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MiR-142-5p reverses the resistance to gefitinib through targeting HOXD8 in lung cancer cells

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Abstract. – OBJECTIVE: To investigate the role and potential mechanism of micro ribonucleic acid (miR)-142-5p in the acquired resistance to gefitinib in lung cancer cells.

MATERIALS AND METHODS: The drug resistance of PC9/G cells was detected *via* methyl thiazolyl tetrazolium (MTT) assay. Expression levels of miR-142-5p and HOXD8 in PC9 and PC9/G cells were detected *via* quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) and Western blotting. PC9/G cells were transfected with miR-142-5p mimic, while PC9 cells were transfected with miR-142-5p inhibitor. Subsequently, expression changes of HOXD8 were determined using qRT-PCR and Western blotting, cell sensitivity to gefitinib was detected through MTT assay, and the apoptosis was detected *via* flow cytometry. Moreover, Dual-Luciferase reporter assay was conducted to determine the relationship between HOXD8 and miR-142-5p. Finally, potential involvement of HOXD8 in miR-142-5p-regulated gefitinib sensitivity was confirmed *via* rescue tests.

RESULTS: PC9/G cells were more significantly resistant to gefitinib compared with its parental PC9 cells. MiR-142-5p was down-regulated in PC9/G cells, while that of HOXD8 was up-regulated in PC9/G cells. Transfection of miR-142-5p mimic could inhibit the expression level of HOXD8 in PC9/G cells and reverse its resistance to gefitinib. Conversely, transfection of miR-142-5p inhibitor could upregulate HOXD8 in PC9 cells and promote its resistance to gefitinib. According to the Dual-Luciferase reporter assay, miR-142-5p could suppress the expression of HOXD8 through the targeted binding to the HOXD8 3'UTR. Moreover, miR-142-5p could regulate mitochondrial apoptosis pathway by targeting HOXD8. Finally, rescue tests confirmed that miR-142-5p regulated the sensitivity of PC9 cells to gefitinib by acting on the target gene HOXD8.

CONCLUSIONS: Down-regulation of miR-142-5p induces the resistance of lung cancer PC9 cells to gefitinib by upregulating HOXD8.

Key Words:
MiR-142-5p, HOXD8, Lung cancer, Gefitinib, Drug resistance.

Introduction

Lung cancer is the leading cause of cancer death in human, seriously threatening human health. Non-small cell lung cancer (NSCLC) pathologically includes lung adenocarcinoma, lung squamous carcinoma, and large cell carcinoma, accounting for approximately 85% of the total. At present, the 5-year survival rate of NSCLC patients is still lower than 20% in spite of the continuous improvement of operation, chemotherapy, radiotherapy, immunotherapy, etc[1]. In recent years, individualized molecular therapy based on genotyping has become a milestone treatment for NSCLC, the most representative one of which is epidermal growth factor receptor (EGFR)-based therapy[2]. Unfortunately, the secondary resistance to EGFR-tyrosine kinase inhibitor (TKI) occurs within 1 year in most lung cancer patients receiving effective treatment.

Micro ribonucleic acids (miRNAs) are a kind of non-coding single-stranded RNAs with 20-24 nucleotides in length, which can post-transcriptionally regulate target genes by binding to their 3'-untranslated region (3'UTR). Each miRNA specifically recognizes its target genes and regulate their expressions, thereby affecting the functions associated with the target gene. Generally, one miRNA can bind to multiple target genes and regulate their functions, while multiple miRNAs can also jointly regulate the expression of the same target gene. MiRNAs can be involved in the regulation of many biological processes in the human body, including cell growth, differentiation, proliferation, and apoptosis[3,4].

MiR-142 is one of the common miRNAs abnormally expressed in malignant tumors[5,6]. The precursors of miR-142 forms two single-stranded small molecules, miR-142-3p and miR-142-5p, during the maturation. Nowadays, it has been found that miR-142-5p can significantly inhibit

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the tumor proliferation, and its level is closely related to the tumor grade. Wang et al[7] have proven that miR-142-5p is lowly expressed in NSCLC tissues, and restoring the expression of miR-142-5p in tumor cells can inhibit cell proliferation and metastasis. However, the role of miR-142-5p in gefitinib resistance in lung cancer remains unclear. In this study, therefore, the association between miR-142-5p and gefitinib resistance and its related mechanism were explored.

Materials and Methods

Cell Lines, Culture, and Reagents

Human lung adenocarcinoma PC9 cells were purchased from Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China). Cells were cultured in the Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone, South Logan, UT, USA), 100 U/mL penicillin and 100 μg/mL streptomycin under 5% CO_2 at 37°C. Gefitinib-resistant lung cancer PC9/G cell line was produced from parental PC9 cells. Briefly, PC9 cells were cultured with 2 μM/L gefitinib for 6 months to acquire resistance to gefitinib, and the drug resistance was detected and proved through the methyl thiazolyl tetrazolium (MTT) assay. Gefitinib was withdrawn from resistant PC9/G cells obtained at 1 week before experiment, and the cells were cultured in the drug-free medium containing serum. Gefitinib was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cell Viability Assay

The cells were digested with trypsin and resuspended, and then 100 μL of cells were added into each well of a 96-well plate (6000 cells/well). On the next day, gefitinib at gradient concentrations was added, and gefitinib-free wells added were used as the control group, with 3 replicates in each concentration. After drug intervention for 48 h, cell proliferation was detected using MTT assay kit.

RNA Isolation and Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

The total RNA was extracted from cells using the TRIzol method (Invitrogen, Carlsbad, CA, USA), and the concentration and purity of RNA extracted were determined using the micro-nucleic acid analyzer. 1 g of RNA was reversely transcribed into complementary deoxyribose nucleic acid (cDNA), and then 2 μL of the RT products were taken for PCR, with glyceraldehyde 3-phosphate dehy-drogenase (GAPDH) and U6 as the internal references. The primer sequences were as follows: miR-142-5p: F: 5'-UGUUUUGAGCGGGGGUCAAGAGC-3', and R: 5'-CUCUCAUUUGCUAUAUUCA-3'. HOXD8: F: 5'-GTTTTGAACCGCCCTTGTAA-3', and R: 5'-GTGAGGCTATCGCTTTCCTG-3'. GAPDH: F: 5'-AGGTGGAGGAGTGGGTGTCGCTGTT-3', and R: 5'-CCGGGAAACTGTGGCGTGATGG-3'. U6: F: 5'-CTCGCTTCGGCAGCACA-3'; and R: 5'-AACGCTTCACGAATTTGCGT-3'. The fluorescence qPCR was performed as follows: 95°C for 10 min, 60°C for 20 min and 72°C for 20 min, for a total of 40 cycles. With GAPDH as the control, the data were analyzed and calculated based on the Ct value, so as to determine the relative expression level of the target miRNA.

Western Blotting

Western blotting was performed as follows: after treatment, the cells were collected, and the protein was extracted using the radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China). The total protein concentration was measured using the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA). Then, 20 μg of protein samples in each group were taken for 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and horizontally transferred onto a polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) using the wet method. After blockage with 5% skim milk at room temperature for 2 h, membranes were incubated with the primary antibody diluted with Tris Buffered Saline-Tween-20 (TBST) at 4°C overnight. After TBST washing for 3 times, the protein samples were incubated with the secondary antibody for 2 h, and the membrane was washed again for 3 times. Enhanced chemiluminescence (ECL) reaction substrate was added for X-ray exposure, and the optical density of bands was analyzed using the image analysis software, with β-actin as the reference protein.

Transfection

At 24 h before transfection, the cells were inoculated into a 6-well plate at a density of about 60%, and a total of 1 mL of serum-containing medium was contained in each well. On the day of transfection, 5 μL of Lipofectamine 2000 was added into 250 μL of serum-free medium and mixed evenly to be the working solution A, followed by standing at room temperature for 5 min. Then, miR-142

mimic or miR-142 NC was added into 250 μL of serum-free medium and mixed evenly to be the working solution B (final concentration: 100 nM), followed by standing at room temperature for 5 min. The working solution A and B were mixed evenly and incubated at room temperature for 25 min. Then, the original medium in the cells to be transfected was aspirated, and the mixed working solution was added, followed by culture in the incubator at 37°C. The mixture was shaken once every 30 min, and the working solution was replaced with the serum-containing medium after 3 h, followed by culture in the incubator for later use.

Dual-luciferase Reporter Assay

The target genes of miR-142 were predicted using the TargetScan. The database showed that HOXD8 contained potential binding sequences paired with miR-142-5p at its mRNA 3'UTR. The HOXD8 3'UTR sequence fragments containing the target sequences complementary to miR-142 were synthesized as wild-type HOXD8 vector. Mutated HOXD8 3'UTR fragments containing the binding sites to miR-142 were cloned into the pGL3-luciferase gene, and thus mutant-type HOXD8 vector was constructed. Then, the cells were inoculated into a 24-well plate (1×10^5 cells/well), and co-transfected with miR-124 mimics/NC and wild-type/mutant-type luciferase vector using Lipofectamine 2000. After transfection for 48 h, the cells were collected, and the luciferase activity was measured using the Dual-Luciferase reporter assay kit (Promega, Madison, WI, USA).

Statistical Analysis

Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA). Data were represented as mean ± SD (Standard Deviation). The *t*-test was used for analyzing measurement data. Differences between two groups were analyzed by the Student's *t*-test. Comparison between multiple groups was done using One-way analysis of variance (ANOVA) test, followed by Post-Hoc Test (Least Significant Difference). $p<0.05$ indicated the significant difference.

Results

Expression of MiR-142 Was Significantly Low in PC9/G Cells

The gefitinib-resistant cells were induced by low-dose gefitinib. To ensure the reliability of subsequent experiments, the sensitivity of PC9 and PC9/G cells to gefitinib was detected through MTT assay. It was found that PC9 cells were highly sensitive to gefitinib, and the cell growth was significantly inhibited with the gradual increased concentration of gefitinib to 2 μM/L. On the contrary, PC9/G cells were less sensitive to gefitinib, and the cell growth was not significantly inhibited after treatment of 2 μM/L gefitinib for 48 h compared with control group, suggesting that PC9/G cells were resistant to gefitinib (Figure 1A). The half maximal inhibitory concentration (IC_{50}) of PC9 and PC9/G cells was 1.5 μM and 4.4 μM, respectively (Figure 1B). Moreover, the expression of miR-142-5p in PC9 and PC9/G cells was determined using qRT-PCR. MiR-142-5p is highly expressed in various cancer tissues, including lung cancer tissues; however, its expression in drug-resistant cells has not been reported yet, which, therefore, was examined in this study. The results

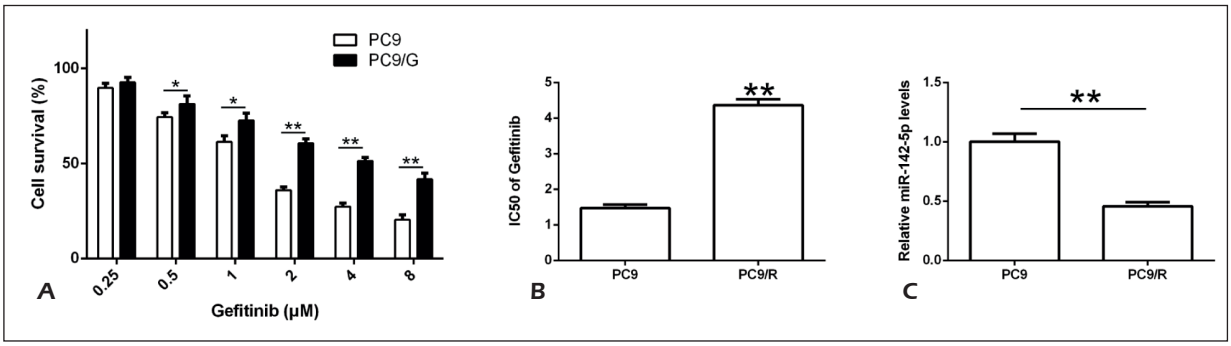

Figure 1. PC9/G cells were significantly resistant to gefitinib. **A**, Cell viability of acquired gefitinib resistant lung cancer cell line (PC9/G) and its parental cell line (PC9) was determined by MTT assay after treated with indicated concentrations of gefitinib for 48 h. **B**, Gefitinib IC50 was determined according to the cell viability curve. **C**, The expression levels of miR-142-5p was detected in the above cells by qRT-PCR. Data are presented as means ± SD (n = 3). $^{**}p<0.01$ versus control group.

manifested that the expression level of miR-142-5p in PC9/G cells was significantly lower than that in PC9 cells, indicating that the down-regulated expression of miR-142-5p may be involved in cell resistance to gefitinib (Figure 1C).

MiR-142-5p Induced Sensitivity of Lung Cancer Cells to Gefitinib

It was observed *via* qRT-PCR that the expression level of miR-142-5p was significantly lower in PC9/G cells than that in PC9 cells. To study the effect of miR-142-5p on sensitivity of PC9 and PC9/G cells to gefitinib, miR-142-5p mimic or miR-142-5p inhibitor was transfected to in cells. Their transfection efficiency was first detected. After transfection of miR-142-5p mimic in PC9 cells, the expression level of miR-142-5p was significantly upregulated (Figure 2A, $p<0.05$), while transfection of miR-142-5p inhibitor could significantly down-regulate the expression of miR-142-5p in PC9/G cells (Figure 2B, $p<0.05$). Furthermore, MTT assay showed that the sensitivity of PC9 cells to gefitinib declined after transfection of miR-142-5p inhibitor compared with control group (Figure 2C, $p<0.05$), while the sensitivity to gefitinib was enhanced after up-regulation of miR-142-5p expression in PC9/G cells (Figure 2D, $p<0.05$). Besides, the IC_{50} in the two

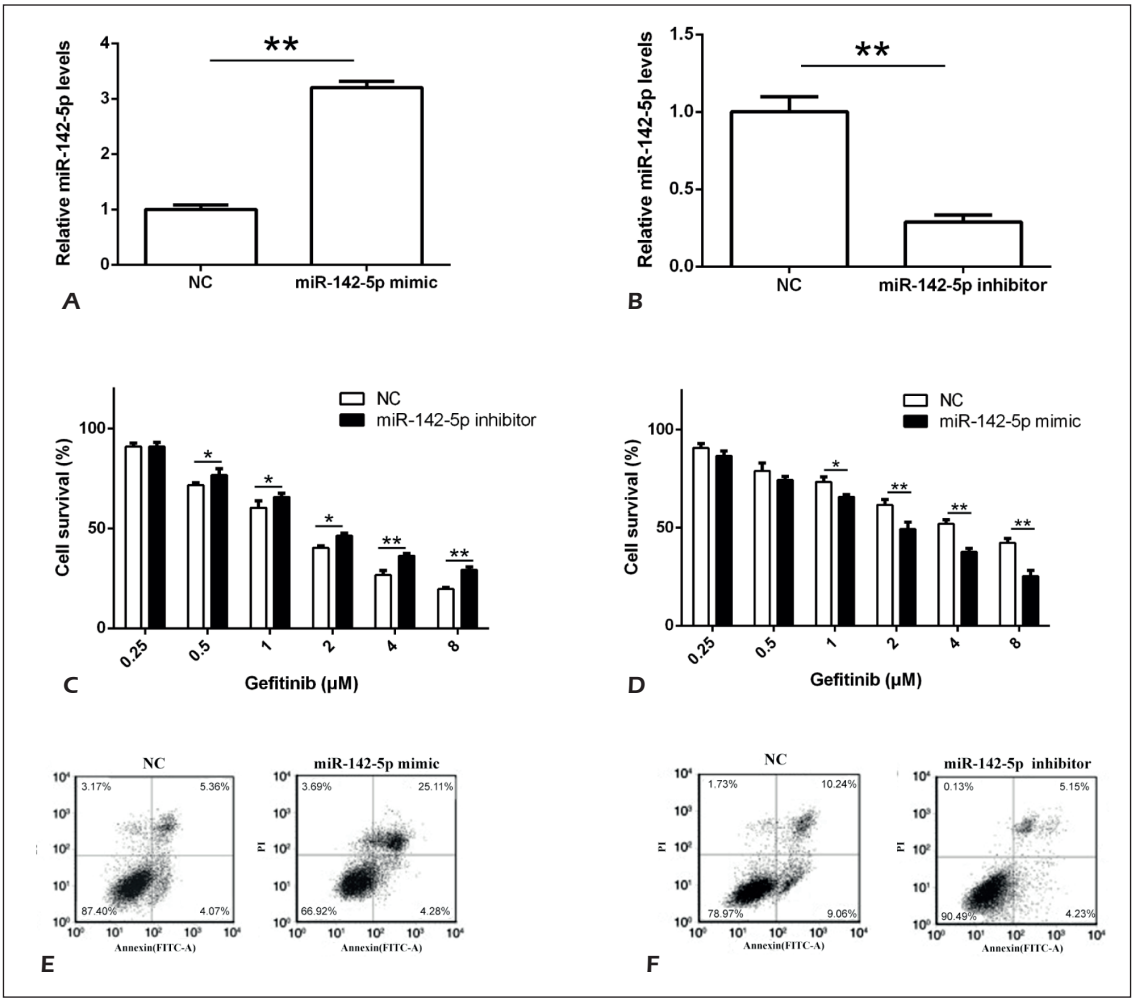

Figure 2. MiR-142-5p induced sensitivity of lung cancer cell lines to gefitinib. **A**, Expression of miR-142-5p in PC9/G cells transfected with miR-142-5p mimic or miR-142-5p negative control of mimic (NC). **B**, Expression of miR-142-5p in PC9 cells transfected with miR-142-5p inhibitor or miR-142-5p NC. **C**, The viable PC9 cells transfected with miR-142-5p inhibitor and miR-142-5p NC were detected by MTT after treated by indicated does of gefitinib. **D**, The viable PC9/R cells transfected with miR-142-5p mimic and NC were detected by MTT after treated by indicated does of gefitinib. **E**, After treatment with 1 μM gefitinib for 24 h, The apoptosis rates of PC9/G cells transfected with miR-142-5p mimics or NC were detected by flow cytometry. **F**, After treatment with 0.5 μM gefitinib for 24 h, The apoptosis rates of PC9 cells transfected with miR-142-5p inhibitor or NC were detected by flow cytometry. Data are presented as means ± SD (n = 3). *$p<0.5$, **$p<0.01$ versus control group.

groups was 2.1 μM and 2.0 μM, respectively. To sum up, miR-142-5p can regulate the sensitivity of lung cancer cells PC9 and PC9/G to gefitinib. To further explore the effect of miR-142-5p on apoptosis, apoptosis in each group was detected after transfection through flow cytometry. The apoptosis rate of PC9 cells transfected with miR-142-5p inhibitor significantly decreased after treatment with gefitinib compared with control group, while that of PC9/G cells transfected with miR-142-5p mimics significantly increased (Figure 2E, 2F), demonstrating that miR-142-5p can promote the pro-apoptotic effect of gefitinib.

MiR-142-5p Targeted HOXD8 and Regulated its Expression

In this experiment, the bioinformatics prediction website TargetScan was used to predict the possible target genes of miR-142. It was found that HOXD8 contained potential binding sequences pairing with miR-142-5p at its mRNA 3'UTR. Liu et al[8] showed that HOXD8 tends to be highly expressed in lung cancer tissues compared with that in normal tissues, suggesting that HOXD8 serves as an oncogene in lung cancer. To explore differential expression of HOXD8 between PC9 and PC9/G cells, its expression in the two kinds of cells was detected using qRT-PCR and Western blotting. The results manifested that the expression of HOXD8 in PC9G/R cells was evidently higher than that in PC9 cells (Figure 3A, $p<0.05$), indicating that HOXD8 may be involved in promoting the gefitinib resistance of PC9 cells. Then, the regulatory effect of miR-142-5p on HOXD8 was further verified in PC9/G and PC9 cells. The results showed that the protein expression of HOXD8 could be reduced after PC9/G cells were transfected with miR-142-5p mimic, while it was up-regulated after PC9 cells were transfected with miR-142-5p inhibitor (Figure 3B, $p<0.05$). To verify that the HOXD8 3'UTR can bind to the miR-142-5p sequence in a targeted manner, Dual-Luciferase reporter assay was performed. The results revealed that transfection of miR-142-5p mimic could evidently suppress the luciferase activity of HOXD8 3'UTR, but that in mutated HOXD8 3'UTR was not altered (Figure 3C and 3D, $p<0.05$). The above results indicated that miR-142-5p directly acts on the HOXD8 3'UTR and inhibits the expression of HOXD8.

MiR-142-5p Regulated the Sensitivity of Lung Cancer to Gefitinib Through Targeting HOXD8

To further verify whether the gefitinib resistance of PC9/G cells mediated by miR-142-5p depends on HOXD8, rescue tests were further conducted. Co-transfection efficacy in

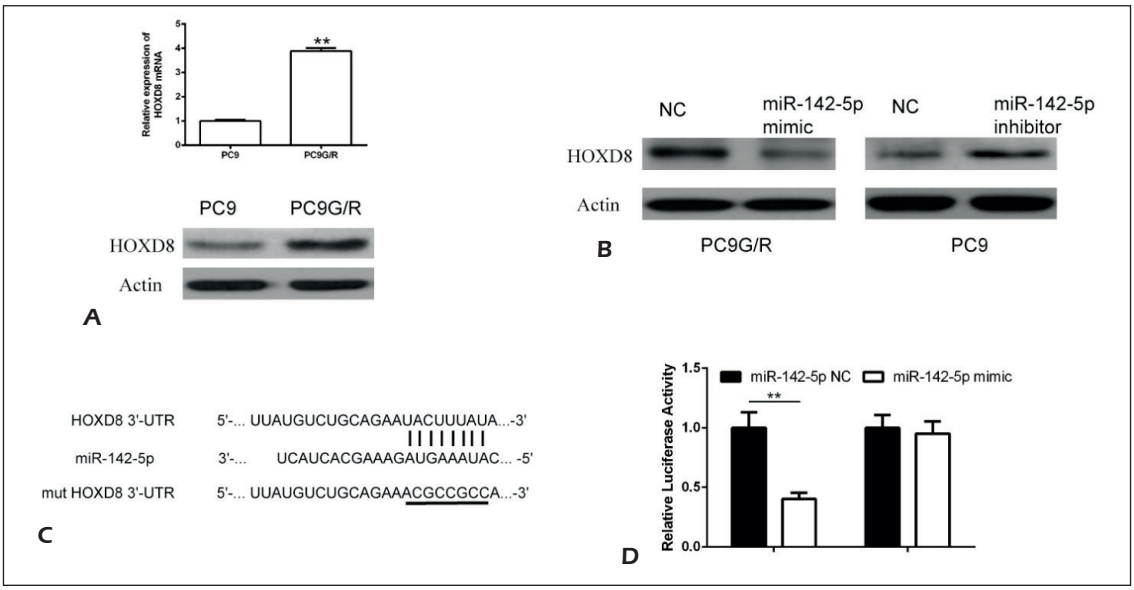

Figure 3. MiR-142-5p directly targeted HOXD8 and regulated its expression. **A**, The expression levels of HOXD8 was detected in the above cells by qRT-PCR and Western blotting. **B**, Overexpression of miR-142-5p decreased the HOXD8 protein level in PC9/G cells and knockdown of miR-142-5p increased the protein level in PC9 cells. **C**, The predicted binding site of miR-142-5p in the 3'-UTR of HOXD8. **D**, Luciferase activity of mimic group was inhibited significantly compared to mutant construct groups.

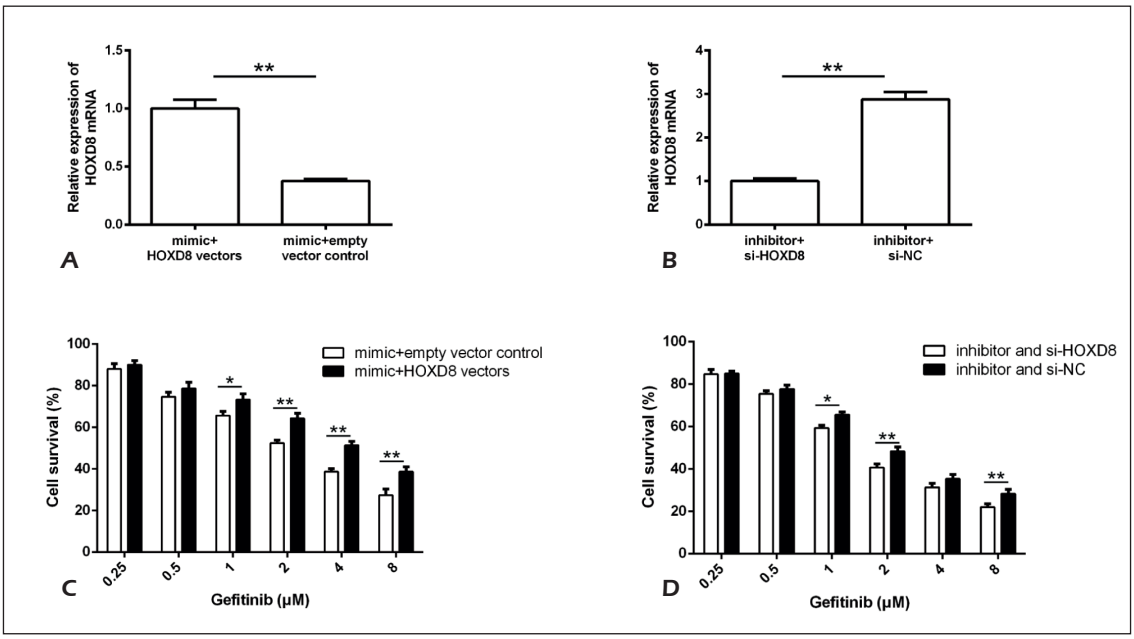

Figure 4. MiR-142-5p targeted HOXD8 to regulate sensitivity of gastric cancer to gefitinib. **A**, qRT-PCR was performed to detect the mRNA level of HOXD8 in PC9/G cells transfected with miR-142-5p mimic and HOXD8 vectors, or miR-142-5p mimic and empty vector control. **B**, qRT-PCR was performed to detect the mRNA level of HOXD8 in PC9 cells transfected with miR-142-5p inhibitor and si-HOXD8, or miR-142-5p inhibitor and si-NC. **C**, Cell viability of PC9/G cells was determined by MTT assay after co-transfected with indicated reagents. **D**, Cell viability of PC9 cells was determined by MTT assay after co-transfected with indicated reagents. Data are presented as means ± SD (n = 3). *p<0.05, **p<0.01 versus control group.

each group was shown in Figure 4A (p<0.05), suggesting the successful transfection. To explore sensitivity changes to gefitinib in each group after transfection, cell proliferation was determined again through MTT assay. It was observed that PC9/G cells co-transfected with miR-142-5p mimic and pcDNA-HOXD8 were more remarkably resistant to gefitinib than those co-transfected with miR-142-5p mimic and empty vector control, and the IC_{50} was 4.3 μM and 2.2 μM, respectively. Besides, PC9 cells co-transfected with miR-142-5p inhibitor and si-NC were more remarkably resistant to gefitinib than those co-transfected with miR-142-5p inhibitor and si-HOXD8, and the IC_{50} was 2.5 μM and 1.3 μM, respectively (Figure 4B, p<0.05). To sum up, HOXD8 can partially reverse the drug resistance caused by the down-regulation of miR-142-5p, while up-regulation of HOXD8 can restore the resistance of PC9/G cells overexpressing miR-142-5p to gefitinib. It can be seen that HOXD8 is an important downstream effector molecule of miR-142-5p. The above data indicated that miR-142-5p can regulate the sensitivity of PC9 cells to gefitinib through targeted inhibition on HOXD8.

MiR-142-5p Regulated Mitochondrial Apoptosis Pathway Through Targeting HOXD8

As above revealed, overexpression of miR-142-5p could partially restore the sensitivity of PC9/G cells to gefitinib and promote apoptosis. To further explore its potential molecular mechanism, changes of apoptosis genes were detected through Western blotting. First, the changes in apoptosis proteins in PC9/G cells transfected with miR-142-5p mimic after treatment with gefitinib were examined. The results manifested that the expressions of Bax, cleaved-caspase3, and cleaved-PARP were remarkably up-regulated in PC9/G cells transfected with miR-142-5p mimic after treatment with gefitinib compared with control group. The opposite results were obtained in PC9 cells. The expressions of Bax, cleaved-caspase3, and cleaved-PARP were remarkably down-regulated in PC9 cells transfected with miR-142-5p inhibitor after treatment with gefitinib compared with control group (Figure 5A). It is indicated that miR-142-5p can promote gefitinib-induced apoptosis by activating the mitochondria-dependent apoptosis pathway. To further investigate the involvement

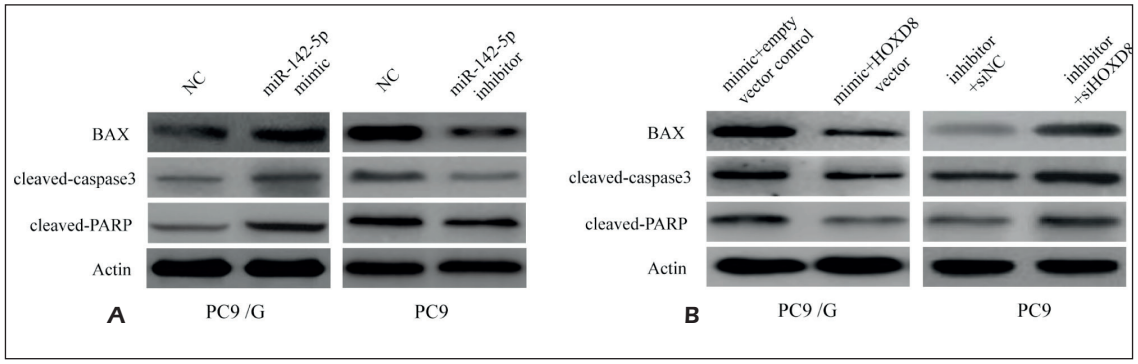

Figure 5. MiR-142-5p regulated mitochondrial apoptosis pathway through targeting HOXD8. **A**, Western blotting was used to detect protein levels of apoptosis genes in PC9 cells transfected with miR-142-5p mimic and PC9/G cells transfected with miR-142-5p inhibitor. **B**, Western blotting was used to detect protein levels of apoptosis genes in PC9/G and PC9 cells transfected with indicated reagents.

of HOXD8 in miR-142-5p-regulated apoptosis, rescue tests were also performed. It was found that the expressions of Bax, cleaved-caspase3, and cleaved-PARP in cells co-transfected with miR-142-5p mimic and pcDNA-HOXD8 were significantly down-regulated. Similarly, PC9 cells were co-transfected with miR-142-5p inhibitor and si-HOXD8 or miR-142-5p inhibitor and si-NC. It was found that transfection of si-HOXD8 could partially reverse the downregulated miR-142-5p -induced down-regulation of Bax, cleaved-caspase3, and cleaved-PARP (Figure 5B). These results indicated that miR-142 regulated the mitochondrial apoptosis pathway by targeting HOXD8.

Discussion

EGFR-TKI targeted therapy has numerous advantages than traditional chemotherapy, which has become an effective treatment for advanced NSCLC. However, its clinical application is greatly limited due to the primary and acquired resistance of gefitinib. Therefore, deeply studying the mechanism of gefitinib resistance in NSCLC cells will help reduce the difficulty of clinical treatment of lung cancer. It has been found that miRNAs not only regulate the occurrence and development of tumors, but also participate in the formation of EGFR-TKI resistance of lung cancer, which are expected to become molecular markers for predicting EGFR-TKI resistance. Among them, the role of miR-142 has attracted increasingly more attention in colon cancer, kidney cancer, and lung cancer. Xiao et al[9] found that miR-142-3p is lowly expressed in NSCLC tissues, and it can inhibit tumor proliferation through targeted inhibition on HMGB1 expression. Moreover, miR-142-5p has been studied in lung cancer where it has an anti-tumor effect on NSCLC. Overexpression of miR-142-5p in lung cancer cells can inhibit the proliferation, invasion, and metastasis of tumor cells, suggesting that miR-142-5p is expected to become a therapeutic target for lung cancer in the future[10-14]. Wang et al[10] further found that miR-142-5p can inhibit lung cancer cells through the targeted inhibition on PIK3CA. Therefore, the role of miR-142-5p in gefitinib resistance of lung cancer and its specific mechanism were explored in the present study.

The gefitinib-resistant PC9/G cells were successfully constructed first, and the expression of miR-142-5p was detected. The expression of miR-142-5p was significantly lower in PC9/G cells than that in parental PC9 cells. Furthermore, overexpression of miR-142-5p in PC9/G cells could significantly reverse gefitinib resistance and promote apoptosis, while knockdown of miR-142-5p in PC9 cells could significantly enhance gefitinib resistance and inhibit apoptosis. Thus, miR-142-5p was involved in regulating gefitinib resistance of NSCLC cells. Bioinformatics predicted that HOXD8 contained potential binding sequences pairing with miR-142-5p at its mRNA 3'UTR, suggesting that HOXD8 may be one of the potential target genes of miR-142-5p. HOXD8, a member of the HOX gene family, plays an important regulatory role in embryonic development, cell differentiation, tumor cell pro-

liferation, metastasis, and even apoptosis. The abnormal expression of HOXD8 is associated with the occurrence and development of various tumors, such as colon cancer and ovarian cancer. Sun et al[15] reported that the high expression of HOXD8 is closely related to cisplatin resistance of ovarian cancer SKOV3 cells. It is also found that HOXD8 has a higher expression level in patients with recurrent ovarian cancer and cisplatin resistance.

To study the regulatory relation between miR-142-5p and HOXD8, HOXD8 level in lung cancer cells with altered level of miR-142-5p was first determined. The results revealed that the expression of miR-142-5p was negatively correlated with that of HOXD8. The expression of HOXD8 significantly declined after overexpression of miR-142-5p. The targeted regulatory relation between miR-142-5p and HOXD8 was verified *via* dual-luciferase reporter assay, and it was observed that miR-142-5p could directly regulate HOXD8. In the rescue tests, the miR-142-5p-mediated gefitinib resistance was reversed by regulating HOXD8 expression in lung cancer cells. Moreover, miR-142 regulated the mitochondrial apoptosis pathway through HOXD8.

In this paper, miR-142-5p level in gefitinib-resistant PC9/G cells was lower than that in gefitinib-sensitive PC9 cells, while the expression of HOXD8 was higher in PC9/G cells than that in PC9 cells. Both of them exerted important roles in the resistance of lung cancer cells to gefitinib. HOXD8 was able to reverse the regulatory effects of miR-142-5p on the sensitivity of cells to gefitinib by influencing the apoptosis pathway.

Conclusions

In summary, we first revealed that miR-142-5p regulates the sensitivity of cells to gefitinib through the target gene HOXD8 in PC9 and PC9/G cells by regulating the apoptosis pathway. This study clarifies new explanations about the mechanism of TKI resistance in lung cancer and may provide new potential therapeutic targets for intervention in the gefitinib resistance.

Conflict of Interests

The authors declare that they have no conflict of interests.

References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019; 69: 7-34.
2) Liu AM, Zhu Y, Huang ZW, Lei L, Fu SZ, Chen Y. Long noncoding RNA FAM201A involves in radioresistance of non-small-cell lung cancer by enhancing EGFR expression via miR-370. Eur Rev Med Pharmacol Sci 2019; 23: 5802-5814.
3) Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005; 120: 15-20.
4) Vishnoi A, Rani S. MiRNA biogenesis and regulation of diseases: an overview. Methods Mol Biol 2017; 1509: 1-10.
5) Yao R, Xu L, Wei B, Qian Z, Wang J, Hui H, Sun Y. MiR-142-5p regulates pancreatic cancer cell proliferation and apoptosis by regulation of RAP1A. Pathol Res Pract 2019; 215: 152416.
6) Gao W, Pang D, Yu S. Serum level of miR-142-3p predicts prognostic outcome for colorectal cancer following curative resection. J Int Med Res 2019; 47: 2116-2125.
7) Wang Z, Liu Z, Fang X, Yang H. MiR-142-5p suppresses tumorigenesis by targeting PIK3CA in non-small cell lung cancer. Cell Physiol Biochem 2017; 43: 2505-2515.
8) Liu Y, Miao L, Ni R, Zhang H, Li L, Wang X, Li X, Wang J. MicroRNA-520a-3p inhibits proliferation and cancer stem cell phenotype by targeting HOXD8 in non-small cell lung cancer. Oncol Rep 2016; 36: 3529-3535.
9) Xiao P, Liu WL. MiR-142-3p functions as a potential tumor suppressor directly targeting HMGB1 in non-small-cell lung carcinoma. Int J Clin Exp Pathol 2015; 8: 10800-10807.
10) Wang Z, Liu Z, Fang X, Yang H. MiR-142-5p suppresses tumorigenesis by targeting PIK3CA in non-small cell lung cancer. Cell Physiol Biochem 2017; 43: 2505-2515.
11) Sempere LF, Liu X, Dmitrovsky E. Tumor-suppressive microRNAs in lung cancer: diagnostic and therapeutic opportunities. ScientificWorldJournal 2009; 9: 626-628.
12) Su YH, Zhou Z, Yang KP, Wang XG, Zhu Y, Fa XE. MIR-142-5p and miR-9 may be involved in squamous lung cancer by regulating cell cycle related genes. Eur Rev Med Pharmacol Sci 2013; 17: 3213-3220.
13) Wan J, Ling X, Peng B, Ding G. MiR-142-5p regulates CD4+ T cells in human non-small cell lung cancer through PD-L1 expression via the PTEN pathway. Oncol Rep 2018; 40: 272-282.
14) Islam F, Gopalan V, Vider J, Lu CT, Lam AK. MiR-142-5p act as an oncogenic microRNA in colorectal cancer: clinicopathological and functional insights. Exp Mol Pathol 2018; 104: 98-107.
15) Sun P, Song Y, Liu D, Liu G, Mao X, Dong B, Braicu EI, Sehouli J. Potential role of the HOXD8 transcription factor in cisplatin resistance and tumour metastasis in advanced epithelial ovarian cancer. Sci Rep 2018; 8: 13483.