Published OnlineFirst December 14, 2015; DOI: 10.1158/1940-6207.CAPR-15-0306

Research Article

Cancer Prevention Research

# Piperlongumine Suppresses Growth and Sensitizes Pancreatic Tumors to Gemcitabine in a Xenograft Mouse Model by Modulating the NF-kappa B Pathway

Yongwei Wang, Xiangsong Wu, Yinan Zhou, Hongchi Jiang, Shangha Pan, and Bei Sun

## Abstract

Pancreatic cancer is an aggressive malignancy, which generally respond poorly to chemotherapy. Hence, novel agents that are safe and effective are highly needed. The aim of this study was to investigate whether piperlongumine, a natural product isolated from the fruit of the pepper Piper longum, has any efficacy against human pancreatic cancer when used either alone or in combination with gemcitabine *in vitro* and in a xenograft mouse model. *In vitro*, piperlongumine inhibited the proliferation of pancreatic cancer cell lines, potentiated the apoptotic effects of gemcitabine, inhibited the constitutive and inducible activation of NF-κB, and suppressed the NF-κB–regulated expression of c-Myc, cyclin D1, Bcl-2, Bcl-xL, Survivin, XIAP, VEGF, and matrix metalloproteinase-9 (MMP-9). Furthermore, in an *in vivo* xenograft model, we found piperlongumine alone significantly suppressed tumor growth and enhanced the antitumor properties of gemcitabine. These results were consistent with the downregulation of NF-κB activity and its target genes, decreased proliferation (PCNA and Ki-67), decreased microvessel density (CD31), and increased apoptosis (TUNEL) in tumor remnants. Collectively, our results suggest that piperlongumine alone exhibits significant antitumor effects against human pancreatic cancer and it further enhances the therapeutic effects of gemcitabine, possibly through the modulation of NF-κB– and NF-κB–regulated gene products. *Cancer Prev Res; 9(3); 234–44. ©2015 AACR.*

## Introduction

Pancreatic cancer remains one of the most aggressive and lethal human malignancies having an alarmingly low 5-year survival rate, even with the best treatment available today, of less than 5% (1, 2). In the year 2013 in the United States alone, almost 45,220 men and women were diagnosed with pancreatic cancer and 38,460 died of disease (3). The poor prognosis of pancreatic cancer is mostly due to the lack of ability for early detection, the aggressive biologic behavior of the tumor and the insensitivity to chemotherapy (4). Gemcitabine, erlotinib, and abraxane are the only FDA-approved agents to treat pancreatic cancer. However, they produce a poor response rate and are associated with multiple adverse events and chemoresistance. Hence, there is an urgent need for novel strategies involving less toxic agents that can overcome the resistance and enhance the effect of chemotherapeutic drugs.

Linkage between the activation of master transcription factor NF-κB and the promotion of pancreatic cancer has been shown by various lines of evidence (5–7). First, constitutive activation of NF-κB has been observed in various pancreatic cancer cell lines, animal models, and human pancreatic cancer tissues (8, 9). Second, NF-κB activation can promote pancreatic cancer growth due to its ability to modulate the expression of genes involved in cell proliferation, invasion, angiogenesis, metastasis, and suppression of apoptosis (8, 10–15). Third, NF-κB activation has also been linked with chemoresistance in pancreatic cancer (16). Together, these findings implicate a role of activated NF-κB in development of pancreatic cancer and thus the agents that can block NF-κB activation have therapeutic efficacy against pancreatic cancer.

Piperlongumine (Fig. 1A), an alkaloid/amide component, is one such agent. Derived from the plant species Piper longum Linn, piperlongumine has been shown to possess numerous key biologic activities such as anti-platelet aggregation (17), antibacterial and insecticidal (18), anti-atherosclerotic (19), anxiolytic (20), antidepressant (21), antidiabetic (22, 23), anti-inflammatory (23), and antitumor capabilities (24). Among the multivalent effects, the potential of piperlongumine as a novel anticancer agent have gained considerable attention recently. Emerging evidence suggests that piperlongumine is capable of killing multiple types of cancer cells, including Burkitt lymphoma (25), head and neck cancer (26), ovarian cancer (27), glioblastoma multiforme (28), breast cancer (29), prostate cancer (30), and colon cancer (31). This alkaloid was also found to sensitize tumors to chemotherapeutic agents such as cisplatin and paclitaxel (26, 27). Piperlongumine inhibits the growth of tumor cells with no apparent toxicity in nonmalignant human cells (32–34). The antitumor effect of piperlongumine may be associated with its ability to target the cellular stress response through direct inhibition of Glutathione S-transferase pi 1 (GSTP1; ref. 33), activate ERK signaling (31, 35), target p38 signaling (36), suppress STAT3 phosphorylation (34), generate reactive oxygen species (ROS;

Department of Pancreatic and Biliary Surgery, The First Affiliated Hospital of Harbin Medical University, Harbin, China.

**Corresponding Author:** Bei Sun, The First Affiliated Hospital of Harbin Medical University, Harbin, China. Phone: 86-158-4638-2335; Fax: 86-451-5364-3849; E-mail: sunbei70@163.com

**doi:** 10.1158/1940-6207.CAPR-15-0306

Published OnlineFirst December 14, 2015; DOI: 10.1158/1940-6207.CAPR-15-0306



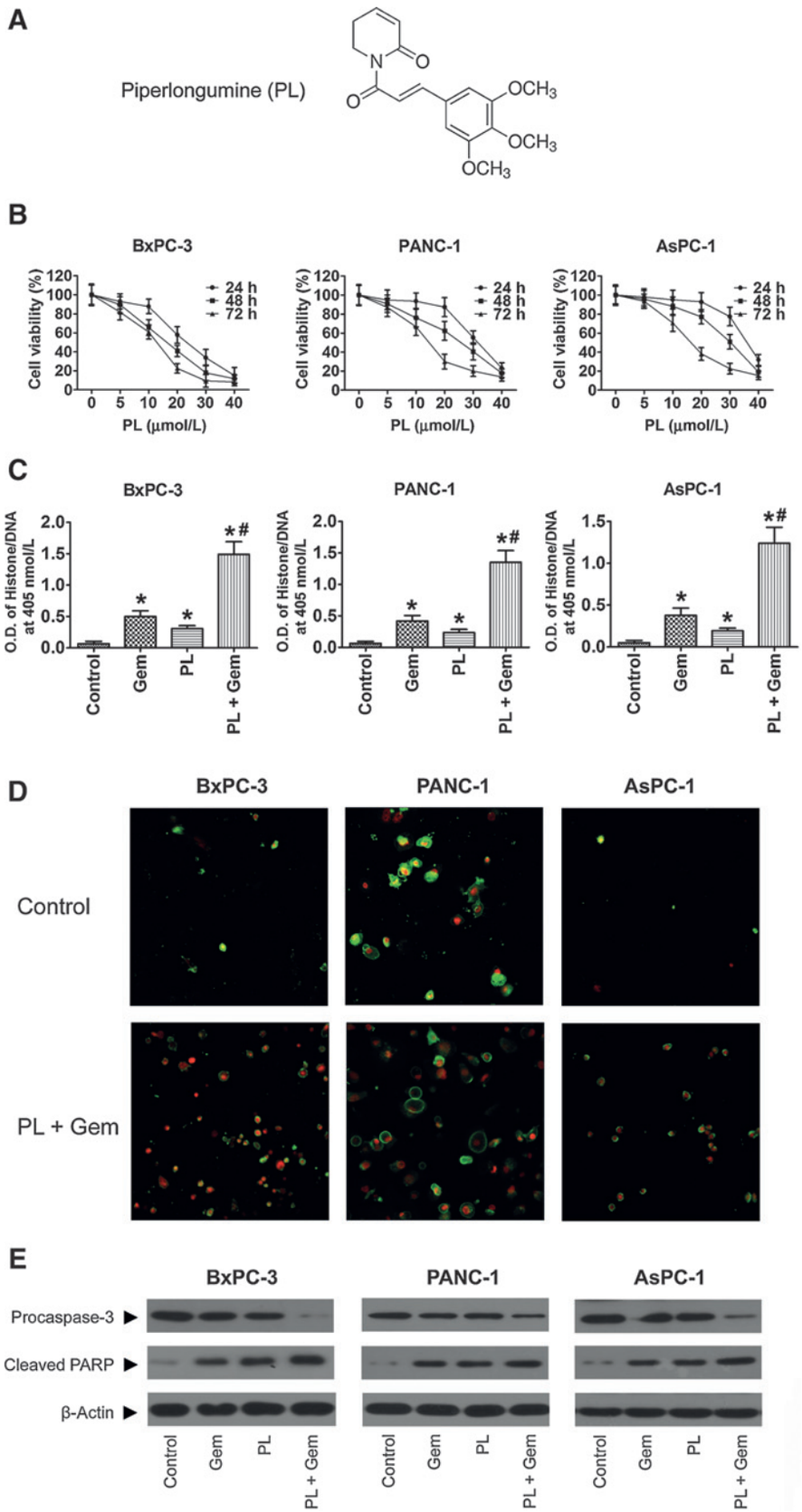



**Figure 1.**
Piperlongumine suppresses the proliferation and potentiates the apoptotic effects of gemcitabine in pancreatic cancer cells *in vitro*. A, chemical structure of piperlongumine. B, cell viability assay. Three pancreatic cancer cells (BxPC-3, PANC-1, and AsPC-1) were grown in the absence or presence of increasing concentration of piperlongumine for 24, 48 and 72 hours and cell viability was measured by CCK-8 assay. Data shown are representative of at least three independent experiments. C, cell apoptosis assay. Cells were pretreated with piperlongumine or left untreated as control for 24 hours before incubating with or without gemcitabine for 48 hours, and then apoptosis was quantitated using a Cell Death Detection ELISA kit. D, cells were viewed under a laser scanning confocal microscopy. Representative photographs were taken from Annexin V/PI-stained pancreatic cancer cells under certain treatment. E, Western blotting analysis. Whole-cell extracts were prepared, separated on SDS-PAGE, and subjected to Western blot analysis to monitor the expression of procaspase-3 and PARP. β-Actin was detected as protein loading control. Gem = Gemcitabine, PL = Piperlongumine, PL + Gem = Piperlongumine + Gemcitabine. *, $P < 0.05$ relative to control; #, $P < 0.05$ relative to single agent.

Published OnlineFirst December 14, 2015; DOI: 10.1158/1940-6207.CAPR-15-0306



ref. 28), inhibit ubiquitin-proteasome system (37), arrest the cell cycle (38), inhibit the PI3K–Akt–mTOR signaling axis (38), and downregulate NF-κB activation (39). All these reports suggest that piperlongumine may have a strong potential for future use in cancer prevention and treatment. However, the potential efficacy of piperlongumine against pancreatic cancer is not known. It is also not known whether piperlongumine can potentiate the effect of gemcitabine in pancreatic cancer.

In the present report, we investigated whether piperlongumine alone could inhibit the growth of human pancreatic cancer cells both *in vitro* and in a xenograft mouse model, and whether piperlongumine could sensitize human pancreatic cancer to gemcitabine. Our observations indicate for the first time that piperlongumine inhibited the *in vitro* proliferation of various pancreatic cancer cells, potentiated gemcitabine-induced apoptosis, and significantly enhanced the antitumor effects of gemcitabine in human xenograft pancreatic cancer model, which was correlated with inhibition of NF-κB activity and downregulation of NF-κB downstream gene products.

## Materials and Methods

### Materials

Piperlongumine was purchased from Sigma-Aldrich, Inc. Gemcitabine (Gemzar) was purchased from Eli Lilly. Antibodies used in this study included Antibodies against Survivin, procaspase-3, c-Myc, Bcl-2, Bcl-xL, VEGF, PCNA, and XIAP (Santa Cruz Biotechnology), Antibodies against Ki-67 and CD31 (Abcam Inc.), and Antibodies against cyclin D1, PARP (Cell Signaling Technology, Inc.). Nuclear Extract Kit and Trans-AM NF-κB p65 ELISA Kit were obtained from Active Motif (Carlsbad).

### Treatment of cells

The subconfluent cells (60%–70% confluent) were treated with various concentrations of piperlongumine alone, gemcitabine alone or their combinations (as indicated in the figure legends) in complete cell culture medium and cells treated with vehicle (DMSO) served as control. The following experiments were repeated thrice.

### Cell culture

The pancreatic cancer cell lines PANC-1, BxPC-3, and AsPC-1 were obtained from the ATCC and were cultured in DMEM and RPMI-1640 medium, respectively, supplemented with FBS (10%), penicillin (100 U/mL) and streptomycin (100 μg/mL; Irvine Scientific). All cells were maintained at 37°C in humidified air with 5% $CO_2$. (All reagents were from HyClone China Ltd., China). The above-mentioned cell lines were obtained directly from the ATCC that performs cell line characterizations and passaged in our laboratory for fewer than 1.5 years after receipt. The method of characterization used by the ATCC is short tandem repeat profiling.

### Cell viability assay

The viability of treated cells was determined by using the Cell Counting Kit-8 (CCK-8) Kit (Dojindo Laboratories) following the instructions outlined by the manufacturer and as previously described by us (40). Briefly, cells were plated at a density of 3 to 5 × $10^3$ cells per well with 200 μL of medium in 96-well microtiter plates. After treatment, CCK-8 solution (10 μL) was added to each well and the plates were incubated at 37°C for 90 minutes. The absorbance of the cell suspension was measured with a microplate reader at a wavelength of 450 nm. The highest concentration of piperlongumine and gemcitabine as single agents and in combination do not interfere with the CCK-8 assay reagents, in the absence of cells (data not shown). Medium containing 10% CCK-8 served as a control.

### Apoptosis assay

Apoptosis was evaluated by using the Cell Death Detection ELISA Kit following the instructions outlined by the manufacturer. Cells were also visualized under a laser scanning confocal microscope (LSM-510, Carl Zeiss Jena GmbH) to detect apoptosis.

### Western blot analysis

To determine the levels of protein expression, cell lysates were prepared and subjected to Western blot analysis as described previously (40). Briefly, cells were washed twice in PBS, sonicated in RIPA buffer and homogenized. Debris was removed by centrifugation at 12,000 × *g* at 4°C for 10 minutes and protein concentration was determined using the BCA Protein assay according to the manufacturer's instructions. Samples containing equal amounts of protein (50 μg) were separated by electrophoresis on 10% or 15% polyacrylamide SDS gels (100 V for 1 to 2 hours) and transferred to polyvinylidene difluoride membranes by electroblotting (100 V for 1 hour at 4°C). The running time and voltage as well as transfer time and voltage may require some optimization depending on the circumstances. The membranes were then blocked by incubating with 5% skim milk in TBST buffer (TBS plus 0.1% Tween 20) for 2 hours and incubated with the appropriate primary antibody with gentle agitation overnight at 4°C. The membranes were then washed several times and incubated with the appropriate horseradish peroxidase (HRP)–conjugated secondary antibody for 1 hour at room temperature. The membranes were incubated with appropriate secondary antibody conjugated with horseradish peroxidise for 1 hour at room temperature. Proteins were visualized using an enhanced chemiluminescence system and captured on X-ray films. β-Actin was simultaneously determined as a loading control.

### Plasmids and transfections

The methodology has been described previously (41). In brief, BxPC-3 cells were seeded in 6-well plates and incubated for 24 hours at 37°C. The cells were then transfected with NF-κB p65 cDNA, control empty vector (pcDNA3.0), NF-κB p65 siRNA and control siRNA, respectively, using Lipofectamine 2000 (Invitrogen). The transfections were performed following the instructions outlined by the manufacturer. After transfection, cells were treated as designed before being harvested for the following studies.

### Xenograft mouse model

Animal studies were in accordance with the institutional guide for the care and use of laboratory animals. Our experimental protocol was reviewed and approved by the Animal Care and Use Committee of Harbin Medical University (Harbin). Male nude BALB/c mice (4–6-weeks-old) were obtained from Shanghai Experimental Animal Center of the Chinese Academy of Sciences. Tumors were established by subcutaneous injection of 5 × $10^6$ BxPC-3 cells into the flanks of mice. Tumor size was measured

Published OnlineFirst December 14, 2015; DOI: 10.1158/1940-6207.CAPR-15-0306



every 3 days with calipers and the volume was estimated according to the formula: $V = (\pi/6) \times (\text{larger diameter}) \times (\text{smaller diameter})^2$. When tumors reached around 100 $mm^3$, the mice were randomly assigned to four groups (each group had 8 mice): (i) control (DMSO was dissolved in 200 μL PBS, once daily by i.p. injection); (ii) piperlongumine (10 mg/kg, once daily by i.p. injection); (iii) gemcitabine (100 mg/kg, twice weekly by i.p. injection); and (iv) piperlongumine and gemcitabine, following the same schedule of individual drugs. The doses of piperlongumine and gemcitabine selected for this experiment were based on preliminary experiments and previous studies (26, 34, 42). The mice were closely monitored for 24 days, then euthanized, and the tumors were removed. Each tumor was divided into two pieces: one fixed in 10% buffered formalin, and the other kept at −80°C for further analysis.

### PCNA and Ki-67 immunohistochemistry

The methodology has been described previously (40). In brief, paraffin-embedded tissue sections (5 μm) were immunostained with an anti-PCNA or anti-Ki-67 Ab. The number of PCNA or Ki-67–positive cells was counted in randomly selected ten microscopic fields at ×400 magnification.

### Quantification of apoptosis in tumor sections

The methodology has been described previously (40). In brief, paraffin-embedded tissue sections (5 μm) were stained with the TUNEL (terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling) agent (Roche), and the number of TUNEL-positive cells was counted in randomly selected ten microscopic fields at ×400 magnification.

### Tumor microvessel density

The methodology has been described previously (43). In brief, tumor sections prepared from tumors 4 weeks after treatment were immunostained with anti-CD31 Ab. The number of microvessels was counted in randomly selected ten microscopic fields at ×400 magnification, and the microvessel density was recorded.

### Immunohistochemical analysis for cyclin D1, MMP-9, and VEGF in tumor samples

The expression of cyclin D1, MMP-9, and VEGF were evaluated by immunohistochemical analysis as described previously (9).

### NF-κB DNA-binding activity assay

The NF-κB DNA-binding activity was determined using the Trans-Am NF-κB /p65 ELISA Kit following the instructions outlined by the manufacturer and as described previously (4). Briefly, nuclear and cytosolic fractions were prepared from cells by using the Nuclear Extraction Kit following the instructions outlined by the manufacturer. Briefly, nuclear lysate protein from each group was added to a 96-well plate with an immobilized oligonucleotide containing the specific consensus sequence (5′-GGGACTTTCC-3′) for NF-κB/p65 binding. Following incubation for 1 hour at room temperature to facilitate the binding, a primary antibody specific for NF-κB was added to each well, followed by a HRP–conjugated secondary antibody. The absorbance was taken at 450 nm using an ELISA plate reader.

### Statistical analysis

Data are represented as mean values ± SD. Statistical comparisons were made with a one-way ANOVA followed by the Dunnett *t* test for multiple comparisons. A *P* value of less than 0.05 was considered significant.

## Results

The purpose of this study was, first, to determine whether piperlongumine, a natural product isolated from the fruit of the pepper Piper longum has potential in the treatment of pancreatic cancer when used either alone or in combination with gemcitabine and, second, to explore the molecular mechanism(s) behind these effects. For this, we used three different well-characterized human pancreatic cancer cell lines. To monitor tumor growth *in vivo*, one of these cell lines, BxPC-3 was subcutaneously injected and used in a mouse xenograft model.

### Piperlongumine suppresses the proliferation and potentiates the apoptotic effects of gemcitabine in pancreatic cancer cells *in vitro*

To investigate the effect of piperlongumine on the proliferation of human pancreatic cancer cells, we used BxPC-3, PANC-1, and AsPC-1 cells, all of which exhibit differential resistance pattern to conventional chemotherapeutic drugs. The cells were assessed for viability using a CCK-8 assay following exposure to increasing concentrations of piperlongumine (0–40 μmol/L) for 24, 48, or 72 hours. As shown in Fig. 1B, piperlongumine suppressed the growth of all three human pancreatic cancer cells in a dose- and time-dependent manner.

To determine whether piperlongumine could enhance the apoptotic effects, we treated pancreatic cancer cells with piperlongumine combined with gemcitabine and then examined these cells with a histone DNA ELISA assay. Our results showed that treatment with either piperlongumine or gemcitabine alone induced apoptosis in all three cell lines tested. Relative to single agents, piperlongumine pretreatment followed by gemcitabine treatment led to significantly higher apoptosis in the cancer cells (Fig. 1C). Laser scanning confocal microscopy confirmed the increased apoptotic effect as shown in the representative photographs (Fig. 1D).

Next, BxPC-3, PANC-1, and AsPC-1 cells were treated with piperlongumine combined with gemcitabine and then caspase-3 activation and PARP cleavage were examined by Western blot analysis. Our data showed that piperlongumine and gemcitabine alone induced caspase-3 activation and PARP cleavage in all three pancreatic cancer cells and the combination was more effective (Fig. 1E).

### Piperlongumine inhibits constitutive as well as gemcitabine-induced NF-κB activation in pancreatic cancer cells

Our results indicate that piperlongumine can potentiate gemcitabine-induced apoptosis in pancreatic cancer cells. How the effects were potentiated was investigated. Because NF-κB has been associated with both proliferation and apoptotic resistance, we next examined the effects of piperlongumine, gemcitabine, and the combination on NF-κB activity using an ELISA-based DNA-binding assay. Consistent with previous studies (9, 40, 43, 44), gemcitabine alone was able to induce NF-κB DNA-binding activity (Fig. 2B). In addition, our results showed that NF-κB p65 siRNA transfection significantly inhibited gemcitabine-induced NF-κB DNA-binding activity whereas NF-κB p65 cDNA transfection enhanced activation of NF-κB DNA-binding activity stimulated by gemcitabine

Published OnlineFirst December 14, 2015; DOI: 10.1158/1940-6207.CAPR-15-0306



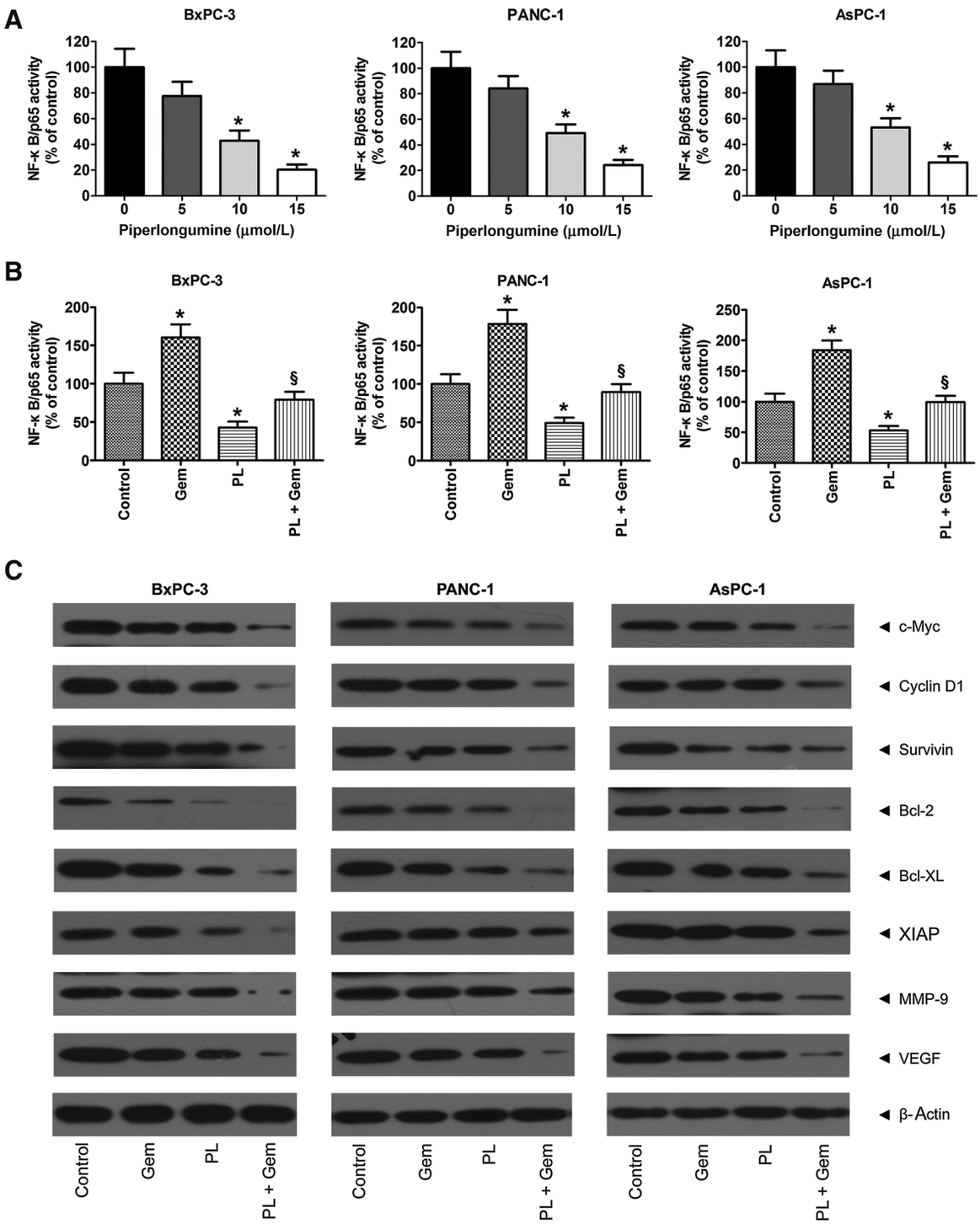



**Figure 2.**
Piperlongumine inhibits NF-κB and NF-κB–regulated gene products. A, NF-κB DNA-binding activity assay. Cells were treated with indicated concentration of piperlongumine for 4 hours, and then nuclear extracts were prepared. NF-κB DNA-binding activity on respective nuclear extracts was detected by ELISA-linked DNA-binding assay. B, cells were pretreated with piperlongumine or left untreated as control for 24 hours before incubating with or without gemcitabine for 48 hours, and then nuclear extracts were prepared and tested for NF-κB DNA-binding activity by ELISA-linked DNA-binding assay. C, Western blotting analysis. Cells were pretreated with piperlongumine or left untreated as control for 24 hours before incubating with or without gemcitabine for 48 hours, and then whole-cell extracts were prepared, separated on SDS-PAGE, and subjected to Western blot analysis to monitor c-Myc, Cyclin-D1, Survivin, Bcl-2, Bcl-XL, XIAP, MMP-9, and VEGF by Western blot. β-Actin was detected as protein loading control. The immunoblots shown here are representative of at least three independent experiments with similar results. Gem = Gemcitabine, PL = Piperlongumine, PL + Gem = Piperlongumine + Gemcitabine. *, $P < 0.05$ relative to control; §, $P < 0.05$ relative to gemcitabine.

Published OnlineFirst December 14, 2015; DOI: 10.1158/1940-6207.CAPR-15-0306



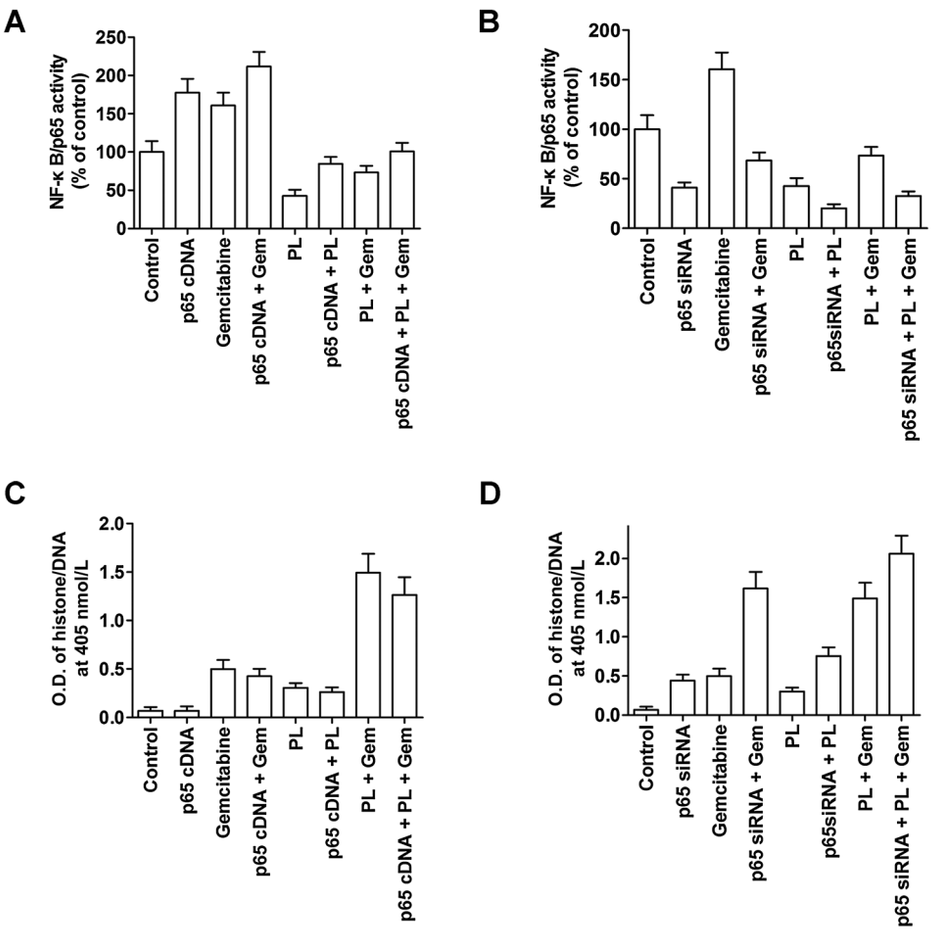



**Figure 3.**
Effect of p65 cDNA or p65 siRNA transfection in BxPC-3 cells. Transiently p65 cDNA or siRNA-transfected cells were pretreated with piperlongumine or left untreated as control for 24 hours before incubating with or without gemcitabine for 48 hours, and then nuclear extracts were prepared. A and B, NF-κB DNA-binding activity assay. NF-κB DNA-binding activity on respective nuclear extracts was determined by ELISA-linked DNA-binding assay. C and D, cell apoptosis assay. Apoptosis was quantitated using a Cell Death Detection ELISA kit. PL = Piperlongumine, Gem = Gemcitabine, PL + Gem = Piperlongumine + Gemcitabine.

(Fig. 3A and B). As shown in Fig. 2A, we further found that treatment with piperlongumine caused a concentration-dependent decrease in NF-κB DNA-binding activity in all three pancreatic cancer cells. More importantly, piperlongumine was also found to significantly suppress gemcitabine- or p65 cDNA transfection–induced activation of NF-κB DNA-binding activity (Fig. 2B and 3A), which indicate that piperlongumine is a potent modulator of constitutive as well as inducible NF-κB activation in pancreatic cancer cells.

Numerous proteins, including c-Myc, cyclin D1, Bcl-2, Bcl-xL, Survivin, and XIAP, are regulated by NF-κB at the transcriptional level and associated with chemoresistance. To investigate whether piperlongumine exerts its effects through modulation of NF-κB–regulated proteins, we examined the effects of piperlongumine, gemcitabine, and the combination on the expression of NF-κB–regulated gene products implicated in cell proliferation (c-myc and cyclin D1), antiapoptosis (Bcl-2, Bcl-xL, Survivin, and XIAP), angiogenesis (VEGF), and metastasis(MMP-9). Our results showed that piperlongumine, alone or in combination with gemcitabine, significantly decreased the expression of all of these molecules compared with vehicle in all three pancreatic cancer cells (Fig. 2C). All these results suggest that potentiation of gemcitabine-induced apoptosis by piperlongumine in pancreatic cancer cells may be mechanistically linked with inactivation of NF-κB and its downstream genes.

### The apoptosis-inducing and -chemosensitizing properties of piperlongumine are mediated by the NF-κB pathway

NF-κB p65 cDNA or siRNA was transfected into BxPC-3 cells, and then treated with piperlongumine and gemcitabine according to the above-mentioned protocol of pretreatment with piperlongumine followed by gemcitabine treatment. Our results showed that overexpression of NF-κB by transfection of p65 cDNA enhanced the expression and activation of NF-κB p65 and protected cells from apoptosis in piperlongumine-treated and untreated BxPC-3 cells (Fig. 3A and C). Conversely, suppression of NF-κB by transfection of p65 siRNA abrogated NF-κB DNA-binding activity and augmented gemcitabine-induced apoptosis (Fig. 3B and D). Furthermore, we found that piperlongumine plus p65 siRNA exerted a more robust suppression of NF-κB DNA-binding activity and an augmented effect on the induction of apoptosis (Fig. 3B and D). Piperlongumine plus gemcitabine were more effective than gemcitabine alone at inhibiting NF-κB activation and inducing apoptosis in p65 cDNA-transfected BxPC-3 cells (Fig. 3A and C). These data provide direct evidence for the functional importance of NF-κB in piperlongumine-induced apoptosis and chemosensitization.

### Piperlongumine potentiates the antitumor effects of gemcitabine in xenograft nude mice model

On the basis of these results, we decided to examine the therapeutic potential of piperlongumine and gemcitabine, alone

Published OnlineFirst December 14, 2015; DOI: 10.1158/1940-6207.CAPR-15-0306



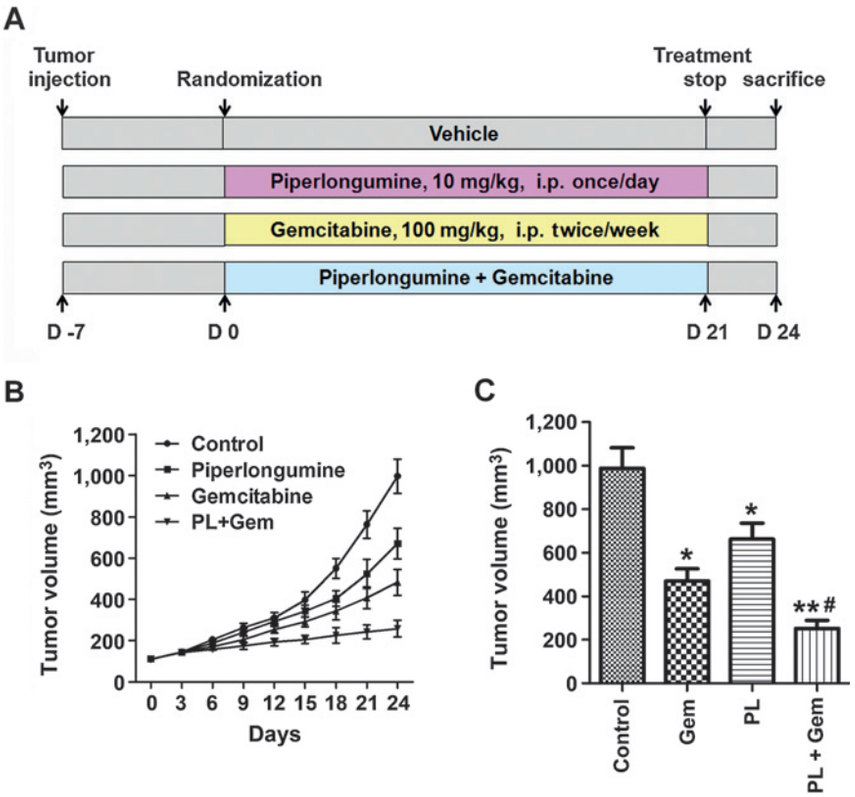



**Figure 4.**
Piperlongumine potentiates the effect of gemcitabine to inhibit the growth of pancreatic cancer in nude mice. Tumors were generated by injecting BxPC-3 cells into the subcutaneous tissue of the right flanks of nude mice. When tumor volume reached an average volume of approximately 100 $mm^3$, the mice were randomly assigned to four treatment groups (8 mice/group) as described in Materials and Methods. Treatment started after randomization and continued up to 24 days. The animals were euthanized on the last day of treatment, and the tumors were removed. A, flow chart representation of experimental design for *in vivo* studies. B, tumor volume was measured using Vernier calipers and calculated by formula: $V = (\pi/6) \times (\text{larger diameter}) \times (\text{smaller diameter})^2$ at indicated time points. C, tumor volumes in mice measured on the last day of the experiment at autopsy using Vernier calipers and calculated by formula: $V = (\pi/6) \times (\text{larger diameter}) \times (\text{smaller diameter})^2$; *, $P < 0.05$; **, $P < 0.01$ relative to control; #, $P < 0.05$, relative to single agent.

or in combination, on pancreatic tumor growth in a xenograft-implanted nude mice model. The treatment schema is summarized in Fig. 4A. Tumors were generated by injecting BxPC-3 cells into the subcutaneous tissue of the right flanks of nude mice. When tumor volume reached around 100 $mm^3$, the mice were assigned to four groups as described under Materials and Methods. Treatment started after randomization and continued up to 24 days. We found that piperlongumine alone significantly inhibited the growth of the tumor ($P < 0.05$ when compared with control; Fig. 4B and C). Gemcitabine alone was also very effective ($P < 0.05$ when compared with control; $P > 0.05$ when compared with piperlongumine alone group); and piperlongumine in combination with gemcitabine was more effective in reducing the tumor burden. The tumor volume in the combination of piperlongumine and gemcitabine group was not only highly significantly lower than the vehicle-treated control group ($P < 0.01$), but also lower than piperlongumine alone group ($P < 0.05$) and gemcitabine alone group ($P < 0.05$) on day 24 (Fig. 4B and C).

### Piperlongumine reduces microvessel density and cell proliferation and induces apoptosis in pancreatic tumor tissues

To understand potential mechanisms of the tumor-suppressive activities, we examined the effect of piperlongumine alone and in combination with gemcitabine on microvessel density, cell proliferation, and apoptosis in tumors removed from each group of treatment. As shown in Fig. 5G and H, piperlongumine or gemcitabine treatment caused a significant reduction of microvessel density when compared with controls, which was further reduced when both compounds were combined. Tumor cell proliferation was assessed using immunohistochemistry for the PCNA and Ki-67 protein. Piperlongumine or gemcitabine treatment resulted in a significant decrease in proliferation rate (Fig. 5A–D), which was further decreased after combined treatment. Likewise, TUNEL staining showed a significant increase in the percentage of TUNEL-positive cells in the group of piperlongumine in combination with gemcitabine compared with controls or single-agent–treated groups (Fig. 5E and F). Together, our results suggest that piperlongumine can augment the antitumor effects of gemcitabine by further suppressing tumor angiogenesis and promoting tumor cell apoptosis.

### Piperlongumine inhibits NF-κB DNA-binding activity and NF-κB–regulated gene products in pancreatic tumor tissues

We also evaluated the effect of piperlongumine and gemcitabine on NF-κB levels in pancreatic tumor tissues. Our results clearly show that the constitutive activation of NF-κB in pancreatic cancer tissue was downregulated by piperlongumine, but most importantly, the gemcitabine treatment resulted in the induction of NF-κB DNA-binding activity similar to those observed in our *in vitro* studies, and this induction was abrogated in tumors treated with piperlongumine (Fig. 6A).

NF-κB is known to regulate the expression of genes involved in proliferation (c-myc and cyclin D1), antiapoptosis (Bcl-2, Bcl-XL, Survivin, and XIAP), invasion/metastasis (MMP-9), and angiogenesis (VEGF). Whether piperlongumine and gemcitabine can modulate the expression these gene products in pancreatic tumor tissues was also determined by Western blot analysis. As shown in Fig. 6B, piperlongumine alone or in combination with gemcitabine, significantly decreased the expression of all of these

Published OnlineFirst December 14, 2015; DOI: 10.1158/1940-6207.CAPR-15-0306



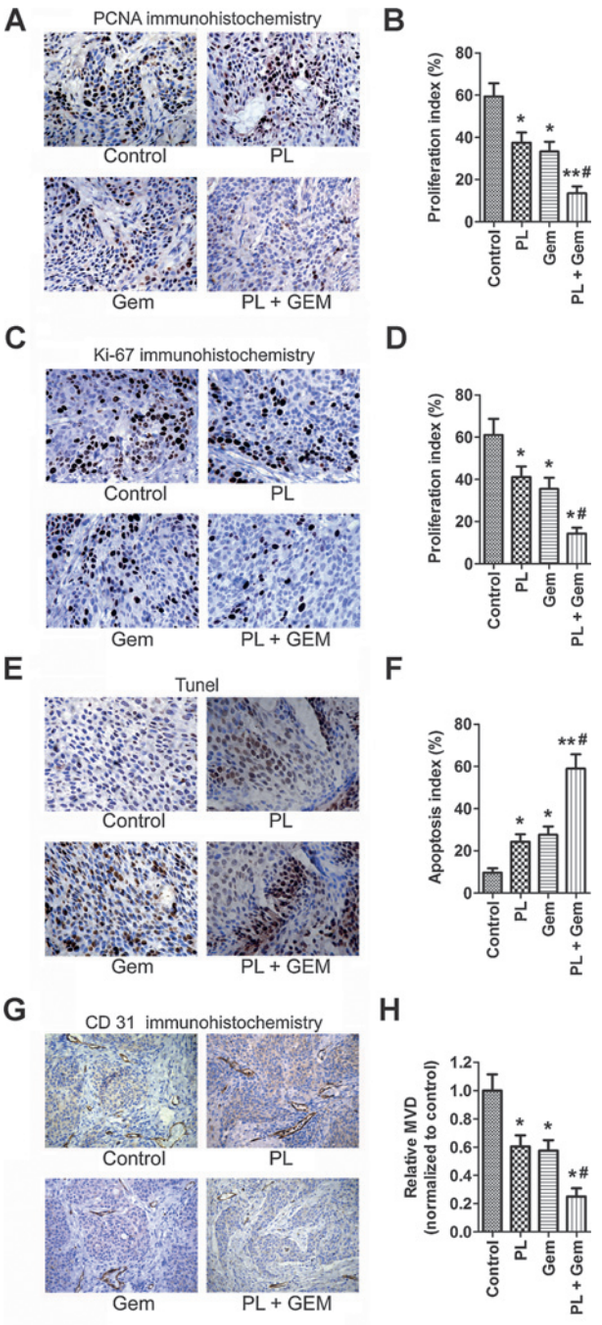



**Figure 5.**
Piperlongumine reduces microvessel density and cell proliferation and induces apoptosis in pancreatic tumor tissues. Tumoral cell proliferation (A and C), apoptosis (E), and microvessel density (G). Immunohistochemistry was performed to measure cell proliferation by PCNA and Ki-67 staining, apoptosis by TUNEL assay and microvessel density by CD31 staining in tumor tissues prepared from subcutaneous tumors 24 days after treatment with vehicle, piperlongumine, gemcitabine, or shikonin + gemcitabine. B, D, F, and H, quantification of Ki-67-, PCNA-, TUNEL- and CD31-positive tumor cells. Tumor slides from treated and untreated animals were visualized under microscope at a magnification of × 400, and Ki-67-, PCNA-, TUNEL-, and CD31-positive tumor cells were quantified. Gem = Gemcitabine, PL = Piperlongumine, PL + Gem = Piperlongumine + Gemcitabine.*, $P < 0.05$; **, $P < 0.01$ relative to control; #, $P < 0.05$, relative to single agent.

molecules compared with vehicle in the xenografts. The Western blot results also showed that piperlongumine, alone or in combination with gemcitabine, significantly decreased the expression of procaspase-3 and induced cleavage of PARP-1 in mouse tumors (Fig. 6B)

Whether modulation of cyclin-D1, MMP-9, and VEGF can also be detected by immunohistochemical methods. As shown in Fig. 6C, immunohistochemical analysis revealed that piperlongumine reduced the expression of all these proteins whether alone or with gemcitabine, which is consistent with the data obtained from western blotting experiments.

Collectively, these data suggest that modulation of NF-κB activity and NF-κB–regulated gene products is, at least, one of the molecular mechanisms by which piperlongumine exerts its effects *in vivo*, which is consistent with our *in vitro* findings.

## Discussion

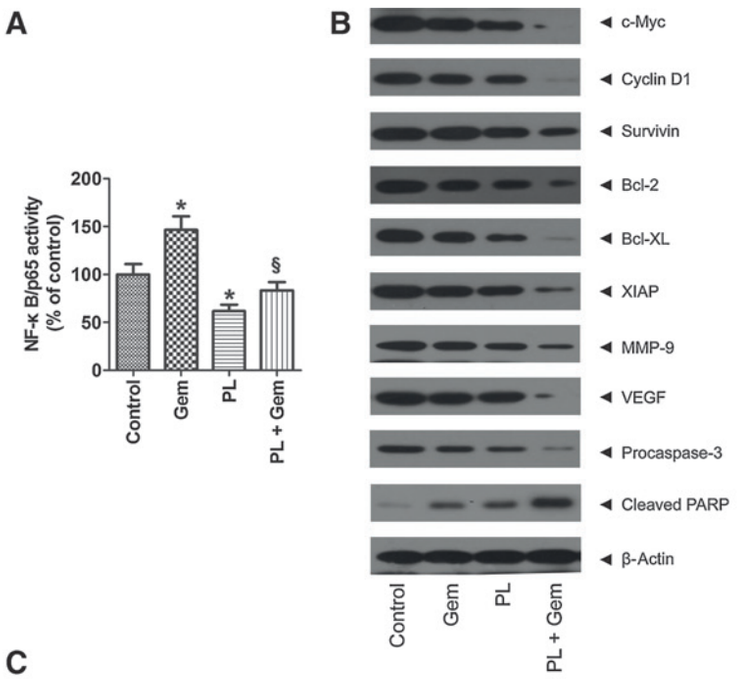
Pancreatic cancer is among the most aggressive human malignancies with a 5-year survival rate of less than 5% despite the best treatment available today. Therefore, novel therapeutic agents that are nontoxic and highly effective to improve the current standard of care of this disease are highly desirable. The present study was designed to investigate whether piperlongumine, a natural product isolated from the fruit of the pepper Piper longum, can potentiate the therapeutic effect of gemcitabine against human pancreatic cancer. In all three pancreatic cancer cell lines, piperlongumine inhibited proliferation, potentiated the cytotoxic effect of gemcitabine, and inhibited constitutive and gemcitabine-induced activation of NF-κB. In a pancreatic cancer xenograft model, piperlongumine argumented the antitumor activity of gemcitabine; this effect was associated with the downregulation of NF-κB activation and NF-κB–regulated gene products, decreased tumor cell proliferation (PCNA and Ki-67), decreased microvessel density (CD31), and increased tumor cell apoptosis (TUNEL).

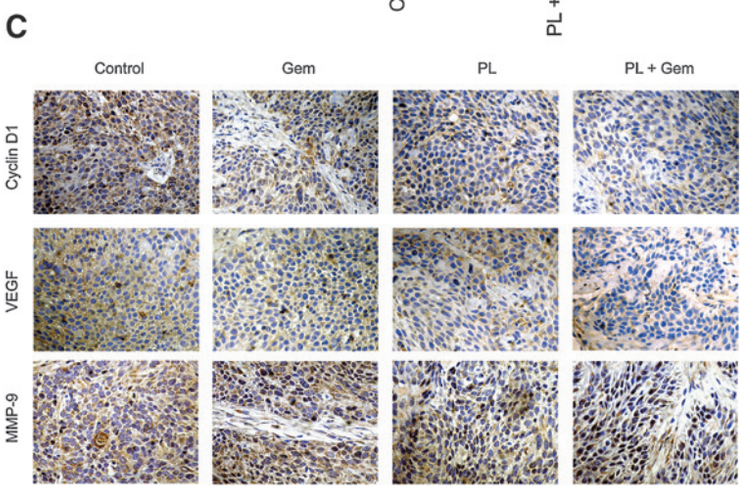
We observed that piperlongumine can suppress the proliferation of all three pancreatic cancer cell lines in a dose- and time-dependent manner. This is the first report to describe the growth-inhibitory effects of piperlongumine in pancreatic cancer cells. The detailed molecular mechanism(s) by which piperlongumine exerts its inhibitory effects on cell proliferation were also investigated. We found that piperlongumine downregulated the expression of cell proliferative (c-myc and cyclin D1) and anti-apoptic (Bcl-2, Bcl-Xl, Survivin, and XIAP) gene products. We also found that piperlongumine downregulated the constitutive activation of NF-κB in pancreatic cancer cell lines. These results on downregulation of NF-κB activation by piperlongumine are consistent with previous reports that piperlongumine inhibited activation of NF-κB in other cancer cell lines (30, 39) and with reports that resveratrol (45), genistein (46), dihydroartemisinin (44), zyflamend (47), γ-tocotrienol (48), curcumin (49), shikonin (9), and 3,3′-Diindolylmethane (50), all present within piperlongumine, are associated with NF-κB inactivation.

Specifically, our results also demonstrated that piperlongumine has a potential to enhance the effect of gemcitabine in human pancreatic cancer cells *in vitro*. We found that this effect may be mediated through the downregulation of cell survival proteins such as Bcl-2, Bcl-Xl, XIAP, and survivin by piperlongumine. Interestingly, we also observed for the first time that piperlongumine suppressed both constitutive and gemcitabine-induced

Published OnlineFirst December 14, 2015; DOI: 10.1158/1940-6207.CAPR-15-0306





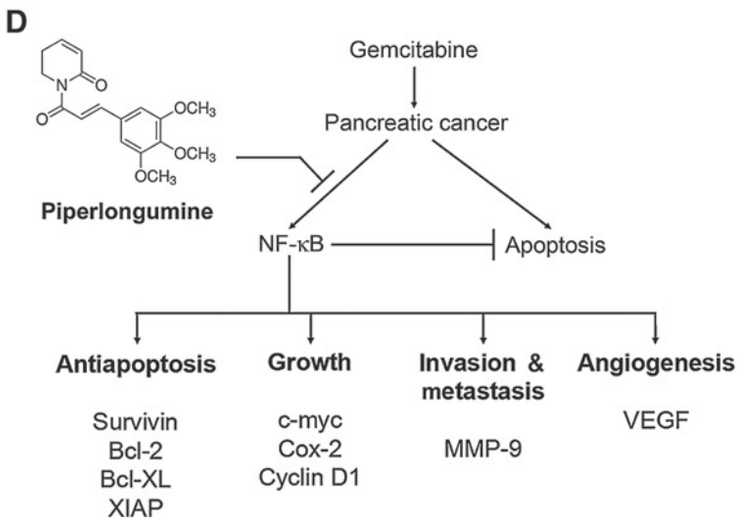




**Figure 6.**
Piperlongumine inhibits NF-κB and NF-κB–regulated gene products in BxPC-3 xenograft tumors. Nuclear extracts and total protein extracts were prepared from subcutaneous tumors 24 days after treatment with vehicle, gemcitabine, piperlongumine, or piperlongumine + gemcitabine. A, NF-κB DNA-binding activity assay. NF-κB DNA-binding activity on respective nuclear extracts was detected by ELISA-linked DNA-binding assay. B, Western blotting analysis. Whole-cell extracts were prepared, separated on SDS-PAGE, and subjected to Western blot analysis to monitor the expression of c-Myc, Cyclin-D1, Survivin, Bcl-2, Bcl-XL, XIAP, VEGF, MMP-9, procaspase-3, and PARP. β-Actin was detected as protein loading control. The immunoblots shown here are representative of at least three independent experiments with similar results. C, immunohistochemical analysis. The expressions of Cyclin-D1, VEGF, and MMP-9 were examined by using immunohistochemical staining. D, proposed mechanisms of piperlongumine action in augmenting the efficacy of gemciabine in pancreatic cancer. Gem = Gemcitabine, PL = Piperlongumine, PL + Gem = Piperlongumine + Gemcitabine; *, $P < 0.05$ relative to control; §, $P < 0.05$ relative to gemcitabine.

Published OnlineFirst December 14, 2015; DOI: 10.1158/1940-6207.CAPR-15-0306



NF-κB activation. NF-κB has been linked with chemoresistance, so it is very likely that the inactivation of NF-κB by piperlongumine is mechanistically associated with sensitization of pancreatic cancer cells to gemcitabine-induced killing. Furthermore, using NF-κB cDNA transfection and NF-κB p65 siRNA approach, we demonstrated that NF-κB activation performs a crucial function in protecting cells against the cytotoxic effects of gemcitabine, which provides direct support for a critical role for NF-κB signaling in piperlongumine-mediated cytotoxic synergism with gemcitabine in pancreatic cancer cells and further strengthens the notion that NF-κB activation plays an important role in the development of the chemoresistance phenotype in pancreatic cancer cells.

We next determined whether piperlongumine potentiates the antitumor effects of gemcitabine *in vivo* in a subcutaneous pancreatic cancer model. Our results clearly showed that the i.p. administration of piperlongumine or gemcitabine alone suppressed the growth of human pancreatic tumors in nude mice. Piperlongumine was very well tolerated by the animals. We also found that the piperlongumine + gemcitabine combination had a much more effective and potent antitumor effect than either agent alone. When examined for the mechanism by which piperlongumine exerts its effects against human pancreatic cancer, we noticed decreased proliferation as documented by PCNA and Ki-67 immunostaining, decreased microvessel density as documented by CD31 immunostaining, and increased apoptosis as documented by increased TUNEL staining within tumors, in tumor remnants of combination treatment with piperlongumine and gemcitabine. Further investigation also revealed that piperlongumine inhibited constitutive and gemcitabine-induced NF-κB activation. Piperlongumine, alone or in combination with gemcitabine, significantly downregulated the expression of NF-κB–regulated gene products such as c-Myc, Cyclin D1, Bcl-2, Bcl-xL, Survivin, and XIAP in pancreatic tumor samples. These results are in good agreement with our molecular studies *in vitro*, indicating that the suppression of NF-κB and its downstream targets is, at least, one of the molecular mechanisms by which piperlongumine may exert its effects against human pancreatic cancer (Fig. 6D).

In summary, the results from our cell-based and *in vivo* studies suggest for the first time that piperlongumine has great potential for the treatment of pancreatic cancer, and it can further potentiate the efficacy of gemcitabine through the suppression of NF-κB and its downstream regulated genes that are implicated in proliferation, angiogenesis, invasion, metastasis, and chemoresistance. On the basis of our findings, further in-depth studies, including clinical trials, are required to fully appreciate the value of piperlongumine as an anticancer agent in pancreatic cancer.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Authors' Contributions

**Conception and design:** Y. Wang, X. Wu, B. Sun
**Development of methodology:** X. Wu, H. Jiang, S. Pan, B. Sun
**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** Y. Zhou, H. Jiang, S. Pan, B. Sun
**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** Y. Wang, X. Wu, Y. Zhou, B. Sun
**Writing, review, and/or revision of the manuscript:** Y. Wang, B. Sun
**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** Y. Zhou, S. Pan

## Grant Support

This work was supported in part by China Postdoctoral Science Foundation (2015M571449, to Y.W. Wang); and the research Fund for The First Affiliated Hospital of Harbin Medical University (2015B05; to Y.W. Wang); and the National Natural Science Foundation of China (81372613; to B. Sun).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 9, 2015; revised October 22, 2015; accepted November 24, 2015; published OnlineFirst December 14, 2015.

## References

1. Penchev VR, Rasheed ZA, Maitra A, Matsui W. Heterogeneity and targeting of pancreatic cancer stem cells. Clin Cancer Res 2012;18:4277–84.
2. Chen S, Wang G, Niu X, Zhao J, Tan W, Wang H, et al. Combination of AZD2281 (Olaparib) and GX15–070 (Obatoclax) results in synergistic antitumor activities in preclinical models of pancreatic cancer. Cancer Lett 2014;348:20–8.
3. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013;63:11–30.
4. Wang Y, Zhou Y, Zhou H, Jia G, Liu J, Han B, et al. Pristimerin causes G1 arrest, induces apoptosis, and enhances the chemosensitivity to gemcitabine in pancreatic cancer cells. PloS ONE 2012;7:e43826.
5. Aggarwal BB. Nuclear factor-kappaB: the enemy within. Cancer Cell 2004;6:203–8.
6. Maier HJ, Schmidt-Strassburger U, Huber MA, Wiedemann EM, Beug H, Wirth T. NF-kappaB promotes epithelial-mesenchymal transition, migration and invasion of pancreatic carcinoma cells. Cancer Lett 2010;295:214–28.
7. Liu L, Salnikov AV, Bauer N, Aleksandrowicz E, Labsch S, Nwaeburu C, et al. Triptolide reverses hypoxia-induced epithelial-mesenchymal transition and stem-like features in pancreatic cancer by NF-kappaB downregulation. Int J Cancer 2014;134:2489–503.
8. Weichert W, Boehm M, Gekeler V, Bahra M, Langrehr J, Neuhaus P, et al. High expression of RelA/p65 is associated with activation of nuclear factor-kappaB-dependent signaling in pancreatic cancer and marks a patient population with poor prognosis. Br J Cancer 2007;97:523–30.
9. Wang Y, Zhou Y, Jia G, Han B, Liu J, Teng Y, et al. Shikonin suppresses tumor growth and synergizes with gemcitabine in a pancreatic cancer xenograft model: involvement of NF-kappaB signaling pathway. Biochem Pharmacol 2014;88:322–33.
10. Liptay S, Weber CK, Ludwig L, Wagner M, Adler G, Schmid RM. Mitogenic and antiapoptotic role of constitutive NF-kappaB/Rel activity in pancreatic cancer. Int J Cancer 2003;105:735–46.
11. Greten FR, Weber CK, Greten TF, Schneider G, Wagner M, Adler G, et al. Stat3 and NF-kappaB activation prevents apoptosis in pancreatic carcinogenesis. Gastroenterology 2002;123:2052–63.
12. Holcomb B, Yip-Schneider M, Schmidt CM. The role of nuclear factor kappaB in pancreatic cancer and the clinical applications of targeted therapy. Pancreas 2008;36:225–35.
13. Diehl JA, Benzeno S. Cyclin D1 and pancreatic carcinoma: a proliferative agonist and chemotherapeutic antagonist. Clin Cancer Res 2005;11:5665–7.
14. Wang Z, Kong D, Banerjee S, Li Y, Adsay NV, Abbruzzese J, et al. Down-regulation of platelet-derived growth factor-D inhibits cell growth and angiogenesis through inactivation of Notch-1 and nuclear factor-kappaB signaling. Cancer Res 2007;67:11377–85.
15. Niu J, Chang Z, Peng B, Xia Q, Lu W, Huang P, et al. Keratinocyte growth factor/fibroblast growth factor-7-regulated cell migration and invasion

Published OnlineFirst December 14, 2015; DOI: 10.1158/1940-6207.CAPR-15-0306



through activation of NF-kappaB transcription factors. J Biol Chem 2007;282:6001–11.
16. Arlt A, Gehrz A, Muerkoster S, Vorndamm J, Kruse ML, Folsch UR, et al. Role of NF-kappaB and Akt/PI3K in the resistance of pancreatic carcinoma cell lines against gemcitabine-induced cell death. Oncogene 2003;22: 3243–51.
17. Fontenele JB, Leal LK, Silveira ER, Felix FH, Bezerra Felipe CF, Viana GS. Antiplatelet effects of piplartine, an alkamide isolated from Piper tuberculatum: possible involvement of cyclooxygenase blockade and antioxidant activity. J Pharm Pharmacol 2009;61:511–5.
18. Yang YC, Lee SG, Lee HK, Kim MK, Lee SH, Lee HS. A piperidine amide extracted from Piper longum L. fruit shows activity against Aedes aegypti mosquito larvae. J Agric Food Chem 2002;50:3765–7.
19. Son DJ, Kim SY, Han SS, Kim CW, Kumar S, Park BS, et al. Piperlongumine inhibits atherosclerotic plaque formation and vascular smooth muscle cell proliferation by suppressing PDGF receptor signaling. Biochem Biophys Res Commun 2012;427:349–54.
20. Cicero Bezerra Felipe F, Trajano Sousa Filho J, de Oliveira Souza LE, Alexandre Silveira J, Esdras de Andrade Uchoa D, Rocha Silveira E, et al. Piplartine, an amide alkaloid from Piper tuberculatum, presents anxiolytic and antidepressant effects in mice. Phytomedicine 2007;14:605–12.
21. Lee SA, Hong SS, Han XH, Hwang JS, Oh GJ, Lee KS, et al. Piperine from the fruits of Piper longum with inhibitory effect on monoamine oxidase and antidepressant-like activity. Chem Pharm Bull 2005;53:832–5.
22. Rao VR, Muthenna P, Shankaraiah G, Akileshwari C, Babu KH, Suresh G, et al. Synthesis and biological evaluation of new piplartine analogues as potent aldose reductase inhibitors (ARIs). Eur J Med Chem 2012;57: 344–61.
23. Lee W, Yoo H, Kim JA, Lee S, Jee JG, Lee MY, et al. Barrier protective effects of piperlonguminine in LPS-induced inflammation *in vitro* and *in vivo*. Food Chem Toxicol 2013;58:149–57.
24. Glasauer A, Chandel NS. Targeting antioxidants for cancer therapy. Biochem Pharmacol 2014;92:90–101.
25. Han SS, Son DJ, Yun H, Kamberos NL, Janz S. Piperlongumine inhibits proliferation and survival of Burkitt lymphoma *in vitro*. Leukemia Res 2013;37:146–54.
26. Roh JL, Kim EH, Park JY, Kim JW, Kwon M, Lee BH. Piperlongumine selectively kills cancer cells and increases cisplatin antitumor activity in head and neck cancer. Oncotarget 2014;5:9227–38.
27. Gong LH, Chen XX, Wang H, Jiang QW, Pan SS, Qiu JG, et al. Piperlongumine induces apoptosis and synergizes with cisplatin or paclitaxel in human ovarian cancer cells. Oxid Med Cell Longev 2014;2014: 906804.
28. Liu JM, Pan F, Li L, Liu QR, Chen Y, Xiong XX, et al. Piperlongumine selectively kills glioblastoma multiforme cells via reactive oxygen species accumulation dependent JNK and p38 activation. Biochem Biophys Res Commun 2013;437:87–93.
29. Jin HO, Lee YH, Park JA, Lee HN, Kim JH, Kim JY, et al. Piperlongumine induces cell death through ROS-mediated CHOP activation and potentiates TRAIL-induced cell death in breast cancer cells. J Cancer Res Clin Oncol 2014;140:2039–46.
30. Ginzburg S, Golovine KV, Makhov PB, Uzzo RG, Kutikov A, Kolenko VM. Piperlongumine inhibits NF-kappaB activity and attenuates aggressive growth characteristics of prostate cancer cells. Prostate 2014;74:177–86.
31. Randhawa H, Kibble K, Zeng H, Moyer MP, Reindl KM. Activation of ERK signaling and induction of colon cancer cell death by piperlongumine. Toxicol *In Vitro* 2013;27:1626–33.
32. Adams DJ, Dai M, Pellegrino G, Wagner BK, Stern AM, Shamji AF, et al. Synthesis, cellular evaluation, and mechanism of action of piperlongumine analogs. Proc Natl Acad Sci U S A 2012;109:15115–20.
33. Raj L, Ide T, Gurkar AU, Foley M, Schenone M, Li X, et al. Selective killing of cancer cells by a small molecule targeting the stress response to ROS. Nature 2011;475:231–4.
34. Bharadwaj U, Eckols TK, Kolosov M, Kasembeli MM, Adam A, Torres D, et al. Drug-repositioning screening identified piperlongumine as a direct STAT3 inhibitor with potent activity against breast cancer. Oncogene 2015;34:1341–53.
35. Adams DJ, Boskovic ZV, Theriault JR, Wang AJ, Stern AM, Wagner BK, et al. Discovery of small-molecule enhancers of reactive oxygen species that are nontoxic or cause genotype-selective cell death. ACS Chem Biol 2013;8:923–9.
36. Wang Y, Wang JW, Xiao X, Shan Y, Xue B, Jiang G, et al. Piperlongumine induces autophagy by targeting p38 signaling. Cell Death Dis 2013;4:e824.
37. Jarvius M, Fryknas M, D'Arcy P, Sun C, Rickardson L, Gullbo J, et al. Piperlongumine induces inhibition of the ubiquitin-proteasome system in cancer cells. Biochem Biophys Res Commun 2013;431:117–23.
38. Shrivastava S, Kulkarni P, Thummuri D, Jeengar MK, Naidu VG, Alvala M, et al. Piperlongumine, an alkaloid causes inhibition of PI3 K/Akt/mTOR signaling axis to induce caspase-dependent apoptosis in human triple-negative breast cancer cells. Apoptosis 2014;19:1148–64.
39. Han JG, Gupta SC, Prasad S, Aggarwal BB. Piperlongumine chemosensitizes tumor cells through interaction with cysteine 179 of IkappaBalpha kinase, leading to suppression of NF-kappaB-regulated gene products. Mol Cancer Ther 2014;13:2422–35.
40. Wang YW, Wang SJ, Zhou YN, Pan SH, Sun B. Escin augments the efficacy of gemcitabine through down-regulation of nuclear factor-kappaB and nuclear factor-kappaB-regulated gene products in pancreatic cancer both *in vitro* and *in vivo*. J Cancer Res Clin Oncol 2012;138:785–97.
41. Cheng ZX, Sun B, Wang SJ, Gao Y, Zhang YM, Zhou HX, et al. Nuclear factor-kappaB-dependent epithelial to mesenchymal transition induced by HIF-1alpha activation in pancreatic cancer cells under hypoxic conditions. PloS ONE 2011;6:e23752.
42. Makhov P, Golovine K, Teper E, Kutikov A, Mehrazin R, Corcoran A, et al. Piperlongumine promotes autophagy via inhibition of Akt/mTOR signalling and mediates cancer cell death. Br J Cancer 2014;110:899–907.
43. Kong R, Sun B, Jiang H, Pan S, Chen H, Wang S, et al. Downregulation of nuclear factor-kappaB p65 subunit by small interfering RNA synergizes with gemcitabine to inhibit the growth of pancreatic cancer. Cancer Lett 2010;291:90–8.
44. Wang SJ, Gao Y, Chen H, Kong R, Jiang HC, Pan SH, et al. Dihydroartemisinin inactivates NF-kappaB and potentiates the anti-tumor effect of gemcitabine on pancreatic cancer both *in vitro* and *in vivo*. Cancer Lett 2010;293:99–108.
45. Harikumar KB, Kunnumakkara AB, Sethi G, Diagaradjane P, Anand P, Pandey MK, et al. Resveratrol, a multitargeted agent, can enhance antitumor activity of gemcitabine *in vitro* and in orthotopic mouse model of human pancreatic cancer. Int J Cancer 2010;127:257–68.
46. Banerjee S, Zhang Y, Wang Z, Che M, Chiao PJ, Abbruzzese JL, et al. *In vitro* and *in vivo* molecular evidence of genistein action in augmenting the efficacy of cisplatin in pancreatic cancer. Int J Cancer 2007;120:906–17.
47. Kunnumakkara AB, Sung B, Ravindran J, Diagaradjane P, Deorukhkar A, Dey S, et al. Zyflamend suppresses growth and sensitizes human pancreatic tumors to gemcitabine in an orthotopic mouse model through modulation of multiple targets. Int J Cancer 2012;131:E292–303.
48. Kunnumakkara AB, Sung B, Ravindran J, Diagaradjane P, Deorukhkar A, Dey S, et al. {Gamma}-tocotrienol inhibits pancreatic tumors and sensitizes them to gemcitabine treatment by modulating the inflammatory microenvironment. Cancer Res 2010;70:8695–705.
49. Kunnumakkara AB, Guha S, Krishnan S, Diagaradjane P, Gelovani J, Aggarwal BB. Curcumin potentiates antitumor activity of gemcitabine in an orthotopic model of pancreatic cancer through suppression of proliferation, angiogenesis, and inhibition of nuclear factor-kappaB-regulated gene products. Cancer Res 2007;67:3853–61.
50. Banerjee S, Wang Z, Kong D, Sarkar FH. 3,3'-Diindolylmethane enhances chemosensitivity of multiple chemotherapeutic agents in pancreatic cancer. Cancer Res 2009;69:5592–600.

Published OnlineFirst December 14, 2015; DOI: 10.1158/1940-6207.CAPR-15-0306



Cancer Prevention Research

# Piperlongumine Suppresses Growth and Sensitizes Pancreatic Tumors to Gemcitabine in a Xenograft Mouse Model by Modulating the NF-kappa B Pathway

Yongwei Wang, Xiangsong Wu, Yinan Zhou, et al.

*Cancer Prev Res* 2016;9:234-244. Published OnlineFirst December 14, 2015.