Polyunsaturated Fatty Acids ω-3 and ω-6 Regulate the Proliferation Invasion and Angiogenesis of Human Gastric Cancer Through COX/PGE Signaling Pathway

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Abstract

Background: To investigate the effect of polyunsaturated fatty acids ω-3 and ω-6 and their metabolites prostaglandin PGE2 and PGE3 on the proliferation, invasion and neovascularization of gastric cancer.

Methods: RT-PCR and ELISA were used to detect the gene and protein expression of COX-1 and COX-2 in gastric cancer cell lines, respectively. The effects of ω-3, ω-6, PFG2 and PEG3 on the proliferation, invasion and neovascularization of gastric cancer cells were detected by cell proliferation, invasion and neovascularization assay in vitro. COX-2 siRNA was synthesized by short gene interfering RNA (siRNA) technique and transfected into gastric cancer cells, and the expression of COX-2 protein in gastric cancer cell lines was detected again by Western blot. The effects of COX-2 gene silencing on proliferation, invasion and neovascularization of gastric cancer cells were detected by WST-1 assay, transwell chamber assay and gastric cancer neovascularization assay, respectively.

Results: COX-2 was only expressed in MKN74 and MKN45 cell lines, while COX-1 was expressed in four gastric cancer cell lines. In gastric cancer cell lines with positive COX-2 expression, ω-6 and PEG2 could significantly enhance the proliferation, invasion and neovascularization of gastric cancer cells, and after transfection with COX-2 siRNA, the effects of ω-6 and PEG2 on enhancing the proliferation, invasion and neovascularization of gastric cancer cells were significantly attenuated; ω-3 and PEG3 could inhibit the proliferation, invasion and neovascularization of gastric cancer cells. In gastric cancer cell lines with negative COX-2 expression, ω-6 and PEG2 had no significant effect on the proliferation, invasion and neovascularization of gastric cancer; ω-3 and PEG3 could significantly inhibit the proliferation, invasion and neovascularization of gastric cancer.

Conclusion: ω-6 PUFAs reinforce the metastatic potential energy of gastric cancer cells via COX-2/PGE2; ω-3 PUFAs inhibit the metastatic potential energy of gastric cancer via COX-1/PGE3.

Background

Gastric cancer is one of the common malignant tumors, with an incidence of 17.6/100,000 worldwide, about 1.1 million new cases per year, accounting for 5.6% of all new cases of malignant tumors and ranking fifth, and it also ranks fourth due to 770,000 deaths it causes [1]. The main cause of death in patients with gastric cancer is metastasis, the liver is the most common hematogenous metastatic organ of gastric cancer, and the incidence of liver metastasis in gastric cancer ranges from 17–29% [2, 3]. Patients with liver metastasis from gastric cancer have a very poor prognosis and a 5-year survival rate of less than 10% [4]. Although there are many studies on liver metastasis of gastric cancer, the molecular mechanism of liver metastasis of gastric cancer has not been elucidated so far, and there is no effective treatment in clinical practice. Therefore, it is important to deeply study the mechanism of liver metastasis of gastric cancer and take targeted interventions to improve the survival rate and quality of life of patients with gastric cancer.

Recent studies have confirmed that polyunsaturated fatty acids (PUFAs) are closely related to the occurrence and progression of tumors [5]. PUFAs are a class of fatty acids containing double bonds on the carbon chain [6], which, in addition to providing energy for the body, are also involved in the composition of cell membrane lipids and are important substances in the regulation of cellular metabolism and cell signaling. PUFAs are classified into ω-3 system, ω-6 system, ω-7 system, and ω-9 system according to the position of the first double bond in the carbon chain as counted from the methyl end [7]. Among them, ω-3 and ω-6 PUFAs are the most common. The ω-3 and ω-6 PUFAs are the main components of various biofilm structures, which play an indispensable role in maintaining the normal physiological metabolism of the human body and are essential fatty acids in the human body. The ω-3 PUFAs have good immunomodulatory and immunomodulatory effects and can inhibit local chronic inflammatory responses by regulating the cell microenvironment, stabilizing cell membranes, and regulating cell proliferation and differentiation, which in turn play a role in the prevention and treatment of tumors [8–10]. The applicant's previous studies have shown that, PUFAs can affect the invasion, proliferation and neovascularization of gastric cancer cells, and the role of PUFAs is closely related to their metabolites prostaglandin (PGE) and cyclooxygenase (COX) on the nuclear membrane of tumor cells in vivo, while the expression of PGE and COX is closely related to lymphatic metastasis of gastric cancer [11]. The ω-3 and ω-6 PUFAs, as essential fatty acids in the human body, can play a role in inhibiting tumor invasion by reducing ω-6 PUFAs containing foods and appropriately increasing ω-3 PUFAs containing foods in the daily diet [12].Cyclooxygenase (COX) is an essential enzyme for the synthesis of prostaglandin (PG) and a key rate-limiting enzyme in the initial step of PG synthesis. Cyclooxygenase has two isozymes, COX-1 and COX-2. COX-1 is a structural enzyme that is expressed in most normal tissues, and COX-1 promotes prostaglandin production, thereby maintaining normal human function [13]; COX-2 is an inducible enzyme that is rarely expressed in normal tissues, but often highly expressed in tumor cells, such as melanoma, colon cancer, breast cancer, liver cancer, cervical cancer, esophageal cancer, pancreatic cancer, and gastric cancer [14]. At present, studies have confirmed that dietary polyunsaturated fatty acid is closely related to the occurrence and metastasis of gastric cancer [15]. Among them, ω-6 PUFA (arachidonic acid) can bind to cyclooxygenase 2 (COX-2) to produce prostaglandin (PG) E2 and enhance cancer cell invasion; ω-3 PUFA (eicosapentaenoic acid) can bind to cyclooxygenase 1 (COX-1) to produce PGE3, and inhibit the activity of COX-2, reduce the production of PGE2, and inhibit cancer cell invasion [16].

Currently, there are few studies on the role of ω-3 PUFA and ω-3 PUFA in gastric cancer metastasis and their mechanisms. However, the anti-tumor effect of ω-3 PUFAs and the tumor promoting effect of ω-6 PUFAs are complex processes involving multiple factors, multiple levels and interrelated, and there are still many issues to be elucidated. For this reason, this study focused on exploring the mechanism of ω-3 PUFAs, ω-6 PUFAs and their intermediate metabolites PEG2 and PEG3 on gastric cancer progression and metastasis, elucidating the biological characteristics and mechanism of PUFAs affecting gastric cancer metastasis, exploring the molecular targets affecting gastric cancer metastasis, and providing a theoretical basis and new way for the clinical application of ω-3 and ω-6 PUFAs in the prevention and treatment of gastric cancer.

Materials And Methods

Cell lines and culturing condition

The cell lines derived from human gastric carcinoma were examined: MKN45, MKN74 and NUGC-4 cell lines were obtained from Japanese Riken Cell Bank (Tsukuba, Japan). All cell lines were maintained in RPMI 1640 (Sigma Chemical Co., St. Louis, MO) addited with 10% heat-inactivated fetal bovine serum (FBS). Human umbilical vein endothelial cells (HUVECs) were obtained from Kurabo Co. (Osaka, Japan). HUVECs were maintained in HuMedia-EG2 medium supplemented with 2% FBS, 5 ng/mL basic fibroblast growth factor, 10 μg/mL heparin, 10 ng/mL epidermal growth factor, and 1 μg/mL hydrocortisone according to the supplier's instructions (Kurabo Co.). All cells were incubated at 37°C in a humidified atmosphere of 5% CO2 in air.

RT-PCR Analysis of COX-1and COX-2 mRNA Expression

Total RNA was extracted from gastric cancer cell lines by an Isogen Kit (Nippon Gene, Tokyo, Japan), and quantities were determined spectrophotometrically. The 1μg of total RNA aliquots was reversetranscribed into cDNA using the SuperScript III system (Invitrogen, San Diego, CA) in a PCR Thermal Cycler (model TP3000; Perkin-Elmer, Norwalk, CT). Reaction mixture aliquots (1μL) were used as templates for PCR analysis. Amplification reactions were performed in a DNA Thermal Cycler.

The primer sequences and PCR conditions showed in Table 1. The amplified DNA fragments were resolved by electrophoresis in 1.5% agarose gels containing ethidium bromide.

Enzyme-linked immunosorbent assay for COX-1, 2 proteins measurement

To determinate the COX-1, 2 proteins measurement HUVECs and cells of the three gastric cancer cell lines (MKN74, MKN45, NUGC-4) were seeded at a density of 2×10^5 cells/mL cells into 12-well plates and cultured overnight, following which the medium in each well was replaced and the cells cultured for a further 48 h. Cell numbers were the determined, and the culture media were harvested and microfuged at 1500 rpm for 15 min to remove the particles. The supernatant liquid were frozen at -80°C until use in ELISA assay. The concentration of COX-1, 2 in the supernatants per 2×10^5/mL cells were measured by ELISA kit (R&D Systems) according to the manufacturer's instructions.

Design and synthesis of siRNA and transfection into colon cancer cells

Two specific siRNAs were designed based on the coding region gene sequence of the human COX-2 gene, and the COX-2 siRNA sequences were 5'-GCCAAGGAGUGC UAAAGAA-3' and 5'-CCAACACAGAAAUUGU-3'; and the control siRNA sequences were 5'-UUCUCCGAACGUGUCACGUTT-3' and 5'-ACGUGACACGU CGGAGAATT-3'. After counting the two kinds of gastric cancer cells, they were seeded in cell culture dishes with a diameter of 35 mm at a density of 2×10^5 cells/well and cultured overnight, followed by

replacement with fresh culture medium containing 10% fetal bovine serum without antibiotics for another 24 h before transfection. 500 μL of Opti-MEM® I reduced serum medium was used to dilute 200 nmol/L COX-2 siRNA or control siRNA, while 10 μL of LipofectAMINE^{TM}2000 was diluted with the same reagent. After standing at room temperature for 5 min, the two were quickly mixed and then allowed to stand at room temperature for 20 min. Then, the mixture of siRNA-Lipofect AMINE^{TM}2000 (diluted by adding 1 mL culture medium) at the concentration of 100 pmol/L was directly added to each cultured cell, followed by mixing well and placement in an incubator at 37°C for transfection. After 48 h of transfection, cells were collected for Western blotting assay to verify the silencing effect of COX-2 gene.

Western blot was used to detect the effect of COX-2 gene silencing on COX-2 protein expression in gastric cancer cells

COX-2-expressing gastric cancer cells at 1 × 10^6 cells/mL in the logarithmic growth phase were aspirated, the cells were lysed with Cell-Lysis buffer, total protein was extracted and centrifuged at 500 × g for 15 min at 4°C, and then the supernatant was collected to determine the protein concentration using the Bradford method. A total of 30 μg of sample protein was mixed well with an appropriate amount of solid-phase pH gradient strip solution, and electrophoresis was performed using a 10% SDS-PAGE gel for 2 h. The proteins on the gel plate after electrophoresis were transferred to PVDF membranes, followed by blocking with 5% skimmed milk powder for 2 h at room temperature and washing the membranes three times with TBST buffer. The membrane was immersed in blocking solution containing rabbit anti-human COX-2 monoclonal antibody (dilution ratio of 1:800), followed by reaction at room temperature for 2 h and the membrane washing three times with TBST solution; then the membranes were immersed in horseradish peroxidase-labeled goat anti-rabbit or anti-mouse IgG (dilution ratio of 1:2000) solution, respectively, followed by placement overnight at 4°C, washing three times with TBST buffer, color development by ECL method and scanning by computer. The gray value of the target band was determined using the image analysis software Image J. The relative expression level of the target protein was expressed as the ratio of the gray value of the target protein and the internal reference protein band, followed by plotting after statistical analysis.

WST-1 assay examined the effects of ω-3 PUFA, ω-6 PUFA, PEG2 and PEG on proliferation of gastric cancer cell

Gastric cancer cells expressing and not expressing COX-2 in the logarithmic growth phase were taken, and each group of cells was added to a 96-well culture plate at a density of 1 × 10^4 cells/100 μL, respectively, with five replicate wells in each group, and the cells were cultured overnight to adhere and grow. The culture medium was replaced, and after another 72 h of culture, 100 μL CellTiter 96 aqueousone solution reagent was added to each well and placed in a 37°C incubator for 4 h of reaction, and then the absorbance (D value) of the cells in each well at a wavelength of 490 nm was measured with a microplate reader to reflect the proliferation of the cells. The cell growth curve was plotted with time as the abscissa and the average D-ordinate.

Transwell chamber assay examined the effects of ω-3 PUFA, ω-6 PUFA, PEG2 and PEG on invasion of gastric cancer cell

The *in vitro* invasion assay was performed using BioCoat Matrigel Invasion Chambers (Becton Dickinson, Bedford, MA) according to the manufacturer's instructions. Briefly, gastric cancer cells expressing and not expressing COX-2 in the logarithmic growth phase were used to adjust the single cell density to 2.0 × 10^5cells/mL with different culture media containing 5% fetal bovine serum, the cells were seeded into transwell chambers with Matrigel at the bottom, and the chambers were placed in 24-well cell culture plates, with 5 replicates for each group of cells. After 12 h of culture, chambers were removed, and cells that did not cross the membrane were wiped off with a cotton swab, rinsed three times with PBS, fixed in 4% formaldehyde solution for 5 min and stained with Diff-Quick's solution. Result interpretation: The number of penetrating cells within five fields was counted separately for each filter membrane under a light microscope (×100), and the average number of cells per field was calculated, in order to reflect the invasive ability of the cells.

ω-3 PUFA,ω-6 PUFA□PEG2 and PEG3 influence angiogenesis in vitro

HUVEC and human fibroblasts were seeded in a 24-well culture plate in a certain proportion and cultured together. On the second day, the culture medium was replaced and ω-3 PUFA, ω-6 PUFA, PEG2 and PEG at different concentrations were added, and then a transwell chamber with 0.45 μm microwells was placed in the 24-well culture plate. The bottom of the chamber was covered with polycarbonate membrane and transwell chambers with the wells contained 2×10^4/ml gastric cancer cells to form a co-culture system. The culture medium was changed every day, and after 11 days of co-culture, the culture medium was removed and the culture wells were washed three times with PBS solution and fixed with formaldehyde for 30 min, followed by vascular staining with CD31 antibody and then by natural drying. Neovascularization in 10 different areas was photographed under a microscope and then each photograph was analyzed with vascular analysis software (Kurabo Co.); the total area or length of blood vessels in each photograph was calculated, and the standard amount of new blood vessels was expressed in pixels.

Angiogentic activity during co-cultivation with gastric cancer cells, and regulation of polyunsaturated fatty acid

To further investigate the effect of different gastric cancer cells on tubule formation by HUVECs. Transfected or non-transfected gastric cancer cells (MKN45 or NUGC-4) were co-cultured with HUVECs and fibroblasts using a double chamber method in 24-well plates. MKN45 or NUGC-4 cells (2×10^4 cells/mL) were planted in transwell chambers, consisting of polycarbonate membranes with 0.45μm pores, and the cells adhere overnight. Then, the transwell chambers were placed in the HUVEC/fibroblast co-incubate in 24-well plates, and the medium exchanged every two days. Cells were incubated for 12 days, and HUVEC tubule formation was determined as above described.

Statistical analysis

Statistical comparisons were made using Student's *t*-test for paired observations or one-way ANOVA with a *post hoc* test (Dunnett multiple comparison) for multiple group comparisons. Statistical significance was indicated by $p < 0.05$. Data are presented as mean ± SD. Each experimentwas carried out in triplicate.

Results

Expression of COX-1 and COX-2 in gastric cancer cells

COX-1 and COX-2 mRNA levels were determined in all gastric cancer cell lines by RT-PCR. The results showed that all gastric cancer cell lines were expressed COX-1 mRNA. COX-2 mRNA was detected in MKN45 and MKN74 cells (Fig. 1). Consistent with RT-PCR observations, COX-1 and COX-2 protein secreted into culture liquid supernatant was measured by ELISA. COX-2 proteins are 390.16 ± 22.19 and 423.05 ± 17.73 pg/mL/2×10^5 cells in MKN74 and MKN45 cultured supernatant, respectively, but not detected in NUGC-4 cells cultured supernatant. The secreted COX-1protein was determined in cultured liquid supernatant of MKN45, MKN74 and NUGC-4 cells. The secreted level of COX-1 in MKN74 (560.72 ± 43.09) and MKN45 (623.15 ± 38.59) is higher than NUGC-4 cells (25.93 ± 21.05). MKN74 and MKN45 respectively compared with control, *P* value less than 0.01 (Fig. 2).

Effect of COX-2 siRNA transfection on secretion of COX-2 proteins in gastric cancer cells

MKN45 and MKN74 gastric cells were transfected with siRNA which specifically targets for COX-2 gene, the expressions of COX-2 proteins was detected by immunoblotting. The results revealed that COX-2 gene silencing led to a near total loss of COX-2 expression, compared with the untransfected and control siRNA groups and positive control β-actin, the expressions of COX-2 proteins in MKN45, MKN74 cancer cells were significantly inhibited (Fig. 3A). MKN45 and MKN74 cells transfected with COX-2 siRNA there were significant inhibited expession of PGE2 (Fig. 3B).

Effects of ω-3 PUFA, ω-6 PUFA, PEG2 and PEG3 on gastric cancer cell proliferation

In gastric cancer cell line MKN45 expressing COX-2. The cell proliferation curve showed that there was difference in the proliferation of MKN45 gastric cancer cells following treatment with ω-6 and PEG2 after 24 h cultured; the proliferation of MKN45 cells in 0 and 50 μM of ω-6 and PEG2 group was significantly enhanced than those in control groups after 48, 72, 96, 120 h (**P* < 0.01, compared with control groups), and meanwhile, the proliferation of MKN45 cells were significantly inhibited by ω-3 and PEG3 in a concentration-dependent manner (compared with the control groups, respectively, **P* < 0.01, as shown in Fig. 4A and Fig. 4B). After being transfected with COX-2 siRNA for 24 h, MKN45 cells were and the

proliferation of cancer cells was measured by WST-1 assay. The results showed that after COX-2 gene silencing, the proliferation of MKN45 cells were significantly inhibited (compared with control groups, *P < 0.01). As the same time, ω-3 and PEG3 could also inhibited the proliferation of MKN45, but ω-6 and PEG2 there were no significant change (compared with COX-2 siRNA groups, *P< 0.01, as shown in Fig. 4C and 4D). The proliferation of NUGC-4 cells was significantly inhibited by the presence of ω-3 and PEG3. Moreover, there were not significant change in the presence of ω-6 and PEG2 (compared with the control groups respectively, *P< 0.01, Fig. 4E and 4F).

Effects of ω-3 PUFA, ω-6 PUFA, PEG2 and PEG3 on gastric cancer cell invasion

The results of trans-well invasion assay showed that the COX-2 positive cell MKN45, ω-3 and PEG3 inhibited the invasion of MKN45 cells in a dose-dependent manner, and ω-6 and PEG2 there was not significant effect on invasive capability of MKN45 cells (*P< 0.01, Fig. 5A). MKN45 cells transfected with COX-2 siRNA, the invasive ability of MKN45 there are not significant changes in the presence of ω-6 and PEG2. PGE3 and ω-3 could significant reduced invasion ability of MKN45 cells compared with the control (*P< 0.01, as shown in Fig. 5B). The invasion of gastric cancer cell NUGC-4 was inhibited by ω-3 and PEG2 in a concentration dependent manner compared with the control (*P< 0.01), and ω-6 and PGE2 there are not significantly influenced the invasiveness of NUGC-4 cells (*P< 0.01, as shown in Fig. 5C).

Effect of ω-3 PUFA, ω-6 PUFA, PEG2 and PEG3 on HUVECs tube formation

To measure the role of ω-3 PUFA,ω-6 PUFA□PEG2 and PEG3 in tube formation by HUVEC, we examined the effect of ω-3 PUFA, ω-6 PUFA, PEG2 and PEG3 on HUVEC tube formation using an angiogenesis assay. HUVECs tube formation was significantly promoted in a dose-dependent manner following presence of ω-6 PUFA and PEG2 (compared with control *P< 0.01). On the contrary, HUVEC tube formation was also significantly inhibited by ω-3 PUFA and PEG3 compared with control (*P < 0.01, compared with control, Fig. 6A).

Effect of gastric cancer cells and presence of ω-3 PUFA, ω-6 PUFA, PEG2 and PEG3 on tube formation

In order to further pursue the effect of PUFA and its metabolites PGE2, 3 on angiogenesis, and focusing on the interaction between tumor cell and stromal cell by characterizing the angiogentic activity in co-cultured system consisting of HUVECs, fibroblasts, and MKN45 or NUGC-4 gastric cancer cells. HUVEC tube formation was significantly enhanced by coculture with MKN45 cells compared with NUGC-4 cells (*P< 0.01). In MKN45 co-cultured system, ω-6 PUFA and PEG2 significantly promoted HUVEC tube formation in a dose-dependent manner (compared with MKN45 only, *P< 0.01), but this promoted action was inhibited by COX-2 siRNA. Furthermore, HUVEC tube formation were decreased by ω-6 PUFA and PEG2 in MKN45 and NUGC-4 co-cultured system (compared with control, *P < 0.01, as shown in Fig. 6B and 6C).

Discussion

The ω-3 and ω-6 polyunsaturated fats are the most common polyunsaturated fats. They play an indispensable role in maintaining the normal physiological metabolism of the human body and are essential fatty acids for the human body. ω-3 PUFAs mainly include: Alpha linolenic acid (ALA) and eicosapentaenoic acid (EPA); ω-6 PUFAs mainly include: linoleic acid (LA) and arachidonic acid (AA). The conversion and utilization of ω-3 PUFAs and ω-6 PUFAs in the human body is a complex process: Cyclooxygenase can promote the conversion of AA and EPA into prostaglandin, thromboxane A (TXA) and other products [17]. Recent studies have shown that, ω-3 PUFAs have an inhibitory effect on the occurrence and progression of malignant tumors, while ω-6 PUFAs have a promoting effect; their mechanism of action may be related to the regulation of cyclooxygenase and prostaglandin synthetase (PGES), the main enzymes in the function and reaction of prostaglandin E3, and prostaglandin E2, metabolites of ω-3 PUFAs and ω-6 PUFAs [18, 19]. ω-6 PUFAs bind to COX-2 in the human body to generate PGE2, while PGE2 can induce cell proliferation and stimulate the expression of BLC-2 protein (BLC-2 protein inhibits apoptosis) to imbalance cell proliferation and apoptosis and promote the occurrence of tumors; PGE2 can also promote extracellular matrix degradation and produce thromboxane to promote platelet aggregation, which is conducive to the invasion and metastasis of cancer cells. While ω-3 PUFAs produce PGE3 after binding to COX-1, PGE3 can inhibit the production of PGE2, and can inhibit phospholipase A2 (PLA2), phosphatidylinositol-specific phospholipase C (PI-PLC), nuclear factor-κB and COX-2 activities, which in turn reduce the proliferation and invasion of tumor cells and play a role in inhibiting the growth and metastasis of malignant tumors [20–24].

Previously, we retrospectively analyzed the clinical data of 115 patients with radical resection of colorectal cancer and found that the positive rate of prostaglandin E2 expression in colorectal cancer tissues was 87.8%, which was significantly higher than that in normal colorectal mucosal tissues, and correlated with the depth of invasion, lymph node and liver metastasis of colorectal cancer; it was positively correlated with the expression of cyclooxygenase 2; the 5-year cumulative survival rate was 63.6% in patients with double negative PGE2 and COX-2, and 37.8% in patients with double positive expression [25]. Thus, PGE2 and COX-2 downstream of ω-6 PUFAs can be used as important markers for clinical evaluation of metastasis of colorectal cancer and are important for patient prognosis assessment.

ω-6 PUFAs rely on the catalytic effect of COX-2 to generate PGE2 in the body, which can stimulate the expression of Bcl-2 protein to imbalance cell proliferation and apoptosis and thus promote tumor progression. PGE2 can also enhance the degradation of extracellular matrix, which further promotes the invasion and metastasis of cancer cells [19]. ω-6 PUFAs in the microenvironment can up-regulate PGE2 production in colorectal cancer cells and promote the transformation of myeloid-inhibiting cells (MDSC) into M2 macrophages [26]; hypoxia-inducible factor-1α (HIF-1α) secreted by M2 macrophages promotes tumor invasion and metastasis by inducing the expression of COX-2 and PGE2 in stromal cells and tumor cells in the hypoxic microenvironment [27]. HIF-1α derived from M2 macrophages elevates the secretion of CXCR4 in cancer cells to promote colorectal liver metastasis [28]. While ω-3 PUFAs produce

prostaglandin E3 (PGE3) in response to COX-1, PGE3 inhibits the proliferation and invasion of tumor cells by down-regulating the expression of phospholipase A2 phosphatidylinositol-specific phospholipase C (PI-PLC), nuclear factor (NF-κB) and COX-2; PGE3 can also inhibit the metastasis of colorectal cancer by down-regulating colorectal cell adhesion factors and the formation of new blood vessels, and ω-3 PUFAs upstream of PGE3 have potential application value in the treatment of colorectal cancer as a target of anti-tumor angiogenesis [29, 30].

On the basis of previous studies, this experiment focused on exploring the role of ω-3, ω-6, PGE2, and PGE3 in gastric cancer metastasis, and the results showed that, the expression of PGE2 and COX-2 in gastric cancer cell lines was closely related to their liver metastasis, that is, PGE2 and COX-2 were expressed in cell lines MKN45 and MKN74 with high liver metastasis, while PGE3 and COX-1 were expressed in cell lines with high and low-high liver metastases. The ω-6 PUFAs in the tumor microenvironment are converted into PGE2 that promotes tumor growth by binding to COX-2 in gastric cancer cells, and PGE2 can progressively enhance the proliferation, invasion and neovascularization of gastric cancer cells over the increase of concentration. On the one hand, ω-3 PUFAs can inhibit the activity of COX-2 and reduce the production of PGE2, thereby inhibiting the proliferation, invasion and neovascularization of tumor cells; on the other hand, ω-3 PUFAs can compete with ω-6 PUFAs to bind to COX-1 to produce PGE3, which can significantly inhibit the proliferation, invasion and neovascularization of tumor cells. In addition, after silencing COX-2 gene, ω-6 inhibits the proliferation, invasion and neovascularization of gastric cancer cells. In order to detect the effect of unsaturated fatty acids in tumor microenvironment on gastric cancer neovascularization, we used gastric cancer cells and stromal cells to construct a co-culture system to culture gastric tumor neovascularization in vitro, and detected the effect of gastric cancer cells with different expression of COX-2 on neovascularization. The effect of MKN45 on the neovascularization of HUVEC in COX-2 positive gastric cancer cells was significantly stronger than that in COX-2 negative gastric cancer cells NUGC-4; ω-6 PUFA could promote the neovascularization of COX-2 positive gastric cancer cells, while ω-3 could inhibit the neovascularization of COX-1 positive gastric cancer cells. The above results demonstrated that the effect of ω-3 and ω-6 PUFA on gastric cancer metastasis was mainly achieved by regulating the physiological functions of COX and PGE.ω-6 enhances the metastatic potential energy of gastric cancer cells by being converted into PGE2 that promotes tumor growth after binding to COX-2; ω-3 can inhibit the activity of COX-2 and reduce the production of PGE2 on the one hand, thereby inhibiting the metastatic potential energy of gastric cancer; on the other hand, ω-3 can compete with ω-6 to bind to COX-1 to produce PGE3 so as to inhibit the metastatic potential energy of gastric cancer. Taking PUFA and its intermediate metabolites as interference factors, the in vitro simulation experiment and exploration experiment of tumor internal environment using co-culture system can more objectively and truly reproduce and observe the effect of PUFA on the microenvironment of gastric cancer cells, which plays an irreplaceable important role in understanding the specific growth, invasion and metastasis mechanism of tumor cells, and also lays an important theoretical foundation for the next in vivo experiment and clinical trials.

At present, there are few studies on ω-3 PUFA, ω-6 PUFA and PGE2, 3 in gastric cancer, and the mechanism and clinical significance of the effect of polyunsaturated fatty acids on the occurrence,

development and metastasis of gastric cancer remain to be more deeply and comprehensively studied. Through further in vitro and in vivo experiments at a later stage, our team will find a suitable ratio of two fatty acids or a suitable concentration of COX-2 inhibitor, in order to inhibit the invasion and metastasis of tumor cells, and finally provide a new way for clinical prevention and treatment of gastric cancer.

Conclusion

ω-6 PUFAs reinforce the metastatic potential energy of gastric cancer cells via COX-2/PGE2; ω-3 PUFAs inhibit the metastatic potential energy of gastric cancer via COX-1/PGE3

Abbreviations

ω-3 PUFAs
ω-3 polyunsaturated fatty acids
ω-6 PUFAs
ω-6 polyunsaturated fatty acids
COX-1
cyclooxygenase 1
COX-2
cyclooxygenase 2
PGE2
prostaglandin E2
PGE3
prostaglandin E2
HUVEC
human umbilical vein endothelial cell
PG
prostaglandin
ALA
Alpha linolenic acid
EPA
eicosapentaenoic acid
LA
linoleic acid
AA
arachidonic acid
TXA
thromboxane A
PGES
prostaglandin synthetase

PLA2
phospholipase A2
PI-PLC
phosphatidylinositol-specific phospholipase C
NF-κB
nuclear factor

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have read the manuscript and approved the final version.

Competing interests

The authors declare that they have no competing interests.

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Contributions

JCM, CSZ and WQL designed the project, JCM and CSZ wrote the manuscript, LL, JD and CWP finished the Figs. 1 and 2, BLC finished Fig. 3, YZC finished Fig. 4, JCM and YPW finished the Fig. 5 and Fig. 6, all authors reviewed the manuscript. The authors read and approved the final manuscript.

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Tables

Table 1 is not available with this version.

Figures

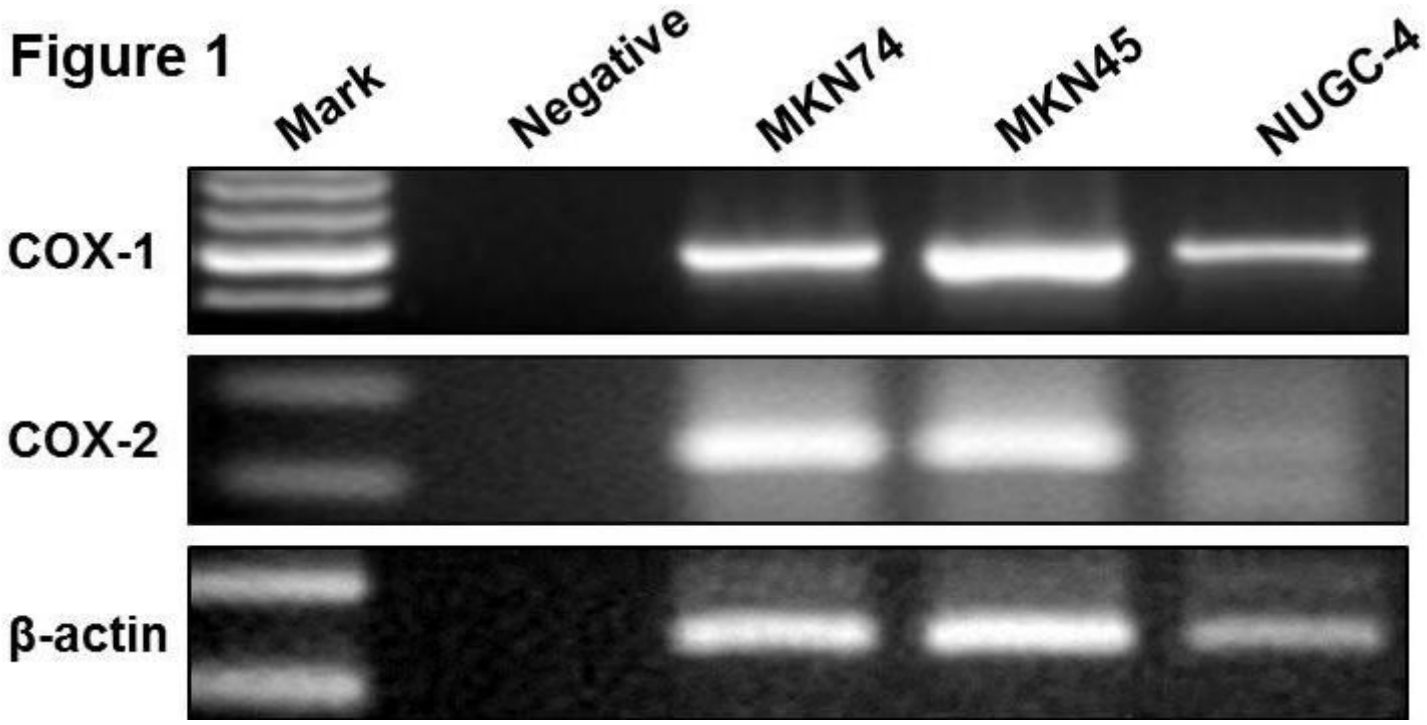

Figure 1

The Expression of COX-1 and COX-2 in gastric cancer cells COX-1 and COX-2 mRNA in gastric cancer cell lines were measured by RT-PCR. PCR products stained with ethidium bromide were s displayed to 1.5% agarose gel electrophoresis. β-actin served as a loading control.

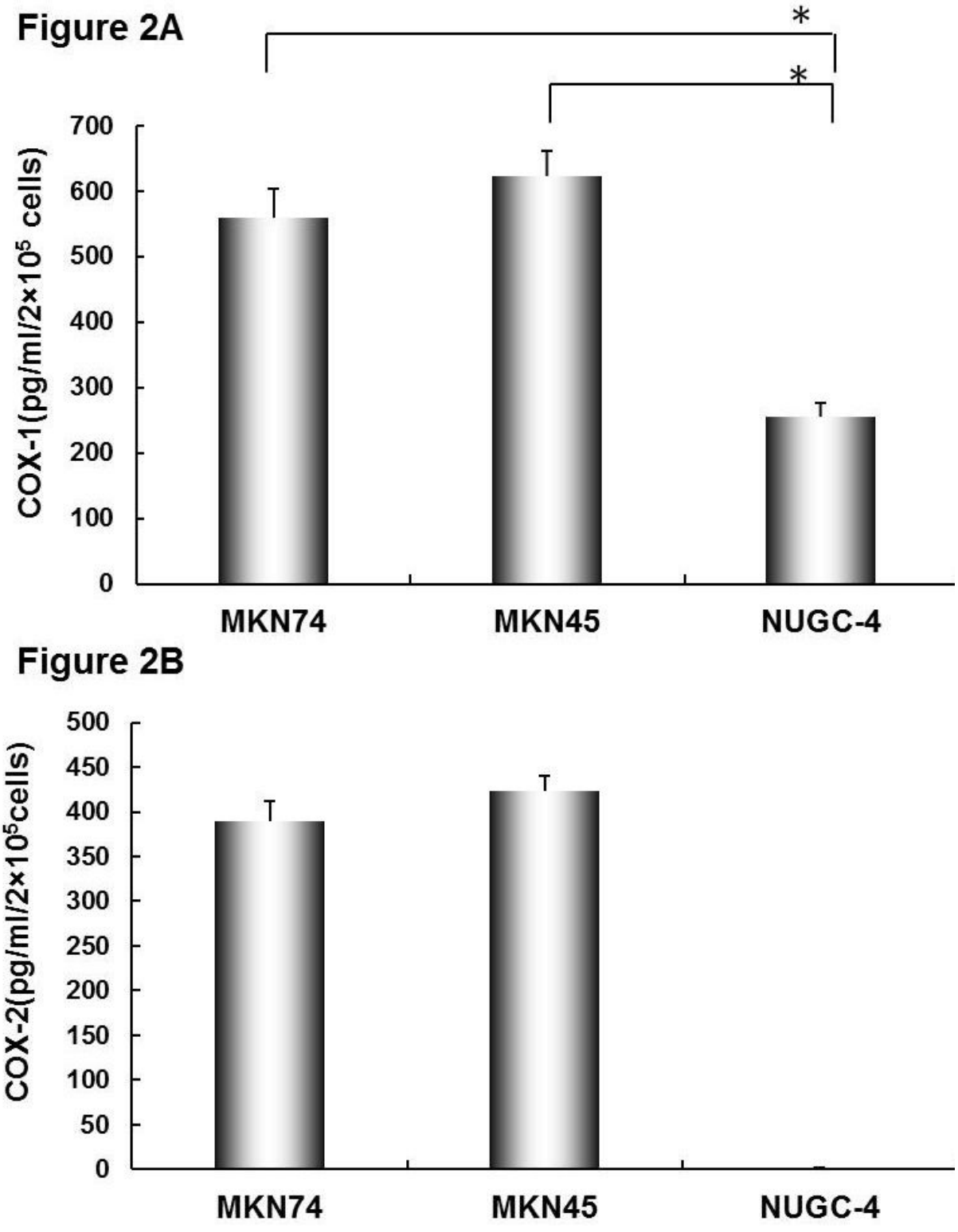

Figure 2

The secreted levels of COX-1 and COX-2 in gastric cancer cell lines COX-1 and COX-2 proteins concentration in MKN74, MKN45 and NUGC-4 cells culture medium was determined by ELISA. The values are expressed as mean±SD. Multiple comparisons were analysised by one-way ANOVA followed by student-Newman-Keuls test, *P<0.01.

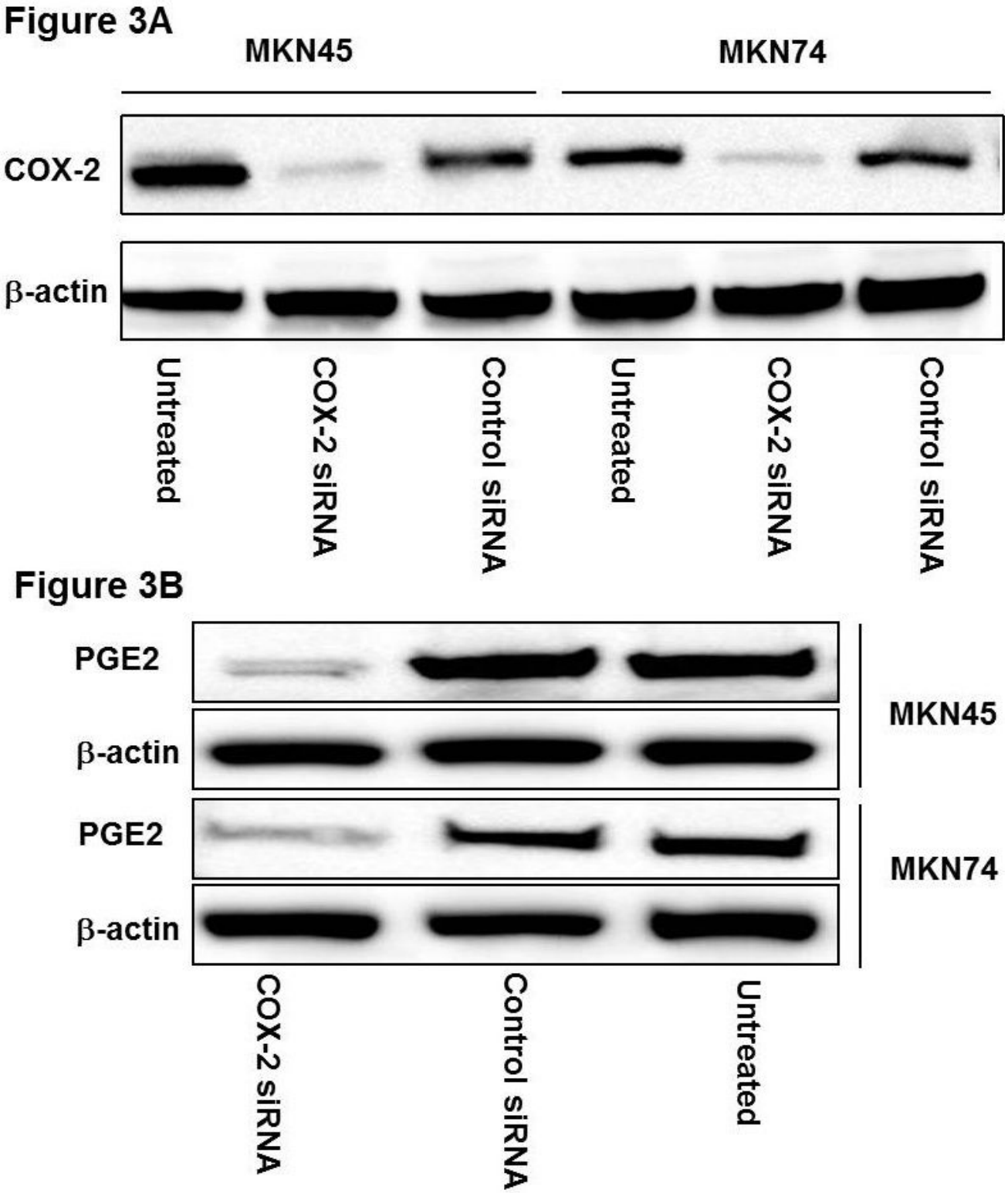

Figure 3

The expression of COX-2 protein in gastr cancer cell line after silencing of CXCL12 gene Knockdown of COX-2 by COX-2 siRNA was confirmed by immunoblotting in expressed COX-2 gastric cancer cell lines: MKN74 and MKN45. COX-2 siRNA duplex oligoribonucleotides were transfected into cells for 48 h; the proteins were extracted and then western blot (A). After transfected with COX-2 siRNA in MKN45 and MKN74 cells there were significant inhibited expession of PGE2 (B).

Figure 4

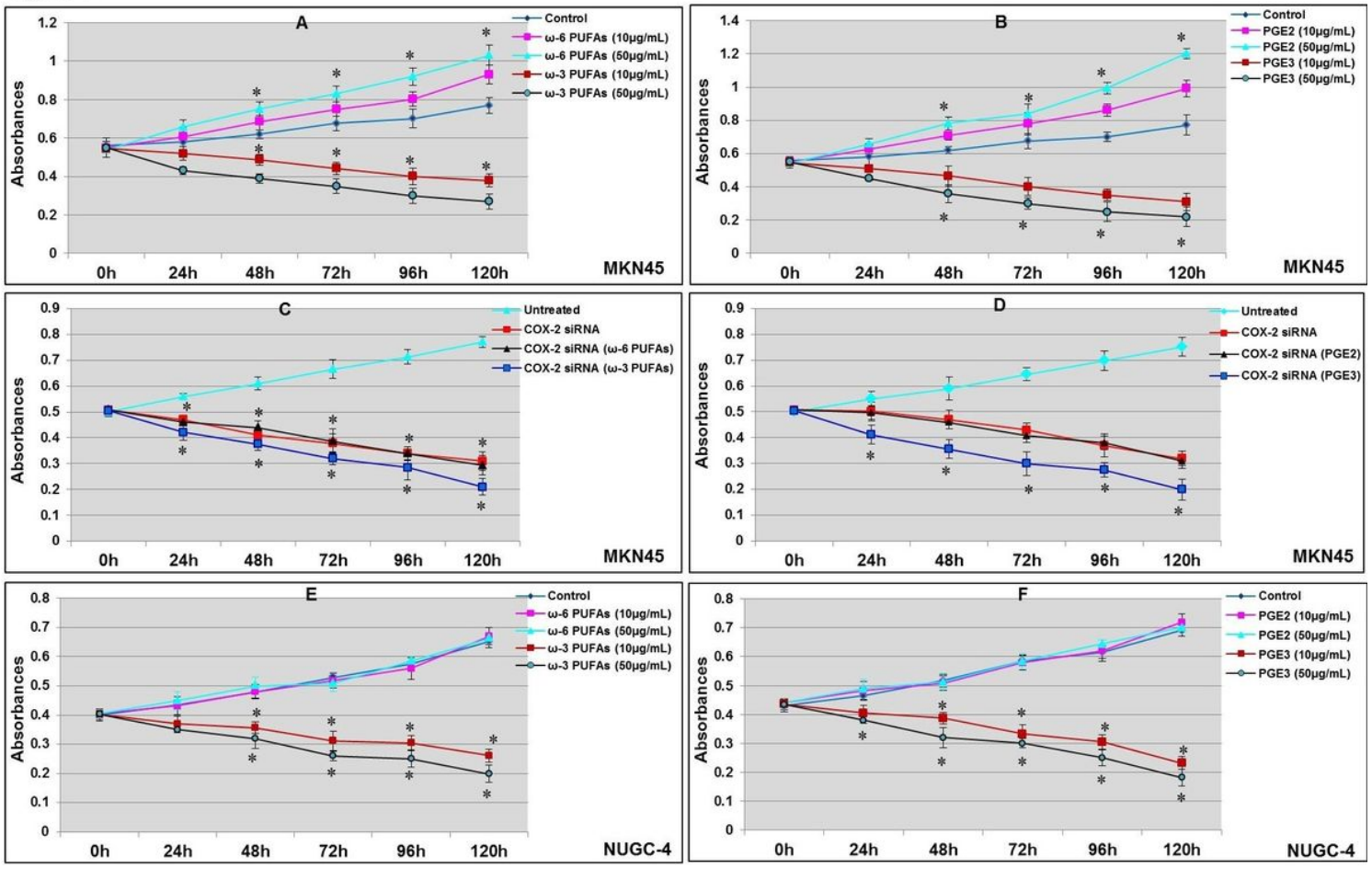

Figure 4

Effects of ω-3 PUFA, ω-6 PUFA, PEG2 and PEG3 on proliferation of gastric cancer cell MKN45 gastric cancer cells incubated for 24 h; the proliferation of cancer cells was measured by WST-1 assay. The proliferation of MKN45 cells in ω-3 PUFA (A) and PGE3 (B) were significantly inhibited (compared with control groups, *P<0.01); ω-6 PUFA (A) and PGE2 (B) were significantly promoted (compared with control groups, *P<0.01). MKN45 cells transfected with COX-2 siRNA, the proliferation of MKN45 cells were significantly inhibited (compared with control group, *P<0.01). PEG3 and ω-3 could also inhibited the proliferation of MKN45 (compared with COX-2 siRNA group, *P<0.01). But ω-6 and PEG2 there were no significant change (C and D). The proliferation of NUGC-4 cells was significantly inhibited by the presence of ω-3 and PEG3. Moreover, there were not significant change in the presence of ω-6 and PEG2 (compared with the control groups respectively, *P<0.01, Figure 4E and 4F). Multiple comparisons used the method of one-way ANOVA and followed by the SNK test. Values are expressed as mean ± SD. Bars indicated SD, *P<0.01.

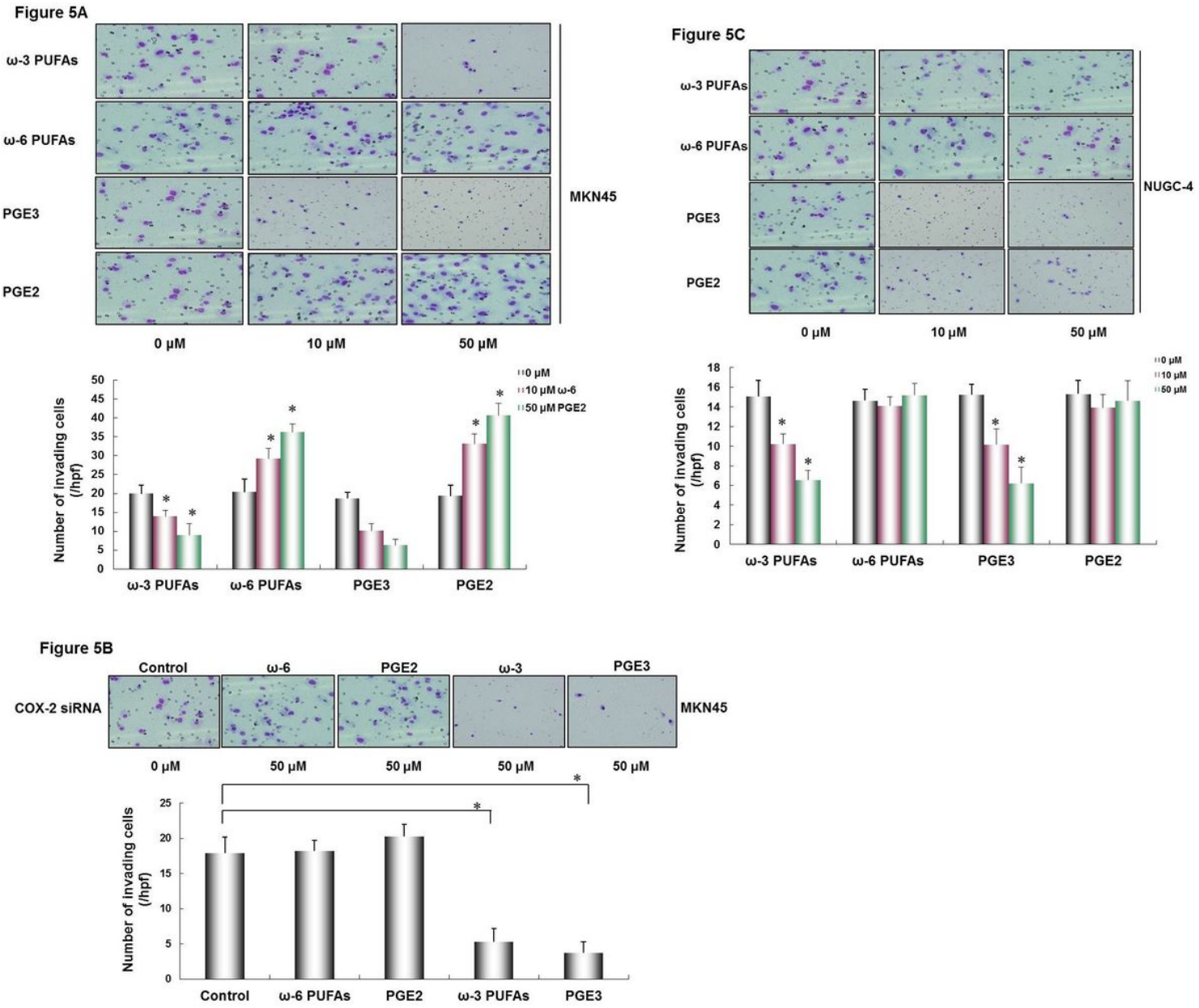

Figure 5

Effects of ω-3 PUFA, ω-6 PUFA, PEG2 and PEG3 on gastric cancer cell invasion PEG3 and ω-3 inhibited the invasion of MKN45 cells, and ω-6 and PEG2 there was not significant effect on invasive ability of MKN45 cells (A). MKN45 cells transfected with COX-2 siRNA there are not significant changes in the presence of ω-6 and PEG2 in MKN45 cells. PGE3 and ω-3 could significant reduced invasion capability of MKN45 cells compared with the control (B). The invasion of NUGC-4 cells was inhibited by ω-3 and PEG2 in a concentration dependent manner compared with the control (*P<0.01), and ω-6 and PGE2 there are not significantly influenced the invasiveness of NUGC-4 cells (C). Multiple comparisons used the method of one-way ANOVA and followed by the SNK test. Columns, relative invading number. Bars indicate SD, *P<0.01.

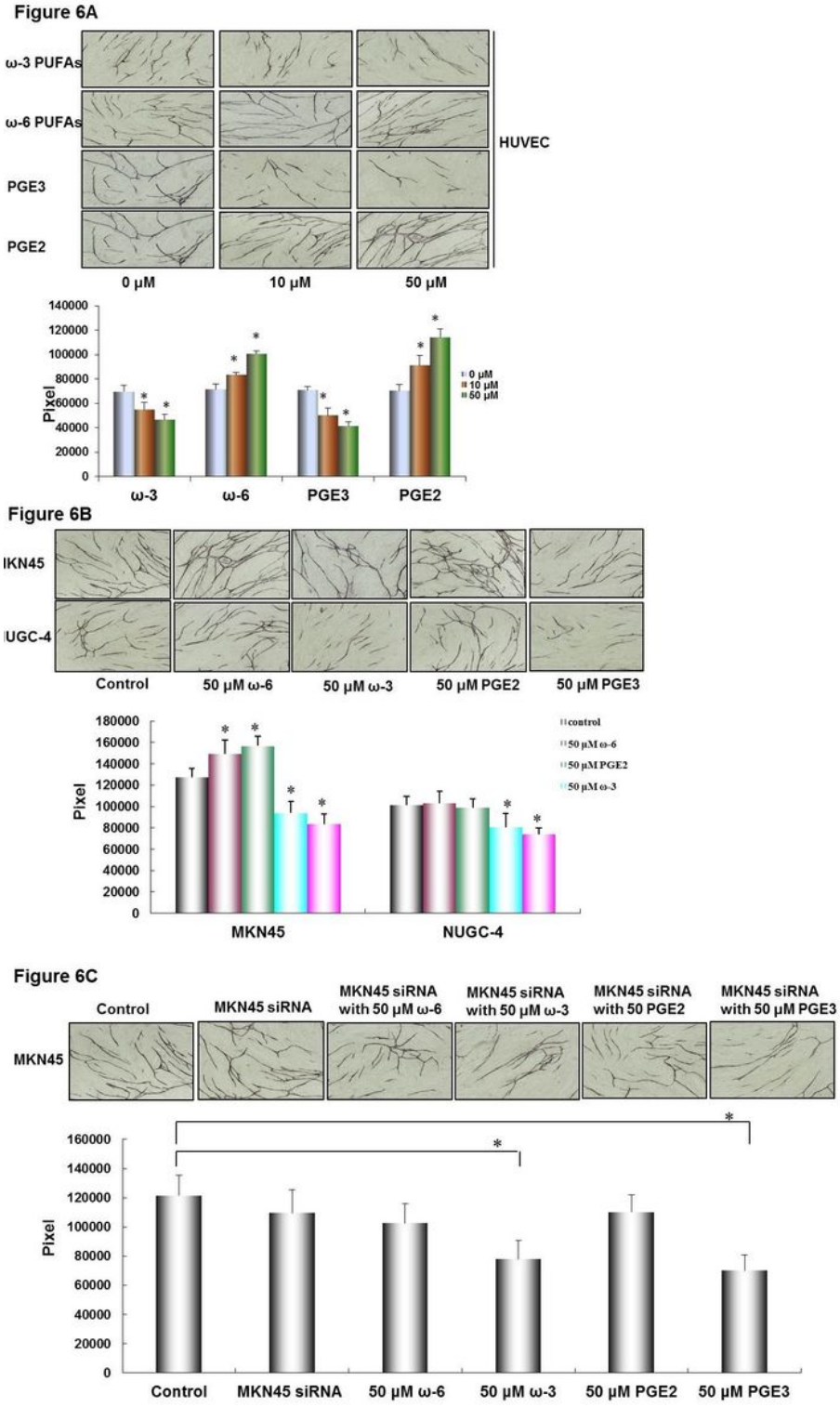

Figure 6

Effect of ω-3, ω-6 PUFA, PEG2, PEG3 and gastric cancer cells on HUVECs tube formation HUVECs tube formation was significantly promoted by ω-6 PUFA and PEG2. On the contrary, HUVEC tube formation was also significantly inhibited by ω-3 PUFA and PEG3 (A). HUVEC tube formation was significantly enhanced by coculture with MKN45 cells compared with NUGC-4 cells. In MKN45 co-cultured system, ω-6 PUFA and PEG2 significantly promoted HUVEC tube formation, but this promoted action was inhibited by

COX-2 siRNA. HUVEC tube formation was decreased by ω-6 PUFA and PEG2 in MKN45 and NUGC-4 co-cultured system (B and C). Multiple comparisons used the method of one-way ANOVA and followed by the SNK test. Bars indicate SD, *P<0.01.