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The proteasome-dependent degradation of ALKBH5 regulates ECM deposition in $PM_{2.5}$ exposure-induced pulmonary fibrosis of mice

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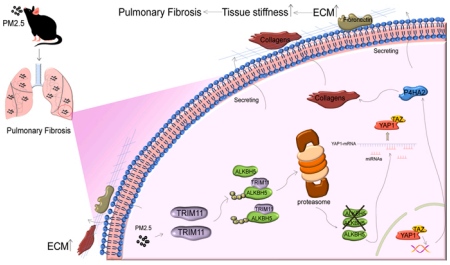
HIGHLIGHTS

- Long-term exposure to $PM_{2.5}$ induced pulmonary fibrosis through the YAP1 signals activation.
- $PM_{2.5}$-induced pulmonary fibrosis was associated with decreases of ALKBH5 proteins levels.
- ALKBH5 was recognized by TRIM11 and then degraded through the proteasome pathway, subsequently activated YAP1 signals.

GRAPHICAL ABSTRACT



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ABSTRACT

Long-term inhalation of fine particulate matter ($PM_{2.5}$) can cause serious effects on the respiratory system. It might be attributed to the fact that $PM_{2.5}$ could directly enter and deposit in lung tissues. We established models of $PM_{2.5}$ exposure in vivo and in vitro to explore the adverse effects of ambient $PM_{2.5}$ on pulmonary and its potential pathogenic mechanisms. Our results showed that $PM_{2.5}$ exposure promoted the deposition of ECM and the increased stiffness of the lungs, and then led to pulmonary fibrosis in time- and dose- dependent manners. Pulmonary function test showed restrictive ventilation function in mice after $PM_{2.5}$ exposure. After $PM_{2.5}$ exposure, ALKBH5 was recognized by TRIM11 and then degraded through the proteasome pathway. ALKBH5 deficiency ($ALKBH5^{-/-}$) aggravated restrictive ventilatory disorder and promoted ECM deposition in lungs of mice induced by PM2.5. And the YAP1 signaling pathway was more activated in $ALKBH5^{-/-}$ than WT mice after $PM_{2.5}$ exposure. In consequence, decreased ALKBH5 protein levels regulated miRNAs and then the miRNAs-targeted YAP1 signaling was activated to promote pulmonary fibrosis induced by $PM_{2.5}$.

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1. Introduction

The harm of air pollution to human health, especially fine particulate matter ($PM_{2.5}$) is still a public health issue of global concern. Nowadays, the annual limit of the WHO air quality guidelines was 5 μg/m^3 (https://www. who.int). In 2019, more than 90% of the world's population experienced annual average $PM_{2.5}$ concentrations exceeded the 10 μg/m^3, 4.14 million deaths and 118 million lost DALYs attributed to long-term exposure to $PM_{2.5}$. $PM_{2.5}$ was considered as the leading risk factor among all environmental and occupational risks (Institute, 2020). $PM_{2.5}$ had demonstrated to be a risk factor of various respiratory diseases, such as the pulmonary fibrosis, chronic obstructive pulmonary disease (COPD) and emphysema (Gu et al., 2017; Shull et al., 2021; Sun et al., 2020; Wang et al., 2019b). Previous research demonstrated that each increment of 10 μg/m^3 in 2-day moving average of $PM_{2.5}$ concentrations was significantly associated with a 0.29% increase in mortality from respiratory diseases (Chen et al., 2017b).

Pulmonary fibrosis (PF) is characterized by uncontrolled extensive deposition of collagen fibers, which eventually leads to organ scars and respiratory failure with high mortality and limited treatment options (Sun et al., 2020). Lung extracellular matrix (ECM) is the microenvironment in which tissue cells live, and its dynamic balance is the basis for providing the mechanical stiffness and elastic recoil required for normal lung function (Redente et al., 2020; Upagupta et al., 2018). It is a crucial key of the onset of a fibrotic scar (Dong and Ma, 2016). Studies demonstrated that there was increased matrix stiffness in lung of human pulmonary fibrosis compared with normal lung tissue, and the average Young's modulus in PF fibrotic lung tissue was approximately 8-fold compared with normal lung tissue (Booth et al., 2012; Liu et al., 2015). Meanwhile, increased stiffness of ECM promotes fibrous changes in the lungs. This process might affect the changes of the lung shape and activity of cells, as well as the fibroblast factors expression, such as transforming growth factor beta 1 (TGF-β1), the small GTPase RhoA and Rho kinase (RhoA/ROCK), megakaryoblastic leukemia factor-1 (MKL-1) (Burgstaller et al., 2017; Huang et al., 2012; Upagupta et al., 2018; Zhou et al., 2013).

Yes-associated protein (YAP1) and transcriptional co-activator with PDZ-binding motif (TAZ) are the central effectors in HIPPO pathway, which are considered as "an on–off mechanosensing switch for ECM stiffness" (Yamashiro et al., 2020). Recent studies had shown that YAP1 activation was the pathological hub in multiple organ fibrosis, including lung (Alsamman et al., 2020; Francisco et al., 2020; Haak et al., 2019; Strippoli et al., 2020). Prolyl-4- hydroxylase A subunit 2 (P4HA2) is a crucial enzyme catalyzing collagen formation. Functionally, P4HA2 excessive expression enhanced the collagens deposition in vivo and in vitro (Feng et al., 2017), and promoted increased stiffness of extracellular matrix (ECM) (Pankova et al., 2019; Toss et al., 2018). Especially, recent research had demonstrated that the activation of YAP1 could drive the expression of P4HA2 (Pankova et al., 2019). What`s more, epigenetic mechanism studies had proved that the levels of YAP1 were regulated by microRNAs (miRNAs) (Jin et al., 2019; Jin et al., 2020), and miRNAs dysregulation was associated with a variety of respiratory diseases induced by $PM_{2.5}$ (Dai et al., 2021; Wang et al., 2019a). Therefore, it is hypothesized that the mechanism of $PM_{2.5}$ promoting the increase of ECM stiffness of lung might relate with the miRNAs-modulated YAP1/ P4HA2 signaling pathway activation.

MiRNAs dysregulation is impacted by multiple regulatory factors, and nowadays observations manifested a critical role played by AlkB homolog 5 (ALKBH5), which had brought effect to maturation of miRNAs (Jin et al., 2020; Zhu et al., 2019). In addition, recent studies had proved that ALKBH5 was closely related to lung injury and fibrosis of the extrapulmonary organs, such as non-small cell lung cancer (NSCLC) (Jin et al., 2020), lung squamous cell carcinoma (Xu et al., 2020), and renal fibrosis (Ning et al., 2020). Moreover, previous studies demonstrated that exposure to chemicals was associated with reduction of ALKBH5 expression, such as arsenite-induced oxidative stress increased (Zhao et al., 2019), black phosphorus quantum dots (BPQDs)-ferroptosis of lung cells (Ruan et al., 2021).

The amount of cellular proteins are controlled by multiple mechanisms, especially ubiquitin-proteasome system, which its deregulation is associated with respiratory diseases (Katzen and Beers, 2020; Weathington et al., 2013), including pulmonary fibrosis (Inui et al., 2021). Ubiquitin-proteasome system has been determined that the cooperation of ubiquitin-related enzymes is used to gradually label the target proteins. Then the target proteins are bound to E3-ubiquitin ligases, following delivered to the proteasome and eliminated, which is an important link in maintaining cell protein homeostasis (Roque et al., 2020). ALKBH5 protein accumulation was potentially regulated by ubiquitin-proteasome system, because endogenous ubiquitylation sites investigate demonstrated ALKBH5 could have ubiquitination sites (K147) (Wagner et al., 2011). Subsequently, recent evidences are accumulating to demonstrate that exposure to exogenous chemicals is associated with abnormal conditions of proteasome pathway (Mallampalli et al., 2020; Pomatto et al., 2018). Our previous study declared an increase inflammation in the lungs after $PM_{2.5}$ exposure (Zhou et al., 2019). Tripartite Motif Containing 11(TRIM11) is defined as E3 ubiquitin ligases, which can bind to its specific substrates and degrade them through the proteasome pathway, and is closely related to the inflammation and lung injury (Liu et al., 2016). Meanwhile, ALKBH5 is predicted to be a substrate of TRIM11 through Ubibrowser database. Therefore, an assumption is reasonable that ALKBH5 protein might be regulated in a TRIM11-dependented proteosome pathway manner.

Here, $PM_{2.5}$ exposure mice model was established to evaluate the PF and explore it's potential molecular mechanism. Our results showed that ALKBH5 protein decreased in a TRIM11-dependented proteasome pathway manner, subsequently affected miRNAs-modulated YAP1 signaling pathway activity, then promoted the deposition of extracellular matrix and led to pulmonary fibrosis. ALKBH5 knockout mice were used to study the key role of ALKBH5 in pulmonary fibrosis after $PM_{2.5}$ exposure. Our findings could deeply explain the potential pathological mechanisms of PF induced by $PM_{2.5}$.

2. Materials and methods

2.1. Animals and $PM_{2.5}$ exposure Study design

6-week-old male C57BL/6 mice, body weights were 18–23 g, were obtained from the Beijing Vital River Laboratory Animal Technology Co., Ltd, (Beijing, China). They were acclimated for a week before the exposure. During the acclimation and exposure, all mice were treated humanely under the standard temperature (20.0–23.0 ℃) and humidity (48.0%-51.3%), provided free distilled water and commercial standard food. The body weights of mice were recorded once a week.

According to the principle of random grouping, 72 mice were divided into 3 groups and kept in different exposure chambers: filtered air (FA), unfiltered air (UA) and concentrated $PM_{2.5}$ air (CA). The FA and UA mice were respectively exposed to clean air in the chamber with high-efficient particulate air (HEPA) filter, atmosphere in the chamber without HEPA-filters. The CA mice received concentrated $PM_{2.5}$ ambient air by a $PM_{2.5}$ concentration enrichment system (Beijing Huironghe Technology Co., Ltd, Beijing, China). The aerosol monitor (TSI instrument Co., Ltd, Minneapolis, USA) and the distribution of the particulate matters by ultraviolet aerodynamic particle sizer (TSI instrument Co., Ltd, Minneapolis, USA) was used to monitor the concentrations of $PM_{2.5}$ in the exposure chambers. The exposure protocol was 6 h/d (from 9:00 a.m. to 15:00 p.m.), 7 days/week for 8 weeks or 16 weeks at the exactly same time. During the remaining time, the mice were placed into FA chamber.

Wild type (WT) and $ALKBH5^{-/-}$ (alkbh5 gene knockout, KO) mice were generously gifted by Dr. Hailin Wang from Chinese Academy of Sciences (CAS). Twenty-four male WT and 24 male $ALKBH5^{-/-}$ mice (18.5–24 g, 6-week-old) were divided randomly into two groups (FA and