Cyclooxygenase-2-dependent and -independent effects of celecoxib in pancreatic cancer cell lines

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Abstract

Cyclooxygenase-2 (COX-2) is involved in inhibition of apoptosis, potentiation of cell growth, and angiogenesis and as such is a target for drug development. The COX-2 enzyme is frequently overexpressed in pancreatic cancer. The aim of this study was to determine the effects of celecoxib on the growth inhibition and induction of apoptosis by gemcitabine in pancreatic cancer cell lines. Baseline expression of COX-2 enzyme was determined by Western blot analysis in five human pancreatic cancer cell lines. Cells were treated with gemcitabine (100 nmol/L), celecoxib (1, 10, and 50 μmol/L), and the combination. No potentiation in growth inhibition was observed in MIAPaCa cells (low COX-2 expression). However, growth inhibition and apoptosis were significantly increased with celecoxib in the BxPC-3 cells that have a high COX-2 expression. Significant down-regulation of nuclear factor-κB activation was observed in BxPC-3 cells treated with celecoxib and gemcitabine. Moreover, down-regulation of COX-2 mRNA and protein expression was also observed in the BxPC-3 cells treated with the combination as compared with the untreated and the celecoxib-treated and gemcitabine-treated cell lines. We conclude that celecoxib potentiates gemcitabine-induced growth inhibition and apoptosis in pancreatic cell lines. In addition to inhibition of the COX-2 enzyme, the celecoxib and gemcitabine combination down-regulated nuclear factor-κB activation, which in turn may have contributed to the induction of apoptosis and the down-regulation of transcription of the COX-2 enzyme. [Mol Cancer Ther 2004;3(11):1421–6]

Introduction

Pancreatic cancer remains a leading cause of cancer mortality. Gemcitabine (2′,2′-difluorodeoxycytidine) is currently considered the optimal treatment for patients with newly diagnosed metastatic pancreatic cancer. Unfortunately, the impact of gemcitabine-based chemotherapy regimens in pancreatic cancer has been at best modest (1). Therefore, novel approaches combining gemcitabine with biological agents such as celecoxib in advanced pancreatic cancer are being pursued.

The cyclooxygenase (COX) isoenzymes catalyze the rate-limiting step in the conversion of arachidonic acid into prostaglandins (2). Three isoforms of the COX enzymes have been identified. COX-2, an inducible enzyme, is overexpressed in malignancies including pancreatic cancer (3). Recent evidence suggests that COX-2 has a central role in the development and growth of cancer (3). Increased invasiveness (4) and promotion of angiogenesis (5) have been associated with COX-2 overexpression. COX-2 has antiapoptotic effects (6, 7). In pancreatic cancer, an association between COX-2 expression and perineural invasion has been reported (8). The complex regulation of COX-2 protein expression is mediated through both transcriptional and post-transcriptional mechanisms.

Ras mutations are present in up to 90% of pancreatic cancer (9). Expression of mutant k-Ras activates the Raf/mitogen-activated protein kinase pathway, resulting in up-regulation of COX-2 transcription (10). Furthermore, mutant Ras activates the Akt/protein kinase B pathway (11), resulting in stabilization of COX-2 mRNA (12) and activation of the nuclear factor-B (NF-B) transcriptional factor. Furthermore, prostaglandins such as prostaglandin E2 are known to activate NF-B (13), resulting in further transcription of the COX-2 gene (Fig. 1). In turn, NF-B transcriptionally up-regulates the expression of the COX-2 gene (14).

Selective and nonselective COX-2 inhibitors have been shown to induce apoptosis as well as potentiate the growth inhibitory effects of chemotherapeutic agents including gemcitabine in pancreatic cancer cell lines (15–17). Prostaglandins can reverse the growth inhibitory effects of COX-2 inhibitors (7). Therefore, COX-2 is a potential target for chemoprevention and therapy. Celecoxib, a selective COX-2 inhibitor, has been shown previously to inhibit the growth of human pancreatic cancer cell lines (3). In addition to COX-2 inhibition, celecoxib promotes apoptosis through COX-2-independent pathways involving the inhibition of phosphoinositide 3-kinase activation of the Akt pathway (18–20). Clinical trials indicate an improved safety profile of celecoxib in comparison with nonsteroidal anti-inflammatory drugs (NSAID) with respect to gastrointestinal toxicity (21).

The first aim of this study was to investigate the growth inhibitory effects of gemcitabine with celecoxib in pancreatic cancer cell lines. The second aim of the study was to evaluate the contribution of the COX-2 expression on the growth inhibition of the celecoxib and gemcitabine.
combination. To evaluate this effect, we compared the effects of the two drugs in four pancreatic cancer cell lines with different baseline COX-2 expression levels. The third aim of the study was to determine the COX-2-independent effects of celecoxib. Because celecoxib is also known to inhibit the Akt pathway and to decrease prostaglandin synthesis, we hypothesized that celecoxib inhibits NF-κB activation leading to apoptosis and down-regulation of COX-2 mRNA and protein expression. Our hypothetical diagram is represented in Fig. 1.

Materials and Methods

Cell Culture, Drugs, and Reagents

Human pancreatic cancer cell lines BxPC-3, MIAPaCa, PANC-1, MOH-1, and HPAC were used in this study. BxPC-3 cells were grown in RPMI 1640 with 10% fetal bovine serum. MIAPaCa and PANC-1 cells were grown as a monolayer cell culture in DMEM containing 4.5 mg/mL d-glucose and L-glutamine supplemented with 10% fetal bovine serum, and MOH-1 and HPAC cells were grown in DMEM/F-12 (1:1) with 10% calf serum. Celecoxib and gencitabine were generous gifts from Pfizer (New York, NY) and Eli Lilly Research Laboratories (Indianapolis, IN), respectively. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), isopropanol, and DMSO were respectively. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), isopropanol, and DMSO were purchased from Sigma Chemical (St. Louis, MO). Apoptosis detection kit was purchased from Roche Applied Science (Indianapolis, IN). Cell culture media were purchased from Life Technologies (St. Louis, MO). Apoptosis detection kit was purchased from Roche Applied Science (Indianapolis, IN). Cell culture media were purchased from Life Technologies (Grand Island, NY).

Cell Viability Assay

The viability of cells treated with gencitabine, celecoxib, or the combination was determined by the standard MTT reduction assay. BxPC-3, HPAC, PANC-1, and MIAPaCa cells were plated (5-10,000/well) in 96-well plate and incubated overnight at 37°C. Celecoxib was dissolved in DMSO and added to cell culture medium at a volume-to-volume concentration not exceeding 0.1%. The effects of celecoxib (1, 10, or 50 μmol/L), gencitabine (100 nmol/L), and the combination on BxPC-3, HPAC, PANC-1, and MIAPaCa cell lines were studied. The MTT assay was done in triplicates for each drug concentration used. After the required drug treatment time, aliquots of 100 μL MTT (1 mg/ mL) were added to each well and incubated for 2 hours at 37°C. The supernatant was removed and isopropanol (100 μL) was then added. The color intensity was measured by kinetic microplate reader (Molecular Devices, Sunnyvale, CA) at 595 nm. DMSO-treated cells were considered untreated control and assigned a value of 100%. Linearity of the color intensity relative to cell number within the range expected in the study was determined at the outset.

Apoptosis Assay

The Cell Death Detection ELISA kit (Roche Applied Science) was used to detect apoptosis in treated BxPC-3 and MIAPaCa cells. The assay is based on a photometric enzyme immunoassay for the qualitative and quantitative determination of cytoplasmic histone-associated DNA fragments (mononucleosome and oligonucleosome). The assay uses anti-histone b10 antibodies that bind to H2A, H2B, H3, and H4 histones and anti-DNA peroxidase antibodies that react with ssDNA and dsDNA. Cells seeded in 96-well plates were treated with celecoxib (1 and 2 μmol/L), gencitabine (100 nmol/L), or the combination. The cells were trypsinized and ~10,000 cells were added to lysis buffer (500 μL) and incubated at room temperature for 0.5 hour. The cells were centrifuged at 20,000 × g for 10 minutes and the supernatant (100 L) was transferred into anti-histone-coated microtiter plate and incubated at room temperature for 90 minutes. The plate was washed twice with washing solution (200 L) provided with the kit. A solution containing anti-DNA peroxidase (100 L) dissolved in incubation buffer was added to the same plate and incubated for 90 minutes. After removal of the unbound antibodies, the nucleosomes were quantified by the peroxidase reaction using 2,2′-azino-di(3-ethylbenzthiazol- lin-sulfonat) as substrate. A microtiter plate reader at 492 nm read the color intensity.

Immunoblotting for the Expression of COX-2 Protein

BxPC-3, MIAPaCa, MOH-1, HPAC, and PANC-1 cells were used to determine the baseline expression of the COX-2 enzyme. The influence of treatment on COX-2 expression was also determined. BxPC-3 cells were treated with gencitabine (100 nmol/L) and/or celecoxib (1 and 2 μmol/L) for 48 hours, harvested by scraping the cells from culture plates, and collected by centrifugation. Cells were resuspended in Tris buffer (125 mmol/L, pH 6.8), sonicated twice for 10 seconds, and lysed using an equal volume of 8% SDS. Cell extracts were boiled for 10 minutes and chilled on ice. Protein concentration was then measured using BCA Protein Assay Kit (Pierce, Rockford, IL). The samples were loaded on 10% SDS-PAGE for separation and electrophoretically transferred to a nitrocellulose

Figure 1. A hypothetical model for NF-κB and COX-2 pathway interaction. NF-κB activation via Akt signaling and/or exposure to gencitabine results in increased transcription of the COX-2 gene and inhibition of apoptosis. The COX-2 enzyme catalyzes the production of prostaglandins (PGE2), which in turn can activate NF-κB.
membrane. Each membrane was incubated with monoclonal antibody against COX-2 (1:1,000, Cayman Chemical Co., MI) and polyclonal anti-β-actin (1:2,000, Sigma Chemical). Blots were washed with phosphate buffer containing 0.05% Tween and incubated with secondary antibodies conjugated with peroxidase. The signal intensity was then measured using chemiluminescence detection system (Pierce). Autoradiograms of the Western blots were scanned with Gel Doc 1000 image scanner (Bio-Rad, Hercules, CA) that was linked to a Macintosh computer.

Reverse-Transcription PCR for COX-2 mRNA Expression

The influence of treatment on COX-2 mRNA was determined by reverse transcription-PCR. BxPC-3 cells were treated with gemcitabine (100 nmol/L) and/or celecoxib (1 and 2 μmol/L) for 48 hours. Culture medium was removed and Trizol (2 mL) was added. The cells were scraped and the lysate was passed through a pipette several times. Chloroform (20 μL) was added and was mixed, incubated at room temperature for few minutes and then centrifuged at 12,000 × g for 10 minutes. The pellet was washed with 80% ethanol and dissolved in RNase-free water. Total RNA (2 g) was reverse transcribed using DTT (0.1 mmol/L), deoxynucleotide triphosphates (1 mmol/L), and random primers (5 pmol/L), SuperScript II (10 units/L, Invitrogen, Grand Island, NY). Reverse transcription-PCR for COX-2 and -actin amplification was done using Taq polymerase (Invitrogen) with COX-2 primer (Sigma Chemical). The PCR conditions were 94°C for 1 minute, 53°C for 1 minute, and 72°C for 2 minutes for 35 cycles. PCR products were subjected to electrophoresis on 2% agarose gel and were stained with ethidium bromide.

Electrophoretic Mobility Shift Assay for NF-κB Activation

BxPC-3 cells were treated with gemcitabine (100 nmol/L) and/or celecoxib (1 and 2 μmol/L) for 48 hours. The cells were suspended in Triton X-100 lysis buffer (500 μL) containing Tris-HCl (20 mmol/L, pH 7.5), (100 mmol/L) MgCl2, levamisole (50 mmol/L), sodium butyrate (200 mmol/L), phenylmethylsulfonyl fluoride (100 mmol/L), and protein inhibitor (Roche Applied Science), which contain a broad spectrum of serine, cysteine, and metalloproteases. The cells were lysed with 20 strokes in a Dounce homogenizer ( Kontes Glass Co., New Jersey, NJ) and centrifuged at 3,000 × g for 15 minutes at 4°C. The nuclear pellet was resuspended in an equal volume of Tris-HCl (10 mmol/L, pH 7.4) and MgCl2 (5 mmol/L) followed by equal volume of NaCl (1 mol/L), Tris-HCl (10 mmol/L, pH 7.4), and MgCl2 (5 mmol/L). The nuclear suspension was incubated on ice for 30 minutes and centrifuged at 10,000 × g for 20 minutes at 4°C. The supernatant was quantified using BCA assay (Pierce).

EMSA was done using the Odyssey Infrared Imaging System with NF-κB IRDye-labeled oligonucleotide from LI-COR, Inc. (Lincoln, NE). The DNA binding reaction was set up using nuclear extract (5 μg) mixed with oligonucleotide and gel shift binding buffer consisting of 20% glycerol, MgCl2 (5 mmol/L), EDTA (2.5 mmol/L), DTT (2.5 mmol/L), NaCl (250 mmol/L), Tris-HCl (50 mmol/L, pH 7.5), and polydeoxyinosinic-polydeoxycytidylic acid (0.25 mg/mL). The reaction was incubated at room temperature in dark for 30 minutes. Orange G loading dye (2 μL, 10×) was added to each sample and loaded on the pre-run 8% polyacrylamide gel and ran at 30 mA for 1 hour. NF-κB p65 antibody and unlabeled NF-κB DNA were used to confirm the supershift and the specificity of NF-κB DNA binding activity.

The gel was scanned and the signals were quantified using Odyssey Infrared Imaging System, and Odyssey software (LI-COR). Comparison between untreated and treated cells was done via t test. Statistical significance was assumed for P ≤ 0.05.

Results

Baseline Expression of COX-2 in Human Pancreatic Cancer Cells

Figure 2 shows the immunoblot for COX-2 expression in BxPC-3, MIAPaCa, MOH-1, HPAC, and PANC-1 cell lines. COX-2 expression was highest in the BxPC-3 cell line followed by MIAPaCa, HPAC, MOH-1, and PANC-1.

Effects of Celecoxib and Gemcitabine on the Viability of Pancreatic Cancer Cells

Viability of BxPC-3, MIAPaCa, HPAC, and PANC-1 pancreatic cancer cells treated with celecoxib (1, 10, or 50 μmol/L), gemcitabine (100 nmol/L), and the combination was determined by the MTT assay. In the BxPC-3 cell line, a potentiation of the growth inhibition of gemcitabine by celecoxib was observed with the 1 μmol/L concentration of celecoxib (Fig. 3A). Although a potentiation of growth inhibition of gemcitabine by celecoxib was observed with the 1 μmol/L concentration of celecoxib (Fig. 3A), at the 5 μmol/L concentration, a reduction in growth inhibition of gemcitabine by celecoxib was noted (Fig. 3B). HPAC (Fig. 3C), and PANC-1 (Fig. 3D) cell lines was observed, these did not reach statistical significance. Hence, we selected BxPC-3 and MIAPaCa as higher and lower sensitivity cell lines, respectively, for further studies.
in apoptosis was noticed with celecoxib, gemcitabine, or the combination (Fig. 4B). Therefore, we selected the BxPC-3 cell line for further studies evaluating the effects of celecoxib on the COX-2 expression and NF-κB activation in an attempt to study further the mechanisms underlying the potentiation of apoptosis by celecoxib.

Modulation of COX-2 Expression in BxPC-3 Cells Treated with Celecoxib and Gemcitabine

The expression of COX-2 protein was determined in BxPC-3 cells treated with gemcitabine (100 nmol/L), celecoxib (1 and 2 μmol/L), or the combination (Fig. 5). Neither gemcitabine nor celecoxib resulted in a significant alteration in COX-2 protein expression as measured by immunoblotting. In contrast, the treatment with gemcitabine plus celecoxib resulted in a significant down-regulation of COX-2 expression. To confirm the down-regulation of COX-2, the level of COX-2 mRNA expression was evaluated in BxPC-3 cells treated with gemcitabine (100 nmol/L), celecoxib (1 and 2 μmol/L), or the combination. A significant down-regulation of COX-2 mRNA was seen in the BxPC-3 cells treated with the combination of the two drugs (Fig. 6).

Activation of NF-κB in BxPC-3 Cells Treated with Celecoxib and Gemcitabine

Because NF-κB plays a critical role in cell survival and COX-2 expression, the NF-κB activation was determined in BxPC-3 cells treated with gemcitabine (100 nmol/L), celecoxib (1 and 2 μmol/L), or the combination (Fig. 7). Gemcitabine treatment activated NF-κB at 48 hours, whereas celecoxib resulted in the down-regulation of NF-κB activation at 48 hours. However, a significant inhibition of NF-κB was observed in cells treated with the combination of the two drugs for 48 hours, and these results were consistent with our hypothesis as presented in Fig. 1.

Discussion

The COX-2 enzyme is a modulator of carcinogenesis, apoptosis (6), and angiogenesis (4) in pancreatic cancer and as such is a rational target for drug development. The selective COX-2 inhibitor (NS-398) and NSAIDs are known to potentiate the growth inhibitory effects of gemcitabine in pancreatic cancer cell lines (15). The growth inhibitory effects of gemcitabine and NSAIDs is thought to be mainly due to inhibition of cell cycle progression without a significant impact on apoptosis (15). The relationship

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Figure 3. Effect of celecoxib (☐), gemcitabine (100 nmol/L; □), and the combination (■) on cell viability by the MTT assay. (A) BxPC-3, (B) MIAPaCa, (C) HPAC, and (D) PANC-1 human pancreatic cancer cells were treated as described under Materials and Methods. Cell viability was calculated relative to untreated cells. There was a significant reduction in cell viability in the BxPC-3 cells treated with gemcitabine and celecoxib compared with cells treated with either agent alone (P = 0.004). In the MIAPaCa, PANC-1, and HPAC cells, a nonsignificant increase in growth inhibition was observed with the combination over cells treated with either agent alone (P = 0.073, 0.067, and 0.054, respectively).

Figure 4. Induction of apoptosis in BxPC-3 (A; top, day 1; bottom, day 2) and MIAPaCa (B; top, day 1; bottom, day 2) human pancreatic cancer cells. Cells were treated with gemcitabine (100 nmol/L), celecoxib (1 μmol/L), or the combination as described in Materials and Methods. There was a significant potentiation of apoptosis observed in BxPC-3 cells treated with gemcitabine and celecoxib as compared with cells treated with either drug alone (P = 0.0092). No potentiation of apoptosis was observed in the MIAPaCa cell line (P = 0.23). Control, untreated cells; Gem, gemcitabine-treated cells; Cel, celecoxib-treated cells; Gem + Cel, cells treated with the combination of gemcitabine and celecoxib.

Figure 5. Western blot analysis of the expression of COX-2 in BxPC-3 cell line untreated and treated with celecoxib (1 or 2 μmol/L), gemcitabine (100 nmol/L), or the combination. Significant down-regulation of COX-2 expression was observed in cells treated with gemcitabine and celecoxib. Control, untreated cells; G, gemcitabine-treated cells; C, celecoxib-treated cells; G + C, cells treated with the combination of gemcitabine and celecoxib.
between COX-2 expression and the antitumor activity of COX inhibitors is still controversial. Yip-Schneider et al. (17) compared the growth inhibitory effects of indomethacin, sulindac, and NS-398 in three pancreatic cancer cell lines with differing baseline COX-2 expression. Significantly higher growth inhibition was observed in the BxPC-3 cell line as compared with the PaCa-2 cell line, which does not express the COX-2 enzyme. Using a similar design, Molina et al. (16) found no relation between baseline COX-2 expression and growth inhibition with sulindac or NS-398.

In the present study, we evaluated the effects of celecoxib and gemcitabine on the growth and apoptosis in pancreatic cancer cell lines with different baseline COX-2 expression. In the BxPC-3 cell line, celecoxib potentiated the growth inhibition of gemcitabine. The effect of the gemcitabine-celecoxib combination on the growth of MIAPaCa, PANC-1, and HPAC cells was less evident. Therefore, it seems that the antitumor effects of celecoxib are partly mediated through inhibition of the COX-2 enzyme and that a low baseline expression of COX-2 enzyme may decrease the benefit from combining celecoxib with gemcitabine. Future clinical trials incorporating celecoxib with chemotherapeutic agents may consider measurement of baseline COX-2 expression as a possible predictor of response.

The growth inhibition of celecoxib in BxPC-3 and MIAPaCa cell lines was concentration dependent. Higher concentrations of celecoxib exceed the levels required to inhibit prostaglandin production by the COX-2 enzyme. Therefore, the observed increase in growth inhibition at these high concentrations of celecoxib suggests that mechanisms other than COX-2 inhibition may be involved in the effects of celecoxib. This observation is supported by previous studies demonstrating the ability of NSAIDs to inhibit the growth of colorectal carcinoma cell lines that lacked COX-1 and COX-2 enzyme expression (22, 23).

The mechanism of growth inhibition observed with celecoxib and gemcitabine seemed to significantly differ between BxPC-3 and MIAPaCa cell lines. In the BxPC-3, the combination resulted in a significant increase in the proportion of cells undergoing apoptosis. In contrast, no increase in apoptosis was observed in the MIAPaCa cell line, and the growth inhibitory effects were likely because of cell cycle arrest as reported previously. If the predominant effect of COX-2 inhibitors including celecoxib in a given pancre-

NF-κB is a transcriptional factor that is involved in a wide spectrum of cellular functions including apoptosis and cell cycle control. NF-κB is retained in the cytoplasm by the inhibitory protein IκB (24). Several mediators in the signal transduction pathway including Akt are known to activate NF-κB through phosphorylation of IκB (12). Activated NF-κB translocates to the nucleus, resulting in the transcription of several genes (e.g., COX-2). In turn, prostaglandin production (13) can further activate NF-B (Fig. 1). Therefore, activation of NF-κB can result in an exaggerated and sustained effect through the activation of the COX-2 pathway. Celecoxib is known to inhibit the phosphoinositide 3-kinase/Akt pathway in addition to inhibiting prostaglandin production (18, 19, 25). NF-κB has been shown to inhibit apoptosis in response to chemotherapeutic agents (26) and to promote the transcription of the COX-2 gene (14). Therefore, we assayed the effects of celecoxib, gemcitabine, and the combination on NF-κB activation. Gemcitabine resulted in a stimulation of NF-κB activation. The combination of gemcitabine and celecoxib significantly inhibited NF-κB activation, resulting in an increase in the proportion of cells undergoing apoptosis and down-regulation of the transcription of COX-2 enzyme.

K-Ras mutations is known to increase COX-2 expression through the stabilization of the COX-2 mRNA (11) and the activation of the Akt/NF-κB pathway (12). The BxPC-3 cell line used in this study has wild-type Ras gene. Therefore, the observed down-regulation of NF-κB activation and

### Figure 6
Reverse transcription-PCR analysis of the expression of COX-2 mRNA in BxPC-3 cells treated with celecoxib (1 or 2 μmol/L), gemcitabine (100 nmol/L), or the combination. Significant down-regulation of COX-2 mRNA expression was observed in the cells treated with gemcitabine and celecoxib. Control, untreated cells; G, gemcitabine-treated cells; C, celecoxib-treated cells; G + C, cells treated with the combination of gemcitabine and celecoxib.

### Figure 7
Determination of NF-κB activation by EMSA in BxPC-3 cells treated with celecoxib (1 or 2 μmol/L), gemcitabine (100 nmol/L), or the combination. Significant down-regulation of NF-κB activation was observed in the cell line treated with gemcitabine and celecoxib. Top, NF-κB DNA binding activity; bottom, quantitation of signals. Control, untreated cells; G, gemcitabine-treated cells; C, celecoxib-treated cells; G + C, cells treated with the combination of gemcitabine and celecoxib.
COX-2 expression in the BxPC-3 cell line might not apply to pancreatic cell lines with k-Ras mutations. Preclinical models evaluating the impact of the dysregulation of the different pathways involved in signal transduction including the k-Ras mutations on the observed COX-2-independent effects of celecoxib should be evaluated in future trials.

Although all the NSAIDs and COX-2-specific inhibitors share the ability to inhibit prostaglandin production, their effect on COX-2 expression is variable. Meade et al. (27) showed that NSAIDs induced COX-2 expression in mammary epithelial cells and colon carcinoma cell lines. A similar effect was observed with sulindac and NS-398 in BxPC-3 cell line (16). The induction of COX-2 expression was attributed to the peroxisome proliferative response elements (27), which are induced by COX inhibitors. Elder et al. (28) reported up-regulation of COX-2 expression in colorectal cancer cell lines by NS-398. This up-regulation was mediated through activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway (28). In our study, celecoxib did not induce COX-2 expression, indicating that the COX-2-independent effects of COX inhibitors vary among cell lines.

In conclusion, we found that celecoxib could potentiate the growth inhibition induced by gemcitabine in pancreatic cancer cell lines. The observed potentiation of growth inhibition and induction of apoptosis is potentially clinically significant considering that it was observed at nanomolar concentrations of gemcitabine that are 100-fold below the peak plasma concentration (10–25 μmol/L) in humans (29). The effects of the gemcitabine and celecoxib are mediated through inhibition of the COX-2 enzyme and the NF-κB pathway and these observations are consistent with our hypothesis as presented in Fig. 1. Elucidating the COX-2-independent effects of celecoxib could facilitate the identification of patients who may benefit from addition of celecoxib to standard cytotoxic therapy.

References
Molecular Cancer Therapeutics

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