DOCTORAL THESIS

$PM_{2.5}$ Exposure and Its Association with Respiratory, Cardiac and Hepatic Diseases

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ABSTRACT

Airborne particulate matter, especially with an aerodynamic diameter less than 2.5 μm ($PM_{2.5}$), can go directly to the respiratory bronchioles of the lung, and result in respiratory diseases. More recently, the epidemiological studies indicated that $PM_{2.5}$ exposure was also associated with cardiovascular diseases, hepatic diseases and obesity. However, the underlying molecular mechanisms involved in $PM_{2.5}$-elicited damages are not well elucidated. In this thesis, the effects of $PM_{2.5}$ on respiratory, cardiac and hepatic systems were investigated in both *in vitro* and *in vivo* models by using multi omics and biological analyses.

As an industrial city of China, Taiyuan has suffered from serious $PM_{2.5}$ pollution for years due to large coal combustion and active industrial activities. An ambient exposure system was established in Taiyuan, which enabled to mimic real atmospheric conditions of $PM_{2.5}$ exposure. Compared with traditional intratracheal instillation method, the installed $PM_{2.5}$ exposure system not only eliminated the possible artificial effects on animals but also maintained the real physiochemical characteristics of $PM_{2.5}$. The case studies indicated that real-ambient $PM_{2.5}$ exposure induced a variety of physiological damages in aged mice and Sprague-Dawley (SD) rats. Some previously uncharacterized effects were firstly found to be associated with the real-ambient $PM_{2.5}$ exposure. In this thesis, both conventional intratracheal instillation method and established $PM_{2.5}$ exposure system were applied in animal studies and the efficiency were

compared in these two methods.

In *in vitro* studies, $PM_{2.5}$ collected from Taiyuan were exposed to lung BEAS-2B cells, liver L02 cells and heart neonatal rat cardiomyocytes (NRCMs) cells. The results indicated that $PM_{2.5}$ exposure greatly decreased the cell viability and induced oxidative stress in all three kinds of cells. The metabolomic analysis in BEAS-2B cells showed that tricarboxylic acid (TCA) cycle and glycolysis were significantly affected by $PM_{2.5}$ exposure. In addition, obvious metabolic phenotype remodeling between oxidative phosphorylation and glycolysis were found in BEAS-2B cells, NRCMs and L02 cells after treated with $PM_{2.5}$. Notably, high fat was found to enhance the effects of $PM_{2.5}$ on the BEAS-2B cells in energy metabolic reprogramming. These *in vitro* studies suggested that energy metabolism disorder might be a potential mechanism of $PM_{2.5}$-induced toxicities.

To investigate the effects of $PM_{2.5}$ exposure on the lung, heart and liver, high-fat diet (HFD)-induced obese mice model and Sirtuin3 knockout (Sirt3 KO) mice model were used. In the obese mice model, though the body weight and blood glucose were not increased, the respiratory rate, blood pressure, heart rate, heart weight and liver weight were significantly elevated in the obese mice after exposure to $PM_{2.5}$ through intratracheal instillation method, suggesting that 4-week $PM_{2.5}$ exposure did not exacerbate obesity but obesity increased the vulnerability to the harmful effects of $PM_{2.5}$. In the lung, the results from

metabolomic and gene expression analysis indicated that cotreatment of $PM_{2.5}$ and HFD promoted glycolysis but inhibited fatty acid oxidation, glutamine metabolism and TCA cycle, resulting in the reduction of oxidative phosphorylation and adenosine triphosphate (ATP) production. In the heart, the metabolomic and gene expression analysis indicated that cotreatment of $PM_{2.5}$ and HFD elevated the metabolites and genes related to oxidative stress and inflammation and altered substrate preference from glycolysis to fatty acid oxidation. The metabolic reprogramming in the lung and heart indicated that energy metabolism was disturbed by $PM_{2.5}$ combined with obesity. Considering that mitochondria were the major sources of endogenous reactive oxygen species (ROS), the disorder of energy metabolism would aggravate oxidative stress and inflammation to induce more severe adverse effects on the lung and heart, further proving that energy metabolism dysfunction might be an important contributing factor to $PM_{2.5}$-induced cardiopulmonary toxicities. In the liver, $PM_{2.5}$ showed nonalcoholic steatohepatitis (NASH) symptom in the obese mice. The multi-omics analysis showed that the purine metabolism, glutathione (GSH) metabolism, cysteine and methionine metabolism related to oxidative stress, arginine and proline metabolism and tryptophan metabolism associated with inflammation as well as lipid metabolism related to hepatic steatosis were significantly disturbed by combined effects of $PM_{2.5}$ and HFD.

In the Sirt3 KO mice model, the deficiency of Sirt3, which was one

nicotinamide adenine dinucleotide (NAD^+)-dependent protein deacetylase, might affect many important cellular processes, including redox balance, immunity and energy production. To clarify the roles of Sirt3 in $PM_{2.5}$-induced energy metabolism disorder, oxidative stress and inflammation, the wild type (WT) and Sirt3 KO mice were exposed to the real-ambient $PM_{2.5}$ exposure system for 15 weeks. The physiological results suggested that $PM_{2.5}$ decreased the heart rate and catecholamines to induce heart failure and increased ROS and pro-inflammatory cytokines to induce liver dysfunction in the Sirt3 KO mice. The results from metabolomic, lipidomic and proteomic analysis showed that the increase of fatty acid oxidation and decrease of glycolysis in the heart as well as decrease of arginine and proline metabolism, purine metabolism and cysteine and methionine metabolism in the liver of Sirt3 KO mice were consistent with the results found in the obese mice model. The similar results found in Sirt3 KO mice model and obese mice model proved that the installed $PM_{2.5}$ exposure system was not only efficient in establishing animal models for $PM_{2.5}$ toxicological studies but also more representative than traditional intratracheal instillation method. Furthermore, the overexpression of Sirt3 in L02 cells alleviated $PM_{2.5}$-induced cell death, oxidative stress and inflammation, suggesting that $PM_{2.5}$-induced energy metabolism disorder in the heart and oxidative stress and inflammation in the liver were regulated by Sirt3.

In summary, our results found that, beyond oxidative stress and inflammation,

energy metabolism dysfunction was also an important contributing factor to $PM_{2.5}$-elicited toxicities. Of note, Sirt3 gene was found to play critical role in mediation of $PM_{2.5}$-induced adverse effects. The results of this thesis may provide new insights into the health risk of $PM_{2.5}$ to pulmonary, cardiac and hepatic diseases.

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