was found that PM elicited a severe inflammatory response, with an endogenous rise of FGF10 in BALF. Recombinant FGF10 was administered to mice exposed to PM, revealing that this molecule could effectively alleviate lung inflammation and inhibit cell apoptosis.

Fibroblast growth factors (FGF) participate in numerous cellular activities, such as proliferation, differentiation and migration [17]. In particular, FGF10 is key for the transduction of mesenchymal signals into epithelial cells, as well as bronchopulmonary development, and cardiac development and homeostasis [10, 18]. Furthermore, FGF10 acts as a paracrine messenger by binding to a specific FGF receptor 2b (Fgfr2b) through heparin/heparan sulfate molecules [19]. It has been reported that FGF10 can mobilize interstitial cells in the lung to reduce LPS-induced lung injury [20], while another study demonstrated that FGF10-FG-FR2 signaling mediates the differentiation of basal cells, promoting the repair of the alveolar epithelial barrier injury caused by influenza virus infection [21]. Indeed, this molecule appears to mediate the natural response of promoting the steady maintenance of cells intrinsic to lung structure upon injury. Previous study found that FGF10 prevented mechanicallyinduced DNA fragmentation in alveolar epithelial cells via Grb-SOS/RAS/Raf-1/ERK1/2 signaling, resulting in reduced apoptosis [22]. FGF10 also improved epithelial integrity, ame-



**Figure 6.** FGF10 reduces PM-induced apoptosis and proapoptotic protein expression, and increases cell proliferation in BEAS-2B cells. Cells were treated with FGF10 (10 ng/ml), were used to treat BEAS-2B cells at one hour before stimulation with PM, and subsequently stimulated for 24 hours. A. The apoptosis of BEAS-2B cells stimulated by PM and FGF10 detected by flow cytometry in the (1) vehicle, (2) FGF10 (10 ng/ml), (3) PM (200 ug/ml) and (4) PM+FGF10 groups. B. The *in vitro* proliferation assay by CCK-8 demonstrate that FGF10 significantly promoted cellular proliferation in BEAS-2B cells. C. The results of the protein expression of cleaved csapase-3, Bcl-2, Bax and GAPDH as loading control in the four groups, as determined by western blot. The results are presented as mean  $\pm$  standard error of the mean (SEM; \*\**P* < 0.01 vs Vehicle group; #*P* < 0.05, ##*P* < 0.01 vs PM group, *n*=3).

liorating high-altitude edema [23]. However, the impact of FGF10 on PM-induced airway inflammation has not been reported to date. In the present study, the effect of FGF10 on the PM-induced lung injury model was evaluated

throughout various experiments *in vivo* and *in vitro*.

An animal model of airway inflammation was established to investigate the FGF10-mediated

attenuation of PM-induced airway inflammation. The exposure to PM was corroborated to trigger a severe inflammatory response, as evidenced by the increased levels of protein and inflammatory cytokines in BALF, the thickening of the alveolar septa as observed under H&E microscopy, and the greater apoptosis levels, as reflected by the increased cleaved caspase-3 levels. PM exposure was found to upregulate pro-inflammatory factors in BALF, such as IL-6, IL-8, TNF-α and PGE2, which favor inflammation and alveolar epithelial damage. FGF10 administration effectively reduced the inflammatory factors and total protein in BALF. Most importantly, FGF10 attenuated the histopathological changes, inflammation and apoptosis associated with PM exposure, and increased cellular proliferation, and this was in harmony with presently available research.

Epithelial cells are a major cell type in lung tissues, which mediate repair and intervene in the pathophysiology of CRD [24-26]. The dysfunction of epithelial barriers has been recognized as a key component in the pathogenesis of multiorgan failure [27]. Thus, BEAS-2B cells were used to investigate the protective role of FGF10 in PM-induced acute lung injury. Similar to the results from the *in vivo* experiments, FGF10 effectively hindered the PM-induced increase in inflammatory factors and inhibited cell apoptosis. Together, these results indicate that FGF10 has a significant protective effect on PM-induced lung injury.

The present study has certain limitations: First, since FGF10 has widespread effects, the intraperitoneal route selected for the administration was bound to lead to systemic FGFR2, resulting to a series of side effects, despite the satisfactory results. Therefore, in the future, the investigators intend to improve the administration method by using atomization for local administration, which should improve the results. Second, BEAS-2B cells were used for the in vitro assays, which are virus-transformed, immortalized bronchial epithelial cells. In the future, primary epithelial cells from mice should be utilized. Future studies should also investigate the participation of signaling mediated by high mobility group box 1-receptor for advanced glycation end-products (HMGB1-RAGE) in the context of PM-induced lung injury models. Indeed, this component has been recently found to be implicated in this context, with the rapid release from the nucleus into the extracellular matrix, mediating apoptosis and inflammatory activation. Thus, in the future, the investigators will focus in elucidating the molecular mechanisms underlying the protective role of FGF10 in PM-induced lung injury.

#### Conclusion

In summary, the present study was able to successfully construct short-term PM-induced lung injury models both in vivo and in vitro. The treatment with PM elicited a severe inflammatory response with increased IL-6, IL-8, TNF- $\alpha$  and PGE2 levels in BALF, and cell apoptosis. FGF10 administration effectively alleviated the inflammation and reduced the expression of inflammatory factors, demonstrating for the first time that exogenous recombinant FGF10 ameliorates PM-mediated pulmonary injury through the alleviation of inflammation, the reduction of cell apoptosis, and increasing cell proliferation. These present results suggest that FGF10 can effectively alleviate airway inflammation. These may serve as a foundation for the potential use of FGF10 atomization or inhalation as a clinically useful treatment approach for airway inflammation.

#### Acknowledgements

This study was supported by Wenzhou Technology Foundation (Y20180125), Zhejiang Provincial Natural Science Foundation (LZ15-H010001), Technology Bureau of Wenzhou (Nos. Y20180189), Zhejiang Provincial Science Technology Department Foundation (202036-1781), Zhejiang Provincial Science Technology Department Foundation (2015103253), The National Nature Science Foundation of China (81570075, 81770074), and The National Key Research and Development Program of China (2016YFC1304000). We would like to thank Bellusci Saverio from Justus-Liebig University Giessen for the careful revision of the manuscript.

#### Disclosure of conflict of interest

#### None.

Address correspondence to: Jinming Wu, Department of Gastroenterology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China. Tel: +86-13587694167; Fax: +860577-55578033; E-mail: wzfydw@163.com; Nian Dong and Chengshui Chen, Department of Respiratory and Critical Care Medicine, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325006, China. Tel: +86-15067810816; Fax: +86-0577-55578033; E-mail: 154598090@ qq.com (ND); Tel: +86-13806889081; Fax: +86-0577-55578033; E-mail: chenchengshui@wmu. edu.cn (CSC)

#### References

- [1] Wang C, Xu J, Yang L, Xu Y, Zhang X, Bai C, Kang J, Ran P, Shen H and Wen F. Prevalence and risk factors of chronic obstructive pulmonary disease in China (the China Pulmonary Health [CPH] study): a national cross-sectional study. Lancet 2018; 391: 1706-1717.
- [2] Adeloye D, Chua S, Lee C, Basquill C, Papana A, Theodoratou E, Nair H, Gasevic D, Sridhar D and Campbell H. Global and regional estimates of COPD prevalence: systematic review and meta-analysis. J Glob Health 2015; 5: 020415.
- [3] Lin J, Wang W, Zhou X, Wang C, Huang M, Cai S, Chen P, Lin Q, Zhou J and Gu Y. The level of asthma control in China from a national asthma control survey. Chin J Tuberc Respir Dis 2017; 40: 494-498.
- [4] Yang H, Li S, Sun L, Zhang X, Hou J and Wang Y. Effects of the ambient fine particulate matter on public awareness of lung cancer risk in China: evidence from the internet-based big data platform. JMIR Public Health Surveill 2017; 3: e64.
- [5] Shi J, Chen R, Yang C, Lin Z, Cai J, Xia Y, Wang C, Li H, Johnson N and Xu X. Association between fine particulate matter chemical constituents and airway inflammation: a panel study among healthy adults in China. Environ Res 2016; 150: 264-268.
- [6] Abrahamowicz M, Schopflocher T, Leffondré K, du Berger R and Krewski D. Flexible modeling of exposure-response relationship between long-term average levels of particulate air pollution and mortality in the American Cancer Society study. J Toxicol Environ Health A 2003; 66: 1625-1654.
- [7] De Rose V, Molloy K, Gohy S, Pilette C and Greene CM. Airway epithelium dysfunction in cystic fibrosis and COPD. Mediators Inflamm 2018; 2018: 1309746.
- [8] De Grove K, Provoost S, Brusselle G, Joos G and Maes T. Insights in particulate matter-induced allergic airway inflammation: focus on the epithelium. Clin Exp Allergy 2018; 48: 773-786.
- [9] Xu F, Cao J, Luo M, Che L, Li W, Ying S, Chen Z and Shen H. Early growth response gene 1 is essential for urban particulate matter-induced

inflammation and mucus hyperproduction in airway epithelium. Toxicol Lett 2018; 294: 145-155.

- [10] Yuan T, Volckaert T, Chanda D, Thannickal VJ and De Langhe SP. Fgf10 signaling in lung development, homeostasis, disease, and repair after injury. Front Genet 2018; 9: 418.
- [11] Prince LS. FGF10 and human lung disease across the life spectrum. Front Genet 2018; 9: 517.
- [12] Wu J, Chu X, Chen C and Bellusci S. Role of fibroblast growth factor 10 in mesenchymal cell differentiation during lung development and disease. Front Genet 2018; 9: 545.
- [13] Halim NS, Aizat WM and Yahaya BH. The effect of mesenchymal stem cell-secreted factors on airway epithelial repair. Regen Med 2018; 14: 15-31.
- [14] Volckaert T and De Langhe SP. Wnt and FGF mediated epithelial-mesenchymal crosstalk during lung development. Dev Dyn 2015; 244: 342-366.
- [15] Guan WJ, Zheng XY, Chung KF and Zhong NS. Impact of air pollution on the burden of chronic respiratory diseases in China: time for urgent action. Lancet 2016; 388: 1939-1951.
- [16] Simoni M, Baldacci S, Maio S, Cerrai S, Sarno G and Viegi G. Adverse effects of outdoor pollution in the elderly. J Thorac Dis 2015; 7: 34-45.
- [17] Hubert F, Payan SM and Rochais F. FGF10 signaling in heart development, homeostasis, disease and repair. Front Genet 2018; 9: 599.
- [18] Ndlovu R, Deng LC, Wu J, Li XK and Zhang JS. Fibroblast growth factor 10 in pancreas development and pancreatic cancer. Front Genet 2018; 9: 482.
- [19] Itoh N and Ohta H. Fgf10: a paracrine-signaling molecule in development, disease, and regenerative medicine. Curr Mol Med 2014; 14: 504-509.
- [20] Tong L, Zhou J, Rong L, Seeley EJ, Pan J, Zhu X, Liu J, Wang Q, Tang X and Qu J. Fibroblast growth factor-10 (FGF-10) mobilizes lung-resident mesenchymal stem cells and protects against acute lung injury. Sci Rep 2016; 6: 21642.
- [21] Quantius J, Schmoldt C, Vazquez-Armendariz Al, Becker C, El Agha E, Wilhelm J, Morty RE, Vadasz I, Mayer K and Gattenloehner S. Influenza virus infects epithelial stem/progenitor cells of the distal lung: impact on Fgfr2bdriven epithelial repair. PLoS Pathog 2016; 12: e1005544.
- [22] Upadhyay D, Correa-Meyer E, Sznajder JI and Kamp DW. FGF-10 prevents mechanical stretch-induced alveolar epithelial cell DNA damage via MAPK activation. Am J Physiol Lung Cell Mol Physiol 2003; 284: L350-L359.

- [23] She J, Goolaerts A, Shen J, Bi J, Tong L, Gao L, Song Y and Bai C. KGF-2 targets alveolar epithelia and capillary endothelia to reduce high altitude pulmonary oedema in rats. J Cell Mol Med 2012; 16: 3074-3084.
- [24] Lachapelle P, Li M, Douglass J and Stewart A. Safer approaches to therapeutic modulation of TGF- $\beta$  signaling for respiratory disease. Pharmacol Ther 2018; 187: 98-113.
- [25] Pettersen CA and Adler KB. Airways inflammation and COPD: epithelial-neutrophil interactions. Chest 2002; 121: 142S-150S.
- [26] Pilette C, Colinet B, Kiss R, André S, Kaltner H, Gabius HJ, Delos M, Vaerman JP, Decramer M and Sibille Y. Increased galectin-3 expression and intra-epithelial neutrophils in small airways in severe COPD. Eur Respir J 2007; 29: 914-922.
- [27] Fink MP and Delude RL. Epithelial barrier dysfunction: a unifying theme to explain the pathogenesis of multiple organ dysfunction at the cellular level. Crit Care Clin 2005; 21: 177-196.