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PM2.5 deregulated microRNA and inflammatory microenvironment in lung injury

Guangyan Liu [a], Yunxia Li [b], Jiaming Zhou [c], Jia Xu [a], Biao Yang [a,*]

[a] Department of Pathogen Biology, Shenyang Medical College, No. 146, Huanghe North Street, Shenyang, People's Republic of China
[b] Department of Respiratory Medicine, Affiliated Center Hospital of Shenyang Medical College, No. 5, Nanqi West Road, Shenyang, People's Republic of China
[c] Franklin and Marshall College, 415 Harrisburg Ave, Lancaster City, PA, USA

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Alan Jeffrey Hargreaves

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ABSTRACT

PM2.5 negatively affects human health, particularly lung injury. However, the role of PM2.5-regulated miRNAs in lung injury remains unknown. MiRNA array results showed mmu-miR-467c-5p regulated Prdx6 expression to adapt to lung injury condition, and deregulated miRNAs regulated macrophages to build a localized inflammatory microenvironment. In addition, miRNAs were transferred into adjacent alveolar epithelial cells, regulating the expressions of cell injury signaling pathway-targeted genes, and accelerating local lung tissue injury. NO and RAGE were increased in the coculture supernatant, and SPD was decreased. PM2.5 exposure induced local lung injury, promoted inflammation in local lung tissues, increased capillary permeability in the lung tissue, and rearranged the local lung tissue structure. We also confirmed in AECOPD patients TNF-α and IL-1β levels are obviously higher than healthy person. These findings provide new mechanistic insights regarding PM2.5 and targeted miRNAs in the inflammatory microenvironment, which increases our knowledge of PM2.5-lung injury interactions.

1. Introduction

In recent years, with rapid industrialization worldwide, air quality has substantially declined, and haze often occurs. Air pollution particles damage human health and negatively impact the natural environments, especially particulate matter 2.5 (PM2.5, a median aerodynamic diameter < 2.5 μm), which has become a widespread public health problem. Epidemiological and experimental research confirmed that PM2.5 damaged lung function, increased incidences of respiratory diseases and local hospital admission rates (Zheng et al., 2020; Gu et al., 2020). PM2.5 also induced many adverse health consequences, including asthma (Ko et al., 2007), chronic obstructive pulmonary disease (COPD) (Burnett et al., 2014), respiratory infections (Lin et al., 2005), and lung cancer (Pope et al., 2002; Laden et al., 2007). Similarly, air pollution particles increase the incidence and mortality rates from chronic cardiovascular diseases (Chen et al., 2008). However, the mechanism and consequences of these gene expression changes in lung injury remain unclear.

MiRNAs are short noncoding RNA molecules of 20–25 nucleotides long. MiRNAs play important roles in silencing endogenous genes and regulating posttranscriptional gene expression (Bartel, 2009). Exosomes deliver miRNAs to the target cell cytoplasm. miRNAs control protein expression through translation inhibition and mRNA degradation and play roles in epigenetic regulation of pathophysiological processes such as immune responses, protein metabolism, cell injury, tumor occurrence and metastasis, and drug resistance. Different miRNA disorders exist in various animal models of lung injury (Zhou et al., 2011; Huang et al., 2014). Studying the role of miRNAs will help clarify the pathophysiological mechanisms of lung injury and reveal potential therapeutic targets.

PM2.5 exposure can alter miRNA expression. Single nucleotide polymorphisms in gene-encoding miRNAs alter the relationship between PM2.5, sICAM-1 (soluble Intercellular Adhesion Molecule 1) and VCAM-1 (Vascular Cell Adhesion Molecule 1), which are related to atherosclerosis and cardiovascular disease (Liang et al., 2019). Thus far, however, little experimental evidence exists to show that PM2.5 exposure affects miRNA expression in the lung tissue and the biological consequences of this exposure. In the other hand, many studies have conducted in-depth analyses of the relationship between PM2.5 and human

* Correspondence to: Department of Pathogen Biology, Shenyang Medical College, No. 146, Huanghe North Street, Shenyang, Liao Ning, People's Republic of China.
E-mail addresses: canjian_85@163.com (G. Liu), 782186096@qq.com (Y. Li), jzhou2@fandm.edu (J. Zhou), toxujia@163.com (J. Xu), y49781775@126.com (B. Yang).

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health, mainly including the cytotoxic and oxygen free-radical effects (Dergham et al., 2015; Wang et al., 2016; Yang et al., 2018). The regulatory role of most miRNAs in PM2.5-induced inflammation is largely unknown. Because PM2.5 mechanisms in human health remain unclear, research on the correlation between PM2.5 and human health is important.

We hypothesized that PM2.5 exposure induces differential miRNA expression in lung injury and that these changes participate in pulmonary macrophage-epithelial cell crosstalk. MiRNA microarray analysis showed that PM2.5 deregulated the expression of several miRNAs associated with inflammation, oxidative stress and epigenetic modification both in vivo and in vitro. To provide a more direct and valuable basis for treating lung disease at the molecular level, we designed experiments and analyzed the miRNA expression, characteristic function and correlation in human and animal cells and tissues, thus contributing to human life and health.

2. Material and methods

2.1. Animals and PM2.5 administration

BALB/c mice that were purchased from Vital River. CP (Beijing, China), were used at 8 weeks of age (18–22 g), and they were randomly divided into PM2.5 exposed group and neutral saline exposed group. PM2.5 samples were prepared as described from our previous studies, and the analysis of PM2.5 was performed (Ma et al., 2015). All studies were performed according to protocols reviewed and approved by the Ethics Committee of Shenyang Medical College, China (No.2015052902). BALB/c mice received the same food, water and were kept at the same ambient conditions in Laboratory Animal Care Center. After four weeks acclimation, all mice were executed PM2.5 and neutral saline exposure. We performed the exposure experiments using the Mouth-nose Exposed system (Beijing Huironghe Technology Co.,Led, China).

The PM2.5-exposed group animals were exposed to concentrated PM2.5 with the concentration of 750 μg/cm^3 for 6 h per day, 5 days per week for a total of 4 wk, and the control group animals were treated with neutral saline in the same condition. Twenty-four hours following the last exposure, mice were euthanized, and blood serum and lung tissue samples were collected for further analysis.

2.2. Histological and apoptosis assay

The lung tissues were inflated and fixed with 10% neutral formalin overnight. Lungs were dehydrated with increasing ethanol (EtOH) concentrations and then embedded in paraffin. Five-micrometer-thick paraffin sections were cut from each tissue specimen, and then haematoxylin and eosin (H&E) staining and apoptosis assay (Elabscience Biotechnology Co.,Ltd, China) was performed as the instructions.

2.3. Mouse miRNAs microarray

The Agilent Mouse miRNA Microarray was experiment and data analysis have been performed in Shanghai OE Biotech CO., Ltd. Total RNA was quantified by the NanoDrop ND-2000 (Thermo Scientific) and the RNA integrity was assessed using Agilent Bioanalyzer 2100 (Agilent Technologies). The sample labeling, microarray hybridization and washing were performed. Total RNA were dephosphorylated, denaturated and then labeled with Cyanine-3-CTP. After purification the labeled RNAs were hybridized onto the microarray. After washing, the arrays were scanned with the Agilent Scanner G2505C (Agilent Technologies).

Feature Extraction software (version10.7.1.1, Agilent Technologies) was used to analyze array images to get raw data. Next, Genespring software (version 13.1, Agilent Technologies) was employed to finish the basic analysis with the raw data. To begin with, the raw data was normalized with the quantile algorithm. The probes that at least 100.0% of samples in any 1 condition out of 2 conditions have flags in "Detected" were chosen for further data analysis. Differentially expressed miRNAs were then identified through fold change as well as P value calculated using t-test. The threshold set for up- and down-regulated genes was a fold change > = 2.0 and a P value < = 0.05. Target genes of differentially expressed miRNAs were the intersection predicted with 3 databases (Targetscan, microRNAorg, PITA). Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were applied to determine the roles of these target genes.

2.4. Immuno-histochemistry

To determine the levels of Prdx6 (Peroxiredoxin 6) expression in lung tissues, we performed the Immuno-histochemistry. The lung tissue was placed in 4% paraformaldehyde 2 hrs at RT, and then overnight at 4 ℃. After fixation, paraffin-embedded tissues were sectioned at 5 μm and Prdx6 antibody (Cell Signaling Technology, Inc., USA) was used to detect in the lung tissue. The density was quantified by calculating the expression of Prdx6 in positive raito under microscope with Image J software (NIH, USA).

2.5. Cell culture and treatments

Pulmonary epithelial cells (BEAS-2B) and monocyte cells (U937) that were obtained from the China Infrastructure of Cell Line Resource, were grown in RPMI-1640 medium (Hyclone, USA), with 10% fetal bovine serum (Hyclone, USA) and 1% penicillin-streptomycin (Hyclone, USA) and kept at 37 ℃ in 5% CO_2.

Coculture experiments were set up using transwell plates with 6 well plate, 0.4 μm pore size polycarbonate membrane inserts (Corning, USA). On the one hand, to detected the effect of proliferation in monocytes, we seeded the U937 cells and grown to confluence in the lower chamber before coculture. Appropriate numbers (BEAS-2B) were added to the upper chamber. In the other hand, to detected the damage effect that induced by miRNAs of PM2.5-exposed the pulmonary epithelial cells, we seeded the BEAS-2B cell and grown in the lower and up chamber. During coculture, the PM2.5 suspension was added into the upper chamber. Then the experimental samples were collected for this research.

Cell viability was assessed with CellTiter 96 AQueous One Solution Reagent (Promega, USA) according to the manufacturer's instructions. The cells were seeded at a density of 1.0 × 10^4 per well in triplicate in 96-well plates, tackled with different concentration PM2.5 in 0, 25, 100, 500, 1000 μg/ml, and cultured in a CO_2 incubator for 24 h. Alternatively, Absorbance was taken on a infinite M200 Pro Reader (TECAN, Switzerland) at a wavelength of 490 nm after PM2.5 exposure.

2.6. Human miRNA microarray

The Agilent Human miRNA Microarray experiment and data analysis of the 6 samples were conducted by OE Biotechnology Co., Ltd. (Shanghai, China). Operation process is similar to the Agilent Mouse miRNA Microarray. Target genes of differentially expressed miRNAs were the intersection predicted with 2 databases (miRDB, miRWalk). GO analysis and KEGG analysis were applied to determine the roles of these target genes. Hierarchical Clustering was performed to show the distinguishable miRNAs expression pattern among samples.

2.7. Small RNA isolation and miRNA qPCR

Pulmonary epithelial cell (BEAS-2B) was grown in RPMI-1640 medium (Hyclone, USA), with 10% fetal bovine serum (Umibio, China)

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