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Potential molecular mechanism of cardiac hypertrophy in mice induced by exposure to ambient $PM_{2.5}$

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ABSTRACT

Cardiac hypertrophy could be induced by ambient fine particulate matter ($PM_{2.5}$) exposure. Since cardiac hypertrophy represents an early event leading to heart dysfunction, it is necessary to explore the molecular mechanisms, which are largely unknown. In the present study, an ambient particulate matter exposure mice model was established to explore its adverse effects related to the heart and the potential mechanisms. Forty-eight male C57BL/6 mice were randomly subjected to three groups: filtered air group, unfiltered air group and concentrated air group, and were exposed for 8 and 16 weeks, 6 h/day, respectively. In vitro experiments, the cardiac muscle cell line (HL-1) was treated with $PM_{2.5}$ (0, 25, 50 and 100 μg/mL) for 24 h. In the present study, cardiac hypertrophy was occurred in vivo and vitro after exposure to $PM_{2.5}$. Mechanistically, circ_0001859 could sponge miR-29b-3p, which could interact with 3′UTRs of Ctnnb1 (gene name of β-catenin). And Ctnnb1 expression was transcriptionally inhibited by si-circ_0001859 or miR-29b-3p mimic in HL-1 cells. Additionally, miR-29b-3p inhibitor could also make a reversion about the inhibition effect of circ_0001859 silencing on Ctnnb1 mRNA level in HL-1 cells. Functionally, knockout of circ_0001859 or overexpression of miR-29b-3p could inhibit LEF1/IGF-2R pathway and alleviate the progress of hypertrophy induced by $PM_{2.5}$ in HL-1 cells. And miR-29b-3p inhibitor could reverse the inhibition effect of circ_0001859 silencing on hypertrophic response induced by $PM_{2.5}$ in HL-1 cells. Consequently, the data demonstrated that circRNA_0001859 promoted the process of cardiac hypertrophy through suppressing miR-29b-3p leading to enhance Ctnnb1 level, and activated downstream pathway molecules LEF1/IGF-2R.

1. Introduction

Global prevalence of cardiovascular diseases (CVDs) has been on the rise and constitutes the leading causes of morbidity and mortality around the world (Joseph et al., 2017). According to the World Health Organization (WHO) data, almost 17.9 million people have died annually of CVDs in 2019, accounting for nearly 32% of all death (https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)), which are the top cause of global death. And the number may reach up to 23.3 million by 2030 (https://www.who.int/cardiovascular_diseases/about_cvd/en/https://www.who.int/cardiovascular_diseases/about_cvd/en/). Therefore, a deeper understanding of the risk factors of CVDs is the key step to take innovative measurements. An exhaustive body of literature has emerged to show that a host of factors can cause CVDs, including heredity, hypertension, obesity, aging and environmental pollution (Moore and Shah, 2020; Ekroos et al., 2020; Wang et al., 2020a). Specifically, in numerous epidemiological studies, CVDs could be caused by both short-term and long-term $PM_{2.5}$ exposure

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(Ljungman et al., 2019; Feng et al., 2016). Moreover, the WHO's comprehensive 2013 Review of evidence on health aspects of air pollution (REVIHAAP) provided confirmation of a causal link between exposure to $PM_{2.5}$ and CVDs (WHO Regional Office for Europe, 2013). In Addition, as an important risk factor for CVDs, cardiac hypertrophy is an adaptive process of the heart that occurs in response to stresses (Yildiz et al., 2020). Long-term stress could lead to irreversible hypertrophic responses, which may have more serious effect on structure or function of heart. However, the current epidemiological evidences are hard to explain the mechanism of cardiac hypertrophy induced by $PM_{2.5}$ exposure. Therefore, study on the mechanism of cardiac hypertrophy might provide strategy of finding new targets for effective prevention and treatment.

Previous study found the potentially biological mechanisms of $PM_{2.5}$-mediated cardiac hypertrophy accompanied by macrophage activation (Su et al., 2020). It has been evidenced that macrophage M1 polarization can be induced by the activated β-catenin signaling pathway (Yuan et al., 2020). Meanwhile, in vitro study, inhibition of β-catenin signaling could abolish cardiomyocyte hypertrophy (Lin et al., 2020). Although there was evidence that $PM_{2.5}$ caused increases of β-catenin mRNA and protein in pulmonary arterial hypertension rat models (Cong et al., 2018), the upstream regulatory molecular mechanism of β-catenin has been remained unclear. Additionally, abundant studies have showed that epigenetics occupies an important position in cardiac hypertrophy, involving DNA methylation, RNA methylation, histone modifications, chromatin structure and non-coding RNAs (Greco and Condorelli, 2015; Lei et al., 2020). Circular RNAs (CircRNAs), a member of non-coding RNAs with no 5′-3′ polarity, are generated from back-splicing of precursor mRNA (pre-mRNA) (Vicens and Westhof, 2014; Jeck and Sharpless, 2014). CircRNAs are characterized by conserved sequences, widely spread with stable structure, and cell- or tissue-specific expression, which play an essential regulatory role in several physiological and pathological processes (Memczak et al., 2013). Recently, a few studies evidenced that $PM_{2.5}$ could alter circRNAs expression in lung tissue and BEAS-2B cells (Zhong et al., 2019; Li et al., 2020b, 2020e). It has been reported that the relative level of β-catenin could be regulated by circRNAs (Hu et al., 2019). The functions of circRNAs have been confirmed, which may have potential to associate with their parent genes because of sharing the same pre-mRNA (Shen et al., 2019). Therefore, it is a hypothesis that homologous circRNAs might regulate its parent gene β-catenin and then develop the cardiac hypertrophy induced by $PM_{2.5}$ exposure.

Notably, growing evidences have demonstrated that circRNAs could regulate gene level via different mechanisms, including as microRNAs (miRNAs) sponges, binding RNA-related proteins and regulating splicing or transcription (Meng et al., 2017; Xue et al., 2020). MiRNAs, endogenously expressed small single-stranded RNAs (approximately 23 nucleotides), are now accepted to play an essential role in the regulation of gene expression (Lu and Rothenberg, 2018). MiRNAs deregulation has been revealed in various diseases induced by $PM_{2.5}$ (Chen et al., 2021; Li et al., 2020c). Meanwhile, accumulating evidences indicate that miR-29 is down-regulated with cardiac hypertrophy (Li et al., 2021; Kaur et al., 2014). Screening of starBase database revealed miR-29b-3p had putative binding sites in both circ_0001859 and Ctnnb1. It was speculated that circ_0001859 sponged miR-29b-3p to indirectly regulate Ctnnb1 in the process of cardiac hypertrophy induced by $PM_{2.5}$.

Here, a mice model of ambient $PM_{2.5}$ exposed was established to elucidate in vivo effect and possible epigenetic mechanism of $PM_{2.5}$ on cardiac hypertrophy. The cardiac morphological and histological changes as well as expressions of markers associated with hypertrophy was evaluated. The upstream ceRNAs mechanism of β-catenin signals was explored in mice after $PM_{2.5}$ exposure. The data showed that cardiac hypertrophy was found, which was regulated with the activation of β-catenin/LEF1/IGF-2R pathway in vivo and in vitro after $PM_{2.5}$ exposure. Mechanically, mRNA level of β-catenin was regulated by circ_0001859 adsorbing miR-29b-3p. These results may provide a better understanding of the possible mechanism of $PM_{2.5}$-induced cardiac hypertrophy and might further deepen the current knowledge on the role of circ_0001859 in the process.

2. Materials and methods

2.1. Animals and treatment

Forty-eight male C57BL/6 mice (6-week-old) were purchased from Vital River Laboratory (Beijing Vital River Laboratory Animal Technology Co., Ltd, Beijing, China). The mice were housed in cages with room temperature (21–24 °C), relative humidity (50%) and 12 h light-dark cycle. All mice were fed ad libitum standard chow and distilled water. The weights of mice were monitored weekly.

After one week of acclimatization, the mice were randomly divided into 3 groups (16 mice/group): filtered air (FA) group, unfiltered air (UA) group and concentrated air (CA) group. Mice in FA group breathed clean air filtered by three-layers of high-efficient particulate air filters which could remove particulate matter in outdoor airflow. Mice in UA group were placed in the similar chambers and exposed to real time ambient air delivered through pipes. CA mice were exposed in concentrated $PM_{2.5}$ atmosphere by performing with a system of $PM_{2.5}$ concentration enrichment (Beijing Huironghe Technology Co., Ltd, Beijing, China). The mice were exposed different air for 8 or 16 weeks, 6 h/day,7 day/week, respectively. And they were kept in filtered air except during the exposure period. During the exposure, the concentrations of ambient $PM_{2.5}$ were monitored by Aerosol Detector DUSTTRAKTM II-Model 8530 (TSI instrument Co., Ltd, Minneapolis, USA). The size distribution of particulate matters was analyzed by Aerodynamic Particle Sizer Spectrometer 3321 (TSI instrument Co., Ltd, Minneapolis, USA).

Echocardiography was used to measure cardiac function of the mice that were anesthetized with 1% pentobarbital sodium (0.01 mL/g body weight) after the final exposure to $PM_{2.5}$ for 24 h. And after the mice were sacrificed, the hearts were washed in PBS, weighed, fixed, cut into pieces and stored at −80 °C for subsequent experiments.

2.2. Echocardiographic assessment

Echocardiography with a Vevo 2100™ high-resolution small animal ultrasound system (Visual Sonics, Toronto, Ontario, Canada) was used to assess mice cardiac function. Briefly, after shaving from the chest, mice were anesthetized by inhalation of 1.5% maintenance of isoflurane. For each parameter of heart function and structure, three consecutive cardiac cycles were recorded and three values averaged.

2.3. Histological analysis

FITC-conjugated wheat germ agglutinin (WGA) was used to measure cardiomyocyte cross-sectional area (CSA) of the mice. After that, the slides were examined under the fluorescence microscope (Olympus Optical Co., Ltd, Tokyo, Japan). Then, staining with hematoxylin and eosin (H&E) was used to evaluate the histopathological changes of heart under the light microscope as described previously.

2.4. $PM_{2.5}$ preparation

The $PM_{2.5}$ high volume sampler system TH-1000 H (Tianhong, Wuhan, China) was used to collect $PM_{2.5}$. Sampling using PTFE membrane (250 * 200 mm) was performed continuously at a flow rate of 1.05 m^3/min for 12 h every day from November 2017 to February 2018, which synchronized with the duration of the animals' exposure. The $PM_{2.5}$ suspension was obtained by thoroughly mixing clipped PTFE membrane and distilled water under sonication. After filtering into plastic dishes, freeze suspension was frozen at least 6 h at −80 °C and then transferred to the vertical freeze dryer ZX-LGJ-18 (Zhixin, shanghai, China) at least 24 h. After the moisture was sublimated, the