Parthenolide and sulindac cooperate to mediate growth suppression and inhibit the nuclear factor-κB pathway in pancreatic carcinoma cells

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Abstract
Activation of the transcription factor nuclear factor-κB (NF-κB) has been implicated in pancreatic tumorigenesis. We evaluated the effect of a novel NF-κB inhibitor, parthenolide, a sesquiterpene lactone isolated from the herb feverfew, in three human pancreatic tumor cell lines (BxPC-3, Panc-1, and Mia PaCa-2). Parthenolide inhibited pancreatic cancer cell growth in a dose-dependent manner with substantial growth inhibition observed between 5 and 10 μmol/L parthenolide in all three cell lines. Parthenolide treatment also dose-dependently increased the amount of the NF-κB inhibitory protein, IκB-α, and decreased NF-κB DNA binding activity. We have previously shown that nonsteroidal anti-inflammatory drugs (NSAIDs) suppress the growth of pancreatic cancer cells. To determine whether inhibition of the NF-κB pathway by parthenolide could sensitize pancreatic cancer cells to NSAID inhibition, BxPC-3, Panc-1, and Mia PaCa-2 cells were treated with parthenolide and the NSAID sulindac, either alone or in combination. Treatment with the combination of parthenolide and sulindac inhibited cell growth synergistically in Mia PaCa-2 and BxPC-3 cells and additively in PANc-1 cells. In addition, treatment with the parthenolide/sulindac combination lowered the threshold for apoptosis. Increased levels of IκB-α protein were detected, especially in Mia PaCa-2 cells, after treatment with parthenolide and sulindac compared with each agent alone. Similarly, decreased NF-κB DNA binding and transcriptional activities were detected in cells treated with the combination compared with the single agents, demonstrating cooperative targeting of the NF-κB pathway. These data provide preclinical support for a combined chemotherapeutic approach with NF-κB inhibitors and NSAIDs for the treatment of pancreatic adenocarcinoma. [Mol Cancer Ther 2005;4(4):587–94]

Introduction
Cancer of the exocrine pancreas is the fourth leading cause of cancer-related deaths in the United States with nearly identical rates of incidence and mortality (1). The statistics for pancreatic cancer are dismal as exemplified by a 5-year survival rate of <3% and a median survival time of <6 months. Difficulty in achieving early diagnosis and the aggressive nature of this type of cancer contribute to the low survival rate of patients with pancreatic cancer. Despite advances in surgery, radiotherapy, and chemotherapy, >90% of patients with pancreatic cancer die of chemoinsensitive disease. Even the most effective drug, gemcitabine, is able to induce only a 5% response rate (1).

The elucidation of novel proteins and pathways that promote pancreatic tumor growth will aid in the identification of promising molecular targets for chemotherapy. RelA, the p65 subunit of the transcription factor nuclear factor-κB (NF-κB), is constitutively activated in human pancreatic adenocarcinoma tissue and in pancreatic tumor cell lines (2). NF-κB regulates the expression of genes involved in the inflammatory response, growth control, and apoptosis (3, 4). Normally, NF-κB is sequestered in the cytoplasm by the inhibitory IκB proteins (5). Inducible phosphorylation of the IκB proteins by IκB kinases (IKK) targets them for degradation thus allowing translocation of NF-κB into the nucleus and activation of NF-κB–inducible genes. The NF-κB–inducible gene encoding urokinase-type plasminogen activator, a protease critical for tumor invasion and metastasis, is overexpressed in pancreatic tumor cells, demonstrating NF-κB–dependent transcriptional activation in pancreatic tumor cells (6). Stat3 and NF-κB activation also prevents apoptosis during pancreatic carcinogenesis (7). Taken together, these findings suggest that constitutive NF-κB activation may contribute to pancreatic tumorigenesis, and therefore the chemotherapeutic potential of NF-κB inhibitors should be evaluated.

Compounds known as sesquiterpene lactones isolated from extracts of Mexican Indian medicinal plants inhibit NF-κB (8). Parthenolide, a sesquiterpene lactone isolated from the herb feverfew (Tanacetum parthenium), has anti-inflammatory properties and is in clinical use for the
treatment of migraines (9). In breast cancer cells expressing constitutively active NF-κB, parthenolide inhibits NF-κB DNA binding activity and increases sensitivity to the chemotherapeutic agent paclitaxel (10). In the present study, we evaluated the effect of parthenolide on the growth of human pancreatic cancer cell lines and show that parthenolide-induced growth suppression is associated with inhibition of NF-κB activity in pancreatic tumor cells.

We have previously shown that nonsteroidal anti-inflammatory drugs (NSAID), known to inhibit the enzyme cyclooxygenase (COX), suppress the growth of pancreatic cancer cells via both COX-dependent and COX-independent mechanisms (11–14). NSAIDs may therefore be effective for the treatment of pancreatic cancer. However, for resistant cancers such as pancreatic cancer, combination chemotherapy targeting pathways critical for growth and survival will likely be required. To determine whether inhibition of the NF-κB pathway by parthenolide could sensitize pancreatic cancer cells to NSAID inhibition, we evaluated the effectiveness of parthenolide in combination with the NSAID sulindac. The combination suppressed pancreatic cancer cell growth and induced apoptosis to a greater degree than either agent alone. Combination treatment also resulted in higher levels of the inhibitory IkB-α protein and decreased NF-κB DNA binding and transcriptional activities relative to each agent alone, suggesting joint targeting of the NF-κB pathway by parthenolide and sulindac.

Materials and Methods

Cell Culture and Treatments

BxPC-3, Mia PaCa-2, and Panc-1 cells were obtained from the American Type Culture Collection (Rockville, MD) and maintained as recommended. Parthenolide (Sigma-Aldrich, St. Louis, MO) was dissolved in ethanol (40 mmol/L stock) and stored at −20°C. Sulindac (Sigma-Aldrich) was dissolved in DMSO and stored at −20°C. Recombinant human tumor necrosis factor α (TNF-α; R&D Systems, Minneapolis, MN) was dissolved in PBS containing 0.1% bovine serum albumin (10 μg/mL) and stored at −20°C.

Cell Growth

Cells were plated in duplicate at 4 × 10^4/mL in 96-well plates. The following day, parthenolide was added to achieve the indicated final concentrations. Growth was assayed 48 hours after parthenolide addition using a colorimetric cell proliferation kit, 2,3-bis-[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt (Roche, Indianapolis, IN). Percent cell growth was calculated relative to the growth of control-treated cells (100%). Growth was also monitored by performing cell counts as follows. Cells were plated in 6-well plates; 72 hours after drug addition, cells were trypsinized, stained with trypan blue, and counted in duplicate using a hemacytometer. Percent cell growth was expressed relative to control-treated cells (100%). Drug additivity or synergy was determined by data analysis using CalcuSyn software (Biosoft, Cambridge, United Kingdom) based on the method of Chou and Talalay (15) for dose effect analysis. Combination index (CI) values were determined as a quantitative measure of drug interaction indicating either an additive (CI = 1), synergistic (CI < 1), or antagonistic (CI > 1) effect.

Electrophoretic Mobility Shift Assay

Cells were plated in 6-well plates and grown to 50% to 70% confluence. Cells were incubated with parthenolide at the indicated concentrations for 3 hours. Where indicated, cells were treated with TNF-α (5 ng/mL) for 10 minutes before harvesting. Whole cell lysates were prepared and incubated with radiolabeled probes specific for NF-κB or SP-1 (Promega, Madison, WI) as the internal control as described previously (16). Supershifts were done by incubating the complexes with antibodies specific for the p65 and p50 subunits of NF-κB on ice for 10 minutes. DNA-protein complexes were separated by electrophoresis and visualized by autoradiography.

Western Blotting

Where indicated, cells were treated with TNF-α (5 ng/mL) for 5 minutes before harvesting. Cells were lysed in radioimmunoprecipitation assay buffer (PBS, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, and 1 mmol/L Na3VO4), and the supernatants were obtained. Cell lysates (10 μg total protein) were resolved by SDS-PAGE on 4% to 20% gradient gels (Invitrogen, Carlsbad, CA) and transferred to Immobilon P membranes (Millipore, Billerica, MA). The blots were probed with phospho-specific (Ser52) IκB-α and total IκB-α antibodies (Cell Signaling Technology, Beverly, MA) according to the manufacturer’s protocol followed by enhanced chemiluminescence (Perkin-Elmer Life Sciences, Boston, MA) to detect phosphorylated and total protein levels, respectively. To detect Bcl-xL protein levels, a primary antibody specific for Bcl-xL was employed (Trevigen, Gaithersburg, MD).

Apoptosis

Apoptosis was measured using the Cell Death Detection ELISA (Roche) to quantitatively determine the amount of cytoplasmic histone–associated DNA fragments produced by cells undergoing apoptosis. Cell lysates were placed into streptavidin-coated microtiter plates and incubated with anti–histone-biotin and anti–DNA-peroxidase antibodies for 2 hours. After washing and incubation with substrate, the plates were read at 405 nm to quantitate the amount of nucleosomes bound to the plate. Relative apoptosis was determined by a ratio of the average absorbance of the treatment wells to the average absorbance of the control wells. Apoptosis was also measured by the terminal deoxynucleotidyltransferase–mediated nick-end labeling method to detect fragmented DNA as recommended by the manufacturer (APO-DIRECT, BD Biosciences, San Diego, CA). After labeling DNA breaks with FITC-UTP, positively stained cells were detected by flow cytometry and quantified using CellQuest software (BD Biosciences).
Transfection

Cells were plated in 6-well plates. The following day, cells were transfected with 1 μg of the NF-κB-luciferase reporter construct (3x NF-κB-luc; ref. 17) in 3 μL FuGENE 6 transfection reagent (Roche). Three hours later, cells were treated with sulindac and/or parthenolide and then TNF-α (5 ng/mL) was added after two more hours. Cells were lysed 24 hours post-transfection in passive lysis buffer followed by one freeze-thaw cycle (Promega). The substrate luciferin was added to the cell extracts and samples were read in a luminometer to determine luciferase activity. Percent luciferase activity was normalized relative to total protein.

Statistical Analysis

Statistical significance between control- and parthenolide-treated cells or between the combination treatments and either single agent alone was determined by a two-tailed Student’s t test with a 95% confidence interval.

Results

Effect of Parthenolide on Pancreatic Cancer Cell Growth

Three human pancreatic tumor cell lines (BxPC-3, MIA PaCa-2, and PANC-1) were treated with increasing concentrations of parthenolide for 48 h. Relative growth was assessed using a colorimetric proliferation assay and expressed relative to control-treated cells. Parthenolide inhibited pancreatic cancer cell growth in a dose-dependent manner with substantial growth inhibition observed between 5 and 10 μM parthenolide in all three cell lines (Fig. 1). The MIA PaCa-2 cells were the most sensitive to parthenolide-induced growth inhibition (IC_{50} = 2 μM) compared with BxPC-3 (IC_{50} = 6 μM) and PANC-1 cells (IC_{50} = 8 μM). Thus, parthenolide is an effective inhibitor of pancreatic cancer cell growth in vitro.

Effect of Parthenolide on the NF-κB Pathway

Parthenolide has been previously reported to inhibit NF-κB binding activity (8). NF-κB is normally sequestered in the cytosol in its inactive form by the inhibitory IκB proteins. Inducible phosphorylation of IκB proteins by IKKs targets them for polyubiquitination and subsequent degradation, leading to the activation of NF-κB. To study the effect of parthenolide on the NF-κB pathway, pancreatic tumor cells were treated with parthenolide for 3 hours followed by stimulation with TNF-α. TNF-α treatment facilitated the detection of changes in phosphorylated and total IκB-α levels by Western blot analysis (Fig. 2). Levels of phosphorylated IκB-α decreased with increasing concentrations of parthenolide in BxPC-3 and PANC-1 cells but increased in MIA PaCa-2 cells. In each of the three pancreatic cell lines, parthenolide dose-dependently resulted in the accumulation of IκB-α protein, suggesting that parthenolide suppresses growth in pancreatic cancer cells by elevating the level of IκB proteins that inhibit NF-κB possibly by modulating IKK activity.

The ability of parthenolide to inhibit the DNA binding activity of NF-κB in pancreatic tumor cell lines was also examined. BxPC-3, PANC-1, and MIA PaCa-2 cells were treated with increasing concentrations of parthenolide for 3 hours. Whole cell extracts were prepared, and DNA binding by NFκB was analyzed in electrophoretic mobility shift assays with NFκB and SP-1 (internal control) probes (Fig. 3A). Constitutive NFκB binding was observed in all three exponentially growing pancreatic cancer cell lines. Treatment with parthenolide inhibited NFκB binding activity in the three cell lines at concentrations similar to those required to repress cell growth and elevate IκB-α levels. Lower concentrations of parthenolide were effective in MIA PaCa-2 cells compared with PANC-1 and BxPC-3 cells. The NFκB/DNA complex 1 was shown by antibody supershift assay to consist of p65/p50 subunit heterodimers whereas complex 2 consisted of p50 subunit homodimers (Fig. 3B).

Growth Effects of Parthenolide in Combination with the NSAID Sulindac

We have previously shown that NSAIDs such as sulindac inhibit the growth of pancreatic cancer cells in vitro (11). To study the effect of NFκB inhibition on the response of pancreatic cells to NSAIDs, cells were treated with parthenolide and sulindac, alone or in combination, for 3 days (drug concentrations close to the IC_{50} were chosen). Cell growth was determined by performing cell counts relative to control-treated cells. Parthenolide in combination with sulindac inhibited the growth of pancreatic tumor cell lines greater than either agent alone (Fig. 4). Data analysis by the Chou and Talalay method showed synergistic inhibition by the drug combinations in MIA PaCa-2 and BxPC-3 cells and additive inhibition in PANC-1 cells.
Aphosphorylated and total Iα were prepared and analyzed by Western blot to detect phosphorylated and total IκB-α protein levels. Representative blot.

Figure 3. Parthenolide decreases NF-κB DNA binding activity in pancreatic cancer cells. A, BxPC-3, Panc-1, or MIA PaCa-2 cells were incubated with the indicated concentrations of parthenolide for 3 h. Whole cell lysates were prepared for electrophoretic mobility shift assay with radiolabeled NF-κB or SP-1 (internal control) specific probes. Representative experiment. B, supershifts were performed by incubating the complexes with antibodies specific for the p65 or p50 subunits of NF-κB.

Figure 2. Effect of parthenolide on the phosphorylation and total level of the NF-κB inhibitor protein, IκB-α. Pancreatic tumor cells were pretreated with parthenolide for 3 h followed by stimulation with TNF-α (5 ng/mL). TNF-α treatment facilitated the detection of changes in phosphorylated and total IκB-α levels by Western blot analysis. Lysates were prepared and analyzed by Western blot to detect phosphorylated and total IκB-α protein levels. Representative blot.

Figure 4. Growth effects of parthenolide in combination with the NSAID sulindac. BxPC-3, Panc-1, and MIA PaCa-2 cells were incubated with parthenolide and/or sulindac at the indicated concentrations. On day 3 after drug addition, cell growth was monitored by cell counts. Percent cell growth was expressed relative to control treated cells (100%). Columns, average from at least two independent experiments counted in duplicate; bars, ±SD. *, P < 0.05 versus each agent alone.

Apoptosis Is Induced by the Combination of Sulindac and Parthenolide

Cells were treated with sulindac and/or parthenolide, and apoptosis was quantitated by an ELISA detecting DNA fragmentation. In BxPC-3 and PaCa-2 cells, apoptosis could be detected after 24 or 48 hours of treatment, respectively (Table 1). The drug combinations induced greater relative apoptosis than either agent alone. Apoptosis was also measured by terminal deoxynucleotidyltransferase-mediated nick-end labeling followed by flow cytometric analysis. The percent of apoptotic PaCa-2 and BxPC-3 cells increased following treatment with the drug combination for 48 and 72 hours, respectively, confirming the ELISA results (Table 1). Thus, the combination of parthenolide and sulindac is able to lower the apoptotic threshold in two of the pancreatic cancer cell lines. In contrast, little apoptosis was induced in Panc-1 cells following treatment with the agents alone or in combination for up to 72 hours (data not shown).

Effect of Parthenolide and/or Sulindac on the Level of Phosphorylated and Total IκB-α Proteins

To determine the possible mechanism of parthenolide- and sulindac-induced growth inhibition and apoptosis, effects on the NF-κB pathway were investigated. BxPC-3, Panc-1, and MIA PaCa-2 cells were incubated with parthenolide and sulindac, alone or in combination, for 3 or 24 hours followed by the addition of TNF-α. Cell lysates were prepared and analyzed by Western blot to detect phosphorylated and total IκB-α proteins (Fig. 5). Treatment with the combination of parthenolide and sulindac for 3 hours decreased the amount of phosphorylated IκB-α in Panc-1 and MIA PaCa-2 cells but had little effect in BxPC-3 cells. After 24 hours, phosphorylated IκB-α levels were lower in all three cell lines. Sulindac alone and parthenolide alone increased the total amount of IκB-α in each of the cell lines. In BxPC-3 cells, the level of total IκB-α following treatment with the combination was similar to that induced by sulindac alone. In Panc-1 and especially in MIA PaCa-2 cells, total IκB-α levels were further elevated by the combination of parthenolide and sulindac, correlating with the lower levels of phospho-IκB-α. To confirm these results, cells were also treated with parthenolide and/or sulindac in the presence of the proteosome inhibitor MG132 that prevents IκB-α degradation, and similar effects on the level of phospho-IκB-α were observed (data not shown). These results suggest that the inhibitory effects of the combination may be, in part, mediated by the agents jointly targeting the NF-κB pathway.

NF-κB DNA Binding and Transcriptional Activities Decrease following Treatment with Sulindac and Parthenolide

To monitor effects on NF-κB activity, BxPC-3 and MIA PaCa-2 cells were treated with parthenolide and/or sulindac for 3 hours followed by the addition of TNF-α.
Table 1. Apoptosis induced by parthenolide and/or sulindac

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ELISA (relative apoptosis)</th>
<th>TUNEL (% apoptotic cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BxPC-3, control</td>
<td>1.0</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>parth, 5 μmol/L</td>
<td>5.9 ± 1.1</td>
<td>0.5 ± 0.6</td>
</tr>
<tr>
<td>sul, 250 μmol/L</td>
<td>2.1 ± 0.5</td>
<td>—</td>
</tr>
<tr>
<td>sul, 500 μmol/L</td>
<td>7.7 ± 3.1</td>
<td>15.2 ± 0.9</td>
</tr>
<tr>
<td>parth 5 + sul 250</td>
<td>12.0 ± 3.2*</td>
<td>—</td>
</tr>
<tr>
<td>parth 5 + sul 500</td>
<td>12.7 ± 4.5</td>
<td>30.0 ± 6.9*</td>
</tr>
<tr>
<td>MIA PaCa-2, control</td>
<td>1.0</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>parth, 2.5 μmol/L</td>
<td>2.4 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>sul, 250 μmol/L</td>
<td>0.9 ± 0.1</td>
<td>—</td>
</tr>
<tr>
<td>sul, 500 μmol/L</td>
<td>1.8 ± 0.4</td>
<td>2.8 ± 2</td>
</tr>
<tr>
<td>parth 2.5 + sul 250</td>
<td>4.3 ± 0.6*</td>
<td>—</td>
</tr>
<tr>
<td>parth 2.5 + sul 500</td>
<td>4.1 ± 1.3</td>
<td>13.1 ± 0.8*</td>
</tr>
</tbody>
</table>

NOTE: Two different methods were used to measure apoptosis in BxPC-3 and MIA PaCa-2 cells treated with the indicated concentrations of parthenolide and/or sulindac. Apoptosis was measured by an ELISA to detect DNA fragmentation after treatment for 24 h (BxPC-3) or 48 h (PaCa-2). Relative apoptosis is expressed as a percentage of apoptosis induced by TNF-α, set equal to 1. As shown, the mean ± SD from at least two independent experiments. Apoptosis was also assessed by TUNEL followed by flow cytometry after drug treatment for 48 h (PaCa-2) or 72 h (BxPC-3). The percent of apoptotic cells was shown as the mean ± SD from two independent experiments.

Abbreviations: TUNEL, terminal deoxynucleotidyltransferase-mediated nick-end labeling; parth, parthenolide; sul, sulindac.

*p < 0.05 versus each agent alone.

Whole cell lysates were prepared for analysis by electrophoretic mobility shift assay to detect binding to NF-κB or SP-1 probes (Fig. 6A). In both BxPC-3 and MIA PaCa-2 cell lines, the combination further reduced NF-κB DNA binding activity, correlating with the growth inhibitory effects of the combination. Similar results were obtained in the absence of TNF-α (data not shown).

To confirm that inhibition of DNA binding activity observed in two of the pancreatic carcinoma cell lines resulted in decreased transcriptional activity, the reporter gene NF-κB-luciferase was transfected into BxPC-3, PANC-1, and MIA PaCa-2 cells. Parthenolide and/or sulindac were added 3 hours later followed by the addition of TNF-α to amplify the luciferase signal. Lysates were prepared 24 hours post-transfection to measure luciferase activity (Fig. 6B). In BxPC-3 cells, luciferase activity was slightly lower in combination-treated cells compared with cells treated with the single agents. Combination-treated PANC-1 and MIA PaCa-2 cells exhibited substantially less luciferase activity compared with cells treated with the single agents, confirming cooperative inhibition of NF-κB-directed gene expression by parthenolide and sulindac. Similar results were obtained in the PANC-1 cell line in the absence of TNF-α (data not shown).

To further support our data obtained with the NF-κB reporter system, we evaluated the effect of parthenolide/sulindac on the expression of an endogenous NF-κB-regulated gene. The expression of the antiapoptotic protein Bcl-xL is known to be transcriptionally regulated by NF-κB in pancreatic cancer cells (18). Following treatment with the combination of parthenolide and sulindac, the level of Bcl-xL was decreased in PANC-1 and MIA PaCa-2 cells but not BxPC-3 cells (Fig. 6C). These results show that in PANC-1 and MIA PaCa-2 cells, changes in the level of endogenously expressed Bcl-xL mimic the modulation of the NF-κB pathway by parthenolide and sulindac.

**Discussion**

The transcription factor NF-κB is a central regulator of a variety of genes that control the inflammatory response, cell growth, and apoptosis (19). In addition, NF-κB regulates the expression of genes involved in angiogenesis, invasion, and metastasis and is therefore an attractive therapeutic target (20). Basal levels of NF-κB activity or induced activation of the NF-κB pathway may also result in preexisting or acquired resistance to anticancer therapies by promoting survival and oncogenic pathways.

The aberrant activation of NF-κB in pancreatic cancer suggests that specific NF-κB inhibitors should be evaluated for their ability to suppress pancreatic tumor growth. The sesquiterpene lactone parthenolide specifically inhibits NF-κB binding by targeting the IkB kinase complex (21). We tested the effect of parthenolide treatment in three human pancreatic tumor cell lines. Parthenolide potently inhibited the growth of BxPC-3, PANC-1, and MIA PaCa-2
cells in a dose-dependent manner in vitro. Furthermore, parthenolide effectively inhibited NF-κB binding at similar concentrations. Of the three cell lines, PaCa-2 cells had the lowest amount of basal NF-κB binding activity and were also the most sensitive to parthenolide treatment. These results show that parthenolide is an effective NF-κB inhibitor that suppresses pancreatic cancer cell growth in vitro.

Targeting NF-κB in combination with other critical growth-regulating molecules/pathways may overcome the innate chemoresistance of cancer cells and provide additional therapeutic options or improved response. For example, parthenolide increases the sensitivity of breast cancer cells to paclitaxel by decreasing the expression of NF-κB–inducible antiapoptotic genes (10). Similarly, inhibition of NF-κB sensitizes pancreatic carcinoma cells to apoptosis induced by the topoisomerase-2 inhibitors VP16 and doxorubicin (22). Inhibition of the NF-κB pathway, but not the Akt survival pathway, decreases the resistance of pancreatic carcinoma cell lines against gemcitabine-induced apoptosis (23). In the present study, we evaluated the efficacy of treating pancreatic cancer cells with parthenolide to inhibit the NF-κB pathway in combination with the NSAID sulindac. We have previously shown that NSAIDs, known to inhibit the enzyme COX, suppress pancreatic carcinoma cell growth in vitro via both COX-dependent and COX-independent mechanisms (11–14). However, despite their ability to suppress pancreatic cell growth, the NSAIDs do not induce substantial apoptosis in the pancreatic tumor cells, thus illustrating the characteristic resistance of pancreatic cancer cells to undergoing apoptosis (12). The combination of parthenolide and sulindac was not only able to exert additive and synergistic growth inhibitory effects in pancreatic carcinoma cells but also lowered the threshold of apoptosis in two of the pancreatic carcinoma cell lines evaluated. In PANC-1 cells, despite growth inhibitory effects, the combination did not induce measurable apoptosis. We have evidence that in PANC-1 cells treated with the combination, growth suppression is associated with cell cycle alterations.8

Overall in the human pancreatic cancer cell lines evaluated, growth inhibition by sulindac and parthenolide correlated with modulation of the NF-κB pathway. Each of the agents individually inhibited the NF-κB pathway; however, in combination, greater effects were observed on inhibitor IκB-α accumulation and NF-κB DNA binding and transcriptional activities. Modulation of the NF-κB pathway by the combination in PANC-1 and Mia PaCa-2 cells was also mirrored in the level of endogenously expressed Bcl-xL, whose expression is known to be regulated by NF-κB. In addition to its role in apoptosis, Bcl-xl has recently been shown to enhance metastasis in breast cancer cells (24). In contrast, no change in the level of Bcl-xL was observed in BxPC-3 cells despite decreased NF-κB binding and transcriptional activities by the combination. This discrepancy can be explained by noting that in the latter assays, NF-κB activity is monitored with isolated NF-κB binding sites or promoter regions; whereas, the promoter region of Bcl-xL contains multiple regulatory elements in addition to NF-κB (25). Thus, in BxPC-3 cells, the activity of NF-κB may be masked by the action of other transcription factors involved in the regulation of Bcl-xL expression.

A parthenolide affinity reagent was shown to inhibit the activity of the upstream kinase responsible for phosphorylating the IκB proteins, known as IκB kinase-β (IKK-β); ref. 26). Similarly, sulindac was shown to prevent TNF-α–mediated increases in IKK activity in two colon carcinoma cell lines and specifically inhibited IKK-β activity in COS cells transfected with wild-type or constitutively active

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8 Unpublished data.
Based on the results from our study, parthenolide should be considered for use as an effective and potent inhibitor of NF-κB in cancers influenced by this pathway. The NSAID sulindac is also able to mediate its inhibitory effects in a COX-independent manner by targeting the NF-κB pathway in both COX-2–positive (BxPC-3) and COX-2–negative (PANC-1 and PaCa-2) pancreatic carcinoma cells. Furthermore, treatment with the combination of parthenolide and sulindac results in greater inhibition of the NF-κB pathway and correspondingly, pancreatic cancer cell growth. Compounds targeting the NF-κB pathway can sensitize pancreatic tumor cells by counteracting resistance mechanisms and therefore deserve further evaluation as chemotherapy for and possibly chemoprevention of pancreatic cancer.

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* Unpublished data.
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