EXOSOMES MEDIATE MIR-21 TO PROMOTE CISPLATIN RESISTANCE IN LUNG CANCER BY REGULATING PTEN

Liqun Liang[1,#], Junmei Jia[1,#,*], Hongchun Kang[2], Bin Song[3]
[1]Oncology Department, The First Affillated Hospital of Shanxi Medical University, Shanxi Province, China - [2]Department of Pharmacy, Chongqing Three Gorges Central Hospital, Chongqing City, China.
[#]*Contribute equally to this research and should be considered as co-first author*

ABSTRACT

Objective: *This study was designed to investigate the potential molecular mechanisms by which exosomes mediate the transfer of drug resistance between lung cancer cells by microRNA (miRNA, miR-21).*

Methods: *Exosomes from cell supernatants were extracted by kit, their morphology and size were observed by electron microscopy, and exosome-specific proteins were detected by Western blot. The uptake of PKH26-labeled exosomes by cells transfected with green fluorescent plasmids was observed by laser confocal microscopy. MiR-21 mimic/inhibitor or PTEN overexpression plasmids were transfected into cells to regulate the expression levels of miR-21 and PTEN in cells. Expression levels of miR-21 and PTEN in lung cancer cells were determined by RT-qPCR, Northern blot and Western blot. The CCK-8 method was used to detect cell activity under cisplatin treatment and to calculate IC50. The clone formation experiment verified the sensitivity of different cells to cisplatin.*

Results: *Compared with the parent cells A549, the expression of miR-21 in A549/DDP was significantly increased, and the regulation of miR-21 level in lung cancer cells could affect the sensitivity of cells to cisplatin. MiR-21 levels in exosomes (exo/DDP) derived from A549/DDP were significantly higher than in those derived from A549 cells. The results showed that exo/DDP could be absorbed by A549 cells. CCK-8 and clone formation experiments showed that after incubating with exo/DDP, the sensitivity of A549 cells to cisplatin decreased significantly and IC50 increased significantly. RT-qPCR and Western blot results showed that after co-incubating exo/DDP in A549 cells, the expression of miR-21 was significantly increased, while that of PTEN was decreased. Meanwhile, by regulating the expression levels of miR-21 and PTEN, this experiment further confirmed that miR-21 could regulate the expression of PTEN, thus affecting the sensitivity of lung cancer cells to cisplatin.*

Conclusion: *Exosomes secreted by cisplatin-resistant lung cancer cells can transmit miR-21, thereby affecting the level of miR-21 in recipient cells. This, in turn, regulates the expression of cell PTEN and induces cisplatin resistance in lung cancer cells. This study provides a new approach and target to reverse drug resistance in lung cancer.*

Keywords: *Exosomes, miR-21, lung cancer, cisplatin-resistant cells.*

DOI: 10.19193/0393-6384_2021_2_148

Received *March 15, 2020;* ***Accepted*** *October 20, 2020*

Introduction

Lung cancer is one of the most common malignant tumours in the world, with obvious clinical symptoms[1, 2] and extremely high mortality. The average survival time of lung cancer patients is only a few months, and the one-year survival rate is less than 20%[3]. At present, drug resistance in the course of chemotherapy is an important reason for the poor prognosis of lung cancer[4]. Therefore, to improve the prognosis of lung cancer patients and explore the potential mechanism of chemotherapy resistance of lung cancer, it is crucial to find effective therapeutic targets. Cisplatin, a common and effective anticancer drug, is widely used to treat malignant tumours, including lung cancer, breast cancer, bladder cancer and head and neck squamous cell cancer[5, 6]. Cisplatin mainly inhibits DNA replication and transcription by cross-linking with DNA, thus inducing the death of cancer cells[7]. Recently, some researchers believe

that tumour microenvironment plays an important role in tumour therapy[8]. Moreover, exosome (exo) plays an important role in the tumour microenvironment. In different tumour types, exosomes released by drug-resistant cells can induce tumour cells to generate chemotherapy resistance[9-11].

Exosomes are microvesicles from polyvesicles, generally between 30 nm and 150 nm in diameter. They can participate in intercellular signal communication by transmitting intracellular active components, including proteins, messenger RNA (mRNA), microRNA (miRNA) and long non-coding RNA[12, 13]. Studies have shown that exosomes can mediate miRNA to promote tumour development, induce tumour invasion and metastasis, and generate chemoradiotherapy resistance[14]. In breast cancer, exosomes can promote breast cancer metastasis by activating the NF-κB signalling pathway through the mediation of miR-222[11]. In ovarian cancer, exosomes secreted by tumour-associated fibroblasts mediate miR-21 to target APAF1 in tumour cells, thereby inhibiting tumour cell apoptosis and inducing taxol resistance in tumour cells[15]. In gastric cancer, exosomes secreted by tumour-associated macrophages induce cisplatin resistance by mediating miR-21[16]. However, the mechanism of miR-21 in exosomes in the generation of cisplatin resistance in lung cancer cells has not been elucidated.

In this study, we investigated the role of miR-21 in drug resistance transmission by using a cisplatin-resistant cell line model of lung cancer combined with exosome function, providing a new potential therapeutic target for lung cancer patients.

Materials and methods

Materials

Human lung adenocarcinoma cell A549 was purchased from the Shanghai Institute of the Chinese Academy of Sciences. Lung adenocarcinoma cisplatin-resistant cell line A549/DDP was constructed by parental sensitive cell A549 gradually induced with low doses of cisplatin. PCR primers, miR-12 mimic, miR-21 inhibitor and PTEN overexpression plasmids were all synthesized by Shanghai Gene Pharma Co., Ltd.

Cell culture and transfection

Cells were cultured in DMEM (containing 10% foetal bovine serum) at 5% CO_2 and 37°C saturated humidity. According to the instructions of the transfection reagent Lipofectamine2000 (Invitrogen, USA), miR-21 mimic, miR-21 inhibitor and pcDNA3.1-PTEN plasmids were transfected into the corresponding cell lines. The transfection efficiency was determined by the cell fluorescence intensity 48 h after transfection, and subsequent experiments were conducted. These included:

- miR-21 mimic sequence: 5 '-UAGCUUAUCAGACUGAUGUUGA-3' and
- miR-21 inhibitor sequence: 5 '-UCAACAUCAGUCUGAUAAGCUA-3'.

Cell activity determination and clone formation experiment

Cell viability was measured using a CCK-8 kit (Beyotime, Shanghai). After digestion with trypsin, cells were inoculated into 96-well plates at a density of 5000 cells per well. The cells were adherent overnight and cultured for 48 h with different concentrations of cisplatin. Then, CCK-8 reagent was added and incubated at 37°C for 4 h. The absorbance value of each hole at 490 nm was detected by microplate, and the cell activity was recorded and calculated. The experiment was set up six double holes and repeated three times independently. Lung cancer cells were inoculated in a 6-well plate at a density of 200 cells per well. The cells were attached to the wall and cultured with different concentrations of cisplatin for 48 h. The normal medium was replaced for another two weeks. After the six-well plate was washed with PBS, 1% crystal violet dye (containing 30% formaldehyde) was added. After 20 min, [what] was rinsed with water and air-dried. Then photos were taken and [what?] were counted.

Exosome extraction and electron microscopy identification

A549 and A549/DDP cells were cultured in a 20 ml culture dish. After the cell density reached 50%, they were replaced with a complete medium containing 10% foetal bovine serum excluding exosomes. When the cell density reached 90%, the cell supernatant was collected. An exosome extraction kit (QIAGEN, Germany) was used to extract exosomes from cell supernatants according to the instructions.

The 20 ul exosome suspension was fixed with 4% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.4) for 2 h. The fixed exosomes were dropped onto a carbon-coated copper grid, allowed to sit for 5 min and then immersed in a 2% solution of phosphotungstic acid for 1 min. The images were then observed and photographed with an electron microscope at an acceleration voltage of 80 kV.

Exosome uptake experiment

Exo/DDP from A549/DDP was labelled with a PKH26 red fluorescent dye (Sigma, USA) as described in the instructions, and a plasmid vector with green fluorescence (GFP) was transfected into A549 cells. PKH26-labelled exosomes were added to the fluorescent A549 cells and co-incubated for 24 h. The exosomes were then observed and photographed under a laser confocal microscope.

Real-time quantitative reverse transcription PCR

Cell RNA was extracted with Trizol (Vazyme, Shanghai) according to instructions. Reverse transcription of mRNA and miRNA was performed using Takara's reverse transcription kit, followed by real-time quantitative PCR using Takara's SYBR Premix EX Taq kit. Primer sequences are shown in Table 1.

Primer	Sequence (5'-3')
miR-21 forward	AGAAATGCCTGGGTTTTTTTGGTT
miR-21 reverse	TTGGGAATGCTTTTCAAAGAAGGT
U6 forward	CTCGCTTCGGCAGCACACA
U6 reverse	AACGCTTCACGAATTTGCGT
PTEN forward	ACCCCTTCATTGACCTCAACTA
PTEN reverse	TCTCGCTCCTGGAAGATGGTGA
β-actin forward	ATGTGCAAGGCCCGCTTCG
β-actin reverse	TTAATGTCACGCACGATTTCC

Table 1: Primer Sequences.

Western blot

The total cell protein was extracted with RIPA containing protease inhibitors, and the protein concentration was determined with the BCA kit (KeyGEN). The 30 μg protein samples were separated by electrophoresis in 10% SDS polyacrylamide gel and transferred to PVDF membrane. The strips were sealed with 5% skim milk for 1 hour and incubated overnight at 4oC with the primary antibody. The next day, the secondary antibody was incubated at room temperature for 2 h and exposed to colour on the BioRAD gel imager using ECL kit. PTEN, antibodies -actin, and secondary antibodies were all purchased from CST (USA).

Northern blot

The total RNA extracted from cells was quantitatively denatured by Nanodrop, and 20-μg samples were added to each well. RNA was separated in 1% denatured agarose formaldehyde gel, transferred to a nylon membrane, fixed by UV lamp and observed RNA integrity. Then it was hybridized with Digoxin-labelled cDNA probe (PCR DIG probe synthesis kit, Roche, Germany). After the hybridization, the membrane was washed and tablet was developed.

Statistical analysis

In this study, GraphPad Prism7 software was used for statistical analysis. The measurement data are expressed as mean ± standard deviation ($\bar{x}\pm s$). Mean differences between groups were compared by paired t-test or independent sample t-test.

Results

miR-21 level in lung cancer cells affects cell sensitivity to cisplatin

To explore the function of miR-21 in lung cancer cisplatin-resistant cells A549/DDP, we first identified cisplatin resistance in two cell lines. CCK-8 results showed that there was a significant difference in cisplatin sensitivity between the two cells (IC50: A549 vs A549/DDP = 5.178 μM vs 23.72 μM; $p<.05$) (Figure 1A).

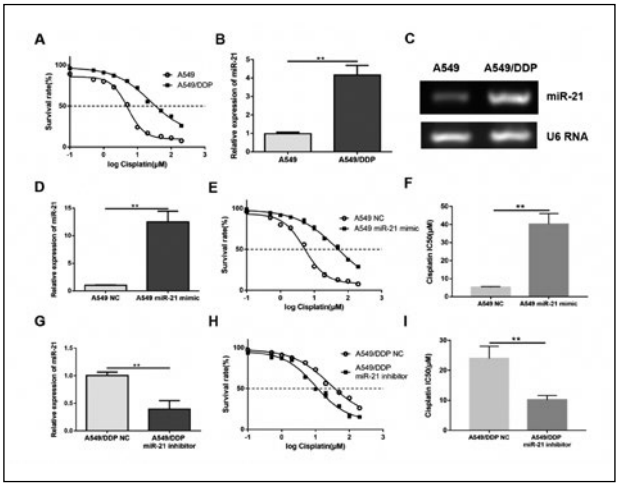

Figure 1: miR-21 level in lung cancer cells affects cell sensitivity to cisplatin.

(A) the sensitivity of cells to cisplatin was detected by the CCK-8 method and IC50 was calculated (A549 vs A549/DDP = 5.178μM vs 23.72 μM). (B) RT-qPCR was used to detect the expression of miR-21 in the cells. (C) Northern blot analysis showed that miR-21 expression in drug-resistant cells A549/DDP was significantly higher than that in parent cells A549. (D) RT-qPCR was used to verify the expression of miR-21 after transfection. (E-F) IC50 of cisplatin cells was determined by the CCK-8 method (A549 NC vs A549 miR-21 mimic = 6.172 μM vs 40.22 μM). (G) RT-qPCR was used to verify the expression of miR-21 in cells. (H–I) cisplatin IC50 of A549/DDP cells (NC vs miR-21 inhibitor = 21.94 μM vs 10.09 μM). $^{**}p<.01$.

Subsequently, the expression of miR-21 in the two cell lines was detected by RT-qPCR and Northern blot. The results showed that miR-21 expression

in cisplatin-resistant cells A549/DDP was significantly up-regulated compared with that in parent cells A549 (Figure 1B-C). To evaluate whether miR-21 can affect the sensitivity of lung cancer cells to cisplatin, we transfected miR-21 mimics into A549 cells to increase miR-21 expression (Figure 1D). The results of cell viability experiments showed that after the expression of miR-21 was up-regulated, cisplatin inhibited cells significantly, and IC50 of A549 miR-21 mimic cells to cisplatin was significantly higher than that of parental cells (Figure 1E-F).

At the same time, we transfected miR-21 inhibitor into A549/DDP cells to inhibit the expression of miR-21 (Figure 1G). These results were consistent with expectations. Cisplatin resistant cells A549/DDP recovered their cisplatin sensitivity after miR-21 expression was inhibited (Figure 1H-I). These results indicated that the level of miR-21 in lung cancer cells affects the sensitivity of cells to cisplatin.

MiR-21 is highly expressed in exosomes secreted by cisplatin-resistant cell lines of lung cancer

To investigate whether exosome-derived miR-21 is associated with cisplatin resistance in lung cancer cells, we collected cell supernatants of A549 and A549/DDP and extracted exosomes from them. In this study, exosome morphology and size were observed by electron microscopy (Figure 2A), and exosome-specific proteins were detected by Western blot (Figure 2B). The exosomes obtained in this study were bilayer structures with sizes between 30 nm and 150 nm.

Western blot showed a high expression of specific protein CD63 on exosome membrane and low expression of endoplasmic reticulum-specific protein Calnexin. MiRNAs were extracted from exosomes and the contents of miR-21 exosomes were determined by RT-qPCR and Northern blot. As shown in Figure 2C-D, miR-21 levels in exosomes from drug-resistant strains were higher than those from A549 cells.

Exosomes secreted by cisplatin-resistant cell lines can transmit drug resistance

To further explore whether exosomes can transmit cellular drug resistance, we incubated the exosomes (exo/DDP) from drug-resistant cells A549/DDP with the parent cells A549, with the exosomes secreted by A549 itself (exo/A549) with A549 as a negative control.

We observed the changes in the sensitivity of cells to cisplatin after co-incubation. Laser confocal scanning microscope images showed exo/DDP uptake by A549 cells (Figure 3A). After co-hatching, a cell viability experiment and a clone formation experiment were conducted. The results showed that after hatching with exo/DDP, the sensitivity of A549 cells to cisplatin decreased significantly and IC50 increased significantly (Figure 3B-E). All the above experiments confirmed that exosomes secreted by cisplatin-resistant cell line A549/DDP could transmit drug resistance.

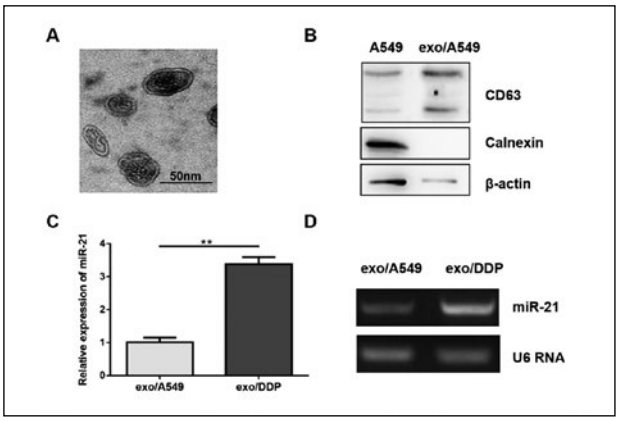

Figure 2: miR-21 was highly expressed in exosomes secreted by cisplatin-resistant cell lines of lung cancer.
(A) exosomes were extracted from the cell supernatant and observed under an electron microscope with a scale of 50 nm. (B) exosomes were identified by Western blot using exosome-specific protein CD63 and endoplasmic reticulum-specific protein Calnexin, with β-actin as an internal reference. (C) RT-qPCR was used to detect miR-21 levels in cell-derived exosomes. (D) the level of miR-21 in exosomes was detected by Northern blot. $^{**}p<.01$.

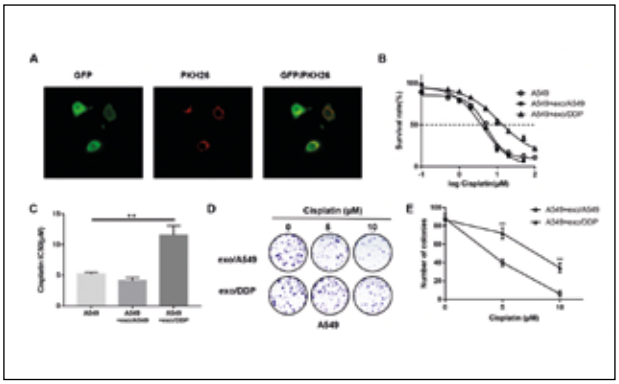

Figure 3: Exosomes secreted by cisplatin-resistant cell lines can transmit drug resistance.
(A) co-incubation experiment of exosomes and cells. Images of exosomes uptake were taken by laser confocal microscopy. The stable expression of green fluorescent protein (GFP) in A549 cells, and exosomes derived from A549/DDP were labelled with red fluorescent dye (PKH26). (B–C) the CCK-8 method was used to detect and calculate the changes of cisplatin IC50 in A549 cells after co-incubation with exosomes from different sources (exo/A549 vs exo/DDP = 4.108 μM vs 11.48 μM). (D–E) clone formation experiment to observe the changes of cisplatin sensitivity of A549 cells after co-incubation with exosomes from different sources. $^{**}p<.01$

Exosomes secreted by drug-resistant cells can transmit miR-21 and affect the expression of PTEN in cells

To reveal the mechanism of exosomes transmitting cell drug resistance, RT-qPCR analysis and detection found that after incubating with drug-resistant cell-derived exosomes exo/DDP, miR-21 expression in recipient cells A549 was significantly increased (Figure 4A). Bioinformatics search results showed that miR-21 may bind to the 3' non-coding region (3' UTR) of tumour suppressor gene PTEN mRNA (Figure 4B) to inhibit PTEN expression. RT-qPCR and Western blots analysis showed that the mRNA and protein levels of PTEN in A549 cells were significantly reduced after incubation with exo/DDP (Figure 4C-D). These data indicate that exosomes can transmit miR-21 and affect the expression level of PTEN in recipient cells.

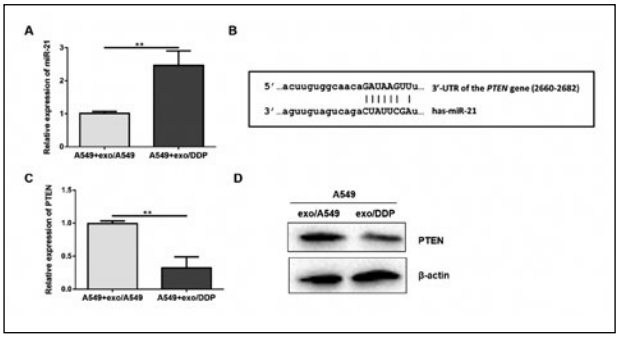

Figure 4: Exosomes secreted by drug-resistant cells can transmit miR-21 and affect the expression of PTEN in cells.

(A) miR-21 expression level in recipient cells A549 after incubation with exosomes (exo/DDP) from A549/DDP by RT-qPCR. (B) The predicted binding site of miR-21 to the 3'UTR region of human PTEN gene. (C) RT-qPCR was used to detect the mRNA expression level of PTEN in cells. (D) The level of PTEN protein in cells was detected by Western blot. $^{**}p<.01$.

miR-21 induces cisplatin resistance in lung cancer cells by inhibiting PTEN expression

To confirm that miR-21 in lung cancer cells can induce cisplatin resistance by inhibiting PTEN, we up-regulated the expression level of miR-21 in A549 cells and down-regulated miR-21 in A549/DDP cells. RT-qPCR and Western blot results showed that both mRNA and protein levels of PTEN were inhibited after miR-21 was up-regulated, while PTEN levels were significantly increased after miR-21 was down-regulated (Figure 5A-C). These results proved that miR-21 could inhibit the expression of PTEN in lung cancer cells.

Subsequently, we transfected pcDNA3.1-PTEN overexpressed PTEN in drug-resistant cell line A549/DDP cells, and we verified the mRNA and protein levels of PTEN in transfected plasmid A549/DDP cells (Figure 5D) by RT-qPCR and Western blot. CCK-8 and clonogenesis experiments showed that after upregulation of PTEN, the resistance of A549/DDP cells to cisplatin significantly decreased, and IC50 significantly decreased (Figure 5F-I). These results suggest that miR-21 can regulate the expression of PTEN in cells by targeting the 3'UTR region of PTEN, thus affecting the sensitivity of lung cancer cells to cisplatin.

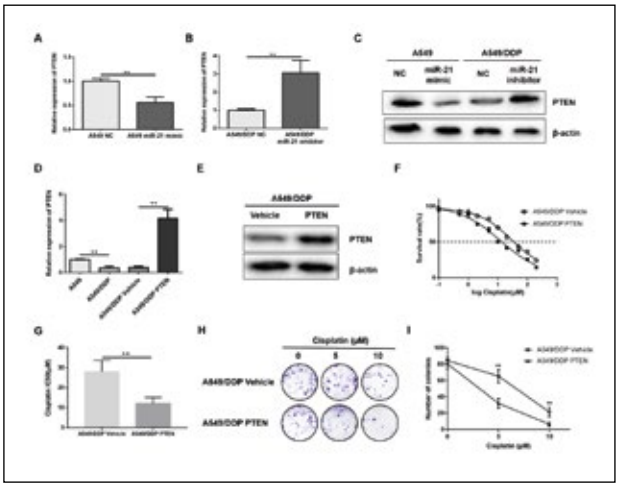

Figure 5: miR-21 induced cisplatin resistance in lung cancer cells by inhibiting PTEN expression.

(A-B) RT-qPCR was used to verify the mRNA level of PTEN in cells. (C) Western blot analysis of PTEN protein expression in cells. (D) RT-qPCR results showed that the mRNA level of PTEN in A549/DDP cells was lower than that in A549 cells. (E) The protein level of PTEN in cells was detected by Western blot. (F-G) IC50 value of cisplatin after A549/DDP transfection was detected by the CCK-8 method (pcDNA3.1-no-load vs pcDNA3.1-PTEN = 28.13 μM vs 11.62 μM). (H-I) the changes in cisplatin sensitivity of A549/DDP cells after transfection with plasmids were observed in the clonal formation experiment. $^{**}p<.01$.

Discussion

Current studies suggest that miRNA can alter the expression of target genes by interfering with endogenous RNA, thereby promoting or inhibiting tumour progression[17]. MiR-21, one of the most studied miRNA, is overexpressed in most cancers and shows carcinogenic activity[18]. Studies have shown that miR-21 can promote the growth of liver cancer cells and inhibit cell apoptosis in glioblastoma[19]. At the same time, overexpression of miR-21 can induce chemotherapy resistance in tumour cells, such as breast cancer and ovarian cancer[20, 21]. In lung cancer, studies have demonstrated that miR-21 plays an important role in the proliferation, invasion and apoptosis of tumour cells[22]. In this study, we found that miR-21 levels in cisplatin-resistant cells A549/

DDP were significantly up-regulated compared with parental cells. By up-regulating or inhibiting the level of miR-21 in cells, it was further proved that miR-21 was related to the sensitivity of cells to cisplatin. At the same time, this study showed that the expression of miR-21 in recipient cells increased after co-incubating with exosomes of drug-resistant cell origin. This may be one of the mechanisms by which exosomes mediate the development of acquired drug resistance in lung cancer cells.

PTEN is a tumour suppressor gene that negatively regulates intracellular PI3K levels and regulates a variety of cell functions, including cell proliferation, invasion, drug resistance and intracellular transport, by inhibiting the Akt signalling pathway[23]. In NSCLC, the PTEN-PI3K/AKT pathway plays an important role in regulating cell cycle, promoting apoptosis, inducing drug resistance and other processes[24]. LncARSR can promote the resistance of liver cancer cells to adriamycin by regulating the PTEN-PI3K/Akt pathway[25]. Studies have shown that miR-21 can directly target the 3'UTR region of PTEN to regulate its expression[26]. Changes in miR-21/PTEN levels have been observed in gefitinib-resistant lung cancer patients[27]. In this study, the negative regulatory effect of miR-21 on PTEN expression was verified by regulating the expression of miR-21 in lung cancer cells. The final rescue trial in the study further confirmed that miR-21 induced cisplatin resistance in lung cancer cells in a PTEN-dependent manner.

In summary, exosomes secreted by drug-resistant lung cancer cell lines can transmit miR-21, thereby affecting the level of miR-21 in recipient cells, thus regulating the level of cell PTEN to induce cisplatin resistance in lung cancer cells. This study further enriches the research on the mechanism of cisplatin resistance in lung cancer and provides a new approach and target for drug development to reverse the resistance of lung cancer.

References

1) Valentino F, Borra G, Allione P, Rossi L. Emerging targets in advanced non-small-cell lung cancer. Future Oncol 2018; 14: 61-72.
2) Latimer KM. Lung Cancer: Clinical Presentation and Diagnosis. FP Essent 2018; 464: 23-26.
3) Salehi-Rad R, Li R, Paul MK, Dubinett SM, Liu B. The Biology of Lung Cancer: Development of More Effective Methods for Prevention, Diagnosis, and Treatment. Clin Chest Med 2020; 41: 25-38.
4) Sepesi B, Rice DC, Heymach JV, Vaporciyan AA, Swisher SG. Stage I lung cancer-to operate or to radiate? that is the question. J Thorac Dis 2016; 8: 2324-2327.
5) Cao W, Yang Q, Yuan Z, Li H, Wang W, et al. Gemcitabine inhibits cisplatin resistance in cisplatin-resistant A549 cells by upregulating trx-interacting protein and inducing cell cycle arrest. Biochem Biophys Res Commun 2020; 1: 8.
6) Raudenska M, Balvan J, Fojtu M, Gumulec J, Masarik M. Unexpected therapeutic effects of cisplatin. Metallomics 2019; 11: 1182-1199.
7) Tong J, Wang P, Tan S, Chen D, Nikolovska-Coleska Z, et al. Mcl-1 Degradation Is Required for Targeted Therapeutics to Eradicate Colon Cancer Cells. Cancer Res 2017; 77: 2512-2521.
8) Senthebane DA, Rowe A, Thomford NE, Shipanga H, Munro D, et al. The Role of Tumor Microenvironment in Chemoresistance: To Survive, Keep Your Enemies Closer. Int J Mol Sci 2017; 18: 7.
9) Wang ZF, Liao F, Wu H, Dai J. Glioma stem cells-derived exosomal miR-26a promotes angiogenesis of microvessel endothelial cells in glioma. J Exp Clin Cancer Res 2019; 38: 201.
10) Au Yeung CL, Co NN, Tsuruga T, Yeung TL, Kwan SY, et al. Exosomal transfer of stroma-derived miR21 confers paclitaxel resistance in ovarian cancer cells through targeting APAF1. Nat Commun 2016; 7: 11150.
11) Ding J, Xu Z, Zhang Y, Tan C, Hu W, et al. Exosome-mediated miR-222 transferring: An insight into NF-kappaB-mediated breast cancer metastasis. Exp Cell Res 2018; 369: 129-138.
12) Gao D, Jiang L. Exosomes in cancer therapy: a novel experimental strategy. Am J Cancer Res 2018; 8: 2165-2175.
13) Cheng L, Sharples RA, Scicluna BJ, Hill AF. Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood. J Extracell Vesicles 2014; 6; 3.
14) Bach DH, Hong JY, Park HJ, Lee SK. The role of exosomes and miRNAs in drug-resistance of cancer cells. Int J Cancer 2017; 141: 220-230.
15) Au Yeung CL, Co NN, Tsuruga T, Yeung TL, Kwan SY, et al., Exosomal transfer of stroma-derived miR21 confers paclitaxel resistance in ovarian cancer cells through targeting APAF1. Nat Commun 2016; 7: 11150.
16) Zheng P, Chen L, Yuan X, Luo Q, Liu Y, et al. Exosomal transfer of tumor-associated macrophage-derived miR-21 confers cisplatin resistance in gastric cancer cells. J Exp Clin Cancer Res 2017; 36: 53.
17) Qiu Z, Dai Y. Roadmap of miR-122-related clinical application from bench to bedside. Expert Opin Investig Drugs 2014; 23: 347-355.
18) Gulei D, Raduly L, Broseghini E, Ferracin M, Berindan-Neagoe I. The extensive role of miR-155 in malignant and non-malignant diseases. Mol Aspects Med 2019; 70: 33-56.
19) Han Y, Meng F, Venter J, Wu N, Wan Y, et al. miR-34a-dependent overexpression of Per1 decreases cholangiocarcinoma growth. J Hepatol 2016; 64: 1295-1304.
20) Devulapally R, Sekar TV, Paulmurugan R. Formulation of Anti-miR-21 and 4-Hydroxytamoxifen Co-loaded Biodegradable Polymer Nanoparticles and Their Anti-proliferative Effect on Breast Cancer Cells. Mol Pharm 2015; 12: 2080-2092.

21) Niu L, Ni H, Hou Y, Du Q, Li H. miR-509-3p enhances platinum drug sensitivity in ovarian cancer. Gene 2019; 686: 63-67.
22) Hashemi ZS, Khalili S, Forouzandeh Moghadam M, Sadroddiny E. Lung cancer and miRNAs: a possible remedy for anti-metastatic, therapeutic and diagnostic applications. Expert Rev Respir Med 2017; 11: 147-157.
23) Udagawa H, Umemura S, Murakami I, Mimaki S, Makinoshima H, et al. Genetic profiling-based prognostic prediction of patients with advanced small-cell lung cancer in large scale analysis. Lung Cancer 2018; 126: 182-188.
24) Chang H, Cai Z, Roberts TM. The Mechanisms Underlying PTEN Loss in Human Tumors Suggest Potential Therapeutic Opportunities. Biomolecules 2019; 9: 713.
25) Li Y, Ye Y, Feng B, Qi Y. Long Noncoding RNA lncARSR Promotes Doxorubicin Resistance in Hepatocellular Carcinoma via Modulating PTEN-PI3K/Akt Pathway. J Cell Biochem 2017; 118: 4498-4507.
26) Shan G, Tang T, Xia Y, Qian HJ. MEG3 interacted with miR-494 to repress bladder cancer progression through targeting PTEN. J Cell Physiol 2020; 235: 1120-1128.
27) Pan Z, Wang K, Chen Q, Zheng X, Song Z, et al. SFI Enhances Therapeutic Efficiency of Gefitinib: An Insight into Reversal of Resistance to Targeted Therapy in Non-small Cell Lung Cancer Cells. J Cancer 2020; 11: 334-344.

Corresponding Author:
Junmei Jia
Email: mhm636@163.com
(China)