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miRNAs deregulation in serum of mice is associated with lung cancer related pathway deregulation induced by PM2.5☆

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ABSTRACT

Ambient fine particulate matter (PM2.5) as an environmental pollution has been associated with the lung cancer. However, the mechanism of epigenetics such as miRNAs deregulation between PM2.5-exposure and lung cancer has not been elucidated clearly. Twenty C57BL/6 mice were divided randomly into 2 groups and exposed to the filtered air (FA) and the concentrated air (CA), respectively. The FA mice were exposed to filtered air in chambers with a high-efficient particulate air filter (HEPA-filter), and the CA mice were exposed to concentration ambient PM2.5. The total duration of exposure was performed 6 h per day from December 1st, 2017 to January 27th, 2018. The mice exposed 900.21 μg/m^3 PM2.5 for 6 h per day in CA chamber, which was nearly equaled to 225.05 μg/m^3 for 24-h calculatingly. After exposure, the serum miRNAs levels were detected by microarray. Genetic and pathological alterations in lung of mice with/without PM2.5 exposure were detected. 38 differential miRNAs in serum of mice were found after PM2.5 exposure for 8 weeks. Among of them, 13 miRNAs related with lung cancer were consistent in serum and lung of mice. The target genes of 13 deregulated miRNAs including CRK, NR2F2, VIM, RASSF1, CCND2, PRKCA, SIRT1, CDK6, MAP3K7, HIF1A, UBE2V2, ATG10, BAX, E2F1, RASSF5 and CTNNB1, could involve in the pathway of lung cancer developing. Compared with the FA group, the significantly increases of histopathological changes, ROS and DNA damage were observed in lung of mice in CA group. Our study suggested that miRNAs in serum could be identified as candidate biomarkers to predict the lung cancer development during early PM2.5 exposure.

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1. Introduction

Long-term exposure to atmospheric pollution contributes to global disease burden, and is responsible for the increasing morbidity and mortality of cardiovascular and respiratory disease, especially lung cancer. Global Burden of Disease (GBD) 2013 study pointed that ambient particulate matter air pollution was identified as a leading risk factor for global disease burden with an estimated 2.9 million attributable deaths (Forouzanfar et al., 2015). Global population-weighted PM2.5 (PM2.5, particulate matter with aerodynamic diameter 2.5 μm or smaller) increased from 39.7 μg/m^3 to 44.2 μg/m^3, increasing most rapidly from 2010 to 2015 (Cohen et al., 2017). The number of deaths attributable to PM2.5 was estimated to have a 20% increase from 1990 to 2015 (Landrigan et al., 2018). It is well known that air pollution has been a major health threat to respiratory globally. Current study indicated that chronic respiratory diseases represented a substantial health-care burden in China. The International Agency for Research on Cancer (IARC) classified outdoor air pollution and airborne particulates as group 1 carcinogen to humans (Loomis et al., 2014). It is reported that lung cancer resulting from air pollution was a leading cause of disability adjusted life-years (DALYs) and ranked the fourth most common

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causes of mortality in China (Yang et al., 2013).

Prospective cohort data for 1.2 million adults in America illustrated that exposure to low-level PM2.5 had a significant impact on cardiovascular disease while high-level PM2.5 tended to induce lung cancer (CA Pope et al., 2011). Another epidemiological study performed in a cohort of 18.9 million Medicare beneficiaries reported that chronic exposure to PM2.5 for12-month and 60-month was linked to increase 1.13 times and 1.33 times the lung cancer mortality risk, respectively (Pun et al., 2017). Supporting an epidemiology, sociodemographic characteristics play a role in between PM2.5 exposure and developing lung cancer, each increase 10 μg/m^3 average concentration of PM2.5 in 2-year correlated with an increased risk of lung cancer in males (RR = 1.055) and females (RR = 1.149) (Guo et al., 2016). Consist with these studies, clinical and experimental studies given that causal association had been confirmed between PM2.5 exposure and lung cancer, especially non-small cell lung cancer (NSCLC) (Burnett et al., 2014; Eckel et al., 2016; Forouzanfar et al., 2015; Loomis et al., 2013; Urman and Dean Hosgood, 2015).

Evidences suggested that miRNAs deregulation was one of the potential mechanism of malignancies and respiratory diseases induced by particulate matter (PM) (Badyda et al., 2017; Bleck et al., 2013; Yang et al., 2013; Hu et al., 2014). In lung epithelial cells, PM altered miRNA levels and then resulted in pulmonary diseases (Heβelbach et al., 2017). After acute PM2.5 exposure through intratracheal instillations in mice, miRNAs drove immune response and toxic effect in lung by regulating the imbalance of immune cells (Hou et al., 2018). In epidemiologic study, even if immediate and acute pulmonary disease was not diagnosed after PM2.5 exposure, miRNAs deregulation could be found. A global analysis of the circulating miRNA genome in an experimental cross-over study performed in non-smoking subjects who walked in polluted streets 2-h in London suggested that 54 miRNAs deregulation in plasm, in which seven miRNAs were highly expressed in the lung (Krauskopf et al., 2018). miRNAs in detected lung tissue of patients with NSCLC were expected to be independent predictors. During carcinogenesis, evidences showed that anti-oncogenic pathways could be inhibited by amplifying or silencing epigenetically genes encoding a miRNA that targets tumor suppressor genes (Ventura and Jacks, 2009). In serum, the levels of miRNA are stable, reproducible, and consistent among individuals of the same species (Chen et al., 2008). Serum miRNAs had been considered as novel and stable markers for NSCLC developing (Gao et al., 2018a,b). Therefore, it is hypothesized that determination of miRNA levels in serum could explain the relevance of miRNAs and carcinogenesis in lung induced by PM2.5. Previous studies performed miRNAs detection in human after a short-term air pollution exposure (Espin-Perez et al., 2018), however, there were different responses between short-term and long-term PM2.5 exposure. miRNAs deregulation has been found in lung cells exposed to PM (Quezada-Maldonado et al., 2018), it was different from animal study due to the lack of metabolic process in vitro.

In the present study, a mouse model with real-time whole body PM2.5 exposure was established from December 1st, 2017 to January 27th,2018. miRNAs microarray was used to detect the miRNAs expression in serum of mice. Furthermore, we identified the miRNAs expression in lung tissues to clarify these epigenetic changes and evaluated the damages of lung tissue. Our results suggested that deregulated miRNAs in serum were associated with lung cancer related signal pathways deregulation in lung of mice after PM2.5 exposure for 8 weeks. The present study could provide some strategies of early diagnosis on lung cancer induced by PM2.5 and explain the potential mechanism of PM2.5 carcinogenesis.

2. Materials and methods

2.1. Animal treatment

Male C57BL/6 mice, aging 6-week-old, were purchased from Vital River Laboratory (Beijing Vital River Laboratory Animal Technology Co., Ltd, Beijing, China). After one-week acclimatization, 20 mice were divided randomly into 2 groups and exposed to the filtered air (FA) and the concentrated air (CA), respectively. All mice were housed in whole-body inhalation chambers at a temperature of 21 °C–23 °C under a 12 h light/dark cycle, maintained commercial standard chow and allowed free access to drinking distilled water. The FA mice were exposed to filtered air in identical chambers with a high-efficient particulate air filter (HEPA-filter) which can remove particles from ambient air. The CA mice exposed to concentrated air received PM2.5 which were concentrated 6–10 folds compared with outdoor air. The concentrated air was from a PM2.5 concentrated enrichment system (VACES) with a concentration monitor (HRH-7886, Beijing Huironghe Technology Co., Ltd, Beijing, China) located at the Hebei Medical University, Shijiazhuang, China. The real-time concentration of PM in chambers and ambient air were monitored by Aerosol Detector DUSTTRAKTM II-Model 8530 (TSI instrument Co., Ltd, Minneapolis, USA), and analyzed the distribution of the particulate matters using Aerodynamic Particle Sizer Spectrometer 3321 (TSI instrument Co., Ltd, Minneapolis, USA). The total duration of exposure was performed 6 h per day (from 9:00 a.m. to 15:00 p.m.), from December 1st, 2017 to January 27th,2018. All protocols of the experiment were approved by the Laboratory Animal Ethical and Welfare Committee of Hebei Medical University, Shijiazhuang, China. Approval No. is IACUC-Hebmu-20170163.

2.2. Serum and lung tissues sampling and processing

Mice were euthanized under pentobarbital sodium the day after the end of exposure. All mice were decapitated on ice. Blood samples were collected from abdominal aorta, and centrifuged at 3500 rpm for 15min to obtain the serum within 1 h. The supernatant serum was collected and stored quickly at −80 °C for microarray analysis. Left lung tissues were preserved in 4% paraformaldehyde (PFA). Other lung tissues were isolated and kept at −80 °C for further analysis.

2.3. miRNA microarray analysis

Total RNA was extracted from 400 μL serum using TRLzol LS reagent (Invitrogen, USA), according to the manufacturer's instructions (n = 3 each group). RNA quantity and quality were measured by NanoDrop ND-1000 (NanoDrop, Thermo, USA). RNA integrity was assessed by standard denaturing agarose gel electrophoresis. An Agilent miRNA microarray was used to profile the miRNAs in serum. The Whole Mouse miRNA Microarray was a broad view that represents all known miRNAs in the mouse transcriptome. Sequences were compiled from a broad source survey, and then verified and optimized by alignment to the assembled mouse transcriptome. A miRNA microarray was performed by KangChen Bio-tech Inc. (Shanghai, China).

2.4. Bioinformatics analysis

Agilent Feature Extraction software (version 11.0.1.1) was used to analyze acquired array images. Quantile normalization and subsequent data processing were performed with the GeneSpring GX v14.9 software package (Agilent Technologies). After quantile normalization of the raw data, miRNAs that at least 3 out of 6