Abstract. Fine particulate matter (PM2.5) is associated with an increased lung cancer risk. However, the effect of PM2.5 exposure on lung cancer cells is still largely unknown. The present study revealed that A549 lung cancer cells secreted exosomes containing high levels of Wnt3a after treatment with PM2.5. These exosomes activated β-catenin signalling in A549 cells. These exosomes exhibited no effects on migration and invasion, but promoted proliferation of A549 cells via the Wnt3a/β-catenin pathway in vitro. These exosomes promoted A549 tumour progression in a Wnt3a-dependent fashion in vivo. These results demonstrated that PM2.5 has a direct effect on promoting lung tumour development. Inhibition of exosome production by tumour cells or blockade of the Wnt3a/β-catenin pathway represents a promising strategy to impede PM2.5-mediated lung tumour progression.

Introduction

Fine particulate matter (PM2.5) indicates atmospheric aerodynamic equivalent diameter less than or equal to 2.5 micron particles (1,2). Long-term and short-term exposure to PM2.5 directly threatens public health (3,4). PM2.5 has been related to respiratory disease and cardiovascular disease (5-7). Exposure to PM2.5 increases the risk of asthma and exacerbates established asthma (5,6). PM2.5 induces inflammation and mucus hyperproduction in the airway epithelium (7). PM2.5 is necessary for the migration of human bronchial smooth muscle cells, defining a novel role for PM2.5 in airway remodelling in chronic obstructive pulmonary disease (8). Moreover, PM2.5 is associated with the development of atherosclerosis in ApoE-/- mice (9).

Many publications have demonstrated that PM2.5 is associated with cancer incidence. Suggestive evidence has shown an association between ambient air pollution and the incidence of postmenopausal breast cancer in European women (10). Ambient PM2.5 exposure may be a risk factor for hepatocellular carcinoma in the United States (11). PM2.5 was also involved in lung cancer burden (12). Enhanced ability of motility and proliferation were observed after PM2.5 exposure of non-small cell lung cancer cells (13). PM2.5 also induced epithelial-mesenchymal transition of human lung cancer cells (14). In addition, lung cancer stem cell properties were induced by PM2.5 (15). However, the definitive relationship between PM2.5 exposure and lung cancer has yet to be explored.

Exosomes are vesicles smaller than 150 nm in diameter that are enriched in endosome-derived components. Exosomes have a bilayer lipid structure containing transmembrane proteins, and they enclose soluble proteins, RNA and DNA (16,17). Accumulating evidence has well recognized the important role of exosomes as couriers to mediate communication between different cells (18). Tumour-derived exosomes (TEXs) are closely related to tumour development. TEXs educated dendritic cells to promote tumour metastasis via the HSP72/HSP105-TLR2/TLR4 pathway (19). Lnc-Sox2ot of TEXs promoted EMT and stemness by acting as a ceRNA in pancreatic ductal adenocarcinoma (20). Tumour exosomal RNAs promoted lung pre-metastatic niche formation by activating alveolar epithelial TLR3 to recruit neutrophils (21). Wnt10b in cancer-associated fibroblasts has been shown to promote breast cancer cell metastasis (22). In addition, activation of the Wnt signalling pathway was detected in PM2.5-induced pulmonary arterial hypertension of rats (23). Therefore, exosomes from PM2.5-treated lung cancer cells may affect tumour progression through activation of Wnt signalling.

The present study demonstrated that Wnt3a was enriched in exosomes from PM2.5-treated A549 (EXOpm2.5) cells.
(human epithelial cancer cells, which activated Wnt/β-catenin signalling in A549 cells). EXOPM2.5 significantly promoted A549 cell proliferation in a Wnt3a-dependent fashion in vitro. Furthermore, intratumoural injection of EXOPM2.5 accelerated tumour growth and decreased survival rate of mice via Wnt3a. Therefore, these results extend the knowledge of PM2.5 exposure and lung cancer progression.

Materials and methods

Reagents. PM2.5 was purchased from the National Institute of Standards and Technology (Gaithersburg, MD, USA). Human Wnt3a siRNA, negative control (NC) siRNA and antibodies against GRP94 (cat. no. sc-393402), CD63 (cat. no. sc-59284), Tsg101 (cat. no. sc-136111), Alix (cat. no. sc-53540), HSP70 (cat. no. sc-59570), Wnt3a (cat. no. sc-136163) and β-Actin (cat. no. sc-517582) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Human Wnt1, Wnt3a, Wnt4, Wnt7a, Wnt9a and Wnt10b primers were purchased from OriGene Technologies, Inc. (Rockville, MD, USA). An antibody against β-catenin (cat. no. ab6302) was purchased from Abcam (Cambridge, MA, USA). LF3, a specific inhibitor of Wnt/β-catenin signalling (24), was purchased from Selleckchem (Houston, TX, USA). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies, Inc. (Tokyo, Japan). Matrigel matrix basement membrane was purchased from BD Biosciences (San Diego, CA, USA).

Mice and cell line. Female athymic nude mice (aged 6-8 weeks) were purchased from Joint Ventures Sipper BK Experimental Animal Co., Ltd. (Shanghai, China). The mice were maintained in specific pathogen-free facilities with temperature ranging from 22 to 24˚C, humidity ranging from 50 to 60% and 12 h of light/dark cycle at Wenzhou Medical University (Wenzhou, China). Mice had free access to food and water, and all experiments using mice were approved by and performed according to the guidelines of the Animal Ethics Committee of Wenzhou Medical University.

The A549 cell line, a human lung adenocarcinoma cell line, was purchased from the American Type Culture Collection (ATTC; Manassas, VA, USA), and cultured in RPMI-1640 media supplemented with 10% (v/v) fetal calf serum (FBS; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37˚C in a 5% CO2 incubator.

Preparation of PM2.5. PM2.5 (10 mg) was suspended in 1 ml of normal saline, sonicated at 20% power for 3 pulses of 10 sec each (waiting 5 sec between pulses), and then 4 ml of normal saline was added to a final concentration of 2 mg/ml. The PM2.5 solution was aliquoted and stored at 4˚C. Before use, the PM2.5 solution was sonicated at 20% power for 3 pulses of 10 sec each (waiting 5 sec between pulses).

PM2.5 treatment and exosome isolation. A549 cells (2.5x105/ml) were seeded into 6-well plates in total volume of 2 ml/well. After 12 h, the supernatant was discarded, and 2 ml of fresh RPMI-1640 media was added with 100 µg/ml PM2.5. After 24 h, the supernatant collected from all the wells (240 ml in total) was differentially centrifuged at 300 x g for 10 min, 1,200 x g for 20 min, and 10,000 x g for 30 min at 4˚C. The supernatants from the final centrifugation were ultracentrifuged at 100,000 x g for 1 h at 4˚C. After removing the supernatants, the exosomal pellets were washed in a large volume of ice-cold phosphate-buffered saline (PBS) and centrifuged at 100,000 x g for an additional 1 h at 4˚C. The final pellets were resuspended in PBS. The amount of exosomal proteins was assessed by a BCA assay (Thermo Fisher Scientific, Inc.).

Nanoparticle tracking analysis and electronic microscopy. Nanoparticle tracking analysis of exosomes was assessed by NanoSight NS300 Particle Size Analyzer (Malvern Panalytical Ltd., Malvern, UK). To detect morphology of exosomes, exosomes were isolated and diluted in 100 µl of PBS, and 20 µl of the suspension was placed onto Formvar carbon-coated copper grids (Beijing XXBR Technology Co., Ltd., Beijing, China) at room temperature for 1 min. The excess suspension was removed using filter paper. Exosomes were stained with 2% phosphotungstic acid at room temperature for 5 min. The grids were then fixed with 2.5% glutaraldehyde at room temperature for 5 min followed by rinsing with PBS three times. Images were observed with a Philips Tecnai-10 transmission electron microscope operating at 80 kV (Phillips Electronic Instruments, Inc., Mahway, NJ, USA).

Real-time PCR. Total RNA was extracted with TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer’s instructions. The following PCR conditions were used: 1 cycle at 95˚C for 30 sec; and 40 cycles of 5 sec at 95 and 60˚C at 34 sec. Real-time PCR was performed using an Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The following primers were used: Wnt1 forward, 5'-ctctggtggagatctgctcaacc-3' and reverse, 5'-cataacggaacttgactgacagga-3'; Wnt3a forward, 5'-atgactgacttgacagga-3' and reverse, 5'-gtctgataagtcacctgctg-3'; Wnt4 forward, 5'-gtctggtgaagtctgactgctga-3' and reverse, 5'-ccacactagcactgacagga-3'; Wnt7a forward, 5'-aggagaggctcactaatggcgc-3' and reverse, 5'-ccggaatctgactggtgtaa-3'; Wnt9b forward, 5'-gccctgtgcacctgacatcag-3' and reverse, 5'-acaacgctggacaggtctgt-3'; Wnt10b forward, 5'-ctcggatgtctgtgatcag-3' and reverse, 5'-ggatctgacctggttcggc-3'.

Immunofluorescence staining. For detection of β-catenin nuclear translocation, A549 cells were treated with 10 µg/ml exosomes for 0, 30 and 60 min. The cells were then fixed, permeabilized and incubated with rabbit polyclonal antibodies against β-catenin (cat. no. ab6302; Abcam) using a dilution of 1:1,000 at 4˚C overnight. Subsequently, the cells were incubated with Alexa Fluor® 647 conjugated goat anti-rabbit antibodies (cat. no. ab150079; Abcam) using a dilution of 1:200 for 1 h. Cells were counterstained with DAPI to indicate DNA. Stained cells were viewed using a confocal microscope (SP2; Leica, Solms, Germany).

Migration assay. A549 cells (1x10⁴/ml and 2.5x10⁵/ml) were treated with 10 µg/ml exosomes from A549 with mock treatment (EXO_m) or EXOPM2.5 for 24 h at 37˚C. Then, 2x10⁴ cells in 100 µl of serum-free media were seeded into the top chamber. The bottom chamber was filled with 800 µl of medium containing 20% serum. After being cultured for...
18 h at 37˚C, the cells were fixed with methanol for 20 min and washed with PBS three times. The fixed cells were stained with 10 µg/ml DAPI for 30 min and washed with PBS. The stained cells were examined using a fluorescence microscope.

**Invasion assay.** After rehydration using a 6-fold volume of serum-free media, 50 µl of Matrigel was added on an 8-µm polycarbonate membrane in 24-well Transwell plates, and the Matrigel was solidified at 37˚C. Then, 1x10^6 A549 tumour cells were incubated with 10 µg/ml EXO_Ctrl or EXO_PM2.5 for 24 h at 37˚C. Subsequently, 5x10^4 A549 cells in 100 µl of serum-free media were seeded into the top chamber. The bottom chamber was filled with 800 µl of medium containing 20% FBS. After being cultured for 48 h at 37˚C, the cells were fixed with methanol for 20 min and washed with PBS three times. The fixed cells were stained with 10 µg/ml DAPI for 30 min and washed with PBS. The stained cells were examined using a fluorescence microscope.

**In vitro proliferation assay.** A549 cells (2x10^4) were seeded into 96-well plates at 200 µl/well, and 2 µg of exosomes was added. Four hours before the end of culture, 20 µl of CCK-8 was added. The optical density (OD) of each well was read at 450 nm using an automated microplate reader (Sunrise; Tecan Group, Ltd., Mannedorf, Switzerland).

**Western blot analysis.** Exosomes or crude proteins were extracted from cell lysates by RIPA lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China) and then qualified by BCA Protein Assay kit (Beyotime Institute of Biotechnology). A total of 20 µg of proteins was separated by 10% SDS-PAGE and transferred onto a polyvinylidene difluoride membrane (EMD Millipore, Billerica, MA, USA). The membrane was blocked with 5% BSA in TBST, and then incubated with corresponding primary antibodies overnight at 4˚C. After incubating with HRP-coupled secondary antibodies for 1 h at room temperature, the membranes were scanned using a Tanon 4500 Gel Imaging System (Tanon Science and Technology Co., Ltd., Shanghai, China). The following primary antibodies and secondary antibodies were used: Rabbit monoclonal antibodies against GRP94 (dilution 1:1,000; cat. no. ab1086606), rabbit monoclonal antibodies against Tsg101 (dilution 1:1,000; cat. no. ab125011), mouse monoclonal antibodies against Alix (dilution 1:500; cat. no. ab117600), mouse monoclonal antibodies against HSP70 (dilution 1:500; cat. no. ab474555), mouse monoclonal antibodies against Wnt3a (dilution 1:1,000; cat. no. ab81614), mouse monoclonal antibodies against CD63 (dilution 1:500; cat. no. ab193349), rabbit monoclonal antibodies against β-catenin (dilution 1:2,000; cat. no. ab32572), mouse monoclonal antibodies against β-actin (dilution 1:500; cat. no. ab8226), HRP-coupled rabbit polyclonal antibodies against mouse (dilution 1:2,000; cat. no. ab6728) and HRP-coupled goat antibodies against rabbit (dilution 1:2,000; cat. no. ab6721; all were from Abcam).

**RNA interference assay.** For transient silencing of the Wnt3a gene, 40 nM siRNA was transfected into cells (2x10^4/well) using 3 µl of INTERFERin siRNA transfection reagent (Polyplus-Transfection, New York, NY, USA) per well in a 24-well plate. The knockdown efficiency of Wnt3a was confirmed by western blotting.

**In vivo animal studies.** To establish the tumour model, A549 cells (5x10^6) were subcutaneously injected into nude mice. On day 10, the mice were randomly divided into three groups (each group with 5 mice/total 120 mice) and received intratumoural injections of 5 µg of exosomes every other day (total 14 injections). For the survival study, when the largest tumour volume reached 4,000 mm³ (60 mice), the mice were no longer free to move around. For humanitarian reasons, the mice were sacrificed by cervical dislocation after being intraperitoneally injected with 50 mg/kg pentobarbital sodium approved by Animal Ethics Committee of Wenzhou Medical University. The other 60 mice were also sacrificed by this way on day 36. The length and width of tumours were assessed every four days by vernier caliper and the tumour was calculated according to the following formula: Volume = (length x width^2)/2.

**Statistical analysis.** Results were expressed as the mean ± SEM. Statistics were analysed by one-way or two-way analysis of variance (ANOVA) with Newman-Keuls post hoc test. The survival curves were calculated using the Kaplan-Meier method and the log-rank test was used for survival analysis. All statistics were analysed by GraphPad Prism 5.0 software (Graphpad Software, Inc., La Jolla, CA, USA). A P-value of <0.05 was considered to indicate a statistically significant difference.

**Results**

**PM2.5 exposure increases Wnt3a protein level in EXO_PM2.5.** To assess if PM2.5 treatment upregulated expression of Wnt family members, the mRNA expression level of Wnt family members in PM2.5-treated A549 cells was assessed. The Wnt3a mRNA level was greatly increased compared to control cells (Fig. 1A). To investigate if PM2.5 exposure affected Wnt3a protein level in EXO_PM2.5, EXO_PM2.5 and exosomes from A549 cells with mock treatment (EXO_Ctrl) were isolated. Visualization using electron microscopy indicated that EXOCtrl and EXOPM2.5 had similar morphology, and both had diameters ranging from 50 to 150 nm (Fig. 1B), indicating that PM2.5 exposure did not affect exosomal morphology. Nanoparticle tracking analysis revealed that the size distribution of EXO_Ctrl and EXO_PM2.5 was 119±41.7 and 119±41.3 nm (mean ± SD), respectively (Fig. 1C). The protein components of EXO_Ctrl and EXO_PM2.5 were examined. Both exosomes were negative for the endoplasmic reticulum-residing protein, GRP94, and positive for HSP70 as well as the multivesicular body-related proteins, CD63, Tsg101 and Alix (Fig. 1D). According to a previous publication, CD63 was used as a loading control (25). Notably, Wnt3a was only detected in EXO_PM2.5 (Fig. 1D). These results indicated that EXO_PM2.5 has higher Wnt3a protein levels than EXO_Ctrl.

**EXO_PM2.5 induces activation of the Wnt/β-catenin pathway.** β-catenin is a key downstream effector in the Wnt signalling pathway (26). In the off-state, β-catenin is phosphorylated by CK1 and subsequently phosphorylated by GSK-3β (27,28), resulting in destabilization of β-catenin (29). In the on-state,
a Wnt ligand binds to a Frizzled receptor and then prevents β-catenin phosphorylation by GSK-3β, leading to accumulation and increased nuclear import of β-catenin (30). Since Wnt3a was enriched in EXOPM2.5, we investigated whether EXOPM2.5 activated Wnt/β-catenin signalling. Treatment with EXOPM2.5 markedly increased total β-catenin protein in A549 cells (Fig. 2A). In addition, enhanced nuclear translocation of β-catenin was observed in EXOPM2.5-treated A549 cells (Fig. 2B). These results indicated that EXOPM2.5 activated the Wnt/β-catenin pathway.

EXOPM2.5 does not affect A549 cell migration and invasion. Since the Wnt/β-catenin pathway has been implicated in tumour migration and invasion (31,32), we investigated whether EXOPM2.5 promoted A549 cell migration. As revealed in Fig. 3A, EXOPM2.5 treatment had no effect on A549 cell migration (Fig. 3A and B). The role of EXOPM2.5 on invasion of A549 cells was next examined. No difference of invasive ability was observed in A549 cells with or without EXOPM2.5 treatment (Fig. 3C and D). These results indicated that EXOPM2.5 does not alter the migration and invasion abilities of A549 cells.

EXOPM2.5 promotes A549 cell proliferation in a Wnt3a/β-catenin-dependent manner. β-catenin is implicated in tumourigenesis (33), and Wnt3a/β-catenin signalling has also been demonstrated to induce tumour cell proliferation (34). Thus, the effect of EXOPM2.5 on A549 cell proliferation was investigated. EXOPM2.5, but not EXOCtrl, significantly promoted A549 cell proliferation (Fig. 4A). To elucidate the role of Wnt3a in this effect, Wnt3a was knocked down in PM2.5-treated A549 cells by Wnt3a siRNA. Exosomes with low amounts of Wnt3a...
Figure 3. **EXO<sub>PM2.5</sub> does not affect A549 cell migration and invasion.** (A) A549 tumour cells were incubated with 10 µg/ml EXO<sub>Ctrl</sub> or EXO<sub>PM2.5</sub> for 24 h, and the cells were then plated in the top chamber of a Transwell plate. After 18 h, the cells on the bottom of the Transwell filter were imaged and quantified. Magnification, x100. (B) The results of A were statistically analysed (n=5). (C) A549 tumour cells were incubated with 10 µg/ml EXO<sub>Ctrl</sub> or EXO<sub>PM2.5</sub> for 24 h, and cells were then plated in the top chamber, which was precoated with 50 µl of Matrigel. After 48 h, the cells on the bottom of the Transwell filter were imaged and quantified. Magnification, x100. (D) The results of C were statistically analysed (n=5). Data are representative of three independent experiments. NS, not significant; EXO<sub>PM2.5</sub>, exosomes from PM<sub>2.5</sub>-treated A549 cells; EXO<sub>Ctrl</sub>, exosomes from A549 cells with mock treatment.

Exosomes from PM<sub>2.5</sub>-treated A549 cells were obtained from PM<sub>2.5</sub>-treated A549 cells (Fig. 4B). Exosomes from PM<sub>2.5</sub>-treated A549 cells transfected with NC siRNA (EXO<sub>PM2.5</sub>/NC siRNA) promoted A549 cell proliferation (Fig. 4C). However, exosomes from PM<sub>2.5</sub>-treated A549 cells were not able to promote A549 cell proliferation (Fig. 4D).
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EXOPM2.5 promotes A549 cell growth in vivo via Wnt3a. Finally, the effect of EXOPM2.5 on A549 cell progression in vivo was assessed. An A549 tumour model in nude mice was established, and intratumoural injection of EXOPM2.5 was performed every other day. EXOPM2.5 increased A549 cell growth (Fig. 5A) and reduced the survival rate of tumour-bearing mice (Fig. 5B). Immunohistochemical staining of Ki-67 revealed that EXOPM2.5 significantly promoted tumour cell proliferation and reduced the survival rate of tumour-bearing mice (Fig. 5C and D). To dissect the role of Wnt3a in EXOPM2.5 in this process, intratumoural injection of EXOPM2.5/NC siRNA or EXOPM2.5/Wnt3a siRNA was performed every other day. EXOPM2.5/NC siRNA, but not EXOPM2.5/Wnt3a siRNA, increased tumour growth and reduced the survival rate of tumour-bearing mice (Fig. 5E and F). Immunohistochemical results also revealed that EXOPM2.5/Wnt3a siRNA did not promote tumour cell proliferation (Fig. 5G and H). In the survival study, when the largest tumour volume reached 4,000 mm³, for humanitarian reasons, the mice were sacrificed. However, in Fig. 5B, compared with EXO Ctrl or PBS-treated mice, the tumour volume of EXOPM2.5-treated mice reached 4,000 mm³ much earlier. Similarly, in Fig. 5F, compared with EXOPM2.5/Wnt3a siRNA or PBS-treated mice, the tumour volume of EXOPM2.5/NC siRNA-treated mice reached 4,000 mm³ much earlier. Therefore, these results reflected the real tendency of survival time of tumour mice with different treatments. Altogether, these results indicated that EXOPM2.5-induced tumour growth in vivo was dependent on Wnt3a.

**Discussion**

Since humans are required to breathe in air, the PM2.5 pollution in atmosphere directly affects the physiological environment of the respiratory tract, especially the lungs. PM2.5 is implicated in increased risk of lung cancer (35). However, there is no direct evidence of the effect of PM2.5 exposure on lung cancer cells. The present study revealed that PM2.5-treated A549 lung cancer cells produced exosomes containing high levels of Wnt3a, which promoted A549 cell proliferation by activating Wnt/β-catenin signalling. We also detected Wnt3a by flow cytometry after adsorbing exosomes onto latex, but we did not detect Wnt3a this way (data not shown) which suggests that Wnt3a does not exist in exosomes. IL-10 and TGF-β1 are in exosomes, where they can exert immunosuppressive functions via the expression of their receptors on the cell membrane (36,37). Therefore, even if Wnt3a is in exosomes, it still can activate β-catenin signalling. In tumour patients who inhale PM2.5, PM2.5 stimulates lung epithelial cells. PM2.5...
may also induce lung epithelial cells to secrete exosomes containing Wnt3a, which activates Wnt/β-catenin signalling in tumour cells, leading to tumour progression.

In developed countries, the concentration of PM$_{2.5}$ in the atmosphere is generally less than 10 µg/m$^3$, and in developing countries it is generally above 35 µg/m$^3$, and the highest is likely to reach 200-300 µg/m$^3$. An adult breathes more than 20,000 times a day, inhaling ~20 m$^3$ of air. Therefore, an adult inhales at least 200 µg/day of PM$_{2.5}$. The concentration we used was 100 µg/ml in 2 ml and the quality of PM$_{2.5}$ was 200 µg. Thus, in humans, it is likely to be exposed to such a concentration. Notably, at a concentration of 100 µg/ml, we did not observe increased apoptosis of A549 cells (data not shown). On the contrary, PM$_{2.5}$ promoted lung tumor cell proliferation by inducing the cells to secrete exosomes with high levels of Wnt3a. With PM$_{2.5}$ treatment, A549 cells notably upregulated Wnt3a expression. The mechanism of the effect of PM$_{2.5}$ was not investigated in this study. Environmental ultrafine particulate matter has been reported to activate NF-κB and AP-1 (7). Wnt10a and Wnt10b are the target genes.
of NF-κB (38). Bioinformatics analysis predicted binding sites of NF-κB and AP-1 in the Wnt3a promoter, indicating that PM2.5 may promote Wnt3a transcription by activating NF-κB and AP-1. If the mechanism is unveiled in the future, it will be beneficial to identify the specific target to prevent lung cancer progression caused by PM2.5 exposure. Wnt signalling through its receptors (Frizzled) activate β-catenin signalling, which is often called the canonical pathway (39). As a ligand of the canonical pathway, the downstream effector of Wnt3a in EXOPM2.5 is β-catenin, which was supported by the increased protein level and nuclear translocation of β-catenin in EXOPM2.5-treated A549 cells. Use of the LF3 inhibitor confirmed that Wnt3a contained in EXOPM2.5 promoted A549 cell proliferation through activation of β-catenin signalling in vitro. However, the role of β-catenin in EXOPM2.5-mediated tumour inhibition was not ascertained in vivo, but the findings did indicate that all of the effects were Wnt3a-dependent.

The present study demonstrated that EXOPM2.5 significantly promoted A549 cell proliferation in vitro. Exosomes isolated from Wnt3a knockdown EXOPM2.5-treated A549 cells had extremely low levels of Wnt3a and did not induce A549 cell proliferation in vitro. These results indicated that Wnt3a was responsible for EXOPM2.5-mediated A549 cell proliferation in vitro. Inhibition of β-catenin signalling in A549 cells prevented EXOPM2.5-induced A549 cell proliferation in vitro, indicating that Wnt3a contained in EXOPM2.5 activated β-catenin signalling in A549 cells. The mouse tumour model revealed that EXOPM2.5 promoted A549 cell growth and decreased the survival rate of tumour-bearing mice. In the mouse tumour model, EXOPM2.5 did promote A549 cell proliferation. The Wnt/β-catenin pathway was also involved in tumour cell migration and invasion by mediating epithelial-mesenchymal transition of tumour cells (40). However, the migration and invasion promoting effect of EXOPM2.5 on A549 cells in vitro could not be observed.

In summary, PM2.5 exposure induced high expression of Wnt3a in A549 lung cancer cells. Isolated exosomes with a high level of Wnt3a activated β-catenin signalling in A549 cells and promoted their proliferation in vitro. Furthermore, these exosomes also promoted tumour progression in vivo. Therefore, these results indicated that inhibition of the Wnt/β-catenin pathway or exosome secretion may prevent PM2.5-mediated lung cancer progression.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors’ contributions

HX, XJ and YW performed the real-time PCR, the immunofluorescent staining, migration, invasion, cell proliferation and the animal experiments. SL and LC performed the cell culture, the exosome isolation and the western blotting experiments. LD conceived and designed the study. HX wrote the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

All experiments using mice were approved by and performed according to the guidelines of the Animal Ethics Committee of Wenzhou Medical University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References


