[Frontiers in Bioscience E3, 1483-1492, June 1, 2011]

Garcinol-induced apoptosis in prostate and pancreatic cancer cells is mediated by NF-κB signaling

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TABLE OF CONTENTS

1. ABSTRACT

Garcinol, obtained from *Garcinia indica*, is a potent antioxidant. Its anticancer activity has been investigated; however, there is no published report on its action against prostate and pancreatic cancer cells. We have earlier reported its activity against breast cancer cells, and here we tested our hypothesis that garcinol could inhibit cell proliferation and induce apoptosis in prostate as well as pancreatic cancer cells. Using multiple techniques such as MTT, Histone-DNA ELISA, activated caspase assays, clonogenic assays and EMSA, we investigated the mechanism of apoptosis-inducing effect of garcinol in prostate (LNCaP, C4-2B and PC3) and pancreatic (BxPC-3) cancer cells. We found that garcinol inhibited cell growth of all the cell lines tested with a concomitant induction of apoptosis in a dose-dependent manner. Down-regulation of NF-κB signaling pathway appears to be the mechanism of apoptosis-induction because garcinol inhibited constitutive levels of NF-κB activity, which was consistent with down-regulation of NF-κB-regulated genes. A significant decrease in the colony forming ability of all the cell lines was also observed, suggesting the possible application of this compound against metastatic disease. In summary, our results provide pre-clinical evidence to support the use of garcinol against human prostate and pancreatic cancer, thus meriting its further investigation as a potential chemo-preventive and/or therapeutic agent.

2. INTRODUCTION

Cancer remains a serious health problem for the United States as well as for the entire world. Cancer related deaths stand next only to the ones associated with heart diseases. It is estimated that 1 in every 4 deaths in the US is due to cancer (1). The incidence as well as mortality due to cancer has marginally declined in last two decades but this disease still affects the lives of millions of people worldwide. Among the various cancers, prostate cancer stands out as one of the leading causes of cancer-related deaths in men, and although pancreatic cancer has lower incidence rate compared to many other cancers, it ranks fourth in terms of mortality. These statistics suggest that the progress towards prostate and pancreatic cancer treatment has been slow and unsatisfactory. Clearly, novel preventive and/or therapeutic approaches are warranted to combat these cancers. One such approach could be the use of naturally occurring dietary substances that are classically known to be non-toxic to normal cells but effective in the killing of cancer cells. Garcinol, a polyisoprenylated benzophenone derivative, is extracted from *Garcinia indica,* and has been shown to have promising anticancer activity (2) as shown by recent interest (2;3) especially for breast cancer (4;5).

The antioxidant activity of natural compounds is generally regarded as a good indicator of their anticancer

activity. There is ample evidence in the literature to suggest a potent antioxidant activity of garcinol (6-11). Also, garcinol inhibits histone acetyltransferases (3;12), dysfunction of which is known to lead to cancer. Further, there are reports on the anticancer potential of garcinol (11;13-19) but the mechanism remains poorly understood. We recently reported the anticancer activity of garcinol against breast cancer cells (4). Our results showed that garcinol can effectively inhibit cell proliferation and viability of breast cancer cells with minimal to no effect on normal breast epithelial cells. Having established this cancer cell-specific activity of garcinol in breast cancer cells, we sought to evaluate whether such anticancer activity of garcinol might be relevant in prostate and pancreatic cancer models as well. For our investigation we used cell lines with different phenotypes: LNCaP (androgen receptor (AR)-positive prostate cancer cells that are responsive to androgen), C4-2B (AR-positive prostate cancer cells derived from LNCaP cells but unresponsive to androgen), PC3 (AR-negative, aggressive prostate cancer cells) and BxPC-3 (pancreatic cancer cells with wild type k-ras). It is important to note that a recent report has shown induction of death receptors DR4 and DR5 in PC3 cells (20); however no further mechanistic details on the biological activity of garcinol has been reported against prostate and pancreatic cancer cells, which prompted our investigation. Therefore, the studies described here were designed to test whether garcinol could function as an anticancer agent against prostate and pancreatic cancer cells, and investigated the potential biological mechanism of action.

3. MATERIALS AND METHODS

3.1.Cell lines, reagents, and antibodies

Prostate cancer cell lines, LNCaP, C4-2B (derived from LNCaP cells) and PC3 were maintained in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 µg/ml streptomycin. Pancreatic cancer cell line BxPC-3 was maintained in DMEM (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum, 100 units/ml penicillin, and 100µg/ml streptomycin. All cells were cultured in a 5% CO_2-humidified atmosphere at 37°C. Garcinol was purchased from Biomol/Enzo Life Sciences International, Inc. (Plymouth Meeting, PA) and was dissolved in DMSO to make 25mM stock solution. Antibody against human Bcl-2 was purchased from Dako North America, Inc. (Carpinteria, CA); antibody against cleaved caspase-3 was from Cell Signaling (Danvers, MA); antibody against Bcl-xL was from Santa Cruz Biotechnology (Santacruz, CA) while monoclonal antibody to β-actin was purchased from Sigma-Aldrich (St. Louis, MO).

3.2. ell growth inhibition studies by 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

PC3 and BxPC-3 cells were seeded at a density of 2 x 10^3 cells per well while LNCaP and C4-2B cells were seeded at a density of 5 x 10^3 cells per well in 96-well culture plates. After overnight incubation, liquid medium was removed and replaced with a fresh medium containing DMSO (vehicle control) or different concentrations of garcinol diluted from a 25mM original stock solution. After 72 h of incubation, 25µl of 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5mg/ml in phosphate-buffered saline, PBS) was added to each well and incubated further for 2 h at 37°C. Upon termination, the supernatant was aspirated and the MTT formazan, formed by metabolically viable cells, was dissolved in isopropanol (100µl) by mixing for 30 min on a gyratory shaker. The absorbance was measured at 595 nm on Ultra Multifunctional Microplate Reader (TECAN, Durham, NC). Each treatment had eight replicate wells and the amount of DMSO in reaction mixture never exceeded 0.1%. Moreover, each experiment was repeated at least three times.

3.3.Cell Viability studies by trypan blue assay

Cells were seeded in 6-well culture plates and treated with garcinol. Upon completion of incubation, culture medium (with floating dead cells) was collected and pooled with the adherent cells removed from the plate by trypsinization. The cells were briefly spun and re-suspended in the normal culture medium. Cell viability was assessed by adding 50µl of Trypan Blue solution (0.4% in PBS) to 50µl of the cell suspension. After 2 minutes, the number of living cells, which did not retain the dye was counted using a hemocytometer, and was compared to the total number of cells (living + dead) to calculate the viability percentage.

3.4.Histone/DNA ELISA for detection of apoptosis

The Cell Death Detection ELISA Kit (Roche, Palo Alto, CA) was used to detect apoptosis in prostate and pancreatic cancer cells treated with garcinol as described previously (4;21). Briefly, cells were treated with garcinol or DMSO control for 72 h. After treatment, the cytoplasmic histone/DNA fragments from these cells were extracted and incubated in the microtiter plate modules coated with anti-histone antibody. Subsequently, the peroxidase-conjugated anti-DNA antibody was used for the detection of immobilized histone/DNA fragments followed by color development with ABTS substrate for peroxidase. The spectrophotometric absorbance of the samples was determined by using Ultra Multifunctional Microplate Reader (TECAN) at 405 nm.

3.5.Soft agar colonization assays

PC3 / BxPC-3 (3 x 10^4) and LNCaP / C4-2B (5 x 10^4) cells were treated with either DMSO or increasing concentrations of garcinol, allowed to grow for 72 hours and then collected by trypsinization**.** 3 x 104 cells were then plated in 0.5 ml of culture medium containing 0.3% (w/v) top agar layered over a basal layer of 0.7% (w/v) agar (with culture medium and the supplements) in 24-well plates. At the time of seeding, the culture was supplemented with different concentrations of garcinol or the DMSO. After appropriate culture time (3-4 weeks), colonies (>50 cells) were counted. Experiments were carried out in quadruplicate, and results presented are representative of three independent observations.

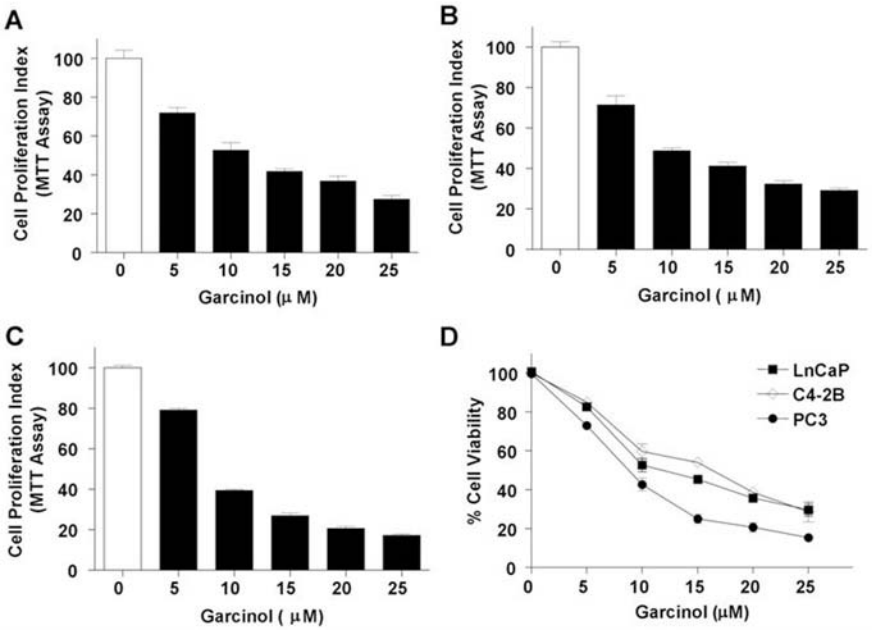


Figure 1. Evaluation of cell proliferation in (A) LNCaP, (B) C4-2B and (C) PC3 prostate cancer cells by MTT assay. (D) Cell viability in garcinol-treated prostate cancer cells, as evaluated with trypan blue staining. Cells were either vehicle-treated (DMSO-control) or treated with increasing concentrations of garcinol for 72 h and then analyzed. The amount of DMSO never exceeded 0.1% during the treatment. Number of cells counted or O.D. value in DMSO (control)-treatment was considered 100% and the number of cells in garcinol-treated cells was calculated relative to this control. Each data point represents Mean ± S.E. of 8 replicates from, at least, three independent experiments.

3.6.Clonogenic assay

To test the survival of breast cancer cells, clonogenic assay was performed as described previously (4;22;23). Briefly, cells were plated in a six-well plate, treated with garcinol for 72 hours, trypsinized, and 1000 viable cells plated in 100 mm petri dishes. The cells were then incubated for 20 days at 37°C in a 5% CO_2/5% O_2/90% N_2 incubator and colonies were stained with 2% crystal violet and counted.

3.7.Western blot assay

Cell lysates were obtained from cells using cold RIPA buffer as described previously (21). Total proteins were fractionated using SDS-PAGE and transferred onto nitrocellulose membrane for Western blotting as described earlier (4;21;24).

3.8.Homogeneous caspase-3/7 assay for apoptosis

Caspase-3/7 homogeneous assay was performed as described previously (4;24) using a kit from Promega (Madison, WI). Cells were treated with garcinol or DMSO control for 72 h. After treatment, 100μl Apo-ONE® caspase-3/7 reagent was added and plates were shaken for 2 minutes, followed by incubation at room temperature for 3h. The fluorescence was then evaluated using ULTRA Multifunctional Microplate Reader (TECAN) at excitation/emission wavelengths of 485/530 nm.

3.9.Electrophoretic mobility shift assay (EMSA)

EMSA was performed by incubating 5μg of nuclear protein extract with IRDye-700–labeled nuclear factor-κB (NF-κB) oligonucleotides (LI-COR, Lincoln, NE) (4;21). The incubation mixture included 2μg of poly dI-dC (poly deoxyinosinic-deoxycytidylic acid) in the binding buffer. The DNA-protein complex formed was separated from free oligonucleotide through 8% native polyacrylamide gel electrophoresis using buffer containing 50mM Tris, 200mM glycine (pH 8.5), and 1mM EDTA, and then visualized by Odyssey Infrared Imaging System using Odyssey Software Release 1.1 (Li-COR, Inc., Lincoln, NE).

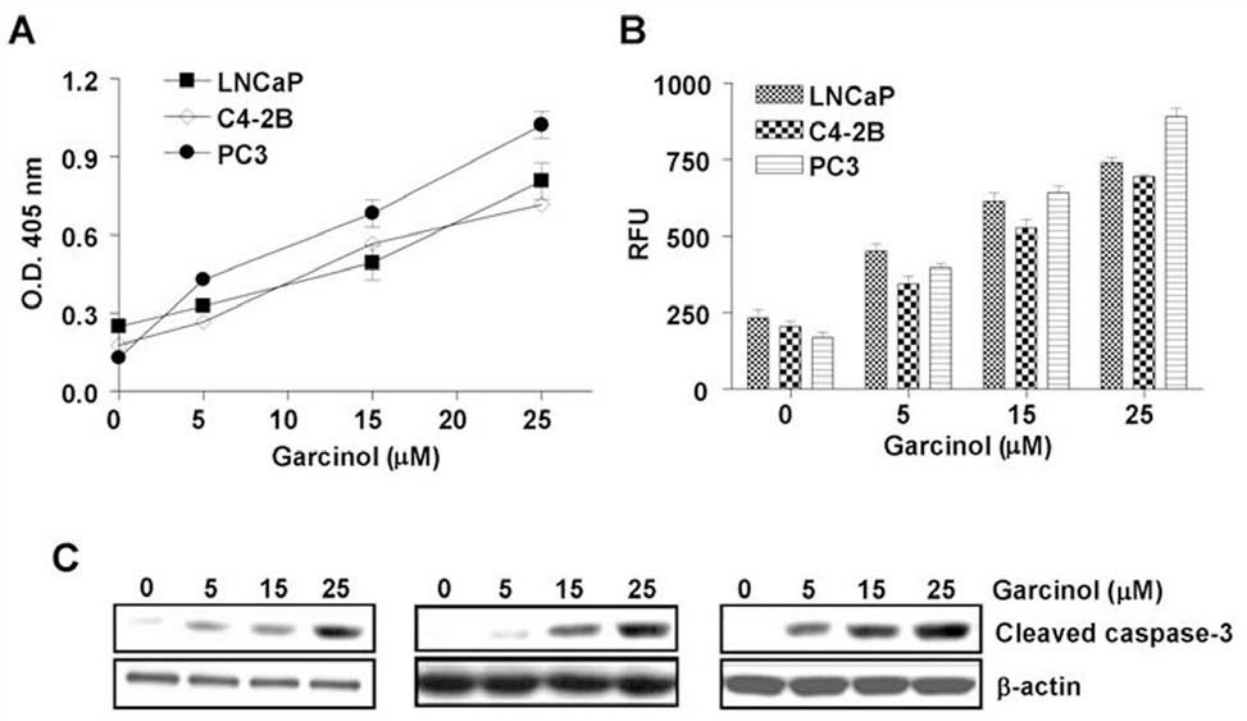
3.10 Data analysis

The experimental results presented in the figures are representative of three or more independent observations. The data are presented as the mean values ± SE. Statistical comparisons between groups were done using one-way ANOVA. Values of $p < 0.05$ were considered to be statistically significant and individual p-values are reported in the figures, as appropriate.

4. RESULTS

4.1.Garcinol inhibits cell growth of prostate cancer cells

The anti-proliferative properties of garcinol were evaluated by exposing prostate cancer cell lines, LNCaP, C4-2B and PC3 to increasing doses of garcinol for 72 hours followed by MTT assay to assess the effect of treatment on cell proliferation. Our results (Figure 1A-C) show that garcinol had a dose-dependent inhibitory effect on the proliferation of all the cell lines. Interestingly, the highly aggressive PC3 cells were found to be particularly sensitive



Fgure 2. Induction of apoptosis in prostate cancer cells by garcinol treatment. Cells were either vehicle-treated (DMSO-control) or treated with increasing concentrations of garcinol for 72 h and assayed for apoptosis by (A) Histone/DNA ELISA method and (B) Homogeneous Caspase-3/7 Assay. (C) Representative western blots showing the effects of increasing concentrations of garcinol on the generation of activated caspase-3 in LNCaP, C4-2B and PC3 prostate cancer cells after 72 hours of treatment. β-actin protein was used as protein loading control for the blot.

to garcinol treatment as exposure to just 10μM garcinol resulted in 61.4% inhibition of cell proliferation ($p<0.01$) as opposed to 47.3% inhibition of LNCaP ($p<0.01$) and 51.3% inhibition of C4-2B cells ($p<0.01$). Cell viability assays were also conducted, using trypan blue, and garcinol was observed to induce dose-dependent killing of all the prostate cancer cells tested (Figure 1D). Again, the garcinol-induced loss of viability was much more in case of PC3 cells. These results suggest that garcinol has anti-proliferative and growth inhibitory effects against prostate cancer cells irrespective of their androgen receptor status and aggressiveness.

4.2.Garcinol effectively induces apoptosis in prostate cancer cells

Since the inhibition of overall cell growth, proliferation and viability of cancer cells by anticancer compounds is known to be accompanied by induction of apoptosis, we assessed the ability of garcinol to induce apoptosis in prostate cancer cells using multiple assays. Histone/DNA ELISA assay (Figure 2A) revealed a dose-dependent induction of apoptotic cell death by garcinol in all the three prostate cancer cell lines tested. To confirm our results, we performed another assay for apoptosis, the caspase-3/7 homogeneous assay. This assay determines the levels of active caspase-3 and/or caspase-7 by fluorescence as a measure of apoptosis. Results from this assay (Figure 2B) also suggested a dose-dependent induction of apoptosis induced by garcinol in all the prostate cancer cells, as evidenced by increased fluorescence reminiscent of active caspase-3/7.

Activation of caspases involves a cleavage, resulting in the production of active caspases from their pro-forms. Thus, the presence of activated caspases in the cells is a good indicator of the induction of caspase-dependent apoptosis. We performed western blot analysis to study the generation of active (cleaved) caspase-3. We used antibody that recognizes only the cleaved (active) form of caspase-3. A dose dependent increase in the cleaved form of caspase-3 was observed in all the three prostate cancer cell lines tested (Figure 2C), suggesting that garcinol treatment results in a caspase-dependent induction of apoptosis. Thus, the use of different methodologies confirmed our findings that garcinol is a potent inducer of apoptosis in prostate cancer cells.

4.3.Garcinol inhibits proliferation and induces apoptosis in pancreatic cancer cells

The results shown above clearly suggest the anticancer effect of garcinol against prostate cancer cells (Figures 1-2). Moreover, we have recently published our findings on the mechanism of anticancer effects of garcinol against breast cancer cells (4). Thus, it appears that garcinol might have beneficial properties against multiple cancers. To further evaluate such anticancer action of garcinol, we exposed pancreatic cancer cell line BxPC-3 to increasing doses of garcinol and studied the effects of treatment on

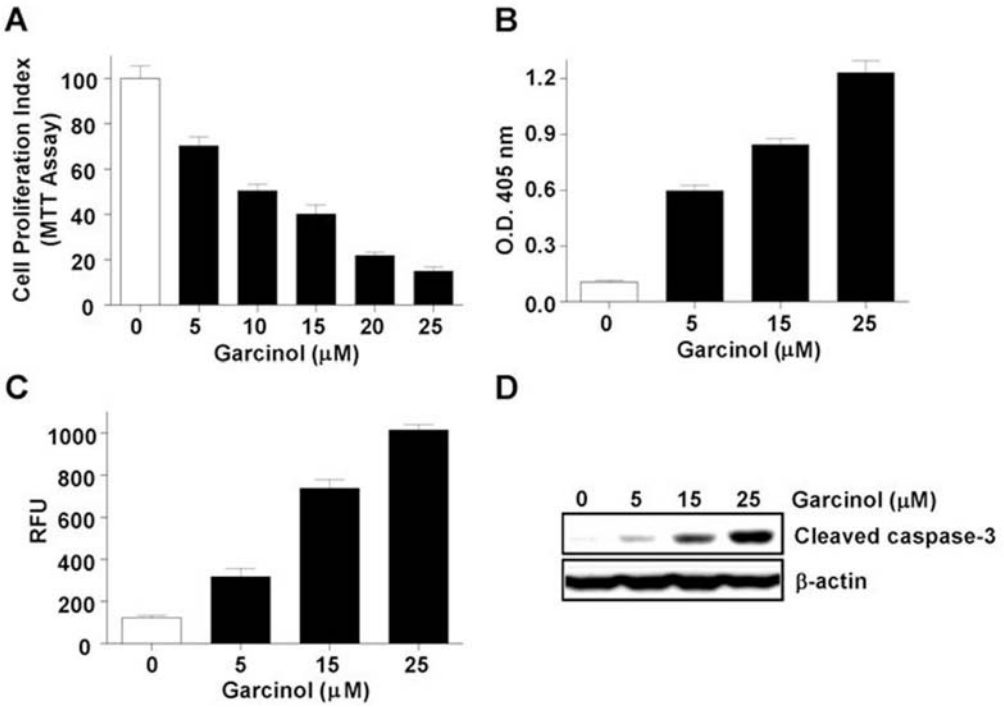


Figure 3. Inhibition of cell proliferation and induction of apoptosis by garcinol in pancreatic cancer cells BxPC-3. (A) Cell proliferation in BxPC-3 cells was evaluated by MTT assay. The amount of DMSO never exceeded 0.1% during the treatment. Number of cells counted or O.D. value in DMSO (control)-treatment was considered 100% and the number of cells in garcinol-treated cells was calculated relative to this control. Apoptosis induction was evaluated by (B) Histone/DNA ELISA method and (C) Homogeneous Caspase-3/7 Assay. (D) Representative western blots showing the effects of increasing concentrations of garcinol on the generation of activated caspase-3 in BxPC-3 cells after 72 hours of treatment. β-actin protein was used as protein loading control for the blot.

proliferation and cell death. Similar to its effects on prostate cancer cells, garcinol induced a dose-dependent inhibition of cell proliferation of BxPC-3 (Figure 3A). A dose as low as 5μM was found to result in 29.7% inhibition ($p<0.05$) and 20μM dose resulted in ~80% inhibition ($P<0.01$). Assay for induction of cell death, as evaluated by Histone/DNA ELISA method (Figure 3B), revealed a dose-dependent induction of apoptosis by garcinol. Caspase-3/7 homogeneous assay (Figure 3C) confirmed the findings that garcinol was very effective in inducing apoptosis in pancreatic cancer cells with all the doses tested. Moreover, treatment of BxPC-3 cells with increasing doses of garcinol also resulted in a dose-dependent increase in activation of caspase-3 as evidenced by western blot analysis for cleaved caspase-3 (Figure 3D). Taken together, these findings clearly demonstrate that garcinol is an effective inhibitor of growth of pancreatic cancer cells and induces significant level of apoptosis in these cells.

4.4.Effect of Garcinol on the clonogenicity of prostate and pancreatic cancer cells

Inhibition of clonogenic potential of cancer cells is a very desirable activity for any putative anticancer agent. To determine the effect of garcinol treatment on clonogenic potential of prostate and pancreatic cancer cells, anchorage-dependent as well as anchorage-independent (soft agar) clonogenic assays were performed. Treatment of prostate cancer cells (LNCaP, C4-2B, PC3) (Figure 4A) and pancreatic cancer cells BxPC-3 (Figure 4C) with 25μM garcinol resulted in a marked reduction in the number of colonies, as determined by crystal violet staining. In soft agar assay, a dose-dependent inhibition by garcinol on the clonogenic potential of all the prostate cancer cell lines was observed (Figure 4B). The number of colonies in control (DMSO-treated) cells varied, with PC3 cells forming the largest number of colonies, which roughly correlates with the *in vitro* growth pattern of these cells consistent with aggressiveness compared to other prostate cancer cells. Treatment of cells with garcinol, particularly at the highest dose tested (25μM), was found to result in almost equal number of colonies in all the three cell lines. These results suggest that the most aggressive PC3 prostate cancer cells are most sensitive to garcinol. The level of inhibition was evaluated to be significant for all the cell lines ($p<0.05$ for LNCaP cells; $p<0.01$ for C4-2B and PC3 cells). A highly significant inhibition of BxPC-3 soft agar colonies by garcinol was also observed (Figure 4D); 25 μM garcinol was found to inhibit the colony forming ability of these pancreatic cancer cells by more than 50%. The results obtained from the clonogenic assays support our observations of MTT assay, as shown in Figures 1 and 3, clearly demonstrating that garcinol significantly inhibits the growth and proliferation of prostate as well as pancreatic cancer cells.

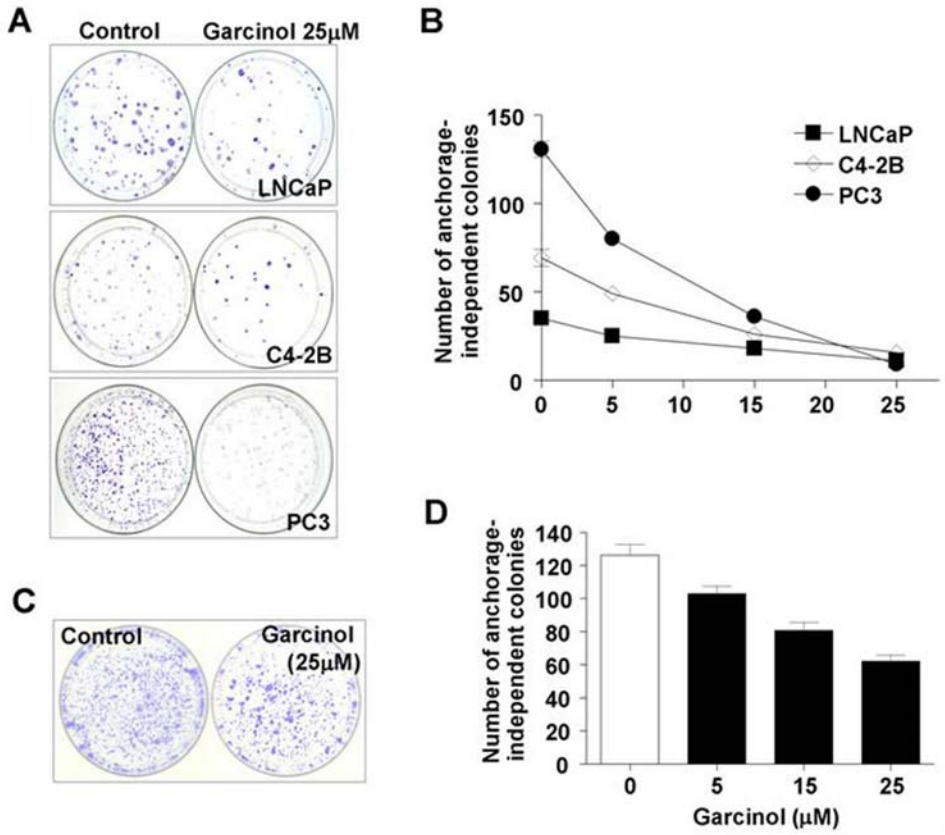


Figure 4. Clonogenic assays for assessing the effects of garcinol on (A-B) LNCaP, C4-2B and PC3 prostate and (C-D) BxPC-3 pancreatic cancer cells. (A and C) Anchorage-dependent and (B and D) Anchorage–independent (soft-agar) assays were performed as described under "Materials and Methods". Experiments were repeated at least three times and representative data is presented.

4.5.Effect of garcinol on NF-κB-DNA binding activity

Next, we studied the effect of garcinol treatment on endogenous DNA-binding activity of NF-κB to assess whether NF-κB is mechanistically involved with the activity of garcinol. Exposure of prostate as well as pancreatic cancer cells to increasing doses of garcinol followed by EMSA using nuclear extracts revealed that garcinol significantly inhibits the DNA-binding activity of NF-κB (Figure 5), which was consistent with densitometric analysis confirming the inhibitory effect of garcinol on NF-κB-DNA binding activity. Exposure to 25μM garcinol resulted in 78% inhibition in LNCaP cells, 84% inhibition in C4-2B cells, 56% inhibition in PC3 cells and 46% inhibition in BxPC-3 cells. All these values were found to be highly significant compared to untreated control ($p<0.01$). It is important to note that NF-κB, a master transcription factor, influences a number of genes that are involved in proliferation, survival and metastasis of cancer cells (25;26), and these results clearly suggest that the inhibition of NF-κB activity by garcinol is one of the mechanism by which garcinol elicits anticancer activity against both prostate and pancreatic cancer cells.

4.6.Effect of garcinol on NF-κB target genes

To further investigate the influence of garcinol on NF-κB signaling pathway, we studied the effect of garcinol on the expression of gene products that are positively modulated by NF-κB (Bcl-2 and Bcl-xL). For these studies, we chose PC3 cells as the representative prostate cancer cells and studied the effect of garcinol on NF-κB gene targets in these cells as well as in the BxPC-3 pancreatic cancer cells. A dose-dependent inhibition in the expression of NF-κB target genes (Bcl-2 and Bcl-xL) were observed after treatment with increasing doses of garcinol (Figure 6). Since NF-κB activity was found to be inhibited by garcinol (Figure 5), these results were expected; however, these results provided convincing proof in support of the functional role of NF-κB down-regulation by garcinol. NF-κB is a master switch that is central to many oncogenic and survival pathways (25-27), and thus the inhibition of its activity by garcinol suggest that garcinol could be a potent anticancer agent against multiple cancers.

5. DISCUSSION

The major conclusions of our present study are: a) garcinol effectively inhibits the proliferation and growth of prostate cancer cells irrespective of AR status and responsiveness; b) induction of caspase-dependent apoptosis seems to be the mechanism for garcinol-induced inhibition of cell growth; c) garcinol also inhibits the growth of pancreatic cancer cells through induction of

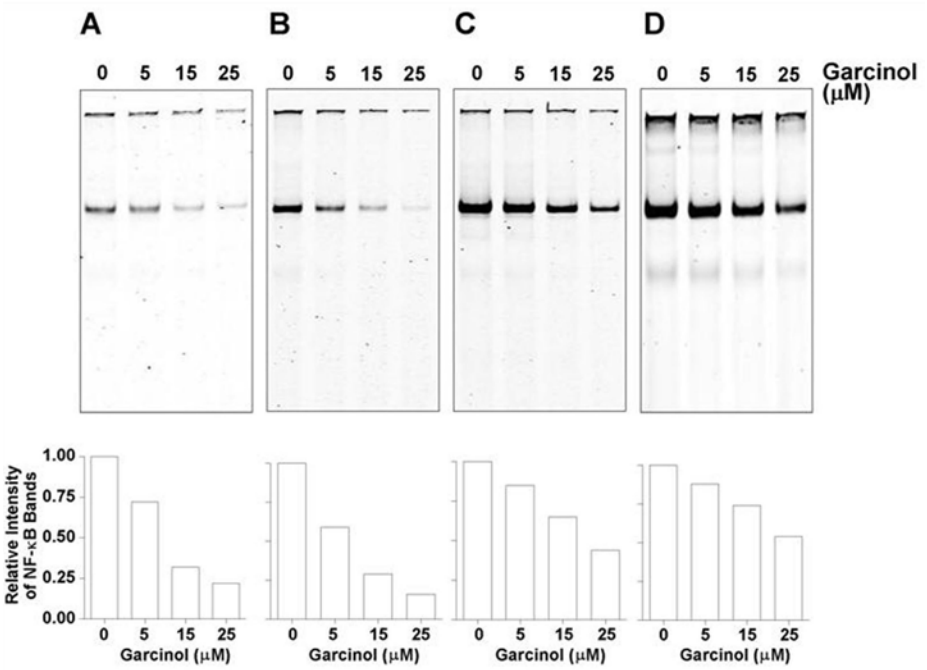


Figure 5. Dose-dependent inhibition of DNA-binding activity of NF-κB by garcinol in (A) LNCaP, (B) C4-2B, (C) PC3 and (D) BxPC-3 cells. Cells were incubated with increasing concentrations of garcinol or DMSO-control for 72 h, and nuclear proteins were subjected to Gel Shift Assay for the evaluation of NF-κB DNA binding activity, as described under "Materials and Methods". Densitometric analysis of NF-κB bands is represented in respective lower panels. The intensity of NF-κB band in the control cells (DMSO-treated) was assigned an arbitrary value of 1.0 and the relative intensities of garcinol-treated cells are presented.

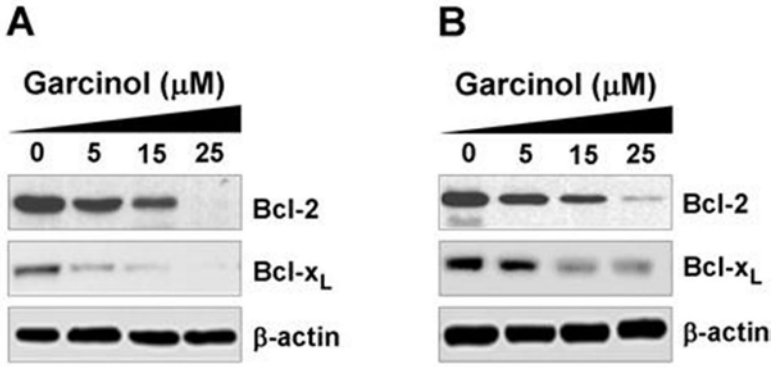


Figure 6. Representative Western blot analysis showing the effects of increasing concentrations of garcinol on NF-κB-regulated proteins in (A) PC3 prostate cancer cells and (B) BxPC-3 pancreatic cancer cells after 72 hours treatment. β-actin protein was used as protein loading control for this experiment.

apoptosis, and d) suppression of NF-κB activity by garcinol could explain its inhibitory effect on prostate as well as pancreatic cancer cells.

Genus Garcinia includes about 200 species found in the tropics, and the antioxidant activity of aqueous extract of the plant has been reported, which is higher than other reported spices and fruits; thus its domestic use in cooking, home remedies and as a beverage is well accepted (28). The rind also contains isogarcinol, hydroxycitric acid, hydroxycitric acid lactone, citric acid and oxalic acid while the fruit contains other compounds such as malic acid, several polyphenols, carbohydrates, anthocyanin, pigments and ascorbic acid (2). Garcinol is the active principal compound of *Garcinia indica*, which crystals out as yellow needles from the hexane extract of the fruit rind. Its strong antioxidant activity has been attributed to the presence of phenolic hydroxyl groups as well as a β-diketone moiety (2;13), and thus garcinol has emerged in recent years as a putative candidate for anticancer studies, which appears to be mediated by the inactivation of NF-κB and its downstream signaling pathways as documented by our results.

Pleiotropic agents, such as those found in nature can target receptor-positive as well as receptor-negative cancer cells. Garcinol comes across as a perfect pleiotropic agent based on our previous studies in breast cancer models (4) where we showed that it can effectively inhibit the estrogen receptor (ER)-positive MCF-7 cells as well as the triple negative MDA-MB-231 cells that are characterized by the absence of ER, progesterone receptor (PR) and the Her-2/neu (ErbB2). Our current results are consistent with previous findings and here we show that garcinol could effectively inhibit the cell growth in AR-positive LNCaP as well as AR-negative PC3 prostate cancer cells (Figures 1-2), suggesting that the anticancer activity of garcinol is independent of AR function because garcinol showed potent activity against AR (LNCaP) positive and responsive cells as well as in AR-positive but AR-nonresponsive (C4-2B) cells. Such activity of garcinol, particularly against AR-unresponsive and AR-negative prostate cancer cells might be of interest in the context of metastasis and the late-stage disease where prostate cancer turns refractory to AR-targeted agents and is very aggressive. Furthermore, garcinol was surprisingly found to be relatively more effective against the AR-negative PC3 cells as compared to AR-positive cells. Moreover, we found significant decrease in the colony forming ability of all the cell lines tested, which suggests that garcinol could be useful for inhibiting metastasis. This finding is likely to have major clinical implications towards the development of garcinol as a therapeutic agent in the future.

The inhibitory action of garcinol against cancer cells is increasingly being realized. A recent report has shown induction of death receptors DR4 and DR5 in multiple cell lines including MDA-MB-231, MCF-7 and PC3 (20), which is consistent with our previous results (4), and further supports similar biological activity of garcinol against prostate and pancreatic cancer and also consistent with overall growth inhibition of cancer cells and inhibition of tumor progression as suggested (29). Therefore, we assessed apoptosis-inducing effects of garcinol, which revealed a dose-dependent induction of apoptosis by garcinol in both prostate and pancreatic cancer cells. In an earlier published report on human leukemia cells, garcinol was reported to display strong growth inhibitory activity through induction of caspase-3 activity in a dose- and time-dependent manner (13) and our previous study on breast cancer cells also established activation of caspase-3 (4). The results described here (Figures 2-3) suggest that garcinol activates caspase-3 in prostate and pancreatic cancer cells as well. Thus, it appears that caspases-mediated cell death is one of the mechanism through which garcinol regulates the growth of cancer cells. These results are really encouraging because they establish that the use of garcinol might not be limited to a specific cancer type. The highly significant inhibitory effect of garcinol on the growth and clonogenicity of prostate as well as pancreatic cancer cells suggests that this compound should be evaluated exhaustively in the relevant model systems for further understanding the mechanism(s) by which it induces its biological effects.

Regulation of cell growth and apoptosis is part of the normal process for the maintenance of homeostasis in which NF-κB plays a very crucial and important role by regulating many signaling pathways including those that are involved in cancer development and progression (30). There are some indirect reports on the inhibitory action of garcinol on NF-κB (10;11) documenting that garcinol could be an effective inhibitor of lipopolysaccharide-induced activation of NF-κB. It is believed that the highly aggressive cancer cells have constitutively higher NF-κB activity (4;31). Our current results confirmed this direct correlation because the basal level of NF-κB activity in PC3 cells was greater than those in less aggressive LNCaP cells (Figure 5). Using the aggressive PC3 cells and AR-positive (LNCAP and C4-2B) prostate cancer cells as well as Bx-PC3 pancreatic cancer cells, we investigated the effect of garcinol on NF-κB-DNA binding activity and observed a dose-dependent inhibition of NF-κB activity in all the cell lines (Figure 5). A number of NF-κB-regulated genes were also found to be down-regulated by garcinol in PC3 and BxPC-3 cells (Figure 6). These results support the pleiotropic effects of garcinol and inactivation of NF-κB is partly responsible for garcinol-induced apoptosis of multiple cancer cell lines. Interestingly, preclinical studies have shown that agents targeting anti-apoptotic Bcl-2 family members have preclinical activity as single agents and in combination with other anti-neoplastic agents (32). In view of such a central role of Bcl-2 family members in pro-survival pathways, their down-regulation by garcinol, as observed in the present study, provides another mechanism through which this compound modulates the cellular apoptotic machinery leading to an efficient induction of apoptosis.

The results presented here clearly show that garcinol is an effective inhibitor of cell growth/proliferation and inducer of apoptosis in human prostate and pancreatic cancer cells. Inactivation of NF-κB signaling and down-regulation of its target genes appears to be one potential mechanism by which garcinol exerts its apoptosis-inducing effects. This report describes anticancer activity of garcinol against multiple cancer models underscoring its true pleiotropic effect. There are no published reports on such activity of garcinol against prostate and pancreatic cancer cells and our results would provide first evidence and rationale for designing further in-depth studies to fully appreciate the value of garcinol for the inhibition of tumor progression and/or treatment of human malignancies.

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Key Words: Garcinol, Prostate cancer, Pancreatic cancer, NF-κB, Apoptosis

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