Zinc oxide nanoparticles inhibit malignant progression and chemotherapy resistance of ovarian cancer cells by activating endoplasmic reticulum stress and promoting autophagy

WENLI GU and CAIHONG YANG

Department of Gynecology, The General Hospital of Ningxia Medical University, Yinchuan, Ningxia 750001, P.R. China

Received April 28, 2023; Accepted August 30, 2023

DOI: 10.3892/etm.2023.12207

Abstract. The mortality rate of ovarian cancer (OC) is high, posing a serious threat to women's lives. Zinc oxide nanoparticles (ZnO-NPs) show great potential in the treatment of cancer. However, the mechanism of ZnO-NPs in inhibiting the malignant proliferation and chemotherapy resistance of OC has remained elusive. In the present study, ZnO-NPs at different concentrations were used to treat SKOV3 cells, and subsequently, analyses including the Cell Counting Kit-8 assay, EDU staining, colony-formation assay, flow cytometry, wound-healing assay, Transwell assay and western blot were used to detect cell proliferation, invasion, migration, epithelial-mesenchymal transition (EMT) and chemotherapy resistance, as well as endoplasmic reticulum stress (ERS)- and autophagy-related indicators. Finally, the mechanisms of action of ZnO-NPs on OC were examined by adding ERS inhibitor 4-phenylbutyric acid (4-PBA) and autophagy inhibitor 3-methyladenine (3-MA). It was found that ZnO-NPs inhibited SKOV3 cell proliferation, facilitated apoptosis and induced cell cycle arrest. Furthermore, ZnO-NPs inhibited the invasion, migration and EMT of SKOV3 cells. ZnO-NPs also inhibited chemotherapy resistance of SKOV3 cells. ZnO-NPs activated ERS and promoted autophagy. The addition of 4-PBA or 3-MA significantly reversed the effects of ZnO-NPs on SKOV3 cells. Overall, ZnO-NPs inhibit the malignant progression and the chemotherapy resistance of SKOV3 cells by activating ERS and promoting autophagy.

Introduction

Ovarian cancer (OC) ranks third in incidence among female reproductive tract malignant tumors and it ranks first in terms of its mortality rate. The onset of OC is insidious, with no typical symptoms in the early stage, and most of the patients are clinically diagnosed in the intermediate and late stages (1). According to global cancer statistics, there were ~300,000 new cases of OC and >180,000 associated deaths worldwide in 2020, with a mortality to morbidity ratio of >0.6 (2). At present, for patients with OC, the main treatment options are total resection without pathological staging or combined surgery to reduce the total tumor and adjuvant chemotherapy after surgery. However, most of these patients relapse after initial treatment and platinum-sensitive patients may develop platinum-resistant recurrent OC as the number of courses increases, with a poor prognosis (3). In addition, there are numerous types of drugs that may be used as second-line chemotherapy for OC, but the efficacy of most of the treatments is poor, and there is no uniform treatment plan for the scenario that the tumor progresses again after second-line treatment (4). Therefore, it is urgent to find effective drugs for the prevention and treatment of the metastasis and drug-resistance of OC.

Nanomaterials are natural or artificial materials in powder or clumps consisting of one or more basic particles with a size ranging from 1-110 nm. They have the advantages of good selectivity, low side effects and long-term stability (5). In recent years, nanomaterial-based therapy has shown promise as an important strategy for the treatment of tumors. Zinc oxide nanoparticles (ZnO-NPs) are common engineering nanomaterials, which have exhibited great potential in tumor treatment due to their biocompatibility, biodegradability and unique physicochemical properties. A previous study has demonstrated that ZnO-NPs may induce apoptosis of breast cancer cells through a mitochondrial apoptotic pathway (6). In addition, ZnO-NPs have been evidenced to possess significant antitumor activity in various types of malignant tumor, including liver cancer, lung cancer, breast cancer, colon cancer, osteosarcoma and cervical cancer (7). Padmanabhan *et al* (8) have confirmed that ZnO-NPs may induce oxidative stress and proteotoxic stress in OC cells, thus promoting the apoptosis of OC cells. Furthermore, the combination of ZnO-NPs with cisplatin and gemcitabine may significantly enhance the pro-apoptotic effect of cisplatin and gemcitabine on non-small cell lung cancer (9). However, the mechanism of ZnO-NPs in inhibiting the malignant progression and the chemotherapy resistance of OC has remained to be elucidated.

As an important cell survival pathway, autophagy has an important role in the occurrence and development of a

Correspondence to: Dr Caihong Yang, Department of Gynecology, The General Hospital of Ningxia Medical University, 804 Shengli South Street, Xingqing, Yinchuan, Ningxia 750001, P.R. China
E-mail: yangch820624@163.com

Key words: zinc oxide nanoparticles, ovarian cancer, malignant progression, chemotherapy resistance, endoplasmic reticulum stress, autophagy

variety of diseases, including cancers (10-12). A large number of studies have confirmed that autophagy has a key function in controlling the tumor microenvironment and exerting tumor-inhibitory effects (13,14). By regulating autophagy, advances in biomaterials tailored for drug delivery have the potential to overcome the limited selectivity and side effects frequently associated with traditional therapeutic agents in tumors (15). In addition, Liu *et al* (16) have confirmed that ZnO-NPs at non-cytotoxic concentrations can promote autophagy, while ZnO-NPs at cytotoxic concentrations can inhibit autophagy. However, the exact regulatory roles of ZnO-NPs in autophagy in OC have remained elusive.

Endoplasmic reticulum stress (ERS) that results from external stimuli or intracellular damage is often associated with autophagy, hypoxia signaling or reactive oxygen species (ROS) responses, and has a close relation with a variety of human diseases, including malignant tumors (17). The relationship between ERS and tumorigenesis has also been a hot topic in recent years. ERS may not only induce apoptosis of tumor cells, but also promote cell survival and lead to drug resistance of tumor cells (18). Therefore, an in-depth understanding of the complexity of ERS may contribute to the discovery of novel drug targets and therapeutic intervention strategies. A previous study has found that ZnO-NPs have an important role in inducing ERS (19). However, it has remained elusive whether the effect of ZnO-NPs on ERS is associated with the malignant proliferation and chemotherapy resistance of OC.

Therefore, the present study investigated the mechanism of ZnO-NPs in the regulation of the malignant development and chemotherapy resistance of OC, intending to provide novel insight for the clinical use of ZnO-NPs as a treatment for OC.

Materials and methods

Cell culture. Human OC SKOV3 cells (cat. no. YS2383C; YaJi Biological) and cisplatin (DDP)-resistant SKOV3/DDP cells (cat. no. YS3657C; YaJi Biological) were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) at 37˚C with 5% CO_2. DDP (1 μg/ml; MedChemExpress) was added to the medium of SKOV3/DDP cells to maintain the chemoresistance of cells. ZnO-NPs were prepared as previously described (20). Different concentrations of ZnO-NPs (1, 10, 20, 30, 40, 50, 60, 70 and 80 μg/ml) were used to treat SKOV3 or SKOV3/DDP cells for 24 h. In order to further explore the effects of ZnO-NPs on ERS and autophagy, ERS inhibitor 4-phenylbutyric acid (4-PBA; 7 mM) was used to pretreat SKOV3 or SKOV3/DDP cells for 4 h (21) and autophagy inhibitor 3-methyladenine (3-MA; 200 μM) was used to pretreat SKOV3 or SKOV3/DDP cells for 1 h (22).

Cell counting kit-8 (CCK8) assay. The viability of cells in all groups was measured using a CCK-8 kit (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions. At the end of the incubation periods, 10 μl CCK-8 solution was added to each well and cells were incubated for an additional 2 h. The absorbance was read at an optical density of 450 nm using a microplate reader. The cell viability was presented as a percentage of the control.

EdU staining assay. Following the indicated treatments, cells were incubated with EdU (20 mmol/l) for 2 h. Subsequently, the cells were fixed with 4% paraformaldehyde for 20 min at room temperature according to the kit instructions (cat. no. ab219801; Abcam). Subsequently, the cells were permeated with 0.5% Triton X-100 for 15 min at room temperature. After the addition of added Click reaction solution, the cells were incubated in the dark for 30 min at room temperature. Finally, images of the cells were acquired under a fluorescence microscope.

Colony-formation assay. Following the indicated treatments, cells were cultured in RPMI-1640 medium at 37˚C for 14 days. Subsequently, the cells were fixed with 4% paraformaldehyde for 15 min at room temperature and stained with 0.1% crystal violet for 15 min at room temperature. The number of colonies (>50 cells) was counted under a microscope.

Flow cytometric analysis. For apoptosis analysis, the Annexin V-FITC Apoptosis Detection kit (Biobud Inc.) was used according to the manufacturer's instructions. The cells were suspended in the binding buffer and then stained with propidium iodide (PI) for 5 min and FITC-conjugated Annexin V for 15 min in the dark at 4˚C. For cell cycle analysis, the Cell Cycle Detection Kit (Keygen Biotech) was used according to the manufacturer's instructions. The cells were fixed in 70% cold ethanol at 4˚C overnight. Following centrifugation (450 x g at 4˚C and 5 min) and washing with PBS, cells were then stained with 500 μl PI RNase at room temperature for 30 min in the dark. Finally, the analysis of cell apoptosis and cell cycle was performed with a flow cytometer (BD LSRFortessa).

Western blot analysis. To obtain the total protein, cells with the indicated treatments were lysed on ice for 30 min with RIPA lysis buffer (Beyotime Institute of Biotechnology) and then centrifuged at 24,080 x g for 10 min at 4˚C. The concentration of proteins was measured by the BCA method (Bio-Rad Laboratories, Inc.). A total of 30 μg protein per lane was separated by 12% SDS-PAGE and then transferred to PVDF membranes (iBlot PVDF Regular Stacks; Invitrogen; Thermo Fisher Scientific, Inc.). After blocking with 5% BSA (Invitrogen; Thermo Fisher Scientific, Inc.), the membranes were probed with primary antibodies at 4˚C overnight and subsequently incubated with secondary antibody (cat. no. ab6721; 1:5,000 dilution; Abcam) for 1 h on the next day. Finally, an Enhanced ECL Chemiluminescent Substrate Kit (Yeasen Biotechnology Co., Ltd.) was used to develop the protein bands and the signal intensity was measured with ImageJ software (v1.53a; National Institutes of Health). The following antibodies were used: 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), phosphorylated-PKR-like endoplasmic reticulum kinase (p-PERK; 1:1,000; cat. no. ab79483; Abcam), PERK (1:1,000; cat. no. ab229912; Abcam), phosphorylated-eukaryotic translation initiation factor 2α (p-eIF2α; 1:1,000; cat. no. ab32157; Abcam), eIF2α (1:1,000; cat. no. ab26197; Abcam), activating transcription factor 4 (ATF4; 1:1,000; cat. no. ab270980; Abcam), C/EBP homologous protein (CHOP; 1:1,000; cat. no. ab11419; Abcam), Caspase 12 (1:1,000; cat. no. ab62484; Abcam), LC3 (1:1,000; cat. no. ab192890; Abcam), Beclin1 (1:1,000;

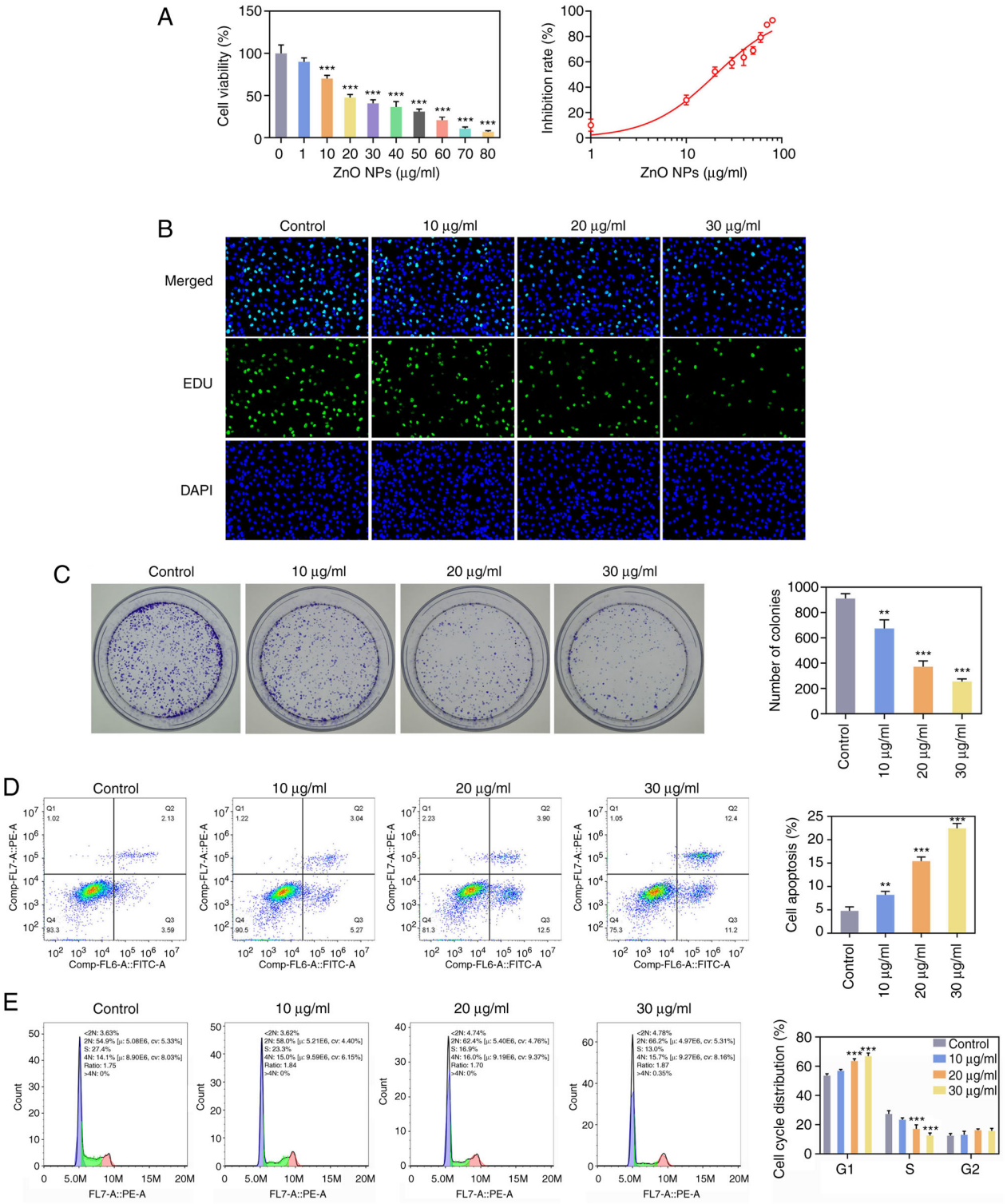

Figure 1. ZnO-NPs inhibit SKOV3 cell proliferation, facilitate cell apoptosis and induce cycle arrest. (A) Cell viability and IC50 were detected by a Cell Counting Kit-8 assay. (B) EdU staining (magnification, x200) and (C) colony formation assay were performed to detect the cell proliferation ability. (D and E) Flow cytometry was used to detect (D) apoptosis and (E) cell cycle. **P<0.01, ***P<0.001 vs. Control. ZnO-NPs, zinc oxide nanoparticles.

cat. no. ab207612; Abcam), p62 (1:1,000; cat. no. ab109012; Abcam) and glyceraldehyde-3-phosphate dehydrogenase (1:1,000; cat. no. ab8245 or ab9485; Abcam).

Wound-healing assay. Cells were inoculated into a six-well plate at a density of 1x10^5 cells/well. A pipette was used to generate a straight line scratch in the center of the plate. After a rinse with PBS, the cells were cultured in a serum-free medium for 24 h. The width of the scratch was observed with a microscope and measured by ImageJ software (v1.53a; National Institutes of Health).

Transwell assay. The cells were planted in 24-well Transwell cell culture chambers (8-μm pore size) pre-coated with or

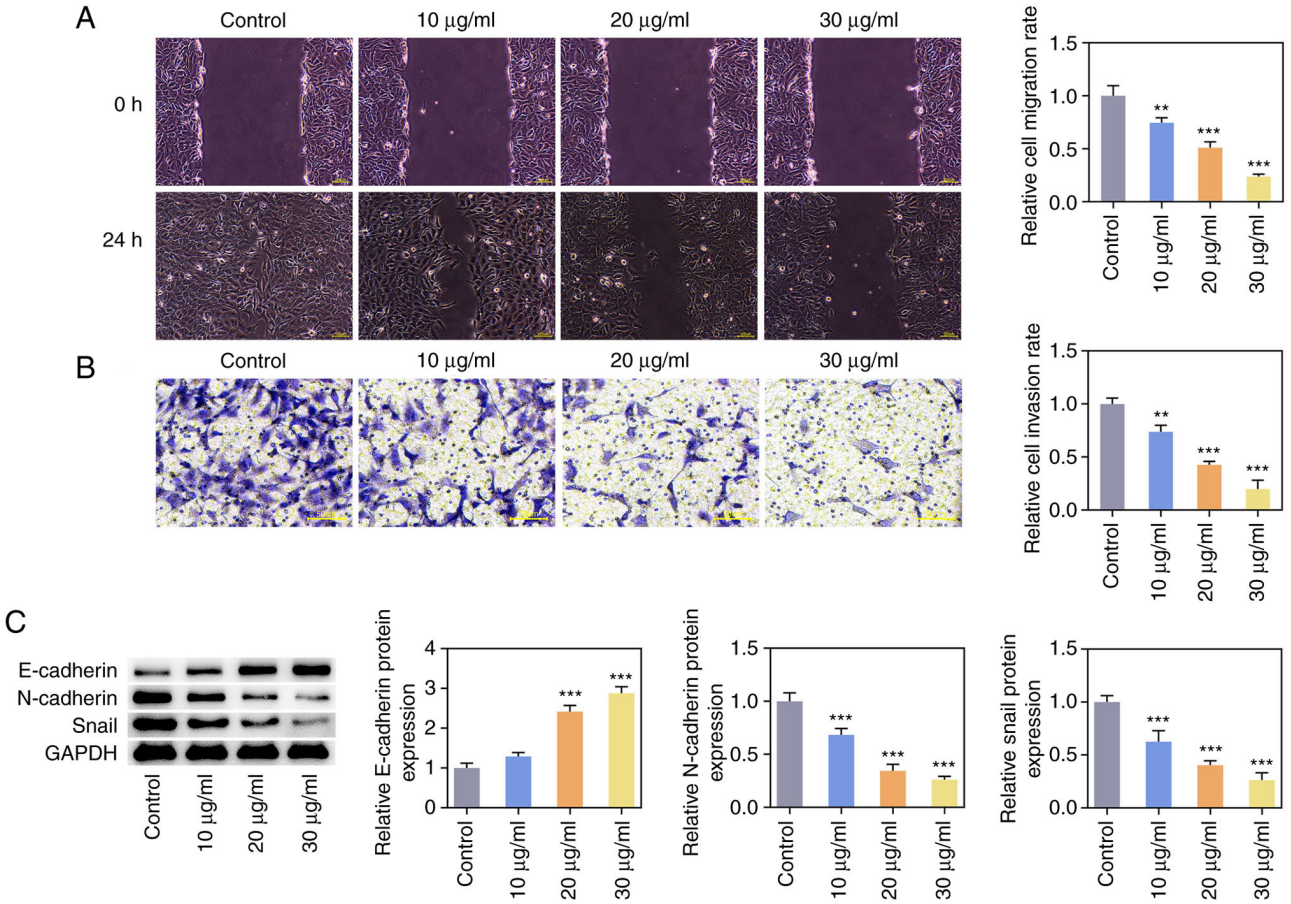

Figure 2. Zinc oxide nanoparticles inhibit invasion, migration and EMT of SKOV3 cells. (A) Wound healing (scale bar, 100 μm) and (B) Transwell assays (scale bar, 50 μm) were used to detect the migration and invasion of ovarian cancer cells. (C) Western blot analysis was used to detect the expression of EMT-related proteins. **P<0.01, ***P<0.001 vs. Control. EMT, epithelial to mesenchymal transition.

without Matrigel® basement membrane gel (Corning, Inc.). In the lower chamber of a 24-well plate, 700 μl of medium containing 15% FBS was added. After the routine culture of cells for 24 h, the membranes were collected and then stained with 0.5% crystal violet for 15 min at room temperature. At last, the cells were photographed under a microscope and evaluated by ImageJ software (v1.53a; National Institutes of Health).

Statistical analysis. All experiments were repeated in triplicate and the data are presented as the mean ± standard deviation. Data of multiple groups were compared by one-way analysis of variance followed by Tukey's test with GraphPad Prism software 8.3.0 (GraphPad; Dotmatics).

Results

ZnO-NPs inhibit SKOV3 cell proliferation, facilitate apoptosis and induce cell cycle arrest. Following the treatment of SKOV3 cells with ascending concentrations of ZnO-NPs, the cell viability and IC50 were detected by a CCK8 assay. The results indicated that the cell activity was significantly decreased with the increase in the concentration of ZnO-NPs, and the IC50 value of ZnO-NPs was 20.24 μg/ml (Fig. 1A). According to this IC50 value, ZnO-NPs at concentrations of 10, 20 and 30 μg/ml were selected for the subsequent experiments. EdU staining and colony-formation assays were applied for the detection of cell proliferation and the results showed that the cell proliferation ability was significantly decreased with increasing concentrations of ZnO-NPs compared to the control group (Fig. 1B and C). Subsequently, flow cytometry was used to detect apoptosis and cell cycle, and the results showed that, compared to the control group, cell apoptosis was significantly increased and cell cycle arrest was noticed in the ZnO-NPs group (Fig. 1D and E).

ZnO-NPs inhibit invasion, migration and epithelial to mesenchymal transition (EMT) of SKOV3 cells. Wound-healing and Transwell assays indicated that ZnO-NPs inhibited the migration and invasion of OC cells in a concentration-dependent manner (Fig. 2A and B). Western blot analysis was used to detect the expression of EMT-related proteins and it was found that the expression of E-cadherin was increased, while the expression of N-cadherin and Snail was decreased in the ZnO-NPs group compared with that in the control group (Fig. 2C).

ZnO-NPs inhibit chemotherapy resistance of SKOV3 cells. A CCK8 assay was used to detect the effect of DDP on the viability of SKOV3 cells treated by different concentrations of ZnO-NPs and the IC50 was calculated. The results indicated that the viability of SKOV3 and SKOV3/DDP cells was significantly decreased with the increase in the concentration of ZnO-NPs.

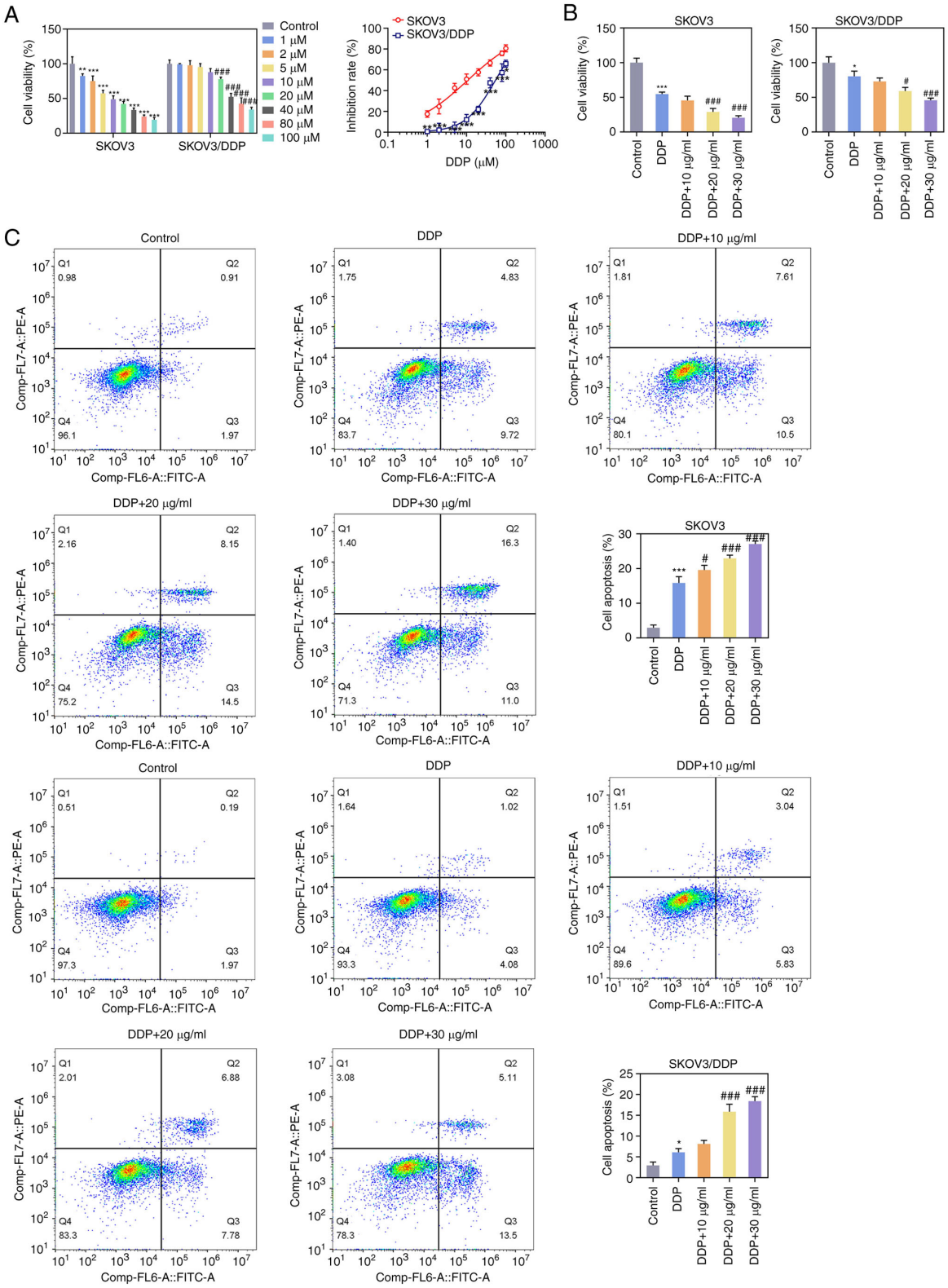

Figure 3. ZnO-NPs inhibit chemotherapy resistance of SKOV3 cells. (A) A CCK8 assay was used to detect the effect of DDP on the viability of SKOV3 cells treated with different concentrations of ZnO-NPs and the IC_{50} was calculated. (B) CCK8 was to measure the viability of SKOV3/DDP cells treated with 10, 20 or 30 μg/ml ZnO-NPs. (C) Flow cytometry was used to detect cell apoptosis. *P<0.05, **P<0.01, ***P<0.001 vs. Control; $^{\#}$P<0.05, $^{\#\#\#}$P<0.001 vs. DDP. DDP, cisplatin; CCK8, Cell Counting Kit-8; ZnO-NPs, zinc oxide nanoparticles.

The IC50 value of ZnO-NP-treated SKOV3 cells exposed to DDP was 10.27 μM and the IC50 value of ZnO-NP-treated SKOV3/DDP cells exposed to DDP was 54.57 μM (Fig. 3A). According to these results, 10 μM DDP was chosen to induce SKOV3 cells and SKOV3/DDP cells. SKOV3 or SKOV3/DDP cells were divided into control, DDP, DDP + 10 μg/ml ZnO-NP, DDP + 20 μg/ml ZnO-NP and DDP + 30 μg/ml ZnO-NP groups. Cell viability was measured with the CCK8 and the

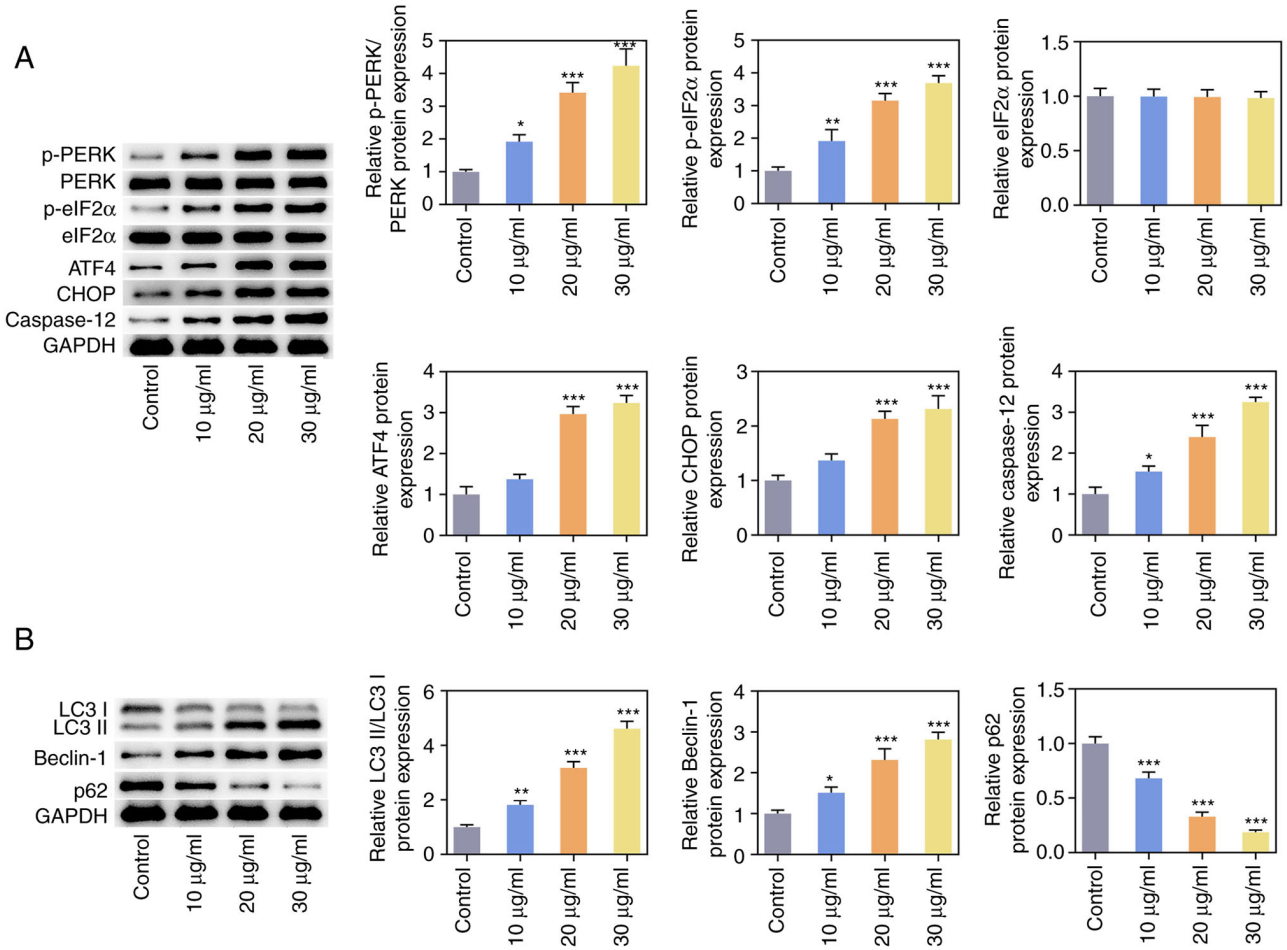

Figure 4. Zinc oxide nanoparticles activate ERS and promote autophagy. (A) Western blot analysis was used to detect the expression of ERS-related proteins. (B) Western blot detected the expression of autophagy-related proteins. *P<0.05, **P<0.01, ***P<0.001 vs. Control. ERS, endoplasmic reticulum stress; p-, phosphorylated; PERK, PKR-like endoplasmic reticulum kinase; eIF2α, eukaryotic translation initiation factor 2α; ATF4, activating transcription factor 4; CHOP, C/EBP homologous protein.

results suggested that SKOV3-cell viability was decreased to 55% and SKOV3/DDP-cell viability was decreased to 80% after DDP treatment. Compared with the DDP group, ZnO-NP treatment further inhibited the viability of SKOV3 and SKOV3/DDP cells (Fig. 3B). The flow cytometry results showed that the apoptosis of SKOV3 and SKOV3/DDP cells was significantly increased in the DDP group compared with the control group. Compared with the DDP group, the apoptosis of SKOV3 and SKOV3/DDP cells was further increased after the administration of ZnO-NPs (Fig. 3C). When SKOV3 cells and SKOV3/DDP cells were treated with the same dose of DDP, the activity of the resistant cell line remained much higher than that of the native cancer cell line, which demonstrated the drug resistance of SKOV3/DDP cells (or reduced sensitivity of SKOV3/DDP cells to DDP). The activity of the SKOV3/DDP resistant cell line decreased significantly after ZnO-NPs treatment, indicating that ZnO-NPs reduced the drug resistance of SKOV3/DDP cells (or the sensitivity of SKOV3/DDP cells to DDP increased after ZnO-NP treatment).

ZnO-NPs activate ERS and promote autophagy. Western blot analysis was used to detect the expression of ERS-related proteins and the results showed that p-PERK, p-eIF2α, ATF4, CHOP and Caspase 12 expression were increased by ZnO-NPs treatment in a concentration-dependent manner compared to the control group (Fig. 4A). Western blot analysis was also employed to detect the expression of autophagy-related proteins. It was found that ZnO-NPs concentration-dependently increased LC3II/I and Beclin-1 expression and decreased P62 expression compared to the control group (Fig. 4B). Since 30 μg/ml ZnO-NPs had the most significant effect, this specific concentration was selected for subsequent experiments.

ZnO-NPs inhibit malignant progression and chemotherapy resistance of SKOV3 cells by activating ERS and promoting autophagy. Subsequently, in order to further explore the effects of ZnO-NPs on ERS and autophagy, the ERS inhibitor 4-PBA and autophagy inhibitor 3-MA were respectively applied to the cells. The cells were divided into the control, ZnO-NPs, ZnO-NPs + 4-PBA and ZnO-NPs + 3-MA groups. EdU staining and colony-formation assays indicated that the cell proliferation capacity in the ZnO-NPs + 4-PBA and ZnO-NPs + 3-MA groups was significantly increased compared with that in the ZnO-NPs group (Fig. 5A and B). Flow cytometry suggested that, compared with the ZnO-NPs group, cell apoptosis in the ZnO-NPs + 4-PBA and ZnO-NPs + 3-MA groups was significantly decreased

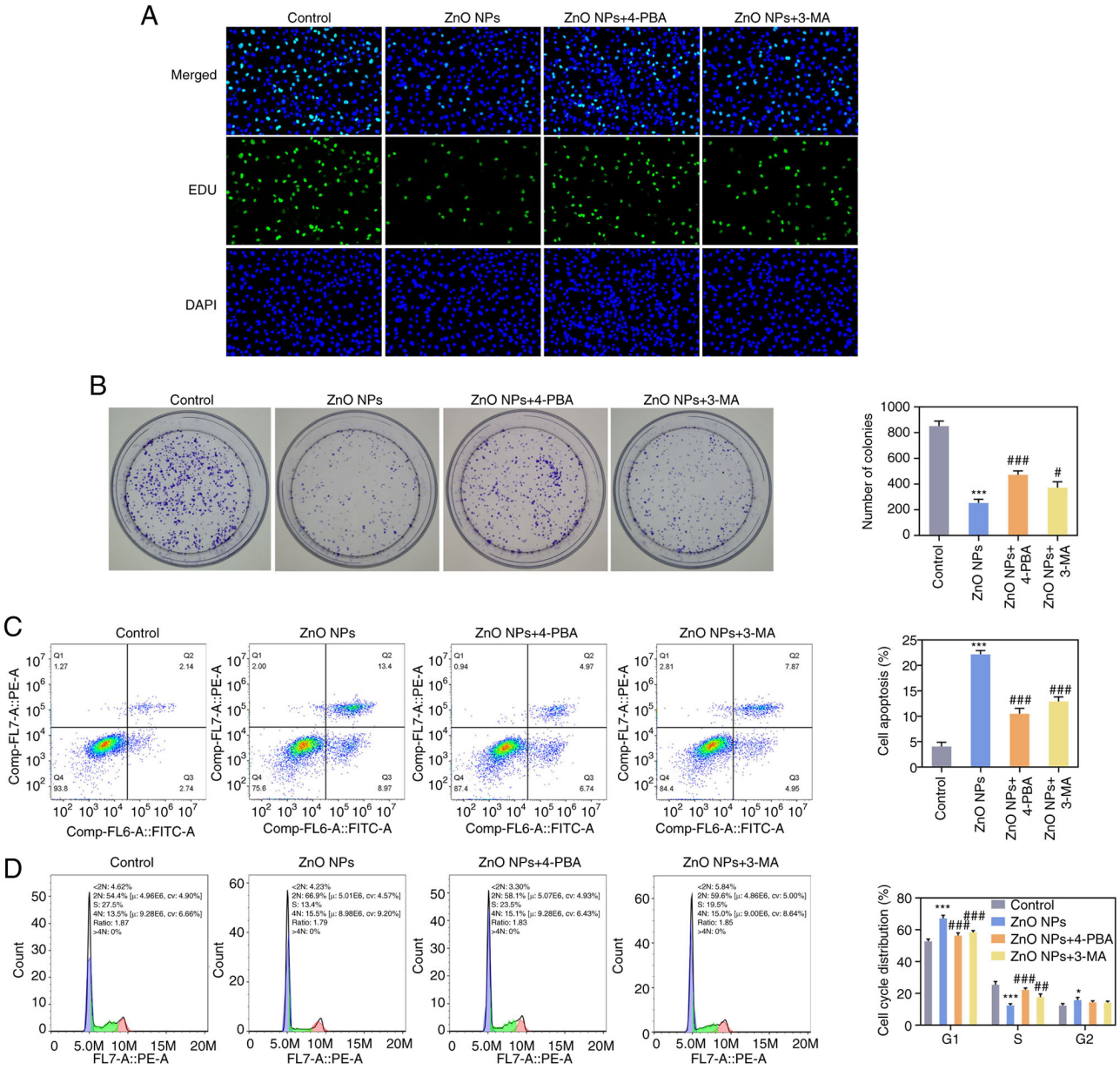

Figure 5. ZnO-NPs inhibit the malignant progression of SKOV3 cells by activating ERS and promoting autophagy. (A) EdU staining (magnification, x200) and (B) a colony-formation assay were used to detect the cell proliferation ability. (C and D) Flow cytometry was used to detect (C) apoptosis and (D) cell cycle. *P<0.05 and ***P<0.001 vs. Control; $^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 vs. ZnO-NPs. ZnO-NPs, zinc oxide nanoparticles; 4-PBA, 4-phenylbutyric acid; 3-MA, 3-methyladenine.

and cell cycle arrest was reduced (Fig. 5C and D). Results from the wound-healing and Transwell assays showed that the invasion and migration abilities in the ZnO-NPs + 4-PBA and ZnO-NPs + 3-MA groups were significantly increased compared with those in the ZnO-NPs group (Fig. 6A and B). Compared with the ZnO-NPs group, the expression of E-cadherin was decreased and the expression of N-cadherin and Snail were increased in the ZnO-NPs + 4-PBA and ZnO-NPs + 3-MA groups (Fig. 6C). In terms of chemotherapy resistance, SKOV3 or SKOV3/DDP cells were divided into the control, DDP, DDP + ZnO-NPs, DDP + ZnO-NPs + 4-PBA and DDP + ZnO-NPs + 3 MA groups. It was found that, compared with the DDP + ZnO-NPs group, the cell viability in the DDP + ZnO-NPs + 4-PBA and DDP + ZnO-NPs + 3-MA groups was significantly increased and cell apoptosis was significantly decreased (Fig. 7A and B).

Discussion

Currently, the main treatment methods for OC are tumor cell reduction and postoperative platinum-based chemotherapy. Although the clinical remission rate of OC has reached 60-80% due to the application of initial surgery and chemotherapy, most patients will relapse and develop drug resistance, which may result in a high propensity for tumor invasion and metastasis. Patients with advanced OC often succumb to distant metastasis after platinum resistance and the 5-year overall survival rate is only ~30% (23-25). Therefore, it is urgent to further study the mechanism of chemotherapy resistance of OC and to find relevant targets or drugs that may effectively inhibit chemotherapy resistance of OC.

ZnO-NPs as nanomaterials can effectively improve the stability of clinical drugs and improve patients' drug

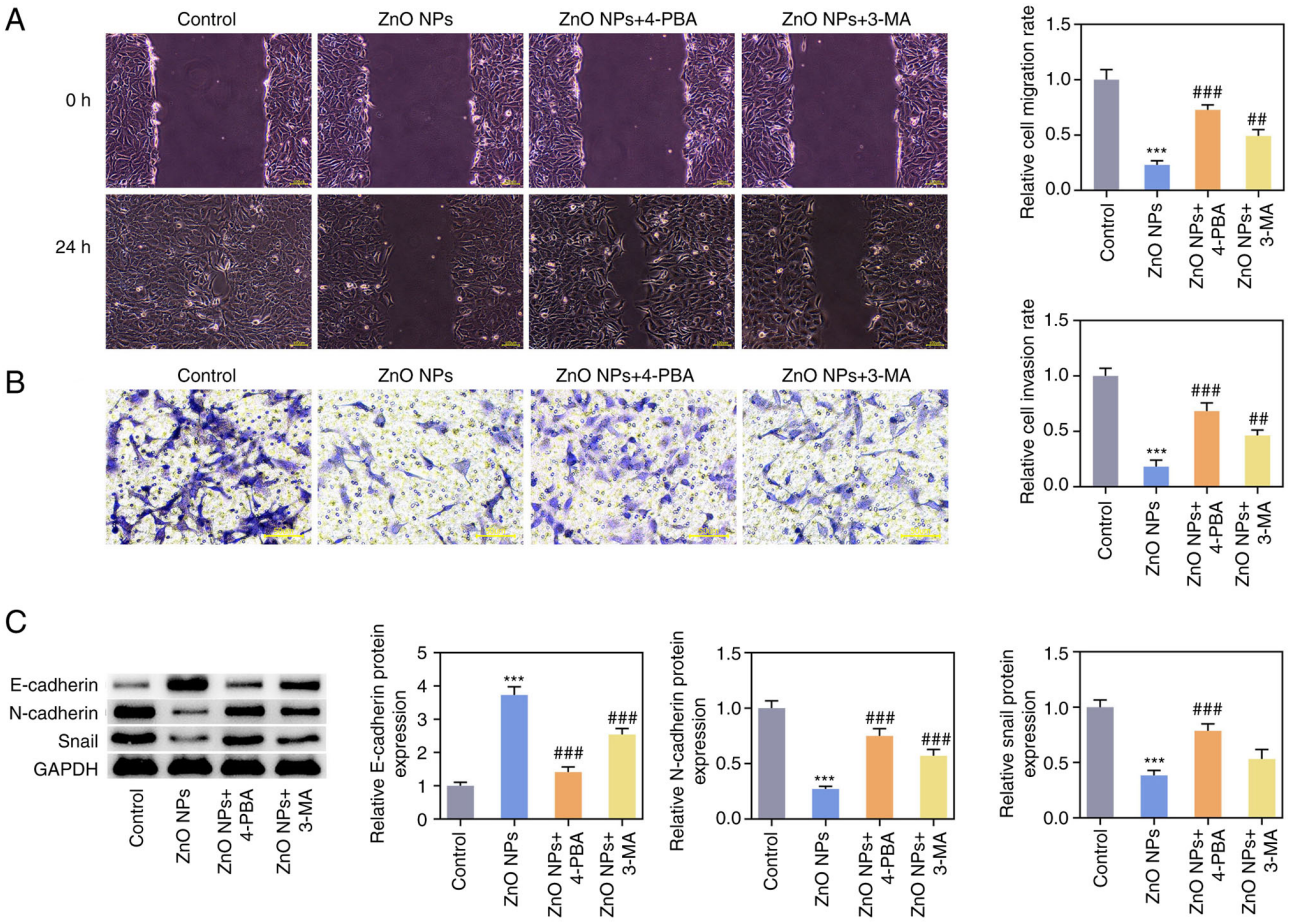

Figure 6. ZnO-NPs inhibit the malignant progression of SKOV3 cells by activating endoplasmic reticulum stress and promoting autophagy. (A) Wound-healing (scale bar, 100 μm) and (B) Transwell assays (scale bar, 50 μm) were used to detect the migration and invasion of ovarian cancer cells. (C) Western blot detected the expression of epithelial-mesenchymal transition-related proteins. ***P<0.001 vs. Control; $^{##}$P<0.01, $^{###}$P<0.001 vs. ZnO NPs. ZnO-NPs, zinc oxide nanoparticles; 4-PBA, 4-phenylbutyric acid; 3-MA, 3-methyladenine.

absorption capacity. ZnO-NPs have an important antitumor role in a variety of cancer types. A study has shown that ZnO-NP treatment may lead to prostate cancer cell apoptosis and death (26). ZnO-NPs stimulate oxidative stress to induce melanoma-like skin lesions and apoptosis of melanoma cells in mice with epidermal barrier dysfunction through the activation of the NF-κB pathway (27,28). A recent study indicated that ZnO-NPs can promote OC cell death, thus inhibiting the progression of OC (29). In the present study, it was confirmed that ZnO-NPs were able to significantly inhibit the activity of SKOV3 cells, inhibit cell invasion and migration, as well as induce apoptosis and cell cycle arrest in OC. A study by Bai *et al* (30) showed that ZnO-NPs may induce apoptosis and autophagy of SKOV3 cells, indicating that ZnO-NPs have a certain inhibitory effect on the proliferation of OC cells, which is consistent with the experimental results of the present study. Furthermore, a previous study by our group has shown that ZnO-NPs are able to inhibit the proliferation and apoptosis of SKOV3 cells (20). For this reason, SKOV3 cells were also used in the present study. In addition, ZnO-NPs were indicated to inhibit the EMT and chemotherapy resistance of SKOV3 cells.

The ER is the main site for lipid synthesis, protein function and calcium ion metabolism (31). Multiple studies have confirmed that ERS is involved in the regulation of malignant tumor proliferation and chemotherapy resistance (17,32). Saikosaponin A can induce cervical cancer cell apoptosis through upregulating G-protein coupled receptor 78, CHOP and caspase-12 expression to activate ERS-dependent pathways (33). Shikotin can promote the resistance of colorectal cancer cells to 5-fluorouracil by activating ERS (34). It has been reported that ERS induces apoptosis of OC cells (35,36). Therefore, exploring the regulation of ERS on OC cells is also a strategy for the treatment of OC. It has been found that ZnO-NPs have an important role in inducing ERS (19). However, the regulatory effect of ZnO-NPs on ERS in OC has not been reported so far, to the best of our knowledge. The present results showed that ZnO-NPs were able to induce ERS, thereby inhibiting the malignant progression and chemotherapy resistance of OC cells. Fang *et al* (37) found that ERS inhibitor tauroursodeoxycholic acid can reduce PERK expression and increase the production of autophagy-related protein LC3-II to restore autophagy after injury, thus reducing kidney injury in mice. Lipopolysaccharide stimulation can activate ERS and inhibit autophagy, while spermidine can reduce acute lung injury and inflammation induced by lipopolysaccharide by inhibiting ERS and activating autophagy (38). In addition, alpha-tomatine can inhibit the proliferation of OC cells and exert a pro-apoptotic role by inhibiting autophagy (39). Zhou *et al* (40) confirmed that microRNA-133a is able to reduce cisplatin resistance of OC cells by downregulating the

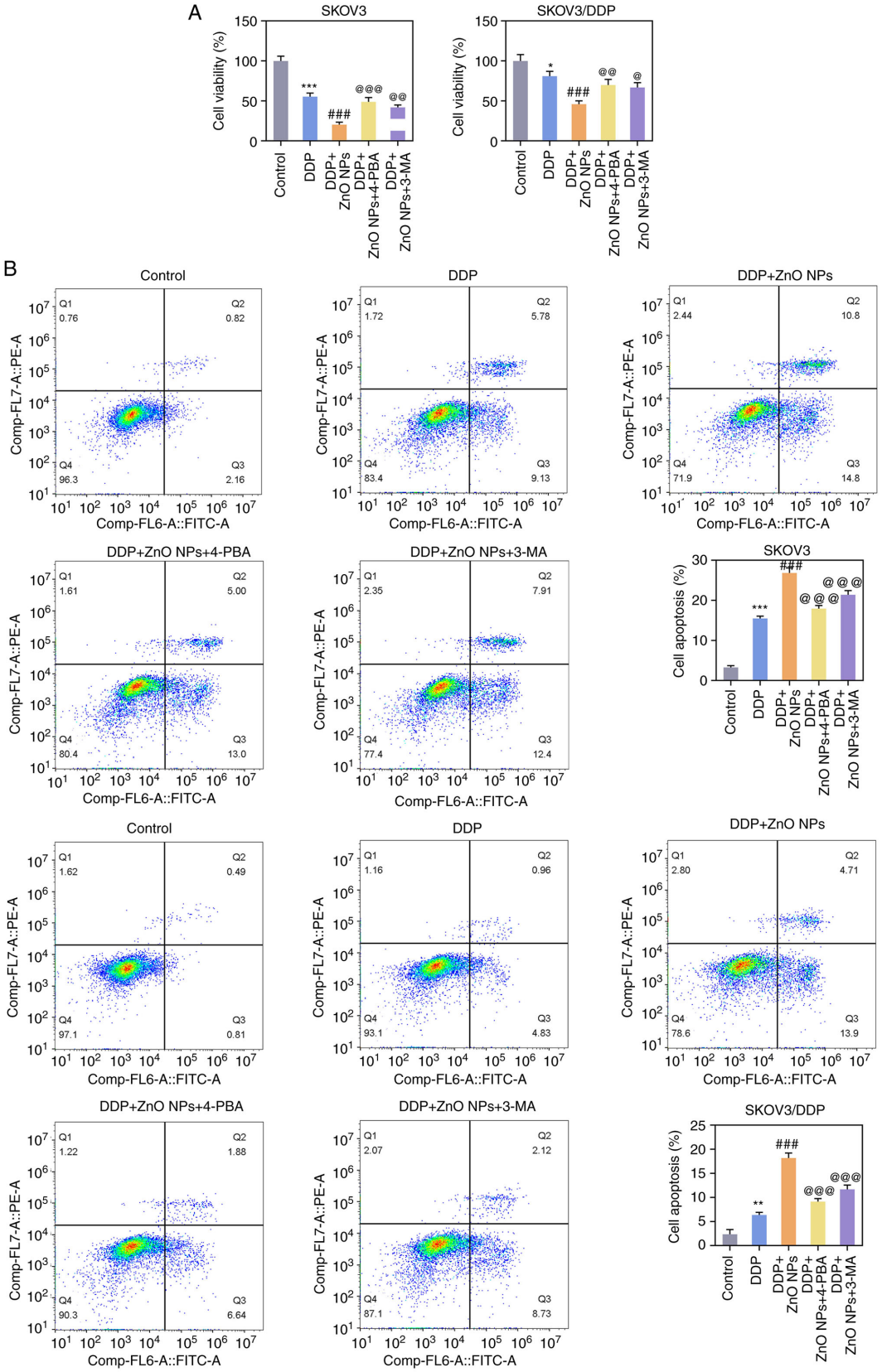

Figure 7. ZnO-NPs inhibit chemotherapy resistance of SKOV3 cells by activating endoplasmic reticulum stress and promoting autophagy. (A) Cell viability was measured by a Cell Counting Kit-8 assay. (B) Flow cytometry was used to detect cell apoptosis. *P<0.05, **P<0.01, ***P<0.001 vs. Control; $^{\#\#\#}$P<0.001 vs. DDP; $^{@}$P<0.05, $^{@@}$P<0.01, $^{@@@}$P<0.001 vs. DDP + ZnO NPs. ZnO-NPs, zinc oxide nanoparticles; 4-PBA, 4-phenylbutyric acid; 3-MA, 3-methyladenine; DDP, cisplatin.

expression of Yamaguchi sarcoma viral homolog 1, thereby inhibiting autophagy. Furthermore, p53 and LC3 expression are upregulated in ZnO-NP-treated cells, indicating that ZnO-NPs can promote apoptosis and induce autophagy, and ZnO-NPs

induced significant cytotoxicity, apoptosis and autophagy in human OC cells through the production of ROS and oxidative stress (41). In the present study, it was found that ZnO-NPs promoted autophagy and inhibited malignant progression and chemotherapy resistance of OC cells by activating ERS.

Of note, the present study has certain limitations. First, the experiment was conducted in only one OC cell line, SKOV3, in future subsequent experiments, the present findings will be verified using other OC cell lines. In addition, the regulatory relationship between ERS and autophagy under ZnO-NPs regulation in OC was not further investigated, which will be the focus of future research by our group.

In conclusion, ZnO-NPs may inhibit malignant progression and chemotherapy resistance of OC cells by activating ERS and promoting autophagy.

Acknowledgements

Not applicable.

Funding

The present study was supported by Ningxia Natural Science Foundation project (grant no. 2023AAC03534).

Availability of data and materials

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

Authors' contributions

CY and WG designed and conceived the study and selected the experiments. WG performed the experiments. WG wrote the manuscript. CY processed the experimental data. CY and WG confirm the authenticity of all the raw data. Both authors have read and approved the final 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.

References

1. Penny SM: Ovarian Cancer: An Overview. Radiol Technol 91: 561-575, 2020.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2020.
3. Orr B and Edwards RP: Diagnosis and treatment of ovarian cancer. Hematol Oncol Clin North Am 32: 943-964, 2018.
4. Ray-Coquard I, Mirza MR, Pignata S, Walther A, Romero I and du Bois A: Therapeutic options following second-line platinum-based chemotherapy in patients with recurrent ovarian cancer: Comparison of active surveillance and maintenance treatment. Cancer Treat Rev 90: 102107, 2020.
5. Mabrouk M, Das DB, Salem ZA and Beherei HH: Nanomaterials for biomedical applications: Production, characterisations, recent trends and difficulties. Molecules 26: 1077, 2021.
6. Yan M and Majd MH: Evaluation of induced apoptosis by biosynthesized zinc oxide nanoparticles in MCF-7 breast cancer cells using Bak1 and Bclx expression. Dokl Biochem Biophys 500: 360-367, 2021.
7. Anjum S, Hashim M, Malik SA, Khan M, Lorenzo JM, Abbasi BH and Hano C: Recent advances in zinc oxide nanoparticles (ZnO NPs) for cancer diagnosis, target drug delivery, and treatment. Cancers (Basel) 13: 4570, 2021.
8. Padmanabhan A, Kaushik M, Niranjan R, Richards JS, Ebright B and Venkatasubbu GD: Zinc Oxide nanoparticles induce oxidative and proteotoxic stress in ovarian cancer cells and trigger apoptosis Independent of p53-mutation status. Appl Surf Sci 487: 807-818, 2019.
9. Hu C and Du W: Zinc oxide nanoparticles (ZnO NPs) combined with cisplatin and gemcitabine inhibits tumor activity of NSCLC cells. Aging (Albany NY) 12: 25767-25777, 2020.
10. Sehrawat A, Mishra J, Mastana SS, Navik U, Bhatti GK, Reddy PH and Bhatti JS: Dysregulated autophagy: A key player in the pathophysiology of type 2 diabetes and its complications. Biochim Biophys Acta Mol Basis Dis 1869: 166666, 2023.
11. Wilson N, Kataura T, Korsgen ME, Sun C, Sarkar S and Korolchuk VI: The autophagy-NAD axis in longevity and disease. Trends Cell Biol 33: 788-802, 2023.
12. Onorati AV, Dyczynski M, Ojha R and Amaravadi RK: Targeting autophagy in cancer. Cancer 124: 3307-3318, 2018.
13. Jain V, Singh MP and Amaravadi RK: Recent advances in targeting autophagy in cancer. Trends Pharmacol Sci 44: 290-302, 2023.
14. Debnath J, Gammoh N and Ryan KM: Autophagy and autophagy-related pathways in cancer. Nat Rev Mol Cell Biol 24: 560-575, 2023.
15. Lopez-Mendez TB, Sánchez-Álvarez M, Trionfetti F, Pedraz JL, Tripodi M, Cordani M, Strippoli R and González-Valdivieso J: Nanomedicine for autophagy modulation in cancer therapy: A clinical perspective. Cell Biosci 13: 44, 2023.
16. Liu L, Wang J, Zhang J, Huang C, Yang Z and Cao Y: The cytotoxicity of zinc oxide nanoparticles to 3D brain organoids results from excessive intracellular zinc ions and defective autophagy. Cell Biol Toxicol 39: 259-275, 2023.
17. Cubillos-Ruiz JR, Bettigole SE and Glimcher LH: Tumorigenic and immunosuppressive effects of endoplasmic reticulum stress in cancer. Cell 168: 692-706, 2017.
18. Sisinni L, Pietrafesa M, Lepore S, Maddalena F, Condelli V, Esposito F and Landriscina M: Endoplasmic reticulum stress and unfolded protein response in breast cancer: The balance between apoptosis and autophagy and its role in drug resistance. Int J Mol Sci 20: 857, 2019.
19. Luo Y, Wu C, Liu L, Gong Y, Peng S, Xie Y and Cao Y: 3-Hydroxyflavone enhances the toxicity of ZnO nanoparticles in vitro. J Appl Toxicol 38: 1206-1214, 2018.
20. Gu WL, Li H, Niu X and Zhou J: Biological fabrication of zinc oxide nanoparticles from Nepeta cataria potentially produces apoptosis through inhibition of proliferative markers in ovarian cancer. Green Process Synth 11: 316-326, 2022.
21. Ma YY, Di ZM, Cao Q, Xu WS, Bi SX, Yu JS, Shen YJ, Yu YQ, Shen YX, and Feng LJ: Xanthatin induces glioma cell apoptosis and inhibits tumor growth via activating endoplasmic reticulum stress-dependent CHOP pathway. Acta Pharmacol Sin 41: 404-414, 2020.
22. Zhang Q, Wang X, Cao S, Sun Y, He X, Jiang B, Yu Y, Duan J, Qiu F and Kang N: Berberine represses human gastric cancer cell growth in vitro and in vivo by inducing cytostatic autophagy via inhibition of MAPK/mTOR/p70S6K and Akt signaling pathways. Biomed Pharmacother 128: 110245, 2020.
23. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, Gaudet MM, Jemal A and Siegel RL: Ovarian cancer statistics, 2018. CA Cancer J Clin 68: 284-296, 2018.
24. Kathawala RJ, Kudelka A and Rigas B: The chemoprevention of ovarian cancer: The need and the options. Curr Pharmacol Rep 4: 250-260, 2018.
25. Giornelli GH: Management of relapsed ovarian cancer: A review. Springerplus 5: 1197, 2016.

26. Virych PA, Zadvorniy TV, Borikun TV, Lykhova OO, Chumachenko VA, Virych PA, Pavlenko VA, Kutsevol NV and Lukianova NY: Effects of dextran-graft-polyacrylamide/ZnO nanoparticles on prostate cancer cell lines in vitro. Exp Oncol 44: 217-221, 2022.
27. Wang P, Hu G, Zhao W, Du J, You M, Xv M, Yang H, Zhang M, Yan F, Huang M, *et al*: Continuous ZnO nanoparticle exposure induces melanoma-like skin lesions in epidermal barrier dysfunction model mice through anti-apoptotic effects mediated by the oxidative stress-activated NF-κB pathway. J Nanobiotechnology 20: 111, 2022.
28. Fan P, Yang C, Wang L, Wang Q, Zhang Y, Zhou J, Weng J and Feng B: ZnO nanoparticles stimulate oxidative stress to induce apoptosis of B16F10 melanoma cells:In vitroandin vivostudies. Biomed Phys Eng Express 7, 2021.
29. Khan MS, Altwaijry N, Jabir NR, Alamri AM, Tarique M and Khan AU: Potential of green-synthesized ZnO-NPs against human ovarian teratocarcinoma: An in vitro study. Mol Biol Rep 50: 4447-4457, 2023.
30. Bai DP, Zhang XF, Zhang GL, Huang YF and Gurunathan S: Zinc oxide nanoparticles induce apoptosis and autophagy in human ovarian cancer cells. Int J Nanomedicine 12: 6521-6535, 2017.
31. Saraswat Ohri S, Mullins A, Hetman M and Whittemore SR: Activating transcription factor-6α deletion modulates the endoplasmic reticulum stress response after spinal cord injury but does not affect locomotor recovery. J Neurotrauma 35: 486-491, 2018.
32. Bahar E, Kim JY and Yoon H: Chemotherapy resistance explained through endoplasmic reticulum stress-dependent signaling. Cancers (Basel) 11: 338, 2019.
33. Du J, Song D, Cao T, Li Y, Liu J, Li B and Li L: Saikosaponin-A induces apoptosis of cervical cancer through mitochondria- and endoplasmic reticulum stress-dependent pathway in vitro and in vivo: Involvement of PI3K/AKT signaling pathway. Cell Cycle 20: 2221-2232, 2021.
34. Piao MJ, Han X, Kang KA, Fernando PDSM, Herath HMUL and Hyun JW: The endoplasmic reticulum stress response mediates shikonin-induced apoptosis of 5-fluorouracil-resistant colorectal cancer cells. Biomol Ther (Seoul) 30: 265-273, 2022.
35. Rezghi Barez S, Movahedian Attar A and Aghaei M: MicroRNA-30c-2-3p regulates ER stress and induces apoptosis in ovarian cancer cells underlying ER stress. EXCLI J 20: 922-934, 2021.
36. Xu J, Bi G, Luo Q, Liu Y, Liu T, Li L, Zeng Q, Wang Q, Wang Y, Yu J and Yi P: PHLDA1 Modulates the endoplasmic reticulum stress response and is required for resistance to oxidative stress-induced cell death in human ovarian cancer cells. J Cancer 12: 5486-5493, 2021.
37. Fang L, Zhou Y, Cao H, Wen P, Jiang L, He W, Dai C and Yang J: Autophagy attenuates diabetic glomerular damage through protection of hyperglycemia-induced podocyte injury. PLoS One 8: e60546, 2013.
38. Baek AR, Hong J, Song KS, Jang AS, Kim DJ, Chin SS and Park SW: Spermidine attenuates bleomycin-induced lung fibrosis by inducing autophagy and inhibiting endoplasmic reticulum stress (ERS)-induced cell death in mice. Exp Mol Med 52: 2034-2045, 2020.
39. Wu H, Li W, Wang T, Rong Y, He Z, Huang S, Zhang L, Wu Z and Liu C: α-Tomatine, a novel early-stage autophagy inhibitor, inhibits autophagy to enhance apoptosis via Beclin-1 in Skov3 cells. Fitoterapia 152: 104911, 2021.
40. Zhou Y, Wang C, Ding J, Chen Y, Sun Y and Cheng Z: miR-133a targets YES1 to reduce cisplatin resistance in ovarian cancer by regulating cell autophagy. Cancer Cell Int 22: 15, 2022.
41. Hollister R: Critical incident stress debriefing and the community health nurse. J Community Health Nurs 13: 43-49, 1996.