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# Global gene expression profiling of human bronchial epithelial cells exposed to airborne fine particulate matter collected from Wuhan, China

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## HIGHLIGHTS

- This is the first transcriptomic study in China evaluating the use of microarray technology to elucidate the cellular response to $PM_{2.5}$.
- We observed that different concentrations of $PM_{2.5}$ had a distinct effect on the gene expression in HBE cells.
- We identified many genes and pathways that altered significantly in HBE cells after $PM_{2.5}$ exposures.

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## ABSTRACT

*Background:* Many studies have linked ambient fine particulate matter ($PM_{2.5}$) air pollution to different cardiopulmonary diseases in the general population. However the complex mechanisms underlying $PM_{2.5}$-induced adverse health effects are not yet to be fully elucidated.
*Method:* In this study, we aimed to identify genes and pathways that may contribute to $PM_{2.5}$-induced lung toxicity in humans through genome-wide approaches. Human bronchial epithelial (HBE) cells, exposed to various concentrations of $PM_{2.5}$ collected from Wuhan, China, showed decreased cell viability in a dose-dependent manner. HBE cells were exposed to 200 μg/ml and 500 μg/ml $PM_{2.5}$ and microarrays were used to obtain a global view of the transcriptomic responses.
*Results:* A total of 970 and 492 genes were identified that significantly changed after 200 μg/ml and 500 μg/ml $PM_{2.5}$ exposures, respectively. $PM_{2.5}$ induced a large number of genes involved in inflammatory and immune response, response to oxidative stress, and response to DNA damage stimulus, which might contribute to $PM_{2.5}$ related cardiopulmonary diseases. Pathway analysis revealed that different dose of $PM_{2.5}$ triggered partially common disturbed pathways. Flow cytometry assay evidenced that there were statistically significant differences in the G1 phase of cell cycle after low or high-dose $PM_{2.5}$ exposure when compared to the unexposed controls. Only high-dose $PM_{2.5}$ significantly increased the proportion of cells in the S phase of cell cycle.
*Conclusion:* We identified many genes and pathways that altered significantly in HBE cells after $PM_{2.5}$ exposures. These findings are important in providing further understanding of the mechanisms underlying $PM_{2.5}$-induced adverse health effects.



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## 1. Introduction

Particulate matter (PM) air pollution, a mixture of solid particles and liquid droplets existing in the air, exerts adverse impact on human health, particularly in developing countries with extensive air pollution (Wang et al., 2012). There is growing concern that long-term exposure to ambient airborne PM is associated with many different cardiopulmonary diseases and it could increase the risk of lung cancer. Inhalable PM can be divided into two classes based on aerodynamic diameter: coarse particles $PM_{10}$ (2.5–10 μm) and fine particles $PM_{2.5}$ (≤2.5 μm). One study demonstrated that the $PM_{10}$ could deposit in the upper respiratory tract while $PM_{2.5}$ could reach the deepest sites of the respiratory system (alveolar lung region) and result in more profound health effects (Dockery, 2009). In the study of six U.S. metropolitan areas, $PM_{2.5}$ was found to have a stronger association with daily mortality than $PM_{10}$ (Pope and Dockery, 2006). It was reported that short-term exposure to a high concentration of $PM_{2.5}$ has been associated with various adverse health effects, including excess mortality, exacerbation of respiratory diseases, as well as acute changes in lung function (Holguin, 2008; Iskandar et al., 2012).

China has experienced a rapid industrialization and transportation development in the past decades, resulting in a serious air pollution problem which is at the higher end of the world air pollution level (Shang et al., 2013). $PM_{2.5}$ air pollution is a continuing challenge to public health in China. In many cities of China, such as Wuhan, levels of ambient airborne $PM_{2.5}$ are much higher than WHO standards (Qian et al., 2001). It was recently demonstrated that $PM_{2.5}$ was the fourth leading risk factor of disease burden in China (Lim et al., 2012). In 2010, the top four causes of death in China were stroke (23.92%), lung disease (14.48%), coronary heart disease (11.71%) and lung cancer (5.19%) (http://www.worldlifeexpectancy.com/country-health-profile/china), all were linked with $PM_{2.5}$.

Some biological mechanisms possibly responsible for adverse respiratory outcomes associated with PM have been described, including immune and inflammatory responses, oxidative stress and DNA damage (Maier et al., 2008; Vattanasit et al., 2013). $PM_{2.5}$ can act directly on many effector cells associated with immune and inflammatory response (Becker and Soukup, 2003). Xu et al. evaluated the relationship between $PM_{2.5}$ exposure and inflammatory responses in mice during and after the Beijing 2008 Olympic Games. They suggested that short-term air quality improvements significantly reduced the overall inflammatory responses (Xu et al., 2012).

Oxidative stress in cells is proved to be a central mechanism by which $PM_{2.5}$ exposure leads to injury, disease and mortality (Ghio et al., 2012). It can induce oxidative DNA lesions and DNA strand breaks (Danielsen et al., 2009; Hanzalova et al., 2010). A series of studies have observed that $PM_{2.5}$ can stimulate the formation of reactive oxygen species (ROS), which could lead to oxidative damage to lipids, proteins and DNA, ultimately resulting in cell death. $PM_{2.5}$ consists of hundreds of different chemicals, such as PAHs and transition metals, many of them can cause DNA damage (Gutierrez-Castillo et al., 2006). Sanchez-Perez et al. (2009) suggested that DNA damage could be the mechanism by which particulate matter promote lung cancer.

In the past decades, numerous investigations on $PM_{2.5}$ and human health have been reported. However, the complex mechanisms underlying $PM_{2.5}$-induced adverse health effects remain incompletely understood. To reveal the potential health effects of $PM_{2.5}$ from China, we analyzed global gene expression profiles of human bronchial epithelial (HBE) cells following acute exposure to $PM_{2.5}$ samples.

## 2. Materials and methods

### 2.1. Particle sample collection and extraction

$PM_{2.5}$ was collected using a TH-1000C II air sampler (Tianhong, Wuhan, China) at a flow rate of 1.05 $m^3$/min for 24 h from ambient air. The sampling occurred in a representative community located in the center of the downtown area of Wuhan, China from March 12th to March 17th in 2012. Cumulative samples were collected onto Teflon coated glass fiber filters and stored at −80 °C until extracted. The filters were cut into small pieces and immerged in 0.9% saline and probe sonicating 3 × 15 min with a sonicator. The particles were dried by lyophilization, then weighed and suspended in the sterile phosphate buffered saline (PBS), and stored at −20 °C.

Chemical composition of the $PM_{2.5}$ we collected was analyzed by State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing, China. Briefly, 57 elements were investigated and the concentration of each was determined: TC, OC, EC, Cl, $NO_3$, $SO_4$, BghiP, IP, DBahA, BbFA, COR, FA, BaA, CHR, PY, BaP, BeP, BkFA, Li, Be, Na, P, K, Sc, V, Cr, Mn, Co, Ni, Cu, Zn, As, Rb, Y, Mo, Cd, Sn, Sb, Cs, La, Ce, Sm, W, Tl, Pb, Bi, Th, U, Zr, Al, Sr, Mg, Ti, Ca, Fe, Ba and Si. Data remained to be published.

### 2.2. Cell culture and particle exposure

HBE cells, an SV40-transformed normal human bronchial epithelial cell line, have been characterized and shown to be non-tumorigenic and to retain features of normal bronchial cells. Therefore, they are useful for $PM_{2.5}$ exposure experiment. The HBE cells for use here were obtained from the Shanghai Institute of Cell Biology of Chinese Academy of Sciences (Shanghai, China). HBE cells were maintained in 5% $CO_2$ at 37 °C in Minimum Essential Medium Eagle's medium (MEM), supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. For particle exposure, cells were seeded at a concentration of $2 \times 10^3$ in triplicate 24 h prior to exposure. A small aliquot of particle suspension was mixed with culture medium by sonication for 20 min, and then applied evenly to the cultured cells with blank samples (unexposed filters) using as controls running parallel (Deng et al., 2013). For cell viability assay, HBE cells were exposed to $PM_{2.5}$ at the concentrations of 100, 200, 300, 400, 500, 600, 700, and 800 μg/ml for 24 h. For microarray and flow cytometry assays, HBE cells were divided into three groups according to the concentration of $PM_{2.5}$, i.e., low dose (200 μg/ml), high dose (500 μg/ml), or control.

### 2.3. Cell viability

Cell viability was evaluated by WST-8 hydrolysis using Cell Counting Kit 8 (CCK-8) (Dojindo Molecular Technologies, Inc., Gaithersburg, MD, USA) according to the manufacturer instructions. After being exposed to various concentrations of $PM_{2.5}$, 10 μl of CCK-8 solution was added to each well, and the cells were incubated for 4 h. The absorbance at 450 nm was then measured with a multimode reader Infinite M200 Pro (Tecan, Switzerland). To rule out interferences between the particle samples and the detection systems or the assay reagents, we used a control with different concentrations of $PM_{2.5}$ but no cells. The viability ratios of non-treated control cells were used to establish the 100% level.

### 2.4. Gene expression profiles

After the HBE cells were treated with $PM_{2.5}$ for 24 h, total RNA was extracted from vehicle control and $PM_{2.5}$ treated cells using Trizol (Invitrogen, Gaithersburg, MD, USA) and further purified with NucleoSpin RNA clean-up protocol (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. After measuring the obtained RNA concentration using a Nanodrop ND 2000 (Thermo, Waltham, MA, USA) and denaturing the RNA by gel electrophoresis, the samples were amplified and labeled using Agilent Quick Amp Labeling kit (Agilent Technologies, Santa Clara, CA, USA). Hybridization was performed at 45 °C for 15 h in hybridization oven. After washing, the arrays were scanned by Agilent G2565CA Microarray Scanner (Agilent Technologies, Santa Clara, CA, USA) and the subsequent data compiled with Agilent Feature Extraction Software (version 10.5.1.1) (Agilent Technologies, Santa Clara, CA, USA).

### 2.5. Microarray data analysis

Gene expression data extracted from Agilent Feature Extraction Software were imported into the Agilent GeneSpring GX v11.0 (Agilent Technologies, Santa Clara, CA, USA) for further analysis. The microarray data sets were normalized in GeneSpring GX using the Agilent FE one-color scenario (mainly quantile normalization). Differentially expressed genes (DEGs) between treatment groups and vehicle control were identified as the ones with a fold-change of more than ±2.5 and the mRNA must be detectable with a processed signal intensity ≥1500 in at least one of the two samples being compared. Annotation and biological interpretation of the identified DEGs were conducted based on the Gene Ontology (GO) database (http://geneontology.org/). The functional classification and biological pathway analysis were conducted based on Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (http://www.genome.jp/kegg/pathway.html) using Molecule Annotation System