Nanotoxicology

ISSN: 1743-5390 (Print) 1743-5404 (Online) Journal homepage: https://www.tandfonline.com/loi/inan20

N^6-methyladenosine-dependent primary microRNA-126 processing activated PI3K-AKT-mTOR pathway drove the development of pulmonary fibrosis induced by nanoscale carbon black particles in rats

Bin Han, Chen Chu, Xuan Su, Ning Zhang, Lixiao Zhou, Mengyue Zhang, Shuaishuai Yang, Lei Shi, Bo Zhao, Yujie Niu & Rong Zhang

To cite this article: Bin Han, Chen Chu, Xuan Su, Ning Zhang, Lixiao Zhou, Mengyue Zhang, Shuaishuai Yang, Lei Shi, Bo Zhao, Yujie Niu & Rong Zhang (2019): N^6-methyladenosine-dependent primary microRNA-126 processing activated PI3K-AKT-mTOR pathway drove the development of pulmonary fibrosis induced by nanoscale carbon black particles in rats, Nanotoxicology, DOI: 10.1080/17435390.2019.1661041

To link to this article: https://doi.org/10.1080/17435390.2019.1661041

View supplementary material

Published online: 10 Sep 2019.

Submit your article to this journal

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=inan20

NANOTOXICOLOGY
https://doi.org/10.1080/17435390.2019.1661041

Taylor & Francis
Taylor & Francis Group

ARTICLE

Check for updates

N^6-methyladenosine-dependent primary microRNA-126 processing activated PI3K-AKT-mTOR pathway drove the development of pulmonary fibrosis induced by nanoscale carbon black particles in rats

Bin Han[a], Chen Chu[a], Xuan Su[a], Ning Zhang[a], Lixiao Zhou[a], Mengyue Zhang[a], Shuaishuai Yang[a], Lei Shi[b], Bo Zhao[c], Yujie Niu[b,d] and Rong Zhang[a,d]

[a]Department of Toxicology, School of Public Health, Hebei Medical University, Shijiazhuang, China; [b]Occupational Health and Environmental Health, School of Public Health, Hebei Medical University, Shijiazhuang, China; [c]Department of Laboratory Diagnosis, Hebei Medical University, Shijiazhuang, China; [d]Hebei Key Laboratory of Environment and Human Health, Hebei Medical University, Shijiazhuang, China

ABSTRACT

The pulmonary fibrosis could be caused by long-term inhalation of carbon black (CB) particles. Studies on the mechanisms of pulmonary fibrosis induced by CB are required to develop the stratagem of prevention and treatment on fibrosis. The RNA-binding protein DiGeorge syndrome critical region gene 8 (DGCR8)-dependent pri-miRNAs processing is regulated by N^6-methyladenosine (m^6A) modification, which targets the downstream signal pathway. However, its role in pulmonary fibrosis has not been known clearly. In the present study, rats inhaled CB at dose of 0, 5 or 30 mg/m^3 for 28 days, 6 h/day, respectively. The rats inhaled CB at dose of 0 or 30 mg/m^3 for 14 days, 28 days and 90 days, respectively. In vitro experiments, the normal human bronchial epithelial cell line (16HBE) was treated with CB (0, 50, 100 and 200 μg/mL) for 24 h. In vitro and vivo study, the levels of fibrosis indicators including α-SMA, vimentin, collagen-I and hydroxyproline in CB treatment groups statistically increased in dose- or time- dependent manners compared with the control. After CB treatment, PI3K-AKT-mTOR pathway was activated and regulated by miRNA-126. We found that both of m^6A modifications of pri-miRNA-126 and its binding with DGCR8 were decreased after CB treatment, which resulted in the reduction of mature miRNA-126 accompanied by accumulation of unprocessed pri-miRNA-126. This work demonstrated that m^6A modification of pri-miRNA-126 and its binding with DGCR8 decreases blocked miRNA-126 maturation, and then activated the PI3K/AKT/mTOR pathway, which drove the fibro genesis in the lung after CB exposure.

ARTICLE HISTORY
Received 7 March 2019
Revised 17 August 2019
Accepted 19 August 2019

KEYWORDS
Carbon black (CB); pulmonary fibrosis; N6-Methyladenosine (m6A); miRNA-126; PI3K/AKT/mTOR signaling pathway

Introduction

Carbon black (CB) is a kind of amorphous carbon produced by incomplete combustion or thermal cracking of hydrocarbons under conditions of insufficient air. CB is widely used due to its good stability and inertness, such as black dye for the manufacture of inks, paints, and reinforcing agent for rubber. In toxicological study, CB is often used as a negative control because of its low toxicity and low solubility (Hubbs et al. 2013). However, study has shown that CB could induce inflammation and histopathological damage in lung (Koike et al. 2008). CB was thought to exacerbate several respiratory diseases in humans, including lung inflammation and fibrosis (Lin et al. 2011; Kamata et al. 2011; Saputra et al. 2014). In our previous study, we found the thickening of the alveolar wall and collagen deposition after CB inhalation for 14 days in mice (Zhang et al. 2014). Though the fibrosis in lung could be induced by CB particles, the mechanism of fibrosis has not been known clearly. 'PI3K/AKT/mTOR signaling pathway' plays an important role in regulating physiological functions such as cell growth, proliferation, cell survival, protein synthesis and transcription (Hay and Sonenberg 2004). Previous studies suggested the PI3K/AKT/mTOR signaling pathway was involved in the development of fibrotic diseases, such as cardiac fibrosis (Yu et al. 2013; Han et al. 2006), liver fibrosis (Patsenker et al. 2011) and renal fibrosis

CONTACT Rong Zhang rongzhang@hebmu.edu.cn Department of Toxicology, School of Public Health, Hebei Medical University, 361 Zhongshan east Rd., Shijiazhuang, Hebei 050017, China

Supplemental data for this article can be accessed here.

© 2019 Informa UK Limited, trading as Taylor & Francis Group

(Geissler and Schlitt 2010). mTOR inhibitors showed fibrotic intervention on pulmonary fibrosis in both of human (Luai et al. 2014) and animals (Bartel 2009; Brennecke et al. 2003; Liu et al. 2010). p70S6k has been shown to be downstream of mTOR, which reflect activation of the mTOR signaling pathway (Brown et al. 1995; Dennis, Fumagalli, and Thomas 1999).

MicroRNAs (miRNAs), a kind of small non-coding RNAs, negatively regulate gene expression by binding to the 3′-untranslated region (3′-UTR) of their target gene mRNAs (Bartel 2009). miRNAs play important roles in almost all of physiology and pathophysiology process, including apoptosis, cell differentiation, proliferation (Brennecke et al. 2003) and cell cycle distribution as well as fibrosis (Liu et al. 2010; Luai et al. 2014; Ji et al. 2015; Wu et al. 2016). In SK-MES-1 cell lines, PI3K/AKT/mTOR pathway was found as a downstream target of miRNA-126 (Wang et al. 2011). Phosphatidyl Inositol 3-kinase (PI3K) could be regulated by miR-126 through a seed sequence in the 3′-UTR region of the corresponding PIK3R2 (PI3KR2, PI3K regulatory subunit 2, also known as p85-β) (Song et al. 2016).

The regulation of miRNAs to target genes is dependent on the metabolism of their primary miRNAs (pri-miRNAs) (Gebert and MacRae 2018). The first step during the process of miRNAs metabolism is that the RNA-binding protein DiGeorge syndrome critical region gene 8 (DGCR8) recognizes the specific structure of the pri-miRNAs (Denli et al. 2004) and then recruits drosha ribonuclease III (DROSHA) (Gregory et al. 2004). A microprocessor complex consisting of DGCR8 and DROSHA produces precursor miRNAs (pre-miRNAs) by cleavage of the RNA duplex (Han et al. 2004; Han et al. 2006). The DGCR8-dependent pri-miRNAs processing was regulated by N^6-methyladenosine (m^6A) modification. It is demonstrated that some miRNAs containing m^6A tags in their pri-miRNA (Alarcón et al. 2015b; Alarcon et al. 2015a). m^6A, as the most abundant internal modification of messenger RNAs (Alarcón et al. 2015b), could be written by methyltransferase-like 3 (METTL3) and methyltransferase-like 14 (METTL14) in intracellular mRNA (Alarcón et al. 2015b; Visvanathan and Somasundaram 2018). Therefore, it is hypothesis that m^6A involved in the metabolism of miRNA-126 and drove the PI3K/AKT/mTOR pathway then lead to pulmonary fibrosis after CB exposure.

Here, we established a pulmonary fibrosis model in rats after CB inhalation and explored its possible mechanism. Our study found that the PI3K/AKT/mTOR signaling pathway was activated by miRNA-126 after CB treatment. The mature processes of miRNA-126 from unprocessed pri-miRNA-126 were inhibited through the decreased m^6A modification of pri-miRNA-126 and its binding with DGCR8 and pri-miRNA-126, which related with the decreases of METTL3 and METTL14 levels during pulmonary fibrosis development. Our results might help to deepen the understanding of the role and function of m^6A modification in pulmonary fibrosis caused by CB. The constructed METTL3/METTL14-m^6A-miRNA126- PI3K/AKT/mTOR axis might serve as a biomarker for pulmonary fibrosis prevention and these results would provide a potential drug target for the treatment of pulmonary fibrosis caused by CB.

Materials and methods

Materials

CB (≥99.8%) was purchased from the CB manufacturing factory (Jiaozuo Chemical Industry Limited Company, Henan, China) and its characteristics were same as our previous studies (Zhang et al. 2014). The size and morphology of the CB were measured using a Tecnai G220 transmission electron microscope (TEM, FEI, USA). The surface morphology of CB was observed by a scanning electron microscope (SEM, Sirion 200, FEI, Holland). All samples were coated with gold particles prior to analysis. The specific surface area of the CB particles was calculated using the Brunauer-Emmett-Teller (BET) adsorption isotherm.

Assay of endotoxin

The presence of endotoxin on the CB was tested using Toxin Sensor™ Chromogenic LAL Endotoxin Assay Kit (Genscript, NJ, USA). The endotoxin could catalyze the activation of a proenzyme in Limulus amebocyte lysate (LAL), which would cleave a substrate to produce a colored end-product. The end-product was measured spectrophotmetrically and

compared to a standard curve. The test was repeated three times.

Study design

Male SD rats (6-8 weeks, 180-220 g) were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Rats were housed under specific pathogen-free conditions on a 12-h light-dark cycle with food and water provided ad libitum. The present protocol was approved by the Committee of the Ethics Animal Experiments of Hebei Medical University (IACUC-Hebmu-20160047) and carried out under the institutional guidelines for ethical animal use.

The rats were randomly divided into control, exposure and recovery groups. Rats individually placed in nose-only exposure tanks, for 6 hours/day, respectively. The rats in 5 mg/m^3 exposure group were exposed for a continuous inhalation for 28 days. And rats in 30 mg/m^3 exposure group were exposed for a continuous inhalation for 14 days, 28 days and 90 days, respectively. Rats in the recovery group were exposed to CB for 90d and recovered for another 14d. At the same time, the control rats were exposed to filtered air for 6 h/day.

For inhalation, CB was dispersed via HRH-DAG768 powder dispersion system (Huironghe Technology, Beijing, China) according to our previous study (Chu et al. 2019). CB powder was transported by a rotating brush onto a 19 L exposure chamber. Briefly, CB powder came from the reservoir, loosened by the rotating brush at 13 L/min feed rate, then mixed with dispersion air at 10 L/min flow rate. Finally, CB was dispersed into practically all individual particles in the chamber.

In order to ensure that all subjects were at the same concentration, the mass concentration of the CB was analyzed by filtering the sample weight and adjusted to 30 mg/m^3. During exposure, the CB powder was sampled on the filter for 40 min under a flow of 1 L/min through a monitoring channel on the exposure tank, 6 samples per day. Rats were weighed twice a week, and daily recorded the basic values of temperature, humidity, carbon dioxide concentration and oxygen content in tanks. The size distribution of CB in the tanks was detected by Aerodynamic Particle Sizer (APS) Spectrometer 3321 (TSI, USA).

The prediction of the deposited alveolar fraction was generated using the Multiple Path Particle Dosimetry Model (MPPD) model version 3.04 (Anjilvel and Asgharian 1995). The deposition of CB in 30 mg/m^3 group was 2.5, 5 and 16 mg/lung by MPPD after CB exposure for 14, 28 and 90 days, respectively. In 5 mg/m^3 group, the deposition of CB was 0.83 mg/lung after exposure for 28 days.

Rats were respectively sacrificed at 14, 28, 90 days and recovery 14 days post-90d-exposure. Lung, kidney, spleen, and liver were weighted and calculated the organ coefficients according to the formula: Organ coefficients = organ weight/body weight.

Cell culture and treatment

The normal human bronchial epithelial cell lines (16HBE) were purchased from the China Center for Type Culture Collection (CCTCC). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% bovine growth serum (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), 100 U/ml penicillin and 100 μg/ml streptomycin (Solarbio, Beijing, China) in 5% CO_2 at 37 °C. CB was heated at 120 °C for 2 h to be sterilized, dissolved in deionized water to a stock solution of 10 mg/mL, and sealed at 4 °C after sterilized. CB was mixed with 100 Hz ultrasonic for 30 min before use. The CB stock solution was diluted to a final concentration by DMEM medium with 0.04% Tween 80 before use. 16HBE cells in logarithmic growth phase were seeded at a density of 5×10^5 cells/well in 6-well cell culture plates. The cells were treated with CB for 24 hours at doses of 50, 100, and 200 μg/mL, respectively. DMEM with 0.04% Tween 80 served as a negative control. Triplex wells were set for each concentration. And all of experiments repeated three times.

Cell transfection

For miR-126 overexpression, 16HBE cells were transfected with 50 nM of miR-126 mimic or miRNA negative control mimic (NC mimic) (Genepharma, Shanghai, China) for 48 h, respectively. For PIK3R2 knockdown, 16HBE cells were transfected with PIK3R2 siRNA (Genepharma, Shanghai, China) for