



# Dysregulated expression of microRNA involved in resistance to osimertinib in *EGFR* mutant non-small cell lung cancer cells

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**Background:** An increasing amount of evidence has confirmed that the altered expression of microRNAs (miRNAs) is critical to the mechanism underlying primary and even acquired resistance to tyrosine kinase inhibitors (TKIs). However, studies on the linkage between the altered miRNAs expression and osimertinib resistance are few, and the effect of miRNAs in this context is still unclear. In the light of this, we hypothesized that the differential expression of multiple miRNAs is the driver in the osimertinib resistance process. Thus, the aim of our study was to find differentially expressed miRNAs in non-small cell lung cancer cells resistant to osimertinib.

**Methods:** An AZD9291(Osimertinib)-resistant cell line model was constructed, and the differential miRNAs between epidermal growth factor receptor (EGFR)-sensitive cell lines A549 and H1975 and the corresponding drug-resistant cell lines were identified via biosynthesis analysis.

**Results:** In the A549 osimertinib-resistant cell line, 93 miRNAs were upregulated and 94 miRNAs were downregulated. In the H1975 osimertinib-resistant cell line, 124 miRNAs were upregulated and 53 miRNAs were downregulated. Finally, 7 significantly different miRNAs were screened using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis.

**Conclusions:** This study on the mechanism of target therapy in lung cancer systematically and comprehensively examined the miRNAs involved in osimertinib resistance. It was found that miR-708-5p, miR-708-3p, miR-10395-3p, miR-7704 miR-34a-5p, miR-19b-1-5p, and miR-219a-5p may play key roles in osimertinib resistance.

**Keywords:** Epidermal growth factor receptor (EGFR); epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs); non-small cell lung cancer (NSCLC); microRNA (miRNA); osimertinib resistance

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## Introduction

Lung cancer is the foremost cause of cancer-related incidence and mortality worldwide (1-3), with non-small cell lung cancer (NSCLC) accounting for approximately 85% of total lung cancer cases (1). Despite the progress

in lung cancer detection and therapy, the 5-year overall survival rate remains poor (4). Epidermal growth factor receptor (*EGFR*) is the most common activating mutation in NSCLC, accounting for approximately 40–55% of cases in the Asian population and being associated with poor

prognosis (5,6). Thus far, the small molecule tyrosine kinase inhibitors (TKIs), including erlotinib, gefitinib, afatinib, and osimertinib, have received approval in the standard first-line treatment of patients with *EGFR*-mutant NSCLC due to their proven benefits in the objective response rate (ORR), progression-free survival (PFS), and tolerability over cytotoxic chemotherapy (7-9). Moreover, osimertinib has shown superior curative effect compared to the earlier generation inhibitors. The Phase III FLAURA Randomized trial evaluated osimertinib as a first-line treatment that produced longer PFS and overall survival (OS) compared to first-generation TKIs (10,11). Consequently, osimertinib is the preferred first-line treatment for *EGFR*-positive NSCLC.

Despite of the satisfactory efficacy of osimertinib, acquired resistance inevitably occurs over time (12,13). Various molecular mechanisms of acquired resistance to osimertinib have been reported, including altered *EGFR* signaling pathway and activation of bypass pathways, such as *RAS/RAF/MEK/ERK* (MAPK), phosphatidylinositol 3-kinase/mammalian target of the rapamycin (mTOR), and *MET*/epidermal growth factor receptor 2 (HER2) amplification; *RET* fusion; and histological/phenotypic transformation (14-18). However, a consensus concerning these mechanisms has not yet been reached. Thus, further analysis on osimertinib resistance may be critical for determining suitable treatment strategies and improving

prognosis.

MicroRNAs (miRNAs), a family of small noncoding RNA molecules of approximately 20–22 nucleotides in length, are essential to the regulation of multiple biological processes, including gene regulation, apoptosis, and maintenance of cell differentiation (19,20). In particular, miRNAs play a key role in regulating gene expression, and miRNAs are commonly dysregulated in cancer (21,22). In terms of lung cancer, miRNAs can be used as diagnostic and prognostic markers, and have a dual role in indicating carcinogenesis and behavior (23-25). Accumulating literature points to the altered expression of miRNAs as a key contributor to the underlying mechanism of primary and even acquired resistance to TKIs (26,27). For example, it has been found that the upregulation of miR-21 mediates gefitinib resistance in NSCLC via the activation of anaplastic lymphoma kinase (ALK) and signal-regulated kinase (ERK) pathway (28). In addition, previous studies have shown that miRNAs can restore the sensitivity of drug-resistant cells, thereby improving the therapeutic effect of *EGFR*-TKIs (29). Indeed, miR-133b was found to be associated with longer PFS when erlotinib was used as second- or third-line therapy for NSCLC (30).

However, studies on the linkage between the altered miRNA expression and resistance to osimertinib, a third-generation *EGFR*-TKI, are few, while the effect of miRNAs in this process remains unclear (25,29,31). We thus hypothesized that differential expression of multiple miRNAs involves in the process of osimertinib resistance as drivers (32). Thus, the aim of our study was to analyze the miRNAs differences in acquired resistance to osimertinib. We present the following article in accordance with the MDAR reporting checklist (available at <https://jtd.amegroups.com/article/view/10.21037/jtd-23-401/rc>).

### Highlight box

#### Key findings

- Our study found that miR-708-5p, miR-708-3p, miR-10395-3p, miR-7704, miR-34a-5p, miR-19b-1-5p, and miR-219a-5p may play key roles in osimertinib resistance.

#### What is known and what is new?

- A growing body of evidence has confirmed that altered miRNA expression is principally involved in the underlying mechanism of primary or even acquired resistance to TKIs.
- However, studies on the linkage between the altered miRNA expression and osimertinib resistance are few, and the effect of the miRNAs in this regard is still unclear. Thus, the aim of our study was to analyze the differential miRNAs in the acquired resistance to osimertinib.

#### What is the implication, and what should change now?

- The dysregulated expression of microRNA is involved in resistance to osimertinib in *EGFR*-mutant non-small cell lung cancer cells, and targeting altered miRNAs constitutes a promising strategy for overcoming osimertinib resistance.

## Methods

### Cell culture

Two lung adenocarcinoma cell lines (A549 and NCI-H1975) were purchased from Procell Biologics Co., Ltd. (Wuhan, China) and H1975 from Beina Chuanglian Biotechnology Co., Ltd. (Beijing, China) cultured in MEM (Dulbecco's Modified Eagle's Medium) medium and Human Resources Processes Manual Introduction (HRPMI)-1640 medium containing 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin respectively. The A549 cell line belong to the *EGFR* wild-type lung adenocarcinoma cell line. H1975

is a L858R mutant with a T790M mutant cell line. All cells were routinely screened for the absence of mycoplasma, and at least 3 independent experiments were performed for each condition.

### ***Drug preparation for in vitro studies***

AZD9291 (Osimertinib) was purchased from Aladdin's official website, was dissolved in dimethyl sulfoxide (DMSO) to prepare 0.4-M stock solutions, which were aliquoted and stored at  $-80^{\circ}\text{C}$  and diluted as necessary for *in vitro* experiments.

### ***Construction of osimertinib-resistant cell lines A549/Osi and H1975/Osi***

The osimertinib-resistant cell lines were established using a stepwise method (33). Specifically, A549 and H1975 cell lines were cultured with increasing concentrations of AZD9291 starting at 0.5 mol/L. This was followed by a stepwise dose escalation every 7 days. After 7 months, A549 and H1975 cells could grow normally in 45  $\mu\text{mol/L}$  and 50  $\mu\text{mol/L}$  of AZD9291, respectively. The surviving cells were osimertinib-resistant cell lines and continued to be cultured in AZD9291-free medium for 2 weeks for subsequent studies.

### ***Functional test of drug-resistant cell lines***

#### **Cell proliferation assay**

Cell proliferation was assessed using cell counting kit 8 (CCK-8) assay to examine the effect of osimertinib on the 2 NSCLC cell lines. Cell suspensions were seeded in 96-well plate at a density of  $3\times 10^3$ , and various concentrations of osimertinib were added. After incubation at  $37^{\circ}\text{C}$  for 72 h, CCK-8 (Invigentech, Irvine, CA, USA) reagent was added to each well and further incubated for 2 h at  $37^{\circ}\text{C}$ . The absorbance of each well was measured at 450 nm using a microplate reader. The half maximal inhibitory concentration ( $\text{IC}_{50}$ ) value was defined as the concentration of AZD9291 needed for 50% reduction of growth. The corrected absorbance of each sample compared with that of the untreated control was calculated. Each experiment was performed independently 3 times.

#### **Cell migration assay**

We evaluated cell migration using scratch tests. In detail, the cells to be tested in the logarithmic growth stage were

collected and seeded into a 6-well plate at a concentration of  $3\times 10^5$  cells/well, and the cell was scraped with a 100- $\mu\text{L}$  sterile micropipette tip. Then, the serum-free medium was added to continue the culture. Finally, migratory cells were observed with an inverted fluorescence microscope (10 $\times$ ) at 0, 24, and 48 h and analyzed by using by Image-Pro Plus 6.0 software (Media Cybernetics, Rockville, MD, USA).

#### **Colony formation assay**

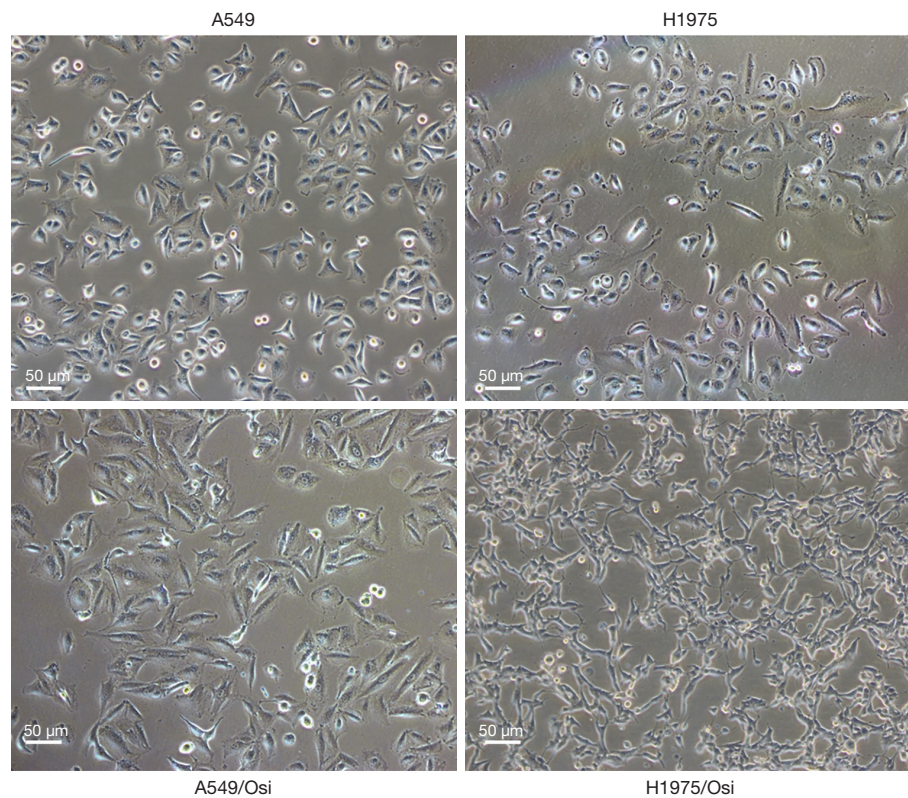
Cells ( $4\times 10^3$ ) were seeded in 6-well plates and cultured for 2 weeks at the above-described conditions. Then, these plates were washed with phosphate-buffered saline (PBS) twice, and colonies were fixed with methanol for 10 min and stained with 0.1% crystal violet. Finally, colony numbers were counted using a microscope.

#### **Transwell migration and invasion assay**

We evaluated cell migration and invasion using scratch tests. Approximately  $5\times 10^4$  cells in serum-free medium were seeded in the upper chamber. Moreover, medium supplemented with FBS was filled into the bottom wells of the chamber. After incubation for 24 h, the cells that had not migrated were removed from the upper surface using cotton swabs, while cells that had invaded into the lower surface were fixed with paraformaldehyde and stained with crystal violet. The invasion assay also required the ABW matrix glue to be stored at  $-80^{\circ}\text{C}$  and placed in a refrigerator at  $4^{\circ}\text{C}$  a day in advance to make it liquid. Serum-free medium and liquid substrate gel were thoroughly mixed at a dilution ratio of 1:8. The 100  $\mu\text{L}$  diluted matrix gel was absorbed and added to the upper chamber of the Transwell chamber. The numbers of invasive cell were counted by capturing different fields using a microscope.

#### **Bioinformatics analysis**

First, miRNA sequencing was performed on the third generation EGFR-TKI-sensitive cell lines and the corresponding constructed drug-resistant cell lines. Second, miRDeep2 software was used to predict and identify new miRNA mature (star miRNA and mature miRNA) and precursor sequences of the species by combining homologous miRNA sequences of closely related species, including RNA fold and other RNA secondary structures, and the expression of new miRNA in each sample was counted. Thirdly, Deseq2 software was used to analyze the differentially expressed genes (DEGs) between the experimental group (drug-resistant cell line) and control



**Figure 1** The parent cells A549 and H1975 are mostly polygonal in shape, with obvious cell outline and easy to grow into clusters, while drug-resistant cells A549/Osi and H1975/Osi cells grow relatively slowly, showing spindle type and irregular cell outline, loss of epithelioid cell morphology, round vacuoles in cytoplasm, and an increase in the intercellular spaces ( $\times 100$ , scale bar =  $50\ \mu\text{m}$ ).

group (normal cell lines), when meet statistical P value  $< 0.05$  and  $|\log_2 \text{ multiples (difference)}| > 1$  conditions for the differences of miRNAs. Compared with normal cells, in drug-resistant strains, miRNAs that meet  $\log_2(\text{difference multiple}) > 1$  are up-regulated genes, and  $\log_2(\text{difference multiple}) < -1$  are down-regulated genes, while those that do not meet the above conditions are non-significant differentially expressed genes. Finally, miRNA target genes were predicted using miRanda and RNAhybrid, and the intersection of the 2 methods was taken as the final result. The R package “clusterProfiler” was used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis based on all target genes of the differential miRNAs labeled as up or down in the differential expression analysis results. According to the PolymiRTS database, the Single Nucleotide Polymorphism Arrays (SNP) of the different known miRNAs were annotated. The above-described bioinformatic analysis steps were entrusted to Shanghai Tianhao Biotechnology Co.,

Ltd. (Shanghai, China) for completion.

### Statistical analysis

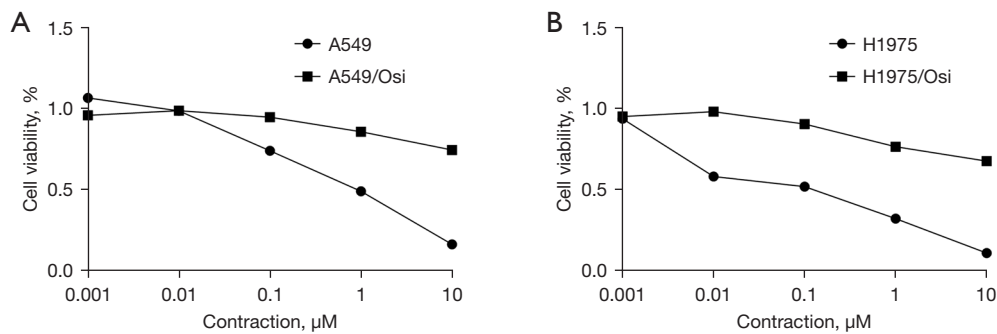
All results are presented as the mean  $\pm$  standard error of the mean (SEM) and were analyzed using SPSS 20.0 software (IBM Corp., Armonk, NY, USA). The standard Student *t*-test was used to determine the significance differences between the drug-resistant cell line and parent cell lines.  $P < 0.05$  denoted statistically significant difference.

## Results

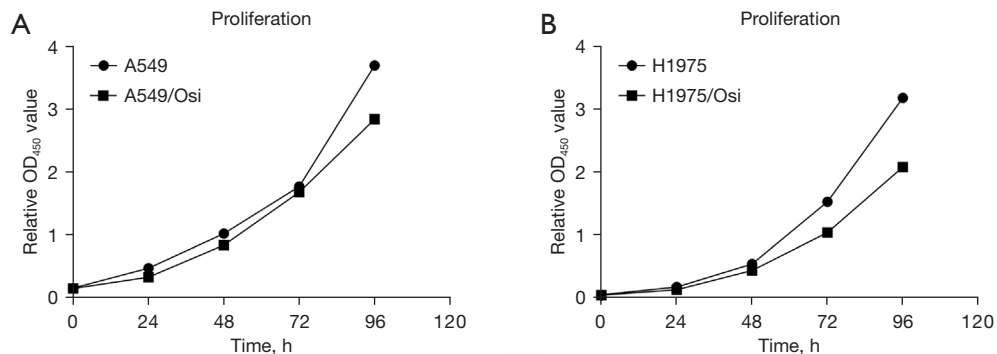
### Morphological observations of drug-resistant cells

A morphological observation of drug-resistant cells is shown in *Figure 1*. Compared with the parent cells, drug-resistant cells tended to be spindle-like and irregularly shaped, with antennae, loss of epithelioid cell morphology, round





**Figure 2** The viability of parent cells A549 (A) and H1975 (B) and drug-resistant cells A549/Osi and H1975/Osi decreased with increasing AZD9291 concentration (0.001, 0.01, 0.1, 1 and 10 μmol/L, respectively). But compared with the parent cells, the decline of drug-resistant cells was not obvious, and the trend was gentle, and gradually tended to be stable.



**Figure 3** The OD of each cell line at different time was determined by enzymometer at 450 nm wavelength. The number of parent cells A549 (A) and H1975 (B) and drug-resistant cells A549/Osi and H1975/Osi increased gradually with the extension of time (24, 48, 72 and 96 h, respectively), and the proliferation ability of drug-resistant cells decreased slightly compared with the parent cells. OD, optical density.

vacuoles in cytoplasm, and an increase in the intercellular spaces with relatively slow growth (Figure 1).

#### Cell viability and proliferation analyses of resistant A549/Osi and H1975/Osi cells

The cell viability and proliferation of resistant cells were measured with CCK8 assay. The results showed that the cell vitality of A549/Osi- and H1975/Osi-resistant cells was significantly increased (Figure 2), while the proliferation ability was slightly decreased and proliferation capacity compared with the parent cells (Figure 3). The mean IC<sub>50</sub> values of A549, A549/Osi, H1975 and H1975/Osi cells were 0.093, 2.165, 0.022 and 2.445, respectively. Therefore, the resistance indexes (RI) of A549/Osi cells and H1975/Osi cells were 23.226 and 109.215, respectively. This promoted the successful construction of AZD9291 resistant cell lines (if RI >5, it is considered that drug-resistant cell lines meet the

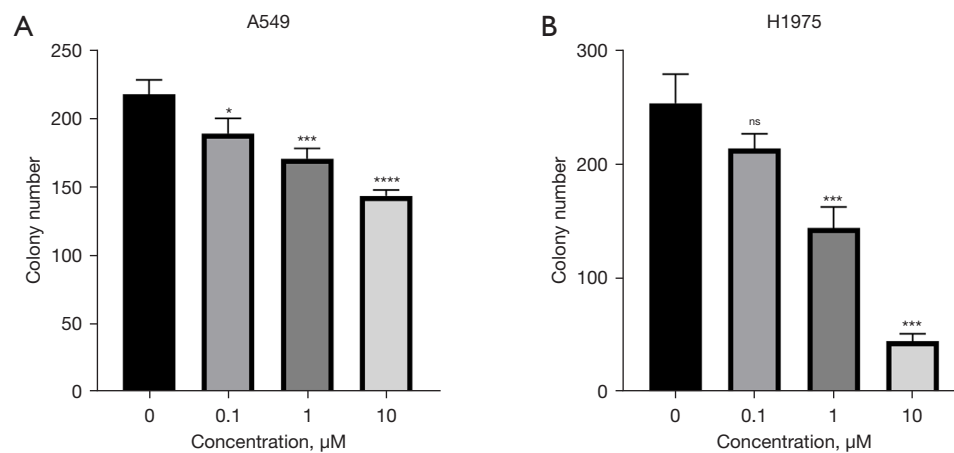
requirements of drug-resistant strains).

#### Clone formation analyses of resistant A549/Osi and H1975/Osi cells

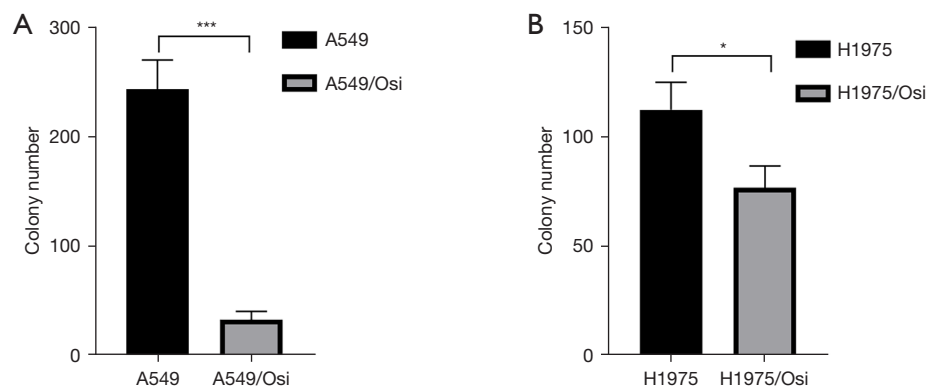
The results of the clone formation experiment showed that the colony number in the parent A549 and H1975 cells decreased with the increase in the concentration and thus in a dose-dependent manner ( $P < 0.05$ ) (Figure 4). In the absence of AZD9291, the number of colonies of A549/Osi and H1975/Osi cells was significantly lower than that of the parent cells ( $P < 0.05$ ) (Figure 5).

#### Migration and invasion capability analyses of resistant A549/Osi and H1975/Osi cells

Experiments on scratch (Figure 6) and Transwell experiments (Figure 7) were conducted to determine the



**Figure 4** The number of colonies of parent cells A549 (A) and H1975 (B) decreased dose-dependent with increasing AZD9291 concentration (0, 0.1, 1 and 10 µmol/L, respectively), and the difference was more significant at 1 and 10 µmol/L ( $P < 0.01$ ). \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$ ; \*\*\*\*,  $P < 0.0001$ ; ns, P value is not statistically significant.



**Figure 5** In the absence of AZD9291, the number of colonies of A549/Osi (A) and H1975/Osi (B) cells was significantly lower than that of the parent cells. EGFR mutant cell lines H1975 decreased less than wild cell lines A549. \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$ .

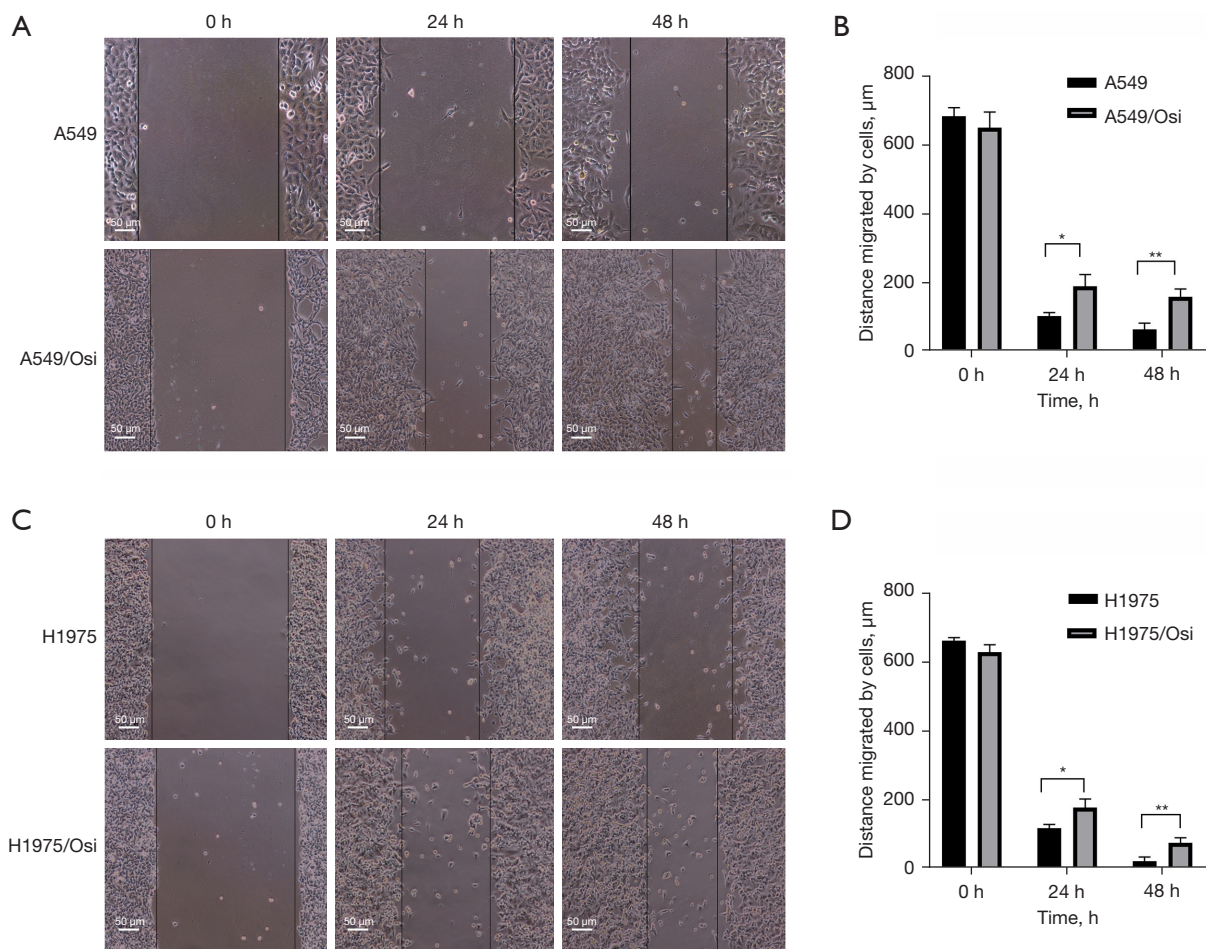
migration and invasion capabilities of cells. The results revealed that the cell migration capacity of A549/Osi- and H1975/Osi-resistant cell was markedly increase compared with that in the parent cells in 24 and 48 h ( $P < 0.05$ ).

#### *Differences in known miRNA expression in resistant A549/Osi and H1975/Osi cells*

miRDeep2 software was used to compare the miRNA mature bodies in each sample and 2,656 miRNA expression levels were determined. Meanwhile, new miRNAs could be predicted and the expression levels of 1,420 potential new miRNAs were determined based on miRNA hairpin structure. By principal component analysis, it was found

that samples in four different groups before and after drug resistance were well clustered, and all were grouped into one group respectively. The difference before and after drug resistance of A549 cell strain was greater than that before and after drug resistance of H1975 cell strain (*Figure 8*).

A total of 140 miRNAs were significantly differentially expressed in A549 cells, of which 64 were up-regulated and 76 were down-regulated after drug resistance (*Figure 9*). miR-708-5p, miR-516a-5p, miR-204-5p, miR-3085-3p and miR-708-3p ranked the top five in terms of up-regulated miRNA difference multiples. miR-3152-5p, miR-5000-5p, miR-122-3p, miR-122-5p, and miR-548w were the top five differences in the down-regulated miRNAs, respectively. In H1975 cells, 42 known miRNAs were differentially



**Figure 6** The parent cells A549 (A), H1975 (C) and the corresponding drug-resistant cells A549/Osi, H1975/Osi were cultured in 6-well plates, and the serum-free medium was added for scratch experiment. It was found that the migration rate of drug-resistant cells was faster than that of the parent cells. Statistical analysis showed that there was a significant difference between the two groups after 24 h ( $P < 0.05$ ), and the difference between the two groups after 48 h was more obvious ( $P < 0.01$ ;  $\times 100$ , scale bar = 50  $\mu\text{m}$ ) (B,D). \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

expressed before and after drug resistance. Compared with normal strains, 27 miRNAs were up-regulated and 15 were down-regulated in drug-resistant cells (Figure 9). Among them, miR-1268a, miR-7704, miR-4508, miR-10400-5p and miR-708-5p ranked top five in the order of up-regulated miRNA difference multiples. miR-127-3p, miR-19b-1-5p, miR-1-3p, miR-194-3p, and miR-25-5p ranked the top five differences in the down-regulated miRNA, respectively.

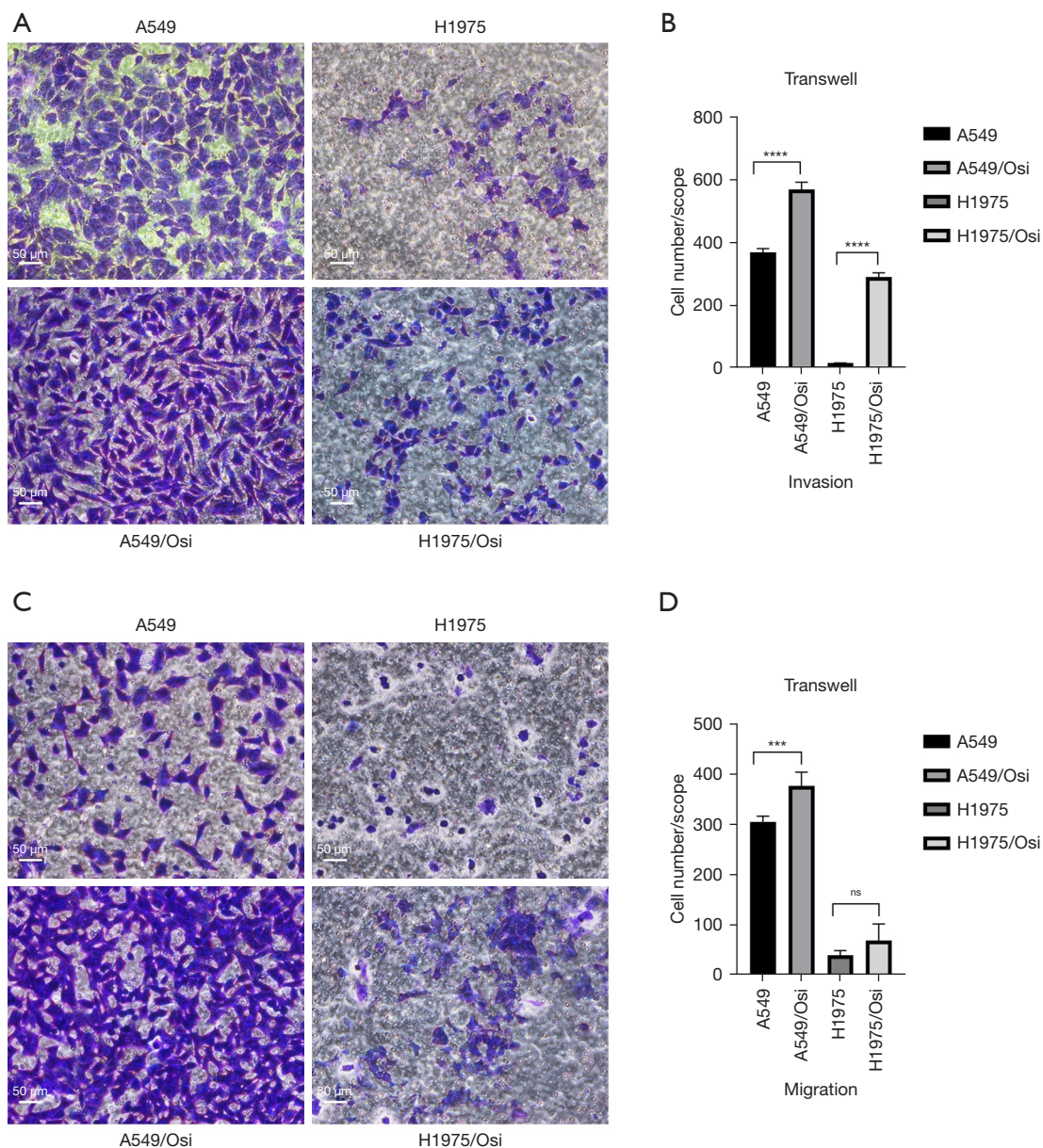
By combining the known miRNAs differentially expressed before and after drug resistance in the two cell lines, it was found that there were 9 miRNAs with significant differences in the two cell lines (Figure 9), and the differentially expressed trend of 7 miRNAs was consistent in the two cell lines. miR-708-5p, miR-708-

3p, miR-10395-3p, miR-7704 and miR-34a-5p were significantly up-regulated in drug-resistant strains, while miR-19b-1-5p and miR-219a-5p were significantly down-regulated in drug-resistant strains. However, the differences between miR-1-3p and miR-138-5p were inconsistent.

#### *Differential Gene Ontology (GO) enrichment in resistant A549/Osi and H1975/Osi cells*

The target genes of differentially expressed miRNA were studied by GO and KEGG enrichment. By GO enrichment analysis, significantly differentially expressed genes were enriched according to biological process (BP), cell component (CC) and molecular function (MF). The most significantly enriched items are selected from the three





**Figure 7** The invasion experiment and migration experiment of each cell, respectively (A,C). After crystal violet staining, blue cells can be seen, and drug-resistant cells are significantly more than the parent cells. Statistical analysis showed that the drug-resistant cells had significant differences in invasion (B) and migration (D) experiments compared with the parent cells ( $\times 100$ , scale bar =  $50 \mu\text{m}$ ). \*\*\*,  $P < 0.001$ ; \*\*\*\*,  $P < 0.0001$ ; ns, P value is not statistically significant.

categories of BP, CC, and MF respectively and shown in the figure (Figure 10).

#### Differential gene KEGG enrichment distribution plot

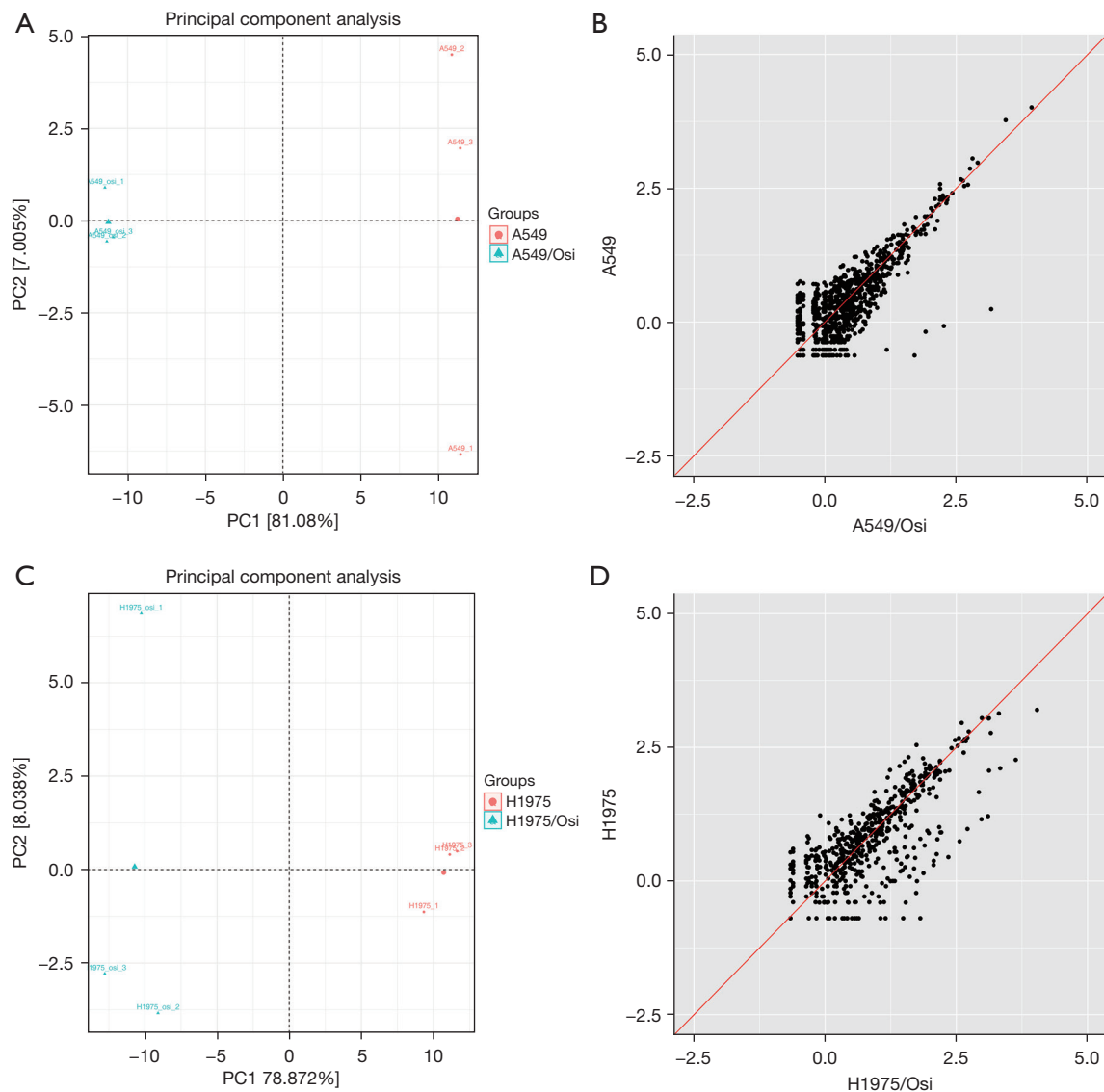
Scatter plots were used to show the results of KEGG enrichment, which were mainly measured by GeneRatio,

P value and the number of genes enriched in this pathway. The results showed that MAPK signaling pathway was significant in both groups of cells (Figure 11).

#### SNP annotation results of known miRNAs

SNPs of miRNAs may affect miRNA-messenger RNA





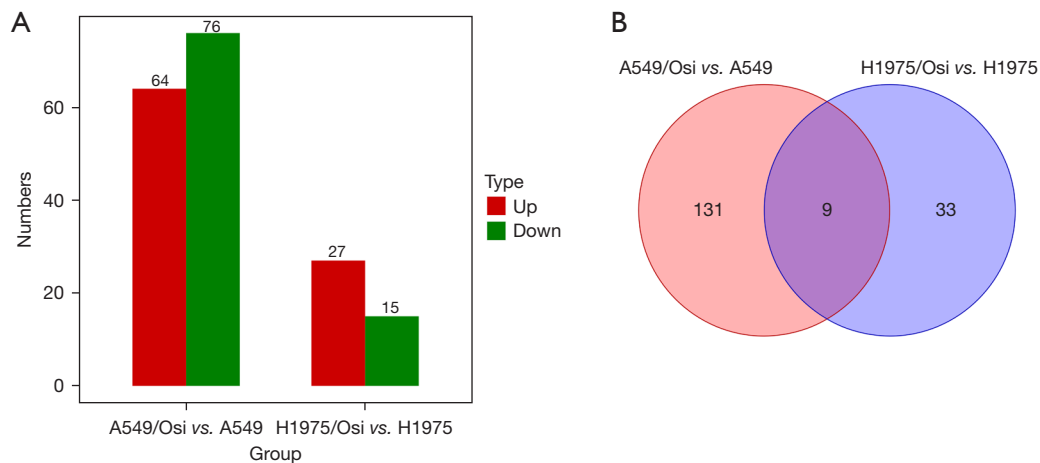
**Figure 8** The scatter plot of the logarithm of the corrected average expression level of a gene in 2 samples or groups. Each point represents the logarithm of the corrected average expression level of a gene in 2 samples or groups. The fewer points there are deviating from the diagonal, the higher the correlation of expression between samples, and the smaller the difference of expression between genes. PC, principal component.

(mRNA) interactions, thereby altering gene expression and disease phenotype. In this study, 2 methods, miRanda and RNAhybrid, were used to predict miRNA target genes, with the intersection of the 2 methods being taken as the final result (Table 1).

## Discussion

Osimertinib is currently the first choice for postoperative

adjuvant therapy and advanced first-line treatment of *EGFR*-sensitive mutated NSCLC, and it is also the standard treatment for T790M mutation after first- and second-generation *EGFR*-TKI resistance. However, unfortunately, in most patients, resistance ultimately develops after an initial response to osimertinib, and the underlying mechanism of this resistance is complex. Most patients' drug resistance mechanism is not clear, and there is no follow-up precise treatment, with the prognosis being poor (34,35).



**Figure 9** The bar chart of the differentially expressed genes. Genes with significantly upregulated differential expression are shown in red and genes, and those with significantly downregulated differential expression are shown in green (A). Venn diagram shows the distribution of differentially expressed genes between comparisons (B).

Individual miRNAs play a key role in targeting and regulating the expression of numerous mRNAs either by promoting mRNA degradation or inhibiting their translation (21). Aberrant miRNAs expression patterns can be used as diagnostic and prognostic markers in NSCLC (36). A large number of studies have reported the role of miRNAs in cancer progression, such as proliferation, invasion, metastasis, metabolism, and drug resistance (24,37-40). Importantly, studies have identified that cancer cells develop drug resistance via multiple complex mechanisms that involve in various miRNAs by intervening in cell-cell interaction (26,27). In this study, through the biosynthesis analysis, we identified the differential expression patterns among miRNAs after cell resistance. Specifically, the expression of miR-708-5p, miR-708-3p, miR-10395-3p, miR-7704, and miR-34a-5p was upregulated while the expression of miR-19b-1-5p and miR-219a-5p was downregulated.

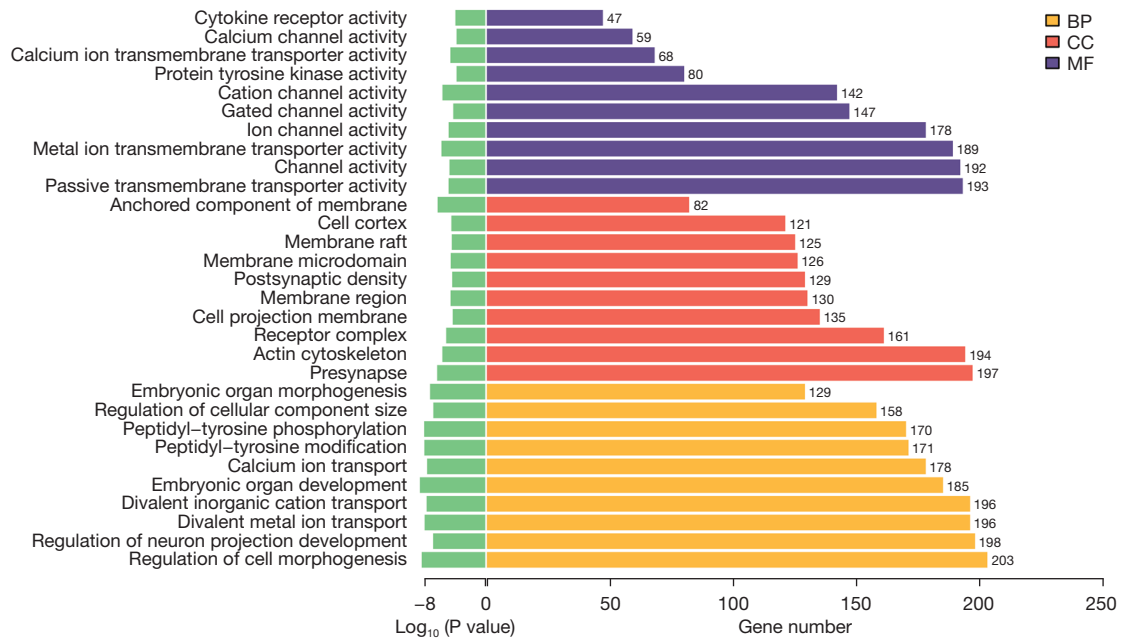
Other research has demonstrated that miR-708 suppresses proliferation, survival, and migration of lung cancer cells (41). Specifically, chemoresistant cells were found to reduce miR-708-5p expression compared to naïve lung cancer cells, and combination treatment with miR-708-5p was shown to partially restore the antiproliferative effects of chemotherapy and fully restored its proapoptotic qualities. Findings from our study were inconsistent with these results, and may possibly be explained by the following. First, different treatments were involved in the different proresistance pathways against chemoresistant

and TKI-resistant cells. Second, the same miRNAs may have different effects that are modulated in a specific setting depending on their direct targets and the activated downstream signaling pathways. Finally, there also may other stress-associated transcription factors promoting miR-708-5p expression.

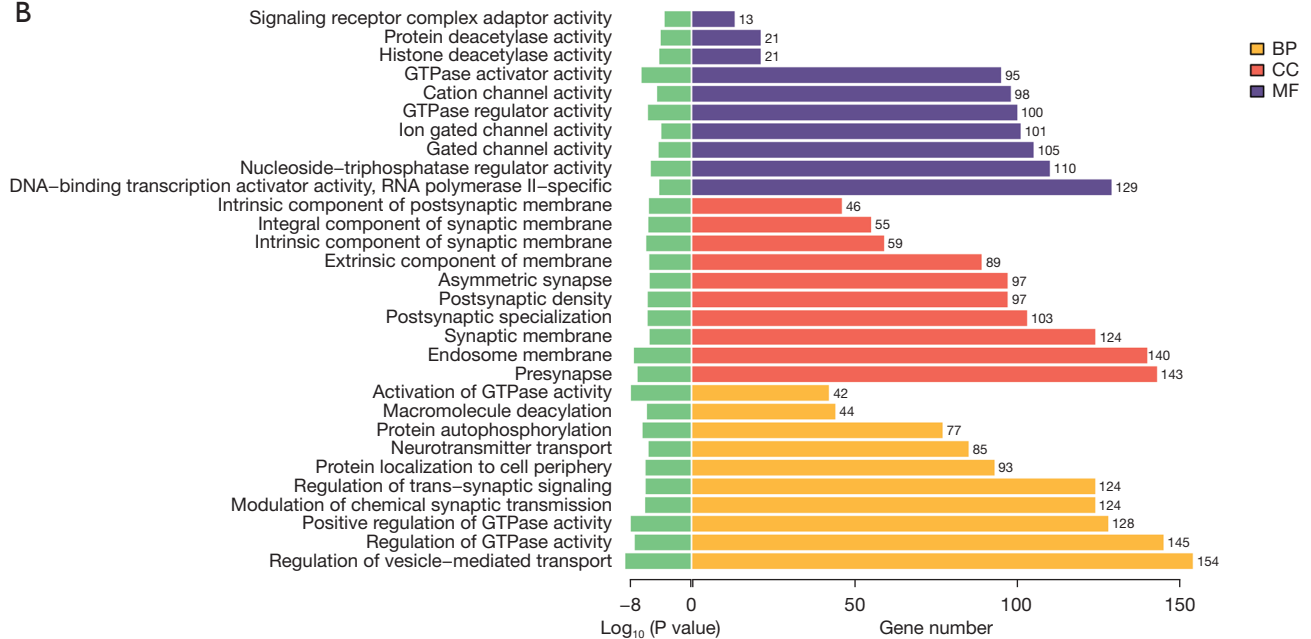
To date, several lines of evidence have shown that miR-34 and miR-219 regulate the survival and motility of cancer cells by activating different multiple targets. miR-34 and miR-219 downregulation is a frequently observed in NSCLC cell lines (42,43). However, our study is the first of its kind to investigate the potential association between miR-219, miR-34, and osimertinib resistance. Therefore, although current studies lack relevant functional assays, it is reasonable to speculate that resistance effects of miRNAs can be combined.

In this study, 7 miRNAs were found to be associated with osimertinib resistance in A549 and H1975 cell lines according to biosynthesis analysis. miR-708-5p, has been shown to repress pro-resistant signaling pathways, including COX-2 and mPGES-1. Moreover, combination treatment of erlotinib (ERL) or paclitaxel (PAC) with miR-708-5p enhances COX-2 and mPGES-1 protein suppression. combination chemotherapeutic and miR-708-5p treatment intensifies the anti-proliferative and pro-apoptotic effects of ERL and PAC (41). It has been confirmed that the regulation of EMT inhibition mediated by miR-34a-5P- and HOTAIR may provide a new therapeutic paradigm

A



B

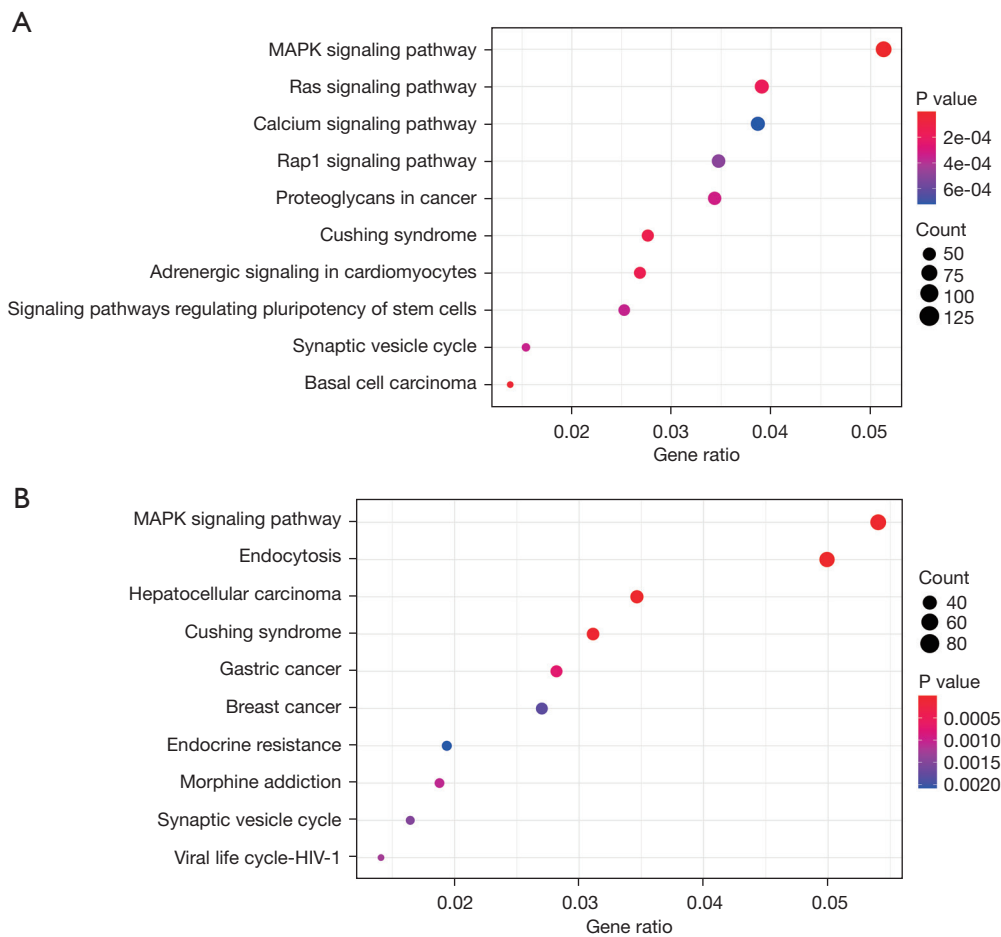


**Figure 10** The GO enrichment. The longer the P value line is in the figure, the larger the P value, and the lower its significance. The color corresponds to the size of the P values, with a deeper color representing smaller and more significant P value, indicating a more reliable the result of GO enrichment. GO, Gene Ontology; CC, cell components; BP, biological process; MF, molecular function.

for lung cancer (44). The reviews of the relevant literature points to miR-708-5p and miR-34a-5p as having particular importance (45). Therefore, miR-708-5p and miR-34a-

5p need to be further analyzed. Further research directions relevant to this study include comparing the expression of serum miRNAs in patients with *EGFR*-sensitive mutant





**Figure 11** The KEGG enrichment. The abscissa is the gene ratio, which represents the ratio between the number of differentially expressed miRNAs under the pathway entry and the total number of differentially expressed genes. The higher the value the gene ratio is, the higher the value of the  $-\log_{10}$  (Q-value), the higher the enrichment degree of the differentially expressed miRNAs in this KEGG pathway. The closer the item in the figure is to the upper right corner, the higher the enrichment degree and the greater the reference value. KEGG, Kyoto Encyclopedia of Genes and Genomes.

**Table 1** SNP annotation results of known miRNAs

miRNA	rs_id	Alleles	miRNA_position
hsa-miR-429	rs370935426	C/T	4
hsa-miR-429	rs375039921	C/T	14
hsa-miR-429	rs368678282	A/G	21
hsa-miR-548al	rs515924	A/G	8
hsa-miR-548al	rs145394973	A/T	13
hsa-miR-1304-3p	rs2155248	A/C	-8
hsa-miR-615-5p	rs375090644	A/T	13
hsa-miR-625-3p	rs368340850	C/G	3

**Table 1** (continued)

Table 1 (continued)

miRNA	rs_id	Alleles	miRNA_position
hsa-miR-625-3p	rs12894182	A/C	19
hsa-miR-3677-3p	rs138871259	A/G	4
hsa-miR-3677-3p	rs373356017	C/T	3
hsa-miR-122-3p	rs41292412	C/T	3
hsa-miR-4746-5p	rs375980769	G/T	19
hsa-miR-4746-5p	rs369043795	C/T	20
hsa-miR-181d-5p	rs201654897	A/G	17
hsa-miR-643	rs371710100	A/G	1
hsa-miR-516a-5p	rs368499496	C/T	20
hsa-miR-1-3p	rs377171105	G/T	22
hsa-miR-941	rs111479365	A/G	12
hsa-miR-941	rs113283070	C/G	7
hsa-miR-941	rs113698386	C/G	15
hsa-miR-941	rs113309089	A/G	12
hsa-miR-941	rs113946605	C/G	15
hsa-miR-941	rs112466552	A/G	12
hsa-miR-941	rs34604519	C/G	15
hsa-miR-941	rs111993072	A/G	12
hsa-miR-941	rs113672516	A/G	6
hsa-miR-941	rs12625454	C/G	15
hsa-miR-941	rs111586745	A/G	12
hsa-miR-941	rs35544770	A/G	6
hsa-miR-944	rs190786647	C/G	16
hsa-miR-944	rs77473380	C/T	15
hsa-miR-145-3p	rs190323149	C/T	6
hsa-miR-7705	rs3202310	C/T	-1
hsa-miR-188-5p	rs186369276	G/T	19
hsa-miR-500a-5p	rs369176381	G/T	8
hsa-miR-676-3p	rs376251880	A/G	17
hsa-miR-664b-3p	rs112159031	A/G	8

miRNA, microRNA; SNP, single-nucleotide polymorphism.

NSCLC before and after osimertinib resistance, and the possibility of animal experiments to determine the role of miRNAs in drug resistance after intervention; whether the tumor can regain its sensitivity to osimertinib.

## Conclusions

In A549 and H1975 cell lines, osimertinib resistance was associated with the upregulation of miR-708-5p, miR-708-3p, miR-10395-3p, miR-7704, and miR-34a-5p expression,

and the downregulation of miR-19b-1-5p and miR-219a-5p expression. These results, in combination with the relevant literature, suggest that miR-708-5p and miR-34a-5p may play a tumor-promoting role in NSCLC and be positively correlated with osimertinib resistance.

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