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Skullcapflavone I suppresses proliferation of human lung cancer cells via down-regulating microRNA-21

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ABSTRACT

Background: Lung cancer is the most leading cause of cancer-related deaths worldwide. Skullcapflavone I is a flavone compound extracted from *Scutellaria baicalensis* Georgi (Wogon). The present study investigated the effects of skullcapflavone I on human lung cancer cell proliferation, as well as underlying possible mechanism.
Methods: Cell proliferation was detected using Trypan blue assay. Cell viability was measured using cell counting kit 8 (CCK-8) assay. Quantitative reverse transcription PCR (qRT-PCR) was performed to assess microRNA-21 (miR-21) expression. Cell transfection was conducted to enhance the levels of miR-21 and protein phosphatase 2A (PP2A). Western blotting was used to evaluate the expressions of proliferation cell nuclear antigen (PCNA), Cyclin D1, PP2A, phosphatidylinositol 3-kinase (PI3K), protein kinase 3 (AKT), mechanistic target of rapamycin (mTOR) and p70S6K.
Results: Skullcapflavone I significantly suppressed the viability and proliferation of A549 and H1975 cells. The expressions of miR-21 in A549 and H1975 cells were drastically decreased after skullcapflavone I treatment. Overexpression of miR-21 remarkably reversed the skullcapflavone I-induced A549 cell viability inhibition. Moreover, skullcapflavone I enhanced the expression of PP2A in A549 cells. Skullcapflavone I inactivated PI3K/AKT/mTOR signaling pathway in A549 cells via up-regulating PP2A. Besides, skullcapflavone I treatment had no significant influence on human normal bronchial epithelial 16HBE cell viability, proliferation and apoptosis.
Conclusion: Skullcapflavone I exerted anti-cancer effect on lung cancer cells by down-regulating miR-21 expression, up-regulating PP2A expression and then inactivating PI3K/AKT/mTOR signaling pathway.

1. Introduction

Cancer is one of the most major public health issues all over the world (Gregurek et al., 2010; Siegel et al., 2017). Lung cancer is the most leading cause of cancer-related deaths worldwide, which accounts for roughly 1.8 million new cases and 1.2 million deaths each year (Hirsch et al., 2017). According to the different histologically features, lung cancer can be divided into small cell lung carcinoma (SCLC, approximately 15–20% of cases) and non-small cell lung carcinoma (NSCLC, approximately 80–85% of cases) (Lonardo et al., 2010). The 5-year survival rates of patients with different types of lung cancer vary from 4% to 17% depending on stage and regional differences (Hirsch et al., 2017; Wang et al., 2016). Despite the diagnosis and therapy of lung cancer have been remarkably improved in recent years, lots of patients still died of this disease (Forde and Ettinger, 2013; Wakelee et al., 2014). Novel and more effective therapy medicines are still urgently needed.

Scutellaria baicalensis Georgi (Wogon) is a popular and multi-purpose herbal medicine widely distributed in many countries all over the world, such as China, Russia, Mongolia, Japan and Korea (Kudo et al., 2017). Skullcapflavones, the flavone compounds isolated from the roots of *Scutellaria baicalensis*, have been aroused more and more attention at home and abroad, due to its wide beneficial effects (Boozari et al., 2015; Chandrasekaran et al., 2011; Tsai et al., 2015). For example, Park et al. (2005) demonstrated that skullcapflavone I induced apoptosis of activated rat hepatic stellate cells. Bui et al. (2017) indicated that skullcapflavone II alleviated ovalbumin-induced allergic rhinitis by blocking Th2 production and mast cell histamine release. Jang et al. (2012) proved that skullcapflavone II suppressed ovalbumin-induced airway inflammation in a mouse model of asthma. However, there is little information available about the effects of skullcapflavone on lung cancer cells.

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Emerging evidences suggested that microRNAs (miRNAs) implicated in the regulation of multiple diseases processes, including cancers (Calin and Croce, 2006; Croce and Calin, 2005). Numerous medicines can suppress the growth of cancers by modulating the expressions of miRNAs (Bae et al., 2011; Kjersem et al., 2014). miRNA-21 (miR-21) is an important anti-apoptotic factor in a variety of cancer cells by targeting the network of tumor-suppressive pathways (Dillhoff et al., 2008; Papagiannakopoulos et al., 2008). Liu et al. (2012) indicated that miR-21 was highly expressed in lung cancer tissues and associated with the recurrence and metastasis of lung cancer. Markou et al. (2016) revealed that miR-21 was a therapeutic target for lung cancer.

In this research, we investigated the effects of skullcapflavone I, a member of skullcapflavones, on lung cancer A549 and H1975 cell proliferation and miR-21 expression. The possible molecular mechanism and potential signaling pathway were also analyzed. These findings will provide evidence for understanding the anti-cancer effect of skullcapflavone I on lung cancer, and may offer new possible therapeutic medicine for lung cancer therapy.

2. Materials and methods

2.1. Cell lines

Human lung cancer cell lines A549 and H1975 were provided by Stem Cell Bank, Chinese Academy of Science (Shanghai, China). Human bronchial epithelial 16HBE cells were received from Procell Life Science & Technology Co., Ltd. (Wuhan, China). Cells were all cultured in Rosewell Park Memorial Institute (RPMI)-1640 medium (Sigma-Aldrich, St Louis, MO, USA) supplemented with 10% (*v*/v) heat-inactivated fetal bovine serum (FBS, Gibco, Life Technology, Carlsbad, CA, USA) and 1% (v/v) penicillin-streptomycin-glutamine (100×) solution (Gibco, Life Technology, Carlsbad, CA, USA). Cultures were maintained in a humidified incubator (Thermo Fisher Scientific, Waltham, MA, USA) with 5% CO_2 at 37 °C.

2.2. Preparation of skullcapflavone I solution

Skullcapflavone I was obtained from Sigma-Aldrich (catalog number: 53382, St Louis, MO, USA) and dissolved into dimethyl sulfoxide (DMSO, Sigma-Aldrich, St Louis, MO, USA) solution to a storage concentration of 20 mg/ml. Then, skullcapflavone I solution was sterilized through 0.22 μm filter (Millipore, Bedford, MA, USA) and stored in −20 °C until use. The chemical structure of skullcapflavone I was illustrated in Fig. 1.

2.3. Cell proliferation assay

Proliferation of A549 and H1975 cells after 20 μg/ml skullcapflavone I treatment was detected using Trypan blue assay with a hemocytometer (Shanghai Anxin Optical Instrument Manufacture CO., Ltd., Shanghai, China). Briefly, cells were seeded into 24-well plate (Corning Incorporation, New York, NY, USA) with 1×10^3 cells per well and treated by 20 μg/ml skullcapflavone I for 6, 12, 24, 36, 48 and 72 h. Cells in control group received same quantity of DMSO solution. After that, the number of living cells in each group was counted under microscope (Nikon, Japan).

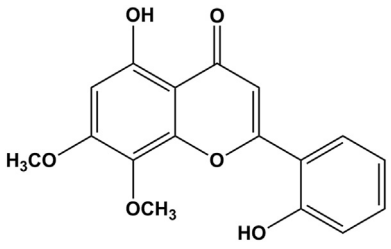

Fig. 1. The chemical structure of skullcapflavone I.

2.4. Cell viability assay

Viability of A549, H1975 and 16HBE cells after 20 μg/ml skullcapflavone I treatment and/or miR-21 mimic transfection were assessed using cell counting kit 8 (CCK-8) assay (Beyotime Biotechnology, Shanghai, China). Briefly, cells were seeded into 96-well plate (Corning Incorporation, New York, NY, USA) with 1×10^4 cells per well and treated by 20 μg/ml skullcapflavone I for 24 h. Cells in control group received same quantity of DMSO solution. Then, 10 μl CCK-8 solution was added into the each well of the plate and the plate was maintained in humidified incubator at 37 °C for 1 h. After that, the absorbance of each well at 450 nm was recorded using a Micro-plate Reader (Bio-Tek Instruments, Winooski, VT, USA). Cell viability (%) was calculated by average absorbance of treatment group/average absorbance of control group × 100%.

2.5. Cell apoptosis assay

Apoptosis of 16HBE cells after 20 μg/ml skullcapflavone I treatment was detected by Annexin V-FITC Apoptosis Detection kit (Beyotime Biotechnology, Shanghai, China). Briefly, 16HBE cells were seeded into 24-well plate with 3×10^4 cells per well and treated by 20 μg/ml skullcapflavone I for 24 h. Cells in control group received same quantity of DMSO solution. Then, cells in each group were collected, washed with kit buffer for three times and stained using 5 μl Annexin V-FITC and 10 μl PI for 20 min at room temperature in the dark. The percentage of apoptotic cells were tested by flow cytometer (Guava Technologies, Hayward, CA, USA).

2.6. Quantitative reverse transcription PCR (qRT-PCR)

qRT-PCR was conducted to measure miR-21 expression in A549 cells after 20 μg/ml skullcapflavone I treatment and/or miR-21 mimic transfection. Total RNAs in A549 cells were isolated using TRIzol™ Plus RNA Purification kit (Invotrogen, Carlsbad, CA, USA). Then, cDNA was reversely transcribed using High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). After that, the expression of miR-21 was assessed using MirVana™ miRNA Detection kit (Invitrogen, Carlsbad, CA, USA) and the expression of U6 was used as endogenous control. The primers for miR-21 were: 5′-GCCCGCTAGCT TATCAGACTGATG-3′ (forward) and 5′-GTGCAGGGTC CGAGGT-3′ (reverse). The primers for U6 were: 5′-TGCGGGTGCTGCTTCGGC AGC-3′ (forward) and 5′-CCAGTGCAGGGTCCG AGGT-3′ (reverse). Data were quantified using $2^{-\triangle\triangle Ct}$ method (Ish-Shalom and Lichter, 2010).

2.7. Cell transfection

miR-21 mimic and its negative control (NC) were designed and provided by GenePharma Corporation (Shanghai, China). The full-length protein phosphatase 2A (PP2A) sequence was constructed into pEX-2 plasmid (GenePharma Corporation, Shanghai, China) and referred as pEX-PP2A. The unloaded pEX-2 plasmid was acted as NC. Cell transfection was performed using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) in line with the manufacturer's instruction. The transfection efficiency of miR-21 mimic was assessed using qRT-PCR and the transfection efficiency of pEX-PP2A was assessed using western blotting.

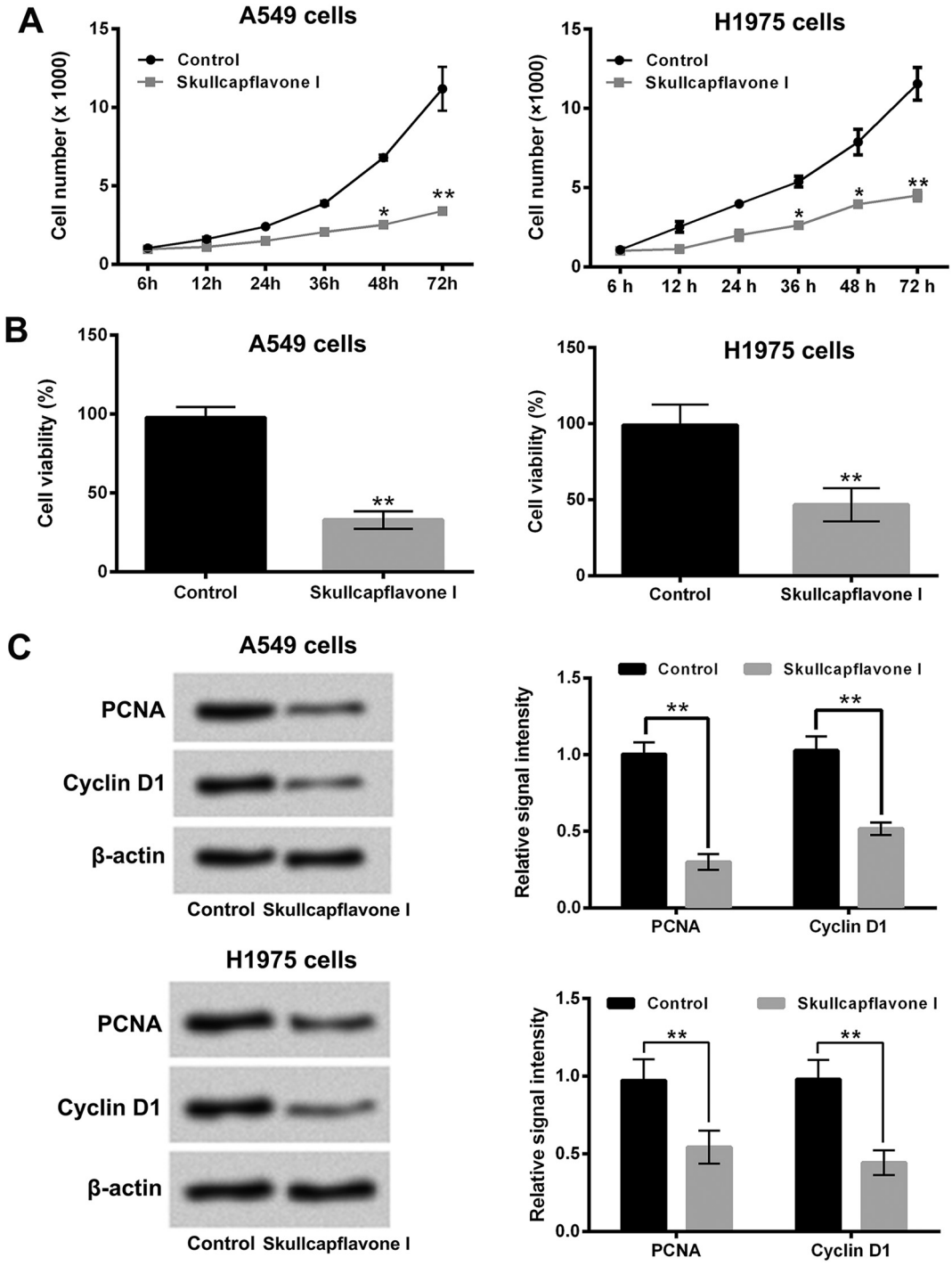

Fig. 2. Skullcapflavone I suppressed lung cancer A549 and H1975 cell viability and proliferation.
(A) Numbers of A549 and H1975 cells after 20 μg/ml skullcapflavone I treatment for 6, 12, 24, 36, 48 and 72 h were measured using Trypan blue assay. (B) Viability of A549 and H1975 cells after 20 μg/ml skullcapflavone I treatment for 24 h was detected using cell counting kit 8 (CCK-8) assay. (C) Western blotting was used to assess the protein expressions of PCNA and Cyclin D1 in A549 and H1975 cells after 20 μg/ml skullcapflavone I treatment for 24 h. PCNA: Proliferation cell nuclear antigen. $^{*}P < .05$; $^{**}P < .01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.8. Western blotting

Total proteins in cells were isolated using M-PER™ Mammalian Protein Extraction Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and quantified using Pierce™ BCA Protein Assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Then, equal concentration of proteins were electrophoresed on polyacrylamide gels using Bio-Rad Bis-Tris Gel System (Bio-Rad Laboratories, Hercules, CA, USA) and transferred onto nitrocellulose (NC) membranes (Millipore, Bedford, MA, USA) following with the manufacturer's protocol. After incubation with 5% bovine serum albumin (BSA, Beyotime Biotechnology, Shanghai, China) for 1 h, the NC membranes were incubated with relevant primary antibodies for 12 h. All primary antibodies were diluted in 1% BSA solution with a dilution of 1:1000. Anti-Proliferation cell nuclear antigen (PCNA) antibody (ab152112), Anti-Cyclin D1 antibody (ab137819), Anti-Bcl-2 antibody (ab196495), Anti-Bax antibody

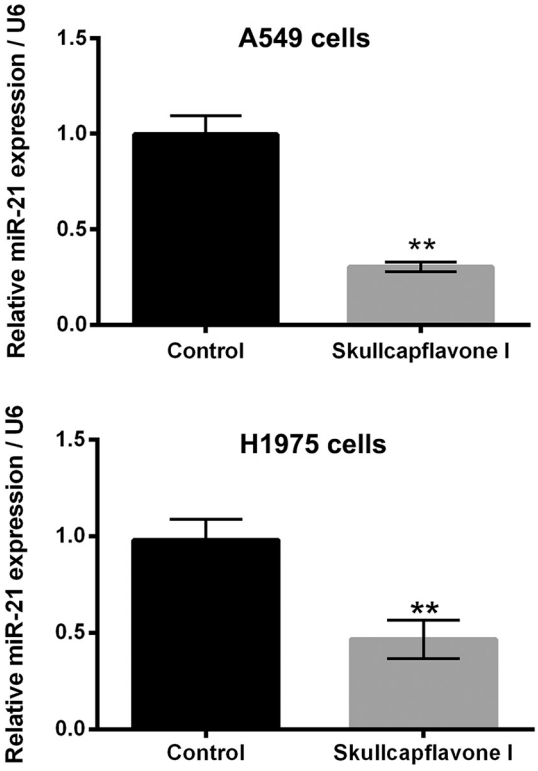

Fig. 3. Skullcapflavone I down-regulated miR-21 expression in A549 and H1975 cells.
The expressions of miR-21 in A549 and H1975 cells after 20 μg/ml skullcapflavone I treatment for 24 h was determined using quantitative reverse transcription PCR (qRT-PCR). miR-21: MicroRNA-21. $^{**}P < .01$.

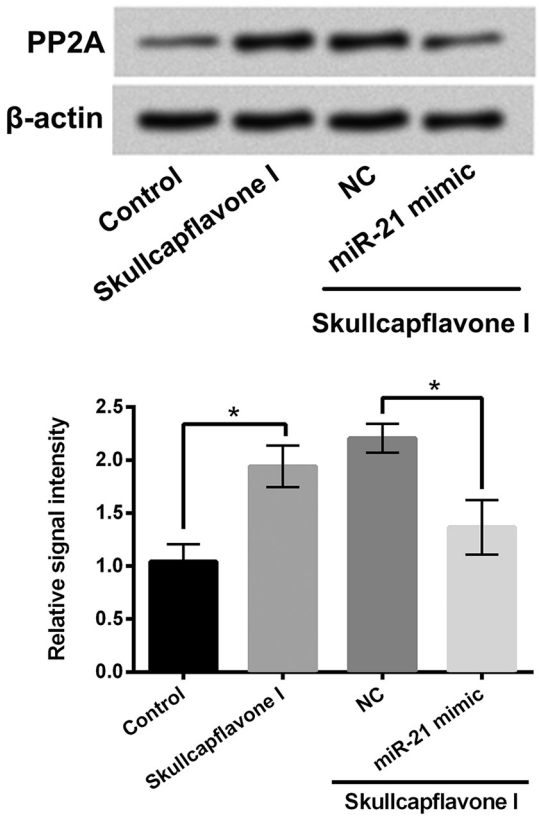

Fig. 5. Overexpression of miR-21 alleviated skullcapflavone I-induced PP2A expression increase in A549 cells.
After 20 μg/ml skullcapflavone I treatment and/or miR-21 mimic transfection, the protein expressions of PP2A in A549 cells were evaluated using western blotting. PP2A: Protein phosphatase 2A; miR-21: MicroRNA-21; NC: Negative control. $^{*}P < .05$.

(ab53154), Ant-Caspase 3 antibody (ab13847), Anti-Caspase 9 anbitody (ab32539), Anti-PP2A antibody (ab137849), Anti-phosphatidylinositol 3-kinase (PI3K) antibody (ab86714), Anti-p-PI3K antibody (ab182651), Anti-protein kinase 3 (AKT) antibody (ab8805), Anti-p-AKT antibody (ab38449), Anti-mechanistic target of rapamycin (mTOR) antibody (ab32028), Anti-p-mTOR antibody (ab84400), Anti-p70S6K antibody (ab9366), Anti-p-p70S6K antibody (ab2571) and Anti-β-actin antibody (ab8226) were all purchased from Abcam Biotechnology (Cambridge, MA, USA). Subsequently, the NC membranes were incubated with Goat anti-Mouse (Rabbit) IgG H&L (HRP) (ab205718, ab205719, Abcam Biotechnology, Cambridge, MA, USA) at room temperature for 1 h. Signals of proteins were recorded using Bio-Rad ChemiDoc™ XRS system (Bio-Rad Laboratories, Hercules, CA, USA), supplemented with 200 μl Immobilon Western Chemiluminescent HRP Substrate (Bio-Rad Laboratories, Hercules, CA, USA) on the surface of membranes. Data were quantified using Image Lab™ Software (Bio-Rad Laboratories, Hercules, CA, USA).

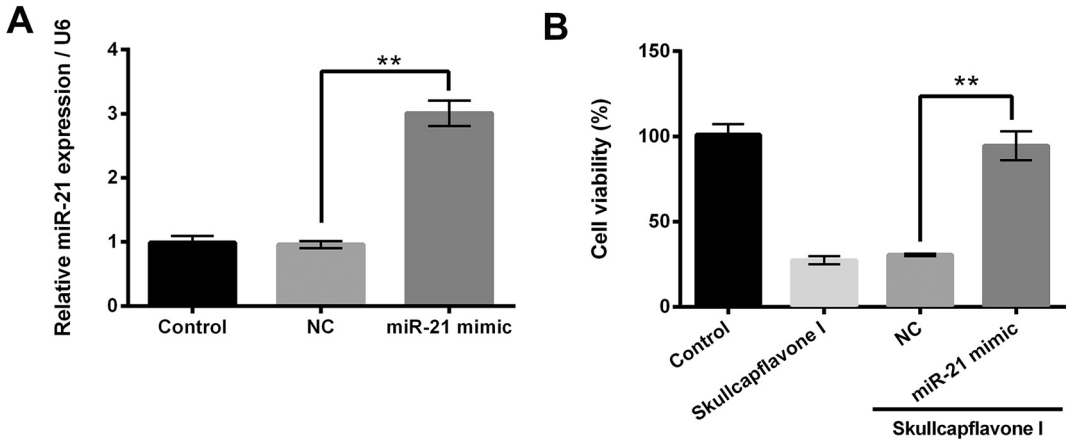

Fig. 4. Skullcapflavone I inhibited A549 cell viability by down-regulating miR-21.
(A) After miR-21 mimic or its negative control (NC) transfection, the expression of miR-21 in A549 cells was measured using quantitative reverse transcription PCR (qRT-PCR). (B) Viability of A549 cells after 20 μg/ml skullcapflavone I treatment and/or miR-21 mimic transfection were detected using cell counting kit 8 (CCK-8) assay. miR-21: MicroRNA-21. $^{**}P < .01$.

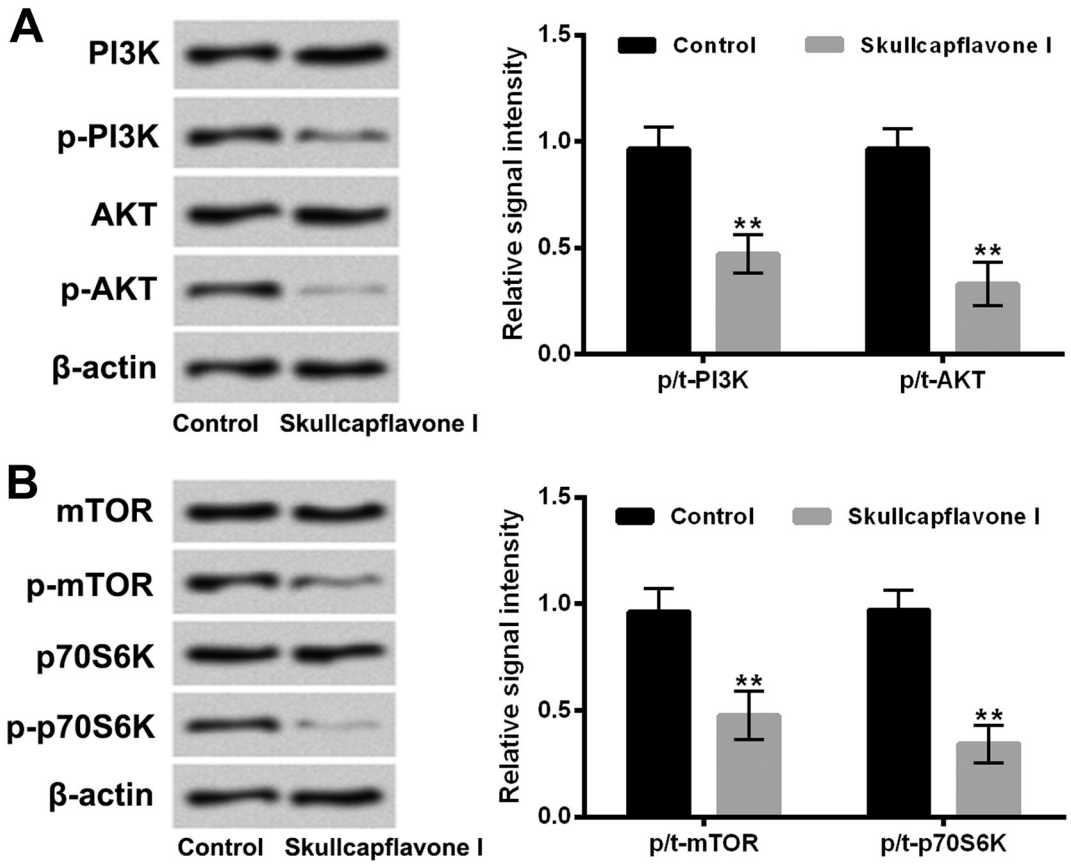

Fig. 6. Skullcapflavone I inactivated PI3K/AKT/mTOR signaling pathway in A549 cells.
(A-B) After 20 μg/ml skullcapflavone I treatment, the protein expressions of PI3K, p-PI3K, AKT, p-AKT, mTOR, p-mTOR, p70S6K and p-p70S6K in A549 cells were analyzed using western blotting. PI3K: Phosphatidylinositol 3-kinase; AKT: Protein kinase 3; mTOR: Mechanistic target of rapamycin. $^{**}P < .01$.

2.9. Statistical analysis

All experiments were repeated at least three times in this research. Results of multiple experiments were presented as the mean ± standard deviation (SD). Statistical analysis was performed using Graphpad 6.0 software (Graphpad, San Diego, CA, USA). The *P*-value was calculated using Student's *t*-test. $P < .05$ was considered to be significant difference.

3. Results

3.1. Skullcapflavone I suppressed lung cancer A549 and H1975 cell viability and proliferation

Viability and proliferation of A549 and H1975 cells after 20 μg/ml skullcapflavone I treatment were measured using CCK-8 assay and Trypan blue assay, respectively. As shown in Fig. 2A, skullcapflavone I treatment reduced the number of A549 and H1975 cells in a time-dependent manner ($P < .05$ or $P < .01$), which indicated that skullcapflavone I inhibited the proliferation of A549 and H1975 cells. Fig. 2B presented that skullcapflavone I treatment significantly suppressed the viability of A549 and H1975 cells ($P < .01$). Moreover, results in Fig. 2C displayed that the expressions of PCNA and Cyclin D1 in A549 and H1975 cells were both remarkably decreased after skullcapflavone I treatment ($P < .01$). These above findings suggested that skullcapflavone I could suppress lung cancer A549 and H1975 cell viability and proliferation.

3.2. Skullcapflavone I down-regulated the expression of miR-21 in A549 and H1975 cells

The expressions of miR-21 in A549 and H1975 cells after 20 μg/ml skullcapflavone I treatment were assessed using qRT-PCR. As presented in Fig. 3, skullcapflavone I treatment dramatically down-regulated the expression of miR-21 in both A549 and H1975 cells ($P < .01$), which implied that miR-21 might participate in the anti-tumor effects of skullcapflavone I on lung cancer A549 and H1975 cells.

3.3. Skullcapflavone I inhibited A549 cell viability by down-regulating miR-21

To analyze the role of miR-21 in skullcapflavone I-induced A549 cell viability inhibition, miR-21 mimic was transfected into A549 cells. Fig. 4A illustrated that miR-21 mimic transfection significantly enhanced the expression of miR-21 in A549 cells ($P < .01$). Compared to skullcapflavone I + NC group, the viability of A549 cells was remarkably increased in skullcapflavone I + miR-21 mimic group (Fig. 4B, $P < .01$), which indicated that skullcapflavone I inhibited A549 cell viability might be via down-regulating miR-21 expression.

3.4. Overexpression of miR-21 alleviated the skullcapflavone I-induced PP2A expression increase in A549 cells

The protein expression of PP2A in A549 cells after skullcapflavone I treatment and/or miR-21 mimic transfection were detected using western blotting. As shown in Fig. 5, skullcapflavone I single treatment significantly up-regulated the expression of PP2A in A549 cells ($P < .05$). Furthermore, miR-21 mimic transfection obviously

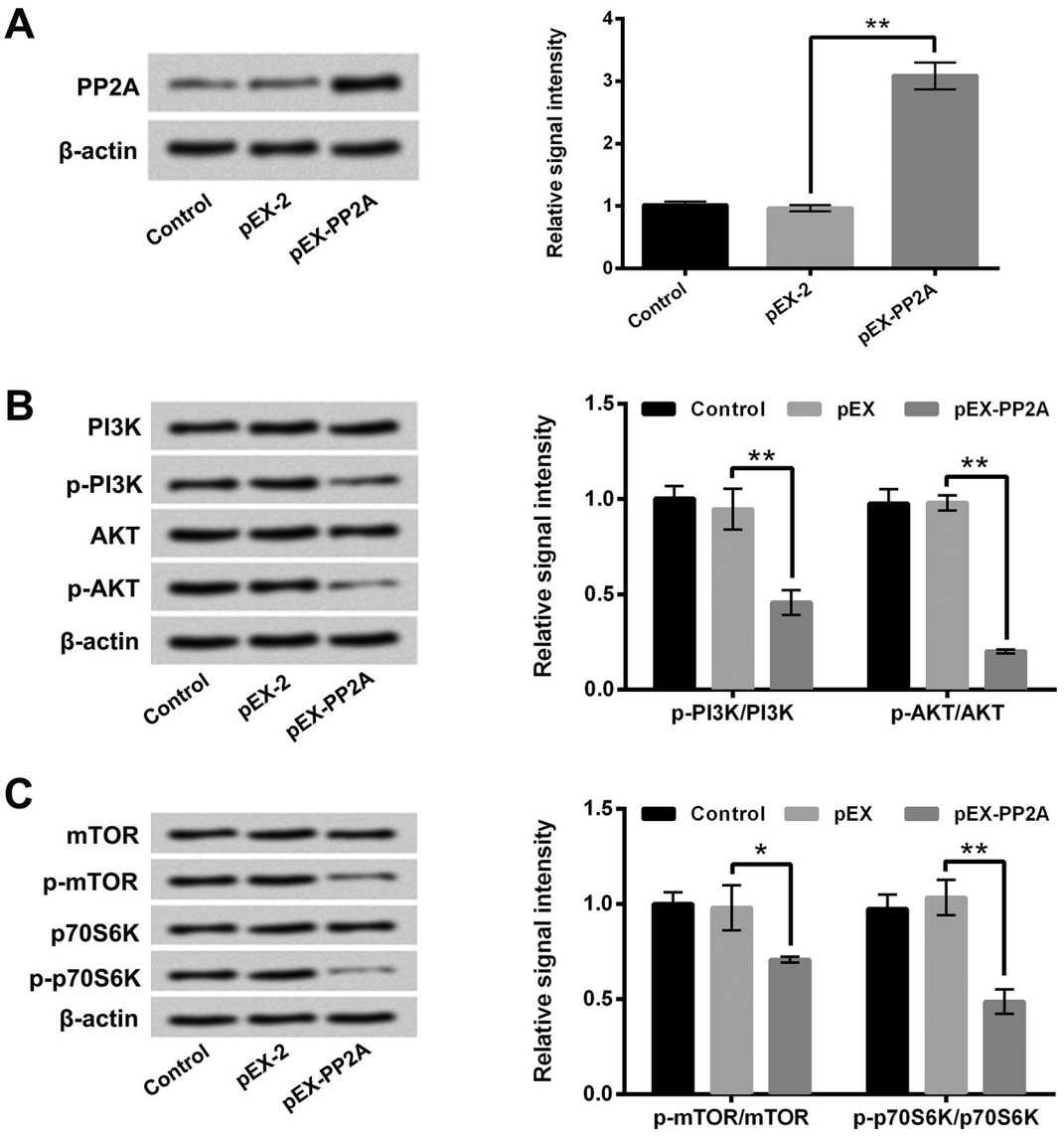

Fig. 7. Overexpression of PP2A inactivated PI3K/AKT/mTOR signaling pathway in A549 cells.
(A) After pEX-2 or pEX-PP2A transfection, the protein expression of PP2A in A549 cells was detected using western blotting. (B–C) After pEX-2 or pEX-PP2A transfection, the protein expressions of PI3K, p-PI3K, AKT, p-AKT, mTOR, p-mTOR, p70S6K and p-p70S6K in A549 cells were analyzed using western blotting. PP2A: Protein phosphatase 2A; PI3K: Phosphatidylinositol 3-kinase; AKT: Protein kinase 3; mTOR: Mechanistic target of rapamycin. *$P < .05$; **$P < .01$.

alleviated the skullcapflavone I-induced PP2A expression increase in A549 cells ($P < .05$). These findings implied that PP2A might be involved in the effects of skullcapflavone I and miR-21 on A549 cells.

3.5. Overexpression of PP2A inactivated PI3K/AKT/mTOR signaling pathway in A549 cells

Further experiments were carried out to explore the effects of skullcapflavone I and PP2A on PI3K/AKT/mTOR signaling pathway in A549 cells. Fig. 6A and B displayed that 20 μg/ml skullcapflavone I treatment dramatically inactivated the PI3K/AKT/mTOR signaling pathway in A549 cells through declining the p-PI3K/PI3K, p-AKT/AKT, p-mTOR/mTOR and p-p70S6K/p70S6K expression rates ($P < .01$). pEX-PP2A was transfected to elevate the expression of PP2A in A549 cells (Fig. 7A, $P < .01$). Fig. 7B and C showed that the expression rates of p-PI3K/PI3K, p-AKT/AKT, p-mTOR/mTOR and p-p70S6K/p70S6K in A549 cells were all obviously decreased after pEX-PP2A transfection ($P < .05$ or $P < .01$). These above findings implied that skullcapflavone I could inactivate PI3K/AKT/mTOR signaling pathway in A549 cells via up-regulating PP2A.

3.6. Skullcapflavone I had no significant influence on human bronchial epithelial 16HBE cell viability, proliferation and apoptosis

Finally, the influences of skullcapflavone I on human bronchial epithelial 16HBE cell viability, proliferation and apoptosis were evaluated. Results in Fig. 8A showed that 20 μg/ml skullcapflavone I treatment had no significant effect on 16HBE cell viability. The PCNA and Cyclin D1 protein levels in 16HBE cells were both not changed after 20 μg/ml skullcapflavone I treatment (Fig. 8B). Besides, Fig. 8C displayed that skullcapflavone I incubation also had no obvious influence on 16HBE cell apoptosis, which accompanied with the Bcl-2, Bax, Caspase 3 and Caspase 9 protein levels were not changed (Fig. 8D). These findings suggested that skullcapflavone I had no significant influence on human bronchial epithelial 16HBE cell viability, proliferation and apoptosis.

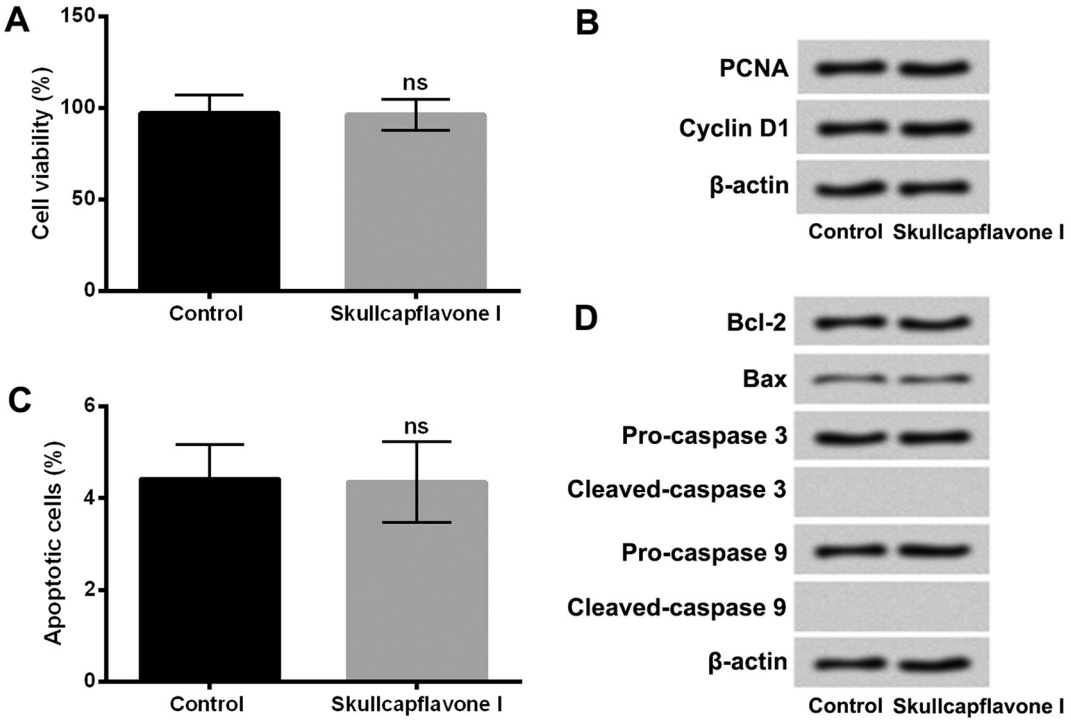

Fig. 8. Skullcapflavone I had no significant influence on human bronchial epithelial 16HBE cell viability, proliferation and apoptosis.
After 20 μg/ml skullcapflavone I treatment, (A) viability of 16HBE cells was detected using cell counting kit 8 (CCK-8) assay, (B) the PCNA and Cyclin D1 protein levels in 16HBE cells were tested by western blotting, (C) apoptosis of 16HBE cells was assessed by Annexin V-FITC Apoptosis Detection kit and (D) the Bcl-2, Bax, Caspase 3 and Caspase 9 protein levels in 16HBE cells were tested by western blotting. PCNA: Proliferation cell nuclear antigen. Ns: no significant.

4. Discussion

Scutellaria baicalensis is a well-known medicinal plant in China, Japan and Korea (Kudo et al., 2017). In this research, we found that skullcapflavone I, a flavone compound extracted from the roots of *Scutellaria baicalensis*, exerted anti-cancer effects on lung cancer A549 and H1975 cells by suppressing cell viability and proliferation. Further results revealed that the expressions of miR-21 in A549 and H1975 cells were distinctly decreased after skullcapflavone I treatment. Overexpression of miR-21 remarkably alleviated the skullcapflavone I-induced A549 cell viability inhibition and PP2A expression increase. In addition, both skullcapflavone I treatment and PP2A overexpression inactivated PI3K/AKT/mTOR signaling pathway in A549 cells.

Abnormal proliferation is one of the most important characteristics of cancers cells (Reya et al., 2001). Therefore, suppressing cancer cell proliferation is considered as an effective method for cancer therapy (Kishimoto et al., 2014). Plant-derived medicines have made their own niche in the treatment of multiple cancers by inhibiting cancer cell proliferation (Fridlender et al., 2015; Pereira et al., 2012). In the present study, we indicated that skullcapflavone I obviously suppressed the proliferation of lung cancer A549 and H1975 cells. The expressions of PCNA and Cyclin D1, which play critical regulatory roles in cancer cell proliferation (Perri et al., 2014; Wang, 2014), were both decreased after skullcapflavone I treatment. Considering that skullcapflavone I had been demonstrated to exert pro-apoptotic effects on activated rat hepatic stellate cells (Park et al., 2005), the findings of our research further suggested that skullcapflavone I could exert anti-cancer effects on various cancer cells.

As small non-coding RNAs in eukaryotic cells, miRNAs can serve as gene regulators capable of altering multiple genes expressions by targeting the 3′ untranslated regions (3′UTR) of the genes (Lee, 2013). A series of plant-derived medicines can exert anti-cancer effects by modulating the expressions of miRNAs (Bae et al., 2011; Mohammadi et al., 2017). miR-21 plays an important anti-apoptotic role in a variety of cancer cells (Dillhoff et al., 2008; Pfeffer et al., 2015). In this research, we found that the expressions of miR-21 in A549 and H1975 cells were dramatically decreased after skullcapflavone I treatment and overexpression of miR-21 significantly reversed the skullcapflavone I-induced A549 cell viability inhibition. These findings suggested that skullcapflavone I inhibited A549 and H1975 cell viability by down-regulating miR-21 and implied that skullcapflavone I exerted anti-cancer effects at least in part by regulating miRNAs expressions in cancer cells.

PP2A is a member of serine/threonine-specific phosphatases family and acts as a tumor suppressor in cells, which possesses very important regulatory activities on multiple cell functions, such as cell differentiation, cell proliferation, cell invasion and cell apoptosis (Chen et al., 2014; Gilan et al., 2015; Gutierrez et al., 2014). Saddoughi et al. (Saddoughi et al., 2013) demonstrated that sphingosine analogue drug FTY720 suppressed lung cancer growth via activating PP2A-receptor-interacting protein kinase 1 (RIPK1)-dependent programmed necrosis. Liu et al. (Liu et al., 2015) indicated that overexpression of SET oncoprotein, an endogenous inhibitor of PP2A, also known as 12PP2A, was associated with tumor progression and poor prognosis in human non-small cell lung cancer. In this study, we revealed that skullcapflavone I significantly up-regulated the expression of PP2A in A549 cells. While miR-21 mimic transfection dramatically attenuated the skullcapflavone I-induced PP2A expression increase. These results suggested that PP2A participated in the effects of skullcapflavone I and miR-21 on lung cancer A549 cells.

The activation of PI3K/AKT/mTOR signaling pathway has been demonstrated in numerous cancer cells, including lung cancer (Fumarola et al., 2014). Switzer et al. (2009) proved that dithiolethione compounds suppressed PI3K/AKT signaling pathway in human lung cancer cells by enhancing PP2A expression. Meng et al. (2015) reported that triptolide co-treatment with hydroxycamptothecin synergistically increased lung cancer A549 cell apoptosis by PP2A-regulated AKT pathway. The results in our research illustrated that both

skullcapflavone I treatment and PP2A overexpression significantly inactivated PI3K/AKT/mTOR signaling pathway in lung cancer A549 cells and implied that skullcapflavone I exerted anti-cancer effect on lung cancer might be through down-regulating miR-21 expression, up-regulating PP2A expression and then inactivating PI3K/AKT/mTOR signaling pathway.

Finally, we also assessed the influence of skullcapflavone I treatment on human bronchial epithelial 16HBE cell viability, proliferation and apoptosis. We discovered that skullcapflavone I treatment had no significantly influences on 16HBE cell viability, proliferation and apoptosis, which suggested that skullcapflavone I could selectively suppress lung cancer cell viability and proliferation, but had no effect on normal lung cells.

To sum up, our research verified that skullcapflavone I exerted anti-cancer effect on lung cancer A549 cells might be through down-regulating miR-21 expression, up-regulating PP2A expression and then inactivating PI3K/AKT/mTOR signaling pathway. We propose that skullcapflavone I could be an efficient therapy medicine for lung cancer. However, further in vitro and in vivo studies are still needed to support this proposal.

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Declaration of Competing Interest

Authors declare that there is no conflict of interests.

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