Article

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# Solanine Mitigates Cisplatin Resistance in Lung Cancer via Modulation of the Beclin1-YAP1 Pathway

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**Background: Cisplatin resistance remains a significant challenge in the treatment of lung cancer, severely limiting its therapeutic efficacy. Solanine has shown potential in sensitizing resistant cancer cells to chemotherapy. The Beclin1-YAP1 signaling axis plays a pivotal role in autophagy regulation and drug resistance. Based on this pathway, the present study aimed to investigate the molecular mechanisms through which solanine overcomes cisplatin resistance in lung cancer.**

**Methods: A549/cisplatin (DDP) cells were divided into four groups: control, DDP, solanine, and DDP + solanine. Cell invasion and proliferation were evaluated using Transwell assays, scratch wound healing assays, and colony formation assays. The expression levels of Beclin1, Yes-associated protein 1 (YAP1), P-glycoprotein (P-gp), lung resistance-related protein (LRP), transcriptional coactivator with PDZ-binding motif (TAZ), phosphorylated signal transducer and activator of transcription 3 (p-STAT3), and mammalian target of rapamycin (mTOR) were analyzed by Western blotting. Additionally, Beclin1 overexpression was employed to validate the impact of solanine.**

**Results: The DDP, solanine, and DDP + solanine groups demonstrated a significant reduction in the number of invading cells and colonies, as well as a marked decrease in cell migration distance compared to the control group ($p < 0.05$). Additionally, the expression of proteins associated with DDP resistance was significantly downregulated. The DDP + solanine group showed a substantial decrease in colony formation, invasion, and cell migration distance compared to either the DDP or solanine groups alone. Additionally, overexpression of Beclin1 was found to attenuate the reversal effect of solanine.**

**Conclusion: Solanine enhances cisplatin sensitivity in A549/DDP cells *in vitro*, potentially through modulation of the Beclin1-YAP1 signaling pathway.**

**Keywords:** solanine; lung cancer; cisplatin; drug resistance; Beclin1-YAP1 pathway

## Introduction

Lung cancer is considered the most prevalent and fatal malignancy worldwide due to its high mortality rate and tendency to develop resistance to therapy. Accounting for a startling 18.4% of all cancer-related deaths, it remains the leading cause of cancer-related deaths globally [1]. Furthermore, according to the 2018 Global Cancer Statistics, lung cancer constitutes 11.6% of all newly diagnosed cancer cases [2]. Data from the American Cancer Society indicate that the 5-year relative survival rate for lung cancer is only 19%, underscoring the grim prognosis. This low survival rate is largely attributed to the aggressive nature of the disease and its frequent diagnosis at advanced stages. Additionally, metastasis, where cancer cells spread to distant organs, is observed in approximately 57% of lung cancer cases, further complicating treatment efforts [3].

Currently, chemotherapy remains the primary treatment for lung cancer, with cisplatin (DDP)-based combination regimens representing the standard first-line approach. However, resistance frequently develops during treatment for lung cancer, reducing the sensitivity of tumor cells to chemotherapeutic agents, diminishing the effectiveness of the drugs, and adversely affecting patient outcomes [4]. Overcoming resistance to platinum-based chemotherapeutics is crucial for enhancing therapeutic efficacy and extending patient survival. Platinum compounds such as cisplatin and carboplatin are widely used in the management of various cancers, including lung cancer. Therefore, identifying effective strategies to prevent or reverse platinum resistance is essential for improving the efficacy of chemotherapy, reducing recurrence rates, and ultimately prolonging the lives of lung cancer patients.

*Solanum nigrum* (black nightshade), a plant from the Solanaceae family, has been widely investigated for its therapeutic properties [5]. $\alpha$-solanine, a bioactive compound from this plant, enhances the expression of the growth arrest-specific transcript 5 (GAS5)/microRNA-18a (miR-18a) pathway in prostate cancer (PCA) cells, thereby suppressing proliferation and improving radiosensitivity [6]. In esophageal cancer cells, $\alpha$-solanine downregulates Survivin and upregulates miR-138 levels, enhancing sensitivity

to 5-fluorouracil or DDP [7]. Additionally, $\alpha$-solanine significantly enhances the inhibitory effects of daunorubicin in Jurkat leukemia cells, suggesting a role in enhancing doxorubicin sensitivity [8]. It also reverses multidrug resistance in leukemia cells by modulating the c-Jun N-terminal kinase (JNK) signaling pathway and suppressing the expression of multidrug resistance protein 1 (MRP1) [9].

This study aimed to investigate solanine's capacity to overcome DDP resistance in lung cancer. By assessing its effects on DDP-resistant lung cancer cell lines, this research aimed to identify the molecular mechanism through which solanine may enhance DDP sensitivity, offering novel insights into strategies to counteract chemotherapy resistance in lung cancer.

Autophagy is a conserved biological process crucial for maintaining cellular homeostasis and promoting survival under various stress conditions, including nutrient deprivation and metabolic stress [10,11]. Research indicates that autophagy plays a dual role in cancer, particularly in mediating resistance to chemotherapeutic agents. Chemotherapy can simultaneously induce apoptosis and autophagy in tumor cells, and the interplay between these processes often determines the final fate of the cell [12]. Beclin1, a central regulator of autophagy, functions as a core subunit of the phosphatidylinositol 3-phosphate (PI3P) complex, mediating the initiation of autophagy [13]. Recent studies have revealed that sirtuin 1 (SIRT1) enhances cisplatin resistance in bladder cancer by promoting Beclin1 deacetylation-dependent autophagy [14]. Moreover, inhibition of autophagy, particularly through targeting autophagy-related 5 (Atg5) or Beclin1, has been shown to sensitize A549 lung cancer cells to cisplatin-induced apoptosis [15].

Beclin1 has also been implicated in activating Yes-associated protein 1 (YAP1), contributing to the development of drug resistance [16]. YAP1 is essential in regulating carcinogenesis, tissue regeneration, organ size, and homeostasis. It contributes to therapy resistance by promoting growth factor signaling, inhibiting apoptosis, modulating DNA damage responses, promoting cell cycle progression, enhancing stem cell-like properties, and inducing epithelial-mesenchymal transition (EMT) [17]. According to recent research, YAP1 contributes to both acquired resistance to targeted molecular treatments and traditional chemotherapy. Inhibiting YAP1 has been proposed as a promising approach to overcoming such resistance [18]. In small-cell lung cancer (SCLC), YAP1 induces multidrug resistance by activating a cluster of differentiation (CD)-associated signaling pathways involving 74 connected signaling pathways [18]. The Beclin1-YAP1 signaling pathway thus represents a key regulatory axis in chemotherapy resistance.

Although solanine has been investigated in other cancer models, its potential role in overcoming cisplatin resistance in lung cancer, particularly through the Beclin1-YAP1 axis, remains unexplored. This study aimed to elucidate the impact of solanine on DDP resistance in lung cancer cells, with an emphasis on the molecular pathways mediated by the Beclin1-YAP1 signaling axis.

## Materials and Methods

### *Cell Culture*

A549 (CL-0016) and cisplatin-resistant A549/DDP (CL-0519) cell lines were obtained from Wuhan Procell Biotechnology Co., Ltd. (Wuhan, China). All cell lines were authenticated by short-tandem repeat (STR) profiling and confirmed to be mycoplasma-free. Cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (11879020, GIBCO BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS, A5670701, GIBCO BRL, Grand Island, NY, USA) and 1% penicillin-streptomycin (15140148, GIBCO BRL, Grand Island, NY, USA). To maintain cisplatin resistance, 1 µg/mL cisplatin (DDP, C2210000, Sigma-Aldrich, St. Louis, MO, USA) was added to the culture medium. Cells were incubated at 37 °C in a humidified atmosphere containing 5% $CO_2$ and subcultured using trypsin when they reached approximately 80% confluence.

### *MTT Assay*

A549/DDP cells in the logarithmic growth phase were harvested, digested with 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA) for 3–5 min at 37 °C, centrifuged at 300 ×g for 5 min to pellet the cells, and then seeded at a density of $3 \times 10^3$ cells per well in 96-well plates. The solanine group was treated with varying concentrations of solanine (0, 3, 6, 9, 12, 15 µM; PHL80074, Sigma-Aldrich, St. Louis, MO, USA) [19], while the control group received an equivalent volume of dimethyl sulfoxide (DMSO). After 48 hours of incubation at 37 °C, 20 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution (C0009S, Beyotime, Shanghai, China) was added to each well. Following a 4-hour incubation, the supernatant was discarded, and 150 µL of DMSO (ST038, Beyotime, Shanghai, China) was added to each well to dissolve the formazan crystals by gentle shaking. Absorbance (A) was measured at 570 nm using a SpectraMax i3x multi-mode microplate reader (Molecular Devices, San Jose, CA, USA). The inhibitory concentration ($IC_{10}$) value was calculated by fitting a dose-response curve using GraphPad Prism 9.5 software (GraphPad Software, San Diego, CA, USA) and was used in subsequent reversal experiments to minimize cytotoxicity while maintaining biological activity.

The inhibition rate of cell growth was calculated using the following formula:

Inhibition rate = $[1 - (A_{solanine} / A_{control})] \times 100\%$.

After establishing the optimal solanine concentration, A549/DDP cells in the logarithmic phase were trypsinized,

centrifuged, and seeded at $3 \times 10^3$ cells per well in 96-well plates. Cells in the DDP group were treated with increasing concentrations of DDP (0, 20, 40, 60, 80, and 100 μM DDP) [20]. In the DDP + solanine group, solanine at the determined reversal concentration was added in combination with DDP. After treatment, absorbance at 570 nm was measured, and the inhibition rate of cell growth was calculated. The half-maximal inhibitory concentration ($IC_{50}$) of DDP was determined using linear regression analysis. The reversal efficiency was calculated using the following formula:

Inhibition rate = $[1 - (A_{DDP + solanine} / A_{DDP})] \times 100\%$,

Reversal fold = $IC_{50}$ of DDP group / $IC_{50}$ of DDP + solanine group.

### *Scratch Assay*

Prior to seeding the cells in 12-well plates, horizontal reference lines were drawn on the bottom of each plate using a marker pen for subsequent alignment and imaging. A549/DDP cells in the logarithmic growth phase were harvested by trypsinization, centrifuged, and resuspended at a density of $5 \times 10^5$ cells per well. Cells were randomly assigned to four groups: control (equal amount of phosphate-buffered saline (PBS)), DDP, solanine, and DDP + solanine. Cells were seeded into 12-well plates and incubated at 37 °C under 5% $CO_2$ until a confluent monolayer was formed. A consistent vertical scratch was made in each well using a 200 μL pipette tip after the cells had covered the bottom of the plate. Detached cells were removed by washing the wells three times with PBS. Images were captured at 0 h, 12 h, and 24 h using an inverted microscope (Olympus IX73, Olympus Corporation, Tokyo, Japan). Cell migration was quantified by measuring the scratch width at each time point using ImageJ software (version 1.53k, National Institutes of Health, Bethesda, MD, USA). The wound closure rate was calculated using the following formula:

Wound closure rate = [(initial wound width – wound width at indicated time) / initial wound width] × 100%.

### *Clonogenic Assay*

Log-phase A549/DDP cells were collected by trypsinization, centrifuged, and resuspended to a concentration of 400 cells per well. Cells were randomly divided into four groups: control, DDP, solanine, and DDP + solanine. Following digestion, cells were seeded into 6-well plates and cultured for 10–14 days, allowing individual colonies to form. Cells were considered countable when they consisted of more than 50 cells. Cells were then fixed with 4% paraformaldehyde and stained with 0.1% crystal violet solution (C0121, Beyotime, Shanghai, China) for 30 minutes. Colony images were captured, and the number of colonies was quantified using an inverted microscope (DMI4000 B, Leica, Wetzlar, Germany).

### *Transwell Assay*

A549/DDP cells in the logarithmic growth phase were digested, centrifuged with 0.25% trypsin-EDTA for 3–5 minutes at 37 °C, then centrifuged at 300 ×g for 5 minutes at 4 °C. The cells were resuspended at a density of $5 \times 10^3$ cells per well in 12-well plates. Cells were randomly divided into four groups: control, DDP, solanine, and DDP + solanine. Cells were seeded into the upper chambers of Transwell inserts (3422, Corning Incorporated, Corning, NY, USA), while the lower chambers were filled with Roswell Park Memorial Institute-1640 (RPMI-1640) medium supplemented with 20% fetal bovine serum as a chemoattractant. Each group was plated in triplicate. Following incubation for 48 hours, non-migrated cells on the upper surface of the membrane were gently removed using a cotton swab. The migrated cells on the lower surface were fixed in 70% methanol for 30 minutes and stained with crystal violet (548-62-9, Macklin Biochemical Co., Ltd., Shanghai, China) for 10 minutes. Migrated cells were imaged under an inverted microscope (DMI4000 B, Leica, Wetzlar, Germany), and the number of cells on the underside of the membrane was counted for each field.

### *Western Blot*

A549/DDP cells were digested, centrifuged, and seeded into 6-well plates at a density of $1 \times 10^6$ cells per well. Cells were randomly divided into four groups: control, DDP, solanine, and DDP + solanine. Following a 48-hour incubation period, cells were harvested and lysed on ice for 30 minutes using radioimmunoprecipitation assay (RIPA) lysis buffer. The lysates were centrifuged to obtain total protein extracts. Protein samples were separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes (FFP39, Beyotime, Shanghai, China). Membranes were blocked with 5% non-fat milk for 1 hour and then incubated overnight at 4 °C with primary antibodies against Beclin1 (11306-1-AP), YAP1 (13584-1-AP), P-glycoprotein (P-gp, 22336-1-AP), lung resistance-related protein (LRP, 16478-1-AP), transcriptional coactivator with PDZ-binding motif (TAZ, 61265), phosphorylated signal transducer and activator of transcription 3 (p-STAT3, 80199-2-RR), STAT3 (10253-2-AP), mammalian target of rapamycin (mTOR, 66888-1-Ig), sequestosome 1 (P62, 18420-1-AP), microtubule-associated protein 1 light chain 3 (LC3, 14600-1-AP) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (60004-1-Ig). All antibodies were obtained from Proteintech (Wuhan, China) and used at a dilution of 1:1000. Following primary antibody incubation, membranes were washed three times with Tris-buffered saline with Tween 20 (TBST) (10 minutes each), incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (SA00001-2, SA00001-1) for 1 hour at room temperature, and washed again with TBST. Bands were visualized using enhanced chemi-

**Table 1. Primer sequence.**

| Gene | Forward sequence | Reverse sequence |
|---|---|---|
| *ABCB1* | GCTGTCAAGGAAGCCAATGCCT | TGCAATGGCGATCCTCTGCTTC |
| *STAT3* | CTTTGAGACCGAGGTGTATCACC | GGTCAGCATGTTGTACCACAGG |
| *LRP* | CAACGGCATCTCAGTGGACTAC | TGTTGCTGGACAGAACCACCTC |
| *TAZ* | GAGGACTTCCTCAGCAATGTGG | CGTTTGTTCCTGGAAGACAGTCA |
| *mTOR* | AGCATCGGATGCTTAGGAGTGG | CAGCCAGTCATCTTTGGAGACC |
| *GAPDH* | GTCTCCTCTGACTTCAACAGCG | ACCACCCTGTTGCTGTAGCCAA |

*ABCB1*, ATP-binding cassette sub-family B member 1; *STAT3*, signal transducer and activator of transcription 3; *LRP*, lung resistance-related protein; *TAZ*, transcriptional coactivator with PDZ-binding motif; *mTOR*, mammalian target of rapamycin; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase.

luminescence (ECL) chemiluminescent substrate (34577, Thermo Fisher Scientific, Waltham, MA, USA), and images were captured using a ChemiDoc MP Imaging System (Bio-Rad, Hercules, CA, USA). Band intensities were quantified using ImageJ software (version 1.8.0, National Institutes of Health, Bethesda, MD, USA), with GAPDH serving as the internal reference for normalization.

### *Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)*

Total RNA was extracted from cells using TRIzol reagent (15596018, Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. Reverse transcription was performed using the PrimeScript™ Reverse Transcriptase (RT) reagent kit (RR047A, Takara, Shiga, Japan) to synthesize complementary DNA (cDNA). qRT-PCR was carried out with TB Green® Premix Ex Taq™ II (RR820A, Takara, Shiga, Japan) on a QuantStudio 5 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The amplification conditions were as follows: initial denaturation at 95 °C for 30 seconds, followed by 40 cycles of 95 °C for 5 seconds and 60 °C for 30 seconds. Relative mRNA expression levels of ATP-binding cassette sub-family B member 1 (*ABCB1*), *STAT3*, *LRP*, *TAZ* and *mTOR* were calculated using the $2^{-\Delta\Delta Ct}$ method and normalized to *GAPDH* expression. The sequences of primers used are listed in Table 1.

### *Plasmid Transfection and Beclin1 Overexpression in A549/DDP Cells*

The pcDNA3.1-Beclin1 overexpression plasmid (21150), containing the full-length Beclin1 gene and verified by complete sequencing, was obtained from Addgene (Watertown, MA, USA). The pcDNA3.1 empty vector (138209, Addgene, Watertown, MA, USA) was used as a negative control (NC). Complete plasmid sequences are provided in the **Supplementary Material**. Double-stranded RNA (dsRNA) was prepared using the annealing procedure according to the manufacturer's instructions. A549/DDP cells in the logarithmic growth phase were seeded into 6-well plates and allowed to adhere until reaching approximately 80% confluence. Following a single wash with phosphate-buffered saline (PBS), the culture medium was replaced with a serum-free medium. Transfection was performed using Lipofectamine 2000 (11668027, Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions, and cells were incubated for 48 hours. Western blot analysis was subsequently used to examine the expression of Beclin1 protein in the transfected cells.

The A549/DDP cells were divided into the following treatment groups:

(1) NC/DDP group: cells transfected with the NC plasmid and treated with DDP.

(2) Ad-Beclin1/DDP group: cells transfected with Ad-Beclin1 and treated with DDP.

(3) NC/DDP + solanine group: NC-transfected cells treated with both DDP and solanine.

(4) Ad-Beclin1/DDP + solanine group: Ad-Beclin1-transfected cells treated with both DDP and solanine.

### *Statistical Analysis*

All data were analyzed using GraphPad Prism 9.5 (GraphPad Software, San Diego, CA, USA). Continuous variables with a normal distribution were expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$). A one-way analysis of variance (ANOVA) was used to compare multiple groups, followed by Tukey's post hoc test for pairwise comparisons. A $p$-value $< 0.05$ was considered statistically significant.

## Results

### *Solanine Inhibits A549/DDP Cell Proliferation and Reverses Drug Resistance*

To validate the drug-resistant phenotype, the $IC_{50}$ values of DDP were measured in both A549 and A549/DDP cells. The $IC_{50}$ of A549/DDP cells (87.08 $\pm$ 1.45 µM) was significantly higher compared to the parental A549 cells (23.38 $\pm$ 2.04 µM), confirming the establishment of DDP resistance (Fig. 1A).

The MTT assay was used to evaluate the proliferation of A549/DDP cells. The $IC_{10}$ concentration of solanine was

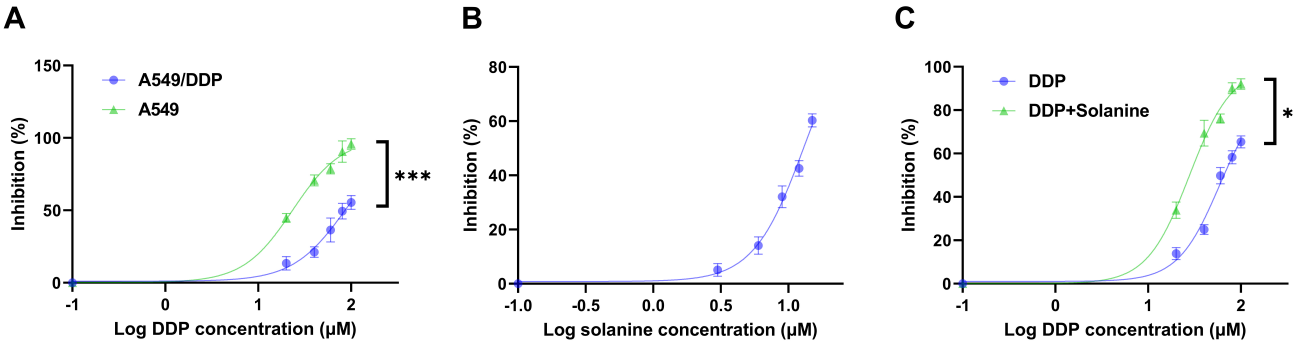


**Fig. 1. Inhibitory effect of solanine and cisplatin on A549/DDP cell proliferation.** (A) Validation of cisplatin (DDP) resistance in A549/DDP cells. (B) Effect of solanine on A549/DDP cell proliferation. (C) Reversal of drug resistance by solanine in A549/DDP cells. n = 3. Data are expressed as mean ± standard deviation (SD). *$p < 0.05$, ***$p < 0.001$.

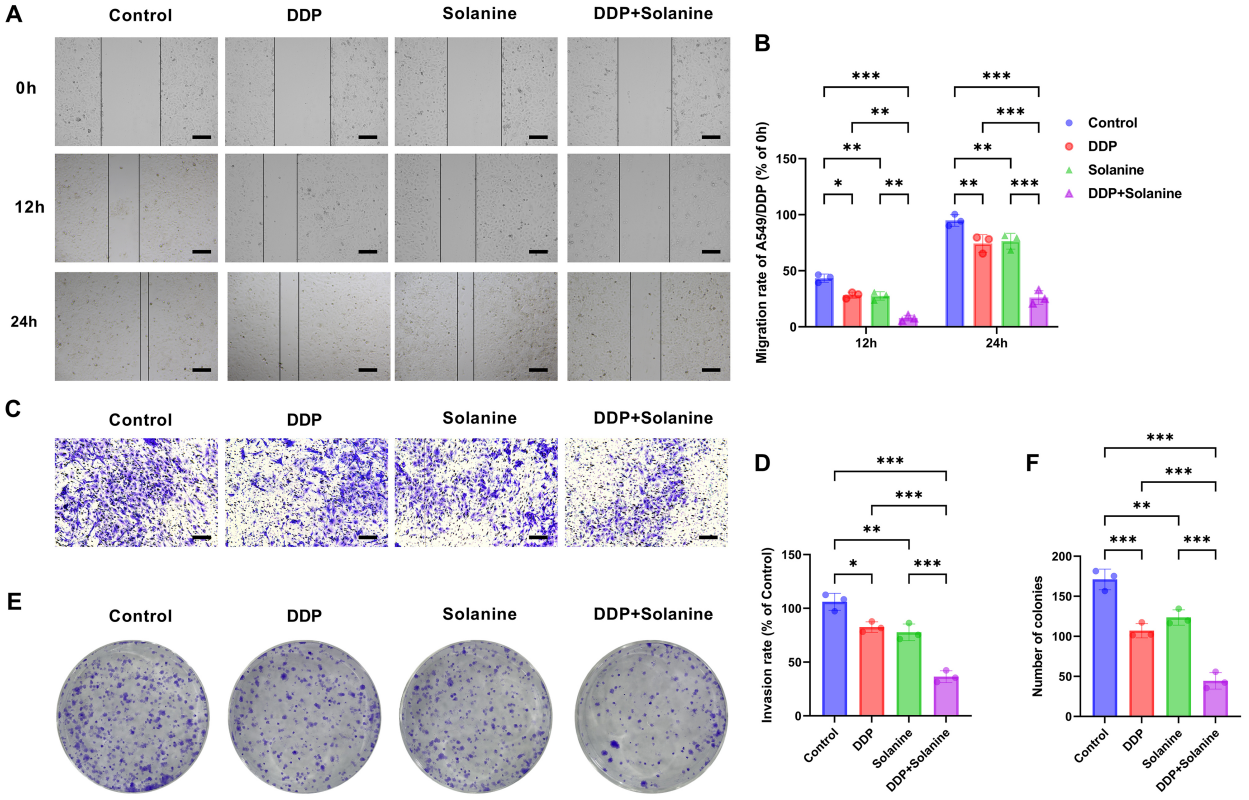


**Fig. 2. Effects of solanine and cisplatin on migration, invasion, and proliferation of A549/DDP cells.** (A,B) Wound healing assay showing cell migration after treatment with solanine and/or cisplatin (DDP). (C,D) Transwell assays showing invasion capacity under different treatments. (E,F) Colony formation assays indicate the long-term proliferative ability of A549/DDP cells under the influence of solanine and DDP. Scale bars: 50 μm (200× magnification). n = 3. Data are expressed as the mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

determined to be 5.01 ± 0.24 μM, a level that provides an effective inhibitory effect without significant cytotoxicity. Therefore, 5 μM solanine was selected for subsequent experiments (Fig. 1B).

Additionally, solanine significantly enhanced the inhibition of A549/DDP cell proliferation in a dose-dependent manner with increasing DDP concentrations ($p < 0.05$). In the DDP + solanine (5 μM) treatment group, the $IC_{50}$ of DDP in A549/DDP cells was significantly reduced to 27.89 ± 1.25 μM, compared to 65.49 ± 1.77 μM in the DDP-only group. This corresponds to a drug resistance reversal fold of 2.34 (Fig. 1C). Therefore, a concentration of 28 μM DDP—approximately corresponding to the $IC_{50}$ value observed in the DDP + solanine (5 μM) group—was selected for use in subsequent experiments to ensure a consistent and adequate inhibitory effect.

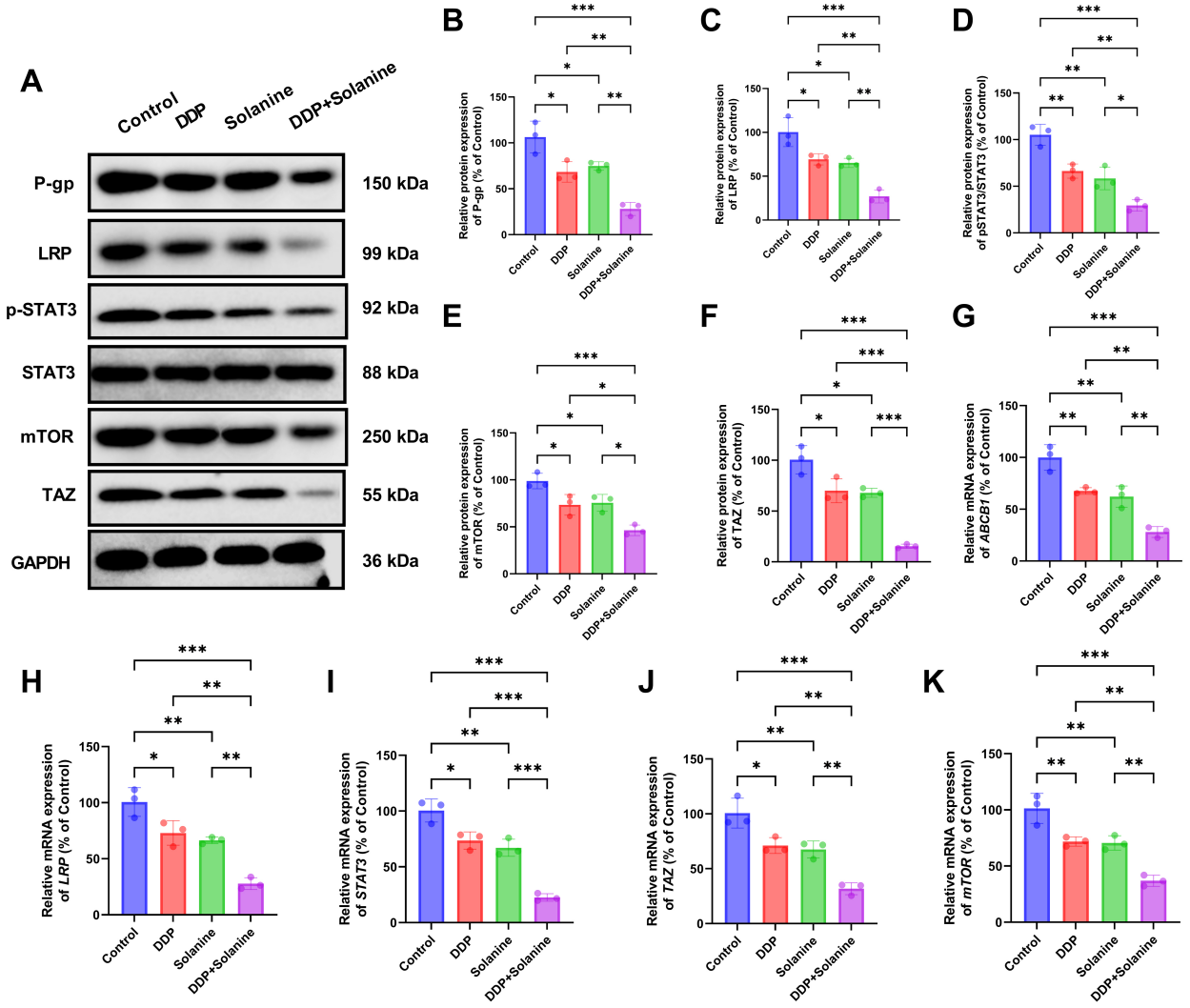


**Fig. 3. Solanine and cisplatin synergistically suppress drug resistance-associated protein expression in A549/DDP cells.** (A–F) Western blot analysis of P-glycoprotein (P-gp), lung resistance-related protein (LRP), phosphorylated signal transducer and activator of transcription 3 (p-STAT3), mammalian target of rapamycin (mTOR), and transcriptional co-activator with PDZ-binding motif (TAZ) protein expression under different treatment conditions. (G–K) qRT-PCR analysis of mRNA expression levels of *ABCB1*, *LRP*, *STAT3*, *mTOR*, and *TAZ* in different treatment groups. n = 3. Data are expressed as mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

### *Solanine Enhances the Inhibition of Migration, Invasion, and Proliferation of A549/DDP Cells by DDP*

The migratory, invasive, and proliferative capacities of A549/DDP cells were assessed using scratch assays, Transwell chamber assays, and colony formation assays. Compared with the control group, both the solanine treatment group and the DDP + solanine group demonstrated significantly reduced cell migration distance (Fig. 2A,B), invasion (Fig. 2C,D), and colony formation (Fig. 2E,F) ($p < 0.05$). Additionally, the DDP + solanine group showed a further significant reduction in these parameters compared to the DDP and solanine single-treatment groups ($p < 0.05$). These results indicate that solanine enhances the inhibitory effects of DDP on A549/DDP cell migration, invasion, and proliferation.

### *Solanine Enhances the Inhibitory Effect of DDP on the Expression of Drug Resistance-Associated Proteins in A549/DDP Cells*

Western blot analysis revealed that the expression levels of drug resistance-related proteins (P-gp, LRP, p-STAT3, mTOR, and TAZ) were significantly decreased in the solanine and DDP + solanine groups compared to the control group ($p < 0.05$). Furthermore, the DDP + solanine group showed significantly lower expression levels of the drug resistance-related proteins compared to the individual DDP and solanine treatment groups (Fig. 3A–F). These protein level findings were further corroborated by qRT-PCR analysis. The mRNA expression levels of *ABCB1*, *LRP*, *STAT3*, *mTOR*, and *TAZ* were significantly reduced in the solanine and DDP + solanine groups compared to the control group ($p < 0.05$), with the greatest reduction observed

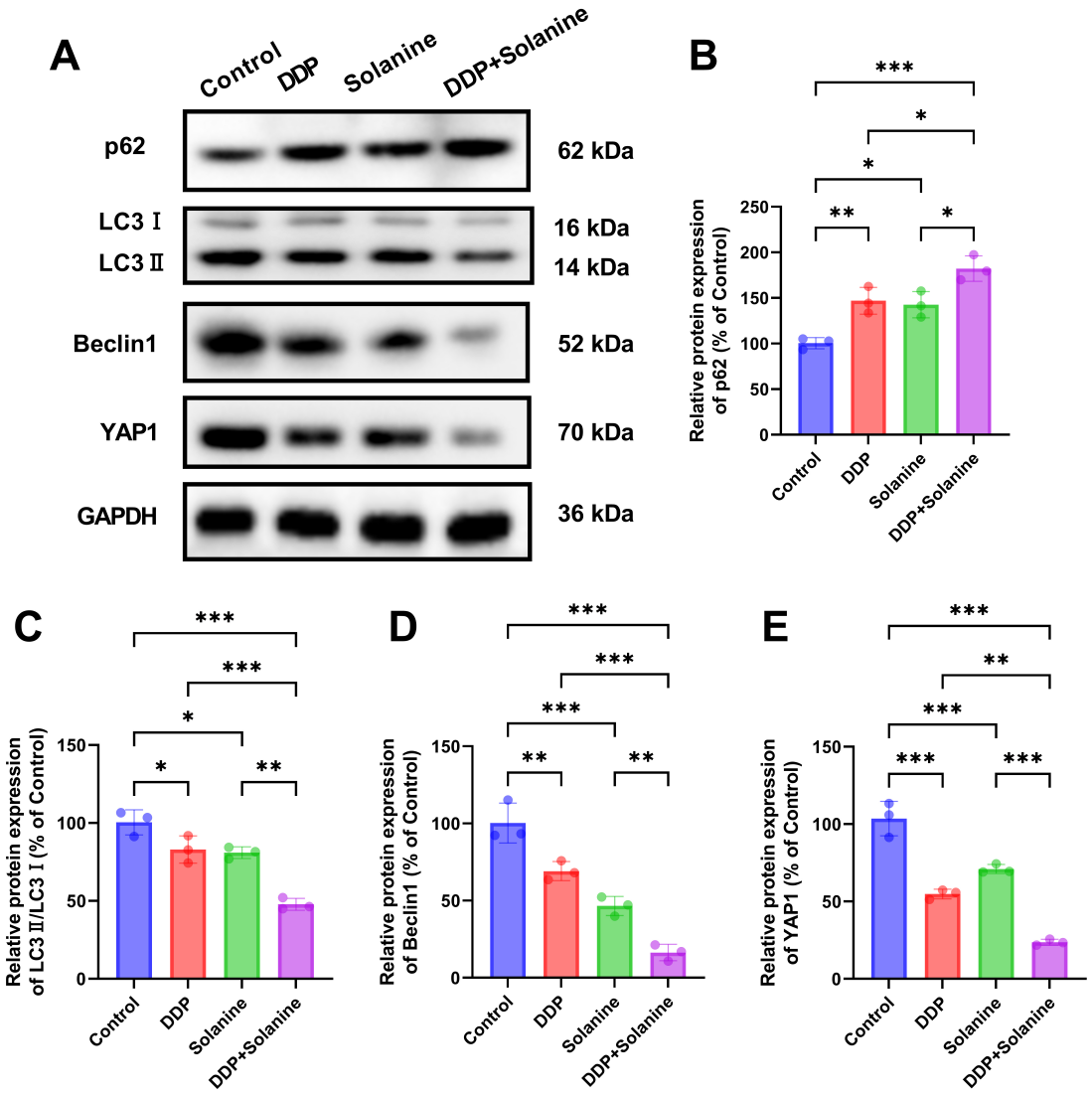


**Fig. 4. Solanine enhances cisplatin-induced autophagy in A549/DDP cells.** (A) Western blot analysis of sequestosome 1 (p62), microtubule-associated protein 1 light chain 3II/I (LC3II/LC3I), Beclin1, and Yes-associated protein 1 (YAP1) protein levels in different treatment groups. (B–E) Quantification of p62, LC3II/LC3I, Beclin1, and YAP1 protein levels normalized to GAPDH. n = 3. Data are expressed as the mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

in the DDP + solanine group (Fig. 3G–K). These results collectively suggest that solanine amplifies DDP's inhibitory effects on the expression of drug resistance-associated proteins in A549/DDP cells.

### *Solanine Enhances the Inhibitory Effect of DDP on Autophagy in A549/DDP Cells*

Western blot analysis showed a significant increase in p62 protein levels in the solanine and DDP + solanine groups compared to the control group (Fig. 4A,B) ($p < 0.05$). Furthermore, the DDP + solanine group showed significantly higher expression of p62 than either the DDP or solanine single-treatment groups (Fig. 4A,B) ($p < 0.05$). In contrast, the expression levels of LC3II/LC3I, Beclin1, and YAP1 were significantly reduced in the solanine and DDP + solanine groups compared to the control group (Fig. 4A,C–E) ($p < 0.05$). The DDP + solanine group exhibited the most significant reduction in LC3II/LC3I, Beclin1, and YAP1 expression compared to the DDP and solanine groups (Fig. 4A,C–E) ($p < 0.05$), indicating a marked suppression of autophagy. These findings suggest that solanine enhances DDP-mediated suppression of autophagy in A549/DDP cells.

### *Overexpression of the Beclin1 Gene Inhibits Solanine-Induced Reversal of DDP Resistance in A549/DDP Cells*

To confirm the transfection efficiency of Beclin1, Western blot analysis was performed. The results showed a significant increase in Beclin1 protein levels in the Ad-Beclin1/DDP group compared to NC/DDP cells, indicating

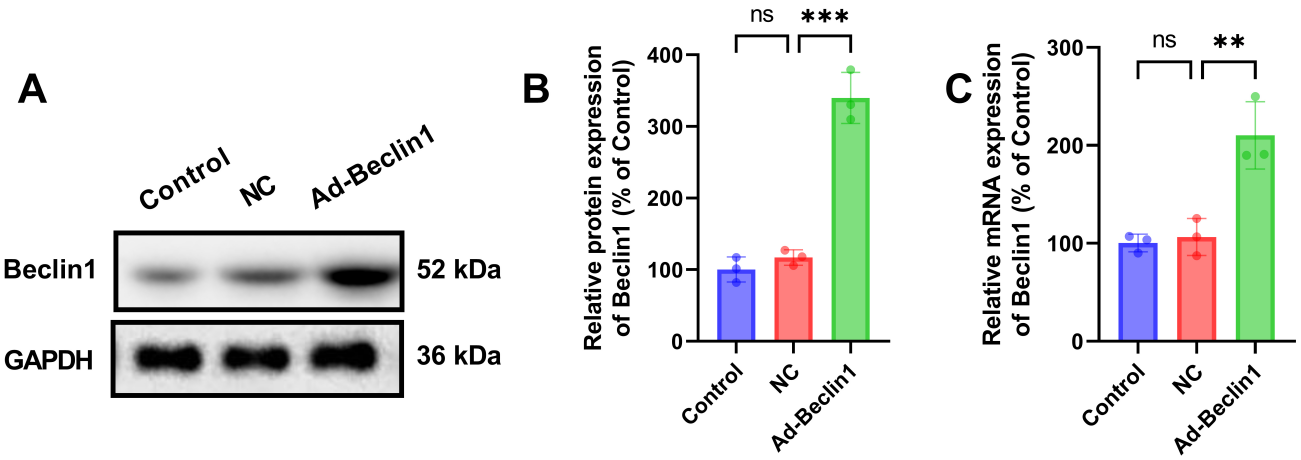


**Fig. 5. Validation of Beclin1 overexpression in A549/DDP cells.** (A,B) Western blot analysis of Beclin1 protein expression in Beclin1-overexpressing A549/cisplatin (DDP) cells. (C) qRT-PCR analysis of *Beclin1* mRNA expression in the Beclin1-overexpressing group. n = 3. Data are expressed as mean ± SD. $^{ns}p > 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. NC, negative control.

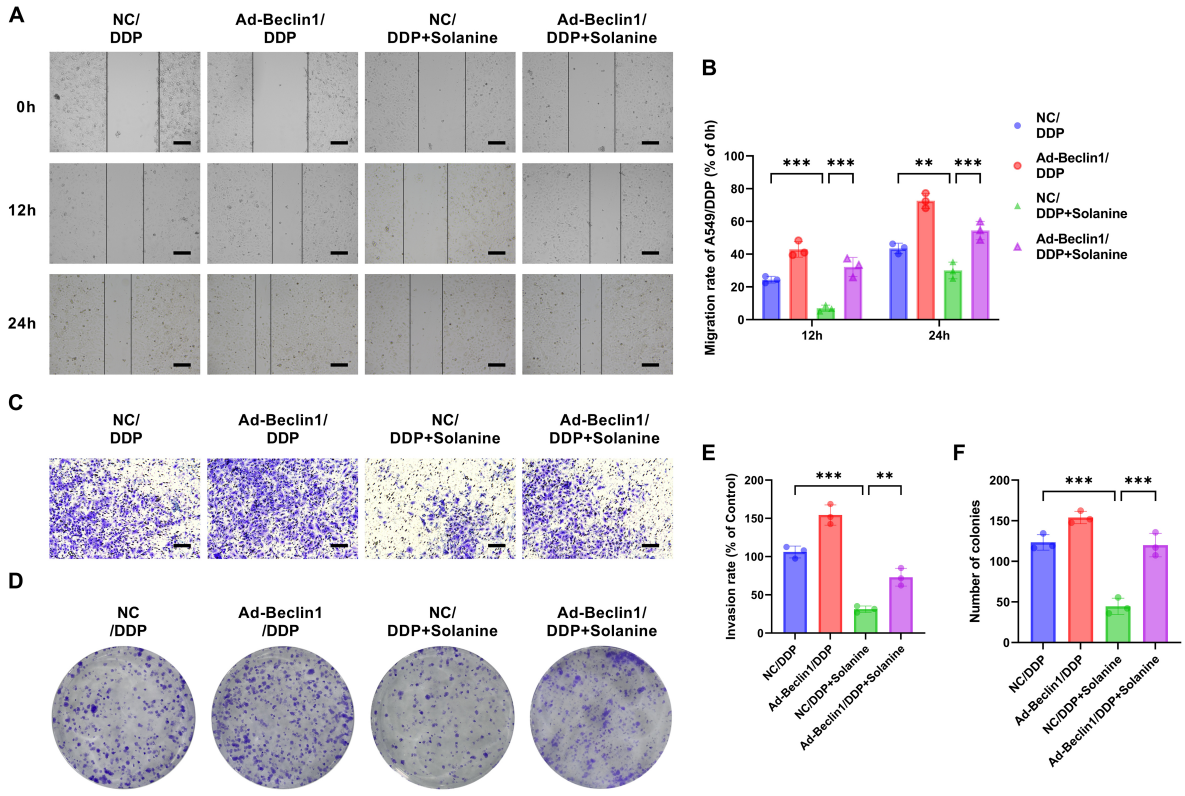


**Fig. 6. Effects of solanine and cisplatin on migration, invasion, and proliferation of A549/DDP cells with Beclin1 overexpression.** (A,B) Wound healing assay showing the migration of Beclin 1-overexpressing A549/cisplatin (DDP) cells following treatment. (C,E) Transwell assay evaluating invasion of A549/DDP cells with Beclin1 overexpression under the influence of solanine and DDP. (D,F) Colony formation assay evaluating the proliferation of A549/DDP cells with Beclin1 overexpression under the influence of solanine and DDP. Scale bars: 50 µm (200× magnification). n = 3. Data are expressed as mean ± SD. $^{**}p < 0.01$, $^{***}p < 0.001$. NC, negative control.

successful overexpression at the protein level ($p < 0.05$; Fig. 5A,B). Furthermore, qRT-PCR analysis revealed a significant elevation in Beclin1 mRNA expression in the overexpression group, further confirming effective transfection ($p < 0.05$; Fig. 5C).

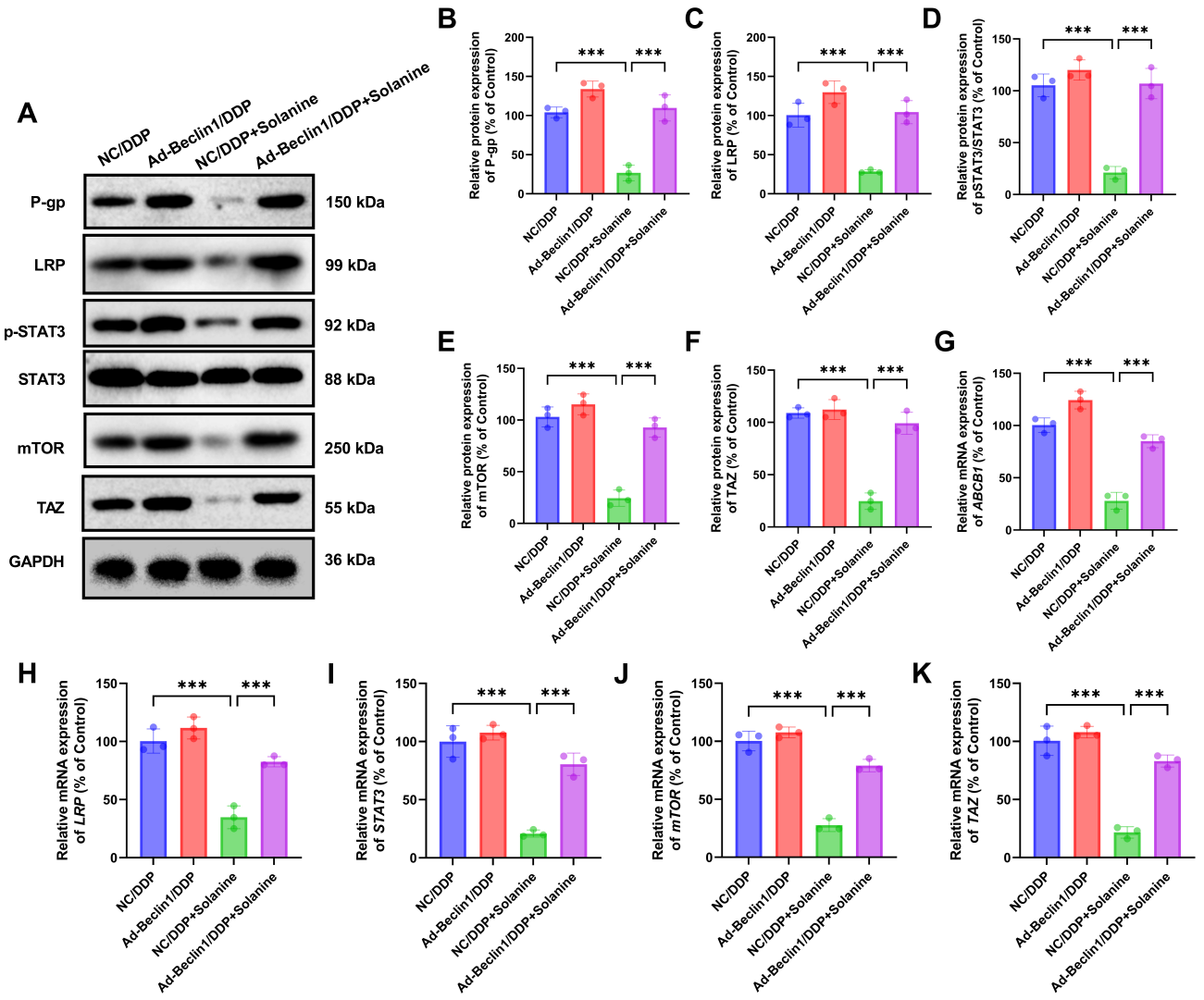


**Fig. 7. Effects of solanine and cisplatin on drug resistance protein expression in A549/DDP cells with Beclin1 overexpression.** (A–F) Western blot analysis of P-gp, LRP, p-STAT3, mTOR and TAZ protein expression in different treatment groups. (G–K) qRT-PCR analysis of *ABCB1*, *LRP*, *STAT3*, *mTOR*, and *TAZ* mRNA expression levels in different treatment groups. n = 3. Data are expressed as the mean ± SD. ***$p < 0.001$. NC, negative control.

To investigate the role of Beclin1 in solanine-mediated reversal of DDP resistance, we compared multiple treatment groups. Compared to the NC/DDP group, the NC/DDP + solanine group showed significantly reduced invasion ($p < 0.05$; Fig. 6A,B), migration ($p < 0.05$; Fig. 6C,E), and proliferation ($p < 0.05$; Fig. 6D,F), indicating that solanine effectively reverses DDP resistance. However, in the Ad-Beclin1/DDP + solanine group, these inhibitory effects were notably attenuated, as evidenced by increased cell proliferation, migration, and invasion compared to the NC/DDP + solanine group. These findings suggest that Beclin1 overexpression impairs the ability of solanine to reverse DDP resistance, identifying Beclin1 as a key regulatory factor in this process. Further investigation is needed to elucidate the underlying molecular mechanisms.

### *Overexpression of the Beclin1 Gene Reverses the Inhibitory Effect of Solanine on Drug Resistance-Related Proteins in A549/DDP Cells*

The impact of Beclin1 overexpression on solanine-mediated suppression of drug resistance-associated proteins (P-gp, LRP, p-STAT3, mTOR, and TAZ) was examined in A549/DDP cells. Compared to the NC/DDP group, the NC/DDP + solanine group showed significantly reduced expression of these proteins ($p < 0.05$; Fig. 7A–F), indicating that solanine effectively suppresses drug resistance-associated pathways. However, the Ad-Beclin1/DDP + solanine group exhibited significantly elevated expression of these proteins compared to the NC/DDP + solanine group ($p < 0.05$), suggesting that Beclin1 overexpression weakened the inhibitory effect of solanine. These protein level changes were corroborated by qRT-PCR results,

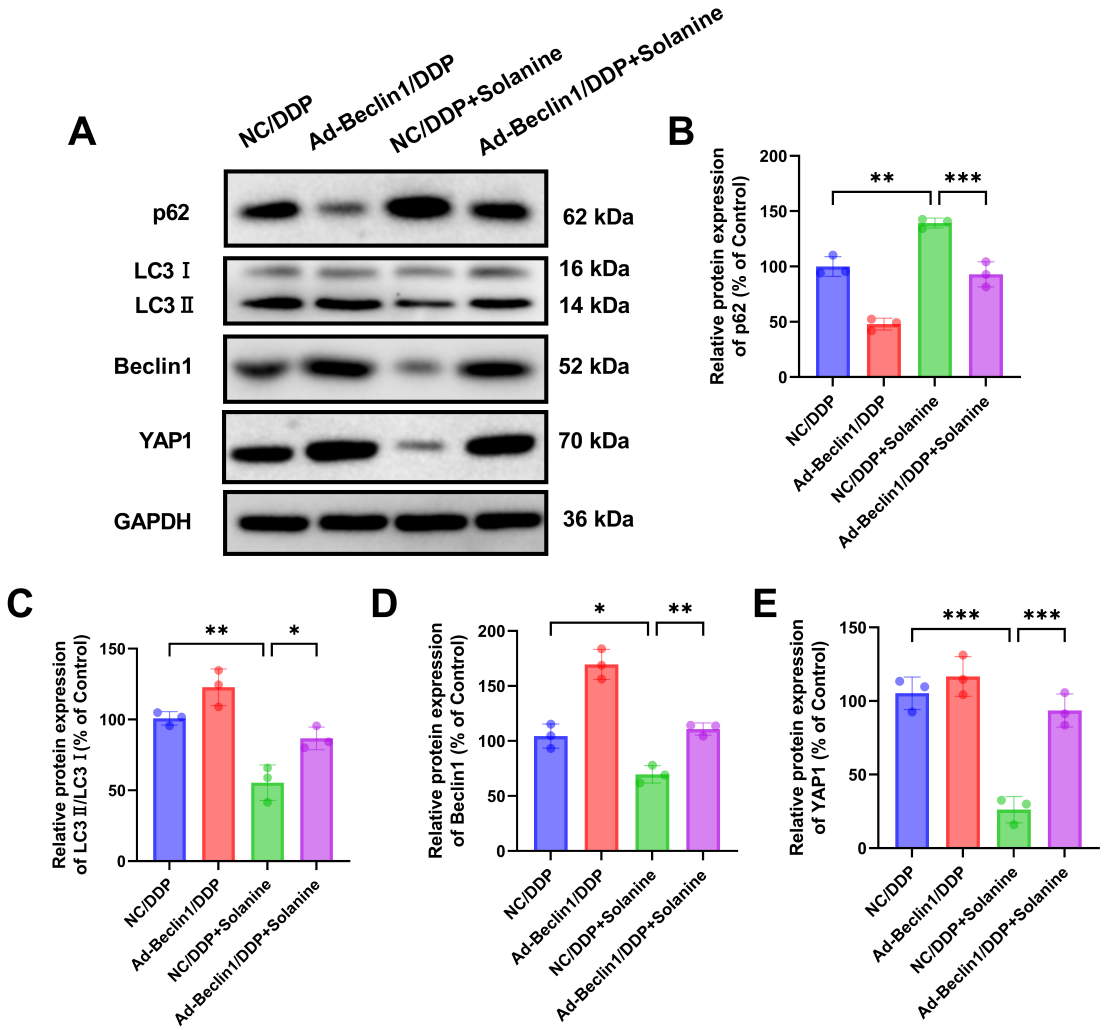


**Fig. 8. Beclin1 overexpression reverses solanine-induced suppression of autophagy in A549/DDP cells.** (A) Western blot analysis of p62, LC3II/LC3I, Beclin1, and YAP1 protein levels in different treatment groups. (B–E) Quantitative analysis of p62, LC3II/LC3I, Beclin1, and YAP1 protein levels normalized to GAPDH. n = 3. Data are expressed as the mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

which showed similar expression trends (Fig. 7G–K). These findings indicate that Beclin1 is a key regulator in solanine-induced reversal of DDP resistance in A549/DDP cells.

### *Overexpression of the Beclin1 Gene Reverses the Inhibitory Effect of Solanine on Autophagy in A549/DDP Cells*

To evaluate the effect of Beclin1 overexpression on solanine-induced suppression of autophagy, the expression of autophagy-related proteins (Beclin1, LC3II/LC3I, p62, and YAP1) was evaluated in A549/DDP cells. Compared to the NC/DDP group, the NC/DDP + solanine group showed reduced expression of Beclin1, YAP1, and LC3II/LC3I, alongside increased p62 levels, indicating that solanine effectively inhibited autophagy. However, in the Ad-Beclin1/DDP + solanine group, expression levels of autophagy-related proteins were significantly elevated than those in the NC/DDP + solanine group ($p < 0.05$; Fig. 8A–E). These results indicate that Beclin1 overexpression mitigates solanine's autophagy-suppressive effects, highlighting its critical regulatory role in autophagy modulation in A549/DDP cells.

## Discussion

Autophagy is essential in the chemotherapy response of cancer cells. Upon exposure to chemotherapeutic agents, tumor cells can undergo both cytotoxic (death-associated) and cytoprotective autophagy. In pathological conditions, autophagy serves as a survival mechanism by recycling intracellular components to meet cellular energy demands. Consequently, autophagy can contribute to chemoresistance by enhancing cancer cell survival. Targeting autophagy has emerged as a promising therapeutic strategy to overcome drug resistance in cancer cells [21].

Beclin1 is a key regulator of autophagy initiation and has been extensively associated with chemotherapeutic drug resistance [22]. In gastric cancer, modulation of Beclin1 expression has been shown to influence apoptosis-related proteins, which are crucial for regulating cell death pathways. Upregulation of Beclin1 promotes apoptosis in malignant cells by activating critical apoptotic pathways. This alteration not only facilitates programmed cell death but also increases the sensitivity of previously resistant gastric cancer cells to chemotherapeutic agents such as DDP [22]. Moreover, Beclin1 activates the YAP1 protein, and together, they contribute to the development of drug resistance [16]. Currently, common drugs used to reverse chemoresistance are associated with severe adverse effects, including cardiotoxicity, hepatotoxicity, nephrotoxicity, and immunosuppression. These side effects limit their clinical applicability. Previous research has demonstrated that the expression of Beclin1 is notably elevated in the DDP-resistant A549/DDP lung cancer cell line compared to the DDP-sensitive A549 cell line. This differential expression suggests a potential role for Beclin1 in mediating DDP resistance, making it a compelling therapeutic target for sensitizing lung cancer cells to chemotherapy [23].

Notably, lung cancer cell lines, including A549 and H1299, exhibit significantly enhanced sensitivity to DDP when treated in combination with solanine. This observation suggests that solanine may serve as a chemosensitizing agent, enhancing the efficacy of DDP in these chemotherapy-resistant cancer cell lines [24]. The present study aimed to investigate the effect of solanine on DDP resistance in A549 lung cancer cells, with a specific focus on the Beclin1-YAP1 signaling axis. The study provides insights into the molecular mechanisms by which solanine modulates this pathway and potentially alters the cellular response to chemotherapy.

Using various experimental methods, we first assessed the combined effects of solanine and DDP on the migration, invasion, and proliferation of A549/DDP cells. These included colony formation assays to evaluate long-term cell proliferation, scratch assays to assess cell motility, Transwell assays to evaluate invasive potential, and MTT assays to determine cell viability. By employing these complementary methodologies, we aimed to gain a comprehensive understanding of how solanine and DDP interact to influence the aggressive behaviors of A549/DDP cells, especially in regard to their capacity to migrate, invade, and proliferate under therapeutic conditions. The results indicated that in the DDP + solanine group, migration distance, number of invaded cells, and colony formation were significantly reduced compared to both the DDP-only and solanine-only groups. These findings provide preliminary evidence that the combined treatment of solanine and DDP effectively suppresses lung cancer cell activity.

LRP plays a role in regulating material exchange between the nucleus and cytoplasm, as well as vesicular transport. By reducing intracellular drug concentrations, LRP contributes to resistance against platinum-based agents, alkylating agents, and other chemotherapeutic drugs. Chemotherapy also induces increased expression of P-gp, which lowers intracellular drug accumulation and enhances drug efflux, thereby reducing cellular sensitivity to chemotherapeutic agents [25]. P-gp and LRP serve as direct indicators of drug resistance sensitivity. In studies involving drug-resistant A2780/DDP cell lines, downregulation of the autophagy-related gene Beclin1 inhibited DDP-induced autophagy, thereby enhancing the sensitivity of resistant cells to DDP [26]. Additionally, YAP1 protein has a known binding site for Beclin1 [16] and is involved in the regulation of chemoresistance-associated proteins, such as p-STAT3 [13], mTOR [27], and TAZ [28].

In the present study, we further observed that the expression levels of drug resistance markers P-gp and LRP were significantly reduced in the DDP + solanine treatment group. Additionally, the expression of Beclin1, YAP1, p-STAT3, mTOR, and TAZ proteins was downregulated. LC3II/I expression was also reduced, while p62 levels were markedly elevated, indicating suppressed autophagic activity in the DDP + solanine group. These findings suggest that solanine directly lowers drug resistance in lung cancer cells, enhancing their sensitivity to DDP, and that solanine is intimately linked to the regulation of chemoresistance. To further validate these results, Beclin1-overexpressing A549/DDP cells were generated, and siRNA transfection was used to manipulate gene expression in A549/DDP cells. The findings demonstrated that overexpression of Beclin1 reversed the inhibitory effects of solanine on A549/DDP cell proliferation, migration, invasion, and drug resistance protein expression. These findings suggest that Beclin1 mediates solanine-induced reversal of DDP resistance in lung cancer cells.

However, this study has limitations. We lack direct evidence establishing a mechanistic link between Beclin1 and YAP1, such as co-immunoprecipitation data, and did not perform YAP1-specific knockdown or inhibition experiments to confirm its role downstream of Beclin1. Moreover, all experiments were conducted *in vitro*, and the absence of *in vivo* validation limits the translational applicability of the findings. Additional studies using animal models are required to evaluate the *in vivo* efficacy and safety profile of solanine.

## Conclusion

This study suggests that solanine may reverse DDP resistance in lung cancer by modulating the Beclin1-YAP1 signaling pathway, potentially influencing autophagy and cell survival. These findings offer new insights into the molecular mechanisms underlying chemosensitizing effects associated with solanine and may inform future therapeutic strategies for overcoming DDP resistance.

## Availability of Data and Materials

Data and materials from this study can be obtained from the corresponding author upon reasonable request.

## Author Contributions

NC designed the experimental framework and performed the main experiments. PC collected, analyzed and interpreted the data. ND contributed to data curation and participated in result discussion and manuscript revision. All authors were involved in the drafting and critical revision of the manuscript. All authors have read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

Not applicable.

## Acknowledgment

Not applicable.

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This research received no external funding.

## Conflict of Interest

The authors declare no conflict of interest.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202537198.117.

## References

[1] Li Y, Yan B, He S. Advances and challenges in the treatment of lung cancer. Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie. 2023; 169: 115891. https://doi.org/10.1016/j.biopha.2023.115891.

[2] Aghababaei F, Hadidi M. Recent Advances in Potential Health Benefits of Quercetin. Pharmaceuticals (Basel, Switzerland). 2023; 16: 1020. https://doi.org/10.3390/ph16071020.

[3] Megyesfalvi Z, Gay CM, Popper H, Pirker R, Ostoros G, Heeke S, *et al*. Clinical insights into small cell lung cancer: Tumor heterogeneity, diagnosis, therapy, and future directions. CA: a Cancer Journal for Clinicians. 2023; 73: 620–652. https://doi.org/10.3322/caac.21785.

[4] Konoshenko M, Lansukhay Y, Krasilnikov S, Laktionov P. MicroRNAs as Predictors of Lung-Cancer Resistance and Sensitivity to Cisplatin. International Journal of Molecular Sciences. 2022; 23: 7594. https://doi.org/10.3390/ijms23147594.

[5] Lin LT, Choong CY, Tai CJ. Solanine Attenuates Hepatocarcinoma Migration and Invasion Induced by Acetylcholine. Integrative Cancer Therapies. 2020; 19: 1534735420909895. https://doi.org/10.1177/1534735420909895.

[6] Zhang X, Yang L, Xu X, Tang F, Yi P, Qiu B, *et al*. A review of fibroblast growth factor 21 in diabetic cardiomyopathy. Heart Failure Reviews. 2019; 24: 1005–1017. https://doi.org/10.1007/s10741-019-09809-x.

[7] Wu J, Wang L, Du X, Sun Q, Wang Y, Li M, *et al*. $\alpha$-solanine enhances the chemosensitivity of esophageal cancer cells by inducing microRNA 138 expression. Oncology Reports. 2018; 39: 1163–1172. https://doi.org/10.3892/or.2018.6187.

[8] Yi YJ, Jia XH, Wang JY, Chen JR, Wang H, Li YJ. Solanine induced apoptosis and increased chemosensitivity to Adriamycin in T-cell acute lymphoblastic leukemia cells. Oncology Letters. 2018; 15: 7383–7388. https://doi.org/10.3892/ol.2018.8229.

[9] Yi YJ, Jia XH, Zhu C, Wang JY, Chen JR, Wang H, *et al*. Solanine reverses multidrug resistance in human myelogenous leukemia K562/ADM cells by downregulating MRP1 expression. Oncology Letters. 2018; 15: 10070–10076. https://doi.org/10.3892/ol.2018.8563.

[10] He J, Dong L, Luo L, Wang K. Redox Regulation of Autophagy in Cancer: Mechanism, Prevention and Therapy. Life (Basel, Switzerland). 2022; 13: 98. https://doi.org/10.3390/life13010098.

[11] Kwon J, Kim J, Kim KI. Crosstalk between endoplasmic reticulum stress response and autophagy in human diseases. Animal Cells and Systems. 2023; 27: 29–37. https://doi.org/10.1080/19768354.2023.2181217.

[12] Biswas U, Roy R, Ghosh S, Chakrabarti G. The interplay between autophagy and apoptosis: its implication in lung cancer and therapeutics. Cancer Letters. 2024; 585: 216662. https://doi.org/10.1016/j.canlet.2024.216662.

[13] Xu H, Qin ZH, Wei Y, Wu J. Editorial: Beclin 1 and autophagy—in memory of Beth Levine (1960-2020). Frontiers in Cell and Developmental Biology. 2022; 10: 1058861. https://doi.org/10.3389/fcell.2022.1058861.

[14] Sun Y, Liu X, Tong H, Yin H, Li T, Zhu J, *et al*. SIRT1 Promotes Cisplatin Resistance in Bladder Cancer via Beclin1 Deacetylation-Mediated Autophagy. Cancers. 2023; 16: 125. https://doi.org/10.3390/cancers16010125.

[15] Chen J, Zhang L, Zhou H, Wang W, Luo Y, Yang H, *et al*. Inhibition of autophagy promotes cisplatin-induced apoptotic cell death through Atg5 and Beclin 1 in A549 human lung cancer cells. Molecular Medicine Reports. 2018; 17: 6859–6865. https://doi.org/10.3892/mmr.2018.8686.

[16] Zhang Q, Fang X, Zhao W, Liang Q. The transcriptional coactivator YAP1 is overexpressed in osteoarthritis and promotes its progression by interacting with Beclin-1. Gene. 2019; 689: 210–219. https://doi.org/10.1016/j.gene.2018.11.068.

[17] Papavassiliou KA, Papavassiliou AG. The Biology and Therapeutic Potential of the Src-YAP Axis in Non-Small Cell Lung Cancer (NSCLC). Cancers. 2022; 14: 6178. https://doi.org/10.3390/cancers14246178.

[18] Zhu J, Wu T, Lin Q. Non-hippo kinases: indispensable roles in YAP/TAZ signaling and implications in cancer therapy. Molecular Biology Reports. 2023; 50: 4565–4578. https://doi.org/10.1007/s11033-023-08329-0.

[19] Tang X, Guo Y, Zhang S, Wang X, Teng Y, Jin Q, *et al*. Solanine Represses Gastric Cancer Growth by Mediating Autophagy Through AAMDC/MYC/ATF4/Sesn2 Signaling Pathway. Drug Design, Development and Therapy. 2023; 17: 389–402. https://doi.org/10.2147/DDDT.S389764.

[20] Teng X, Wang SY, Shi YQ, Fan XF, Liu S, Xing Y, *et al*. The role of emodin in cisplatin resistance reversal of lung adenocarcinoma A549/DDP cell. Anti-cancer Drugs. 2021; 32: 939–949. https://doi.org/10.1097/CAD.0000000000001086.

[21] Al Saihati HA, Rabaan AA. Cellular resistance mechanisms in cancer and the new approaches to overcome resistance mech-

anisms chemotherapy. Saudi Medical Journal. 2023; 44: 329–344. https://doi.org/10.15537/smj.2023.44.4.20220600.

[22] Zheng HC, Zhao S, Xue H, Zhao EH, Jiang HM, Hao CL. The Roles of Beclin 1 Expression in Gastric Cancer: A Marker for Carcinogenesis, Aggressive Behaviors and Favorable Prognosis, and a Target of Gene Therapy. Frontiers in Oncology. 2020; 10: 613679. https://doi.org/10.3389/fonc.2020.613679.

[23] Zhou H, Pan P, Zhao Q, Liu W, Sun Y, Wang J, *et al*. Overcoming Basal Autophagy, Kangai Injection Enhances Cisplatin Cytotoxicity by Regulating FOXO3a-Dependent Autophagic Cell Death and Apoptosis in Human Lung Adenocarcinoma A549/DDP Cells. BioMed Research International. 2022; 2022: 6022981. https://doi.org/10.1155/2022/6022981.

[24] Luo S, Tian GJ, Yu FX, Wen ZD. A narrative review of the anti-tumor studies of solanine. Translational Cancer Research. 2021; 10: 1578–1582. https://doi.org/10.21037/tcr-20-3094.

[25] Li R, Qin J, Wang Z, Lv F, Guo J, Zhu H, *et al*. Dioscin reduced chemoresistance for colon cancer and analysis of sensitizing targets. Biochemical and Biophysical Research Communications. 2023; 638: 94–102. https://doi.org/10.1016/j.bbrc.2022.10.104.

[26] Ma J, Liu L, Ling Y, Zheng J. Polypeptide LTX-315 reverses the cisplatin chemoresistance of ovarian cancer cells via regulating Beclin-1/PI3K/mTOR signaling pathway. Journal of Biochemical and Molecular Toxicology. 2021; 35: e22853. https://doi.org/10.1002/jbt.22853.

[27] Tang H, Zhu S, Chen K, Yuan S, Hu J, Wang H. IL-17A regulates autophagy and promotes osteoclast differentiation through the ERK/mTOR/Beclin1 pathway. PloS One. 2023; 18: e0281845. https://doi.org/10.1371/journal.pone.0281845.

[28] Wijshake T, Zou Z, Chen B, Zhong L, Xiao G, Xie Y, *et al*. Tumor-suppressor function of Beclin 1 in breast cancer cells requires E-cadherin. Proceedings of the National Academy of Sciences of the United States of America. 2021; 118: e2020478118. https://doi.org/10.1073/pnas.2020478118.