Matrine Reverses Multidrug Resistance in Eca-109/VCR Cells Via Inhibiting PI3K/Akt/mTOR Signaling Pathway

Bo Liu  
first affiliated Hospital of Hebei North University

Shuxuan Lin  
Hebei North University

Xiujuan Li  
Hebei North University

Fengxi Liu  
Hebei North University

Junchao Liu  
First affiliated Hospital of Hebei North University

Na Wang  
Hebei North University

Yang Lv  (✉ hbbfxy_lvyang@163.com)  
Hebei North University  https://orcid.org/0000-0001-6914-9776

Research

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Abstract

**Objective** To investigate the effect of matrine on multidrug resistance of Eca-100/VCR cells.

**Methods** Methyl thiazolyl tetrazolium assay was used to assess the cytotoxic activity of matrine to Eca-109/VCR cells and the sensitivity of cells to Vincristine (VCR). Then Eca-109/VCR cells were divided into four groups: control group, matrine group, VCR group and matrine combined with VCR group. Immunofluorescence staining was used to detect P-gp protein expression. Western blot was used to detect MRP1, P-gp, Beclin 1, p62(SQSTM1), LC3, p-mTOR, p-Akt and p-p70S6K protein expression. MDR1 mRNA expression was detected by RT-qPCR. Transmission electron microscopy was used to detect the ultra structural changes.

**Results** Matrine could reverse the resistance of Eca-109/VCR cells to VCR with a reversal index of 3.52. Compared with the other groups, the expression of LC3-II, Beclin 1 increased while P-gp, MRP1, p62(SQSTM1), p-mTOR, p-Akt and p-p70S6K significantly decreased in matrine+VCR group (P<0.05). The expression of MDR1 mRNA decreased in Eca-109/VCR cells treated with matrine, VCR or their combination. The number of autophagic vacuoles in Eca-109/VCR cells increased after matrine treatment.

**Conclusion** Matrine has anticancer and anti multidrug resistance functions in Eca-100/VCR cells, and it may be produced by promoting autophagy which mediated through PI3K/Akt/mTOR signaling pathway.

Introduction

Esophageal carcinoma is the eighth most prevalent cancer and the seventh leading cause of cancer-related death worldwide [1]. In China, the morbidity and mortality of esophageal carcinoma accounted for the fifth and fourth of malignancy, respectively [2]. The treatment of esophageal carcinoma is mainly based on surgical resection. Therapies that combine surgery with radiotherapy, chemotherapy, and chemoradiotherapy are also utilized [3]. As is known to all, chemoradiotherapy is the primary treatment option for patients with advanced cancer but can be limited by severe side effects and drug resistance to chemotherapeutic agents [4, 5]. Therefore, many researchers have turned their attention to traditional Chinese medicines (TCM), to reduce side effects and resist multidrug resistance (MDR) [6, 7]. Matrine, a quinolizidine alkaloid that has been widely used to treat viral hepatitis, cardiac arrhythmia and skin inflammations, exhibits chemotherapeutic potential through its ability to trigger caspase-independent program cell death [8]. The present study aimed to investigate the MDR effect of matrine on human esophageal carcinoma Eca-109/VCR cells and its possible mechanism.

Material And Methods

Cells and cell culture
Eca-109/VCR cell line was obtained from the Life Science Centre of Hebei North University (Zhangjiakou, China). The cells were maintained at 37°C, 5% CO₂ and saturated humidity, in RPMI-1640 medium (Corning, China) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA), 100 U/ml of penicillin and 100 µg/ml of streptomycin. Cells in mid-logarithmic growth were used for experiments. When the density of the cell colonies reached ~90% confluence, the cells were detached with 0.25% trypsin (Amresco, Solon, OH, USA) and transferred to fresh flasks at a ratio of 1:2. VCR (V0129, TCIDevelopment Co., Ltd, Shanghai, China) with a final concentration of 2 µg/mL was added to maintain the resistance of Eca-109/VCR cells. It was discontinued 1 week before the start of the experiment to maintain cell resistance.

Cell Proliferation Inhibition Assay

The MTT assay was used to assess the cytotoxic activity of matrine. The trypsin digested Eca-109/VCR cells were collected and counted under microscope. Then cells (5x10⁴ cells/well) were plated in 96-well culture plates and allowed to adhere overnight at room temperature, following which the cells were treated with various concentrations of matrine (0, 0.25, 0.5, 0.75, 1.0, 1.5 or 2.0 mg/mL). The cells were subsequently incubated for 24 and 48 h then they were washed twice with PBS and treated with 20 µL of sterile MTT dye (5 mg/mL, Qilu Pharmaceutical Co., Ltd, China) for 4 h at 37°C. The cells were washed with PBS and then solubilized with 150 µL of dimethyl sulfoxide (DMSO). The 96-well plates were shaken until the formazan crystals dissolved completely. Then the absorbance value was measured on a microplate reader (SpectraMax M2, Molecular Devices, USA) at 490 nm. The rate of cell inhibition was calculated using equation: the inhibition rate (%) = [1-A490(test)/A490(blank)]×100%. Each experiment was conducted in triplicate and included three replicates.

The Sensitivity Of Cells To Vcr By Mtt Assay

Eca-109 and Eca-109/VCR cells at logarithmic growth stage were used for the experiment. Cells (5x10⁴ cells/well) were plated in 96-well culture plates and allowed to adhere overnight at room temperature, following which the cells were treated with drug. Eca-109 cells were treated with different concentrations of VCR (0, 0.02, 0.04, 0.08, 0.16, 0.32 µg/mL). Four wells were set up in each group, and the final volume of each well was 200µL. Eca-109/VCR control group and Eca-109/VCR+ matrine (1.0mg/mL matrine treated for 24h) group were set up in Eca-109/VCR cell lines. The two groups were treated with different concentrations of VCR (0.5, 1, 1.5, 2, 3 µg/mL) for 24h, and four wells were set in each group, the final volume of each well was 200 µL. The other experimental steps were the same as the above cell proliferation inhibition assay. Then 50% inhibition concentration (IC50), resistant index, and reversal index were calculated to evaluate MDR reversal effect. The inhibitory concentration 50% (IC50) was determined by a non-linear regression analysis of the concentration-response curve using the Hill equation. Resistant index = IC50 (Eca-109/VCR) / IC50(Eca-109). Reversal index = IC50 (Eca-109/VCR + VCR ) / IC50 (Eca-109/VCR + VCR + matrine).
**Immunofluorescence Staining**

Eca-109/VCR cells were divided into four groups: control group, matrine (1.0 mg/mL) group, VCR (2 µg/mL) group and matrine (1.0 mg/mL)+VCR (2 µg/mL) group. Cells of each group were plated onto chamber slides at 5x10^4 cells per chamber for 24 h. Then, they were fixed with 4% paraformaldehyde for 30 min at room temperature (RT) and subsequently permeabilized with 0.25% Triton X-100 for 20 min at RT. The slides were then treated with 0.5% bovine serum albumin (BSA; Beyotime Institute of Biotechnology, Jiangsu, China) in 0.1% Tween-20 for 30 min at RT, primary antibodies P-gp (cat. no ab170903, dilution, 1:1000; Boaosen Biotechnology Co., Ltd, Beijing, China) was added and incubated on the slides overnight at 4°C. Secondary antibodies rhodamine-labeled affinity purified antibody (Anti-rabbit IgG (H+L) Ab, 5230-0332, dilution, 1:500; Seracare, USA) in the dark at RT for 60 min and washed with PBS three times. Finally, the cover slip was sealed with Fluorescent Mounting Media (S2100, Beijing Solarbio Technology Co., Ltd. Beijing, China). Negative controls were also employed to offset the disturbance of the primary or secondary antibody. The results were observed and recorded by fluorescence microscopy (Leica TCS-ST2 Instrument, Japan). The projected cell area was evaluated using ImageJ software. The assays were performed in triplicate in three independent experiments.

**Western Blot**

Eca-109/VCR cells were divided into four groups: control group, matrine (1.0 mg/mL) group, VCR (2 µg/mL) group and matrine (1.0 mg/mL)+VCR (2 µg/mL) group. Western blot was used to detect the MDR effect of matrine on Eca-109/VCR cells. Furthermore, the PI3K/Akt/mTOR pathway plays an important role in regulating the cell cycle, apoptosis and autophagy. Therefore, we next investigated whether the effects of matrine on Eca-109/VCR cells were mediated by this pathway. Western blot methods was performed as previously described[9]. Briefly, cells were lysed in RIPA lysis buffer (Beyotime, China). Cell protein lysates were separated by 10% SDS-polyacrylamide gel electrophoresis. Proteins were transferred to PVDF membranes (Immobilon 0.45 µm, Millipore, USA), and immersed in a blocking solution containing 5% fat-free milk and 0.1% Tween-20 for 1h. After blocking, membranes were incubated with P-gp (cat. no ab170903, dilution, 1:1000; Boaosen Biotechnology Co., Ltd, Beijing, China), MRP1 (cat. no ab233383, dilution, 1:1000; Boaosen Biotechnology Co., Ltd, Beijing, China), p-mTOR (cat. no ET1608-5, dilution, 1:1000; Hangzhou HuaAn Biotechnology Co., Ltd, Hangzhou, China), p-Akt (cat. no ET1607-73, dilution, 1:1000; Hangzhou HuaAn Biotechnology Co., Ltd, Hangzhou, China), p-p70S6K-T421/S424 (cat. no AP0502, dilution, 1:1000; ABclonal Technology, Wuhan, China), LC3 (cat. no PM036, dilution, 1:1000; Medical & Biological Laboratories Co., Ltd, Japan), p62 (SQSTM1)(cat. no PM045, dilution, 1:1000; Medical & Biological Laboratories Co., Ltd, Japan), Beclin 1 (cat. no PD017, dilution, 1:1000; Medical & Biological Laboratories Co., Ltd, Japan) overnight at 4°C and then with diluted horseradish peroxidase-conjugated secondary antibody (sc-2004, dilution, 1:1000, Santa Cruz) for 2h at room temperature. After washing, the resulting bands were visualized using the standard ECL procedure, quantified by densitometry, and normalized to the corresponding β-actin (cat. no AC026, dilution, 1:1000; ABclonal Technology, Wuhan, China) bands.
**Total RNA extraction and qRT-PCR**

Total RNA from Eca-109/VCR cells in different groups was isolated with TRIzol® reagent (Invitrogen, Thermo Fisher Scientific, Inc.) and were then converted to cDNA with a PrimeScript RT Reagent kit (cat. no RR036A, Takara Biotechnology Co., Ltd., Dalian, China), according to the manufacturer's protocol. SYBR Premix Ex Taq (cat. no RR820A, Takara Biotechnology Co., Ltd.) was used for the RT-qPCR assays to detect the expression levels of MDR1 in cells. The sequences of the primers were as follows: MDR1: 5′-GAGTGTCTGTCCGTCTTC-3′ and 5′-TGCTCCGTCACATCA-3′; GAPDH (glyceraldehyde-3-phosphate dehydrogenase): 5′-GTCTCCTCTGACTTCAACAGCG-3′ and 5′-ACCACCCTGTTGCTGTAGCCAA-3′. qRT-PCR was performed on the ABI 7300 system (Applied Biosystems, Foster City, CA, USA) in 30-µL reactions using the following program: 40 cycles of 95°C for 120 s, 60°C for 30 s and 72°C for 30 s. All samples were amplified in triplicate in one assay run simultaneously. Transcription levels were normalized against GAPDH, and relative gene expression was quantified using the $2^{-\Delta\Delta C_t}$ method.

**Transmission Electron Microscope (TEM) Sample Preparation Of Culture Cell**

Cell suspension with concentration of $5 \times 10^4$ cells/mL was prepared and inoculated in 50 mL culture flasks. After cultured overnight, culture medium was discarded. In the experiment group (Eca-109/VCR cells treated with 1.0 mg/mL Matrine for 48 h), 1.5 mL cell suspension and 1.5 mL culture medium were added to reach the terminal volume of 3 mL. In the control group, same terminal volume of culture medium was added. After 24 h digestion with trypsin, cells and supernatant were collected into a centrifuge tube. The centrifuge condition is rotation speed 1500 r/min and time 5 min. The sample was washed with PBS for 3 times, and fixed at room temperature using glutaraldehyde, followed by osmic acid. After fixation with aqueous fixatives, samples are dehydrated in increasing concentrations of acetone, followed by propylene oxide. The samples were embedded, and 60 - 80 nm sections were stained with uranyl acetate followed by lead citrate to enhance contrast.

**Statistical analysis**

Data analysis was performed by SPSS 20.0. Data were described as the mean ± SD. Student’s t-test, paired t-test and ANOVA were conducted to analyse quantitative variables. A $P$-value $\leq 0.05$ was considered statistically significant.

**Results**

**Effect of matrine on proliferation inhibition rate of Eca-109/VCR cells**
Matrine inhibits the cell viability of Eca-109/VCR. The effect of various concentrations of matrine (0, 0.25, 0.5, 0.75, 1.0, 1.5 or 2.0 mg/mL) on the inhibition rate of Eca-109/VCR following exposure for 24 or 48 h is demonstrated in Fig. 1. At concentrations <1.0 mg/mL, matrine inhibited the cell viability of Eca-109/VCR cells obviously, whereas at concentrations higher than 1.0 mg/mL, the inhibitory effect was weak and cell growth tended to be flat. Furthermore, there was little toxicity to cells at concentrations <1.0 mg/mL. Therefore, concentrations of 1.0 mg/mL was selected for the following research experiments.

The Sensitivity Of Cells To Vcr By Mtt Assay

Cell survival rates were detected by MTT assay to observe the VCR resistance phenotype of cells exposed to VCR. The results showed that the IC50 value of VCR on Eca-109 (Fig. 2A) and Eca-109/VCR cells were 0.216µg/mL and 2.948µg/mL, respectively. The difference between them was statistically significant (P<0.01). The resistance index was 13.65. The results indicated that Eca-109/VCR cell line was a good model of drug resistance cells and could be used in subsequent experiments.

When matrine was added, the sensitivity of Eca-109/VCR cells to VCR increased, and the IC50 of VCR was decreased to 0.837 µg/mL. Compared with the non-matrine treated group, the difference was statistically significant (P<0.01), which suggesting that matrine could reverse the resistance of Eca-109/VCR cells to VCR with a reversal index of 3.52 (Fig. 2B).

Immunofluorescence Staining

Immunofluorescence staining was performed to show the distribution patterns of P-gp protein in each group. Under the confocal-microscope observation, P-gp protein was stained with red fluorescence, which was expressed in the membrane and cytoplasm of Eca-109/VCR cells (Fig. 3A). Analysis of the fluorescence intensity revealed that compared with other groups, the expression of P-gp in matrine+VCR group significantly decreased and the difference was statistically significant (Fig. 3B, P<0.01).

Western Blot Results

The results for experiment of drug resistance showed that compared with the other three groups, the expression of P-gp and MRP1 protein in matrine +VCR group significantly decreased (Fig. 4A) and the difference was statistically significant (P<0.05). The results for PI3K/Akt/mTOR pathway examination (Fig. 4B) showed that in matrine +VCR group, the expression of LC3-II (In our actual experiment, what was detected was LC3-II according to the molecular weight displayed by Marker), Beclin 1 protein was higher while the expression of p62(SQSTM1), p-mTOR, p-Akt and p-p70S6K protein was lower than other groups (P<0.05). Taken together, these data suggested that matrine combined with VCR has anticancer and inhibit MDR and this process may be produced by promoting autophagy which mediated through PI3K/Akt/mTOR signaling pathway.
RT-q PCR results

RT-qPCR results (Fig. 5) showed that compared with the control group, the relative expression of MDR1 mRNA decreased in Eca-109/VCR cells treated with Matrine, VCR or their combination. Among them, the expression in matrine +VCR group was lowest, and the difference was statistically significant ($P<0.05$).

The Ultrastructural Changes In Eca-109/vcr Cells By Tem

In the control group, the nuclear membrane of Eca-109/VCR cells was intact, and the organelle morphology in the cytoplasm was basically normal. After treatment of Eca-109/VCR cells with Matrine solution of 1.0 mg / ml for 48 h, nuclear was edema or pyknosis, and a large number of vacuolated autophagy with double membrane structure containing organelles were formed in the cytoplasm, and mitochondria with damaged structure was seen (Fig. 6).

Discussion

Drug resistance is one of the main hurdles to effective therapy of numerous tumors, which is taken into account during chemotherapy. Even a small increase in chemotherapy drug can cause severe toxicity to dose-limiting normal tissue. Thus, to accomplish higher curability with least toxicity during chemotherapy, various natural products and their derivatives have been considered as potential sources of chemosensitizer. Matrine is a principal active drug monomer in Sophora flavescens and has been used as an anti-cancer herbal medicine for hundreds of years in China with a broad range of pharmacological effects [10], without apparent side effects or toxicity. More up to date studies have found that matrine has effective anti-tumor activity, such as in hepatocellular carcinoma [11], lung cancer [12, 13], gastric carcinoma [14], pancreatic cancer [15], glioma [16], and et al. However, the precise mechanisms of these antitumor effects, particularly those on esophageal cancer, have not been clear. In this study, we proposed a hypothesis by which matrine has anticancer and anti-multidrug resistance functions in esophageal carcinoma, and this process may be produced by autophagy mediated by PI3K / Akt / mTOR signaling pathway.

Autophagy, an evolutionarily conserved mechanism, is a critical cellular pathway to maintain cellular homeostasis by degrading unnecessary proteins and organelles. This degradation results in the release of nucleosides, amino acids, proteins, lipids, fatty acids, and sugars into the cell’s cytoplasm for recycling. Although autophagy induction or inhibition has been reported to be implicated in the antitumour activity of matrine against several solid tumours [13, 17], the role of autophagy and the underlying mechanism remain unobvious in esophageal carcinoma. MDR is a refractory outcome of chemotherapy and is defined as the resistance of cancer cells to multiple chemotherapeutic drugs with different structures and mechanisms of action [18]. MDR is a major cause of chemotherapy failure and responsible for increasing cancer-related mortality. The major factors responsible for MDR are: increased drug export, reduced drug accumulation, alterations in drug targets and signal transduction molecules, increased repair of drug-
induced DNA damage, and evasion of apoptosis, etc [19]. Among them, drug efflux mediated by P-gp is the classic mechanism of MDR.

P-gp is a 170 kDa protein which is encoded by the MDR1 gene. This transporter is found in normal cells of various tissues including the brain, kidney, liver, pancreas and gastrointestinal tract, etc. P-gp transports anticancer drugs such as vincristine, paclitaxel, epirubicin and mitoxantrone against the concentration gradient using energy derived from hydrolysis of ATP. Chemotherapeutic agents can stimulate P-gp expression in cancer cells and thereby cause resistance to chemotherapy [20]. Some studies have demonstrated that P-gp downregulation or efflux inhibition can increase the sensitivity of resistant cells to chemotherapeutic drugs. Studies have shown that matrine, as a monomer of Traditional Chinese medicine, can reverse P-gp mediated multidrug resistance. Zhi Chen et al [21] evaluated the reversing effect of matrine on chemoresistant leukemia K562/ADR cells. Their data indicated that matrine had potent reversal properties augmenting cytotoxicity of cancer medicines on K562/ADR cells as well as apoptotic rates induced by doxorubicin. Moreover, matrine inhibited drug-exporting activity and expression of P-gp on both mRNA and protein levels. In similarly designed studies using MDR breast cancer MCF-7/ADR [22] and leukemia K562/DOX cell lines [23], similar conclusions were reached that matrine reverses P-gp mediated MDR. Our experiments’ results showed that compared with the other three groups, the expression of P-gp protein in matrine +VCR group significantly decreased and the difference was statistically significant, which suggested that matrine, alone or combined with VCR could resist MDR by inhibiting P-gp mediated drug efflux.

Recent mechanistic investigations have demonstrated that autophagy pathways are involved in the development of MDR [24]. Studies have explored approaches of using autophagy to resist MDR during anticancer therapy, but the mechanisms underlying the relationship between autophagy and MDR have not been fully studied.

The conversion of microtubule-associated protein light chain 3 (LC3) proteins (LC3-I to LC3-II) are involved in the formation of autophagosomes, and is now widely applied in monitoring autophagy. Also, the amount of LC3-II is clearly correlated with the amount of autophagosomes, and thus serves as a relatively accurate marker of autophagy [25]. The autophagic pathway proceeds through several phases, such as initiation, vesicle elongation, autophagosome maturation and autophagosome–lysosome fusion. Beclin 1 can intervene at every major step in autophagic pathways that is mediated by its interacting partners [26]. As a selective substrate, p62(SQSTM1) accumulation is a result of autophagy inhibition, and when autophagy was suppressed, Beclin 1 will decrease at the same time [27]. Therefore, the regulation of the p62(SQSTM1) and Beclin 1 complex is crucial for autophagy control.

Exogenous or endogenous stresses could activate autophagy by targeting different signal regulators. Among these, the blockage of PI3K/Akt/mTOR signalling pathway plays a critical role in regulating autophagy [28, 29]. The PI3K/AKT/mTOR pathway is one of the comprehensive signaling pathways that regulate cell proliferation, growth, metabolism, and cell survival and is one of the most periodically deregulated pathways in cancer [30]. Among this pathway, targeting mTOR has been proved to be
effective for leukaemia therapy [31]. The inactivation of the phosphorylation of Akt, mTOR and their downstream substances, p70S6K and 4EBP1, were found in matrine-treated AML cells. The aberrant activation of mTOR/p70S6K pathway plays an important role in tumorigenesis and phosphorylated p70S6K by mTOR has higher activity to promote translation than p70S6K [32]. Akt is a central player in PI3K/Akt/mTOR signal transduction as its phosphorylation can lead to the activation and signaling through the mTORC1 (mechanistic target of rapamycin complex 1)[33]. Moreover, mTOR serves as a cellular sensor of nutrient, growth factor availability, energy levels, and stress signals, which is positively regulated by nutrient and growth factors. Under cell stress conditions, such as nutrient deprivation, growth factor shortage, and hypoxia, and so on, Akt/mTOR axis is inhibited to induce autophagy.

In this study, RT-qPCR showed that compared with the control group, the relative expression of MDR1 mRNA decreased in Eca-109/VCR cells treated with matrine, VCR or their combination. Among them, the expression in matrine+VCR group was lowest. Immunofluorescence staining results also showed that the expression of P-gp in matrine+VCR group significantly decreased and the difference was statistically significant. The data of Western blot also showed that compared with the other groups, the expression of P-gp and MRP1 protein in matrine +VCR group significantly decreased and the difference was statistically significant. The results above indicated that matrine had anti-multidrug resistance functions in esophageal carcinoma. TEM results showed that after treatment with matrine, nuclear was edema or pyknosis, and a large number of vacuolated autophagy with double membrane structure containing organelles were formed in the cytoplasm, and mitochondria with damaged structure was seen, which demonstrated that matrine could promote autophagy in Eca-109/VCR cells. Moreover, the results for PI3K/Akt/mTOR pathway examination showed that in matrine +VCR group, the expression of LC3-II, Beclin 1 protein was higher while the expression of p62(SQSTM1), p-mTOR, p-Akt and p-p70S6K protein was lower than other groups. Taken together, these data suggest that matrine combined with VCR has anticancer function and induced autophagy via the PI3K/Akt/mTOR pathway in Eca-109/VCR cells.

In conclusion, Matrine has anticancer and anti-multidrug resistance functions in Eca-100/VCR cells, and this process may be produced by autophagy mediated by PI3K / Akt / mTOR signaling pathway. These results may provide preliminary guidance for the treatment of esophageal carcinoma using matrine.

Declarations

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Conflicts of Interest
The authors declare that they have no competing interests regarding the publication of this manuscript.

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**Figures**

**Figure 1**

MTT results assay demonstrating the cytotoxicity of matrine in Eca-109/VCR cells. (Eca-109/VCR cells were treated with matrine at concentrations of 0, 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 mg/mL for 24 and 48 h. Matrine inhibited the cell viability of Eca-109/VCR in a dose-and time-dependent manner.)
Figure 2

The sensitivity of cells to VCR by MTT assay. (2A, the sensitivity of Eca-109 cells to VCR; 2B, the sensitivity of Eca-109/VCR cells to VCR.)

Figure 3

P-gp

Analysis results of the fluorescence intensity of P-gp expression in different group

Figure 3
Distribution of P-gp in Eca-109/VCR cells by immunofluorescence staining, ×400. (A, P-gp protein was stained with red fluorescence, which was expressed in the membrane and cytoplasm of Eca-109/VCR cells; B, Analysis results of the fluorescence intensity of P-gp in different group, * P<0.05, ** P<0.01 vs the control group)

Figure 4

Western blot results. (3A, Experiment for drug resistance showed that compared with the other three groups, the expression of P-gp and MRP1 protein in matrine + VCR group significantly decreased, P<0.05; 3B, Experiment for PI3K/Akt/mTOR pathway showed that in matrine + VCR group, the expression of LC3-II, Beclin 1 protein was higher while the expression of p62(SQSTM1), p-mTOR, p-Akt and p-p70S6K protein was lower than other groups, P<0.05.)
Figure 5

RT-qPCR results of MDR1 mRNA expression in Eca-109/VCR(* P<0.05 vs the control group, # P<0.05 vs matrine group, ▲ P<0.05 vs VCR group ).

Figure 6

Morphological changes of Eca-109/VCR cells by TEM. (A. cells of the control group; B. After treatment with matrine, a large number of vacuolated autophagy were formed in the cytoplasm; C. After treatment with matrine, cell nuclear and cytoplasm was edema)