# miR-181 regulates cisplatin-resistant non-small cell lung cancer via downregulation of autophagy through the PTEN/PI3K/AKT pathway

JIANGANG LIU[1], YINGRU XING[2] and LING RONG[3]

[1]Department of Medical Oncology, The People's Hospital of Bozhou, Bozhou, Anhui 236804;
[2]Affiliated Tumor Hospital, Huainan Eastern Hospital Group, Huainan, Anhui 232001; [3]Department of
Respiratory Medicine, The People's Hospital of Bozhou, Bozhou, Anhui 236804, P.R. China

**Abstract.** A number of miRNAs have been found to be abnormally expressed or mutated in numerous cancers and thus, are considered to act as oncogenes or tumor suppressor genes. The aim of the present study was to investigate the effect of miR-181 on cisplatin-resistant non-small cell lung cancer (NSCLC). In patients with cisplatin-resistant NSCLC, miR-181 expression was found to be markedly decreased. In addition, in the cisplatin-resistant human lung adenocarcinoma cell line A549/DDP, miR-181 downregulation promoted cell growth and metastasis and inhibited cell apoptosis, whereas miR-181 overexpression exerted the opposite effects. Furthermore, miR-181 downregulation suppressed LC3 and ATG5 protein expression in A549/DDP cells through suppression of the PTEN/PI3K/AKT/mTOR pathway, whereas miR-181 overexpression recovered LC3 and ATG5 protein expression by promoting PTEN/PI3K/AKT/mTOR signaling. In turn, PTEN inhibitors reduced the anticancer effects of miR-181 overexpression on A549/DDP cell growth via the regulation of autophagy through the PI3K/AKT/mTOR pathway. Therefore, miR-181 may be a novel and important regulator of cisplatin-resistant NSCLC by serving a role in the regulation of apoptosis, as an established rate-limiting miRNA target.

## Introduction

Non-small cell-lung cancer (NSCLC) is the most common pathological type of lung cancer, accounting for 80-85% of total lung cancer cases (1). The majority of NSCLC patients have already developed multiple extrapulmonary metastases at diagnosis due to the typically occult nature of disease onset (2). Chemotherapy is the preferred main treatment for such patients (2) and plays an important role in reducing tumor load, relieving patient symptoms, improving quality of life and prolonging survival (3).

As a common cytotoxic antitumor drug, cisplatin has been used as the first-line chemotherapeutic agent for NSCLC over the past several decades (4). Cisplatin promotes cell apoptosis through DNA injury and altering cell metabolic state (4). However, resistance to cisplatin severely affects the effectiveness of chemotherapy. In addition, cisplatin is inevitably associated with common adverse reactions, including alopecia, nausea, vomiting, bone marrow suppression and liver function impairment (5).

Chemoresistance includes primary and acquired resistance. Primary resistance is the manifestation of low sensitivity or insensitivity at the first application of a certain chemotherapeutic drug. This type of chemoresistance may be attributed to the tumor cell characteristics (6). Acquired resistance is described as tumor cells exhibiting lowered sensitivity to a certain chemotherapeutic drug after several applications and is a common type of drug resistance in the clinical setting (7).

The PI3K/AKT/mTOR signaling pathway may stimulate cell growth at multiple phases of the cell cycle. Furthermore, it can control cellular processes through its downstream targets, thus initiating and regulating cancer development (8). Activation of this signaling pathway eventually leads to inhibition of cancer cell autophagy and promotes cancer cell development (9). In addition, the PI3K/AKT/mTOR signaling pathway renders cancer cells resistant to multiple cancer treatments, leading to a poor prognosis for numerous types of cancer (8). The autophagy-associated gene phosphatase and tensin homolog (PTEN) is a key factor in regulating autophagosome formation (10). Namely, PTEN is a positive regulatory molecule of autophagy that blocks the inhibitory effect of PI3K/PKB on autophagy, thereby activating autophagy (10).

In recent years, a new class of small-molecule RNAs termed microRNAs (miRNA/miRs) have been identified in a number of eukaryotes. miRNAs may promote target mRNA

*Correspondence to:* Dr Yingru Xing, Affiliated Tumor Hospital, Huainan Eastern Hospital Group, Huainan, Anhui 232001, P.R. China
E-mail: austxingyr@126.com

Dr Ling Rong, Department of Respiratory Medicine, The People's Hospital of Bozhou, Bozhou, Anhui 236804, P.R. China
E-mail: rzxr3072249@126.com

Table I. Characteristics of patients with NSCLC.

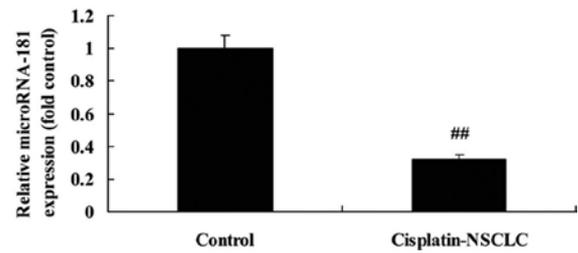| Variables | NSCLC (n=6) |
| --- | --- |
| Age (years) | |
| ≤55 | 3 |
| >55 | 3 |
| Sex | |
| Female | 3 |
| Male | 3 |
| Tumor size (cm) | |
| ≤3.0 | 2 |
| >3.0 | 4 |
| Edmondson grade | |
| I | 0 |
| II | 1 |
| III | 5 |
| Serum AFP levels (ng/ml) | 75.2±216.8 |
| Albumin (g/l) | 38.2±4.5 |
| Bilirubin ($\mu$mol/l) | 19.4±11.2 |

NSCLC, non-small cell lung cancer.



Figure 1. miR-181 expression in patients with cisplatin-induced NSCLC. Control, paracarcinoma tissue group; cisplatin-NSCLC, cisplatin-resistant NSCLC. [##]$p<0.01$ vs. paracarcinoma tissue group. NSCLC, non-small-cell lung cancer.

degradation or inhibit protein translation and regulate endogenous gene expression. The key roles of miRNAs in gene regulation are generally achieved through complete or incomplete complementary pairing with the 3' untranslated region of their target mRNA (11) and increasing evidence indicates that miRNAs may regulate gene expression, modification, transcription and translation (12). Additionally, recent studies have uncovered that miRNA polymorphism, abnormal miRNA expression and gene expression that affects drug absorption may result in continuous tumor drug resistance. Furthermore, metabolism and distribution channels, as well as receptors specifically participating in clinical functions, may also exert the aforementioned effects (12). Notably, Liu *et al* (13) suggested that miR-181b modulates glioblastoma cell growth and apoptosis and Su *et al* (14) reported that miR-181 regulates myeloid differentiation and the development of acute myeloid leukemia. Therefore, the present study aimed to elucidate the effect of miR-181 on cisplatin-resistant NSCLC.

## Materials and methods

*RNA extraction and quantitative reverse transcription PCR.* Cisplatin-induced NSCLC in patients (n=6) were collected, and cisplatin-induced NSCLC and paracarcinoma tissue were collected and saved at -80˚C. The present study was approved by the Ethics Committee of The People's Hospital of Bozhou (n. PHBZ/L-2015008). All patients provided informed written consents to participate in this study. The charascteristics of the patients involved in the present study are shown in Table I. Total RNA was extracted from serum and cell using TRIzol (Invitrogen Life Technologies, Carlsbad, CA, USA). Reverse transcription was carried out into cDNA using the M-MLV Reverse Transcription system (Takara Biotechnology Co., Ltd., Dalian, China). The ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) was used to perform qPCR with the SYBR-Green PCR kit (TransGen Biotech, Inc., Beijing, China). PCR primers used were as follows: miR-181: 5'-GCGGCAACATTCAACGC TGTCGGTGAGT-3' and 5'-GTCGTATCCAGTGCGTGT CGTGGAGTCGGCAATTG-3'; U6: 5'-CTCGCTTCGGCA GCACA-3' and 5'-AACGCTTCACGAATTTGCGT-3'. PCR was performed at 95˚C for 10 min, followed by 40 cycles of 95˚C for 25 sec and 60˚C for 30 sec. The relative quantification was calculated using the $2^{-\Delta\Delta Ct}$ method.

*Cell culture and transfections.* A549/DDP cells were purchased from Nanjing KeyGen Biotech Co., Ltd. (Nanjing, China), and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), 800 ng/ml cisplatin at 37˚C in humidified 5% $CO_2$ atmosphere. miR-181: AACAUUCA ACGCUGUCGGUGAGU, anti-miR-181: ACCAUCGACC GUUGAUUGUACC and negative control: UUCUCCGAA CGUGUCACGUTT mimics were purchased from Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). Transfection with 100 nM of mR-181, anti-miR-181 and negative control mimics was performed with Lipofectamine 2000 reagent (Invitrogen).

*MTT assay and apoptosis assay.* After transfections for 24, 48 and 72 h, MTT (5 mg/ml, 20 $\mu$l) was added to the cell and incubated for 4 h at 37˚C. Dimethyl sulfoxide (DMSO) was added to the cell and incubated for 20 min at 37˚C. Absorbance was detected at 492 nm using a multi-well plate reader.

After transfections for 72 h, the cells were washed with phosphate-buffered saline (PBS) and resuspended in 100 $\mu$l binding buffer. The cells were stained with 5 $\mu$l of Annexin V-fluorescein isothiocyanate and 5 $\mu$l of propidium iodide (PI) (BD Biosciences, Franklin Lakes, NJ, USA) for 15 min at room temperature. Apoptosis rate was detected using BD FACSCalibur flow cytometer (BD Biosciences).

*Western blot analysis.* Total proteins were extracted from cells using RIPA assay (Pierce, Rockford, IL, USA) and quantified using the BCA method (Pierce). Protein (50 $\mu$g) was separated on 8-10% SDS-polyacrylamide gel, and then transferred to a PVDF membrane (Amersham Biosciences, Chicago, IL, USA). The membrane was blocked with 5%-skim milk powder in TBST and incubated with Bax, LC3, ATG5, PTEN, PI3K, p-Akt, p-mTOR and GAPDH (1:500; Santa
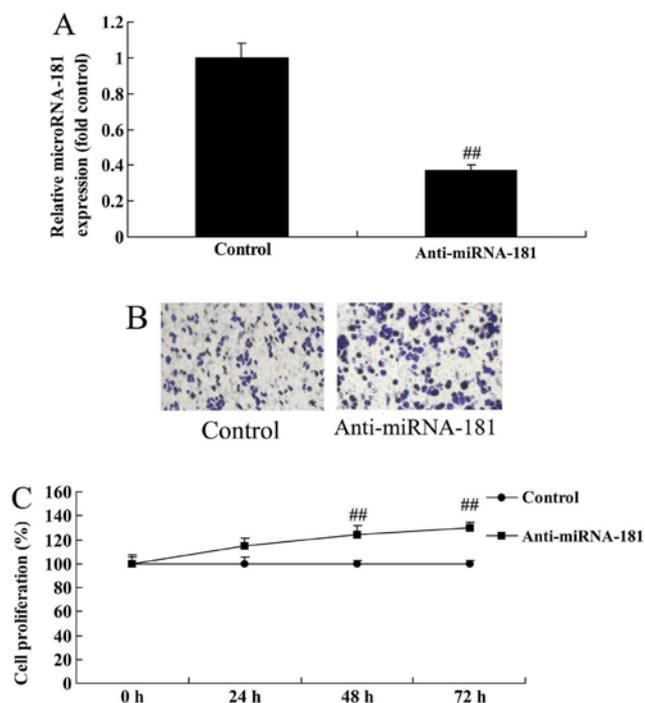
Figure 2. Downregulation of miR-181 promotes cell growth and metastasis of A549/DDP cells. (A) miR-181 expression, (B) metastasis and (C) cell growth of A549/DDP cells. Control, control negative group; anti-miR-181, miR-181 downregulation group. ##p<0.01 vs. paracarcinoma tissue group.

Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4˚C, followed by incubation with secondary antibody (1:2,000; Santa Cruz Biotechnology) for 1 h at room temperature. Protein bands were revealed with a chemiluminescence kit (Pierce) and analyzed using Gel-Pro Analyzer software (Version 4.0, Media Cybernetics, Rockville, MD, USA).

*Statistical analysis*. All values are depicted as mean ± standard deviation (SD). Statistical analyses were performed using one-way ANOVA. P<0.05 was considered to indicate a statistically significant difference using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA).

## Results

*miR-181 expression in patients with cisplatin-resistant NSCLC*. We first investigated miR-181 expression in patients with cisplatin-resistant NSCLC. As demonstrated in Fig. 1, miR-181 expression was obviously decreased in patients with cisplatin-resistant NSCLC, compared with that in the normal group. These results demonstrated that miR-181 expression may be a significant factor in cisplatin-resistant NSCLC patients.

*Decreased miR-181 expression promotes the growth and metastasis of A549/DDP cells*. We subsequently investigated whether miR-181 affects cell growth and metastasis in the cisplatin-resistant human lung adenocarcinoma cell line A549/DDP. As demonstrated in Fig. 2A, anti-miR-181 mimics decreased miR-181 expression in A549/DDP cells compared with the control group, whereas miR-181 downregulation promoted the growth and metastasis of A549/DDP cells compared with the control group (Fig. 2B and C).
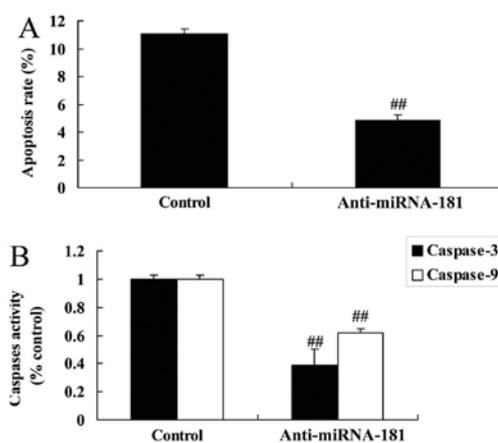


Figure 3. Downregulation of miR-181 inhibits apoptosis and caspase-3/9 activity of A549/DDP cells. miR-181 downregulation inhibited (A) apoptosis and (B) caspase-3/9 activity of A549/DDP cells. Control, control negative group; anti-miR-181, miR-181 downregulation group. ##p<0.01 vs. paracarcinoma tissue group.
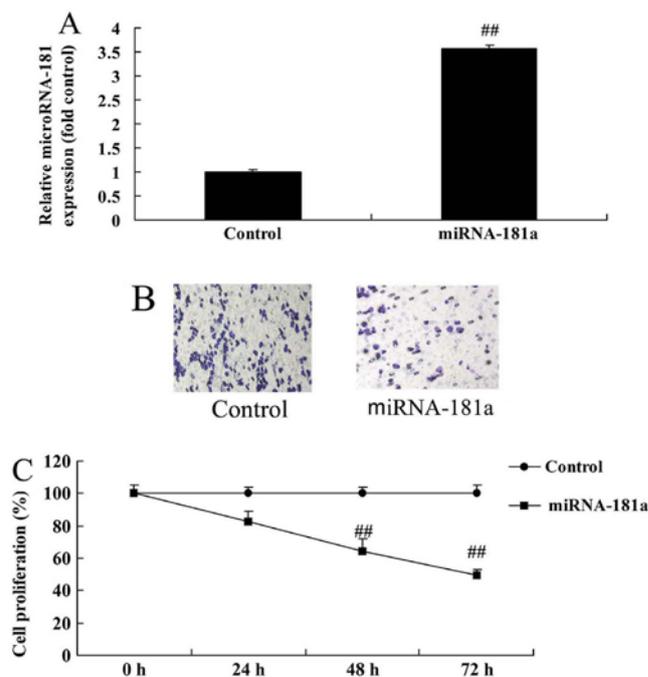


Figure 4. Overexpression of miR-181 inhibits cell growth and metastasis of A549/DDP cells. miR-181 (A) expression, (B) metastasis and (C) cell growth of A549/DDP cells. Control, control negative group; miR-181, miR-181 overexpression group. ##p<0.01 vs. paracarcinoma tissue group.

*miR-181 downregulation inhibits apoptosis and caspase-3/9 activity in A549/DDP cells*. Furthermore, miR-181 downregulation inhibited apoptosis and caspase-3/9 activity in A549/DDP cells compared with the control group, whereas miR-181 upregulation promoted caspase-3/9 expression to induce apoptosis in A549/DDP cells (Fig. 3A and B).

*miR-181 overexpression inhibits the growth and metastasis of A549/DDP cells*. Cell growth and metastasis were measured in A549/DDP cells to determine whether they are affected by miR-181 overexpression. As depicted in Fig. 4, miR-181 overexpression inhibited the growth and metastasis of A549/DDP
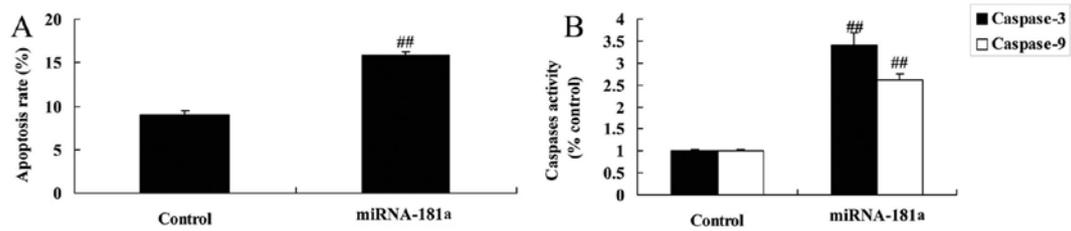
Figure 5. miR-181 overexpression induces apoptosis and caspase-3/9 activity of A549/DDP cells. miR-181 overexpression induced (A) apoptosis and (B) caspase-3/9 activity of A549/DDP cells. Control, control negative group; miR-181, miR-181 overexpression group. ##p<0.01 vs. paracarcinoma tissue group.
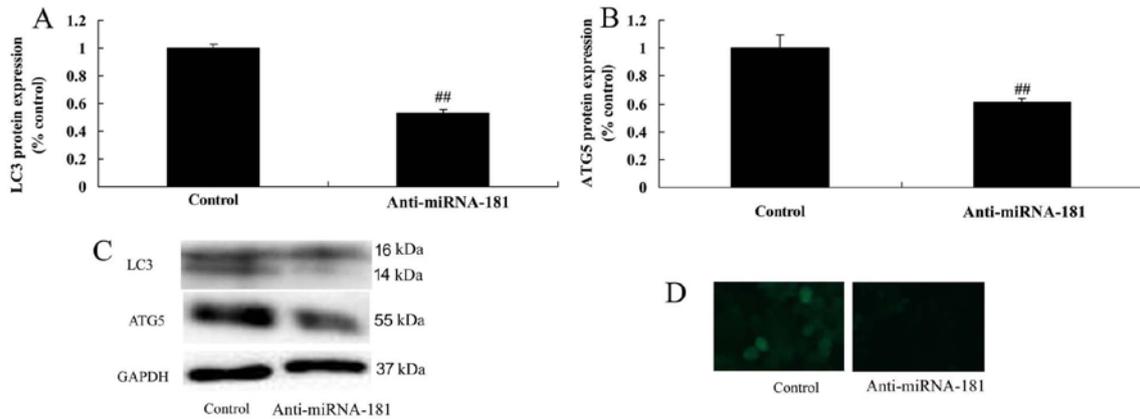


Figure 6. Downregulation of miR-181 reduces autophagy of A549/DDP cells. (A and B) miR-181 downregulation reduced LC3 and ATG5 protein expression, (C) LC3 and ATG5 protein expression using western blot analysis and (D) LC3 assay staining of A549/DDP cells. Control, control negative group; anti-miR-181, miR-181 downregulation group. ##p<0.01 vs. paracarcinoma tissue group.
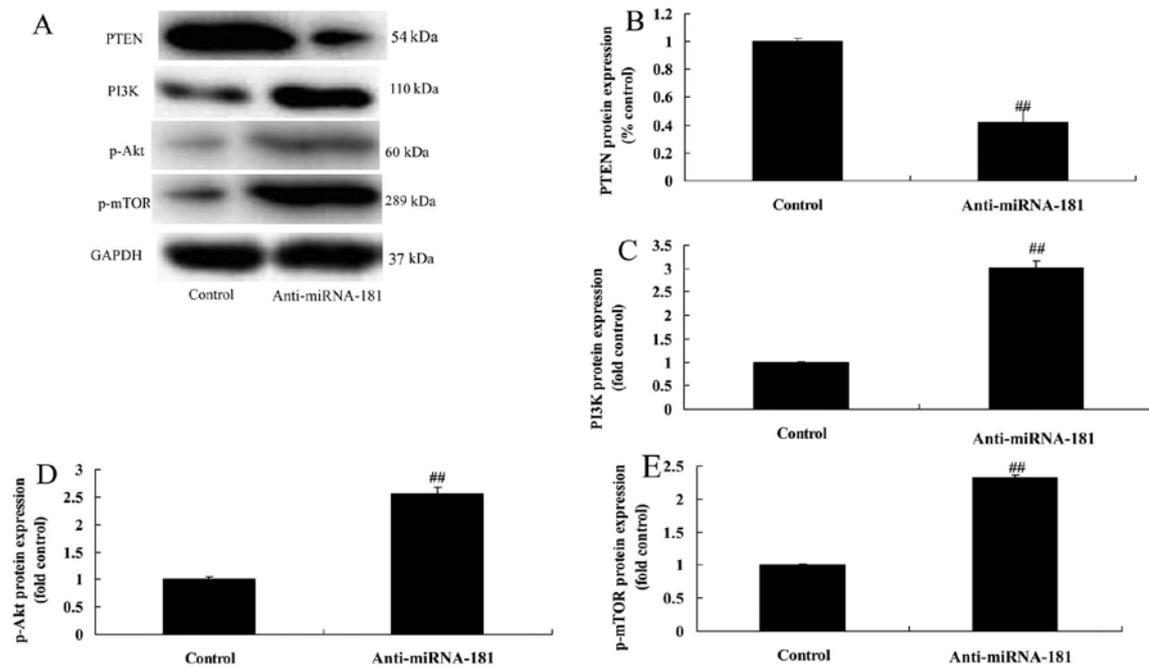


Figure 7. Downregulation of miR-181 reduces PTEN/PI3K/Akt/mTOR pathway. miR-181 downregulation reduced PTEN protein expression and induced PI3K, p-AKT and p-mTOR protein expression in A549/DDP cells compared with the control group by (A) western blot analysis and (B-E) by statistical analysis. Control, control negative group; anti-miR-181, miR-181 downregulation group. ##p<0.01 vs. paracarcinoma tissue group.

cells compared with the control group, indicating that miR-181 overexpression may suppress cancer cell growth in patients with cisplatin-resistant NSCLC.

*miR-181 overexpression induces apoptosis and caspase-3/9 activity in A549/DDP cells.* miR-181 overexpression was found to induce apoptosis and caspase-3/9 activity in A549/DDP cells
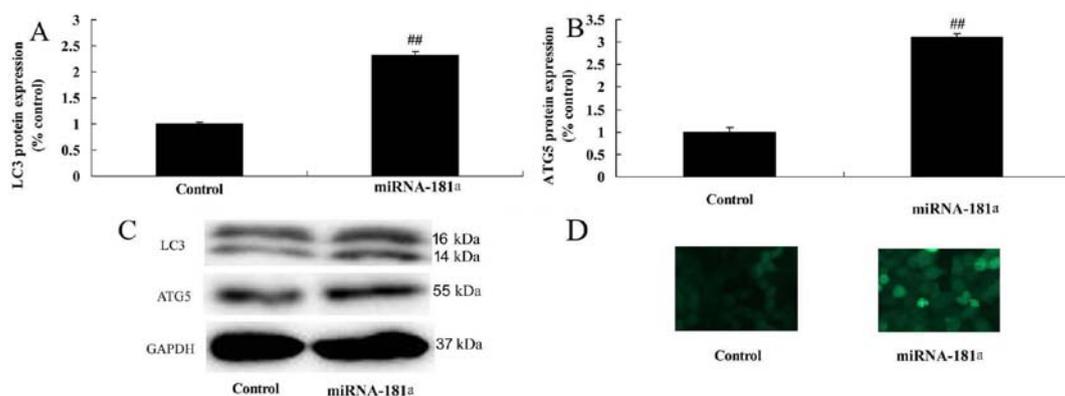
Figure 8. Overexpression of miR-181 promotes autophagy of A549/DDP cells. miR-181 overexpression (A and B) induced LC3 and ATG5 protein expression, (C) LC3 and ATG5 protein expression using western blot analysis and (D) LC3 assay staining of A549 cell/DDP. Control, control negative group; miR-181, miR-181 overexpression group. $^{\#\#}$p<0.01 vs. paracarcinoma tissue group.
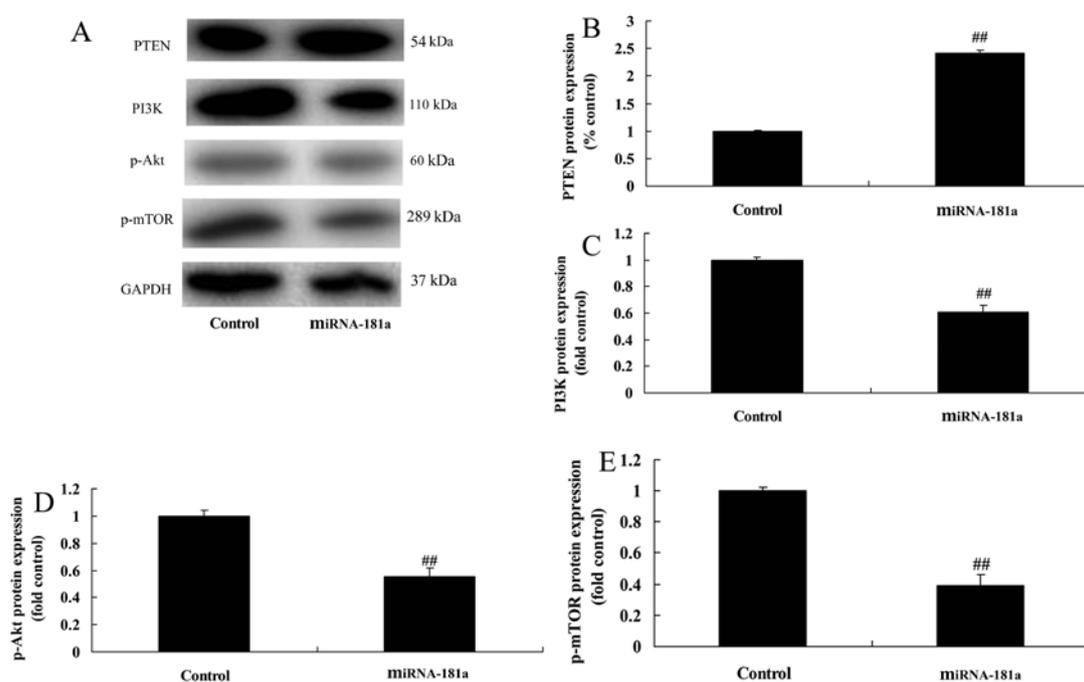


Figure 9. Overexpression of miR-181 promotes PTEN/PI3K/Akt/mTOR pathway. The overexpression of miR-181 promoted PTEN protein expression and suppressed PI3K, p-AKT and p-mTOR protein expression in A549/DDP cells compared with the control group by (A) western blot analysis and (B-D) by statistical analysis. Control, control negative group; miR-181, miR-181 overexpression group. $^{\#\#}$p<0.01 vs. paracarcinoma tissue group.

compared with the control group (Fig. 5). Therefore, miR-181 overexpression may induce apoptosis of cisplatin-resistant NSCLC cells, although the underlying mechanism requires further investigation.

*miR-181 downregulation reduces autophagy in the A549/DDP cells.* It was demonstrated that miR-181 downregulation reduced autophagy and suppressed LC3 and ATG5 protein expression in the A549/DDP cells compared with the control group (Fig. 6), which may thus promote tumor progression.

*miR-181 downregulation reduces PTEN/PI3K/AKT/mTOR pathway signaling.* miR-181 downregulation reduced PTEN protein expression and induced PI3K, p-AKT and p-mTOR

protein expression in A549/DDP cells compared with the control group (Fig. 7). These data provide evidence that miR-181 knockdown may promote the proliferation and migration of A549/DDP cells.

*miR-181 overexpression promotes autophagy in A549/DDP cells.* Overexpression of miR-181 promoted autophagy and induced LC3 and ATG5 protein expression in A549/DDP cells compared with the control group (Fig. 8).

*miR-181 overexpression promotes PTEN/PI3K/AKT/mTOR pathway signaling.* The overexpression of miR-181 promoted PTEN protein expression and suppressed PI3K, p-AKT and p-mTOR protein expression in A549/DDP cells compared with the control group (Fig. 9).
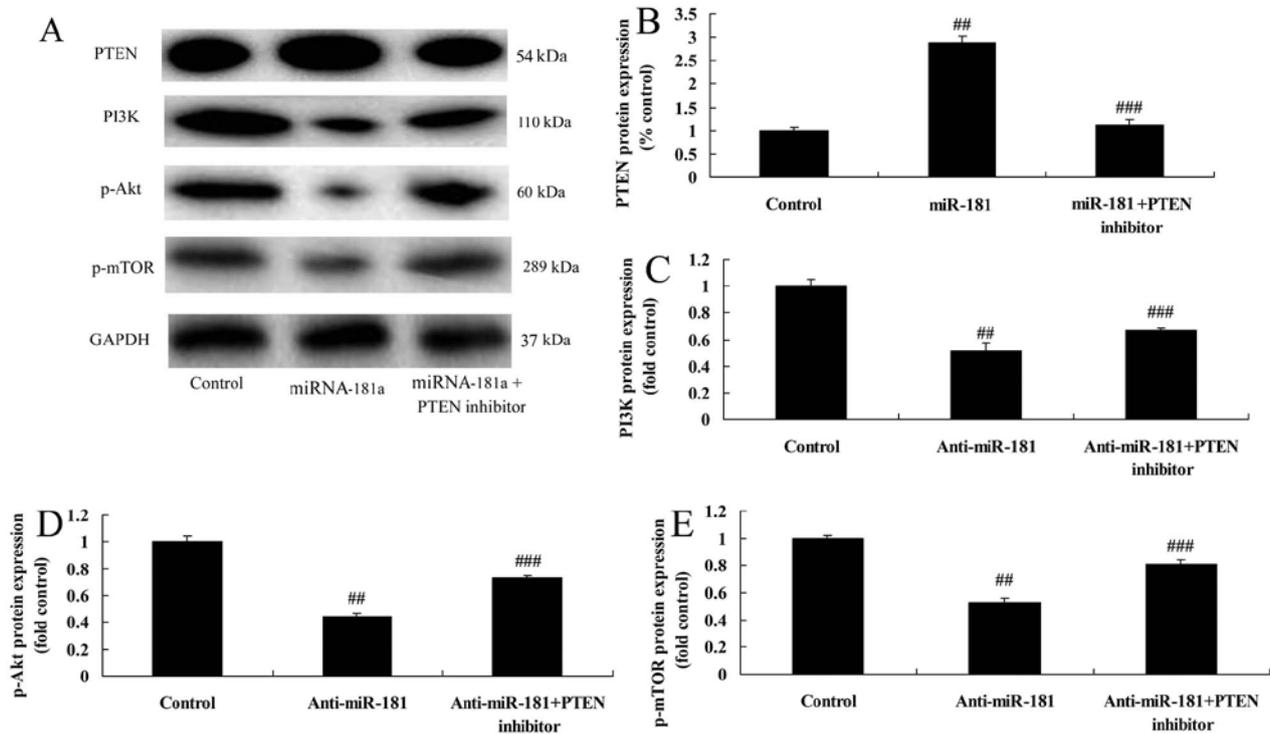
Figure 10. PTEN inhibitor reduces the anticancer effects of miR-181 on the PTEN/PI3K/Akt/mTOR pathway of A549/DDP cells. The PTEN inhibitor reduced the anticancer effects of miR-181 overexpression on the promotion of PTEN protein expression and inhibition of PI3K, p-AKT and p-mTOR protein expression in A549/DDP cells, compared with the miR-181 overexpression group by (A) western blot analysis, and (B-D) by statistical analysis. Control, control negative group; anti-miR-181, miR-181 downregulation group; anti-miR-181 + PTEN inhibitor, miR-181 downregulation + PTEN inhibitor group. $^{\#\#}p<0.01$ vs. paracarcinoma tissue group; $^{\#\#\#}p<0.01$ vs. miR-181 downregulation group.
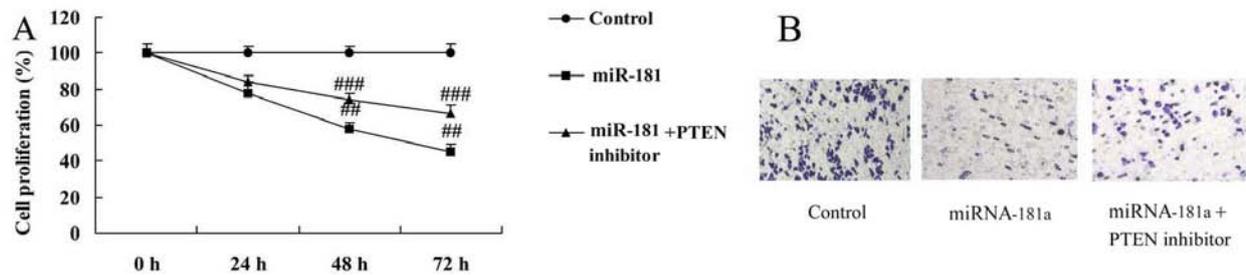


Figure 11. PTEN inhibitor reduces the anticancer effects of miR-181 on cell growth and metastasis of A549/DDP cells. PTEN inhibitor reduced the anticancer effects of miR-181 on (A) cell growth and (B) metastasis of A549/DDP cells. Control, control negative group; anti-miR-181, miR-181 downregulation group; anti-miR-181 + PTEN inhibitor, miR-181 downregulation + PTEN inhibitor group. $^{\#\#}p<0.01$ vs. paracarcinoma tissue group; $^{\#\#\#}p<0.01$ vs. miR-181 downregulation group.

*PTEN inhibitors reduce the anticancer effects of miR-181 on the PTEN/PI3K/AKT/mTOR pathway in A549/DDP cells.* To further confirm these results, we used a PTEN inhibitor to reduce the PTEN/PI3K/AKT/mTOR pathway signaling in A549/DDP cells induced by miR-181 overexpression. As depicted in Fig. 10, the PTEN inhibitor reduced the anticancer effects of miR-181 overexpression on the promotion of PTEN protein expression and inhibition of PI3K, p-AKT and p-mTOR protein expression in A549/DDP cells, compared with the miR-181 overexpression group.

*PTEN inhibitors reduce the anticancer effects of miR-181 on the growth and metastasis of A549/DDP cells.* PTEN inhibitors reduced the anticancer effects of miR-181 overexpression on the inhibition of cell growth and metastasis in A549/DDP cells, compared with the miR-181 overexpression group (Fig. 11).

*PTEN inhibitors reduce the anticancer effects of miR-181 on apoptosis and caspase-3/9 activity in A549/DDP cells.* PTEN inhibitors reduced the elevated levels of apoptosis and caspase-3/9 activity in A549/DDP cells induced by miR-181 overexpression, compared with miR-181 overexpression group (Fig. 12).

*PTEN inhibitors reduce the anticancer effects of miR-181 on the autophagy of A549/DDP cells.* Finally, PTEN inhibitors reduced autophagy and suppressed LC3 and ATG5 protein expression in A549/DDP cells following miR-181 overexpression, compared with the miR-181 overexpression
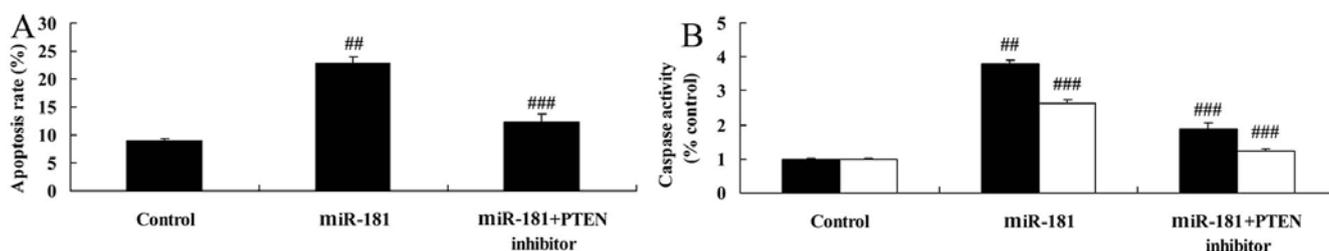
Figure 12. PTEN inhibitor reduces the anticancer effects of miR-181 on apoptosis and caspase-3/9 activity of A549 cell/DDP. PTEN inhibitor reduced the anticancer effects of miR-181 on (A) apoptosis and (B) caspase-3/9 activity of A549/DDP cells. Control, control negative group; anti-miR-181, miR-181 downregulation group; anti-miR-181 + PTEN inhibitor, miR-181 downregulation + PTEN inhibitor group. ##p<0.01 vs. paracarcinoma tissue group; ###p<0.01 vs. miR-181 downregulation group.
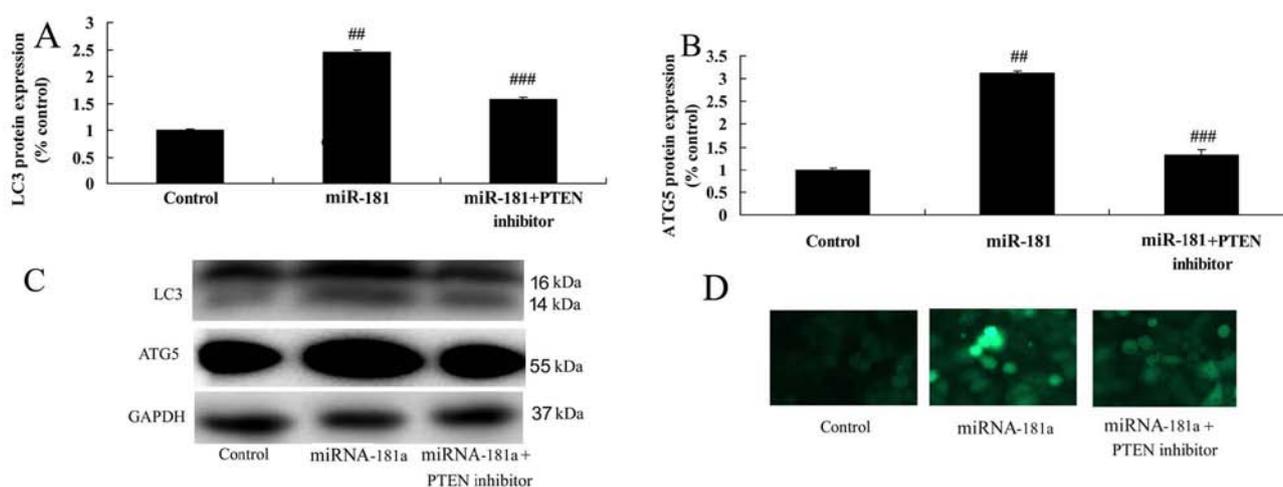


Figure 13. PTEN inhibitor reduces the anticancer effects of miR-181 on autophagy of A549/DDP cells. PTEN inhibitor reduced the anticancer effects of miR-181 on (A and B) LC3 and ATG5 protein expression, (C) LC3 and ATG5 protein expression using western blot analysis and (D) LC3 assay staining of A549/DDP cells. Control, control negative group; anti-miR-181, miR-181 downregulation group; anti-miR-181 + PTEN inhibitor, miR-181 downregulation + PTEN inhibitor group. ##p<0.01 vs. paracarcinoma tissue group; ###p<0.01 vs. miR-181 downregulation group.

group (Fig. 13). In addition, PTEN/PI3K/AKT/mTOR pathway signaling was inversely correlated with miR-181 levels in patients with cisplatin-resistant NSCLC.

## Discussion

NSCLC is the most common pathological type of lung cancer, and due to its typically occult onset, the majority of NSCLC patients present with distant metastasis at the time of diagnosis. As a result, chemotherapy is considered as the main treatment for such patients at present (15). Cisplatin is a classical chemotherapeutic drug used in the first-line treatment of NSCLC, which can effectively reduce tumor load in patients, improve their quality of life and prolong their survival (16). However, a proportion of patients may be sensitive to cisplatin during the first treatment, while others gradually develop drug resistance (16). To overcome this, the dose of cisplatin must be increased, leading to more severe toxic side effects and substantial reductions in patient quality of life and clinical prognosis (2). Therefore, the molecular and biological mechanisms underlying the development of cisplatin resistance must be investigated. In particular, it is crucial to elucidate the cause of tumor tolerance to chemotherapy in order to individualize the treatment of

NSCLC patients (17). The present study demonstrated that miR-181 expression was effectively decreased in patients with cisplatin-resistant NSCLC compared with a normal group, suggesting that miR-181 is an important indicator of cisplatin-resistant NSCLC. Pichler et al (18) reported that miR-181a was associated with poor clinical outcome in patients with colorectal cancer.

Radiotherapy and chemotherapy are considered effective methods for the treatment of NSCLC in clinical practice. Ionizing radiation and chemotherapeutic drugs may induce NSCLC cell death through multiple signaling pathways (19). Research on the role of type I programmed cell death, namely apoptosis, in the combined effects of radiochemotherapy has made progress. However, our understanding of the role of type II programmed cell death, namely autophagy, remains limited (19). Under physiological conditions, autophagy is a process that involves the degradation of autologous long-life proteins and waste organelles through the formation of autophagosomes and participation of lysosomes. In addition, autophagy serves to recycle amino acids as well as proteins to maintain cellular homeostasis (20). Excessive cell stimulation, long stimuli duration and large-scale autophagic degradation products promote programmed cell death (20). In the present study, miR-181 downregulation was found to promote

cell growth and metastasis and to inhibit cell apoptosis in A549/DDP cells, whereas miR-181 overexpression inhibited cell growth and metastasis and induced apoptosis of the cells.

Our understanding of the importance of autophagy and apoptosis in the development and prevention of human disease has improved. In addition, the association between the two has been investigated (21). Notably, it has been observed that apoptosis-regulating genes, such as Bcl-2 family members (Bcl-2 and CASP9) can also regulate autophagy (22), while autophagy-associated proteins, such as Atg5, beclin 1 and Atg4D, are also involved in cell apoptosis (22). Additionally, caspase-8 activation may promote apoptosis and the autophagic degradation of caspase-8 aids to suppress the apoptotic death of mammalian cells. The effect of autophagy on pre-death cells is also associated with apoptosis (23). In other words, autophagy may be an upstream event prior to apoptosis, which may be essential for apoptosis initiation (23). The present study indicated that miR-181 downregulation suppressed LC3 and ATG5 protein expression, whereas miR-181 overexpression restored LC3 and ATG5 protein expression in A549/DDP cells. Wu *et al* (24) reported that miR-181 inhibits proliferation and promotes apoptosis of chondrocytes in osteoarthritis through suppression of PTEN.

The PI3K/AKT/mTOR signaling pathway is activated in multiple cancers and the activated signaling can promote tumor cell differentiation, growth and survival (9). Mechanisms activating PI3K/AKT/mTOR signaling include a loss of function of the tumor inhibitor PTEN, proliferation and mutation of PI3K and/or AKT, activation of growth factor receptors, and stimulation of carcinogens (25). Signals are transmitted to mTOR, which can regulate protein translation after the signaling pathway is activated. mTOR is a downstream target gene of AKT, which is generally considered to be a regulator of autophagy through regulating a series of autophagy-associated genes (25). Moreover, miR-181 overexpression promoted, while miR-181 downregulation reduced, PTEN/PI3K/AKT/mTOR pathway signaling in A549/DDP cells. Similarly, Strotbek *et al* (26) reported that miR-181 promotes AKT signaling in luminal breast cancer.

PTEN deficiency can be found in tumor patients. In particular, it was discovered that the expression rate of PTEN in NSCLC tissue was lower compared with that in healthy individuals. Thus, it may be hypothesized that PTEN mutations lead to activation of the AKT pathway and inhibition of autophagy, and play a role in tumor initiation and progression (27,28). Similarly, it has been demonstrated that expression of PTEN protein in NSCLC is lower compared with that in healthy individuals, from which it was suggested that PTEN tumor regulation may be mediated through the regulation of autophagy (29). Thus, alterations in PTEN expression may directly affect tumorigenesis through autophagy. This likely occurs via the catalysis of PIP3 to PIP2 to inhibit AKT activity, which would thereby relieve the inhibition of the PI3K/AKT/mTOR pathway on autophagy (30). In the present study, PTEN inhibitors reduced the anticancer effects of miR-181 overexpression on the growth of A549/DDP cells, likely via the regulation of autophagy through the PI3K/AKT/mTOR pathway.

In conclusion, the present study demonstrated that miR-181 overexpression inhibited cell growth and metastasis and induced apoptosis and autophagy in A549/DDP cells through the PTEN/PI3K/AKT/mTOR pathway. Thus, miR-181 may help elucidate the potential molecular mechanisms underlying chemotherapy drug resistance in NSCLC and provide a basis for novel therapeutic strategies for the treatment of NSCLC in the clinical setting.

## References

 1. Yamamoto N, Goto K, Nishio M, Chikamori K, Hida T, Maemondo M, Katakami N, Kozuki T, Yoshioka H, Seto T, *et al*: Final overall survival in JO22903, a phase II, open-label study of first-line erlotinib for Japanese patients with EGFR mutation-positive non-small-cell lung cancer. Int J Clin Oncol 22: 70-78, 2017.
 2. Krzakowski M, Lucas C and Gridelli C: Fractionated scheme of oral vinorelbine as single-agent therapy or in combination with cisplatin concomitantly with thoracic radiotherapy in stage III non-small-cell lung cancer: Dose-escalation phase I trial. Clin Lung Cancer 15: 266-273, 2014.
 3. Kelley MJ, Jha G, Shoemaker D, Herndon JE II, Gu L, Barry WT, Crawford J and Ready N: Phase II Study of dasatinib in previously treated patients with advanced non-small cell lung cancer. Cancer Invest 35: 32-35, 2017.
 4. Dingemans AM, Bootsma G, van Baardwijk A, Reymen B, Wanders R, Brans B, Das M, Hochstenbag M, van Belle A, Houben R, *et al*: A phase I study of concurrent individualized, isotoxic accelerated radiotherapy and cisplatin-vinorelbine-cetuximab in patients with stage III non-small-cell lung cancer. J Thorac Oncol 9: 710-716, 2014.
 5. Kentepozidis N, Economopoulou P, Christofyllakis C, Chelis L, Polyzos A, Vardakis N, Koinis F, Vamvakas L, Katsaounis P, Kalbakis K, *et al*: Salvage treatment with irinotecan/cisplatin versus pemetrexed/cisplatin in patients with non-small cell lung cancer pre-treated with a non-platinum-based regimen in the first-line setting: A randomized phase II study of the Hellenic Oncology Research Group (HORG). Clin Transl Oncol 19: 317-325, 2017.
 6. Belani CP, Yamamoto N, Bondarenko IM, Poltoratskiy A, Novello S, Tang J, Bycott P, Niethammer AG, Ingrosso A, Kim S, *et al*: Randomized phase II study of pemetrexed/cisplatin with or without axitinib for non-squamous non-small-cell lung cancer. BMC Cancer 14: 290, 2014.
 7. Okamoto K, Okamoto I, Takeda M, Kobayashi S, Takeda K, Nakamatsu K, Nishimura Y and Nakagawa K: A phase I study of split-dose cisplatin and etoposide with concurrent accelerated hyperfractionated thoracic radiotherapy in elderly patients with limited-disease small cell lung cancer. Jpn J Clin Oncol 44: 743-748, 2014.
 8. Zhu Q, Liang X, Dai J and Guan X: Prostaglandin transporter, SLCO2A1, mediates the invasion and apoptosis of lung cancer cells via PI3K/AKT/mTOR pathway. Int J Clin Exp Pathol 8: 9175-9181, 2015.
 9. Wang R, Zhang Q, Peng X, Zhou C, Zhong Y, Chen X, Qiu Y, Jin M, Gong M and Kong D: Stellettin B induces G1 arrest, apoptosis and autophagy in human non-small cell lung cancer A549 cells via blocking PI3K/Akt/mTOR pathway. Sci Rep 6: 27071, 2016.
10. Guo Y, Chang H, Li J, Xu XY, Shen L, Yu ZB and Liu WC: Thymosin alpha 1 suppresses proliferation and induces apoptosis in breast cancer cells through PTEN-mediated inhibition of PI3K/Akt/mTOR signaling pathway. Apoptosis 20: 1109-1121, 2015.
11. Wu N, Zhang C, Bai C, Han YP and Li Q: MiR-4782-3p inhibited non-small cell lung cancer growth via USP14. Cell Physiol Biochem 33: 457-467, 2014.
12. Voortman J, Goto A, Mendiboure J, Sohn JJ, Schetter AJ, Saito M, Dunant A, Pham TC, Petrini I, Lee A, *et al*: MicroRNA expression and clinical outcomes in patients treated with adjuvant chemotherapy after complete resection of non-small cell lung carcinoma. Cancer Res 70: 8288-8298, 2010.
13. Feng B, Chen S, Gordon AD and Chakrabarti S: miR-146a mediates inflammatory changes and fibrosis in the heart in diabetes. J Mol Cell Cardiol 105: 70-76, 2017.
14. Matysiak M, Fortak-Michalska M, Szymanska B, Orlowski W, Jurewicz A and Selmaj K: MicroRNA-146a negatively regulates the immunoregulatory activity of bone marrow stem cells by targeting prostaglandin E2 synthase-2. J Immunol 190: 5102-5109, 2013.

15. Kwon JH, Kim JH, Lee JA, Shin HC, Kim HJ, Song HH, Jung JY, Kim HY, Choi DR, Kim HS, et al: Phase II study with fractionated schedule of docetaxel and cisplatin in patients with advanced non-small cell lung cancer. Cancer Chemother Pharmacol 66: 889-897, 2010.
16. Thatcher N, Hirsch FR, Luft AV, Szczesna A, Ciuleanu TE, Dediu M, Ramlau R, Galiulin RK, Bálint B, Losonczy G, et al; SQUIRE Investigators: Necitumumab plus gemcitabine and cisplatin versus gemcitabine and cisplatin alone as first-line therapy in patients with stage IV squamous non-small-cell lung cancer (SQUIRE): An open-label, randomised, controlled phase 3 trial. Lancet Oncol 16: 763-774, 2015.
17. Niho S, Kubota K, Nihei K, Sekine I, Sumi M, Sekiguchi R, Funai J, Enatsu S, Ohe Y and Tamura T: Dose-escalation study of thoracic radiotherapy in combination with pemetrexed plus Cisplatin followed by pemetrexed consolidation therapy in Japanese patients with locally advanced nonsquamous non-small-cell lung cancer. Clin Lung Cancer 14: 62-69, 2013.
18. Pichler M, Winter E, Ress AL, Bauernhofer T, Gerger A, Kiesslich T, Lax S, Samonigg H and Hoefler G: miR-181a is associated with poor clinical outcome in patients with colorectal cancer treated with EGFR inhibitor. J Clin Pathol 67: 198-203, 2014.
19. Sirichanchuen B, Pengsuparp T and Chanvorachote P: Long-term cisplatin exposure impairs autophagy and causes cisplatin resistance in human lung cancer cells. Mol Cell Biochem 364: 11-18, 2012.
20. Yang J, Zhou Y, Cheng X, Fan Y, He S, Li S, Ye H, Xie C, Wu W, Li C, et al: Isogambogenic acid induces apoptosis-independent autophagic cell death in human non-small-cell lung carcinoma cells. Sci Rep 5: 7697, 2015.
21. Oh BS, Shin EA, Jung JH, Jung DB, Kim B, Shim BS, Yazdi MC, Iranshahi M and Kim SH: Apoptotic effect of galbanic acid via activation of caspases and inhibition of Mcl-1 in H460 non-small lung carcinoma cells. Phytother Res 29: 844-849, 2015.
22. Li YR, Li S, Ho CT, Chang YH, Tan KT, Chung TW, Wang BY, Chen YK and Lin CC: Tangeretin derivative, 5-acetyloxy-6,7,8,4'-tetramethoxyflavone induces G2/M arrest, apoptosis and autophagy in human non-small cell lung cancer cells in vitro and in vivo. Cancer Biol Ther 17: 48-64, 2016.
23. Hwang KE, Kim YS, Jung JW, Kwon SJ, Park DS, Cha BK, Oh SH, Yoon KH, Jeong ET and Kim HR: Inhibition of autophagy potentiates pemetrexed and simvastatin-induced apoptotic cell death in malignant mesothelioma and non-small cell lung cancer cells. Oncotarget 6: 29482-29496, 2015.
24. Wu XF, Zhou ZH and Zou J: MicroRNA-181 inhibits proliferation and promotes apoptosis of chondrocytes in osteoarthritis by targeting PTEN. Biochem Cell Biol 95: 437-444, 2017.
25. Song BQ, Chi Y, Li X, Du WJ, Han ZB, Tian JJ, Li JJ, Chen F, Wu HH, Han LX, et al: Inhibition of notch signaling promotes the adipogenic differentiation of mesenchymal stem cells through autophagy activation and PTEN-PI3K/AKT/mTOR pathway. Cell Physiol Biochem 36: 1991-2002, 2015.
26. Strotbek M, Schmid S, Sánchez-González I, Boerries M, Busch H and Olayioye MA: miR-181 elevates Akt signaling by co-targeting PHLPP2 and INPP4B phosphatases in luminal breast cancer. Int J Cancer 140: 2310-2320, 2017.
27. Trigka EA, Levidou G, Saetta AA, Chatziandreou I, Tomos P, Thalassinos N, Anastasiou N, Spartalis E, Kavantzas N, Patsouris E, et al: A detailed immunohistochemical analysis of the PI3K/AKT/mTOR pathway in lung cancer: Correlation with PIK3CA, AKT1, K-RAS or PTEN mutational status and clinico-pathological features. Oncol Rep 30: 623-636, 2013.
28. Nicoś M, Krawczyk P, Jarosz B, Sawicki M, Trojanowski T and Milanowski J: Prevalence of NRAS, PTEN and AKT1 gene mutations in the central nervous system metastases of non-small cell lung cancer. Brain Tumor Pathol 34: 36-41, 2017.
29. He Y, Mo Q, Luo B, Qiao Y, Xu R, Zuo Z, Deng J, Nong X, Peng G, He W, et al: Induction of apoptosis and autophagy via mitochondria- and PI3K/Akt/mTOR-mediated pathways by E. adenophorum in hepatocytes of saanen goat. Oncotarget 7: 54537-54548, 2016.
30. Liu Z, Wang L, Zhang LN, Wang Y, Yue WT and Li Q: Expression and clinical significance of mTOR in surgically resected non-small cell lung cancer tissues: A case control study. Asian Pac J Cancer Prev 13: 6139-6144, 2012.