CLIC1-mediated autophagy confers resistance to DDP in gastric cancer

Zhen-Liang Nong[a,b,c,d], Kun Zhao[e], Ye Wang[a,b,c,d], Zhu Yu[a,b,c,d], Cong-jun Wang[a,b,c,d] and Jun-Qiang Chen[a,b,c,d]

Gastric cancer has been a constant concern to researchers as one of the most common malignant tumors worldwide. The treatment options for gastric cancer include surgery, chemotherapy and traditional Chinese medicine. Chemotherapy is an effective treatment for patients with advanced gastric cancer. Cisplatin (DDP) has been approved as a critical chemotherapy drug to treat various kinds of solid tumors. Although DDP is an effective chemotherapeutic agent, many patients develop drug resistance during treatment, which has become a severe problem in clinical chemotherapy. This study aims to investigate the mechanism of DDP resistance in gastric cancer. The results show that intracellular chloride channel 1 (CLIC1) expression was increased in AGS/DDP and MKN28/DDP, and as compared to the parental cells, autophagy was activated. In addition, the sensitivity of gastric cancer cells to DDP was decreased compared to the control group, and autophagy increased after overexpression of CLIC1. On the contrary, gastric cancer cells were more sensitive to cisplatin after transfection of CLIC1 siRNA or treatment with autophagy inhibitors. These experiments suggest that CLIC1 could alter the sensitivity of gastric cancer cells to DDP by activating autophagy. Overall, the results of this study recommend a novel mechanism of DDP resistance in gastric cancer. *Anti-Cancer Drugs* 35: 1–11 Copyright © 2023 The Author(s). Published by Wolters Kluwer Health, Inc.

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[a]Department of Gastrointestinal Surgery, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region, [b]Guangxi Laboratory of Enhanced Recovery after Surgery for Gastrointestinal Cancer, [c]Guangxi Clinical Research Center for Enhanced Recovery after Surgery, [d]Guangxi Zhuang Autonomous Region Engineering Research Center for Artificial Intelligence Analysis of Multimodal Tumor Images and [e]Department of Hepatobiliary Surgery, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region, People's Republic of China

Correspondence to Jun-Qiang Chen, Department of Gastrointestinal Surgery, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region Tel: +86 17377593039; e-mail: chenjunqiang@gxmu.edu.cn

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Introduction

Gastric cancer is one of the four most commonly observed malignant tumors globally. More than 1 million cases of gastric cancer are diagnosed, and 783 000 people die of it annually, meaning one out of every 12 deaths worldwide is due to gastric cancer [1]. The treatment for early gastric cancer is generally surgery, and the chances of survival are good. Because the symptoms of gastric cancer are usually not apparent in the early stages, local or distant metastasis is present at the time of diagnosis [2]. For these patients, chemotherapy becomes a crucial means of treatment. As one of the most widely used chemotherapeutic drugs, cisplatin plays a vital role in chemotherapy and has a curative effect on various types of cancers, including gastric cancer. However, the repeated occurrence of drug resistance in clinical treatments has led to an inadequate outcome of chemotherapy. Therefore, investigating the chemotherapy resistance of cisplatin will help improve its chemotherapeutic effect on gastric cancer patients, prognosis and survival rate. However, the potential mechanism of cisplatin resistance is still unclear and needs to be explained. Given this, exploring the mechanism of cisplatin resistance in gastric cancer is extremely necessary.

Intracellular chloride channel 1 (CLIC1) protein is known to be expressed in the cell membrane, cytoplasm and nucleus [3]. According to the reports, CLIC1 expression is elevated in many cancers, such as ovarian, gastric, gallbladder, liver and bladder [4–10]. As CLIC1 plays a vital role in the occurrence and development of malignant tumors, more and more studies on CLIC1 have come up in recent years [11]. Dehghan-Nayeri *et al.* [12] reported that during early diagnosis of leukemia patients and evaluation of the efficacy, CLIC1 is an important marker and might help assess the prognosis. Our previous study also found that the resistance of gastric cancer to vincristine was associated with CLIC1 expression [13]. Wu and Wang [14] also found that CLIC1 promoted drug resistance in JeG3 cells through the upregulation of MRP1. Thus, the evidence suggests that CLIC1 is involved in the chemotherapy resistance of tumors.

Autophagy is an evolutionarily conservative catabolic process that takes place in the cytoplasm. The organelles are isolated into a bilayer vesicle and transported to lysosomes for massive degradation [15–18]. The lysosome's decomposed products are recovered and transported to

Table 1 The primer sequences

Name	Sequence
CLIC1-F	ACCGCAGGTCGAATTGTTC
CLIC1-R	ACGGTGGTAACATTGAAGGTG
β-actin-F	CTACCTCATGAAGATCCTCACCGA
β-actin-R	TTCTCCTTAATGTCACGCACGATT

the cytoplasm through the lysosomal membrane. Many studies have shown that autophagy revives a vital function in the development and treatment of cancer and is related to the emergence of drug resistance in tumor cells [19–22]. Tumors of some kinds of cancers have high levels of autophagy, whereas others gain resistance by enhancing autophagy [23]. Zhang *et al* [24]. reported that HMGB1 causes resistance in prostate cancer cells to gemcitabine by activating autophagy. Therefore, autophagy may be the key to acquiring drug resistance during chemotherapy for the tumor.

In this study, cisplatin-resistant strains of gastric cancer cells were induced *in vitro*, and CLIC1 and autophagy were significantly increased. Subsequently, we discussed the relationship between CLIC1 and autophagy in drug resistance. We found that silencing CLIC1 inhibits autophagy in drug-resistant strains of gastric cancer, thereby reducing cisplatin resistance in gastric cancer cells. On the contrary, autophagy was significantly activated when CLIC1 was overexpressed in gastric cancer cells, and an apparent drug resistance appeared. The results of this study also provide us with potential targets for gastric cancer therapy.

Materials and methods

Cell cultures

We purchased the MKN28 cell line and AGS cell line from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Ham's F-12 media was used, which contains fetal bovine serum, streptomycin, and penicillin. We cultured the cells in an incubator at a temperature of 37 °C and 5% CO_2 concentration in air circulation. MKN28/DDP and AGS/DDP cells were induced *in vitro* by continuous incrementation and intermittent high-dose shocks with low concentrations of cisplatin (KeyGEN BioTECH, Jiangsu, China) for 6 months.

Cell Counting Kit-8

Cells were placed in 96-well plates, with 3000 cells per well, and cultured for 6 h. After confirming cell adherence, cisplatin was added as planned, that is, in 10 concentration gradients, and five wells were set for each concentration. The cells were then cultured in an incubator for 24 h. The Cell Counting Kit-8 (CCK-8) (MedChemExpress, US) was used to check cell viability. Absorbance at 450 nm was used, from which semi-inhibitory concentration (IC_{50}) was calculated, and this was repeated three times.

Lentivirus-mediated RNA interference and overexpression

We used CLIC1-interfering RNA sequences and negative control siRNA sequences consistent with our previous studies [13]. The synthesized siRNA was inserted into a lentiviral vector. We purchased the lentiviral vectors for CLIC1 overexpression and negative control (NC) vectors from GeneCopoeia. The lentiviral vector was then transfected into 293T cells, and the supernatant containing the virus was collected and concentrated to determine the final titer of the virus produced. The recombinant lentivirus was transduced into the cells, and the culture medium was changed after 16 h. The transfected cells were then observed via fluorescence microscopy 48 h later.

RT-PCR

First, we used the RNA extraction kit (Vazyme, China) to extract the total RNA from the cells and then reverse-transcribed the RNA into cDNA and diluted it up to 100 µl. The ChamQ SYBR real time quantitative PCR (QRT-PCR) Master Mix (Vazyme) was used for QRT-PCR detection. The thermal cycling parameters were as follows: initial denaturation conditions were 95 °C denaturation for 30 s, 95 °C denaturation for 10 s and 60 °C denaturation for 30 s, for a total of 40 cycles. The mRNA expression differences were normalized to β-actin expression and recorded as multiples of increase over the specified controls. The primer sequences are shown in Table 1.

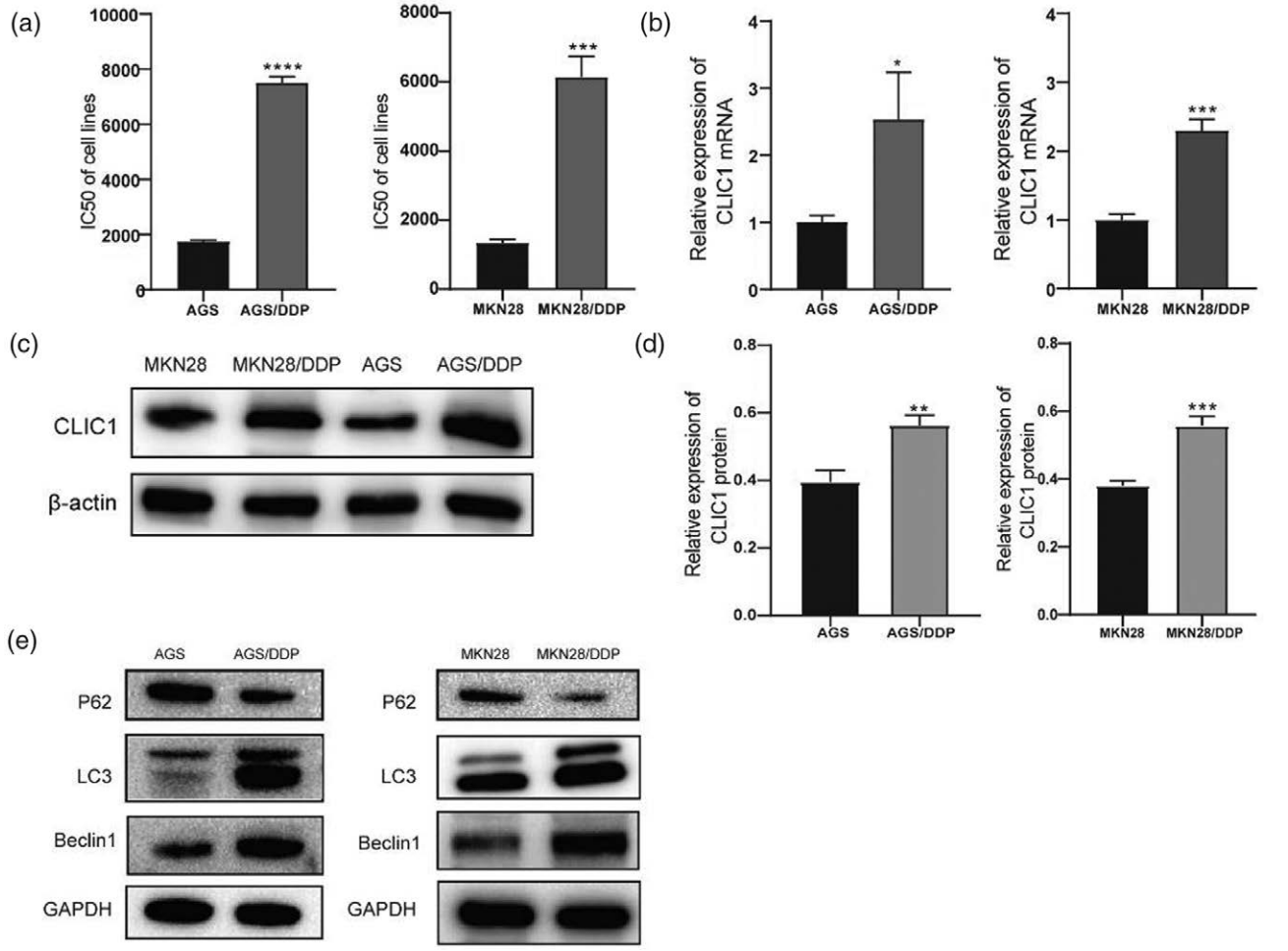
Western blot

RIPA lysis buffer (Beyotime, China) and phenylmethylsulfonyl fluoride (PMSF) (Solarbio, China) were used for cell lysis in a ratio of 100:1. We used the bicinchoninic acid method to determine the protein concentration of the extracted samples (Beyotime, China). Proteins were isolated using SDS-PAGE and transferred onto a polyvinylidene fluoride (PVDF) membrane. Then, the PVDF membrane was blocked with 5% skimmed milk diluted in Tris-buffered saline (TBS). The primary antibodies specific to mammalian target of rapamycin (mTOR) (PTG, Wuhan, China), Phospho-mTOR (PTG), unc-51-like kinase 1 (ULK1) (PTG), Phospho-ULK1 (PTG), autophagy related 13 (PTG), LC3 (CST, USA), P62 (CST, USA), glyceraldehyde-3-phosphate dehydrogenase (PTG), Beclin-1 (PTG, Wuhan, China), beta-polyclonal antibody to beta actin (PTG) and CLIC1 (Sant Cruz, USA) were added and incubated overnight at 4 °C, then incubated at room temperature with the corresponding secondary antibody for 1 h. The enhanced chemiluminescence kit (Tanon, China) was used as the luminescent agent, and li-Cor Biosciences (Lincoln, NE) was used to detect bands which were then quantified using the Image J software.

Cell invasion and migration assay

Serum-free media was used to dissolve the matrix gel and dilute it to 300 µg/ml for the cell invasion assay. A total of

Fig. 1



CLIC1 and autophagy were highly expressed in cisplatin-resistant cell lines MKN28/DDP and AGS/DDP. (a) The IC50 for cisplatin in AGS, AGS/DDP, MKN28, and MKN28/DDP cell lines ($P<0.01$); (b) The mRNA level of CLIC1 in AGS, AGS/DDP, MKN28 and MKN28/DDP cell lines ($P<0.05$); (c,d) The protein level of CLIC1 in AGS, AGS/DDP, MKN28 and MKN28/DDP cell lines ($P<0.01$); (e) The protein level of autophagy in AGS, AGS/DDP, MKN28 and MKN28/DDP cell lines. ($P<0.01$). CLIC1, intracellular chloride channel 1; IC50, semi-inhibitory concentration.

100 µl was added to each transwell chamber, and the matrix was left to solidify at 37 °C for 1 h. MKN28, AGS and drug-resistant cells were suspended in serum-free media. The cell number was counted, and the cell suspension was diluted to 10000 cells/50 µl. 200 µl of MKN28, MKN28/DDP, AGS and AGS/DDP cells with a concentration of 3×10^5 cells/ml were seeded in each compartment, respectively. However, 800 µl of Ham's F-12 containing 10% fetal bovine serum was added to the wells and incubated for 24 h. After 24 h, 0.1% crystal violet (Beyotime, China) was used to stain the cells that crossed the membrane and counted in five microscope fields which were randomly selected from each filter. The matrix glue was not prepared for the cell migration test, but the rest of the protocol was the same as the invasion test.

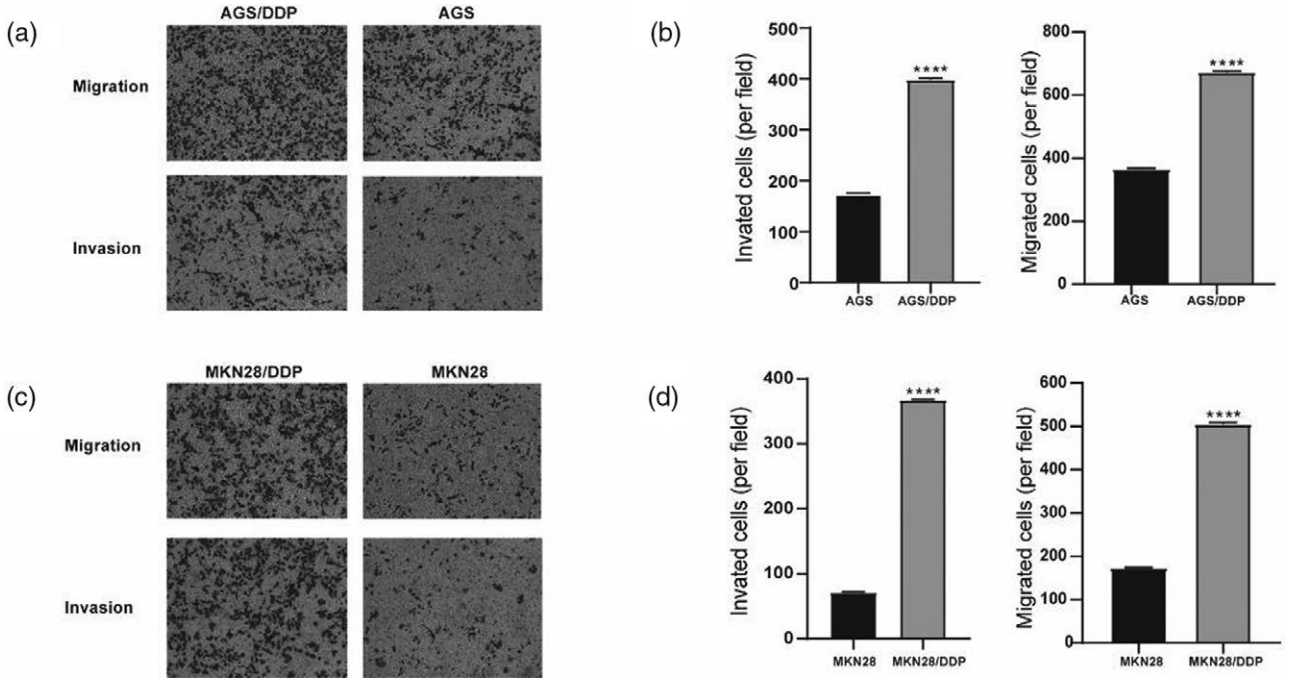
LC3-GFP-RFP to assess autophagy levels

Ad-mCherry-GFP-LC3B was purchased from Beyotime to assess autophagy levels. The cells were inoculated into petri dishes, and about 40% of their growth was supplemented with a specific amount of mole venom. About 12 h after infection, the culture medium containing the virus was removed and 2 ml of fresh complete culture medium was added to each well. After continuous culture for 24 h, cell growth and fluorescence protein expression were observed.

In-vivo cisplatin sensitivity test

We commissioned Guangxi Yisheng Biotechnology Co., Ltd. to conduct the zebrafish experiments. Reproduction of zebrafish embryos occurs naturally in pairs. The embryos were isolated and cleaned at 6 and 24 h after fertilization, and suitable embryos were selected according to their stage of development. Embryos were incubated at 28 °C. Cells from the NC group and CLIC1-overexpressed group were digested and centrifuged successively and suspended in a new medium to prepare a $1–2\times10^7$ cells/ml concentration

Fig. 2



The invasion and metastasis in cisplatin-resistant cell lines MKN28/DDP and AGS/DDP were enhanced. (a,b) Cell migration and invasion experiments displayed that the number of AGS/DDP group cells that passed through the membrane was more than that of the AGS group ($P<0.01$); (c,d) Cell migration and invasion experiments displayed that the number of MKN28/DDP group cells that passed through the membrane was more than that of MKN28 group ($P<0.01$).

for future use. The cells were labeled with red fluorescence. Then the two groups of cells were injected into the yolk sac of 2-day-old zebrafish. About 500 cells were injected into each fish. After injection, zebrafish were incubated at 28 °C for 1 h and then transferred to a 35 °C incubator for 24 h. After 24 h, a cisplatin injection was prepared with normal saline, and 2.5 ng of the solution or normal saline was injected into the yolk sac of zebrafish in the two groups, respectively, and treated for 72 h. Zebrafish in the NC group and overexpressed group were observed and imaged under a fluorescence microscope for 24 h and 96 h, respectively, and the fluorescence intensity was measured. After the experiment, the young zebrafish were euthanized by freezing at –80 °C for preservation. The procedure for euthanizing zebrafish was followed by the American Veterinary Medical Association (AVMA) guidelines.

The 5-week-old male thymic BALB/C nude mice were raised under specific conditions. They were subcutaneously injected into the axillary region with the NC or overexpressed group's AGS cells (6.5×107 in 200 µl medium) and then checked for tumor growth every 2 days. On the eighth day, when the implanted tumor grew to the expected size, cisplatin was injected intraperitoneally every 3 days until day 28. The dose of cisplatin was measured at 3 mg/kg each time [25]. The mice were sacrificed on the 30th day. Finally, we extracted RNA and protein from the tumors for QRT-PCR and western blot experiments, and a few primary tumors were paraffin-embedded for immunostaining analysis of CLIC1 protein expression.

Statistical analysis

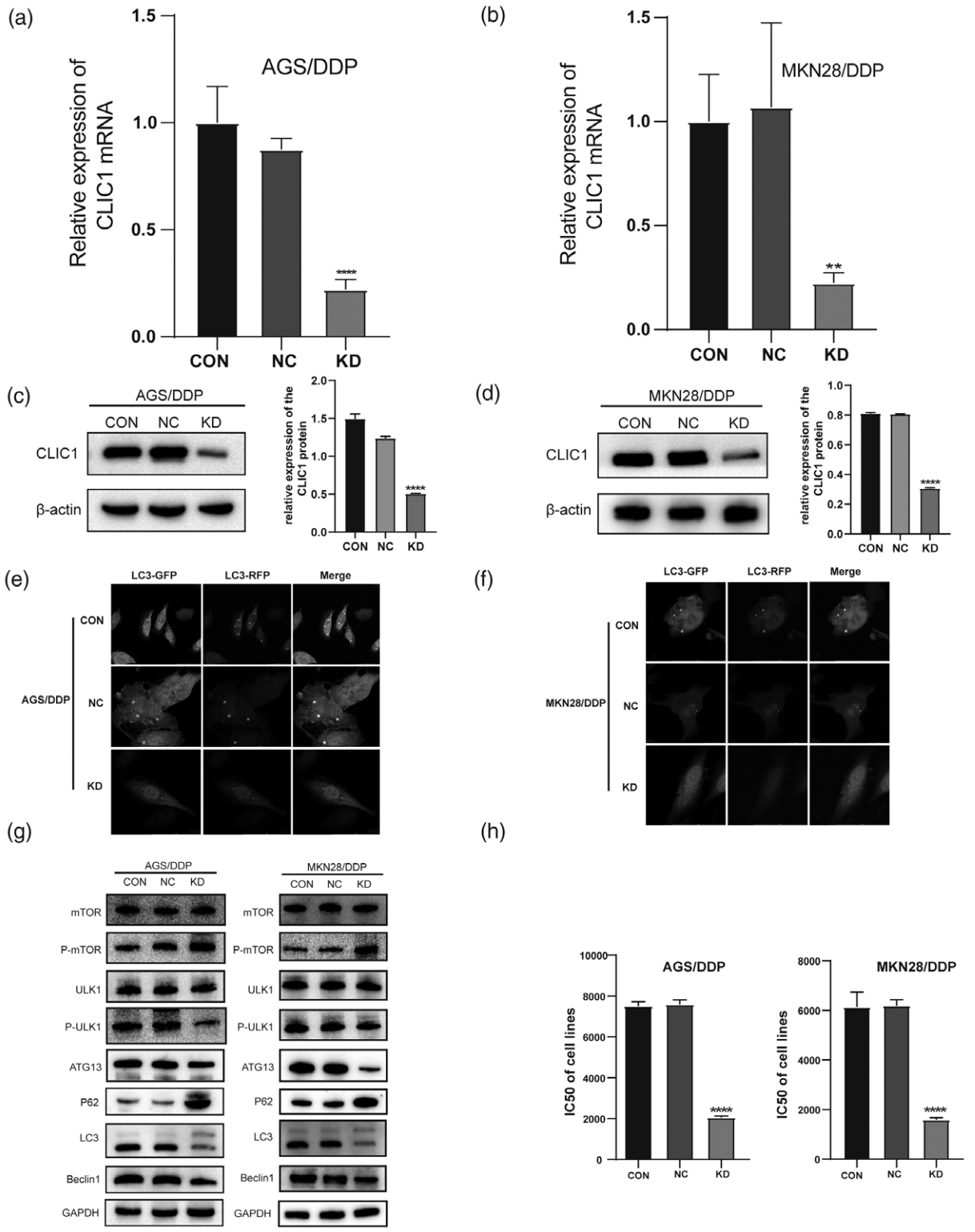
We used mean ± SD to represent the data. T-test was used to compare the two groups, and the analysis of variance (ANOVA) was used to analyze the mean values between multiple groups. The difference was regarded as statistically significant when $P<0.05$. Data were collected and analyzed for at least three sets of experiments.

Results

CLIC1 and autophagy are highly expressed in cisplatin-resistant cell lines MKN28/DDP and AGS/DDP

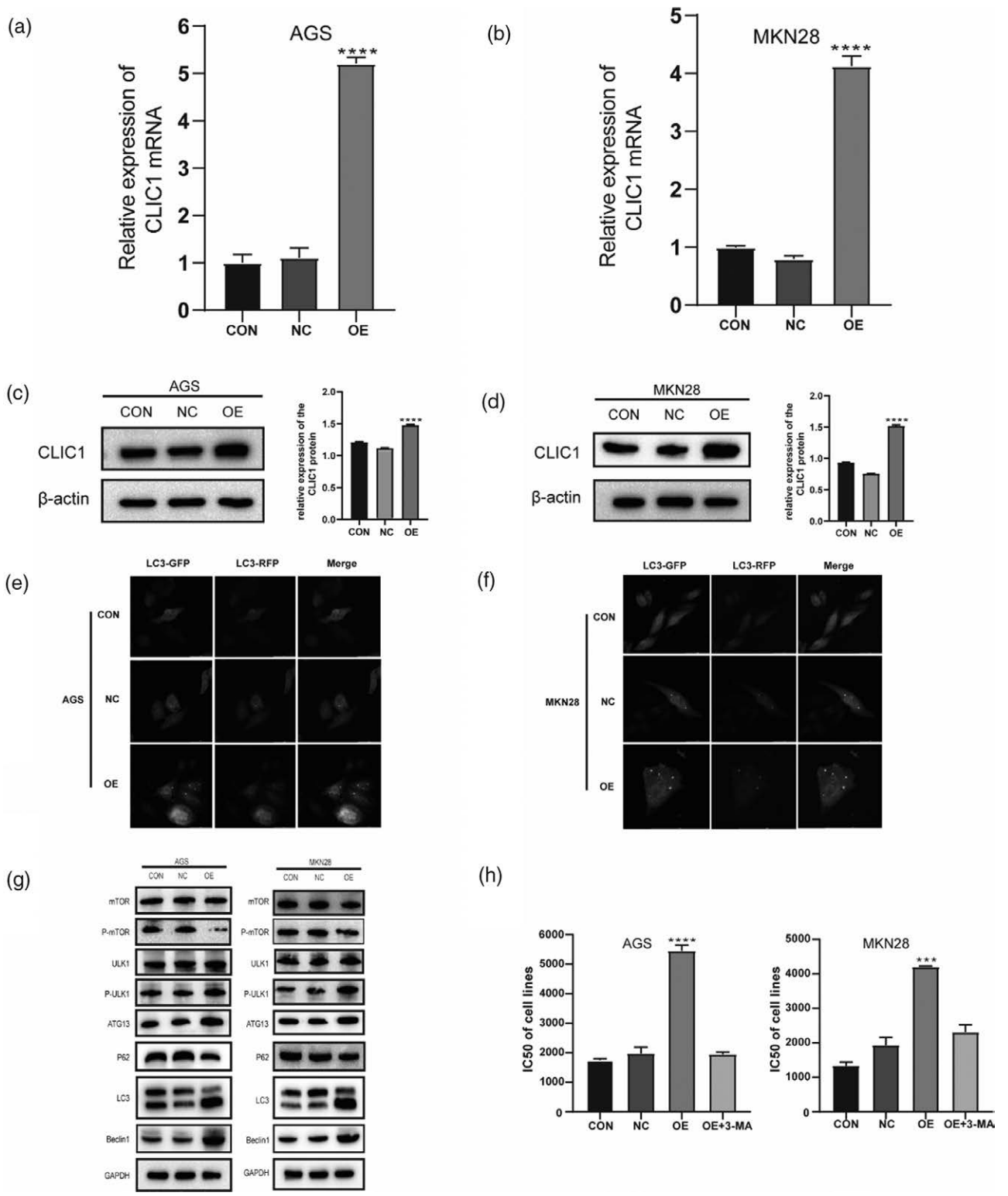
To confirm the resistance of MKN28 and AGS cells to cisplatin, we used the CCK8 method for IC_{50} detection for MKN28 and AGS cell lines and drug-resistant MKN28/DDP and AGS/DDP cell lines. The IC_{50} for MKN28 and MKN28/DDP to cisplatin were 1340 ± 84.14 ng/ml and 6135 ± 493.81 ng/ml, respectively. The IC_{50} for AGS and AGS/DDP to cisplatin were 1746 ± 45.25 ng/ml and 7503 ± 180.39 ng/ml, respectively, with statistically significant differences ($P<0.05$, Fig. 1a). Furthermore, we tested the expression levels of CLIC1 in parent cells and drug-resistant cells. qRT-PCR results showed that the expression of CLIC1 in AGS/DDP was remarkably higher as compared to AGS ($P<0.05$, Fig. 1b), and the same result was found

Fig. 3



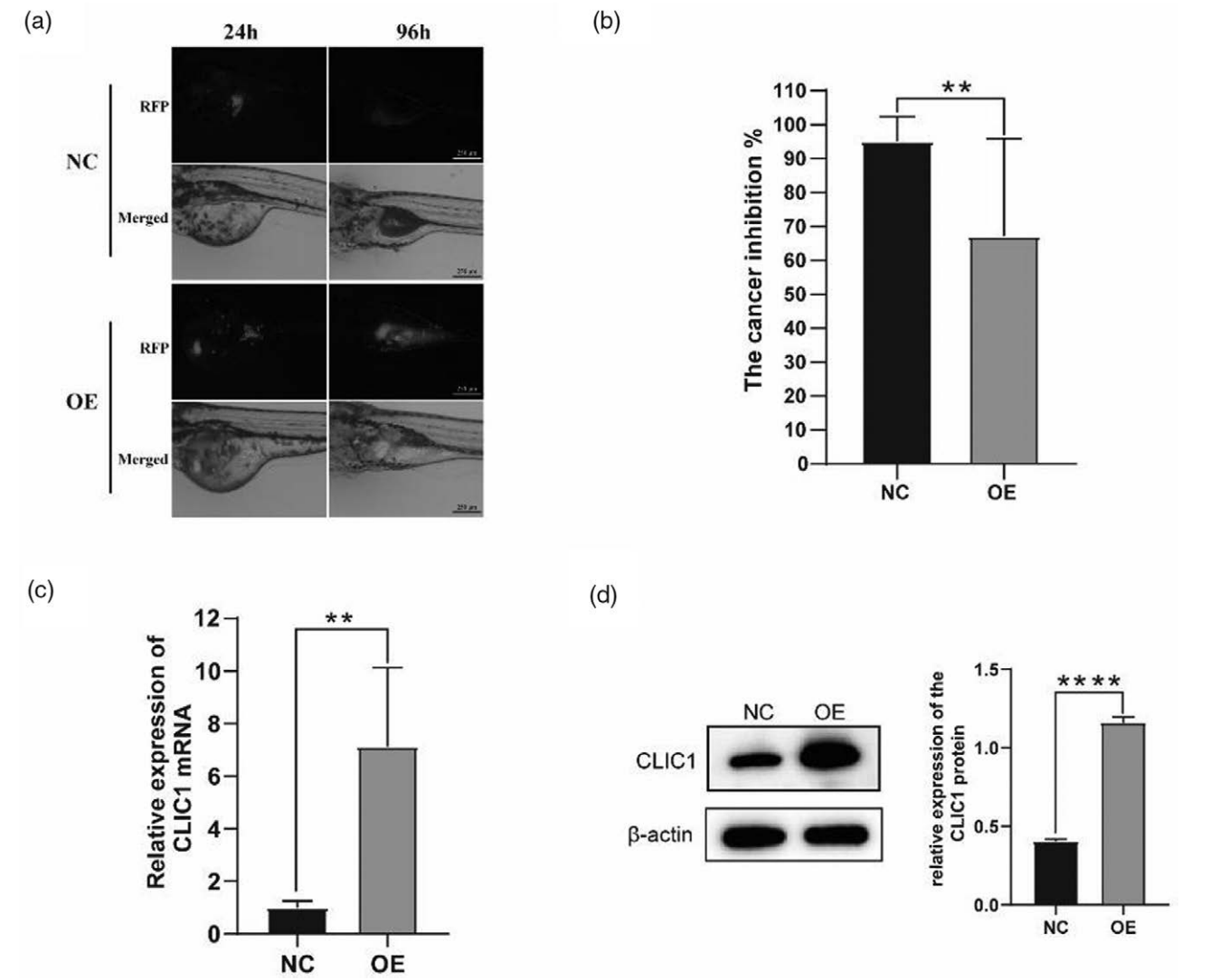
Silencing CLIC1 reverses DDP resistance in gastric cancer cells. (a) The CLIC1 mRNA expression in AGS/DDP after silencing ($P<0.01$); (b) The CLIC1 mRNA expression in MKN28/DDP after silencing ($P<0.01$); (c) The CLIC1 protein level in AGS/DDP after silencing ($P<0.01$); (d) The CLIC1 protein level in MKN28/DDP after silencing ($P<0.01$); (e,f) The level of autophagosome in AGS/DDP and MKN28/DDP after silencing; (g) The protein level of autophagy in AGS/DDP and MKN28/DDP after silencing; (h) The IC50 of cisplatin in AGS/DDP and MKN28/DDP cell lines after silencing ($P<0.01$). CLIC1, intracellular chloride channel 1; IC50, semi-inhibitory concentration.

Fig. 4



Overexpressed CLIC1 enhances DDP resistance in gastric cancer cells by activating autophagy. (a) The CLIC1 mRNA expression in AGS after overexpression ($P<0.01$); (b) The CLIC1 mRNA expression in MKN28 after overexpression ($P<0.01$); (c) The CLIC1 protein level in AGS after overexpression ($P<0.01$); (d) The CLIC1 protein level in MKN28 after overexpression ($P<0.01$); (e,f) The level of autophagosome in AGS and MKN28 after overexpression; (g) The protein level of autophagy in AGS and MKN28 after overexpression; (h) Overexpression or use of autophagy inhibited AGS and MKN28 cells against cisplatin IC50. CLIC1, intracellular chloride channel 1; IC50, semi-inhibitory concentration.

Fig. 5



Effects of CLIC1 expression on the resistance in AGS to DDP in zebrafish. (a,b) The fluorescence intensity of the OE group was higher than the NC group ($P<0.01$); (c) The CLIC1 mRNA expression in the NC and OE group ($P<0.01$); (d) The CLIC1 protein level in the OE and NC group ($P<0.01$). CLIC1, intracellular chloride channel 1; NC, negative control; OE, overexpressed.

in MKN28/DDP ($P<0.01$, Fig. 1b). Western blot indicated that the protein expression levels of CLIC1 in MKN28/DDP and AGS/DDP were higher than in their parent cell lines ($P<0.01$, Fig. 1c,d). Meanwhile, we also used western blot to detect the expression of autophagy in MKN28, MKN28/DDP, AGS and AGS/DDP cell lines, respectively. It was found that autophagy was activated, and its expression was elevated in drug-resistant cell lines (Fig. 1e). This clearly suggests that CLIC1 was highly expressed in MKN28/DDP and AGS/DDP, and autophagy was activated.

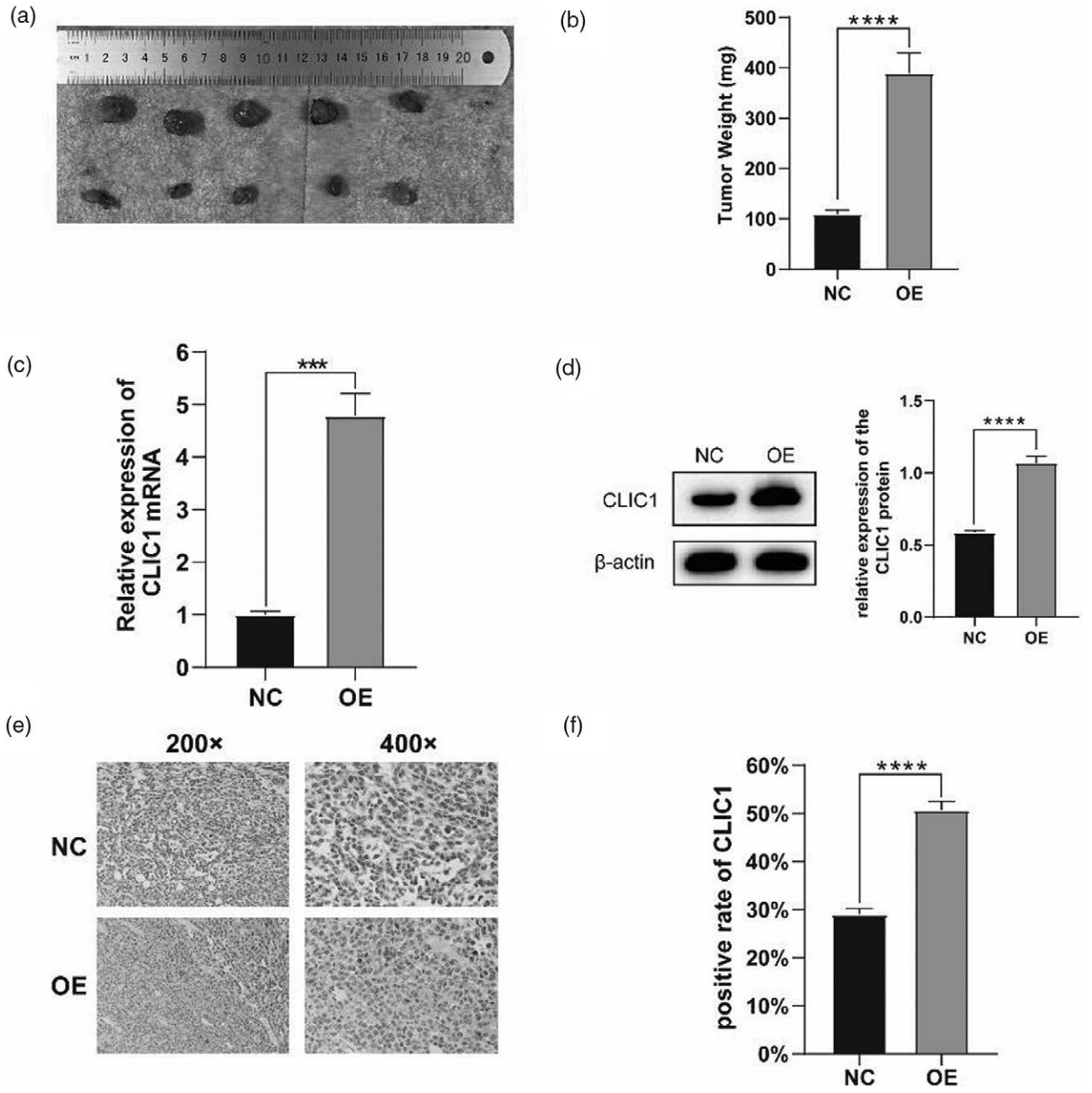
Enhanced invasion and metastasis in cisplatin-resistant cell lines MKN28/DDP and AGS/DDP

To evaluate the invasion and migration ability of drug-resistant cell lines, we conducted invasion and migration experiments on the two drug-resistant cell lines. Compared to the control (CON) group, the number of AGS/DDP cells passing through the membrane was significantly increased (Fig. 2a,b). MKN28/DDP also obtained similar results ($P<0.01$, Fig. 2c,d). These outcomes demonstrated that the invasion and migration abilities of drug-resistant cell lines were more substantial than their parents.

Silencing CLIC1 reverses DDP resistance in gastric cancer cells

We transfected MKN28/DDP and AGS/DDP cells with lentivirus-mediated CLIC1 siRNA to silence the expression of CLIC1 and then observed the changes in drug resistance in those cells. We also checked the expression

Fig. 6



Effects of CLIC1 expression on the resistance in AGS to DDP in nude mice. (a,b) The volume weight of orthotopically transplanted tumors in the NC group was vastly lower than in the OE group ($P<0.01$); (c) The CLIC1 mRNA expression in the OE and NC group ($P<0.01$); (d–f) The CLIC1 protein level in the OE and NC group ($P<0.01$). CLIC1, intracellular chloride channel 1; NC, negative control; OE, overexpressed.

levels of CLIC1 in the two drug-resistant cell lines by QRT-PCR and western blot after transfection with lentivirus-mediated CLIC1-siRNA. The expression of CLIC1 in the drug-resistant strains had drastically decreased as compared to the blank CON and the NC group ($P<0.01$, Fig. 3a–d). In addition, LC3 adenovirus was used to detect the level of autophagosomes after AGS/DDP and MKN28/DDP silencing (Fig. 3e,f). Similarly, we tested the expression of autophagy and found that autophagy was inhibited in drug-resistant strains. Its expression decreased in the CLIC1-siRNA group (Fig. 3g). Subsequently, we measured the IC_{50} of cells by the CCK8 method. It was found that in the MKN28/DDP group, the IC_{50} of cisplatin in the CLIC1-siRNA group (1588.67 ± 68.52 ng/ml) was significantly decreased (the CON group was 6135 ± 493.81 ng/ml, the NC group was 6194 ± 197.79 ng/ml) ($P<0.01$, Fig. 3h). Similar results were shown by the AGS/DDP group ($P<0.01$, Fig. 3h).

These findings indicate that CLIC1 silencing can reverse DDP resistance in MKN28/DDP and AGS/DDP cells and inhibit autophagy.

Overexpressed CLIC1 enhances DDP resistance of gastric cancer cells by activating autophagy

We used lentivirus to establish the expression vectors so as to investigate overexpression of CLIC1 can improve cisplatin resistance in MKN28 and AGS cells. We constructed the lentivirus-mediated CLIC1 overexpression vectors and successfully transfected them into cells. Finally, the CLIC1 expression level in MKN28 and AGS was tested using western blot and QRT-PCR. The expression of CLIC1 in the overexpressed group was remarkably increased than in the CON and the NC group ($P < 0.01$, Fig. 4a–d). We then used LC3 adenovirus to detect the level of autophagosomes after AGS and MKN28 overexpression (Fig. 3e,f). We also used a western bolt to detect the expression of autophagy. We found that autophagy was activated, and its expression was significantly increased in the overexpressed group as compared to the NC and the CON groups ($P < 0.01$, Fig. 4g). Then, we calculated the IC_{50} for the MKN28-overexpressed group and found that compared with the NC and the CON groups, the IC_{50} for the overexpressed group was remarkably increased (CON vs. NC vs. overexpressed: 1340 ± 84.14 ng/ml vs. 1931.33 ± 186.45 ng/ml vs. 4201 ± 16.87 ng/ml, $P < 0.01$, Fig. 4h), and the same results were obtained for the AGS-overexpressed group (CON vs. NC vs. overexpressed: 1746 ± 45.25 ng/ml vs. 1977.33 ± 171.24 ng/ml vs. 5450.67 ± 162.6 ng/ml, $P < 0.01$, Fig. 4h). These outcomes suggest that overexpressing CLIC1 can improve DDP resistance in MKN28 and AGS cells.

To confirm whether autophagy is reported in CLIC1-mediated drug resistance of MKN28 and AGS cells, we designed a set of experiments in which an autophagy inhibitor (3-methyladenine) was used to inhibit autophagy in the overexpressed group, and then the IC_{50} was measured. The results showed that when autophagy inhibitors were involved, IC_{50} was significantly reduced in the hindered overexpressed group ($P < 0.01$, Fig. 4h). This suggests that overexpression of CLIC1 mediates DDP resistance in MKN28 and AGS cells by activating autophagy.

Effects of CLIC1 expression on the in-vivo resistance of AGS cells to DDP

To verify if CLIC1 can also influence the resistance to DDP in AGS cells *in vivo*, we constructed an in-vivo model of zebrafish. We first isolated the NC and CLIC1-overexpressed group cells of AGS and entrusted the Guangxi Yisheng Biotechnology Co., Ltd. to conduct the zebrafish experiment. They injected tumor cells into zebrafish yolk sacs. 2.5 ng cisplatin was injected into zebrafish yolk sacs 24 h after the initial injection and treated with the drug for 72 h. Subsequently, zebrafish in the NC and CLIC1-overexpressed groups were observed and observed under a microscope for 24 and 96 h, respectively, and the fluorescence intensity was measured. The fluorescence intensity for the overexpressed group was higher than the NC group ($P < 0.01$, Fig. 5a,b). Then we checked the expression of CLIC1 in the overexpressed and NC groups, and the results are shown in Fig. 5c,d ($P < 0.01$).

We also constructed a nude mouse model. AGS cells in the NC and CLIC1-overexpressed groups were injected into the nude mouse xenograft model, and cisplatin was used on day 8. Figure 6a,b shows that after cisplatin treatment, the average weight of tumors in the NC and overexpressed groups was 109.8 ± 2.1 mg and 398 ± 37.9 mg, respectively, indicating that the resistance of the overexpressed group to cisplatin was significantly increased ($P < 0.001$). We then used a western blot and qRT-PCR to test the expression of CLIC1 in the tumor tissues. The results showed that CLIC1 expression was higher in the overexpressed group than in the NC group (Fig. 6c,d). Immunohistochemistry also showed that the positive rate in the overexpressed group was higher than in the NC group ($P < 0.001$; Fig. 6e,f). The above animal experiments indicated that DDP resistance in AGS cells was significantly increased when CLIC1 expression was elevated.

Discussion

Gastric cancer is a commonly occurring tumor, and 8.8% of patients associated with cancer deaths each year are gastric cancer patients [26]. Surgery and chemotherapy are crucial treatment methods for patients with gastric cancer, among which chemotherapy is indispensable for advanced patients. However, in recent years, more and more patients evidently show drug resistance in the course of chemotherapy, which leads to unsatisfactory results and affects the prognosis. It has been reported that about 10–30% of patients with malignant tumors are resistant to first-line chemotherapy agents [27–29]. Therefore, drug resistance is a severe obstacle affecting the prognosis, making further research on it essential to find new therapeutic targets.

Friedli *et al.* [30] reported that the CLIC family consists of at least six members, namely CLIC1 to CLIC6, among which CLIC1 is the most studied and the first researched family member. In recent years, CLIC1 has attracted more and more researchers' interest for its comprehensive function in the occurrence and development of cancer [31]. It has been reported that CLIC1 can facilitate tumor metastasis in various types of cancers, including gastric cancer [32–35], and silencing CLIC1 can inhibit the migration and invasion of gastric cancer cells [36]. CLIC1 has been known to contribute to vincristine resistance in gastric cancer cells [13] and also promotes floxuridine and methotrexate resistance in human

choriocarcinoma [14]. However, it is unclear whether CLIC1 is involved in DDP resistance in gastric cancer or not. Our study showed that CLIC1 was positively expressed in cisplatin-resistant cell lines AGS/DDP and MKN28/DDP. Silencing CLIC1 could potentially reduce the cisplatin resistance of gastric cancer cells, and overexpressing CLIC1 could raise cisplatin resistance, which indicates that CLIC1 can affect the cisplatin sensitivity in gastric cancer cells.

Autophagy is an evolutionarily conservative biological process that maintains cell homeostasis by degrading proteins and damaged organelles [18]. In malignant transformation and carcinogenesis, autophagy can recover macromolecules to remove damaged organelles, thus, preventing the occurrence of tumors. However, when cancer develops, autophagy can be used as a survival technique to deal with metabolic stress, such as nutritional deficiency, hypoxia, lack of growth factors, chemotherapy or some targeted treatments that may mediate drug resistance in anticancer therapy [37,38]. Autophagy dysfunction is associated with many types of cancers, and autophagy enhancement is observed after chemotherapy and radiotherapy in various cancer cells [39,40]. Studies have shown that tumors can enhance their resistance to anticancer therapy, including radiotherapy, chemotherapy and targeted therapy, by upregulating autophagy [41,42]. It has been reported that HMGB1 and some miRNA can activate or inhibit autophagy during chemotherapy, thus, regulating drug resistance in tumor cells [24,43,44]. All this evidence indicates that autophagy is closely related to chemotherapy resistance in tumors. However, the regulatory relationship between autophagy and CLIC1-mediated cisplatin resistance has not been studied extensively, so we conducted this study.

In this study, we found that the expression of CLIC1 and autophagy were remarkably increased in drug-resistant strains, so we silenced the CLIC1 in two drug-resistant strains, AGS/DDP and MKN28/DDP, and found that the autophagy products in drug-resistant strains decreased and their sensitivity to cisplatin increased. Subsequently, we overexpressed CLIC1 in AGS and MKN28 cell lines and found that autophagy and drug resistance in the two cell lines increased significantly. Still, there was no significant increase in drug resistance when an autophagy inhibitor was added. Therefore, we believe that CLIC1 can activate autophagy and mediate cisplatin resistance in gastric cancer cells. We also detected mTOR pathway-related proteins, and the results suggested that the expression of mTOR pathway-related proteins was also significantly changed. Therefore, we suggest that CLIC1 regulates autophagy through the mTOR pathway.

To sum up, our study demonstrated that CLIC1 is directly linked to resistance to DDP in gastric cancer cells. When CLIC1 expression is elevated in gastric cancer cells, the mTOR pathway changes, thus activating autophagy, and finally inducing DDP resistance in these cells. Therefore, the results of our study provide a new mechanism for the production of DDP resistance in gastric cancer and, thus, a potential target for the treatment of gastric cancer patients.

Acknowledgements

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The research protocol was approved by the Animal Ethics Committee of the First Affiliated Hospital of Guangxi Medical University.

Conflicts of interest

There are no conflicts of interest.

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