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AIMS AND SCOPE

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WJGO mainly publishes articles reporting research results and findings obtained in the field of gastrointestinal oncology and covering a wide range of topics including liver cell adenoma, gastric neoplasms, appendiceal neoplasms, biliary tract neoplasms, hepatocellular carcinoma, pancreatic carcinoma, cecal neoplasms, colonic neoplasms, colorectal neoplasms, duodenal neoplasms, esophageal neoplasms, gallbladder neoplasms, *etc*.

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ORIGINAL ARTICLE

Basic Study

Deltonin enhances gastric carcinoma cell apoptosis and chemosensitivity to cisplatin *via* inhibiting PI3K/AKT/mTOR and MAPK signaling

Lin Yang, Ya-Nan Liu, Yi Gu, Qi Guo

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Abstract

BACKGROUND

As an active ingredient derived from *Dioscorea zingiberensis* C.H. Wright, deltonin has been reported to show anti-cancer effects in a variety of malignancies.

AIM

To investigate the role and mechanism of action of deltonin in promoting gastric carcinoma (GC) cell apoptosis and chemosensitivity to cisplatin.

METHODS

The GC cell lines AGS, HGC-27, and MKN-45 were treated with deltonin and then subjected to flow cytometry and 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium bromide assays for cell apoptosis and viability determination. Western blot analysis was conducted to examine alterations in the expression of apoptosis-related proteins (Bax, Bid, Bad, and Fas), DNA repair-associated proteins (Rad51 and MDM2), and phosphatidylinositol 3-kinase/protein kinase B/mammalian target of the rapamycin (PI3K/AKT/mTOR) and p38-mitogen-activated protein kinase (MAPK) axis proteins. Additionally, the influence of deltonin on GC cell chemosensitivity to cisplatin was evaluated both *in vitro* and *in vivo*.

RESULTS
Deltonin treatment weakened viability, enhanced apoptosis, and dampened DNA repair in GC cell lines in a dose-dependent pattern. Furthermore, deltonin mitigated PI3K, AKT, mTOR, and p38-MAPK phosphorylation. HS-173, an inhibitor of PI3K, attenuated GC cell viability and abolished deltonin inhibition of GC cell viability and PI3K/AKT/mTOR and p38-MAPK pathway activation. Deltonin also promoted the chemosensitivity of GC cells to cisplatin *via* repressing GC cell proliferation and growth and accelerating apoptosis.

CONCLUSION
Deltonin can boost the chemosensitivity of GC cells to cisplatin *via* inactivating p38-MAPK and PI3K/AKT/mTOR signaling.

Key Words: Deltonin; Gastric carcinoma; Cisplatin; Apoptosis; Chemotherapy; Axis

Core Tip: Chemoradiotherapy is currently the mainstay of clinical treatment for advanced gastric carcinoma (GC). However, chemoradiotherapy is difficult to achieve the desired results due to the challenges of early diagnosis of GC and the characteristics of distant metastasis and drug resistance. This study attempted to enhance the efficacy of GC clinical treatment from a pharmacological mechanism perspective.

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INTRODUCTION

Gastric carcinoma (GC) is a digestive tract malignancy prevalent worldwide, ranking second in cancer-related deaths[1]. Currently, it is still associated with a high incidence and mortality rate in developing countries[2]. There are several risk factors for GC, including diet patterns, smoking and drinking, family/genetic history, and *Helicobacter pylori* infection[3-5]. At present, chemoradiotherapy is the main clinical treatment for advanced GC. However, owing to the challenges in the early diagnosis of GC and the features of distant metastasis and drug resistance in the advanced stage, it is difficult for radiotherapy to achieve the expected results[6]. This experiment attempted to enhance the efficacy of GC clinical treatment from the perspective of drug mechanism.

Deltonin, an active ingredient in traditional Chinese medicine, is derived from *Dioscorea zingiberensis* C.H. Wright, and shows anti-cancer effects on many malignancies like colon cancer and breast cancer[7]. For instance, deltonin activates autophagy through the protein kinase B/mammalian target of the rapamycin (AKT/mTOR) axis and prevents FaDu, a head and neck squamous cell carcinoma cell line, from proliferating through cell cycle arrest and apoptosis induction, thus boosting cell apoptosis[8]. Moreover, through reactive oxygen species (ROS)-mediated mitochondrial disorders and extracellular signal-regulated kinase/AKT axis, deltonin restrains human breast carcinoma cell proliferation and promotes cell apoptosis[9]. Although previous studies have demonstrated that deltonin functions in most cancers, there are few studies on its role in GC cells and the relevant mechanisms.

The phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR signaling pathway is activated in multiple tumors and regulates various processes such as tumor cell growth, apoptosis, migration, invasiveness, autophagy, and survival[10]. Currently, this signaling pathway is deemed to be a crucial therapeutic target for tumors. Some studies have verified that apigenin inhibits the PI3K/AKT/mTOR axis to suppress liver cancer cell proliferation, thus eliciting autophagy in liver cancer cells and facilitating cell apoptosis[11]. Diallyl disulfide inhibits the PI3K/AKT/mTOR signaling pathway to elicit G2/M phase arrest of human osteosarcoma cells, as well as their apoptosis and autophagic death[12]. p38 mitogen-activated protein kinases (p38-MAPK), as a type of serine/threonine MAPK, participate in the signaling cascades of cytokines and stress cell responses and influence the occurrence, metastasis, and drug resistance of tumor cells[13,14]. For instance, diosgenin suppresses ovarian cancer cell activity by modulating the PI3K/AKT/p38-MAPK axis-associated protein profiles[15]. Another example is inotilone, which inhibits lung carcinoma cell migration and invasiveness through the ROS-mediated PI3K/AKT/p38-MAPK axis[16]. Thus, both p38-MAPK and PI3K/AKT/mTOR signals play essential regulatory roles in multiple malignancies. Nevertheless, whether deltonin influences drug resistance and disease progression in GC *via* the two signaling pathways still needs further investigation.

This study aimed at investigating the underlying anti-tumor function of deltonin in GC cells. Our experiments revealed that deltonin boosted cell apoptosis and improved the chemosensitivity of GC cells to cisplatin. Furthermore, deltonin inhibited PI3K/AKT/mTOR and p38-MAPK signaling pathway activation. Thus, our work provides a new therapeutic avenue to explore novel drugs for patients with GC undergoing end-stage chemotherapy.

MATERIALS AND METHODS

Cell culture

The culture medium of GC (AGS, HGC-27, and MKN-45) and human gastric epithelial (GES-1) cell lines, all from the Chinese Academy of Sciences, Shanghai, China, was RPMI1640 medium (Thermo Fisher Scientific, MA, United States) + 1% penicillin/streptomycin (Thermo Fisher Scientific) + 10% fetal bovine serum (FBS; Invitrogen, CA, United States), and the culture condition was 37 °C and 5% CO_2. Cells in logarithmic growth phase were trypsinized using 0.25% trypsin (Thermo Fisher HyClone, United States) and then harvested through centrifugation at 170 *g* for 5 min.

Cell treatment

The three GC cell lines were treated with cisplatin (Cat. No. 15663-27-1, Sigma-Aldrich, United States; 5 μg/mL)[17], deltonin (Cat. No. HYN2283, MedChemExpress; 0, 0.625, 1.25, 2.5, 5, 10, and 20 μM)[9,18], and/or HS-173 (a PI3K inhibitor; Cat. No. HY-15868, MedChemExpress; 1 μM)[19], or 740 Y-P (a PI3K activator; Cat. No. HY-P0175, MedChemExpress; 20 μM)[20]. Thereafter, the cells were harvested in preparation for the following experiments.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

The three GC cell lines in logarithmic growth phase were inoculated into 96-well plates (4×10^3 cells/well, 100 μL) and incubated for 24 h under conditions of 100% humidity, 37 °C, and 5% CO_2 in air. They were then treated with cisplatin, deltonin, and/or the PI3K inhibitor HS-173; the control group was treated with phosphate buffered saline (PBS) of the same volume. Each group contained five replicates. Cells were immersed in 50 μL of 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium bromide (MTT) (5 g/L) (Beyotime Biotechnology, Shanghai, China) after 24-h culture, and the supernatant was aspirated following 4-h incubation at 37 °C. The cells were treated with DMSO at 150 μL per well, and then placed on a plate shaker. Ultimately, a microplate reader was used to examine each well's OD value at 450 nm at 24, 48, and 72 h.

Western blot analysis

After cell treatment mentioned in section 2.2 and cultivation in 6-well plates, the cells were subjected to two PBS washes and 30 min of lysis in 200 μL RIPA (Beyotime Biotechnology, Shanghai, China). Thereafter, the lysates were collected for a 15-min centrifugation at 14000 rpm to obtain total protein. Protein concentrations were measured using Bradford dye (Bio-Rad). Following 2 h of separation on a polyacrylamide gel by electrophoresis at a voltage maintained at 100 V, the protein samples were electroblotted onto polyvinylidene fluoride membranes (Millipore, Bedford, MA, United States). They were then blocked with 5% nonfat-dried milk for 1 h at room temperature (RT), followed by three 10-min Tris-buffered saline with 0.1% Tween® 20 detergent (TBST) rinses and overnight incubation at 4 °C with primary antibodies at 1:1000 dilution that were procured from Abcam (MA, United States): Anti-Bax (ab32503), anti-Bid (ab32060), anti-Bak (ab32371), anti-Fas (ab133619), anti-Rad51 (ab133534), anti-MDM2 (ab16895), anti-PI3K (ab32089), anti-mTOR (ab134903), anti-p-mTOR (ab137133), anti-p-PI3K (ab182651), anti-AKT (ab8805), anti-p-AKT (ab38449), anti-p38-MAPK (ab170099), anti-p-p38-MAPK (ab178867), and anti-β-actin (ab115777). Following TBST washes, the membranes were subjected to 1 h of RT incubation with horseradish peroxidase-labeled anti-rabbit secondary antibody (1:300 dilution). Thereafter, TBST was used to rinse the membranes again thrice (10-min rinses). Eventually, the membranes were imaged and the staining intensity was assessed using BeyoECL Plus (Beyotime Biotechnology, Shanghai, China) and ImageJ, respectively.

Flow cytometry

The human GC cell lines in logarithmic growth phase were harvested and prepared as single-cell suspensions for inoculation in a 25 cm^2 culture flask. Following adherent culture overnight, the original medium was replaced with fresh medium containing 0.3% FBS for the experimental group and a comparable volume of PBS medium for the control group, followed by 24 h of incubation with 5% CO_2 at 37 °C and cell supernatant collection. Thereafter, the cells were subjected to cold PBS flushing for 3 times, trypsinization using EDTA-free trypsin, and harvesting. Then, the cells were treated as instructed in the Annexin V-PI Apoptosis Detection Kit (Yeasen Biotech Co., Ltd.) protocol. Subsequently, flow cytometry was performed within 1 h for analyzing cell apoptosis.

In vivo experiments in nude mice

We acquired 12 female athymic BALB/c nude mice (6 wk old with a weight of 22-24 g) from Shandong University Experimental Animal Center (Jinan, China) and reared them under normal specific pathogen-free conditions (24 °C, 12-h/12-h light/dark regime, and free access to food and water). Then, AGS cells were administered hypodermically at 2×10^6 cells/0.1 mL PBS into mouse right back according to a previous study[21]. Seven days later, the animals were randomly distributed to one of the following groups: Sham (treated with normal saline *via* intraperitoneal injection), cisplatin (once every 3 d at 3 mg/kg, for 3 times)[22,23], deltonin (once every 3 d at 50 mg/kg, for 3 times)[8], and cisplatin (once every 3 d at 1.5 mg/kg, for 3 times) + deltonin (once every 3 d at 25 mg/kg, for 3 times). During the following 28 d after drug treatment, a caliper was used for measuring the tumor volume ($0.5 \times \text{length} \times \text{width}^2$) weekly. Four weeks later, the nude mice were sacrificed using 30 mg/kg phenobarbital sodium, and the tumor was resected and weighed. The animal experiments were approved by the Ethics Review Committee of the Second Affiliated Hospital of Soochow University (approval No. SZSH-2020-042), and were implemented strictly following the Declaration of Helsinki and the Regulations of the People's Republic of China on the Management of Laboratory Animals issued on October 31, 2017.

Immunofluorescence assay

Tumor tissue specimens were treated with 4% paraformaldehyde and then paraffin-embedded. Tumor sections were prepared (4 μm in thickness), dewaxed using gradient alcohol, and rehydrated. Following RT sealing with bovine serum albumin (5%) for half an hour, the sections were incubated with anti-p-PI3K/AKT/mTOR/p38 MAPK antibodies (ab191606, ab131443, ab109268, and ab38238) at RT for 1 h. After washing with PBS, they were incubated with the Cy3-(ab98416) or fluorescein isothiocyanate-labelled goat anti-rabbit secondary antibody (ab6717) for 60 min at RT. All antibodies were procured from Abcam. Following nuclei labeling with 4',6-diamidino-2-phenylindole (Beyotime Technology, Shanghai, China), a confocal immunofluorescence microscope (Leica LSM 800, Wetzlar, Germany) was used to visualize the images.

Statistical analysis

SPSS16.0 from SPSS Inc. (Chicago, IL, United States) was used for performing all statistical analyses, and $P < 0.05$ indicated statistical significance. Between-group differences were analyzed by unpaired, two-sided Student's *t*-tests, and multi-group differences were determined by one-way ANOVA followed by Tukey's post-hoc tests. All data are described as the mean ± SD.

RESULTS

Deltonin prevents GC cell proliferation and accelerates apoptosis

GES-1, AGS, HGC-27, and MKN-45 cells were all treated with 0-20 μM of deltonin for 24 h, after which their viability was examined using MTT assays. GC cell viability was observed to significantly decrease when the dose of deltonin exceeded 2.5 μM, while only 20 μM of deltonin exerted an inhibitory effect on GES-1 viability ($P < 0.05$ *vs* control, Figure 1A). The IC_{50} values were gauged for AGS, HGC-27, and MKN-45 cells following treatment with deltonin at different concentrations; the IC_{50} values were 3.487, 2.343, and 2.78 for AGS, HGC-27, and MKN-45 cells, respectively (Figure 1B). The GC cells were treated with 2.5 μM deltonin and then subjected to the MTT assay to examine cell viability at different time points. Deltonin inhibited GC cell viability in a time-dependent manner ($P < 0.05$ *vs* control, Figure 1C). Flow cytometry analysis revealed that deltonin treatment promoted cell apoptosis ($P < 0.05$ *vs* control, Figure 1D). And as indicated by Western blot analysis, deltonin treatment enhanced the protein levels of pro-apoptotic markers Bax, Bak, Bid, and Fas but reduced those of Rad51 and MDM2, which are associated with DNA repair processes ($P < 0.05$ *vs* control, Figure 1E). Western blot assays also indicated that deltonin (2.5 μM) treatment markedly lowered PI3K/AKT/mTOR and p38-MAPK protein levels in GC cells (including AGS and HGC-27), with the expression gradually decreasing with time (0, 24, 48, and 72 h) ($P < 0.05$ *vs* control, Figure 1F and G). Additionally, these proteins presented decreased expression in GC cells in a deltonin concentration-dependent manner (0, 2.5, 5, and 10 μM) ($P < 0.05$ *vs* control, Figure 1H and I). The above results demonstrated the ability of deltonin to exert an inhibitory effect on GC cell growth and enhance apoptosis while inactivating p38-MAPK and PI3K/AKT/mTOR axes in these cells.

Repressing PI3K/AKT/mTOR and p38-MAPK signaling suppresses deltonin-mediated anti-tumor effects

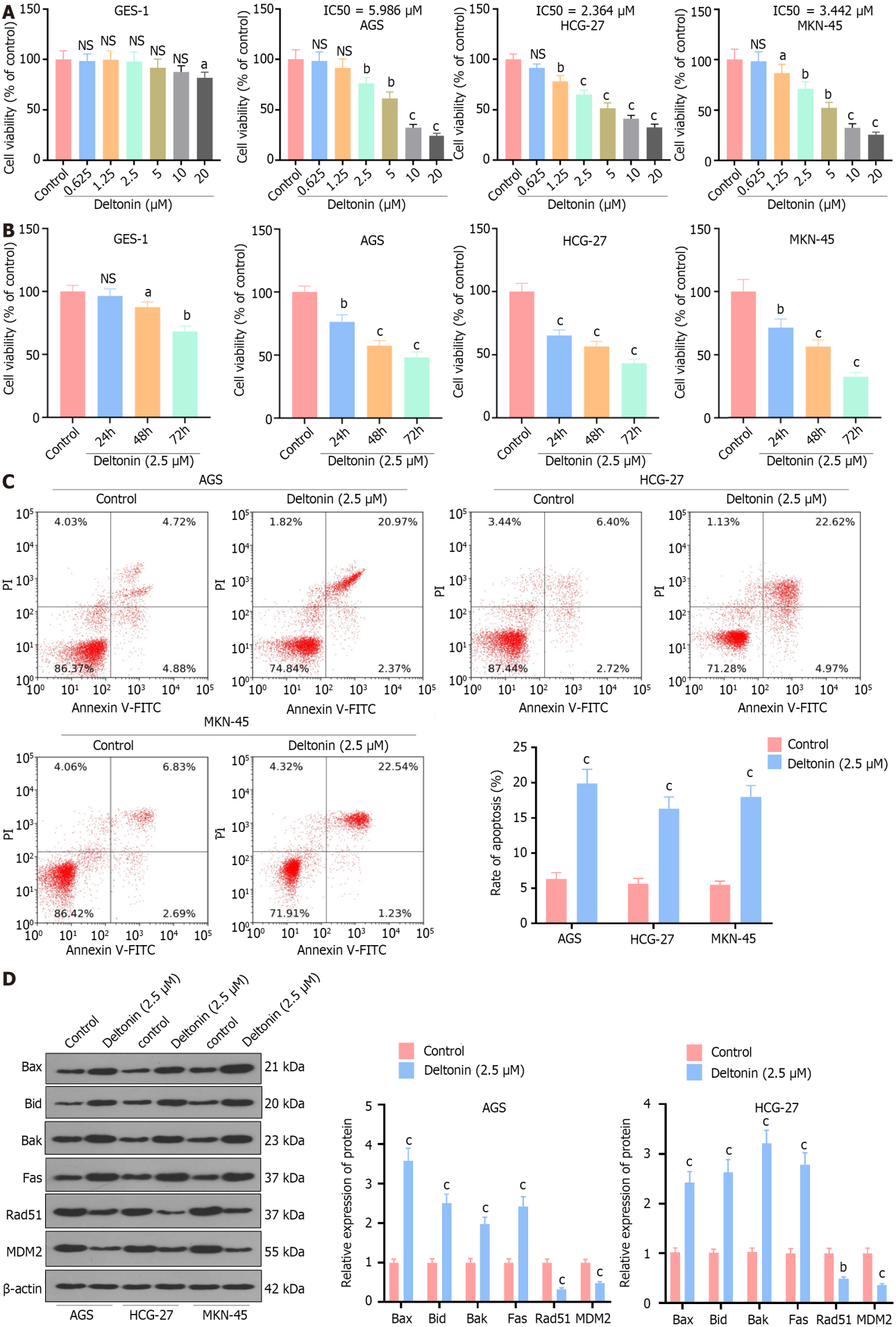
GC cells treated with deltonin (2.5 μM) and HS-173 (0.8 nM) showed remarkably lower viability compared to the control ($P < 0.05$, Figure 2A and B). Nevertheless, deltonin + HS-173 exerted no additional influence on cell viability compared to the HS-173 alone group ($P > 0.05$, Figure 2A and B). The determination of apoptosis-related protein profiles also determined that deltonin and HS-173 individually increased the expression of Bax, Bak, Bid, and Fas, whereas co-treatment with HS-173 and deltonin barely influenced their expression levels ($P < 0.05$, Figure 2C and D). Western blot analysis also showed that phosphorylated PI3K/AKT/mTOR and p38-MAPK protein levels were substantially reduced with deltonin or HS-173 treatment, whereas the administration of deltonin and HS-173 exerted no inhibitory effect on p38-MAPK and PI3K/AKT/mTOR axes (*vs* HS-173 group alone, $P > 0.05$, Figure 2E and F). Therefore, deltonin may repress GC cell viability by suppressing p38-MAPK and PI3K/AKT/mTOR signaling.

Impact of activating PI3K/AKT/mTOR and p38-MAPK signaling on deltonin-mediated effects

Next, we treated GC cells (AGS and HGC-27) with deltonin (2.5 μM) and the PI3K activator 740 Y-P (20 μM), and found that deltonin notably enhanced cell viability *vs* the control, wherein cell viability was inhibited by the addition of deltonin ($P < 0.05$, Figure 3A and B). Furthermore, Western blot analysis showed reduced expression of apoptosis-related proteins (Bax, Bak, Bid, and Fas) in the 740 Y-P group, while the deltonin + 740 Y-P group showed increased expression of these proteins in comparison to 740 Y-P alone treatment ($P < 0.05$, Figure 3C and D). Western blot analysis also indicated augmented PI3K, AKT, mTOR, and p38-MAPK phosphorylation in AGS and HGC-27 cells in the 740 Y-P group, whereas deltonin co-treatment suppressed such increased phosphorylation ($P < 0.05$ *vs* 740 Y-P group, Figure 3E and F). Together, these results suggest that activating PI3K/AKT/mTOR and p38-MAPK signaling may facilitate cell proliferation and weaken the anti-cancer effects of deltonin.

Deltonin enhances chemosensitivity of GC cells to cisplatin

AGS and HGC-27 cells were treated with 2.5 μM of deltonin or 5 μg/mL of cisplatin or cisplatin (2.5 μg/mL) + deltonin (1.25 μM). Treatment with cisplatin or deltonin considerably attenuated cell viability, whereas cisplatin + deltonin co-treatment reduced cell viability compared to the cisplatin alone group ($P < 0.05$, Figure 4A and B). According to flow cytometry analysis, the apoptosis of cisplatin- or deltonin-treated cells was dramatically increased compared to the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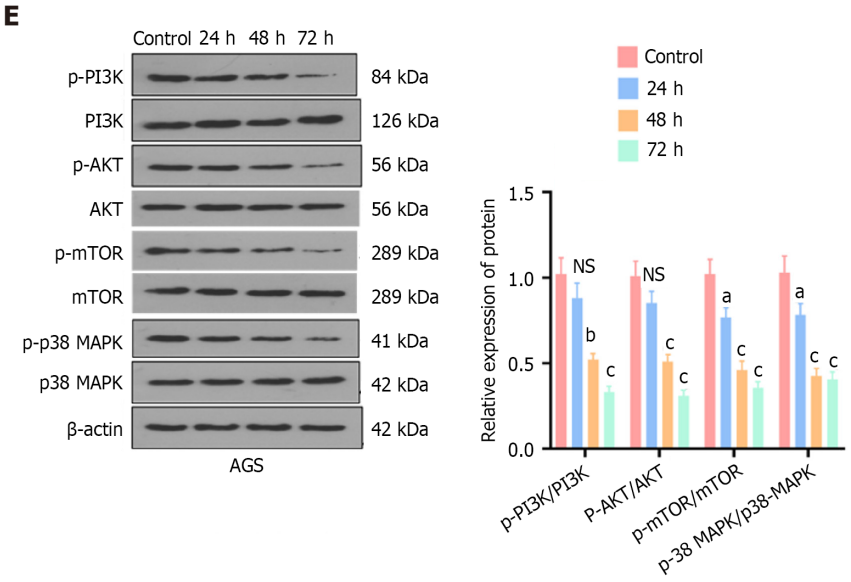
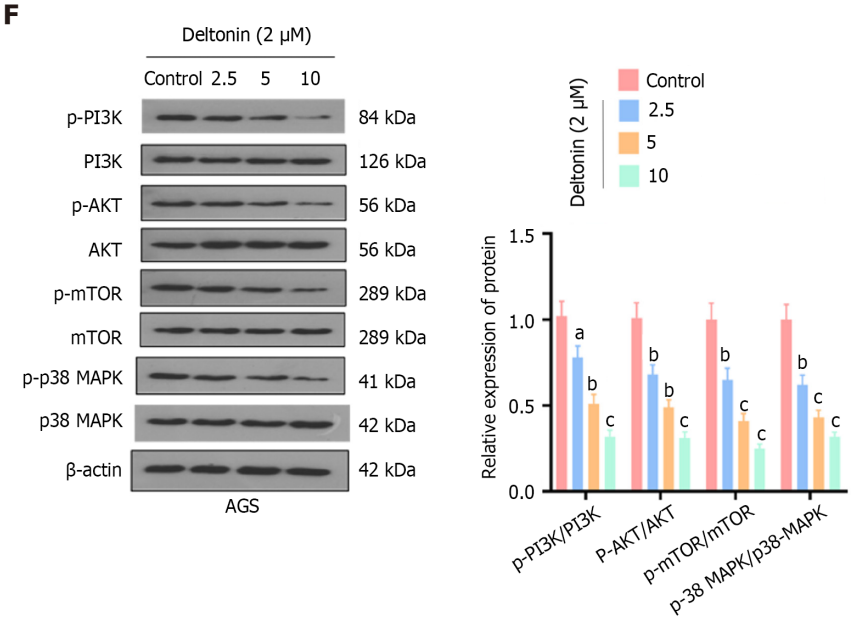
IC50 = 5.986 μM
AGS
IC50 = 2.364 μM
HCG-27
IC50 = 3.442 μM
MKN-45
Cell viability (% of control)
Control
0.625
1.25
2.5
5
10
20
Deltonin (μM)
B
GES-1
AGS
HCG-27
MKN-45
24h
48h
72h
Deltonin (2.5 μM)
C
AGS
HCG-27
MKN-45
Control
Deltonin (2.5 μM)
PI
Annexin V-FITC
4.03%
4.72%
86.37%
4.88%
1.82%
20.97%
74.84%
2.37%
3.44%
6.40%
87.44%
2.72%
1.13%
22.62%
71.28%
4.97%
4.06%
6.83%
86.42%
2.69%
4.32%
22.54%
71.91%
1.23%
Rate of apoptosis (%)
D
Bax
Bid
Bak
Fas
Rad51
MDM2
β-actin
21 kDa
20 kDa
23 kDa
37 kDa
37 kDa
55 kDa
42 kDa
Relative expression of protein


E
Control 24 h 48 h 72 h
p-PI3K
PI3K
p-AKT
AKT
p-mTOR
mTOR
p-p38 MAPK
p38 MAPK
β-actin
84 kDa
126 kDa
56 kDa
56 kDa
289 kDa
289 kDa
41 kDa
42 kDa
42 kDa
AGS
Control
24 h
48 h
72 h
Relative expression of protein
1.5
1.0
0.5
0.0
NS
NS
a
a
b
c
c
c
c
c
c
c
p-PI3K/PI3K
P-AKT/AKT
p-mTOR/mTOR
p-38 MAPK/p38-MAPK


F
Deltonin (2 μM)
Control 2.5 5 10
p-PI3K
PI3K
p-AKT
AKT
p-mTOR
mTOR
p-p38 MAPK
p38 MAPK
β-actin
84 kDa
126 kDa
56 kDa
56 kDa
289 kDa
289 kDa
41 kDa
42 kDa
42 kDa
AGS
Deltonin (2 μM)
Control
2.5
5
10
Relative expression of protein
1.5
1.0
0.5
0.0
a
b
c
b
b
c
c
b
c
c
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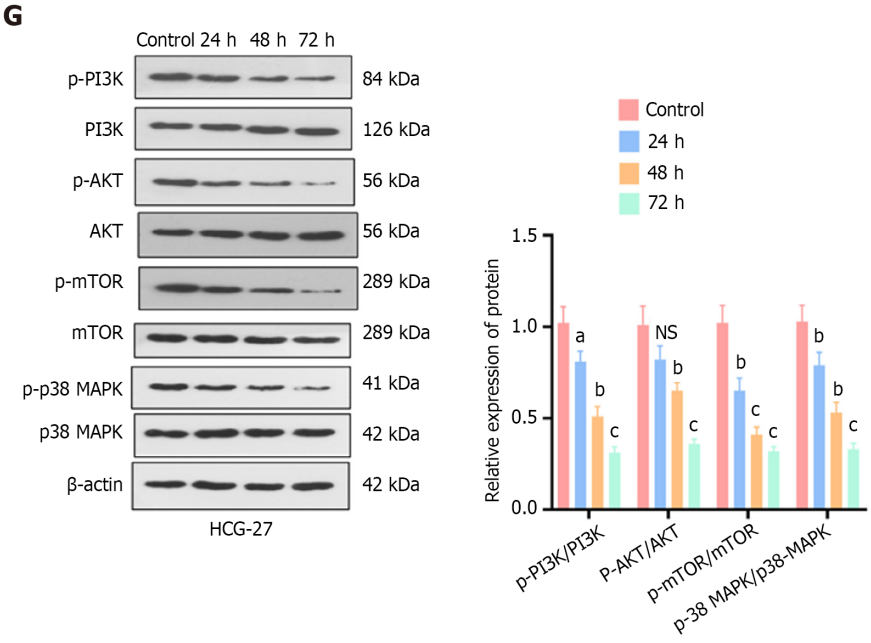
P-AKT/AKT
p-mTOR/mTOR
p-38 MAPK/p38-MAPK


G
Control 24 h 48 h 72 h
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PI3K
p-AKT
AKT
p-mTOR
mTOR
p-p38 MAPK
p38 MAPK
β-actin
84 kDa
126 kDa
56 kDa
56 kDa
289 kDa
289 kDa
41 kDa
42 kDa
42 kDa
HCG-27
Control
24 h
48 h
72 h
Relative expression of protein
1.5
1.0
0.5
0.0
a
b
c
NS
b
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b
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P-AKT/AKT
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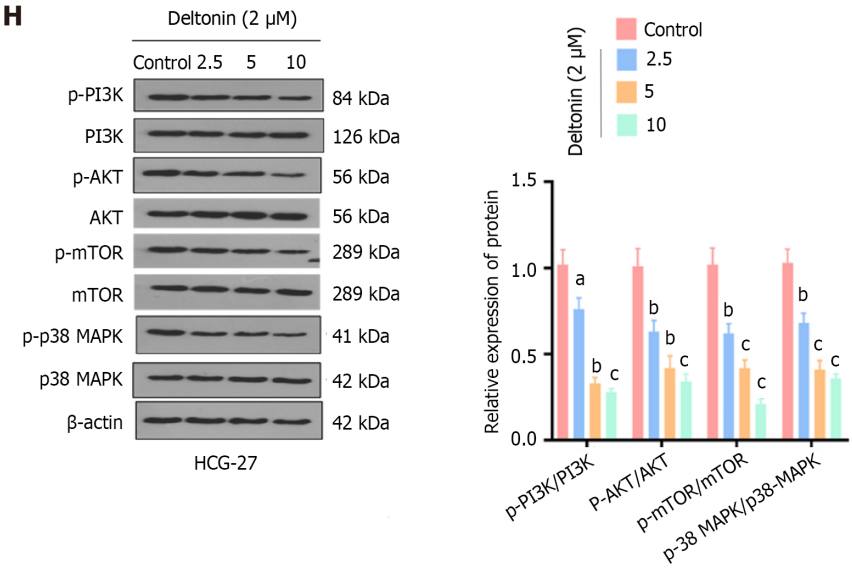

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Figure 1 Deltonin inhibits gastric carcinoma cell proliferation and expedites their apoptosis. A: Gastric carcinoma (GC) cell lines AGS, HGC-27, and MKN-45 were treated with deltonin (0 μM, 0.625 μM, 1.25 μM, 2.5 μM, 5 μM, 10 μM, and 20 μM) for 24 h. 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium bromide (MTT) assay was used to examine cell viability; B: IC_{50} values of AGS, HGC-27, and MKN-45 cells treated with deltonin of different concentrations; C: Flow cytometry analysis of apoptosis of AGS, HGC-27, and MKN-45 cells treated with 2.5 μM deltonin for 24 h; D: Western blot analysis of expression of apoptosis-concerned proteins (Bax, Bak, Bid, and Fas) and DNA repair-associated proteins (Rad51 and MDM2) in GC cells; E: Western blot analysis of protein expression in AGS cells treated with 2.5 μM of deltonin for 0 h, 24 h, 48 h, and 72 h; F: Western blot analysis of protein expression in AGS cells treated with deltonin (0, 2.5, 5, and 10 μM) for 24 h; G: Western blot analysis of protein expression in HGC-27 cells treated with 2.5 μM of deltonin for 0 h, 24 h, 48 h, and 72 h; H: Western blot analysis of protein expression in HGC-27 cells treated with deltonin (0, 2.5, 5, and 10 μM) for 24 h; NS: $P > 0.05$, $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ *vs* control group. $n = 3$. NS: No significance; PI3K: Phosphatidylinositol 3-kinase; AKT: Protein kinase B; mTOR: Mammalian target of the rapamycin; p38-MAPK: p38-mitogen-activated protein kinase.

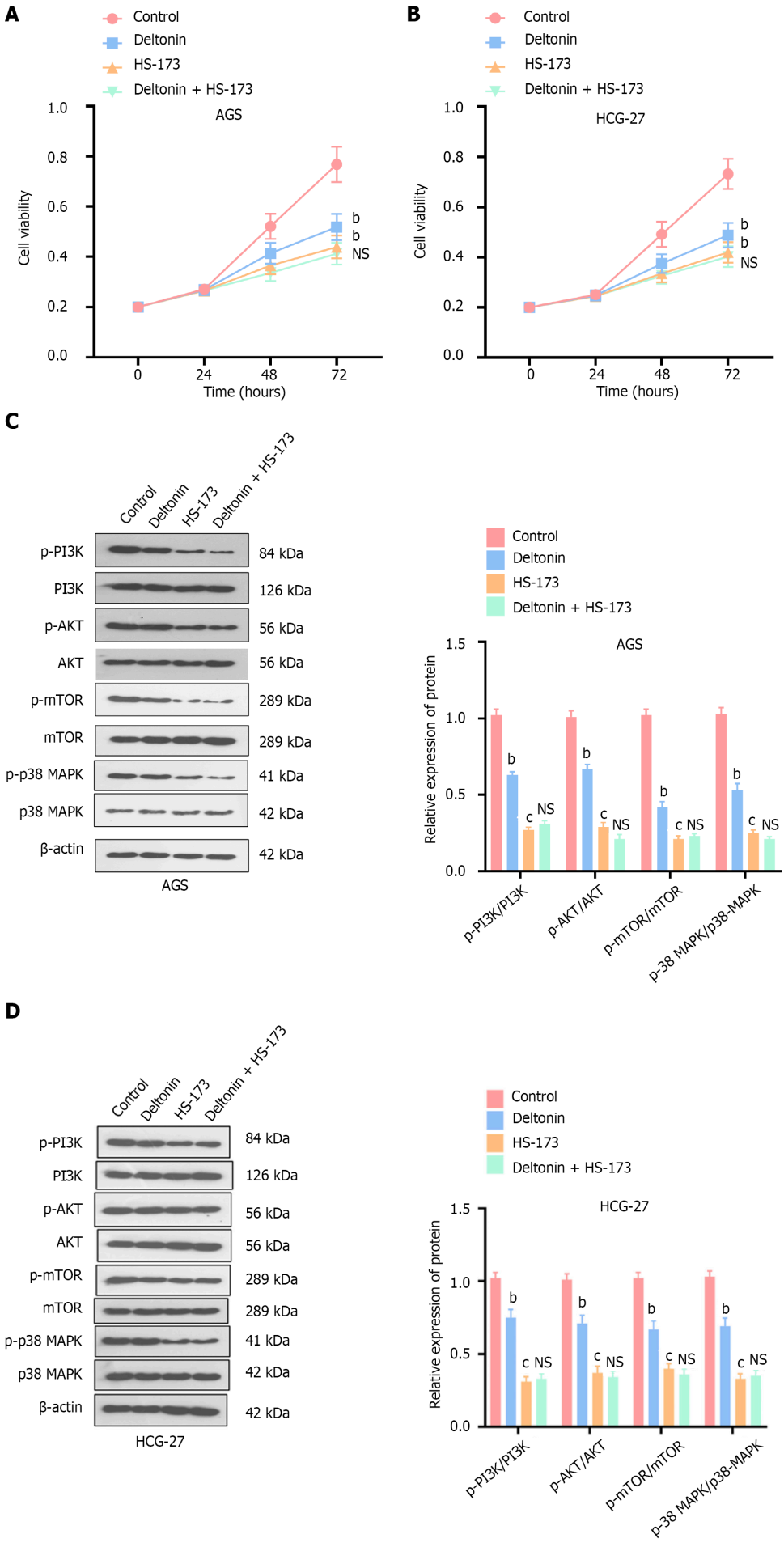
control ($P < 0.05$, Figure 4C and D), and it was further enhanced in the cisplatin + deltonin group ($P < 0.05$, Figure 4C and D, *vs* cisplatin group). Western blot analysis also showed elevated Bax and Bid and reduced Rad51 protein expression in cisplatin- or deltonin-treated cells *vs* the control. Moreover, Bax and Bid protein expression in the cisplatin + deltonin group was further increased, while Rad51 expression was considerably reduced in comparison to the expression levels in the cisplatin alone group ($P < 0.05$, Figure 4E and F). Based on the above findings, deltonin may exert a pro-apoptotic effect and promote the chemosensitivity of GC cells to cisplatin.

Deltonin increases chemosensitivity of GC cells to cisplatin in vivo through PI3K/AKT/mTOR and p38-MAPK signaling inhibition

To further verify the function and mechanism of deltonin in chemosensitivity of GC cells to cisplatin, we conducted *in vivo* experiments in nude mice. The tumor-bearing mice were intervened with saline, deltonin (50 mg/kg), cisplatin (3 mg/kg), or deltonin (25 mg/kg) + cisplatin (1.5 mg/kg). Treatment with deltonin or cisplatin both reduced tumor volume and weight compared to the sham group ($P < 0.05$, Figure 5A-C), but failed to reduce mouse body weight ($P > 0.05$, Figure 5D). Interestingly, the joint application of deltonin + cisplatin further mitigated the mouse tumor volume and weight in comparison to cisplatin treatment alone ($P < 0.01$, Figure 5A-C), but barely altered the body weight ($P > 0.05$, Figure 5D). We then carried out immunofluorescence assays to determine PI3K/AKT/mTOR and p38-MAPK phosphorylation levels in the tumor tissues. Both deltonin and cisplatin reduced the levels of phosphorylated p38-MAPK and PI3K/AKT/mTOR, and their combination further reduced the levels compared to the cisplatin alone group (Figure 5E-H). These findings suggest that deltonin enhances chemosensitivity of GC cells to cisplatin by suppressing p38-MAPK and PI3K/AKT/mTOR signaling activation (Figure 6).

DISCUSSION

GC is a prevalent internal gastrointestinal malignancy with a high clinical fatality rate[24]. The current methods are ineffective for early GC diagnosis, owing to which GC is often diagnosed at the end stage when it is accompanied by distant metastasis and chemotherapy resistance. Moreover, surgical treatment and drug chemotherapy display poor efficacy[25]. Cisplatin is a frequently used chemotherapy drug for many malignant tumor diseases and is also extensively adopted in the context of GC[26,27]. Regarding the primary mechanism of cisplatin in cancer treatment, it triggers DNA damage in tumor cells. Unfortunately, cisplatin treatment can easily contribute to the drug resistance of tumor cells and influence the function of chemotherapy[28]. Hence, probing the drug action mechanisms in GC has great clinical implications for its treatment. Here, we discovered that deltonin hinders p38-MAPK and PI3K/AKT/mTOR signaling


A
Control
Deltonin
HS-173
Deltonin + HS-173
AGS
Cell viability
1.0
0.8
0.6
0.4
0.2
0.0
0
24
48
72
Time (hours)
b
b
NS
B
Control
Deltonin
HS-173
Deltonin + HS-173
HCG-27
Cell viability
Time (hours)
b
b
NS
C
Control
Deltonin
HS-173
Deltonin + HS-173
p-PI3K
PI3K
p-AKT
AKT
p-mTOR
mTOR
p-p38 MAPK
p38 MAPK
β-actin
84 kDa
126 kDa
56 kDa
56 kDa
289 kDa
289 kDa
41 kDa
42 kDa
42 kDa
AGS
Control
Deltonin
HS-173
Deltonin + HS-173
AGS
Relative expression of protein
1.5
1.0
0.5
0.0
b
c
NS
p-PI3K/PI3K
p-AKT/AKT
p-mTOR/mTOR
p-38 MAPK/p38-MAPK
D
Control
Deltonin
HS-173
Deltonin + HS-173
p-PI3K
PI3K
p-AKT
AKT
p-mTOR
mTOR
p-p38 MAPK
p38 MAPK
β-actin
84 kDa
126 kDa
56 kDa
56 kDa
289 kDa
289 kDa
41 kDa
42 kDa
42 kDa
HCG-27
Control
Deltonin
HS-173
Deltonin + HS-173
HCG-27
Relative expression of protein
b
c
NS
p-PI3K/PI3K
p-AKT/AKT
p-mTOR/mTOR
p-38 MAPK/p38-MAPK

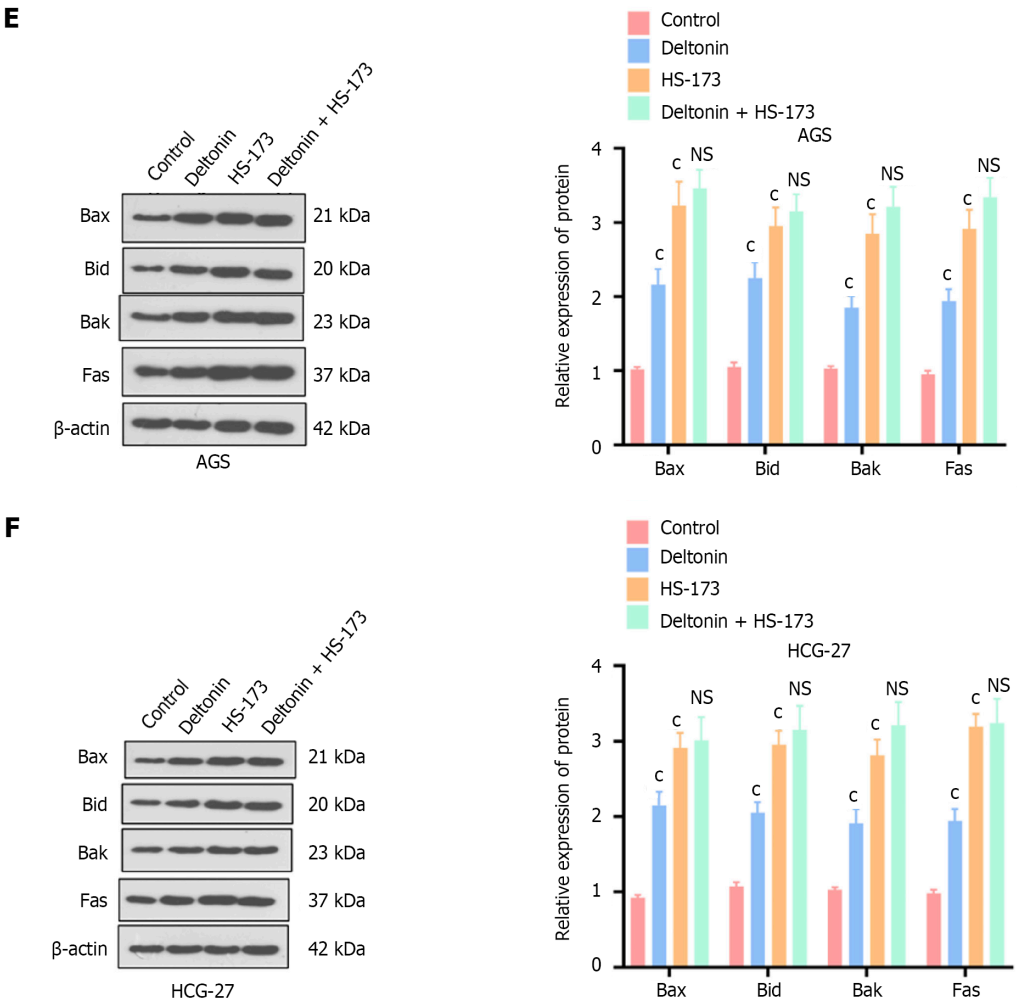


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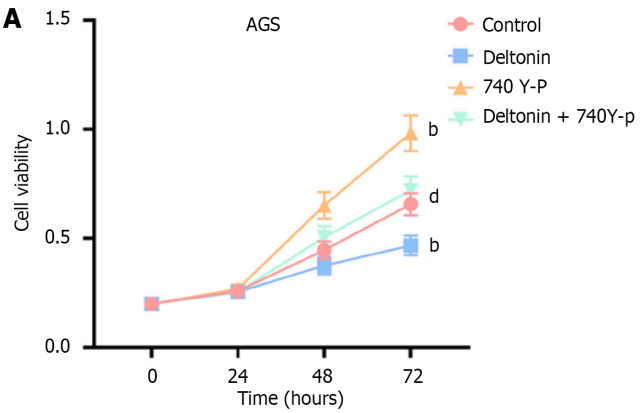
Figure 2 Deltonin attenuates gastric carcinoma cell viability by dampening the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of the rapamycin mitogen-activated protein kinase pathways. HGC-27 and AGS cells were treated with 2.5 μM of deltonin and/or 0.8 nM of HS-173 for 24 h. A and B: 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium bromide assay for cell viability examination; C and D: Western blot analysis of the profiles of apoptosis-correlated proteins; E and F: Western blot confirmation of the protein profiles of phosphatidylinositol 3-kinase/protein kinase B/mammalian target of the rapamycin p38-mitogen-activated protein kinase. $^{b}P < 0.01$, $^{c}P < 0.001$ *vs* control group; NS: $P > 0.05$ *vs* HS-173 group, $n = 3$. PI3K: Phosphatidylinositol 3-kinase; AKT: Protein kinase B; mTOR: Mammalian target of the rapamycin; p38-MAPK: p38-mitogen-activated protein kinase.

activation to boost GC cell apoptosis and promote their chemosensitivity to cisplatin.

Deltonin is known as an anti-tumor drug that curbs tumor cell angiogenesis to restrain tumor growth and facilitate apoptosis[29]. Deltonin inhibits AKT and p38-MAPK signaling pathway activation to further inhibit mouse colon cancer cell proliferation and bolster tumor cell apoptosis[18]. Furthermore, the intake of deltonin significantly suppresses colon cancer C26 cell proliferation in tumor-bearing mice, restricts tumor angiogenesis, and elicits cell apoptosis, thus prolonging the life cycle of the mice[30]. All the above studies confirm that deltonin enhances cancer cell apoptosis and represses cancer in a multitude of tumor diseases, which aligns with the observations in this study. Here, we demonstrated that deltonin considerably inhibits proliferation, boosts apoptosis, and dampens DNA repair in GC cells.

Chemotherapy is a prevailing method for GC, effectively extending patients' life. Cisplatin is a typical drug used in GC chemotherapy. Nonetheless, GC resistance is a leading contributor to chemotherapy failure[31,32]. Many studies have evaluated drug resistance in GC, including the most complicated molecular and drug mechanisms[33]. For instance, ten-eleven translocation-2 (TET2), a DNA demethylase, modulates interleukin (IL)-6 levels in the tumor microenvironment *via* histone acetylation, thus influencing cell resistance, and TET2 overexpression notably mitigates cisplatin resistance in GC cells[34]. Curcumin also augments the sensitivity of GC cells to adriamycin and other chemotherapy drugs by down-regulating the nuclear factor-kappaB (NF-κB) axis in human GC SGC-7901 cells and a downstream anti-apoptotic target gene of NF-κB[35]. Most of the prior studies have investigated the tolerance of chemotherapeutic drugs in GC from the aspect of molecular and drug mechanisms. Here, we unveiled that deltonin efficaciously augmented the chemosensitivity of GC cells to cisplatin and thereby boosted the anti-tumor function of cisplatin *via* eliciting apoptosis and DNA damage.

PI3K/AKT/mTOR and p38-MAPK signals were initially considered as factors that could regulate inflammation and immune response and affect inflammatory reactions, cell proliferation, differentiation, apoptosis, and other cellular processes[36,37]. Recent evidence has also demonstrated the pro-oncogenic functions of p38-MAPK and PI3K/AKT/mTOR in several tumors[38,39]. For instance, an *in vitro* experiment on GC cells has revealed that blocking PI3K/AKT/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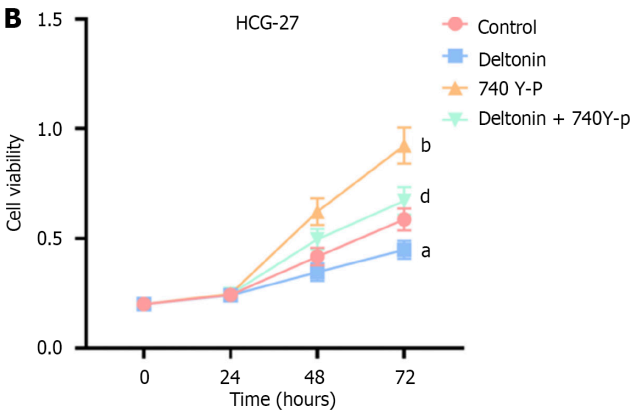
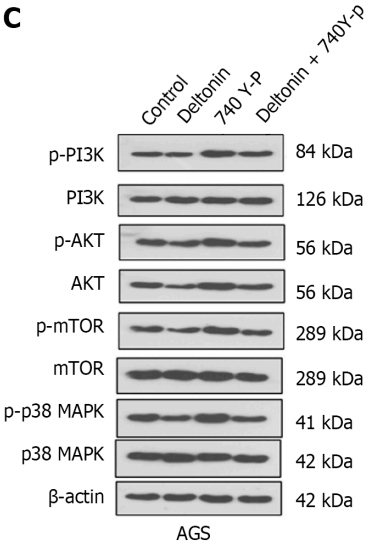
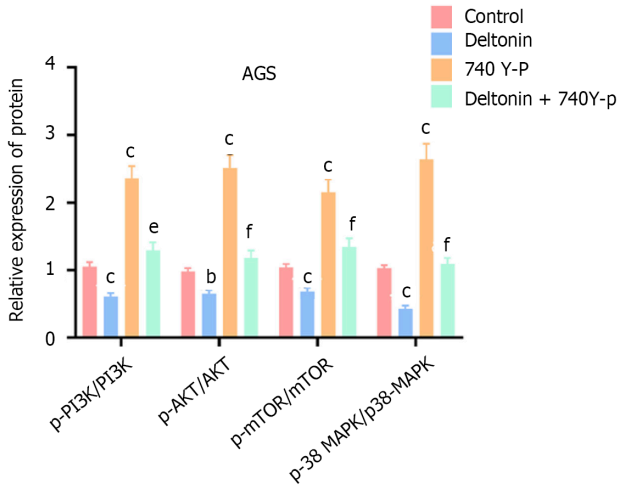
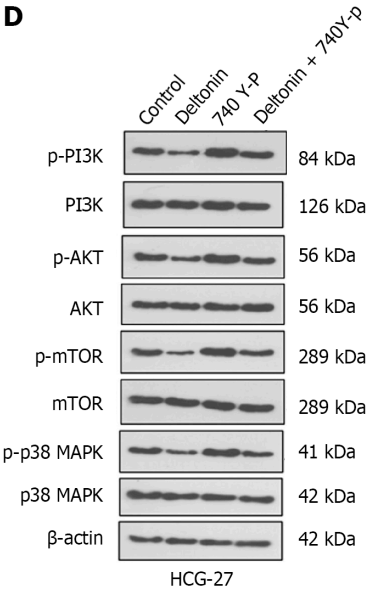
AGS
Control
Deltonin
740 Y-P
Deltonin + 740Y-p
Cell viability
Time (hours)
b
d
b


B
HCG-27
Control
Deltonin
740 Y-P
Deltonin + 740Y-p
Cell viability
Time (hours)
b
d
a


C
Control
Deltonin
740 Y-P
Deltonin + 740Y-p
p-PI3K
PI3K
p-AKT
AKT
p-mTOR
mTOR
p-p38 MAPK
p38 MAPK
β-actin
84 kDa
126 kDa
56 kDa
56 kDa
289 kDa
289 kDa
41 kDa
42 kDa
42 kDa
AGS


Control
Deltonin
740 Y-P
Deltonin + 740Y-p
AGS
Relative expression of protein
p-PI3K/PI3K
p-AKT/AKT
p-mTOR/mTOR
p-38 MAPK/p38-MAPK


D
Control
Deltonin
740 Y-P
Deltonin + 740Y-p
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PI3K
p-AKT
AKT
p-mTOR
mTOR
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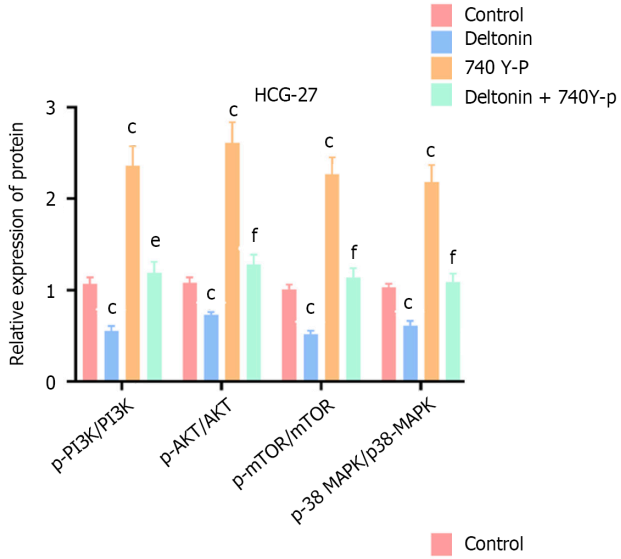
42 kDa
HCG-27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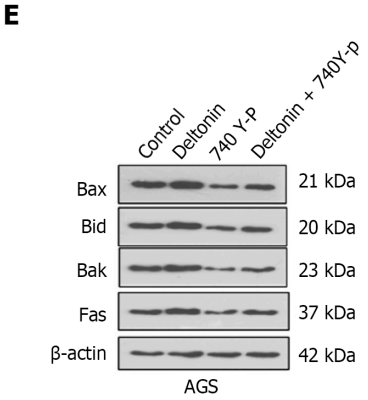
p-38 MAPK/p38-MAPK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Control
Deltonin
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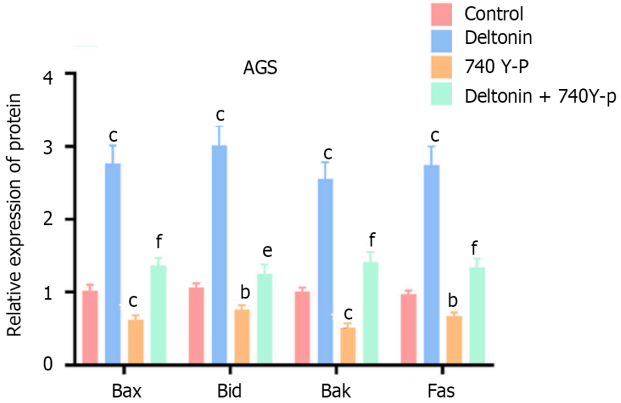
Deltonin + 740Y-p
Bax
Bid
Bak
Fas
β-actin
21 kDa
20 kDa
23 kDa
37 kDa
42 kDa
AGS


Control
Deltonin
740 Y-P
Deltonin + 740Y-p
AGS
Relative expression of protein
Bax
Bid
Bak
Fas

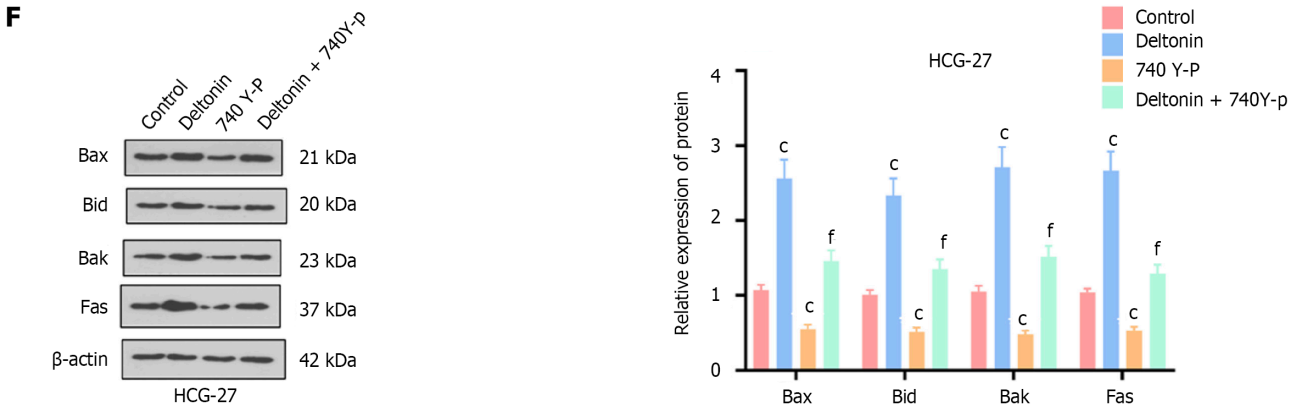


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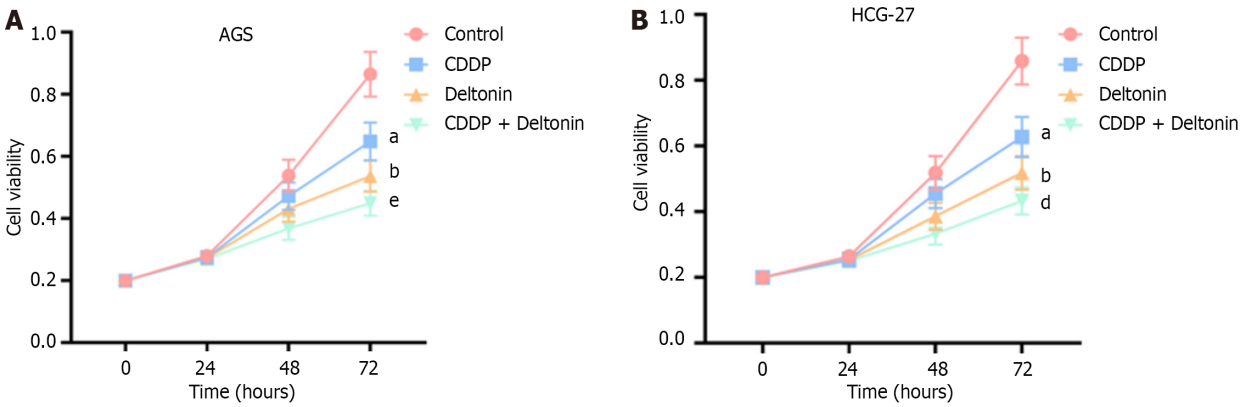
Figure 3 Influence of phosphatidylinositol 3-kinase/protein kinase B/mammalian target of the rapamycin p38-mitogen-activated protein kinase signaling pathway activation on the effects mediated by deltonin. HGC-27 and AGS cells were treated with 2.5 μM of deltonin and/or 20 μM of phosphatidylinositol 3-kinase activator 740 Y-P for 24 h. A and B: 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium bromide assay for cell viability; C and D: Western blot verification of the profiles of apoptosis-concerned proteins; E and F: Western blot determination of the protein profiles of phosphatidylinositol 3-kinase/protein kinase B/mammalian target of the rapamycin and p38-mitogen-activated protein kinase. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ *vs* control group; $^{d}P < 0.05$, $^{e}P < 0.01$, $^{f}P < 0.001$ *vs* 740 Y-P group, $n = 3$. PI3K: Phosphatidylinositol 3-kinase; AKT: Protein kinase B; mTOR: Mammalian target of the rapamycin; p38-MAPK: p38-mitogen-activated protein kinase.

mTOR signaling activation augments the resistance of GC cells to paclitaxel and promotes their apoptosis[40]. Afatinib dampens p38-MAPK and PI3K/AKT/mTOR signaling activation, thereby eliciting GC cell apoptosis and bolstering their resistance to chemotherapy[41]. All these conclusions align with our current study findings. Here, we discovered that deltonin significantly hinders p38-MAPK and PI3K/AKT/mTOR signaling activation, thereby bolstering GC cell apoptosis and attenuating the resistance of GC cells to cisplatin.

CONCLUSION

In summary, through a series of experiments, we uncovered that treating GC cells (AGS, HGC-27, and MKN-45) with deltonin results in reduced proliferation ability and increased apoptosis rate; of these, HGC-27 cells exhibited the best proliferation capability and the lowest apoptosis rate. Therefore, we exploited AGS and HGC-27 cells for further experiments and analyses. Our experiments demonstrated the ability of deltonin to promote GC cell apoptosis and chemosensitivity to cisplatin by lowering PI3K/AKT/mTOR and p38-MAPK-associated protein levels, offering novel insights into the mechanism of drug action. Nevertheless, further investigations are required to understand how deltonin represses these two axes, and *in vivo* experiments should be conducted using both male and female nude mice and other GC cell lines.



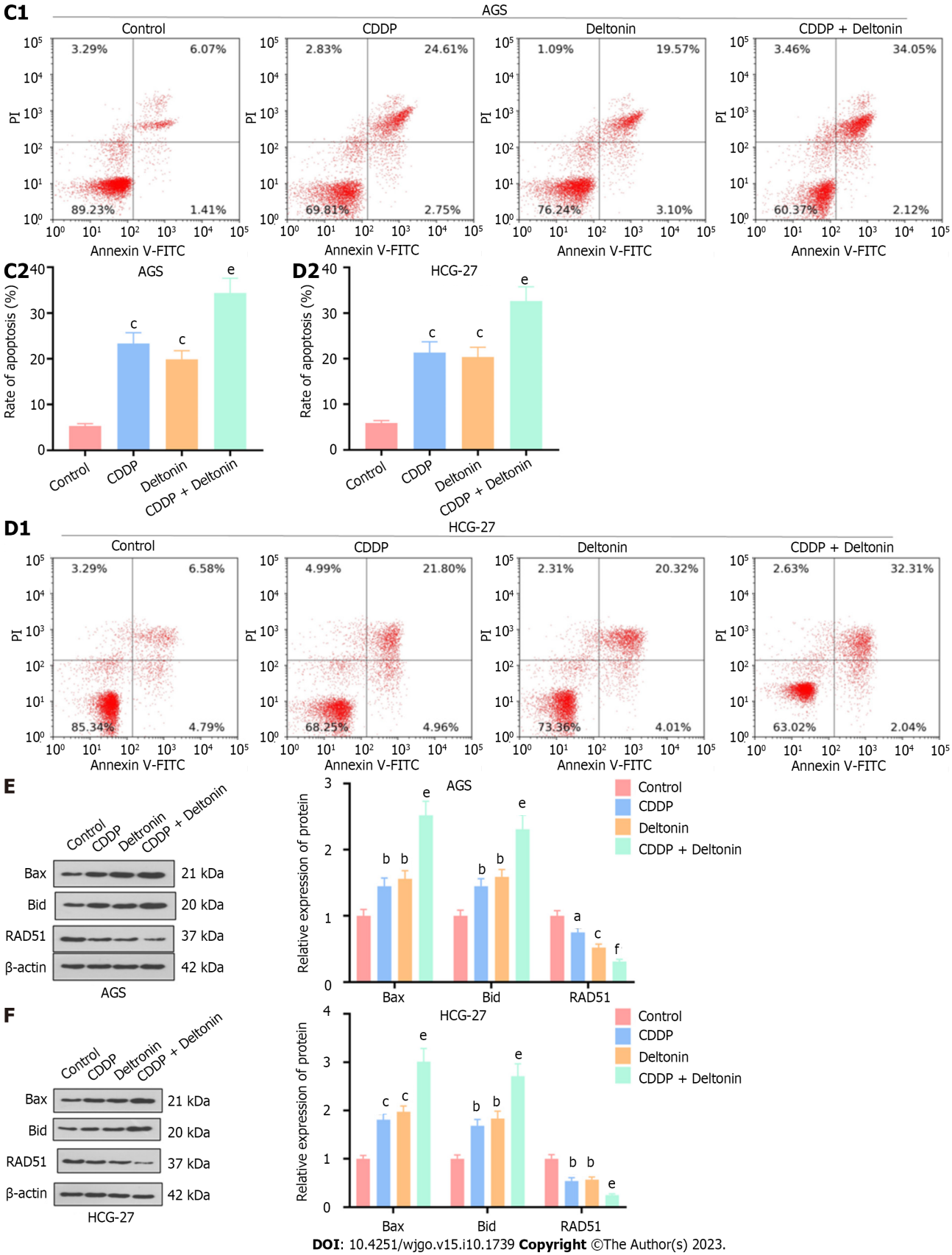


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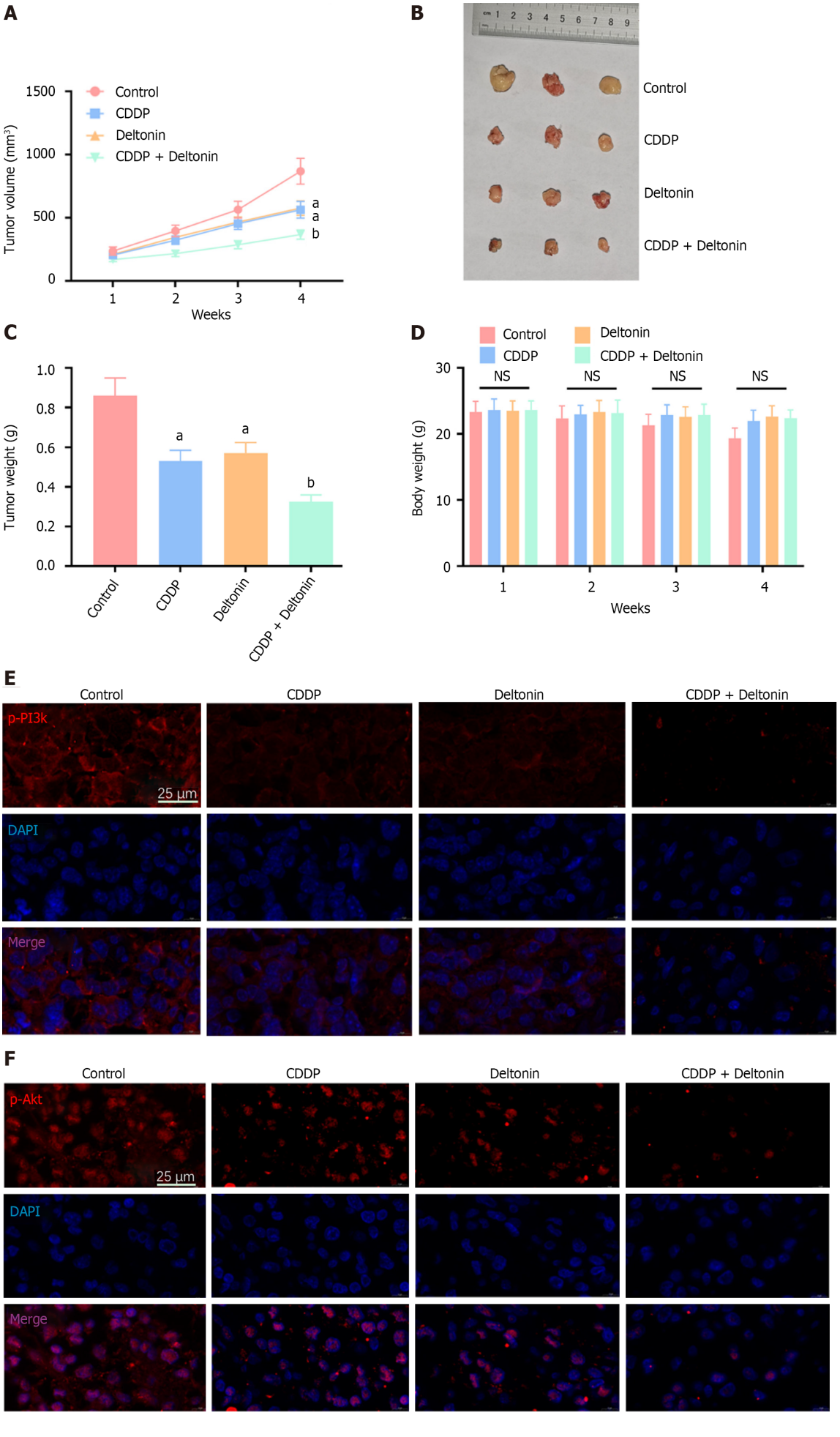
Figure 4 Deltonin enhances the chemosensitivity of gastric carcinoma cells to cisplatin. AGS and HGC-27 gastric carcinoma cells were treated with 2.5 μM of deltonin or 5 μg/mL of cisplatin or deltonin (1.25 μM) plus cisplatin (2.5 μg/mL) for 24 h. A and B: 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium bromide assay for examination of cell viability; C and D: Flow cytometry analysis of cell apoptosis; E and F: Western blot analysis of expression of apoptosis-correlated proteins (Bax and Bid) and the DNA repair-associated protein Rad51. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ *vs* control group; $^{d}P < 0.01$, $^{e}P < 0.01$, $^{f}P < 0.001$ *vs* cisplatin group, $n = 3$. CDDP: Cisplatin.


A
Control
CDDP
Deltonin
CDDP + Deltonin
Tumor volume (mm³)
1500
1000
500
0
1
2
3
4
Weeks
a
a
b
B
Control
CDDP
Deltonin
CDDP + Deltonin
C
Tumor weight (g)
1.0
0.8
0.6
0.4
0.2
0.0
a
a
b
Control
CDDP
Deltonin
CDDP + Deltonin
D
Control
CDDP
Deltonin
CDDP + Deltonin
Body weight (g)
30
20
10
0
NS
NS
NS
NS
1
2
3
4
Weeks
E
Control
CDDP
Deltonin
CDDP + Deltonin
p-PI3k
25 μm
DAPI
Merge
F
Control
CDDP
Deltonin
CDDP + Deltonin
p-Akt
25 μm
DAPI
Merge

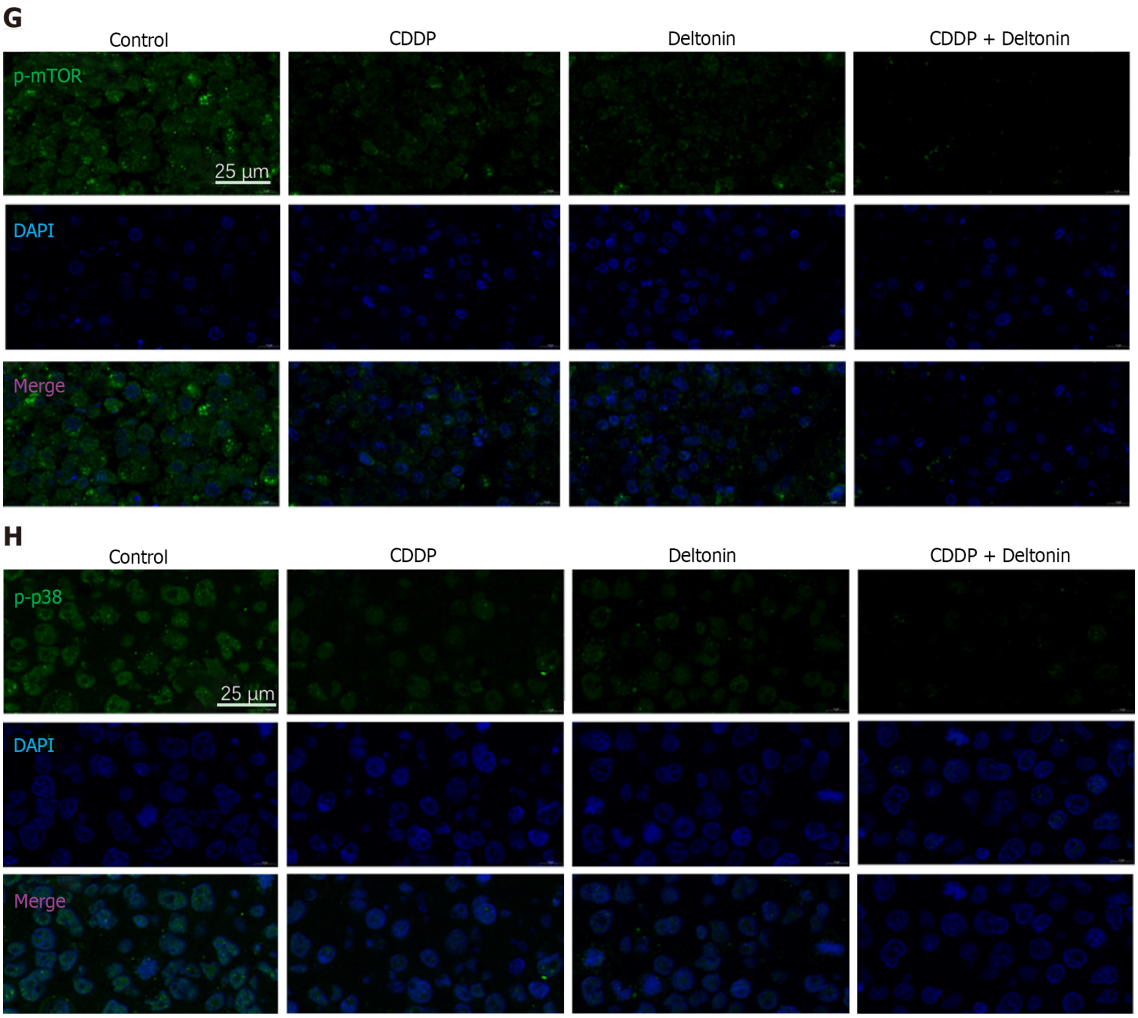


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Figure 5 Deltonin augmentes the chemosensitivity to cisplatin *in vivo* by dampening the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of the rapamycin and mitogen-activated protein kinase signaling pathways. Tumor-bearing mice were treated with saline, deltonin (50 mg/kg), and cisplatin (3 mg/kg) or deltonin (25 mg/kg) + cisplatin (1.5 mg/kg). A: The tumor volume was calculated during the 28 d; B and C: On the 28th d, the mice were sacrificed, the tumor images were taken, and the tumor weight was gauged; D: The body weight of the nude mice in different groups was figured out; E-H: Immunofluorescence measurement of the levels of phosphorylated phosphatidylinositol 3-kinase/protein kinase B/mammalian target of the rapamycin and p38-mitogen-activated protein kinase in the tumor tissues. NS: $P > 0.05$, $^{a}P < 0.01$ *vs* sham group, $^{b}P < 0.01$ *vs* cisplatin group, $n = 3$. NS: No significance; PI3K: Phosphatidylinositol 3-kinase; AKT: Protein kinase B; mTOR: Mammalian target of the rapamycin; DAPI: 4',6-diamidino-2-phenylindole; CDDP: Cisplatin.

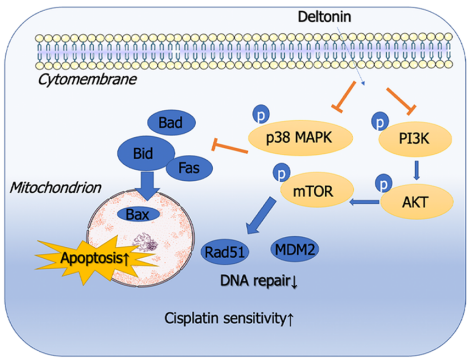

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Figure 6 Mechanism diagram. Deltonin bolsteres the apoptosis of gastric cancer cells and enhances their chemosensitivity to cisplatin *via* the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of the rapamycin and mitogen-activated protein kinase signaling pathways. PI3K: Phosphatidylinositol 3-kinase; AKT: Protein kinase B; mTOR: Mammalian target of the rapamycin; p38-MAPK: p38-mitogen-activated protein kinase.

ARTICLE HIGHLIGHTS

Research background

Despite being the main clinical treatment modality for advanced gastric cancer (GC), chemoradiotherapy is still difficult to achieve the expected effect due to the early diagnosis of GC and the characteristics of distant metastasis and drug resistance. Deltonin, an active ingredient in traditional Chinese medicine, shows anti-cancer effects on many malignancies.

Research motivation

This study attempted to optimize the treatment strategies for advanced GC and enhance the therapeutic effect on patients from a pharmacological mechanism perspective.

Research objectives

Here, we investigated the role and mechanism of action of deltonin in promoting GC cell apoptosis and chemosensitivity to cisplatin.

Research methods

In this study, gastric cancer cell lines (AGS, HGC-27, and MKN-45 cells) were treated with deltonin. Then, apoptosis was observed, and the expression of apoptosis-related proteins (Bax, Bid, Bad, and Fas), DNA repair-related proteins (Rad51 and MDM2), and phosphatidylinositol 3-kinase/protein kinase B/mammalian target of the rapamycin (PI3K/AKT/mTOR-MAPK) proteins was detected by Western blot analysis. In addition to this, the effect of deltonin on the chemosensitivity of GC cells to cisplatin was evaluated by *in vivo* and *in vitro* experiments

Research results

Treating GC cells (AGS, HGC-27, and MKN-45) with deltonin resulted in reduced proliferation ability and increased apoptosis rate; of these, HGC-27 cells exhibited the best proliferation capability and the lowest apoptosis rate. Our experiments demonstrated the ability of deltonin to promote GC cell apoptosis and chemosensitivity to cisplatin by lowering PI3K/AKT/mTOR and p38-MAPK-associated protein levels, offering novel insights into the mechanism of drug action.

Research conclusions

Deltonin enhances the chemosensitivity of GC cells to cisplatin by inhibiting the p38-MAPK and PI3K/AKT/mTOR signaling pathways.

Research perspectives

This study has verified that deltonin is able to regulate GC cell apoptosis as well as chemosensitivity to cisplatin through the PI3K/AKT/mTOR and p38-AMPK signaling pathways by *in vivo* and *in vitro* experiments. Such results provide a new direction for drug therapy of gastric cancer. However, the study of the regulatory role of the pathways in this study was limited and could not fully elucidate its mechanism of action. Therefore, further analysis as well as nude mouse experiments and more cellular experiments are needed to excavate the mechanism.

FOOTNOTES

Author contributions: Yang L and Liu YN contributed equally to this work and are co-first authors. Yang L and Liu YN conceived and designed the experiments; Yang L, Liu YN, Gu Y, and Guo Q performed the experiments; Yang L, Liu YN, Gu Y, and Guo Q contributed to the statistical analysis; Yang L, Liu YN, and Guo Q wrote the paper; and all authors read and approved the final manuscript.

Institutional review board statement: Our study was approved by the Ethics Review Committee of the Second Affiliated Hospital of Soochow University (approval No.: SZSH-2020-042).

Institutional animal care and use committee statement: The animal experiments were approved by the Ethics Review Committee of the Second Affiliated Hospital of Soochow University (approval No. SZSH-2020-042).

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The data sets used and analyzed during the current study are available from the corresponding author on reasonable request.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Country/Territory of origin: China

ORCID number: Qi Guo 0000-0002-2996-5987.

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