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MicroRNA-506-3p reverses gefitinib resistance in non-small cell lung cancer by targeting Yes-associated protein 1

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Abstract. Epidermal growth factor receptor-tyrosine kinase inhibitors, such as gefitinib, have been found to be clinically effective in the treatment of patients with non-small cell lung cancer (NSCLC). However, the therapeutic effect of gefitinib is often limited by the development of gefitinib resistance. MicroRNAs (miRNAs), a group of small non-coding RNAs, have been demonstrated to be frequently dysregulated in human malignancies. For instance, the downregulation of miR-506-3p has been reported in NSCLC patients. The aim of the present study was to determine the role and underlying molecular mechanism of miR-506-3p in the regulation of gefitinib sensitivity in NSCLC. A gefitinib-resistant PC-9 (PC-9GR) cell line was established, and reduced miR-506-3p expression was observed in PC-9GR cells as compared with that in parental cells. The results of cell cytotoxicity and cell apoptosis assays indicated that PC-9GR cells were more sensitive to gefitinib following the transfection with an miR-506-3p mimic, while transfection with an miR-506-3p antagonist reduced the sensitivity of PC-9GR cells to gefitinib. It was further revealed that Yes-associated protein 1 (YAP1) was directly suppressed by miR-506-3p in PC-9GR cells. The elevated sensitivity of PC-9GR cells to gefitinib following transfection with the miR-506-3p mimic was counteracted by the overexpression of YAP1. Furthermore, an inverse correlation between the miR-506-3p and YAP1 mRNA levels was detected in lung adenocarcinoma specimens. Collectively, the results of the present study suggested that the downregulation of miR-506-3p contributes to gefitinib resistance, and thus, the restoration of miR-506-3p may be a potential therapeutic approach for overcoming NSCLC gefitinib resistance.

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Introduction

Lung cancer is the leading cause of cancer-associated mortality, with non-small cell lung cancer (NSCLC) accounting for ~85% of lung cancer cases worldwide (1). Despite recent developments in cancer treatment, the prognosis for patients with lung cancer has seen limited improvement, with the 5-year survival rate for NSCLC remaining at 9-14% since 2010 (2). Due to the discovery of epidermal growth factor receptor tyrosine kinase receptor inhibitors (EGFR-TKIs), such as gefitinib and erlotinib, a new generation of agents has been made available for the treatment of patients with NSCLC, whose tumor harbors activating mutations in the EGFR gene (3-5). However, the majority of patients who initially respond to treatment with TKIs, later develop acquired resistance, which limits the treatment options (6). Several mechanisms of acquired resistance to TKIs have been identified, including secondary EGFR T790 M mutation, MET amplification, HER2 amplification, KRAS mutation and loss of PTEN (7,8). Further elucidation of the mechanisms underlying gefitinib resistance is imperative for the development of therapeutic strategies.

MicroRNAs (miRNAs) are small non-coding endogenous RNAs that mainly bind to the 3'-untranslated region (3'-UTR) of target mRNAs, resulting in the degradation of mRNA or the blockade of protein translation (9). miRNAs exert a wide range of biological functions, including proliferation, differentiation, metabolism and apoptosis (9,10). The involvement of miRNAs in gefitinib resistance in NSCLC has been previously reported (11). A study demonstrated that miR-138-5p was downregulated in gefitinib-resistant NSCLC cell models, and that the overexpression of miR-138-5p enhanced gefitinib sensitivity via the suppression of G protein-coupled receptor 124 (12). In another previous study, the elevation of miR-127 promoted a shift from the epithelial to the mesenchymal phenotype and increased gefitinib resistance in lung cancer cells (13). The aim of the present study was to identify and investigate novel miRNAs that are potentially involved in gefitinib resistance in NSCLC.

Materials and methods

Clinical specimens and cell culture. A total of 25 pairs of lung adenocarcinoma and adjacent non-cancerous specimens were resected from patients (14 male and 11 female) aged

56-67 years old between 2015 and 2017 diagnosed by clinicians at Taizhou Central Hospital (Taizhou, China). There were 13 patients at stage I, 4 patients at stage II, 6 patients at stage III and 2 patients at stage IV, classified by TNM staging (14). All samples were frozen immediately after surgery and stored at -80˚C. Written informed consent was obtained from each patient. The study was approved by the Ethics Committee of Taizhou Central Hospital.

The 293 cell line, human normal epithelial cell line BEAS-2B and the NSCLC cell line PC-9 were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). PC-9 and 293 cells were cultured in RPMI 1640, supplemented with 10% fetal bovine serum (HyClone; GE Healthcare Life Sciences, Logan, UT, USA) in an incubator with 5% CO_2 at 37˚C. The 293 cells were applied in the experiments of luciferase assays, BEAS-2B cells were used for the reconstruction of pcDNA3-AP1 and PC-9 cells were used in all other experiments.

Establishment of the gefitinib-resistant cell line. Gefitinib-resistant cells were obtained by continuously exposing PC-9 cells to gefitinib, as previously described (12). Briefly, PC-9 cells were first exposed to 0.2 μmol/l gefitinib (LC Laboratories, Woburn, MA, USA) for 48 h, washed and cultured in drug-free medium until 80% confluence was reached, followed by re-exposure to increasing concentrations of gefitinib. Gefitinib-resistant PC-9 (PC-9GR) cells were obtained after 6 months of continuous exposure. The resistant cells were able to grow in 10 μmol/l gefitinib and were maintained in 2 μmol/l gefitinib-containing medium. In order to eliminate gefitinib, PC-9GR cells were cultured in drug-free medium for 1 week prior to all experiments.

Cell growth inhibition assay. The effects of gefitinib on cells were determined using the MTT assay. Briefly, exponentially growing cells were seeded in 96-well plates at the density of $3x10^3$/well with 100 μl in each well, and cultured in 37˚C overnight. On the following day, gefitinib was dissolved in dimethyl sulfoxide (DMSO) and added to each well at different concentrations (1, 2, 4, 8 and 16 μM), while the cells in the control groups were treated with the equivalent volume of DMSO. Cells in each group were incubated for 48 h. Following incubation, 10 μl MTT was added to each well, and cells were incubated for a further 3 h. The optical density at 492 nm was measured using a microplate reader (Beckman Coulter, Inc., Brea, CA, USA).

Cell apoptosis. Cells ($2x10^5$ cells/well) were seeded into 12-well plates and cultured for 48 h at 37˚C. Subsequently, flow cytometry was used for the detection of the cell apoptosis by Annexin V-fluorescein isothiocyanate (FITC) /propidium iodide (PI) staining. Cells were washed with cold PBS and fixed in ice-cold 70% ethanol overnight at -20˚C. The next day, cells were stained by Annexin V-FITC and PI (Roche Diagnostics, Basel, Switzerland) at room temperature for 15 min in the dark. After incubation for 1 h at 37˚C, cell apoptosis was measured using a flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). The cell number at each phase was analyzed by FlowJo software version 7.6.3 (FlowJo LLC, Ashland, OR, USA).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from tissues and cells using TRIzol (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), while miRNeasy Mini Kit (Qiagen, Inc., Valencia, CA, USA) was applied for the extraction of miRNA from tissues and cells. RNA concentration was tested using NanoDrop 2000 (Thermo Fisher Scientific, Inc., Wilmington, DE, USA). RNA was reverse transcribed into cDNA using the First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.). mirVanaqRT-PCR miRNA Detection kit and SYBR Green I (Applied Biosystems; Thermo Fisher Scientific, Inc.) were conducted using an ABI 7500 Real Time PCR System (Thermo Fisher Scientific, Inc.). The primers were as follows: YAP, forward 5'-TAGCCCTGCGTAGCCAGTTA-3', reverse 5'-TCATGCTTAGTCCACTGTCTGT-3'; GAPDH, forward 5'-GGAGCGAGATCCCTCCAAAAT-3', reverse 5'-GGCTGT TGTCATACTTCTCATGG-3'; miR-506-3p, forward 5'-TGC GGTAAGGCACCCTTCTGAGTA-3', reverse 5'-CCAGTG CAGGGTCCGAGGT-3'; U6, forward 5'-CTCGCTTCGGCA GCACA-3', reverse 5'-AACGCTTCACGAATTTGCGT-3'. The thermocycling conditions were as follows: 95˚C for 3 min, 40 cycles of 95˚C for 10 sec, 60˚C for 15 sec and 72˚C for 31 sec. The relative expression level was determined using the $2^{-\Delta\Delta Cq}$ method (15). GAPDH and U6 were used as internal controls for Yes-associated protein 1 (YAP1) and miR-506-3p, respectively.

Transfection with miR-506-3p mimic and inhibitor. miR-506-3p mimics (50 nM), miR-506-3p inhibitor (100 nM) or their corresponding miR-negative control (miR-NC; Guangzhou RiboBio Co., Ltd., Guangzhou, China) were transfected into the cells using Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol.

Transfection with pcDNA3-YAP1. YAP1 was amplified by PCR using cDNA obtained from BEAS-2B cells, and cloned into pcDNA3 (Thermo Fisher Scientific, Inc.) to generate pcDNA3-YAP1. pcDNA3-YAP1 and negative control which were used for gain-of-function experiments were transfected into cells ($1x10^5$) using Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol.

Western blot analysis. Briefly, cells were lysed in radioimmunoprecipitation assay buffer (Thermo Fisher Scientific, Inc.), and protein concentration was determined using the BCA Protein Assay kit (Thermo Fisher Scientific, Inc.). Protein lysate (15 μg) was separated on 10% SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes (Thermo Fisher Scientific, Inc.). Next, the PVDF membranes were blocked in 5% non-fat milk (Yili, Beijing, China) for 2 h at room temperature, and then incubated with monoclonal rabbit anti-YAP1 (ab5277; 1:1,000; Abcam, Cambridge, MA, USA), B-cell lymphoma 2 (Bcl-2; ab32142; 1:1,000; Abcam), Bcl-2-associated X protein (Bax; ab32503; 1:1,000; Abcam) or monoclonal mouse anti-GAPDH (ab9485; 1:1,000; Abcam) antibody for 3 h at room temperature. Subsequently, membranes were incubated with goat anti-rabbit secondary antibody (ab97080; 1:20,000; Abcam) for 1 h at room temperature. Following washing with Tris-buffered saline, the bands were detected

using the ECL Western Blotting kit (Thermo Fisher Scientific, Inc.). Image-Pro Plus software (version 6.0; Media Cybernetics, Inc., Rockville, MD, USA) was used to analyze the relative protein expression, represented as the density ratio vs. GAPDH.

Plasmid construction and dual-luciferase reporter assay. YAP1 3'-UTR sequences (5'-ACUUUUCUAAAUGUA GUGCCUUU-3') were amplified by PCR using cDNA from 293 cells, and cloned into pGL3 basic vectors (Promega Corporation, Madison, WI, USA) between *Kpn*I and *Xho*I restriction enzyme sites. The Quick Change site-directed mutagenesis kit (Agilent Technologies, Inc., Santa Clara, CA, USA) was used for the mutation of the putative binding site of miR-506-3p in the YAP1 3'-UTR-containing vector, with the YAP1 3'UTR mutant sequence as follows: 5'-ACU UUUCUAAAUGUAGUGCGAUU-3'. For dual-luciferase reporter assay, PC-9GR cells were seeded in 24-well plates at the density of $1x10^5$/well and cultured at 37˚C overnight. On the following day, cells transfected with YAP1 3'UTR WT plasmids or YAP1 3'UTR MUT plasmids were also transfected with miR-NC mimics or miR-506-3p mimic, and luciferase reporter plasmids containing *Renilla* vector (pRL-TK; Promega Corporation). At 48 h post-transfection, the luciferase activity was determined using the Dual-Luciferase Reporter Assay System (Promega Corporation). Luciferase activity was normalized to the *Renilla* luciferase activity to determine the transfection efficiency.

Statistical analysis. All data were analyzed using Graphpad Prism software (version 6; Graphpad Software, Inc., La Jolla, CA, USA), and are presented as the mean ± standard deviation. Differences between two groups were evaluated using the Student's t-test. Differences among three or more groups were compared using one-way analysis of variance, followed by the Newman-Keuls test. The association between miR-506-3p and YAP1 was analyzed by the Pearson correlation analysis. A value of $P<0.05$ was considered to indicate a statistically significant difference. All experiments were performed in triplicate.

Results

PC-9GR cells are relatively insensitive to gefitinib treatment, compared with PC-9 cells. To study the underlying molecular mechanism of gefitinib resistance, a PC-9GR cell model was established by long-term exposure of PC-9 cells to gefitinib. PC-9 and PC-9GR cells were treated with increasing concentrations of gefitinib for 48 and 96 h, in order to test their sensitivity to gefitinib. Compared with parental PC-9 cells, gefitinib exhibited a markedly weaker cytotoxic efficacy on PC-9GR cells following exposure to doses of 2, 4, 8 and 16 μM for 48 h (Fig. 1A). In addition, the difference in the response to gefitinib treatment became more evident after 96-h treatment, as a low dose of 1 μM gefitinib was able to induce marked viability reduction in PC-9 cells, but not in PC-9GR cells (Fig. 1B). In order to investigate the role of miR-506-3p in mediating gefitinib resistance, miR-506-3p levels were compared between PC-9 and PC-9GR cells. Using RT-qPCR, miR-506-3p was found to be significantly downregulated in PC-9GR cells (Fig. 1C), suggesting that miR-506 may be involved in gefitinib resistance.

miR-506-3p inhibits the proliferation of PC-9GR cells. Next, the function of miR-506-3p in controlling the growth of PC-9GR cells was explored using mimic transfection to elevate the miR-506-3p levels. As expected, transfection with miR-506-3p mimics elevated the expression of miR-506-3p in PC-9GR cells, as compared with that in cells transfected with miR-NC mimics (Fig. 2A). The overexpression of miR-506-3p was found to induce significant growth arrest in PC-9GR cells cultured in gefitinib-free medium in a time-dependent manner (Fig. 2B). Notably, miR-506-3p elevation also markedly inhibited the growth of PC-9 cells, but to a smaller extent (Fig. 2C). These observations indicated that miR-506-3p is pivotal for the proliferation of PC-9GR cells.

Downregulation of miR-506-3p drives gefitinib resistance in PC-9 cells. In order to investigate the role of miR-506-3p in the development of gefitinib resistance, miR-506-3p expression levels were elevated or inhibited by the transfection of PC-9GR cells with miR-506-3p mimic or antagonist, respectively. The response of these cells to gefitinib treatment was then detected. Transfection with miR-506-3p antagonist significantly reduced miR-506-3p expression in PC-9GR cells, as compared with that observed in miR-NC-transfected cells (Fig. 3A). The overexpression of miR-506-3p was demonstrated to enhance gefitinib-induced cytotoxicity, whereas transfection with miR-506-3p antagonist inhibited this cytotoxicity in PC-9GR cells (Fig. 3B). Flow cytometric analysis further revealed that compared with gefitinib group, the overexpression of miR-506-3p significantly increased cell apoptosis in response to gefitinib treatment (8 μM), while the inhibition of miR-506-3p reduced the cell apoptosis rate (Fig. 3C and D). Western blot analysis indicated that Bcl-2 protein expression levels were decreased following miR-506-3p overexpression and increased following miR-506-3p inhibition. By contrast, the Bax expression levels were increased following miR-506-3p overexpression and decreased following miR-506-3p inhibition (Fig. 3E). In addition, treatment with a low concentration of gefitinib (1 μM) was able to inhibit cell proliferation in PC-9GR cells with miR-506-3p overexpression (Fig. 3F), suggesting that miR-506-3p sensitized PC-9 cells to gefitinib.

YAP1 is a target gene of miR-506-3p in PC-9 cells. Previous studies have demonstrated that YAP1 was directly suppressed by miR-506-3p in breast and liver cancer (16,17). In the current study, YAP1 mRNA and protein expression levels were found to be elevated in PC-9GR cells, as compared with those in PC-9 cells (Fig. 4A and B). However, overexpression of miR-506-3p significantly reduced YAP1 expression in PC-9GR cells (Fig. 4C and D). The results of dual-luciferase reporter assay revealed that miR-506-3p mimic transfection markedly decreased the luciferase activity of PC-9 cells transfected with YAP1 3'UTR-wild type (Fig. 4E), suggesting that YAP1 was a target gene of miR-506-3p in NSCLC cells.

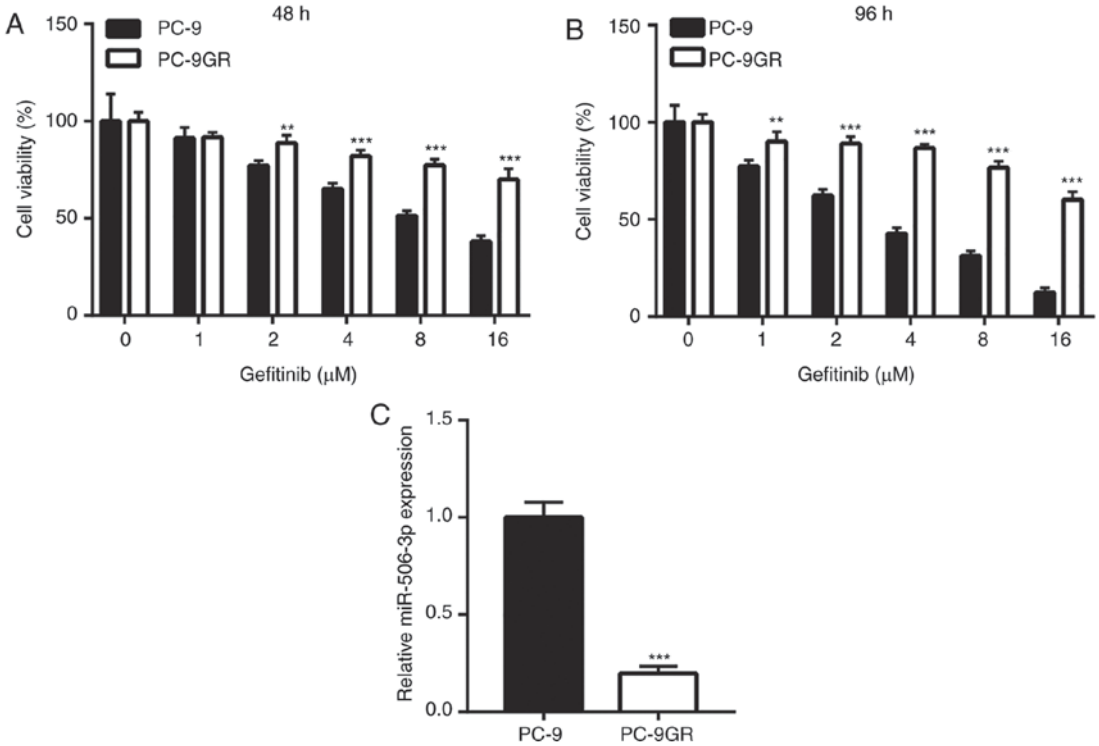

Figure 1. miR-506-3p was downregulated in PC-9GR cells, which were relatively insensitive to gefitinib, when compared with PC-9 cells. The viability reduction was significantly higher in PC-9 cells following treatment with increasing concentrations of gefitinib (1, 2, 4, 8 and 16 μM) for (A) 48 h and (B) 96 h, as compared with that in PC-9GR cells. (C) In PC-9GR cells, the expression level of miR-506-3p was decreased compared with that in PC-9 cells. **P<0.01 and ***P<0.001, vs. PC-9 cells at the same concentration. miR, microRNA; PC-9GR, gefitinib-resistant PC-9.

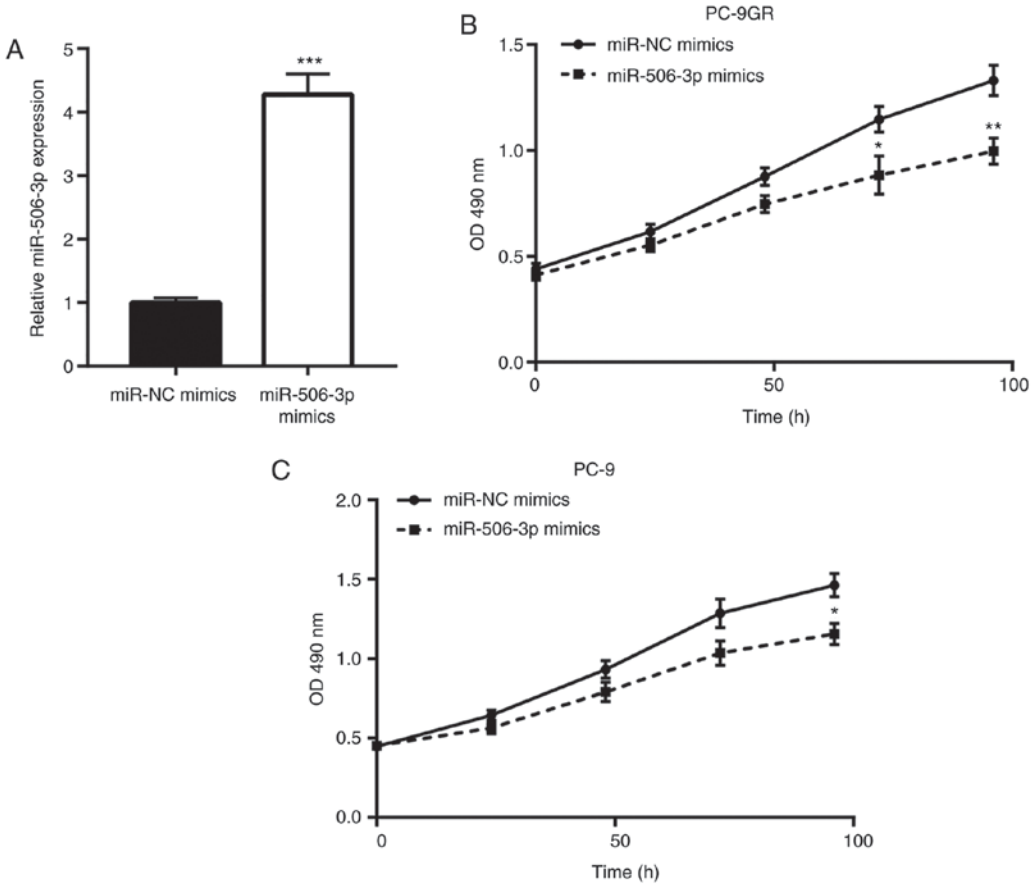

Figure 2. Overexpression of miR-506-3p inhibited the growth of PC-9 and PC-9GR cells. (A) Transfection with miR-506-3p mimics elevated the miR-506-3p expression in PC-9GR cells. The elevation of miR-506-3p induced growth arrest in (B) PC-9GR and (C) PC-9 cells. *P<0.05, **P<0.01 and ***P<0.001, vs. miR-NC group. miR, microRNA; PC-9GR, gefitinib-resistant PC-9; NC, negative control.

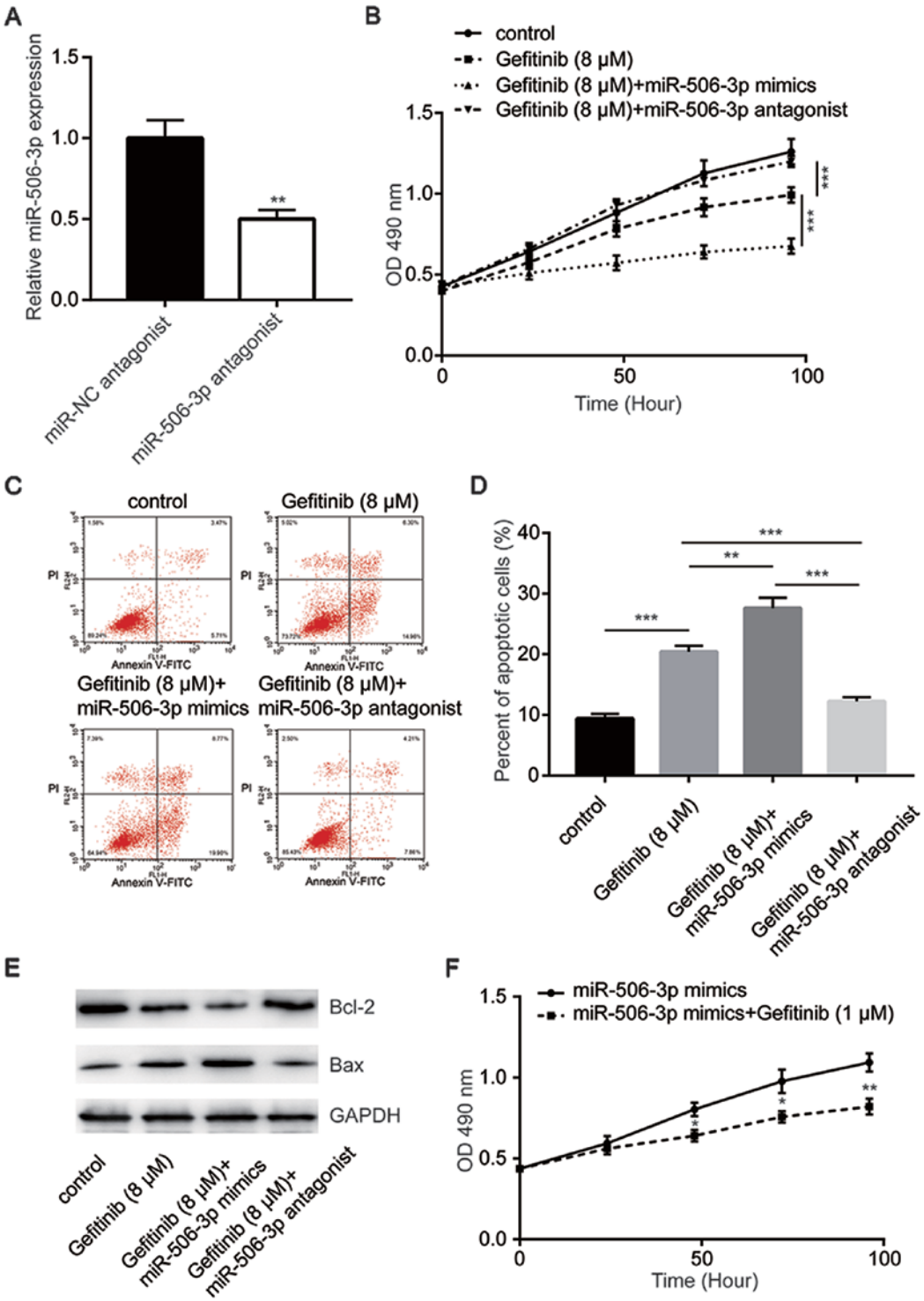

Figure 3. miR-506-3p-mediated gefitinib sensitivity in PC-9GR cells. (A) Transfection with miR-506-3p antagonist decreased the miR-506-3p expression levels in PC-9GR cells. (B) miR-506-3p mimics enhanced gefitinib (8 μM for 48 h) treatment-induced reduction in the viability of PC-9GR cells, while the miR-506-3p antagonist reversed the cell viability reduction. (C) Flow cytometric assay and (D) quantitative analysis of cell apoptosis, demonstrating that miR-506-3p mimics enhanced gefitinib (8 μM for 48 h) treatment-induced apoptosis in PC-9GR cells, while the miR-506-3p antagonist reversed cell apoptosis. (E) miR-506-3p mimics enhanced gefitinib (8 μM for 48 h) treatment-induced Bcl-2 reduction and Bax elevation in PC-9GR cells, while miR-506-3p antagonist reversed the expression alteration of Bcl-2 and Bax. (F) After treatment for 48 h, 1 μM gefitinib induced growth arrest in PC-9GR cells transfected with miR-506-3p mimics, although this concentration did not alter the viability of untransfected PC-9GR cells. *P<0.05, **P<0.01 and ***P<0.001, vs. corresponding control group. miR, microRNA; PC-9GR, gefitinib-resistant PC-9; NC, negative control; Bax, Bcl-2-associated X protein.

YAP1 is involved in miR-506-3p-mediated gefitinib sensitivity in PC-9 cells. In order to investigate the association between YAP1 expression and miR-506-3p-associated gefitinib sensitivity in NSCLC cells, YAP1 overexpression was induced in PC-9GR cells by the transfection with pcDNA3-YAP1 plasmid (Fig. 5A). As shown in Fig. 5B, the overexpression of YAP1 was able to partially reverse the elevated sensitivity to gefitinib in PC-9GR cells transfected with miR-506-3p mimics

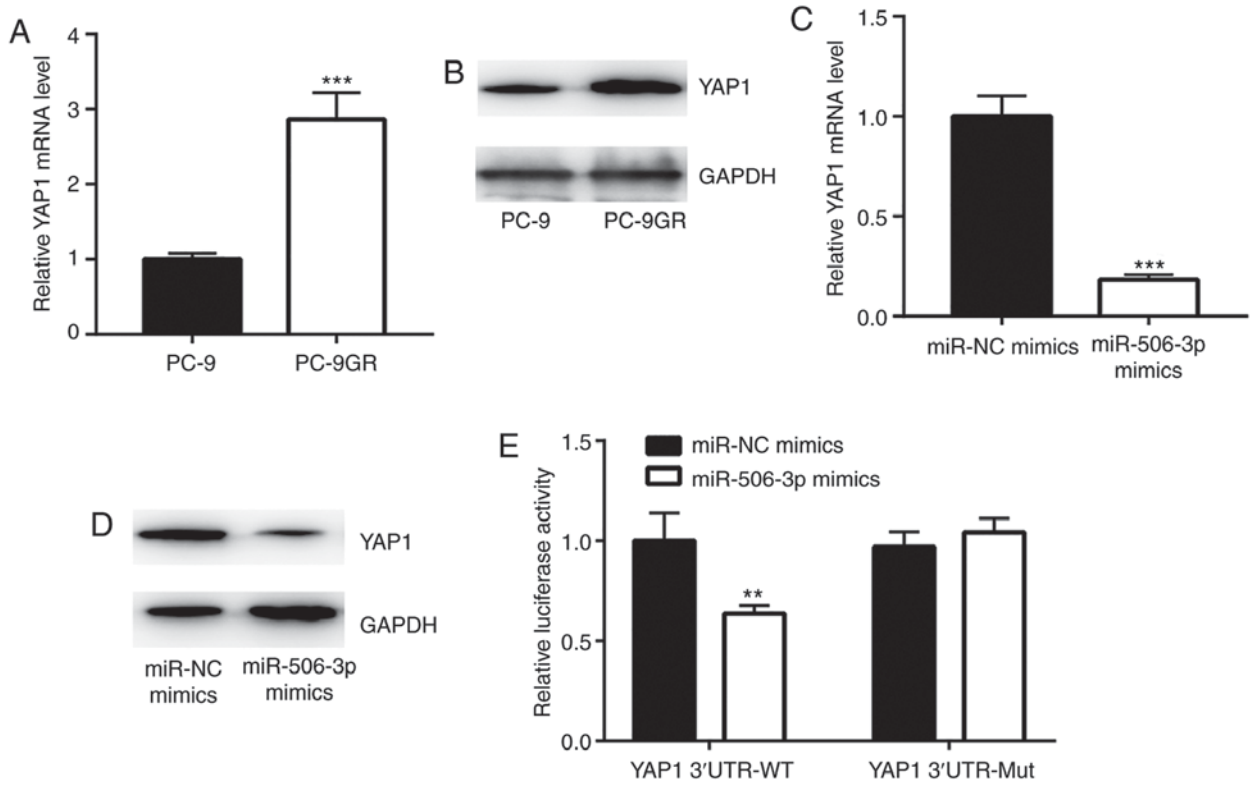

Figure 4. YAP1 is downregulated in PC-9GR cells and is a target gene of miR-506-3p. (A) mRNA and (B) protein levels of YAP1 were elevated in PC-9GR cells, as compared with those in PC-9 cells. (C) mRNA and (D) protein levels of YAP1 in PC-9GR cells were decreased by overexpression of miR-506-3p. (E) Transfection with miR-506-3p mimics reduced the luciferase activity of PC-9GR cells transfected with YAP1 3'UTR-WT. **P<0.01 and ***P<0.001, vs. corresponding control group. miR, microRNA; PC-9GR, gefitinib-resistant PC-9; NC, negative control; YAP1, Yes associated protein 1; 3'UTR, 3'-untranslated region; WT, wild-type; Mut, mutated.

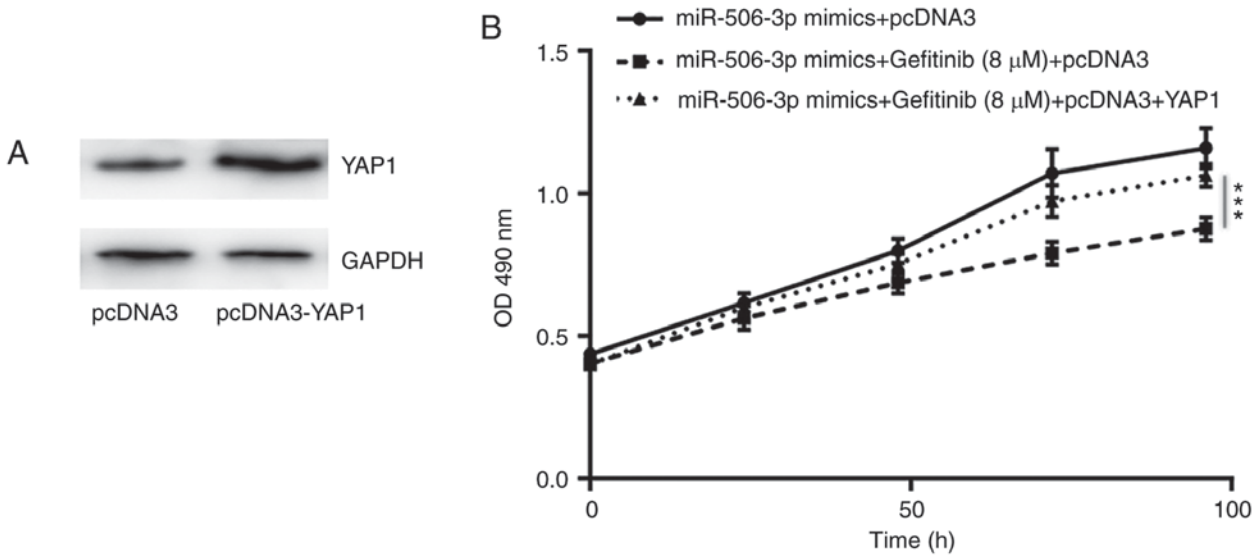

Figure 5. YAP1 was involved in miR-506-3p-mediated gefitinib sensitivity in PC-9GR cells. (A) Transfection with pcDNA3-YAP1 elevated the YAP1 protein level in PC-9GR cells. (B) miR-506-3p overexpression sensitized PC-9GR cells to gefitinib treatment, whereas simultaneous overexpression of YAP1 partially reversed this phenomenon. ***P<0.001. miR, microRNA; PC-9GR, gefitinib-resistant PC-9; NC, negative control; YAP1, Yes associated protein 1.

(Fig. 5B), indicating that YAP1 was pivotal in the regulatory role of miR-506-3p in gefitinib sensitivity.

miR-506-3p expression is downregulated in tumor tissues of patients with NSCLC and correlated with YAP1 mRNA levels. To evaluate the role of miR-506-3p and YAP1 expression levels in NSCLC cells, RT-qPCR was used to detect the mRNA levels of miR-506-3p and YAP1 in 26 pairs of tumor tissues and matched normal tissues from patients with NSCLC. As compared with the normal tissues, a significant decrease in

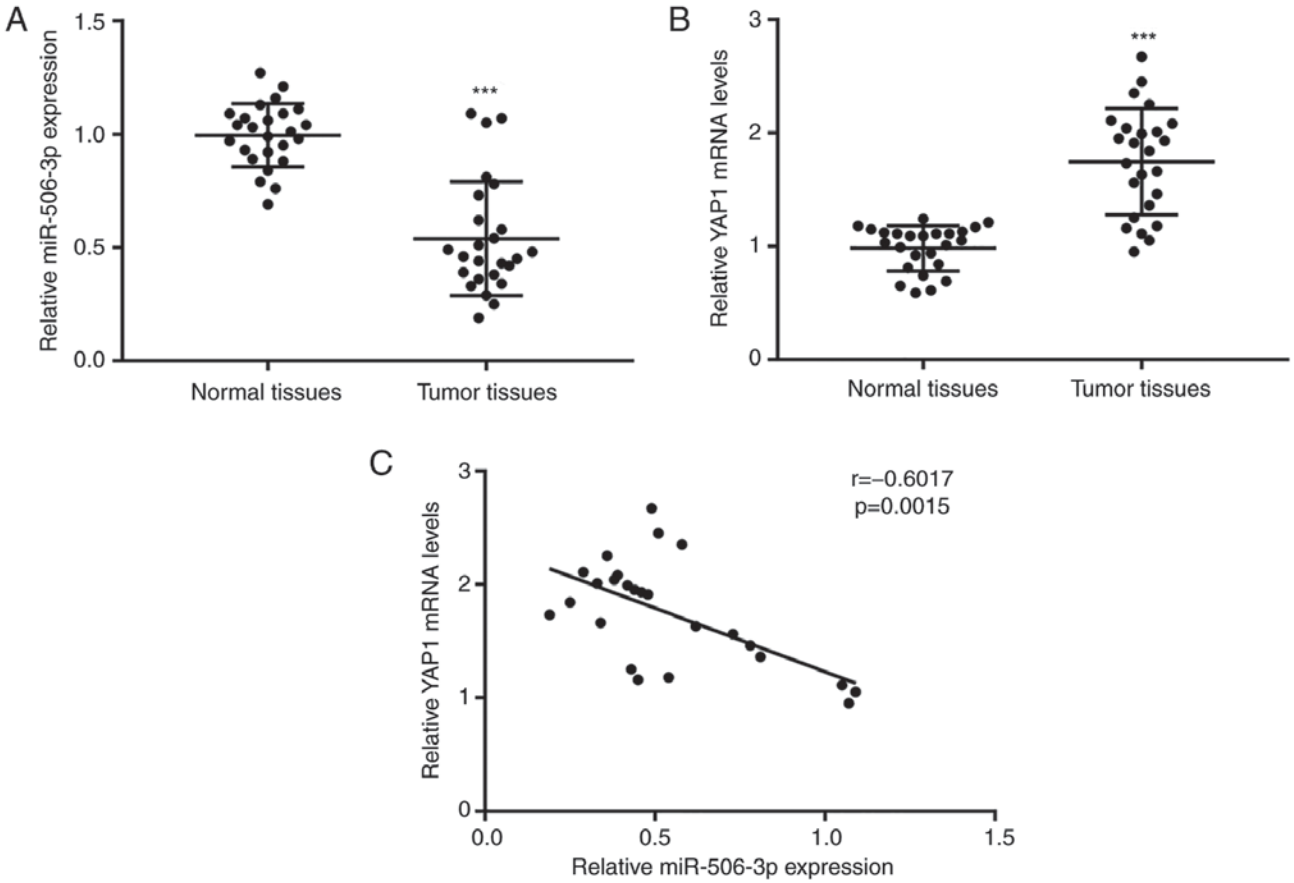

Figure 6. Negative correlation between miR-506-3p expression and YAP1 mRNA levels in NSCLC. (A) miR-506-3p was downregulated and (B) YAP1 mRNA was upregulated in tumor tissues obtained from 25 patients with NSCLC, as compared with the adjacent normal tissues. (C) Pearson correlation analysis indicated that the expression of miR-506-3p was inversely associated with YAP1 mRNA levels in tumor tissues from NSCLC patients. ***P<0.001, vs. normal tissues. miR, microRNA; YAP1, Yes associated protein 1; NSCLC, non-small cell lung cancer.

miR-506-3p expression was observed in tumor tissues, while YAP1 expression was found to be elevated (Fig. 6A and B). Pearson correlation analysis revealed a strong negative correlation (r=-0.6017, P=0.0015) between the miR-506-3p expression and YAP1 mRNA levels in tumor tissues (Fig. 6C). These results further validated the regulatory association between miR-506-3p and YAP1 in NSCLC tumor tissues.

Discussion

Numerous studies have reported aberrant expression of miR-506-3p in multiple cancer types, including breast, pancreatic and colorectal cancer (18-20). In lung cancer, research on the expression of miR-506-3p has provided conflicting results. Yin *et al* (21) reported that miR-506-3p was upregulated in lung cancer tissue in 83% of patients with lung cancer; however, the elevation in miR-506-3p expression inhibited tumor growth in lung cancer cells *in vivo* (17). Another recent study detected miR-506-3p downregulation in NSCLC and revealed that miR-506-3p inhibited NSCLC progression (22). In the present study, the downregulation of miR-506-3p expression in NSCLC was confirmed, and the role of miR-506-3p in mediating gefitinib sensitivity in NSCLC cells was identified.

Various mechanisms for the development of gefitinib resistance have been identified (23-25). Altered expression of several miRNAs has also been associated with gefitinib sensitivity, via the regulation of their target genes in NSCLC cells (12,26,27). The expression of miR-506-3p was identified to be a predictor of the response to chemotherapy in multiple cancer types (19,28,29). However, the function of miR-506-3p in gefitinib sensitivity remains unknown. The results of the present study demonstrated a decrease in the miR-506-3p expression levels in PC-9GR cells, as compared with those in the parental cells. The cell proliferation assay demonstrated that miR-506-3p mimics inhibited the growth of both PC-9GR and PC-9 cells compared with miR-NC mimics, which is consistent with the findings of a previous study on the anti-proliferative function of miR-506-3p in NSCLC cells (22). Notably, the overexpression of miR-506-3p markedly increased the cell viability reduction and apoptosis following gefitinib treatment in PC-9GR cells, while the inhibition of miR-506-3p was demonstrated to have the opposing effect. Bcl-2 and Bax are known regulators of cell apoptosis. miR-506-3p overexpression increased the gefitinib-induced Bcl-2 reduction and Bax elevation in PC-9GR cells, suggesting enhanced cytotoxicity. By contrast, the inhibition of miR-506-3p decreased the Bcl-2 expression and increased the Bax expression following gefitinib treatment. In addition, in PC-9GR cells transfected with the miR-506-3p antagonist, treatment with 1 μM gefitinib induced growth

arrest in PC-9GR cells, although this concentration did not inhibit cell growth in untransfected PC-9GR cells. These data collectively revealed a gefitinib-sensitizing role of miR-506-3p in NSCLC cells.

The Hippo/YAP pathway is a well-defined signaling pathway in mediating cell growth and controlling organ size (30). In cancer cells, YAP1 is often elevated, contributing to drug resistance through the maintenance of stemness (31,32). Specifically, the elevation of YAP1 has also been found to be involved in the development of gefitinib resistance in NSCLC cells (33,34). In the PC-9GR cell line established in the present study, a significant elevation of mRNA and protein YAP1 levels was observed, as compared with the same levels in PC-9 cells, suggesting that YAP1 may also be implicated in gefitinib resistance. Furthermore, YAP1 has been identified as a target gene of miR-506-3p in breast and liver cancer (35,36). In the present study, it was observed that the overexpression of miR-506-3p decreased YAP1 expression in PC-9GR cells. In addition, dual-luciferase reporter assay confirmed YAP1 as a target gene of miR-506-3p in PC-9GR cells. Notably, YAP1 elevation partially reversed miR-506-3p mimic-induced hypersensitivity of PC-9GR cells to gefitinib treatment, suggesting that YAP1 served a pivotal role in the mediation of miR-506-3p in gefitinib sensitivity in NSCLC cells. Furthermore, the analysis of miR-506-3p and YAP1 expression levels in specimens collected from NSCLC patients indicated a negative correlation between these levels, providing evidence of the regulatory association of miR-506-3p and YAP1 in NSCLC.

In conclusion, the results of the present study demonstrated that the downregulation of miR-506-3p contributes to gefitinib resistance in NSCLC cells via the regulation of YAP1. This newly identified miR-506-3p/YAP1 axis in NSCLC provided insights for the development of a miR-506-based therapeutic approach to overcome gefitinib resistance in patients with NSCLC. However, the lack of reasonable correlation between miR-506-3p/YAP1 and EGFR-TKIs is a limitation of the present study, which will be investigated in our future research.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JZ and LT performed the experiments, and analyzed the data. LJ conceived the study, analyzed the data and prepared the manuscript.

Ethics approval and consent to participate

The current study was approved by the ethics committee of Taizhou Central Hospital. Each patient was informed and consented to participate.

Patient consent for publication

Each patient consented to the publication of the clinical data.

Competing interests

The authors declare that they have no competing interests.

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