Britannin inhibits cell proliferation, migration and glycolysis by downregulating KLF5 in lung cancer

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Abstract. Lung cancer is a harmful type of malignancy and the leading cause of cancer-associated mortality. It is therefore imperative to develop novel drugs effective for treating this cancer. The Traditional Chinese Medicine compound Britannin has been previously reported to inhibit the development of certain cancers, such as pancreatic, breast and liver cancer. Moreover, Kruppel-like factor 5 (KLF5) has been identified an on oncogene in lung cancer. In the present study, the possible regulatory effects and underlying mechanism of Britannin in lung cancer were investigated. A549 and 16HBE cells were treated with different concentrations of Britannin. Subsequently, Cell counting kit-8, EdU staining and colony formation assays were used to detect the proliferative ability of these cells. Cell migration was detected by wound healing and Transwell assays, respectively. XF96 extracellular flux analyzer was used to analyze the extent of extracellular acidification and oxygen consumption rate in cells, whereas assay kits were used to detect glucose and lactic acid levels in the cell supernatant. The targeting effect between Britannin and the KLF5 protein was investigated using molecular docking technology. The protein expression levels of KLF5 in cells challenged with Britannin was detected by western blotting. Finally, overexpression of KLF5 in A549 cells was performed before cell proliferation, migration and the glycolysis rate were measured to explore the regulatory effects of Britannin. Britannin was found to inhibit the proliferation, migration and glycolysis of lung cancer cells, during which the protein expression levels of KLF5 were decreased. This suggests that Britannin regulated the expression of KLF5 in A549 cells. Overexpression of KLF5 reversed the inhibitory effects of Britannin on the proliferation, migration and glycolysis in lung cancer cells. In conclusion, these results suggest that Britannin can inhibit cell proliferation, migration and glycolysis by downregulating KLF5 expression in lung cancer cells.

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Introduction

Lung cancer refers to a malignant tumor originating from the bronchial mucosal epithelium (1). Lung cancer is one of the most common and fastest-growing type of malignant tumor at present (2). Currently, it is the main cause of human cancer deaths, accounting for ~30% of all cancer deaths worldwide (3). Radiotherapy and chemotherapy remain effective treatments for early, middle and advanced stages of lung cancer (4). The 5-year survival rate of patients with lung cancer after radiotherapy and chemotherapy is 15% (5). In the treatment of patients with lung cancer, standard platinum-based doublet therapy, which is the traditional chemotherapy regimen, has not made a breakthrough in the improvement of the survival rate (6). In addition, there can be serious adverse reactions, including cutaneous adverse reactions and diabetic ketoacidosis, to certain drugs, including poziotinib and pembrolizumab (7,8). Therefore, the development of alternative effective treatments is an important topic in lung cancer research.

Glycolysis, also known as Warburg effect, refers to the transformation of glucose into lactate in cancer cells under the aerobic conditions (9). Increasing studies have unveiled the significance of glycolysis in tumor progression of lung cancer (10,11).

Traditional Chinese Medicine (TCM) combined with chemotherapy and radiotherapy forms an important part of the lung cancer treatment system in China (12). Increasing numbers of TCM preparations, including Liqi, *Ganoderma lucidum* and *Prunella vulgaris* have been reported to exert anticancer effects with few side effects. Nowadays, TCM has also been extensively applied to the clinical treatment of lung cancer and TCM has great potential to provide candidate drugs for the disease (13,14). Britannin compound is a key bioactive component from the Chinese herb *Inula*, which has traditionally been used for the effects of eliminating phlegm and being an anti-emetic. Britannin has been reported to reduce the activity of the acute lymphoblastic leukemia cell

line MOLT-4 by decreasing the proliferative capacity of cells whilst promoting cell cycle arrest (15). In addition, Britannin can inhibit the invasion and migration of 4T1 breast cancer cells, thereby inhibiting lung metastasis of these cells (16). However, to the best of our knowledge, a study on the effects of Britannin in lung cancer has not yet been reported.

Kruppel-like factor 5 (KLF5) is a transcription factor that is expressed in various tissues and functions as a pivotal regulator in cell proliferation, differentiation and survival (17). Research has revealed that abnormal expression of KLF5 is observed in a number of different types of cancer, such as breast, prostate and bladder cancer (17). Notably, increasing evidence has shown that KLF5 also exerts tumor-promoting activities in lung cancer (18-21).

Therefore, in the present study, the influence of Britannin on the proliferation, migration, and glycolysis of lung cancer cells were analyzed, before its mechanism of action was investigated.

Materials and methods

Molecular docking. The three-dimensional (3D) structure of the Kruppel-like factor 5 (KLF5) protein (PDB ID: 2EBT) was obtained using the PDB database (https://www.rcsb.org/). Thereafter, the KLF5 protein structure file was edited using the PyMOL software (version 2.2.0; Delano Scientific LLC) to remove the excess water molecules and delete small ligands so that only the protein structure remained. Since the downloaded protein structure contained ligands, the original ligands such as water molecules and other bound ligands were deleted from the structure and the original ligand positions were set as docking sites. The PubChem database (http://pubchem.ncbi.nlm.nih.gov) was used to obtain the 3D structure of Britannin, which was then imported into OpenBabel software (version 2.2.1; Open Babel development team) for hydrogenation and conversion into the 'mol2' file format (6). AutoDock software (version 4.2; Scripps Research Institute) was utilized to display the specific docking energy value. The position with the lowest binding energy (-6.3 kcal/mol) was selected for visualization. Finally, the results were analyzed using the Protein-Ligand Interaction Profiler (version 2.3.0; https://plip-tool.biotec.tu-dresden.de/plip-web).

Cell culture. Lung carcinoma cell A549 cells (cat. no. B211337) and bronchial epithelial 16HBE cells (cat. no. MZ-1420) purchased from Ningbo Mingzhou Biotechnology Co., Ltd. Cells were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) at 37˚C with 5% CO_2. Different concentrations of Britannin (5, 10 and 20 μM; purity, 99.90%; cat. no. HY-N3005; MedChemExpress) dissolved in DMSO were used to treat A549 or 16HBE cells for 24 h at 37˚C and untreated cells were used as negative controls.

Cell counting kit-8 (CCK-8) assay. A549 cells were seeded into 96-well plates ($8x10^4$ cells/well) for 24 h at 37˚C. Different concentrations of Britannin (5, 10 and 20 μM) were then added to the cells and incubated for 24 h at 37˚C. Subsequently, 10 μl CCK-8 reagent (Beyotime Institute of Biotechnology) was added for 3 h of incubation after the removal of the original culture medium. The optical density of the samples were measured at 450 nm using a plate reader (Infinite® 200 PRO; Tecan Group, Ltd.).

EdU staining. Cell proliferation capacity was determined by 5-ethynyl-2'-deoxyuridine (EdU) staining (Beyotime Institute of Biotechnology). A549 cells were seeded into 12-well chambers, treated with the aforementioned concentrations of Britannin and incubated for 24 h at 37˚C. The cells were then incubated with 200 μl EdU (500 μM) at 37˚C for 2 h. Next, cells were fixed with 4% paraformaldehyde for 15 min at room temperature and permeabilized with 0.3% Triton X-100 for 10 min. Cells were then labeled with 2 μg/ml Hoechst 33342 (Beyotime Institute of Biotechnology) for 30 min at room temperature. EdU-positive cells were imaged using a fluorescence microscope (Leica Microsystems GmbH).

Colony formation assay. A549 cells were seeded into 6-well plates ($5x10^3$ cells/well) before the aforementioned concentrations of Britannin were added to the cells. The culture medium was replaced every 3 days. After 14 days at 37˚C, the cells were washed with PBS, fixed in 4% paraformaldehyde at room temperature for 15 min and then stained with 0.05% crystal violet at room temperature for 20 min to visualize cell colonies under a BX53 light microscope (Olympus Corporation). Colonies (≥50 cells) were counted with Image J software v.1.52 (National Institutes of Health).

Wound healing assay. A549 cells were seeded into 6-well plates ($5x10^5$ cells/well) before the aforementioned concentrations of Britannin were added to the cells. Subsequently, the samples were scratched using a 200-μl pipette tip. Following removal of the floating cells using PBS, the cells were cultured in serum-free DMEM containing the aforementioned treatments. Cells were imaged at 0 and 24 h under a light microscope. ImageJ software v.1.52 (National Institutes of Health) was used to measure the scratch areas. The recovery rate of the wound was calculated using the following equation: [(Width at 0 h-width at 24 h)/width at 0 h] x100.

Transwell migration assay. A549 cells at a density of $3x10^4$/well in serum-free medium were added to the upper Transwell chamber (8-μm pore diameter; Merck KGaA) whilst the bottom chamber was filled with complete DMEM containing 7% FBS. The cells were the incubated for 24 h at 37˚C before cells on the upper side of the filter membrane were removed using a cotton swab. Cells that passed through the membrane were fixed with 70% methanol for 15 min at room temperature, stained with 0.1% crystal violet at room temperature for 30 min and imaged using a light microscope (Olympus Corporation). Cell migration rates were determined using ImageJ software (version 1.48; National Institutes of Health).

Western blotting. A549 cells ($1x10^5$ cells/well) were seeded into 6-well plates. After the cells were treated with the aforementioned concentrations of Britannin, the proteins were extracted using RIPA buffer (Beyotime Institute of Biotechnology). The supernatant was used to determine protein concentration by bicinchoninic acid

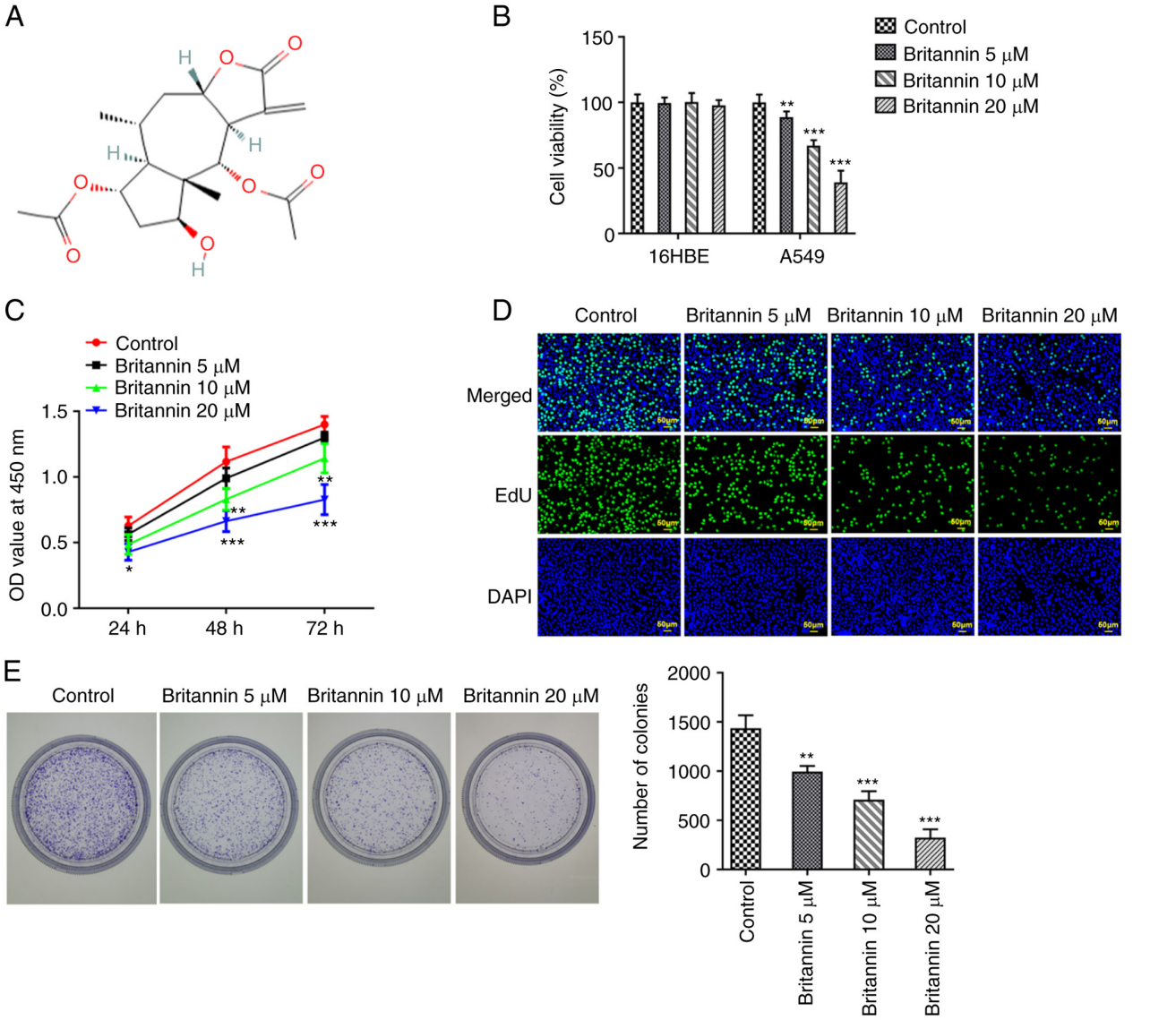

Figure 1. Britannin inhibits the proliferation of A549 lung cancer cells. (A) The chemical structure of Britannin. Cell counting kit-8 assays were used to detect (B) viability and (C) proliferation of Britannin-treated cells. Proliferation of Britannin-treated cells was measured using (D) EdU staining and (E) colony formation assays. *P<0.05, **P<0.01 and ***P<0.001 vs. Control.

protein concentration determination kit (cat. no. RTP7102; Real-Times (Beijing) Biotechnology Co., Ltd.). Proteins (6 μg/lane) were subjected to SDS-PAGE on 10% gels and then transferred onto a PVDF membrane (0.45 μm; MilliporeSigma). Membranes were blocked with 5% BSA (Beyotime Institute of Biotechnology) for 2 h at room temperature and incubated with primary antibodies overnight at 4˚C, followed by incubation with secondary antibodies for 1 h at room temperature, specifically Goat Anti-Rabbit IgG H&L (HRP; 1:2,000; cat. no. ab6721; Abcam). Primary antibodies used were as follows: E-cadherin (1:1,000; cat. no. ab227639; Abcam); N-cadherin (1:1,000; cat. no. ab76011; Abcam); Snail (1:1,000; cat. no. ab216347; Abcam); KLF5 (1:1,000; cat. no. ab137676; Abcam); and GAPDH (1:1,000; cat. no. ab181602; Abcam). Bands were visualized using ECL Western Blotting Detection Reagent (cat. no. RPN2106; GE Healthcare) and ImageJ software (version 1.8.0; National Institutes of Health) was used for the semi-quantification of protein expression levels.

RNA isolation and reverse transcriptase-quantitative PCR (RT-qPCR). Total RNA was extracted from Britannin-treated 16HBE cells or A549 cells subjected to Britannin treatment and transfection of Oe-KLF5 or Oe-NC using the RNeasy Kit® and DNase Set (DNAse H; Qiagen China Co., Ltd.) assay kits according to the manufacturer's protocols. RNA concentration was measured using a NanoDrop photometer (NanoDrop Technologies; Thermo Fisher Scientific, Inc.). A total of 1 μg RNA was reverse-transcribed to cDNA using the iScript™ RT kit (Bio-Rad Laboratories, Inc.). The temperature protocol was 15 min at 37˚C, 5 sec at 85˚C and 30 min at 4˚C. Amplification of cDNA was the performed using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.) with the iQ SYBR® Green Supermix (Bio-Rad Laboratories, Inc.) according to the manufacturer's instructions. The thermal cycling conditions used were as follows: Initial denaturation at 95˚C for 5 min; followed by 40 cycles of 95˚C for 10 sec, 57-60.5˚C for 10 sec and 72˚C for 30 sec. GAPDH served as the internal control gene for normalization and the expression

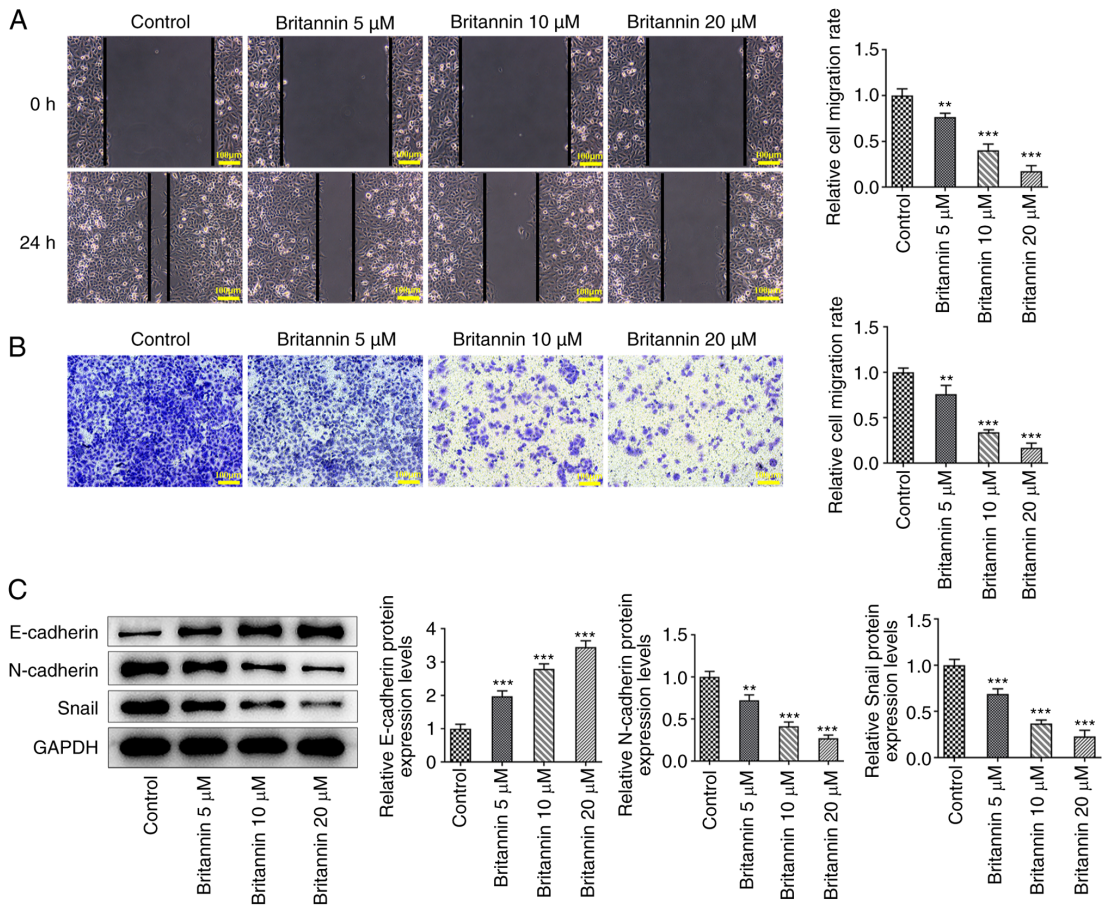

Figure 2. Britannin inhibits the migration of lung cancer cells. Migratory abilities of Britannin-treated A549 cells were detected by (A) wound healing assays and (B) Transwell assays. A549 cells were treated with Britannin and the (C) protein expression levels of E-cadherin, N-cadherin and Snail were detected by western blotting. **P<0.01 and ***P<0.001 vs. Control.

levels were quantified using the $2^{-\Delta\Delta Cq}$ method (22). The PCR primers used were as follows: KLF5 forward (F), 5'-AGC TACAATACGCTTGGCCT-3' and reverse (R). 5'-ATGTGT GTTACGCACGGTCT-3' and GAPDH F, 5'-AATGGGCAG CCGTTAGGAAA-3' and R, 5'-GCGCCCAATACGACC AAATC-3'.

Detection of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). A Seahorse Bioscience XF96 Extracellular Flux Analyzer (Agilent Technologies, Inc.) was used to detect the cellular ECAR and OCR. In brief, A549 cells ($2x10^4$ cells/well) subjected to Britannin treatment and transfection of Oe-KLF5 or Oe-NC were seeded into 96-well cell culture XF microplates and incubated overnight at 37˚C for further testing according to the manufacturer's instructions.

Lactate production and glucose uptake assay. A549 cells subjected to Britannin treatment and transfection of Oe-KLF5 or Oe-NC were seeded into a 24-well plate ($2x10^5$ cells/ml). Aliquots of media from each well were assessed for the concentration of lactate present using a lactate test kit (cat. no. A019-2; Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions. For glucose uptake assay, A549 cells were cultured under normoxic conditions in glucose-free DMEM (Gibco; Thermo Fisher Scientific, Inc.) for 16 h at 37˚C and were then incubated with 25 mmol/l high-glucose DMEM (Gibco;

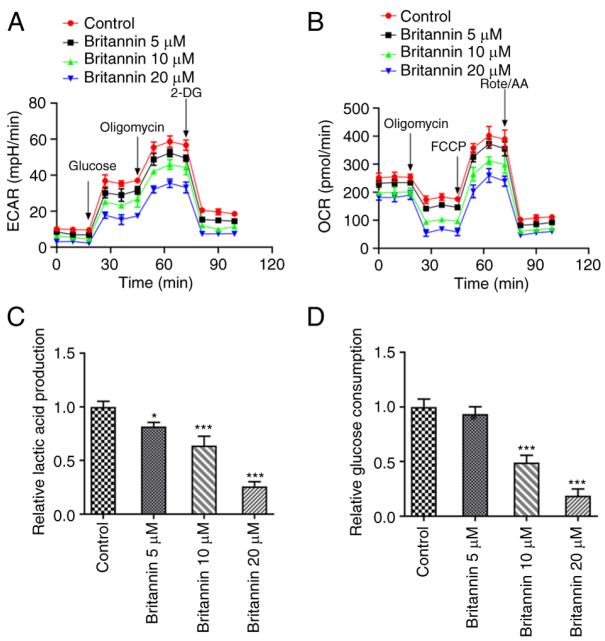

Figure 3. Britannin inhibits the glycolysis of lung cancer cells. The XF96 extracellular flux analyzer was used to detect the (A) ECAR and (B) OCR in Britannin-treated A549 cells. The (C) lactic acid production and (D) glucose consumption levels in the cell supernatant were measured using the respective assay kits. *P<0.05 and ***P<0.001 vs. Control. ECAR, extracellular acidification rate; OCR, oxygen consumption rate; 2-DG, 2-deoxy-D-glucose; Rote, rotenone; AA, antimycin A; mpH/min, milli-pH.

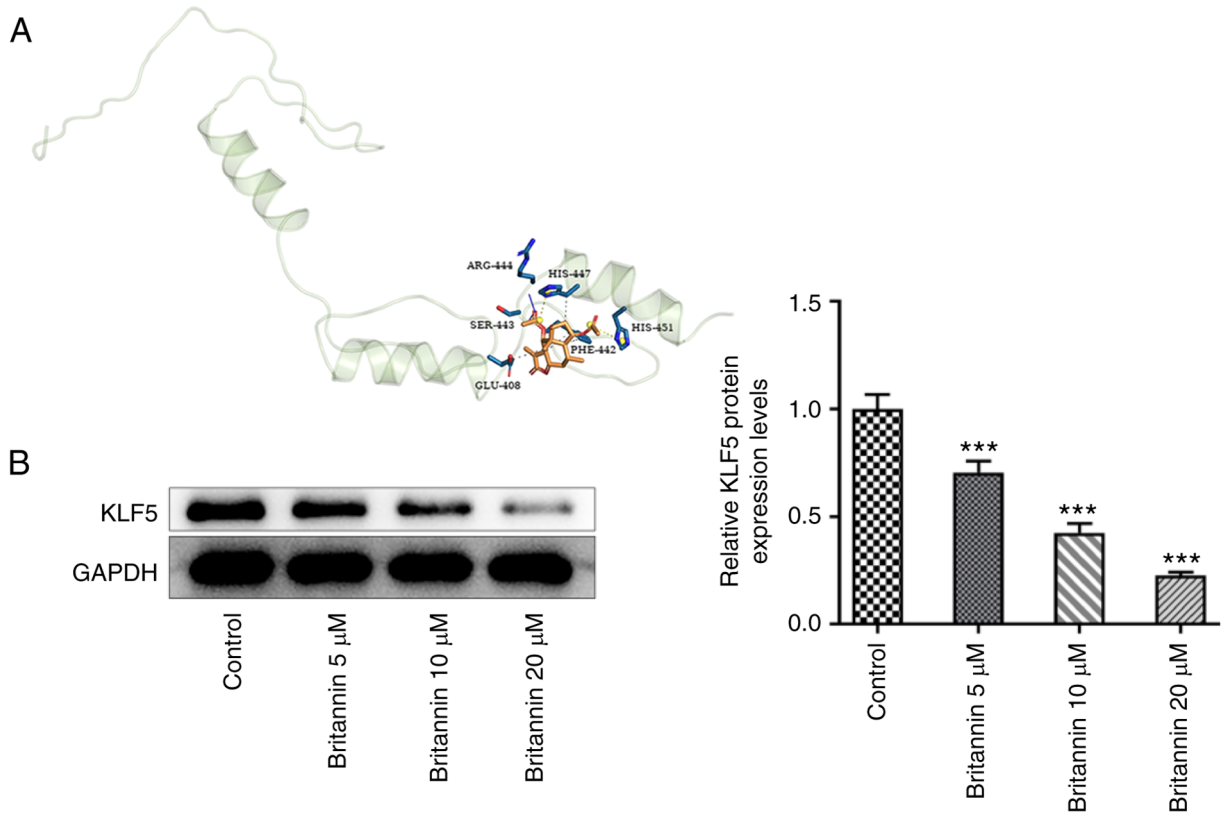

Figure 4. Britannin downregulates the expression level of KLF5. (A) Molecular docking was used to study the interaction between Britannin and KLF5. (B) A549 treated with Britannin were analyzed by western blotting to determine the protein expression levels of KLF5. ***P<0.001 vs. Control. KLF5, Kruppel-like factor 5.

Thermo Fisher Scientific, Inc.) for 24 h at 37˚C. Then, the intracellular glucose levels were measured using a fluorescence-based glucose assay kit (cat. no. ab136956; BioVision, Inc.) according to the manufacturer's instructions. Values were normalized accordingly to calculate lactic acid production and glucose consumption.

Cell transfection. Cells were transfected with pcDNA3.1 containing KLF5 (Oe-KLF5; GeneChem, Inc.) and negative control plasmid (Oe-NC; GeneChem, Inc.) using the Lipofectamine® 2000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.) at final concentration of 50 nM according to the manufacturer's instructions. After 48 h at 37˚C, RT-qPCR and western blotting were used to evaluate the transfection efficiency. Further experiments were performed after 48 h.

Statistical analysis. All data were expressed as mean ± standard deviation. One-way analysis of variance with Tukey's post hoc test was performed for multiple comparisons using GraphPad Prism software (version 5; Dotmatics). A two-tailed unpaired Student's t-test was applied for comparisons between two groups. P<0.05 was considered to indicate a statistically significant difference. Each experiment was conducted at least three times.

Results

Britannin inhibits the proliferation of lung cancer cells. The chemical formula for Britannin was shown in Fig. 1A. Different concentrations of Britannin were used to treat 16HBE and A549 cells for 24 h, then a CCK-8 assay was used to detect whether Britannin had an effect on cell viability. The results demonstrated that Britannin was not toxic to 16HBE cells, but the viability of A549 cells was significantly decreased with the increase of Britannin concentrations compared with that in the control group (Fig. 1B). Considering that Britannin exerted no effect on the viability of 16HBE cells, A549 cells whose viability was greatly diminished by Britannin were selected for the ensuing experiments to further work out the role of Britannin in lung cancer. A549 cells were then treated with different concentrations of Britannin for 24, 48 and 72 h before cell proliferation was analyzed using CCK-8 assay. The results demonstrated that with the increase of Britannin treatment time, the inhibitory ability of Britannin on the viability of A549 cells was significantly enhanced compared with that in the untreated cells (Fig. 1C). EdU staining and colony formation assay were next performed to measure the extent of cell proliferation, where it was demonstrated that the proliferation of A549 cells treated with Britannin was markedly decreased in a dose-dependent manner compared with that in the control group (Fig. 1D and E).

Britannin inhibits the migration of lung cancer cells. The migratory ability of A549 cells was subsequently detected by wound healing and Transwell assays. It was demonstrated that the migratory ability of cells was significantly decreased with increasing concentrations of Britannin treatment (Fig. 2A and B). The expression of epithelial-mesenchymal

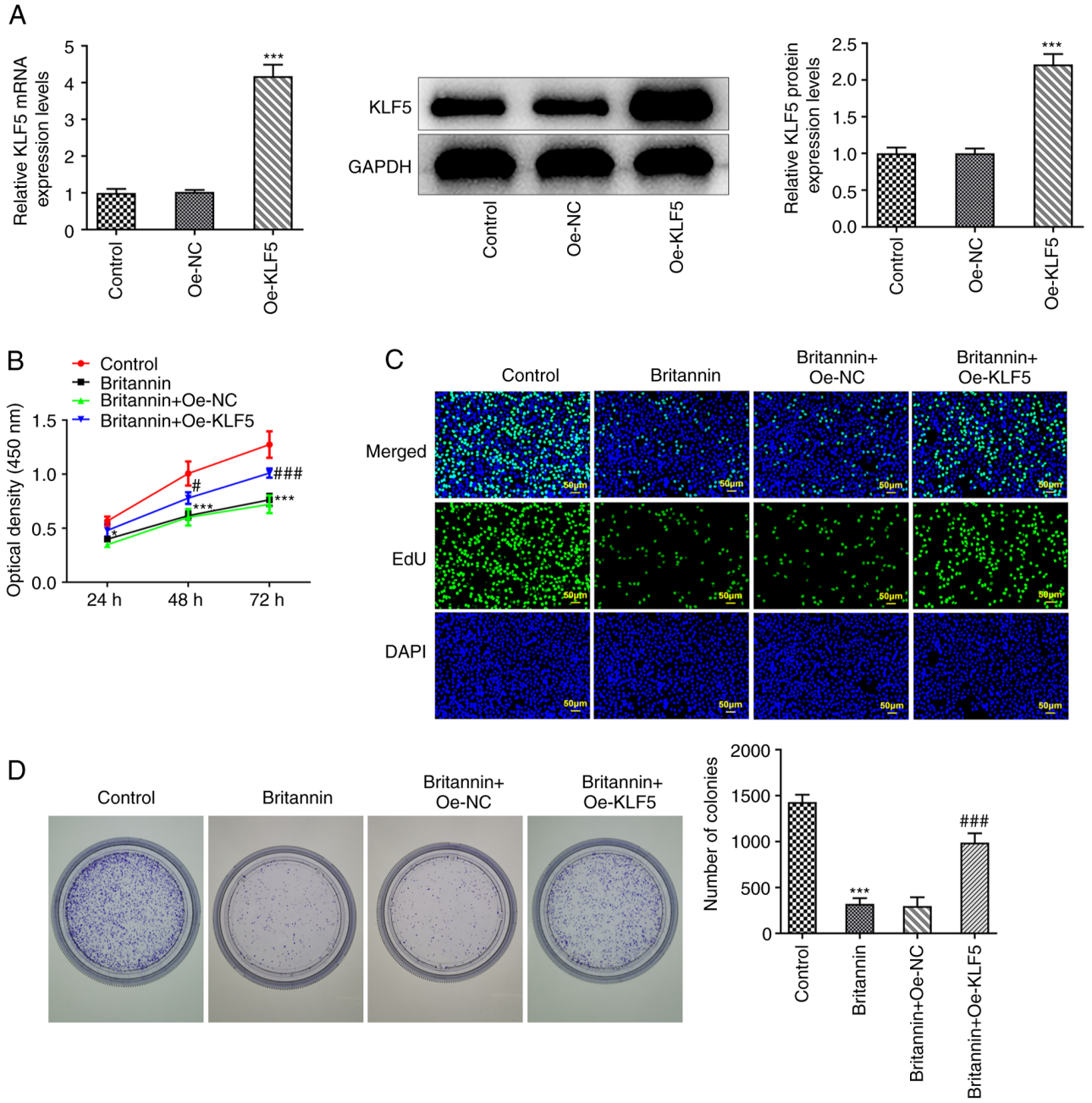

Figure 5. Overexpression of KLF5 reverses the inhibitory effects of Britannin on the proliferation of lung cancer cells. (A) Successful transfection of A549 cells with Oe-KLF5 was detected by reverse transcription-quantitative PCR and western blotting. (B) Cell proliferation was detected by cell counting kit-8 assay. Cell proliferation was measured using (C) EdU staining and (D) colony formation assays. *P<0.05 and ***P<0.001 vs. Control; $^{\#}$P<0.05 and $^{\#\#\#}$P<0.001 vs. Britannin + Oe-NC. Oe, overexpression; NC, negative control; KLF5, Kruppel-like factor 5.

transition (EMT)-related proteins E-cadherin, N-cadherin and Snail was then detected by western blotting. Compared with those in the control group, the protein expression levels of E-cadherin were increased significantly in Britannin-treated cells, whilst those of N-cadherin and Snail were decreased significantly (Fig. 2C).

Britannin inhibits glycolysis in lung cancer cells. The XF96 extracellular flux analyzer was used to detect ECAR and OCR in Britannin-treated cells. These results demonstrated that compared with those in the control group, ECAR and OCR levels in the Britannin-treated groups were decreased in a dose-dependent manner (Fig. 3A and B). The lactic acid production and glucose consumption levels in the cell supernatant were next measured using assay kits, where the results demonstrated that with the increase in the treatment dose of Britannin, the lactic acid and glucose consumption levels were decreased in a dose-dependent manner (Fig. 3C and D). Compared with those in the untreated cells, lactic acid production was significantly decreased by all doses of Britannin treatment whereas the glucose consumption level was significantly decreased by 10 and 20 μM of Britannin.

Britannin downregulates the protein expression level of KLF5. KLF5 has been increasingly identified as an oncogene in lung cancer. A molecular docking strategy was used to study the

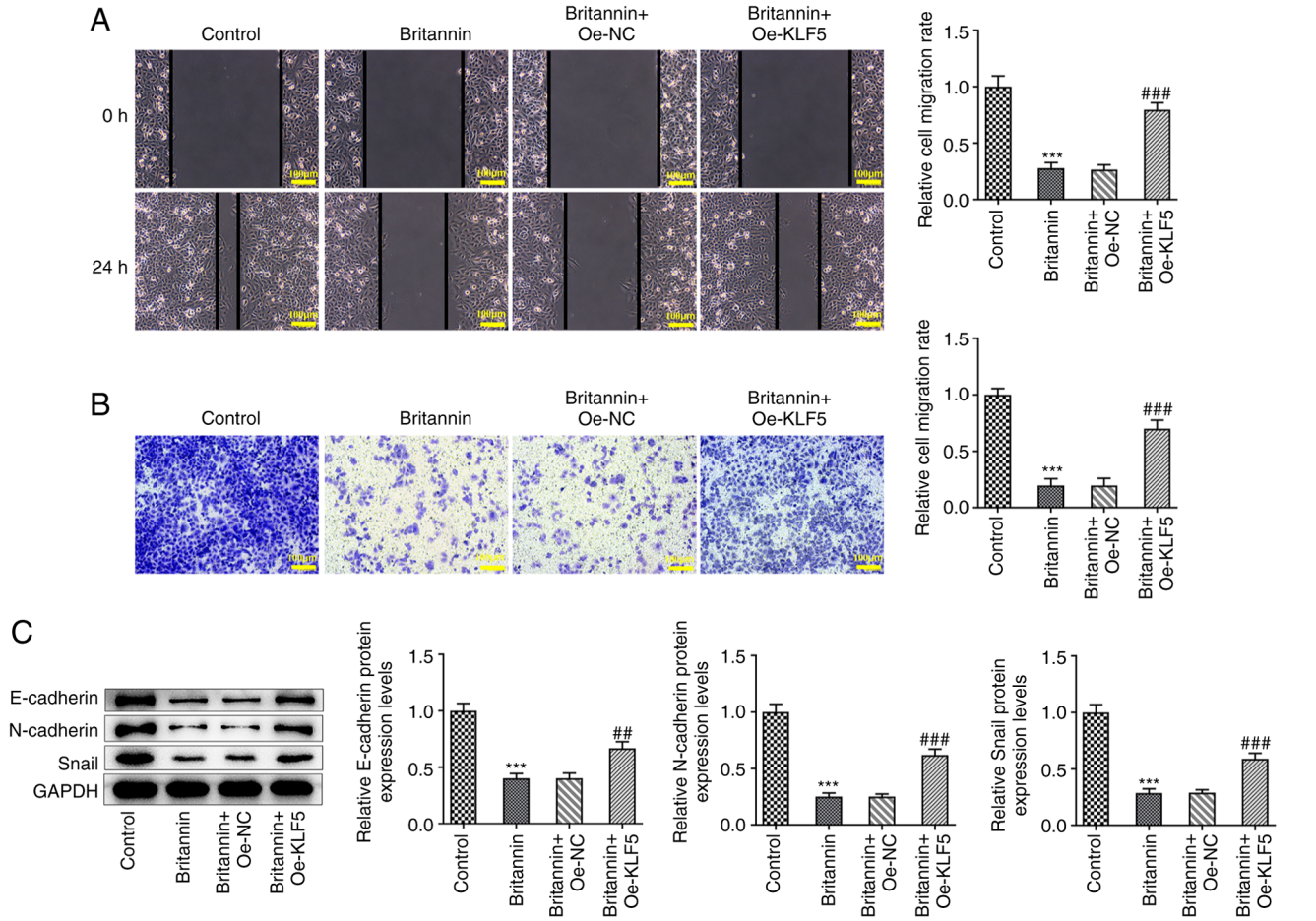

Figure 6. Overexpression of KLF5 reverses the inhibitory effects of Britannin on the migration of lung cancer cells. Migratory abilities of cells were detected by (A) wound healing and (B) Transwell assays. A549 cells were treated with Britannin and the (C) protein expression levels of E-cadherin, N-cadherin and Snail were detected by western blotting. ***P<0.001 vs. Control; $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 vs Britannin + Oe-NC. Oe, overexpression; NC, negative control; KLF5, Kruppel-like factor 5.

interaction between ligand and receptor and to predict the binding mode and affinity between Britannin and KLF5. It was demonstrated that Britannin could target KLF5 (Fig. 4A). In addition, after Britannin treatment, the expression of the KLF5 protein in A549 cells was decreased significantly in a dose-dependent manner, compared with that in untreated cells (Fig. 4B).

Overexpression of KLF5 reverses the inhibitory effects of Britannin on the proliferation, migration and glycolysis of lung cancer cells. The regulatory mechanism of Britannin in A549 cells was then explored. A KLF5 overexpression plasmid was constructed and the successful transfection of Oe-KLF5 was verified by RT-qPCR and western blotting (Fig. 5A). The cells were then divided into the following treatment groups: i) Control; ii) Britannin; iii) Britannin + Oe-NC; and iv) Britannin + Oe-KLF5. CCK-8, EdU staining and colony formation assays were used to analyze the degree of cell proliferation and the results demonstrated that compared with that in the Britannin + Oe-NC group, cell proliferation was found to be markedly increased in the Britannin + Oe-KLF5 group (Fig. 5B-D).

Wound healing and Transwell assay results demonstrated that the overexpression of KLF5 significantly reversed the inhibition of A549 cell migration caused by Britannin treatment (Fig. 6A and B). Western blotting results demonstrated that compared with those in the Britannin + Oe-NC group, the protein expression levels of E-cadherin, N-cadherin and Snail were significantly increased in the Britannin + Oe-KLF5 group (Fig. 6C).

The results of ECAR and OCR indices detected using the XF96 extracellular flux analyzer demonstrated that overexpression of KLF5 markedly reversed the inhibitory effect of Britannin on ECAR and OCR (Fig. 7A and B). In addition, lactate production and glucose consumption levels were significantly increased in the Britannin + Oe-KLF5 group compared with those in the Britannin + Oe-NC group (Fig. 7C and D). These results suggest that overexpression of KLF5 reversed the inhibitory effects of Britannin on the proliferation, migration and glycolysis of lung cancer cells.

Discussion

Britannin, which can be extracted from the plant *Inula japonica* Thunb. and has been used in TCM, has been previously reported to have anti-inflammatory, anti-oxidative stress, antitumor and organ protective effects (23). In the present study, Britannin was demonstrated to inhibit the viability and proliferation of A549 lung cancer cells. It was also demonstrated

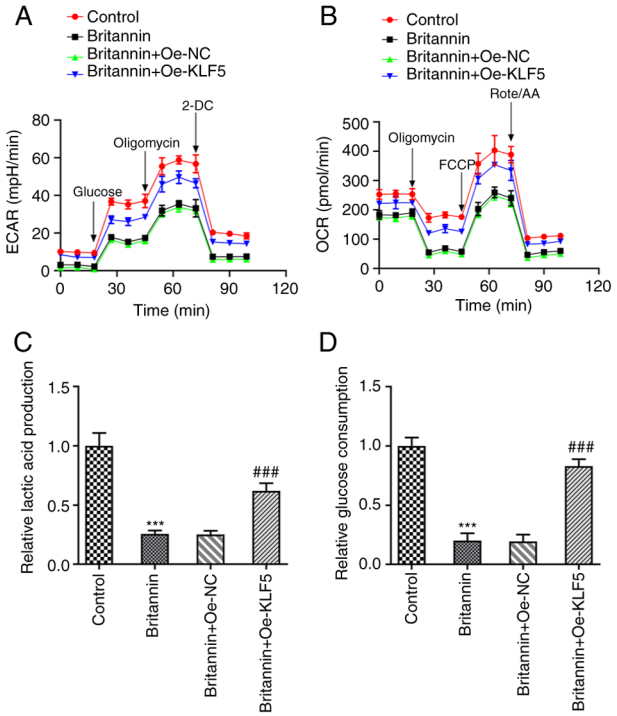

Figure 7. Overexpression of KLF5 reverses the inhibitory effects of Britannin on the glycolysis of lung cancer cells. The XF96 extracellular flux analyzer detected (A) ECAR and (B) OCR in Britannin-treated A549 cells. The (C) lactic acid production and (D) glucose consumption levels in the cell supernatant were measured using the respective assay kits. ***P<0.001 vs. Control; ###P<0.001 vs. Britannin + Oe-NC. Oe, overexpression; NC, negative control; KLF5, Kruppel-like factor 5; 2-DG, 2-deoxy-D-glucose; Rote, rotenone; AA, antimycin A; mpH/min, milli-pH.

that Britannin can significantly inhibit cell migration and the expression levels of EMT-related proteins. To the best of our knowledge, there have been no previous studies on the effects of Britannin on lung cancer, though Britannin reportedly has been demonstrated to exert therapeutic effects in other types of cancer. In particular, a previous study reported that Britannin inhibited the proliferation and migration of pancreatic cancer cells whilst inhibiting the NF-κB pathway, thereby inhibiting pancreatic cancer tumor growth (24). In liver cancer cells, Britannin has been found to induce apoptosis and autophagy through the activation of AMP-activated protein kinase regulated by reactive oxygen species, thereby inhibiting the growth and metastasis of liver cancer cells (25). These previous findings, together with the results of the present study, suggest that Britannin can serve an inhibitory role in the growth and migration of a number of tumor types.

It has previously been reported that KLF5 is expressed in paracancer and lung cancer tissues in patients with lung cancer. Specifically, the expression levels of KLF5 are abnormally elevated in lung cancer tissues, indicating that KLF5 is an oncogene in lung cancer (18). Furthermore, α-Catulin has been reported to promote cancer stemness by antagonizing the WW domain-containing E3 ubiquitin protein ligase 1-mediated degradation of KLF5 in lung cancer (19). Testis development-related 1 serves a carcinogenic role in non-small cell lung cancer by targeting the microRNA (miR)-214-5p/KLF5 axis (20). KLF5-induced γ-butyrobetaine hydroxylase 1-antisense RNA 1 has been found to upregulate maternal embryonic leucine zipper kinase expression to activate focal adhesion kinase (FAK) signaling, by sponging miR-27a-5p, contributing to the malignant phenotype of non-small cell lung cancer (21). These results suggest that KLF5 is an oncogene in lung cancer. The regulatory mechanism of Britannin was next explored. Through the use of molecular docking technology, it was demonstrated that Britannin targeted the KLF5 protein. Therefore, this may explain how Britannin inhibited the expression of KLF5 and the development of lung cancer in the present study. Overexpression of KLF5 in A549 cells in the present study significantly reversed the inhibitory effects of Britannin on the proliferation and migration of lung cancer cells.

Metabolic reprogramming is an important characteristic of tumor cells, which is mainly caused by increased glycolysis and decreased oxidative phosphorylation, in a process called 'aerobic glycolysis' or the 'Warburg effect' (9). Such metabolic changes have been reported in primary and metastatic cancers, and accelerates the various stages of tumor occurrence and development (26). The Warburg effect is considered a marker of advanced malignant tumors (27). A previous study reported that Britannin mediated the apoptosis and glycolysis of T cell lymphoblastic lymphoma cells through AMPK-dependent autophagy (28). In addition, KLF5 knockdown was found to inhibit hypoxia-induced cisplatin resistance by inhibiting hypoxia inedible factor-1α-dependent glycolysis in lung cancer cells (29). KLF5 can also directly bind to the phospholipase A and acyltransferase 3 promoter to activate its transcription, which promotes glycolysis in pancreatic cancer (30). Therefore, this suggests that KLF5 is associated with glycolysis, such that Britannin may serve a regulatory role in the glycolysis of lung cancer cells through KLF5. In the present study, Britannin was demonstrated to inhibit the ECAR and OCR in A549 cells, in addition to the level of lactic acid production and glucose consumption in the cell supernatant, suggesting that Britannin can inhibit the glycolysis of A549 lung cancer cells. Furthermore, overexpression of KLF5 reversed the inhibitory effect of Britannin on the glycolysis of lung cancer cells.

The present study has certain limitations. All the experiments were conducted in only one lung cancer cell line. Additional lung cancer cell lines will need to be used in future experiments. In addition, KLF5 expression in normal lung cells was not analyzed in the present study and the impact of Britannin on the protein expression levels of KLF5 in normal cells was not determined. Since this was not the focus of the present study, whether KLF5 is affected by Britannin in normal lung cells should be analyzed in future experiments.

To conclude, it was found in the present study that Britannin inhibited cell proliferation, metastasis and glycolysis by downregulating KLF5 expression in lung cancer cells. These findings offer a reference for the further investigation of the mechanism underlying the therapeutic effect of Britannin in lung cancer.

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Availability of data and materials

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

Authors' contributions

XW designed the experiments. YW, BY and MQ performed the experiments and wrote the article. FL and XW analyzed the experimental data and confirm the authenticity of all the raw data. All the authors agreed to the publication of the article. All authors read and approved the final version of the manuscript.

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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