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# Particulate matter exposure induces the autophagy of macrophages via oxidative stress-mediated PI3K/AKT/mTOR pathway

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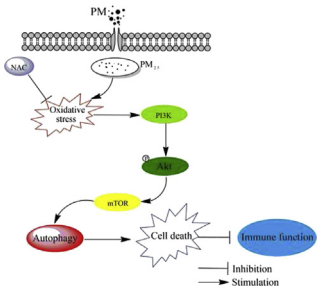
## HIGHLIGHTS

- $PM_{2.5}$ exposure decreased numbers of immune cells in SD rats BALF.
- $PM_{2.5}$ could induce the autophagy of pulmonary macrophages in SD rats and Raw264.7 cells.
- $PM_{2.5}$ exposure led to the activation of oxidative stress in Raw264.7 cells.
- $PM_{2.5}$ induced cell autophagy via the oxidative stress-mediated PI3K/AKT/mTOR pathway.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Many epidemiological investigations have consistently demonstrated the immunotoxicity of fine particulate matter ($PM_{2.5}$), but the underlying molecular mechanism remains unclear and needs to be elucidated. In this work, the immune cells, including pulmonary macrophages of SD rats and Raw264.7 cells, were applied to further investigate the effect of $PM_{2.5}$ on cell autophagy of macrophages, thus clarified the possible molecular mechanism of immunotoxicity caused by $PM_{2.5}$. SD rats were exposed to summer (0.2, 0.6, 1.5 mg $kg^{-1}$ b.w.) and winter (0.3, 1.5, 2.7 mg $kg^{-1}$ b.w.) $PM_{2.5}$ adopting the intratracheal instillation method. The exposure was performed one time every 3 days and continued for 2 months. The data showed that $PM_{2.5}$ exposure decreased numbers of immune cells in pulmonary macrophages of SD rats. In addition, $PM_{2.5}$ could induce the cell autophagy through the increased LC3 and decreased p62 mRNA and protein levels of pulmonary macrophages in SD rats and Raw264.7 cells in a concentration-dependent manner. Strikingly, $PM_{2.5}$-induced oxidative stress was observed. However, NAC supplement (the ROS inhibitor) significantly reversed $PM_{2.5}$-caused effects. Additionally, the PI3K/AKT/mTOR pathway was activated in $PM_{2.5}$-treated cells and NAC had an important inhibitory effect. These results demonstrated that $PM_{2.5}$ exposures induced autophagy of pulmonary macrophages via the oxidative stress-mediated PI3K/AKT/mTOR pathway, which may

*Abbreviations:* $PM_{2.5}$, Particulate Matter 2.5; SD rats, Sprague Dawley Rats; BALF, Bronchial Alveolar Lavage Fluid; MDC, Monodansylcadaverine; SDS-PAGE, Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis; PVDF, Polyvinylidene Fluoride; NAC, N-acetylcysteine.

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contribute to explain the molecular mechanism of immunotoxicity caused by $PM_{2.5}$ and provide the theoretical foundation for environment toxicology of $PM_{2.5}$.



## 1. Introduction

The global air pollution has become a major threat to human health. Day-to-day variations in air pollution have been linked to increased risks of cardiopulmonary morbidity and mortality (Brook Rd Fau – Rajagopalan et al., 2010; Pope et al., 2004). Especially, fine particulate matter ($PM_{2.5}$) with an aerodynamic diameter less than 2.5 μm mostly originates from coal combustion, vehicle exhaust and the open sources in Chinese cities (Jin et al., 2016; Li et al., 2015). Epidemiological and experimental investigations have demonstrated $PM_{2.5}$ exposure was closely associated with the immunotoxicity (Rivas-Santiago et al., 2015; Zhang et al., 2015a,b). Therefore, the studies about potential mechanisms of $PM_{2.5}$-induced immunotoxicity were very important. However, the current studies were limited and need further investigation.

It is well-known that alveolar macrophages are the immune cells from lung and mainly distribute in the respiratory tract and alveolar surface, which exert the defense function by against foreign body (Hickman-Davis et al., 2001). Particles inside of the lungs mainly rely on alveolar macrophages to engulf foreign particles, which then can be removed by lysosome (Gwyer Findlay and Hussell, 2012; Wang et al., 2016). As a result, the decrease of macrophages causes the damage of immune system that further results in body's immunity weaken (McMurran et al., 2016). Therefore, there are the significance concerns between macrophages and health of human body. This study mainly focuses on the effect of $PM_{2.5}$ exposure on cell autophagy of macrophages and classifies relevant mechanisms.

Autophagy is the cellular process that disposes damaged or unwanted proteins and organelles via the self-digestion to generate intracellular nutrients and maintain the intracellular homeostasis during various cell stresses (Azad et al., 2009). These substrates are enfolded into massive cytoplasmic by double-membraned, with the extension and fusion of membrane edge, which results in the formation of the autophagosome (Yang and Klionsky, 2010). Further autophagosome fuses with lysosomes to form autolysosomes to degrade the substrates by lysosomal hydrolases, the degradation products are recycled back into cytosol and reused to enhance cell survival during nutrient deprivation (Abounit et al., 2012; Chen et al., 2008). The process of autophagy is completed by a series of autophagy related proteins that play a diverse role in different stages. As the markers, microtubule associated protein 1 light chain 3 (LC3) always keeps steady on the autophagosome membrane to reflect the number of autophagosome until the fusion with lysosomes (Van Limbergen et al., 2009).

A lot of evidences showed that the PI3K/AKT/mTOR pathway played an important role in the modulation of cell autophagy (Lin et al., 2016). As the major modulator of autophagy, kinase mammalian target of rapamycin (mTOR) receives inputs from different signaling pathways, especially from those that sense the energetic state of the cell to trigger or halt the synthesis of proteins (Le Sage et al., 2016; Liu et al., 2015a,b). Recently, it was confirmed that oxidative stress could trigger autophagy through several distinct mechanisms involving Atg4, catalase, and the mitochondrial electron transport chain (mETC) (Azad et al., 2009; Deng et al., 2013). In this study, we determined the effect of $PM_{2.5}$ on the activation of oxidative stress and PI3K/AKT/mTOR pathway and their roles on $PM_{2.5}$-induced cell autophagy.

Taiyuan as a capital city of Shanxi Province is a center of coal based electricity production and many chemicals industries. The city and neighboring with many main cities currently face serious problems of ambient $PM_{2.5}$ pollution in China (Jin et al., 2015). The present study demonstrated that $PM_{2.5}$ exposure can cause the damage of immune cells, which mainly attributed to the abduction autophagy of macrophages via the oxidative stress mediated activation of PI3K/AKT/mTOR pathway.

## 2. Materials and methods

### 2.1. Materials

Dulbecco's modified Eagle's medium (DMEM) culture medium, penicillin, streptomycin, and fetal bovine serum (FBS) were purchased from Thermo (Beijing, China). Monodansylcadaverin (MDC) were obtained from Sigma (St. Louis, MO). N-acetylcysteine (NAC), microtubule-associated protein 1 light chain 3 (LC3), Dimethylsulfoxide (DMSO), 2′7′-dichlorofluorescein diacetate (DCFH-DA) and antibodies for LC3, p62, mTOR, PI3K, *p*-Akt, GAPDH were purchased from Sangon Biology (Shanghai, China). Assay kits for SOD, glutathione (GSH), catalase (CAT) and Wright-Giemsa were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

### 2.2. Sampling and preparation of $PM_{2.5}$

$PM_{2.5}$ samples were collected during summer of June 2012 and winter of January 2013 at Taiyuan, a site of Shanxi urban background for atmospheric pollution. As described in our previous study (Jin et al., 2015), the sampling sites were located in Taiyuan city, Shanxi province, China (30°15′N latitude, 112°33′E longitude). $PM_{2.5}$ high volume air sampler (Thermon Anderson, USA) was placed on the rooftop of a building about 25 m tall and there were no any obstacles and no large pollution sources near the building. $PM_{2.5}$ concentrations were measured using DustTrakTM II Aerosol Monitor (TSI Inc., USA), while daily $PM_{2.5}$ samples were collected on quartz fiber filters (QFFs) with a pump flow rate of 1.13 $m^3$/min. The filters loading samples were cut and surged in Milli-Q water with sonication. To obtain $PM_{2.5}$ suspensions, the above suspensions were freeze-dried in vacuum, weighed, and stored at −20 °C.

### 2.3. Animals and intratracheal instillation

Sprague Dawley rats weighing 180–220 g were purchased from the Experimental Animal Center of Chinese Military Medical Sciences Academy (No. SCKX-2006-0008). The animals were provided a comfortable living conditions, housed at pathogen-free and had clean-air room with restricted access. All the animal studies were conformed to the principles for laboratory animal research outlined by the Animal Welfare Act (Pub. L. 89-544). The animal protocol used in this study was approved by the Institutional Animal Care and Use Committee of Shanxi University (Taiyuan, Shanxi Province, China). Rats were divided randomly into seven equal groups of six animals and anesthetized with diethyl ether. The trachea was intubated with an 18-gauge angiocatheter (Becton–Dickinson).