

# Silencing GOLPH3 gene expression reverses resistance to cisplatin in HT29 colon cancer cells via multiple signaling pathways

ZHI-PING ZHOU<sup>1\*</sup>, LIN-PEI WANG<sup>1\*</sup>, ZHONG-SHI HONG<sup>1</sup>, CHENG-ZHI QIU<sup>1</sup>, MING-ZHEN WANG<sup>2</sup>,  
ZHI-XIONG CHEN<sup>1</sup>, LONG-FENG TANG<sup>1</sup>, WAI-SHI YU<sup>1</sup> and CHUN-XIAO WANG<sup>1</sup>

<sup>1</sup>Department of General Surgery, The Second Affiliated Hospital of Fujian Medical University, Quanzhou, Fujian 362000;

<sup>2</sup>Department of Proctology, The Fifth Hospital of Xiamen City, Xiamen, Fujian 361101, P.R. China

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**Abstract.** Golgi phosphorylated protein (GOLPH)3 is overexpressed in colorectal cancer tissues and promotes the proliferation of colon cancer cells. A previous study by the authors demonstrated that GOLPH3 was associated with poor prognosis in colorectal cancer. However, the association between GOLPH3 gene overexpression and resistance to platinum-based drugs in colon cancer remains unknown. In the present study, the association between GOLPH3 overexpression and resistance of HT29 colon cancer cells to cisplatin and the mechanism underlying the development of chemoresistance were investigated. HT29 cells were divided into five groups. The expression of GOLPH3 mRNA was measured in the control and siRNA transfection groups. Reverse transcription-quantitative polymerase chain reaction analysis, cell proliferation, colony formation assay, tumor sphere formation and apoptosis (Annexin V) assays, western blotting and a nude mouse tumorigenicity assay were performed. HT29 cells were resistant to 10  $\mu$ M cisplatin treatment, whereas the expression of GOLPH3, P-glycoprotein, phosphorylated extracellular signal-regulated kinase (pERK)1/2 and  $\beta$ -catenin protein was significantly upregulated compared with the control group. With cisplatin treatment, silencing GOLPH3 gene expression downregulated the expression of these proteins, reduced cell proliferation and tumorigenicity, induced apoptosis and reversed the resistance of HT29 cells to cisplatin. In addition, the change in pERK1/2 and  $\beta$ -catenin

expression demonstrated that the mechanism of GOLPH3 overexpression involved in cisplatin resistance was associated with activation of the mitogen-activated protein kinase/ERK and Wnt/ $\beta$ -catenin signaling pathways in HT29 cells. The tumorigenicity experiment in nude mice also demonstrated that silencing GOLPH3 expression increased the sensitivity of HT29 cells to cisplatin *in vivo*. Therefore, overexpression of GOLPH3 may be involved in the resistance of HT29 colon cancer cells to cisplatin chemotherapy by activating multiple cell signaling pathways.

## Introduction

Colorectal cancer is one of the most common malignancies of the digestive system worldwide. However, the majority of the patients in China are at an advanced stage at initial diagnosis (1), therefore, adjuvant chemotherapy is required in a number of cases. Currently, folinic acid, 5-fluorouracil and oxaliplatin (FOLFOX) is the first-line chemotherapeutic regimen for colorectal cancer. However, a number of patients eventually succumb to the disease due to resistance to chemotherapeutic drugs (2,3). Therefore, to prolong the survival of patients with colorectal cancer, it is crucial to determine the mechanism underlying the development of drug resistance and find an effective treatment method to increase drug sensitivity.

The Golgi phosphorylated protein (GOLPH)3 gene, which is located on chromosome 5p13, encodes a ~34-kDa protein of the Golgi complex. Previous research demonstrated that GOLPH3 is a proto-oncogene (4-7). Previous studies by the authors revealed that GOLPH3 mRNA was overexpressed in colorectal cancer tissues and may promote the proliferation of colon cancer cells through activating the phosphoinositide 3-kinase/Akt/mechanistic target of rapamycin kinase and Wnt/ $\beta$ -catenin signaling pathways *in vitro*. Furthermore, the overexpression of GOLPH3 may serve as an important marker for predicting poor prognosis in colorectal cancer (8-10). To the best of our knowledge, GOLPH3-associated resistance to chemotherapy and its underlying mechanism in human colon cancer have not been previously reported.

Based on previous research on the association between the overexpression of GOLPH3 and colorectal cancer, it

**Correspondence to:** Dr Cheng-Zhi Qiu, Department of General Surgery, The Second Affiliated Hospital of Fujian Medical University, 34 Zhongshan North Road, Quanzhou, Fujian 362000, P.R. China  
E-mail: qchengzhi@sohu.com

\*Contributed equally

**Abbreviations:** GOLPH3, Golgi phosphorylated protein 3; pERK, phosphorylated extracellular signal-regulated kinase; FOLFOX, folinic acid, 5-fluorouracil and oxaliplatin

**Key words:** GOLPH3, drug resistance, cisplatin, colorectal cancer

was hypothesized that the overexpression of GOLPH3 may be involved in the resistance of colon cancer cells to platinum-based chemotherapy. Therefore, the aim of the present study was to investigate the effects of overexpressing GOLPH3 on resistance to cisplatin in HT29 human colon cancer cells, and the underlying mechanism by siRNA transfection and in a nude mouse tumor transplantation model.

## Materials and methods

**Cell lines and culture.** The human colorectal cancer cell line, HT29, was purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in RPMI-1640 medium (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) supplemented with 10% fetal bovine serum in 5% CO<sub>2</sub> at 37°C. The cells were harvested in the logarithmic growth phase.

**Selection of cisplatin concentration.** HT29 cells were divided into four groups according to cisplatin concentration (0, 2.5, 5 and 10 µM). Cisplatin was obtained from Shandong Qilu Pharmaceutical Factory (Shandong, China).

**Cell grouping.** HT29 cells were divided into five groups as follows: Control group; transfection group and experimental groups 1, 2 and 3. The cells in the control group were untreated. In the transfection group, the cells were transfected with 50 nM siRNA-GOLPH3 (Zimmer AG, Shanghai, China). Group 1 consisted of HT29 cells that were treated with cisplatin. Group 2 comprised siRNA-GOLPH3-transfected HT29 cells that were treated with cisplatin. Group 3 involved HT29 cells that were treated with cisplatin and 50 µM extracellular signal-regulated kinase (ERK)1/2 inhibitor PD98059 (Cell Signaling Technology Inc., Danvers, MA, USA). Each experimental group was maintained in RPMI-1640 containing 10 µM cisplatin for 24 h at 37°C.

**RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR).** The expression of GOLPH3 mRNA in the control and siRNA transfection groups was assessed. Total RNA was extracted from cultured cells using TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA, USA). Total RNA (2 µg) from each sample was reverse transcribed into cDNA using the SuperScript III Reverse Transcriptase kit (Invitrogen, Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. RT-qPCR was performed using the SYBR Ex Taq kit (Takara Bio, Inc., Otsu, Japan) and the ABI 9700 RT-qPCR detection system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The RT-qPCR conditions were initial denaturation for 20 sec at 95°C, followed by 45 cycles at 95°C for 10 sec, annealing at 60°C for 20 sec and extension for 20 sec at 72°C. All PCR primers were synthesized by Zimmer AG. The primer sequences for each gene were as follows: GOLPH3 forward, 5'-AGGCGACTCCAAGGAAA-3' and reverse, 5'-TGATGTGTGAACCTCGCG-3' and GAPDH forward, 5'-GGTCATAAGCTTGCGTTGATTAAG-3' and reverse, 5'-CTACGGAAACCTTGTACGACTTT-3'. Using GAPDH as an internal reference, the relative expression level was calculated with the 2<sup>-ΔΔC<sub>q</sub></sup> method.

**Cell proliferation (MTT) assay.** The cells were seeded into 96-well plates at a density of 10<sup>5</sup> cells/well and maintained for 24 h at 37°C in an anchorage-dependent manner. Next, four double cells were set up in each group, and four control wells that only contained cells without any other treatment were set up in each group. After a 48-h culture, 10 µl 5 mg/ml MTT (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was added to each well, followed by further culture for 4 h, after which time the culture medium was discarded. A total of 150 µl DMSO (Sigma-Aldrich; Merck KGaA) was added to each well, and the wells were mixed by oscillation in the dark. OD<sub>490</sub> was measured with a microplate reader (Huadong Electronics, Nanjing, China). The aforementioned procedure was repeated three times. The following formula was used: Rate of growth inhibition in cancer cells = (1 - mean OD<sub>490</sub> in treatment groups/mean OD<sub>490</sub> in control groups) x 100%.

**Colony formation assay.** The cells in the logarithmic growth phase were inoculated into 6-well plates (500 cells/well) containing complete culture medium. The medium was changed every 3 days over the next 3 weeks. Once the colonies became visible, the culture was terminated, and the cells were washed with PBS and fixed in 100% methanol. After staining with 0.5% crystal violet dye for 30 min, the cells were washed three times with PBS, and the colonies (>50 cells) were counted under an inverted microscope. The aforementioned steps were repeated three times, and the mean cell count was calculated.

**Tumor sphere formation assay.** HT29 cells were trypsinized, and then single dispersed cells were suspended in DMEM-F12 (Sigma-Aldrich; Merck KGaA) containing 20 ng/ml basic fibroblast growth factor, 20 ng/ml epidermal growth factor (PeproTech, Rocky Hill, NJ, USA), B27 and 5 µg/ml insulin, and were subsequently seeded onto 24-well ultra-low attachment plates (1,000 cells/well). The cells were then incubated at 37°C in DMEM-F12 for 6-7 days and supplemented with fresh medium every 2-3 days. Finally, the visible spheres were counted and images were captured (magnification, x200; Olympus Corporation, Tokyo, Japan).

**Apoptotic (Annexin V) assay.** The culture medium was centrifuged at 1,000 x g for 5 min. The supernatant was discarded, and the cells were resuspended gently in PBS. A total of 5-10 million resuspended cells were centrifuged at 1,000 x g for 5 min, and the supernatant was discarded. The cells were resuspended in a mixture containing 195 µl Annexin binding buffer, 5 µl Pacific Blue Annexin V (Beckman Coulter, Inc., Brea, CA, USA) and 10 µl propidium iodide (PI; Sigma-Aldrich; Merck KGaA) working solution for 10-20 min at room temperature, placed in an ice bath and detected by flow cytometry (FC500; Beckman Coulter, Inc.).

**Western blot analysis.** Each cell line in the logarithmic growth phase was washed with PBS. The cells were lysed in RIPA lysis buffer (containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% SDS, 1 mM EDTA, 1% Triton X-100, 1 mM PMSF, and 1 mM protease inhibitor cocktail) for 20 min on ice with occasional vortex mixing. The concentration of the proteins was determined using a BCA assay kit (Wegene Bio, Shanghai, China). The protein lysates (30 µg per lane) were

separated by 10% SDS-PAGE and transferred electrophoretically to nitrocellulose membranes (Beijing Solarbio Science & Technology Co., Ltd.). The membranes were blocked in phosphate-buffered saline with 0.1% Tween-20 (PBST) plus 3% BSA (Sigma-Aldrich; Merck KGaA) for 1 h at room temperature. The blots were incubated with antibodies against GOLPH3 (1:1,000; catalog no. ab 98023); P-glycoprotein (p-gp; 1:500; catalog no. ab 103477) (both from Abcam, Cambridge, UK);  $\beta$ -catenin (1:500; catalog no. sc-59737; Santa Cruz Biotechnology, Inc., Dallas, TX, USA); ERK1/2 (1:500; catalog no. ab 17942); pERK1/2 (1:500; catalog no. ab 65142) (both from Abcam); GAPDH (1:1,000; catalog no. sc-365062); and  $\beta$ -actin (1:1,000; catalog no. sc-47778) (both from Santa Cruz Biotechnology, Inc.) at 4°C overnight. The membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibodies (both 1:5,000; catalog nos. sc-516102 and sc-2357, respectively; Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. The bands were visualized with a chemiluminescence detection kit (catalog no. 32109; Thermo Fisher Scientific, Inc.), and the results were analyzed with ImageJ software (version 1.48; National Institutes of Health, Bethesda, MD, USA). The relative intensity of the target protein was calculated as gray value of the target band/gray value of the GAPDH (or  $\beta$ -actin) band. GAPDH (or  $\beta$ -actin) was used as a loading control. All experiments were performed independently in triplicate.

**Nude mouse tumorigenicity assay.** A total of 16 Balb/c nude mice (6 weeks old) were purchased from Beijing Vital River Laboratory Animal Technology Company (Beijing, China). All animal experiments were approved by the Animal Experimental Ethics Committee of China Three Gorges University (ethics approval no. 2016070A; Yichang, Hubei, China). All mice were free to eat and drink, and the temperature was kept at 22±2°C with a humidity of 50-60%. The lighting was controlled in the room with alternating cycles of 12 h-light (8:00-20:00) and 12 h-darkness (20:00-8:00). All experimental procedures involving animals were performed in accordance with the Guide for the Care and Use of Laboratory Animals and conformed to the institutional ethical guidelines for animal experimentation. Nude mice were handled and cared for at the Experimental Animal Center of China Three Gorges University.

The female nude mice (16-17 g) were randomly divided into four groups (n=4/group) as follows: Control group, transfection group and experimental groups 1 and 2. The control group consisted of mice that were xenografted with HT29 cells. The transfection group comprised mice that were xenografted with siRNA-GOLPH3-transfected HT29 cells. Group 1 involved mice that were xenografted with HT29 cells and injected intraperitoneally with cisplatin. Group 2 consisted of mice that were xenografted with siRNA-GOLPH3-transfected HT29 cells and injected intraperitoneally with cisplatin. The cells were trypsinized, collected by centrifugation (400 x g for 5 min at 4°C) and suspended in PBS. Subsequently, 0.2 ml of DMEM (Shanghai Biotend Biotechnology, Shanghai, China) containing 10<sup>7</sup> cells was injected subcutaneously into the axillary region of each mouse. The mice were housed in a pathogen-free environment. When an appreciable tumor had formed subcutaneously at 7 days after injection, each nude

mouse was weighed. Nude mice in experimental groups 1 and 2 received an intraperitoneal injection of cisplatin (3 mg/kg, every 3 days), while nude mice in the control and transfection groups were treated with normal saline instead of cisplatin. Tumor growth was monitored every 3 days, and tumor volume was calculated using the formula,  $V = 0.5ab^2$ , where 'a' and 'b' are the length and width of the tumor, respectively, measured with a sliding caliper. The mice were sacrificed after 30 days, and the tumors were resected and weighed.

**Statistical analysis.** SPSS software (version 19.0; SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Numerical data are expressed as the mean ± standard deviation. The Student's t-test was used to compare each experimental group with the control group. The differences between experimental groups were compared by one-way analysis of variance. The differences between paired experimental groups were compared by the Least Significant Difference t-test. Statistical significance was set at  $P < 0.05$ .

## Results

**Detection of GOLPH3 expression in transfected HT29 cells.** Compared with the control group (HT29 cells) (1.002±0.223), the expression of GOLPH3 mRNA in the siRNA-GOLPH3 transfection group (0.162±0.062) significantly decreased ( $P < 0.01$ ). Western blotting revealed that the GOLPH3 expression in the control and transfection groups was 1.003±0.094 and 0.099±0.112, respectively, and the difference was statistically significant ( $P < 0.001$ ), confirming the transfection efficiency (Figs. 1 and 2).

**Detection of cell proliferation and tumorigenicity in the transfection group.** The OD<sub>490</sub> value of the transfection group (0.715±0.074) was significantly lower compared with the control group (1.007±0.130) as demonstrated by the MTT assay ( $P < 0.01$ ). This indicated that the proliferation of HT29 cells significantly decreased following the silencing of GOLPH3 mRNA expression (Fig. 3). The colony formation assay demonstrated that the number of colonies in the control group (463.300±43.020) was significantly higher compared with the transfection group (341.700±54.930) ( $P < 0.05$ ), indicating that silencing GOLPH3 mRNA expression significantly reduced the tumorigenicity of HT29 cells (Fig. 4).

**Detection of apoptosis in the transfection group.** The apoptosis rate of the transfection group (15.520±2.921%) was significantly higher compared with the control group (1.843±1.298%), as shown by flow cytometry with Annexin V-fluorescein isothiocyanate (FITC)/PI ( $P < 0.01$ ) (Fig. 5). This finding also indicated that the GOLPH3 gene was effectively silenced.

**Involvement of the GOLPH3 in the resistance of HT29 cells to cisplatin.** Compared with the control group, an increasing concentration of cisplatin (2.5, 5 and 10  $\mu$ M) increased the expression of GOLPH3, P-gp,  $\beta$ -catenin and pERK1/2 in HT29 cells (Fig. 6 and Table I). The difference in protein expression levels was most significant between the 10  $\mu$ M cisplatin treatment group and the control group ( $P < 0.01$ ). Therefore, 10  $\mu$ M cisplatin was selected for subsequent experiments.

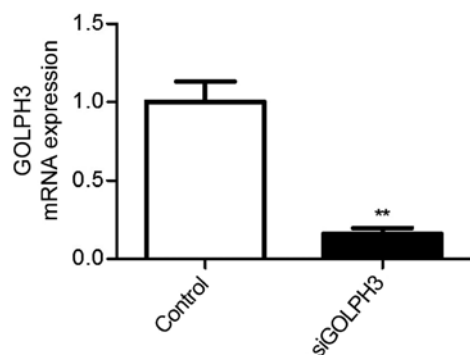


Figure 1. Comparison of GOLPH3 mRNA expression in the control and transfection groups. GOLPH3 expression was normalized to 18s rRNA expression and analyzed using Student's t-test. \*\* $P < 0.01$  vs. the control group. GOLPH3, Golgi phosphorylated protein 3; si, small-interfering.

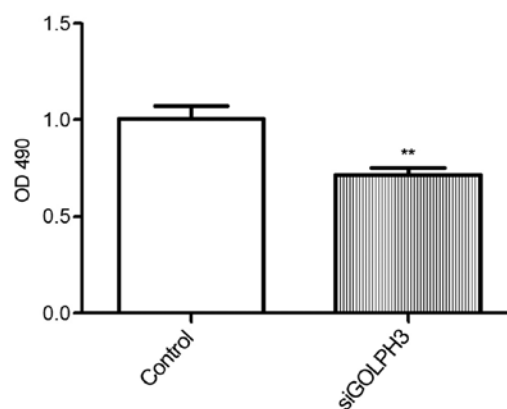


Figure 3. Comparison of the proliferation of colon cancer cells in the transfection and control groups. Student's t-test was used for analysis. \*\* $P < 0.01$  vs. the control group. GOLPH3, Golgi phosphorylated protein 3; OD, optical density; si, small-interfering.

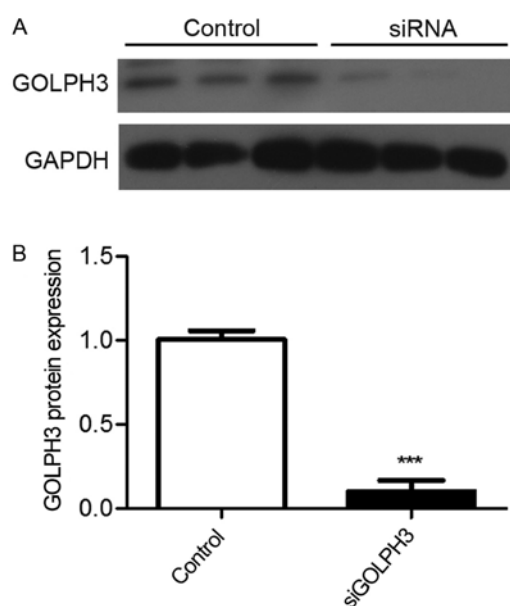


Figure 2. Comparison of GOLPH3 protein expression in the control and transfection groups. GAPDH expression was used to normalize GOLPH3 expression, and Student's t-test was used for analysis. \*\*\* $P < 0.001$  vs. the control group. GOLPH3, Golgi phosphorylated protein 3. si, small-interfering.

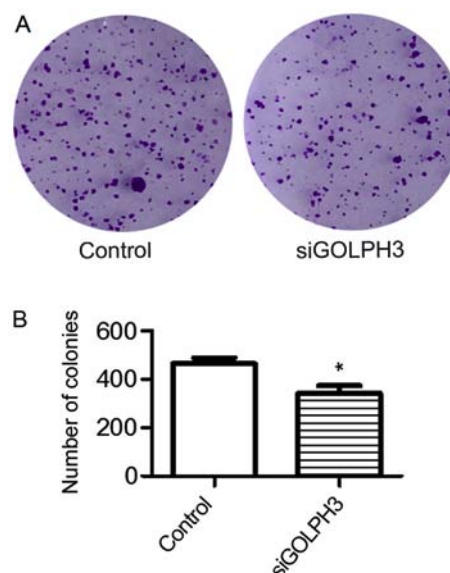


Figure 4. Comparison of the number of HT29 colonies between the transfection and control groups. Student's t-test was used for analysis. \* $P < 0.05$  vs. the control group. GOLPH3, Golgi phosphorylated protein 3; si, small-interfering.

P-gp expression was significantly increased in the 10  $\mu$ M cisplatin group compared with the control group ( $P < 0.01$ ), indicating that HT29 cells developed resistance to 10  $\mu$ M cisplatin. Furthermore, the expression of GOLPH3, pERK1/2 and  $\beta$ -catenin proteins was upregulated in the 10  $\mu$ M cisplatin treatment group ( $P < 0.01$ ), which also suggested that the resistance of HT29 cells to cisplatin was associated with GOLPH3 expression, mitogen-activated protein kinase (MAPK)/ERK and the Wnt/ $\beta$ -catenin cell signaling pathway.

**Effects of silencing GOLPH3 expression on the proliferation of HT29 cells under cisplatin treatment.** Under cisplatin treatment, cell proliferation was examined using the MTT assay (Fig. 7). The OD<sub>490</sub> of experimental groups 1 and 2 ( $0.746 \pm 0.085$  and  $0.236 \pm 0.071$ , respectively) was significantly lower compared with the control group ( $1.000 \pm 0.127$ ) ( $P < 0.05$ ), and the OD<sub>490</sub> of experimental group 2 was significantly lower compared with experimental group 1 ( $P < 0.001$ ). The colony

counts in the control and experimental groups 1 and 2 were  $604.70 \pm 39.70$ ,  $442.30 \pm 34.270$  and  $126.30 \pm 45.650$ , respectively, and the differences among all groups were significant ( $P < 0.05$ ). These results confirmed that silencing of GOLPH3 decreased the proliferation of HT29 cells under cisplatin treatment (Figs. 7 and 8).

**Effects of silencing GOLPH3 on the tumorigenicity of HT29 cells under cisplatin treatment.** Under cisplatin treatment, the tumor sphere counts in the control and experimental groups 1 and 2 were  $264.30 \pm 47.990$ ,  $212.80 \pm 30.380$  and  $30.750 \pm 14.50$ , respectively. The tumor sphere count in experimental group 2 was significantly lower compared with the control group and experimental group 1 ( $P < 0.001$ ). However, there was no significant difference between experimental group 1 and the control group ( $P > 0.05$ ). These results demonstrated that silencing GOLPH3 expression decreased the tumorigenicity of HT29 cells under cisplatin treatment (Fig. 9).

Table I. Protein expression levels following treatment with different concentrations of cisplatin.

Cisplatin concentration ( $\mu$ M)	GOLPH3	pERK1/2	$\beta$ -catenin	P-gp
0	1.020 $\pm$ 0.119	1.020 $\pm$ 0.045	1.020 $\pm$ 0.067	1.020 $\pm$ 0.241
2.5	1.735 $\pm$ 0.367 <sup>a</sup>	1.725 $\pm$ 0.501 <sup>b</sup>	1.798 $\pm$ 0.724 <sup>c</sup>	2.129 $\pm$ 0.586 <sup>d</sup>
5	2.377 $\pm$ 0.498 <sup>e</sup>	2.589 $\pm$ 0.166 <sup>f</sup>	2.756 $\pm$ 0.478 <sup>g</sup>	3.118 $\pm$ 0.662 <sup>h</sup>
10	3.249 $\pm$ 0.463 <sup>i</sup>	3.146 $\pm$ 0.361 <sup>j</sup>	3.554 $\pm$ 1.222 <sup>k</sup>	3.693 $\pm$ 0.706 <sup>l</sup>

<sup>a</sup>P=0.129; <sup>b</sup>P=0.177; <sup>c</sup>P=0.482; <sup>d</sup>P=0.110; <sup>e</sup>P=0.007; <sup>f</sup>P=0.005; <sup>g</sup>P=0.054; <sup>h</sup>P=0.006; <sup>i</sup>P<0.0001; <sup>j</sup>P=0.001; <sup>k</sup>P=0.008; <sup>l</sup>P=0.001 vs. 0  $\mu$ M. One-way analysis of variance was used for statistical analysis. GOLPH3, Golgi phosphorylated protein 3; P-gp, P-glycoprotein; ERK, extracellular signal-regulated kinase.

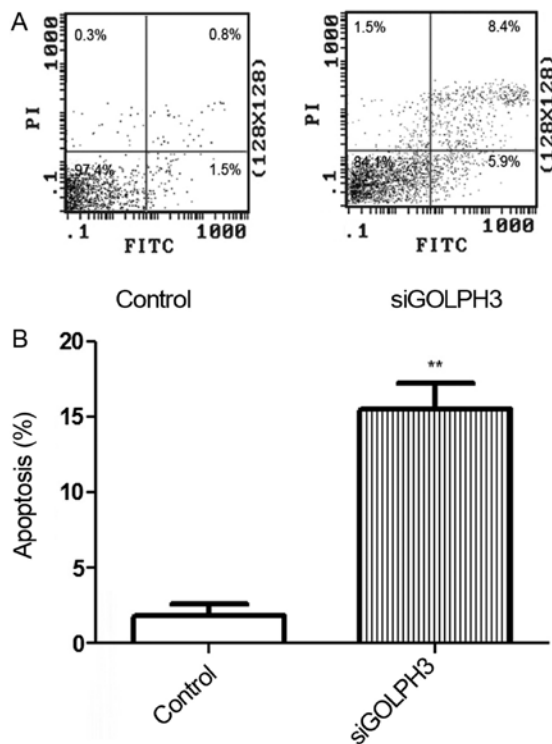


Figure 5. Comparison of apoptosis rates in the transfection and control groups. Student's t-test was used for analysis. \*\*P<0.01 vs. the control group. GOLPH3, FITC, fluorescein isothiocyanate; Golgi phosphorylated protein 3, PI, propidium iodide; si, small-interfering.

**Effects of silencing GOLPH3 on the apoptosis of HT29 cells under cisplatin treatment.** The apoptosis rates of experimental group 1 and experimental group 2 (23.890 $\pm$ 6.363 and 59.400 $\pm$ 2.392%, respectively) were significantly higher compared with the control group (1.843 $\pm$ 1.298) by flow cytometry with Annexin V-FITC/PI (P<0.01). The apoptosis rate of experimental group 2 was significantly higher compared with experimental group 1 (P<0.001) (Fig. 10). This finding demonstrated that silencing GOLPH3 gene expression induced apoptosis in HT29 cells under cisplatin treatment.

**Effects of silencing GOLPH3 on protein expression under cisplatin treatment.** Compared with experimental group 1, GOLPH3 and P-gp expression was significantly downregulated in experimental group 2 following the silencing of GOLPH3

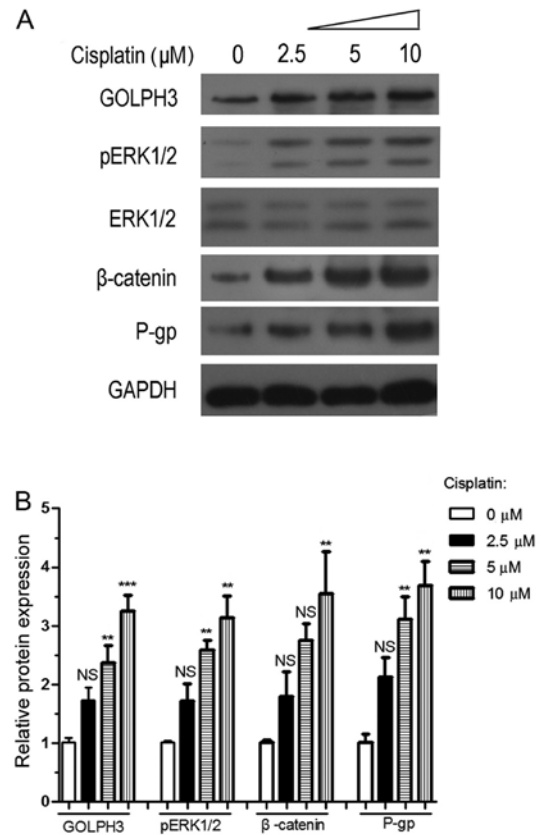


Figure 6. Effects of cisplatin on the expression of GOLPH3, P-gp,  $\beta$ -catenin and pERK1/2 proteins. (A) Protein expression following treatment with different concentrations of cisplatin. (B) Comparison of protein expression levels following treatment with different concentrations of cisplatin. GAPDH was used for normalization, and expression levels were analyzed by one-way analysis of variance. NS, P>0.05, \*\*P<0.01 and \*\*\*P<0.001 vs. the control group. GOLPH3, Golgi phosphorylated protein 3; P-gp, P-glycoprotein; ERK, extracellular signal-regulated kinase; NS, non-significant; p, phosphorylated.

under cisplatin treatment (P<0.01) (Fig. 11 and Table II). This result suggested that silencing GOLPH3 expression reversed resistance to cisplatin in HT29 cells. There was no significant difference in ERK1/2 protein expression between the control group, experimental groups 1 and 2. However, pERK1/2 and  $\beta$ -catenin expression in experimental group 2 was significantly lower compared with experimental group 1 (P<0.01). This indicated that silencing GOLPH3 expression significantly reduced  $\beta$ -catenin and pERK1/2 expression (active component



Table II. Protein expression in experimental groups 1 and 2.

Groups	GOLPH3	pERK1/2	β-catenin	P-gp
Control	1.000±0.173	1.004±0.271	1.000±0.078	1.014±0.346
Cisplatin	2.734±0.440 <sup>a</sup>	4.353±0.956 <sup>b</sup>	2.472±0.444 <sup>c</sup>	4.269±0.454 <sup>d</sup>
Cisplatin + siGOLPH3	0.249±0.084 <sup>e,f</sup>	1.124±0.410 <sup>g,h</sup>	0.993±0.052 <sup>i,j</sup>	1.842±0.383 <sup>k,l</sup>

Experimental group 1, the cells were treated with cisplatin. Experimental group 2, the cells were treated with cisplatin and transfected with siGOLPH3. <sup>a</sup>P=0.001; <sup>b</sup>P=0.001; <sup>c</sup>P=0.002; <sup>d</sup>P<0.0001 vs. control; <sup>e</sup>P=0.009 vs. control; <sup>f</sup>P<0.0001 vs. cisplatin treatment group; <sup>g</sup>P=0.821 vs. control; <sup>h</sup>P=0.001 vs. cisplatin treatment group; <sup>i</sup>P=0.972 vs. control; <sup>j</sup>P=0.002 vs. cisplatin treatment group; <sup>k</sup>P=0.043 vs. control; <sup>l</sup>P<0.0001 vs. cisplatin treatment group. Least Significant Difference t-test was used for statistical analysis. ERK, extracellular signal-regulated kinase; GOLPH3, Golgi phosphorylated protein 3; P-gp, P-glycoprotein; p, phosphorylated; si, small-interfering.

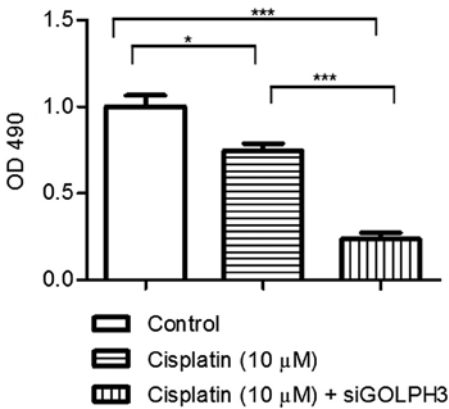


Figure 7. Inhibitory effect of cisplatin on the proliferation of HT29 cells. Least Significant Difference t-test was used for analysis. \*P<0.05 and \*\*\*P<0.001. GOLPH3, Golgi phosphorylated protein 3; OD, optical density; si, small-interfering.

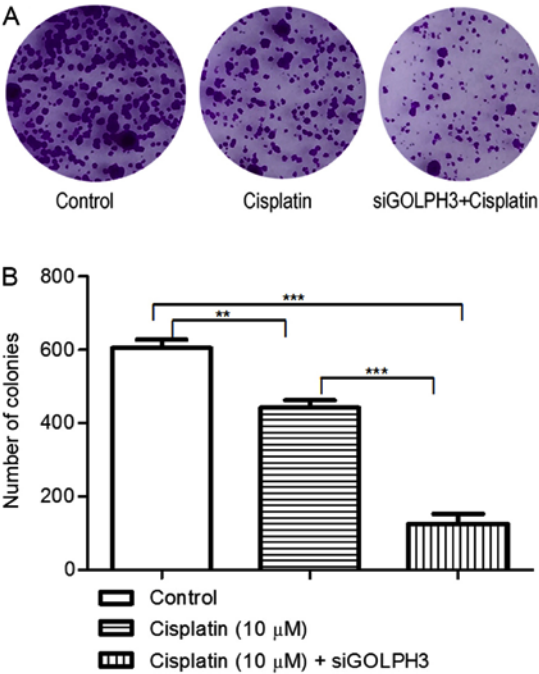


Figure 8. Comparison of the numbers of HT29 colonies under cisplatin treatment. (A) Images of cell colonies in each group. (B) Comparison of the number of colonies in each group. Least Significant Difference t-test was used for analysis. \*\*P<0.01 and \*\*\*P<0.001. GOLPH3, Golgi phosphorylated protein 3; OD, optical density; si, small-interfering.

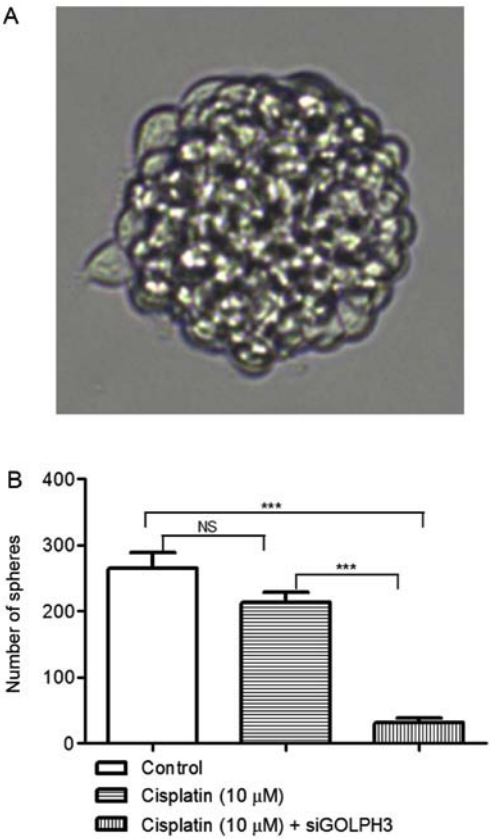


Figure 9. Comparison of tumorigenicity of colon cancer cells under cisplatin treatment. (A) Tumor sphere of HT29 cells in serum-free medium. Magnification, x200. (B) Comparison of tumor sphere counts in each group. Least Significant Difference t-test was used for analysis. NS, P>0.05 and \*\*\*P<0.001. GOLPH3, Golgi phosphorylated protein 3; NS, non-significant; si, small-interfering.

of ERK1/2) and inhibited the Wnt/β-catenin and MAPK/ERK cell signaling pathways under cisplatin treatment. Therefore, GOLPH3 overexpression is involved in resistance to cisplatin by activating the MAPK/ERK and Wnt/β-catenin cell signaling pathways in HT29 cells.

*Effects of blocking the MAPK/ERK signaling pathway on protein expression under cisplatin treatment.* The MAPK/ERK signaling pathway inhibitor PD98059 was used in a further study to analyze the effects of cisplatin treatment on HT29 cells. β-catenin and P-gp expression in experimental group 3

Table III. Protein expression in experimental groups 1 and 3.

Groups	$\beta$ -catenin	P-gp
Control	1.001 $\pm$ 0.072	1.004 $\pm$ 0.319
Cisplatin	2.303 $\pm$ 0.345 <sup>a</sup>	3.430 $\pm$ 0.335 <sup>b</sup>
Cisplatin + PD98059	1.150 $\pm$ 0.117 <sup>c,d</sup>	1.525 $\pm$ 0.189 <sup>e,f</sup>

<sup>a</sup>P=0.001; <sup>b</sup>P<0.0001 vs. control; <sup>c</sup>P=0.428 vs. control; <sup>d</sup>P=0.001 vs. experimental group 1; <sup>e</sup>P=0.069 vs. control; <sup>f</sup>P=0.001 vs. experimental group 1. Least Significant Difference t-test was used for statistical analysis. P-gp, P-glycoprotein.

Table IV. Comparison of volume of transplanted tumor in nude mice in each group.

Groups	Volume (cm <sup>3</sup> )
Control	2.083 $\pm$ 0.110
siGOLPH3	1.738 $\pm$ 0.121 <sup>a</sup>
Cisplatin	1.573 $\pm$ 0.224 <sup>b</sup>
Cisplatin + siGOLPH3	0.625 $\pm$ 0.189 <sup>c,d</sup>

The values are presented as the mean  $\pm$  standard deviation. <sup>a</sup>P=0.034; <sup>b</sup>P=0.003; <sup>c</sup>P<0.0001 vs. control, one-way analysis of variance; <sup>d</sup>P<0.0001 vs. experimental group 1, Student's t-test. GOLPH3, Golgi phosphorylated protein 3; si, small-interfering.

was significantly lower compared with experimental group 1 (P<0.01) (Fig. 12 and Table III). This finding suggested that blocking the MAPK/ERK signaling pathway partially reversed resistance to cisplatin in HT29 cells.

*Silencing GOLPH3 gene expression improves sensitivity of tumor-bearing nude mice to cisplatin.* The nude mouse tumor transplantation experiment was terminated at 30 days following the start of the experiment. The volume of the axillary subcutaneous tumors in the siRNA group and experimental groups 1 and 2 was significantly lower compared with the control group (P<0.05; Fig. 13 and Table IV). In experimental group 2, the tumor volume of the nude mice was significantly lower compared with experimental group 1 in the same period after 15 days (P<0.001). These results verified that silencing GOLPH3 expression improves the sensitivity of the transplanted tumors in nude mice to cisplatin chemotherapy, and that the overexpression of GOLPH3 is involved in resistance to platinum compounds.

## Discussion

Platinum chemotherapeutic drugs exert their pharmacological effects by targeting DNA. For example, the effect of cisplatin on DNA formation is mainly the crosslinking between Pt-AG and Pt-GG chains, and crosslinking can also take place between the chains and DNA-protein, which can block DNA replication and transcription at the nucleic acid level, inhibit the division of tumor cells and induce apoptosis (11,12). The mechanisms of

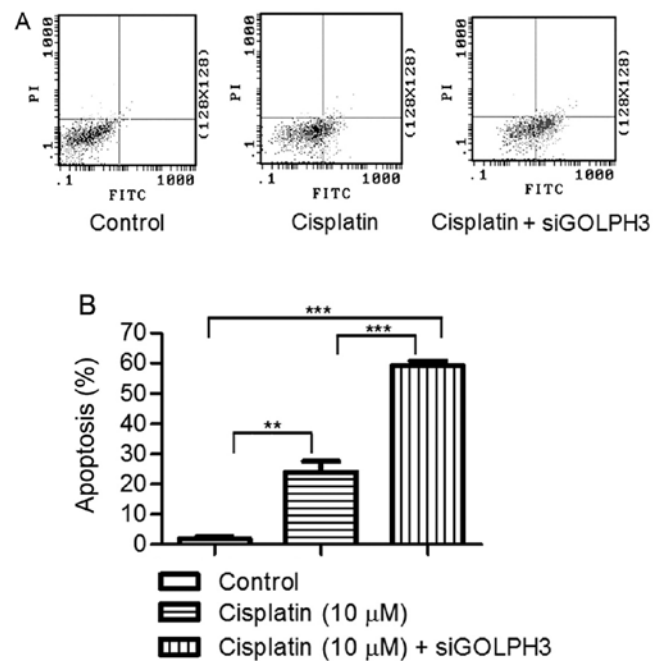


Figure 10. Comparison of the apoptosis of HT29 cells under cisplatin treatment in different groups. (A) Analysis of apoptosis of HT29 cells. (B) Comparison of apoptosis rates in each group. Least Significant Difference t-test was used for analysis. \*\*P<0.01 and \*\*\*P<0.001. FITC, fluorescein isothiocyanate; GOLPH3, Golgi phosphorylated protein 3; PI, propidium iodide; si, small-interfering.

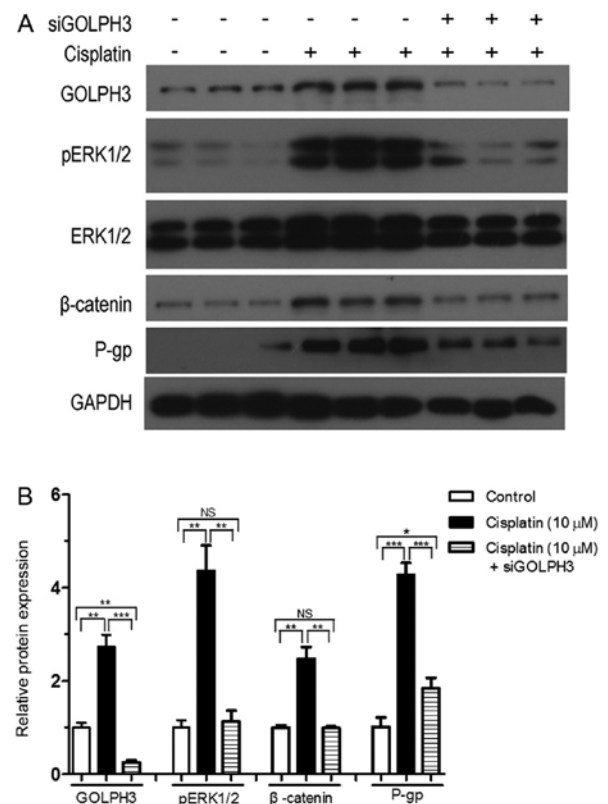


Figure 11. Protein expression in experimental groups 1 and 2 under cisplatin treatment. (A) Protein expression bands. (B) Comparison of protein expression in each group. Least Significant Difference t-test was used for analysis, and GAPDH was used for normalization. NS, P>0.05, \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001. GOLPH3, Golgi phosphorylated protein 3; P-gp, P-glycoprotein; ERK, extracellular signal-regulated kinase; NS, non-significant; si, small-interfering.

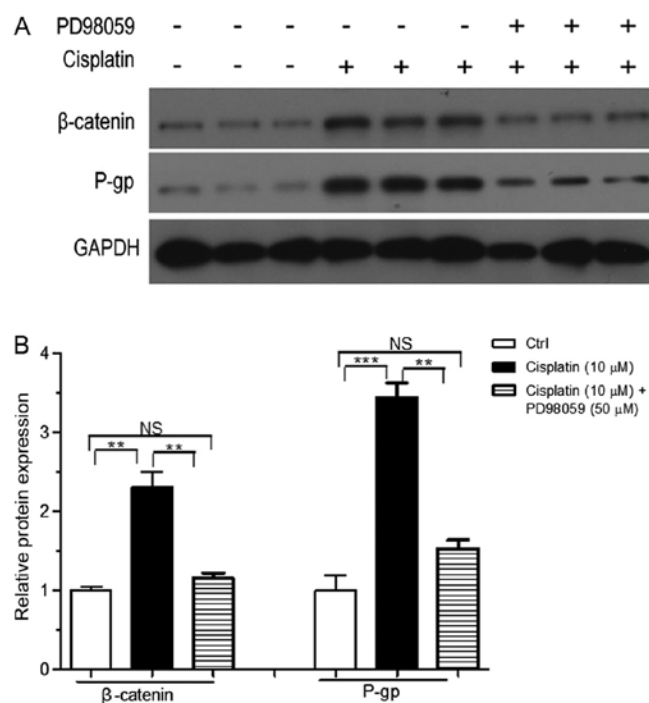


Figure 12. Protein expression in experimental groups 1 and 3. (A) Protein expression bands. (B) Comparison of protein expression in each group. significant difference t-test was used for analysis, and GAPDH was used for normalization. NS,  $P > 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ . P-gp, P-glycoprotein; NS, non-significant.

cisplatin resistance in tumor cells are as follows: i) Abnormal nucleotide excision repair system (13,14); ii) activation of the drug detoxification mechanism (15,16); iii) abnormal gene expression (17,18); iv) reduction in the movement of drugs into cancer cells and increased pump activity (19,20) and v) abnormal activation of cellular signaling pathways (21-23). Among these mechanisms, abnormal gene expression and cell signaling pathways have an important role in resistance to chemotherapy, and the selection of chemotherapeutics in colon cancer is becoming a research hotspot. GOLPH3, a new oncogene, is overexpressed in colorectal cancer tissues and is associated with the activation of cell signaling pathways. Therefore, GOLPH3 may be involved in resistance to chemotherapy.

The data of the present study demonstrated that, the expression of GOLPH3, P-gp, pERK1/2 and β-catenin proteins was upregulated in HT29 cells under cisplatin treatment, causing the emergence of drug resistance. Silencing GOLPH3 expression downregulated the expression of GOLPH3, P-gp, pERK1/2 and β-catenin, increased the sensitivity of HT29 cells to cisplatin, reduced tumorigenicity and reversed resistance to cisplatin. These results confirmed that GOLPH3 overexpression is involved in resistance of HT29 cells to cisplatin. The results of the nude mouse tumorigenesis experiment verified this conclusion. The change in pERK1/2 and β-catenin expression indicated that the mechanism underlying the involvement of GOLPH3 overexpression in cisplatin resistance was associated with activation of the MAPK/ERK and Wnt/β-catenin signaling pathways in HT29 cells.

The effect of the MAPK signaling pathway on the chemoresistance of cancer cells to platinum-based drugs has

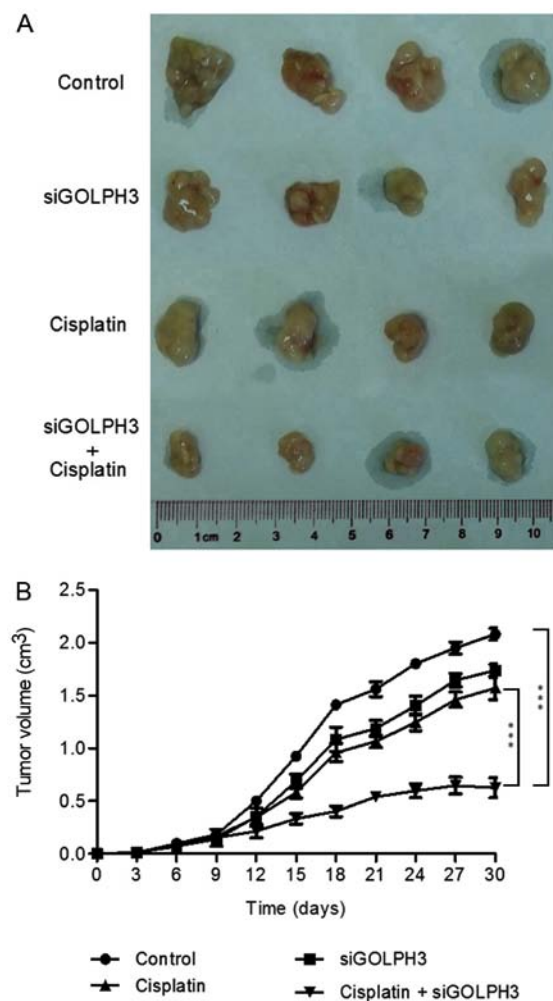


Figure 13. Comparison of the volume of subcutaneous tumors in nude mice in each group. (A) Subcutaneous tumor specimens from the nude mice in each group. (B) Growth curve of subcutaneous transplanted tumor in nude mice in each group. GOLPH3, Golgi phosphorylated protein 3; si, small-interfering. \*\*\* $P < 0.001$ .

attracted increasing attention. Excessive activation of the ERK1/2 signaling pathway is significantly associated with platinum-based drug resistance in a number of tumors (24,25). Liu *et al* (26) found that high-mobility group box 1 protein (a regulator of autophagy) was released in colorectal cancer cell lines following chemotherapy with oxaliplatin and decreased the sensitivity of colorectal cancer cells to oxaliplatin by activating the MEK/ERK pathway. Zhao *et al* (27) confirmed that silencing WEE1 expression reversed multidrug resistance and increased the sensitivity of HepG2/DDP cells to cisplatin. The underlying mechanism was associated with inhibition of the MAPK/ERK signaling pathway and the downregulated expression of genes that are associated with multidrug resistance.

The expression of β-catenin and P-gp was significantly decreased after the MAPK/ERK signaling pathway was blocked by PD98059. This indicated that blocking the MAPK/ERK signaling pathway partly reversed the resistance of HT29 cells to cisplatin. In a previous study by the authors, GOLPH3 activated the Wnt/β-catenin signaling pathway to promote the proliferation of colon cancer cells (10), which was confirmed by the present study.



Platinum-based drugs mainly exert their tumoricidal activity through DNA damage. It has been demonstrated that the DNA-PK-GOLPH3-MYO18A pathway is activated after DNA damage and is able to improve cell survival (28). The DNA-PK-GOLPH3-MYO18A pathway links response to DNA damage directly with the Golgi apparatus. If any components of the pathway are removed, the tumoricidal efficacy of the drugs is enhanced, cell proliferation is inhibited and the apoptosis rate is increased (29). Therefore, GOLPH3 overexpression may activate the DNA-PK/GOLPH3/MYO18A pathway in HT29 cells, which protects cancer cells against DNA damage caused by platinum drugs and leads to resistance to chemotherapy.

In summary, GOLPH3 overexpression is implicated in the resistance of HT29 cells to cisplatin and its action may be mediated through activation of the Wnt/ $\beta$ -catenin and MAPK/ERK signaling pathways. Silencing GOLPH3 expression partly reverses drug resistance. Our findings suggest that GOLPH3 is a potential target for reversing drug resistance in colon cancer.

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### Availability of data and materials

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

### Authors' contributions

CZQ conceived and designed the study. LPW, ZSH, MZW and ZXC performed the experiments. ZPZ and LPW participated in the design of the study, and ZPZ and CZQ were responsible for the investigation and analysis of the original data. ZPZ, CZQ and LPW wrote the manuscript. WSY and LFT contributed to data collection and analysis. MZW and CXW contributed to conducting the statistical analysis and drafting the manuscript. All have authors read and approved the manuscript.

### Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Three Gorges University (Yichang, Hubei, China). All of the animal experiments were performed according to the National Institutes of Health (Bethesda, MD, USA) guidelines on the use of experimental animals, and were approved by the

Ethics Committee of the Institutional Review Board of the Three Gorges University on Animal Experiments.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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