OXFORD

SOT | Society of Toxicology
academic.oup.com/toxsci

TOXICOLOGICAL SCIENCES, 2021, 1–15

https://doi.org/10.1093/toxsci/kfab133
Advance Access Publication Date: 4 November 2021
Research Article

Downloaded from https://academic.oup.com/toxsci/advance-article/doi/10.1093/toxsci/kfab133/6420722 by rongzhang@hebmu.edu.cn on 22 November 2021

N6-Methyladenosine Modification of CDH1 mRNA Promotes $PM_{2.5}$-Induced Pulmonary Fibrosis via Mediating Epithelial Mesenchymal Transition

Jie Ning,[*] Hairong Du,[†] Yaling Zhang,[*] Qingping Liu,[*] Tao Jiang,[*] Yaxian Pang,[*] Xiaochen Tian,[‡] Liqun Yan,[§] Yujie Niu,[¶,‖] and Rong Zhang[*,‖,1]

[*]Department of Toxicology, School of Public Health, Hebei Medical University, Shijiazhuang 050017, PR China; [†]Department of Communicable Disease Prevention and Control, Guangming District Center for Disease Control and Prevention, Shenzhen, Guangdong 518016, PR China; [‡]Department of Orthopaedic Surgery, Shijiazhuang People's Hospital, Shijiazhuang 050011, PR China; [§]Departments of Radiology, Second Hospital of Hebei Medical University, Shijiazhuang 050000, PR China; [¶]Department of Occupation Health and Environmental Health, School of Public Health, Hebei Medical University, Shijiazhuang 050017, PR China and [‖]Hebei Key Laboratory of Environment and Human Health, School of Public Health, Hebei Medical University, Shijiazhuang 050017, PR China

Jie Ning and Hairong Du contributed equally to this study.

[1]To whom correspondence should be addressed at Department of Toxicology, Hebei Medical University, 361 Zhongshan East Road, Shijiazhuang, Hebei 050017, PR China. Fax: +86-311-86265605. E-mail: rongzhang@hebmu.edu.cn.

ABSTRACT

The association between ambient airborne fine particulate matter ($PM_{2.5}$) exposure and respiratory diseases has been investigated in epidemiological studies. To explore the potential mechanism of $PM_{2.5}$-induced pulmonary fibrosis, 60 mice were divided into 3 groups to expose to different levels of $PM_{2.5}$ for 8 and 16 weeks: filtered air, unfiltered air, and concentrated $PM_{2.5}$ air, respectively. BEAS-2B cells were treated with 0, 25, 50, and 100 μg/ml $PM_{2.5}$ for 24 h. The biomarkers of pulmonary fibrosis, epithelial-mesenchymal transition, N6-methyladenosine (m6A) modification, and metabolism of mRNAs were detected to characterize the effect of $PM_{2.5}$ exposure. The results illustrated that $PM_{2.5}$ exposure induced pathological alteration and pulmonary fibrosis in mice. The expression of E-cadherin was decreased whereas vimentin and N-cadherin expression were increased in a dose- and time-dependent manner after $PM_{2.5}$ exposure. Mechanistically, $PM_{2.5}$ exposure increased the levels of METTL3-mediated m6A modification of CDH1 mRNA. As a target gene of miR-494-3p, YTHDF2 was upregulated by miR-494-3p down-regulation and then recognized m6A-modified CDH1 mRNA to inhibit the E-cad expression, consequently induced the EMT progression after $PM_{2.5}$ exposure. Our study indicated that $PM_{2.5}$ exposure triggered EMT progression to promote the pulmonary fibrosis via miR-494-3p/YTHDF2 recognized and METTL3 mediated m6A modification.

Key words: $PM_{2.5}$; pulmonary fibrosis; epithelial-mesenchymal transition; m6A RNA methylation; METTL3; YTHDF2.

© The Author(s) 2021. Published by Oxford University Press on behalf of the Society of Toxicology.
All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

According to the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2019 (Christopher *et al.*, 2020), air pollution is the 4th leading risk factor for attributable disability-adjusted life years. Global attributable deaths to ambient particulate matters were 2.92 million. Ambient airborne fine particulate matter ($PM_{2.5}$, aerodynamic diameter $\leq$2.5 μm), a major risk factor for poor health worldwide, has been associated with the development and exacerbation of respiratory diseases (Loxham *et al.*, 2019), including asthma, pulmonary fibrosis, chronic obstructive pulmonary diseases, and lung cancer (Burney and Amaral, 2019; Conti *et al.*, 2018; Hao *et al.*, 2015). Pulmonary fibrosis was a progressive fibrotic prototype of chronic pulmonary diseases (Richeldi *et al.*, 2017), which was the 3rd leading cause of death worldwide. Accumulating evidence indicated that $PM_{2.5}$ exposure could reduce respiratory capacity and induce pulmonary fibrosis (Gehring *et al.*, 2013; Sun *et al.*, 2020).

Pulmonary fibrosis was largely attributed to fibrogenic agent, including inhaled particulate matters, chemicals, and fibers (Cheng *et al.*, 2019; Dong *et al.*, 2016; Gao *et al.*, 2020). Previous literature reported that $PM_{2.5}$ exposure could reduce lung function and trigger fibrosis by epithelial-mesenchymal transition (EMT) (Tang *et al.*, 2017). EMT was considered as principal source of fibroblasts and contributed to fibroblasts population. EMT is the transformation from epithelial cells into the mesenchymal-like cells (Marmai *et al.*, 2011; Su *et al.*, 2020), which is characterized as the loss of E-cadherin (E-cad, encoded by CDH1 mRNA, epithelial marker), as well as the increase expression of vimentin (mesenchymal marker) and N-cadherin (N-cad, encoded by CDH2 mRNA) (Giannelli *et al.*, 2016; Kalluri and Neilson, 2003; Tang *et al.*, 2017). However, some studies had questioned that the epithelial cells hardly went into the interstitium and converted into interstitial fibroblasts (Li *et al.*, 2010; Nieto *et al.*, 2016). Recent studies have showed that the epithelial cells did not transformed into interstitial fibroblasts but displayed a EMT state in renal fibrosis and pulmonary fibrosis models (Lovisa *et al.*, 2015; Wang *et al.*, 2021). The special EMT state was named partial EMT which epithelial cells remained in their original anatomical position whereas lost its epithelial markers (Lovisa *et al.*, 2016). Although above studies suggested that the EMT progression was related to pulmonary fibrosis, the mechanisms whereby the EMT involved in pulmonary fibrosis after $PM_{2.5}$ exposure were unclear.

Epigenetic modification and metabolism of mRNAs performed biological functions during the development of pulmonary fibrosis and EMT progression (Han *et al.*, 2020; Lin *et al.*, 2019). N6-methyladenosine (m6A) is one of the most prevalent internal mRNAs modification (Dominissini *et al.*, 2012; Roundtree *et al.*, 2017). m6A modification is abundant in eukaryotic mRNAs, which plays a role in mediating RNA metabolism and regulatory functions (Gilbert *et al.*, 2016). Deregulation of m6A modification has been closely associated with pulmonary physiological and pathological processes (Choe *et al.*, 2018; Li *et al.*, 2021b; Ma *et al.*, 2021). YTHDF proteins are critical for regulating stress granules formation in response to diverse stimuli (Ries *et al.*, 2019). Especially, YTHDF2 protein plays a role in partition into stress granules. YTHDF1 could synergize with YTHDF3 protein to promote translation of m6A-modified mRNA, whereas YTHDF2 protein regulated degradation of m6A-modified mRNA (Shi *et al.*, 2017). In addition, it has been reported that YTHDF1 accelerated translation of Snail mRNA in m6A-regulated EMT in cancer cells (Lin *et al.*, 2019). However, it is still unclear the biological effect of YTHDF2 protein on m6A-mediated EMT progression in $PM_{2.5}$-induced pulmonary fibrosis.

As the first characterized m6A reader protein, YTHDF2 modulated the EMT progression through METTL3-mediated m6A mRNA modification in smoking-related lung cancer (Cheng *et al.*, 2021). Previous study found that miRNAs overexpression could increase the m6A abundance by targeting m6A regulatory enzymes, and then promoted the cell reprogramming of pluripotent mouse embryonic fibroblasts (Chen *et al.*, 2015). It was evidenced that miRNAs-targeted m6A modifications were involved in EMT progression at gene post-transcriptional levels (Jin *et al.*, 2020). Chronic $PM_{2.5}$ exposure stimulated the release of miRNAs derived from epithelial cells and drove the EMT phenotype with proliferation and migration of pulmonary epithelia (Xu *et al.*, 2019b). Therefore, it is hypothesized that $PM_{2.5}$ exposure might dysregulate miRNAs and subsequently contribute to EMT-related gene expression by targeting YTHDF2 mRNA.

Here, we hypothesize that the m6A level of m6A modification and expression of miRNA could dramatically regulate EMT progression and development of pulmonary fibrosis in mice induced by $PM_{2.5}$ exposure.

MATERIALS AND METHODS

Animal experiments

Six-week-old C57BL/6 male mice were purchased from Vital River Laboratory (Beijing Vital River Laboratory Animal Technology Co., Ltd, Beijing, China). After equilibrated for a week, 60 mice were randomly divided into 3 groups and exposed to filtered air (FA), unfiltered air (UA), and concentrated $PM_{2.5}$ air (CA) for 8 and 16 weeks. The FA mice were housed in cages with high-efficient particulate air filters (HEPA filters). The UA mice were performed with a pipeline which transported unfiltered ambient air and exposed to ambient air. The CA mice received the concentrated $PM_{2.5}$ air which was generated using a $PM_{2.5}$ concentrated enrichment system as we described previously (Chu *et al.*, 2019). The exposure time was 6 h per day, 7 days per week for 8 weeks or 16 weeks, respectively. The mice stayed in individual ventilated cages which were equipped with HEPA filters for the rest of the time except for the exposure period. The concentration of particulate matters in chambers and outdoors was monitored with an Aerosol Detector DUSTTRAKTM II (TSI instrument, Shoreview, Minnesota). The particle size spectrum in chambers was analyzed using an Aerodynamic Particle Sizer Spectrometer 3321 (TSI instrument). The gas levels of NO_2, SO_2, CO, and O_3 in cages were measured with monitors GasAlertMicro5 PID (BW Canada).

All mice were kept access to free feeding of commercial diet and distilled water. At the end of exposure, mice were anesthetized by pentobarbital sodium and the lung tissues were separated for further analysis. The animal experiments were approved by the Institution Animal Care and Use Committee (IACUC) of Hebei Medical University (IACUC-Hebmu-20170163) and carried out in accordance with the relevant guidelines.

Ambient $PM_{2.5}$ extraction and preparation

During the exposure period, the ambient $PM_{2.5}$ was collected on the Teflon filter by High-Volume Air samples (Thermo Scientific) at a pump flow rate of 1.05 l/min. The Teflon filters were cut into small pieces, then extracted by an ultrasonic processing in deionized water for 30 min and shook on a shaker for 20 min with 3 repetitions (Thomson *et al.*, 2015). The $PM_{2.5}$

Downloaded from https://academic.oup.com/toxsci/advance-article/doi/10.1093/toxsci/kfab133/6420722 by rongzhang@hebmu.edu.cn on 22 November 2021