AZD9291-resistant non-small cell lung cancer cell-derived exosomal lnc-MZT2A-5:1 induces the activation of fibroblasts

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Background: AZD9291 resistance is still a challenge in the treatment of non-small cell lung cancer (NSCLC) and fibroblasts in the tumor microenvironment (TME) play a key role in the malignant phenotype of NSCLC. The study aimed to investigate the role of exosomes derived from AZD9291-resistant cells on the phenotypes of lung fibroblasts and the underlying mechanism.

Methods: The supernatants and exosomes of wild type and AZD9291-resistant NSCLC (H1975/PC9) cells were collected, and co-cultured with lung fibroblasts (MRC-5 cells) respectively. Transwell and quantitative real-time PCR (qRT-PCR) assays were used to evaluate migration and inflammation levels. Exosomes were collected by ultracentrifugation, and identified by nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM) and western blots. Microarray was used to screen dysregulated exosomal lncRNAs from the resistant cells. Candidate lncRNAs were selected by bioinformatical annotation of their target genes and verified by qRT-PCR. The target lncRNA was then selected for further confirmation.

Results: Both the supernatant and exosomes from resistant cells significantly promoted the migration of MRC-5 cells, and the exosomes also upregulated mRNA levels of inflammation cytokines. Microarray identified 159 dysregulated exosomal lncRNAs. Fifteen candidate lncRNAs were selected following the biological roles of their target genes. qRT-PCR validation indicated that lnc-MZT2A-5:1 had the highest fold change. Finally, we found that lnc-MZT2A-5:1 could promote the migration ability and inflammation cytokines expression level of MRC-5 cells.

Conclusions: Our study clarified that lnc-MZT2A-5:1 from AZD9291-resistant NSCLC cell lines could promote the activation of MRC-5 cells, thus to uncover a new mechanism for AZD9291 resistance and provide new potential targets for the treatment of NSCLC.

Keywords: AZD9291 resistance; non-small cell lung cancer (NSCLC); exosome; lnc-MZT2A-5:1; fibroblasts

Introduction

Lung cancer is a leading cause of death worldwide, with 1.8 million people diagnosed and 1.6 million people dying from the disease every year (1). Non-small cell lung cancer (NSCLC), consisting of squamous cell carcinoma, large cell carcinoma and adenocarcinoma, is the predominant histologic subtype, accounting for approximately 85% of all lung cancer cases (2). The 5-year overall survival (OS) rate of patients with NSCLC is about 17.8% (3).

The effective application of various targeted therapies

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and immunotherapy in some patients with advanced NSCLC has made great progress (4). Instead of traditional surgical interventions, targeted therapy and immunotherapy has changed the course of treatment for most patients, and molecular testing is now the standard recommendation for those with advanced lung adenocarcinoma (5). Osimertinib (AZD9291) is a third-generation tyrosine kinase inhibitors (TKIs) which selectively targets activating EGFR mutations and T790M-resistance mutation by formation of a covalent bond to the C797 residue of mutant EGFR (6). Despite the great success made by AZD9291 not only in first-line therapy but also in salvage therapy for T790M secondary mutations, acquired resistance inevitably emerges, limiting its long-term clinical benefits (7). However, the mechanism of drug resistance of AZD9291 is still not fully understood (8). Therefore, a better understanding of the drug resistance mechanism of targeted therapy and personalized treatment of lung cancer patients have become research hotspots in this field.

Exosomes range in diameter from 30 to 150 nm and are released into the extracellular environment by fusing with cell membranes (9). While at first, it was thought that exosomes only function when cells remove unnecessary molecules, many studies have subsequently shown they can transfer their contents (such as proteins and non-coding RNA) from one cell to another, thereby regulating cellular intercommunication (10,11). Exosomes may participate in or interfere with the continuous crosstalk between cells or the local/distant host environment, thereby affecting tumor progression.

In normal tissue, fibroblasts are embedded in extracellular matrix (ECM), in an inactive quiescent state. While in tumor microenvironment (TME), fibroblasts are activated, termed as carcinoma-associated fibroblasts (CAFs) (12). Fibroblasts in the TME play a key role in the malignant phenotype of NSCLC (13). Recent studies have shown that exosomes are involved in regulating the sensitivity of tumor cells to chemotherapy drugs (14). Drug-resistant cancer cells can encapsulate chemotherapeutic agents in exosomes and transport anticancer drugs from tumor cells, which confers resistance to sensitive cells (15). In breast cancer cells, exosome-derived miR-221/222 could enhance the resistance of ER-positive breast cancer cells to tamoxifen, and exosome-derived miR-522-3p could increase the resistance of PC9 cells (16,17). Exosomes can also enhance drug resistance of tumor cells by inducing epithelial mesenchymal transition (EMT) or enhancing anti-apoptotic signaling pathways (18). However, the relationship between exosomes and lung cancer

phenotypes has not yet been elucidated in AZD9291-resistant NSCLC cells.

In this study, we found that supernatant and exosomes derived from wild type or AZD9291-resistant H1975 and PC9 cells could promote the migration of MRC-5 cells. Moreover, we identified the differentially expressed lncRNAs in the exosomes by microarray analysis. Five candidate lncRNAs were then selected for further investigation according to the bioinformatics analysis of their predicted targets. qRT-PCR was performed to verify the expression levels of the five candidate lncRNAs, and we found that lnc-MZT2A-5:1 and lnc-CCDC103-7:1 were significantly increased in exosomes derived from AZD9291-treated H1975 and PC9 cells. Interestingly, we found that the overexpression of lnc-MZT2A-5:1 but not lnc-CCDC103-7:1 could significantly promote the migration and inflammation of MRC-5 cells. In summary, we identified new mechanisms of AZD9291 resistance and provided new potential targets for the treatment of NSCLC. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/atm-21-5186).

Methods

Cells and cell culture

Human MRC-5 was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in Minimum Essential Medium (MEM; Gibco, Carlsbad, USA) supplemented with 10% fetal bovine serum (Gibco, Carlsbad, USA). Human NSCLC cell lines PC9 and H1975 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). The AZD9291-resistant H1975 and PC9 cell line were established in a previous study (19), while NSCLC cell lines and the resistant cell lines were cultured in RPMI-1640 medium (Gibco, Carlsbad, USA) supplemented with 10% FBS. All cells were incubated at 37 ℃ in a humidified atmosphere containing 5% CO_2.

Migration assay

For cell migration assays, a total of 1×10^5 MRC-5 cells in 100 μL serum-free medium were seeded on transwell plates with inserts (Corning, USA). For detecting exosomes or cell supernatant function, equal quantities of supernatant or exosomes of wild type or AZD9291-resistant H1975/ PC9 cells were added into the inserts. We added 600 μL complete medium into the bottom chambers, and the cell

inserts were then incubated for 24 h and fixed and stained.

Isolation and determination of exosomes

Wild type or AZD9291-resistant H1975/PC9 cells were seeded in a 10 cm dish and cultured for 48 h, then the supernatant collected and filtered. Exosomes in the supernatant were isolated by centrifugation. In brief, a total of 500 mL culture medium was centrifuged at 2,000 ×g for 10 min at 4 ℃ to remove dead cells, and 10,000 ×g for 30 min at 4 ℃ to remove cell debris. Finally, exosomes were isolated by centrifugation at 110,000 ×g for 90 min at 4 ℃. The exosomes were washed with PBS twice, and stored in PBS at –80 ℃. The number and size distribution of exosomes were analyzed by nanoparticle tracking analysis (NTA), and microvesicles were detected by transmission electron microscopy (TEM).

Western blot

Cell total proteins were extracted by RIPA lysis buffer, and samples were separated by SDS-PAGE. Proteins were transferred onto a polyvinylidene fluoride membrane, which was incubated with primary antibody at 4 ℃ overnight. Protein expression was performed using an enhanced chemiluminescence kit (GE Healthcare, Pittsburgh, USA), while the density of the bands on blots was analyzed by Image J software (National Institutes of Health). The antibodies against CD81 (cat. no. ab109201), CD63 (cat. no. ab134045), HSP27 (cat. no. ab109376), and TSG101 (cat. no. ab125011) were from Abcam (Cambridge, UK).

RNA extraction and quantitative real-time PCR

Trizol reagent (Invitrogen, Carlsbad, USA) was used to extract Total RNA from cells, which was then reverse transcribed into cDNAs using the Reverse Transcription kit (RiboBio, Guangzhou, China). Quantitative real-time PCR (qRT-PCR) was performed to amplify the cDNA template using a SYBR Premix Dimmer Eraser kit (RiboBio, Guangzhou, China) on the ABI 7500 system, and the relative mRNA levels of genes were determined by using the comparative $2^{-\Delta\Delta Ct}$ method. All primer sequences are listed in Table S1.

Microarray and bioinformatics analysis

Exosomal lncRNAs microarray analysis was performed at the Shanghai Biotechnology Corporation (Shanghai, China), using Agilent Human miRNA 8*60K V21.0 microarray (Agilent Technologies, USA). Quantile normalization and subsequent data processing were performed using Quantile algorithm, Gene Spring Software 12.6 (Agilent Technologies). The expression of lncRNAs with a FC abs ≥2 was considered significantly changed, and genes in the upstream and downstream of lncRNA within the range of 10 kb were selected as cis-target genes for lncRNAs. According to the prediction of trans-target genes, those genes having complementary or similar sequence with lncRNAs were selected, the complementary energy between the two sequences was then calculated by RNAplex, and genes with e≤–30 were selected as the trans-target genes of lncRNAs. Go and KEGG analysis of predicted target genes were performed through DAVID (https://david.ncifcrf.gov/) (20).

Cell transfection

lnc-MZT2A-5:1, lnc-CCDC103-7:1, and their negative control (NC) were synthesized by RiboBio (Guangzhou, China), and equal amounts of each were transfected in MRC-5 cells according to the instructions of the Lipofectamine 2000 kit (Invitrogen, Carlsbad, USA). Cells were then harvested after 36 hours for analysis.

Statistical analysis

All data are presented as the mean ± SD. and were analyzed with SPSS version 22.0 (SPSS, Chicago, USA), and each experiment was performed at least three times (biological replicate). Differences between two groups or multiple groups were respectively performed with the Student's *t*-test or one-way ANOVA followed by Bonferroni *post-hoc* tests. Comparisons of data among multiple groups were performed by one way analysis of variance followed by Tukey's *post hoc* tests, and P<0.05 was considered statistically significant.

Results

Supernatant of AZD9291-resistant NSCLC cells promoted the migration of MRC-5 cells

To detect the mechanism of AZD9291 resistance, we co-cultured MRC-5 cells with the supernatant of NSCLC (H1975 and PC9 cells) sensitive or resistant to AZD9291. We found that the supernatant of wild type cells could
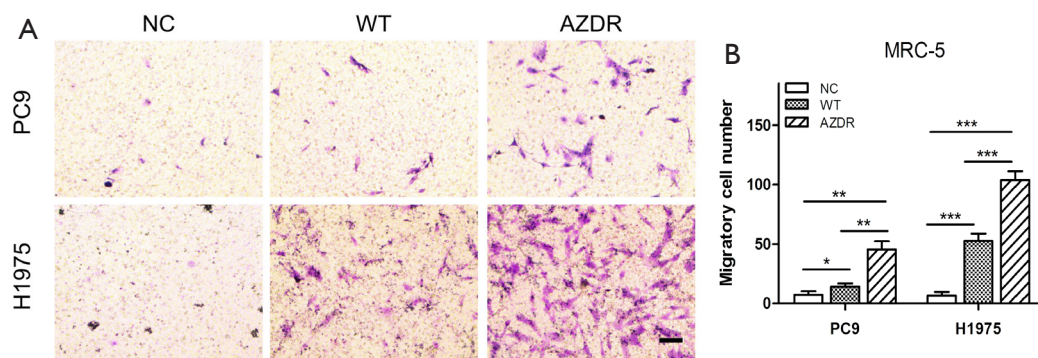
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Figure 1 The supernatant of wild type and AZD9291-resistant H1975 and PC9 cells promoted the migration of MRC-5 cells. (A) Representative images of transwell assays analysis of MRC-5 cells co-cultured with or without supernatant of wild type or AZD9291-resistant H1975/PC9 cells stained by crystal violet. (B) Statistical analysis of transwell assays (n=3). A length of 50 μm was shown by scale bar and statistical significance was distinguished with P value: *, P<0.05; **, P<0.01; ***, P<0.001. NC, negative control; WT, wild type.
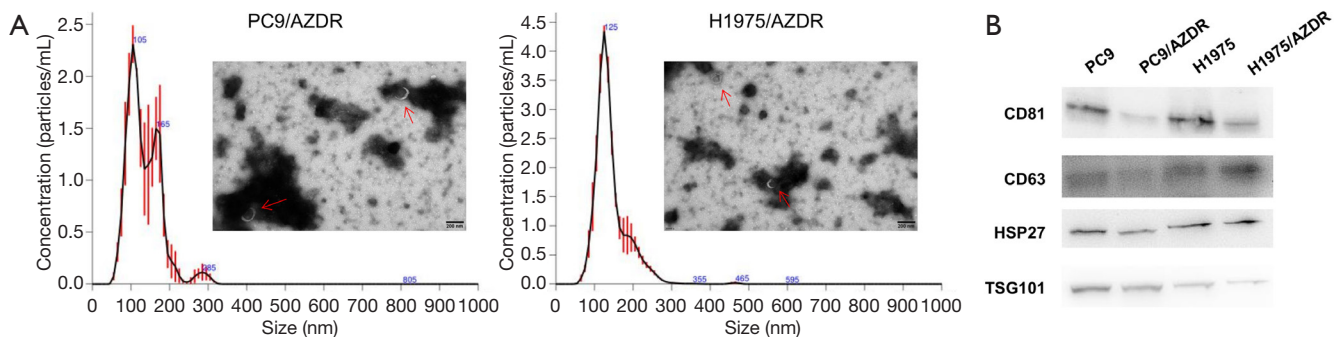

Figure 2 Characterization of exosomes derived from wild type or AZD9291-resistant H1975/PC9 cells. (A) NTA analysis of the size distribution, and TNM images of exosomes shown by the red arrows. (B) Western blotting analysis for exosomal markers (CD81, CD63, Hsp27, and TSG101) of exosomes. NTA, nanoparticle tracking analysis.

significantly promote the migration of MRC-5 cells compared to the NC group. while the supernatant from the resistant cells could further promote the migration of MRC-5 cells compared to the wild type group (*Figure 1*). Those data indicated that the AZD9291-resistant NSCLC cells could promote the migration of MRC-5 cells by some secreted factors in the supernatant.

Identification of exosomes derived from NSCLC cells

To explore the mechanism underlying the regulation of supernatant of AZD9291-resistant NSCLC cells on the migration of MRC-5 cells, exosomes were isolated and purified from the supernatant of wild type and AZD9291-resistant PC9/H1975 cells, respectively. The diameter of exosomes was detected by TEM and NTA, and all exosomes

showed typical nanoparticles wrapped by lipid bilayer membranes. The size of these nanoparticles ranged from 50 to 200 nm (*Figure 2A*). In addition, we also analysed the protein markers of isolated exosomes by Western blot, and s shown in *Figure 2B*, the proteins of CD81, CD63, TSG101, and Hsp27 were positively expressed in all exosomes. These results indicate that we successfully isolated exosomes secreted by wild type and AZD9291-resistant NSCLC cells.

Exosomes derived from wild type and AZD9291-resistant NSCLC cells promoted the migration and inflammation of MRC-5 cells

To explore the biological effects of exosomes derived from wild type and AZD9291-resistant NSCLC cells on MRC-5 cells, we next co-cultured MRC-5 cells with or
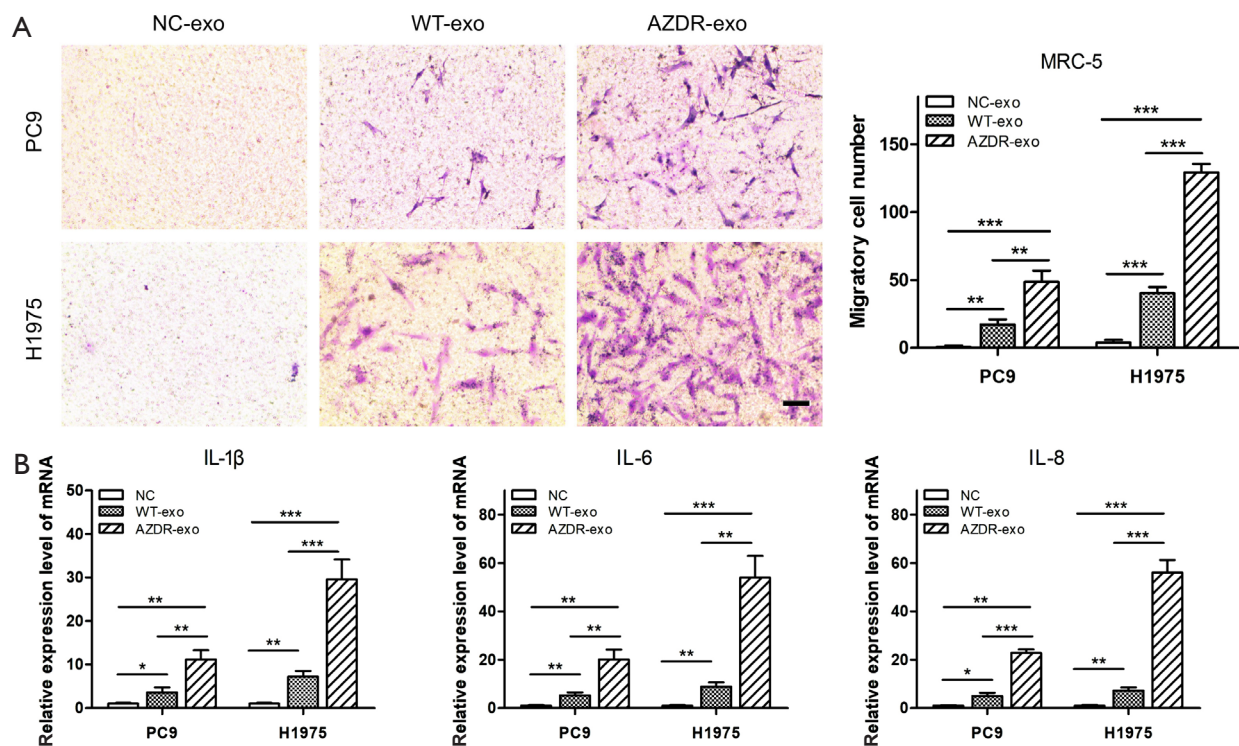
Figure 3 Exosomes derived from wild type or AZD9291-resistant NSCLC cells promoted the migration and inflammation of MRC-5 cells. (A) Representative images and statistical analysis of transwell assays analysis of MRC-5 cells co-cultured with or without exosomes derived from wild type or AZD9291-resistant H1975/PC9 cells (n=3) stained by crystal violet. (B) The mRNA levels of IL-1β, IL-6, and IL-8 in MRC-5 cells co-cultured with or without exosomes derived from wild type or AZD9291-resistant H1975/PC9 cells (n=3). A length of 50 μm was shown by scale bar and statistical significance was distinguished with P value: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. NSCLC, non-small cell lung cancer.

without exosomes derived from wild type or AZD9291-resistant PC9/H1975 cells. Interestingly, we found that the administration of exosomes derived from both wild type and AZD9291-resistant PC9/H1975 cells could promote the migration of MRC-5 cells (*Figure 3A*), and exosomes derived from AZD9291-resistant PC9/H1975 cells had a more profound effect compared to that from the wild type (*Figure 3A*). Furthermore, we examined the mRNA levels of inflammatory cytokines in exosome co-cultured MRC-5 cells. Consistently, exosomes derived from both wild type and AZD9291-resistant PC9/H1975 cells could increase the mRNA levels of inflammatory cytokines (IL-1β, IL-6, and IL-8) of MRC-5 cells, and the exosomes derived from AZD9291-resistant PC9/H1975 cells had a more profound effect compared to those from the wild type (*Figure 3B*). These results indicated that exosomes secreted by AZD9291-resistant NSCLC cells were more capable of

promoting migration and inflammation of MRC-5 cells.

Exosomal lnc-MZT2A-5:1 promoted the migration and inflammation of MRC-5 cells

We further explored the molecular mechanisms underlying the activation of MRC-5 cells by ADZ9291-resistant NSCLS cell derived exosomes. Given that exosomes encapsulate abundant lncRNAs and affect the communication between cells (21), we wanted to know whether lncRNAs in exosomes were involved in the effect of ADZ9291-resistant NSCLS cells on MRC-5 cells. Therefore, we performed lncRNA microarray analysis on the exosomes derived from wild type or ADZ9291-resistant NSCLS cells (*Figure 4A*). We identified a total of 80 significantly up-regulated and 79 significantly down-regulated lncRNAs (FC abs ≥2), and the top 30 lncRNAs

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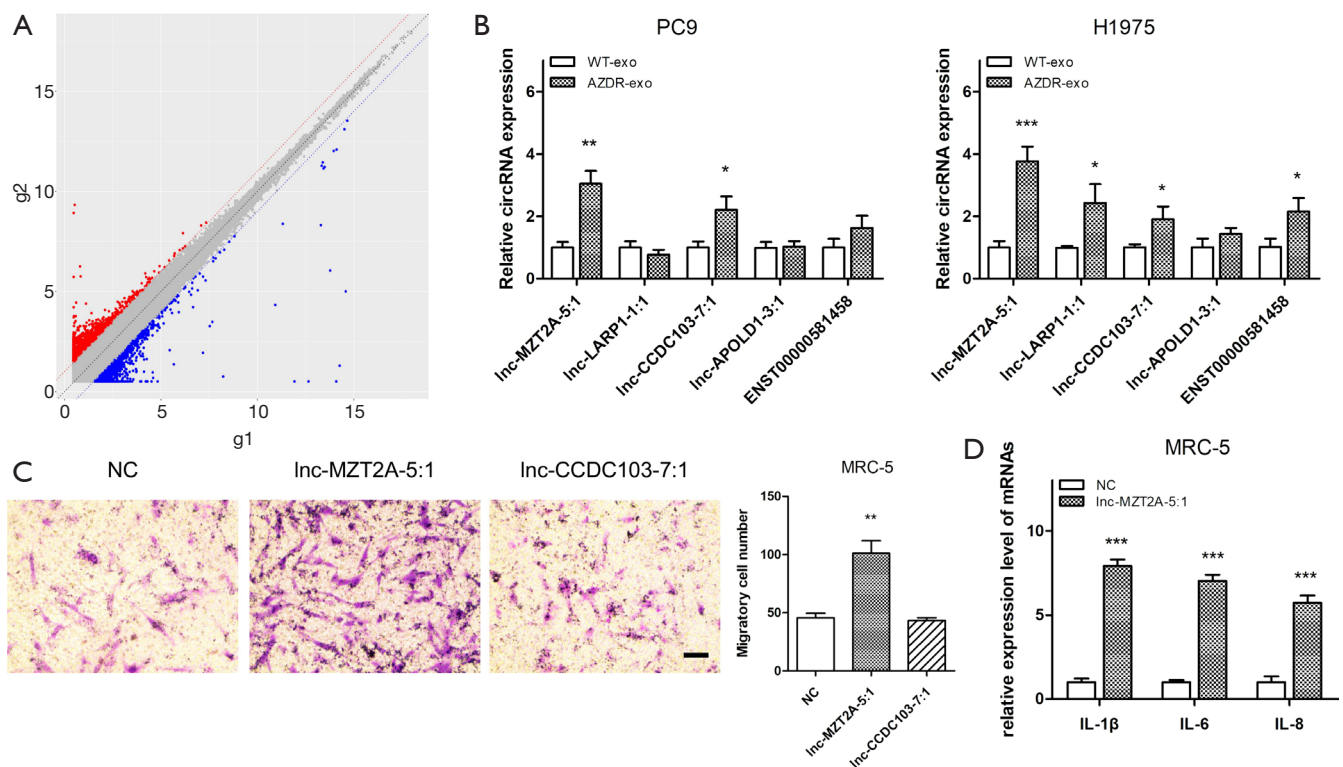

Figure 4 Exosomal lnc-MZT2A-5:1 promoted the migration and inflammation of MRC-5 cells. (A) Scatter plot of differentially expressed lncRNAs in exosomes derived from wild type and AZD9291-resistant NSCLC cells stained by crystal violet (red dot: significantly increased; blue dot: significantly decreased). (B) The expression levels of five candidate lncRNAs in exosomes derived from wild type or AZD9291-resistant NSCLC cells. (C) Representative images and statistical analysis of transwell assays analysis of MRC-5 cells overexpressed with or without lnc-MZT2A-5:1 or lnc-CCDC103-7:1 (n=3), stained by crystal violet. (D) The mRNA levels of IL-1β, IL-6, and IL-8 in MRC-5 cells overexpressed with or without lnc-MZT2A-5:1 (n=3). A length of 50 μm was shown by scale bar and statistical significance was distinguished with P value: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. NSCLC, non-small cell lung cancer.

with the highest FC abs are shown in *Table 1*. lncRNAs are considered correlated with either neighboring (cis) or distal (trans) protein-coding genes (22). We predicted the cis- and trans-target genes of the dysregulated lncRNAs, and GO enrichment and KEGG pathway analyses were performed by DAVID on these target genes. As shown in *Table 2*, part of the target genes are enriched in the pathways of NSCLC and small cell lung cancer. We speculated that certain lncRNAs are likely to regulate the expression of their target genes and participated in regulation of the malignant progression of lung cancer. The corresponding 15 lncRNAs which may be involved in the malignant progression of lung cancer are shown in *Table 3*. Furthermore, we found that five of the 15 lncRNAs were also among the top 30 changed lncRNAs, as shown in *Table 1*. Therefore, we selected the five lncRNAs (lnc-MZT2A-5:1, lnc-LARP1-1:1, lnc-CCDC103-7:1, lnc-APOLD1-3:1, and

ENST00000581458), which are most likely to be involved in the progression of lung cancer, for further study.

Interestingly, our qRT-PCR results showed that only lnc-MZT2A-5:1 and lnc-CCDC103-7:1 were significantly increased in exosomes derived from ADZ9291-resistant PC9 and H1975 cells compared to the corresponding wild type (*Figure 4B*). Next, we overexpressed lnc-MZT2A-5:1 and lnc-CCDC103-7:1 in MRC-5 cells and found that the overexpression of lnc-MZT2a-5:1 but not lnc-CCDC103-7:1 could significantly promote the migration of MRC-5 cells through transwell assay (*Figure 4C*). Accordingly, overexpression of lnc-MZT2a-5:1 could also increase the mRNA levels of IL-1β, IL-6, and IL-8, suggesting the increase of inflammation in MRC-5 cells (*Figure 4D*). These data indicated that exosomal lnc-MZT2a-5:1 may be involved in the malignant progression of NSCLC caused by ADZ9291-resistant.

Table 1 Top 30 dysregulated exosomal lncRNAs in resistant cells

No.	LncRNAs	Fold change	Fold change (abs)	Regulation	Source
1	lnc-MZT2A-5:1	5.571	5.571	Up	LNCipedia
2	ENST00000610936	0.235	4.250	Down	ENSEMBL_GENCODE
3	lnc-RP11-1012A1.4.1-1:1	4.172	4.172	Up	LNCipedia
4	NONHSAT047203	3.991	3.991	Up	NONCODE
5	lnc-UGGT2-4:1	3.957	3.957	Up	LNCipedia
6	ENST00000566521	3.909	3.909	Up	ENSEMBL_GENCODE
7	NR_029628	3.599	3.599	Up	RefSeq
8	lnc-FAM84B-1:1	3.547	3.547	Up	LNCipedia
9	lnc-LARP1-1:1	3.506	3.506	Up	LNCipedia
10	ENST00000422118	0.297	3.365	Down	ENSEMBL_GENCODE
11	lnc-CCDC103-7:1	3.324	3.324	Up	LNCipedia
12	lnc-PION-4:1	0.301	3.319	Down	LNCipedia
13	lnc-APOLD1-3:1	3.299	3.299	Up	LNCipedia
14	lnc-RNASEH1-7:2	0.306	3.272	Down	LNCipedia
15	ENST00000508993	3.235	3.235	Up	ENSEMBL_GENCODE
16	ENST00000517511	3.113	3.113	Up	ENSEMBL_GENCODE
17	ENST00000436077	0.345	2.901	Down	ENSEMBL_GENCODE
18	NR_110135	2.893	2.893	Up	RefSeq
19	ENST00000606221	2.858	2.858	Up	ENSEMBL_GENCODE
20	ENST00000609586	0.353	2.834	Down	ENSEMBL_GENCODE
21	NR_108042	2.833	2.833	Up	RefSeq
22	lnc-AC037487.1-4:1	2.815	2.815	Up	LNCipedia
23	ENST00000581458	2.807	2.807	Up	ENSEMBL_GENCODE
24	ENST00000484322	2.798	2.798	Up	ENSEMBL_GENCODE
25	ENST00000417800	0.358	2.791	Down	ENSEMBL_GENCODE
26	lnc-ARRDC3-6:1	2.775	2.775	Up	LNCipedia
27	lnc-FAM181B-8:1	2.757	2.757	Up	LNCipedia
28	lnc-SORBS2-1:1	0.363	2.754	Down	LNCipedia
29	ENST00000584036	2.719	2.719	Up	ENSEMBL_GENCODE
30	lnc-XIAP-2:1	0.368	2.716	Down	LNCipedia

Table 2 Lung cancer-related pathways and target genes

Pathway ID	Description	Count	Target genes	Target types
hsa05223	Non-small cell lung cancer	1	*FHIT*	Cis-targets
hsa05222	Small cell lung cancer	1	*FHIT*	
hsa05222	Small cell lung cancer	8	*E2F2, CDK4, XIAP, APAF1, PIAS2, PIK3CD, SKP2, CYCS*	Trans-targets
hsa05223	Non-small cell lung cancer	5	*E2F2, PRKCA, CDK4, PIK3CD, STK4*	

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Table 3 15 candidate lncRNAs

No.	lncRNAs	Fold change	Fold change (abs)	Target genes
1	lnc-MZT2A-5:1	5.571	5.571	CYCS, PIAS2
2	lnc-LARP1-1:1	3.506	3.506	XIAP, SKP2
3	lnc-CCDC103-7:1	3.324	3.324	CDK4, PIAS2
4	lnc-APOLD1-3:1	3.299	3.299	XIAP
5	ENST00000581458	2.807	2.807	APAF1
6	lnc-FAM110C-2:2	2.640	2.640	PIAS2, XIAP
7	lnc-PTPRG-5:1	2.621	2.621	FHIT
8	lnc-RADIL-5:1	2.201	2.201	PIAS2
9	lnc-PRND-2:1	2.194	2.194	CDK4, XIAP
10	lnc-PPM1N-1:1	2.170	2.170	CYCS
11	lnc-SHFM1-1:1	0.474	2.109	PRKCA
12	lnc-GNAQ-8:1	0.481	2.080	XIAP
13	lnc-ANKRD50-6:1	0.486	2.059	SKP2
14	ENST00000447617	0.488	2.050	PIK3CD, XIAP
15	lnc-TAOK3-12:1	0.496	2.015	E2F2, STK4

Discussion

Ahe platinum-based dual-drug combination dosing regimen has become the treatment of choice for advanced NSCLC. With in-depth study of the molecular mechanism of tumorigenesis, molecular targeted therapy such as EGFR-TKIs has made breakthrough progress in controlling the progression of advanced NSCLC (23). Osimertinib has been widely used in the clinical treatment of NSCLC with T790M mutation, but the occurrence of drug resistance affects its long-term efficacy. Therefore, the exploration of osimertinib resistance mechanisms in NSCLC has become an important focus of research in the field. Here, we found that the supernatant and exosomes derived from wild type and AZD9291-resistant H1975/PC9 cells could promote the migration of MRC-5 cells. We also identified the differentially expressed exosomal lncRNAs by microarray and found that lnc-MZT2A-5:1 could significantly promote the migration and inflammation of MRC-5 cells. Our study identified new mechanisms of AZD9291 resistance and provided new potential targets for the treatment of NSCLC.

The TME is composed of ECM proteins, cytokines, microvesicles, and stromal cells and plays a crucial role in regulating drug resistance in NSCLC (21). As key mediators of intercellular information transmission, exosomes show promising potential in the sensitivity of tumor cells to drugs. Previous studies reported that exosomal H19 facilitated erlotinib resistance in NSCLC via the miR-615-3p/ATG7 axis (24), and that exosomal lncRNA urothelial carcinoma-associated 1 (UCA1) was involved in gefitinib resistance in NSCLC (25). Additionally, Deng *et al.* reported the molecular mechanisms of osimertinib resistance in resistant and sensitive H1975 cells (26). However, few studies have reported the relationship of exosomal lncRNAs between osimertinib resistant NSCLC cells and MRC-5 cells. In the present study, we identified that exosomal lnc-MZT2A-5:1 was significantly increased in exosomes derived from AZD9291-resistant NSCLC cells compared to that derived from wild type cells and could promote the migration and inflammation of MRC-5 cells, providing potential therapeutic targets for NSCLC.

The ECM is a complex network composed of elastic fibers, collagen, and non-collagen glycoproteins, and is the microenvironment for cell growth. Activated fibroblasts can secrete large amounts of inflammatory factors and participate in tumor progression, and can promote drug resistance in gastric cancer (27), esophageal cancer (28), prostate cancer (29), and tongue cancer (30). However, there are few studies reporting the relationship between

inflammation and drug resistance in lung cancer. Our research shows that AZD9291-resistant NSCLC cells-derived exosomes can promote the migration and inflammation of MRC-5 cells. Importantly, the exosomes derived from wild type NSCLC could also promote the migration and inflammation of MRC-5 cells, although this had a weaker influence compared to that derived from AZD9291-resistant NSCLC cells. These data provide a new molecular mechanism for the rapid development of drug resistance in lung cancer.

Previous studies have shown that after cisplatin treatment, lung cancer cells secreted more exosomes that can carry miRNAs, which may in turn confer resistance to the cells (31). There are also many studies showing that exosomes may change the drug sensitivity of tumors by transferring miRNA (32,33). However, there are few studies on lncRNA in drug-resistant cell-derived exosomes. To further study the specific mechanism of AZD9291-resistant NSCLC cell-derived exosomes to promote the progression of NSCLC, we performed microarray analysis of lncRNAs in exosomes derived from wild type and AZD9291-resistant NSCLC cells, and identified the exosomal lnc-MZT2A-5:1 was significantly increased and could promote the migration and inflammation of MRC-5 cells. Although the molecular mechanism underlying the regulation of lnc-MZT2A-5:1 on MRC-5 cells is still unclear, our prediction of cis- and trans-target genes related to differentially expressed lncRNAs provides an idea for further exploration. For example, growing evidence suggests that FHIT, a cis-targets of dysregulated lncRNAs, is a candidate tumor suppressor for NSCLC (34). Moreover, CYCS, a predicted target gene of lnc-MZT2A-5:1, was reported to be involved in the regulation of autophagy and apoptosis of NSCLC cells (35). Therefore, based on the predicted target genes, we will further explore the molecular mechanisms underlying the exosomal lncRNAs regulating the biological functions of MRC-5 cells in the future.

In conclusion, our results indicate that exosomes derived from wild type and AZD9291-resistant NSCLC cells can promote the malignant phenotype of MRC-5 cells. More importantly, microarray was performed to analyze exosomal lncRNAs, and showed that lnc-MZT2A-5:1 was significantly up-regulated in the exosomes derived from AZD9291-resistant NSCLC cells and could promoted the activation and migration of MRC-5 cells compared to that derived from wild type NSCLC cells. Our study identified new mechanisms of AZD9291 resistance and provided new potential targets for the treatment of NSCLC.

Footnote

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Data Sharing Statement: Available at https://dx.doi.org/10.21037/atm-21-5186

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Table S1 Primer sequences used for qRT-PCR

No.	lncRNAs	Forward (5'-3')	Reverse (5'-3')
1	lnc-MZT2A-5:1	AAGACCAGCCTGACCAACAC	CTGCCACCATTTCCTTTGTT
2	lnc-LARP1-1:1	AGGGAGGATCTTGCATTCCT	AAGAACGAAAAGGGTGAGCA
3	lnc-CCDC103-7:1	GCCACAGAGCAGCCTAACAT	CAGCACCATCTCAGCAAGAA
4	lnc-APOLD1-3:1	GCCCATCCGAACAAGAACTA	CTACCAAACATTCCCCCTCA
5	ENST00000581458	GGCAAAACTCCCTCATCAGA	TGACCAGCACCTCCACATAA
6	GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG