Identification of Epigenetic Alterations in Response to Air Pollution as Potential Biomarkers for Assessing Health Risks

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ABSTRACT

Introduction: Exposure to air pollutants, mainly composed of PM2.5, is one of the leading causes of death worldwide. Long-term PM2.5 exposures have been shown to link numerous chronic diseases including cardiovascular and respiratory diseases and cancers. Therefore, an early surveillance of PM2.5 exposure appears important for healthcare improvement and preventing air-pollution-associated diseases. PM2.5 is known to contribute to multiple cellular damages such as mitochondrial dysfunction and ROS (Reactive Oxygen Species) production, which caused irreversible DNA damage and epigenomic changes. Although PM2.5 exposure alters various epigenetic modifications including DNA and histone methylations have been reported, the evidences of global epigenetic alterations including noncoding RNAs expressions are still limited. In this study, we have profiled and analyzed global transcriptomes in a PM2.5 exposure mouse model. Our aim is to identify specific epigenetic alternations, instead of genetic mutations, which can rapidly response to PM2.5 exposure and further provides early predictive biomarkers for sensing early signs of air pollution-associated diseases.

Method: Samples of PM2.5 were collected from Kaohsiung area and administrated into B6 mice with dosage of 25mg/mouse via oropharyngeal aspiration route twice a week for a total of 24 weeks. After treatment, blood samples from the PM2.5-treated mice (n=4) or PBS controls (n=4) were used to isolate total RNAs from buffy coats. In the period of treatment, physiological changes of the mice including body weights, blood pressures, the weights of pancreases and lungs were monitored. Total RNAs were applied to RNA sequencing and pathway analysis, gene ontology (GO) and gene set enrichment analysis (GSEA), to reveal PM2.5-associated cellular and metabolic pathways.

Results: In the PM2.5 exposure mouse model, we found the organs of the treated mice including pancreases and lungs were significantly increased. In addition, the blood pressures and heart rates in the PM2.5-treated mice were also elevated. We also noted that increased levels of angiogenesis, infiltration of leucocytes and activation of macrophages in the treated mice as compared to the controls. These observations indicated exposure of PM2.5 causes systemic effects and may associate with pro-inflammatory responses. In the genome-wide transcriptome profiling, we identified distinct PM2.5-associated cellular and metabolic signaling pathways, including the pathways in cancer, diabetes, and immune responses, supporting the PM2.5 exposures cause body-wide stress and immune responses.

Conclusion: We applied the PM2.5 exposure mouse model to evaluate physiological effects. By genome-wide transcriptomic analysis, we identified PM2.5-associated cell responses and signaling pathways, which provide opportunities to develop potential biomarkers for assessing the risk of air pollutant exposures.

EXPERIMENTAL DESIGN

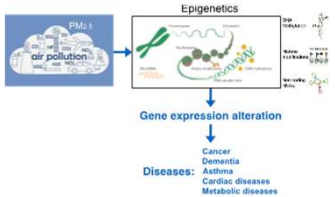

Figure 1. Diagram of the relationship between air pollution (PM2.5) and epigenetic alterations, which consequently affect gene expression and disease progression.

Figure 2. PM2.5 sample collection and extraction. PM2.5 was obtained through high-volume impaction using a Digitel DHA-80 operated at a flow rate of 500 L/min. Samples were collected on fiberglass filters. Fiberglass filters used for collecting PM2.5 were wetted with 70% ethanol in a glass measuring beaker and then sonicated for 30 min at room temperature. The ethanol was removed using an evaporator. The extracted samples were archived in a −80 ˚C freezer, lyophilized, and stored in a desiccator at −80˚C.

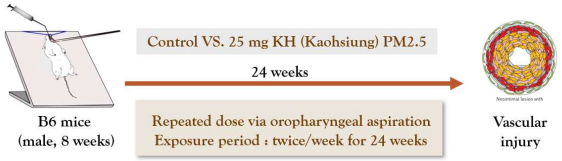

Figure 3. The illustration of PM2.5 administration into mouse.

RESULTS

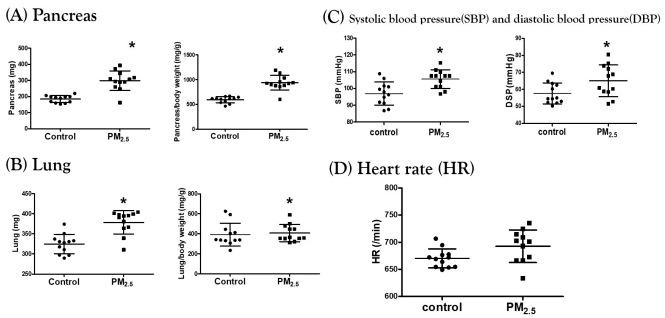

Figure 4. Physiological changes associated with PM2.5 treatment in mice. weight gain of (A) pancreas and (B) lung after PM2.5 treatment. (C) PM2.5 causes of high blood pressure and (D) high heart rate.

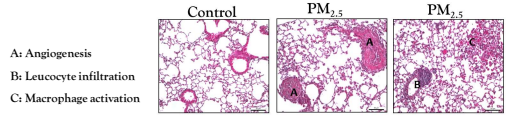

Figure 5. H&E staining of lung tissues from PM2.5-treated mice (PM2.5) and controls

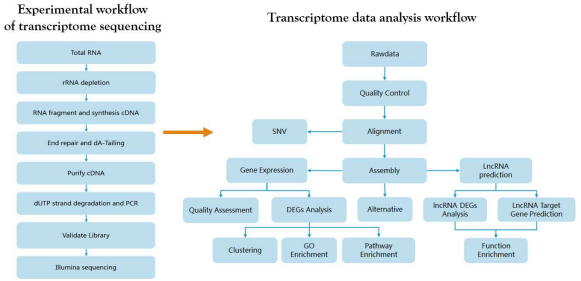

Figure 6. Flow chat of RNA sequencing procedures including RNA QC (quality control) and pathway analysis

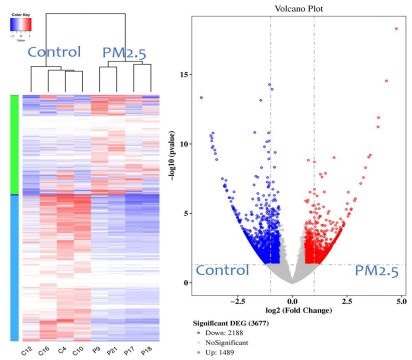

Figure 7. Heat map (left panel) and volcano plot (right panel) indicate differential expression genes (DEGs). C, control; P, PM2.5.

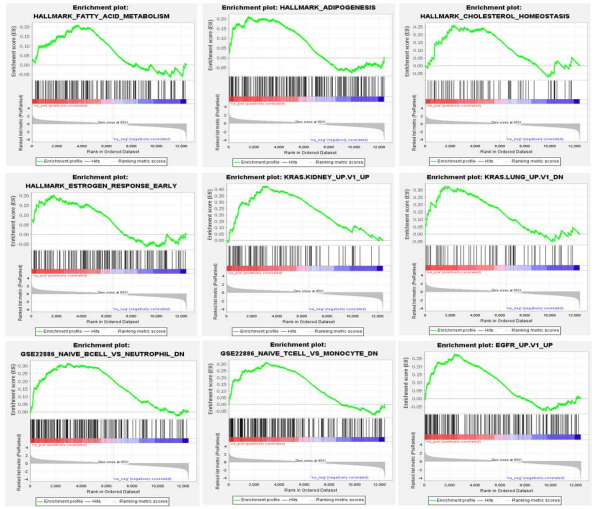

Figure 8. Gene Set Enrichment Analysis (GSEA) of DSGs from Control vs. PM2.5-treated mice. The annotated pathways are indicated on the top of each plot.

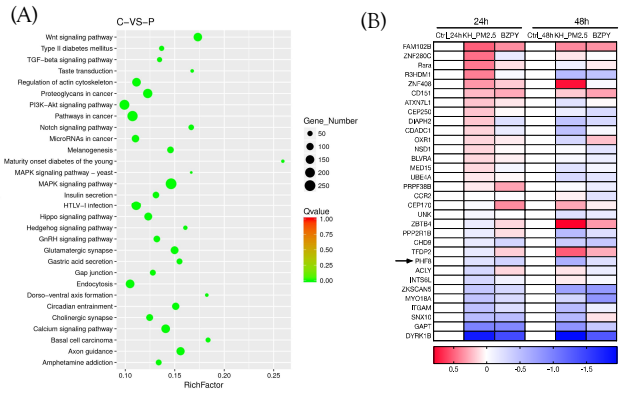

Figure 9. (A) KEGG annotation of DSGs from Control vs. PM2.5-treated mice (C-VS-P). (B) Real-time PCR analysis of the selected genes that showed differential expressions in the RNA-sequencing analysis.

SUMMARY

- PM2.5 causes systemic perturbations of physiological and immunological functions.
- PM2.5 induces distinct cellular and metabolic pathways associated with multiple diseases.
- Transcriptomic analysis provides an opportunity to identify novel biomarkers for assessing PM2.5 exposure.