Overexpression of microRNA-124-5p sensitizes non-small cell lung cancer cells to treatment with 5-fluorouracil via AEG-1 regulation

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Abstract. Chemotherapeutic resistance represents a major obstacle for the treatment of patients with non-small cell lung cancer (NSCLC); however, the associated molecular mechanisms underpinning the development of resistance remain poorly characterized. In the current study, 5-fluorouracil (5-FU)-resistant A549 cells (A549/5-FU) were generated from A549 cells. Reverse transcription-quantitative PCR and western blotting were used to detect microRNA(miR)-124-5p and astrocyte elevated gene 1 (AEG-1) expression levels in cells and tumor tissues. In addition, the cytotoxic effect of 5-FU on the cells was determined using the Cell Counting Kit-8 assay, and the Dual-luciferase reporter assay was used to validate AEG-1 as a target gene of miR-124-5p. Transfection with a miR-124-5p mimic enhanced inhibition of cell viability induced by 5-FU in A549/5-FU cells, whereas miR-124-5p inhibitor transfection partially reversed 5-FU-induced cell viability inhibition in A549 and H1299 cells. A decrease in miR-124-5p expression level was observed in A549/5-FU cells compared with the parental A549 cells. Furthermore, AEG-1 was predicted as a target gene of miR-124-5p, and its expression was increased in A549/5-FU cells compared with A549 cells. Additionally, the upregulation of miR-124-5p was associated with lower expression levels of AEG-1 in A549/5-FU cells, compared with parental A549 cells. Moreover, the Dual-luciferase reporter assay confirmed the ability of miR-124-5p to bind directly to the 3'-untranslated region of AEG-1 mRNA. Notably, the overexpression of AEG-1 reversed the ability of the miR-124-5p mimic to increase the sensitivity of A549/5-FU cells to 5-FU treatment. Additionally, a significant negative correlation between miR-124-5p expression and AEG-1 mRNA levels was detected in 40 pairs of NSCLC tissues and their corresponding adjacent paracancerous tissues. The results of the present study indicated that miR-124-5p may regulate the chemotherapeutic sensitivity of NSCLC cells, and may therefore represent a promising biomarker or therapeutic target for patients with NSCLC.

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Introduction

Lung cancer resulted in >1.7 million mortalities globally in 2018 (1). Non-small cell lung cancer (NSCLC) is the most prevalent lung cancer subtype that accounts for >80% of lung cancer cases (2). Chemotherapy is a standard therapeutic approach for patients with NSCLC that has been demonstrated to increase the overall survival time of patients (3,4). 5-Fluorouracil (5-FU) is one of most commonly used chemotherapeutic agents for patients with NSCLC (5). Mechanistically, it disrupts uracil metabolism in cancer cells and initially inhibits cancer progression (6); however, resistance to 5-FU frequently develops and results in poor therapeutic outcomes and patient mortality (7). Therefore, numerous studies have attempted to investigate the complex mechanisms underpinning the development chemotherapeutic resistance in order to improve the efficacy of chemotherapy treatment regimens and, ultimately, patient outcomes (8,9).

MicroRNAs (miRNAs) are a group of non-coding, single-stranded RNA molecules ubiquitously expressed in human cells (10). In cells, miRNAs serve as negative regulators of gene expression by binding to the 3'-untranslated region (UTR) of target gene mRNAs and inducing mRNA degradation or inhibiting translation (11). Expression of miRNAs is essential for multiple biological processes, such as cell proliferation and differentiation (12,13). However, the aberrant expression of certain miRNAs and the subsequent dysregulation of target gene expression have been associated with the genesis and progression of multiple types of cancer (14,15). Downregulation of miRNA (miR)-124-5p is associated with lymphangiogenesis in human gastric cancer (16); additionally, low expression levels of miR-124-5p have been detected in glioma and colorectal cancer cells (17,18).

Astrocyte elevated gene-1 (AEG-1) was originally identified in primary human fetal astrocytes (19) and it was subsequently revealed that AEG-1 influenced the progression of several types of cancer. In lung cancer, the upregulation of AEG-1 was associated with the epithelial-mesenchymal transition (EMT) and mediated metastasis (20); furthermore,

increased expression of AEG-1 was associated with chemotherapeutic resistance in cancer cells (21,22). However, the role of AEG-1 in the development of resistance to chemotherapy in lung cancer is yet to be elucidated.

The results of the present study revealed that the down-regulation of miR-124-5p conferred chemotherapeutic resistance and may, therefore, represent a novel treatment strategy for patients with NSCLC.

Materials and methods

Patients and tissue samples. A total of 40 pairs of tumors and adjacent noncancerous tissues (>5 cm from tumors) were surgery removed from patients with NSCLC at The Third People's Hospital of Linyi City (Shandong, China) between June 2015 and October 2017. Tissues were stored at -80˚C until further use. The present study was approved by the Ethics Committee of The Third People's Hospital of Linyi City, and all participants provided written informed consent.

Cell culture. A549, H1299 and 293 cell lines were purchased from the American Type Culture Collection and cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Inc.) in a humidified incubator with 5% CO_2 at 37˚C. 5-FU was purchased from Sigma-Aldrich; Merck KGaA, dissolved in dimethyl sulfoxide (DMSO) and diluted in DMEM prior to use.

Generation of 5-FU resistant A549 (A549/5-FU) cells. A549/5-FU cells were generated from A549 parental cells. Briefly, A549 cells were treated with gradually increasing concentrations of 5-FU (0.1-10 μM) in DMEM for >9 months (23). The proliferation of A549 cells was initially inhibited by 5-FU. However, following culture with 5-FU for 8 months, the cell viability assay revealed that the cells were relatively insensitive towards 5-FU compared with cells treated with DMSO in the control group.

Elevation/inhibition of miR-124-5p. miR-124-5p mimic (5'-CGUGUUCACAGCGGACCUUGAU-3'); miR-negative control (NC) (5'-AUUGGAACGAUACAGAGAAGAUU-3'); miR-124-5p inhibitor (5'-AUCAAGGUCCGCUGUGAACACG-3'); and miR-NC inhibitor (5'-CAGUACUUUUGUGUAGUACAA-3') were purchased from Applied Biological Materials, Inc. The miR-124-5p mimic or inhibitor (50 nM) was transfected into the A549, A549/5-FU and H1299 cell lines using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. In brief, miR-124-5p mimic or inhibitor (50 nM) was mixed with Lipofectamine® 2000 in DMEM at 37˚C for 30 min before being added to the cultured cells. The transfection efficacy was assessed 72 h after transfection using RT-qPCR.

Flow cytometry analysis. The percentage of apoptotic A549/5-FU cells was measured using the Annexin V-FITC Apoptosis Detection Kit I (BD Biosciences) according to the manufacturer's protocol. Briefly, 48 h following treatment with 1 μM 5-FU or DMSO, and transfection with miR-NC mimic or miR-124-5p mimic, $5x10^5$ A549/5-FU cells were collected and stained with PI and FITC-Annexin V at room temperature for 30 min. The samples were analyzed using a FACSCalibur flow cytometer (BD Biosciences). The data were analyzed using FlowJo software V. 1.6.0 (FlowJo software LLC). PI^+/Annexin V^+ and PI^-/Annexin V^+ cells were considered to indicate apoptotic cells.

Western blotting. Total protein lysates were extracted from A549, A549/5-FU and H1299 cells using radioimmunoprecipitation assay lysis buffer (Sigma-Aldrich; Merck KGaA). A total of 25 μg/lane of the lysates were separated by SDS-PAGE on an 8% gel. The concentration of lysates was determined using a Bicinchoninic acid Protein Assay kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The separated proteins were subsequently transferred onto a polyvinylidene difluoride membrane and blocked with 5% non-fat milk for 1 h at room temperature. The membrane was incubated with primary antibodies against AEG-1 (cat. no. 14065; 1:1,000; Cell Signaling Technology, Inc.) and β-actin (cat. no. A1978; 1:10,000; Sigma Aldrich, Merck KGaA) overnight at 4˚C. Following incubation, the membrane was washed with TBST (0.2% Tween-20) and incubated with HRP-conjugated secondary antibodies against rabbit (cat. no. ABL3012-2; 1:100,000; AbSci) and mouse (cat. no. ABL3032-2; 1:100,000; AbSci) for 1 h at 4˚C. The protein bands were visualized using Pierce™ Enhanced chemiluminescence Western Blot Substrate (Pierce; Thermo Fisher Scientific, Inc.) and Image Quant LAS 500 (GE Healthcare Life Sciences).

RNA extraction and reverse transcription-quantitative (RT-q) PCR. Total RNA was extracted from lung cancer and adjacent lung tissues, A549, A549/5-FU and H1299 cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). For analysis of miR-124-5p expression, RT was performed using a stem-loop primer and the RevertAid First Strand cDNA kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. For mRNA expression analysis, RNA was reverse transcribed into cDNA using PrimeScript RT Master mix (Takara Bio, Inc.) according to the manufacturer's protocol. qPCR was subsequently performed using a SYBR® Premix Ex Taq kit (Takara Bio, Inc.). miRNA and mRNA expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method (24) and normalized to U6 and β-actin levels, respectively. The thermocycling condition were as follow: 95˚C for 30 sec; 35 cycles of 95˚C for 15 sec and 60˚ for 20 sec. The following primer sequences were used: Stem loop, 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGATCAAG-G-3'; miR-124-5p forward, 5'-TCGGCAGGCGTGTTCACAGCGG-3' and reverse, 5'-CTCAACTGGTGTCGTGGA-3'; U6 forward, 5'-CTCAACTGGTGTC-GTGGA-3' and reverse, 5'-CTCAACTGGTGTCGTGGA-3'; AEG-1 forward, 5'-AAATGGGCGGACTGTTGAAGT-3' and reverse, 5'-CTGTTTTGCACTGC-TTTAGCAT-3'; β-actin forward, 5'-CATGTACGTTGCTATCCAGGC-3' and reverse, 5'-CTCCTTAATGTCACGCACGAT-3'.

Cytotoxicity assay. The Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc.) was used to investigate the cytotoxicity of 5-FU in A549 and H1299 cell lines. Briefly, 1,000 cells/well were seeded in a 96-well plate and incubated

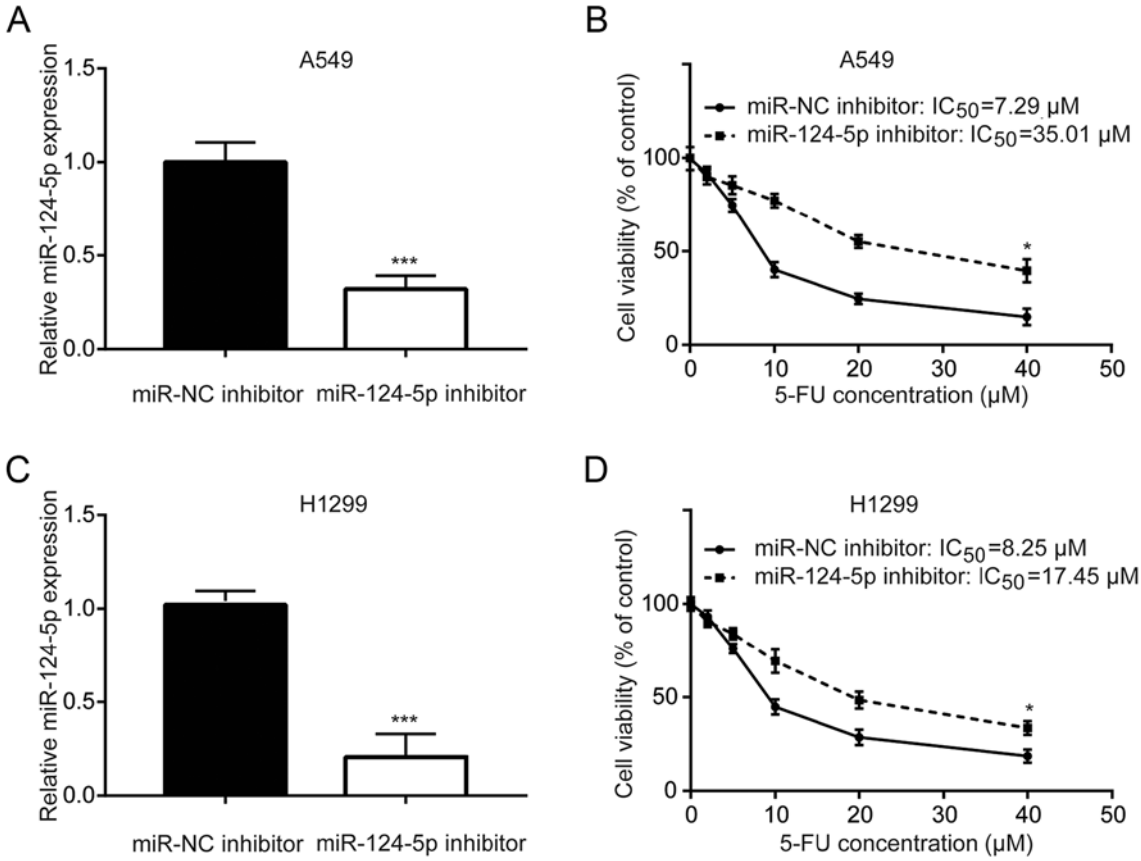

Figure 1. miR-124-5p increases 5-FU sensitivity in A549 and H1299 cells. (A) Transfection with a miR-124-5p inhibitor decreased miR-124-5p expression in A549 cells. (B) Inhibition of miR-124-5p desensitized A549 cells to 5-FU treatment. (C) Transfection with a miR-124-5p inhibitor decreased miR-124-5p expression in H1299 cells. (D) Inhibition of miR-124-5p reduced the sensitivity of H1299 cells to treatment with 5-FU. *P<0.05 and ***P<0.001. miR, microRNA; 5-FU, 5-fluorouracil; NC, negative control; IC_{50}, half-maximal inhibitory concentration.

for 24 h at 37˚C. The cells were treated with DMSO or increasing concentrations of 5-FU (0, 2, 5, 10, 20 and 40 μM) for 72 h; subsequently 10 μl CCK-8 solution was added to each well and incubated for an additional 2 h. DMEM containing the CCK-8 solution was transferred into wells of another 96-well plate, and the absorbance of each well was measured at 450 nm using a microplate reader. The ratio of absorbance of 5-FU- to DMSO-treated wells was used to determine the cytotoxicity. The half maximal inhibitory concentration (IC_{50}) was calculated using the online tool IC_{50} Calculator (https://www.aatbio.com/tools/ic50-calculator).

Dual-luciferase reporter assay. TargetScan (http://www.targetscan.org/vert_72/) was used to predict the potential target genes of miR-124-5p. The AEG-1 3'-UTR was amplified from the cDNA of the 293 cell line and was subsequently cloned into the pmirGLO plasmid (Promega Corporation) in order to synthesize pmirGLO-AEG-1 3'UTR-wild-type (WT). A pmirGLO-AEG-1 3'UTR-mutant (mut) containing two mutations in the predicted miR-124-5p binding site was generated by introducing site-specific mutations in the AEG-1 3'UTR-WT using the Quick Site-Directed Mutagenesis kit (Agilent Technologies, Inc.). A549 cells were transfected with either pmirGLO-AEG-1 3'UTR-WT or 3'UTR-mut, as well as the miR-124-5p mimic or miR-NC mimic, using Lipofectamine® 2000. Following incubation for 48 h at 37˚C, relative luciferase activity was detected using a Dual-Glo Luciferase Assay system (Promega Corporation) according to the manufacturer's protocol. For normalization, the firefly luciferase was normalized to *Renilla* luciferase.

Knockdown and overexpression of AEG-1. Control small interfering (si)RNA (5'-TTCTCCGAACGTGTCACGT-3') and AEG-1 siRNA (5'-AACAGAAGAAGAAGAACCGGA-3') were purchased from Shanghai GenePharma Co., Ltd. Transient silencing was performed on AEG-1 cells; 50 nM AEG-1 siRNA was mixed with Lipofectamine® RNAiMax (Invitrogen; Thermo Fisher Scientific, Inc.) in serum-free DMEM for 5 min at room temperature and added to the A549 and A549/5-FU cells. The cells were used for further experimentation 72 h post-transfection.

Full length AEG-1 cDNA was amplified from A549 cDNA and cloned into a pcDNA3.1 vector (Addgene, Inc.) with PrimeSTAR® GXL DNA Polymerase (Takara Bio, Inc.). The thermocycling conditions were 30 cycles at 98˚C for 10 sec followed by 68˚C for 120 sec. To initiate overexpression of AEG-1, 2 μg pcDNA3.1-AEG-1 was incubated with Lipofectamine® 2000 in serum-free DMEM for 15 min at room temperature and subsequently added to the A549 and A549/5-FU cells. These cells were used for further experimentation 24 h after transfection.

Statistical analysis. The data were analyzed using GraphPad Prism software 6.0 (GraphPad Software, Inc.) and are expressed

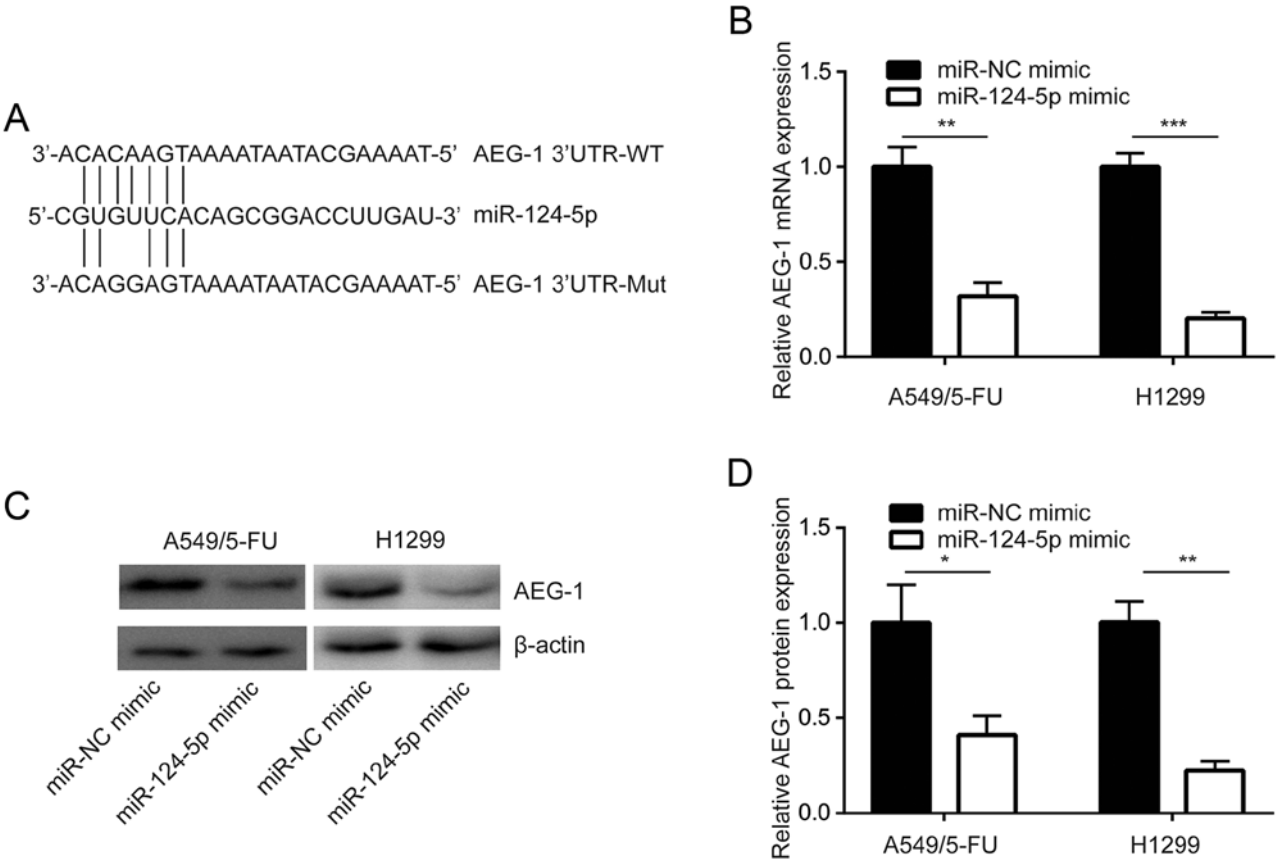

Figure 2. miR-124-5p negatively regulates AEG-1 expression in A549 and H1299 cells. (A) The 3'UTR of AEG-1 mRNA exhibited a binding site complementary to miR-124-5p. Two site mutations were introduced into the putative binding site to construct AEG-1 3'UTR-Mut. (B) Transfection with a miR-124-5p mimic decreased AEG-1 mRNA levels in A549 and H1299 cells. (C) Transfection with a miR-124-5p mimic decreased AEG-1 protein levels in A549 and H1299 cells. (D) Quantitative analysis of AEG-1 protein expression. *P<0.05, **P<0.01 and ***P<0.001. miR, microRNA; 5-FU, 5-fluorouracil; NC, negative control; AEG-1, astrocyte elevated gene-1; 3'UTR, 3'untranslated region.

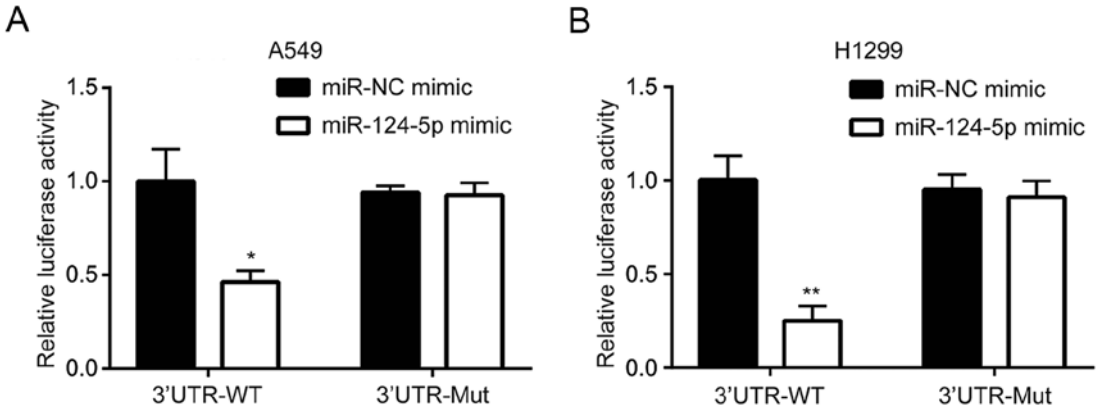

Figure 3. AEG-1 is a direct target of miR-124-5p in A549 and H1299 cells. (A) A dual-luciferase reporter assay revealed that the miR-124-5p mimic significantly repressed the relative luciferase activity of A549 cells transfected with AEG-1 3'UTR-WT. (B) Transfection with a miR-124-5p mimic significantly repressed the relative luciferase activity of H1299 cells transfected with AEG-1 3'UTR-WT. *P<0.05; **P<0.01. miR, microRNA; 5-FU, 5-fluorouracil; NC, negative control; WT, wild-type; AEG-1, astrocyte elevated gene-1; 3'UTR, 3'untranslated region.

as the mean ± SD. Two-tailed paired Student's t-test was used to evaluate statistical differences between two groups. One-way ANOVA followed by the Newman Keul's post-hoc test was used for the analysis of three groups. Pearson's correlation analysis was used to determine the correlation between the expression levels of miR-124-5p and AEG-1 in patient tissues. P<0.05 was considered to indicate a statistically significant difference.

Results

miR-124-5p inhibitor decreases A549 and H1299 cell sensitivity to 5-FU. miR-124-5p has previously been identified as a prognostic predictor for patients with NSCLC (25). As demonstrated in Fig. 1A, transfection with the miR-124-5p inhibitor decreased miR-124-5p expression in A549 cells.

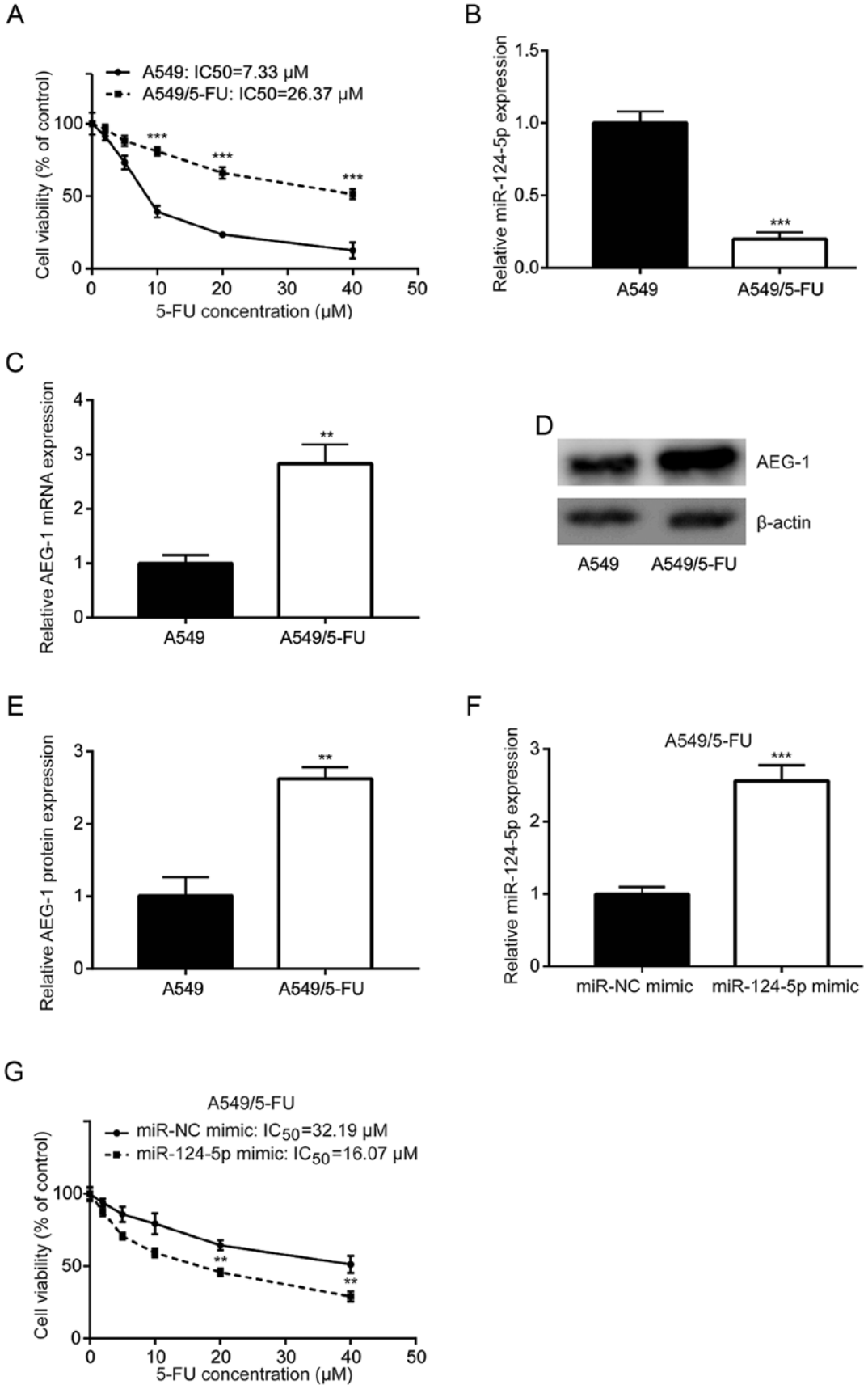

Figure 4. Aberrant expression of miR-124-5p and AEG-1 in A549/5-FU cells. (A) A549/5-FU cells were relatively insensitive to treatment with increasing concentrations of 5-FU compared with A549 cells. (B) In A549/5-FU cells, expression of miR-124-5p was decreased in comparison with parental A549 cells. (C) AEG-1 mRNA levels were increased in A549/5-FU cells compared with A549 cells. (D) Western blotting revealed that the protein expression level of AEG-1 was elevated in A549/5-FU cells compared with A549 cells. (E) Quantification of AEG-1 protein expression level. (F) Transfection with a miR-124-5p mimic resulted in increased miR-124-5p levels in A549/5-FU cells. (G) Transfection with a miR-124-5p mimic sensitized A549/5-FU cells to 5-FU treatment. **P<0.01 and ***P<0.001. miR, microRNA; 5-FU, 5-fluorouracil; NC, negative control; AEG-1, astrocyte elevated gene-1; 3'UTR, 3'untranslated region; IC_{50}, half-maximal inhibitory concentration.

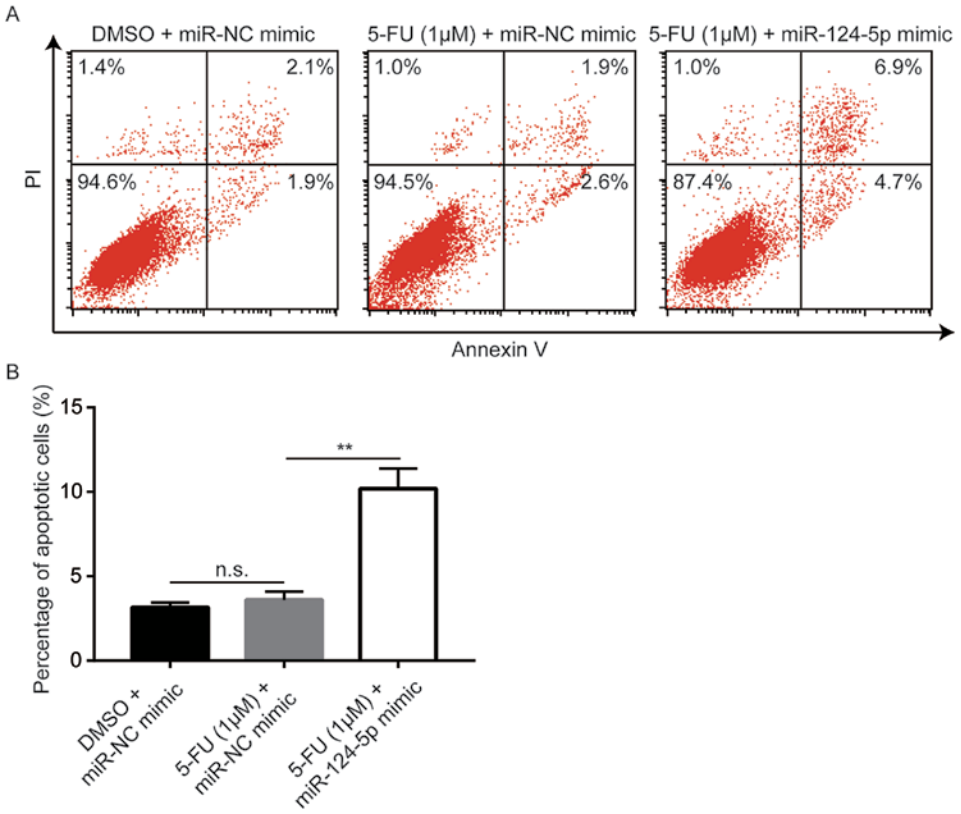

Figure 5. miR-124-5p mimic induced apoptosis in A549/5-FU cells treated with a low concentration of 5-FU, detected using flow cytometry. (A) Representative images of apoptosis in A549/5-FU cells treated with 1 μM 5-FU or 1 μM 5-FU + miR-124-5p mimic. (B) Quantitative analysis of apoptosis indicated that a low concentration of 5-FU (1 μM) did not result in apoptosis, whereas the miR-124-5p mimic in combination with 5-FU (1 μM) resulted in significant apoptosis in A549/5-FU cells. **P<0.01. miR, microRNA; 5-FU, 5-fluorouracil; NC, negative control; PI, propidium iodide; DMSO, dimethyl sulfoxide; n.s., not significant.

Inhibition of miR-124-5p significantly increased the 5-FU IC_{50} value (7.29 vs. 35.01 μM) of A549 cells compared with the NC, suggesting decreased sensitivity of A549 cells to 5-FU (Fig. 1B). Similarly, in another NSCLC cell line H1299, downregulation of miR-124-5p significantly increased the 5-FU IC_{50} value (8.25 vs. 17.45 μM) of H1299 cells compared with the NC (Fig. 1C and D). These results indicated that miR-124-5p may mediate 5-FU sensitivity in A549 and H1299 cells.

miR-124-5p negatively regulates AEG-1 expression in NSCLC cells. TargetScan was used to predict the potential target genes of miR-124-5p, which was determined to be complementary to the 3'-UTR of AEG-1 mRNA, a known sensitizer of chemotherapy (21). This indicated that miR-124-5p may regulate AEG-1 expression (Fig. 2A). In addition, in A549/5-FU cells, overexpression of miR-124-5p reduced AEG-1 mRNA expression (Fig. 2B). Western blot analysis revealed that AEG-1 protein expression was decreased following miR-124-5p overexpression in A549/5-FU cells, which was also demonstrated in H1299 cells (Fig. 2C and D). These results revealed that miR-124-5p negatively regulated the expression of AEG-1 in NSCLC cells.

AEG-1 is a target gene of miR-124-5p in NSCLC cells. The dual-luciferase reporter assay revealed that the miR-124-5p mimic reduced luciferase activity in A549/5-FU cells transfected with AEG-1 3'UTR-WT compared with cells transfected with AEG-1 3'UTR-mut (Fig. 3A). This suggested that AEG-1 was a target gene of miR-124-5p. Similar results were observed in H1299 cells (Fig. 3B).

Downregulation of miR-124-5p and overexpression of AEG-1 in 5-FU-resistant A549 cells. To investigate the mechanism of 5-FU-resistance development in NSCLC, A549/5-FU cells were established by exposing parental A549 cells to gradually increasing concentrations of 5-FU. Cytotoxicity assays revealed that treatment with 5-FU significantly reduced the cell viability of A549, but not A549/5-FU cells (Fig. 4A). In addition, RT-qPCR revealed that miR-124-5p expression was decreased in A549/5-FU cells compared with the parental cells (Fig. 4B), and AEG-1 mRNA expression was increased in A549/5-FU cells compared with A549 cells (Fig. 4C). Western blot analysis revealed similar results in terms of protein expression (Fig. 4D and E). Of note, transfection with the miR-124-5p mimic sensitized A549/5-FU cells to 5-FU treatment (Fig. 4F and G).

miR-124-5p mimic enhances 5-FU-induced cell apoptosis in A549/5-FU cells. 5-FU blocks thymidylate synthase in cancer cells, preventing DNA synthesis and leading to apoptosis (8). Flow cytometric analysis revealed that a low concentration of 5-FU (1 μM) did not induce apoptosis in A549/5-FU cells; however, following miR-124-5p mimic transfection, apoptosis was significantly increased in A549/5-FU cells treated with 1 μM 5-FU (Fig. 5), suggesting that miR-124-5p reversed 5-FU resistance in NSCLC cells via the induction of apoptosis.

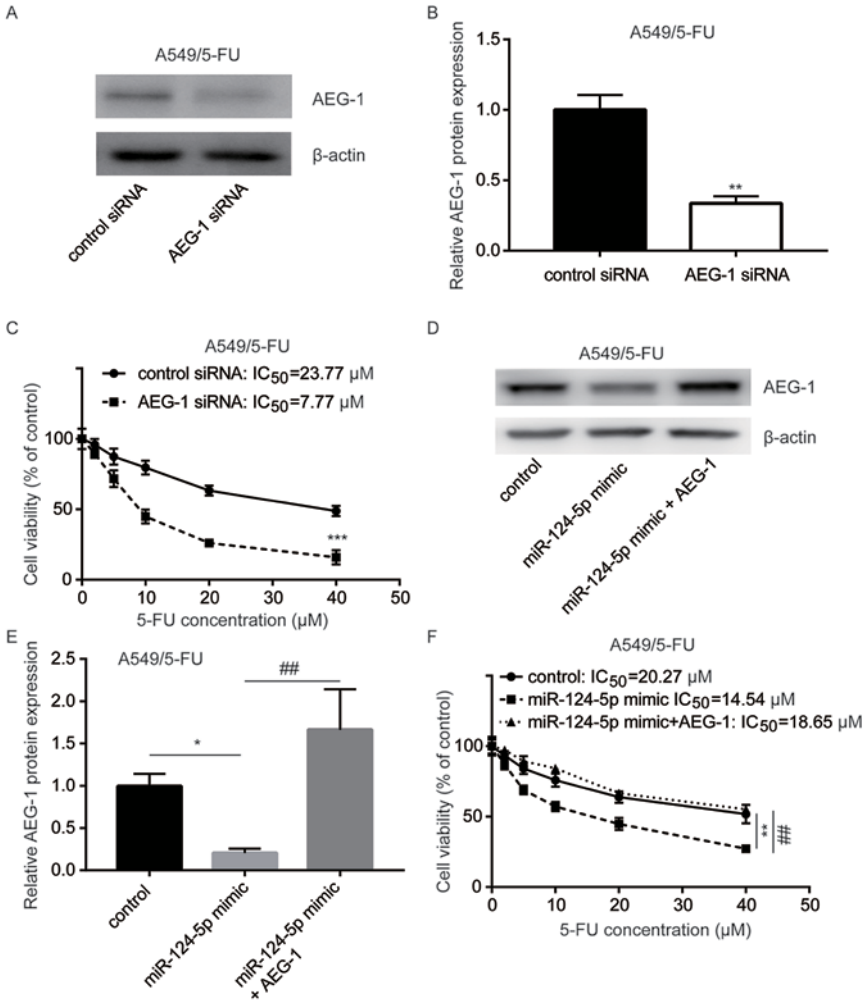

Figure 6. miR-124-5p downregulation mediated 5-FU resistance of A549/5-FU cells by upregulating AEG-1. (A) Transfection of AEG-1 siRNA decreased AEG-1 protein expression levels in A549/5-FU cells. (B) Quantitative analysis of AEG-1 protein expression in A549/5-FU cells; transfection with AEG-1 siRNA induced a significant decrease in AEG-1 expression levels. (C) Silencing of AEG-1 enhanced the cytotoxic effect of 5-FU in A549/5-FU cells. (D) Transfection with recombinant AEG-1 reversed the decreased protein expression of AEG-1 in A549/5-FU cells transfected with a miR-124-5p mimic. (E) Quantitative analysis of AEG-1 protein expression. (F) Overexpression of AEG-1 reversed the enhanced sensitivity to 5-FU in A549/5-FU cells transfected with a miR-124-5p mimic. *P<0.05, **P<0.01 and ***P<0.001 vs. control siRNA; $^{\#\#}$P<0.01 vs. miR-124-5p mimic. miR, microRNA; 5-FU, 5-fluorouracil; NC, negative control; IC_{50}, half-maximal inhibitory concentration; AEG-1, astrocyte elevated gene-1; siRNA, small interfering RNA.

miR-124-5p inhibition mediates 5-FU resistance of A549/5-FU cells by upregulating AEG-1. AEG-1 protein expression in A549/5-FU cells was significantly decreased following transfection with AEG-1 siRNA (Fig. 6A and B). The CCK-8 cytotoxicity assay revealed that silencing of AEG-1 sensitized A549/5-FU cells to 5-FU treatment (IC_{50}, 23.77 vs. 7.77 μM) (Fig. 6C), suggesting that AEG-1 contributed to A549 cell sensitivity to 5-FU. AEG-1 overexpression abrogated the decreased AEG-1 protein expression in A549/5-FU cells transfected with the miR-124-5p mimic (Fig. 6D and E). Compared with the control group (IC_{50}, 20.27 μM), the miR-124-5p mimic sensitized A549/5-FU cells to 5-FU (IC_{50}, 14.54 μM), whereas transfection with the recombinant AEG-1 (IC_{50}, 18.65 μM) decreased 5-FU sensitivity (Fig. 6F). These results suggested that miR-124-5p affected the sensitivity of NSCLC cells to 5-FU by decreasing the expression level of AEG-1.

Expression of miR-124-5p is inversely correlated with AEG-1 expression in NSCLC tumor tissues. RT-qPCR was performed to detect the expression levels of miR-124-5p and AEG-1 in 40 pairs of tumor and adjacent noncancerous tissues collected from patients with NSCLC. The expression of miR-124-5p was significantly decreased in tumor tissues compared with normal tissues (Fig. 7A). In addition, AEG-1 mRNA levels were increased in tumor tissues compared with normal tissues (Fig. 7B). Pearson's correlation analysis revealed a negative correlation between miR-124-5p and AEG-1 mRNA expression levels in tumor tissues (r=-0.485; P=0.001; Fig. 7C).

Discussion

The development of chemoresistance represents a major challenge for the treatment of patients with NSCLC (26). Previous studies have revealed that dysregulation of miRNA expression is associated with the development of chemoresistance (27,28). In addition, several miRNAs have been identified as predictors of the magnitude of the response to chemotherapy in patients with NSCLC (29-31). The present study demonstrated that the downregulation of miR-124-5p

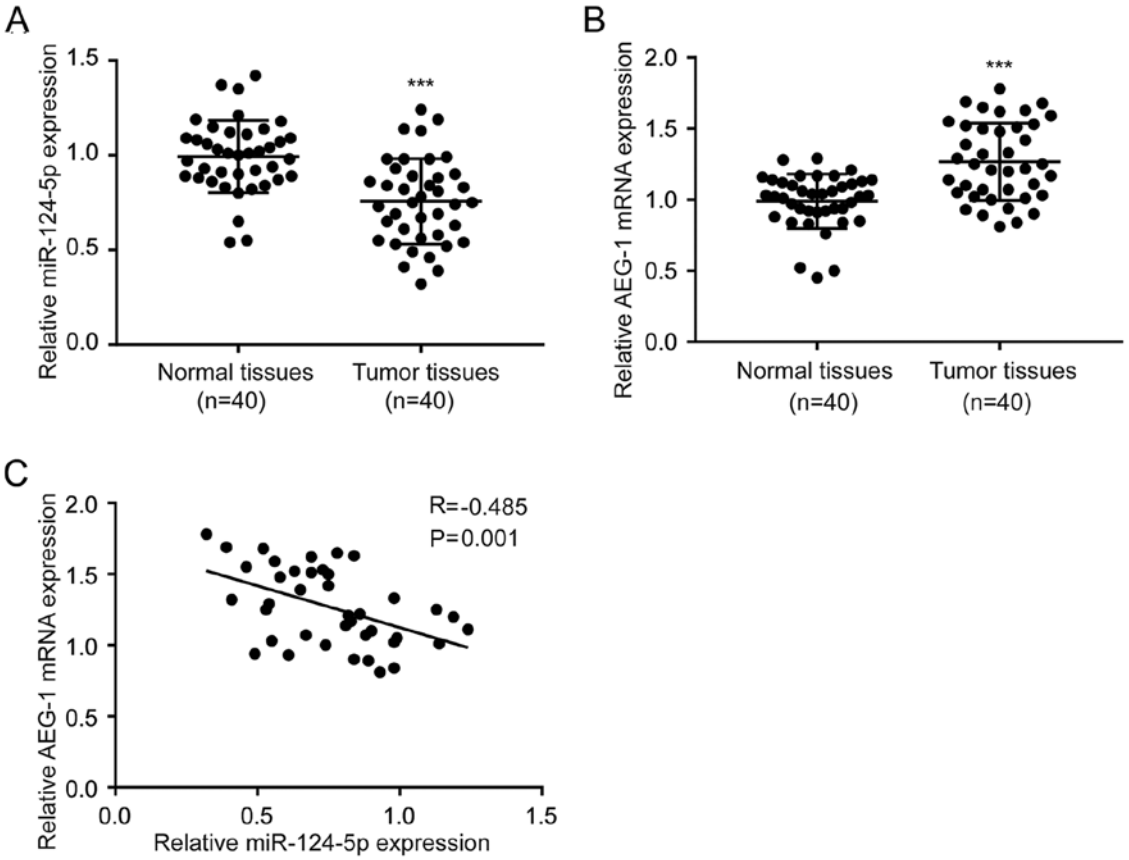

Figure 7. AEG-1 mRNA levels were negatively correlated with miR-124-5p levels in 40 tumor tissues collected from patients with non-small cell lung cancer. (A) RT-qPCR results demonstrated that miR-124-5p expression was significantly decreased in tumor tissues compared with matched normal tissues. (B) RT-qPCR revealed that AEG-1 mRNA expression was significantly increased in tumor tissues compared with matched normal tissues. (C) Expression of miR-124-5p was negatively correlated with AEG-1 mRNA expression in tumor tissues. ***P<0.001. miR, microRNA; AEG-1, astrocyte elevated gene-1; RT-qPCR, reverse transcription-quantitative PCR.

reduced sensitivity to 5-FU treatment and promoted the development of chemoresistance in A549 and H1299 cells via the downregulation of AEG-1.

Previous studies have demonstrated that miR-124-5p serves as a tumor suppressor in several types of cancer. For example, in glioma, miR-124-5p inhibited tumor growth *in vitro* and *in vivo* by downregulating laminin subunit β-1 (17). In colorectal cancer, high expression of miR-124-5p was associated with an increase in overall survival (18). A recent study demonstrated that miR-124-5p was downregulated in NSCLC tissue samples compared with matched paracancerous tissues, and was also associated with radiation sensitivity in NSCLC cells (32). Furthermore, miR-124-5p suppressed NSCLC cell proliferation by directly targeting signal transducer and activator of transcription 3 and protein kinase B (33,34). The results of the present study revealed that miR-124-5p was downregulated in A549/5-FU cells compared with parental A549 cells. Additionally, the CCK-8 assay demonstrated that the miR-124-5p mimic increased A549/5-FU cell sensitivity to 5-FU, whereas inhibition of miR-124-5p reduced A549 cell sensitivity to 5-FU treatment. The miR-124-5p mimic induced apoptosis in A549/5-FU cells treated with 1 μM 5-FU. These results indicated that miR-124-5p may sensitize NSCLC cells to chemotherapeutic treatment with 5-FU.

AEG-1 regulates several key pathways associated with carcinogenesis in a number of organs and tissues (35) and has also been demonstrated to mediate cisplatin resistance in serous ovarian cancer cells (36). Furthermore, AEG-1 is upregulated in NSCLC tissues compared with matched normal tissues, and promotes EMT through the activation of the Wnt signaling pathway (20). Patients with NSCLC with a low-expression level of AEG-1 also exhibited an improved response to postoperative chemotherapy and radiotherapy compared with patients exhibiting high AEG-1 expression (37). Additionally, miR-136 sensitized glioma cells to chemotherapy via the regulation of AEG-1 expression (38). The results of the present study revealed that AEG-1 was upregulated in A549/5-FU cells compared with the parental cells, and that the miR-124-5p mimic decreased AEG-1 mRNA and protein expression levels in A549/5-FU cells. Subsequently, a bioinformatics tool was used to predict that AEG-1 was a target gene of miR-124-5p, which was subsequently validated using a dual-luciferase reporter assay. Finally, RT-qPCR analysis suggested that the expression of miR-124-5p was inversely associated with AEG-1 mRNA expression levels in tumor tissues obtained from patients with NSCLC.

There are limitations of the current study. The role of miR-124-5p was solely investigated *in vitro*. In addition, a single miRNA may target several target genes in cells. In the future, further investigation of the target genes of miR-124-5p should be conducted and the role of miR-124-5p should be examined *in vivo*.

In conclusion, the present study demonstrated that miR-124-5p directly decreased AEG-1 expression and sensitized NSCLC cells to treatment with 5-FU. Therefore, miR-124-5p may serve as a novel target for the treatment of NSCLC.

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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

XT, CZ, WG, BS, BJ and PS designed and performed the experiments. CZ and WG collection the samples and clinical data. XT supervised the study and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All protocols involving human participants were approved by the Ethics Committee of The Third People's Hospital of Linyi City (Linyi, China). Written informed consent was provided by all participants prior to the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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