Research Square

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CLIC1 mediated autophagy confers resistance to DDP in gastric cancer

Zhen-liang Nong
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Kun Zhao
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Ye Wang
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Zhu Yu
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Chong-jun Wang
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Wei-jia Huang
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Jun-qiang Chen (chenjunqiang@gxmu.edu.cn)
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region

Research Article

Keywords: Gastric cancer, CLIC1, Autophagy, DDP, Resistance

Posted Date: June 16th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1740274/v1

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Abstract

As one of the most ordinary malignant tumors worldwide, gastric cancer has been concerned by researchers all the year round. The treatment of gastric cancer includes surgery, chemotherapy, and traditional Chinese medicine. Chemotherapy is an effective treatment for patients with advanced gastric cancer. As a critical chemotherapeutic drug, DDP has been approved to treat many kinds of solid tumors. Although DDP is an effective chemotherapeutic agent, many patients develop drug resistance in treatment, which has become a severe problem in clinical chemotherapy. This study aimed to research the mechanism of DDP resistance in gastric cancer. The results showed that compared to the parental cells, CLIC1 expression was increased in AGS/DDP and MKN28/DDP, and autophagy was activated. In addition, the sensitiveness of gastric cancer cells to DDP was decreased compared to the control group, and the autophagy was enhanced after overexpression of CLIC1. Conversely, gastric cancer cells were more sensitive to cisplatin after transfection of CLIC1siRNA or treatment with autophagy inhibitors. These experiments suggested that CLIC1 could alter the sensitiveness 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.

Introduction

Gastric cancer (GC) is four of the best customary malignant tumors globally. More than 1 million cases of gastric cancer are diagnosed and 783,000 people die of it every year, which means one out of every 12 deaths worldwide is due to gastric cancer [1]. The treatment of early gastric cancer is mainly surgery, and the therapeutic effect is good. Since the symptoms of gastric cancer are usually not apparent in the early stages, local or distance metastasis was present at the time of diagnosis [2]. For these patients, chemotherapy is 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 many types of cancer, including gastric cancer. But there are more and more drug resistances in clinical treatment, leading to chemotherapy's unsatisfactory outcome. Therefore, the study of cisplatin chemotherapy resistance is helpful to improve the chemotherapy effect of gastric cancer patients and the prognosis and survival rate. However, the potential mechanism of cisplatin resistance is still unclear and needs to be elucidated. Given this, exploring the mechanism of cisplatin resistance in gastric cancer is very urgent and essential

Intracellular chloride channel 1 (CLIC1) protein is 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 cancers [4–10]. As CLIC1 recreates a broad function in the occurrence and development of malignant tumors, more and more studies on CLIC1 in recent years [11]. Dehghan-Mayer et al. reported that when early diagnosing leukemia patients and evaluating the efficacy, CLIC1 is an important marker and may help assess prognosis[12]. Our previous study also found that the resistance of gastric cancer to vincristine was related to CLIC1 expression [13], J. Wu et al. also found that CLICl promoted drug resistance of JeG3 cells through up-regulation of MRP1[14]. This suggests that CLIC1 is also involved in chemotherapy resistance of tumors.

Autophagy is an evolutionarily conservative catabolic process in part of the cytoplasm, and organelles are isolated into a bilayer vesicle and transported to lysosomes for massive degradation [15–18]. The lysosome decomposition products are recovered and transported to the cytoplasm through the lysosomal membrane. Many studies have proved that autophagy recreates a vital function in the development and treatment of cancer and is related to the acquisition of drug resistance by tumor cells [19–22]. Some kinds of tumors have high levels of autophagy, while others gain resistance by enhancing autophagy [23]. ZHANG et al. [24] reported that HMGB1 causes prostate cancer cells to be resistant to gemcitabine by activating autophagy. Therefore, autophagy may be the key for the tumor to acquire drug resistance during chemotherapy.

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

Materials And Methods

Cell cultures

We purchased MKN28 cells and AGS cells from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). Ham's F-12 medium used contained fetal bovine serum streptomycin, and penicillin. We cultured cells in an incubator at 37 °C with air having 5% CO2. MKN28/DDP and AGS/DDP cells were induced in vitro by continuous incrementation and intermittent high-dose shock with low concentration cisplatin (KeyGEN BioTECH, Jiangsu, China) for 6 months.

CCK8

Placed cells into 96-well plates with 3000 cells per well and cultivated for 6 hours. After cell adherence, cisplatin was added according to the designed 10 concentration gradients, and 5 multiple Wells were set for each concentration gradient. Then the cells were cultured in an incubator for 24h. The Cell Counting Kit-8 (MedChemExpress, US) was used to detect cell viability. Measure the absorbance at 450 nm, calculate the semi-inhibitory concentration (IC50), and repeat three times.

Lentivirus-mediated RNA interference (RNAi) and overexpression

We used CLIC1 interfering RNA sequences and negative control siRNA sequences consistent with previous studies[13]. The synthesized siRNA was inserted into the lentiviral vector. We purchased lentiviral vectors for CLIC1 overexpression (OE) and negative control vectors (NC) from the Genecopoeia. The lentiviral vector was then transfected into 293T cells, and the supernatant of the virus was collected and

concentrated on determining the final titer of the virus. The recombinant lentivirus was transfected into cells, and the medium was changed after 16 hours. The transfected cells were observed by fluorescence microscopy 48 h later.

RT-PCR

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

Table 1

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

Western blot

RIPA lysis buffer (Beyotime, China) and PMSF (Solarbio, China) were used for cell lysis in a ratio of 100:1. we used the BCA method to determine the protein concentration after extracting protein (Beyotime, China). Proteins were isolated by SDS-PAGE and transmitted onto a PVDF membrane. Subsequently, the PVDF membrane was sealed with 5% skim milk expanded with Tris-buffered saline (TBS), and primary antibodies specific to LC3(CST, USA), P62 (CST, USA), GAPDH (PTG, Wuhan, China), Beclin-1(PTG, Wuhan, China), beta-ACTB (PTG, Wuhan, China) and CLIC1 (Sant Cruz, USA) were incubated overnight at 4℃, then incubated at room temperature with the corresponding secondary antibody for 1 h. The ECL kit (Tanon, China) was used as the luminescent fluid, and using li-Cor Biosciences (Lincoln, NE) detected bands and quantified them with the Image J software.

Cell invasion and migration assay

Used in the serum-free medium to dissolve the matrix gel and diluted to 300μg/ mL for cell invasion assay.100μL was added to each Transwell chamber and the matrix solidified after incubation at 37°C for 1h. MKN28 and AGS cells and drug-resistant cells were suspended in serum-free medium. The cell number was counted and diluted at 10000 cells /50ul. 200μl MKN28, MKN28/DDP, AGS, and AGS/DDP with a density of 3×10^5/ml were seeded in each compartment, respectively, and 800μl of Ham's F-12 contained 10% fetal bovine serum was counted in the downward compartment and incubated for 24 h.

After 24h, used 0.1% crystal violet (Beyotime, China) to stain cells that crossed the membrane and counted in 5 microscope fields randomly selected from each filter. The matrix glue was not prepared for the cell migration test, and the rest was the same as the invasion test.

In vivo cisplatin sensitivity test

We commissioned Guangxi Yisheng Biotechnology Co., Ltd. to conduct zebrafish experiments. Reproduction of zebrafish embryos occurs naturally in pairs. The embryos were cleaned at 6 and 24 hours after fertilization and suitable embryos were selected according to their stage of development. Embryos were incubated at 28℃. The cells of the NC group and CLIC1-OE group were digested and centrifuged successively, and suspending the cells in a new medium to prepare 1-2×107 cells /mL for future use. The cells were labeled with red fluorescence, and then the two groups of cells were respectively injected into the yolk sac of 2-day zebrafish. About 500 cells were injected into each fish. After injection, zebrafish recovered at 28℃ for 1 hour and then transferred to a 35℃ incubator for 24 hours. After 24 hours, cisplatin injection was prepared with normal saline, and 2.5ng or normal saline was injected into the yolk sac of zebrafish in the two groups, respectively, and treated for 72 hours. Zebrafish in the NC group and OE group were observed and photographed under a fluorescence microscope for 24h and 96h, respectively, and the fluorescence intensity was counted. After the experiment, the young zebrafish were frozen and killed at -80℃ for preservation. The procedure of killing zebrafish was under the guidelines of the American Veterinary Medical Association (AVMA).

The 5-week-old male thymic BALB/ C nude mice were raised under certain conditions. It subcutaneously injected the NC or OE group AGS cells (6.5×107 in 200μ L medium) into the axillary region and checked for tumor growth every two days. When the implanted tumor grew to the eighth day, cisplatin was injected intraperitoneally every three days until day 28, and the dose of cisplatin was 3mg /kg each time [25], and then killed the mice on the 30th day. Finally, we extracted RNA and protein from the tumor for QRT-PCR and Western Blot experiments, and some primary tumors were paraffin-embedded for immunostaining analysis of CLIC1 protein expression.

Statistical analysis

Mean ± standard deviation was used to represent data. Using the t-test to compare the two groups and the Analysis of variance (ANOVA) was used to analysis the mean values between multiple groups. The difference was regarded as statistical significance when $P<0.05$. Calculations were made for at least three separate experiments.

Results

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

In order to confirm the resistance of MKN28 and AGS to cisplatin, we used CCK8 method for IC50 detection of MKN28 and AGS and drug-resistant MKN28/DDP and AGS/DDP cell lines. The IC50 of

MKN28 and MKN28/DDP to cisplatin were 1340ng±84.14ng/ mL and 6135±493.81ng/ mL, respectively. The IC50 of AGS and AGS/DDP to cisplatin were 1746ng±45.25ng/ mL and 7503±180.39ng/ mL, respectively, with statistically significant differences (P<0.05, Fig.1a). Furthermore, we tested the expression of CLIC1 in parent cells and drug-resistant cells. qRT-PCR results showed that the expression of CLIC1 in AGS/DDP was remarkably higher than that in AGS (P<0.05, Figure 1b), and the same result was found in MKN28/DDP (P<0.01, Figure 1b). Western blot results indicated that the protein expression level of CLIC1 in MKN28/DDP and AGS/DDP was higher than its parent cells (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 respectively, and it was found that autophagy was activated and its expression was elevated in drug-resistant cell lines (Fig.1e). This suggested that CLIC1 was highly expressed in MKN28/DDP and AGS/DDP, and autophagy was activated.

The invasion and metastasis of cisplatin-resistant cell lines MKN28/DDP and AGS/DDP were enhanced

In order to evaluate the invasion and migration ability of drug-resistant cell lines, we conducted invasion and migration experiments on two drug-resistant cell lines, respectively. Compared with the 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 of drug-resistant cell lines were stronger 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 changes in drug resistance in those cells. Then, we detected the expression of CLIC1 in two drug-resistant cells by QRT-PCR and Western blot after transfection of lentivirus-mediated CLIC1-siRNA. The expression of CLIC1 in drug-resistant strains was extremely descending than the blank control (CON) and the negative control group (NC) (P<0.01, Fig. 3a-d). Similarly, we furthermore tested the expression of autophagy and found that autophagy was inhibited, and its expression decreased in the CLIC1-siRNA group (Fig. 3e). Subsequently, we detected the IC50 of cells by the CCK8 method. It was found that in the MKN28/DDP group, the IC50 of cisplatin in the CLIC1-siRNA group (1588.67±68.52ng/ mL) was significantly decreased (the CON group was 6135±493.81ng/ mL, the NC group was 6194±197.79 ng/ mL) (P<0.01, Fig. 3f). Similar outcomes were found in the AGS/DDP group (P<0.01, Fig. 3f). These consequences recommend that CLIC1 silencing can reverse DDP resistance of 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 to explore whether overexpression of CLIC1 can improve cisplatin resistance of MKN28 and AGS. We established the Lentivirus-mediated CLIC1 overexpression vectors in the issue and successfully transfected them into cells. Finally, the CLIC1 expression in MKN28 and AGS was tested by Western blot and QRT-PCR. The expression of CLIC1 in the overexpressed group (OE) was remarkably increased than in the CON and the NC group (P<0.01, Fig. 4a-

d). We also used Western Bolt to detect the expression of autophagy, and it was found that autophagy was activated and expressed more increased in the OE group than in the NC and the CON groups (P<0.01, Fig. 4e). Then we detected the IC50 of the MKN28-OE group and found that compared with the NC and the CON groups, the IC50 of the OE group was remarkably increased (CON vs NC vs OE: 1340ng±84.14ng/ml vs 1931.33±186.45ng/ml vs 4201±16.87ng/ml, P<0.01, Fig. 4f), the same results were obtained in the AGS-OE group (CON vs NC vs OE: 1746ng±45.25ng/ml vs 1977.33±171.24ng/ml vs 5450.67±162.6ng/ml, P<0.01, Fig. 4f). Those outcomes suggest that overexpressing CLIC1 can improve DDP resistance of MKN28 and AGS cells.

To additionally confirm that autophagy is implicated in CLIC1-mediated drug resistance of MKN28 and AGS cells, we designed a set of experiments in which an autophagy inhibitor (3-MA) was used to inhibit autophagy in the overexpressed group, and then IC50 was detected. The results showed that when autophagy inhibitors inhibited autophagy, IC50 was greatly reduced in the hindered OE group (P<0.01, Fig. 4f). 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 whether the CLIC1 can also influence the resistance of AGS cells to DDP in vivo, we constructed an in vivo model of zebrafish. We first constructed the NC and CLIC1-OE group cells of AGS, and entrusted the Guangxi Yisheng Biotechnology Co., Ltd. to conduct zebrafish experiment. They injected tumor cells into zebrafish yolk sacs. 2.5ng cisplatin was injected into zebrafish yolk sacs 24 hours after injection and treated with the drug for 72 hours. Subsequently, zebrafish in the NC and CLIC1-OE groups were observed and photographed under a microscope for 24h and 96h, respectively, and the fluorescence intensity was counted. The fluorescence intensity of the OE group was stronger than the NC group (P<0.01, Fig. 5 a, b). Then we detected the expression of CLIC1 in the OE and NC groups, and the results were shown in figure 5 c, d (P<0.01).

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

Discussion

Gastric cancer is a high-incidence tumor, and 8.8% of patients associated with cancer deaths each year are gastric cancer patients [26]. Surgery and chemotherapy are essential treatment methods for patients

with gastric cancer, among which chemotherapy is indispensable for advanced patients. However, in recent years, more and more patients have apparent drug resistance in the course of chemotherapy, which leads to unsatisfactory chemotherapy effects 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 significant obstacle affecting prognosis, so it is very urgent to study drug resistance of gastric cancer to provide new therapeutic targets for the clinic.

M. Friedli et al. reported that the CLIC family consists of at least six members, namely CLIC1 to CLIC6 [30], among which CLIC1 is the most studied and the earliest researched family member. In recent years, CLIC1 has attracted more and more researchers' interest in its comprehensive function in the occurrence and development of cancers[31]. There was literature reported that CLIC1 can facilitate tumor metastasis of many types of cancer, including gastric cancer[32–35], silencing CLIC1 can inhibit the migration and invasion of gastric cancer cells [36]. CLIC1 has been found to contribute to vincristine resistance in gastric cancer cells[13] and also can promote FUDR and MTX resistance in human choriocarcinoma[14]. However, it is unclear whether CLIC1 is involved in DDP resistance in gastric cancer. This investigation discovered that CLIC1 was positively expressed in cisplatin-resistant cell lines AGS/DDP and MKN28/DDP. Silencing CLIC1 could reduce the cisplatin resistance of gastric cancer cells, and overexpressing CLIC1 could raise cisplatin resistance, which indicates that CLIC1 can affect the cisplatin sensitivity of gastric cancer cells.

Autophagy is an evolutionarily highly 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 has formed, autophagy can be used as a survival way 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 cancer, and autophagy enhancement after chemotherapy and radiotherapy is observed 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 up-regulating autophagy, resulting in drug resistance [41, 42]. It has been reported that HMGB1 and some miRNA can activate or inhibit autophagy during chemotherapy, thus changing the drug resistance of tumor cells [24, 43, 44]. All this evidence indicates that autophagy is closely related to tumor chemotherapy resistance. However, the regulatory relationship between autophagy and CLIC1-mediated cisplatin resistance has not been further studied, 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 of two drug-resistant strains AGS/DDP and MKN28/DDP, and found that the autophagy products of drug-resistant strains decreased and their sensitivity to cisplatin increased. Subsequently, we overexpressed the expression of CLIC1 in AGS and MKN28, and we found that autophagy and drug resistance of the two cell lines increased significantly, but 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.

To sum up, our study demonstrated that CLIC1 is bound up with the resistance of gastric cancer cells to DDP. When CLIC1 expression is elevated in gastric cancer cells, autophagy can be activated to induce DDP resistance in gastric cancer cells. Therefore, the results of this study provide a new mechanism for gastric cancer to produce DDP resistance and a potential target for the treatment of gastric cancer patients.

Declarations

Funding *The study was supported by the Guangxi Scientific Research and Technology Development Project (grant no.1598011-4), the National Natural Science Foundation of China (grant no.82060430), the Guangxi Clinical Research Center for Enhanced Recovery after Surgery Guangxi Science and Technology Base and Talent Project (grant no.AD19245196) the Guangxi Key Research and Development Project (grant no.AB18126058), and ERAS Research Laboratory of High-incidence Gastrointestinal Tumors in Guangxi (YYZS2020003).*

Competing Interests *No conflicts of interest.*

Author Contributions *All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Zhenliang Nong , Kun Zhao, Ye Wang, Zhu Yu, Chongjun Wang, Weijia Huang, and Jun-qiang Chen. The first draft of the manuscript was written by Zhenliang Nong and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.*

Data Availability *The datasets generated during and/or analysed during the current study are not publicly available due to individual privacy but are available from the corresponding author on reasonable request.*

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

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Figures

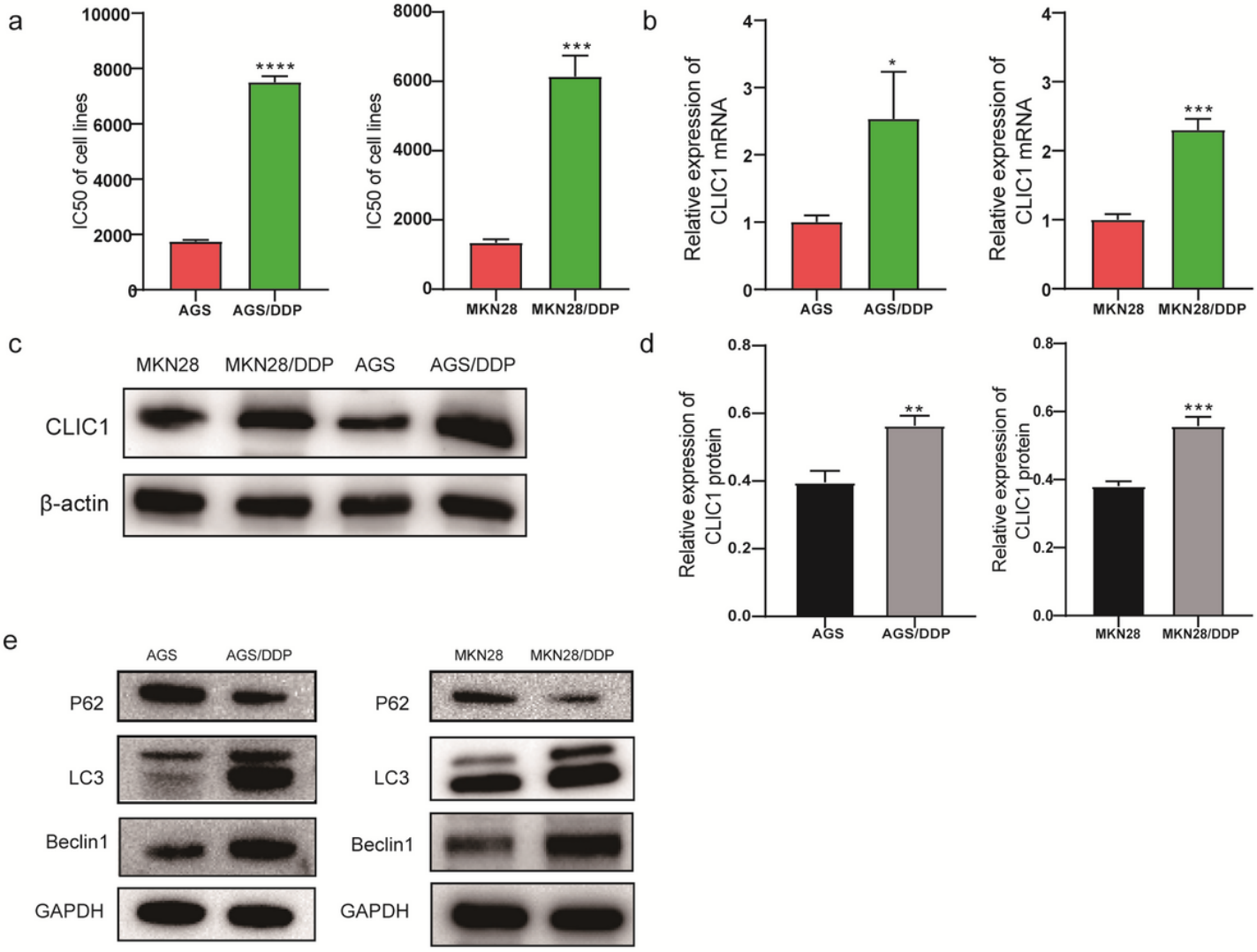

Figure 1

CLIC1 and autophagy were highly expressed in cisplatin-resistant cell lines MKN28/DDP and AGS/DDP. a The IC50 to 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. ($P < 0.01$)

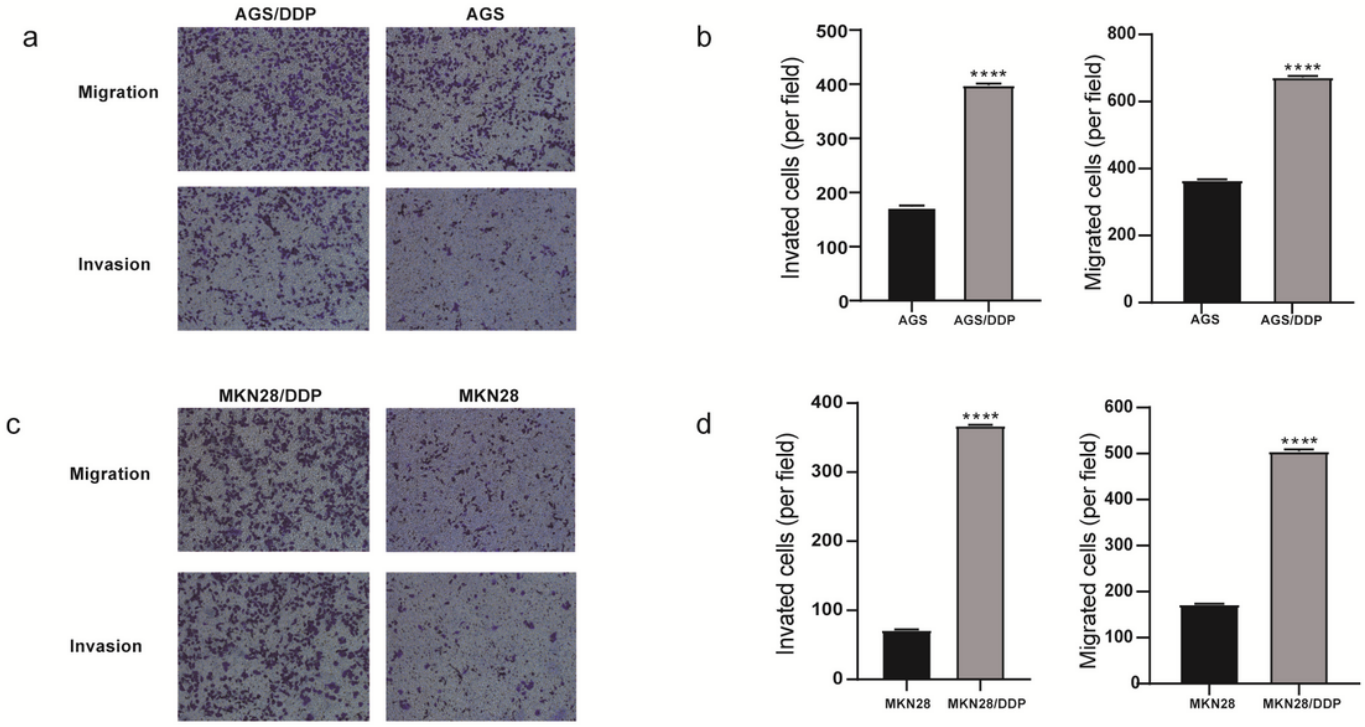

Figure 2

The invasion and metastasis of 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 passed through the membrane was more than that of AGS ($P < 0.01$); c, d : Cell migration and invasion experiments displayed that the number of MKN28/DDP group passed through the membrane was more than that of MKN28 ($P < 0.01$).

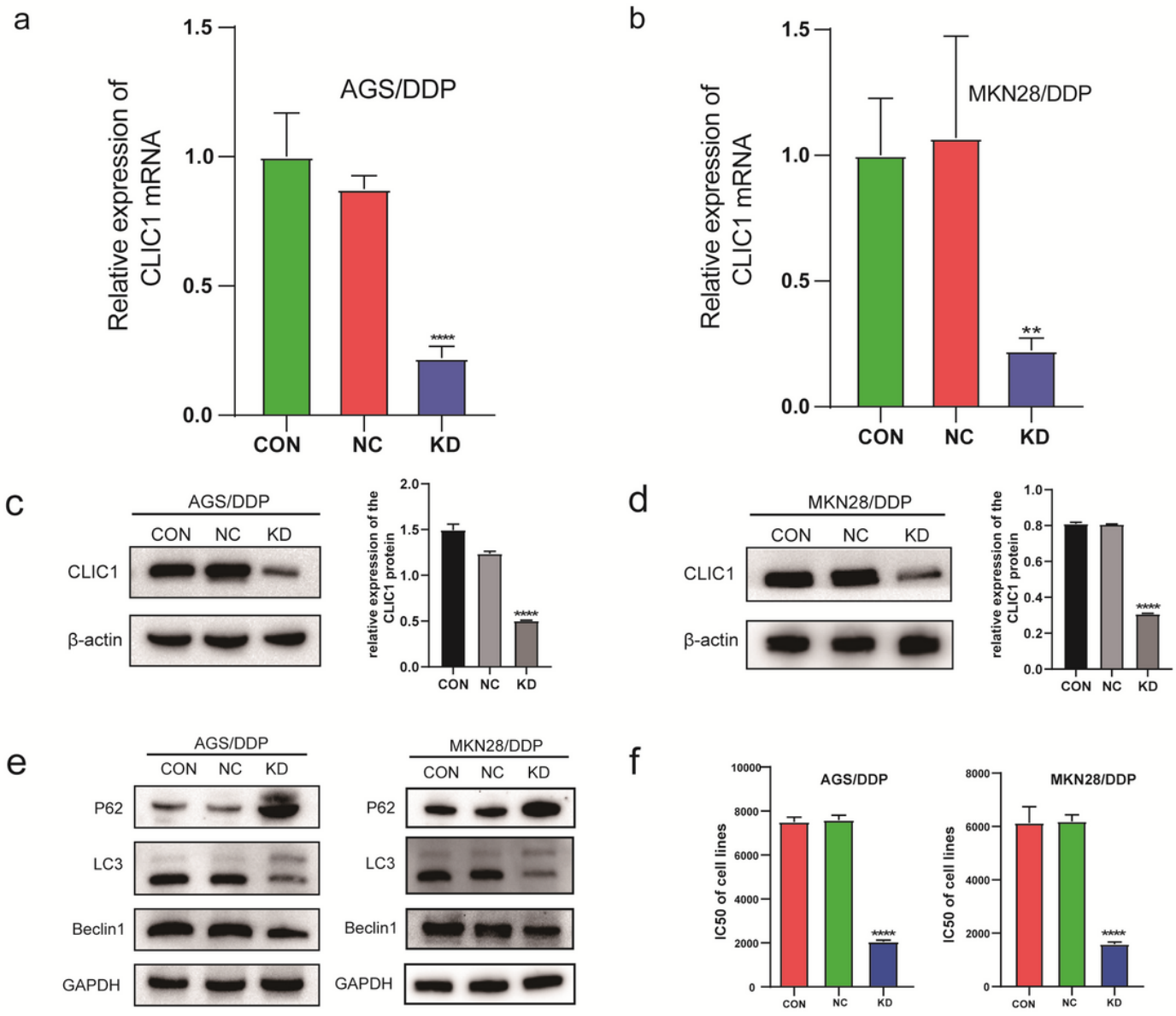

Figure 3

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: The protein level of autophagy in AGS/DDP and MKN28/DDP after silencing; f: The IC50 to cisplatin in AGS/DDP and MKN28/DDP cell lines after silencing ($P < 0.01$).

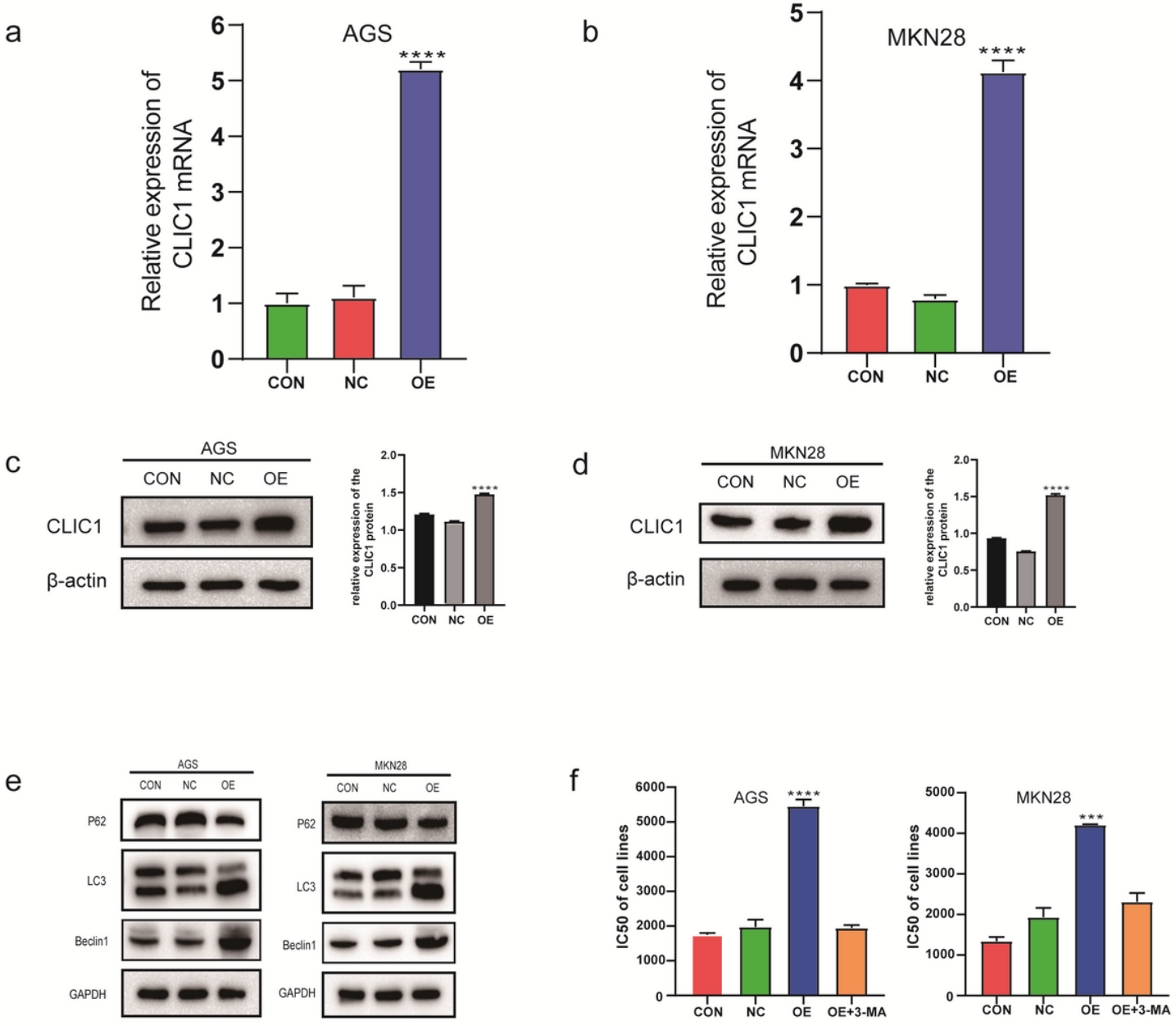

Figure 4

Overexpressed CLIC1 enhances DDP resistance of 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: The protein level of autophagy in AGS and MKN28 after overexpression ($P < 0.01$); f: Overexpression or use of autophagy inhibited AGS and MKN28 cells against cisplatin IC50

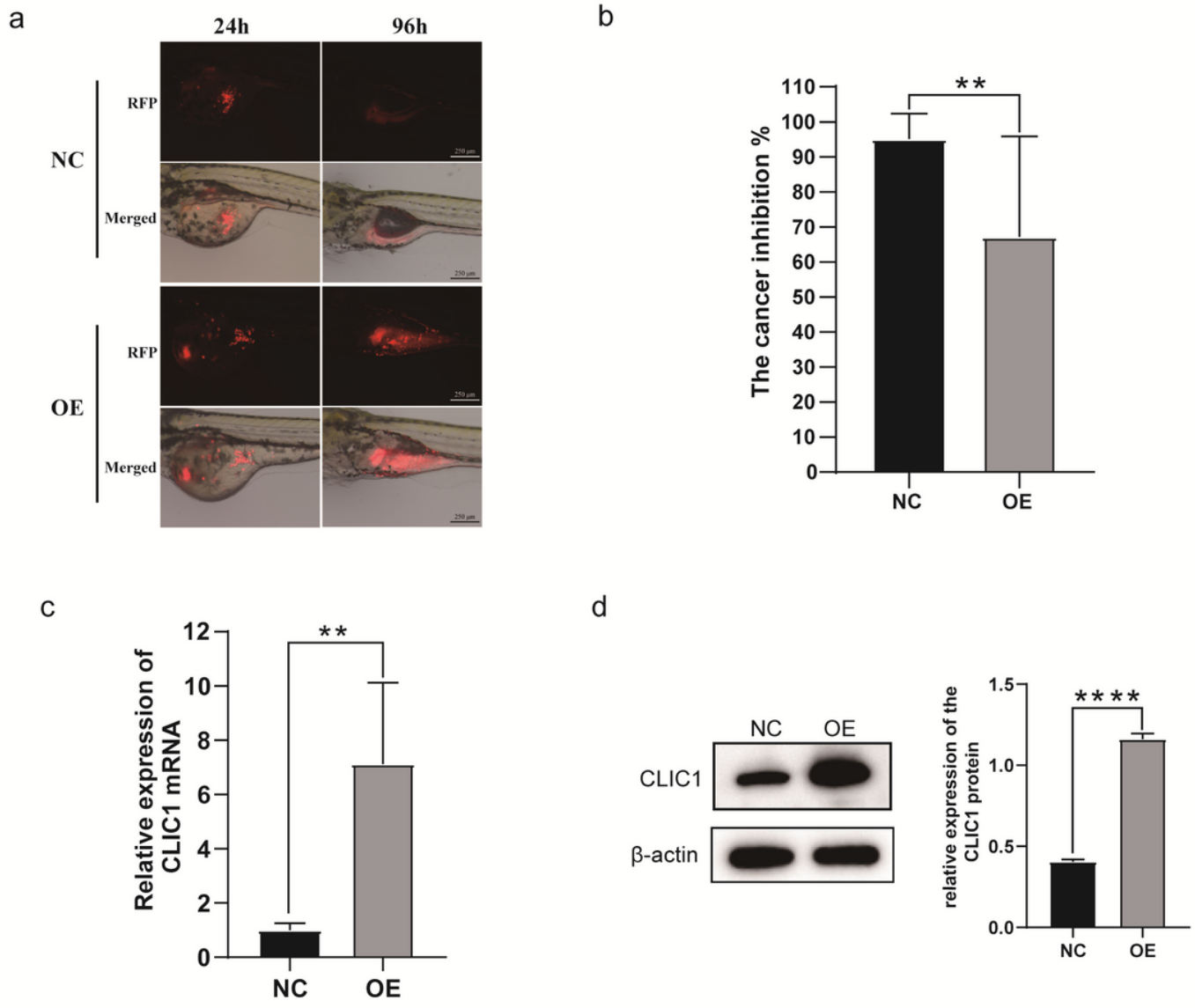

Figure 5

Effects of CLIC1 expression on the resistance of AGS to DDP in zebrafish. a, b: The fluorescence intensity of the OE group was stronger 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 ($P < 0.01$)

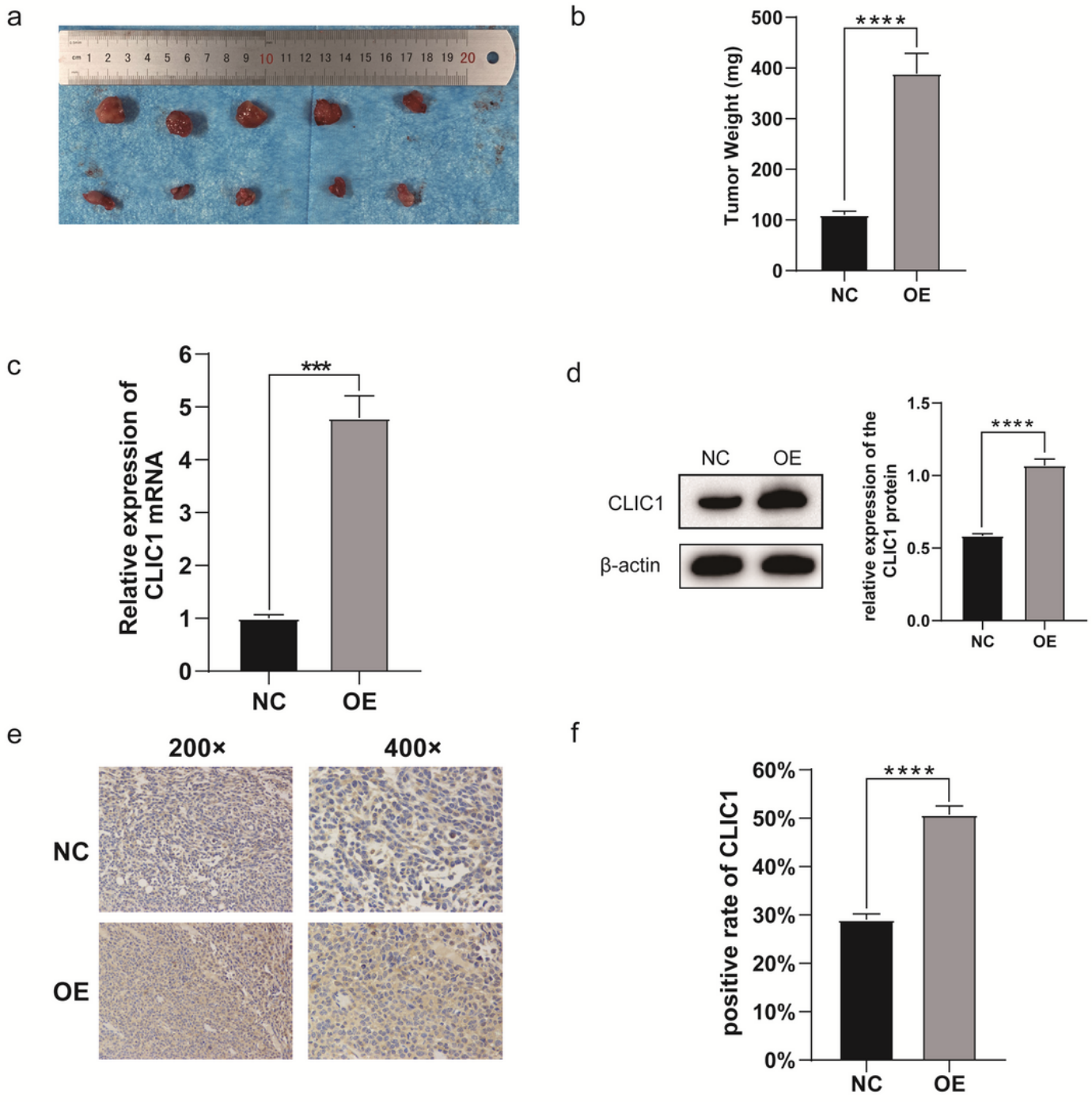

Figure 6

Effects of CLIC1 expression on the resistance of 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 ($P < 0.01$)