European Review for Medical and Pharmacological Sciences 2019; 23: 5831-5839

MiR-145 changes sensitivity of non-small cell lung cancer to gefitinib through targeting ADAM19

Y. WANG, Y.-M. LIAN, C.-Y. GE

Department of Respiratory Medicine, Liaocheng People's Hospital, Liaocheng, China

Abstract. – OBJECTIVE: The aim of this study was to investigate the role of micro-ribonucleic acid (miR)-145 in acquired resistance of non-small cell lung cancer (NSCLC) to gefitinib, and to explore its potential mechanism.

MATERIALS AND METHODS: PC-9 cells were continuously stimulated with low-concentration of gefitinib to induce the formation of acquired gefitinib-resistant PC-9/G cells. The sensitivity of PC-9 and PC-9/G cells to gefitinib was detected *via* Cell Counting Kit-8 (CCK-8) assay. The expressions of miR-145 and adamalysin-19 (ADAM19) in PC-9 and PC-9/G cells were detected *via* quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) and Western blotting. Subsequently, PC-9 and PC-9/G cells were transfected with miR-145 mimics and miR-145 NC, respectively. The changes in ADAM19 expression were detected *via* qRT-PCR and Western blotting. The changes in the sensitivity of cells to gefitinib after transfection were explored *via* CCK-8 assay. Moreover, the influences of miR-145 transfection on cell apoptosis, invasion and migration were detected *via* flow cytometry, wound healing assay and transwell assay, respectively. Target gene and acting site of miR-145 were verified *via* Dual-Luciferase reporter gene assay. Furthermore, targeted regulation of miR-145 on ADAM19 was verified by *in vitro* cellular experiments.

RESULTS: The sensitivity of PC-9/G cells to gefitinib was significantly lower than that of PC-9 cells, with nearly 15-fold difference in half maximal inhibitory concentration (IC-50) ($p<0.05$). QRT-PCR results indicated that miR-145 expression in PC-9/G cells was significantly decreased ($p<0.01$). The results of Western blotting showed that the expression level of ADAM19 in PC-9/G cells was markedly higher than that of PC-9 cells. The overexpression of miR-145 could remarkably reduce the expression level of ADAM19 in PC-9/G cells, increase the sensitivity of PC-9/G cells to gefitinib, and inhibit cell invasion and metastasis. The detection of Luciferase activity revealed that miR-145 could bind to the 3'-untranslated region (UTR) of ADAM19 gene and negatively regulate the protein expression.

CONCLUSIONS: MiR-145 improves the sensitivity of acquired gefitinib-resistant cells to gefitinib. Meanwhile, it inhibits cell invasion and metastasis through negative regulation on ADAM19. Furthermore, the low-expression of miR-145 may become a biomarker and therapeutic target for acquired resistance to gefitinib.

Key Words:
MiRNA-145, ADAM19, Non-small cell lung cancer (NSCLC), Gefitinib, Drug resistance.

Introduction

According to global statistics, lung cancer has become one of the most common malignant tumors with the highest morbidity rate[1]. The vast majority of lung cancer is non-small cell lung cancer (NSCLC). Currently, the therapeutic strategy of NSCLC is still comprehensive therapy based on the operation. Due to the hidden symptoms of NSCLC, many patients are already in the progressive stage (above stage IIIB) when diagnosed. Therefore, they have lost the opportunity of radical operation[2]. Compared with conventional radiotherapy and systemic chemotherapy, epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) has obtained satisfactory efficacy in the treatment of EGFR-mutant NSCLC[3]. However, acquired resistance to EGFR-TKI occurs sooner or later during treatment[4], seriously affecting the therapeutic effect of EGFR-TKI[5]. Increasingly more attention has been paid to the mechanism of acquired resistance to EGFR-TKI. At present, the research mainly focuses on secondary mutation of the EGFR gene, abnormal activation of EGFR downstream pathway, epithelial-mesenchymal transition (EMT), transformation into SCLC and bypass activation of EGFR pathway[6,7]. However, the exact mechanism remains unclear. Micro-ribonucleic acid (miRNA) is a non-coding small-molecule single-stranded RNA with about 18-25 nt in length[8]. A larger number of studies have demonstrated that miRNA is involved in the occurrence and development of a variety of malignant tumors. Some miRNAs, known as oncogenic miRNAs, are overexpressed in tumors to promote

Corresponding Author: Cuiyun Ge, MM; e-mail: jiangqinghe2008@163.com

its occurrence and development. However, some other miRNAs, known as tumor suppressor miRNAs, can inhibit tumor formation and interfere with malignant behaviors of tumors[9]. In the research on the mechanism of acquired resistance to EGFR-TKI, it is reported that miRNA is involved in regulating drug resistance formation[10]. Li et al[11] have found that the overexpression of miR-21 promotes the resistance of NSCLC to EGFR-TKI. No studies have explored the correlation between miR-145 and gefitinib resistance in NSCLC so far. Therefore, this work aims to explore the correlation between miR-145 and gefitinib resistance in lung cancer and its possible mechanism. Our findings may provide some ideas for the treatment of patients with drug-resistant lung cancer.

Materials and Methods

Cell Culture

PC-9 cells were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Drug-resistant PC-9/G cells were produced *via* gefitinib in gradient concentration. First, PC-9 cells were cultured in complete medium containing 0.01 μM gefitinib. The medium was replaced with the drug-free medium after 48 h until cell fusion of 70-80%. This process was constantly repeated with a higher concentration of gefitinib until the cells could grow in gefitinib (0.5 μM). PC-9 and PC-9/G cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), 100 U/L penicillin and 100 μg/L streptomycin in a wet incubator with 5% CO_2 at 37°C. The culture medium was replaced once every 48 h, and the cells were passaged when 80-90% cells were fused.

Transfection of MiR-145

MiR-145 mimics and miR-145 NC were purchased from GenePharma (Shanghai, China). PC-9 and PC-9/G cells were transfected with miR-145 mimics or miR-145 NC for 48 h according to the manufacturer's instruction of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The sequences were as follows: miR-145 mimics forward: 5'-ATCGTCCAGTTTTCCCAGG-3', reverse: 5'-CGCCTCCACACACTCACC-3', miR-145 NC forward: 5'-ATTGGAACGATACAGAGAAGATT-3', reverse: 5'-GGAACGCTTCACGAATTTG-3'.

Luciferase Reporter Gene Assay

PC-9/G cells were first inoculated into 12-well plates at a density of 1×10^5 cells/well. When the cell density reached 60%, the corresponding transfection reagents were prepared, including 50 ng wild-type or mutant-type 3'-untranslated region (UTR) reporter plasmids, miR-145 mimics or miR-145 NC at a final concentration of 20 nM, and Lipofectamine 2000. 48 h after transfection, the corresponding Luciferase activity of PC-9/G cells was detected in strict accordance with the Dual-Luciferase reporter gene assay kit (Beyotime, Shanghai, China).

Real-Time Fluorescence Quantitative Polymerase Chain Reaction (qPCR)

Total messenger RNA (mRNA) was extracted from cells using TRIzol (Invitrogen, Carlsbad, CA, USA). Extracted RNA was reverse transcribed into complementary deoxyribonucleic acid (cDNA) under the following conditions: 25°C for 10 min, 50°C for 30 min, and 85°C for 5 min. Subsequently, detection was performed using the fluorescence qPCR kit (TaKaRa, Otsu, Shiga, Japan). The primer sequences were as follows: miR-145 forward: 5'-GTCCAGTTTTCCCAGGAATCCCT-3', reverse: 5'-GCTGTCAACATACGCTACGTAACG-3'. Adamalysin-19 (ADAM19) forward: 5'-GAACATCCGGATTGCTCTCG-3', reverse: 5'-TGGGTCATGCCAAAGTTGTG-3'. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (internal reference) forward: 5'-GGTCTCCTCTGACTTCAACA-3', reverse: 5'-AGCCAAATTCGTTGTCATAC-3'. Fluorescence qPCR conditions were as follows: 95°C for 5 min, 95°C for 15 s, 60°C for 1 min, for a total of 40 cycles. The temperature in the melting curve was set as 60-95°C, and 3 repeated wells were set for each sample.

Cell Proliferation Assay

PC-9/G cells were first digested with trypsin and inoculated into 96-well plates at a density of 8×10^3 cells/well. The volume of the complete medium was 200 μL. The proliferation of PC-9 and PC-9/G cells after treatment with different concentrations of gefitinib for 48 h was detected using the methyl thiazolyl tetrazolium (MTT) kit (Sigma-Aldrich, St. Louis, MO, USA). Meanwhile, the proliferation of PC-9/G cells at 24 h, 48 h, 72 h and 96 h after transfection with miR-145 mimics or miR-145 NC was also detected.

Colony Formation Assay

PC-9/G cells were inoculated into 6-well plate at a density of 400 cells/well, followed by culture for 24 h. When cells adhered to the wall and spread well, the original medium was replaced with the drug-free medium for 15 days of culture. Then, the cells were washed with Phosphate-Buffered Saline (PBS; Gibco, Grand Island, NY, USA), fixed with 10% formaldehyde (Beyotime, Shanghai, China), and stained with Giemsa. Finally, photography was performed. Cell colonies formed by more than 50 cells indicated 1 clone.

Flow Cytometry

After transfection with miR-145 mimics or miR-145 NC, PC-9/G cells were digested into a single-cell suspension. The cell concentration was adjusted to 5×10^3 cells/mL. 1 mL of cell suspension was centrifuged at 1,000 rpm and 4°C for 10 min. Subsequently, the supernatant was discarded, and 1 mL of cold PBS was added and gently vibrated. After centrifugation at 1,000 rpm and 4°C for 10 min, the supernatant was discarded. The cells were re-suspended in 200 μL buffer. After that, 10 μL of Annexin V-fluorescein isothiocyanate (FITC) and 5 μL propidium iodide (PI) were added and mixed evenly, followed by reaction at room temperature for 10 min in a dark place. Finally, flow cytometry was detected by a flow cytometer (Partec, Arlesheim, Switzerland).

Western Blotting

The total protein was extracted from cells using the lysis buffer. 30 g/well protein samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under 350 mA and transferred to activated polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) for 2 h. After sealing with 5% skim milk powder for 1 h, the membranes were incubated with primary antibody at 4°C overnight. Then, the membranes were washed with Tris-Buffered Saline with Tween-20 (TBST; Thermo Fisher Scientific, Waltham, MA, USA), and incubated with horseradish peroxidase (HRP)-labeled secondary antibody at room temperature for 1 h. After washing again with TBST, the image was developed, and the gray value was analyzed using Image J software (Bethesda, MD, USA). The relative expression level of the target protein was calculated by optical density $_{\text{target protein}}$/optical density $_{\beta\text{-actin}}$.

Wound Healing Assay

PC-9/G cells were inoculated into 6-well plates and cultured. When 90-100% cells were fused, 6-well plates were scratched uniformly in the same width using a 10 μL spearhead vertically to the bottom of the plate. Floating cells were removed using PBS and the serum-free medium was added, followed by incubation in a CO_2 incubator. 0 h and 48 h after scratching, the migration distance in the scratch area was observed under a microscope. Several different fields of view were randomly selected and photographed.

Transwell Migration and Invasion Assay

After transfection, PC-9/G cells were cultured until the logarithmic growth phase. Then, the cells were digested, washed once with PBS and once with the serum-free medium, suspended in serum-free medium and counted. Cell concentration was adjusted to 1×10^5 cells/mL. 600-800 μL of the medium containing 10% serum was added into the lower chamber. Meanwhile, 100-150 μL of cell suspension was added into the upper chamber, followed by incubation in an incubator for 24 h. The chambers were then removed, and the liquid in the upper chamber was sucked dry. The cells were fixed with methanol and stained with Giemsa dye. The number of migrated and invaded cells was counted under the microscope, and cell invasion and migration ability were compared among different groups.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. Measurement data were expressed as mean ± standard deviation ($\bar{\chi}\pm s$). *t*-test was adopted to compare the difference between the two groups. One-way ANOVA was performed to compare the difference among different groups, followed by post-hoc test LSD (Least Significant Difference). $p<0.05$ was considered statistically significant.

Results

PC-9/G Cells Were More Resistant to Gefitinib Than PC-9 Cells, and MiR-145 Expression Was Downregulated in PC-9/G Cells

PC-9 cells were very sensitive to gefitinib due to the deletion mutation of exon 19 in the EGFR gene. Gefitinib-resistant PC-9/G cells were induced by the above method for half a year. The sensitivity of PC-9 and PC-9/G cells to gefitinib was detected *via* CCK-8 assay. The results re-

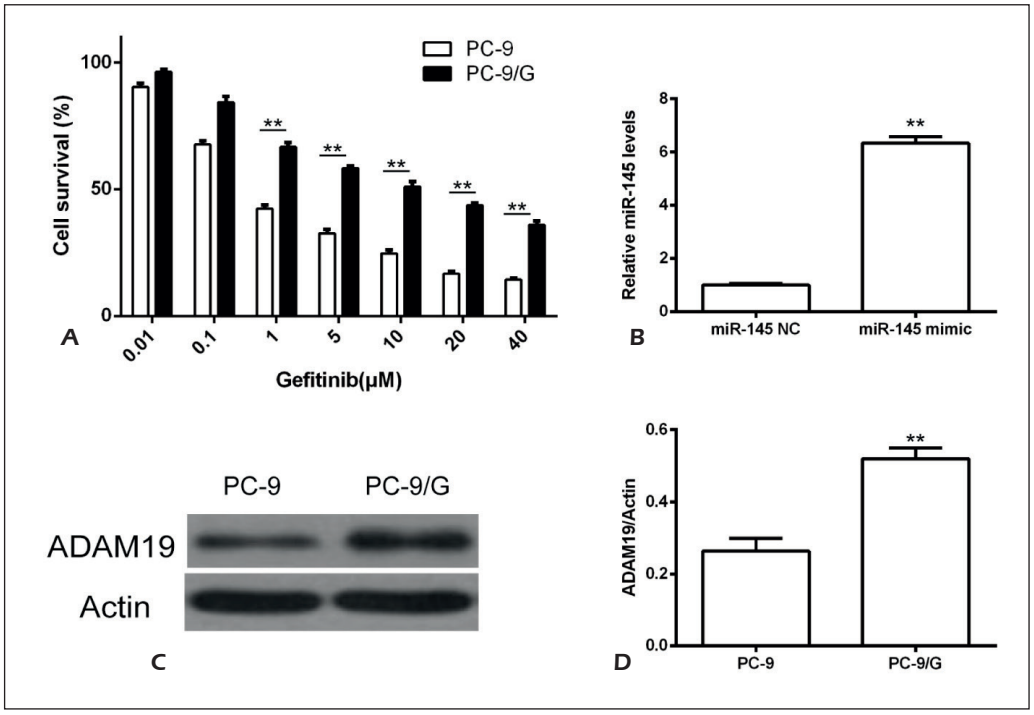

Figure 1. MiR-145 expression in PC-9 and gefitinib-resistant PC-9/G cells. **A**, Cell proliferation curves of PC-9/G and parental cells were determined by CCK8 assay after treated with different concentrations of gefitinib for 48 h. **B**, The expression level of miR-145 in PC-9 and PC-9/G cells was detected. **C-D**, The expression levels of ADAM19 in PC-9 and PC-9/G cells were detected by Western blotting. Data were presented as means ± SD (n=3). $^{**}p<0.01$ *vs.* control group.

vealed that PC-9 cells were very sensitive to gefitinib, half maximal inhibitory concentration (IC-50) of which was 0.66 μM. PC-9/G cells were much more resistant to gefitinib (IC-50: 9.8 μM) when compared with PC-9 cells, and the difference was statistically significant ($p<0.05$) (Figure 1). After confirming the resistance of PC-9/G cells to gefitinib, the expression of miR-145 in PC-9/G and PC-9 cells was further detected *via* RT-PCR. The results showed that the expression of miR-145 in PC-9/G cells was markedly lower (about 1/4) than that of the PC-9 cells, showing a statistically significant difference ($p<0.05$)

Upregulation of MiR-145 Expression Changed the Sensitivity of PC-9/G Cells to Gefitinib and Inhibited Cell Proliferation Ability

The expression of miR-145 was markedly down-regulated in the PC-9/G cells when compared with that of the PC-9 cells. To verify the effect of miR-145 down-regulation on drug resistance, miR-145 was overexpressed in PC-9/G cells. Subsequently, the changes of cell sensitivity to gefitinib were observed. After transfection with miR-145 mimics, miR-145 expression was first detected *via* qRT-PCR. It was found that the expression of miR-145 was significantly up-regulated after transfection compared with that of the control group, suggesting effective transfection (Figure 2A). Then, transfected PC-9/G cells were co-incubated with gefitinib at different gradient concentrations, and its inhibitory rate on transfected cells was observed. The results showed that the sensitivity of cells to gefitinib was significantly increased after overexpression of miR-145 (IC-50: 1.90 μM) (Figure 2B), and the difference was statistically significant ($p<0.01$). In other words, the up-regulation of miR-145 expression could partially restore the sensitivity of PC-9/G cells to gefitinib. The influence of miR-145 overexpression on cell proliferation was further explored. The results of the proliferation assay manifested that the proliferation of cells in the overexpression group was significantly lower than that of the control group (Figure 2C). The results of colony formation assay, which were consistent with the above results, also showed that the number of proliferating cells in the overexpression group was significantly smaller than that of the control group after 15 days of incubation (Figure 2D). All the above results indicated that the up-regulation of miR-145 could markedly increase the sensitivity

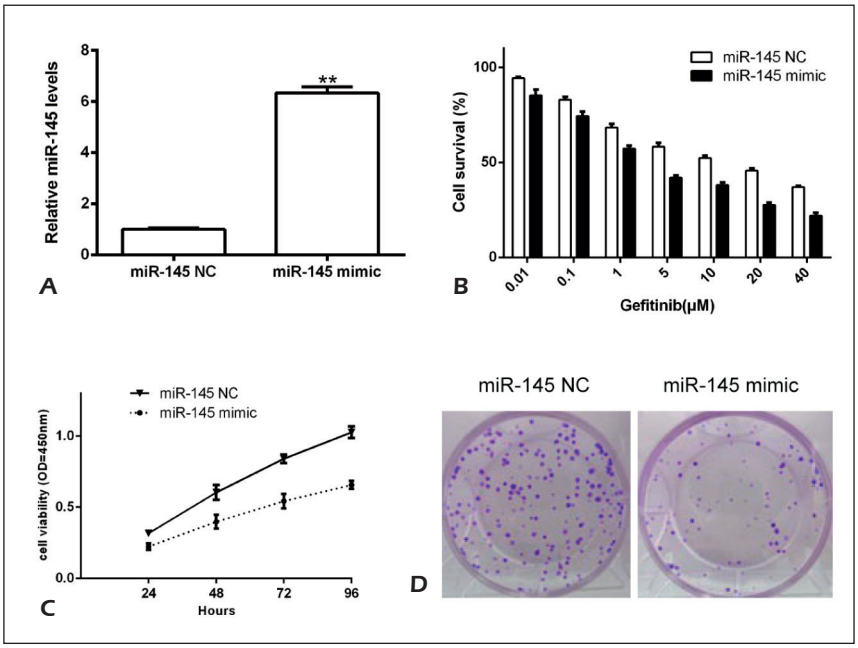

Figure 2. Overexpression of miR-145 reversed gefitinib resistance of PC-9/G cells. **A**, Expression of miR-145 in PC-9/G cells transfected with miR-145 mimics or miR-145 negative control of mimics (NC). **B**, PC-9/G cells were transfected with miR-145 mimics or miR-145 NC. The effects on proliferation were determined by CCK8 assay after treated with different concentrations of gefitinib for 48 h. **C**, The proliferation of PC-9/G cells transfected with miR-145 mimics compared with negative control was determined by CCK8 assay. **D**, Colony formation assay was conducted to determine the proliferation of PC-9/G cells transfected with miR-145 mimics and miR-145 NC. Data were presented as means ± SD (n=3). *p<0.5, **p<0.01 *vs.* control group.

of cells to gefitinib and inhibit the proliferation of PC-9/G cells.

Upregulation of MiR-145 Expression Promoted Apoptosis of PC-9/G Cells

The above results suggested that the overexpression of miR-145 inhibited the proliferation ability of resistant PC-9/G cells *in vitro*. Subsequently, whether such an inhibitory effect on proliferation was mediated by apoptosis induced after miR-145 overexpression was further explored. The apoptosis rate in both groups after transfection was detected *via* flow cytometry. The results demonstrated that the apoptosis rate in miR-145 mimics group (17.58%) was remarkably higher than that of the miR-145 NC group (5.7%) (Figure 3A, 3B). Moreover, Western blotting showed that the expression levels of Bcl-2 associated X protein (Bax), cleaved caspase-3 and cleaved poly-ADP-ribose polymerase (PARP) were remarkably up-regulated after transfection with miR-145 mimics, whereas the expression level of Bcl-2 was remarkably down-regulated (Figure 3C, 3D). The above results suggested that the up-regulation of miR-145 expression inhibited cell proliferation and could restore its sensitivity to gefitinib by promoting apoptosis.

Upregulation of MiR-145 Expression Inhibited the Migration and Invasion Ability of PC-9/G Cells

As one of the characteristics of malignant tumors, invasion and migration are also important causes of death in tumor patients. A large number of studies have found that the migration ability of tumor is often strengthened after drug resistance occurs. Therefore, the influences of miR-145 on migration and invasion of PC-9/G cells were further investigated in this work. The migration ability of cells after up-regulation of miR-145 was detected *via* wound healing assay. The results demonstrated that miR-145 mimics group showed significantly shorter cell migration distance than the control group (p<0.01, Figure 4A, 4B). Meanwhile, transwell migration assay was performed. Interestingly, the number of cells passing through the filter membrane in the lower chamber was markedly declined in the miR-145 mimic group than the control group, and there was a statistically significant difference (p<0.01, Figure 4C, 4D). The above results suggested that the up-regulation of miR-145 expression could markedly inhibit the migration and invasion ability of PC-9/G cells.

MiR-145 Targeted ADAM19 and Regulated its Expression in PC-9/G Cells

Some studies have proved that miR-145 is able to suppress ADAM19 expression in retinoblastoma in a targeted manner, thus inhibiting tumor development. Therefore, we hypothesized that miR-145 could also regulate the expression of ADAM19 in PC-9/G cells in a targeted manner. To verify this hypothesis, the expression levels of ADAM19 in PC-9 cells and PC-9/G cells were detected *via* qRT-PCR and Western blotting. It was found that

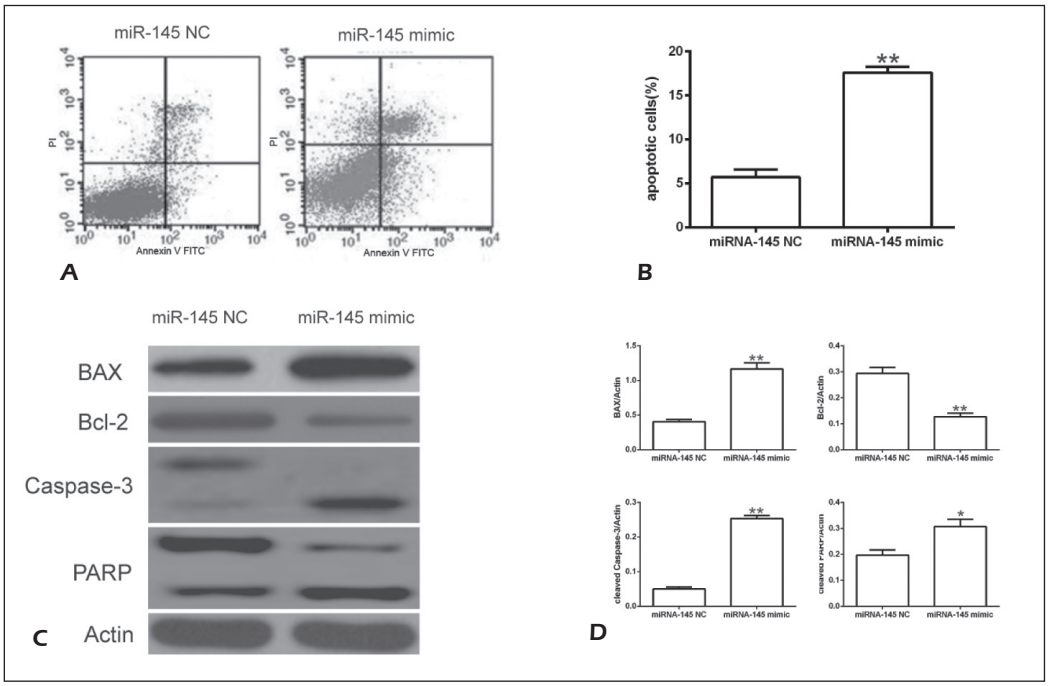

Figure 3. Overexpression of miR-145 induced apoptosis of PC-9/G cells. **A-B**, Flow cytometry assessment showed that the apoptotic rate of PC-9/G cells transfected with miR-145 mimics was significantly increased compared with NC group. **C-D**, The proteins levels of BAX, Bcl-2, cleaved caspase-3 and PARP in PC-9/G cells were determined by Western blotting after transfection with miR-145 mimics or miR-145 NC for 48 h. Data were presented as means ± SD (n=3). **p*<0.05, ***p*<0.01 *vs.* control group.

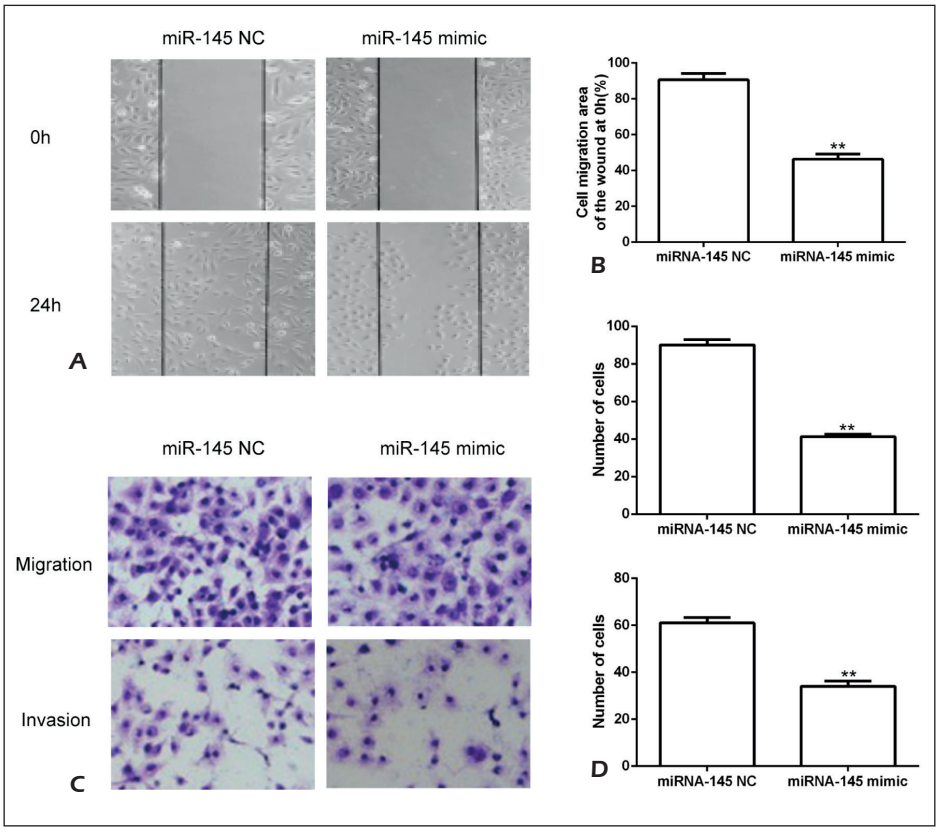

Figure 4. Overexpression of miR-145 suppressed PC-9/G cell migration and invasion. **A-B**, PC-9/G cells were transfected with miR-145 mimics or miR-145 NC for 48 h. The effects on cell migration were determined by cell scratch test. **C-D**, The effects on cell migration and invasion were determined by transwell assay. Data were presented as means ± SD (n=3). **p<0.01 vs. control group.

ADAM19 expression was remarkably increased in PC-9/G cells. Then, the mRNA expression of ADAM19 in PC-9/G cells after the overexpression of miR-145 was explored. As shown in Figure 5A, the mRNA expression level of ADAM19 in PC-9/G cells after overexpression of miR-145 was significantly reduced when compared with that of the control group, displaying a statistically significant difference (*p<0.05). Moreover, the results of Western blotting also manifested that the protein expression of ADAM19 was also remarkably decreased after transfection of miR-145 mimics, and there was a statistically significant difference (p<0.05) (Figure 5B). Comprehensive analysis of the above results demonstrated that in resistant PC-9/G cells, miR-145 might lead to the degradation of ADAM19 mRNA and further down-regulate ADAM19 protein level by targeted binding to ADAM19. Dual-Luciferase reporter gene assay was performed for further confirmation. The constructed pMIR-ADAM19-3'-UTR reporter gene vector was transfected into PC-9/G cells with stable expression of miR-145 and its control miRNA. It was found that after transfection of pMIR-ADAM19-3'-UTR reporter gene vector in PC-9/G cells with stable expression of miR-145, the Luciferase activity was markedly decreased compared with that of the cells with the expression of control miRNA transfected with pMIR-ADAM19-3'-UTR (p<0.05). After cells with stable expression of miR-145 and its control miRNA were transfected with the reporter gene vector cloned with pMIR-ADAM19-MUT-3'-UTR, it was found that the Luciferase activity had no significant changes in PC-9/G cells with stable expression of miR-145 transfected with pMIR-ADAM19-MUT-3'-UTR compared with that in cells with stable expression of control miRNA (p>0.05) (Figure 5C, 5D). The above experimental results suggested that miR-145 targeted ADAM19 and suppressed its expression in PC-9/G cells.

Discussion

MiRNA is a kind of evolutionarily highly-conserved endogenous non-coding single-stranded small RNA. It regulates cell development, differentiation, proliferation, metabolism and apoptosis[6]. Meanwhile, miRNA is related to cell cancerous processes (including growth, proliferation, differentiation, angiogenesis, apoptosis, invasion and metastasis of tumor cells), tumor features, therapeutic effect and prognosis estimation[6-8]. Studies[9] have demonstrated that miR-145, one of the most studied miRNAs currently, is correlated with a variety of tumors. Meanwhile, it is also considered as a tumor marker with therapeutic potential. MiR-145 not only has a close cor-

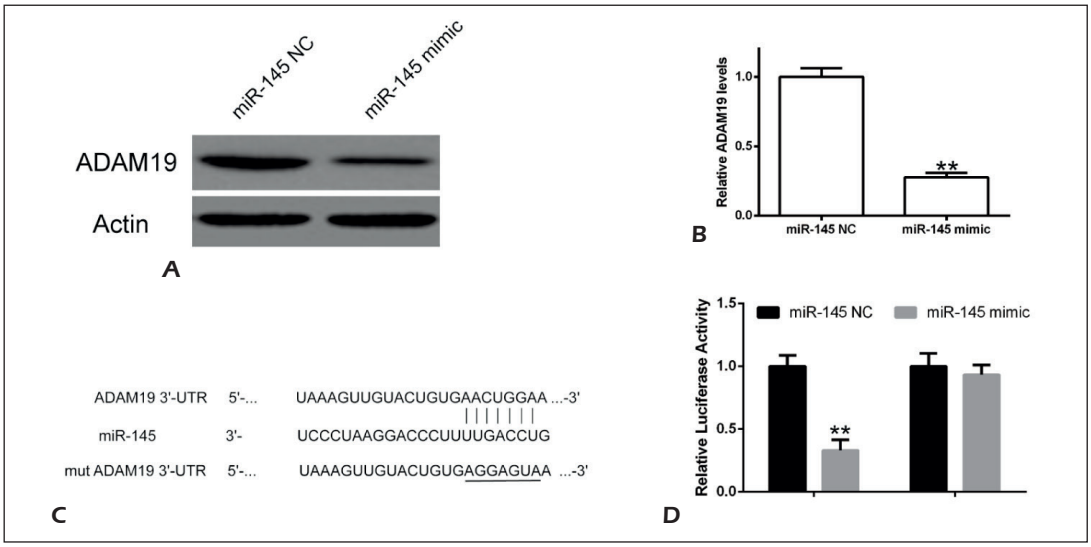

Figure 5. ADAM19 was a functional target of miR-145. **A**, The protein levels of ADAM19 in miR-145 overexpressing PC-9/G cells and control cells were measured by Western blotting. **B**, The relative mRNA levels of ADAM19 in miR-145 overexpressing PC-9/G cells and control cells were measured by qRT-PCR. **C**, The predicted binding sites of miR-145 in the 3'-UTR of ADAM19. **D**, Dual-Luciferase reporter assay was used to determine the binding site. PC-9/G cells treated with miR-145 mimics or miR-145 NC were transfected with pMIR construct containing WT or mutant ADAM19 3'-UTR site. Data were presented as means ± SD (n=3). **p<0.01 *vs.* control group.

relation with the occurrence and development of lung cancer[10,11], but also can regulate a variety of chemotherapeutic drugs[12] and even the sensitivity of lung cancer to molecular targeted drugs[13-15]. Zhan et al[12] have found that the up-regulation of miR-145 can promote the sensitivity of gallbladder cancer to cisplatin. However, the down-regulation of miR-145 expression can promote the resistance of gallbladder cancer to cisplatin. Zhu et al[16] have indicated that miR-145 is often down-regulated in cells of paclitaxel-resistant patients. According to further studies, miR-145 regulates CDK6 and Sp1 expression in a targeted manner, thereby restoring the sensitivity of ovarian cancer cells to paclitaxel. Moreover, Liu et al[17] have revealed that miR-145 can synergistically promote the anti-tumor effect of sunitinib on glioblastoma. The possible underlying mechanism is related to the promotion of glioblastoma cell apoptosis. However, no studies have explored the correlation between miR-145 and gefitinib resistance in lung cancer so far. ADAM is a matrix metalloproteinase (MMP)-related protein family, which belongs to the zinc protease superfamily. ADAMS has similar effects with MMPs, which can degrade extracellular matrix molecules and promote tumor metastasis[18,19]. As an important member of the ADAM family, ADAM19 has various biological functions, such as fertilization, embryonic development, cell adhesion, cell migration, cell signaling, proteolytic shedding and proteolysis[20]. Interestingly, ADAM19 is often highly expressed in a variety of tumors. Further clinical analysis has clarified that the expression level of ADAM19 is related to the malignant degree of tumors[21]. ADAM19 can promote tumor progression in different ways, including promoting proliferation, invasion, metastasis, drug resistance and EMT of tumor cells. Wildeboer et al[22] have confirmed that ADAM19 is highly expressed in primary brain tumors, whose expression level and activity are related to tumor invasion.

There is a large amount of evidence that miRNA plays an important role in regulating the ADAM19 signaling pathway. In colon cancer, miR-30c inhibits the proliferation, invasion and metastasis of cancer cells by suppressing the expression of ADAM19[18]. Moreover, miR-145, as a negative regulator of ADAM19, can directly target ADAM19 and down-regulate its expression in NSCLC. This may eventually inhibit cell invasion and metastasis[23]. Based on the above researches, the role of miR-145 in gefitinib resistance in NSCLC and its related targets were investigated in this study. Moreover, whether miR-145 played a vital role through ADAM19 was also explored.

In this work, the difference in the expression of miR-145 between gefitinib-resistant PC-9/G cells and PC-9 cells was detected *via* qRT-PCR. It was found that the expression level of miR-145 in PC-9/G cells was significantly lower than that of PC-9 cells. This indicated that down-regulation of miR-145 might be involved in the drug resistance of lung cancer cells. To verify this conjecture, miR-145 mimics was overexpressed in PC-9/G cells. The sensitivity of PC-9/G cells to gefitinib was enhanced, and the apoptotic rate was markedly increased when compared with that of the control group. Moreover, cell invasion and metastasis ability were also inhibited. Sun et al[23] has found that miR-145 can inhibit ADAM19 expression in a targeted manner in NSCLC. Therefore, it was speculated that miR-145 might also promote gefitinib resistance by regulating ADAM19. In this study, this conjecture was observed *via* qRT-PCR and Western blotting. The results demonstrated that the up-regulation of miR-145 in PC-9/G cells could remarkably inhibit the expression of ADAM19. To further explore the mechanism of miR-145 in inhibiting ADAM19 expression in PC-9/G cells, the binding site of miR-145 and ADAM19 was verified *via* Luciferase reporter gene assay. The results manifested that miR-145 could directly exert a targeted negative regulation on ADAM19 expression through the predicted site.

This study indicated that low expression of miR-145 promoted the resistance of NSCLC to gefitinib. The underlying mechanism might be inhibiting apoptosis through targeted negative regulation on ADAM19 expression. The overexpression of miR-145 in resistant PC-9/G cells could significantly inhibit tumor proliferation, invasion and metastasis. Furthermore, this study also provided some enlightenment for the study of the reversal of drug resistance in lung cancer to gefitinib based on targeted regulation of miR-145.

Conclusions

We found that miR-145 improved the sensitivity of acquired gefitinib-resistant cells to gefitinib. Meanwhile, it inhibited cell invasion and metastasis through negative regulation on ADAM19. Low-expression of miR-145 might become a bio-

marker and therapeutic target for acquired resistance to gefitinib.

Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.
2) Baltayiannis N, Chandrinos M, Anagnostopoulos D, Zarogoulidis P, Tsakiridis K, Mpakas A, Machairiotis N, Katsikogiannis N, Kougioumtzi I, Courcoutsakis N, Zarogoulidis K. Lung cancer surgery: an up to date. J Thorac Dis 2013; 5 Suppl 4: S425-S439.
3) Gaafar RM, Surmont VF, Scagliotti GV, Van Klaveren RJ, Papamichael D, Welch JJ, Hasan B, Torri V, van Meerbeeck JP. A double-blind, randomised, placebo-controlled phase III intergroup study of gefitinib in patients with advanced NSCLC, non-progressing after first line platinum-based chemotherapy (EORTC 08021/ILCP 01/03). Eur J Cancer 2011; 47: 2331-2340.
4) Shukuya T, Takahashi T, Tamiya A, Ono A, Igawa S, Nakamura Y, Tsuya A, Murakami H, Naito T, Kaira K, Endo M, Yamamoto N. Gefitinib plus paclitaxel after failure of gefitinib in non-small cell lung cancer initially responding to gefitinib. Anticancer Res 2009; 29: 2747-2751.
5) Koyama N, Uchida Y. Clinical significance of erlotinib monotherapy for gefitinib-resistant non-small cell lung cancer with EGFR mutations. Anticancer Res 2013; 33: 5083-5089.
6) Guix M, Faber AC, Wang SE, Olivares MG, Song Y, Qu S, Rinehart C, Seidel B, Yee D, Arteaga CL, Engelman JA. Acquired resistance to EGFR tyrosine kinase inhibitors in cancer cells is mediated by loss of IGF-binding proteins. J Clin Invest 2008; 118: 2609-2619.
7) Onitsuka T, Uramoto H, Nose N, Takenoyama M, Hanagiri T, Sugio K, Yasumoto K. Acquired resistance to gefitinib: the contribution of mechanisms other than the T790M, MET, and HGF status. Lung Cancer 2010; 68: 198-203.
8) Vishnoi A, Rani S. MiRNA biogenesis and regulation of diseases: an overview. Methods Mol Biol 2017; 1509: 1-10.
9) Zhao Y, Liu X, Lu YX. MicroRNA-143 regulates the proliferation and apoptosis of cervical cancer cells by targeting HIF-1alpha. Eur Rev Med Pharmacol Sci 2017; 21: 5580-5586.
10) Sin TK, Wang F, Meng F, Wong SC, Cho WC, Siu PM, Chan LW, Yung BY. Implications of microRNAs in the treatment of gefitinib-resistant non-small cell lung cancer. Int J Mol Sci 2016; 17: 237.
11) Li B, Ren S, Li X, Wang Y, Garfield D, Zhou S, Chen X, Su C, Chen M, Kuang P, Gao G, He Y, Fan L, Fei K, Zhou C, Schmit-Bindert G. MiR-21 overexpression is associated with acquired resistance of EGFR-TKI in non-small cell lung cancer. Lung Cancer 2014; 83: 146-153.
12) Zhan M, Zhao X, Wang H, Chen W, Xu S, Wang W, Shen H, Huang S, Wang J. miR-145 sensitizes gallbladder cancer to cisplatin by regulating multidrug resistance associated protein 1. Tumour Biol 2016; 37: 10553-10562.
13) Liu H, Liu Z, Jiang B, Huo L, Liu J, Lu J. Synthetic miR-145 mimic enhances the cytotoxic effect of the antiangiogenic drug sunitinib in glioblastoma. Cell Biochem Biophys 2015; 72: 551-557.
14) Lin Y, Ge X, Wen Y, Shi ZM, Chen QD, Wang M, Liu LZ, Jiang BH, Lu Y. MiRNA-145 increases therapeutic sensibility to gemcitabine treatment of pancreatic adenocarcinoma cells. Oncotarget 2016; 7: 70857-70868.
15) Yang J, Liu Q, Cao S, Xu T, Li X, Zhou D, Pan L, Li C, Huang C, Meng X, Zhang L, Wang X. MicroRNA-145 increases the apoptosis of activated hepatic stellate cells induced by TRAIL through NF-kappaB signaling pathway. Front Pharmacol 2017; 8: 980.
16) Zhu X, Li Y, Xie C, Yin X, Liu Y, Cao Y, Fang Y, Lin X, Xu Y, Xu W, Shen H, Wen J. miR-145 sensitizes ovarian cancer cells to paclitaxel by targeting Sp1 and Cdk6. Int J Cancer 2014; 135: 1286-1296.
17) Liu H, Liu Z, Jiang B, Huo L, Liu J, Lu J. Synthetic miR-145 mimic enhances the cytotoxic effect of the antiangiogenic drug sunitinib in glioblastoma. Cell Biochem Biophys 2015; 72: 551-557.
18) Zhang Q, Yu L, Qin D, Huang R, Jiang X, Zou C, Tang Q, Chen Y, Wang G, Wang X, Gao X. Role of microRNA-30c targeting ADAM19 in colorectal cancer. PLoS One 2015; 10: e120698.
19) Zhao M, Qiu W, Li Y, Sang QA, Wang Y. Dynamic change of Adamalysin 19 (ADAM19) in human placentas and its effects on cell invasion and adhesion in human trophoblastic cells. Sci China C Life Sci 2009; 52: 710-718.
20) Black RA, White JM. ADAMs: focus on the protease domain. Curr Opin Cell Biol 1998; 10: 654-659.
21) Mochizuki S, Okada Y. ADAMs in cancer cell proliferation and progression. Cancer Sci 2007; 98: 621-628.
22) Wildeboer D, Naus S, Amy Sang QX, Bartsch JW, Pagenstecher A. Metalloproteinase disintegrins ADAM8 and ADAM19 are highly regulated in human primary brain tumors and their expression levels and activities are associated with invasiveness. J Neuropathol Exp Neurol 2006; 65: 516-527.
23) Sun Z, Zhang A, Jiang T, Du Z, Che C, Wang F. MiR-145 suppressed human retinoblastoma cell proliferation and invasion by targeting ADAM19. Int J Clin Exp Pathol 2015; 8: 14521-14527.