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Gene expression profiles and bioinformatics analysis of human umbilical vein endothelial cells exposed to $PM_{2.5}$

Hejing Hu [a, b, 1], Collins Otieno Asweto [a, b, 1], Jing Wu [a, b], Yanfeng Shi [a, b], Lin Feng [a, b], Xiaozhe Yang [a, b], Shuang Liang [a, b], Lige Cao [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

- $PM_{2.5}$ could cause significant variations in gene expression patterns in HUVECs.
- $PM_{2.5}$ changed gene profiles related to stimuli, cellular processes and signaling.
- $PM_{2.5}$ increased JAK-STAT/HIF-1signaling pathway, ER stress and autophagy.
- LIF, CSF3, HMOX1, RPS6, HSPBP1, MOGS, TUBB2A, GABARAP were up-regulated by $PM_{2.5}$.

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ABSTRACT

Cardiovascular system is demonstrated the main target of $PM_{2.5}$ and the objective of this study was to explore the toxic effect and molecular mechanisms caused by $PM_{2.5}$ in primary human umbilical vein endothelial cells (HUVECs) using microarray and bioinformatics analysis. The results showed that 591 genes were differentially expressed triggered by $PM_{2.5}$, of which 174 genes were down-regulated, while 417 genes were up-regulated. Gene ontology analysis revealed that $PM_{2.5}$ caused significant changes in gene expression patterns, including response to stimuli, immune response, and cellular processes. Pathway analysis and Signal-net analysis suggested that endocytosis, chemokine signaling pathway, RNA transport, protein processing in endoplasmic reticulum (ER) and autophagy regulation were the most critical pathways in $PM_{2.5}$-induced toxicity in HUVECs. Moreover, gene expression confirmation of LIF, BCL2L1, CSF3, HMOX1, RPS6, PFKFB, CAPN1, HSPBP1, MOGS, PREB, TUBB2A, GABARAP by qRT-PCR indicated that endocytosis might be involved in the cellular uptake of $PM_{2.5}$ by forming phagosomes, and subsequently inflammation, hypoxia and ER stress was occurred, which finally activated autophagy after $PM_{2.5}$ exposure in HUVECs. In summary, our data can serve as fundamental research clues for further studies of $PM_{2.5}$-induced toxicity in HUVECs.

1. Introduction

Nowadays, ambient outdoor air pollution is recognized as a serious global public health issue, which could increase the risk of cardiovascular disease. Air pollution can induce premature mortality especially from cancer, cardiopulmonary diseases and stroke, both in developed and developing countries (Silva et al., 2013; Shah et al., 2015; World Health Organisation, 2014; Chen and Kan, 2008). $PM_{2.5}$ (particulate matter < 2.5 μm in aerodynamic diameter), also called fine particles, is recommended as the indicator of air particle pollution by WHO (2006). $PM_{2.5}$ can penetrate deeper into the lungs than larger particles and may be largely responsible for the health problems linked to air pollution (Brook et al., 2010; Peters, 2011).

Epidemiological studies suggest that long-term exposure to a 10 $\mu g/m^3$ increase of $PM_{2.5}$, generally results to a no less than 10% increased risk of cardiovascular mortality (Dabass et al., 2016). Moreover, short-term exposure to $PM_{2.5}$ could also lead to both fatal and non-fatal cardiovascular events, even at very low levels

* Corresponding author. School of Public Health, Capital Medical University, Beijing 100069, PR China.
** Corresponding author. 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).
[1] The two authors contribute to this work equally.

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(Kloog et al., 2012; Martinelli et al., 2013; Dabass et al., 2016). The ultrafine particles of $PM_{2.5}$ can translocate from the lungs into the circulation (Nemmar et al., 2002), where endothelial cell acts as a barrier between vessel wall and circulating blood (Alom-Ruiz et al., 2008; Xu et al., 2009; Duan et al., 2013). It has demonstrated that in vitro exposure to $PM_{2.5}$ induces endothelial dysfunction (Davel et al., 2012). Recently, toxicological research have noted that $PM_{2.5}$ could cause a wide battery of systemic responses via blood-borne bioactivity, including hemodynamic alteration (Wellenius et al., 2013), functional impairments in endothelial and vascular smooth muscle, acceleration of atherosclerosis, inflammation and oxidative stress (O'Neill et al., 2005; Sun et al., 2005; Knuckles et al., 2010; Neophytou et al., 2013; Rui et al., 2016). As a result, the vascular system is the critical target of $PM_{2.5}$. Our previous studies had revealed that $PM_{2.5}$ could induce cytotoxicity, followed by oxidative stress and oxidative damage in HUVECs (Hejing Hu et al., 2016).

Toxicogenomics, including gene microarray and bioinformatics, has demonstrated its application to assess toxicity and mechanism of $PM_{2.5}$ on human cell lines, like BEAS-2B cells (Ding et al., 2014), A549 cells (Gualtieri et al., 2012), as well as on rats (Heidenfelder et al., 2009). These studies profiled the differentially expressed genes and evidenced relevance among inflammation, oxidative stress, and DNA damage responses induced by $PM_{2.5}$. However, the underlying mechanism and integrated genome-wide transcriptional analysis of $PM_{2.5}$-induced toxicity in HUVECs are still not well understood. The objective of this study was to examine the in vitro toxicity and mechanisms of $PM_{2.5}$ on HUVECs by toxicogenomic approach.

2. Materials and methods

2.1. Cell culture

The HUVECs line was bought from the Cell Resource Center, Shanghai Institutes for Biological Sciences and 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. The humidified cultivation environment was maintained at 37 °C in 5% CO_2. For experiments, the cells were seeded in culture plates at a density of 1×10^5 cells/mL.

2.2. Exposure to $PM_{2.5}$

The details of $PM_{2.5}$ extraction have been reported in our previous work (Duan et al., 2017). $PM_{2.5}$ was collected on quartz fiber filters from March to June in 2016 by the large-volume air particle sampler (WuhanTianhong TH-1000CII, China), which was installed at the urban building roof of Beijing. $PM_{2.5}$ was sonicated off from the sampled filters with ultrapure water. After filtration, freeze-drying in vacuum and then ultraviolet sterilization, $PM_{2.5}$ was then suspended in ultrapure water. Before the procedure of exposure to HUVECs, the stock suspensions of $PM_{2.5}$ were sonicated for at least 5 min. Cultured HUVECs were treated with $PM_{2.5}$ at the concentrations of 50 μg/mL for 24 h. HUVECs cultured with an equivalent volume of DMEM without $PM_{2.5}$ acted as a control. The number of treatment ($PM_{2.5}$ exposure) and control samples is 3 in each group in this study.

2.3. Microarray analysis

After exposure to $PM_{2.5}$ at the concentration of 50 μg/mL for 24 h, RNA in HUVECs from per treatment group were extracted by TRIzol reagent (Invitrogen, Carlsbad, Canada) and purified with an RNeasy Mini Kit (Qiagen, Hilden, Germany). Subsequently, a UV–Vis Spectrophotometer (Thermo, NanoDrop 2000, USA) was applied in order to measure the quality and amount of RNA at the absorbance of 260 nm. Afterward, the mRNA expression profiling was detected by Affymetrix HTA2.0 (Affymetrix GeneChip®, USA), which included 67,528 gene-level probe sets.

2.4. Bioinformatics analysis

Affymetrix® Expression Console Software (version 1.2.1) was used to perform the microarray analysis. Raw expression data were normalized on a (base 2) logarithmic scale at transcript level using the robust multiarray average method (RMA workflow). Hierarchical clustering was operated to make sure that the samples match to the treatment groups and all the data of transcript expressions formed in the clustering histogram were put through median summarization so as to standardize the values. The official Affymetrix® Transcriptome Analysis Console Software was used to discern out the differentially expressed genes, and Analysis of variance (ANOVA) was applied as the default statistical method to

Table 1
The top 20 differentially expressed up-regulated genes between $PM_{2.5}$ and control groups in HUVECs.

Gene Symbol	Geom mean of intensities (PM2.5)	Geom mean of intensities (control)	Fold Change	ANOVA p-value
LIF	10.5	9.37	2.18	0.03462
GPX1	10.22	9.21	2.03	0.010301
HMOX1	9.65	8.69	1.95	0.016824
CCL20	8.04	7.27	1.7	0.043717
SFN	17.24	16.59	1.58	0.000239
TIMM22	10.97	10.32	1.58	0.013456
VAT1	10.06	9.49	1.49	0.009674
C12orf44	7.57	7.02	1.46	0.034329
SF3B14	8.62	8.08	1.45	0.022141
ST6GALNAC4	7.64	7.1	1.45	0.042661
ARRB2	11.46	10.94	1.44	0.029743
NOC2L	9.11	8.61	1.42	0.003528
EIF3G	10.24	9.73	1.42	0.024523
IGLJ7	7.7	7.23	1.38	0.030899
ETHE1	8.12	7.68	1.36	0.00631
DHPS	7.8	7.37	1.35	0.012965
GPR108	8.72	8.29	1.34	0.024608
AAAS	9.49	9.07	1.34	0.025626
ARFGAP2	7.7	7.3	1.33	0.005023
TMEM208	7.4	7	1.32	0.026578