The sesquiterpene lactone parthenolide in combination with docetaxel reduces metastasis and improves survival in a xenograft model of breast cancer

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Abstract
Parthenolide, a sesquiterpene lactone, shows antitumor activity in vitro, which correlates with its ability to inhibit the DNA binding of the antiapoptotic transcription factor nuclear factor κB (NF-κB) and activation of the c-Jun NH2-terminal kinase. In this study, we investigated the chemosensitizing activity of parthenolide in vitro as well as in MDA-MB-231 cell–derived xenograft metastasis model of breast cancer. HBL-100 and MDA-MB-231 cells were used to measure the antitumor and chemosensitizing activity of parthenolide in vitro. Parthenolide was effective either alone or in combination with docetaxel in reducing colony formation, inducing apoptosis and reducing the expression of prometastatic genes IL-8 and the antiapoptotic gene GADD45α in vitro. In an adjuvant setting, animals treated with parthenolide and docetaxel combination showed significantly enhanced survival compared with untreated animals or animals treated with either drug. The enhanced survival in the combination arm was associated with reduced lung metastases. In addition, nuclear NF-κB levels were lower in residual tumors and lung metastasis of animals treated with parthenolide, docetaxel, or both. In the established orthotopic model, there was a trend toward slower growth in the parthenolide-treated animals but no statistically significant findings were seen. These results for the first time reveal the significant in vivo chemosensitizing properties of parthenolide in the metastatic breast cancer setting and support the contention that metastases are very reliant on activation of NF-κB. [Mol Cancer Ther 2005;4(6):1004–12]

Introduction
Intrinsic or acquired resistance to chemotherapy is a major clinical problem. Several factors including expression levels of multidrug resistance genes, and proapoptotic and antiapoptotic genes determine the response to chemotherapy (1, 2). Antiapoptotic genes that determine response to chemotherapy include cIAP-1, cIAP-2, XIAP, TRAF-1, TRAF-2, Bcl-2, Bfl-1/A1, Bcl-xL, c-FLIP, and Mn-SOD (3, 4). The transcription factors nuclear factor κB (NF-κB), activator protein 1, and signal transducers and activators of transcription 3 are the major transcription factors that control the expression of antiapoptotic and proapoptotic genes that determine the sensitivity of cancer cells to therapy (2, 4, 5). Aberrant activation of these transcription factors, therefore, may lead to changes in antiapoptotic and proapoptotic gene threshold and resistance to chemotherapy.

NF-κB is an extracellular signal–activated transcription factor, which usually resides in the cytoplasm of resting cells due its association with inhibitor of κB proteins (3, 6). On exposure of cells to cytokines and growth factors such as interleukin (IL)-1, tumor necrosis factor (TNF), or epidermal growth factor, a series of signaling events trigger phosphorylation and degradation of inhibitors of κB. NF-κB, liberated from inhibitor of κB, translocates to the nucleus, binds specific response elements in the promoter region of target genes, and regulates gene expression (6). A distinct phosphorylation network controls subcellular distribution and/or activity of activator protein 1 and signal transducers and activators of transcription 3 transcription factors (7, 8).

Constitutive activation of NF-κB is observed in a number of cancers including breast cancer (3). It is likely that constitutively active NF-κB contributes to chemotherapeutic resistance by up-regulating the expression of antiapoptotic genes. Consistent with this possibility, we and others have observed that inhibition of NF-κB through either overexpression of inhibitors of κB or prior exposure to chemical inhibitors, such as parthenolide, sensitize cancer cells to chemotherapeutic drugs such as paclitaxel and CPT11 (9, 10). Parthenolide is the major focus of this investigation. It is derived from the herb feverfew, which is being used for migraine prophylaxis (11). Apart from inhibiting NF-κB, parthenolide inhibits signal transducers and activators of transcription 3 activity (12, 13). Depending on the cell type,
it can activate or repress c-Jun NH2-terminal kinase (JNK), which modulates the activity of activator protein 1 (14–16). We and others have shown the requirement of JNK activation in parthenolide-mediated sensitization of cancer cells to TNF and TNF-related apoptosis inducing ligand (15, 16). The ability of parthenolide to alter the function of three transcription factors makes it an ideal antitumor agent that can sensitize cancer cells to chemotherapy by reducing the level/activity of antiapoptotic proteins. Despite numerous studies showing antitumor activity in vitro, there are few in vivo studies with parthenolide (9, 16–18). In this report, we analyze the chemosensitizing properties of parthenolide in vitro and in a xenograft model of breast cancer focusing on its effects on NF-κB and cytokines and antiapoptotic proteins under its control.

Materials and Methods

Breast Cancer Cell Lines
MDA-MB-231 and HBL-100 cells were obtained from American Type Tissue Culture Collection (Rockville, MD) and grown in MEM plus 10% FCS (9).

In vitro Drug Treatment and Apoptosis Assays
Sensitivity of cells to parthenolide, docetaxel, or combination was measured by clonogenic assay (15). Briefly, cells (100 cells per well in six-well plates, assayed in triplicate) were treated with the indicated drugs for 24 hours. The drugs were removed, cells were washed, and fresh media was added. Number of colonies after 21 days of drug treatment was counted after staining with Giemsa stain. The amount of colony formation for the treated groups was expressed as a percentage of controls (relative colony formation). The carboxyfluorescein FLICA apoptosis detection kit (Immunohistochemistry Technologies, LLC, Bloomington, MN) was used to measure typical apoptosis, atypical apoptosis, and necrosis (19). Briefly, 2 × 105 cells grown overnight on a 60-mm plate were treated with indicated drugs for 48 hours. Both attached and floating cells were collected by trypsinization and incubated with carboxyfluorescein-labeled pan-caspase inhibitor carboxyfluorescein-benzoyloxycarbonyl-valine-alanine-aspartic acid-fluoromethyl ketone for 2 hours at 37°C. Labeled cells were rinsed twice in PBS and resuspended in 300 μL of PBS containing 0.3 μg of propidium iodide. Apoptotic cells were identified by FACScan analysis as previously described (19). All apoptosis assays were done two to five times and representative data are presented in the text.

Northern Blot Analysis
Cells were treated with indicated drugs and RNA was isolated using RNAeasy kit from Qiagen Sciences (Valencia, CA). Northern blotting was done as previously described (20).

Mammary Fat Pad Injection of Tumor Cells and Treatment
MDA-MB-231 cells, which have been passed once through mammary fat pad (TMD231), were used for mammary fat pad injection because these cells formed tumors reproducibly and were metastatic. Cells, 106, were injected into the mammary fat pad of 6- to 7-week-old female nude mice. In the metastasis survival model, tumors (~100 mm3) were removed after 6 weeks and treatment was initiated a day after removal of the tumors and mice were assigned to each treatment group based on average tumor size and weight. Average weight of animals and size of tumors were similar among different groups. In the orthotopic model, the tumors were left in place and treatment commenced 14 days after cell implantation. In both experiments, parthenolide (40 mg/kg as a slurry in 10% ethanol) was given daily by oral gavage. Docetaxel (5 mg/kg in 13% ethanol) was given i.p. once weekly. Experiment was done thrice with 8 to 11 animals per treatment group in each experiment. Lungs, primary tumor regrowth, and/or blood was collected when the animals died or were sacrificed as per the advice of attending Veterinarian or at the end of the experiment. Mice were treated for up to 45 days after tumor removal in the metastasis survival model and the number of mice euthanized at this time point was scored. In the orthotopic mammary fat pad model, mice were treated for 6 weeks, starting 2 weeks after implantation when the tumors were established. The tumors were measured weekly with vernier calipers. Tumor volume was calculated using the following formula: sagittal dimension (mm) × [cross dimension (mm)]2 / 2, and expressed in cubic millimeter (21).

Analysis of Tumor, Lung, and Serum Samples
Lungs were analyzed for metastasis by H&E staining. Metastasis index was calculated as previously described (22). Briefly, the number and size of metastasis in two to five fields per sample were calculated and a score of 4+ was given to a sample with the highest metastasis and relative metastasis in other samples was calculated. LINCOplex multiplex immunoassay system (Linco Research, Inc., St. Charles, MO) was used to measure levels of cytokines in the serum and the samples were analyzed in duplicate.

Immunohistochemistry for p65
Sections, 4 μm in thickness, were deparaffinized and hydrated. Antigen retrieval was done in 140 mmol/L citrate buffer (pH 6.0) in a decloaking chamber (BioCare, Walnut Creek, CA) at 115°C for 3 minutes. The slides were then transferred to boiling deionized water and allowed to cool for 20 minutes at room temperature. The endogenous peroxidase activity was blocked by Peroxo-Block (Zymed, San Francisco, CA) for 2 minutes. The slides were then incubated with rabbit polyclonal p65 antibody (Lab Vision-NeoMarkers, Fremont, CA; diluted 1:100) for 3 hours at room temperature, washed in Optimax (Biogenex, San Ramon, CA), followed by incubation for 10 minutes with horseradish peroxidase polymer conjugate (Zymed). The stain was visualized using Dako liquid DAB plus substrate chromogen solution and hematoxylin Q5 (Vector Laboratories, Burlingame, CA) counterstain. Ten random microscopic fields were identified and the number of nuclei staining for p65 was documented and expressed as percent of total nuclei in the 10 fields. Nuclear counts were done using a hemocytometer counter.
Statistical Analysis
For the metastasis survival model, Prism 3 (version 3.02) for Windows (GraphPad Software, San Diego, CA) was used to generate a Kaplan-Meier survival curves. The log-rank test was then used to compare the survival of the parthenolide alone, docetaxel alone, and docetaxel plus parthenolide groups with the survival of the control group. All analyses used a two-sided $P$ value. For the orthotopic mammary fat pad experiment, analysis was done using SAS Version 8 (Cary, NC). Tumor volume (mm$^3$) was obtained from 80 mice (20 per group) treated in four treatment groups across eight time points. A linear growth curve model was fit using a mixed model with a variance-covariance model that incorporates correlations of observations within a cage and across time. To compare the rates of change of the tumor volume (mm$^3$) between mice treated with parthenolide, docetaxel, parthenolide and docetaxel, and control, the slope estimates from the model were compared. No adjustment for multiple comparisons was made.

To statistically compare the results between groups of the lung metastasis index, nuclear NF-κB staining in tumor cells, and cytokine levels in the serum, the Kruskal-Wallis test (nonparametric ANOVA) with Dunn’s post test for multiple comparisons was done using GraphPad InStat version 3.00 for Windows 95 (GraphPad Software). GraphPad program was also used for statistical analysis of clonogenic and apoptosis assay results.

Results

Parthenolide Increases the Sensitivity of Breast Cancer Cells to Docetaxel In vitro
Previous studies have shown that JNK increases whereas NF-κB reduces the sensitivity of cancer cells to docetaxel (23–25). Because parthenolide could induce JNK and decrease NF-κB in cell type–specific manner (15), we investigated whether parthenolide can increase sensitivity of breast cancer cells to docetaxel. We used clonogenic assay to determine the effect of parthenolide and docetaxel combination on HBL-100 and MDA-MB-231 cells. MDA-MB-231 cells correspond to most aggressive estrogen receptor α–negative breast cancer cells whereas HBL-100 cells are immortalized human breast epithelial cells with integrated SV40 DNA, although there are some doubts about the origin of these cells. A lower number of colonies were formed when cells were treated with combination of parthenolide and docetaxel compared with treatment with either drug (Fig. 1). A lower dose of either drug did not inhibit colony formation by MDA-MB-231 cells. Combination of parthenolide and docetaxel at this dose inhibited colony formation ($P < 0.003$). Drug combination was more effective than either drug at lower doses in inhibiting colony formation by HBL-100 cells ($P = 0.0001$).

Figure 2. The effect of parthenolide and docetaxel on apoptosis of HBL-100 and MDA-MB-231 cells. Apoptosis was measured using carboxy-fluorescein FLICA as described in Materials and Methods. X-axis, propidium iodide staining; Y-axis, active caspase staining. Bottom left, live cells; top left, apoptotic cells; top right, atypical apoptotic cells; bottom right, necrotic cells.
Parthenolide and Docetaxel Combination Enhances Atypical Apoptosis of Cancer Cells

We used carboxyfluorescein FLICA to measure apoptosis of drug-treated cells (15). This assay simultaneously measures cells undergoing typical apoptosis, atypical apoptosis (apoptosis with loss of plasma membrane integrity), and necrosis. However, this assay is not as sensitive as clonogenic assay, thus requiring higher concentration of drugs to detect apoptosis. Docetaxel (5 nmol/L), but not parthenolide (2 μmol/L), induced atypical apoptosis of HBL-100 cells (Fig. 2A). Atypical apoptosis was further enhanced in cells treated with both parthenolide and docetaxel. Total apoptosis/atypical apoptosis/necrosis is shown in Table 1. Parthenolide treatment alone caused apoptosis of MDA-MB-231 cells (Fig. 2B). Cells treated with both parthenolide and docetaxel displayed elevated levels of atypical apoptosis. Thus, increased cell death observed in cells treated with a combination of parthenolide and docetaxel is mostly due to a shift in cell death from typical apoptosis to atypical apoptosis.

Parthenolide and Docetaxel Combination Is Effective in Enhancing Survival and Reducing Metastases in a Xenograft Model of Breast Cancer

To determine whether parthenolide and docetaxel combination is effective in an in vivo metastasis model, we used MDA-MB-231 xenograft model to examine the effect of drugs in an adjuvant setting. In this series of experiments, primary tumors were surgically removed when tumors reached ~100 mm^3 (~6 weeks after implantation) and treatment was initiated a day after surgery. We have observed that surgical removal of primary tumor is necessary to detect metastasis and terminal progression of disease in this model. Experiments were done thrice with 8 to 12 animals per treatment group in each experiment. The 30-day survival data from the first two experiments, in which docetaxel was given at 5 mg/kg once a week and parthenolide at 40 mg/kg daily, are shown in Fig. 3A. It is notable that in the two experiments, the average number of mice alive at 30 days was ~60% in control, docetaxel, and parthenolide (Fig. 3A). However, the average number of mice alive for the combination arm in both experiments was 90%. The unpaired Student’s t test with a two-sided P value showed that this was statistically significant (P = 0.0489). For the long-term survival analysis with mice treated until 45 days after tumor removal, a Kaplan-Meier survival curve was generated and is shown in Fig. 3B. Animals treated with docetaxel and parthenolide showed significantly enhanced survival with 90% of mice alive at day 45 compared with 73% (8 of 11) in the docetaxel alone arm, 64% (7 of 11) in the parthenolide arm, and 34% (4 of 11) in the control arm. The improvement in overall survival in the combination arm was found to be statistically significant (log-rank test, two-sided P = 0.0171). It should also be noted that the survival of animals treated with docetaxel alone or parthenolide alone was not statistically significantly different from control animals but was numerically better. Taken together, these results indicate that parthenolide, when given daily at the doses described, can function as a chemosensitizer in vivo.

Table 1. Parthenolide and docetaxel–induced apoptosis/necrosis of HBL-100 and MDA-MB-231 cells

<table>
<thead>
<tr>
<th>Cell line/drug</th>
<th>% Apoptosis/necrosis ± SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBL-100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>13.5 ± 1.5</td>
<td>—</td>
</tr>
<tr>
<td>Parthenolide 2 μmol/L</td>
<td>13.5 ± 0.5</td>
<td>—</td>
</tr>
<tr>
<td>Docetaxel 5 nmol/L</td>
<td>40.5 ± 1.5</td>
<td>0.006*</td>
</tr>
<tr>
<td>Parthenolide + docetaxel</td>
<td>55.5 ± 3.51</td>
<td>0.008,* 0.008†</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>6.5 ± 0.5</td>
<td>—</td>
</tr>
<tr>
<td>Parthenolide 5 μmol/L</td>
<td>24 ± 2.0</td>
<td>0.02*</td>
</tr>
<tr>
<td>Docetaxel 5 nmol/L</td>
<td>16 ± 3.0</td>
<td>—</td>
</tr>
<tr>
<td>Parthenolide + docetaxel</td>
<td>36 ± 3.0</td>
<td>0.01,* 0.04 †</td>
</tr>
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*a Versus vehicle.
† Versus parthenolide.
‡ Versus docetaxel.

Figure 3. The effect of parthenolide and docetaxel treatment on survival in a metastasis xenograft model. A, 30-day survival rate from two experiments (n = 20–24). B, long-term survival analysis (n = 10–11). Tumor cell implantation, surgery, and treatment protocol are described in Materials and Methods.
We did H&E staining of lungs to determine whether enhanced survival in animals treated with parthenolide plus docetaxel correlates with reduced lung metastasis. A representative staining pattern of lungs from untreated and treated animals is shown in Fig. 4A. Metastasis index is presented in Fig. 4B. Metastasis was lower in animals treated with parthenolide or docetaxel with further reduction in animals treated with parthenolide and docetaxel. These results show a direct correlation between reduced metastasis and enhanced survival. However, although there was a numerical difference, there was no statistical difference ($P = 0.19$).

The ability of parthenolide, docetaxel, and the combination to inhibit NF-κB DNA binding in vivo was assessed by two methods. The amount of NF-κB DNA binding in tumors that recurred in the mammary fat pad was quantified by determining the number of cells in a high power field (400×) that had nuclear staining. A representative staining pattern is shown in Fig. 5A. Five areas per tumor were analyzed; four animals in the control, docetaxel, and combination arms and three in the combination arm had suitable tumors for analysis. The number decreased from 21.15 in control to 13.4 and 13.6 in docetaxel and parthenolide, respectively ($P > 0.2$, single agent versus control) and 10.85 for the combination ($P < 0.01$, versus control; Fig. 5B).

For lung metastases deposits, of every 100 tumor cells seen at 400×, the number of cells to have NF-κB nuclei staining was calculated. The metastatic deposits were randomly identified and the average was 16.6 in control, 12 in docetaxel, 9.1 in parthenolide, and 0.66 in the combination (Fig. 5C). Only the combination was statistically significantly different from control ($P < 0.01$).

### Efficacy of Parthenolide in Orthotopic Mammary Fat Pad Model

To determine the effect of parthenolide, alone or in combination with docetaxel, on primary tumor growth, we treated animals implanted with MDA-MB-231 cells into the mammary fat pad. After 42 days of therapy, docetaxel reduced tumor growth by 15% versus control whereas parthenolide and the combination were more effective as growth was reduced by 25% and 29%, respectively, in this chemoresistant model (Fig. 6). A linear growth curve model was fit using a mixed model with a variance-covariance model that incorporates correlations of observations across time. The rate of change of tumor volume between treatment groups was evaluated by comparing the slope estimates from the model. The rate of change for parthenolide versus control was $-1.52$ ($P = 0.057$), that for docetaxel versus control was $-1.3$ ($P = 0.11$), and for combination $-0.86$ ($P = 0.28$). As such, there was a trend toward slower tumor growth in the parthenolide-treated animals but it did not enhance the activity of docetaxel (as was seen in the metastasis survival model). These results are consistent with a recent observation that NF-κB plays a role in the growth of cancer cells at sites of metastasis but not at primary sites in breast cancer (26).
Animals Treated with Parthenolide and Docetaxel Show Reduced Levels of IFNγ and TNFα but not IL-6 in Serum

To test the significance of docetaxel, parthenolide, and the combination on the ability to decrease NF-κB activity in vivo, we measured the level of mouse cytokines in the serum. Cytokines measured were IL-1β, IL-2, IL-6, IL-10, TNFα, and IFNγ. It should be pointed out that IL-1β, IL-6, TNFα, and IFNγ are NF-κB–inducible genes (27, 28). IL-1β was undetectable. There was no difference in the levels of IL-2, IL-6, and IL-10 (Fig. 7 and data not shown). In contrast, IFNγ was lower in animals treated with parthenolide or docetaxel (P > 0.2, by Kruskal-Wallis test) with further reduction to a statistically significant degree in animals treated with both drugs (P < 0.05; Fig. 7). Both docetaxel and parthenolide reduced TNFα levels with no further reduction in animals treated with both drugs (Fig. 7). The level of reduction did not reach statistical significance. Nonetheless, these results suggest that parthenolide can reduce the levels of cytokines induced by NF-κB in vivo with a greater suppression seen when given with docetaxel. Reduction in these cytokine levels is unrelated to tumor burden because the assay measured only mouse cytokines. Reduction of IFNγ and TNFα in the serum of animals treated with docetaxel was surprising but consistent with the observation that docetaxel decreased anti–NF-κB activity in vivo. These results reveal a unique function for docetaxel in vivo. In addition, the result suggests that parthenolide can inhibit NF-κB activity in vivo and that the combination is able to decrease NF-κB activity even further.

Parthenolide Reduces IL-8 Expression In vitro

Metastasis of MDA-MB-231 cells in xenograft model requires IL-8 and/or CXCR4 (29, 30). In addition, IL-8 is involved in chemotherapy-resistant growth of cancer cells (31). Urokinase-type plasminogen activator is also involved in metastasis of MDA-MB-231 cells (32). All of these genes are regulated by NF-κB (26, 27, 33). Transforming growth factor β1 (TGFβ1) is also suggested to play a role in metastasis of these cells (30). JNK has been shown to suppress TGFβ1 expression and parthenolide induces JNK (34). We have previously shown repression...
of CXCR4 expression by parthenolide in these cells but the effect of parthenolide on IL-8 or TGFβ1 has not been reported (33). Because LINCOplex ELISA was not sensitive enough to identify cancer cell–derived human IL-8 in serum of animals (there is no mouse IL-8 counterpart), we determined whether parthenolide inhibits IL-8 expression in vitro. Parthenolide reduced IL-8 expression in the absence or presence of docetaxel (Fig. 8). TGFβ1 expression was transiently reduced by parthenolide. For unknown reason, parthenolide did not block TGFβ1 expression in the presence of docetaxel. Parthenolide had no effect on urokinase-type plasminogen activator expression.

Among antiapoptotic genes, we have previously shown inhibition of Mn-SOD by parthenolide (9). We observed modest inhibition of cIAP-2 but not of Bcl-xL by parthenolide (data not shown). Parthenolide had no effect on the expression of XIAP (15) and did not influence cleavage of XIAP by docetaxel (data not shown). Similarly, although parthenolide induced posttranslational modification of Bid as we previously reported (15), this modification had no effect on docetaxel-induced cleavage of Bid (data not shown). Interestingly, parthenolide reduced expression of GADD45β, a NF-κB–regulated antiapoptotic gene that reduces apoptosis through inhibition of JNK (ref. 35; Fig. 8). Thus, parthenolide inhibits the expression of some but not all prometastatic and antiapoptotic genes regulated by NF-κB.

Discussion

In this report, we show the chemosensitizing properties of parthenolide in vitro and in vivo in an adjuvant metastasis model. Although previous studies have shown antitumor activity of parthenolide in vitro, in vivo antitumor activity has not been studied. Unlike the results of in vitro studies, parthenolide as a single agent had at most a modicum amount of activity in vivo. However, it is worth noting that its efficacy as a single agent equated to that of docetaxel at the doses and schedule used in these studies. Although we have not well characterized the pharmacokinetics of parthenolide, the dose given to mice was sufficient for reducing the expression of NF-κB–inducible genes and restricting the p65 subunit of NF-κB to the cytoplasm of tumor cells in vivo. Thus, the slight single-agent activity of parthenolide in vivo suggests that minimal inhibition of NF-κB activity alone is not sufficient for significant antitumor activity. Nonetheless, the dose given was sufficient for it to function as a chemosensitizing agent in the metastasis model and cause significant decrease in NF-κB nuclear localization and activity. The fact that two analyses across two different experiments—(a) comparison of 30-day survival from two experiments and (b) the overall survival at 45 days—showed significant results

Figure 6. Efficacy of parthenolide in orthotopic mammary fat pad model. Tumor growth rate in control and treated animals is shown (n = 20 per arm).

Figure 7. Serum levels of IL-6, TNF, and IFN in untreated and drug-treated animals. Serum is from animals in metastasis model and the serum cytokines were measured using LINCOplex ELISA. *, statistically significant difference between treatment and control. TNFα and IL-6 levels did not show statistically significant difference.
strongly supports this conclusion. This very interesting and unique finding is supported by the recent data showing exquisite dependence of breast cancer metastases, but not of primary tumor growth, on NF-κB (26).

Although constitutive NF-κB activation is reported in a variety of cancers (36, 37), its role in specific stages of cancer progression is just beginning to be defined. For example, recent studies suggest the requirement of NF-κB in tumor promotion of the colitis-associated cancer and the malignant phenotype of squamous cell carcinoma (38, 39). In contrast, NF-κB is not required in the early phases of hepatocyte transformation but is required for progression to hepatocellular carcinoma (40). Our study and a study by Huber et al. (26) suggest the requirement of NF-κB in metastasis but not in proliferation of breast cancer cells at primary sites. Thus, anti-NF-κB–based therapies may have cancer type–specific effects.

We observed two distinct effects of parthenolide in vivo: reducing metastasis and increasing the sensitivity to docetaxel. Reduced metastasis in parthenolide-treated animals may be a result of reduced IFNγ production. IFNγ has been shown to reduce the growth of primary breast cancers but increases metastasis (41). Similarly, lower levels of TNFα in parthenolide-treated animals may have resulted in reduced growth of cancer cells at sites of metastasis. TNFα level is elevated in the serum of patients with lymph node–positive breast cancer and it is believed to play a role in angiogenesis (42, 43).

Other possible parthenolide targets that play a role in reducing metastasis include IL-8, TGFβ1, and CXCR4 (Fig. 8).

Exactly how parthenolide sensitizes to docetaxel is not known but may involve direct modulation of anti-apoptotic gene expression or is a consequence of reduced prometastatic gene expression under NF-κB control. MDA-MB-231 cells used in this study express very high levels of NF-κB–regulated antiapoptotic genes, including Bcl-xl, TRAF-1, cIAP-2, and Mn-SOD, compared with breast cancer cells that lack constitutively active NF-κB (9). With the exception of Mn-SOD and cIAP-2, to a lesser extent, parthenolide had minimum effect on the expression of these genes (data not shown). In this study, we have identified GADD45β, an antiapoptotic gene, as a target of parthenolide. Others have shown regulation of proapoptotic proteins Bax and Bak by parthenolide (44). Thus, the chemosensitizing activity of parthenolide could be secondary to reduced expression levels of select antiapoptotic genes. Alternatively, the chemosensitizing action of parthenolide may be an indirect consequence of repression of CXCR4, IL-8, and TGFβ1 expressions. These genes have been shown to modulate antiapoptotic gene expression, apart from playing a role in metastasis (31, 45–47).

Docetaxel is a very important therapy for breast cancer as single agent or in combination with other agents (48, 49). However, docetaxel resistance is observed clinically. Our studies showing the beneficial effects of the docetaxel and parthenolide combination in an adjuvant setting in an animal model raise the hope for a similar combination treatment of docetaxel-resistant breast cancers. We have not observed any apparent toxicity in animals treated with parthenolide alone or in combination with docetaxel even after 10 weeks of continuous treatment. Additional preclinical studies in a syngeneic model may be required to confirm the effectiveness as well as the lack of toxicity of the combination treatment before our studies can be translated to clinic. The other major issue to resolve is improvement in the solubility and bioavailability of parthenolide. Poor solubility prevented us from dosing animals at more than 40 mg/kg of parthenolide. Preliminary pharmacokinetic studies have shown low nanomolar concentrations of parthenolide (~200 nmol/L; data not shown) in serum of treated animals, well below the target plasma concentrations of 5 μmol/L that we were aiming for based on our in vitro data. Efforts to make analogues with better bioavailability but maintaining the same degree of activity are under way. It is also of note that viable cancer cells were seen despite low amounts of NF-κB after treatment with docetaxel and parthenolide. This probably indicates that other survival pathways are important and that combining parthenolide derivatives with improved pharmacologic properties with other agents that block survival pathways will be needed.

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References

Chemosensitizing Activity of Parthenolide

Molecular Cancer Therapeutics

The sesquiterpene lactone parthenolide in combination with docetaxel reduces metastasis and improves survival in a xenograft model of breast cancer

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