

# Combination of $\omega$ -3 Polyunsaturated Fatty Acids with 5-FU Inhibits SGC7901 Gastric Cancer Cell Growth via Rho-ROCK1 Signaling Pathway in Nude Mice

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## Research Article

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# Abstract

**Background:** Gastric cancer is one of the most common malignancy with high mortality rate in the world. Systemic chemotherapy is thought to be an important treatment. However, due to the unsatisfactory efficiency and obvious side effects, it is urgent to detect new therapy strategy for gastric cancer. This study was aimed to investigate the effects and mechanisms of  $\omega$ -3 polyunsaturated acids (PUFAs) combined with 5-FU on the growth of gastric cancer cells in nude mice.

**Methods:** BALB/C nude mice were injected subcutaneously with SGC7901 gastric cancer cells to establish a tumor-bearing mouse model. The tumor growth in vivo was observed. Morphological of tumor specimens was observed by HE staining. The mRNA levels of RhoA, RhoC and ROCK1 in tumor tissues were detected by qPCR, and their protein levels were detected by immunofluorescence and Western Blot. Meanwhile, apoptosis –related proteins were also determined by Western Blot.

**Results:** Compared with the NC control group, the tumor volume and weight in  $\omega$ -3 PUFAs and 5-Fu groups were insignificantly lower, but significantly lower in the combination group. Compared with the abundant blood supply in the NC group, HE staining showed multifocal tumor necrosis in the three intervention groups, and this change was the most prominent in the combination group. And qPCR results showed that the mRNA levels of RhoA in the combination groups were significantly lower than this in the other groups. Immunofluorescence showed that the level of RhoA protein in the three intervention groups decline in varying degrees, especially in the combination group. Western Blot showed that the protein level of RhoA in the three intervention groups were significantly lower than those in the NC control group, especially in the combination group. Meanwhile, the protein level of ROCK1 in both 5-FU group and the combination group were significantly lower, especially in the combination group. Compared with the control group, the levels of Bcl-2 and Caspase-9 decreased in the combination group, the level of cleaved Parp was increased at the same time.

**Conclusion:**  $\omega$ -3 PUFAs combined with 5-FU may inhibit tumor growth through the Rho/ROCK pathway and promote apoptosis by down-regulating the levels of Bcl-2 and Caspase-9 and induce the increase of cleaved Parp level.

## Introduction

Gastric cancer (GC) remains one of the most common and deadly neoplasms in the world. China is one of the regions with the highest incidence of gastric cancer, accounting for 42% of the global gastric cancer patients. It is 456000 new gastric cancer cases in 2018 in China. It has also the highest mortality rate of gastric cancer, accounting for 50% of the world<sup>[1]</sup>. About 90% GC patients died from tumor invasion and metastasis<sup>[2]</sup>. Chemotherapy has been widely used as a treatment for improving the prognosis of GC patients. 5-Fluorouracil (5-Fu), a chemotherapeutic drug commonly used in GC treatment, inhibits thymidylate synthase and infiltrates its metabolites into genomic DNA and RNA. However, 5-FU generally has many adverse effects to which the body is not prone to tolerate usually<sup>[3]</sup>.

Therefore, it is imperative to find better solutions to improve the efficacy and reduce the toxicity of existing anticancer drugs including 5-FU in normal or lower dose conditions.

$\omega$ -3 polyunsaturated fatty acids ( $\omega$ -3 PUFAs) is a dietary fatty acid which is reported to be directly or indirectly associated with the anti-tumor effect by inhibiting tumor cell proliferation, apoptosis, reversing multi-drug resistance, and preventing platelet aggregation<sup>[4]</sup>. Studies in cancer cells and tumor-bearing animal models demonstrated that  $\omega$ -3 PUFA supplementation presented a remarkable anti-cancer activity and played a good role in GC chemoprevention<sup>[5]</sup>. Addition of  $\omega$ -3 PUFAs to the chemotherapy system could not only increase the sensitivity to conventional cancer therapies but reduce the dose of chemotherapeutic drugs<sup>[6]</sup>. However, the mechanisms is still unclear.

Rho-GTPases as the active form after combination of members of the Rho subfamily and GTP have been shown to be associated with tumor invasion and growth, and therefore have become a focus of cancer research<sup>[7, 8]</sup>. Rho-GTPases was molecular switches and have been reported to be involved in cell cytoskeleton organization, migration, transcription, and proliferation. Studies have shown that Rho (including RhoA and RhoC) was activated in breast, colon and lung cancers, as well as in metastatic melanoma<sup>[9]</sup>. Over-expression of the RhoA has been detected in several human tumors, such as those of the urinary tract and cervix. In addition, Rho over-expression also contributes to the malignant phenotype in GC. GTPases binds to effector molecules that activate downstream targets. Rho-associated kinases (ROCKs) were originally recognized as downstream targets of RhoA. ROCK was a serine/threonine kinase that phosphorylates downstream targets, leading to cytoskeletal rearrangement. Active RhoA-GTP interacts with the C-terminal domain of ROCK, promoting the formation of stress fibers and focal adhesions, cell junctions, and cell cycles. Rho/ROCK activity can be regulated by both protein regulator signaling and cell surface receptors. Rho/ROCK signaling is involved in GC invasion. A recent study showed that linoleic acid could induce the expression of ICAM-1 in aortic endothelial cells by affecting the Rho-ROCK pathway, suggesting a potential effect of  $\omega$ -3 PUFAs on the Rho/ROCK pathway and associated oncogenic processes<sup>[10]</sup>. However, the impact of  $\omega$ -3 PUFAs on the Rho/ROCK pathway in GC was poorly known. Since Rho is associated with GC, the present study used it as the starting point to explore the mechanism of  $\omega$ -3 PUFAs combined with 5-FU on Rho/ROCK pathway and GC cell growth in a tumor-bearing BALB/C nude mouse model established by subcutaneous injection of SGC7901 cells, hoping that the results could provide a novel therapeutic target for the treatment of GC.

## Materials And Methods

### Reagents

The hematoxylin and eosin (HE) staining kit (#C0105S), BCA kit (#P0010S), anti-rabbit Alexa Fluor488 immunofluorescence staining kit(#AF1873), anti-Bcl-2(#AF6285),anti-beta actin (#AF5003) antibody and propidium iodide were purchased from the Beyotime Institute of Biotechnology (Haimen, China). Anti-caspase-3 (#14220), 9(#9502), anti-caspase-8 (#4709) and Bax (#5023) antibody were purchased from

Cell Signaling Technology (Boston,USA). Anti-RhoA(#2117), anti-RhOC(#3430) and anti-ROCK1(#4035)antibodies were purchased from Proteintech (Chicago, IL, USA). The quantitative PCR (qPCR) kit was from Bio-Engineering Co., Ltd (Dalian, China). Trizol was purchased from Invitrogen (Carlsbad, CA, USA), and  $\omega$ -3 PUFAs was from Huarui Pharmaceutical Co., Ltd (Wuxi, China). 5-FU was from JinYao Pharmaceutical Co., Ltd (Tianjin, China).

## Establishment of the tumor-bearing mouse model

Thirty-two male BALB/C nude mice weighing 18–22 g (Shanghai Slack Experimental Animal LLC; Animal Quality Certificate: SCXK (Shanghai) 2012-0002) were housed in a specific pathogen-free (SPF) environment. This study was approved by the ethics committee of the First Affiliated Hospital of Wenzhou Medical University (Wenzhou, China).

After one-week acclimatization, the 32 BALB/C nude mice were disinfected on the right thigh dorsal subcutaneous site, followed by inoculation of SGC7901 cells, a high invasion metabais cell line. (purchased from the experimental Animal Centre Cell Bank of Sun Yat-sen University, Guangzhou, China) in the logarithmic growth phase at a density of  $2 \times 10^6$  cells/mouse in 0.2 ml. After inoculation, the mice were transferred back to their cages. The model was considered successful when an induration with the size of a rice grain size appeared at the inoculated area. After successful modeling, the 32 mice were equally randomized into four groups: A, the normal saline (NC) control group; B, the  $\omega$ -3 PUFAs group; C, the 5-FU group; and D, the combined group of  $\omega$ -3 PUFAs + 5-Fu. On the second day after tumor confirmation, the treatment solution was freshly prepared at a dose of 3 g/kg by dissolving 6.4 ml  $\omega$ -3 PUFAs in 80 ml sterile distilled water. Nude mice in group B were given 3 g/kg  $\omega$ -3 PUFAs intragastrically, while group C was treated with intraabdominal injection of 5-FU solution at 30 mg/kg each time (saline diluted), D group was given intragastrically with 3 g/kg  $\omega$ -3 PUFAs and 30 mg/kg 5-FU intraabdominal injection. Group A was given the same amount of normal saline and intraabdominal injection.  $\omega$ -3 PUFAs was given 5 days a week, the dosage of 5-FU was given twice in the morning and in the evening, once a day, once every three days. After the nude mice were sacrificed on 4 weeks, the tumor weight and volume were measured.

## Morphological observation of tumor specimens

The tumor specimens were fixed in 10% formaldehyde at 4°C for 72 h, rinsed with distilled water, dehydrated, cleared in xylene, paraffin embedded, sliced into sections, HE stained, sealed in neutral gum, and finally observed under an optical microscope. Five fields in each group were randomly selected and analyzed.

## qPCR

Total RNA was extracted from the fresh tumor tissue using Trizol according to manufacturer's instructions. The RNA was reverse transcribed to cDNA using a high capacity cDNA reverse transcription kit. cDNA was then amplified based on the primer sequences presented later. GAPDH was used as the reference gene. The primers were synthesized by Beijing Genomics Institute (BGI) Sequencing Technology

Co., Ltd (Beijing, China) after searching for the primers of the target genes and comparing the blast in the NCBI database. The relative mRNA expression levels of RhoA, RhoC and ROCK1 in the NC control group and the three intervention groups were calculated using Real-time PCR method. Primer sequences(5'→3'):RhoA F: GAT TGGCGC TTT TGG GTA CAT, R: AGC AGCTCT CGTAGC CAT TTC;RhoC F: GGA GGT CTA CGT CCCTAC TGT, R: CGC AGT CGA TCA TAG TCT TCC;ROCK1 F: AAC ATG CTG CTG GAT AAA TCT GG,R: TGT ATC ACA TCG TAC CAT GCC T; GADPH F: GCC AGT GGA CTC CAC GAC, R: CAA CTACAT GGT TTA CAT GTT C.

## Immunofluorescence

The prepared sections were washed with PBS for 15 min X 3, fixed with 4% paraformaldehyde for 20 min, rinsed with PBS again for 15 min X 3, blocked for 60 min, washed with PBS for 15 min X 3, and incubated with the appropriate primary antibodies (RhoA, RhoC, and ROCK1; 1:50 dilution) overnight at 4°C. The following day, the sections were recovered to room temperature for 30 min, washed with PBS for 15 min X 3, incubated in a dark place with the appropriate secondary antibody (Alexa Fluor 488 labeled; 1:500 dilution) for 60 min at room temperature, and washed with PBS for 15 min X 3. The nuclei were stained with propidium iodide (PI) for 5 min followed by three 15-min washes with PBS. The anti-fluorescence quenching agent was added, and the sections were observed under a laser confocal microscope (A1 microscope; Nikon, Tokyo, Japan) and photographed. The excitation wavelength/emission wavelength of Alexa Fluor 488 excitation was 499/519nm and that of PI was 536/617nm.

## Western blot

The fresh tissues were homogenized with 500 µl lysis buffer after washing with PBS three times. The resulting homogenate was centrifuged at 12,500 rpm for 20 min. The supernatant was collected and stored at -80°C. Protein concentrations were determined using the bicinchonnic acid (BCA) method. Tris-glycine-based (10%) acrylamide gels were prepared to separate the proteins according to their molecular weights. After the proteins were transferred from the gels to methanol-activated polyvinylidene fluoride (PVDF) transfer membranes at 100 V for 75 min, the membranes were stained with Ponceau red. Non-specific binding was blocked for 90 min at room temperature, and incubated with the appropriate primary antibodies (RhoA and RhoC; 1:500 dilution; β-Actin, 1:800 dilution) overnight at 4°C. The next day, the membranes were washed with 1× TBST for 15 min x 3, incubated with an appropriate secondary antibody for 1 h at room temperature on a shaker, washed with 1× TBST for 15 min x3, exposed to ECL chemiluminescence for 1 min, and scanned. The obtained data were saved for further analysis.

## Statistical analysis

SPSS 21.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Data were plotted using the GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA, USA). Quantitative data are expressed as the mean ± standard deviation (SD), and analyzed using the Student t test. Two-sided P-values  $p < 0.05$  were considered to be statistically significant.

## Results

# Tumor growth in vivo

The mice were sacrificed on day 30 and the tumors were dissected for examination. The volume and weight of the tumors in the  $\omega$ -3 PUFAs and 5-Fu groups were insignificantly lower than those in the NC group (both  $P > 0.05$ ), and significantly lower in the combination group as compared the NC ( $P < 0.05$ ) (Fig. 1).

## Morphological changes of the tumors

Histological examination showed that tumor cells in the NC group were large and irregular, disordered and closely arranged, had high atypia, large nuclear deformities and deep staining (some were bubble-like), multiple vascular cavities, and no large area of cell damage. On the other hand, tumor cells in the other three groups showed loose arrangement, serious damage of cell structure, some cells swelling, vacuoles, rare vascular cavities, and flake-shape or massive necrosis ,especially these in the combined group. (Fig. 2)

## Changes in RhoA, RhoC, and ROCK1 mRNA levels

In order to explore the mechanism of  $\omega$ -3 PUFAs enhancing 5-Fu anti-tumor effect, the mRNA levels of RhoA, RhoC and ROCK1 in tumor tissues were detected by qPCR. As shown in Fig. 3, compared with the NC control group, the mRNA levels of RhoA in the other three intervent groups had different degrees of decline. While it was significantly lower in the combination group ( $P < 0.05$ ), however, there were no significant differences in relative levels of RhoC and ROCK1 mRNA between the three intervention groups and the control group ( $P > 0.05$ ). (Fig. 3)

## Changes in RhoA, RhoC, and ROCK1 protein levels by immunofluorescence

The nuclei of the tumor tissue stained with PI were detected by immunofluorescence. The results showed that in the three intervention groups the fluorescence intensity of RhoA-, RhoC-, ROCK1-positive cells were lower than those in the NC group. While the positive cells of RhoA in the combination group much less than those in the NC group. (Fig. 4)

## Changes in RhoA, RhoC, and ROCK1 protein levels in tumors

As  $\omega$ -3 PUFAs may affect the invasion and apoptosis of GC by affecting the expression of RhoA, RhoC, and ROCK1, we examined the protein expression of RhoA, RhoC, and ROCK1 in homogenized tumor tissues. Western Blot showed that the protein expression of RhoA in the three intervention groups were significantly lower than those in the NC control group ( $P < 0.05$ ), especially in the combination group( $P < 0.01$ ). Meanwhile,ROCK1 in both 5-FU group and the combination group were significantly lower( $P < 0.05$ ),especially in the combination group( $P < 0.01$ ). but no significant difference in the expression of RhoC protein was observed as compared the NC group ( $P > 0.05$ ) (Fig. 5).

## Changes in apoptosis-related proteins in tumors

Levels of apoptosis-related proteins Capase-3, Capase-8, Capase-9, BCL-2, Bax and cleaved Parp were detected by Western blot. The result showed both 5-Fu and the combined treatment up-regulated the level of cleaved Parp and down-regulated the level of Caspase-9 and Bcl-2, while the levels of other proteins were no significant difference in all groups (Fig. 6).

## Discussion

The incidence of GC is high in China. As many GC patients are already in the intermediate and late stages at the time of diagnosis, chemotherapy often becomes the mainstay of treatment for these patients. However, the severe adverse effects from the currently available chemotherapy drugs were often intolerable and prevent many chemotherapy patients from enduring to the end. It is therefore an urgent task to find new therapeutic targets for the treatment of GC patients.  $\omega$ -3 PUFA, an eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) either alone or their combination, are reported to have an anti-tumor effect by inducing apoptosis of cancer cells<sup>[11, 12]</sup>. Study showed that  $\omega$ -3 PUFAs inhibited the proliferation of colorectal cancer cells and induce their apoptosis in vitro<sup>[13]</sup>. The administration of  $\omega$ -3 PUFAs prevented the occurrence of colorectal cancer induced by AOM/DSS in mice. PUFAs may also improve the effectiveness of tumor therapy by increasing the sensitivity of tumor cells to conventional treatment. However, the specific mechanism was still unknown. Therefore, BALB/C nude mice were selected as experimental animals in this study, and the GC SGC7901 cell line was used to establish a GC subcutaneous xenograft tumor model. Using  $\omega$ -3 PUFAs lipid emulsion and chemotherapy drug 5-FU for drug intervention in nude mice, the impact of  $\omega$ -3 PUFAs lipid emulsion on tumor tissue growth and its anti-tumor effect combined with 5-Fu were observed to explore the possible mechanism.

Compared with the NC control group,  $\omega$ -3 PUFAs fish oil emulsion or 5-FU infusion alone showed no obvious inhibitory effect on the growth of the transplanted tumors in the nude mouse model, but the volume and weight of the transplanted tumors were significantly decreased in the combined treatment group. The HE staining result showed that the tumor tissues had varying degrees of cell necrosis accompanied with the decreased number of small blood vessels and tissue structural destruction in the three intervention groups, especially these in the combined group, suggesting that  $\omega$ -3 PUFAs fish oil emulsion could increase the inhibitory effect of 5-fu on the growth of GC.

In recent years, the relationship between  $\omega$ -3 PUFAs and tumor proliferation, invasion and metastasis has aroused increasing attention. Tumor proliferation invasion and metastasis are affected by various factors. RhoA and RhoC in the Rho family not only play a key regulatory role in tumor cell proliferation, invasion and metastasis but also changed the morphology of vascular endothelial cells and promoted tumor angiogenesis<sup>[14]</sup>. Meanwhile, ROCK, the downstream molecule of RhoA and RhoC, is regulated by the interaction between Rho GTP enzymes, and promotes the generation of actin-mediated contractile force through phosphorylation of a variety of downstream target proteins to control cell movement and metastasis<sup>[15, 16]</sup>. It was found in our study that combination  $\omega$ -3 PUFAs with 5-FU reduced the levels of RhoA both in the mRNA or protein level. Some studies also found that the levels of RhoA, RhoC and ROCK1 was closely related to the proliferation, invasion and metastasis of breast, prostate and liver

cancer cells<sup>[17, 18]</sup>, Therefore, in vivo, we considered  $\omega$ -3 PUFAs combined with 5-FU could inhibited the level of RhoA and ROCK1 proteins, which may probably further affect the proliferation, invasion and apoptosis of GC cells<sup>[19]</sup>. However, we did not observe the variation of RhoC which suggested Rhoc was not probably related to the proliferation, invasion and metastasis of the GC cells at least in this study.

Induction of tumor cell apoptosis plays an important role in tumor therapy and it is an important target of many therapeutic strategies. In previous studies, Marnie<sup>[20]</sup> found DHA DOX-treated mice had 50% smaller tumors than control mice and the level of Bcl-2 decreased by 24%, thought the supplementation of DHA may amplificate the effect of DOX on pro-apoptosis in MDA-MB-231 Breast Cancer Cells. It was also reported that the combination of  $\omega$ -3 PUFAs fish oil emulsion and 5-FU significantly reduced the expression level of anti-apoptotic protein Bcl-2 in tumor tissues, and increased the expression level of Bax protein<sup>[21]</sup>. And our research found that treatment with 5-FU alone or combined with  $\omega$ -3 PUFAs decreased the level of Bcl-2, and the latter has a more pronounced effect, which indicated the addition of  $\omega$ -3 PUFAs may enhance the killing effect of 5-FU on tumor cells. Related studies showed that Bcl-2 family promoted and inhibited apoptosis by regulating the release of mitochondrial downstream effector molecules<sup>[22]</sup>. In the colon cancer rat model, apoptosis of the animals fed with fish oil was enhanced and the anti-apoptotic protein Bcl-2 was expressed at a lower level. Bcl-2 is located in the mitochondrial endoplasmic reticulum, and overexpression can inhibit the activity of caspase<sup>[17, 23]</sup>. By increasing the permeability of cell membrane, Bax induced apoptosis, released cytochrome C from mitochondria, and activated caspase-3 and the caspase activation pathway, leading to the shearing of Parp protein<sup>[12, 24]</sup>. Most caspases normally exists in inactive precursor form when sites in the pro-enzyme molecules are cleaved by aspartate proteases to form polymers that are activated and then play a role in promoting apoptosis. Among that, Casepase-9 is located upstream of the apoptosis cascade reaction and is an important starting factor in the apoptosis pathway of the cell-dependent mitochondrial apoptosis. In this study, we also found treatment with 5-FU alone or combined with  $\omega$ -3 PUFAs activated the protein of caspase-9 and result in the cleaving of Parp, which is considered to be an important indicator of apoptosis and also commonly considered as a substrate for the action of caspases. However, adding  $\omega$ -3 PUFAs alone has no significant effect on apoptosis, which probably means  $\omega$ -3 PUFAs is only a role in promoting the chemotherapy effect of 5-FU or its dose is too low to play the role of apoptosis alone in this study. In addition, changes in some apoptosis proteins such as Caspase-3, Caspase-8, Bax were not observed in all intervent groups, which also should be suggested for a further study. Above that, It suggested  $\omega$ -3 PUFAs fish oil emulsion combined with 5-FU may affect the mitochondrial apoptosis pathway to inhibit the growth of mouse xenografts.

In summary,  $\omega$ -3 PUFAs combined with 5-FU may inhibit proliferation, invasion by reducing the levels of RhoA and ROCK1, ultimately affecting the growth of GC and promoting apoptosis of tumor cells by down-regulating the expression of bcl-2, caspase-9 and up-regulating cleaved Parp. This study may provide in vivo experimental data for clinical treatment of GC.

## Declarations



# Ethics approval and consent to participate

This study was carried out in accordance with the recommendations of Wenzhou University

## Consent for publication

Not Applicable

## Availability of data and materials

Availability of data and materials can be obtained from corresponding author

## Competing interests

All authors have no conflict of interest

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## Authors' contributions

Jiang Yiyan done the experiment, Wang Keke, Lou Zhefeng, Hong Dan analytical data Min Tao wrote the paper.

## Acknowledgements

Not Applicable

## Authors' information (optional)

Not Applicable

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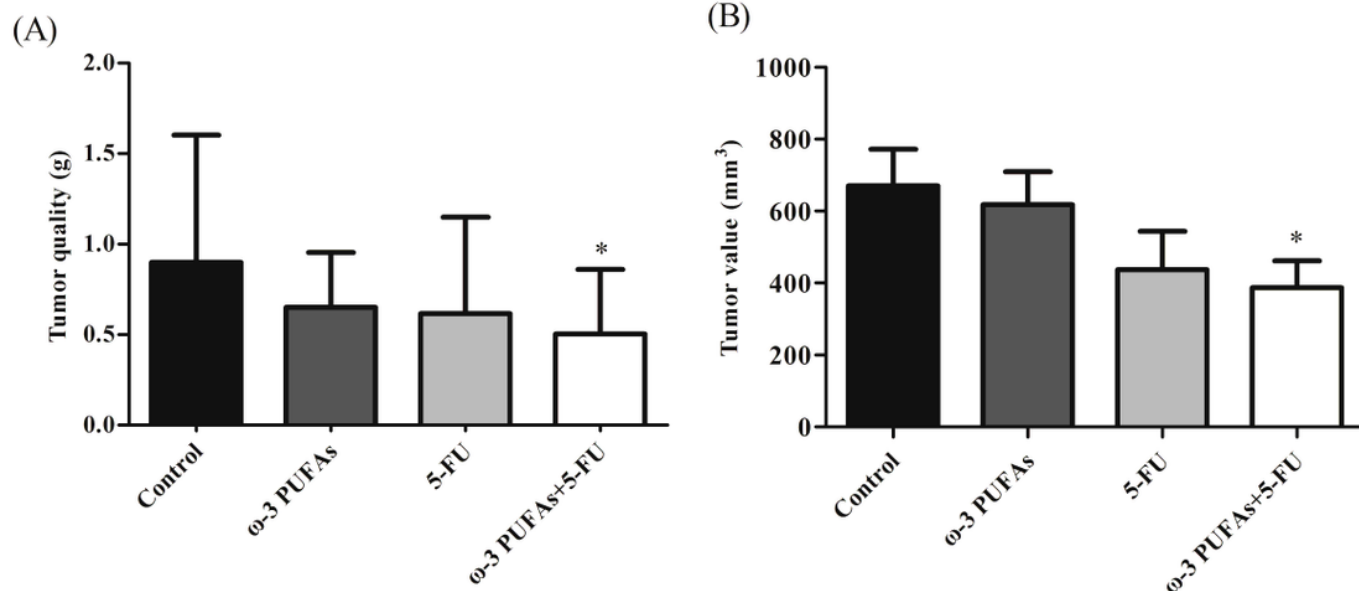
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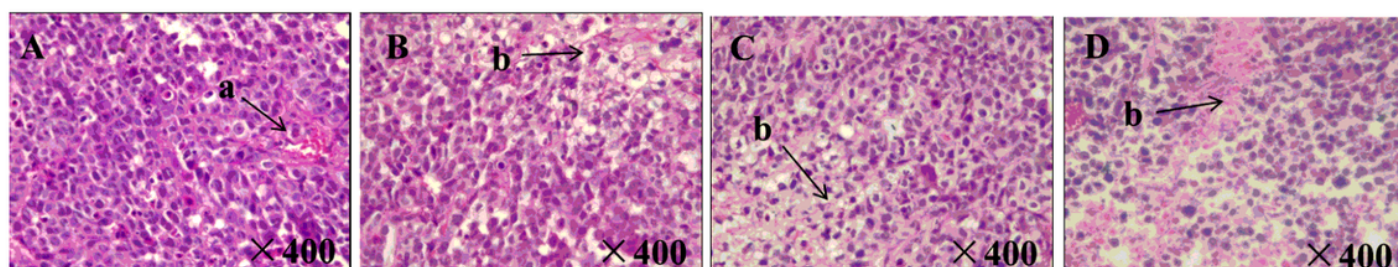
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## Figures



**Figure 1**

The inhibitory effect of ω-3 PUFAs+5-FU on the growth of xenograft tumors derived from SGC7901 cells in nude mice. A: Tumor weight in each group of nude mice treated for 30 days. B: Tumor volume in each group of nude mice. The volume and weight of xenograft tumors in ω-3 PUFAs+5-FU group were lower than those in the NC control group. Data are expressed as the mean ± SEM (\*P<0.05).



**Figure 2**

HE staining results of tumor tissue paraffin sections in each group. A: NC control group. B: ω-3 PUFAs group. C: 5-FU group. D: ω-3 PUFAs+5-FU group. Combined treatment group had more necrotic areas compared with the NC control group.

SGC7901 cells

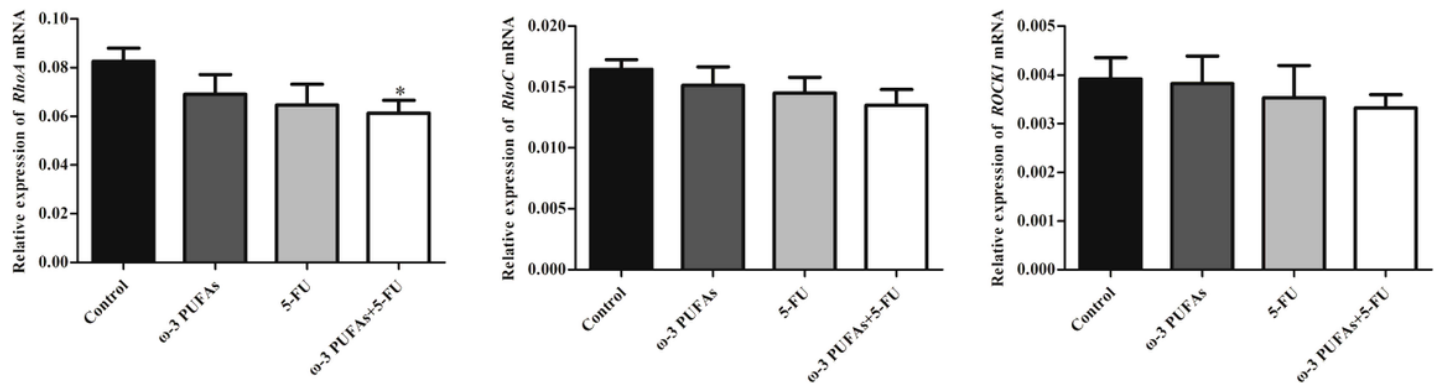


Figure 3

The inhibitory effect of  $\omega$ -3 PUFAs or 5-FU alone, or combination of both on RhoA, RhoC and ROCK1 mRNA levels in xenograft tumors. The expression of RhoA mRNA was lower in  $\omega$ -3 PUFAs + 5-Fu group than that in the NC control group. Data are expressed as the mean  $\pm$ SEM (\* $P$ <0.05).

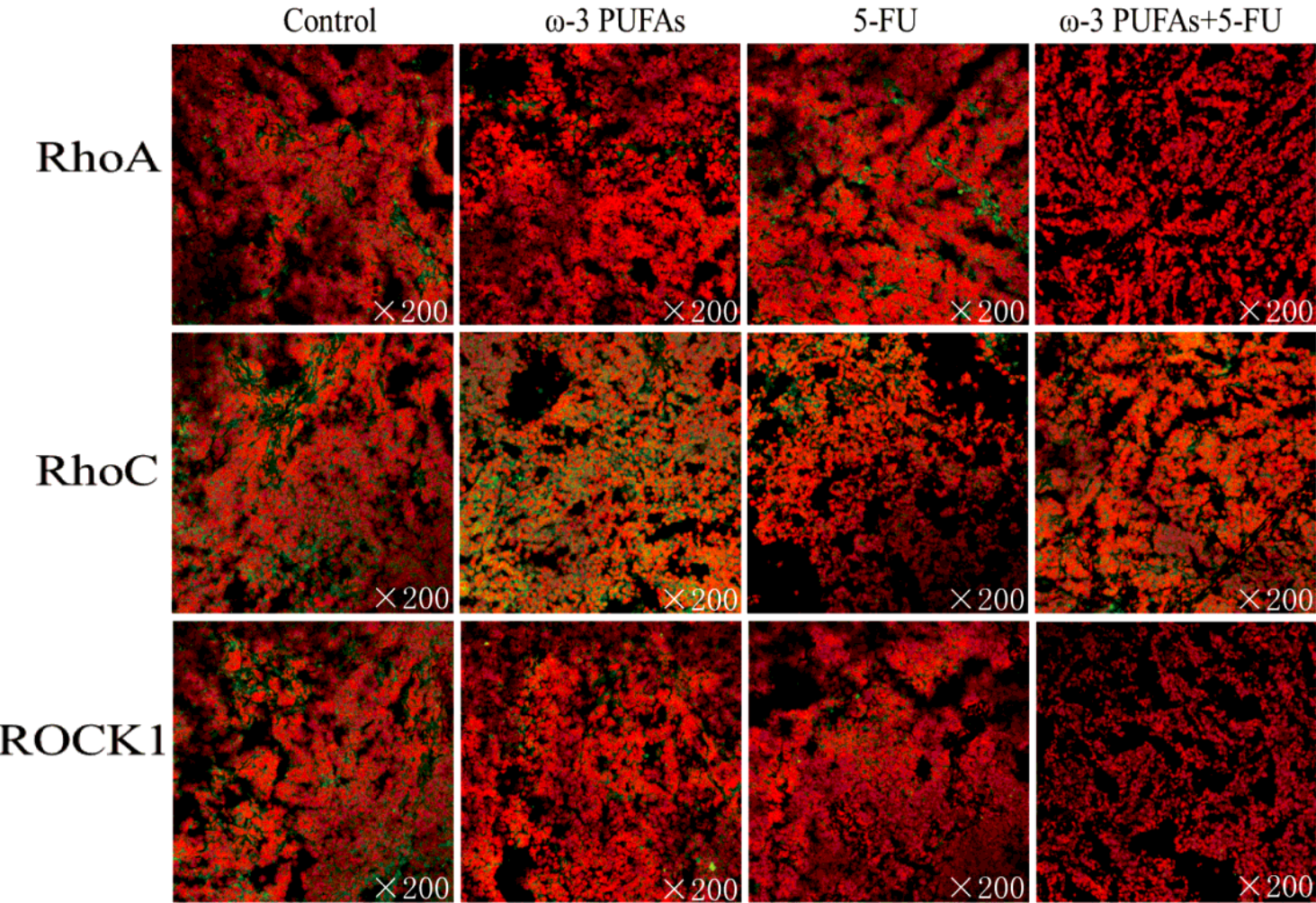
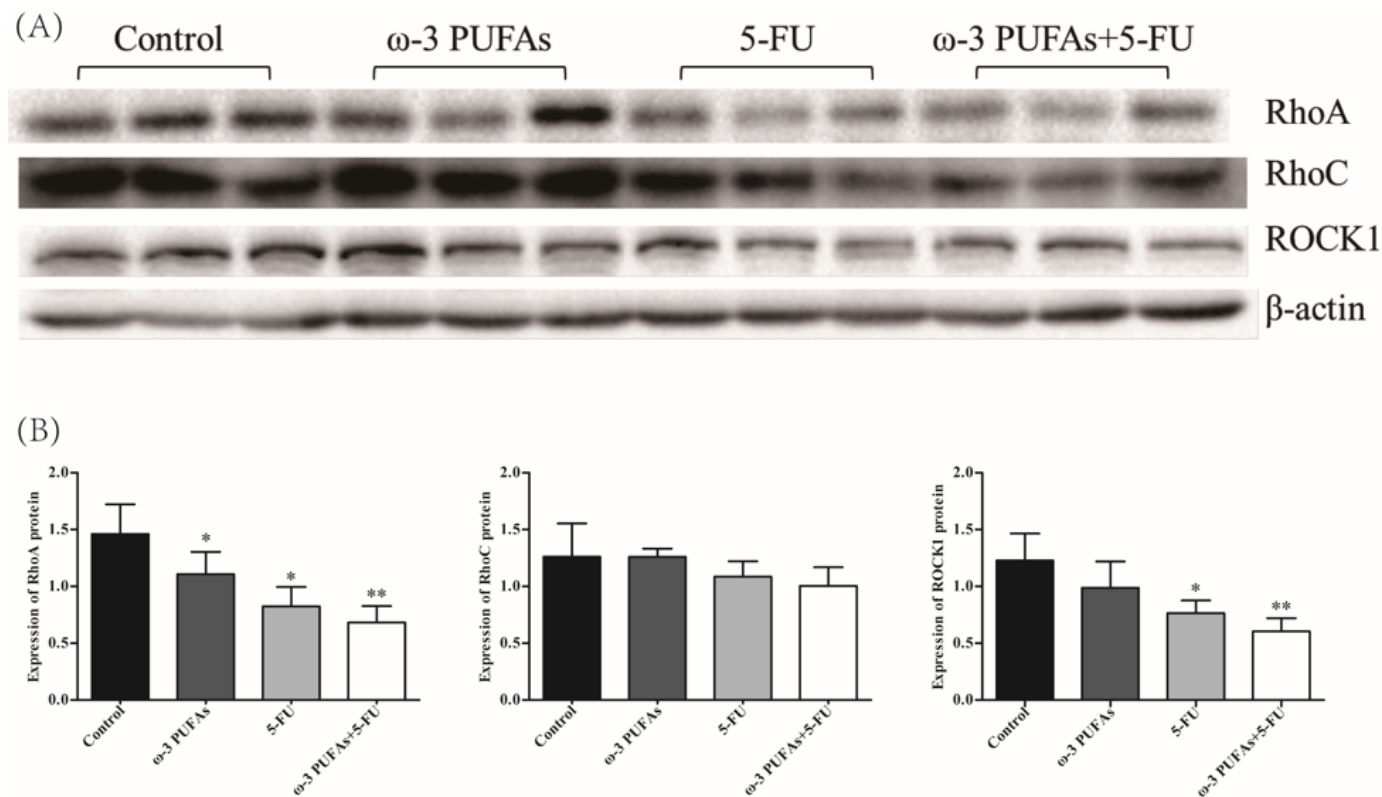


Figure 4

Immunofluorescence results of RhoA, RhoC and ROCK1 protein in transplanted tumor tissues. RhoA-positive (blue dots) cells in  $\omega$ -3 PUFAs+5-FU group were less than those in the NC control group.

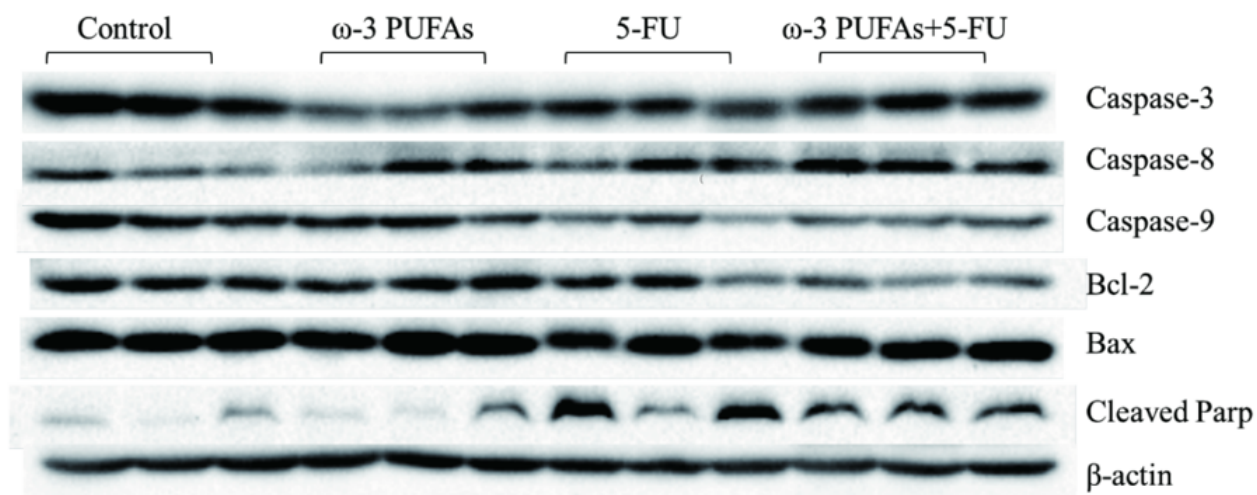


**Figure 5**

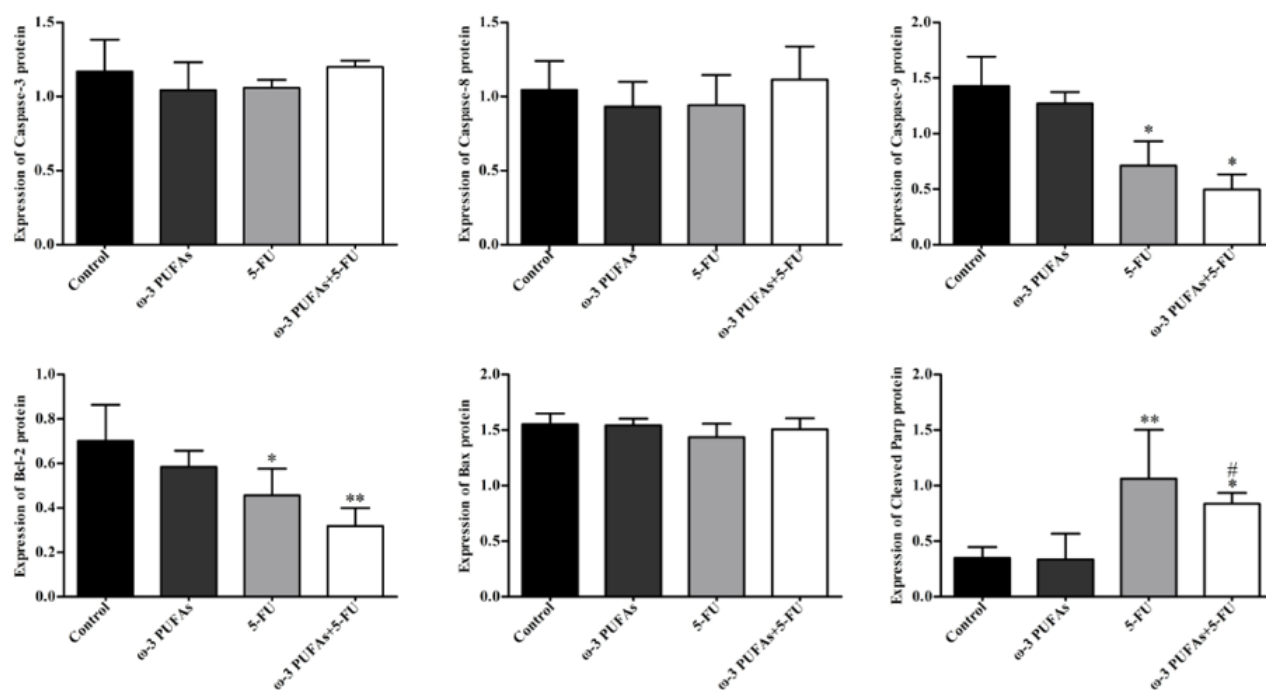
The inhibitory effect of  $\omega$ -3PUFAs or 5-FU alone, or combination of both on RhoA, RhoC and ROCK1 protein levels. A: The experimental groups: NC control,  $\omega$ -3 PUFAs, 5-FU and  $\omega$ -3 PUFAs+5-FU. B was the quantification for A. the protein expressions of RhoA in the three intervention groups were significantly lower than those in the NC control group , especially in the combination group. Meanwhile, the protein expressions of ROCK1 in both 5-FU group and the combination group were significantly lower,especially in the combination group. Data are expressed as the mean  $\pm$ SEM (\*P<0.05, \*\*P<0.01).



(A)



(B)



**Figure 6**

The inhibitory effect of ω-3PUFAs or 5-FU alone, or combination of both on apoptosis-related protein levels. A: The experimental groups: NC control, ω-3 PUFAs, 5-FU and ω-3 PUFAs+5-FU. B was the quantification for A. the levels of Bcl-2 and Caspase-9 proteins decreased in both 5-FU alone and the combination group, especially in the combination group. While the expression of cleaved Parp protein was increased in these two groups. Data are expressed as the mean ±SEM (\*P<0.05, \*\*P<0.01).