Prostaglandin E₂ Enhances Pancreatic Cancer Invasiveness through an Ets-1–Dependent Induction of Matrix Metalloproteinase-2

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ABSTRACT

Accumulating evidence suggests an important role for cyclooxygenase-2 (COX-2) in the pathogenesis of a wide range of malignancies. Here we tested the hypothesis that the COX-2 product prostaglandin E₂ (PGE₂) increases cellular invasive potential by inducing matrix metalloproteinase-2 (MMP-2) expression and activity through an extracellular signal-regulated kinase (ERK)/Ets-1–dependent mechanism in pancreatic cancer. PANC-1 and MIA PaCa-2 pancreatic cancer cells were treated with PGE₂ or rofecoxib, a selective COX-2 inhibitor. MMP-2 expression and activity were assessed using Western blot analysis and zymography, respectively. MMP-2 promoter activity was analyzed with a luciferase-based assay. Ets-1 activity was assessed using gel shift assays. Ets-1 expression was specifically silenced using RNA interference. Cellular invasive and migratory potentials were determined using a Boyden chamber assay with or without Matrigel, respectively. Exogenous PGE₂ induced MMP-2 expression and activity and increased ERK1/2 phosphorylation, Ets-1 binding activity, and MMP-2 promoter activity. PGE₂ also increased cellular migratory and invasive potentials. The mitogen-activated protein kinase kinase inhibitor PD98059 and Ets-1 silencing each abrogated the effect of PGE₂ on cellular invasive potential but not on cellular migratory potential. Rofecoxib suppressed MMP-2 expression and activity, Ets-1 binding activity, MMP-2 promoter activity, and cellular migratory and invasive potentials. These results suggest that PGE₂ mediates pancreatic cancer cellular invasive potential through an ERK/Ets-1–dependent mechanism. Ets-1 expression and activity. They also suggest that COX-2 inhibition may represent a strategy to inhibit invasive potential in pancreatic cancer.

INTRODUCTION

Cyclooxygenase-2 (COX-2) overexpression has been reported to be present in 74% to 100% of human pancreatic cancer specimens (1–3). COX-2 is the inducible isof orm of COX, the enzyme catalyzing the rate-limiting step in the metabolic pathway that transforms arachidonic acid into the prostaglandins (PGs) and thromboxanes, whereas COX-1 is constitutively expressed in most tissues (4). Among the products of COX-2, prostaglandin E₂ (PGE₂), in particular, may play a biologically important role in cancer pathogenesis. Many cancers that overexpress COX-2 have high intratumoral levels of PGE₂ (5–7), and PGE₂ recently was shown to up-regulate the invasive potential of colorectal carcinoma cells (8).

We have reported previously that matrix metalloproteinase-2 (MMP-2) activity is an important determinant of pancreatic cancer cellular invasive potential (9). Ets-1 is a member of the Ets family of transcription factors that share a conserved Ets domain (10). It has been reported that Ets-1 is overexpressed in a variety of human malignancies, including cancers of the ovary (11), breast (12), colon and rectum (13), and pancreas (14). Because of its roles in the transcriptional regulation of MMPs, including MMP-2 (15, 16), Ets-1 is a candidate mediator of cancer invasion and metastasis.

In this study, we tested the hypothesis that PGE₂ increases pancreatic cancer cellular invasive potential through an Ets-1–dependent induction of MMP-2 expression and activity. We also tested the efficacy of COX-2 inhibition in inhibiting Ets-1–dependent MMP-2 induction and cellular invasive potential.

MATERIALS AND METHODS

Materials. Culture media and fetal bovine serum (FBS) were obtained from Life Technologies, Inc. (Rockville, MD). PGE₂, rofecoxib (RFX), and PD98059 were obtained from Sigma (St. Louis, MO), Merck (Montreal, Canada), and Calbiochem (La Jolla, CA), respectively. Anti-MMP-2 and antiantigen monoclonal antibodies were obtained from NeoMarkers, Inc. (Fremont, CA). Anti-COX-2, anti–Ets-1, anti–Oct-1, anti–extracellular signal-regulated kinase (ERK) 1/2, and anti–phospho-ERK1/2 monoclonal antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The BCA assay kit and the DAB liquid substrate kit were obtained from Sigma, and the Vascular ABC protein detection system kit was obtained from Vector Laboratories (Burlingame, CA). All of the other reagents were purchased from Sigma unless otherwise specified.

Cell Culture. Human pancreatic cancer cells (PANC-1, MIA PaCa-2) were obtained from the American Type Culture Collection (Manassas, VA). Cells were maintained in DMEM containing 10% FBS in 75-cm² culture flasks kept in a humidified (37°C, 5% CO₂) chamber. For the experiments described below, cells were trypsinized and harvested on reaching 80% to 90% confluency.

Treatment of Cells with PGE₂ or Rofecoxib. Cells (2 × 10⁵) were seeded onto wells of 24-well plates, and conditioned media were harvested after 24 hours of incubation. Either PGE₂ (10 μmol/L) or RFX (5 μmol/L) subsequently was added. Cells were harvested for the assays described below after incubation periods ranging from 15 minutes to 24 hours. Neither PGE₂ nor RFX at the concentrations used affected cellular viability, as determined by trypan blue exclusion tests performed on cells following 24 hours of incubation with these agents.

PGE₂ ELISA. Cells (1 × 10⁶) in 0.5-mL culture media were seeded onto 24-well plates. At 24 hours after cell seeding, conditioned media were harvested, and PGE₂ concentrations were determined using an ELISA assay kit according to instructions supplied by the manufacturer (Cayman Chemical, Ann Arbor, MI). Samples were assayed in triplicate in each of three independent experiments.

Western Blot Analysis. Cells (2 × 10⁴) were harvested and rinsed with PBS. Cell extracts were prepared using lysis buffer (20 mmol/L Tris-HCl (pH 7.5), 0.1% Triton X, 0.5% sodium deoxycholate, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, and 10 μg/mL leupeptin) centrifugation at 12,000 × g and 4°C. Total protein concentration was measured using the BCA assay. Cellular extracts containing 50-μg total protein were subjected to 10% SDS-PAGE, and the proteins were transferred electrophoretically to polyvinylidene difluoride membranes (Invitrogen, Carlsbad, CA). After blocking with PBS containing 0.2% casein for 1 hour at room temperature, membranes were incubated with 1 μg/mL antibody in PBS containing 0.2% Tween-20 overnight at 4°C. The Vascular ABC kit and DAB liquid substrate were used for chromogenic detection, according to the manufacturer’s instructions. Band intensities were quantified using Image Pro Plus 4.0 (Media Cybernetics, Silver Spring, MD) and normalized to those of actin. Blots were performed triplicate. Mean densitometric values (±SD) are given.

Zymography. Cells (1 × 10⁶) were seeded onto wells of 24-well plates and allowed to adhere in the presence of serum. Media subsequently were replaced by 0.5 mL of serum-free medium per well. After 24 hours of incubation, the
conditioned media were harvested for zymography. Zymography was carried out as described previously (17). In brief, 25 μL of the conditioned medium for each sample were subjected to 10% SDS/PAGE with 1 mg/mL gelatin incorporated into the gel mixture. Following the electrophoresis, the gels were soaked in 2.5% Triton X to remove SDS, rinsed with 10 mmol/L Tris (pH 8.0), and transferred to a bath containing 50 mmol/L Tris (pH 8.0), 5 mmol/L CaCl2, and 2 μmol/L ZnCl2 at 37°C for 17 hours. The gels were stained with 0.1% Coomassie blue in 45% methanol and 10% acetic acid. Experiments were repeated three times.

Cloning of MMP-2 Promoter. The human MMP-2 promoter was cloned from human placental DNA by PCR using primers (5'-CACACCCACCAGA-3' repeated three times). Each sample was subjected to 10% SDS/PAGE with 1 mg/mL gelatin incorporated into the gel mixture. Following the electrophoresis, the gels were soaked in 2.5% Triton X to remove SDS, rinsed with 10 mmol/L Tris (pH 8.0), and transferred to a bath containing 50 mmol/L Tris (pH 8.0), 5 mmol/L CaCl2, and 2 μmol/L ZnCl2 at 37°C for 17 hours. The gels were stained with 0.1% Coomassie blue in 45% methanol and 10% acetic acid. Experiments were repeated three times.

Luciferase Assay. The luciferase assay was conducted as described previously (19). Empty pGL3 vector (pGL3e) was used for control transfections. Cells (4 x 10⁵ cells/well) were plated onto wells of 12-well cell culture dishes and cultured for 12 hours in serum-containing medium. One microgram of pGL3-MMP2, pGL3-MMP2Δ, or pGL3e was transfected into the pGL3 vector (Promega, Madison, WI). A deletion mutant MMP-2 promoter (1081-bp fragment covering positions −1024 to +57 relative to the MMP-2 transcription initiation site) was made from the full-length MMP-2 promoter, by digestion with EcoRI, filled with Klenow fragment of DNA polymerase I, and religated. The vectors containing the inserts are designated pGL3-MMP2 (containing full-length promoter) and pGL3-MMP2Δ (containing deletion mutant promoter); sequencing was performed to confirm correct insert orientation.

Nuclear Extract Preparation. Nuclear extracts were obtained using a modification of a previously described method (20). Harvested cells were lysed with buffer A (10 mmol/L HEPES, 10 mmol/L KCl, 0.1 mmol/L EDTA, 1.5 mmol/L MgCl2, 0.2% NP40, 1 mmol/L DTT, and 0.05 mmol/L phenylmethylsulfonl fluoride), followed by vortexing to shear the cytoplasmic membranes. Nuclear proteins were extracted with high-salt buffer C (20 mmol/L HEPES, 25% glycerol, 1.5 mmol/L MgCl2, 0.1 mmol/L EDTA, 420 mmol/L NaCl, 1 mmol/L DTT, and 0.05 mmol/L phenylmethylsulfonyl fluoride). Total nuclear protein was determined by BCA assay.

Electrophoretic Mobility Shift Analysis. Ets-1 and Oct-1 transcription factor activities were assessed by electrophoretic mobility shift analysis (EMSA) using double-stranded oligonucleotides corresponding to the Ets-1 or Oct-1 consensus sequences (Ets-1, 5'-GATCTCGAGGAGAAGTTCGA-3'; Oct-1, 5'-TGTCGAATGCAAATCACTAGAA-3'; Santa Cruz Biotechnology), respectively. Probes were end-labeled with T4 polynucleotide kinase in the presence of [γ-32P]ATP. Labeled probes were purified by gel filtration. Nuclear extracts from 5 x 10⁶ cells were incubated with 1 μg of labeled probe in a binding buffer containing 50 mmol/L Tris (pH 8.0), 5 mmol/L CaCl2, and 2 μmol/L ZnCl2 at 37°C for 17 hours. The gels were stained with 0.1% Coomassie blue in 45% methanol and 10% acetic acid. Experiments were repeated three times.

Small Interfering RNA Synthesis. Small interfering RNAs (siRNAs) were synthesized and high-performance purified (Qiagen, Valencia, CA). Ets-1 sense (ACUUUGCUACCAUCCCGUAC-3') and antisense (GUACGGGAUUGUAGCAAGU-dTT) siRNAs and control siRNAs (sense, AGGAGAUAUUCGAGGCGUU-dTT; antisense, AAGGCUCGAAUAUUCUCCU-dTT), bearing no homology with any known human genes, were dissolved in buffer [100 mmol/L potassium acetate, 30 mmol/L HEPES-potassium hydroxide, and 2 mmol/L magnesium acetate (pH 7.4)] to a final concentration of 20 μmol/L, heated to 90°C for 60 seconds, and incubated at 37°C for 60 minutes before use.

Small Interfering RNA Transfection. Cells (5 x 10⁵) were plated onto wells of six-well cell culture plates and allowed to adhere for 24 hours. Eight microliters siPORT Amine transfection reagent (Ambion Inc., Austin, TX) per well were added to serum-free DMEM for a final complexing volume of 200 μL, vortexed, and incubated at room temperature for 20 minutes. Two microliters of siRNA solution were added to the diluted siPORT Amine transfection reagent, gently mixed, and incubated at room temperature for 15 minutes. The transfection agent/siRNA complex was added to the wells containing 800 μL DMEM with 10% FBS and incubated in normal cell culture conditions for 6 hours, after which time 1 mL DMEM with 10% FBS was added. All of the assays were performed after 48 hours.

Invasion and Migration Assays. The BD Biocoat Matrigel invasion chamber (BD Bioscience, San Jose, CA) was used according to the manufacturer’s instructions. In brief, 2.5 x 10⁴ cells in serum-free media were seeded onto the Matrigel-coated filters, and in the lower wells 5% FBS was added as the chemoattractant. After 24 hours incubation, the filters were stained with Diff-Quik kit (BD Bioscience), and the number of cells that had penetrated the filter was counted under magnification (randomly selected high-powered fields counted in each well).
power fields). The counting was performed for three fields in each sample. In addition, internal controls (antibody to MMP-2 antibody) were included in each experiment.

To examine COX-2 expression and endogenous PGE₂ activity, Western blot analysis was employed. PGE₂ increased MMP-2 expression in PANC-1 and MIAPaCa-2 cells, respectively, whereas RFX reduced MMP-2 promoter activities by 81% and 124% (P < 0.05) in PANC-1 and MIAPaCa-2 cells, respectively, whereas RFX reduced MMP-2 activity by 48% and 55% (P < 0.05), respectively, in untreated control cells (P < 0.05). The graph depicts intensity of bands representing MMP-2 activities.

**RESULTS**

**COX-2 Expression and the Effect of Rofecoxib on PGE₂ Synthesis.** First, we examined COX-2 expression and endogenous PGE₂ production in the pancreatic cancer cell lines using Western blot analysis and PGE₂ ELISA, respectively. For PANC-1 and MIAPaCa-2 cells, COX-2 expression was confirmed; the selective COX-2 inhibitor RFX (5 μmol/L) reduced PGE₂ production by 61% and 70%, respectively, in these cell lines (P < 0.05; Fig. 1).

**Effects of PGE₂ and Rofecoxib on MMP-2 Expression and Activity.** To determine the effects of PGE₂ on MMP-2 expression and activity, cells were administered exogenous PGE₂ (10 μmol/L). In parallel experiments, RFX (5 μmol/L) was administered to suppress endogenous PGE₂ production. As shown in Fig 2A, exogenous PGE₂ increased MMP-2 protein expression by 85% and 122% (P < 0.05) in PANC-1 and MIAPaCa-2 cells, respectively. In contrast, RFX reduced MMP-2 expression by 48% and 55% (P < 0.05) in PANC-1 and MIAPaCa-2 cells, respectively. The effects of PGE₂ and RFX on MMP-2 gelatinolytic activity paralleled those on MMP-2 expression in PANC-1 and MIAPaCa-2 cells, as shown on zymography (Fig. 2B).

**Effects of PGE₂ and Rofecoxib on MMP-2 Transcriptional Activity.** Because of the observed alterations in MMP-2 expression induced by PGE₂ and by RFX, we next sought to determine the effects of PGE₂ and RFX on MMP-2 transcriptional activity using a luciferase-based reporter assay. The full-length MMP-2 promoter was incorporated into the pGL3 luciferase vector and transfected into the cells together with the pRL-CMV plasmid. The resulting luciferase activity was measured at 24 hours. As shown in Fig. 3A, PGE₂ increased MMP-2 promoter activities by 81% and 124% (P < 0.05), respectively, in PANC-1 and MIAPaCa-2 cells. To localize the responsive element in the MMP-2 promoter sequence, the truncated MMP-2 promoter with deletion of the region spanning positions −1659 to −1025 relative to the MMP-2 transcription initiation site was constructed, and the effects of this deletion on PGE₂-induced luciferase activity was analyzed. As shown in Fig. 3B, deletion of aforementioned region reduced baseline promoter activities 39% and 34% in PANC-1 and MIAPaCa-2, respectively (P < 0.05), and abolished the effect of PGE₂ on promoter activity. This result implicates the region spanning positions −1659 to −1025 of the MMP-2 promoter in PGE₂-mediated induction of MMP-2 transcription.

**Effect of PGE₂ on Transcription Factor Ets-1 Binding Activity.** Ets-1 is a known transcriptional regulator of MMP-2 expression (15, 22), which binds to the region spanning −1255 to −1248 of the...
MMP-2 promoter (18). Because of our observation that PGE2 up-regulates MMP-2 promoter activity, we sought to determine whether PGE2 up-regulates Ets-1 binding activity. As shown in Fig. 4A, PGE2 increased Ets-1 binding activities in PANC-1 and MIAPaCa-2 cells. Oct-1 binding was used as a loading control for nuclear extracts. In contrast, RFX reduced Ets-1 binding activities at 24 hours in PANC-1 and MIAPaCa-2 cells, respectively, whereas RFX suppressed MMP-2 promoter activities by 41% and 46%, respectively. (*P < 0.05 versus untreated control). B. Cells were transfected with pGL3-MMP2Δ2, containing a deletion mutant of the MMP-2 promoter (deletion of the region spanning positions –1659 to –1025 relative to the MMP-2 transcription initiation site), and the luciferase activity in cell lysates was assayed at 24 hours. Deletion of the aforementioned region resulted in reduction of baseline promoter activity by 39% and 34% in PANC-1 and MIAPaCa-2, respectively (*P < 0.05) and abolished the effect of PGE2 on promoter activity.

Effects of Ets-1 Gene Silencing on PGE2-Induced Increase in MMP-2 Expression. To determine whether PGE2-mediated MMP-2 induction is Ets-1 dependent, we suppressed Ets-1 expression using RNA interference. At 48 hours after transfection of siRNA, Ets-1 expression was determined using Western blot analysis. As shown in Fig. 5A, Ets-1 expression was suppressed by 79% and 83% in PANC-1 and MIAPaCa-2 cells, respectively, as compared with their untransfected counterparts. There were no significant differences in Ets-1 expression between untransfected cells and cells transfected with control siRNA bearing no homology with any human genes. Forty-eight hours after the transfection, we treated cells with PGE2 and assayed MMP-2 expression 24 hours subsequently. Ets-1 silencing reduced baseline MMP-2 expression by 63% and 66% (P < 0.05) in PANC-1 and MIAPaCa-2 cells, respectively, and abolished PGE2-mediated MMP-2 induction in PANC-1 and MIAPaCa-2 cells (Fig. 5B). This finding suggests that Ets-1 is critical for PGE2-mediated MMP-2 expression.

Role of ERK Signaling in PGE2-Mediated Ets-1 Binding Activity. PGE2 has been reported to activate ERK signaling (23). Therefore, we next tested the role of ERK activation in mediating PGE2-induced Ets-1 activation. Cells were treated with PGE2 (10 μmol/L) in the presence or absence of the mitogen-activated protein kinase (MAPK) kinase inhibitor PD98059, and ERK1/2 phosphorylation subsequently was analyzed. PGE2 induced ERK1/2 phosphorylation within 30 minutes after its administration (Fig. 6A); PD98059 inhibited PGE2-mediated ERK1/2 phosphorylation (Fig. 6B). We then determined the effect of inhibiting ERK1/2 activation on the PGE2-induced increase in Ets-1 binding activity using EMSA. The induction of Ets-1 binding by PGE2 was abolished by PD98059, as shown in Fig. 6C. These findings suggest that PGE2-induced Ets-1 binding activity is mediated by ERK signaling.

Effects of PGE2 on Cellular Invasion and Migration. We previously have shown that MMP-2 activity is associated with pancreatic cancer invasive potential (9, 24). Here we tested the effects of PGE2 and RFX on cellular invasive and migratory potentials. PGE2 induced 2.8-fold and 2.9-fold increases in PAC-1 and MIAPaCa-2 cellular invasiveness through Matrigel, respectively, relative to untreated cells (P < 0.05). In contrast, RFX induced 63% and 66% reductions in cellular invasiveness in MIAPaCa-2 and PAC-1, respectively, relative to untreated controls (P < 0.05). PGE2 induced 3.2-fold and 3.7-fold increases in PAC-1 and MIAPaCa-2 cellular migration, respectively (P < 0.05); RFX induced 55% and 58% reductions in cellular migration, respectively (Fig. 7A; P < 0.05). Ets-1 silencing reduced PAC-1 and MIAPaCa-2 cellular invasiveness by 30% and 34%, respectively, and abrogated PGE2-mediated increases in cellular invasiveness by 65% and 63%, respectively, relative to PGE2-treated control cells (P < 0.05). Interestingly, Ets-1 silencing altered neither baseline cellular migration nor PGE2-induced increase in cellular migration in PAC-1 and MIAPaCa-2 cells (Fig. 7B). The effects of MAPK kinase inhibition on cellular invasiveness and migration were similar to those of Ets-1 gene silencing. PD98059 (40 μmol/L) reduced baseline PAC-1 and MIAPaCa-2 cellular invasiveness by 40% and 44%, respectively (P < 0.05), and abrogated PGE2-induced increases in cellular invasiveness by 63% and 74%, respectively (P < 0.05), relative to PGE2-treated control cells. There was no significant effect of PD98059 on cellular migration (Fig. 7C).

To examine the relationship between observed alterations in invasiveness and in MMP-2 expression, Matrigel invasion assays were conducted in the presence of MMP-2 neutralizing antibody. PGE2-mediated increases in PAC-1 and MIAPaCa-2 cellular invasiveness through Matrigel were abrogated by anti-MMP-2 antibody but not by control IgG (Fig. 7D). These findings suggest that PGE2-mediated effects on pancreatic cancer cellular invasiveness through Matrigel are at least in part MMP-2 dependent.

DISCUSSION

There is much evidence that implicates important roles for COX-2 and PGE2 in carcinogenesis and progression for a wide range of malignancies (3, 25–29), including pancreatic cancer (2, 3). However, there are few data on the downstream targets by which COX-2 and PGE2 mediate these processes. In this study, we
identified Ets-1 as an important mediator of PGE$_2$-induced induction of MMP-2 expression. Our study is the first to establish the association between PGE$_2$ and Ets-1 in cancer; it also provides evidence that COX-2 may mediate cancer invasion by inducing MMP-2.

The potentially important roles of COX-2 and PGE$_2$ in cancer invasion are supported not only by the observed relationship between COX-2 overexpression and cancer invasion or metastasis but also by other in vitro data. Tsujii _et al._ (30) showed that forcibly overexpressing COX-2 increases MMP-2 expression/activation and cellular invasiveness in colon cancer cells. Administration of COX-2 inhibitors also has been reported to suppress MMP-2 expression and invasiveness in cells derived from a range of cancers, including those of the colon and rectum (31, 32), breast (33), lung (34), and pancreas (35).

Our study builds on these previously reported findings by revealing a potential mechanism by which COX-2–derived PGE$_2$ mediates MMP-2 expression and cellular invasiveness. We hypothesized that Ets-1 might play a role in PGE$_2$-mediated MMP-2 induction. Ets-1 has been reported to be overexpressed in variety of malignancies, including pancreatic cancers (14), and to regulate the transcription of many MMPs, including MMP-2 (15, 22, 36, 37). A recently reported study localized an Ets-1 binding site to the MMP-2 promoter (18). Our findings that PGE$_2$ induces Ets-1 expression and invasiveness in cells derived from a range of cancers, including those of the colon and rectum (31, 32), breast (33), lung (34), and pancreas (35).

Our study builds on these previously reported findings by revealing a potential mechanism by which COX-2–derived PGE$_2$ mediates MMP-2 expression and cellular invasiveness. We hypothesized that Ets-1 might play a role in PGE$_2$-mediated MMP-2 induction. Ets-1 has been reported to be overexpressed in variety of malignancies, including pancreatic cancers (14), and to regulate the transcription of many MMPs, including MMP-2 (15, 22, 36, 37). A recently reported study localized an Ets-1 binding site to the MMP-2 promoter (18). Our findings that PGE$_2$ induces Ets-1
binding activity and MMP-2 promoter activity and that ETS-1 silencing abolishes the effect of PGE$_2$ on MMP-2 expression clearly support our hypothesis.

We further explored up-stream regulation of Ets-1 binding activity. PGE$_2$ previously has been reported to activate MAPK signaling in cancer cell lines (8, 23). Nonsteroidal anti-inflammatory drugs also are reported to suppress ERK1/2 phosphorylation (38). MAPK signaling is reported to enhance Ets-1/Ets-2 binding activity (39). These data, taken together with ours, suggest that Ets-1 binding activity is modified by MAPK signaling provoked by PGE$_2$. The precise mechanisms by which ERK regulates Ets-1 DNA binding affinity remain to be defined. Some studies suggest that Ets-1 protein is induced by MAPK signaling (40, 41). However, our study, although Ets-1 binding activity peaked at 4 hours after administration of PGE$_2$, no changes in Ets-1 protein expression levels were observed to occur during this period (data not shown). This finding suggests the presence of other mechanisms regulating Ets-1 binding activity. Ets-1 is known to interact with other transcription factors such as nuclear factor κB (42, 43), activator protein 1 (44), and Sp1 (45), each of which can modulate its DNA binding activity. Further studies are required to determine the role of interaction between Ets-1 and these other transcription factors in the context of ERK/Ets-1 signaling.

Our data show that PGE$_2$ increases not only MMP-2–dependent cellular invasiveness through Matrigel but also cellular migratory motility through phosphatidylinositol 3’-kinase/Akt signaling (8). In our study, neither Ets-1 gene silencing nor MAPK kinase inhibition affected PGE$_2$–mediated cellular migration. This finding suggests that the effects of PGE$_2$ on MMP-2 expression and on cellular migration are regulated through separate signaling pathways. They further suggest that PGE$_2$ may activate multiple signaling pathways that have synergetic effects on cancer invasion.

The relationship between COX-2 expression and cancer invasion has been observed in several human malignancies (25, 46), including pancreatic cancer (1, 2). Our findings provide a mechanistic explanation for these observations. Detailed information on the molecular signaling pathways driving cancer invasion is important for two reasons: First, key components of these signaling pathways represent candidate biomarkers that may predict patient outcomes more reliably than traditional morphologic criteria; and second, understanding these pathways may suggest potential therapeutic targets for inhibiting invasion, a process that is a major contributor to death among patients with cancers such as those of the pancreas.

In summary, our finding indicates that COX-2–derived PGE$_2$ promotes pancreatic cancer cellular invasive potential through an ERK/Ets-1–dependent induction of MMP-2. This information provides a mechanistic rationale for the observed relationship between COX-2 overexpression and pancreatic cancer progression; targeting this pathway may be a potential therapeutic strategy to inhibit the progression of this highly invasive cancer.

Fig. 6. The effect of PGE$_2$ on ERK1/2 phosphorylation and the effect of PD98059 on Ets-1 binding activity. A, ERK1/2 phosphorylation. Cells were serum starved overnight and then treated with PGE$_2$ (10 μmol/L). ERK1/2 phosphorylation was assayed using Western blot analysis with anti-phosphorylated-ERK1/2 antibody. Equal loading of proteins in each lane was confirmed using anti-ERK1/2 antibody. ERK1/2 phosphorylation was induced by PGE$_2$ within 15 minutes of administration in PANC-1 and MIAPaCa-2 cells. B, the effect of PD98059 on PGE$_2$–induced ERK1/2 phosphorylation. Cells were treated with PGE$_2$ (10 μmol/L) in the presence or absence of PD98059 (40 μmol/L) for 30 minutes, after which ERK1/2 phosphorylation was assayed. PD98059 abolished the effect of PGE$_2$ on ERK phosphorylation in PANC-1 and MIAPaCa-2 cells. C, the effect of PD98059 on Ets-1 binding activity. Cells were treated with PGE$_2$ (10 μmol/L) in the presence or absence of PD98059 (40 μmol/L) for 4 hours, after which Ets-1 binding activity was assayed using EMSA. PGE$_2$–induced increases in Ets-1 binding activity were decreased with PD98059 in PANC-1 and MIAPaCa-2 cells.
Fig. 7. The effects of PGE$_2$ and RFX on cellular invasiveness and migration. A, the effect of PGE$_2$ and RFX. Cellular invasiveness and migration were assayed using Boyden chambers with or without Matrigel, respectively, as described in the Materials and Methods section. Left, PGE$_2$ (10 µmol/L) increased cellular invasiveness through Matrigel by 178% and 191% in PANC-1 and MIAPaCa-2 cells, respectively (P<0.05, versus control), and RFX (5 µmol/L) reduced cellular invasiveness by 63% and 66% (P<0.05, versus control), respectively. Right, PGE$_2$ increased cellular migration by 224% and 268% in PANC-1 and MIAPaCa-2 cells, respectively (P<0.05, versus control), and RFX reduced cellular migration by 55% and 58%, respectively (P<0.05, versus control). B, the effect of Ets-1 gene silencing. Left, Ets-1 gene silencing reduced PANC-1 and MIAPaCa-2 cellular invasiveness by 30% and 34%, respectively (P<0.05, versus untransfected control) and abrogated PGE$_2$-induced increases in invasion by 65% and 63%, respectively (P<0.05, versus PGE$_2$-treated untransfected control cells). Right, Ets-1 gene silencing had no effect on either PANC-1 and MIAPaCa-2 baseline cellular migration or PGE$_2$-induced increases in cellular migration (P<0.05, versus corresponding control cells that received no PGE$_2$). C, the effect of PD98059. Left, PD98059 (40 µmol/L) reduced PANC-1 and MIAPaCa-2 cellular invasiveness by 40% and 44%, respectively (P<0.05, versus untreated control), and abrogated PGE$_2$-induced increases in invasion by 63% and 74%, respectively (P<0.05, versus PGE$_2$-treated controls). Right, PD98059 had no effect on either PANC-1 or MIAPaCa-2 baseline cellular migration or PGE$_2$-induced increases in cellular migration (P<0.05, versus corresponding control cells that received no PGE$_2$). D, the effect of MMP-2 neutralizing antibody on cellular invasiveness. Cellular invasiveness was assayed in the presence of anti-MMP-2 antibody or control IgG. PGE$_2$-induced increases in cellular invasiveness were abrogated by anti-MMP-2 antibody but not by control IgG (P<0.05, versus PGE$_2$-treated control).

ACKNOWLEDGMENTS

We thank Jan Rounds for her excellent secretarial assistance.

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*Cancer Res* 2004;64:7439-7446.