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Oridonin elevates sensitivity of ovarian carcinoma cells to cisplatin via suppressing cisplatin-mediated autophagy

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ABSTRACT

Background: Cisplatin resistance has been found to contribute to the failure of ovarian carcinoma treatment. Oridonin is a natural en-kaurane tetracyclic diterpenoid compound discovered in *Rabdosia rubescene* (Henmsl.) Hara. Herein, we tested whether oridonin could exert chemo-sensitization activity on cisplatin-resistant ovarian carcinoma cells.

Methods: Firstly, A2780CP cells and SKOV3/DDP cells were exposed to cisplatin and/or oridonin treatment. Cell counting kit-8 (CCK-8) kit and Dead Cell Apoptosis Kit with Annexin V-FITC and PI were carried out to test cell viability and apoptosis, respectively. Then, pBeclin-1 was transfected to overexpress Beclin-1. 3-Methyladenine (3-MA) acted as autophagy inhibitor, while rapamycin acted as autophagy activator. Finally, the influence of cisplatin and/or oridonin treatment on human normal ovarian epithelial IOSE 364 cell viability and apoptosis were also detected.

Results: Oridonin incubation notably elevated the cisplatin-caused reduction of A2780CP and SKOV3/DDP cell viabilities and enhancement of cell apoptosis. Cisplatin-caused A2780CP and SKOV3/DDP cell autophagy was dramatically inhibited by oridonin. Overexpression of Beclin-1 mitigated the influence of oridonin on cisplatin-caused A2780CP and SKOV3/DDP cell autophagy. Inhibition of cell autophagy by 3-MA promoted the oridonin + cisplatin-caused A2780CP and SKOV3/DDP cell apoptosis, while activation of cell autophagy by rapamycin had opposite effects. Oridonin and cisplatin co-treatment had no significant effects on IOSE 364 cell viability and apoptosis.

Conclusion: The chemo-sensitization activity of oridonin on cisplatin-resistant ovarian carcinoma cells was verified in this study. Oridonin elevated sensitivity of ovarian carcinoma cells to cisplatin via suppressing cisplatin-mediated autophagy.

1. Introduction

Ovarian carcinoma is a common genital system malignancy in women [1]. There are about 13,980 ovarian carcinoma-related deaths each year in the United States [2]. Depending on difference of histogenetic principles, ovarian carcinoma can be subdivided into epithelial tumor (approximately 60% of cases), germ cell tumor (approximately 30% of cases) and sex-cord stromal tumor (approximately 8% of cases) [1,3]. For ovarian carcinoma, the most effective therapeutic method is still surgery resection combined with adjuvant chemotherapy based on cisplatin or paclitaxel [4,5]. However, the occurrence of drug resistance is a very serious problem [6]. Earlier research reported that drug resistance was responsible for treatment failure and death of > 90% patients with metastatic ovarian carcinoma [7].

Nowadays, more and more compounds isolated from plants have been reported to possess anti-cancer and chemo-sensitization activities [8,9]. Oridonin (CAS number: 28957-04-2) is a natural en-kaurane tetracyclic diterpenoid compound discovered in *Rabdosia rubescene* (Henmsl.) Hara [10]. Oridonin has been extensively studied in recent years. From the year 2014, there are roughly 200 papers can be retrieved. In terms of its chemo-sensitization activity, He *et al.* indicated that oridonin could enhance the sensitivity of human gastric cancer cells to cisplatin through causing cell apoptosis [11]. Xiao *et al.* demonstrated that oridonin could be an effective chemo-sensitizer to suppress gefitinib-resistant non-small cell lung cancer cell proliferation and metastasis [12]. For ovarian carcinoma, Ma *et al.* reported that oridonin could weaken cisplatin drug resistance in human ovarian carcinoma cells by promoting cell apoptosis and lowering cell migration

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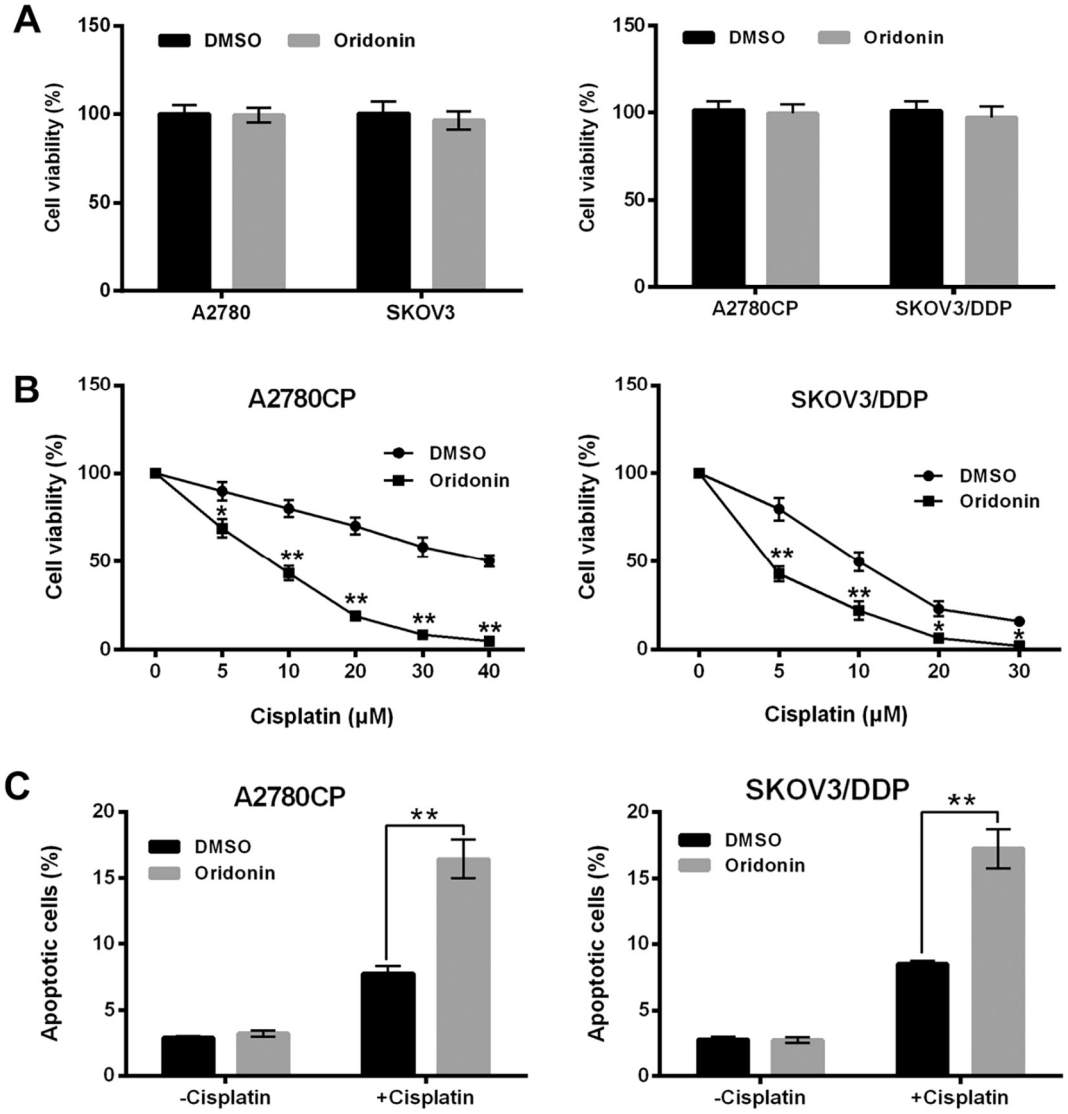

Fig. 1. Oridonin elevated cisplatin-caused ovarian carcinoma cell viability inhibition and apoptosis.
(A) A2780, SKOV3, A2780CP and SKOV3/DDP cells were exposed to 10 μM oridonin for 48 h respectively. CCK-8 assay showed that 10 μM oridonin for 48 h treatment had no significant influence on A2780, SKOV3, A2780CP and SKOV3/DDP cell viability. (B) A2780CP and SKOV3/DDP cells were exposed to 5, 10, 20, 30 or 40 μM cisplatin and/or 10 μM oridonin treatment for 48 h. CCK-8 assay displayed that 10 μM oridonin treatment promoted the 5–40 μM cisplatin treatment-caused A2780CP and SKOV3/DDP cell viability inhibition. (C) Followed by 10 μM cisplatin and/or 10 μM oridonin treatment for 48 h, Annexin V and PI staining with flow cytometry analysis showed that 10 μM oridonin treatment elevated the 10 μM cisplatin treatment-caused A2780CP and SKOV3/DDP cell apoptosis. N = 3. Data were presented as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$.

[13], which illustrated that oridonin possessed chemo-sensitization activity on ovarian carcinoma. To further understanding the molecular mechanism related to the chemo-sensitization activity of oridonin on ovarian carcinoma, more experimental researches are still needed.

Cisplatin (CAS number: 15663-27-1) is a widely used clinical first-line chemotherapy drug for ovarian carcinoma [14,15]. It can bind to ovarian carcinoma cell DNA, cause cross-linking, disrupt DNA function and thereby repress cell mitosis and induce cell apoptosis [16]. Previous studies proved that the occurrence and development of cisplatin resistance contributed to the failure of ovarian carcinoma treatment [17,18]. Wang *et al.* reported that elevation of cell autophagy was connected with the ovarian carcinoma cell resistance to cisplatin treatment [19]. They propose that as an intracellular protein self-degradation process, autophagy can offer ovarian carcinoma cells with a survival-promoting pathway in response to unfavorable environment, such as cisplatin treatment [19]. Moreover, ovarian carcinoma cells can active autophagy to promote growth and aggressiveness [20].

In the current research, two human ovarian carcinoma cisplatin-resistant cell lines, A2780CP and SKOV3/DDP, were constructed to probe whether oridonin exert chemo-sensitization activity on cisplatin-treated ovarian carcinoma cells via modulating cell autophagy. We believe that the outcomes of our research will offer experimental evidences for further comprehending the chemo-sensitization activity of oridonin on cisplatin-resistant ovarian carcinoma.

2. Materials and methods

2.1. Cell lines

A2780CP and SKOV3/DDP cells, were constructed from A2780 and SKOV3 cells (National Infrastructure of Cell Line Resource, Beijing, China), respectively. Briefly, A2780 cells and SKOV3 cells were cultivated at 37 °C with 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM, High Glucose, 10566-016, Gibco, CA, USA) or McCoy's 5A

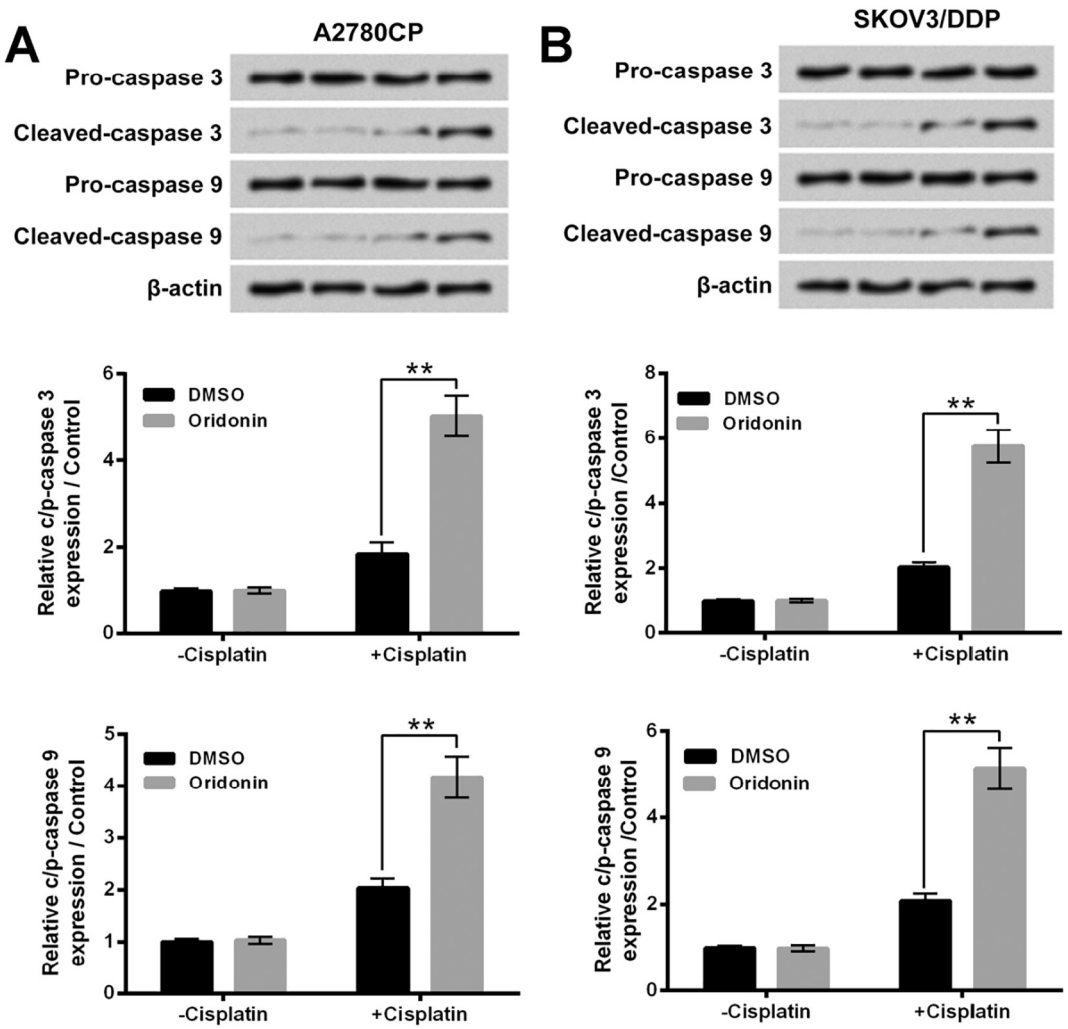

Fig. 2. Oridonin elevated cisplatin-caused up-regulation of c/p-caspase 3 and c/p-caspase 9 expression rates in ovarian carcinoma cells.
(A and B) A2780CP and SKOV3/DDP cells were exposed to 10 μM cisplatin and/or 10 μM oridonin treatment for 48 h. Western blotting analysis showed that 10 μM oridonin treatment elevated the 10 μM cisplatin treatment-caused up-regulation of c/p-caspase 3 and c/p-caspase 9 expression rates. N = 3. The most representative results were shown. Data were presented as mean ± SD. $^{**}P < 0.01$.

Media (Modified with Tricine, 16600-082, Gibco) respectively including 10% fetal bovine serum (FBS, SH30396, HyClone, UT, USA) in 75 cm^2 flask. Then, cells in logarithmic growth phase were grown in DMEM or McCoy's 5A Media containing 10% FBS and cisplatin (purity > 99%, HY-17394, MedChem Express, NJ, USA). The concentration of cisplatin was gradually increased to 2.5 μM. Finally, A2780CP and SKOV3/DDP cell lines that can fully tolerate 2.5 μM cisplatin treatment were constructed (the IC50 of cisplatin for A2780CP cells were 41.47 ± 1.24 μM, the IC50 of cisplatin for SKOV3/DDP cells were 9.52 ± 1.68 μM). In order to ensure the accuracy of the experiments, A2780CP and SKOV3/DDP cells were continuously cultured in DMEM or McCoy's 5A Media containing 10% FBS and 2.5 μM cisplatin for 1 month. Then, the following experiments were carried out. A2780CP and SKOV3/DDP cells at 10–15 passage were used in our experiments.

Human normal ovarian epithelial IOSE 364 cells were a gift from Dr. Auersperg at University of British Columbia (Vancouver, BC, Canada) and grown at 37 °C with 5% CO_2 in Roswell Park Memorial Institute (RPMI)-1640 medium (R8758, Sigma-Aldrich, MO, USA) containing 10% FBS in 75 cm^2 flask.

2.2. Experiment treatment

Oridonin (purity > 99.85%) was received from MedChem Express (HY-N0004). Oridonin powder was diluted in dimethyl sulfoxide (DMSO, ST038, Beyotime Biotechnology, Shanghai, China) to 100 mM. A2780CP, SKOV3/DDP and IOSE 364 cells were treated by 10 μM oridonin for 48 h in our experiments [21,22].

3-Methyladenine (3-MA) and rapamycin were also received from MedChem Express (HY-19312 and HY-10219). 3-MA (10 mM) and rapamycin (10 μM) acted as autophagy inhibitor or activator respectively added into culture medium at the same time with oridonin and/or cisplatin treatment [23,24].

2.3. Achievement of Beclin-1 overexpression

The full-length sequence of Beclin-1 was inserted in pEX-2 plasmid (Invitrogen, CA, USA) and named as pBeclin-1. Empty pEX-2 plasmid was served as negative control (NC). Lipofectamine 3000 reagent (L3000-008, Invitrogen) was utilized for pBeclin-1 or pEX-2 transfection. Transfection efficiency was evaluated by determining Beclin-1 protein levels in A2780CP and SKOV3/DDP cells.

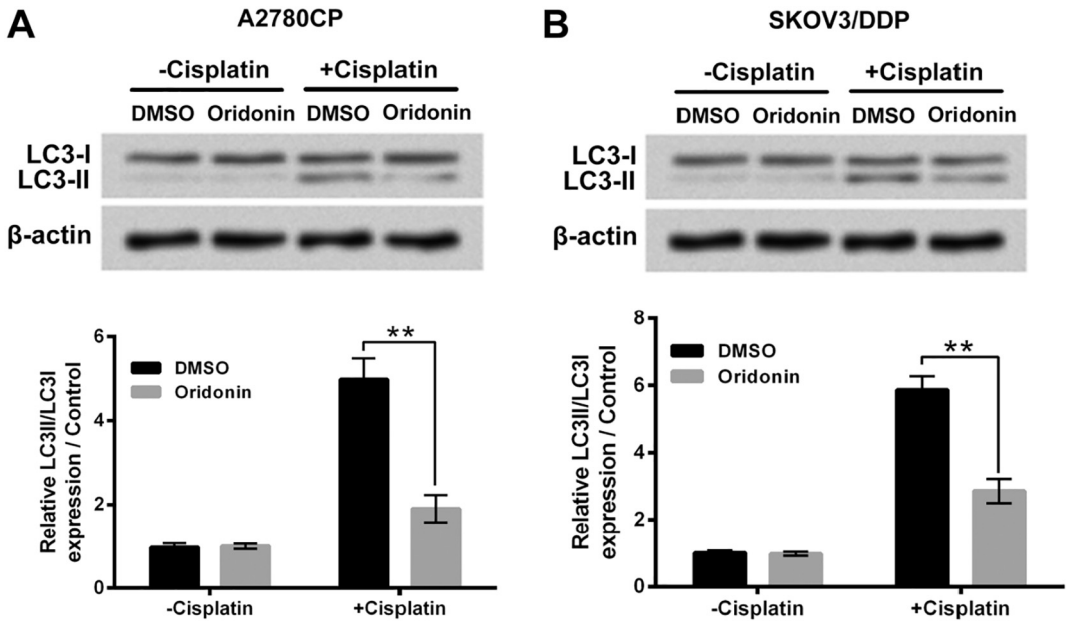

Fig. 3. Oridonin inhibited cisplatin-caused ovarian carcinoma cell autophagy.
(A and B) Followed by 10 μM cisplatin and/or 10 μM oridonin treatment for 48 h, western blotting analysis showed that 10 μM oridonin treatment reduced the 10 μM cisplatin treatment-caused up-regulation of LC3-II/LC3-I expression rates in A2780CP and SKOV3/DDP cells. N = 3. The most representative results were shown. Data were presented as mean ± SD. $^{**}P < 0.01$.

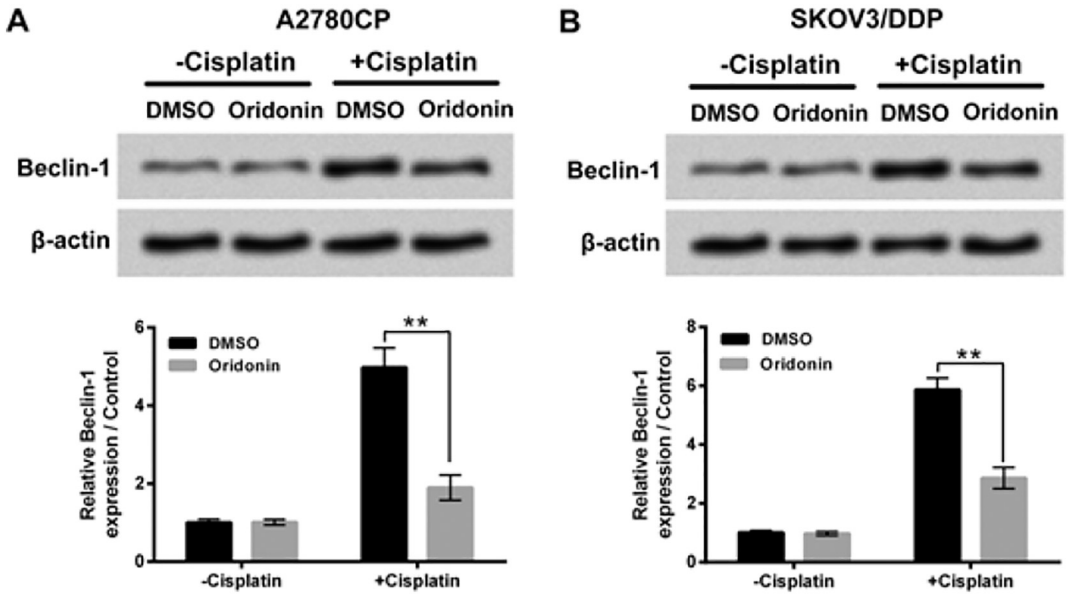

Fig. 4. Oridonin weakened cisplatin-caused up-regulation of Beclin-1 in ovarian carcinoma cells.
(A and B) Followed by 10 μM cisplatin and/or 10 μM oridonin treatment for 48 h, western blotting analysis showed that 10 μM oridonin treatment weakened the 10 μM cisplatin treatment-caused up-regulation of Beclin-1 protein levels in A2780CP and SKOV3/DDP cells. N = 3. The most representative results were shown. Data were presented as mean ± SD. $^{**}P < 0.01$.

2.4. Assessment of cell viability

Cell counting kit-8 (CCK-8) kit (HY-K0301, MedChem Express) offers a method to assess the viabilities of A2780CP, SKOV3/DDP and IOSE 364 cells. Cells were cultivated into 96-well plate with 1×10^4 cells/well in 100 μl culture medium with or without 5–40 μM cisplatin and/or 10 μM oridonin treatment. Same dosage of DMSO was added into the control group. After that, 10 μl CCK-8 solution was supplemented into each well. Followed by placing the 96-well plate at 37 °C for 1 h, the absorbance of each group was determined using Micro-plate Reader (Molecular Device, CA, USA) at 450 nm. Cell viability (%) was represented as the percentage of control.

2.5. Measurement of cell apoptosis

Dead Cell Apoptosis Kit with Annexin V-FITC and PI (V13242, Invitrogen) offers a method to measure A2780CP, SKOV3/DDP and IOSE 364 cell apoptosis. Cells were cultivated into 6-well plate with 1×10^5 cells/well in 1 ml culture medium with or without 10 μM cisplatin and/or 10 μM oridonin treatment. Same dosage of DMSO was added into the control group. After that, adherent and floating cells were collected according to experimental group and rinsed with kit

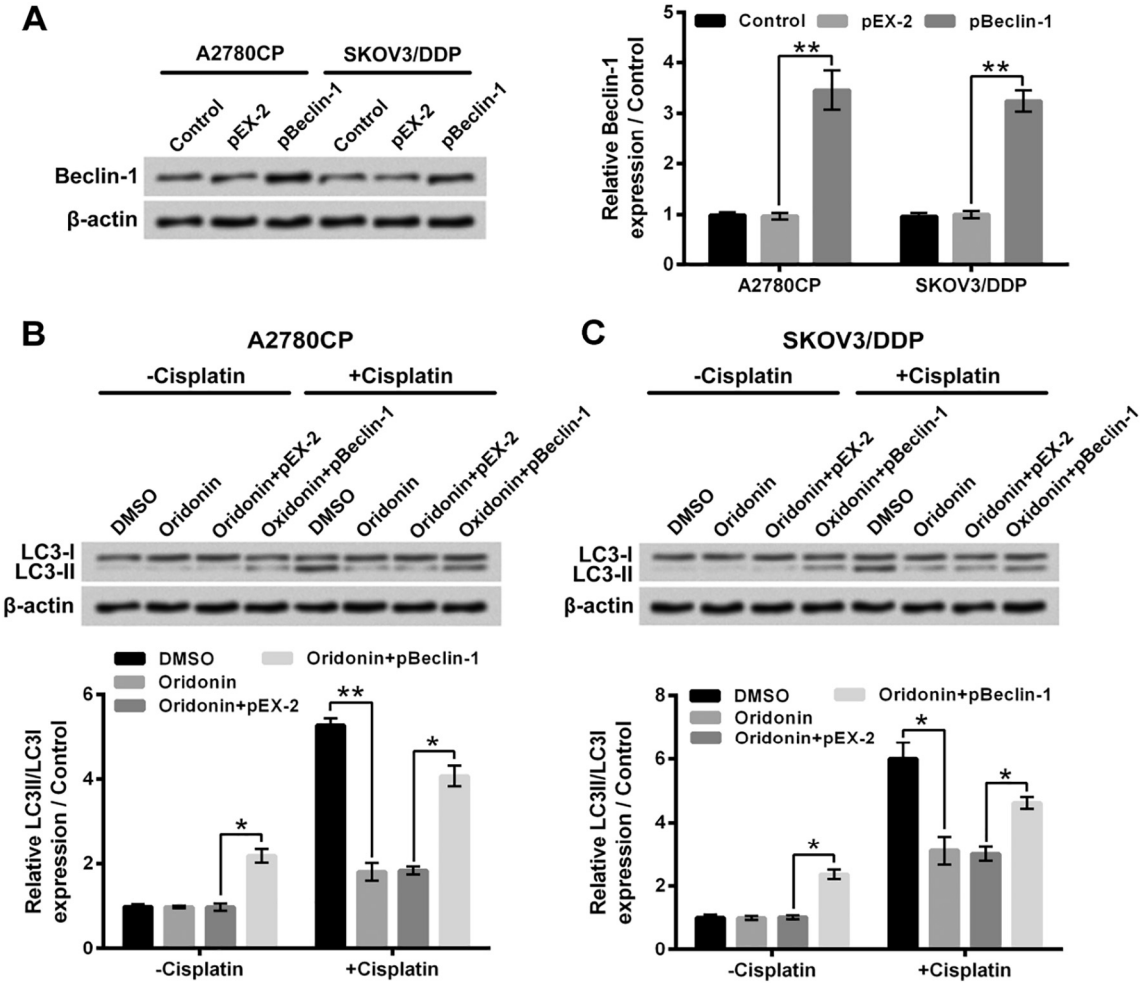

Fig. 5. Overexpression of Beclin-1 mitigated the influence of oridonin on cisplatin-caused ovarian carcinoma cell autophagy.
(A) After pBeclin-1 transfected into A2780CP and SKOV3/DDP cells, western blotting analysis showed that the Beclin-1 protein levels were increased. (B) Followed by 10 μM cisplatin and/or 10 μM oridonin treatment or pBeclin-1 transfection, western blotting analysis showed that the LC3-II/LC3-I expression rates in A2780CP and SKOV3/DDP cells were increased. N = 3. The most representative results were shown. Data were presented as mean ± SD. $*P < 0.05$, $**P < 0.01$.

buffer. Then, 200 μl of cells in kit buffer were mixed with 5 μl Annexin V-FITC solution and 1 μl PI solution at 37 °C for 25 min in the dark. The percentages of apoptotic A2780CP or SKOV3/DDP cells were measured using flow cytometer (Attune Nxt, Thermo Fisher Scientific, MA, USA).

2.6. Western blotting analysis

Total proteins were extracted from A2780CP and SKOV3/DDP cells with the help of Cell Lysis Buffers (No. 635656, Clotech, CA, USA) including Protease and Phosphatase Inhibitor Cocktail (P1045, Beyotime Biotechnology). The concentration of total proteins was calculated by using BCA Protein Assay kit (P0011, Beyotime Biotechnology). Western blotting was carried out using Bio-Rad Bis-Tris Gel system (Bio-Rad Laboratories, CA, USA) as previous described [25]. Briefly, total proteins with equal concentration were electrophoresed on polyacrylamide gels and transferred onto polyvinylidene difluoride (PVDF) membrane (Millipore, MA, USA) that were incubated with relevant antibodies. Anti-Caspase 3 antibody (#9662), anti-Caspase 9 antibody (#9502), anti-microtubule-associated protein 1A/1B-light chain 3 (LC3) antibody (#4108), anti-Beclin-1 antibody (#3738) and anti-β-actin antibody (#4970) were all supplied by Cell Signaling Technology (MA, USA). All primary antibodies were diluted into 1% bovine serum albumin (BSA, Sigma-Aldrich) solution with 1: 1000. Followed by blocking with 5% BSA solution for 1 h at room temperature, the PVDF membranes were incubated with primary antibodies for 12 h at 4 °C. After that, the PVDF membranes carrying proteins and primary antibodies were incubated with anti-mouse IgG (H + L) DyLight™ 680 conjugate secondary antibody (#5470) or anti-rabbit IgG (H + L) DyLight™ 680 conjugate secondary antibody (#5366, Cell Signaling Technology) for 1.5 h at room temperature protected from light. The signals of proteins were recorded and the intensities of bands were quantified using OdysseyClx Infrared imaging system (Licor Biosystems, NE, USA) and Odyssey software, respectively.

2.7. Statistical analysis

Prism 6.0 software (Graphpad Software, CA, USA) was utilized for statistical analysis. Data were represented as mean ± standard deviation (SD) from three independent experiments. *P*-values between two groups were determined with the help of Student's *t*-test. *P*-values between more than three groups were determined with the help of one-way analysis of variance (ANOVA) with Tukey *t*-test. $P < 0.05$ was regarded as significant difference.

3. Results

3.1. Oridonin elevated sensitivity of ovarian carcinoma cells to cisplatin treatment

Firstly, we detected the viabilities of A2780, SKOV3, A2780CP and

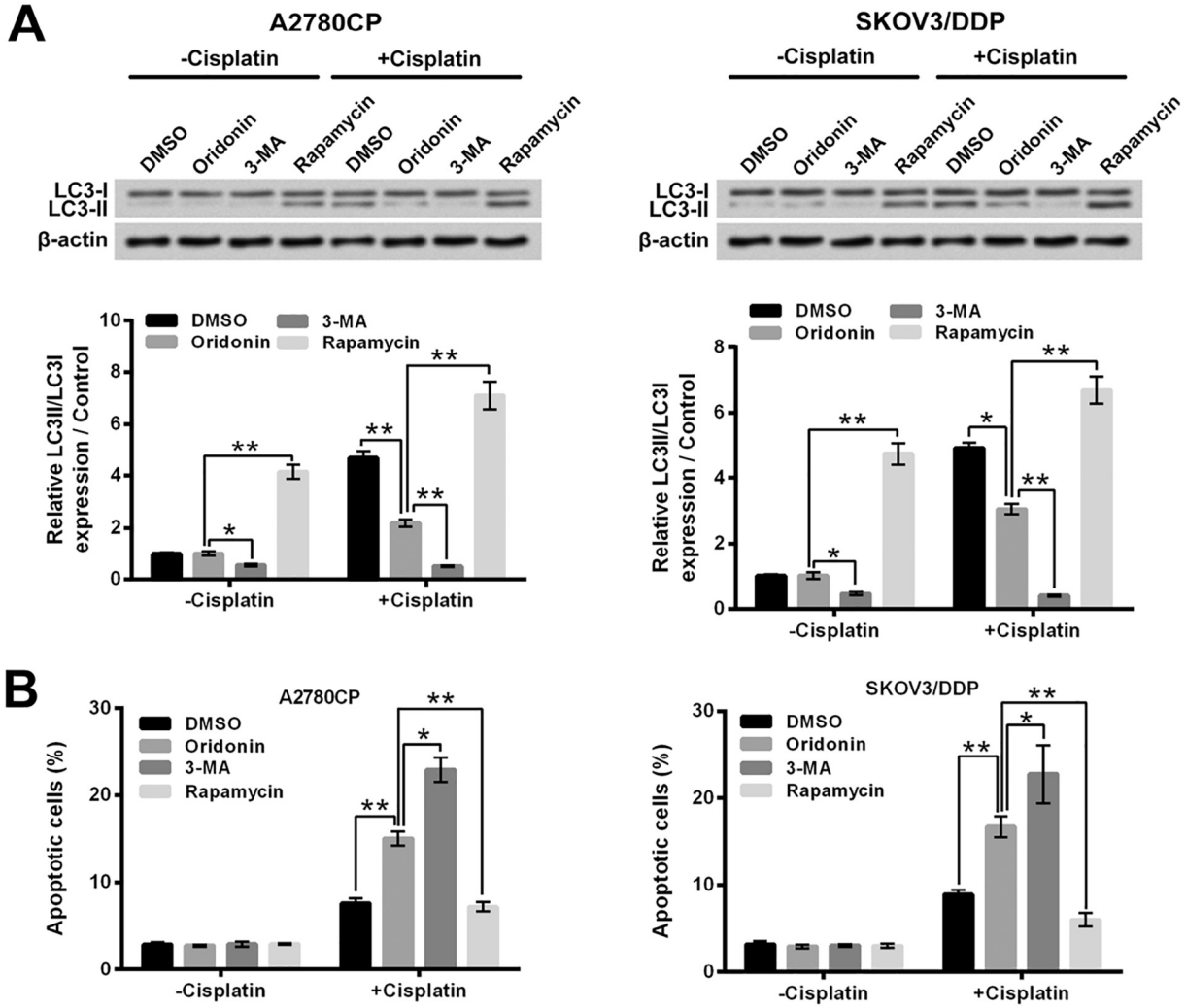

Fig. 6. Oridonin promoted cisplatin-caused ovarian carcinoma cell apoptosis via inhibiting cell autophagy.
A2780CP and SKOV3/DDP cells were exposed to 10 μM cisplatin, and/or 10 μM oridonin, 10 mM 3-MA or 10 μM rapamycin treatment, (A) western blotting analysis showed that the LC3-II/LC3-I expression rates were decreased in 3-MA co-treatment group and increased in rapamycin co-treatment group, (B) Annexin V and PI staining with flow cytometry analysis showed that the apoptosis of A2780CP and SKOV3/DDP cells were increased in 3-MA co-treatment group and decreased in rapamycin co-treatment group. N = 3. The most representative results were shown. Data were presented as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$.

SKOV3/DDP cells after 10 μM oridonin treatment for 48 h. Fig. 1A showed that 10 μM oridonin treatment for 48 h had no significant influence on both A2780, SKOV3 and A2780CP, SKOV3/DDP cell viabilities. Then, we tested the viabilities of A2780CP and SKOV3/DDP cells after 5–40 μM cisplatin and/or 10 μM oridonin co-treatment. Data in Fig. 1B displayed that 10 μM oridonin co-treatment remarkably promoted the 5–40 μM cisplatin treatment-caused reduction of A2780CP and SKOV3/DDP cell viabilities ($P < 0.05$ or $P < 0.01$). 10 μM cisplatin treatment was chosen for further experiments. Fig. 1C exhibited that 10 μM oridonin treatment had no obvious influence on A2780CP and SKOV3/DDP cell apoptosis, but significantly promoted the apoptosis of A2780CP and SKOV3/DDP cells caused by 10 μM cisplatin treatment ($P < 0.01$). Besides, the expression rates of cleaved-caspase 3 and pro-caspase 3 (c/p-caspase 3), as well as cleaved-caspase 9 and pro-caspase 9 (c/p-caspase 9) in A2780CP and SKOV3/DDP cells were both dramatically elevated after co-treatment with 10 μM cisplatin and 10 μM oridonin, relative to single 10 μM cisplatin treatment (Fig. 2A and B, $P < 0.01$). These outcomes illustrated that oridonin could elevate sensitivity of ovarian carcinoma cells to cisplatin treatment.

3.2. Oridonin inhibited cisplatin-caused ovarian carcinoma cell autophagy

LC3 is a key participant of cell autophagy. During autophagy, a cytosolic form of LC3 (LC3-I) is conjugated to phosphatidylethanolamine to from LC3-phosphatidylethanolamine conjugate (LC3-II), which is recruited to autophagosomal membranes [26]. To measure the autophagy of A2780CP and SKOV3/DDP cells after 10 μM oridonin and/or 10 μM cisplatin treatment, the expression rate of LC3-II and LC3-I (LC3-II/LC3-I) were tested by using western blotting. Results in Fig. 3A and B displayed that 10 μM oridonin single treatment had no obvious influence on LC3-II/LC3-I expression rates in A2780CP and SKOV3/DDP cells, while 10 μM cisplatin single treatment notably elevated the LC3-II/LC3-I expression rates in A2780CP and SKOV3/DDP cells. By contrast with 10 μM cisplatin single treatment group, the LC3-II/LC3-I expression rates in A2780CP and SKOV3/DDP cells were notably lowered in 10 μM cisplatin + 10 μM oridonin co-treatment group ($P < 0.01$). These outcomes hinted that oridonin could inhibit cisplatin-caused ovarian carcinoma cell autophagy.

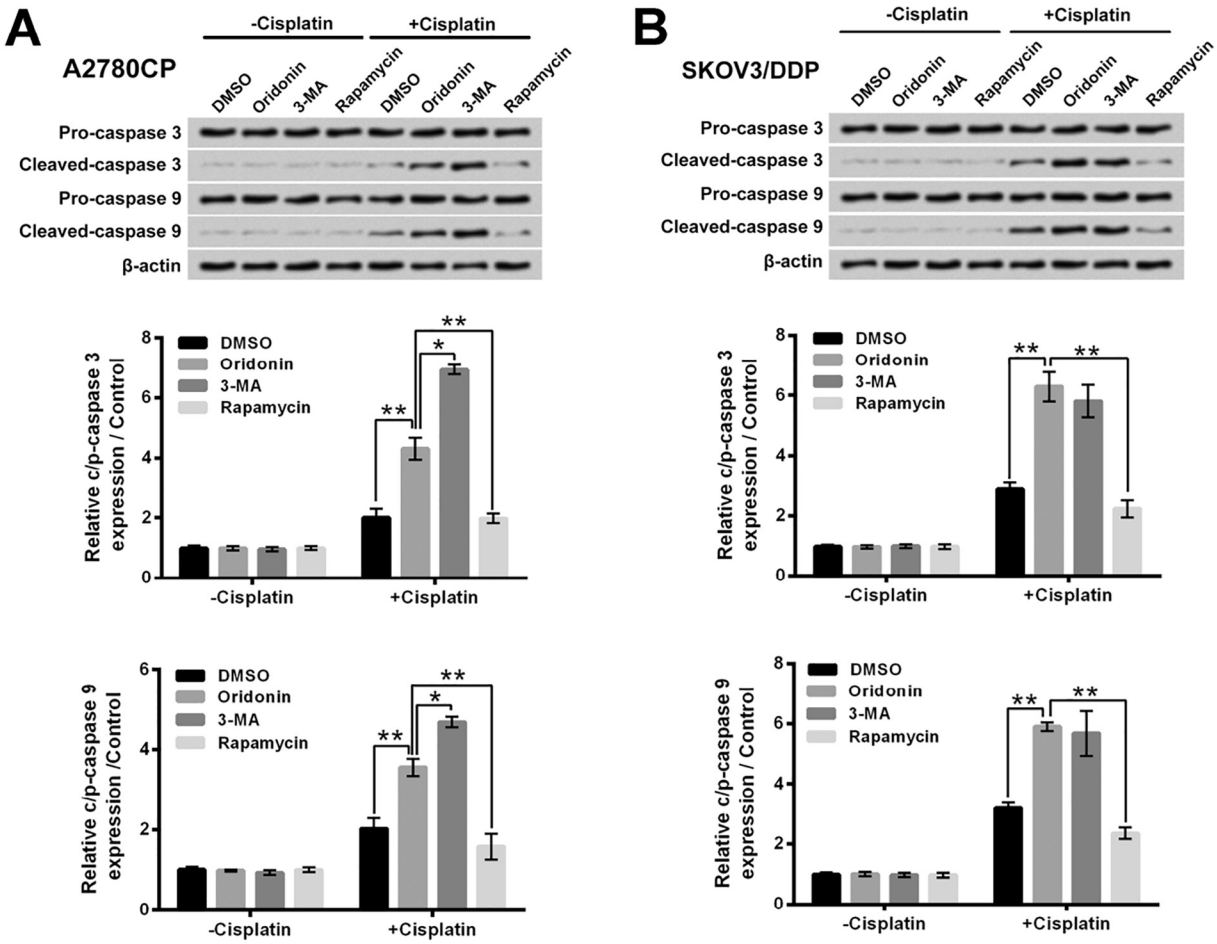

Fig. 7. Inhibition of cell autophagy took part in the up-regulation of the c/p-caspase 3 and c/p-caspase 9 expression rates in ovarian carcinoma cells.
(A and B) A2780CP and SKOV3/DDP cells were exposed to 10 μM cisplatin, and/or 10 μM oridonin, 10 mM 3-MA or 10 μM rapamycin treatment, western blotting analysis showed that the c/p-caspase 3 and c/p-caspase 9 expression rates were increased in 3-MA co-treatment group and decreased in rapamycin co-treatment group. N = 3. The most representative results were shown. Data were presented as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$.

3.3. Oridonin weakened cisplatin-caused up-regulation of Beclin-1 in ovarian carcinoma cells

Besides, Beclin-1 is a core component of autophagy machinery that plays critical roles in the regulation of cell autophagy by engaging in the formation of autophagosome [27]. We also measured the Beclin-1 protein levels in A2780CP and SKOV3/DDP cells after 10 μM oridonin and/or 10 μM cisplatin treatment. Data in Fig. 4A and B showed that 10 μM oridonin single treatment also had no obvious influence on Belcin-1 protein levels in A2780CP and SKOV3/DDP cells. 10 μM cisplatin single treatment notably elevated the Belcin-1 protein levels in A2780CP and SKOV3/DDP cells. Relative to 10 μM cisplatin single treatment group, the Belcin-1 protein levels in A2780CP and SKOV3/DDP cells were remarkably reduced in 10 μM cisplatin + 10 μM oridonin co-treatment group ($P < 0.01$). These outcomes further illustrated that oridonin could inhibit cisplatin-caused ovarian carcinoma cell autophagy and implied that down-regulation of Beclin-1 might take part in this process.

3.4. Overexpression of Beclin-1 mitigated the influence of oridonin on cisplatin-caused ovarian carcinoma cell autophagy

In order to investigate the role of Beclin-1 down-regulation in A2780CP and SKOV3/DDP cell autophagy caused by oridonin and/or cisplatin, pBeclin-1 was transfected into A2780CP and SKOV3/DDP cells to overexpress Beclin-1. Fig. 5A showed that the Beclin-1 protein levels in both A2780CP and SKOV3/DDP cells were dramatically boosted by pBeclin-1 transfection ($P < 0.01$). Results in Fig. 5B and C exhibited that pBeclin-1 transfection both significantly elevated the LC3-II/LC3-I expression rates in A2780CP and SKOV3/DDP cells after 10 μM oridonin treatment ($P < 0.05$). Moreover, by contrast with 10 μM oridonin + 10 μM cisplatin + Pex-2 group, the LC3-II/LC3-I expression rates in A2780CP and SKOV3/DDP cells were notably enhanced in 10 μM oridonin + 10 μM cisplatin + pBeclin-1 group ($P < 0.05$). These outcomes illustrated that oridonin inhibited cisplatin-caused ovarian carcinoma cell autophagy at least be achieved via suppressing Beclin-1 protein level.

3.5. Oridonin promoted cisplatin-caused ovarian carcinoma cell apoptosis via inhibiting cell autophagy

Subsequently, we want to analyze the relationship between elevation of cell apoptosis and reduction of cell autophagy caused by 10 μM oridonin + 10 μM cisplatin co-treatment. 3-MA is an effective autophagy inhibitor that can suppress formation of autophagosome [28]. Rapamycin is an effective autophagy activator that can simulate cell starvation [29]. In our research, 3-MA and rapamycin were utilized to probe whether reduction of cell autophagy was the reason for elevation of cell apoptosis and increased sensitivity of A2780CP and SKOV3/DDP cells to cisplatin treatment. Fig. 6A showed that the LC3-II/LC3-I expression rates in A2780CP and SKOV3/DDP cells were both noticeably decreased by 10 mM 3-MA incubation and enhanced by 10 μM

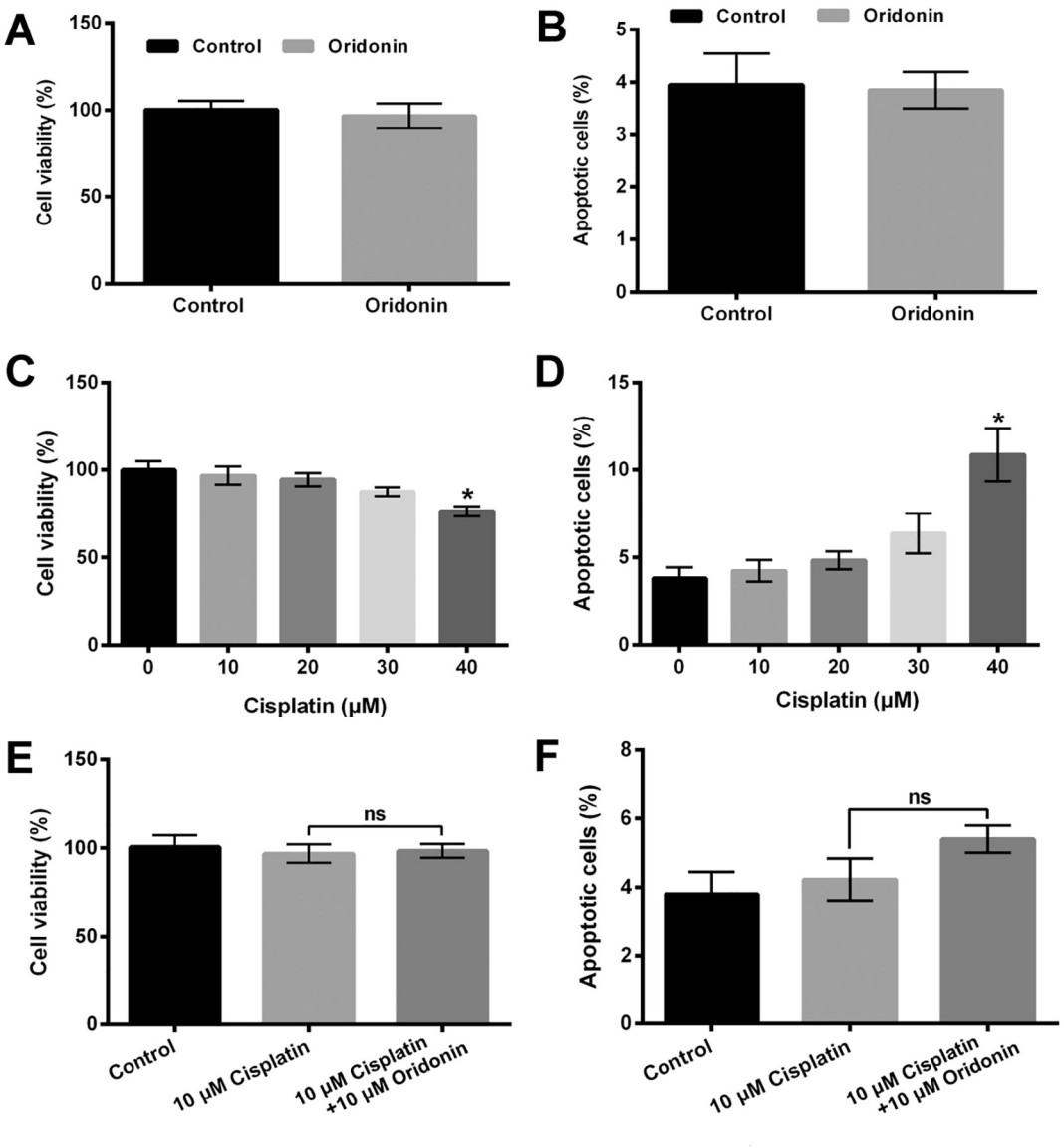

Fig. 8. Oridonin and cisplatin co-treatment had no significant effects on human normal ovarian epithelial IOSE 364 cell viability and apoptosis.
Followed by 10 μM oridonin treatment for 48 h, (A) CCK-8 assay showed that the viability of IOSE 364 cells was not changed, (B) Annexin V and PI staining with flow cytometry analysis showed that the apoptosis of IOSE 364 cells was also not changed. Followed by 10–40 μM cisplatin treatment for 48 h, (C) CCK-8 assay showed that 10–30 μM cisplatin treatment had no significant effects on IOSE 364 cell viability, while 40 μM cisplatin treatment reduced the viability of IOSE 364 cells, (D) Annexin V and PI staining with flow cytometry analysis showed that 10–30 μM cisplatin treatment had no significant effects on IOSE 364 cell apoptosis, while 40 μM cisplatin treatment promoted the apoptosis of IOSE 364 cells. Followed by 10 cisplatin and/or 10 μM oridonin co-treatment for 48 h, (E) CCK-8 assay showed that the viability of IOSE 364 cells was not changed, (F) Annexin V and PI staining with flow cytometry analysis showed that the apoptosis of IOSE 364 cells was also not changed. N = 3. Data were presented as mean ± SD. NS: Non-significant.

rapamycin incubation ($P < 0.05$ or $P < 0.01$). By contrast with 10 μM oridonin + 10 μM cisplatin treatment group, the LC3-II/LC3-I expression rates in A2780CP and SKOV3/DDP cells were also reduced in 10 μM oridonin + 10 μM cisplatin + 10 mM 3-MA treatment group, but enhanced in 10 μM oridonin + 10 μM cisplatin + 10 μM rapamycin treatment group ($P < 0.01$). Moreover, compared to 10 μM oridonin + 10 μM cisplatin treatment group, the apoptosis of A2780CP and SKOV3/DDP cells were enhanced in 10 μM oridonin + 10 μM cisplatin + 10 mM 3-MA treatment group (Fig. 6B, $P < 0.05$), but reduced in 10 μM oridonin + 10 μM cisplatin + 10 μM rapamycin treatment group ($P < 0.01$), which were accompanied with the increased expression rates of c/p-caspase 3 and c/p-caspase 9 in 10 μM oridonin + 10 μM cisplatin + 10 mM 3-MA treatment group (Fig. 7A and B, $P < 0.05$) and decreased expression rates of c/p-caspase 3 and c/p-caspase 9 in 10 μM oridonin + 10 μM cisplatin + 10 μM rapamycin treatment group ($P < 0.01$). These outcomes exhibited that oridonin elevated sensitivity of ovarian carcinoma cells to cisplatin treatment was achieved by suppressing cisplatin-mediated cell autophagy.

3.6. Oridonin and cisplatin co-treatment had no significant effects on IOSE 364 cell viability and apoptosis

Finally, we tested the influences of oridonin and/or cisplatin treatment on human normal ovarian epithelial IOSE 364 cell viability and apoptosis. Fig. 8A and B showed that 10 μM oridonin treatment had no significant influences on IOSE 364 cell viability and apoptosis. 10–30 μM cisplatin also had no obvious effects on IOSE 364 cell viability and apoptosis, while 40 μM cisplatin reduced the viability of IOSE 364 cells (Fig. 8C and D, $P < 0.05$) and promoted IOSE 364 cell apoptosis ($P < 0.05$). By contrast with 10 μM cisplatin treatment

group, the viability and apoptosis of IOSE 364 cells were not changed in 10 μM oridonin + 10 μM cisplatin co-treatment group (Fig. 8E and F). These results illustrated that 10 μM oridonin + 10 μM cisplatin co-treatment had no obvious toxicity to normal ovarian epithelial cells.

4. Discussion

Due to the high level of acquired resistance to cisplatin-based chemotherapy and low 5-year survival rate (< 40%), ovarian carcinoma has become the most aggressive genital system tumor in women [2,30]. It believes that searching for effective chemo-sensitizer that can alleviate cisplatin resistance is of great important value for ovarian carcinoma therapy. Herein, we further verified the chemo-sensitization effect of oridonin on cisplatin-resistant ovarian carcinoma A2780CP and SKOV3/DDP cells. We discovered that oridonin could elevate the sensitivity of ovarian carcinoma cells to cisplatin-caused apoptosis. What's more, we discovered that suppression of cell autophagy caused by oridonin played key roles in the elevation of ovarian carcinoma cell apoptosis stimulated by cisplatin.

As an intracellular protein self-degradation process capable of upholding cellular metabolism under unsuitable environment, high level of autophagy can rescue tumor cells from death when treated by anticancer drugs [31]. Therefore, autophagy inhibition was found to be beneficial for cancer treatment by increasing the sensitivity of cancer cells to death [32,33]. Earlier experiments have proved that the induction of cancer cell apoptosis is primarily responsible for cisplatin anticancer activity to ovarian carcinoma [19,34]. Enhancement of autophagy has been demonstrated to contribute to cisplatin resistance for ovarian carcinoma, which will reduce ovarian cancer cell apoptosis [35]. In the present study, we discovered that oridonin single treatment had on significant effects on A2780CP and SKOV3/DDP cell viability. However, oridonin co-treatment with cisplatin remarkably promoted the cisplatin-caused reduction of A2780CP and SKOV3/DDP cell viability and enhancement of cell apoptosis. The expressions of pro-apoptotic proteins cleaved-caspase 3 and cleaved-caspase 9 were increased in A2780CP and SKOV3/DDP cell after oridonin and cisplatin co-treatment. In terms of analyzing cell autophagy, we found that oridonin co-treatment significantly inhibited the A2780CP and SKOV3/DDP cell autophagy via reducing the LC3-II/LC3-I and Beclin-1 protein expression rate (level). These outcomes illustrated that oridonin elevated apoptosis of ovarian carcinoma cells response to cisplatin treatment might be achieved by suppressing cell autophagy.

Beclin-1, also known as autophagy-associated protein 6 (ATG6), is a key regulator of cell autophagy [36]. Previous experiments indicated that Beclin-1 was closely associated with the oridonin-caused cancer cell autophagy [37,38]. Herein, we discovered that overexpression of Beclin-1 noticeably promoted A2780CP and SKOV3/DDP cell autophagy via elevating LC3-II/LC3-I protein expression rate, which illustrated that oridonin inhibited cisplatin-caused ovarian carcinoma cell autophagy at least in part via declining Beclin-1 protein level.

Subsequently, cell autophagy inhibitor 3-MA and activator rapamycin were supplemented in our experiments to further affirm the roles of cell autophagy in the oridonin-elevated sensitivity of ovarian carcinoma cells to cisplatin treatment. We found that 3-MA incubation dramatically promoted the oridonin + cisplatin-caused A2780CP and SKOV3/DDP cell apoptosis. On the contrary, rapamycin incubation had opposite effects. These findings confirmed that increase of cell autophagy was important to oridonin-elevated A2780CP and SKOV3/DDP cell apoptosis. Besides, we also found that oridonin and/or cisplatin treatment had no significant effects on human normal ovarian epithelial IOSE 364 cell viability and apoptosis, which suggested that oridonin and cisplatin co-treatment had no obvious toxicity to normal ovarian cells.

Taken together, in consistent with the previous study [13], we further verified the chemo-sensitization activity of oridonin on cisplatin-treated ovarian carcinoma cells *in vitro*. We found that oridonin elevated sensitivity of ovarian carcinoma cells to cisplatin via suppressing cisplatin-mediated autophagy. Considering that Ma *et al.* also reported that oridonin reversed cisplatin drug resistance in ovarian carcinoma cells also via inhibiting matrix metalloproteinases (MMPs)-mediated cell migration [13], we propose that oridonin might be a good chemo-sensitizer for enhancing cisplatin-resistant ovarian carcinoma cell apoptosis and repressing cell metastasis. More in vivo researches are still needed in future to confirm our proposal.

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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.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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