



lncRNA Gm16410 Mediates PM_{2.5}-Induced Macrophage Activation *via* PI3K/AKT Pathway

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Frontiers in Cell and Developmental Biology

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Specialty section:

This article was submitted to Frontiers in Cell and Developmental Biology

Frontiers in Cell and Developmental Biology

Received: October 2020

Accepted: February 2021

Published: March 2021

Citation:

Xu JB, Xu HG, Ma K, Wang Y, Niu B, Zhang L and Li FS (2021) lncRNA Gm16410 Mediates PM_{2.5}-Induced Macrophage Activation *via* PI3K/AKT Pathway. *Front. Cell. Dev. Biol.* 9:618045. doi: 10.3389/fcell.2021.618045

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PM_{2.5} refers to atmospheric particulate matters with a diameter of less than 2.5 μm. The deposit of PM_{2.5} in lung cells can cause oxidative stress, leading to changes in macrophage polarity, which can subsequently cause pulmonary inflammation. Long-chain non-coding RNA (lncRNA) is a class of transcripts that regulate biological processes through multiple mechanisms. However, the role of lncRNA in PM_{2.5}-induced lung inflammation has not been established. In this study, the biological effects and associated mechanism of lncRNA in PM_{2.5}-induced change in macrophage polarity were investigated. The lncRNA-mediated PM_{2.5}-induced macrophage inflammation and lung inflammation-associated injury were also determined. Mice were exposed to chronic levels of PM_{2.5}, and changes in the expression of lncRNA in the lung were measured by lncRNA microarray. lncRNAs that showed significant changes in expression in response to PM_{2.5} were identified. lncRNA showing the biggest change was subjected to further analysis to determine its functional roles and mechanisms with respect to macrophage activation. The result showed that a significant reduction in expression of one lncRNA, identified as lncGm16410, was observed in the lung of mice and RAW264.7 cells following exposure to PM_{2.5}. lncGm16410 suppressed PM_{2.5}-induced macrophage activation *via* the SRC protein-mediated PI3K/AKT signaling pathway. PM_{2.5} promoted lung inflammation by downregulating the expression of lncGm16410, enhancing the activation of macrophages. Thus, lncGm16410 might provide new insight into the prevention of PM_{2.5} injury.

Keywords: lncGm16410, PM_{2.5}, macrophage activation, inflammation, PI3K/Akt

INTRODUCTION

Pollution of the environment by fine particulate matter (PM_{2.5}, aerodynamic diameter < 2.5 μm) has become one of the major issues to threaten public health (Gao et al., 2019). PM_{2.5} contains mainly polycyclic aromatic hydrocarbons, transition metals, and endotoxin, all of which are known toxic chemicals (Deng et al., 2013; Ming et al., 2017). These particles can easily translocate from the alveoli to the bloodstream to affect the cardiovascular system, so long-term exposure to high concentrations of PM_{2.5} increases the risk of cardiovascular and respiratory illnesses (Huang et al., 2017; Kirrane et al., 2019). In recent years, much progress has been made in the prevention and

control of PM_{2.5}, but PM_{2.5} remains a potential risk factor (Xie and Li, 2019). Despite the plethora of studies that have been conducted to investigate the toxicity of PM_{2.5}, the exact mechanism of PM_{2.5}-induced injury is still difficult to ascertain.

Long non-coding RNAs (lncRNAs) are a set of non-protein coding RNAs with a minimum length of 200 bases. lncRNAs modulate biological processes that are crucial for the growth and differentiation of cells as well as for the development of tumors (Ren et al., 2018). Recently, several studies have found that lncRNAs participate in the metastasis of tumor cells by competitively regulating miRNAs (Lin C. et al., 2018). For example, lncRNA AFAP1-AS1 was found to be the antisense DNA strand of the AFAP1 encoding gene locus, which acts as an adaptor protein that regulates actin integrity and links members of the SRC family proteins and other signaling proteins to actin filaments (Wu et al., 2013). These findings support the hypothesis that lncRNAs play an important role in various biological processes in cells injured by PM_{2.5}.

The main effects of PM_{2.5} exposure on cardiopulmonary diseases are inflammatory response and oxidative stress, probably due to the stimulation of macrophages by PM_{2.5} particles deposited in the alveoli and the release of some oxidants and cytokines. Several studies have demonstrated that the PI3K/AKT signaling pathway plays an important role in the activation of macrophage (Liu et al., 2017). Therefore, it has become important to investigate the role of lncRNAs in the process of cardiopulmonary disease caused by macrophage activation and the effect of lncRNAs on PM_{2.5}-regulated macrophage inflammatory response.

Most previous studies on PM_{2.5} used tracheal instillation, an approach that is different from and that does not simulate the real situation of human exposure. In this study, a dynamic poisoning cabinet was used to construct a mice model, to determine changes in the expression of lncRNAs in mice in response to chronic PM_{2.5} exposure and establish the role of lncRNA in PM_{2.5}-induced lung injury and inflammation. The findings suggested that lncRNA could reduce the extent of PM_{2.5}-induced lung inflammation and suppress PM_{2.5}-induced activation of macrophage.

MATERIALS AND METHODS

Reagents

PrimeScriptTM RT reagent kit with gDNA Eraser (Perfect Real Time) (Code No.: RR047A) and SYBR[®] Premix Ex TaqTM II (Tli RNaseH Plus) (Code No.: RR820A) were purchased from TaKaRa. BCA protein assay kit (KGP902) and ECL detection kit were purchased from KeyGENE (Nanjing, China). Cell culture dishes were purchased from Guangzhou Jet Bio-Filtration Co., Ltd. (Guangzhou, China). Antibodies against β -actin (60008-1-Ig, mouse), interleukin (IL)-6 (21865-1-AP, rabbit), tumor necrosis factor alpha (TNF- α ; 60291-1-Ig, mouse), protein kinase B (AKT; 10176-1-AP, rabbit), phosphoinositide 3-kinase (PI3K; 60225-1-Ig, mouse), inducible nitric oxide synthase (iNOS; 18985-1-AP, rabbit), arginase-1 (ARG1; 16001-1-AP, rabbit), SRC (11097-1-AP, rabbit), and IL-10 (20850-1-AP, rabbit) were obtained

from Proteintech (Wuhan, China). Antibodies against IL-1 β (bs-20448R, rabbit), IL-12 (bs-1789R, rabbit), and P-AKT (bs-0876R, rabbit) were purchased from Bioss (Beijing, China). Antibody against P-PI3K (4228, rabbit) was purchased from CST. Dasatinib inhibitor was obtained from MCE (New Jersey, United States). lipofectamineTM 3000 Reagent was purchased from Thermo ScientificTM (United States). Mouse IL-6 ELISA kit was purchased from Boster (Wuhan, China).

Extraction and Composition of PM_{2.5}

PM_{2.5} was collected from a large-capacity air sampler of Langfang (Hebei, China) from October 2015 to March 2016. The collection of PM_{2.5} was performed as described previously (Wang et al., 2017). Refer to **Supplementary Material 1** for the component analysis of PM_{2.5}.

Animal Experiments and Experiment Design

All animal experiments were approved by the Animal Experimental Committee of Dalian Medical University. Male Balb/c mice (6 weeks old) were obtained from Changsheng Biotechnology Co., Ltd. (Shenyang, China) (Approval No.: 2015-009). The mice were housed in a clean room (23 \pm 1 C and humidity of 45–55%) provided with conventional food and water and maintained under a diurnal cycle of 12-h light and 12-h darkness. To construct the PM_{2.5} exposure model, 16 mice were divided into two groups with eight animals per group. The first group was kept as healthy control; the environment of the control group and the PM_{2.5} content in the environment were provided in **Supplementary Material 2**. The second group was exposed to PM_{2.5} (PM_{2.5} group). The PM_{2.5} exposure was performed using a dynamic poisoning cabinet (HOPE-MED8050, Tianjin Hepu technology co. Ltd) that simulated the atmospheric environment. According to the standard concentration limit of environmental PM_{2.5} and the ventilation of mice (The body weight in mice is about 30 g, the tidal volume is about 0.1–0.23 ml/kg, and the respiratory frequency is 136–230 times/min, the volume of poisoning warehouse is about 0.3 m³). The exposure concentration of 300 mg/m³ used in this experiment is about 225 mg/m³ of human living ambient air environment PM_{2.5} concentration. The concentration of PM_{2.5} in the flowing air was 300 mg/m³ for 4 months exposure, and the mice were exposed for 5 h/day for 5 days/week. At the end of the exposure period, blood was taken from the eyeball, and the lung tissue was removed and stored directly in liquid nitrogen for further experiments.

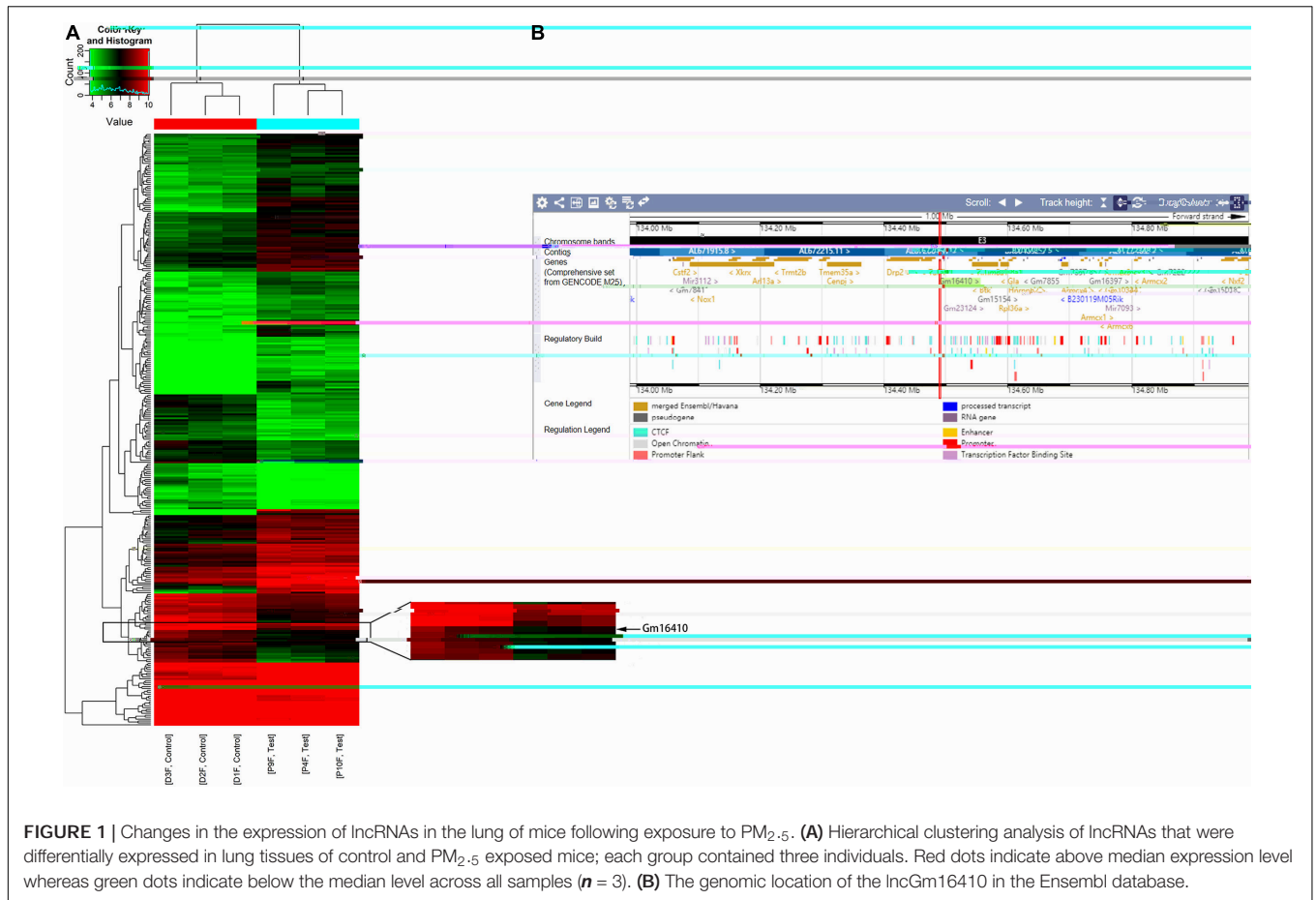
Cell Culture and CCK8 Assay

RAW264.7 and THP-1 cells were cultured in DMEM (Gibco, CA, United States) or 1640 (Gibco) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 mg/ml streptomycin in humidified air at 37 C with 5% CO₂. First, RAW264.7 cells were resuspended, then diluted to 5 \times 10⁴ cells/ml, and then 100 μ l of the cell suspension was added to each well of a 96-well plate. These cells were either control cells or those that overexpressed lncGm16410, and they were treated with

different concentrations of PM_{2.5} for a specific time, followed by the addition of 10 μl of Cell Counting Kit-8 (CCK8) reagent and incubation at 37 °C and in the dark for 2 h. After, the OD value of each well was measured by absorbance at 450 nm. Cell survival rates were expressed relative to that of the control.

lncRNA Microarray Analysis

Microarray profiling was carried out by Shanghai Kangcheng Biotechnology Company. Briefly, the lung sample was labeled



together, these results indicated that lncGm16410 was involved in the regulation of cell growth when the macrophages were exposed to PM_{2.5}.

lncGm16410 Regulates Macrophage Activation Under PM_{2.5} Exposure

Recent studies have found that macrophages can be polarized into M1 or M2 subgroups upon activation by environmental stimulators, and the subsequent change in macrophage activity plays a significant role in the pathogenesis of pulmonary inflammation (Becerra-Diaz and Strickland, 2018; Li et al., 2018). First, the CCK8 method was used to determine the effect of PM_{2.5} on the growth of RAW264.7 and THP-1 cells, and the median lethal dose was used to study the impact of PM_{2.5} on macrophage activity. The median lethal doses of PM_{2.5} for RAW264.7 and THP-1 cells were experimentally determined to be 400 and 80 mg/ml, respectively (Figure 3A). After 12 h of PM_{2.5} exposure, the macrophage M1 marker protein was slightly upregulated, peaking after 24 h, while the macrophage M2 marker protein was upregulated after 48 h of exposure (Figures 3B,C). However, after 24 h of exposure, the macrophage M1 and M2 marker proteins in THP-1 macrophages were significantly upregulated (Figures 3D,E). Unsurprisingly, the results of qRT-PCR also demonstrated a significant increase in M1- and M2-related

markers following PM_{2.5} exposure (Figure 3F). Whether lncGm16410 might affect the PM_{2.5}-induced upregulated levels of M1 and M2 was then investigated. To test this, control RAW264.7 and those that overexpressed lncGm16410 were exposed to PM_{2.5}, and the changes in macrophage M1 and M2 levels were measured. After 24 h of exposure to PM_{2.5}, the mRNA and protein levels of M1 and M2 in the cells that overexpressed lncGm16410 were reduced (Figures 3G–I). Compared with the control cells, the increased expression of M1 and M2 proteins in the PM_{2.5}-treated cells was also detected by an immunofluorescence assay. Immunofluorescence assay also demonstrated that overexpression of lncGm16410 reduced the levels of M1 and M2 proteins in the cytoplasm (Figure 3J). These results indicated that lncGm16410 affected the phenotypic transformation of macrophages and confirmed that lncGm16410 could participate in the process of PM_{2.5}-induced macrophage activation.

lncGm16410 Suppresses the Regulation of SRC Expression Under PM_{2.5} Exposure

According to the lncRNA microarray analysis, we found that, of the several genes adjacent to the lncGm16410, such as Taf7l, Timm8a1, and Btk, only Btk was slightly upregulated.

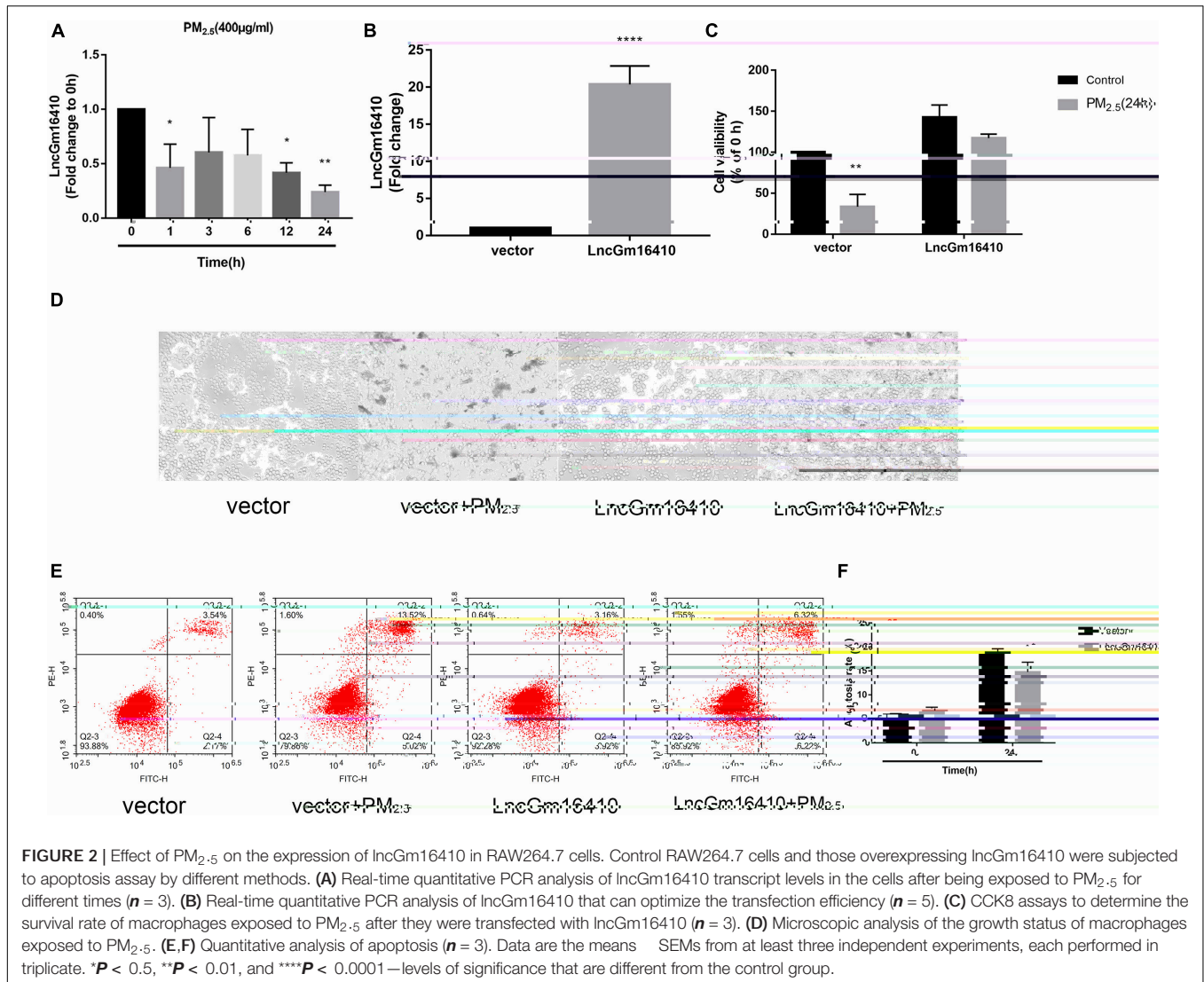


FIGURE 2 | Effect of PM_{2.5} on the expression of lncGm16410 in RAW264.7 cells. Control RAW264.7 cells and those overexpressing lncGm16410 were subjected to apoptosis assay by different methods. **(A)** Real-time quantitative PCR analysis of lncGm16410 transcript levels in the cells after being exposed to PM_{2.5} for different times (*n* = 3). **(B)** Real-time quantitative PCR analysis of lncGm16410 that can optimize the transfection efficiency (*n* = 5). **(C)** CCK8 assays to determine the survival rate of macrophages exposed to PM_{2.5} after they were transfected with lncGm16410 (*n* = 3). **(D)** Microscopic analysis of the growth status of macrophages exposed to PM_{2.5}. **(E,F)** Quantitative analysis of apoptosis (*n* = 3). Data are the means ± SEMs from at least three independent experiments, each performed in triplicate. **P* < 0.5, ***P* < 0.01, and *****P* < 0.0001—levels of significance that are different from the control group.

The result was not statistically significant, which indicates that lncGm16410 may not act as a cisacting gene. Recent studies have shown that exposure to particulate pollutants in the environment can lead to the activation of SRC protein-mediated signaling pathways, which in turn might trigger autophagy and inflammatory responses (Xu et al., 2016, Xu et al., 2019). To further investigate the changes in SRC protein expression in response to PM_{2.5}, the expression levels of SRC in RAW264.7 and THP-1 cells were measured following exposure to PM_{2.5}. Compared with the control cells, the levels of SRC protein in the PM_{2.5}-exposed cells displayed a rapid and sustained increased level of SRC after PM_{2.5} treatment (Figures 4A–D), suggesting that PM_{2.5} could indeed increase the expression of SRC. The effect of lncGm16410 on SRC expression in PM_{2.5}-exposed cells was then determined. The expression of SRC was attenuated in the cells that overexpressed lncGm16410 (Figures 4E,F). Changes in the expression of SRC were also confirmed by immunostaining in the cytoplasm of cells that overexpressed lncGm16410 (Figure 4G). lncGm16410

was involved in the regulation of PM_{2.5}-induced changes in SRC expression.

SRC-Mediated Macrophage Activation via Activating PI3K/AKT Signaling

Although lncGm16410 suppressed SRC expression under PM_{2.5} exposure, it is unclear whether SRC was involved in PM_{2.5}-mediated macrophage activation. Dasatinib is an inhibitor of SRC protein (Hermida-Prado et al., 2019). To determine whether SRC might be involved in PM_{2.5}-mediated macrophage activation, the cells were treated with dasatinib before they were exposed to PM_{2.5} (Figures 5A,B), and the changes in macrophage polarity markers were measured. Compared with control cells, cells that were treated with dasatinib exhibited reduced levels of M1 and M2 expression as revealed by western blot (Figures 5C–F). The inhibitory effect of dasatinib on the expression of NOS2 and ARG1 was also confirmed by immunostaining (Figure 5G). The result indicated that SRC was involved in PM_{2.5}-induced

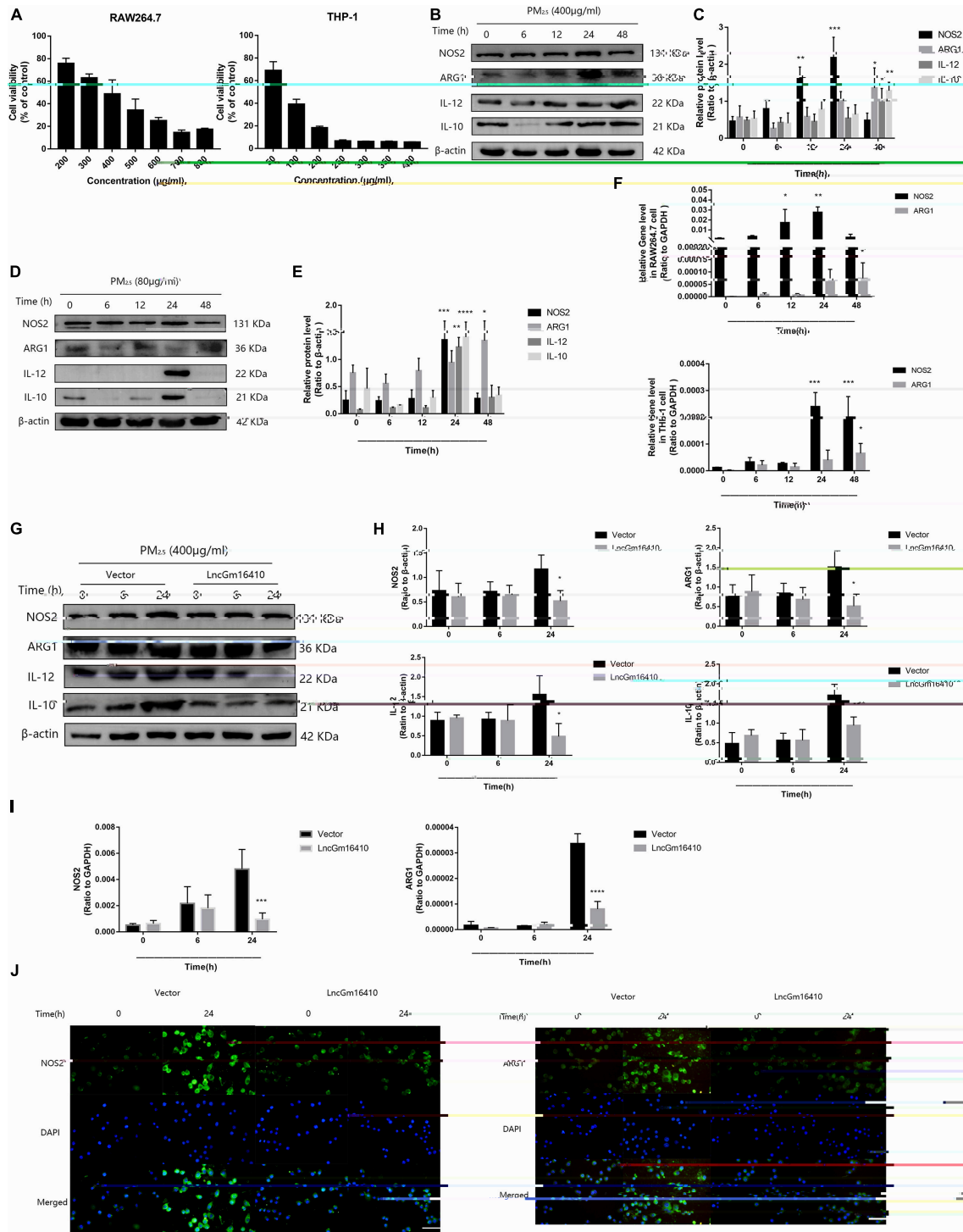
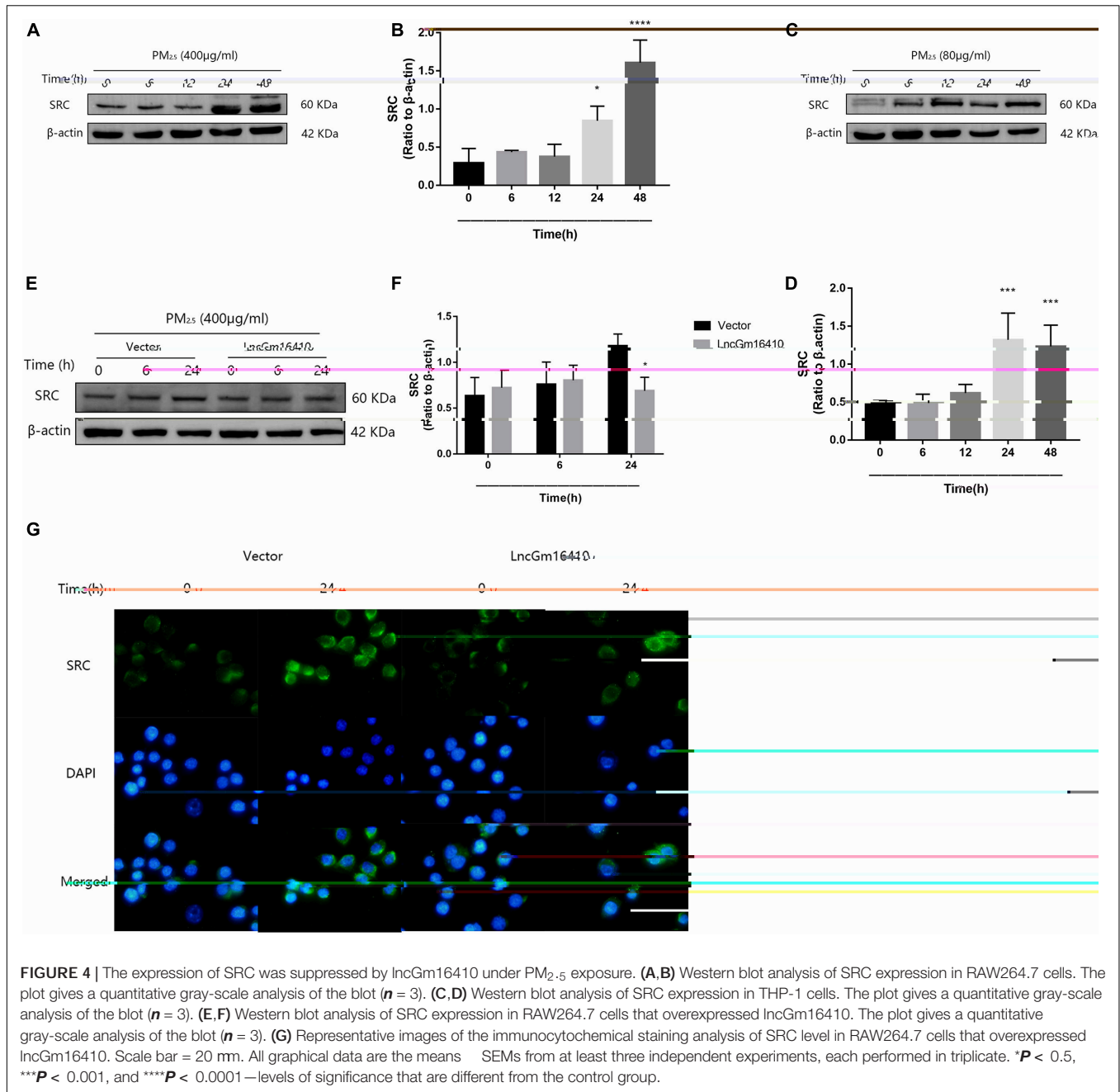


FIGURE 3 | Effect of lncGm16410 overexpression on the activation of RAW264.7 cells following exposure to PM_{2.5}. **(A)** Survival rates of RAW264.7 (*n* = 6) and THP-1 (*n* = 4) cells after exposure to different concentrations of PM_{2.5}. **(B,C)** Western blot analysis of the M1 marker NOS2 and IL-12 and the M2 marker ARG1 and IL-10 in RAW264.7 cells. The plot gives a quantitative gray-scale analysis of the blot (*n* = 3). **(D,E)** Western blot analysis of the M1 marker NOS2 and IL-12 and the M2 marker ARG1 and IL-10 in THP-1 cells. The plot gives a quantitative gray-scale analysis of the blot (*n* = 3). **(F)** Real-time quantitative PCR analysis of NOS2 and ARG1 in RAW264.7 and THP-1 cells (*n* = 4). **(G,H)** Western blot analysis of the M1 marker NOS2 and IL-12 and the M2 marker ARG1 and IL-10 in RAW264.7 cells that overexpressed lncGm16410. The plot on the right gives a quantitative gray-scale analysis of the blot (*n* = 3). **(I)** Real-time quantitative PCR analysis of NOS2 and ARG1 in RAW264.7 cells after overexpression of lncGm16410 (*n* = 3). **(J)** Representative images of immunocytochemical staining analysis of the expression levels of NOS2 and ARG1 in RAW264.7 cells that overexpressed lncGm16410. Scale bar = 20 µm. All graphical data are the means ± SEMs from at least three independent experiments, each performed in triplicate. **P* < 0.5, ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001—levels of significance that are different from the control group.



macrophage activation. As an important molecule in the signal transduction pathway, SRC can shuttle between different cellular compartments to affect cellular processes by mediating the PI3K/AKT signaling pathway (Cheng et al., 2014; Cho et al., 2016). Therefore, to further study the mechanism by which SRC regulates macrophage activity upon exposure to PM_{2.5}, the expression levels of P-PI3K, P-AKT, and nuclear factor kappa B (NF-κB), proteins that are involved in the PI3K/AKT signaling pathway, were measured by western blot. Macrophages that were exposed to PM_{2.5} displayed reduced expression of P-PI3K, P-AKT, and NF-κB when the cells were treated with dasatinib (Figures 5H–K), suggesting that SRC might be involved in the

activation of the PI3K/AKT signaling pathway through regulating the phosphorylation of PI3K and AKT.

lncGm16410 Alleviated Inflammation Caused by Macrophage Activation Under PM_{2.5} Exposure

According to accumulated evidence, the main consequence of macrophage activation is the secretion of related inflammatory factors (He et al., 2017; Zhang et al., 2018b). Therefore, changes in the levels of IL-6, TNF-α, and IL-1β of macrophages under PM_{2.5} exposure were measured. Compared with the control

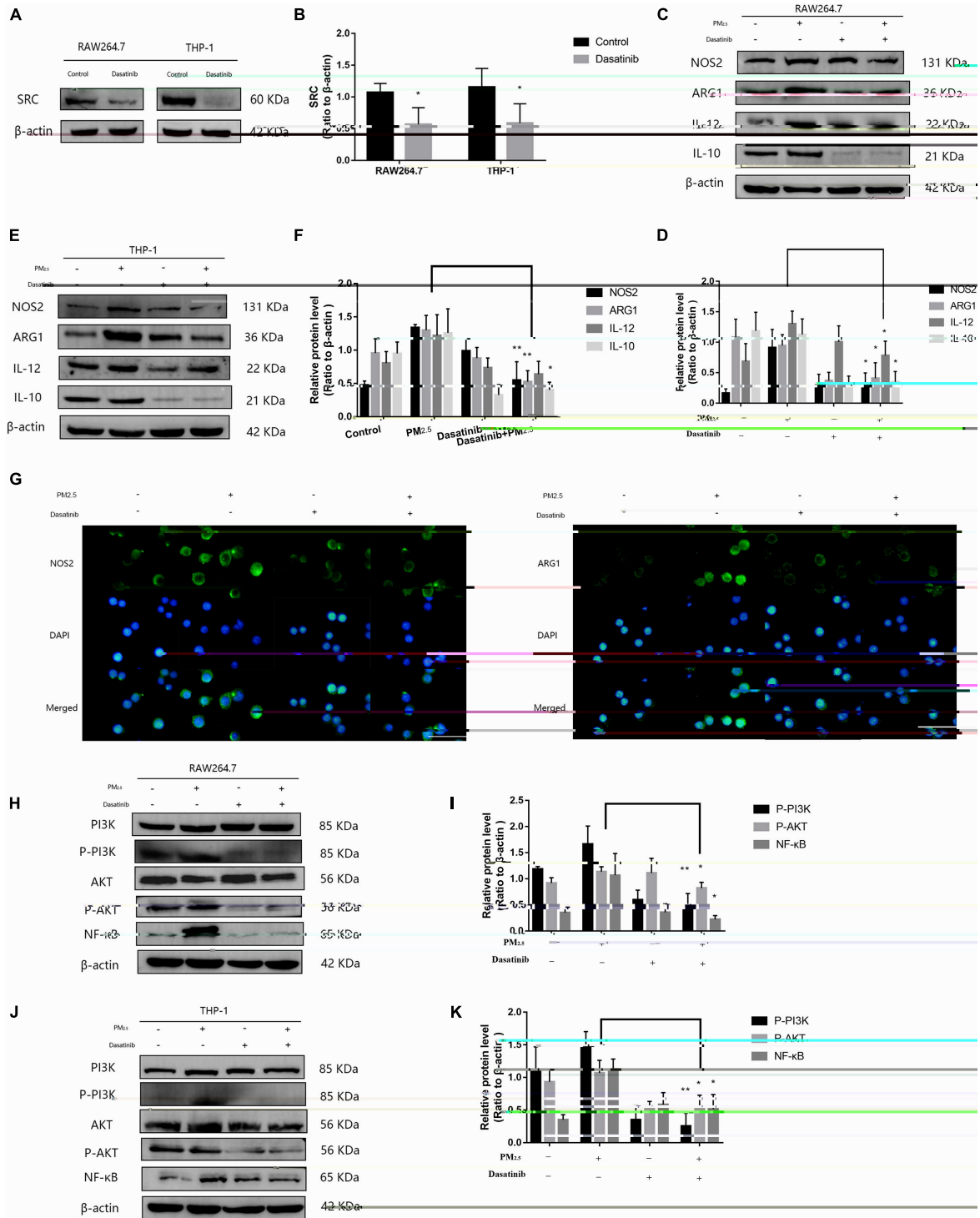
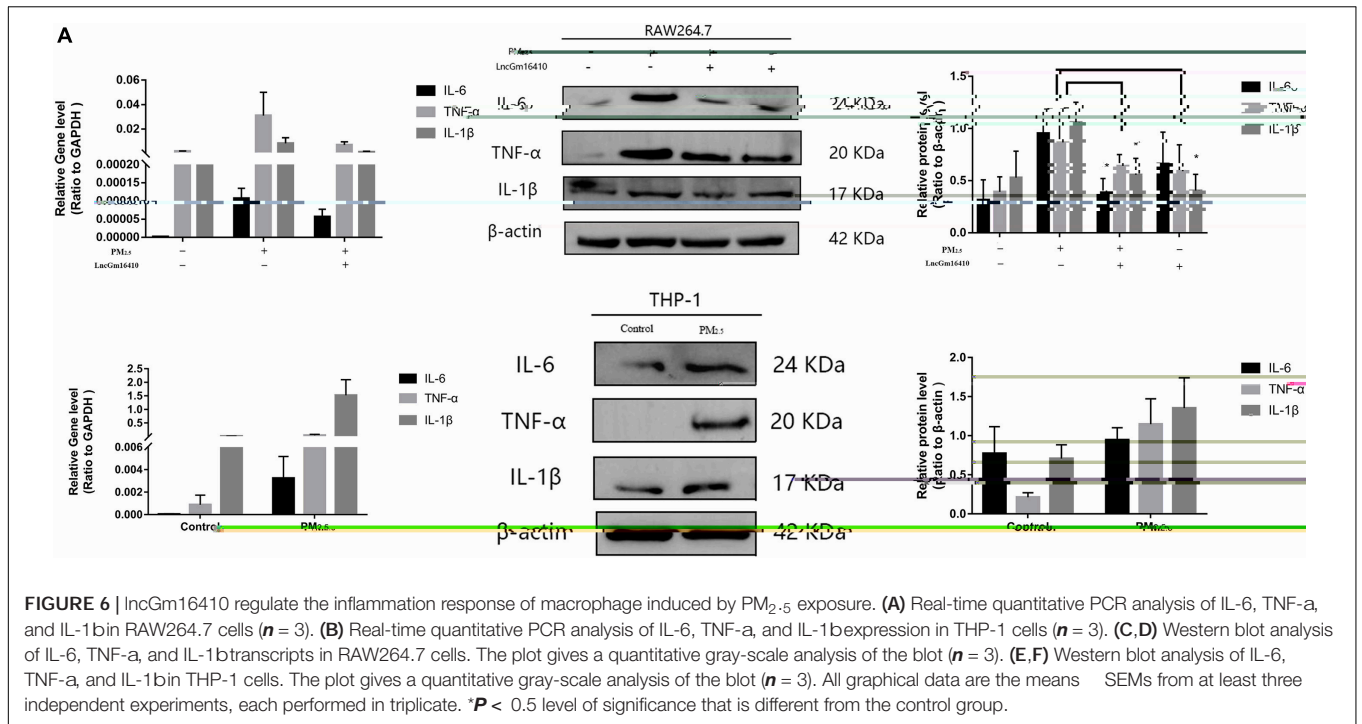
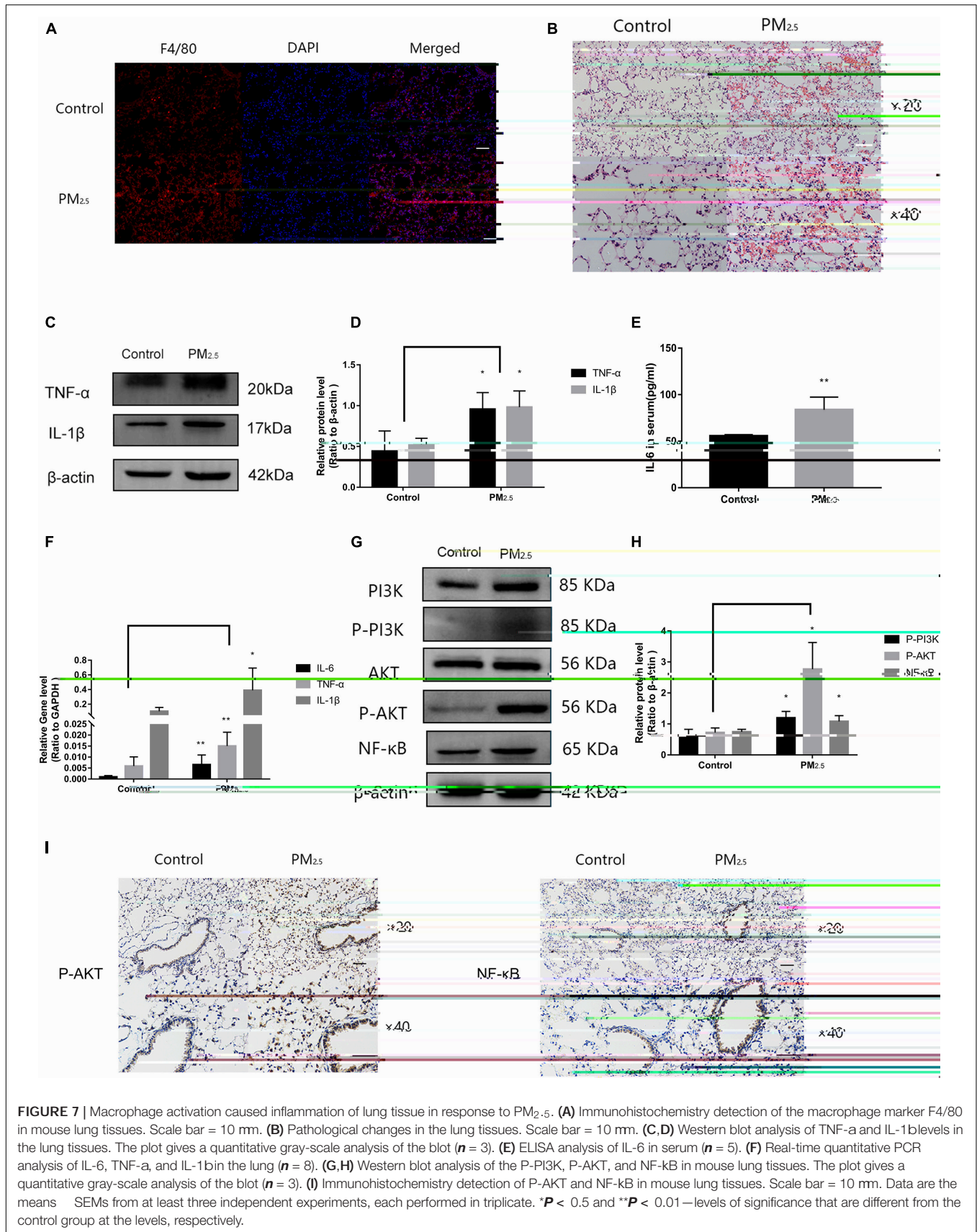


FIGURE 5 | SRC-mediated macrophage activation via activating PI3K/AKT signaling. (A,B) Western blot analysis of SRC expression in RAW264.7 and THP-1 cells. The plot gives a quantitative gray-scale analysis of the blot ($n = 4$). **(C,D)** Western blot analysis of the M1 marker NOS2 and IL-12 and the M2 marker ARG1 and IL-10 in RAW264.7 cells. The plot gives a quantitative gray-scale analysis of the blot ($n = 3$). **(E,F)** Western blot analysis of the M1 marker NOS2 and IL-12 and the M2 marker ARG1 and IL-10 in THP-1 cells. The plot gives a quantitative gray-scale analysis of the blot ($n = 3$). **(G)** Representative images of immunocytochemical staining analysis of NOS2 and ARG1 levels in RAW264.7 cells. Scale bar = 20 μm. **(H,I)** Western blot analysis of P-PI3K, P-AKT, and NF-κB in RAW264.7 cells. The plot gives a quantitative gray-scale analysis of the blot ($n = 3$). **(J,K)** Western blot analysis of P-PI3K, P-AKT, and NF-κB levels in THP-1 cells. The plot gives a quantitative gray-scale analysis of the blot ($n = 3$). All graphical data are the means ± SEMs from at least three independent experiments, each performed in triplicate. * $P < 0.05$ and ** $P < 0.01$ —levels of significance that are different from the control group.



cells, the expression of inflammatory factors in the PM_{2.5}-exposed cells was upregulated (Figures 6A–F). PM_{2.5} exposure led to a slight decrease in the levels of related inflammatory cytokines in RAW264.7 cells that overexpressed IncGm16410 (Figure 6A). In addition, RAW264.7 cells that overexpressed IncGm16410 treated with PM_{2.5} also displayed reduced levels of IL-6 and IL-1 β (Figures 6C,D). These results indicated that overexpression of IncGm16410 may reduce inflammation by regulating macrophage activity upon exposure to PM_{2.5}.



microvesicles (Soni et al., 2016a; Allard et al., 2018). When a PM_{2.5} suspension is instilled into the trachea of mice, the microvesicles released by macrophages can induce an increase in the number of BALF neutrophils as well as an increase in the expression of related inflammatory proteins (Soni et al., 2016b). Many studies have been carried out to find a way to prevent PM_{2.5}-induced injuries in macrophages. However, the mechanism and process of these injuries are still unclear.

lncRNAs are widely expressed as non-coding RNA, and increasing evidence suggests that lncRNAs play a major role in human physiological and pathophysiological processes (Chen et al., 2018). lncRNA microarray analysis of lung tissue samples taken from PM_{2.5}-treated Balb/c mice revealed 201 upregulated and 106 downregulated lncRNAs in response to PM_{2.5} exposure (Figure 1A). The same profile of lncRNA expression was observed in RAW264.7 macrophages following exposure to PM_{2.5} (Figure 2A). This indicated that lncGm16410 may be involved in PM_{2.5}-induced injuries, both in the lung and in the macrophage. Indeed, the data obtained from the macrophage CCK8 and apoptosis experiments *in vitro* (Figure 2C), as well as microscopic analysis of the lung tissue, suggested that lncGm16410 might be involved in the activation of macrophages in response to the PM_{2.5}-induced injuries sustained by the lung.

Notably, we observed that the downregulation of lncGm16410 expression in macrophages was consistent with lncGm16410

expression in the lung. Our data suggested that the initiation of inflammation induced by PM_{2.5} recruited monocytes to the lungs where they then differentiated into macrophages, ultimately initiating an inflammatory cascade. According to our data, the recruitment of a large number of macrophages to the lung not only increased the expression of lncGm16410 in the lung, but the PM_{2.5}-activated alveolar macrophages also caused rapid and sustained inflammation as well as the production of cytokines and chemokines. These cytokines and chemokines released by the macrophages then diffused into the lung, causing tissue damage.

Under the stimulation of environmental factors, macrophages will carry out two different forms of inflammatory and anti-inflammatory functions (Saradna et al., 2018). M1 macrophages participate in the classic activation pathway and mainly produce immune-stimulating cytokines and other inflammatory response effectors (Sica et al., 2015). M2 macrophages are mainly involved in the upregulation of ARG1 (Nandakumar et al., 2017; Tardito et al., 2019). They have the immunomodulatory capacity, and they also play a major role in tissue remodeling, tumor progression, and mediating the regression of inflammation (Sica and Mantovani, 2012). In the present study, macrophage M1 phenotype-associated proteins NOS2 and IL-12 were found to be abundantly expressed after 24 h of exposure to PM_{2.5}, suggesting that PM_{2.5} might stimulate the macrophage to produce an inflammatory response. PM_{2.5} also induced a slow but late increase in the levels of macrophage M2

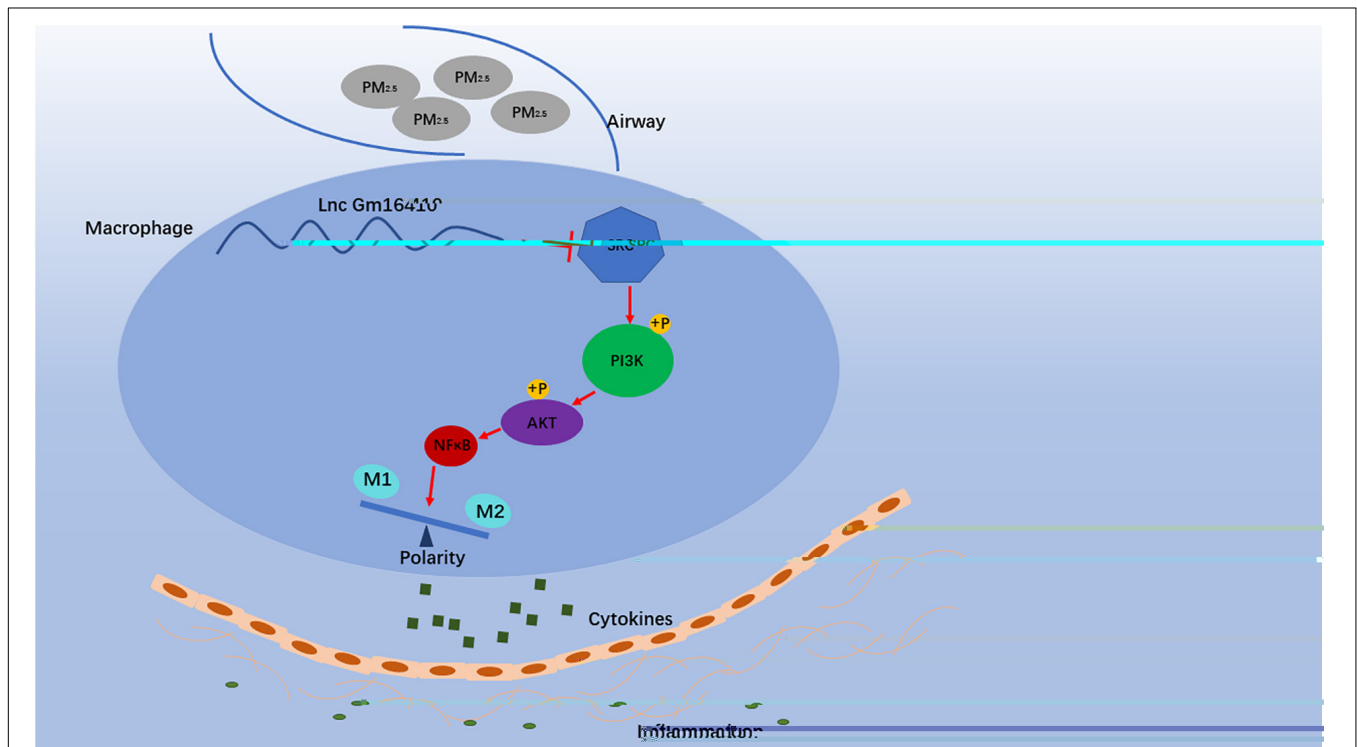


FIGURE 8 | Schematic diagram showing the mechanisms through which lncGm16410 mediates PM_{2.5}-induced macrophage activation. When macrophage is exposed to PM_{2.5}, lncGm16410 suppresses the regulation of SRC expression, leading to a subsequent increase in the latter. The interactions among lncGm16410, SRC, and PI3K/AKT will dictate the balance of M1/M2 macrophages. Macrophage apoptosis and activation result in the overproduction of inflammatory factors and their subsequent release into the lung. This process, in turn, causes inflammation in the lung.

phenotype-associated proteins ARG1 and IL-10, demonstrating that macrophages were fully activated upon PM_{2.5} exposure (Figure 3B) and that both M1 and M2 macrophages were involved in macrophage injury induced by PM_{2.5}. This study mainly focused on the role of lncGm16410 in macrophage activation and showed that lncGm16410 prevented the changes in M1/M2 phenotypes and a decrease in cell viability induced by PM_{2.5}, indicating a preventative effect of lncGm16410 on macrophage activation. Furthermore, lncGm16410 was found to be involved in macrophage-mediated inflammation responses, suggesting a key role for lncGm16410 in pneumonia (Figures 6A,Q).

A recent study on lncRNAs outlines the important roles of these RNAs in the regulation of SRC expression (Zhang S. et al., 2018). In the current study, the expression of SRC in macrophages was upregulated in response to PM_{2.5} exposure. However, overexpression of lncGm16410 counteracted this effect (Figure 4E). One possible explanation for this could be that lncGm16410 downregulated the expression of SRC in macrophages. After exposure to PM_{2.5}, inhibition of SRC in macrophages resulted in different degrees of reduction in the expression of macrophage phenotypic proteins (Figures 5C,F). This suggested that SRC could regulate PM_{2.5}-induced macrophage activity. Taken together, these results verified that lncGm16410 affects macrophage activation by downregulating the expression of SRC in response to PM_{2.5} exposure. SRC was recently found to be involved in PI3K/AKT signaling pathway (Lu et al., 2003; Riggins et al., 2003; Yang et al., 2017; Beadnell et al., 2018). Our data showed that activation of the PI3K/AKT signaling pathway was also inhibited when the activity of SRC was reduced by the inhibitor dasatinib (Figures 5H,J). Although further experiments are needed to clarify the detailed mechanism, the current result does suggest a connection between SRC and PI3K/AKT signaling pathway.

Based on a previous study from our laboratory, acute exposure to PM_{2.5} can cause lung tissue inflammation and oxidative stress (Wang et al., 2017). However, mice exposed to PM_{2.5} exhibited only a small amount of red blood cells and exudates in the alveoli in their lung tissue (Figure 7B). One possible explanation for this phenomenon could be that chronic exposure to PM_{2.5} simulated in this experiment was not a traditional tracheal instillation method but was carried out in the form of dynamic exposure. Although this exposure method did not cause any obvious pathological changes in the lung tissue exposed to PM_{2.5}, it still resulted in some changes to the levels of inflammation-associated proteins and genes in the lung. According to the results of this study, we found that PM_{2.5} exposure can induce the expression of inflammation-related factors in the murine- and human-derived macrophages (RAW264.7 cells and THP-1 cells) and mouse lung tissue, which may cause lung tissue injury and then lead to lung inflammation in mice. As this study adopted a dynamic PM_{2.5} exposure method, mice were exposed to PM_{2.5} through inhalation for 4 months, which can better simulate the long-term exposure of humans to PM_{2.5} in the environment. Simultaneously, by comparing relevant epidemiological studies

(Zhao et al., 2017; Lin H. et al., 2018; Qi et al., 2020) this study found that PM_{2.5} exposure in the environment increases the risk of lung disease and affects the quality of life and life expectancy.

In *in vitro* studies, the macrophages were transfected with a plasmid that overexpressed lncGm16410. The results were consistent with the decrease in inflammatory cytokines observed in the lncGm16410-overexpressing cells (Figures 6A,Q). The reduced mRNA and protein levels of inflammatory factors such as TNF- α shown by lncGm16410-overexpressing cells indicated that the lncGm16410 may suppress the level of inflammatory responses in the lung by reducing the expression of TNF- α in macrophages (Figure 7C). These results firmly demonstrated the involvement of lncGm16410 in macrophage activation and macrophage inflammation via PI3K/AKT.

Our analyses of PM_{2.5}-exposed mice suggested that a decrease in lncGm16410 expression coincides with macrophage apoptosis and activation (Figure 8). Thus, both *in vivo* and *in vitro* results supported the speculation that lncGm16410 may serve as a potential marker of pneumonia caused by PM_{2.5} and may be considered a new therapeutic strategy for lung inflammation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by all animal experiments were approved by the Animal Experimental Committee of Dalian Medical University.

AUTHOR CONTRIBUTIONS

JX and HX were responsible for the overall organizing of the experiments. JX, HX, KM, YW, and BN performed the experiments. JX wrote the manuscript. FL and LZ designed the experiments. All the authors read and approved the final manuscript.

FUNDING

Financial support was provided by the National Natural Science Foundation of China (Grant No. 51372029).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcell.2021.618045/full#supplementary-material>

REFERENCES

- Allard, B., Panariti, A., and Martin, J. G. (2018). Alveolar macrophages in the resolution of inflammation, tissue repair, and tolerance to infection. *Front Immunol.* 9:1777. doi: 10.3389/fimmu.2018.01777
- Beadnell, T. C., Nassar, K. W., Rose, M. M., Clark, E. G., Danysh, B. P., Hofmann, M. C., et al. (2018). Src-mediated regulation of the PI3K pathway in advanced papillary and anaplastic thyroid cancer. *Oncogenesis* 7:23. doi: 10.1038/s41389-017-0015-5
- Becerra-Diaz, M., and Strickland, A. B. (2018). Androgen and androgen receptor as enhancers of M2 macrophage polarization in allergic lung inflammation. *J. Immunol.* 201, 2923–2933. doi: 10.4049/jimmunol.1800352
- Chen, J., Huang, X., Wang, W., Xie, H., Li, J., Hu, Z., et al. (2018). lncRNA CDKN2BAS predicts poor prognosis in patients with hepatocellular carcinoma and promotes metastasis via the miR-153-5p/ARHGAP18 signaling axis. *Aging (Albany NY)* 10, 3371–3381. doi: 10.18632/aging.101645
- Cheng, S. E., Lee, I. T., Lin, C. C., Hsiao, L. D., and Yang, C. M. (2014). Thrombin induces ICAM-1 expression in human lung epithelial cells via c-Src/PDGFR/PI3K/Akt-dependent NF-kappaB/p300 activation. *Clin. Sci. (Lond.)* 127, 171–183. doi: 10.1042/cs20130676
- Cho, R. L., Yang, C. C., Lee, I. T., Lin, C. C., Chi, P. L., Hsiao, L. D., et al. (2016). Lipopolysaccharide induces ICAM-1 expression via a c-Src/NADPH oxidase/ROS-dependent NF-kappaB pathway in human pulmonary alveolar epithelial cells. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 310, L639–L657. doi: 10.1152/ajplung.00109.2014
- Deng, X., Zhang, F., Rui, W., Long, F., Wang, L., Feng, Z., et al. (2013). PM2.5-induced oxidative stress triggers autophagy in human lung epithelial A549 cells. *Toxicol. In Vitro* 27, 1762–1770. doi: 10.1016/j.tiv.2013.05.004
- Feng, S., Duan, E., Shi, X., Zhang, H., Li, H., Zhao, Y., et al. (2019). Hydrogen ameliorates lung injury in a rat model of subacute exposure to concentrated ambient PM2.5 via Aryl hydrocarbon receptor. *Int. Immunopharmacol.* 77:105939. doi: 10.1016/j.intimp.2019.105939
- Gao, J., Yuan, J., Wang, Q., Lei, T., Shen, X., Cui, B., et al. (2019). Metformin protects against PM2.5-induced lung injury and cardiac dysfunction independent of AMP-activated protein kinase alpha2. *Redox Biol* 28:101345. doi: 10.1016/j.redox.2019.101345
- He, M., Ichinose, T., Yoshida, S., Ito, T., He, C., Yoshida, Y., et al. (2017). PM2.5-induced lung inflammation in mice: differences of inflammatory response in macrophages and type II alveolar cells. *J. Appl. Toxicol* 37, 1203–1218. doi: 10.1002/jat.3482
- Hermida-Prado, F., Villaronga, M. A., Granda-Diaz, R., Del-Rio-Ibáñez, N., Santos, L., Hermosilla, M. A., et al. (2019). The SRC inhibitor dasatinib induces stem cell-like properties in head and neck cancer cells that are effectively counteracted by the mithralog EC-8042. *J. Clin. Med.* 8:1157. doi: 10.3390/jcm8081157
- Huang, K., Liang, F., Yang, X., Liu, F., Li, J., Xiao, Q., et al. (2019). Long term exposure to ambient fine particulate matter and incidence of stroke: prospective cohort study from the China-PAR project. *BMJ* 367:l6720. doi: 10.1136/bmj.l6720
- Huang, Q., Chi, Y., Deng, J., Liu, Y., Lu, Y., Chen, J., et al. (2017). Fine particulate matter 2.5 exerted its toxicological effect by regulating a new layer, long non-coding RNA. *Sci. Rep.* 7:9392. doi: 10.1038/s41598-017-09818-6
- Kirrane, E. F., Luben, T. J., Benson, A., Owens, E. O., Sacks, J. D., Dutton, S. J., et al. (2019). A systematic review of cardiovascular responses associated with ambient black carbon and fine particulate matter. *Environ. Int.* 127, 305–316. doi: 10.1016/j.envint.2019.02.027
- Li, X., Lei, Y., Wu, M., and Li, N. (2018). Regulation of macrophage activation and polarization by HCC-derived exosomal lncRNA TUC339. *Int. J. Mol. Sci.* 19:2958. doi: 10.3390/ijms19102958
- Lin, C., Zhang, S., Wang, Y., Wang, Y., Nice, E., Guo, C., et al. (2018). Functional role of a novel long noncoding RNA TTN-AS1 in esophageal squamous cell carcinoma progression and metastasis. *Clin. Cancer Res.* 24, 486–498. doi: 10.1158/1078-0432.ccr-17-1851
- Lin, H., Qian, Z., Guo, Y., Zheng, Y., Ai, S., Hang, J., et al. (2018). The attributable risk of chronic obstructive pulmonary disease due to ambient fine particulate pollution among older adults. *Environ. Int.* 113, 143–148. doi: 10.1016/j.envint.2018.01.029
- Liu, R., Fan, T., Geng, W., Chen, Y. H., and Ruan, Q. (2017). Negative immune regulator TIPE2 promotes M2 macrophage differentiation through the activation of PI3K-AKT signaling pathway. *PLoS One* 12:e0170666. doi: 10.1371/journal.pone.0170666
- Lu, Y., Yu, Q., Liu, J. H., Zhang, J., Wang, H., Koul, D., et al. (2003). Src family protein-tyrosine kinases alter the function of PTEN to regulate phosphatidylinositol 3-kinase/AKT cascades. *J. Biol. Chem.* 278, 40057–40066. doi: 10.1074/jbc.M303621200
- Ma, Q. Y., Huang, D. Y., Zhang, H. J., Wang, S., and Chen, X. F. (2017). Exposure to particulate matter 2.5 (PM2.5) induced macrophage-dependent inflammation, characterized by increased Th1/Th17 cytokine secretion and cytotoxicity. *Int. Immunopharmacol.* 50, 139–145. doi: 10.1016/j.intimp.2017.06.019
- Ming, L., Jin, L., Li, J., Fu, P., Yang, W., Liu, D., et al. (2017). PM2.5 in the Yangtze River Delta, China: chemical compositions, seasonal variations, and regional pollution events. *Environ. Pollut.* 223, 200–212. doi: 10.1016/j.envpol.2017.01.013
- Nandakumar, V., Hebrink, D., Jenson, P., Kottom, T., and Limper, A. H. (2017). Differential macrophage polarization from pneumocystis in immunocompetent and immunosuppressed hosts: potential adjunctive therapy during pneumonia. *Infect. Immun.* 85:e00939–16. doi: 10.1128/iai.00939-16
- Qi, J., Ruan, Z., Qian, Z. M., Yin, P., Yang, Y., Acharya, B. K., et al. (2020). Potential gains in life expectancy by attaining daily ambient fine particulate matter pollution standards in mainland China: a modeling study based on nationwide data. *PLoS Med* 17:e1003027. doi: 10.1371/journal.pmed.1003027
- Ren, J., Ding, L., Zhang, D., Shi, G., Xu, Q., Shen, S., et al. (2018). Carcinoma-associated fibroblasts promote the stemness and chemoresistance of colorectal cancer by transferring exosomal lncRNA H19. *Theranostics* 8, 3932–3948. doi: 10.7150/thno.25541
- Riggins, R. B., DeBerry, R. M., Toosarvandani, M. D., and Bouton, A. H. (2003). Src-dependent association of Cas and p85 phosphatidylinositol 3'-kinase in v-crk-transformed cells. *Mol. Cancer Res.* 4, 428–437.
- Saradna, A., Do, D. C., Kumar, S., Fu, Q. L., and Gao, P. (2018). Macrophage polarization and allergic asthma. *Transl. Res.* 191, 1–14. doi: 10.1016/j.trsl.2017.09.002
- Sica, A., Erreni, M., Allavena, P., and Porta, C. (2015). Macrophage polarization in pathology. *Cell. Mol. Life Sci* 2, 4111–4126. doi: 10.1007/s00018-015-1995-y
- Sica, A., and Mantovani, A. (2012). Macrophage plasticity and polarization: in vivo veritas. *J. Clin. Invest.* 122, 787–795. doi: 10.1172/jci59643
- Soni, S., Wilson, M. R., Dea, K. P., Yoshida, M., Katbeh, U., Woods, S. J., et al. (2016a). Alveolar macrophage-derived microvesicles mediate acute lung injury. *Thorax* 71:1020. doi: 10.1136/thoraxjnl-2015-208032
- Soni, S., Wilson, M. R., O'Dea, K. P., Yoshida, M., Katbeh, U., Woods, S. J., et al. (2016b). Alveolar macrophage-derived microvesicles mediate acute lung injury. *Thorax* 71, 1020–1029.
- Tardito, S., Martinelli, G., Soldano, S., Paolino, S., Pacini, G., Patane, M., et al. (2019). Macrophage M1/M2 polarization and rheumatoid arthritis: a systematic review. *Autoimmun. Rev.* 18:102397. doi: 10.1016/j.autrev.2019.102397
- Tian, Y., Liu, H., Wu, Y., Si, Y., Song, J., Cao, Y., et al. (2019). Association between ambient fine particulate pollution and hospital admissions for cause specific cardiovascular disease: time series study in 184 major Chinese cities. *BMJ* 367:l6572. doi: 10.1136/bmj.l6572
- Wang, H., Song, L., Ju, W., Wang, X., Dong, L., Zhang, Y., et al. (2017). The acute airway inflammation induced by PM2.5 exposure and the treatment of essential oils in Balb/c mice. *Sci. Rep.* 7:44256. doi: 10.1038/srep44256
- Wu, W., Bhagat, T. D., Yang, X., Song, J. H., Cheng, Y., Agarwal, R., et al. (2013). Hypomethylation of noncoding DNA regions and overexpression of the long noncoding RNA, AFAP1-AS1, in Barrett's esophagus and esophageal adenocarcinoma. *Gastroenterology* 144, 956–966.e4. doi: 10.1053/j.gastro.2013.01.019
- Xie, Z., and Li, Y. (2019). Value assessment of health losses caused by PM2.5 pollution in cities of atmospheric pollution transmission channel in the Beijing(-)Tianjin(-)Hebei region, China. *Int. J. Environ. Res. Public Health* 16:1012. doi: 10.3390/ijerph16061012
- Xu, H., Xu, X., Wang, H., Qimuge, A., Liu, S., Chen, Y., et al. (2019). LKB1/p53/TIGAR/autophagy-dependent VEGF expression contributes to PM2.5-induced pulmonary inflammatory responses. *Sci. Rep.* 9:16600. doi: 10.1038/s41598-019-53247-6

