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$PM_{2.5}$-induced alteration of DNA methylation and RNA-transcription are associated with inflammatory response and lung injury

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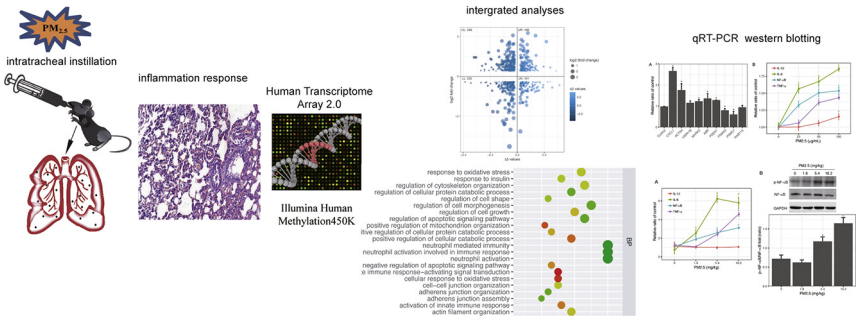
HIGHLIGHTS

- $PM_{2.5}$ induced lung toxicity and inflammation injury in rat after intratracheal instillation.
- $PM_{2.5}$ induced genome wide DNA methylation changes at CpG sites that resided in critical function and disease related genes in human bronchial epithelial cells.
- The differentially methylated and expressed genes were functionally related to cellular community, motility, cell growth, development and differentiation and signal transduction pathways.
- The neutrophil activation involved in immune response was the most critical signaling pathway involved in $PM_{2.5}$-associated lung toxicity.

GRAPHICAL ABSTRACT



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ABSTRACT

The mechanisms of systemic pulmonary inflammation and toxicity of fine particulate matter ($PM_{2.5}$) exposure remains unclear. The current study investigated the inflammatory response and lung toxicity of $PM_{2.5}$ in rats following intratracheal instillation of $PM_{2.5}$. After repeated (treated every 3 days for 30 days) $PM_{2.5}$ exposure, total protein (TP), lactate dehydrogenase (LDH) activity and inflammatory cytokines including interleukin 6 (IL-6), interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α) levels in bronchoalveolar lavage fluid (BALF) were markedly elevated. The expression levels of IL-6, IL-1β, TNF-α and NF-κB in rat lung tissue and BEAS-2B cells were significantly upregulated after $PM_{2.5}$ exposure. Histopathological evaluation suggested that the major pathological changes were alveolar wall thickening and inflammatory cell infiltration of the lungs. Genome wide DNA methylation and RNA-transcription analysis was performed on human bronchial epithelial cells (BEAS-2B) to explore the potential mechanisms in vitro. $PM_{2.5}$ induced genome wide DNA methylation and transcription changes. Differentially methylated CpGs were located in gene promoter region linked with CpG islands. Integrated analysis with DNA methylation and transcription data indicated a clear bias toward transcriptional alteration by differential methylation. Disease ontology of differentially methylated and expressed genes addressed their prominent role in respiratory disease. Functional enrichment revealed their involvement in inflammation or immune response, cellular community, cellular motility, cell growth, development and differentiation, signal transduction and responses to exogenous stimuli. Gene expression validation of ACTN4, CXCL1, MARK2, ABR, PSEN1, PSMA3, PSMD1 verified their functional participation in critical biological processes and supported the

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microarray bioinformatics analysis. Collectively, our data shows that $PM_{2.5}$ induced genome wide methylome and transcriptome alterations that could be involved in pulmonary toxicity and pathological process of respiratory disease, providing new insight into the toxicity mechanisms of $PM_{2.5}$.

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1. Introduction

Ambient air pollution, particularly $PM_{2.5}$ (fine particles with diameters that are 2.5 μm and smaller) has been confirmed to be the fourth leading risk factor according to the 2010 disease burden assessment in China (Yang et al., 2013). It has become a major public concern because of its adverse effects on health. Many epidemiologic studies have proposed that $PM_{2.5}$ exhibits a strong association with increased risk of respiratory disease, cardiovascular disorders, type II diabetes mellitus and even autism spectrum disorders (Achilleos et al., 2017; Morales-Suarez-Varela et al., 2017; Weinmayr et al., 2015). Given the fact that $PM_{2.5}$ has high deposition efficiency but lower clearance efficiency in alveolus, lung is a major target for the actions of $PM_{2.5}$ (Chen et al., 2016).

DNA methylation plays a critical role in the regulation of gene expression during pathological processes (Ahuja et al., 2016; Vogel Ciernia and LaSalle, 2016). $PM_{2.5}$ has been shown to induce DNA methylation changes, both in vitro and vivo, the consequences of which are believed to contribute to disease susceptibility and severity (Bind et al., 2012; Dai et al., 2017; Leclercq et al., 2017; Nawrot et al., 2018). A cohort study of over 700 individuals revealed that $PM_{2.5}$ especially black carbon (BC) and sulfates (SO4), reduced the methylation of two types of repetitive elements including long interspersed nucleotide element-1 (LINE-1) and Alu (Madrigano et al., 2011). The hypomethylation of repetitive element has been reported to be related to immune and inflammatory responses (Yuksel et al., 2016). Particle matter also altered the blood DNA methylation patterns over time, especially levels of 5-hydroxymethylcytosine (5hMC) (Sanchez-Guerra et al., 2015). $PM_{2.5}$ increased the exhaled nitric oxide, a biomarker of airway inflammation, that was associated with a decrease in the DNA methylation of nitric oxide synthase isoform 2A (NOS2A) (Chen et al., 2015). Acute exposure to $PM_{2.5}$ elevated the protein levels of the inflammatory meditators tumor necrosis factor alpha (TNF-α) and soluble intercellular adhesion molecule-1 (sICAM-1) by decreasing their DNA methylation (Wang et al., 2018).

Inflammation has been proposed to be one of the major causal factors linked to the adverse health effects of $PM_{2.5}$ on both cardiovascular and pulmonary system (Kim et al., 2015). However, while DNA methylation changes have been explored through cohort study or experiment research, the research has largely focused on repetitive elements in blood DNA or specific candidate genes. A comprehensive study of genome wide DNA methylation and transcription changes following $PM_{2.5}$ exposure is lacking.

In this study, we investigated the inflammatory response and pathological changes in the lungs of rats after intratracheal instillation of $PM_{2.5}$. Microarray technology was performed to profile the genome-wide DNA methylation and transcription changes in human airway epithelial cells (BEAS-2B). The aim of this study was to clarify the inflammatory injury induced by $PM_{2.5}$ and investigate the potential epigenetic mechanism of pulmonary toxicity. Our findings provide a novel epigenetic perspective to address the biological mechanism by which $PM_{2.5}$ can cause pulmonary toxicity.

2. Materials and methods

2.1. $PM_{2.5}$ preparation and chemical characterization

The $PM_{2.5}$ was collected from Capital Medical University within the city of Beijing, China. Specifically, a large-volume air sampler (TH-1000C II, Tianhong, Wuhan, China) with a flow rate of 1.05 m^3/min was used for $PM_{2.5}$ sampling. Quartz fiber filters (Pall, USA) covered with $PM_{2.5}$ were sonicated in ultrapure water for six 30-min cycles for the extraction. The suspension media was freeze dried under a vacuum after freezing at −80 °C for 12 h. The carbonaceous species and concentration of polycyclic aromatic hydrocarbons (PAHs) of $PM_{2.5}$ were examined by a thermal/optical carbon aerosol analyzer (Model 2001A, Sunset Laboratory, Forest Grove, USA) and GC–MS instrument (7890A-5975C: Agilent, USA). Detail information on the $PM_{2.5}$ collection, extraction and chemical characterization has been reported in our previous research (Li et al., 2017).

2.2. Cell culture and $PM_{2.5}$ exposure

BEAS-2B cells were purchased from the Cell Resource Center, Shanghai Institutes for Biological Sciences. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA), 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified incubator with 5% CO_2 at 37 °C. For experiments, BEAS-2B cells were plated at a density of 1×10^5 cells/mL in 6-well plates. The $PM_{2.5}$-exposure group was treated with 50 μg/mL $PM_{2.5}$ for 24 h. In contrast, the equivalent volume of DMEM without $PM_{2.5}$ was used as control.

The applied $PM_{2.5}$ concentrations (50 μg/mL) were based on cell viability experiment and multiple path particle dosimetry (MPPD) model reported by our previous study (Li et al., 2017). Briefly, the cell viability of BEAS-2B cells was maintained at 90% after exposure to 50 μg/mL for 24 h; multiple path particle dosimetry (MPPD) model was used to estimate the deposited concentrations of inhaled particles on tracheal-bronchial epithelium area with ambient $PM_{2.5}$ concentrations as described (Avino et al., 2016). The human airway morphometry parameter including functional residual capacity (FRC), tidal volume (VT) and breathing frequency was provided by ICRP (ICRP, 1994). The baseline settings of MPPD inputs were shown in Additional file Table S1. Accordingly, dosage 50 μg/mL of $PM_{2.5}$ was calculated with the real daily average $PM_{2.5}$ concentrations (147.59 μg/m^3) during the sampling period.

2.3. Microarray preparation

Illumina HumanMethylation450K BeadChip (Illumina, Inc., San Diego, CA) was used to determine the methylation status of over 485,000 CpG sites covering 99% of RefSeq genes. The whole genomic DNA was isolated and bisulfite converted using the commercially available kit (Qiagen, Valencia, CA; Zymo Research, Irvine, CA).

Affymetrix GeneChip Human Transcriptome Array 2.0 (Affymetrix GeneChip, USA) was performed to quantify genome wide transcription levels. Total RNA of each group was extracted and purified using TRIzol reagent and RNeasy Mini Kit (Invitrogen, Carlsbad, Canada; Qiagen, Hilden, Germany) according to the manufacturer's protocol. The quality and concentration of DNA and RNA samples were assessed using a NanoDrop (Thermo, NanoDrop 2000, USA).

2.4. Data analysis and quality control

Illumina GenomeStudio software (version 2011.1) Methylation module was used to extract the raw data from the chip image. The normalization was performed by Subset-quantile Within Array

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