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Garcinol Inhibits Cell Proliferation and Promotes Apoptosis in Pancreatic Adenocarcinoma Cells

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Garcinol, or polyisoprenylated benzophenone, isolated from the rind of fruiting bodies of Garcinia indica, has been used in traditional medicine for its potential antiinflammatory and antioxidant properties. The objective of this study was to investigate the effect of garcinol on pancreatic cancer (PaCa) cell viability and proliferation. For this, 2 human PaCa cell lines, BxPC-3 and Panc-1, with wild and mutant k-ras, respectively, were treated with garcinol (0–40 \( \mu \text{M} \)). Garcinol significantly (\( P < 0.05 \)) inhibited cell growth (trypan blue exclusion) by induction of apoptosis in a dose- and time-dependent manner. Flow cytometric analysis revealed G0–G1 phase cell cycle arrest in both cell lines. The molecular mechanism of garcinol’s action on PaCa cells was investigated by targeting signaling moieties involved in apoptosis (X-IAP, cIAP, caspase-3, 9, and PARP cleavage), transcription factor NF-\( \kappa \)B, beclin-1, and autophagy. Garcinol significantly (\( P < 0.05 \)) augmented antiproliferative, proapoptotic, autophagic, and antiangiogenic effects in both PaCa cell types relative to untreated cells. These effects were more pronounced in Panc-1. This is the first report on the therapeutically relevant effect of garcinol on PaCa. Further studies are warranted, based on our findings.

INTRODUCTION

Pancreatic cancer (PaCa) is the fourth leading cause of cancer deaths in the United States, with a dismal overall 5-yr survival rate of approximately 5%. Of the estimated 43,140 new PaCa patients who would be diagnosed in 2010 in the United States, up to 36,800 will succumb to this disease (1). This unfortunate prognosis is mainly attributable to the indolent nature of the tumor growth evading early detection, aggressive local invasion, metastases, and poor response to chemotherapy. Curative surgery along with other alternative treatment modalities such as chemo-, immuno-, and radiation therapy have limited proven advantage in terms of patient survival benefit since metastasis often occurs after potential restorative surgical intervention. Thus alternatives with superior therapeutic advantage to restrain pancreatic tumor growth are urgently needed.

Emerging evidence from preclinical models attest that non-nutritive dietary components may augment therapeutic modalities in cancer or, alternatively, as remedial chemopreventive agents they may affect the tumor behavior by promoting apoptosis and affecting progression of tumor growth by involvement in differential regulation of molecular signaling pathways. In this context, the nuclear transcription factor NF-\( \kappa \)B, constitutively active in PaCa, has been well characterized in promoting cell proliferation and inhibiting apoptosis. In this study, we evaluated a novel bioactive dietary compound, Garcinol (also called camboginol; Fig. 1A), which is derived from Garcinia indica for potential use in therapeutic strategy against PaCa. This yellow-colored polyisoprenylated benzophenone is present in the rind of the fruit (cv. Kokum) and makes up 2–3% w/w of it. Using short- and medium-term bioassay protocols, garcinol has been categorized as a putative cancer chemopreventive agent by Ito et al. (2) and Ohnishi et al. (3). Subsequently, based on changes in gene expression patterns using 3-dimensional microarray systems, Hokaiwado et al. (4) confirmed garcinol as a candidate chemopreventive agent. This was later substantiated by reports in the literature indicating that garcinol is effective in preventing the development of aberrant crypt foci in male Fischer rats (5) and inhibits 4 nitroquinoline-1-oxide-induced tongue carcinogenesis (6). Also, the growth inhibitory effect of garcinol on colon cancer cells HT-29 and HCT-116 was found to be more than that of normal immortalized cells (7). In addition, garcinol has proven antibacterial (8,9), antioxidative (10), free radical scavenging, antilulcer, and antiglycation activities (10,11). Mechanistically, induction of apoptosis in human leukemia cell lines by garcinol was shown to involve the release of cytochrome \( c \) into the cytosol, procaspase-9 processing, activation of caspase-3, and degradation of PARP and DNA fragmentation divuling (12,13). Other studies revealed that garcinol reduces cell invasion and survival through the inhibition of FAK’s downstream signaling, which is a major signaling mediator of integrin-mediated cell-matrix contact-regulated cellular proliferation.
proliferation and migration and subsequently induces apoptosis through downregulation of Src, ERK, and Akt survival signaling (14). Another intracellular molecular target of garcinol action involves its inhibitory effect on histone acetyltransferase as reported by Balasubramanian et al. (15). Additionally, garcinol reduces the expression of LPS-induced inflammatory mediators such as iNOS and COX-2 and showed promising and clinically significant analgesic and antiinflammatory effects in arthritic patients (16,17).

To this date, the chemopreventive efficacy of garcinol in PaCa has not been examined. Given the potential of garcinol as a chemopreventative and antiinflammatory agent, this study was designed to examine the potential of garcinol in inhibiting pancreatic carcinoma cell growth. Antitumor effects through downregulation of NF-κB and its downstream target genes for use in therapeutic intervention in retarding cell invasion, proliferation, angiogenesis, and promoting apoptosis were evaluated from a mechanistic standpoint.

MATERIALS AND METHODS

Cell Culture

The human pancreatic carcinoma cell lines BxPC-3 and Panc-1 were obtained from American Type Culture Collection (Manassas, VA). The cell lines were maintained in continuous exponential growth by twice-a-week passage in RPMI-1640 medium (Cellgro, Manassas, VA; BxPC-3 cells) and Dulbecco modified Eagle’s medium (DMEM; Cellgro, Manassas, VA; Panc-1 cells), respectively, and supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 10 mg/ml streptomycin in a humidified incubator containing 5% CO₂ in air at 37°C. Each cell line was split regularly before attaining 70–80% confluence.

Antibodies were obtained from the following commercial sources: Caspase-3 was purchased from Cell Signaling (Beverly, MA); XIAP, cIAP from R&D Systems (Minneapolis, MN), anti-Bcl-xL antibody from Trevigen, Inc. (Gaithersburg, MD);
anti-PARP antibody was from Biomol Research (Plymouth, PA), and anti-β-actin antibody was from Sigma Chemical Co. (St. Louis, MO). Garcinol (Biomol International, Plymouth, PA) was dissolved in DMSO to make a 20 mM stock solution.

Cell Proliferation and Viability

Cells were harvested using 0.025% trypsin at specific time points (24, 48, and 72 h) after garcinol treatment and proliferation inferred by counting the cells with a haemocytometer under a microscope using Trypan blue. Viable cells excluded the dye. Proliferation was expressed as number of cells counted for each treatment group relative to the cell number in absence of garcinol (control). For cell viability assay, cells were seeded at a density of 3 × 10^3 cells/well in 96-well microtiter culture plates. After overnight incubation, the medium was removed and replaced with fresh medium containing different concentrations of garcinol (0–40 µM) diluted from a 20 mM stock. After specific time points (24, 48, and 72 h), 20 µl of MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] solution was added to each well and incubated further for 1 h. Color development due to reduction of tetrazolium in MTS to formazan is directly proportional to the number of living cells. This was measured spectrophotometrically at 595 nm on a plate reader (Bio-Tek Instruments, Winooski, VT). Cell proliferation was quantified as per the manufacturer’s protocol (Promega, Madison, WI). Cell viability has been expressed as a percentage, for each treatment group relative to control in absence of garcinol.

Quantification of Apoptosis

The Cell Apoptosis ELISA Detection Kit (Roche, Palo Alto, CA) was used to detect apoptosis according to manufacturer’s protocol. Briefly, after treatment of BxPC-3 and Panc-1 cells with garcinol (48 h), the cytoplasmic histone/DNA fragments from cells were extracted and bound to immobilized antihistone antibody. Subsequently, a peroxidase-conjugated anti-DNA antibody was used for the detection of immobilized histone/DNA fragments. After addition of the peroxidase substrate, the absorbance by the samples was determined at 405 nm with an Ultra Multiplate Reader (Bio-Tek Instruments).

Morphological Changes

Morphological changes characteristic of apoptosis were determined by DAPI (4’, 6-diamidino-2-phenylindole) staining as per manufacture’s protocol (Invitrogen, Carlsbad, CA). Briefly, 5 × 10^5 cells were seeded into 6-well plates containing 1–2 ml medium. After 24–36 h, garcinol (0–40 µM) was added and incubated for another 48 h. Cells were harvested by trypsinization, washed with PBS, and subsequently incubated for 30 min with DAPI at room temperature in the dark for 30 min. Prior to microscopic analysis, the cells were stained with Prolong Gold Antifade reagent and visualized under a fluorescence microscope (Nikon Eclipsia, 80i, Melville, NY) with an Excitation maximum at 358 nm and an Emission maximum at 461 nm.

Cell Cycle Analysis

After seeding the cells in 6-well plates, they were synchronized in the G1 phase of the cell cycle by serum starvation for 24 h. Thereafter, the cells resumed the cell cycle in media containing serum and garcinol. After treatment with garcinol, the cells were collected at 1100 rpm, the pellet resuspended in 500 µl of PBS, and incubated with RNase (20 µg/ml, final concentration) for 30 min at 37°C, stained with propidium iodide (50 µg/ml, final concentration) for 1 h, and analyzed using FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ).

Caspase Activity

Caspase-3 and -9 activity was measured in whole-cell lysates prepared from garcinol-treated samples using a commercially available assay kit (R&D Assay System, Minneapolis, MN) according to manufacturer’s instructions.

Wound Healing Assay

Cells were cultured in 35 mM dishes and maintained until 90% confluence was achieved. Using a sterile pipette tip, a scratch was made on the dish creating a wound through the cells. Microphotographic images were captured at the beginning and at regular intervals during cell migration to close the wound. The images were compared to determine the migration rate of the cells.

VEGF, MMP-9, IL-8, PGE2 Assay

VEGF, MMP-9, IL-8, and PGE2 activity were measured in conditioned media collected from garcinol-treated samples using a commercially available assay kit (R & D Assay System, Minneapolis, MN) according to manufacturer’s instructions.

Protein Extraction and Western Blot Analysis

The pancreatic cancer cells, BxPC-3 and Panc-1, were plated in a 100-mm dish and allowed to attach for 36 h. Garcinol was directly added to cell cultures at the indicated concentrations and incubated for 48 h. Control cells were incubated in the medium with an equivalent concentration of DMSO. After the incubation period, the cells were harvested in PBS. Cellular lysates were prepared by suspending the cells in 200 µl of lysis buffer (150 mM NaCl, 1 mM EGTA, 0.1% Triton X-100, 0.1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 2 µg/ml leupeptin, and 2 µg/ml aprotinin). The cells were disrupted by sonication and extracted at 4°C for 30 min at maximal microfuge speed to remove debris. For Western blot analysis, each extract prepared as described equivalent to 35–50 µg total protein was separated on SDS-PAGE, electro-transferred onto nitrocellulose membranes, and probed with specific antibodies. Detection of specific proteins (PARP, X-IAP, and cIAP Pan)
was carried out with an enhanced chemiluminescence Western blotting kit according to manufacturer’s instructions (Pierce Biotechnology, Rockford, IL).

**Electrophoretic Mobility Shift Assay**

Nuclear extracts were prepared according to the method described by Chaturvedi et al. (18). Briefly, the cells were washed with cold PBS and suspended in 0.15 ml of lysis buffer [10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 1 mM PMSF, 2 μg/ml leupeptin, 2 μg/ml aprotinin, and 0.5 mg/ml benzamidine]. The cells were allowed to swell on ice for 20 min, and then 4.8 μl of 10% Nonidet P-40 was added. The tubes were vortexed for a few seconds and centrifuged. The nuclear pellet was resuspended in nuclear extraction buffer [20 mM HEPES (pH 7.9), 0.4M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, 2 μg/ml leupeptin, 2 μg/ml aprotinin, and 0.5 mg/ml benzamidine] and incubated on ice. The tubes were then centrifuged for 5 min in a microfuge at 4°C, and the supernatant (nuclear extract) was collected in a cold eppendorf tube and stored at −70°C for later use. The protein content was measured by BCA method.

EMSA was performed by incubating 10 μg of nuclear extract with IRDye–700 labeled NF-κB oligonucleotide. The incubation mixture included 2 μg of poly (dl-dC) in a binding buffer. The DNA-protein complex formed was separated from free oligonucleotide on 8.0% native polyacrylamide gel using buffer containing 50 mM Tris, 200 mM glycine, pH 8.5, and 1 mM EDTA, and then visualized by Odyssey Infrared Imaging System using Odyssey Software Release 1.1.

**Statistical Analysis**

All the experiments were done in triplicate for each of the observations. Each of the data represents the mean ± SD from 3 separate experiments. To analyze the statistical significance of the differences found in the data, an analysis of variance (ANOVA) was done. Statistical significance was assumed at \( P < 0.05 \).

**RESULTS**

**Effect of Garcinol on PaCa Cell Proliferative Status and Viability**

To evaluate the effect of garcinol on viability of PaCa cells in vitro, cells were cultured in 10% FBS-containing medium for 24, 48, and 72 h with increasing concentrations of garcinol (0–40 μM). Garcinol suppressed cell proliferation (as inferred from Trypan blue exclusion assay Fig. 1B) and significantly reduced the percentage of metabolically viable cells (as inferred by MTS Fig. 1C) relative to vehicle control, in a dose- and time-responsive manner. A 50% loss of cell viability of BxPC-3 cells was evident when incubated with 20 μM garcinol for 72 h. In contrast, in Panc-1 cells 10 μM of garcinol at 72 h caused 50% loss of viable cells (\( P < 0.05 \)). The phenotypic changes showing loss of viable cells are depicted in Fig. 1D. Taken together these results suggest that garcinol is an effective inhibitor of PaCa cell viability and interferes with cell proliferation irrespective of their molecular genetic make up. However, BxPC-3 cells harboring wild type k-ras oncogene and mutated p53 was relatively more resistant to the effect of garcinol as compared to Panc-1 cells with mutant k-ras and wild type p53.

**Effect of Garcinol on Apoptosis in PaCa Cells**

To investigate whether the loss of cell viability by garcinol treatment could in part be due to induction of apoptosis, we appraised apoptosis by assessment of nuclear morphological changes by ELISA-Histone DNA analysis, and additionally examined the extent of sub-G1 DNA by flow cytometry of fixed cells. As shown in Fig. 2A, a significant increase in apoptotic cells was observed with increase in concentration of garcinol. This closely parallels the loss of cell viability by garcinol treatment (48 h) in both BxPC-3 and Panc-1 cells. Complementing these findings, cells with nuclear morphology typical of apoptosis was scored in at least 500 cells in garcinol-treated samples after DAPI staining and fluorescence microscopy (Fig. 2B). The observed morphological changes correlated well with garcinol treatment in a dose-dependent manner in both cell lines investigated.

Further quantification of apoptosis in both PaCa cell lines following garcinol treatment by measuring sub-G1 DNA content revealed markedly increased accumulation of cells in the sub-G1 phase (Fig. 3A and 3B). Once again, we noticed a higher percentage of Panc-1 cells (0.3% vs. 36% cells at 0 and 40 μM concentrations garcinol, respectively) being sensitized by garcinol compared to BxPC-3 cells (2% vs. 10% cells at 0 and 40 μM concentrations of garcinol, respectively). This effect was dose-dependent. These results show that PaCa cells undergo apoptosis following garcinol treatment, and a good correlation exists between apoptosis and loss of cell viability and reduced proliferation of cells.

**Effect of Garcinol on Disruption of Cell Cycle in PaCa Cells**

The effect of garcinol treatment on cell cycle progression in PaCa cultured for 24 h was investigated by flow cytometry. Garcinol treatment resulted in a significant dose-dependent increase of cell population in the G0–G1 phase of the cell cycle in BxPC-3 cells (18% vs. 61% cells at 0 vs. 40 μM garcinol concentrations, respectively; Fig. 3A) and in Panc-1 cells (32% vs. 56% cells at 0 vs. 40 μM garcinol concentrations, respectively; Fig. 3B). This increase in cell population in the G0–G1 phase was found to be associated with a concomitant decrease in cell population in the S phase, whereas the population of cells in G2-M phase did not change significantly compared to the corresponding controls. Overall, these results support the notion that the observed decline in cell population by garcinol was due to cell cycle arrest and induction of apoptosis.

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FIG. 2. Induction of apoptosis by garcinol treatment (0–40 µM, 48 h). Histone DNA ELISA (A) and DAPI staining (B) for BxPC-3 cells (upper panel) and Panc-1 cells (lower panel). \( P < 0.05 \) compared to control cells.

FIG. 3. Effect of garcinol (20 and 40 µM, 24 h) on BxPC-3 (A) and Panc-1 (B) cells showing % cells in different phases of cell cycle by flow cytometry. A representative flow histogram for 40 µM garcinol treatment is shown in the upper panel of the figure.
Effects of Garcinol on MMP-9, VEGF, and Cell Invasion Abilities

MMP-9 and VEGF are pivotal molecules in angiogenesis, invasion, and metastasis of cancer cells; hence, we investigated whether garcinol can inhibit MMP-9 and the endogenous production of VEGF in pancreatic cancer cells. Garcinol (20 μM) was found to reduce the basal MMP-9 activity by 72% and 88% in BxPC-3 and Panc-1 cells, respectively, relative to control, following 48 h of treatment (Fig. 4A). We therefore examined, using in vitro wound healing assay, cell migration by creating scratch wounds in confluent monolayers of PaCa cells. After 24-h incubation in 10% serum-containing media supplemented with 20 μM garcinol concentration, the number of cells migrating from the wound edge decreased in a concentration-dependent manner (data not shown). It was noted that in BxPC-3 cells, 20 μM of garcinol at 24 h significantly reduced cell migration compared to untreated, as illustrated in representative microphotographs of cells migrating into the wound (Fig. 4B, upper panel). In contrast, in Panc-1 cells, substantial inhibition of cell migration could be seen at 10 μM garcinol concentration (data not shown). In addition to the effect on the invasive property in Panc-1 cells, the wound was observed to enlarge with time (Fig. 4B, lower panel), with distinct morphological changes similar to apoptotic cells. These results demonstrate that the invasive ability of PaCa cells as affected by garcinol involves regulation by MMP-9.

Interleukin (IL)-8, a cytokine of the CXC chemokine family, reportedly also plays an important role in tumor progression and metastasis. Hence, we determined its activity upon garcinol treatment. The level of IL-8 production was suppressed by approximately 78% in BxPC-3 cells and by 84% in Panc-1 cells compared to the basal level after 20 μM garcinol treatment for 48 h (Fig. 5A).

The inhibitory effect of garcinol on production of VEGF was approximately 90% of basal level in Panc-1 and BxPC-3 cells after 48 h of 20 μM garcinol treatment (Fig. 5B), indicating that garcinol may be a potent suppressor of tumor neovascularization by attenuating VEGF production. PGE2 plays a role in augmenting VEGF in human PaCa cells through an autocrine mechanism. BxPC-3 overexpressing COX-2 enzyme revealed significant reduction in PGE2 levels in upon garcinol treatment at 10 and 20 μM concentration (P < 0.05; Fig. 5C). Taken together, the above-mentioned results indicate that garcinol is predictively effective in reducing angiogenesis and along with
other invasive mediators, such as MMP-9 and IL-8, may lead to inhibition of tumor progression.

**Garcinol Inhibits Apoptotic Molecules in PaCa Cells**

The molecular mechanism by which garcinol induces apoptosis in PaCa cells was investigated using either BxPC-3 or Panc-1 as a representative cell type. Inhibitors of apoptosis proteins (IAP) are overexpressed in human PaCa and are thought to antagonize cell death by interacting with and repressing caspases. We therefore assessed protein expression levels by Western immunoblotting using total cellular protein extract from garcinol-treated cells. Relative to control, XIAP and cIAP protein expression was downregulated in cells in a dose-dependent manner when exposed to garcinol for 48 h (Fig. 6C). This provides a mechanistic evidence of promotion of apoptosis induction by garcinol treatment in PaCa cells.

Caspases are important mediators of apoptosis induced by various agents including phytochemicals and apoptotic stimuli and, therefore, we determined the enzyme activity levels of caspase-3 and caspase-9 in BxPC-3 and Panc-1 cells treated with garcinol. As shown under Figs. 6A and 6B, garcinol significantly inhibited caspases activity. Caspases are sequentially activated by proteolytic processing of their inactive procaspase forms into two smaller units, which in turn leads to cleavage of key cellular proteins including DNA repair enzyme poly ADP-ribose polymerase (PARP). As shown under Fig. 6C, the Western immunoblotting indicate that garcinol treatment resulted in the cleavage of PARP in a dose-dependent manner in BxPC-3 and Panc-1 cells, which was evident by appearance of the 85kDa cleaved intermediate. These results suggest that garcinol induces apoptosis in PaCa cells that may be mediated, at least in part, by the mitochondrial pathway.

**Garcinol Inhibits Activation of NF-κB**

Constitutively active NF-κB in pancreatic tumors contributes to the survival of cells by inhibiting apoptosis. To investigate whether garcinol could abrogate constitutively expressed NF-κB, cells were treated with 15 μM garcinol for increasing time points (0–48 h). As shown under Fig. 6D, garcinol resulted in a concentration-dependent downregulation of NF-κB DNA
binding ability in both BxPC-3 and Panc-1 cells. These results are consistent with downregulation of XIAP proteins because the latter are transcriptionally regulated by NF-κB.

**DISCUSSION**

Based on the current understanding of the severity and complexity of pancreatic cancer progression, a paradigm to arrest tumor cells from proliferating through perturbation of their inter- and intracellular survival signaling cascade mechanisms, leading to promotion of apoptosis, is warranted. In this study, we report the potential of one such novel compound, garcinol, which may have promise for its future use not only as a chemopreventive agent but also as a chemosensitizer in pancreatic cancer therapy. Using paired cell lines with differences in molecular signatures, we report herein that garcinol caused both time- and dose-dependent inhibition of PaCa cell proliferation via G0-G1 phase cell cycle arrest in both cell lines. In this context, based on flow cytometry observations, garcinol resembles indole 3-carbinol (I3C), the principle identifiable active component of Brassica vegetables that also induces a block in G1 cell cycle in breast and prostate cancer cells and inhibits cell proliferation and programmed cell death (19). The growth inhibitory properties of garcinol have been reported in several site-specific experimental tumors and cell lines of human origin for neck and mouth, colon, and cervical cancers (2,3,5).

Apoptosis induction is a central feature in evaluating anti-carcinogenic and chemopreventative properties of treated cells. Using multiple strategic approaches to evaluate apoptosis tendency, we found garcinol to be effective in inducing apoptosis in pancreatic cancer cells. This gives a reasonable indication of its potential in therapeutic intervention. Antiapoptotic molecules, such as X-linked inhibitor of apoptosis (X-IAP) and cellular inhibitor of apoptosis (cIAP) are expressed in PaCa that block apoptosis by inhibiting caspases. Our results indicate that garcinol effectively abrogates these molecules in PaCa. Further investigation of the caspase cascade, notably lowering the activity of caspase-3 and -9 could relate to X-IAP inhibition by garcinol,
which further downstream caused PARP cleavage, considered as a hallmark of apoptosis. Further studies are underway to understand how garcinol drives apoptosis by exploring the Bcl2 family proteins.

Garcinol has been shown to possess antiinflammatory effects (16,17). In pancreatic tumors, cyclooxygenase-2 (COX-2) and its synthesized product, prostaglandin E2 (PGE2), is commonly overexpressed and predictably considered as a promising pharmacological and chemotherapeutic target for the treatment of pancreatic cancer (20,21,22). The effect of garcinol on PGE2 production in interleukin-1β stimulated A549 human lung carcinoma cell lines and in human whole blood stimulated with lipopolysaccharides has been reported recently (23). In agreement with these reports, we observed that garcinol significantly downregulated the level of PGE2 in BxPC-3 cells, known to have moderately high expression of COX-2, the rate-limiting enzyme in the synthesis of prostaglandins from arachidonic acid. Since the promoter sequence of COX-2 contains binding sites for NFκB, it is likely that garcinol influences downregulation of COX-2 and hence, PGE2 levels, via the inhibition of NF-kB binding activity. The significance of garcinol-mediated inhibition of PGE2 for future translation in clinics can be appreciated in relation to the concluding results of a recent Phase I clinical protocol evaluating the effect of a combination of gemcitabine and celecoxib (Celebrex) with radiotherapy. In the clinical trial, the combination was accompanied with greater toxicity than with gemcitabine and radiotherapy alone. This raises obvious concern over the use of a combination of gemcitabine, radiotherapy, and celecoxib (24) as a therapeutic strategy. Our observations highlight the therapeutic benefit of garcinol as a natural inhibitor of PGE2, which provoke avenues for further investigations for inclusion of garcinol as a natural adjuvant in PaCa treatment regimens.

In pancreatic cancer specimens, the constitutive activation of NF-κB significantly contributes to prosurvival activity of tumor cells and is considered as a potential target for treatment (25,26,27). The involvement of NF-κB in a wide spectrum of cellular functions such as cell cycle control, differentiation, apoptosis, and immune and inflammatory response, is well established (26,28). On the contrary, several conventional cancer chemotherapeutic agents, including cisplatin, are known to activate NF-κB, resulting in dual activity, that is, loss of sensitivity of cells to undergo apoptosis and the emergence of resistant phenotype contributing to unfavorable clinical outcome (29). Several laboratory studies have shown that by inhibiting NF-κB, one can enhance therapeutic effectiveness of agents against PaCa (27). In this study, we report that garcinol was effective in inhibiting constitutively active NF-κB and, therefore, we strongly believe it to be a promising candidate in disrupting proliferation and antiapoptotic survival mechanism, which otherwise effectively contribute to proliferation and survival of PaCa cells.

The role of MMP-9 and VEGF in tumor metastasis and angiogenesis is well established. MMP-9 has been acknowledged to be pivotal to invasion of tumor cells, and we have shown that garcinol causes inhibition of MMP-9 activity. These findings additionally complement the wound healing assay, designed to study migration of cancer cells, revealing inhibition of migration subsequent to garcinol treatment and relative to untreated cells. NF-κB is an essential transcription factor associated with MMP-9 induction, since NF-κB binding site is present in the promoter of the MMP-9 gene. Additionally, PGE2 and VEGF molecules that are also regulated by NF-κB have been shown to mediate tumor invasion and could be seen modulated by garcinol affecting neovascularization of tumors.

Pancreatic cancer is characterized by increased expression profile of proinflammatory cytokines, which in addition to survival of cancer cells, also induces an element of systemic inflammatory response, which is conducive to pancreatic carcinoma growth (30). Among proinflammatory cytokines, IL-8 plays an important role in tumor angiogenesis, invasiveness, growth, and metastasis and contributes to the aggressive biology of human pancreatic cancer. Li et al. demonstrated that constitutive NF-κB and AP-1 activation in pancreatic cancer contributes to the overexpression of IL-8 (31). Additionally, as suggested by Shi et al., hypoxic environment also upregulates the IL-8 gene via cooperation of NF-κB and AP-1, contributing to the progression and metastasis of human pancreatic cancer (32). In any event, given the potential of IL-8 as a cause and also a potential modifier of pancreatic carcinogenesis and our observation that garcinol represses IL-8 secretion, this research provides promising insights and important targets for further investigation.

In conclusion, despite the need for further evidence, garcinol shows promise in restricting cancer cell growth by modulating molecules involved in cell proliferation, antiapoptosis, and invasion. Furthermore, the suppression of NF-κB by garcinol augmented apoptosis and inhibited PGE2 and IL-8 secretion. Overall, these results show that garcinol may have potential in pancreatic cancer chemoprevention and may also delay remission and improve long-term care. However, a number of dietary agents as well as drugs including gemcitabine, the current standard clinical therapeutic agent for PaCa, although very effective in cell culture, results in a tumor response rate of 12% and offers a median survival time of 5 mo. As such, the results displayed by garcinol in this in vitro model, although very promising, need to be verified in animal models prior to extrapolation to humans.

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