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ORIGINAL RESEARCH

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The role of proteasome inhibitor MG132 in cisplatin resistant ovarian cancer

Bailing ZHANG[1,2,3], Na GUO[1,2,*]

[1]The Department of Obstetrics and Gynecology, West China Second University Hospital of Sichuan University, 610041 Chengdu, Sichuan, China
[2]Key laboratory of Birth Defects and Related Diseases of Women and Children (Sichuan University), Ministry of Education, 610041 Sichuan, Chengdu, China
[3]The Department of Laboratory Medicine, The Second Affiliated Hospital of Nanchang University, 330006 Nanchang, Jiangxi, China

***Correspondence**
guona507@aliyun.com
(Na GUO)

Abstract

Platinum based combined chemotherapy have been proved to be the most effective drugs for the ovarian cancer treatment, but it is difficult to treat cisplatin resistant ovarian cancer. Carbobenzoxy-L-leucy-L-Leucyl-L-Leucinal (MG132) is a reversible tripeptide aldehyde proteasome inhibitor, the purpose of this study was to observe the effect of MG132 on cisplatin resistant ovarian cancer SKOV3 cell and OVCAR-3 cell the expression of autophagy and apoptosis related factors. The cells were divided into four groups: control, MG132, cisplatin, MG132 and cisplatin combination groups. Cell growth was detected by cell counting kit-8 (CCK-8) assay. The apoptotic rates of cells and the cell cycle were detected by a flow cytometer (FCM). The Beclin1, Light chain 3 (LC3) and Caspase3 was detected by western blotting and reverse transcription-polymerase chain reaction (RT-PCR). Detection of apoptotic bodies by 4,6-Diamidino-2-phenylindole dihydrochloride (DAPI) staining. CCK-8 assay demonstrated that cell survival rate in the combination groups was lower than monotherapy group. FCM showed that apoptotic rates in the combination groups was higher than monotherapy group ($p < 0.05$). Western blotting and RT-PCR detected that Beclin1, LC3 and Caspase3 in the combination group were higher than monotherapy group ($p < 0.05$). DAPI staining showed the production of apoptotic bodies in the combination group and MG132 group. In conclusion, MG132 can inhibit the growth of cisplatin resistant ovarian cancer SKOV3 and OVCAR-3 cells, its inhibitory effect is related to apoptosis and autophagy, and it is expected to be a synergistic antitumor effect with cisplatin.

Keywords

MG132; Cisplatin; Resistant ovarian cancer; Apoptosis; Autophagy

1. Background

Ovarian malignant tumor has the highest mortality in female reproductive malignant tumors, due to the onset hidden and easy to dissemination in the abdominal cavity, approximately 80% of patients at advanced stage, the recurrent rate is 70% [1]. The prognosis is poor, the 5 years survival rate is about 35–40% for advanced ovarian cancer [2]. Platinum compounds were proved to be the most effective drug for ovarian cancer treatment, but for the recurrence and metastasis, especially resistant to platinum recurrence and metastasis of ovarian cancer, which further treatment is difficult, so looking for new drug interaction sites, high efficiency and low toxicity has become a research hotspot in recent years [3]. Recently, researchers reported that autophagy was tightly associated to chemoresistance and played a key role in ovarian cancer recurrence [4].

Ubiquitin proteasome pathway (UPP) is the important protein degradation mechanisms in eukaryotic cells. It can selectively degrade some bioactive proteins in cells, so as to regulate physiological processes such as cell metabolism, differentiation and proliferation [5]. Selectively degrade oncogenes and tumor suppressor gene products, activators or inhibitors, apoptosis regulatory proteins, *etc.*, so as to regulate cell mutation and tumorigenesis. Under normal circumstances, more than 80% of proteins in cells are degraded by UPP [6]. Specifically blocking UPP pathway can significantly affect the degradation of proteins, and then affect cell cycle process and induce apoptosis. Many mechanisms are involved in the drug resistance in tumors, and proteasome inhibitors are newly discovered antitumor drugs in recent years. By blocking protein degradation mediated by ubiquitin proteasome pathway, proteasome inhibitors can induce apoptosis and have an antitumor effect which interfere with the balance of protein synthesis and degradation in cells. They also can enhance sensitivity of chemotherapeutic drugs and radiotherapy to tumor cells. Carbobenzoxy-L-leucy-L-Leucyl-L-Leucinal (MG132) is a reversible aldehyde proteasome inhibitor. MG132 can inhibit the growth of tumor cells through apoptosis pathway. However, there are few studies on the effect of MG132 on ovarian cancer. There is no report on the study of cisplatin resistant ovarian cancer. The purpose of this research is to observe the growth of ovarian cancer SKOV3 and OVCAR-3 cells which are cisplatin resistant, after treatment with MG132,

to observe autophagy and apoptosis related factors expression, and to preliminarily explore the mechanism of MG132 inhibiting the growth ovarian cancer cell, in order to provide new ideas for the treatment of cisplatin resistant ovarian cancer.

2. Materials and methods

2.1 Drugs and Reagents

Cisplatin was obtained from MedChem Express (Cat# HY-17394, USA), MG132 was obtained from MedChem Express (Cat# HY-13259, USA) , Cell Counting Kit-8 (CCK-8) was obtained from Sigma Chemicals (Cat# 96992, USA). Dimethyl sulfoxide (DMSO) was obtained from Ding Guo Chang Sheng (Cat# DH105-1, Beijing, China). Annexin V-FITC/PI apoptosis detection kit was obtained from MultiSciences (Cat# 70-AP101-100, Zhejiang, China). Cell cycle and apoptosis detection kit was obtained from Qi Hai Fu Tai (Cat# C001, Shanghai, China). DAPI dye was obtained from Absin (Cat# abs47047616, Shanghai, China). Mouse anti-human Beclin1antibodies was obtained from Boster (Cat# PB9076, USA), Goat anti-human cyserinly aspartatespecific protease 3 (Caspase3) antibodies was obtains from Merck Millipore (Cat# MAB10753, USA), goat anti-human LC3 antibodies was obtains from Sigma-Aldrich (Cat# ABC232, USA), and mouse anti-human β-actin antibodies was obtained from Santa (Cat# sc-8432, USA). RevertAid First Strand cDNA Synthesis Kit were obtained from Fermentas (Cat# K1622, Shanghai, China), TRIzol was obtained from Coolaber (Cat# RE600, Beijing, China) and UltraSYBR mixture was obtains from Kangwei (Cat# CW0957H, Jiangsujing, China). RPMI-1640 culture (Cat# WH190730CE, EallBio, Beijing, China), medium containing 10% fetal bovine serum (Cat# NT9670B, EallBio, Beijing, China), L-glutamine (2 mmol/L), penicillin (100 U/mL), and streptomycin (100 U/mL) at 37 ℃ in a humidified 5% CO_2 atmosphere.

2.2 CCK-8 assay

Cisplatin resistant ovarian cancer cell line SKOV3 and OVCAR-3 cells were taken as 10×10^4/mL diluted at 100 μL/well concentration and was inoculated on 96 well plate, the culture plate was placed in the incubator for 8, 16, 24, 36 and 48 hours. The test was set at MG132 group at the conentration of 1.0 μg/mL; with cisplatin group at 15 μg/mL concentrations; and with MG132 at 1.0 μg/mL combination with cisplatin at 15 μg/mL concentrations, the above cells are respectively set with 4 secondary holes (at the same time, the blank group of culture medium without serum and cells is set), and continue to be cultured in the incubator for 8, 16, 24, 36 and 48 hours. Before the detection of the tested cells, 10 μL CCK-8 is added to each hole, and then take 100 μL 1640 was added 10 μL CCK-8 used as control. Then put the cells of each test group into the cell incubator for 4 hours, measure the absorbance (d) value of each hole at 450 nm on the enzyme labeling instrument, and repeat each group of experiments for 3 times. Cell survival rate (%) = (D experimental group-blank group)/(d control group-D blank group) $\times$ 100%.

2.3 Flow cytometry

The cisplatin resistant ovarian cancer SKOV3 and OVCAR-3 cells were divided into four groups: control, cisplatin, MG132, MG132 and cisplatin combination groups. The apoptotic rates of cells and the cell cycle were detected by FCM after treatment with MG132 at 0.4 μg/mL concentrations for 48 hours; with cisplatin at 20 μg/mL concentrations for 48 hours; and with cisplatin at 20 μg/mL combined with MG132 at 0.4 μg/mL concentrations for 48 hours. Thereafter, approximately 20 μL of MTT was added. After incubation at 37 ℃ for 4 hours, 150 μL of DMSO was added. In a microplate reader at a wavelength of 570 nm were detected. The formula was: cell inhibitory rate (%) = (1-(optical density of the experimental samples/optical density of the control)) $\times$ 100% (mean T $\pm$ standard deviation).

2.4 DAPI staining

The cisplatin resistant ovarian cancer SKOV3 cells were treated as 2.5×10^6 cells/mL inoculated into 6-well plates and cultured in 37 ℃ cell incubator for 24 hours. MG132 at 0.4 μg/mL, cisplatin at 20 μg/mL, MG132 at 0.4 μg/mL + cisplatin at 20 μg/mL was added respectively, and the control group was set. After continuous culture for 48 hours, the medium was discarded and washed twice with Phosphate Buffered Saline (PBS). 1% paraformaldehyde was added to the cells of each group and fixed at 4 ℃ overnight. DAPI staining solution was added to the cells of each group for 2 min, and the production of apoptotic bodies was observed immediately under fluorescence microscope.

2.5 Western blot analysis

The expression of Beclin1, Caspase3 and LC3 was detected after treatment with MG132 at 0.4 μg/mL, cisplatin at 20 μg/mL, MG132 at 0.4 μg/mL + cisplatin at 20 μg/mL, for 48 hours. The cells were harvested and lysed in 100 μL of lysis buffer for 10 minutes; the extracts were centrifuged at 1200 rpm for 5 minutes. After the addition of the 5$\times$ loading buffer, incubated at 95 ℃ for 5 minutes and resolved using Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis (SDS-PAGE). Anti-Beclin1 (1:1000 dilution), anti-LC3 (1:1000 dilution) and anti-Caspase3 (1:1000 dilution) antibodies were probed. Add the diluted secondary antibody (goat anti-mouse 1:5000) and incubate at room temperature for 1 hour.

2.6 Reverse transcription-polymerase chain reaction (RT-PCR)

The mRNA expression of Beclin1, Caspase3 and LC3 was detected by RT-PCR after treatment with MG132 at 0.4 μg/mL, cisplatin at 20 μg/mL, MG132 at 0.4 μg/mL + cisplatin at 20 μg/mL, respectively, for 48 hours. The total RNA was fragmented and labeled, the labeled RNA was hybridized to probe the hybridization chamber gasket slides after purification. The slides were scanned using an Agilent microarray scanner after washing.

2.7 Statistical analysis

All data were expressed as the mean ± SD and analyzed using SPSS 13.0 (Tianyan Rongzhi Software, Beijing, China). The linear t test was used for statistical analysis, and $p < 0.05$ was considered statistically significant.

3. Results

3.1 MG132 and combination with DDP decreased the viability of cisplatin resistant ovarian cancer cells

The concentration of MG132 at 1.0 μg/mL for 8, 16, 24, 36 and 48 hours, the average survival rates of cisplatin resistant ovarian cancer SKOV3 cells were 61.49%, 50.66%, 36.66%, 17.17% and 9.00% respectively. (Fig. 1A); the average survival rates of cisplatin resistant ovarian cancer OVCAR-3 cells were 61.49%, 45.68%, 25.65%, 12.33% and 6.57% respectively. (Fig. 1B). The concentration of cis-diamminedichloroplatinum (DDP) at 15 μg/mL for 8, 16, 24, 36 and 48 hours, the average survival rates of cisplatin resistant ovarian cancer SKOV3 cells were 12.40%, 7.87%, 5.36%, 2.85% and 0.59% respectively (Fig. 1A); the average survival rates of cisplatin resistant ovarian cancer OVCAR-3 cells were 12.40%, 9.00%, 4.32%, 1.27% and 0.23% respectively (Fig. 1B). The concentrations of MG132 at 1.0 μg/mL combined with cisplatin (15 μg/mL) for 8, 16, 24, 36 and 48 hours, the average survival rates of cisplatin resistant ovarian cancer SKOV3 cells were 91.22%, 86.21%, 77.61%, 56.89% and 31.20% respectively (Fig. 1A); the average survival rates of cisplatin resistant ovarian cancer OVCAR-3 cells were 91.22%, 75.97%, 65.90%, 45.68% and 23.65% respectively (Fig. 1B).

3.2 MG132 induced cisplatin resistant ovarian cancer cells apoptosis

The results of cell cycle detection by FCM showed that for SKOV3 cell, MG132 could increase the proportion of S phase ($p < 0.05$). Cisplatin did not significantly inhibit the proportion of S phase cells, After the combination of drugs treatment, the proportion of S phase increased ($p < 0.05$). (Fig. 2A). The results of cell cycle detection by FCM showed that for OVCAR-3 cell, MG132 could increase the proportion of S phase ($p < 0.05$). Cisplatin did not significantly inhibit the proportion of S phase cells, After the combination of drugs treatment, the proportion of S phase increased ($p < 0.05$). (Fig. 2B).

The results of FCM showed that the apoptosis rates of cells for SKOV3 cell in control group were 7.91% ± 1%, 68.3% ± 0.6% in the MG132 group, 41.49% ± 0.4% in the cisplatin group and 85.58% ± 0.48% in the combined group. The apoptosis rate in the MG132 group was higher than that in the control group ($p < 0.05$) and in the combined group was higher than monotherapy group ($p < 0.05$). (Fig. 3A). The results of FCM showed that the apoptosis rates of cells for OVCAR-3 cell in control group were 10.02% ± 0.9%, 35.65% ± 0.8% in the MG132 group, 19.08% ± 0.3% in the cisplatin group and 50.32% ± 0.5% in the combined group. The apoptosis rate in MG132 group was higher than control group ($p < 0.05$) and in the combined group was higher than monotherapy group ($p < 0.05$). (Fig. 3B).

DAPI staining indicates apoptosis through dark blue dots in the figure (Fig. 4). The results show that apoptotic bodies are produced in MG132 group, cisplatin group and combined group, indicating that MG132, cisplatin and combined drugs can promote apoptosis.

3.3 MG132 enhances cisplatin resistant ovarian cancer sensitivity via inducing apoptosis and autophagy factors

After different drugs treated on cisplatin resistant ovarian cancer SKOV3 cell for 48 hours, the results of western blotting showed that Beclin1 protein in each group was as follows: control group: 0.25811127; MG132 group: 0.50081055; cisplatin group: 0.38939371; MG132 + DDP group: 0.61533215, in the MG132 group was higher than control group ($p < 0.05$) and in the combined group was higher than monotherapy group ($p < 0.05$) (Fig. 5A); LC3 protein in each group was as follows: control group: 0.04763507; MG132 group: 0.20750625; cisplatin group: 0.10208789; MG132 + DDP group: 0.25554062, MG132 group was higher than control group ($p < 0.05$) and in the combined group was higher than monotherapy group ($p < 0.05$) (Fig. 5B); Caspase 3 protein in each group was as follows: control group: 0.0378465; MG132 group: 1.03940873; cisplatin group: 0.070011463; MG132 + DDP group: 1.3482177, MG132 group was higher than control group ($p < 0.05$) and in the combined group was higher than monotherapy group ($p < 0.05$) (Fig. 5C). Western blot protein expression, using β-actin levels as loading controls (Fig. 5D).

The results of RT-PCR showed that the mRNA expression of Beclin1 in each group for SKOV3 cell was: control group: 55.91591 ± 3.155260; MG132 group: 184.48823 ± 5.05300; cisplatin group: 86.5527 ± 1.3072; combined group: 272.47796 ± 3.51542, MG132 group was higher than control group ($p < 0.05$) and in the combined group was higher than monotherapy group ($p < 0.05$) (Fig. 6A); The results of RT-PCR showed that the mRNA expression of Beclin1 in each group for OVCAR-3 cell was: control group: 52.1356 ± 2.4456; MG132 group: 176.56623 ± 2.13265; cisplatin group: 55.26547 ± 0.63265; combined group: 265.32654 ± 2.36589, MG132 group was higher than control group ($p < 0.05$) and in the combined group was higher than monotherapy group ($p < 0.05$) (Fig. 6D); The mRNA relative expression of LC3 in each group for SKOV3 was: control group: 0.11502 ± 0.044856; MG132 group: 5.97657 ± 0.09631; cisplatin group 2.41336 ± 0. 09976; combined group: 11.042039 ± 1.00211, MG132 group was higher than control group ($p < 0.05$) and in the combined group was higher than monotherapy group ($p < 0.05$) (Fig. 6B); The mRNA relative expression of LC3 in each group for OVCAR-3 was: control group: 0.23 568 ± 0. 056589; MG132 group: 3.56897 ± 0.09631; cisplatin group 0.956845 ± 0.089547; combined group: 5.23154 ± 1.23568, MG132 group was higher than control group ($p < 0.05$) and in the combined group was higher than monotherapy drug group ($p < 0.05$) (Fig. 6E). The mRNA relative expression of caspase 3 in each group for SKOV3 was: control group: 0.14258 ± 0.00790 ; MG132 group: 7.49635 ± 0.32674; cisplatin group:

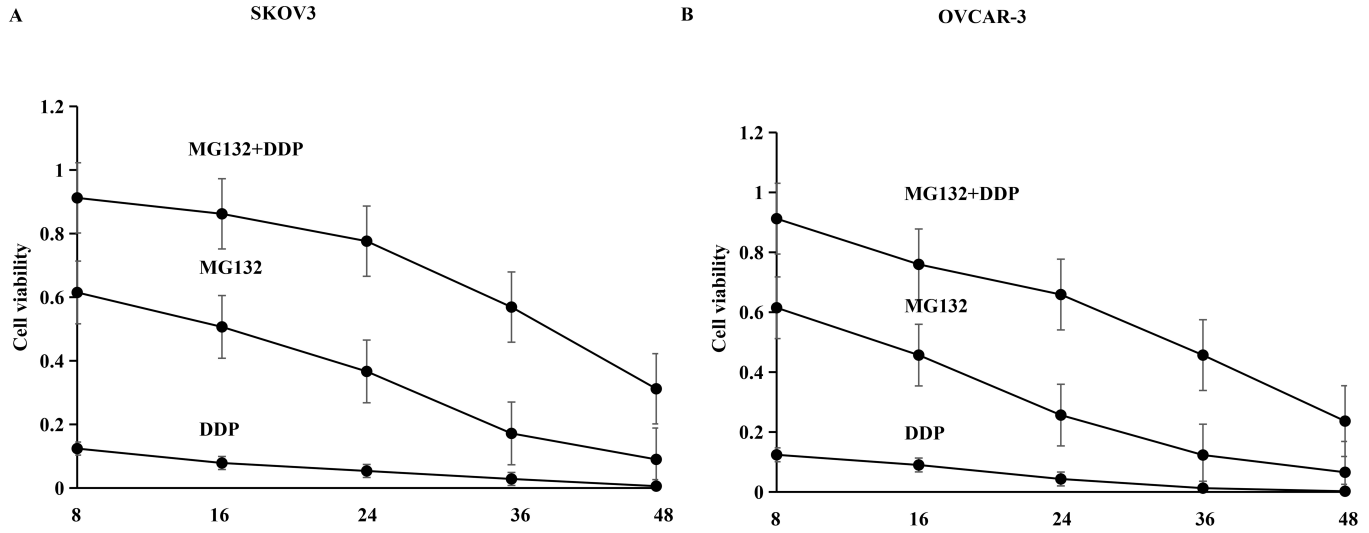

FIGURE 1. Cell growth was detected by cell CCK-8 assay. A. The concentration of MG132 at 1.0 μg/mL with concentration of cisplatin at 15 μg/mL treated after 8, 16, 24, 36 and 48 hours for cisplatin resistant ovarian cancer SKOV3 cells. The cell survival rate in the combined group was significantly lower than that in the MG132 group and DDP group; B. The concentration of MG132 at 1.0 μg/mL with concentration of cisplatin at 15 μg/mL treated after 8, 16, 24, 36 and 48 hours for cisplatin resistant ovarian cancer OVCAR-3 cells. The cell survival rate in the combined group was significantly lower than that in the MG132 group and DDP group. Each group of experiments was repeated three times.
MG132: carbobenzoxy-L-leucy-L-Leucyl-L-Leucinal; DDP: cis-diamminedichloro-platinum.

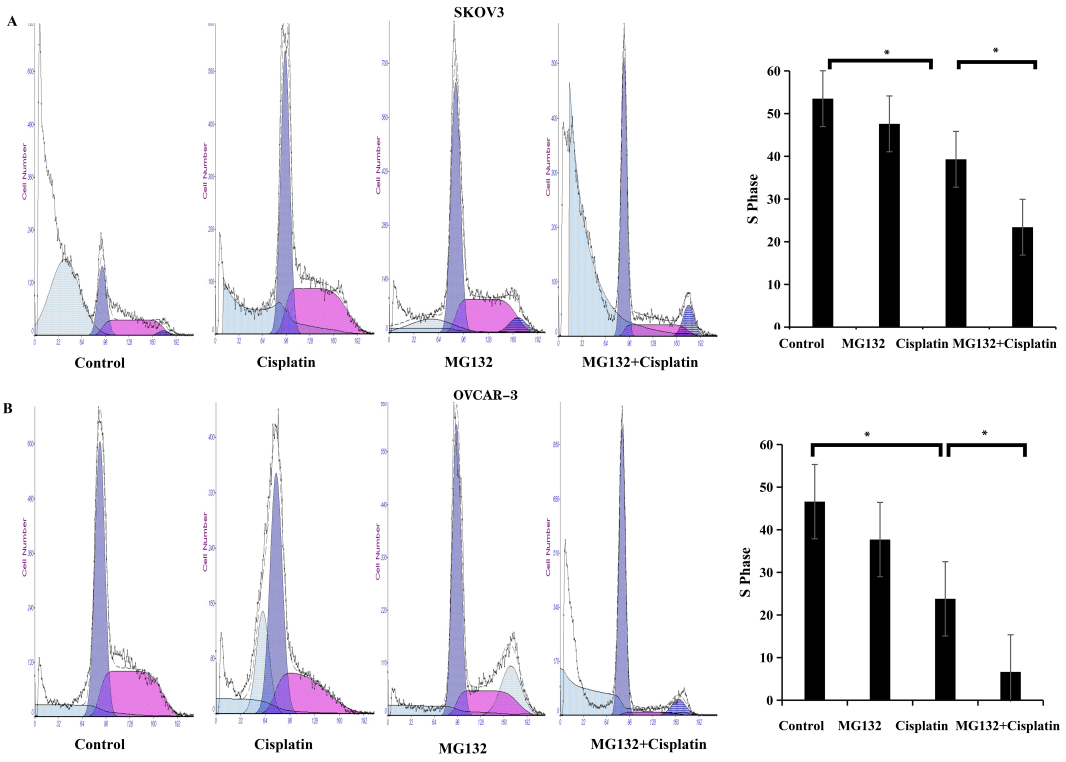

FIGURE 2. Cell cycle detection. A. The results of cell cycle detection by FCM showed that for SKOV3 cell, MG132 could increase the proportion of S phase ($p < 0.05$). Cisplatin did not significantly inhibit the proportion of S phase cells, After the combination of drugs treatment, the proportion of S phase increased ($p < 0.05$). B. The results of cell cycle detection by FCM showed that for OVCAR-3 cell, MG132 could increase the proportion of S phase ($p < 0.05$). Cisplatin did not significantly inhibit the proportion of S phase cells, After the combination of drugs treatment, the proportion of S phase increased ($p < 0.05$). Each group of experiments was repeated three times.
MG132: carbobenzoxy-L-leucy-L-Leucyl-L-Leucinal.

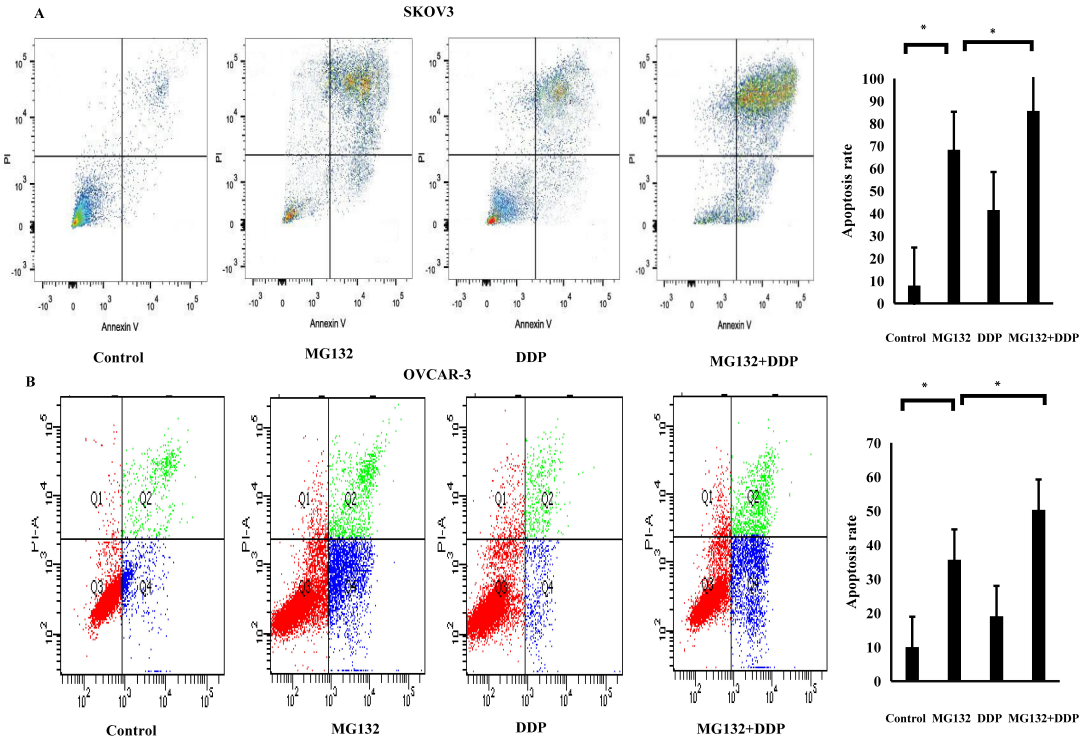

FIGURE 3. FCM showed that the apoptosis rates of cells in the four groups. A. For SKOV3 cell, the apoptosis rate in the MG132 group was higher than that in the control group ($p < 0.05$) and in the combined group was higher than that in the single drug group ($p < 0.05$). B. For OVCAR-3 cell, the apoptosis rate in the MG132 group was higher than that in the control group ($p < 0.05$) and in the combined group was higher than that in the single drug group ($p < 0.05$). Each group of experiments was repeated three times.
MG132: carbobenzoxy-L-leucy-L-Leucyl-L-Leucinal. DDP: cis-diamminedichloro-platinum.

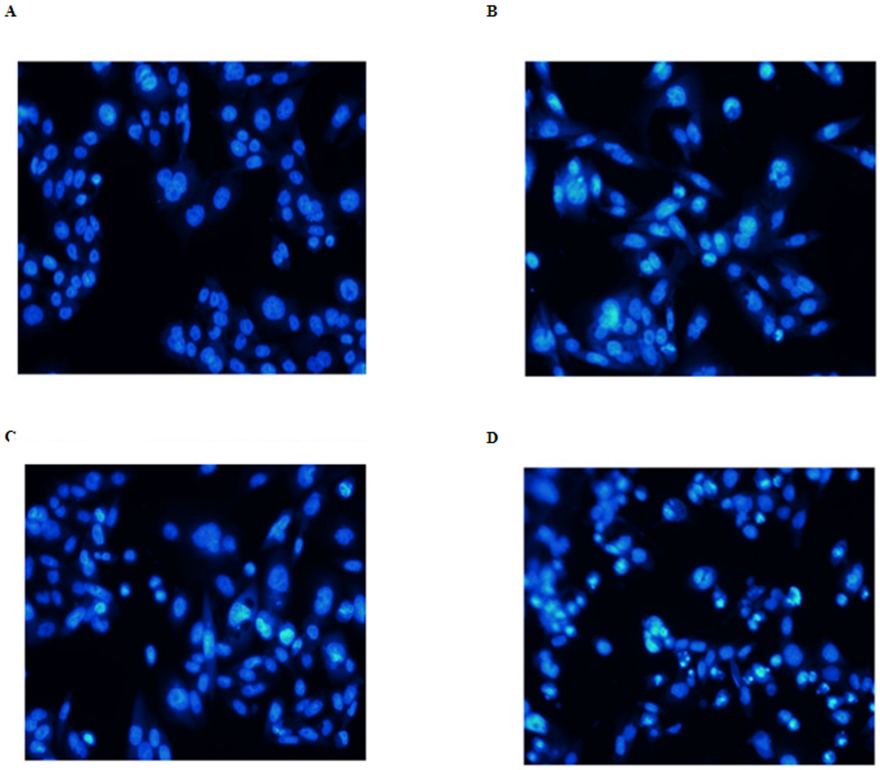

FIGURE 4. DAPI staining indicates apoptosis through dark blue dots in the figure. A. Control group; B. MG132 group; C. Cisplain group; D. Combination group.

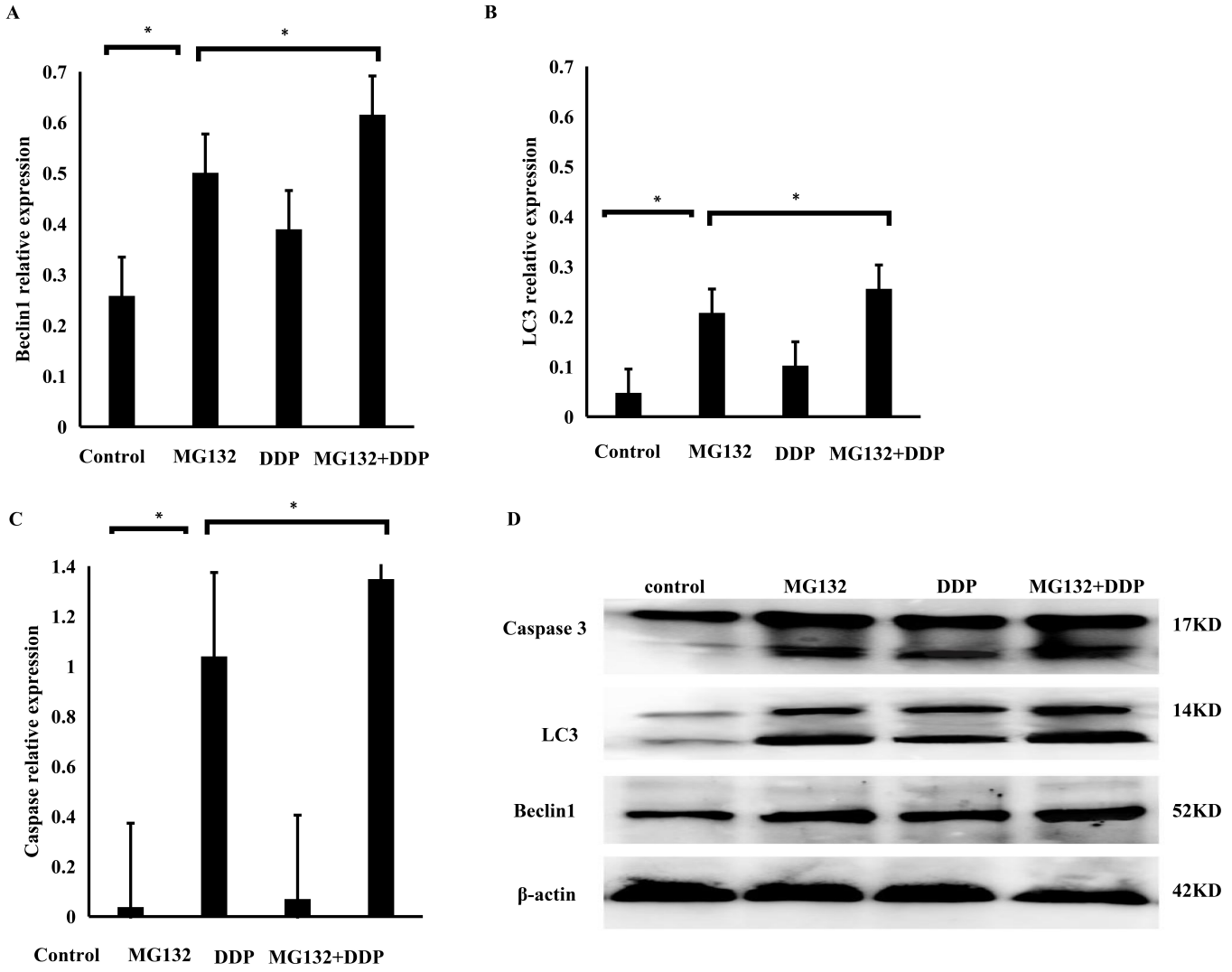

FIGURE 5. Expression of different factors after treated with different drugs. A Beclin1 protein expression in the MG132 group was higher than that in the control group ($p < 0.05$) and in the combined group was higher than that in the single drug group ($p < 0.05$); B. LC3 protein expression in the MG132 group was higher than that in the control group ($p < 0.05$) and in the combined group was higher than that in the single drug group ($p < 0.05$); C. Caspase 3 protein expression in the MG132 group was higher than that in the control group ($p < 0.05$) and in the combined group was higher than that in the single drug group ($p < 0.05$); D. Western blot protein expression, using β-actin levels as loading controls. Each group of experiments was repeated three times.
MG132: carbobenzoxy-L-leucy-L-Leucyl-L-Leucinal; DDP: cis-diamminedichloro-platinum.

2.97934 ± 0.14451; combined group: 16.44695 ± 1.48795, MG132 group was higher than control group ($p < 0.05$) and in the combined group was higher than monotherapy group ($p < 0.05$) (Fig. 6C); The mRNA relative expression of caspase 3 in each group for OVCAR-3 was: control group: 0.80258 ± 0.023568; MG132 group: 5.49635 ± 0.56589; cisplatin group: 0.97934 ± 0.563256; combined group: 13.44695 ± 1.23568, MG132 group was higher than control group ($p < 0.05$) and in the combined group was higher than monotherapy group ($p < 0.05$) (Fig. 6F).

4. Discussion

Apoptosis and autophagy are called type I and type II programmed cell death respectively, which play a key role in the pathogenesis of tumor cells. As a result of the change of the ratio of anti-apoptotic protein to pre-apoptotic protein in programmed cell death cells, ubiquitin proteasome can interfere with this balance, resulting in promoting or inhibiting apoptosis [7]. Autophagy is a process that highly regulates the large-scale degradation of long-lived proteins and organelles [8]. It can regulate the homeostasis of the body by eliminating dysfunctional cells and complementing the ubiquitin proteasome degradation system [9]. Studies have shown that autophagy provides tumor cells with a way to escape apoptotic cell death, resulting in tumor drug resistance [10]. Similarly, inhibition of autophagy can enhance the sensitivity by apoptosis inducers. The inhibitory effect of autophagy on tumor cells is not a simple autophagic cell death, but a biological process with strict regulation of a series of

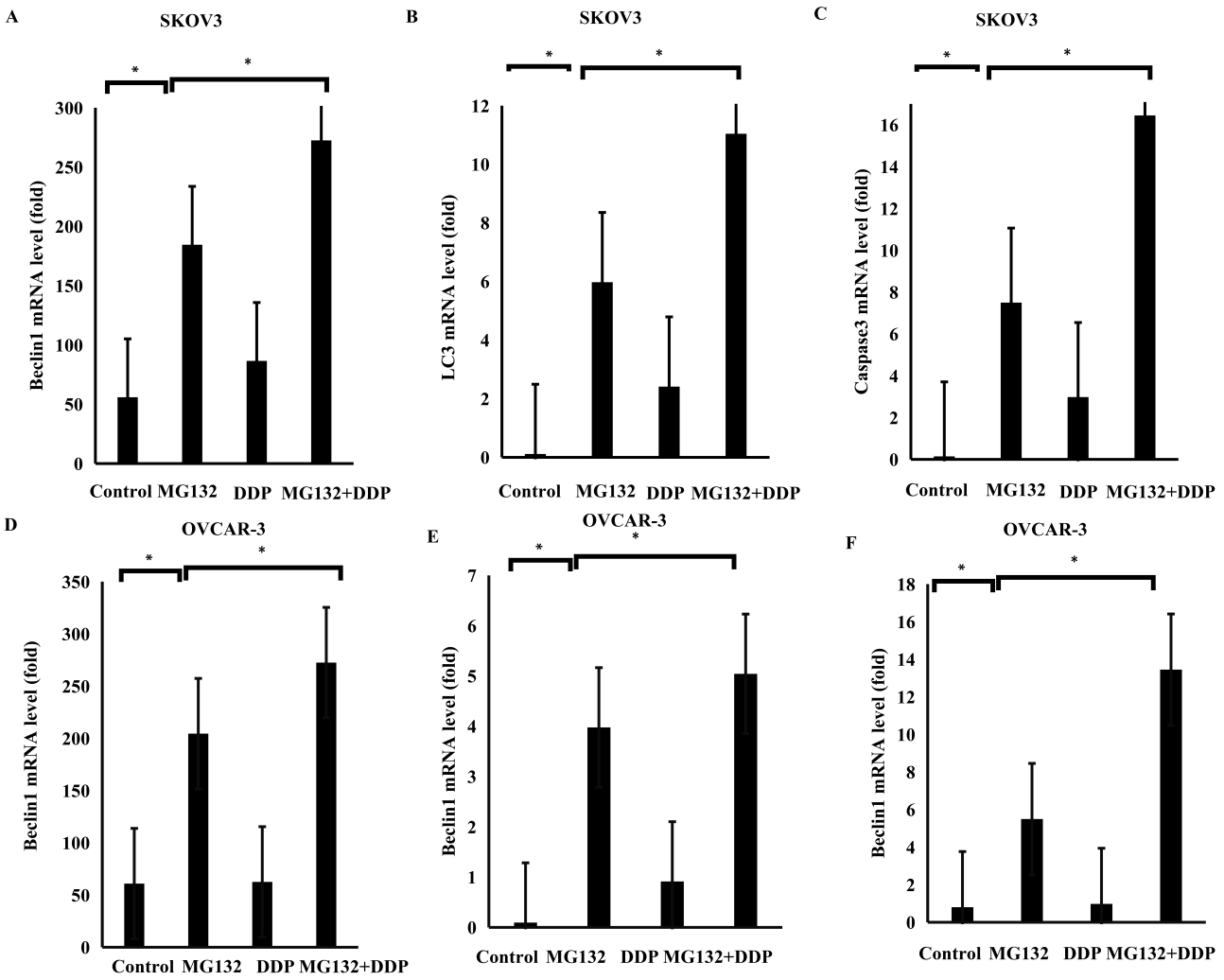

FIGURE 6. The mRNA expression of different factors after treated with different drugs. A Beclin1 mRNA expression in the MG132 group was higher than that in the control group for SKOV3 cell ($p < 0.05$) and in the combined group was higher than that in the single drug group ($p < 0.05$); B. LC3 mRNA expression in the MG132 group was higher than that in the control group ($p < 0.05$) and in the combined group was higher than that in the single drug group for SKOV3 cell ($p < 0.05$); C. Caspase 3 mRNA expression in the MG132 group was higher than that in the control group ($p < 0.05$) and in the combined group was higher than that in the single drug group for SKOV3 cell ($p < 0.05$); D. Beclin1 mRNA expression in the MG132 group was higher than that in the control group ($p < 0.05$) and in the combined group was higher than that in the single drug group for OVCAR-3 cell ($p < 0.05$); E. LC3 mRNA expression in the MG132 group was higher than that in the control group for OVCAR-3 cell ($p < 0.05$) and in the combined group was higher than that in the single drug group ($p < 0.05$); F. Caspase 3 mRNA expression in the MG132 group was higher than that in the control group for OVCAR-3 cell ($p < 0.05$) and in the combined group was higher than that in the single drug group ($p < 0.05$). Each group of experiments was repeated three times.
MG132: carbobenzoxy-L-leucy-L-Leucyl-L-Leucinal. DDP: cis-diamminedichloro-platinum.

genes and multiple mechanisms. Many oncogenes, tumor suppressor genes and various regulatory factors play an anti-tumor effect by participating in the autophagy pathway.

Beclin1 is located on human chromosome 17q21, it is the first confirmed mammalian autophagy gene. It has the same amino acid of 24.4% as yeast autophagy related genes 6. It is considered to be a homologous gene of mammalian atg6 [9–11]. Beclin1 is the first protein identified to connect autophagy and apoptosis pathways [12]. Therefore, Beclin1 is considered to be a key factor involved in the formation of mammalian autophagy. Microtubule associated protein 1 light chain 3 (LC3) is a homologue of yeast autophagy related gene 8 (Atg8) in mammals [13]. LC3 has been used as a specific marker protein of autophagy, it can reflect the number of autophagy and the degree of autophagy [14].

Proteasome inhibitors can block the protein degradation mediated by UPP, interfere with the balance of protein synthesis and degradation in cells, induce apoptosis and play an anti-tumor role [15]. The effects on tumor cells and normal cells are different. The sensitivity is 100–1000 times higher than that of normal cells [16]. When proteasome inhibitors act on normal cells, cell cycle checkpoints can suspend cell activity and continue to divide after proteasome function is restored, which does not affect the physiological function of normal

cells, while tumor cells are prone to apoptosis, especially rapidly differentiated cells. Therefore, proteasome inhibitors are a kind of antitumor drugs with a new mechanism [17]. Proteasome inhibitors can also increase the sensitivity of tumor cells to chemotherapeutic drugs and radiotherapy. They still have good therapeutic effects on drug-resistant tumors. When they act together with other chemotherapeutic drugs, they have better inhibitory effects on tumors [18].

Cisplatin is widely used for malignant tumors, it has been, used as an anti-tumor agent, 15% of patients affected with metastatic tumors for 3 months median duration [19].

CCK-8 was used to detect the cell survival rate of each group. After treating with different drugs, the cell survival rate decreased with the increase of times for cisplatin resistant ovarian cancer SKOV3 and OVCAR-3 cells. The cell survival rate in the combined drug group was significantly lower than monotherapy group, indicating that MG132 and cisplatin had a synergistic effect and significantly reduced the cell survival rate.

FCM showed that MG132 could decrease the proportion of cells in S phase, significantly reduce the proportion of cells and significantly increase the apoptosis rate, indicating that MG132 promoted the apoptosis of cells. The apoptosis rate of the combined drug group was higher than monotherapy group, and the difference was statistically significant, indicating that MG132 can promote cisplatin resistant ovarian cancer cell apoptosis and reduce cell drug resistance, which further proved that MG132 and cisplatin have a synergistic effect.

Western blotting and RT-PCR showed that the protein and mRNA expression of Beclin1, LC3 and Caspase 3 in cells of each group, there were significant differences among control group, MG132 group, cisplatin group and combined group, and the protein and mRNA expression in combined group was higher than monotherapy group. We confirmed that MG132 can promote cisplatin resistant ovarian cancer cells apoptosis and autophagy.

We have studied and concluded that MG132 combined with cisplatin had a synergistic antitumor effect, and it is an effective antitumor drug for cisplatin resistant ovarian cancer [20]. Some research suggest that cisplatin resistance ovarian cancer cells can be overcome by the combination of cisplatin and the proteasome inhibitors [21]. Other article suggest that pro-caspase-3 can induce ovarian cancer cells apoptosis by combination with proteasome inhibitor [22].

5. Conclusions

In conclusion, the growth of the cisplatin resistant ovarian cancer SKOV3 and OVCAR-3 cells can be inhibited by MG132 according to induce apoptosis and autophagy. MG132 is expected to be a synergistic antitumor effect with cisplatin. The purpose of this research is to find out the most effective drug for the treatment of ovarian cancer, improve the survival rate of patients.

ABBREVIATIONS

UPP, ubiquitin proteasome pathway; RT-PCR, reverse transcription-polymerase chain reaction; DMSO, dimethyl sulfoxide; FCM, flow cytometer; MG132, carbobenzoxy-L-leucy-L-Leucyl-L-Leucinal; CCK-8, cell counting kit-8; Caspase 3, cyserinly aspartatespecific protease 3.

AUTHOR CONTRIBUTIONS

NG—performed the statistical analysis and drafted the manuscript. BZ—participated in the design of the study. Both authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

ACKNOWLEDGMENT

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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