International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2022.806.816

Research Article
Curcumin Improving Drug Resistance of MDA-MB-231/DDP Tumor Treatment by Enhancing Autophagy

[1]Ming-Qin Qian, [2]Xian-De Ma and [2]Guo-Wei Pan

[1]College of Integrated Traditional and Western Medicine, Liaoning University of Traditional Chinese Medicine, Shenyang110032, People Republic of China
[2]Teaching and Experiment Center, Liaoning University of Traditional Chinese Medicine, Shenyang 110032, People Republic of China

Abstract

Background and Objective: The poor prognosis of Triple-Negative Breast Cancer (TNBC) is a difficult problem in the clinical treatment of breast cancer. The mechanism of curcumin on improving the drug resistance of breast cancer by regulating the autophagy of the MDA-MB-231/DDP resistant cell line was studied in this research. **Materials and Methods:** The drug resistance of MDA-MB-231 cells was induced by Cisplatin (DDP) *in vitro*. MDA-MB-231/DDP cells were randomly divided into four groups including blank control group, curcumin group, DDP group and curcumin+DDP group. After treatment with curcumin (10 μmol L^{-1}) or/and DDP (5 μg mL^{-1}) for 48 hrs, the proliferation activities were analyzed by the method of CCK8 and the apoptosis rates were detected by flow cytometry. The expression of LC3 I, LC3 II, Beclin-1, Atg1, 4, 7, 13, TRAP1, p-Akt and P70S6K were detected by the method of Western Blot. MDA-MB-231/DDP tumour bearing mice were grouped as above. The tumour bearing mice were given curcumin at a dosage of 100 mg kg^{-1}/day and/or DDP 6 mg kg^{-1}/day intraperitoneal injection once a day for 7 days. The expression of TRAP1, P-Akt and p70S6K were detected by immunohistochemistry. TUNEL assay was used to detect the apoptosis rates in mice tumour tissue. The expressions of LC3 I, LC3 II, Beclin-1, Atg1, 4, 7, 13, TRAP1, P-Akt and p70S6K in tumour tissues of mice were detected by Western Blot. **Results:** Compared with the blank control group, curcumin combined with DDP could increase the apoptosis rate of MDA-MB-231/DDP cells and MDA-MB-231/DDP of tumour bearing mice significantly. Curcumin combined with DDP could promote the autophagy of MDA-MB-231/DDP cells and tumour bearing mice by regulating the expression of LC3 I, LC3 II, Beclin-1, Atg1, 4, 7 and 13. Curcumin combined with DDP could down-regulate the TRAP1 protein expression level in MDA-MB-231/DDP cells and tumour bearing mice significantly. Curcumin combined with DDP could up-regulate the protein in co-expression of LC3 I, LC3 II and TRAP1 in MDA-MB-231/DDP cells and tumour-bearing mice. **Conclusion:** Curcumin can inhibit the over activation of the P-Akt/p70S6K signalling pathway in MDA-MB-231/DDP cells and tumour bearing mice. Curcumin may inhibit the over-activation of the Akt/p70S6K signalling pathway, promote the autophagy of MDA-MB-231 cells, reduce the drug resistance of MDA-MB-231/DDP tumour bearing mice to DDP and induce autophagy death of tumour cells.

Key words: Curcumin, autophagy, drug resistance, cisplatin, breast cancer

Citation: Qian, M.Q., X.D. Ma and G.W. Pan, 2022. Curcumin improving drug resistance of MDA-MB-231/DDP tumor treatment by enhancing autophagy. Int. J. Pharmacol., 18: 806-816.

Corresponding Author: Xian-De Ma, Guo-Wei Pan, Teaching and Experiment Center, Liaoning University of Traditional Chinese Medicine, 79th Chongshan East Road, Shenyang, 110032, Liaoning, People Republic of China

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Triple-Negative Breast Cancer (TNBC) is defined as breast cancer patients with negative for Estrogen Receptor (ER), Progesterone Receptor (PR) and human epidermal growth factor receptor 2 (HER-2). The 5-year survival rate of patients with TNBC is less than 80%[1], which is mostly occurred in young patients with high rates of visceral and brain metastases[2], poor pathological grading, rapid cell proliferation and resistance to chemotherapeutic drugs. Therefore, the poor prognosis of TNBC is a difficult problem in the clinical treatment of breast cancer. Chemotherapy, surgery and radiotherapy are regarded as the most effective means of cancer treatment and play an irreplaceable role in the clinical treatment of cancer. Therefore, it is important to elucidate the mechanism of TNBC resistance and to explore the key targets for inducing TNBC resistance[3]. Human breast cancer cell line MDA-MB-231 is a TNBC cell line, which is often used in basic research. Cisplatin (DDP) is a platinum-based drug of tumour chemotherapy regimens and has an excellent effect in the treatment of most tumours. However, the use of DDP alone in the treatment of TNBC has severe side effects, which limited the application of DDP[4,5]. It is prone to easy resistance to drugs and cannot fully play the key role of chemotherapy in the treatment of tumours. Combination therapy has become a new approach to the treatment of TNBC[6,7].

Autophagy is a biological process in which the organism engulfs its cytoplasmic proteins or organelles, encapsulates them into vesicles, fuses with lysosomes to form autophagy lysosomes and degrades the encapsulated contents. In this way, the organism can achieve the metabolic needs of cells itself and the renewal of some organelles[8]. Under normal physiological conditions, autophagy is beneficial to maintain cellular homeostasis, however, when the organism is subjected to stress injury, autophagy is excessively up-regulated to prevent accumulation of damage in protein and organelles and even induce autophagy death. Autophagias death refers to the autophagy process in which cytosol and organelles are isolated into vesicles of a bilayer membrane, transport to lysosomes/vacuoles for degradation and recycle large macromolecules[9]. LC3-II and Beclin-1 are involved in the formation of autophagy. Beclin-1 is one of the key promoter genes of autophagy which acts on the initial stage of autophagy[10] and LC3-II is a structural protein localized in the autophagosome membrane that showed a positive correlation with the autophagy. So, LC3-II is an important indicator of autophagy activity and autophagy formation[11]. Autophagy may play a dual role in the development of neoplasms, including protective autophagy and autophagy cell death.

Curcumin is the main component of Curcuma, a natural plant medicine that has many pharmacological effects such as anti-inflammatory, anti-oxidation and anti-tumour effects[12]. Studies indicated that curcumin has an obvious effect on the treatment of breast cancer[13] but the mechanism remains unclear. Curcumin can reduce the survival rate of human ovarian cancer SK-OV-3 and A2780 cell lines by inhibiting protective autophagy[14]. And the combination of curcumin and gefitinib can induce autophagy, autophagy cell death and autophagy-mediated apoptosis in non-small cell lung cancer H157 cells and NCI-H1299 cells[15]. All the above research indicated that curcumin was involved in the development of autophagy of tumour cells. In this paper, the effects of curcumin combined with DDP on autophagy of drug-resistant breast cancer cells were investigated and the mechanism of curcumin in reducing the resistance of TNBC to DDP was explored.

MATERIALS AND METHODS

Study area: This study was carried out at Liaoning University of Traditional Chinese Medicine, Shenyang, China from March, 2020 to April, 2021.

Animals: Nude mice, female, 20±2 g, purchased from Beijing HFK Bioscience Co., Ltd (Reference No.: SCXK (Beijing) 2016-0002), were raised adaptively for seven days, fed and drank normally and all operations met the ethical requirements.

MDA-MB-231 cell line was purchased from Shanghai Tongpai Biotech Co., Ltd.

Chemical and reagents: Curcumin was purchased from Shanghai Aladdin Biochemical Technology Ltd., Co. (CAS 458-37-7), Cisplatin was purchased from Qilu Pharmaceutical Ltd., Co. (H37021358), L-15 medium was purchased from HyClone Ltd., Co. (Cat No.: SH30525.01), Fetal Bovine Serum (FBS) was purchased from PAN Biotech (Cat No.: P30-3302), Trypsin was purchased by Gibco Ltd., Co. (Cat No.: 25200056), Annexin V-FITC/PI Apoptosis Kit and TUNEL cell apoptosis kit were purchased from Nanjing Jiancheng Biotechnology Ltd., Co., LC3 I, LC3 II, Beclin-1, Atg1, 4, 7, 13, TRAP1, P-Akt, p70S6K antibodies were purchased from Abcam Ltd., Co.

Instrument: CO_2 incubator and microplate reader (MK3) were from Thermo Ltd., Co., DMi8 fluorescent microscope was from Leica Ltd., Co., Biological Safety Cabinet (HFsafe LC A2) was from Lixin Instrument (Shanghai) Co., Ltd. Electrophoresis apparatus (ESP-300) was from Shanghai Tanon Technology Co., Ltd. Flow cytometry (Beckman CytoFlex) was from Beckman Ltd., Co., Gel Imaging Analysis System (Model SF4200) was from Shanghai Tanon Technology Co., Ltd.

Preparation of MDA-MB-231/DDP: A triple-negative MDA-MB-231 cisplatin-resistant breast cancer cell line was established by gradually increasing the concentration and inducing intermittently as the established method with minor modification[16]. Briefly, the initial dose of DDP was 100 ng mL^{-1} followed by a 2 weeks induction cycle in which 250 ng mL^{-1} was escalated to a final concentration of 4 μg mL^{-1}.

***In vitro* test:** MDA-MB-231/DDP cells were randomly divided into four groups including blank control group, curcumin group, DDP group and curcumin+DDP group. In this research, cells were seeded into 96-well plates with 10 replicate wells per group, 6 replicate wells per group of cells seeded into 6-well plates and 6 vials per group of cells were seeded into flasks.

The cells of the blank control group were not treated and fed normally. In the other groups, after the cells were attached to the wall, the L-15 medium containing curcumin of 10 μmol L^{-1}, DDP of 5 μg mL^{-1}, curcumin (10 μmol L^{-1}) and DDP (5 μg mL^{-1}) were replaced and cultured for 24 hrs, respectively.

The proliferative activity of the cells in each group was detected by the method of CCK8. After incubating for 24 hrs at 37°C, 10 μL of CCK-8 working fluid were added into each well and incubated at 37°C for 4 hrs. Then, the OD value at 450 nm was detected by the plate reader.

Apoptosis analyses were achieved by flow cytometry. After the cells which were washed twice with PBS were digested with trypsin, cells from each well were collected into a Falcon tube. All the cells were washed twice with pre-cooled PBS buffer and the suspension of 1×106 cells mL^{-1} was prepared with binding buffer (1×). 100 μL of cell suspension was taken from each tube and 5 μL of Annexin V-FITC and 5 μL of PI were added. After mixing, the mixture was incubated at room temperature and away from light for 15 min. After incubation, 400 μL of binding buffer (1×) was added into each test tube for apoptosis analysis by flow cytometry.

The autophagy-related proteins including LC3 I, LC3 II, Beclin-1, Atg1, 4, 7, 13, TRAP1, p-Akt and P70S6K were detected by the method of Western Blot. The cells in the cell culture flask were washed with PBS twice and the flasks were placed on the ice for protein extraction. 0.5 mL of WB and IP cell lysate was added into each flask which was repeatedly blown with a pipette to make the cells fall off and then lazed on the ice surface for 15 min. The lysate was collected and centrifuged with 14000 g at 4°C for 15 min to obtain the total protein (supernatant). After finishing the total protein quantification by the BCA method, the protein concentration was adjusted to 6.25 mg mL^{-1}. Protein loading buffer (5×) was added to the adjusted protein solution at a ratio of 4:1 (v/v) and then the solution was bathed in boiling water for 5 min. The SDS-Page gel was prepared for vertical electrophoresis with 10 μL of sample per lane. The proteins in the gel were transferred to the PVDF membrane and sealed with 5% BSA for 1 h. The membrane was cut according to the expected molecular weight of the protein, then the corresponding antibody working solution was added and incubated at 4°C overnight. On the next day, TBST buffer was used to wash the membrane 6 times (5 min for each time) after the antibody working solution was removed. Then add the working fluid of the second antibody and incubate at room temperature for 2 hrs. After incubation, the second antibody working fluid was removed and the membrane was washed with TBST buffer 6 times (5 min for each time). The PVDF membrane was placed in the gel imaging analysis system and covered with ECL luminescent solution. After the image was collected, the grey value of protein bands was measured. The ratio of the grey value of target protein to that of internal reference protein was used as the relative expression level of the target protein.

***In vivo* test:** The 0.2 mL of MDA-MB-231/DDP cells in the logarithmic growth phase was injected subcutaneously into the right anterior axilla of nude mice at a concentration of 1×10^7 cells mL^{-1} to obtain tumour-bearing mice. The mice were fed normally. When the subcutaneous tumour was 1 cm in diameter, the tumour-bearing mice were randomly divided into four groups including the blank control group, curcumin group, DDP group and curcumin+DDP group (n = 10).

Nude mice of curcumin group (100 mg kg^{-1}/day), DDP group (6 mg kg^{-1}/day) and curcumin (100 mg kg^{-1}/day)+ DDP group (6 mg kg^{-1}/day) were intraperitoneally injected corresponding drugs once a day for consecutive 7 days. Normal saline was injected into the nude mice of the blank

control group with the same volume as that of the other groups and all the mice were fed normally. After the last dose, mice were sacrificed by anaesthesia and the complete tumour tissues were removed for analysis.

The diameter and weight of all the tumour tissues were determined to evaluate the therapeutic efficacy firstly. And the apoptosis in tumour tissues was determined by the method of TUNEL subsequently. Briefly, half of tumor tissue was cut and fixed in 4% paraformaldehyde solution for more than 24 hrs. Paraffin sections were prepared routinely. After dewaxing and hydration, 50 μL of TdT enzyme solution containing 45 μL of equilibration buffer, 1 μL of FITC-12-Dutp and 4 μL of TdT Enzyme was onto the tissue membrane of each section and the reaction was followed for 60 min at 37°C in the dark. After being washed with PBS solution, DAPI solution was dropped and restrained for 10 min. Cell apoptosis was observed under a fluorescence microscope and the number of apoptotic cells was counted after the sections were washed with PBS and sealed. Mean±SD was used to analyze the apoptosis of tumour tissues (n = 5).

Expression of TRAP1, P-Akt and p70S6K were determined by immunohistochemistry. Briefly, Paraffin sections were boiled in citrate buffer for 20 min to repair the antigen after dewaxed and hydrated. Than 3% H_2O_2 was added onto the slice to remove the endogenous peroxidase. Normal goat serum was added for sealing and then the first anti-body working solution was added and incubated overnight at 4°C. On the next day, after the slice was taken out and rewarmed for 2 hrs, a second antibody working solution was added and incubated at 37°C for 2 hrs. After washing, DAB chromogenic agent was added for 10 min. Hematoxylin was dyed for 1 min after rinsing with water and then 1% HCl in ethanol (v/v) was added before the slices were sealed. OD values of the samples were determined and relative protein contents were analysed as mean±SD (n = 5).

Finally, the autophagy-related proteins including LC3 I, LC3 II, Beclin-1, Atg1, 4, 7, 13, TRAP1, P-Akt and p70S6K were determined by the method of Western Blot. Briefly, 100 mg of tumour tissues were cut into a tube. The 1 mL of WB and IP cell lysates were added and homogenated by a high-speed electric homogenizer on an ice bath to get the total protein. The protein quantification, concentration adjustment and test procedures are the same as those *in vitro* tests.

Data analysis: SPSS 22.0 software was used to analyze the experimental data. The data were statistically analyzed by one-way ANOVA and the comparison between groups was performed by LSD, with a statistical significance of $p<0.05$.

RESULTS

Effect on the proliferation of MDA-MB-231/DDP cells *in vitro*: The cell proliferation activity of each group was detected by CCK-8 assay and the absorbance value of each group represented their cell proliferation activity. *In vitro* test indicated in Fig. 1 that the proliferation activity of curcumin+ DDP group was significantly lower than that of the blank control group, curcumin group and DDP group ($p<0.01$).

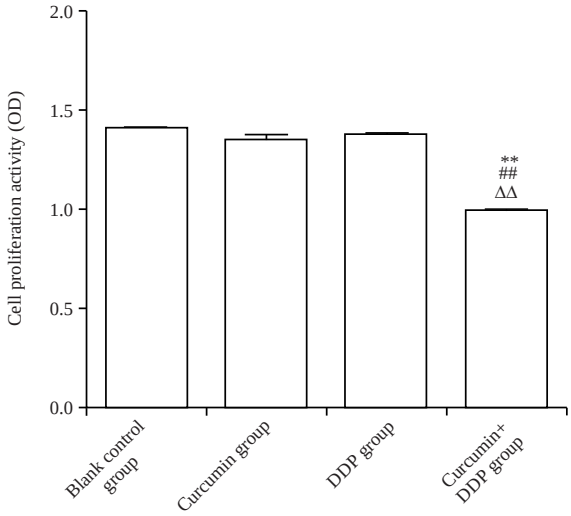

Fig. 1: Proliferative activity on MDA-MB-231/DDP cells

**p<0.01 vs. blank control group, ##p<0.01 vs. Curcumin group, ΔΔp<0.01 vs. DDP group

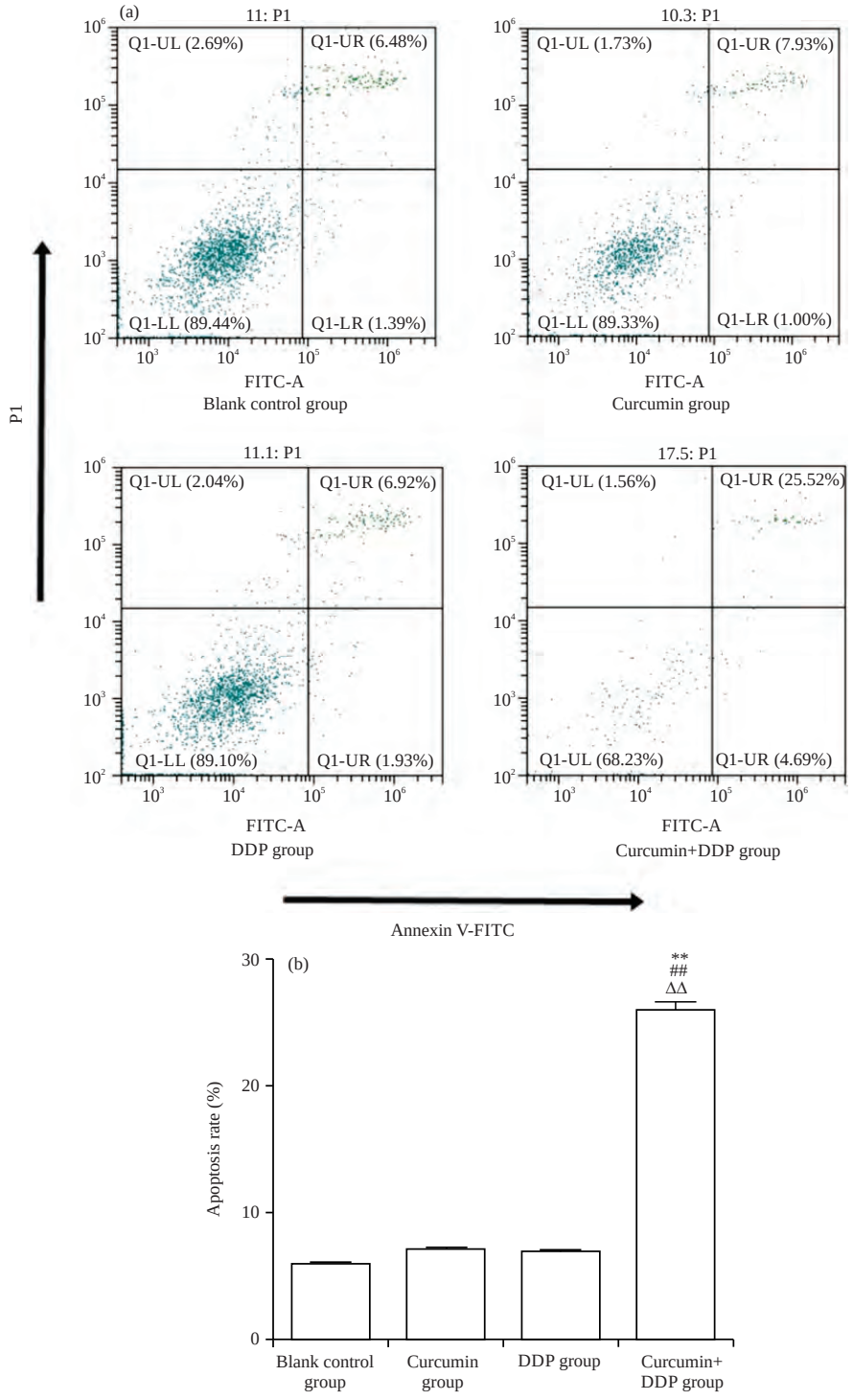

Fig. 2(a-b): Effect on apoptosis of MDA-MB-231/DDP cells (a) Flow cytometry results and (b) Apoptosis rates of MDA-MB-231/DDP cells

**p<0.01 vs. blank control group, ##p<0.01 vs. Curcumin group, ΔΔp<0.01 vs. DDP group

Effect on apoptosis rate of MDA-MB-231/DDP cells *in vitro*: Flow cytometry was used to detect the apoptosis rate of cells in each group. PI single staining (upper left quadrant) was dead cells, annexin V single staining (lower right quadrant) was early apoptotic cells and double staining (upper right quadrant) was middle apoptotic cells The double negative staining area (lower left quadrant) was granular impurities such as cell debris in Fig. 2a. The apoptosis rates of the blank

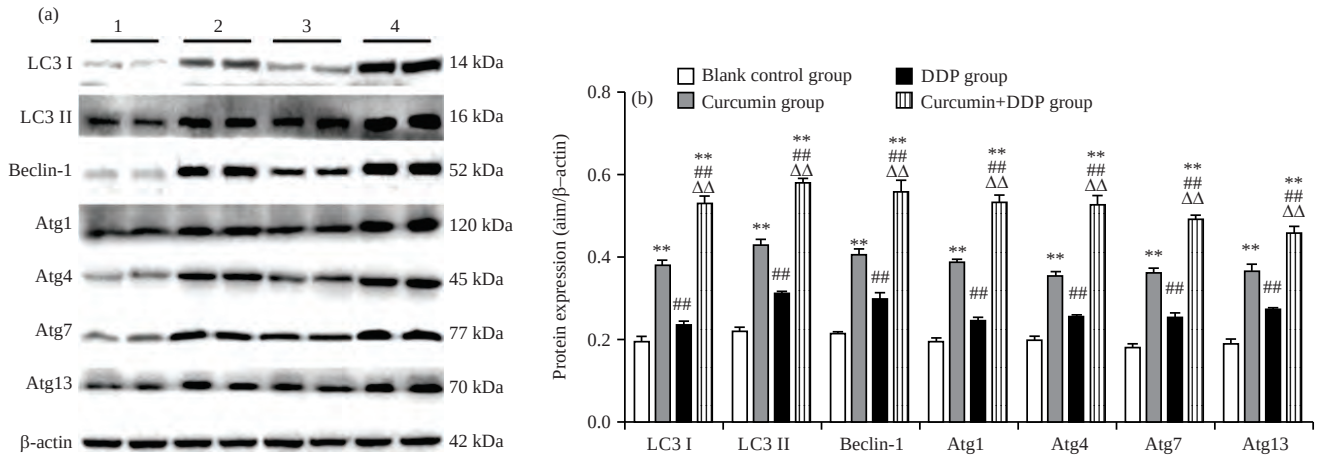

Fig. 3(a-b): Western blot analysis of apoptosis-related proteins *in vitro* (a) Western blot results of and (b) Expression levels of LC3 I, LC3 II, Beclin-1, Atg1, 4, 7, 13 *in vitro*

**p<0.01 vs. blank control group, ##p<0.01 vs. Curcumin group, ΔΔp<0.01 vs. DDP group

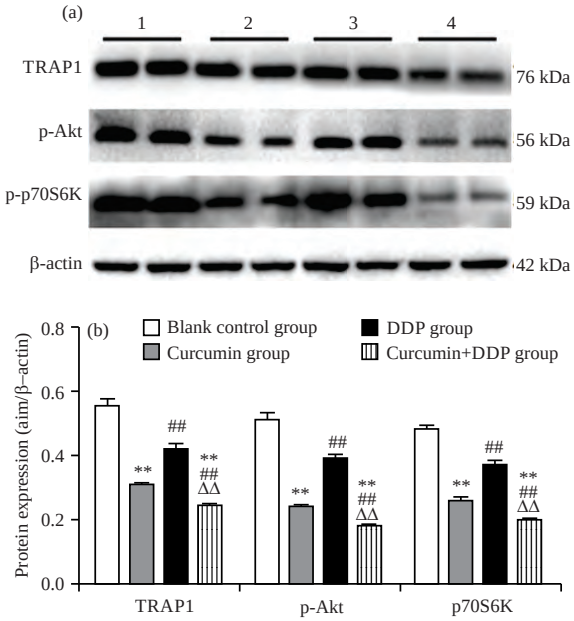

Fig. 4(a-b): Western blot analysis of apoptosis-related proteins *in vitro* (a) Western blot results of (b) Expression levels of TRAP1, p-Akt and p70S6K *in vitro*

**p<0.01 vs. blank control group, ##p<0.01 vs. Curcumin group, ΔΔp<0.01 vs. DDP group

control group, curcumin group, DDP group and curcumin+DDP group were 5.89±0.55, 7.06±0.55, 6.98±0.23 and 25.93±1.80%, respectively. Apoptosis analysis in Fig. 2b was performed and the apoptosis rate of the curcumin+DDP group was significantly higher than that of the blank control group, curcumin group and DDP group (p<0.01).

Western blot analysis of apoptosis-related proteins *in vitro*: Western blot was used to detect the expression levels of LC3 I, LC3 II, Beclin-1, Atg1, 4, 7 and 13 in each group in Fig. 3a. The ratio of the grey value of the target protein band to the grey value of the corresponding internal reference was used as the relative expression level of the target protein.

Autophagy related proteins were analyzed and the results in Fig. 3b indicated that the expression levels of LC3 I, LC3 II, Beclin-1, Atg1, 4, 7 and 13 protein in the curcumin group and curcumin+DDP group were significantly higher than those in the blank control group and DDP group (p<0.01). Compared with the curcumin group, the expression

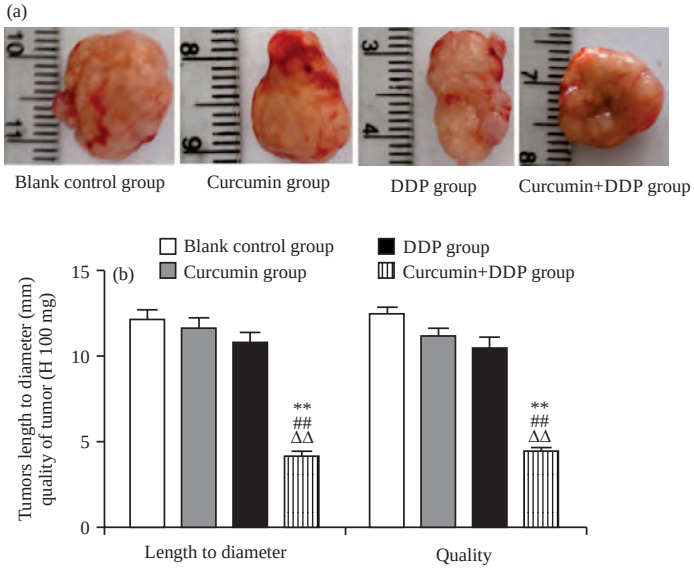

Fig. 5(a-b): Effect on MDA-MB-231/DDp tumour-bearing mice (a) Appearance and (b) Length and weight of tumour in nude mice
**p<0.01 vs. blank control group, ##p<0.01 vs. Curcumin group, ΔΔp<0.01 vs. DDP group

level of LC3 I, LC3 II, beclin-1, Atg1, 4, 7 and 13 in the curcumin+DDP group were significantly increased (p<0.01). The expression of TRAP1, p-Akt and p70S6K protein was also detected by Western blot assay in Fig. 4a. As shown in Fig. 4b, the expression levels of TRAP1, p-Akt and p70S6K protein in the curcumin group, DDP group and curcumin+ DDP group were significantly lower than those in the blank control group (p<0.01). Compared with the curcumin group, the expression levels of TRAP1, p-Akt and p70S6K protein in the DDP group were significantly higher (p<0.01) and the expression level of TRAP1, p-Akt and p70S6K protein in the curcumin+DDP group was significantly lower (p<0.01). Compared with the DDP group, the expression levels of TRAP1, p-Akt and p70S6K protein were significantly decreased in the curcumin+DDP group (p<0.01).

Effect on MDA-MB-231/DDP tumour-bearing mice: The tumour tissues of mice in Fig. 5a in each group were completely dissected and the length, diameter and mass of the tumour were measured. The tumour length and weight Fig. 5b were evaluated among the different groups. And these two indexes of the curcumin+DDP group were significantly lower than those of the blank control group, curcumin group and DDP group (p<0.01).

Apoptosis analysis in tumour tissues of MDA-MB-231/DDP tumour-bearing mice: TUNEL assay in Fig. 6a was used to detect the level of apoptosis in tumour tissues of mice in each group. Three random fields were selected for each slice and the number of positive cells in each field was calculated. The average number of positive cells represented the level of apoptosis in the sample. The results in Fig. 6b indicated that compared with the blank control group, curcumin group and DDP group, the apoptosis level of nude mice in the curcumin+ DDP group was significantly higher (p<0.01).

Immunohistochemistry analysis of TRAP1, p-Akt and P70S6K in MDA-MB-231/DDP tumour-bearing mice: Immunohistochemical method was used to detect the expression levels of TRAP 1, p-Akt and P70S6K in tumour tissues. The positive products were brown and expressed in the cytoplasm or cell membrane in Fig. 7a. Three random fields were selected from each section to measure the average optical density of each field. The average optical density represented the relative protein expression level in the sample. Immunohistochemistry analysis in Fig. 7b indicated that the expression levels of TRAP1, p-Akt and p70S6K in tumour tissues of the curcumin group, DDP group and curcumin+DDP group were significantly lower than those of the blank control group (p<0.01). Compared with the curcumin group, the expression levels of TRAP1, p-Akt and p70S6K in tumour tissue were significantly higher in the DDP group (p<0.01) but lower in the Curcumin+DDP group (p<0.01). Compared with the DDP group, the expression levels of TRAP1, p-Akt and p70S6K in tumour tissue of curcumin+DDP group were significantly decreased (p<0.01).

Western blot analysis of apoptosis-related proteins in MDA-MB-231/DDP tumour-bearing mice: Western blot in

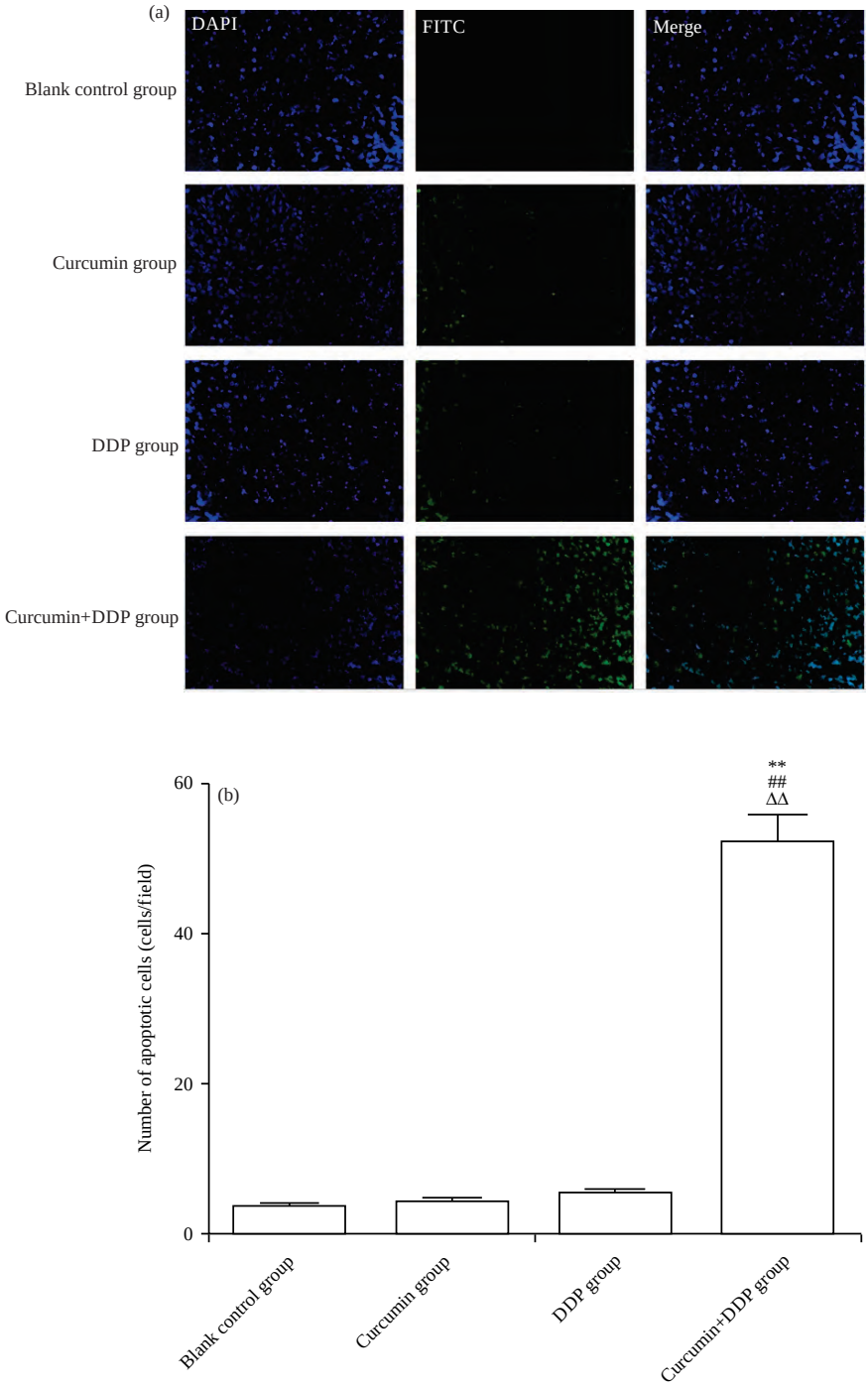

Fig. 6(a-b): Apoptosis analysis in tumour tissues of DA-MB-231/DDP tumour-bearing mice (a) TUNEL assay results and (b) Apoptosis in tumor tissues of MDA-MB-231/DDP tumor-bearing mice

**p<0.01 vs. blank control group, ##p<0.01 vs. Curcumin group, ΔΔp<0.01 vs. DDP group

Fig. 8a was used to detect the expression levels of LC3 I, LC3 II, Beclin-1, Atg1, 4, 7 and 13 in tumour tissues. The relative expression level of the target protein was expressed as the ratio of the grey value of the target protein band to the grey value of the corresponding internal reference. Figure 8b, LC3 I, LC3 II, Beclin-1, Atg1, 4, 7 and 13 protein expression levels in tumour tissue of curcumin group and curcumin+DDP group were significantly higher than those of the blank

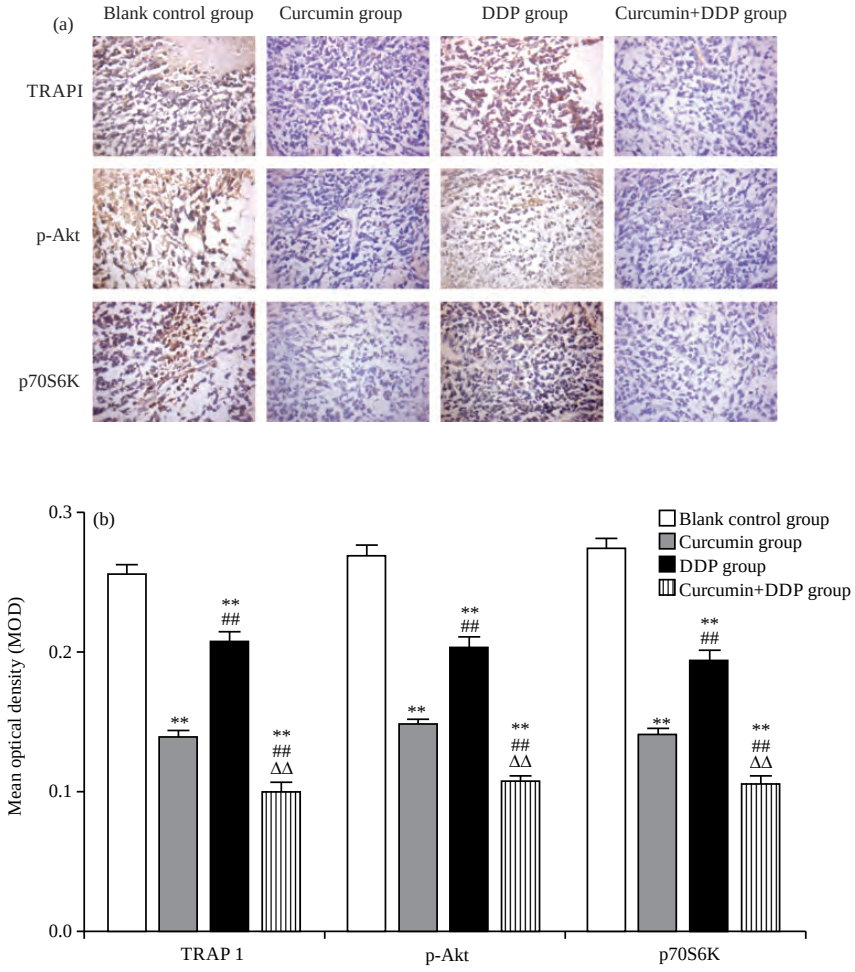

Fig. 7(a-b): Immunohistochemistry analysis in MDA-MB-231/DDP tumour-bearing mice (a) Micrograph and (b) Immunohistochemistry analysis of TRAP1, P-Akt and p70S6K

**p<0.01 vs. blank control group, ##p<0.01 vs. Curcumin group, ΔΔp<0.01 vs. DDP group

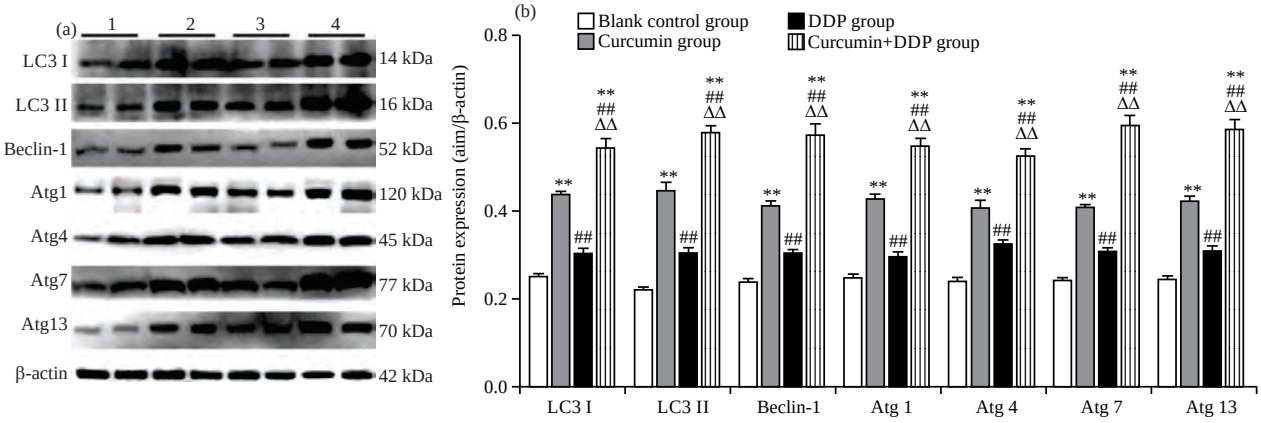

Fig. 8(a-b): Western blot analysis of apoptosis-related proteins in MDA-MB-231/DDP tumour-bearing mice (a) Western blot results and (b) Analysis of apoptosis-related proteins in MDA-MB-231/DDP tumour-bearing mice

**p<0.01 vs. blank control group, ##p<0.01 vs. Curcumin group, ΔΔp<0.01 vs. DDP group

control group and DDP group ($p<0.01$). Compared with the curcumin group, the expression levels of these apoptosis-related proteins in tumour tissue of mice treated with the curcumin+DDP group were significantly higher ($p<0.01$).

DISCUSSION

Autophagy plays a crucial role in the homeostasis of the intracellular environment. Autophagy has dual effects. When autophagy is normally activated, it can accelerate the degradation of intracellular proteins. Some misfolded proteins and damaged organelles can be degraded into metabolic elements and recycled to maintain the intracellular homeostasis to resist energy deficiency and promote cell survival but when the autophagy is excessively activated, it can cause cell lysis and autophagy death[17].

The process of autophagy is mediated by a series of autophagy-related proteins. Microtubule-associated protein 1 light chain 3 (LC3) is the characteristic autophagy marker. After processing, the carboxyl terminus of LC3 is excised to produce LC3 I, which covalently binds with phospholipids on the membrane of the autophagosome through ubiquitin-like modification to form LC 3 II. LC 3 II is the key protein of cell autophagy which content has positively correlated with the number of autophagosomes. Beclin 1 is an essential molecule for the formation of autophagy and its gene is a specific gene for mammals involved in autophagy. Beclin 1 indicated a positive relationship with the activity of autophagy which could modulate autophagy membrane synthesis and substance transport[18]. Atg is a group of evolutionarily conserved genes involved in the intracellular formation process of autophagy[19]. At present, more than 30 Atg genes have been found. When the upstream signal of autophagy was activated, dephosphorylation of Atg1, Atg13 induced the formation of Atg1, 11, 7, 20, 24 complexes and Atg8, 13 complexes, which can recruit other Atg proteins to form the bilayer structure of autophagy precursor[20].

Tumour necrosis factor receptor-associated protein 1 (TRAP1) is a member of the HSP90 family located in cell mitochondria. It plays an important role in maintaining normal cell homeostasis, stabilizing cell mitochondrial function and regulating cell metabolism[21]. TRAP1 is a characteristic protein that showed a higher expression in tumours tissues than that in normal tissues[22,23]. It has also been reported that the expression of TRAP1 is closely related to cellular mitochondrial autophagy[24,25]. In addition, TRAP1 acts as a key target upstream of the Akt signal mediating autophagy levels through modulation of the Akt signal.

In this research, curcumin has a good anti-tumour effect on both the MDA-MB-231/DDP cell line and the MDA-MB-231/DDP tumour-bearing mice. This is consistent with previous studies. However, in combination with DDP, it is the good anti-tumour effect. The results on autophagy-related protein and TRAP1/Akt/p70S6K signalling pathway showed that curcumin had no confirmed anti-tumor effect. But it could significantly regulate the expression level and phosphorylation level of autophagy-related protein and TRAP1/Akt/p70S6K signalling pathway. Curcumin could inhibit the overactivation of the Akt/p70S6K signalling pathway by inhibiting the expression level of TRAP1 protein. Thereby attenuating the inhibition of the expression level of autophagy-related proteins by the Akt/p70S6K signalling pathway and the expression level of autophagy-related protein was up-regulated. It can promote the level of autophagy in the tumour tissues of MDA-MB-231/DDP cell lines and MDA-MB-231/DDP tumour-bearing mice, inducing the autophagy death of tumour cells.

CONCLUSION

Curcumin can down-regulate the expression of TRAP1 and inhibit the over-activation of the PI3K/Akt/p70S6K signalling pathway. It can increase the apoptosis rate and the autophagy of MDA-MB-231/DDP cells and tumour-bearing mice. Curcumin could inhibit the over-activation of the Akt/p70S6K signalling pathway by inhibiting the expression level of TRAP1 protein. Curcumin may inhibit the over-activation of the Akt/p70S6K signalling pathway, promote autophagy of MDA-MB-231 cells, reduce the resistance of MDA-MB-231/DDP tumour-bearing mice to DDP and induce autophagy death of tumour cells.

SIGNIFICANCE STATEMENT

This study explored the mechanism of curcumin improving cisplatin resistance in MDA-MB-231 cells and tumour bearing mice, which will provide a new treatment idea for breast cancer in chemotherapy. At the same time, the relationship between autophagy and drug resistance was discussed and promoting autophagy may be an effective way to reduce drug resistance.

ACKNOWLEDGMENT

This research was supported by the Natural Science Foundation of Liaoning Province (2019-KF-01-14).

REFERENCES

1. Payandeh, M., M. Sadeghi, E. Sadeghi and M. Aeinfar, 2015. Clinicopathology figures and long-term effects of tamoxifen plus radiation on survival of women with invasive ductal carcinoma and triple negative breast cancer. Asian Pac. J. Cancer Prev., 16: 4863-4867.
2. DeSantis, C.E., J. Ma, A.G. Sauer, L.A. Newman and A. Jemal, 2017. Breast cancer statistics, 2017, racial disparity in mortality by state. CA: A Cancer J. Clinicians, 67: 439-448.
3. Noh, J.M., D.H. Choi, S.J. Huh, W. Park and J.H. Yang *et al*., 2011. Patterns of recurrence after breast-conserving treatment for early stage breast cancer by molecular subtype. J. Breast Cancer, 14: 46-51.
4. Byrski, T., J. Gronwald, T. Huzarski, E. Grzybowska and M. Budryk *et al*., 2010. Pathologic complete response rates in young women with BRCA1-positive breast cancers after neoadjuvant chemotherapy. J. Clin. Oncol., 28: 375-379.
5. Vollebergh, M.A., E.H. Lips, P.M. Nederlof, L.F.A. Wessels and M.K. Schmidt *et al*., 2011. An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients. Ann. Oncol., 22: 1561-1570.
6. Pathiranage, V.C., J.N. Lowe, U. Rajagopalan, M.K. Ediriweera and K. Senathilake *et al*., 2020. Hexane extract of *Garcinia quaesita* fruits induces apoptosis in breast cancer stem cells isolated from triple negative breast cancer cell line MDA-MB-231. Nutr. Cancer, 73: 845-855.
7. Colín-Val, Z., N.E. López-Díazguerrero and R. López-Marure, 2021. DHEA inhibits proliferation, migration and alters mesenchymal-epithelial transition proteins through the PI3K/Akt pathway in MDA-MB-231 cells. J. Steroid Biochem. Mol. Biol., Vol. 208. 10.1016/j.jsbmb.2021.105818.
8. Gao, H., M.B. Khawar and W. Li, 2020. Essential role of autophagy in resource allocation during sexual reproduction. Autophagy, 16: 18-27.
9. Klionsky, D.J., 2005. The molecular machinery of autophagy: Unanswered questions. J. Cell Sci., 118: 7-18.
10. Fukui, M., N. Yamabe, H.J. Choi, K. Polireddy, Q. Chen and B.T. Zhu, 2015. Mechanism of ascorbate-induced cell death in human pancreatic cancer cells: Role of bcl-2, beclin 1 and autophagy. Planta Med., 81: 838-846.
11. Deng, Q., Z. Wang, L. Wang, L. Zhang, X. Xiang, Z. Wang and T. Chong, 2013. Lower mRNA and protein expression levels of LC3 and beclin1, markers of autophagy, were correlated with progression of renal clear cell carcinoma. Jpn. J. Clin. Oncol., 43: 1261-1268.
12. Kotha, R.R. and D.L. Luthria, 2019. Curcumin: Biological, pharmaceutical, nutraceutical and analytical aspects. Molecules, Vol. 24, No. 16. 10.3390/molecules24162930.
13. Sabzi, A., A. Rahmani, M. Edalati, H. Kahroba, M.R. Dadpour, R. Salehi and A. Zarebkohan, 2020. Targeted co-delivery of curcumin and doxorubicin by citric acid functionalized Poly (ϵ-caprolactone) based micelle in MDA-MB-231 cell. Colloids Surf. B: Biointerfaces, Vol. 194. 10.1016/j.colsurfb. 2020.111225.
14. Liu, L.D., Y.X. Pang, X.R. Zhao, R. Li and C.J. Jin *et al*., 2019. Curcumin induces apoptotic cell death and protective autophagy by inhibiting AKT/mTOR/P70S6K pathway in human ovarian cancer cells. Arch. Gynecol. Obstet., 299: 1627-1639.
15. Chen, P., H.P. Huang, Y. Wang, J. Jin and W.G. Long *et al*., 2019. Curcumin overcome primary gefitinib resistance in non-small-cell lung cancer cells through inducing autophagy-related cell death. J. Exp. Clin. Cancer Res., Vol. 38. 10.1186/s 13046-019-1234-8.
16. Sheng, J.Y., B.L. Shi and H.F. Chen, 2016. Establishment and appraisal of DDP resistant variant of triple negative breast cancer cell line MDA-MB-231. Cancer Res. Prev. Treat., 43: 175-180.
17. Mizushima, N. and M. Komatsu, 2011. Autophagy: Renovation of cells and tissues. Cell, 147: 728-741.
18. Yang, R., Z. Song, S. Wu, Z. Wei, Y. Xu and X. Shen, 2018. Toll-like receptor 4 contributes to a myofibroblast phenotype in cardiac fibroblasts and is associated with autophagy after myocardial infarction in a mouse model. Atherosclerosis, 279: 23-31.
19. Yorimitsu, T. and D.J. Klionsky, 2005. Autophagy: Molecular machinery for self-eating. Cell Death Differ., 2: 1542-1552.
20. Mizushima, N., 2010. The role of the Atg1/ULK1 complex in autophagy regulation. Curr. Opin. Cell Biol., 22: 132-139.
21. Xie, S., X. Wang, S. Gan, X. Tang, X. Kang and S. Zhu, 2021. The mitochondrial chaperone trap1 as a candidate target of oncotherapy. Front. Oncol., Vol. 10. 10.3389/fonc. 2020.585047.
22. Costantino, E., F. Maddalena, S. Calise, A. Piscazzi and V. Tirino *et al*., 2009. TRAP1, a novel mitochondrial chaperone responsible for multi-drug resistance and protection from apoptotis in human colorectal carcinoma cells. Cancer Lett., 279: 39-46.
23. Choi, H., C. Merceron, L. Mangiavini, E.L. Seifert, E. Schipani, I.M. Shapiro and M.V. Risbud, 2016. Hypoxia promotes noncanonical autophagy in nucleus pulposus cells independent of mTOR and HIF1A signaling. Autophagy, 12: 1631-1646.
24. Caino, M.C., Y.C. Chae, V. Vaira, S. Ferrero and M. Nosotti *et al*., 2013. Metabolic stress regulates cytoskeletal dynamics and metastasis of cancer cells. J. Clin. Invest., 123: 2907-2920.
25. Chen, J.F., Q.S. Wu, Y.X. Xie, B.L. Si and P.P. Yang *et al*., 2017. TRAP1 ameliorates renal tubulointerstitial fibrosis in mice with unilateral ureteral obstruction by protecting renal tubular epithelial cell mitochondria. FASEB J., 31: 4503-4514.