Alisol B 23-acetate inhibits the viability and induces apoptosis of non-small cell lung cancer cells via PI3K/AKT/mTOR signal pathway

YANG LIU[1], XI-CHAO XIA[1], LIU-YANG MENG[2], YU WANG[1] and YUE-MEI LI[1]

[1]Department of Medicine, Pingdingshan University; [2]Emergency Department, Pingdingshan Second People's Hospital, Pingdingshan, Henan 467000, P.R. China

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Abstract. The aim of the present study was to investigate the effects of alisol B 23-acetate (AB23A) on inhibiting the viability and inducing apoptosis of human non-small cell lung cancer (NSCLC) cells and the anticancer mechanisms of AB23A *in vitro*. The viability of A549 cells following treatment with different doses of AB23A was examined using a Cell Counting Kit-8 assay. Subsequently, apoptosis and the cell cycle were detected using flow cytometric analysis. The effect of AB23A on migration and invasion of A549 cells was detected by wound healing and Transwell assays. Western blotting was performed to determine the relative expression of Bax/Bcl-2, phosphatidylinositol 3-kinase (PI3K), protein kinase B (AKT) and mammalian target of rapamycin (mTOR). AB23A markedly inhibited the viability enhanced apoptosis of A549 cells and arrested the cell cycle in G1 phase. Additionally, AB23A upregulated the ratio of Bax/Bcl-2 in the A549 cells in a concentration-dependent manner. The results of wound healing and Transwell assays indicated that AB23A also suppresses the migration and invasion ability of A549 cells. Furthermore, AB23A reduced the protein levels of phosphorylated AKT, PI3K and mTOR. In conclusion, AB23A exerted anti-cancer activity via inhibiting cells viability, migration and invasion, and promoting apoptosis. Therefore, AB23A is a potential antitumor drug for the treatment of NSCLC.

Introduction

Non-small cell lung cancer (NSCLC), which accounts for >85% of newly diagnosed cases of lung cancer, is the leading cause of morbidity and cancer-associated mortality worldwide (1). Although there are a large number of clinical treatment options, the effectiveness of treatment for patients with NSCLC is unfavorable, with a 5-year relative survival rate of 18% (1). Chemotherapy, adjuvant radiotherapy and biological targeted therapy following surgical excision have improved the survival rate of patients with cancer. Chemotherapy is the most effective and common treatment method. Although a significant number of patients initially respond favorably to chemotherapy, the majority of them exhibit severe side-effects and *de novo* or acquired resistance to chemotherapeutic drugs, and conventional chemotherapeutic drugs do not provide a marked survival advantage for patients (2,3). Therefore, there is an urgent requirement to develop a novel drug that can effectively treat NSCLC with fewer side-effects.

Metastasis and proliferation of cancer cells are the main causes of deterioration of patients with NSCLC as cancer cells can survive beyond the normal life span of a cell, have increased proliferation and resistance to chemotherapy and facilitate metastatic activity (4). In addition, defective apoptosis is recognized as the major criterion that contributes to the initiation and progression of cancer. The key proteins in this process are BCL2 associated X (Bax) and B-cell lymphoma (Bcl-2). Consequently, induction of apoptosis and inhibition of cell viability are promising strategies for treatment of cancer. The process is associated with various signaling pathways, including that of phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR). A previous study reported that the PI3K/Akt/mTOR pathway is involved with different cellular processes, from cell growth or survival, to cell necrosis or apoptosis (5). Notably, natural products are considered a promising source for the development of novel anticancer drugs due to their potential effectiveness and low toxicity (6).

Chinese herbal medicine has gradually become an important modern clinical therapeutic approach for human diseases due to the strong pharmacological properties, which contribute to cancer chemotherapy (7). Alisol B 23-acetate (AB23A), a triterpenoid compound, exists naturally in the rhizomes of *Alisma orientalis* (8) and has been identified to have anti-cancer biological functions (9). Furthermore, AB23A had been demonstrated to possess anti-proliferative activity (10) and induced Bax gene nuclear translocation and

Correspondence to: Dr Yang Liu, Department of Medicine, Pingdingshan University, 2 Chongwen Road, Xinhua, Pingdingshan, Henan 467000, P.R. China
E-mail: l1503688@163.com

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apoptotic in PC-3 cells (4). In addition, a number of studies have demonstrated that AB23A has anti-hepatitis virus (11) and anti-bacterial (12) pharmacological activity. In human renal proximal tubular cells, alisol B-induced autophagy mediates apoptosis and nephrotoxicity through the PI3K/AKT/mTOR signaling pathway (13). However, the anticancer mechanism of AB23A remains unclear.

In the present study, the effects of AB23A on A549 cells were systematically investigated, including those on cell viability, migration and invasion, the cell cycle, apoptosis and the activity of the PI3K/AKT/mTOR signaling pathways. The results demonstrated that AB23A may be a promising compound for the treatment of NSCLC. To the best of our knowledge, this study is the first to demonstrate that AB23A exerts anticancer effects on NSCLC and to investigate the possible corresponding molecular mechanism.

Materials and methods

Materials. AB23A (High pressure liquid chromatography ≥98%) was purchased from Shanghai Moqi Biological Technology Co., Ltd. (Shanghai, China). The Cell Counting Kit-8 (CCK-8; cat. no. C0039) was purchased from Beyotime Institute of Biotechnology (Haimen, China). The propidium iodide (PI)/RNase staining kit and the Annexin V-FITC/7AAD kit were all purchased from BD Biosciences (San Jose, CA, USA); All primary antibodies, including Bax (cat. no. ab53154; 1:1,000), Bcl-2 (cat. no. ab196495; 1:1,000), AKT (cat. no. ab38449; 1:1,000), phosphorylated (p)-AKT (cat. no. ab18206; 1:500), PI3K (cat. no. ab86714; 1:1,000), p-PI3K (cat. no. ab125633; 1:1,000), mTOR (cat. no. ab63552; 1:500), p-mTOR (cat. no. ab1093; 1:1,000) and GAPDH (cat. no. ab9484; 1:5,000), and horseradish peroxidase-conjugated anti-mouse IgG (cat. no. ab205719; 1:10,000) or anti-rabbit IgG (cat. no. ab205718; 1:5,000) secondary antibodies were purchased from Abcam (Cambridge, UK).

Cell culture. The human NSCLC cell line A549 and normal human lung epithelial cell line BEAS-2B were obtained from the American Type Culture Collection (Manassas, VA, USA). BEAS-2B cells were cultured in bronchial epithelial cell growth medium (Lonza Group, Ltd., Basel, Switzerland). A549 cells were cultured in Dulbecco's Modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin in a standard incubator supplied with 5% CO_2 at 37˚C.

AB23A treatment experiment. AB23A were dissolved in dimethyl sulfoxide (DMSO). The A549 cells and BEAS-2B cells were seeded in 12-well plates at a density of $6x10^5$ cells/well. AB23A at concentrations of 6 and 9 mM or the vehicle (vehicle control, 1% DMSO) was added to the culture medium. The cells were then harvested for each experiment.

Cell growth rate assay. A CCK-8 assay was conducted to measure cell viability and proliferation. Briefly, A549 cells ($2x10^4$ cells/well) and BEAS-2B cells ($5x10^3$ cells/well) in the exponential growth were placed in a 96-well plate overnight. At a confluence of 70-80%, the A549 and BEAS-2B cells were incubated with different concentrations of AB23B (0, 6 and 9 mM) for different times (12, 24 and 48 h). Following treatment, the CCK-8 reagent was added to each well for 2 h at 37˚C according to the manufacturer's protocol. The growth rate of the A549 and BEAS-2B cells was determined by measuring the absorbance at 450 nm among the three group under a microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and was calculated as follows: Growth rate (%)=(mean experimental absorbance/mean control absorbance) x100.

Cell apoptosis assay. Quantification of apoptotic cells was conducted using flow cytometry. Briefly, A549 cells were pretreated with AB23A for 24 h and then were harvested and stained with an Annexin-V-FITC/7-AAD kit (BD Biosciences) according to the manufacturer's protocol. The data acquisition and analysis were performed with CellQuest 5.0 software (BD Biosciences).

Cell cycle analysis. The cell cycle distributions of the A549 cells was determined using flow cytometry. A total of $1x10^6$ cells were cultured in combination with various concentration of AB23A for 24 h. Subsequently, the cells were harvested and washed with ice-cold PBS, then fixed in 70% ethanol for 2 h at -20˚C. The cells were then washed again with PBS and incubated with the PI/RNase solution for 25 min at 37˚C. The cell cycle distributions were detected using a FACSCAN laser flow cytometer equipped with CellQuest software version 5.0 (BD Biosciences).

Wound healing assay. The migration ability of the A549 cells was evaluated using a wound healing assay. When 70-80% of the 6-well plate was covered, the cells were vertically scraped with a 200 μl pipette tip. Subsequently, the cells were treated with various concentrations of AB23A. Images of three randomly selected fields along the scraped line in each well were captured using a camera (Nikon Corporation, Tokyo, Japan). Following incubation for 24 and 48 h, further images of the selected fields were captured. The relative width of the wound in the 6 and 9 mM groups compared with the vehicle control (0 mM) group at 0, 24 and 48 h was determined using ImageJ version 1.51j8 software (National Institutes of Health, Bethesda, MD, USA).

Transwell assay. The invasion activity of the A549 cells were assessed using a 24-well Transwell assay. An insert that was precoated with 100 μl Matrigel and dried for 30 min at 37˚C was placed in the upper chamber. Subsequently, A549 cells ($2x10^4$ cells/well) were suspended in DMEM with 0.5% FBS and various concentrations of AB23A, and deposited into the upper chamber of each well; DMEM with 10% FBS was added to the lower chambers. After 24 and 48 h incubation, the cells remaining on the upper surface of the membranes were scraped off and the invasive cells on the lower surface of the membranes were fixed in 4% paraformaldehyde at 4˚C and stained with 0.1% crystal violet (Beyotime Institute of Biotechnology) for 25 min at room temperature. Images of three randomly selected fields were captured with a camera (Nikon Corporation). The invasion activity of the A549 cells was assessed by counting the number of cells under a light microscope (magnification, x200).

Protein isolation and western blot analysis. Following treatment with various concentrations of AB23A (0, 6 and 9 mM) for 24 and 48 h, the A549 cells were harvested, washed with ice-cold PBS and lysed in radioimmunoprecipitation assay lysis buffer (Thermo Fisher Scientific, Inc.) for 30 min. The cell lysates were separated with centrifugation at 3,000 x g at 4˚C for 15 min and then the supernatants were collected. The protein concentration was determined by a bicinchoninic acid (BCA) protein assay (Thermo Fisher Scientific, Inc.). Equivalent amounts of samples containing 40 μg protein from each lysate were resolved by 10% SDS-PAGE. Subsequently, the protein was transferred onto a polyvinylidene difluoride membrane (Thermo Fisher Scientific, Inc.). After blocking with 5% non-fat milk in PBS containing 0.1% Tween-20 (TPBS) at room temperature for 2 h, the membranes were incubated overnight at 4˚C with specific primary antibodies. The membranes were then washed with TPBS and incubated with the corresponding secondary antibodies for 1 h at room temperature. After washing the membranes again with TPBS, the protein bands were detected by an enhanced chemiluminescence method using a Tanon 4200R automatic chemiluminescence image analysis system (Tanon Science and Technology Co., Ltd., Shanghai, China) and ImageJ version 1.51j8 software was used to quantify protein expression.

Statistical analysis. Statistical analyses were performed with SPSS 19.0 software (IBM, Corps., Armonk, NY, USA). Data were presented as the mean ± standard deviation of at least three independent experiments. Statistical differences were determined by a Student's t-test or one-way analysis of variance with Dunnett's multiple comparison post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Effects of AB23A on the growth rate of human NSCLC cells, A549 and normal human lung epithelial cells, BEAS-2B. To evaluate the cytotoxicity of AB23A on A549 and BEAS-2B cells, a CCK-8 assay was used to measure cell growth. As presented in Fig. 1A, the growth rate of the A549 cells was significantly reduced by the various concentrations of AB23A (6 and 9 mM) in a time-dependent manner (P<0.01). Therefore, AB23A was cytotoxic to the A549 cells. The results also revealed that AB23A inhibited the growth rate of the A549 cells in a dose-dependent manner. The growth rate of the A549 cells was reduced to 50% following treatment with 9 mM AB23A for 24 h (Fig. 1B). As presented in Fig. 1C, the growth rate of the BEAS-2B cells exhibited no significant change following treatment with the various concentrations of AB23A (6 and 9 mM) in a time-dependent manner. However, AB23A also demonstrated some cytotoxicity on the BEAS-2B cells, which lead to a slight decrease in its activity, although there was no statistically significance difference.

AB23A induces arrest of A549 cell cycle progression. AB23A induces an inhibitory effect through specific disturbances of cell cycle-associated events. To determine the effect of AB23A treatment on the progression of A549 cells cycle, flow cytometry was performed. As presented in Fig. 2, treatment with AB23A (6 and 9 mM) for 24 h led to a significant increase in the proportion of cells at the G0/G1 phase (P<0.01) and

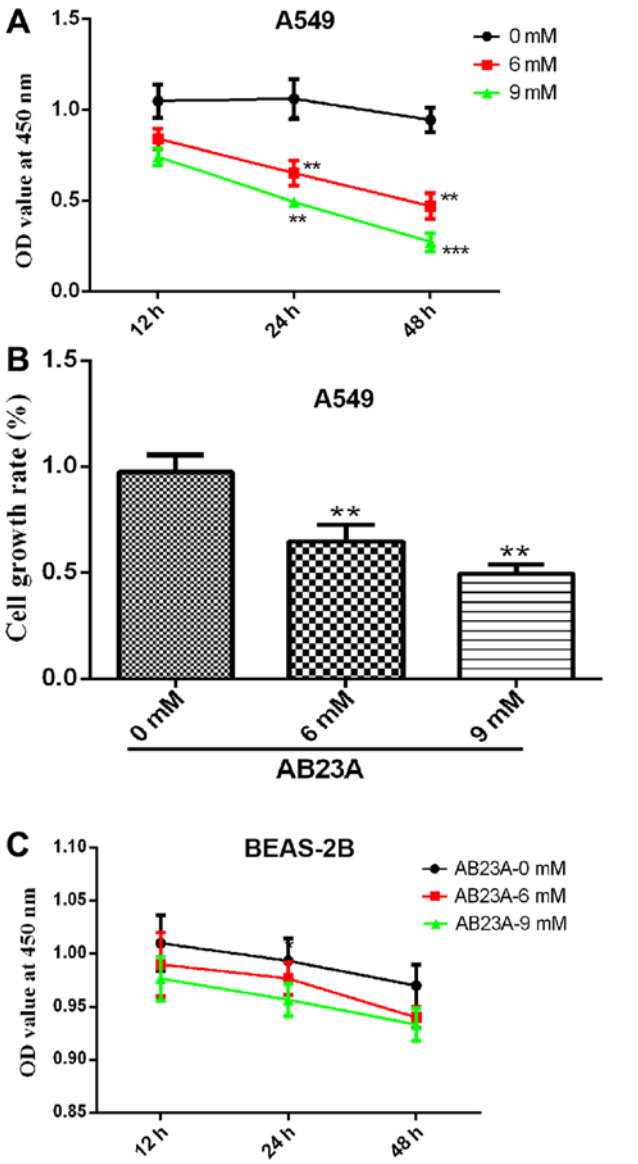

Figure 1. Effects of AB23A on the growth rate of A549 and BEAS-2B cells. (A) The growth rate of A549 cells following exposure to AB23A (0, 6 and 9 mM) for 12, 24 and 48 h. (B) Histograms summarizing the results following treatment with 9 mM AB23A at 24 h in A. (C) The growth rate of BEAS-2B cells following exposure to AB23A (0, 6 and 9 mM) for 12, 24 and 48 h. Data represent the mean ± standard deviation of at least three independent experiments. **P<0.01 and ***P<0.001 vs. the vehicle control group (0 mM). OD, optical density; AB23A, alisol B 23-acetate.

significantly reduced arrest in the S phase in a dose-dependent manner (P<0.05).

AB23A induces A549 cell apoptosis. After the cells were treated with different concentrations of AB23A (6 and 9 mM) for 24 h, the percentage of apoptotic cells was detected using flow cytometry. Treatment with 9 mM AB23A induced a significant increase in the percent of apoptotic cells (P<0.001; Fig. 3). To clarify this effect, the expression levels of the apoptosis-associated proteins Bax and Bcl-2 were detected using western blotting analysis. The levels of Bcl-2 expression were markedly downregulated and those of Bax expression were significantly elevated following treatment with various concentrations of AB23A (6 and 9 mM) for 24 h (P<0.05;

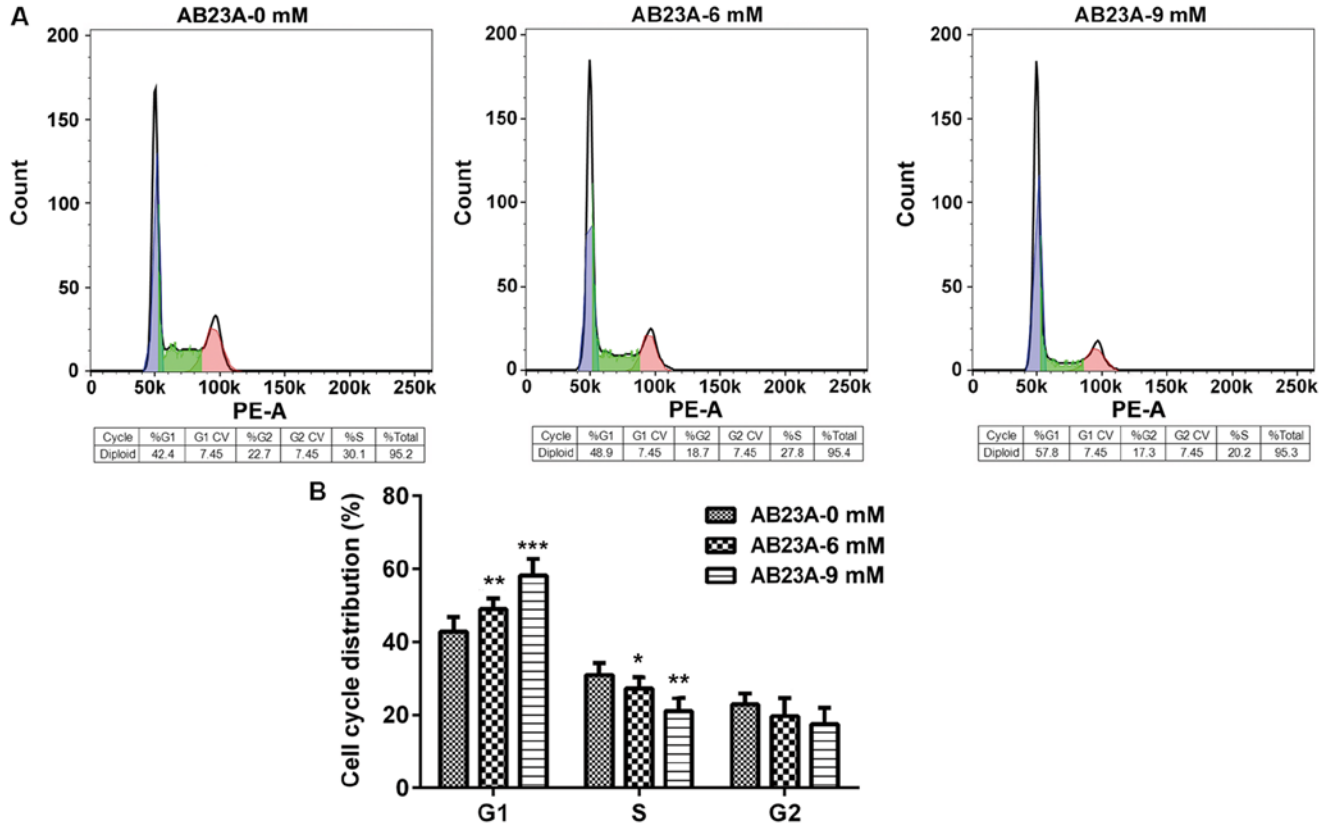

Figure 2. Effects of AB23A on cell cycle in A549 cells. A549 cells were permeabilized and stained with propidium iodide for cell cycle analysis by flow cytometry. (A) Representative picture of cell cycle analysis. (B) Histograms summarizing the results presented in A. The percentage of cells in G0/G1, S and G2/M was determined from the histogram displaying the cell number. Data are presented as the mean ± standard deviation of three independent experiments. *P<0.05, **P<0.01 and ***P<0.001 vs. the vehicle control group (0 mM). AB23A, alisol B 23-acetate.

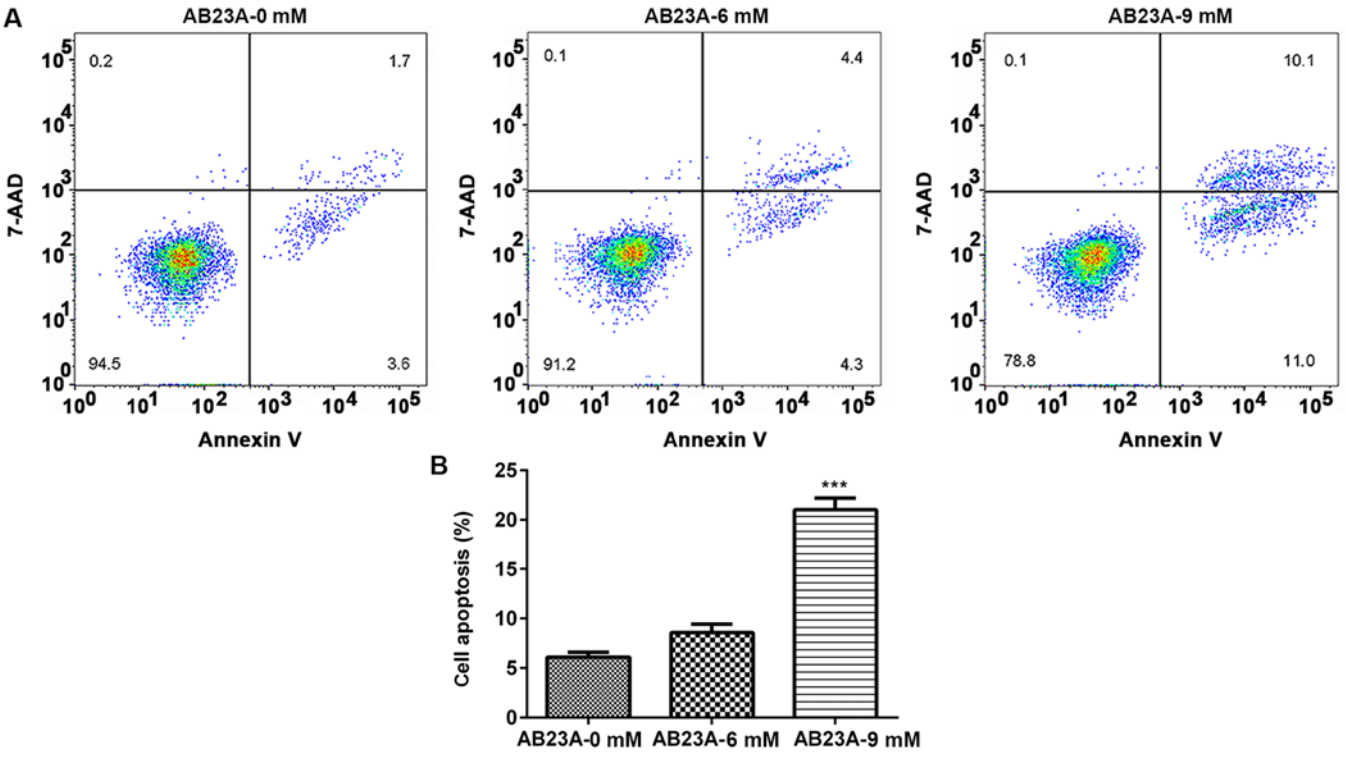

Figure 3. Effects of AB23A on apoptosis in A549 cells. (A) A549 cells were cultured in the presence of AB23A (0, 6 and 9 mM) for 24 h. Then, cells were harvested, washed with ice-cold PBS and stained using the Annexin-V-fluorescein isothiocyanate/7AAD kit. (B) Quantified histograms display the effect of AB23A on A549 apoptosis. Data are presented as the mean ± standard deviation of three independent experiments. ***P<0.001 vs. the vehicle control group (0 mM). AB23A, alisol B 23-acetate.

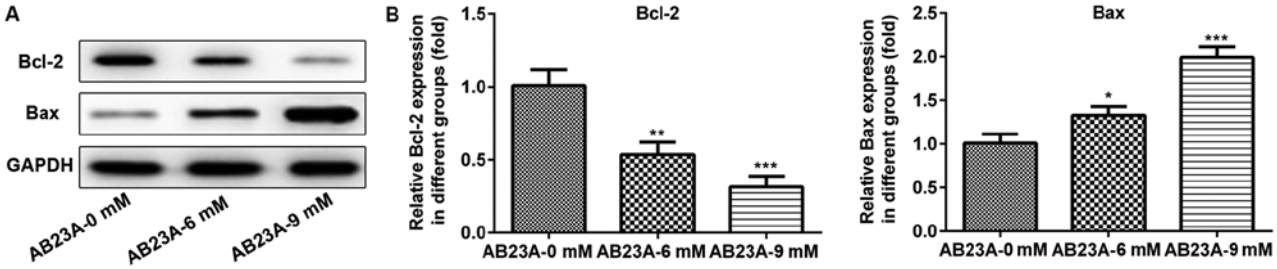

Figure 4. Effects of AB23A on the protein levels of Bax and Bcl-2 in A549 cells. (A) Representative pictures of western blots demonstrating the levels of Bax and Bcl-2. (B) Histograms summarizing the results presented in A. Data are presented as the mean ± standard deviation of three independent experiments. *P<0.05, **P<0.01 and ***P<0.001 vs. the vehicle control group (0 mM). Bcl-2, B-cell lymphoma; Bax, BCL2 associated X; AB23A, alisol B 23-acetate; AB23A, alisol B 23-acetate.

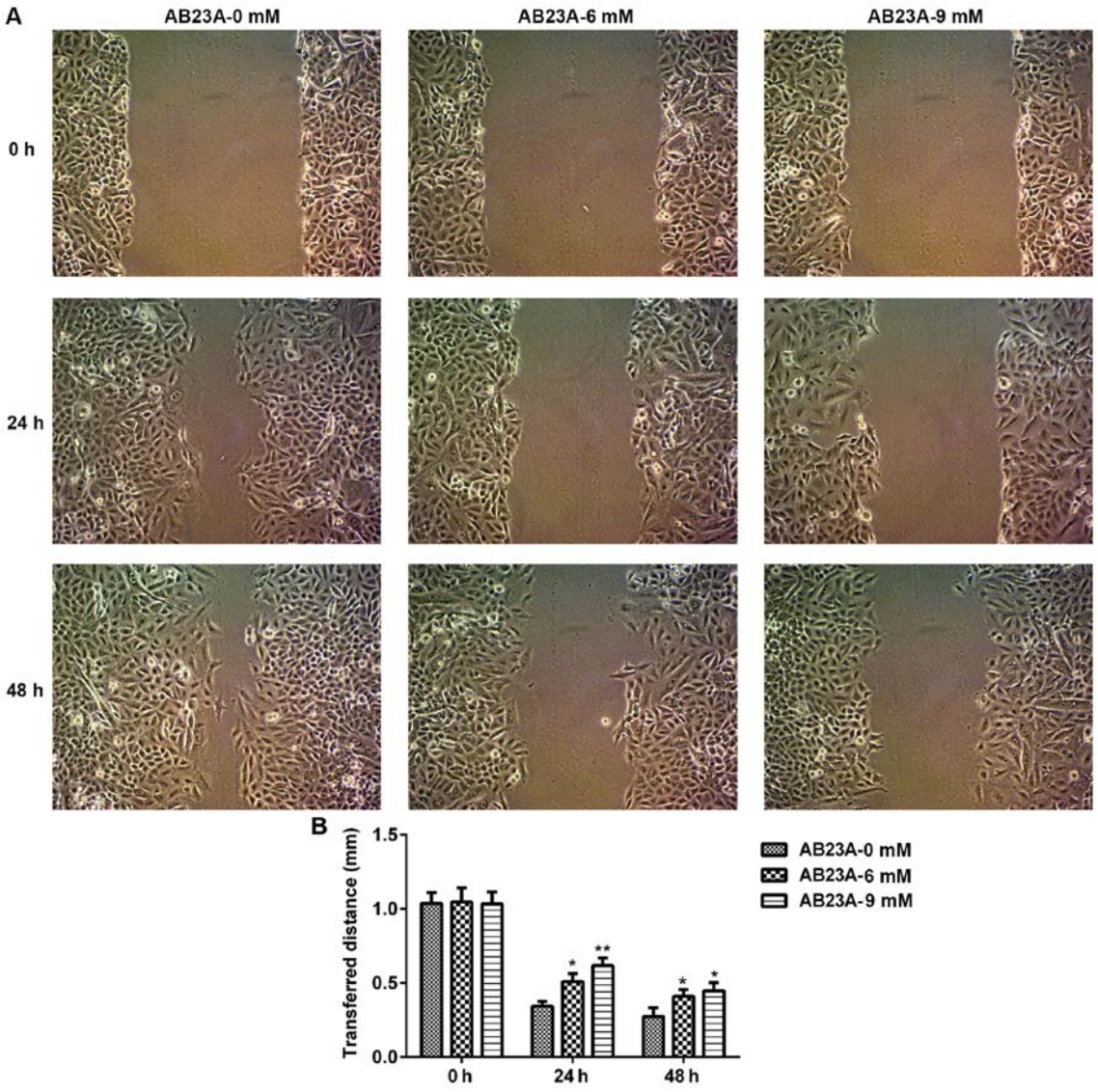

Figure 5. Effects of AB23A on migration in A549 cells. (A) Representative pictures of wound healing demonstrated that AB23A inhibited significantly cell migration following exposure to AB23A (0, 6 and 9 mM) for 24 and 48 h (magnification, x200). (B) Histograms summarizing the results presented in A. Data are presented as the mean ± standard deviation of three independent experiments. *P<0.05 and **P<0.01 vs. the vehicle control group (0 mM). AB23A, alisol B 23-acetate.

Fig. 4). These results suggested that AB23A induces apoptosis of NSCLC cells.

AB23A suppresses A549 cell migration and invasion. The migration and invasion abilities of A549 cells are important for NSCLC metastasis; therefore, the impact of AB23A on the abilities of the A549 cells was assessed using a wound healing assay and Transwell assay. The mobility and invasion of the A549 cells were significantly reduced following treatment with AB23A (6 and 9 mM) for 24 and 48 h (P<0.05; Figs. 5 and 6).

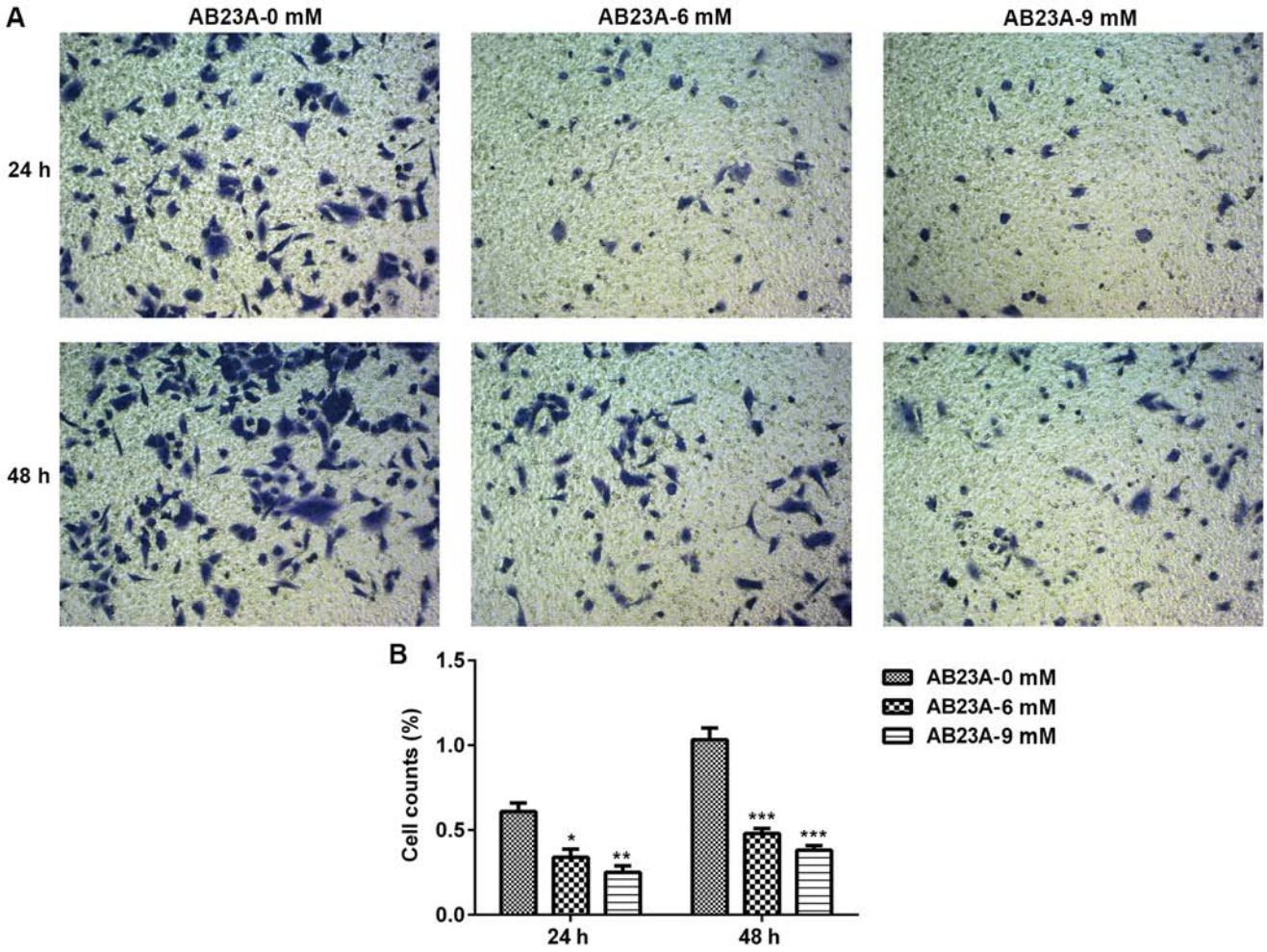

Figure 6. Effects of AB23A on invasion in A549 cells. (A) Representative pictures of Transwell assay demonstrated that AB23A inhibited significantly cells invasion following exposure to AB23A (0, 6 and 9 mM) for 24 and 48 h (magnification, x200). (B) Histograms summarizing the results presented in A. Data are presented as the mean ± standard deviation of three independent experiments. *P<0.05, **P<0.01 and ***P<0.001 vs. the vehicle control group (0 mM). AB23A, alisol B 23-acetate.

AB23A reduces the phosphorylation levels of PI3K, AKT and mTOR in A549 cells. To investigate the mechanism of the apoptosis induced by AB23A, western blot analysis was performed to detect proteins in the PI3K/AKT/mTOR signaling pathways. As presented in Fig. 7, upon treatment with AB23A (6 and 9 mM) for 24 and 48 h, the phosphorylation levels of PI3K, AKT and mTOR were remarkably reduced in a dose-and time-dependent manner, however, the levels of PI3K, AKT and mTOR were not significantly affected.

Discussion

The present study demonstrates, for the first time to the best of our knowledge, the therapeutic effects of AB23A on NSCLC cells. The majority of patients with NSCLC are treated with chemotherapy drugs, including platinum-based drugs; however, the side-effects of chemotherapy drugs reduced the survival rate of patients. Toxicity, drug resistance and a high risk of death have been observed in the clinic as side-effects of chemotherapy drugs used to treat NSCLC (14). Resistance of NSCLC to chemotherapy drugs has become a difficult issue to overcome. In such a severe situation, the natural products used in traditional Chinese medicine exhibit clear advantages, including few side-effects. Therefore, it is imperative to develop novel chemotherapy drugs to overcome refractory chemotherapy resistance and the side-effects of the current chemotherapeutic drugs.

Traditional Chinese medicine has the advantages of low cost, wide safety range, low toxicity, broad spectrum and numerous targets. Therefore, a number of researchers have investigated the rich resources of Chinese medicinal herbs (15). A previous study demonstrated that AB23A can inhibit nitric oxide production without cytotoxic effects (16). AB23A is commonly used for treatment of a number of diseases, including diabetes, pyelonephritis, inflammation and cancer (17,18). The results of the present study revealed that the growth rate was reduced to 50% when the A549 cells were treated with 9 mM AB23A for 24 h, which indicates that AB23A has an inhibitory effect on cell vitality. In addition, in the present study, the inhibitory effect of AB23A on the activity of normal human lung epithelial cells (BEAS-2B) is not obvious, which indicates the low toxicity of AB23A to human body. However, it cannot be excluded that a high dose of AB23A will cause cytotoxicity to normal cells. Additionally, although the A549 and BEAS-2B growth rate reduced a little between 12 and 48 h in untreated cells (AB23A 0 mM), there was no significant difference and

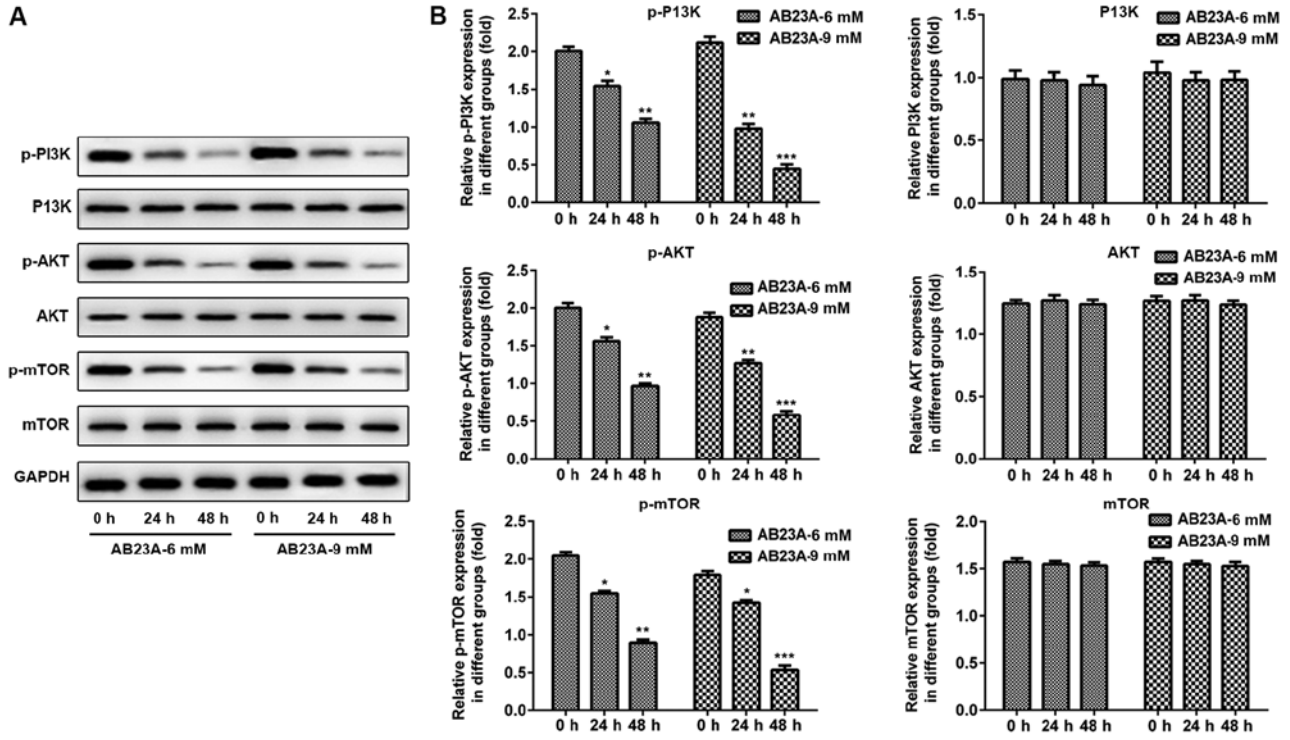

Figure 7. Effects of AB23A on PI3K/AKT/mTOR signaling pathways in A549 cells. (A) Representative pictures of western blots demonstrating the levels of total and phosphorylated of PI3K, AKT and mTOR. (B) Histograms summarizing the results presented in A. Data are presented as the mean ± standard of three independent experiments. *P<0.05, **P<0.01 and ***P<0.001 vs. the vehicle control group (0 h). mTOR, mammalian target of rapamycin; AKT, protein kinase B; PI3K, phosphatidylinositol 3 kinase; p, phosphorylated; AB23A, alisol B 23-acetate.

the survival rate of the cells was all >95%. The present study hypothesized that the change in cell viability may be due to the slight effect of the DMSO solution on A549 cells. These all requires further research.

A previous study demonstrated that AB23A inhibits the proliferation of human breast cells through inducing apoptosis (19). AB23A also induces apoptosis, blocked the G1 phase, and inhibits migration and invasion of ovarian cancer cells (20). The apoptosis-associated genes Bax and Bcl-2 serve a vital role in the initiation and maintenance of apoptosis (21). Interference with cell cycle progression is one of the characteristics of numerous anticancer drugs. AB23A has been demonstrated to induce cell cycle arrest in the G1 phase in human colon cancer and ovarian cancer cell lines (20,22). The results of the present study indicate that AB23A markedly induces cell cycle arrest in the G0/G1 phase in A549 cells. In addition, the results of the western blotting and flow cytometric analyses demonstrate that AB23A induces apoptosis and elevates the protein levels of Bax/Bcl-2 in a dose-dependence manner. Therefore, analysis of apoptosis and the cell cycle may be vital for developing AB23A as a potential anticarcinogen.

Activation of migration, invasion and metastasis is a crucial characteristic of malignancy, which is one of the hallmark capabilities of cancer (23). The high morbidity and mortality rates of patients with NSCLC are closely associated with the alterations observed in the biological behavior of lung cancer cells. Due to the high metastasis rate of cancer cells, NSCLC is difficult to eradicate. Therefore, inhibition of the biological behavior of NSCLC cells is a promising treatment for lung cancer. Wound healing and Transwell assays have been widely used to assess cancer cells metastasis and invasiveness *in vitro* (24,25). In the present study, the results revealed that AB23A may markedly suppress the migration and invasion of A549 cells in a concentration-dependent manner following treatment for 24 and 48 h. Additionally, cell apoptosis was evaluated following treatment for 24 h, and apoptosis significantly increased in a concentration-dependent manner. When detecting the invasion and migration of A549 cells, it was observed that the cytotoxicity of AB23A on A549 cells was effected by inducing apoptosis and reducing cell growth, invasion and migration. Determining the underlying mechanisms requires further study.

The PI3K/AKT/mTOR pathway serves critical roles in diverse cellular processes, from proliferation, transcription, migration or survival to cell death or apoptosis, which are involved in cancer (26). Out of the molecules associated with this pathway, AKT is an important regulator of cellular activities that can be activated by PI3K and that phosphorylate a number of key pro-cancer factors to promote cell viability and inhibit apoptosis. mTOR belongs to the PI3K-related kinase family and is a vital kinase for regulating cell proliferation, apoptosis and viability (27). AKT is localized upstream of mTOR and the suppression of AKT phosphorylation can result in an significant reduction in phosphorylation of downstream mTOR (28). It is well known that mTOR serves an important role in regulating autophagy in mammalian signaling (29) and mTOR signaling is very important for cellular growth in response to a number of physiological conditions through regulating downstream effectors to control the translation and transcription of a variety of proteins (30). These key signaling molecules from this pathway serve a vital role in regulating cell survival and apoptosis. Drugs that target the PI3K/Akt/mTOR signaling pathway have the

potential to inhibit survival pathways and induce apoptosis in cancer cells (16). Previous studies have reported that apigenin could inhibit the growth and proliferation, promote apoptotic cell death, induce cell cycle arrest via the PI3K/Akt/mTOR signaling pathway (31,32). To further clarify the possible mechanism by which AB23A induces apoptosis of NSCLC cells, the impact of AB23A on the expression levels of PI3K, AKT and mTOR was investigated in the present study. Upregulation of Akt phosphorylation by the activation of PI3K and mTOR can integrate upstream activating signals through the PI3K/AKT pathway in return to result in phosphorylation (33). In the present study, AB23A induced apoptosis and inhibited the viability of the A549 cells via inhibition of the PI3K/AKT/mTOR signal pathway. Furthermore, alterations in the expression of phosphorylated PI3K/AKT/mTOR proteins and alterations in the cell phenotype in response to the AB23A stimulus were investigated. The results demonstrated that following treatment with AB23A (6 and 9 mM) for 24 and 48 h, the protein levels of p-PI3K, p-AKT and p-mTOR were significantly reduced in the A549 cells, but there was no significant difference in the levels of PI3K, AKT and mTOR. In conclusion, treatment of NSCLC cells with AB23A has an anti-tumor effect, reduces cell viability and induces apoptosis via the activation of the PI3K/AKT/mTOR signaling pathway. The results reveal that AB23A could be an effective anticancer drug for the treatment of NSCLC.

In conclusion, the present study demonstrated that the anticancer activity of AB23A in A549 cells is mediated by the induction of apoptosis, interference with the cell cycle and suppression of cell activities, accompanied by inhibition of the PI3K/AKT/mTOR pathway *in vitro*. However, further studies are required to elucidate the underlying mechanism of action of AB23A and its anticancer activity *in vivo* also requires further evaluation. The results of this study may provide a novel therapeutic strategy for the treatment of human NSCLC.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

YL designed the experiments and drafted the manuscript. YL, XCX and LYM performed the experiments. YL, YW and YML analyzed the data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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