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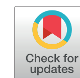
Integrative analysis of methylome and transcriptome variation of identified cardiac disease-specific genes in human cardiomyocytes after $PM_{2.5}$ exposure



Xiaozhe Yang [a, b], Lin Feng [a, b], Yannan Zhang [a, b], Yanfeng Shi [a, b], Shuang Liang [a, b], Tong Zhao [a, b], Baiyang Sun [a, b], Junchao Duan [a, b, **], Zhiwei Sun [a, b, *]

[a] Department of Toxicology and Sanitary Chemistry, School of Public Health, Capital Medical University, Beijing 100069, PR China
[b] Beijing Key Laboratory of Environmental Toxicology, Capital Medical University, Beijing 100069, PR China

HIGHLIGHTS

- $PM_{2.5}$ exposure induced genome wide variation of DNA methylation pattern in AC16.
- $PM_{2.5}$ induced DMGs and DEGs were clustered in cardiac relative Go terms and pathways.
- Integrative analysis of methylome/transcriptome identified 14 cardiac specific genes.
- PPI network identified 6 hub genes and qRT-PCR verified 5 cardiac relative genes.

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ABSTRACT

$PM_{2.5}$ exposure is strongly linked to cardiac disease. Subtle epigenetic or transcriptional alterations induced by $PM_{2.5}$ might contribute to pathogenesis and disease susceptibility of cardiac disease. It is still a major challenge to identify biological targets in human genetics. Human cardiomyocytes AC16 was chosen as cell model. Epigenetic effect of $PM_{2.5}$ in AC16 was analyzed using Illumina HumanMethylation 450 K BeadChip. Meanwhile the transcriptomic profiling was performed by Affymetrix® microarray. $PM_{2.5}$ induced genome wide variation of DNA methylation pattern, including differentially methylated CpGs in promoter region. Then gene ontology analysis demonstrated differentially methylated genes were significantly clustered in pathways in regulation of apoptotic process, cell death and metabolic pathways, or associated with ion binding and shuttling. Correlation of the methylome and transcriptome revealed a clear bias toward transcriptional suppression by hypermethylation or activation by hypomethylation. Identified 386 genes which exhibited both differential methylation and expression were functionally associated with pathways including cardiovascular system development, regulation of blood vessel size, vasculature development, p53 pathway, AC-modulating/inhibiting GPCRs pathway and cellular response to metal ion/inorganic substance. Disease ontology demonstrated their prominent role in cardiac diseases and identified 14 cardiac-specific genes (*ANK2*, *AQP1* et al.). PPI network analysis revealed 6 novel genes (*POLR2I, LEP, BRIX1, ADCY6, INSL3, RARS*). Those genes were then verified by qRT-PCR. Thus, in AC16, $PM_{2.5}$ alters the methylome and transcriptome of genes might be relevant for $PM_{2.5}$-/heart-associated diseases. Result gives additional insight in $PM_{2.5}$ relative cardiac diseases/associated genes and the potential mechanisms that contribute to $PM_{2.5}$ related cardiac disease.

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1. Introduction

Atmospheric fine particulate matter ($PM_{2.5}$) has been listed as an important air pollutant (Anderson et al., 2012). Meanwhile, a large number of epidemiological studies have confirmed that long-term exposure to $PM_{2.5}$ are associated with heart diseases (Hansen

* Corresponding author. Department of Toxicology and Sanitary Chemistry, School of Public Health, Capital Medical University, Beijing 100069, PR China.
** Corresponding author. Department of Toxicology and Sanitary Chemistry, School of Public Health, Capital Medical University, Beijing 100069, PR China.
E-mail addresses: jcduan@ccmu.edu.cn (J. Duan), zwsun@ccmu.edu.cn (Z. Sun).

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et al., 2012; Kaufman et al., 2016; Landrigan et al., 2018). Cardiac diseases became a global public health subject and $PM_{2.5}$ linked cardiac diseases increased disease burden over the world. Most recently, WHO reported that ischaemic heart disease (IHD) account about 36% of the deaths attributable to ambient air pollution globally (Landrigan et al., 2018). However the biological response and potential mechanism of $PM_{2.5}$ effect on heart were poorly understood.

DNA methylation is an important representative of epigenetics and plays a potential role in the development of diseases. PM induced epigenetic alteration including DNA methylation is widely reported (Bind et al., 2012; Bellavia et al., 2013). So DNA methylation could be involved in $PM_{2.5}$ linked pathogenetic process of cardiac diseases. A meta-analysis of epigenome-wide association studies conducted in previous literature identified that 51 (88%) of the CpG sites methylation were associated with at least one related cardiometabolic entity (Ligthart et al., 2016). In another study, epigenetic regulation of ANRIL promoter methylation was reported as a key factor in later coronary heart disease risk in children. This study also suggested that the early life environment may act through epigenetic processes to have influence on later coronary heart disease risk markers, such as increased blood pressure, heart rate and arterial pulse wave velocity (PWV, a measure of arterial stiffness) (Murray et al., 2016). Currently, gene transcriptional alteration has been demonstrated to play a key role in the pathological process of disease including heart diseases (Bisgaier et al., 2018). A recent report about transcriptional variation induced by $PM_{2.5}$ also revealed the underling mechanism of adverse effects on human (Hou et al., 2018). These findings revealed the key gene and toxicological pathways and improved our understanding of $PM_{2.5}$ exposure induced adverse effects on human health. Usually, DNA methylation was evident associated with gene expression. And transcsriptiome variation involved in $PM_{2.5}$ exposure is widely reported (Encarnacion-Medina et al., 2017; Mei et al., 2018). Despite the reports about significant adverse effects of $PM_{2.5}$ on human health, comprehensive analysis of methylome and transcriptome variation on cardiac research is still missing.

A single DNA methylation or gene expression microarray profiling is less sufficient to reveal the biology of $PM_{2.5}$ related heart diseases. Thus, in present study, human cardiomyocytes AC16 was exposed to $PM_{2.5}$ and the aim of present study was to explore the biological complexity of cardiac diseases by integrating DNA methylation and gene expression profiling. Additionally, 14 differentially expressed heart-related genes were filtered, which were either hypo- or hypermethylated. And 6 hub genes were identified after PPI network analysis. Those key genes were then verified by qRT-PCR. The integrated signatures may later reveal key gene and potential pathway induced by exposure to $PM_{2.5}$, which may especially affect the development of heart diseases.

2. Materials and methods

2.1. P.M.$_{2.5}$ preparation

$PM_{2.5}$ samples was consecutive collected using a high-volume sampler particle collector (TH-1000CII, Wuhan Tianhong, China), from NO.10 Xitoutiao Youanmen, Fengtai District Beijing, China in summer. The site for collecting $PM_{2.5}$ was in university campus adjacent moderate road and commercial activities. We prepared $PM_{2.5}$ samples according to the way of Imrich et al. (2000). And then, $PM_{2.5}$ samples were cut into filter strips. Firstly immersing them in a sonicator (KQ-700 V, 700 W) with water in it and then sonicated for 30 min. $PM_{2.5}$ samples then were kept at −80 °C before using. In order to count and adjust the dose of $PM_{2.5}$ samples, 100 μL aliquots of $PM_{2.5}$ suspensions were dropt on the filters and then air drying. The filters were weighted on a microbalance before drying. The filters were weighed again after 48 h. According the decreases of filters weights, we can count the concentration of the $PM_{2.5}$ suspension. Then put the extract $PM_{2.5}$ together, and adjusted the concentration at 5 mg/mL, and stored at −80 °C. Chemical characterization was performed in our previous study (Zhang et al., 2017).

2.2. Human cardiomyocytes culture and $PM_{2.5}$ exposure

The Cell Resource Center, Shanghai Institutes for Biological Sciences (SIBS, China) provided the human cardiomyocytes (AC16) cell line. AC16 were cultured in DMEM (Corning, USA) contained 100 mg/mL streptomycin and 100 U/mL penicillin, 10% fetal bovine serum (Corning, USA), and cultured in cell incubator humidified atmosphere of 95% air and 5% CO_2. In the next experiment, we seeded the human cardiomyocytes AC16 at cell density of 1×10^5/mL in 6-well plates. AC16 cells grew adherently for the time of 24 h, then exposed to DMEM which $PM_{2.5}$ suspended in it at the concentration of 50 μg/mL or the pure DEME as control group for another 24 h. $PM_{2.5}$ was sonicated for 5 min before treating. Control group was treated with an equivalent volume of DMEM. Five replicate wells were designed in each group. After 24 h exposure the genomic DNA or RNA was extracted for DNA methylation and gene expression microarray experiment.

2.3. DNA methylation microarray profiling

We isolated the whole genomic DNA and bisulfite converted it using commercially available kit (Qiagen, Hilden, Germany). Genome wide DNA methylation alteration profiling was conducted using the Illumina HumanMethylation450 K BeadChip (Illumina, Inc., San Diego, USA). Only samples of high purity were used for the analysis. Methylome microarray profiling was conducted to analyze 485,000 CpG sites methylation status covering 99% of the RefSeq gene. The methylation status of each sample was decided by β value which ranged from lowest value of methylation ($\beta = 0$) to the highest value ($\beta = 1$). Differential methylation sites (DMSs) were screened by the limma method. The DMS was defined as differential CpG sites with β values more than 0.1or less than −0.1 and *p*-value <0.05. Next we do GO (Gene Ontology and Kyoto KEGG (Encyclopedia of Genes and Genomes) pathway analysis of differential methylation genes (DMGs), which are the genes containing DMSs (Ming et al., 2006). The sample used for the microarray was from three replicate samples of control and $PM_{2.5}$ groups.

2.4. Gene expression microarray profiling

For the transcriptional microarray profiling, We isolated the total RNA from three replicate samples of control and $PM_{2.5}$ (50 μg/mL) groups according the way of Li et al. (2017). Affymetrix® microarray was used to do the transcriptomic profiling. The detail of gene expression microarray profiling was reported in our previous research (Feng et al., 2017). And we obtained the differential expression genes (DEGs) for next integrative analysis of methylome and transcriptome variation.

2.5. Cross-analysis of DNA methylation and gene expression

Differential DNA methylation is usually closely associated with altered gene expression. So DMGs and DEGs were intersected and we conducted functional enrichment analysis for the overlaps of DMGs and DEGs. Overlapped genes were confirmed based on specific gene symbols from the methylome and transcriptome datasets which showed significant congruence (both *p*-value

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