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$PM_{2.5}$ triggers autophagic degradation of Caveolin-1 via endoplasmic reticulum stress (ERS) to enhance the TGF-β1/Smad3 axis promoting pulmonary fibrosis

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ABSTRACT

Air pollution is highly associated with respiratory diseases. However, the influence and mechanism of particulate matter with aerodynamic equal to or less than 2.5 μm ($PM_{2.5}$) in lung homeostasis remain unclear. Herein, we demonstrated the induction of pulmonary fibrosis (PF) by $PM_{2.5}$ exposure. The animal model showed that $PM_{2.5}$ exposure could activate the oxidative stress and inflammation response, promoting epithelial-mesenchymal transition and accumulation of collagen, high expression of pro-fibrotic factors, and pathological characteristics of fibrosis. The proteomic analysis indicated that $PM_{2.5}$ exposure decreased the expression of caveolin-1 (Cav-1), and many differential proteins were enriched in the TGF-β1/Smad, endoplasmic reticulum stress (ERS) and autophagy pathways. Combining in vivo and in vitro experiments, it was found that $PM_{2.5}$ exposure could reduce Cav-1 protein levels and activate TGF-β1/Smad3 signaling pathways through ERS and autophagy pathways, thereby inducing cell apoptosis and promoting pulmonary fibrosis. However, inhibiting ERS could alleviate the occurrence of autophagy, and blocking the autophagy system could increase the level of Cav-1 protein and inhibit TGF- β 1/Smad3 signaling pathway to improve pulmonary fibrosis. Therefore, we demonstrated that the exposure of $PM_{2.5}$ could enhance the ERS induced-autophagy-mediated Cav-1 degradation, thus activating the TGF-β1/Smad3 axis to promote pneumonocytes apoptosis and overproduction of extracellular matrix (ECM), finally aggravating PF. Moreover, our findings revealed that intermittent exposure to high doses of $PM_{2.5}$ was more toxic than continuous exposure to low dose.

1. Introduction

The high occurrence of pulmonary disorders is significantly associated with particulate matter with aerodynamic equal to or less than 2.5 μm ($PM_{2.5}$) exposure, including fibrosis and inflammation (Li et al., 2013; Gu et al., 2017; Shou et al., 2020; Tahara et al., 2021). $PM_{2.5}$ is an air pollutant characterized by small particle size, large surface area, and strong ability to absorb toxins (Du et al., 2018; Kim et al., 2018). Because of these properties, the smallest airways, such as the alveolar tissue, can be significantly invaded by $PM_{2.5}$. Many compositions can be found in $PM_{2.5}$, including polycyclic aromatic hydrocarbons, automobile exhaust particles, black carbon, nitrate, sulfate, and metals (Hu et al., 2017). Compared to other pollutants, $PM_{2.5}$ can easily deposit in the lung tissue of mice after exposure (Hu et al., 2017). Additionally, various biological processes are dramatically affected by $PM_{2.5}$ exposure, such as pulmonary function, coagulation balance, and cytokine production (Jiang et al., 2017). The incidence of pulmonary fibrosis (PF), a common pulmonary disorder, is continuously increasing. PF is a kind of lung dysfunction caused by interstitial lung inflammation and collagen fibers deposition (Gross and Hunninghake, 2001; Tahara et al., 2021; Thannickal et al., 2004), then progressive respiratory failure. However, the cellular and molecular mechanisms of $PM_{2.5}$-induced PF remain unclear.

Endoplasmic reticulum stress (ERS) refers to the spontaneous regulation process of ER to restore homeostasis after physiological dysfunction caused by changes in ER homeostasis. It can be coupled with signaling pathways such as oxidative stress, ubiquitin proteasome pathway (UPP) and inflammatory molecular signals. It has been proven that ERS can be caused by changes in metabolic conditions, toxic

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substances, protein folding, glycoylation disorders, protein delivery disorders to the Golgi apparatus, and calcium imbalances (Wang and Kaufman, 2016). It is becoming increasingly important to study the influence of ERS on human health. $PM_{2.5}$, as an exogenous pollutant, can induce the ERS (Liu et al., 2022a), which is a signaling response system and a self-protection molecular mechanism. In ERS, the cells activate unfolded protein response (UPR) to reduce cellular damage, unfolded or misfolded proteins to re-establish ER homeostasis. During the long-term ERS process, UPR is not sufficient to clear an increasing amount of unfolded proteins, nor can it remove damaged organelles. Autophagy is activated to restore ER homeostasis, thereby promoting cell survival. However, over activated autophagy induced by excessive or prolonged ERS will ultimately lead to cell death (Senft and Ronal, 2015; Mehrbod et al., 2019).

Autophagy comprises a highly conserved catabolic progress among eukaryotes.

Autophagy refers to the activation of lysosomal pathways in cells to maintain cellular homeostasis when stress stimuli occur, thereby clearing abnormal proteins and damaged organelles (Mukhopadhyay et al., 2014). The main feature of autophagy is the formation of autophagosomes with double layer vesicular structure, which encapsulate some misfolded proteins and damaged organelles, and are transported to lysosomes for recycling and degradation (Wang et al., 2019). Dysfunction of autophagosomal contents can lead to cell apoptosis (Levine and Kroemer, 2008; Levine et al., 2011; Mizushima and Komatsu, 2011). Therefore, autophagy can present dual roles. On the one hand, physiological autophagy can promote normal biological progress to support cell proliferation (Katoh and Katoh, 2017); on the other hand, excessive autophagy can induce cell death (Levine and Kroemer, 2008). Research has found that autophagy is involved in various stress responses, such as viral infections and inflammatory reactions, as well as pathological mechanisms such as respiratory system diseases, hematological system diseases, and immune system diseases (Moosavi and Djavaheri-Mergny, 2019). Although the contribution of autophagy-induced cell apoptosis to PF has been reported, how $PM_{2.5}$ exposure contributes to PF needs further investigation.

Caveolus are flask shaped invaginations rich in lipids, structural proteins (Caverns), and various signaling molecules that exist in the plasma membrane. There are various forms of caveolin in the body, which can be “embedded” in the cell membrane, present in the cytoplasm, and secreted outside the cell (Siwaponanan et al., 2022). Caveolin-1 (Cav-1) is a structural protein of the caveolus that is involved in various cellular functions, including endocytosis and signaling transduction. Cav-1 also plays an important role in different fibrotic diseases (Gvaramia et al., 2013). For example, in carbon tetrachloride-induced liver fibrosis, Cav-1 expression in liver tissues is significantly reduced, and its inhibition accelerates the development of fibrosis, inflammation, and oxidative stress response induced by TGF-β1 (Ji et al., 2018). Cav-1 is also downregulated in bleomycin-injured mouse lungs and lung sections of idiopathic PF patients. Meanwhile, Cav-1 overexpression can inhibit the bleomycin-induced upregulation of IL-1β and caspase-1, inhibit inflammasome activity, and prevent the bleomycin-mediated PF (Lin et al., 2019). Additionally, Cav-1 is downregulated in silicosis fibrosis (He et al., 2022), muscle fibrosis (Batista et al., 2021), atrial fibrosis (Zhang et al., 2021), and renal tubulointerstitial fibrosis (Yu et al., 2021). Shihata et al. found that Cav-1 is a potential target for many fibrotic diseases, including heart, lung, kidney, and liver fibrosis (Shihata et al., 2017). These studies have indicated that Cav-1 has a negative regulatory effect on fibrosis. The latest research has found that transfecting the CAV-1 overexpression plasmid into alveolar cells through electroporation can effectively reduce the degree of bleomycin induced pulmonary fibrosis, and at the same time, the recruitment of neutrophils and macrophages caused by injury will also be weakened (Lin et al., 2019). Studies have reported that CAV-1 derived peptides have been shown to effectively inhibit bleomycin induced pulmonary fibrosis in mice (Amarnath et al., 2019). Therefore, evaluating the therapeutic effect and potential of Cav-1 in fibrotic diseases is crucial.

Furthermore, TGF-β1 is a transforming growth factor that regulates cell growth, cell survival, differentiation, and metastasis and is involved in tissue fibrosis, autoimmune diseases, and tumor formation (Meng et al., 2016). TGF-β is tightly connected to the occurrence and development of tissue fibrosis, and its overproduction leads to excessive deposition of scarring and tissue fibrosis (Hewlett et al., 2018). Besides, increased TGF-β expression has been found in different diseases, including renal fibrosis, liver fibrosis, systemic sclerosis, and PF (Inui et al., 2021). TGF-β can also induce Smad2/3 phosphorylation, promoting fibroblast differentiation (Huang et al., 2019). Hence, inhibiting TGF-β/Smad3 signaling transduction can improve PF (Andugulapati et al., 2020; Park et al., 2021). Pirfenidone, TGF- β inhibitors (Conte et al., 2014), are the only drugs approved by some countries for the treatment of idiopathic pulmonary fibrosis. Hence, detecting key components of TGF-β-induced fibrosis has therapeutic targeting potential.

Our research objective is to reveal the regulatory relationships of ERS, autophagy, Cav-1 degradation, and TGF-β signaling pathways in the occurrence and development of PF induced by $PM_{2.5}$. Moreover, previous studies have explored the health effects of acute and chronic exposure to $PM_{2.5}$. However, the effect of different exposure modes on health at the same average exposure dose had not been reported. Here, we compared the effects of two different exposure modes on the occurrence and development of PF, namely intermittent exposure to high concentrations of $PM_{2.5}$ and continuous exposure to low concentrations of $PM_{2.5}$, with equal total exposure mass. It will provide guidance on the protective measures of personnel working and outdoor activities with $PM_{2.5}$ environmental pollution.

2. Materials and methods

2.1. Animal model establishment and grouping

Here, 6-week-old male Wistar rats (Beijing Vital River Laboratory Animal Technology Co., Ltd., China) were used for respiratory exposure to $PM_{2.5}$. Before establishing the animal model, rats were kept in SPF animal houses with filtered air for one week. After rats were adapted to the air environment, they were placed in the $PM_{2.5}$ real-time online concentrated animal whole-body exposure system ($PM_{2.5}$ RTCES; Beijing Huironghe Technology Co., Ltd., China) for $PM_{2.5}$ respiratory exposure. $PM_{2.5}$ RTCES can concentrate $PM_{2.5}$ in the airline in real-time; simulate the real environment where people or animals are exposed to $PM_{2.5}$; strictly control the related factors (temperature, humidity, airflow) that affect the deposition of $PM_{2.5}$ during inhalation exposure; efficiently concentrate the fine particulate matter 2–10 times. Additionally, particles larger than 2.5 μm in the air are removed, then passed into the animal whole-body exposure system for real-time exposure of experimental animals. The concentration multiple can also be dynamically adjusted, and the concentration of $PM_{2.5}$ can be measured in the animal exposure chamber in real-time. Therefore, establishing pathogenic animal models using this system can reflect real human or animal exposure to environmental $PM_{2.5}$, providing more accurate data. Our preliminary research results and epidemiological studies at home and abroad have shown that particulate matter pollution levels in the atmosphere environment presents obvious seasonal time and space distribution, namely the pollution levels during the winter heating period was more serious than that non-heating period in spring and summer. Given the high incidence of respiratory disease in winter heating period in national conditions, this study chooses the winter heating period exposure experiments on animals.

Rats were separated into three differentially treated groups. Rats that remained in an SPF animal house with a high-efficiency particulate air filter (HEPA) to clean the air and exposed to $PM_{2.5}$-free air were defined as the control group. Rats exposed to the 4-fold ambient atmospheric $PM_{2.5}$ concentration and were continuously exposed for 8 h every day were defined as the 4-fold concentration constant exposure group