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Mechanism of YY1 mediating autophagy dependent ferroptosis in PM2.5 induced cardiac fibrosis

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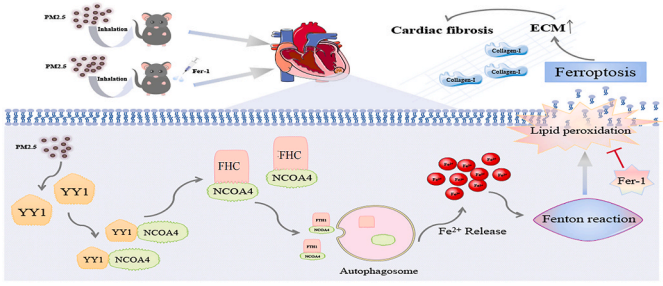
HIGHLIGHTS

- Long-term exposure to PM2.5 induced cardiac fibrosis through ferroptosis activation.
- PM2.5-induced cardiac fibrosis was associated with the increase of ferritinophagy.
- YY1 regulated the ferritinophagy pathway by recognizing NCOA4.

GRAPHICAL ABSTRACT



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ABSTRACT

Epidemiological studies have demonstrated strong associations between exposure to ambient fine particulate matter (PM2.5) and cardiac disease. To investigate the potential mechanism of cardiac fibrosis induced by PM2.5, we established PM2.5 exposure models *in vivo* and *in vitro*, and then cardiac fibrosis was evaluated. The ferroptosis and ferritinophagy was detected to characterize the effects of PM2.5 exposure. The results indicated that PM2.5 exposure could induce cardiac fibrosis in mice. YY1 was induced by PM2.5 exposure and then increased NCOA4, a cargo receptor for ferritinophagy, which interacted with FHC and promoted the transport of ferritin to the autophagosome for degradation. The release of large amounts of free iron from ferritinophagy led to lipid peroxidation directly via the Fenton reaction, thereby triggering ferroptosis. Moreover, siNCOA4 could partly restore the FHC protein level in HL-1 cells and inhibit the occurrence of downstream ferroptosis. Functionally, NCOA4 knockdown inhibited ferroptosis and alleviated HL-1 cell death induced by PM2.5. Ferroptosis inhibitor (Ferrostatin-1) could reverse the promoting effect of ferritinophagy mediated ferroptosis on cardiac fibrosis induced by PM2.5 exposure in mice. Our study indicated that PM2.5 induced cardiac fibrosis through YY1 regulating ferritinophagy-dependent ferroptosis.

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1. Introduction

Air pollution was a major contributor to the global burden of disease and has a huge impact on population health, leading to an average loss of 20 months of life expectancy globally, almost as high as the dangers of tobacco use (22 months). Air pollution has become the fourth highest risk factor for mortality (2020, Brauer et al., 2021). There was evidence that PM2.5 was the major air pollutant that posed serious threat to global public health (Rajagopalan et al., 2018). A 2010 statement from the American Heart Association stated that the available scientific evidence showed a consistent causal relationship between cardiovascular disease (CVD) and PM2.5 exposure (Brook et al., 2010). The following studies have confirmed the effects of both short-term and long-term exposure to PM2.5 on adverse cardiovascular (Li et al., 2015; Zhang et al., 2021). Long-term exposure to PM2.5 increased the risk of adverse cardiovascular events and led to the development of underlying diseases (Kaufman et al., 2020). In a large cohort study, the risk of ischemic heart disease (IHD) mortality increased by 16% per 10 μg/m^3 increase in PM2.5 concentration (Hayes et al., 2020). In addition, a time series study in 184 major Chinese cities indicated that the number of hospital admissions for heart failure (HF) and arrhythmia increased by 0.27% and 0.29% per 10 μg/m^3 PM2.5 increment, respectively (Tian et al., 2019). PM2.5 is closely associated with the increased incidence and mortality of cardiovascular diseases in humans, but the mechanism was still not well known.

Cardiac fibrosis was a common pathological character during the development of various cardiac diseases. It was characterized by excessive deposition of extracellular matrix (ECM) proteins in the myocardium that distort the structure of the myocardium, which could lead to mechanical and electrical dysfunction, and then promote the development of arrhythmias and cardiac dysfunction (Schafer et al., 2017; Li et al., 2018). In our previous study, chronic PM2.5 exposure could cause cardiac fibrosis in mice (Su et al., 2020). PM2.5 exposure could trigger oxidative stress and induce cardiovascular damage. Epidemiological studies indicated that malondialdehyde (MDA) levels increased by 3.7% per 10 μg/m^3 individual PM2.5 increment (Sørensen et al., 2003). The levels of oxidative damage DNA, nuclear bases and lipid peroxidation products were increased in urine of subjects who had been exposed at work to environmental air pollution (Møller and Loft, 2010). In addition, some studies have shown that the changes in cardiac function caused by PM2.5 are related to the role of reactive oxygen species (ROS) (Wilson et al., 2018; Miller, 2020). PM2.5 exposure reversibly elevated heart rate and blood pressure, induced cardiac systolic dysfunction of older mice, and reversibly induced fibrosis in juvenile and older mice. The mechanism by which PM2.5 exposure resulted in cardiac lesions might involve oxidative stress (Qin et al., 2018).

It has been known oxidative stress could lead to autophagy, apoptosis and necrosis which have been found in heart tissues after PM2.5 exposure (Zhang et al., 2018; Ren et al., 2020). The key pathogenic factor of myocardial fibrosis was the death of terminally differentiated cardiomyocytes (Fang et al., 2019). However, inhibition of autophagy, apoptosis or necrosis only partially improved the survival of PM2.5-treated cardiac cells (Yang et al., 2018; Wang et al., 2019). It was hypothesis that other forms of cell death seemed contribute to PM2.5-induced changes in cardiac structure or normal functions. Microarray data and signaling pathway analysis revealed PM2.5 exposure could trigger ferroptosis in endothelial cells (Wang and Tang, 2019). Ferroptosis was an iron-dependent mode of programmed cell death, which was different from apoptosis and necrosis. Free iron could trigger Fenton reactions and produce highly reactive hydroxyl radicals, and subsequently caused lipid peroxidation of the cell membrane, increased membrane permeability, led to cardiomyocyte damage and heart disease (Gujja et al., 2010).

Circulating Fe^{3+} was transported by transferrin and recognized by the membrane protein transferrin receptor 1 (TFR1) into cells. Ferritin was a complex of ferritin light chain (FTL) and heavy chain 1 (FHC) that stored excess iron (Xie et al., 2016). Ferritin was transported to the autophagosome for degradation, a process known as ferritinophagy (Xie et al., 2016). Ferritinophagy can increase free iron levels and induce ferroptosis by producing ROS through Fenton reaction. Initially thought to be a protein that interacted with androgen receptors (AR), NCOA4 has been shown to be a specific ferritin transport receptor, transporting ferritin to autophagosomes and mediating ferritinophagy (Mancias et al., 2014). NCOA4 abundance could be regulated by the ubiquitin protease HERC2 under physiological conditions (Mancias et al., 2015). However, NCOA4 protein might be regulated differently when environmental pollutants stressed. The interaction surface analysis software PDBePISA (EMBL-EBI, Hinxton, Cambridgeshire, UK) found that there was a relatively stable molecular docking between NCOA4 and transcription factor Yin Yang 1 (YY1) protein. Furthermore, YY1, exerted a key effect on autophagy, could selectively activate or inhibit gene transcription (Zhang et al., 2019). Meanwhile, a study showed that YY1 could relieve dilated cardiomyopathy and fibrosis in Lmna DCM mouse model (Tan et al., 2019), whereas other studies indicated that YY1 could promote pulmonary and renal fibrosis (Lin et al., 2011; Wang et al., 2019; Yang et al., 2019).

In the present study, we established a PM2.5 exposure mice model to demonstrate the effects of ferritinophagy-dependent ferroptosis on cardiac fibrosis induced PM2.5 exposure. After PM2.5 exposure, YY1 was activated by PM2.5 and triggered ferritinophagy through directly interacting and then regulating NCOA4. FHC binding NCOA4 was transferred into autophagosomes for lysosomal degradation, releasing a large quantity of free iron to trigger lipid peroxidation, leading to ferroptosis of cardiac cells and then resulting in cardiac fibrosis. Our results provided insights into the pathogenesis behind iron overload induced cardiac fibrosis and provide a novel perspective for preventing PM2.5 related cardiac fibrosis.

2. Materials and methods

2.1. Animals and treatment

Forty-two 6-week-old male C57BL/6 mice, with weight of 19–21 g, were purchased from Vital River Laboratory (Beijing Vital River Laboratory Animal Technology Co., Ltd, Beijing, China). To acclimatize the laboratory conditions, mice were kept in individual ventilated (IVC) cages for a week at the commencement of the study. The mice were housed at a temperature of 18–25 °C and a relative humidity of 30%–70%, and the light/dark cycle was performed for 12 h. During exposure, mice were free to drink and eat, and were weighed weekly.

Eighteen mice were randomly divided into three groups, which included the filtered air (FA) group, unfiltered air (UA) group and concentrated air (CA) group. The FA mice inhaled air in the chambers with the high-efficient particulate air (HEPA) filters. The UA mice were exposed to ambient air in the chambers without HEPA filter. Mice in the CA group were exposed concentrated PM2.5 air by using a PM2.5 concentration enrichment system (Beijing Huironghe Technology Co., Ltd, Beijing, China). The exposure time of CA group was 6 h/d, 7 d/week for 16 weeks and the remaining time were housed in FA chambers. The PM2.5 concentration in FA group, UA group and CA group were detected by Aerosol Detector DUSTTRAKTM II-Model 8530 (TSI instrument Co., Ltd, Minneapolis, USA), and Aerodynamic Particle Sizer Spectrometer 3321 (TSI instrument Co., Ltd, Minneapolis, USA) was used to monitor particle size distribution.

According to the principle of random sampling, 24 mice were divided into 4 groups: FA-Ferrostatin-1(Fer-1) intervention (FAM) group, FA-dimethyl sulfoxide (DMSO) treatment (FAD) group, CA-Fer-1 intervention group (CAM) and CA-DMSO treatment (CAD) group. Fer-1 was dissolved in DMSO to reach the concentration of 10 mg/ml, and then diluted with 0.9% NaCl into a working solution with a concentration of 0.2 mg/ml. Fer-1 group received intraperitoneal injection of 1 mg/kg Fer-1, and DMSO control were received intraperitoneal injections