

Celecoxib suppresses fibroblast growth factor-2 expression in pancreatic ductal adenocarcinoma PANC-1 cells

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Received January 18, 2016; Accepted March 16, 2016

DOI: 10.3892/or.2016.4924

Abstract. The inhibition of cyclooxygenase (COX)-2 has been reported to suppress growth and induce apoptosis in human pancreatic cancer cells. Nevertheless, the precise biological mechanism of how celecoxib, a selective COX-2 inhibitor, regulates the growth and invasion of pancreatic tumors is not completely understood. It has been shown that fibroblast growth factor-2 (FGF-2) and its receptor levels correlate with the inhibition of cancer cell proliferation, migration and invasion in pancreatic ductal adenocarcinoma (PDAC). Therefore, the aim of the present study was to examine the hypothesis that the antitumor activity of celecoxib in PDAC may be exerted through modulation of FGF-2 function. In the present study, we evaluated the effects of celecoxib on the proliferation, migration, invasion and apoptosis of the PANC-1 cell line. Western blotting and quantitative real-time polymerase chain reaction (qRT-PCR) were used to examine the expression of FGF-2, FGFR-2, ERK1/2 and MMPs. In the present study, FGF-2 and FGFR-2 were expressed in PANC-1 cells and FGF-2 exerted a stimulatory effect on phosphorylated extracellular signal regulated kinase (p-ERK) expression. Celecoxib treatment suppressed FGF-2 and FGFR-2 expression and decreased MMP-2, MMP-9 and p-ERK expression in the PANC-1 cells. Furthermore, celecoxib treatment caused the resistance of PANC-1 cells to FGF-2 induced proliferation, migration and invasion ability, as well as the increase in their

apoptotic rate. Our data provide evidence that targeting FGF-2 with celecoxib may be used as an effective treatment in PDAC.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive human malignancies and it is the fourth leading cause of cancer-related death in the US (1). Due to its late presentation, early local invasion and metastatic potential long-term survivors are rare. Clinical trials performed in PDAC patients using different chemotherapeutic agents have only slightly improved their survival over the last few decades, and the overall 5-year relative survival rate remains at 6.9% (2,3). In addition, more than 80% of patients have advanced regional disease or distant metastasis at the time of diagnosis and tumors are often inoperable. Moreover, those patients who undergo surgical tumor resection exhibit a high incidence of local recurrence, peripheral organic metastasis and peritoneal dissemination (4).

At the molecular level, a high percentage of PDACs overexpress a number of growth factors and their receptors, including the epidermal growth factor family, transforming growth factor family and fibroblast growth factor family (5). Fibroblast growth factors (FGFs) are comprised of ~20 molecules with a wide range of biological actions. They have the ability to stimulate angiogenesis, and are involved in cell differentiation, migration, tissue repair and regeneration (6). The two most extensively studied FGFs are FGF-1 and FGF-2 (7). FGF-2 is the prototypic heparin-binding protein with growth, anti-apoptotic and angiogenic activity (8). It is overexpressed in PDAC (9), and the FGF-2 receptor has been reported to correlate with tumor metastasis, stage and retroperitoneal invasion (10). In addition, low expression of FGF-2 has been associated with longer post-operative survival in PDAC patients (11). In general, FGF-2 can exert its biological effects by binding to FGFR-2, which leads to the activation of a number of signaling cascades; in which the most dominant is the MAPK/ERK pathway (12).

Cyclooxygenases (COXs) are key enzymes in the synthesis of prostaglandins. There are two types of COX isoenzymes: COX-1, which is expressed in many normal tissues and responsible for many physiological functions; and COX-2, an inducible prostaglandin synthase that is upregulated in

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Abbreviations: COXs, cyclooxygenases; FGFs, fibroblast growth factors; PDAC, pancreatic ductal adenocarcinoma; ERK, extracellular signal-regulated kinase; MMPs, matrix metalloproteinases; TIMPs, tissue inhibitor of metalloproteinases

Key words: celecoxib, ERK1/2, FGF-2, invasion, migration, PANC-1

different tumor tissues and in response to inflammation (13). Celecoxib is a selective COX-2 inhibitor that was first used for adjuvant treatment in patients with familial adenomatous polyposis (14). Since the introduction of celecoxib in cancer therapy, a number of studies have investigated molecular targets and the clinical effects of selective COX-2 inhibitors, as well as molecular mechanisms of their antitumor activity, either via selective COX-2 inhibition or COX-2-independent mechanisms of action. It has been shown in pre-clinical studies of pancreatic cancer that selective COX-2 inhibitors exert their antitumor effect by inhibiting cell proliferation and promoting apoptosis (15). Furthermore, although precise biological mechanisms underlying the antitumor effect of COX-2-independent action remain unclear, it is possible that the effect of COX-2 inhibitors may be mediated, at least in part, by the suppression of FGF-2 (16). It has been shown that oral COX-2 inhibitors suppress angiogenesis and the growth of gastrointestinal tumor explants in nude mice, possibly via mechanisms associated with the reduced expression of FGF-2 and VEGF (17). Furthermore, in esophageal adenocarcinoma, treatment of Seg-1 cells with NS-398 *in vitro* significantly reduced FGF-2 expression and induced an antitumor effect (18).

Based on these findings, it is possible that the antitumor mechanisms of celecoxib may be associated with FGF-2 signal regulation. Therefore, the aim of the present study was to examine the hypothesis that selective COX-2 inhibitor celecoxib may synergistically suppress the expression of FGF-2 (possibly through the activation of ERK1/2 and increase in the secretion of MMPs) and the expression of its receptor, FGFR-2; thereby, inhibiting proliferation, invasion and migration, and stimulating the apoptosis of human pancreatic cancer PANC-1 cells *in vitro*.

Materials and methods

Reagents. Dulbecco's modified Eagle's medium (DMEM) was obtained from HyClone (Logan, UT, USA), and penicillin and streptomycin were acquired from Gibco-BRL (Gibco, NY, USA). Celecoxib was obtained from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in anhydrous dimethyl sulfoxide (DMSO) (Sigma, St. Louis, MO, USA) at a concentration of 100 mmol/l for storage solution. Human FGF-2 was obtained from Cell Signaling Technology (Boston, MA, USA), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sigma Chemicals (Sigma). Other reagents were purchased from Nanjing Chemical Reagent Co. (Nanjing, China) unless otherwise described.

Cell culture. PANC-1 cells derived from a human PDAC were acquired from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in DMEM with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) and 1% ampicillin-streptomycin. Cells were grown in a humidified incubator with an atmosphere of 5% CO₂ at 37°C.

Cell viability assay. PANC-1 cells were cultured in 96-well plates at a density of 1x10⁴ cells/well and incubated in medium with 10% FBS. After overnight growth, the cells were treated with 5, 10, 20, 40, 80 and 160 μmol/l of celecoxib in serum-free conditions. The control group was set in serum-free

medium. After incubation for 24, 48 and 72 h at 37°C, 20 μl of MTT solution [5 mg/ml in phosphate-buffered saline (PBS)] was added into each well; and cells were further incubated for 4 h at 37°C. Next, 100 μl of DMSO was added into each well at 37°C. A spectrophotometer (Bio-Rad, Hercules, CA, USA) was used to determine the optical density (OD) value of each well at 490 nm. Each experiment was performed at least in triplicate, and results are presented as a relative ratio to the OD at the beginning of the experiment.

Cell apoptosis assay. In order to quantify apoptosis, the cells were stained with Annexin V and propidium iodide (PI) using an Annexin V-FITC/PI apoptosis kit (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions. In brief, PANC-1 cells were cultured into 6-well plates with DMEM containing 10% FBS for 24 h. Next, the cells were further treated for 24 h with an indicated concentration of celecoxib or FGF-2. After treatment, the cells were digested with trypsin, washed twice with cold PBS, and resuspended in 1X binding buffer at a concentration of 1x10⁵ cells/ml. Next, 100 μl of the solution (1x10⁵ cells) was mixed with 5 μl of Annexin V-FITC and 5 μl of PI, and incubated for 15 min in the dark at room temperature. Finally, 400 μl of 1X binding buffer was added to each sample; then, the cells were kept on ice and immediately subjected to flow cytometry on a FACSCalibur flow cytometer (Becton-Dickinson, San Jose, CA, USA). Cell Quest software (Becton-Dickinson) was used to analyze the data.

Invasion assay. For the cell invasion assay, the cells were pretreated with the indicated celecoxib or FGF-2 concentration for 2 h, digested with trypsin and placed in the upper compartment of a Matrigel (BD Biosciences)-coated chamber, as previously described (19). In brief, equal concentrations of cells (5x10⁴ cells/well) were suspended in 300 μl of DMEM containing 0.1% of BSA, and the cell suspensions were added to the upper compartment of a 24-well Boyden chamber (Millipore Co., Billerica, MA, USA). Another 600 μl of DMEM containing 15% FBS was added to the lower compartment in order to stimulate cell invasion. Then, the cells were incubated for 24 h, and the non-invaded cells were removed from the top compartment of the membrane with a cotton swab. Remaining cells on the lower surface of the membrane were fixed with 4% paraformaldehyde and stained in 0.01% crystal violet solution. The number of invading cells was quantified from six random high-power fields (HPFs) visualized at a magnification of x100.

Scratch migration assay. For the evaluation of PANC-1 cell migration, the cells were cultured in 6-well plates (1x10⁶ cells/well) for 24 h, washed with PBS twice and treated with the indicated concentration of celecoxib or FGF-2 for 2 h. Then, the cells were scraped with the fine end of a 1-ml pipette tip; and the plates were washed twice with PBS to remove the detached cells, and were incubated with DMEM containing 10% FBS. Cell migration was photographed using 10 HPFs at 0- and 24-h post-induction of the injury. Remodeling was measured as the relative diminishing area across the induced injury, and expressed as migration area percentage.

Western blot analysis. For western blot analysis, PANC-1 cells were washed twice with cold PBS and lysed in RIPA

buffer (PBS containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 100 ng/ml of phenylmethylsulfonyl fluoride and 10 μ g/ml of aprotinin). Then, the samples that contained 50 μ g of protein/lane were fractionated on a 10% SDS-polyacrylamide gel by electrophoresis, and the separated proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore) using a semi-dry transfer cell (Bio-Rad). Next, the blots were incubated in Tris-buffered saline (TBS; 10 mmol/l of Tris-HCl pH 8.0 and 150 mmol/l of NaCl) containing 5% (w/v) non-fat dry milk and 0.1% Tween-20 for 2 h at room temperature. Then, the immunoblots were incubated first with different specific primary antibodies, followed by incubation with appropriate secondary antibodies. β -actin was used as a control. Signals were quantified using the public domain NIH ImageJ 1.49 (<http://rsbweb.nih.gov/ij/download.html>; US National Institutes of Health, USA).

The following primary antibodies were used: mouse anti-human FGF-2 antibody (catalog no. ab130094; dilution 1:500; Abcam, Cambridge, MA, USA), rabbit anti-human FGF receptor 2 antibody (catalog no. 11835; dilution 1:500), rabbit anti-human p44/42 MAPK antibody (catalog no. 9102; dilution 1:1,000), rabbit anti-human phospho-p44/42 MAPK antibody (catalog no. 9101; dilution 1:1,000), rabbit anti-human MMP-2 antibody (catalog no. 4022; dilution 1:400), rabbit anti-human MMP-9 antibody (catalog no. 3852; dilution 1:400) (all from Cell Signaling Technology) rabbit anti-human TIMP-1 antibody (catalog no. ab109125; dilution 1:400; Abcam), mouse anti-human β -actin antibody (catalog no. sc-130301; dilution 1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA).

RNA extraction and quantitative real-time PCR (qRT-PCR). TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used for the extraction of total RNA from the PANC-1 cells. Next, cDNA was synthesized using a PrimeScript RT reagent kit and qRT-PCR was carried out using a SYBR-Green PCR kit (both from Takara, Dalian, China), according to the manufacturer's instructions. GAPDH was used as an internal control, and relative expression levels were assessed using the $\Delta\Delta$ Ct method. PCR primers for qRT-PCR assay were as follows (5'-3'): FGF-2-forward, AGAGCGACCCTCACATCAAG and FGF-2-reverse, TCGTTTCAGTGCCACAACG; FGFR-2-forward, TCCTATGACATTAACCGTGTT and FGFR-2-reverse, TTT AACACTGCCGTTTAT; MMP-2-forward, GTGCTGAAG GACACACTAAAGAAGA and MMP-2-reverse, TTGCCATC CTTCTCAAAGTTGTAGG; MMP-9-forward, GCGGAGAT TGGGAACCAGCTGTA and MMP-9-reverse, GACGCGCC TGTGTACACCCACA; TIMP-1-forward, CATCCTGTTGTT GCTGTGGCTGAT and TIMP-1-reverse, GTCATCTTGATC TCATAACGCTGG; MAPK-1-forward, CAGTTCCTTGACCC CTGGTCC and MAPK-1-reverse, GTACATACTGCCGCAG GTCA; GAPDH-forward, GGAGCGAGATCCCTCCAAAAT and GAPDH-reverse, GGCTGTTGTCATACTTCTCATGG. All experiments were performed three times in triplicate.

Statistical analysis. Statistical analyses were performed using the SPSS software package (version 13.0; SPSS, Inc., Chicago, IL, USA). All data are presented as mean \pm SEM. Data were analyzed by one-way analysis of variance (ANOVA), and all statistical tests were two-sided. A P-value of <0.05 was considered to indicate a statistically significant result.

Results

Effect of celecoxib and FGF-2 on the proliferation and apoptosis of PANC-1 cells. In the present study, the influence of celecoxib was examined on PANC-1 cell growth. Celecoxib inhibited cell proliferation in a dose- and time-dependent manner (Fig. 1A), and this effect was preventable through the presence of exogenous FGF-2 (10 ng/ml) in culture medium with celecoxib (Fig. 1B). No inhibitory effect on PANC-1 cell growth was observed within the first 24 h for celecoxib concentrations of up to 40 μ mol/l. Longer periods of incubation reduced MTT signals starting at a concentration of 20 μ mol/l. Hence, for further experiments *in vitro*, celecoxib concentrations between 5 and 10 μ mol/l were used.

The ability of FGF-2 to reverse the inhibitory effect of celecoxib on cell proliferation could be attributable to the inhibition of apoptosis. Celecoxib treatment for 24 h induced a significant 4-fold increase in the apoptotic index, from $4.8\pm 1.1\%$ observed in control untreated cells to $19.84\pm 1.9\%$ in cells treated with celecoxib (10 μ mol/l) for 24 h (Fig. 1C and D). FGF-2 alone had no influence on apoptotic level. However, FGF-2 (10 ng/ml) significantly reduced celecoxib-induced apoptosis from $19.84\pm 1.9\%$ in cells treated with celecoxib (10 μ mol/l) alone to $11.58\pm 2.3\%$ in cells treated with celecoxib and FGF-2.

Celecoxib compromises FGF-2-induced invasion and migration of PANC-1 cells. Next, the effects of celecoxib on the invasion and migration of PANC-1 cells were investigated. As shown in Fig. 2A, celecoxib (10 μ mol/l) strongly inhibited cell invasion and migration. Compared with the control group, the invasion and migration abilities of the PANC-1 cells were significantly decreased ($P<0.05$; Fig. 2B and C). In contrast, treatment with FGF-2 notably enhanced the invasion and migration abilities of the PANC-1 cells. However, when PANC-1 cells were treated with FGF-2 (10 ng/ml) and celecoxib (10 μ mol/l), the enhanced invasion and migration abilities induced by FGF-2 were abolished by celecoxib (Fig. 2).

Celecoxib inhibits FGF-2 and FGFR-2 expression in PANC-1 cells. The treatment of PANC-1 cells with 5 and 10 μ mol/l of celecoxib resulted in a marked suppression in cellular FGF-2 content, as indicated by western blot analysis (Fig. 3). Celecoxib suppressed FGF-2 expression in a dose-dependent manner; that is, after incubation with celecoxib for 12 h, cells expressed a marginally detectable level of FGF-2 (Fig. 3A). However, FGF-2 levels increased rapidly within 8 h of celecoxib (10 μ mol/l) removal, and returned to normal levels. In contrast, incubation of PANC-1 cells with celecoxib (10 μ mol/l) for 12 h resulted in a significant decrease in FGFR-2 expression; and after removal of celecoxib, FGFR-2 inhibition persisted for some time.

Celecoxib suppresses MMP-9 and MMP-2 expression in PANC-1 cells. Matrix metalloproteinases (MMPs) serve as reliable markers for tumor cell invasion and migration. In the present study, PANC-1 cells were treated with or without 10 ng/ml of FGF-2 and with or without 10 μ mol/l of celecoxib; and mRNA and protein levels of MMP-2, MMP-9 and TIMP-1 were examined by qRT-PCR and western blot analysis, respectively. As shown in Fig. 4, FGF-2 treatment

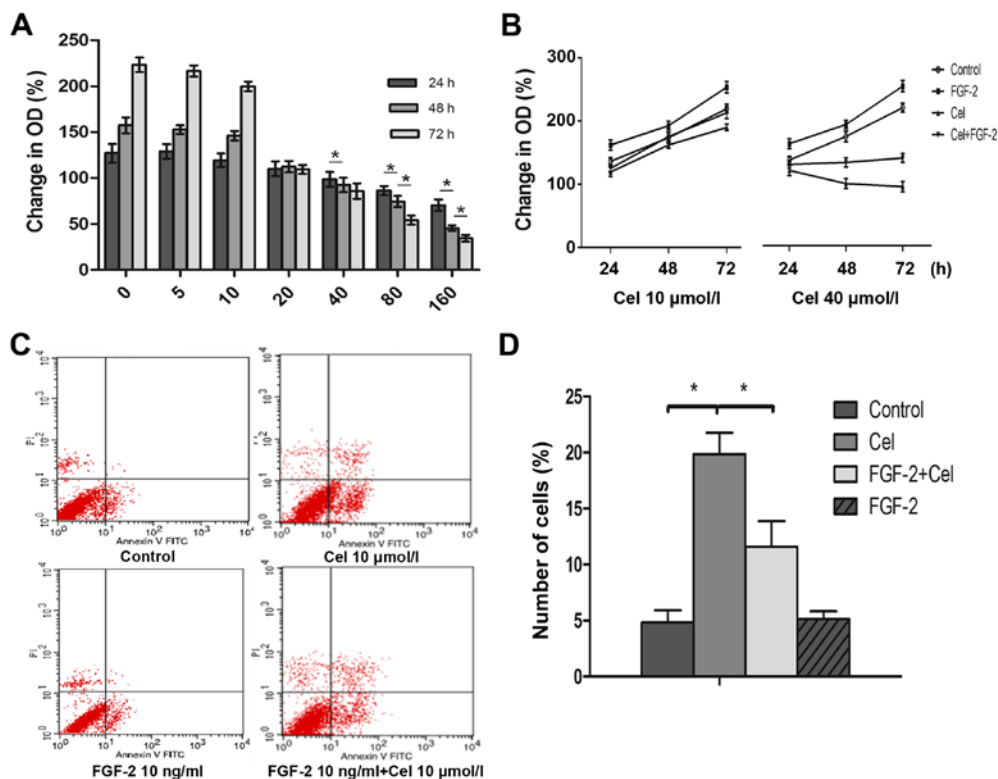


Figure 1. Effect of celecoxib and FGF-2 on the proliferation and apoptosis of PANC-1 cells. (A) Cell viability of PANC-1 cells following treatment with celecoxib at a concentration range of 5-160 $\mu\text{mol/l}$ for 24, 48 and 72 h. (B) Effect of celecoxib (10 and 40 $\mu\text{mol/l}$) and FGF-2 (10 ng/ml) on the proliferation of PANC-1 cells. (C) Effect of celecoxib (10 $\mu\text{mol/l}$) and FGF-2 (10 ng/ml) on the apoptosis of PANC-1 cells. (D) The rate of apoptosis of PANC-1 cells in response to different treatments. Cells were stained with FITC-Annexin V/PI. Data are presented as mean \pm SEM of three independent experiments; * $P < 0.05$ represents a significant difference. Cel, celecoxib.

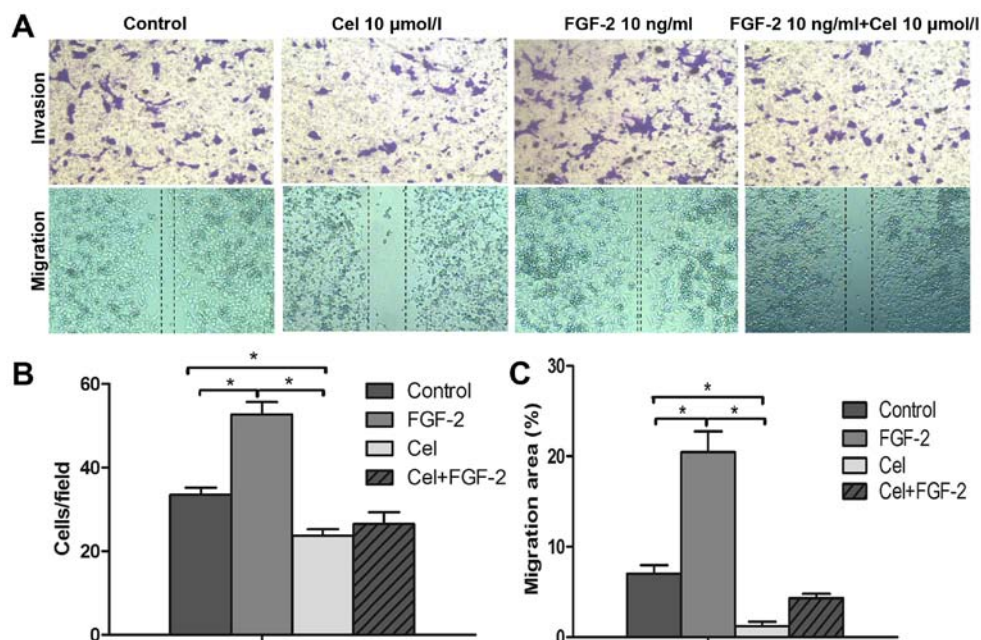


Figure 2. Celecoxib inhibits FGF-2-enhanced PANC-1 cell migration and invasion. (A) The invasive ability of PANC-1 cells on the bottom side of the Transwell membrane at a magnification of $\times 100$ and the scratch-wound migration of PANC-1 cells at a magnification of $\times 10$. (B) Invasion of PANC-1 cells in response to different treatments. The number of invaded cells was quantified by counting the number of cells from six random fields. (C) Migration area percentage of PANC-1 cells in response to different treatments. The data presented are from three independent experiments; * $P < 0.05$ represents a significant difference. Cel, celecoxib.

resulted in increased MMP-2 and MMP-9 protein expression, compared to the untreated control cells. In contrast, western

blot and qRT-PCR analyses revealed that the expression levels of MMP-2 and MMP-9 were significantly decreased in

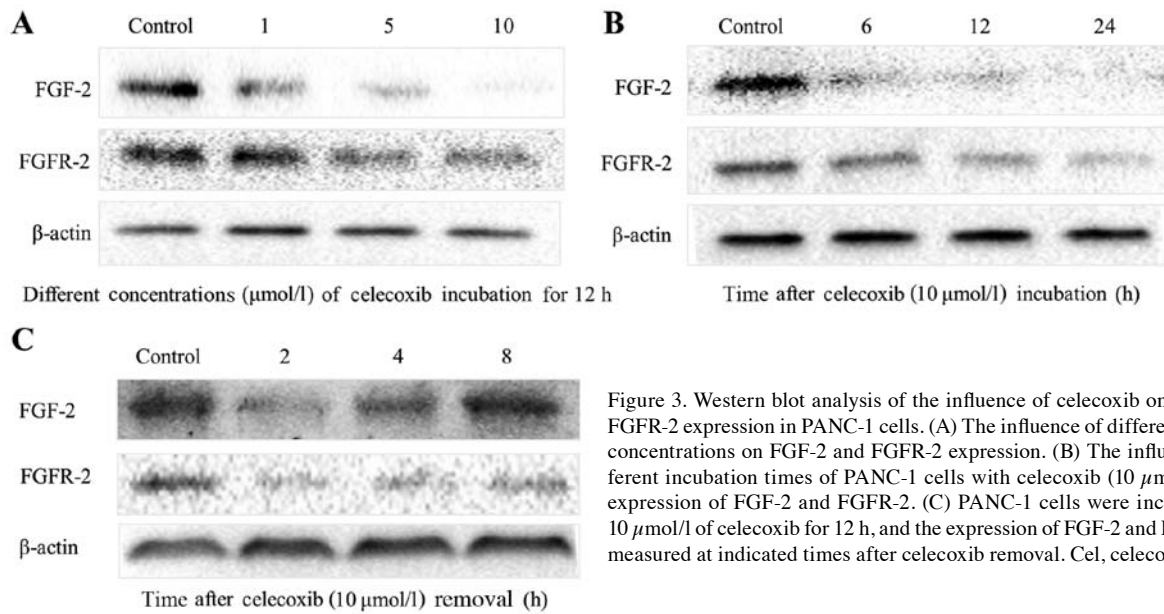


Figure 3. Western blot analysis of the influence of celecoxib on FGF-2 and FGFR-2 expression in PANC-1 cells. (A) The influence of different celecoxib concentrations on FGF-2 and FGFR-2 expression. (B) The influence of different incubation times of PANC-1 cells with celecoxib (10 $\mu\text{mol/l}$) on the expression of FGF-2 and FGFR-2. (C) PANC-1 cells were incubated with 10 $\mu\text{mol/l}$ of celecoxib for 12 h, and the expression of FGF-2 and FGFR-2 was measured at indicated times after celecoxib removal. Cel, celecoxib.

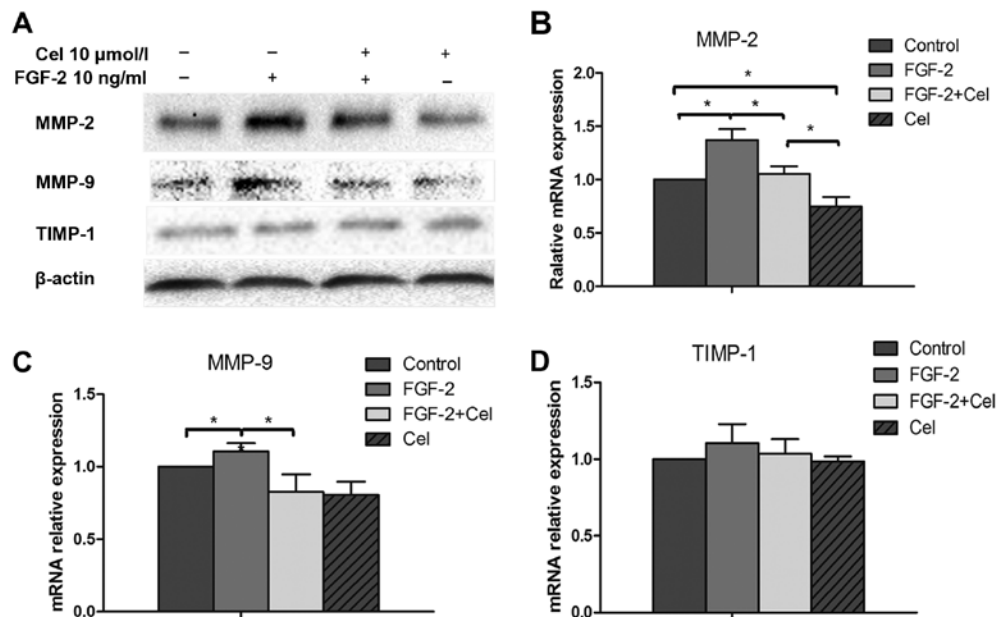


Figure 4. A 24-h celecoxib (10 $\mu\text{mol/l}$) treatment reduces FGF-2 (10 ng/ml)-mediated enhancement of MMP-2 and MMP-9 in PANC-1 cells. (A) Western blot analysis of the expression levels of MMP-2, MMP-9 and TIMP-1. (B) The qRT-PCR analysis of MMP-2 mRNA expression. (C) The qRT-PCR analysis of MMP-9 mRNA expression. (D) The qRT-PCR analysis of TIMP-1 mRNA expression; * $P < 0.05$ represents a significant difference. Cel, celecoxib.

cells exposed to 10 $\mu\text{mol/l}$ of celecoxib ($P < 0.05$; Fig. 4A-C), compared with the control cells. No change in TIMP-1 expression was observed when PANC-1 cells were treated with FGF-2 or celecoxib alone or in combination (Fig. 4D). Celecoxib was also able to suppress the FGF-2-induced enhanced expression of MMP-2 and MMP-9, which is suggestive of its anti-FGF-2-like capacity (Fig. 4A-C).

Celecoxib inhibits FGFR-2 expression and ERK1/2 phosphorylation activated by FGF-2. Mitogenic signaling through FGFRs often involves the activation of the MAPK pathway. Therefore, we sought to determine whether the FGF-2-mediated activation of MAPK was altered as a consequence of changes in FGFR-2 levels. Western blot analysis revealed that FGF-2

treatment caused the increase in its receptor FGFR-2 expression, as well as increased levels of phosphorylated ERK, compared to the control untreated PANC-1 cells (Fig. 5A). In contrast, treatment with celecoxib inhibited FGFR-2 expression and suppressed phosphorylated ERK1/2 altered by FGF-2. In addition, qRT-PCR analyses also revealed that the transcriptional activity of FGFR-2 and MAPK1 decreased in the cells treated with celecoxib, compared to the cells in the control group ($P < 0.05$; Fig. 5B and C).

Discussion

Pancreatic ductal adenocarcinoma (PDAC) is a very aggressive and lethal type of tumor, in which its metastatic invasion

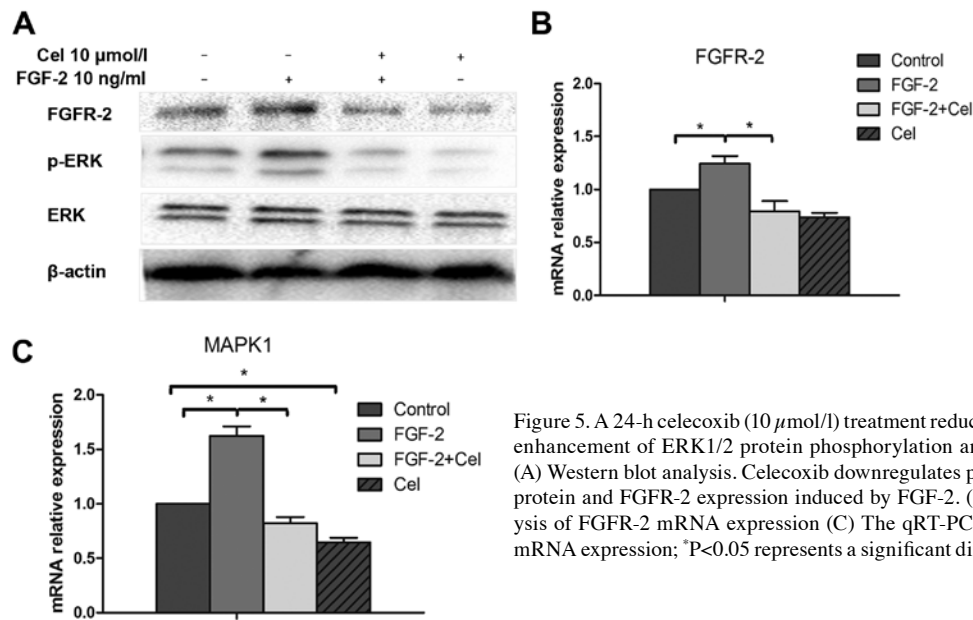


Figure 5. A 24-h celecoxib (10 μ mol/l) treatment reduces the FGF-2-mediated enhancement of ERK1/2 protein phosphorylation and FGFR-2 expression. (A) Western blot analysis. Celecoxib downregulates phosphorylated ERK1/2 protein and FGFR-2 expression induced by FGF-2. (B) The qRT-PCR analysis of FGFR-2 mRNA expression (C) The qRT-PCR analysis of MAPK1 mRNA expression; * P <0.05 represents a significant difference. Cel, celecoxib.

is associated with inflammation (20). The COX-2 enzyme is an important mediator of prostaglandin synthesis and is significantly overexpressed in many different types of cancers (21). COX-2 inhibitors can exert their effects by decreasing the expression of COX-2. Nevertheless, some selective COX-2 inhibitors can also provoke responses in COX-2-negative cancer cells, which could not be explained by COX-2 inhibition. However, the mechanisms of this biological effects are poorly understood (22). Celecoxib, a selective COX-2 inhibitor, has been previously shown to inhibit the growth of human pancreatic cancer cell lines (23). In addition, in pancreatic xenograft experiments, treatment with celecoxib inhibited tumor growth, metastasis and angiogenesis (24). Furthermore, in a clinical assessment study of celecoxib pharmacodynamics in pancreatic cancer patients, celecoxib significantly decreased the level of COX and PGE₂; but this effect did not induce a significant decrease in tumor growth, suggesting that COX and PGE₂ downregulation may be irrelevant for pancreatic cancer growth (25). Moreover, there is evidence suggesting that the mechanism of the antitumor effect of celecoxib may be mediated by targets other than COX-2 such as the downregulation of the expression of survivin (26) or AKT (27). It has been previously reported that PANC-1 cells express a marginally detectable level of COX-2 (28); however, COX-2 inhibitors continue to exert an antitumor effect in these cells. In the present study, we investigated the possible regulation of FGF-2 and its receptor by selective COX-2 inhibitor celecoxib, as well as the role of FGF-2 in the survival, proliferation, invasion and migration of PANC-1 cells. We demonstrated that celecoxib significantly inhibited cell proliferation and stimulated apoptosis in PANC-1 cells. This was accompanied by a profound but reversible suppression of FGF-2 expression in PANC-1 cells. Furthermore, the addition of exogenous FGF-2 to culture medium significantly ameliorated the antiproliferative and pro-apoptotic effects of celecoxib in PANC-1 cells. These data suggest that in PANC-1 cells, COX-2 inhibitor celecoxib suppresses cell proliferation and induces apoptosis, at least in part, via an FGF-2-dependent pathway. FGF-2 is

an important growth and differentiation factor involved in many physiological and pathological processes. Indeed, it has been shown in several studies that the tissue-specific expression of FGFs and FGFRs is critical in the regulation of tissue homeostasis (7-10). The ectopic expression of FGF ligands or aberrant splicing of FGFRs can result in the activation of autocrine signaling pathways and cause uncontrolled cell proliferation (29). Moreover, FGFR-2 overexpression was correlated with increased pancreatic cancer cell proliferation, invasion and early liver recurrence in patients following surgical resection of a tumor (30).

Invasion and metastasis are two hallmarks of cancer, the main causes of cancer-related death, and the most difficult clinical treatment issues, particularly in PDAC (3,4). Numerous studies that explored these processes in PDAC have been conducted over several decades, but tumor metastasis continues to remain the main cause of poor outcome in PDAC patients (31-33). In recent years, studies focusing on the tumor microenvironment suggest that the remodeling of the tumor extracellular matrix (ECM) increases the invasion and metastatic capabilities of tumor cells (32). Among numerous factors involved in ECM remodeling, MMPs secreted by cancer cells have been postulated as one of the major facilitators of tumor invasion (33). Compared to benign tumors, malignant tumors exhibit increased MMP expression levels, which lead to the increased degradation of the ECM and promotion of tumor cell migration and invasion (34). Indeed, the upregulation of the expression of MMPs and TIMPs in cancer tissues results in increased tumor invasion and metastasis (35). The elevated expression of MMP-2 or MMP-9 is associated with the hydrolysis of the ECM and induction of cancer cell invasion through the basement membrane (36). The results of the present study revealed that celecoxib can inhibit FGF-2 signaling pathway-mediated MMP regulation in PANC-1 cells during tumor cell invasion *in vitro*. Our data revealed that the expression levels of MMP-2 and MMP-9 were decreased in the PANC-1 cells when treated with celecoxib. In contrast, the expression levels of MMP-2 and MMP-9 were significantly induced by FGF-2

treatment. It has been reported that many patients with pancreatic cancer overexpress FGF-2, which leads to the activation of the ERK1/2 pathway, increased expression of MMPs and higher invasive potential (9-11). In addition, in the present study, TIMP-1 levels did not change after celecoxib treatment, suggesting that a different pathway is involved in the regulation of TIMP-1 expression in PANC-1 cells.

The MAPK/ERK signaling pathway is one of the most commonly activated pathways in human cancers (37). It has been shown that this pathway, particularly ERK1/2, regulates the expression of MMPs (38). Indeed, a decrease in ERK1/2 phosphorylation may be involved in the downregulation of MMPs and upregulation of the expression of TIMPs (39). In the present study, we showed that ERK1/2 was activated in PANC-1 cells following treatment with FGF-2. In addition, FGF-2 increased PANC-1 cell growth and invasion via binding to its receptor FGFR-2 and phosphorylation of the ERK1/2 protein, which further resulted in increased secretion of MMP-2 and MMP-9. In contrast, treatment with celecoxib suppressed the expression of FGFR-2, inhibited ERK1/2 phosphorylation and acted against the effect of FGF-2; suggesting that the molecular mechanisms of celecoxib inhibition in PANC-1 cell invasion and migration are related to the ERK1/2 pathway and mediated by FGFR-2. Another possible mechanism is the involvement of the Ras-MAPK signaling pathway. Pancreatic adenocarcinoma has the highest incidence of *K-ras* point mutations (70-90% of all cases) (40), and PANC-1 cells have an activating *K-ras* gene and it has been shown that *K-ras* is involved in increasing or activating extracellularly sequestered FGF-2 (41). In addition, oncogenic Ras-induced proliferation can be abolished by addition of an anti-FGF-2 blocking antibody (41). Based on these findings we hypothesized that the antitumor effects of celecoxib may also be associated with *K-ras* regulation in PANC-1 cells.

In conclusion, the present study is the first to report on the relationship between FGF-2 expression and COX-2 inhibitor celecoxib in PANC-1 cells. The antitumor effect of celecoxib was exerted through the inhibition of the expression of FGFR-2 and interruption of the activity of MMPs. These results provide a rational basis for the further evaluation of the efficacy of celecoxib in the treatment of PDAC and perhaps other malignancies, in which FGF2/FGFR2 signaling has an important role.

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