Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2024**; 29(1): 15
https://doi.org/10.31083/j.fbl2901015

Original Research

Huaier Regulates Oxaliplatin Resistance in Colorectal Cancer by Regulating Autophagy and Inhibiting the Wnt/β-catenin Signalling Pathway

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Academic Editor: Taeg Kyu Kwon
Submitted: 15 June 2023 Revised: 4 September 2023 Accepted: 7 September 2023 Published: 17 January 2024

Abstract

Objective: The present study aims to investigate the effect of Huaier on oxaliplatin (OXA) resistance in HCT-8 colorectal cancer (CRC) cells. **Methods**: Oxaliplatin-resistant HCT-8/L CRC cells were used. The Cell Counting Kit-8, western blotting, quantitative real-time polymerase chain reaction, protein extraction kit, immunofluorescence and acridine orange staining assays were used in the study. The experiment results proved that Huaier has an influence on the Wnt/β-catenin signalling pathway, autophagy and drug resistance. The authors of the present study used chloroquine, an autophagy inhibitor and Wnt agonist 1 (a Wnt pathway agonist) to verify the present experiment. **Results**: The results showed that Huaier can regulate autophagy, inhibit the Wnt/β-catenin signalling pathway and reverse the drug resistance of OXA-resistant CRC cells. **Conclusions**: This study proved that Huaier can regulate autophagy, inhibit the Wnt/β-catenin signalling pathway and reverse the drug resistance of OXA-resistant CRC cells.

Keywords: Huaier; drug resistance; colorectal cancer; Wnt/β-catenin; autophagy

1. Introduction

Colorectal cancer (CRC) is one of the major malignant tumours occurring in humans. It has a high morbidity and mortality worldwide [1], and its incidence in China is increasing annually [2]. The drugs used for CRC chemotherapy mainly include 5-fluorouracil, irinotecan and oxaliplatin (OXA) [3]; OXA is a third-generation platinum drug and a standard drug for CRC treatment [4]. However, chemotherapy resistance in CRC has become one of the reasons for treatment failure. Therefore, the causes of chemoresistance failure in CRC need to be explored.

The underlying mechanisms of tumour multidrug resistance are complex and remain unclear [5]. ABCB1, also known as *p-gp* [6]. Various chemotherapeutic drugs can be expelled from cells by binding to different *p-gp* sites, resulting in cell resistance [7]. Therefore, by inhibiting *p-gp* expression, chemotherapeutic drugs in tumour cells cannot be released, which can reverse the cells' drug resistance.

Huaier is a fungus that grows on the trunks of damp locust trees. Its main components are proteoglycans [8], and studies have demonstrated that it has an inhibitory effect on tumour cells. The anticancer mechanism of ear may be associated with a variety of biological activities, such as inhibition of cell proliferation, anti-metastasis, interference with tumour angiogenesis and tumour-specific immunomodulatory effects [9]. Specific mechanisms, such as Huaier, can inhibit cutaneous squamous cell carcinoma cells by regulating the DNA methylation of CDKN2A and TP53 [10]. Huaier can also affect the process of tumour cells through the NF-κB/IκBα, Wnt/β-catenin, methyl ethyl ketone/extracellular signal-regulated kinase (ERK) and other signalling pathways [11–13]. It has been shown to inhibit metastasis and epithelial-mesenchymal transition in triple-negative breast cancer cells, a process caused by the induction of autophagy in triple-negative breast cancer cells [12]. Moreover, it could inhibit pancreatic cancer by inhibiting the Wnt/β-catenin pathway [13]. At the same time, studies have shown that Huaier may be a promising adjuvant treatment for cancer and can be widely used in adjuvant cancer therapy [14]. However, whether it affects autophagy through the Wnt/β-catenin signalling pathway is unknown. The present study explores the mechanism of how Huaier regulates OXA-resistant CRC cells.

2. Materials and Methods

2.1 Chemicals

Huaier was provided by Gaitianli Pharmaceutical (Qidong, Jiangsu, China); the Huaier grain was dissolved in RPMI-1640 medium (Procell Company, Wuhan, China) and sterilised with a 0.22 µm filter (Merck Millipore, Darmstadt, Germany).

The Wnt agonist 1 was purchased from the MedChemExpress Company (Monmouth Junction, NJ, USA), and chloroquine (CQ) was obtained from the Sigma Company (St. Louis, MO, USA).

2.2 Cell Lines and Cell Culture

The HCT8 cells were purchased from the Procell Company, and the resistant cells of HCT8/L were pur-

chased from the Aolu Biological Company (Shanghai, China); all cell lines were cultured in Dulbecco's-Modified-Eagle-Medium-High Glucose supplemented with 10% fetal bovine serum (FBS, Procell Company) and 100 mg/mL of penicillin/streptomycin/glutamine (Procell Company) at 37 °C with 5% CO_2. All cell lines were tested using a mycoplasma kit (Sigma) and were free of mycoplasma contamination. Furthermore, all cell lines were authenticated using short tandem repeat technology performed by the Shanghai Xuanyi Technical Service Centre shortly before use.

2.3 Cell Viability Assay (CCK-8 Assay)

The CCK-8 assay was employed to detect cell viability using a Cell Counting Kit-8 (MCE, NJ, USA). Specifically, cells at the logarithmic stage were digested with trypsin and diluted to 1×10^3–5×10^3 cells/mL; next, the cells were seeded into 96-well plates. This was followed by treatment with Huaier at different doses (0, 6, 9, 12, 15 and 18 mg/mL) for 24 or 48 h; the mixed solution of the Cell Counting Kit-8 per well was then added and placed in an incubator for 2 h. Finally, the plates were measured at an absorbance of 450 nm. This experiment was performed in triplicate.

2.4 Acridine Orange Staining Assay

The cells were seeded in 24-well plates. After 24 h, the culture medium was discarded, and a sensitive strain control group, a drug-resistant strain control group and a drug-resistant strain plus different concentrations of Huaier were cultured for 24 h. Next, the cells were repeatedly washed with phosphate-buffered saline (PBS), added with a 500 µL acridine orange (AO) mixed solution in each well, stained for 15 min, washed twice with PBS and immediately analysed using a fluorescence microscope (Olympus, Tokyo, Japan). The proportion of positive AO staining was determined by counting the number of fluorescent cells.

2.5 Immunofluorescence Staining

The cells were seeded on coverslips. After treatment with Huaier or 10% complete medium, the cells were fixed with 4% paraformaldehyde; 5% bovine serum albumin (BSA, Solarbio, Beijing, China) was used to block for 1 h, and LC3-B antibody (Abclonal, Woburn, MA, USA) was applied for incubation overnight at 4 °C. Next, the cells were incubated with Alexa Fluor 488 (Bioss, Beijing, China) for 1 h and stained with 4′,6-diamidino-2-phenylindole for 5 min. Finally, an anti-fluorescence quenching agent was added. The cells were immediately observed under a fluorescence microscope. The proportion of positive LC3 staining was determined by counting the number of fluorescent cells.

2.6 Quantitative Real-Time PCR (qRT-PCR)

The cells were digested with trypsin and diluted in 6-well plates containing 10% complete medium. Next, the cells were cultured in a 5% CO_2 37 °C incubator overnight. The cells were treated with Huaier at different concentrations (0, 6, 9 and 12 mg/mL) for 24 h. The TRIzol (Biosharp, Shanghai, China) reagent was used to isolate total RNA, and cDNA was synthesised using a polymerase chain reaction (PCR) kit (Sparkjade, Shandong, China). The mRNA expression levels were detected using the SYBR Green qPCR Mix (Sparkjade); the data were analysed using the $2^{-\Delta\Delta Ct}$ method. The specific primers are listed as follows: (1) *p-gp* forward, 5′-TCTATGGTTGGCAACTAACACT-3′ and reverse, 5′-CTCCTGAGTCAAAGAAACAACG-3′; and (2) *GAPDH* forward, 5′-GCACCGTCAAGGCTGAGAAC-3′ and reverse, 5′-TGGTGAAGACGCCAGTGGA-3′.

2.7 Nuclear and Cytoplasmic Protein Extraction Kit

Prior to use, phenylmethylsulfonyl fluoride was added to the cytoplasmic protein extraction reagent A and the nuclear protein extraction reagent at a working concentration of 1 mM. The PBS-washed cells were used 2–3 times, then scraped, blown with a pipette gun and transferred to a centrifuge tube for centrifugation; the supernatant was discarded every 20 µL. For precipitation, cytoplasmic protein extraction reagent A containing PMSF was added, shaken vigorously for 5 s, and soaked in ice for 15 min. Then, 10 µL cell protein extraction reagent B was added into the precipitate, shaken well for 5 s and soaked in ice for 1 min before vigorous shaking again for 5 s at 4 °C. Subsequently, the mixture was centrifuged for 5 min at 15,000 g. A pipette gun was used to transfer the extracted cytoplasmic protein supernatant to a new Eppendorf (EP) tube for analysis. A nuclear protein extraction reagent was added to the remaining precipitate and shaken vigorously for 5 s to completely disperse the cell precipitate. Under the condition of an ice bath, it was shaken forcefully for 30 s/2 min for a total of 30 min and then centrifuged for 10 min at 4 °C and 15,000 g. The extracted supernatant of the nuclear protein was transferred to a new precooled EP tube with a pipette gun for standby.

2.8 Western Blotting Analysis

Cells were lysed using radio-immunoprecipitation assay buffer (Solarbio) to collect proteins. The BCA protein assay kit (Solarbio) was used determined the protein concentration, and the proteins were subsequently separated using sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The proteins were transferred onto polyvinylidene fluoride membranes, 5% BSA was used to block the membranes for 2 h, and the mixture was incubated with primary antibody to *p-gp* (1:1000, CST, MA, USA), LC3B (1:2000, Abclonal), P62 (1:2000, Abclonal), Wnt 3a (1:1000, Bioss), β-catenin (1:10,000, CST), proliferating cell nuclear antigen (1:3000, Abclonal), GAPDH (1:10,000, Abclonal) and β-actin (1:100,000, Abclonal) overnight at 4 °C. Then, the secondary antibody (1:10,000, Abclonal) was incubated for 1 h. Finally, an enhanced

chemiluminescence reagent (Biosharp) was used and developed using a gel imaging machine. The ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used to analyse the protein grey values.

2.9 Statistical Analysis

All data were shown as mean ± standard error of the mean (SEM). A Student's *t*-test, one-way analysis of variance (ANOVA) and two-way ANOVA were performed. The SPSS (version 20, Cary, NC, USA) and GraphPad 8 (San Diego, CA, USA) software were used to analyse the data; a p value of <0.05 was considered statistically significant.

3. Results

3.1 Huaier Inhibits the Activity of HCT-8/L Cells

A comparative plot showed the cell inhibition rates of HCT-8 and HCT-8/L strains in response to different concentrations of OXA. As shown in the **Supplementary Fig. 1**, the IC_{50} value of HCT-8 was 0.5 µg/mL, the IC_{50} value of HCT-8/L was 7.58 µg/mL, and the resistive index was 15.16, indicating that HCT-8/L was a highly OXA-resistant cell line. The CCK-8 assay results showed that (1) Huaier decreased the viability of drug-resistant cells (Fig. 1A,B) and (2) the IC_{50} values for HCT-8 and HCT-8/L cells treated with Huaier for 24 h were 13.92 mg/mL and 15.76 mg/mL, respectively, whereas those treated with Huaier for 48 h were 9.64 mg/mL and 10.97 mg/mL, respectively. Moreover, the inhibitory effect of Huaier on cell viability was significantly enhanced in HCT-8 cells, whereas Huaier weakened the viability of HCT-8/L cells (Fig. 1C,D). Huaier inhibited cell viability; the inhibition of sensitive strains was greater than that of drug-resistant strains.

3.2 Huaier Reverses Drug Resistance of HCT-8/L Cells by Decreasing p-gp Expression

Subsequently, qRT-PCR and western blotting (WB) were used to detect the effect of Huaier on the expression of resistance-associated protein *p-gp* in HCT-8/L cells (Fig. 2A,B). At the protein level, the expression level of *p-gp* in HCT-8/L cells decreased with increasing Huaier concentrations; the expression level of *p-gp* mRNA was significantly lower in HCT8 cells than in HCT8/L cells, whereas the expression of *p-gp* mRNA decreased with the increase in Huaier concentrations (Fig. 2C). The above results showed that Huaier could reverse the drug resistance of HCT-8/L cells by decreasing the *p-gp* expression.

3.3 Huaier Activated the State of Autophagy in HCT-8/L Cells

To investigate the effects of Huaier on autophagy, AO staining assay was performed. Acridine orange is a specific dye that can penetrate acidic organelles, such as autophagic lysosomes. Owing to its sensitivity to pH, AO emits red fluorescence when the pH is low. The AO staining results are presented in Fig. 3A,C. Compared with HCT-8/L cells, HCT-8 cells had more acidic vesicular organelles, which were stained red by AO. After treatment of HCT-8/L cells with different Huaier concentrations, the number of acid vesicular organelles stained red with AO increased significantly.

The findings of the immunofluorescence staining assay further support the AO staining assay results. Although the HCT-8 cytoplasm exhibited LC3 green fluorescence, the staining intensity was weak. Meanwhile, in the Huaier group, the LC3 staining intensity increased with increasing Huaier concentrations (Fig. 3B,D).

Based on the above assays, the effect of Huaier on autophagy was further researched. After 24 h of treatment of HCT-8/L cells with Huaier, the P62 protein expression level significantly decreased, whereas the LC3-II protein expression significantly increased in each group. The expression of LC3-II was higher in the HCT-8 group than in the HCT-8/L group (Fig. 3G,H). However, the P62 protein expression was lower in the HCT-8 group than in the HCT-8/L group (Fig. 3E,F). The above results showed that Huaier can affect the viability of HCT-8/L cells through autophagy.

3.4 Huaier Inhibited the Wnt/β-catenin Signalling Pathway in HCT-8/L Cells

To determine whether Huaier affected the Wnt/β-catenin protein expression of HCT-8/L cells, the Wnt/β-catenin protein was detected using WB assay following treatment with Huaier for 24 h. Cytosolic β-catenin and Wnt3a were less expressed in HCT-8 cells (Fig. 4A–D). Huaier significantly reduced the expression of β-catenin and Wnt3a proteins in the HCT-8/L group.

3.5 Huaier Enhances Autophagy and Reverses Drug Resistance

In this study, the autophagy inhibitor CQ was used to further clarify whether Huaier leads to the transformation of cellular drug resistance by enhancing the degree of autophagy. Acridine orange and immunofluorescence staining assays were performed to determine the effects of Huaier and CQ on autophagy. Chloroquine treatment decreased autophagy in HCT-8/L cells, which was significantly increased in HCT-8/L cells after Huaier treatment; furthermore, when used alongside Huaier, it did not reduce its effect on autophagy in HCT-8/L cells (Fig. 5A–D). The effects of Huaier and CQ on autophagy and drug resistance were detected using WB (Fig. 5E–J). The results showed that Huaier treatment significantly enhanced autophagy. After addition of the autophagy inhibitor CQ, the amount of LC3-II increased. Some studies have indicated that an increase in LC3-II is related to the fusion of autophagosomes and lysosomes, and/or lysosome degradation [15]. As demonstrated in Fig. 5B,D, CQ significantly inhibited autophagy, and the combination of Huaier and CQ promoted autophagy. Huaier reduced the expression of *p-gp*,

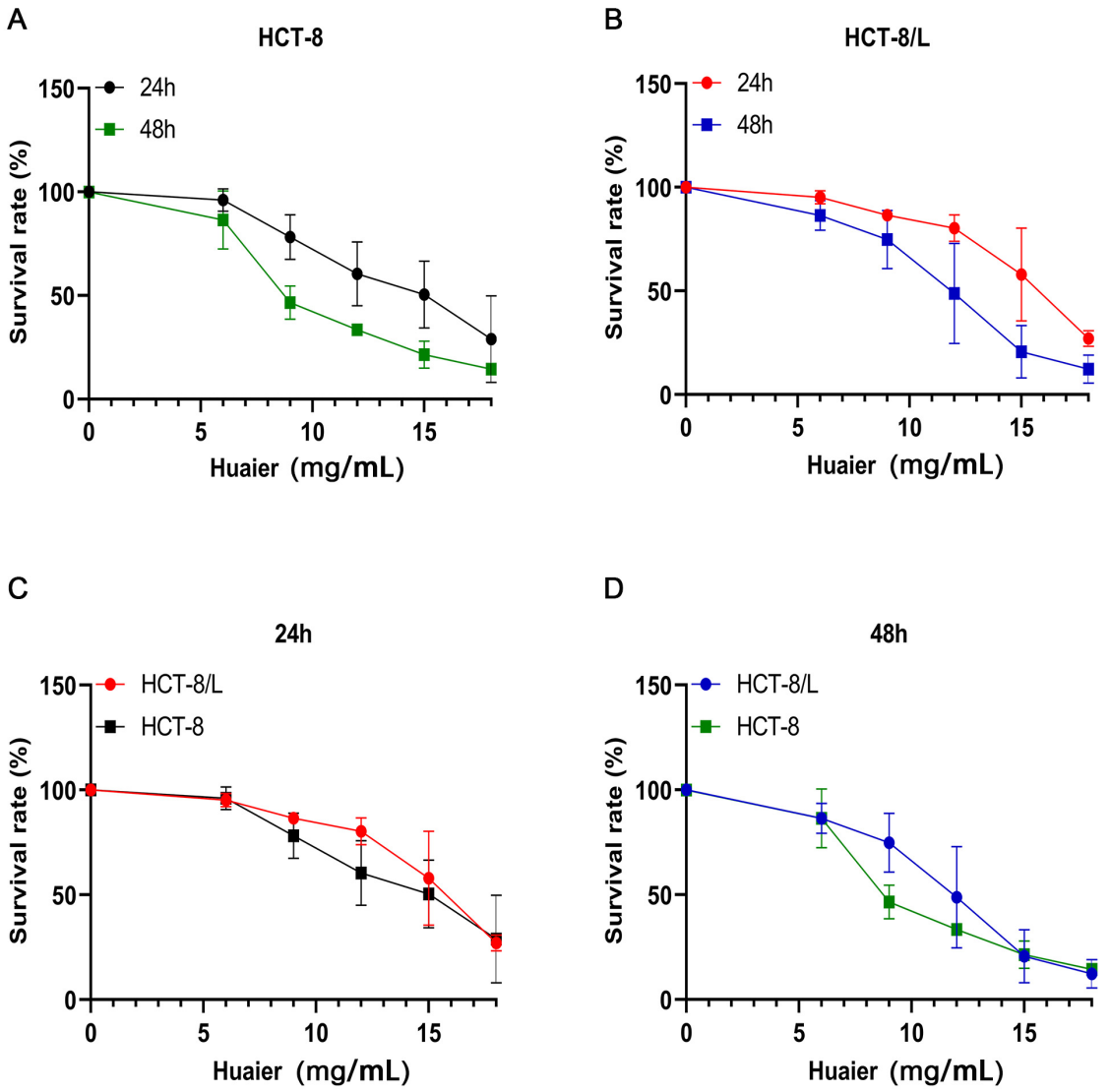

Fig. 1. Huaier reduced the viability of colorectal cancer (CRC) cells. (A) Effect of Huaier on the activity of HCT-8 cells. (B) Effect of Huaier on the activity of HCT-8/L cells. (C) Huaier treatment for 24 h. (D) Huaier treatment for 48 h. Data means ± standard error of measurement (SEM), each group was triplicated, two-way ANOVA analysis.

CQ enhanced the expression of *p-gp*, and the combination of Huaier and CQ reduced the expression of *p-gp* (Fig. 5K).

3.6 Huaier Inhibiting the Wnt/β-catenin Signalling Pathway Activates Autophagy to Reverse Drug Resistance of HCT-8/L Cells

The Wnt agonist 1 was used in the present study to further elucidate that Huaier affected autophagy through inhibiting the Wnt/β-catenin signalling pathway. Acridine orange and immunofluorescence staining were also used to detect the effects of Huaier and Wnt agonist 1 on autophagy. The effects of Huaier and Wnt agonist 1 on autophagy and drug resistance were determined using WB. As presented in Fig. 6A–L, the degree of autophagy was significantly enhanced by Huaier, significantly inhibited by Wnt agonist 1, and promoted by the combination of Huaier and Wnt agonist 1. As demonstrated in Fig. 6M–O, the *p-gp* expression was reduced by Huaier, enhanced by Wnt agonist 1, and reduced by the combination of Huaier and Wnt agonist 1. The above results indicated that Huaier can regulate autophagy and drug resistance in HCT-8/L cells by regulating the Wnt/β-catenin signalling pathway.

3.7 Huaier Inhibits β-catenin Entry into the Nucleus by Affecting the Wnt/β-catenin Signalling Pathway

The β-catenin expression in the nuclei was detected using WB. The results showed that the β-catenin nuclear expression was significantly higher in HCT-8/L cells than in HCT-8 cells; the β-catenin expression decreased after the addition of Huaier (Fig. 7A,B). Subsequently, HCT-8/L resistance to Oxaliplatin was determined following treatment with Huaier. As shown in Fig. 7C, the IC_{50} of

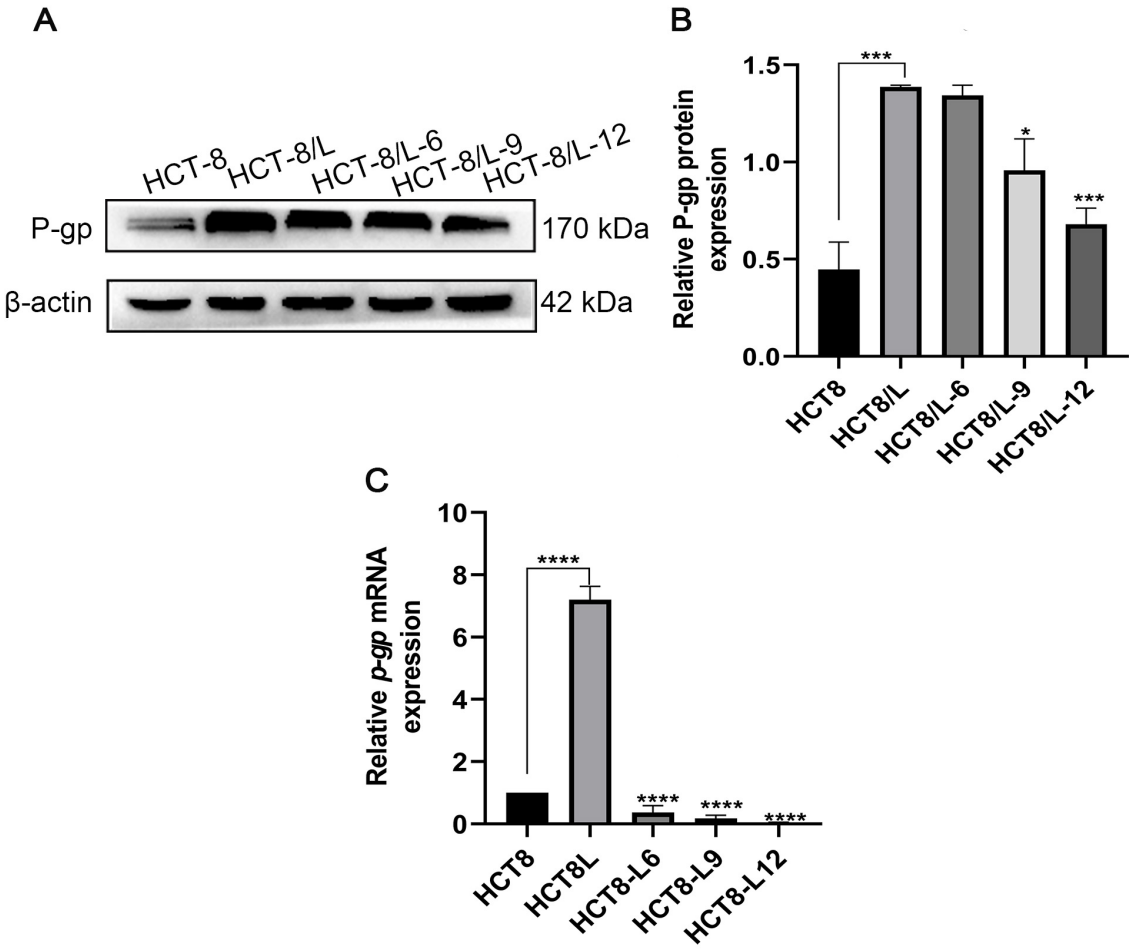

Fig. 2. Effect of Huaier on the expression of *p-gp*. (A) Western blotting results of *p-gp* in HCT-8 and HCT-8/L cells under the effect of different concentrations of Huaier. (B) Effect of Huaier on the relative quantification protein expressions of *p-gp*. (C) On the gene level, the change of *p-gp* and the change of the expression level of *p-gp* in HCT-8/L cells under the effect of different concentrations of Huaier. Data means ± SEM, two-tailed Student's *t*-test, n ≥3. *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$.

HCT-8/L treated with Huaier against oxaliplatin was 3.11 μg/mL, which was less than that of 7.58 μg/mL untreated with Huaier. The results indicated that Huaier inhibited β-catenin entry into the nucleus by affecting the Wnt/β-catenin signalling pathway to reverse drug resistance of HCT-8/L cells.

4. Discussion

Colorectal cancer is the third most common malignant tumour [1]. Currently, OXA is the first-line chemotherapy for its treatment, and patients with CRC also frequently develop OXA resistance. However, the molecular mechanism of OXA resistance is still unclear. At present, with the continuous increase in research on traditional Chinese medicine (TCM), more TCM methods have been reported to have the effects of treating cancer and reversing the drug resistance of tumours.

Huaier plays an anti-tumour role in the treatment of many cancers [12,16], and studies have indicated that Huaier inhibits tumour cell proliferation and invasion [17]. Moreover, Huaier extract could prevent pancreatic cancer through inhibition of the Wnt/β-catenin signalling pathway [13]. Under the action of Huaier, the *p-gp* of hepatoma cells has been shown to decrease, and the sensitivity of hepatoma cells to OXA has been shown to increase [18]. In the present study, the human CRC cell line HCT-8 and the OXA-resistant cell line HCT8/L were used as the research objects to analyse the changes in their proliferative activity and drug resistance before and after intervention with Huaier. The results also revealed that Huaier inhibited the proliferation of tumour cells and drug-resistant cells. Furthermore, an increase in Huaier concentration had an inhibitory effect on cell growth. Based on the WB and qRT-PCR assay results, it can be concluded that the *p-gp* expression level in drug-resistant strains is significantly higher than that in sensitive strains and that it gradually decreases with an increase in the concentration of Huaier. These results suggest that Huaier reduces drug resistance in drug-resistant cells.

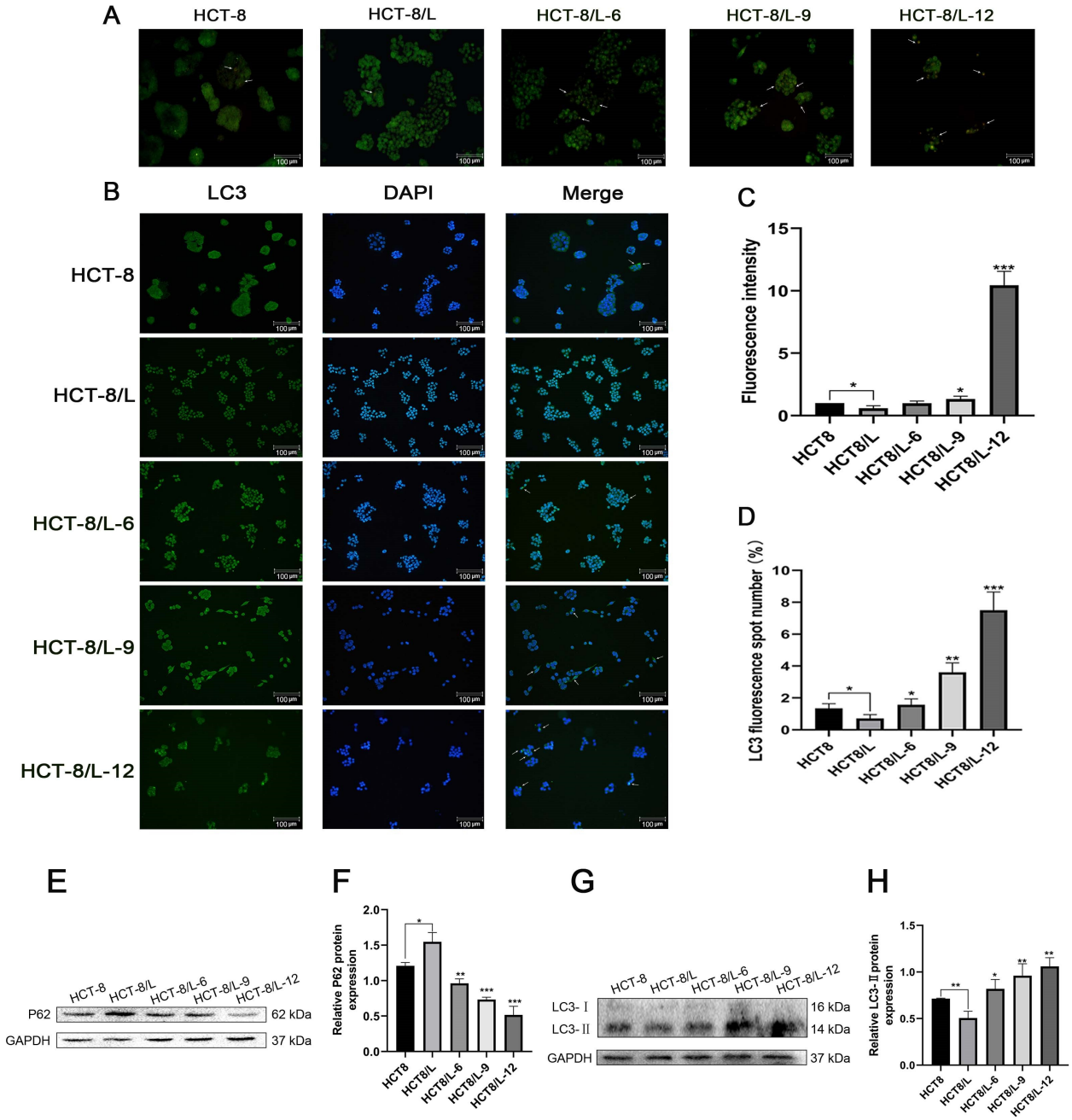

Fig. 3. Effect of Huaier on cell autophagy. (A,C) Acridine orange staining was used to observe the changes of acidic vesicular organelles induced by Huaier (white arrow: acidic vesicular organelles). (B,D) Detection of Huaier-induced autophagy via immunofluorescence staining. (E,F) Western blotting for P62 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) treated with Huaier. (G,H) Western blotting for LC3-II and GAPDH in HCT-8 and HCT-8/L cells treated with Huaier. Data means ± SEM, two-tailed Student's *t*-test, $n \geq 3$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Wei *et al.* [19] reported HES as one of the effective anti-tumour components of Huaier, stating that it can induce apoptosis and autophagy in gastric cancer cells. At the same time, the authors found that mammalian target of rapamycin signalling and ERK signalling may be involved in the anti-gastric cancer effect of hydroxyethyl starch. Zhang *et al.* [20] reported that the use of Huaier in patients with sorafenib-resistant liver cancer significantly enhanced the sensitivity of liver cancer cells to sorafenib. Autophagy plays a two-way role in tumour development [21], and an increasing number of experiments demonstrate that autophagy is related to tumour cell apoptosis and multidrug resistance [22,23]. The present study examined autophagy induced by Huaier through AO staining, immunofluores-

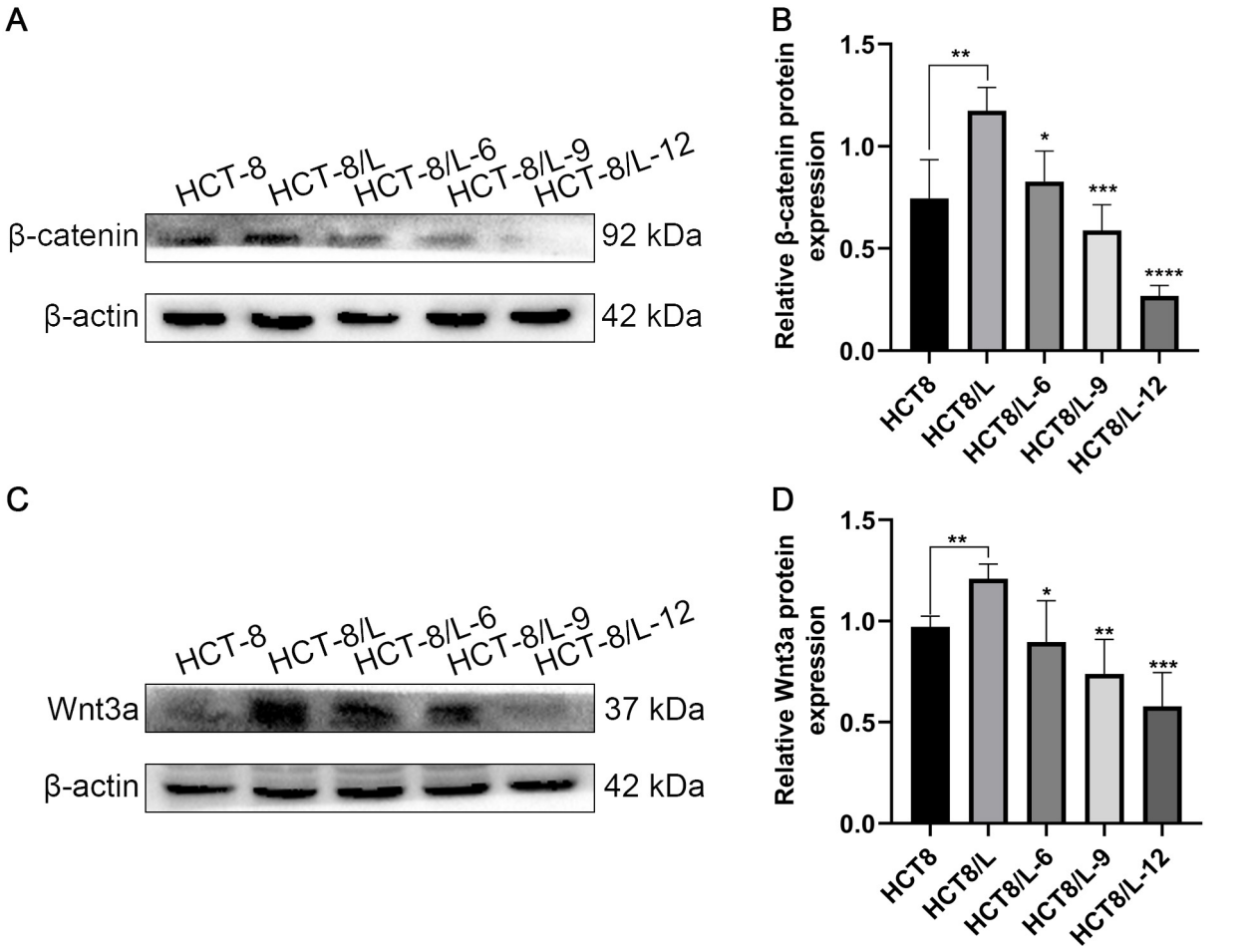

Fig. 4. Effect of Huaier on the Wnt/β-catenin signalling pathway. (A,B) Western blotting for β-catenin and β-actin treated with Huaier. (C,D) Western blotting for Wnt3a and β-actin treated with Huaier. Data means $\pm$ SEM, two-tailed Student's *t*-test, $n \geq 3$. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

cence staining and WB. The results suggested that the level of autophagy increased with an increase in Huaier concentration; this was mainly indicated by the dark colour in AO staining, higher number of autophagic spots in IF staining, higher expression of LC3-II and lower expression of P62 in WB.

Furthermore, it was discovered that under the effect of Huaier, the Wnt3a and β-catenin expression levels in drug-resistant strains decreased significantly, suggesting that Huaier can inhibit the Wnt/β-catenin signalling pathway. Some studies have reported that Wnt/β-catenin inhibits autophagy and p62 expression [24]. Moreover, through the WB assay, it can be concluded that the Wnt/β-catenin signalling pathway is activated in drug resistant strains; specifically, the Wnt3a and β-catenin expression levels are higher in drug-resistant strains than in sensitive strains.

Zou *et al.* [25] found that Huaier inhibited DSS-induced intestinal tumour production in a mouse model by inhibiting pro-inflammatory cytokine levels and signal transducer and activator of transcription 3 stimulation. In this study, autophagy inhibitors (CQ) and pathway agonists (Wnt agonist 1) were used to test whether Huaier can reverse cell resistance by inhibiting the Wnt/β-catenin signalling pathway and upregulating autophagy compared with CQ alone; the combination of CQ and Huaier significantly increased the autophagy level and decreased the *p-gp* expression. When the Wnt agonist 1 was added, the autophagy level of the cells decreased, and the *p-gp* expression level increased, whereas the autophagy level of cells increased, and the *p-gp* expression level decreased when the Wnt agonist 1 was combined with Huaier. Therefore, Huaier can reverse drug resistance by inhibiting the Wnt/β-catenin signalling pathway and enhancing autophagy.

However, there are still some limitations to the present study. Firstly, only *in vitro* studies were performed, and to further confirm the experimental results, subsequent *in vivo* studies will be performed on experimental animals. Secondly, this study found that Huaier could affect colon cancer cells by affecting the Wnt/β-catenin signalling pathway; however, the specific mechanism remains to be explored. Finally, there is a lack of clinical exploration and application.

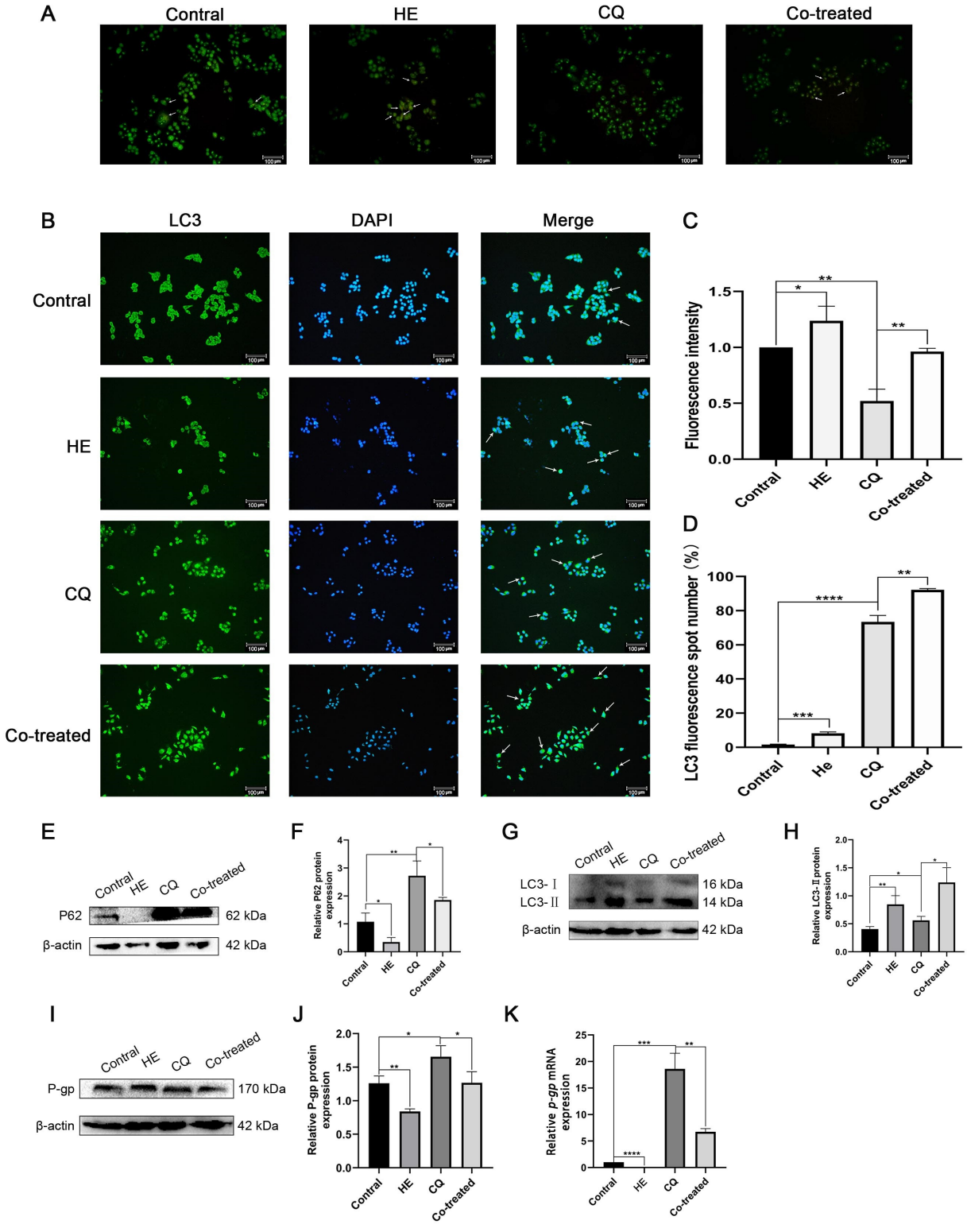

Fig. 5. Effect of Huaier and CQ on HCT-8/L. (A,C) Acridine orange staining was used to observe the changes of acidic vesicular organelles in HCT-8/L cells induced by Huaier and CQ. Control group. (B,D) Detection of Huaier and CQ induced autophagy by immunofluorescence staining. (E,F) Western blotting for P62 and β-actin in HCT-8/L cells treated with Huaier and CQ. (G,H) Western blotting for LC3-II and β-actin in HCT-8/L cells treated with Huaier and CQ. (I,J) Western blotting for *p-gp* in HCT-8/L cells treated with Huaier and CQ. (K) A qRT-PCR for *p-gp* mRNA in HCT-8/L cells treated with Huaier and CQ. CQ, chloroquine. Data means $\pm$ SEM, two-tailed Student's *t*-test, $n \geq 3$. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

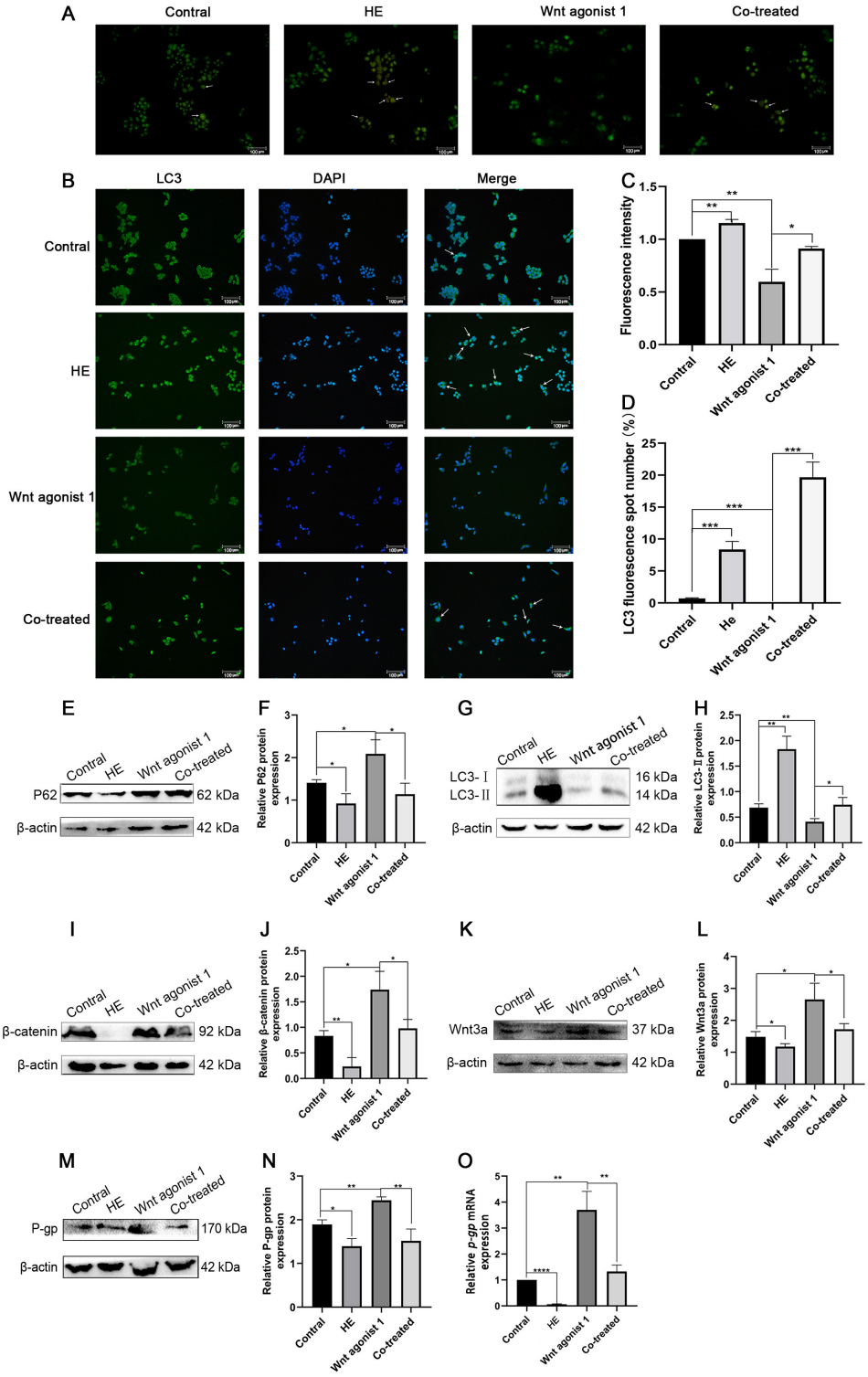

Fig. 6. Effect of Huaier and Wnt agonist 1 on HCT-8/L. (A,C) AO staining was used to observe the changes of acidic vesicular organelles in HCT-8/L cells induced by Huaier and Wnt agonist 1. (B,D) Detection of autophagy induced by Huaier and Wnt agonist 1 via immunofluorescence staining. (E,F) Western blotting for P62 and β-actin in HCT-8/L cells treated with Huaier and Wnt agonist 1. (G,H) Western blotting for LC3-II and β-actin in HCT-8/L cells treated with Huaier and Wnt agonist 1. (I,J) Western blotting for β-catenin and β-actin in HCT-8/L cells treated with Huaier and Wnt agonist 1. (K,L) Western blotting for Wnt3a and β-actin in HCT-8/L cells treated with Huaier and Wnt agonist 1. (M,N) Western blotting for *p-gp* in HCT-8/L cells treated with Huaier and Wnt agonist 1. (O) A qRT-PCR for *p-gp* mRNA in HCT-8/L cells treated with Huaier and Wnt agonist 1. CQ, chloroquine. Data means $\pm$ SEM, two-tailed Student's *t*-test, $n \geq 3$. *$p < 0.015$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

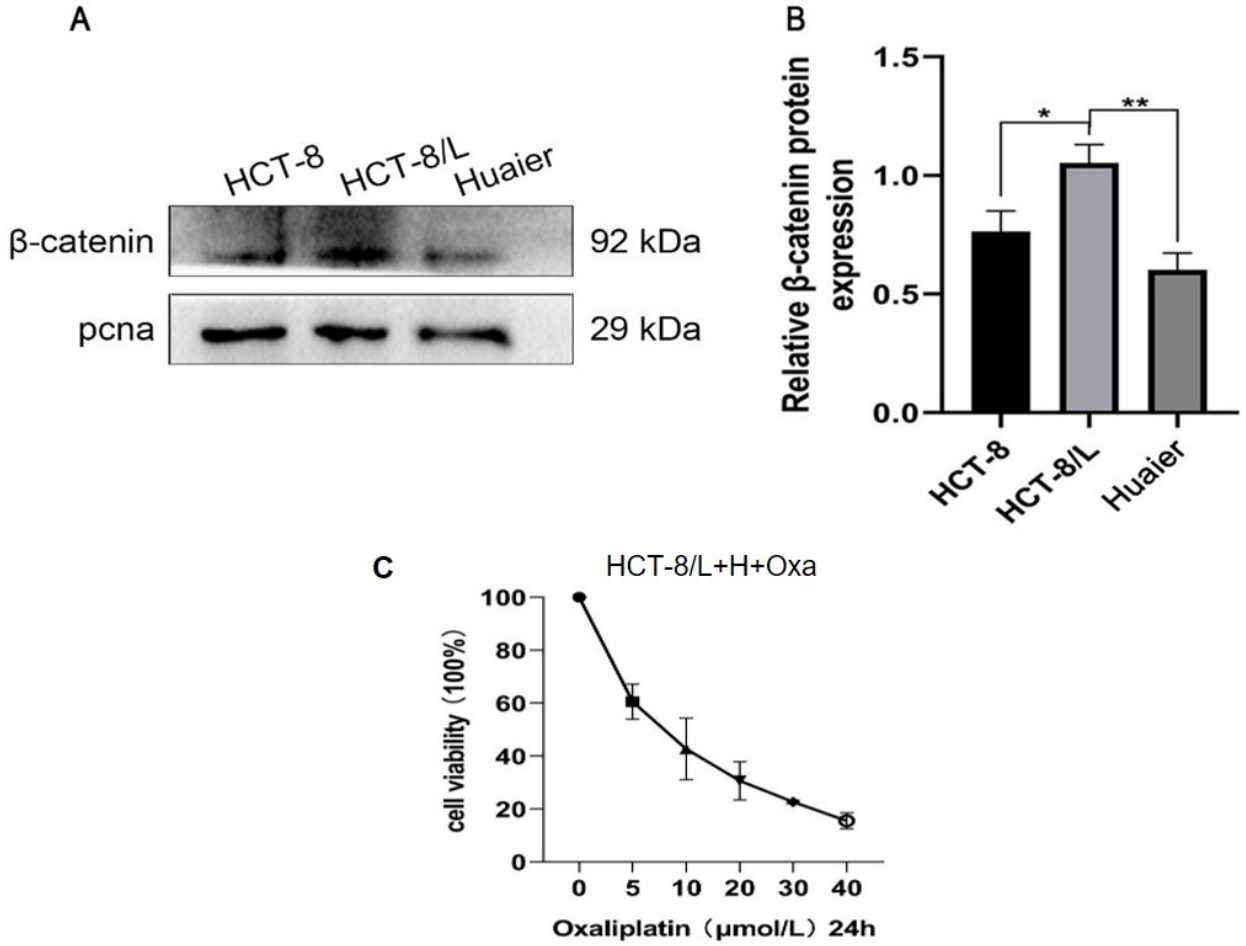

Fig. 7. Effect of Huaier on the entry of β-catenin into the nucleus. (A,B) Western blotting for β-catenin in the nucleus of HCT-8/L cells treated with Huaier. (C) Effect of Oxaliplatin on the activity of HCT-8/L cells after Huaier treatment. Data means ± SEM, two-tailed Student's *t*-test, n ≥3. *$p < 0.05$, **$p < 0.01$.

5. Conclusions

In conclusion, the present study proves that Huaier can regulate the level of autophagy, inhibit the Wnt/β-catenin signalling pathway and reverse the drug resistance of OXA-resistant CRC cells. The present study provides evidence that Huaier is a new drug-resistant therapy for CRC.

Availability of Data and Materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

Conception and design of the work: LWW, XLY; Data collection: QF, MYH; Supervision: QF; Analysis and interpretation of the data: QF, MYH; Statistical analysis: JNZ; Drafting the manuscript: QF, MYH, JNZ; Critical revision of the manuscript: LWW, XLY; Approval of the final manuscript: all authors. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

The present study was supported by the Project Funding of Hebei Provincial Education Department (grant no. ZD2020137), the Key Discipline Construction Project of Hebei Provincial Universities (grant no. Ji Jiao Gao-2013-(4)-2012-37) and basic scientific research business expenses of Chengde Medical College for outstanding students.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2901015.

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