Low-Dose Dietary Phytoestrogen Abrogates Tamoxifen-Associated Mammary Tumor Prevention

Bolin Liu,1 Susan Edgerton,1 Xiaohe Yang,1 Aeree Kim,1 Dalia Ordonez-Ercan,1 Terza Mason,1 Kathy Alvarez,2 Christine McKimmey,1 Naxin Liu,1 and Ann Thor1

1Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma and 2Evanston Northwestern Healthcare Research Institute, Evanston, Illinois

Abstract

Wild-type erbB-2/neu transgenic mice were used to study the interactions between tamoxifen and dietary phytoestrogens (or isoflavones) by dose and form in vivo. Mice were randomized to one of four dietary formulas and implanted with an 8-week continuous-release tamoxifen or placebo pellet at 8 weeks of age. In placebo-treated mice, soy meal diet (but not diets supplemented with low-dose or high-dose isoflavones or a casein diet) resulted in prolongation of tumor latency. In tamoxifen-treated mice fed the soy meal, casein, or high-dose isoflavone enriched diets, the majority (>80%) showed no tumor formation by 60 weeks of age. Of the mice that developed tumors, latency was significantly prolonged. In tamoxifen-treated mice fed the low-dose isoflavone enriched diet, a much higher rate of mammary tumor development (>50%; P < 0.002) and a shorter tumor latency were observed. In vitro studies of human and mouse mammary tumor cell lines confirm that low doses of genistein, co-administered with tamoxifen, promote cell proliferation. This is in contrast to tamoxifen alone or tamoxifen with higher doses of genistein that are growth inhibitory. In summary, low-dose dietary isoflavones abrogated tamoxifen-associated mammary tumor prevention in vivo. These interactions are supported by in vitro data from human and mouse mammary tumor cell lines. These dose-associated interactions likely have relevance to the human use of tamoxifen for prevention or treatment of breast cancer. (Cancer Res 2005; 65(3): 879-86)

Introduction

Higher doses and longer duration of estrogen exposure are associated with increased incidence of breast cancer in population-based studies (1, 2). Estrogen is particularly deleterious in women with an increased risk due to a strong family history of the disease (3). Estrogen promotes mammary epithelial cell growth via classic mechanisms and nonclassic pathways, described in more detail below and in Discussion. Interactions between dietary or supplemental plant-derived estrogens (phytoestrogens, also known as isoflavones) and estrogen signaling, mammary tumorigenesis, or exogenous estrogens/antiestrogens, also known as isoflavones (genistein), 4',7-dihydroxyisoflavone (daidzein), and their respective β-glycosides (11). Phytoestrogen (or soy) consumption has been associated with reduced risk of breast cancer (12, 13), improved cardiovascular health, and increase in bone density at higher levels. The type and combination of individual isoflavones, plant source, as well as extraction method, dose, and consistency of use are likely modifiers of these benefits (12, 14, 15).

Soy derivatives (or phytoestrogens) are increasingly used in cosmetics as an unregulated drug supplement, as a food additive to enhance dietary protein, or as a food texture modifier. Intentional dietary enrichment or supplement use is common in women seeking an alternative to hormonal replacement therapy and a preventive agent against breast cancer, osteoporosis, or cardiovascular disease (16–18). The stage of life during which isoflavones are consumed and the dose and form that are ingested may critically influence its biological effects (19, 20). Even modest amounts (~100 mg) of isolated isoflavones may exert biological effects in vivo (16, 21).

Dietary phytoestrogens may influence signal transduction of both the steroid receptor and the receptor tyrosine kinase pathways (erbB-1, erbB-2, erbB-3, and erbB-4) as well as other biological phenomena. ErbB-2 encodes a transmembrane glycoprotein, p185, with intrinsic tyrosine kinase activity (22). p185 is commonly overexpressed in association with gene amplification in...
30% of primary invasive breast cancers. Cell lineage determines the expression of both receptor tyrosine kinase receptors and ligand binding partners, which in turn determines the combinatorial effect and pathway signaling, apoptosis, cell proliferation, angiogenesis, modulation of transcriptional regulators, and signal transduction (23).

We have previously reported that brief exposure to 17β-estradiol (E2; at 8-16 weeks of age) was associated with mammary tumor induction at a younger age in the wt-erbB-2 transgenic mouse model. E2 exposure resulted in mammary tumors that were more frequently invasive, higher grade, and multifocal (24). The E2-associated phenotypic aggression seemed stable in derived mammary cell lines as mammary tumor-derived cell lines also showed more rapid growth and clonogenicity in soft agar (25). The transgenic mice exposed to short-term tamoxifen (also at an early age, 8-16 weeks) generally failed to develop mammary tumors by 60 weeks of age (tumor prevention, >80%). Similar biological responses to estrogenic hormones have been observed in women, indicating important similarities between the wt-erbB-2 model and human breast cancer. We have also reported phenotypic heterogeneity in this model system with approximately 10% of tumors showing unique histologic growth patterns (26). Again, this is reminiscent of what is observed in human breast cancer. Finally, both this mouse model and human breast cancer often show erbB-2, p53, and epidermal growth factor receptor abnormalities. We therefore believe that the wt-erbB-2 mouse model has biological relevance to human breast cancer.

In addition to studying the influence of estrogen, we have evaluated the effect of diet-based estrogenic agents using both open and closed commercial diets. In particular, we have focused on interactions between exogenous and dietary components. For example, we have reported that mice fed the soy meal diet developed mammary tumors at a later age than casein-fed animals treated with E2 or placebo, whereas no differences were observed by diet for the tamoxifen-treated mice (24).

As an extension of those studies and the conflicting literature regarding the benefits of phytoestrogens, we hypothesized that various doses or forms of dietary phytoestrogens may alter mammary gland development, tumorigenesis, or the preventive effects of tamoxifen. To explore this hypothesis, we extended our in vivo studies of the genetically at risk transgenic mouse and confirmed critical observations using both human and mouse mammary tumor cell lines.

**Materials and Methods**

**Cells and Cell Culture.** The human breast cancer cell line MCF-7 was obtained from the American Type Culture Collection (Rockville, MD) and maintained in DMEM and Ham's F-12 medium (1:1, v/v; Invitrogen Corp., Grand Island, NY) supplemented with 10% fetal bovine serum (Invitrogen). This cell line was cultured in a 37°C humidified atmosphere containing 95% air and 5% CO2 and was split twice a week.

**Mice.** Animal care was provided in accordance with institutional guidelines in our mouse facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care. Protocols and experiments were reviewed and approved by our Institutional Animal Care and Use Committee. Three-hundred eighty-seven virgin female FVB-IgN(MMTV-neu) transgenic mice (wt-erbB-2) were obtained from Jackson Labs (Bar Harbor, ME) at 4 to 5 weeks of age. Mice were placed on one of four different diets on arrival. The diets contained various levels of isoflavones, derived from soy meal or organically extracted genistein and daidzein. These included three separate soy diets: Purina 5001 (a soy meal-based diet containing ~500 µg/g isoflavones; Ralston Purina Co., St. Louis, MO; n = 78), RD D11247 (an isolated isoflavone diet containing ~211 µg/g isoflavones, Research Diets, Inc., New Brunswick, NJ; n = 76), RD D2061301 (an isolated isoflavone diet containing ~500 µg/g isoflavones, Research Diets; n = 58; ref. 27). The remaining mice were placed on casein (milk protein-based) diets, Purina 5K96 (0 µg/g isoflavones; Ralston Purina; n = 99) or RD D11243 (0 µg/g isoflavones, Research Diets; n = 76), which were matched controls to Purina 5001 or RD D11247, respectively. A summary of the dietary composition of these diets is shown (Table 1).

**Table 1.** Composition of mouse diets

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Purina 5001</th>
<th>Purina 5K96</th>
<th>Research Diets D11243</th>
<th>Research Diets D11247</th>
<th>Research Diets D2061301</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)</td>
<td>23.4</td>
<td>19.0</td>
<td>20.3</td>
<td>20.3</td>
<td>20.3</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>4.5</td>
<td>4.5</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>49.0</td>
<td>58.4</td>
<td>66.0</td>
<td>66.0</td>
<td>66.0</td>
</tr>
<tr>
<td>Fiber (%)</td>
<td>5.8</td>
<td>3.6</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>kcal/g</td>
<td>3.3</td>
<td>3.5</td>
<td>3.9</td>
<td>3.9</td>
<td>3.9</td>
</tr>
<tr>
<td>Protein source</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soy meal</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Soy protein (HD-90)</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>yes*</td>
</tr>
<tr>
<td>Casein</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Estimated isoflavone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>concentrations (27)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genistein (µg/g)</td>
<td>214</td>
<td>0</td>
<td>0</td>
<td>137</td>
<td>214</td>
</tr>
<tr>
<td>Daidzein</td>
<td>277</td>
<td>0</td>
<td>0</td>
<td>74</td>
<td>277</td>
</tr>
<tr>
<td>Total isoflavone</td>
<td>491</td>
<td>0</td>
<td>0</td>
<td>211</td>
<td>491</td>
</tr>
</tbody>
</table>

*Combination of soy protein HD-90 and additional genistein and daidzein.
Treatment Groups. At 8 weeks of age, mice were implanted with a single 60-day constant release pellet in the lateral neck. A placebo pellet was implanted in 184 animals. A single 5.0 mg tamoxifen pellet was implanted in 203 mice. All pellets were obtained from Innovative Research of America (Sarasota, FL). Mice were checked twice weekly for tumor formation. Tumor latency was calculated from the date of first palpable tumor. Necropsies were done on each animal and included removal with gross and microscopic examination of mammary glands, examination of lungs (gross and microscopic), and gross examination of other organs except brain and spinal cord. A summary of the mouse treatment randomization, including the number of mice in each group, etc., is provided in a tabular form for convenience (Table 2).

The histologic examination of all palpable or visible tumors was done to confirm tumorigenesis and evaluate tumor histology. The histologic patterns and heterogeneity of these and other mammary tumors have been reported elsewhere (26). Benign mammary glands were also harvested for studies of gland development and gene expression that are ongoing.

Western Blot Analysis. Protein expression levels were determined by Western blot analysis as previously described (28). Briefly, cells were lysed in a buffer containing 50 mmol/L Tris, pH 7.4, 50 mmol/L NaCl, 0.5% NP40, 50 mmol/L NaF, 1 mmol/L Na3VO4, 1 mmol/L phenylmethylsulfonyl fluoride, 25 μg/mL leupeptin, and 25 μg/mL aprotinin. The lysates were centrifuged at full speed in a microcentrifuge for 15 minutes and the supernatants were collected for protein concentration determination, SDS-PAGE, and Western blot analysis with specific antibodies as described in the figure legends.

Establishment of Novel, Mouse Mammary Tumor Cell Lines. Mammary tumors were obtained from the transgenic mice by surgical removal, immediately following euthanasia according to the protocol approved by our Institutional Animal Care and Use Committee. These methods have been previously described in detail (25). In brief, solid tumor tissue was transferred into a tissue culture dish containing PBS. After physical treatment of mammary fat and connective tissues, tumors were minced into small pieces and treated with 0.25% trypsin-EDTA (Invitrogen) at 37°C for 20 minutes. Cells were subsequently centrifuged at 1200 rpm for 5 minutes. After dissociating the supernatant, cells were suspended in DMEM/F12 medium supplemented with 10% fetal bovine serum and 1% antibiotics and antimycotics (Invitrogen). These mammary tumor cells (~1.0 × 10^6 cells/plate) were seeded in tissue culture dishes and kept in a 37°C humidified atmosphere containing 95% air and 5% CO2. The media were changed twice a week to maintain cells in culture. Each line was passaged ~20 times before stability was assumed.

RNA Extraction, cDNA Synthesis, and Reverse Transcription-PCR Analysis. Total RNA was extracted using a modified chloroform/phenol procedure (TRIZOL, Invitrogen). First-strand cDNA was generated using reverse transcriptase (Roche Diagnostics Corp., Indianapolis, IN) following the protocol of the manufacturer, and subsequently amplified by PCR using the Expand High Fidelity PCR System (Roche) and the following primer sets: ERα forward primer, 5'-CCG TCT ACC GCC AGT CCG GCA TC-3', and reverse primer, 5'-CCG TGG GCT CGT TCT CCA GGT AGT A-3'; β-actin forward primer, 5'-GCC CAA CAC CCT CTG CAA TGA GC-3', and reverse primer, 5'-GAC GTA GCA CAG CTT CTC ATT G-3'.

Cell Proliferation Assay. A CellTiter96 AQue nonradioactive cell proliferation kit (Promega Corp., Madison, WI) was used to determine the number of viable cells in the absence or presence of tamoxifen and/or genistein (Sigma Chemical Co., St. Louis, MO). In brief, cells were plated onto 96-well plates. Twenty-four hours later, cells were grown in 100 μL fresh medium as control, or 100 μL fresh medium containing tamoxifen or genistein alone, or 100 μL fresh medium containing the combinations of different doses of tamoxifen and genistein. Cells were incubated at 37°C in a 5% CO2 atmosphere for 72 hours and cell proliferation assay was done to measure the conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium, inner salt (MTS) into a water-soluble formazan by dehydrogenase enzymes in living cells. The absorbance of formazan at 940 nmol/L was recorded by a microplate reader. The percentages of cells surviving from each group relative to controls, defined as 100% survival, were determined by reduction of MTS. All proliferation data shown are the means of at least three independent experiments.

Statistical Methods. Time to first tumor and average time to tumor development were tested using ANOVA. All significant statistics were confirmed using either the Kruskal-Wallis or the Mann-Whitney test. Tumor-free intervals (tumor latency) for survival curves were calculated using the Cox proportional hazards model and differences were tested using the logrank statistic. The first palpable tumor was used to calculate tumor latency for mice that developed either single or multiple mammary tumors. The time until necropsy was used for mice with occult tumors identified by microscopic examination. A two-sided Student’s t test was used to calculate the differences in the number of tumors for each diet combination. Correlations between latency and treatment and/or diet were tested using a Cox proportional hazards model.

Results

Modification of Mammary Tumorigenesis by Dietary Factors in Placebo-Treated Mice. We first evaluated the effects of dietary soy meal as compared with diets enriched with similar or lower doses of supplemental genistein and daidzein and with a control nonestrogenic casein diet on placebo-treated, wt-erbB-2 transgenic mice. Mice maintained on the soy meal diet (Purina 5001) experienced the long mean tumor latency (mean, 39.8 weeks) and the lowest incidence of mammary tumor development by 60 weeks of age (67%; data shown in Fig. 1A and summarized in Table 3). Ninety-three percent of mice fed the high-dose, genistein-enriched diet developed mammary tumors by 60 weeks with a mean tumor latency of 37.3 weeks. All mice fed the low-dose isoflavone enriched diet developed mammary tumors by 60 weeks with a mean tumor latency of 39.5 weeks. All mice fed the nonestrogenic casein control diet also developed mammary tumors with a mean latency of 38.2 weeks. Statistical analysis by dietary factors indicates that the differences in mean latency are insignificant.

Dietary Interactions with Tamoxifen-Associated Tumor Prevention. We next studied mice treated with continuous

<table>
<thead>
<tr>
<th>Table 2. Numbers of mice used for diet and treatment experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diet</strong></td>
</tr>
<tr>
<td>Purina 5001 (soy meal)</td>
</tr>
<tr>
<td>Purina SK96 (casein)</td>
</tr>
<tr>
<td>Research Diets D11243 (casein)</td>
</tr>
<tr>
<td>Research Diets D11247</td>
</tr>
<tr>
<td>(low-dose isolated soy)</td>
</tr>
<tr>
<td>Research Diets D2061301 (high-dose isolated soy)</td>
</tr>
</tbody>
</table>

NOTE: The Purina 5001 diet is based on soy meal as a protein source. The casein control diet for 5001 is SK96 as it contains similar levels of other nutrients. The research diet D11243 is the casein control diet for both low-dose and high-dose isolated soy diets D11247 and D2061301. The latter contains estrogenic activity per phytoestrogen dose to match the activity of the Purina 5001 diet. D11247 was selected because its estrogenic activity per phytoestrogen dose is approximatively half of the soy meal diet Purina 5001 (see Table 1).
tamoxifen (8–16 weeks of age) to determine if interactions between dietary phytoestrogens and tumor prevention would be observed. For all tamoxifen-treated mice ($n = 203$), tumor development was significantly less than the control ($P < 0.0001$) or E$_2$-treated mice (previously reported but shown in Table 3 for comparison; ref. 24). For the tamoxifen-treated mice that developed tumors, the mean tumor latency was significantly longer (42 weeks) than the placebo-treated animals (38.3 weeks); $P < 0.0001$ by ANOVA and Kruskal-Wallis tests.

Of particular importance, tamoxifen-associated mammary tumor prevention was also significantly reduced (to 46.8%) in mice fed the low isoflavone enriched diet as compared with all other diets (87.5% for the casein-fed, 83.7% for the soy meal-fed, and 83.5% for the high-dose isoflavone–fed mice; $P = 0.0001$). These differences can be visualized in a tabular form (Table 3). Despite this modification of tumor prevention by diet, significant differences in the mean tumor-free latency by diet were not observed. For example, in the tamoxifen-treated mice that developed mammary tumors, those maintained on a low-dose isoflavone diet had the shortest tumor-free interval (mean, 39.6 weeks) as compared with soy meal-fed (mean, 40 weeks), casein-fed (mean, 44.7 weeks), and high-dose isoflavone–fed mice (mean, 47.8 weeks; variances between these means are not significantly different). These data show that low-dose isoflavone abrogates tamoxifen-associated mammary tumor prevention in MMTV-wt-erbB-2/neu transgenic mice.

**In vitro Studies of Isoflavone: Tamoxifen Interactions in Mammary Tumor Cell Lines.** The protein expression levels of $h$-actin, of ER isoforms and $h$, and of erbB-2 in five mammary tumor cell lines derived from the same transgenic mice and the human breast cancer cell line MCF-7 cell line are shown (Fig. 2A). All mouse mammary tumor lines were negative for ER$_a$, positive for ER$_h$, and had high levels of erbB-2. This is in contrast to MCF-7, which was positive for both ER$_a$ and ER$_h$ and had a very low level of erbB-2. Our data are consistent with a general observation of inverse correlation between the ER$_a$ status and

### Table 3. Tumor development and latency by diet and treatment

<table>
<thead>
<tr>
<th>Hormonal treatment</th>
<th>Diet</th>
<th>$n$</th>
<th>$n$, with tumors</th>
<th>Mean latency*</th>
<th>% Mice disease-free at 60 wks$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Casein</td>
<td>92</td>
<td>53</td>
<td>38.2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Soy meal</td>
<td>24</td>
<td>4</td>
<td>39.8</td>
<td>39.8</td>
</tr>
<tr>
<td></td>
<td>Low-dose isoflavones</td>
<td>39</td>
<td>24</td>
<td>39.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>High-dose isoflavones</td>
<td>29</td>
<td>25</td>
<td>37.3</td>
<td>7.4</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>Casein</td>
<td>83</td>
<td>6</td>
<td>44.7</td>
<td>87.5</td>
</tr>
<tr>
<td></td>
<td>Soy meal</td>
<td>54</td>
<td>4</td>
<td>40.0</td>
<td>83.7</td>
</tr>
<tr>
<td></td>
<td>Low-dose isoflavones</td>
<td>37</td>
<td>13</td>
<td>39.6</td>
<td>46.8</td>
</tr>
<tr>
<td></td>
<td>High-dose isoflavones</td>
<td>29</td>
<td>4</td>
<td>47.8</td>
<td>83.5</td>
</tr>
<tr>
<td>$E_2$</td>
<td>Casein</td>
<td>93</td>
<td>65</td>
<td>31.4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Soy meal</td>
<td>27</td>
<td>7</td>
<td>35.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Low-dose isoflavones</td>
<td>37</td>
<td>26</td>
<td>30.8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>High-dose isoflavones</td>
<td>27</td>
<td>24</td>
<td>31.4</td>
<td>0</td>
</tr>
</tbody>
</table>

*Mean latency calculated for those animals developing tumors only.

$^c$% Tumor-free at 60 weeks excludes animals euthanized for morphology or molecular biology before 60 weeks.

$^c$ $E_2$ data by diet is provided for comparison.
the expression levels of the epithelial growth factor receptor family members, including epidermal growth factor receptor and erbB-2 (29). Interestingly, a negative correlation between ERα and ERβ expressions has also been reported (30). To further confirm the negativity of ERα in the novel mammary tumor-derived cell lines and to investigate if no expression of ERα is attributed to transcriptional or translational regulation, reverse transcription-PCR analyses were done. The specific ERα signal was only seen in the 15-week mouse mammary benign tissue, which is in agreement with our observation using immunostaining (data not shown), but not in the tumor-derived cell lines (Fig. 2B), suggesting that no expression of ERα in the erbB-2-overexpressing tumor–derived cell lines was regulated at the transcriptional level. CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay Kit was then used to test the effects of tamoxifen and/or genistein by dose on the mammary tumor cell growth of these lines grown in an estrogen-free media (Fig. 2C). When these cells were exposed to tamoxifen alone, cell growth was inhibited in all five cell lines. With tamoxifen plus low doses of genistein (0.1 to 0.3 μmol/L), growth stimulation occurred in four of five cell lines. When higher concentrations of genistein were added to tamoxifen, growth inhibition occurred in all five cell lines. This inhibition was greater than that observed with tamoxifen alone, suggesting that the combination of tamoxifen and higher doses of genistein was synergistic in its ability to inhibit tumor cell growth.

A similar but more detailed experiment was carried out using the human breast cancer cell MCF-7. Any combination of genistein and tamoxifen at lower concentrations (<30 μmol/L for genistein and <10 μmol/L for tamoxifen) resulted in cell proliferation in vitro. If the concentrations of either agent were increased, growth inhibition was observed (Fig. 3A and B). Thus, the biphasic interactions between the different doses of genistein and tamoxifen were observed in both human and mouse mammary tumor cells, similar to our in vivo data using the transgenic mouse model.

### Discussion

The effects of isoflavones on cancer development have been studied in two chemically induced models of mammary carcinogenesis. In both the 7,12-dimethylbenz(a)anthracene-induced Sprague-Dawley and N-nitrosomethylurea induced CD/Crj rat models, dietary isoflavones (either as a single factor or in combination with tamoxifen) produced beneficial antitumor effects (31–33). In rats with fully developed, palpable mammary tumors, the combination of tamoxifen and dietary soy reduced tumor growth (34). In contrast, however, several notable reports have raised concerns about possible deleterious effects of these agents in combination (21, 35, 36). Interactions between tamoxifen and the soy-based diet have also been reported using a combination of tamoxifen and genistein, given to nude mice bearing MCF-7 xenografts (37).

The MMTV-wt-erbB-2 transgenic mouse is a widely utilized model of mammary tumorigenesis (38). Increasing evidence suggests that both hormonal factors and receptor tyrosine kinase signaling are critical to normal homeostasis and tumorigenesis in mammary gland (39). Our previously reported data (24) suggest that estrogenic signaling derived from either exogenous hormones or dietary sources (or abrogation thereof) may modify both ductal/lobular morphogenesis and tumorigenesis. Though we observed no differences in mammary tumorigenesis in tamoxifen-treated mice fed the soy meal or casein (control) diet, we were particularly disturbed by the interaction between tamoxifen and the low-dose isoflavone diet. Our data suggest an abrogation of the memory effects of tamoxifen, with
Results showed that genistein enhanced cell proliferation at low concentrations of tamoxifen, consistent with our previous findings.

In conclusion, isoflavones may act synergistically with tamoxifen in breast cancer by enhancing cell proliferation. This effect is likely due to a reduction in tamoxifen-associated tumor prevention and shortened tumor latency. Similar interactions with genistein by dose were also observed in both human and mouse mammary tumor cell lines.

Given the recognized biphasic activity of phytoestrogens on ER-positive mammary tumor cells, it should not be surprising that isoflavones may promote cell growth at lower doses. We were surprised, however, that tamoxifen was not able to overcome these growth-promoting effects in our model. The molecular mechanism behind these interactions may be multifactorial. Some have postulated that the mechanism of isoflavone protection is early modulation of mammary gland differentiation (41). We have previously reported that for this transgenic model, 8 to 16 weeks of age are the most sensitive to estrogenic influences (24). Other reported mechanisms of soy-mediated or isoflavone-mediated antitumor effects include (1) modification of SERM activity, (2) inhibition of topoisomerase II, (3) inhibition of tyrosine kinase activity, (4) antioxidant activity, (5) induction of apoptosis, (6) induction of cell cycle arrest, and (7) inhibition of angiogenesis. Using the human breast cancer cell lines MCF-7 and MDA-MB-231, we have shown that cell proliferation is enhanced at low concentrations of genistein (5 to 10 μmol/L) in association with mitogen-activated protein kinase activation. Microarray-based studies of benign glands from tamoxifen-treated mice are currently in progress to explore other signaling systems that may be involved in this process.

Dietary soy (or supplemental phytoestrogens) has been the focus of interest of both patients and physicians because of its observed reductive effect on cardiovascular disease, breast cancer, and prostate cancer (12, 14, 15). The intake of soy and other sources rich in phytoestrogens, however, is geographically and culturally dependent. For example, the Japanese consumption of soy provides 20 to 35 mg/d of isoflavones, whereas the U.S. diet provides 1 to 3 mg/d (or approximately one tenth of the Japanese consumption; Kelly patent application no. PCT/US93/00230; refs. 42, 43). In addition to dose and regularity of intake, other data strongly suggest that both the stage of life (or age) during which isoflavone is consumed and the form in which it is ingested may critically influence its biological effects (19, 20). An intake of even a modest amount (≤100 mg) of isoflavones has been shown to exert important biological effects in vivo (16). The significantly lower incidence of hormone-dependent (and post-menopausal) tumors in Asia and Eastern Europe, as compared with Western countries, has been widely attributed to dietary isoflavones (44), Japanese women typically present at an earlier disease stage and have less aggressive breast cancers when compared with the women of United States or Britain (33). In addition, Japanese women show a better overall breast cancer survival than any other ethnic group (45).

The intake of soy-rich foods or derived supplements containing soy protein isolates and/or soy protein concentrates has increased dramatically in United States in the last decade (46). (http://www.cfsan.fda.gov/~dms/fssoypr.html). Breast cancer survivors and individuals who perceive they are at an increased risk of breast cancer, in particular, have shown significant interest in the consumption of soy products, natural herbs, and over-the-counter supplements as disease- or risk-modulating factors (16–18). Although the quantitative data regarding this use are limited, dietary modulation seems remarkably common in newly diagnosed breast cancer patients. In one study, the majority of patients surveyed reported that they changed their diets post-diagnosis (47). Modification of food choice by cancer survivors is typically motivated by the belief that certain foods may prevent cancer (48). In one analysis of women enrolled in a randomized clinical treatment trial, 81% reported dietary supplementation (49). The "healthy" estrogenic properties of soy have also resulted in the use of soy supplementation as an alternative to hormonal replacement therapy. Despite the evidence that suggests widespread usage of soy and derived isoflavone products by women and particularly breast cancer patients, several investigators have raised concerns that soy products may have negative effects on human health and, in fact, may promote breast carcinogenesis (16, 50).

Figure 3. Effects of the interactions between tamoxifen and genistein on MCF-7 human breast cancer cell proliferation. MCF-7 cells (4.0 × 10^3) in 0.1 mL culture medium were plated onto 96-well plates. Twenty-four hours later, cells were grown in 0.1 mL fresh medium as control, or 0.1 mL fresh medium containing either tamoxifen or genistein alone, or 0.1 mL fresh medium containing different doses of tamoxifen and genistein. Same MTS assays were done as in Fig. 2. A, G0, no genistein; G2, genistein 2 μmol/L; G10, genistein 10 μmol/L; G50, genistein 50 μmol/L; B, T0, no tamoxifen; T1, tamoxifen 1 μmol/L; T2, tamoxifen 3 μmol/L; T10, tamoxifen 10 μmol/L.

Antioxidant activity, induction of apoptosis, and inhibition of angiogenesis were observed in the interactions between tamoxifen and genistein. However, inhibition of tyrosine kinase activity was not observed.

a reduction in tamoxifen-associated tumor prevention and shortened tumor latency. Similar interactions with genistein by dose were also observed in both human and mouse mammary tumor cell lines.

Given the recognized biphasic activity of phytoestrogens on ER-positive mammary tumor cells, it should not be surprising that isoflavones may promote cell growth at lower doses. We were surprised, however, that tamoxifen was not able to overcome these growth-promoting effects in our model. The molecular mechanism behind these interactions may be multifactorial. Some have postulated that the mechanism of isoflavone protection is early modulation of mammary gland differentiation (41). We have previously reported that for this transgenic model, 8 to 16 weeks of age are the most sensitive to estrogenic influences (24). Other reported mechanisms of soy-mediated or isoflavone-mediated antitumor effects include (1) modification of SERM activity, (2) inhibition of topoisomerase II, (3) inhibition of tyrosine kinase activity, (4) antioxidant activity, (5) induction of apoptosis, (6) induction of cell cycle arrest, and (7) inhibition of angiogenesis. Using the human breast cancer cell lines MCF-7 and MDA-MB-231, we have shown that cell proliferation is enhanced at low concentrations of genistein (5 to 10 μmol/L) in association with mitogen-activated protein kinase activation. Microarray-based studies of benign glands from tamoxifen-treated mice are currently in progress to explore other signaling systems that may be involved in this process.

Dietary soy (or supplemental phytoestrogens) has been the focus of interest of both patients and physicians because of its observed reductive effect on cardiovascular disease, breast cancer, and prostate cancer (12, 14, 15). The intake of soy and other sources rich in phytoestrogens, however, is geographically and culturally dependent. For example, the Japanese consumption of soy provides 20 to 35 mg/d of isoflavones, whereas the U.S. diet provides 1 to 3 mg/d (or approximately one tenth of the Japanese consumption; Kelly patent application no. PCT/US93/00230; refs. 42, 43). In addition to dose and regularity of intake, other data strongly suggest that both the stage of life (or age) during which isoflavone is consumed and the form in which it is ingested may critically influence its biological effects (19, 20). An intake of even a modest amount (≤100 mg) of isoflavones has been shown to exert important biological effects in vivo (16). The significantly lower incidence of hormone-dependent (and post-menopausal) tumors in Asia and Eastern Europe, as compared with Western countries, has been widely attributed to dietary isoflavones (44), Japanese women typically present at an earlier disease stage and have less aggressive breast cancers when compared with the women of United States or Britain (33). In addition, Japanese women show a better overall breast cancer survival than any other ethnic group (45).

The intake of soy-rich foods or derived supplements containing soy protein isolates and/or soy protein concentrates has increased dramatically in United States in the last decade (46). Breast cancer survivors and individuals who perceive they are at an increased risk of breast cancer, in particular, have shown significant interest in the consumption of soy products, natural herbs, and over-the-counter supplements as disease- or risk-modulating factors (16–18). Although the quantitative data regarding this use are limited, dietary modulation seems remarkably common in newly diagnosed breast cancer patients. In one study, the majority of patients surveyed reported that they changed their diets post-diagnosis (47). Modification of food choice by cancer survivors is typically motivated by the belief that certain foods may prevent cancer (48). In one analysis of women enrolled in a randomized clinical treatment trial, 81% reported dietary supplementation (49). The 'healthy' estrogenic properties of soy have also resulted in the use of soy supplementation as an alternative to hormonal replacement therapy. Despite the evidence that suggests widespread usage of soy and derived isoflavone products by women and particularly breast cancer patients, several investigators have raised concerns that soy products may have negative effects on human health and, in fact, may promote breast carcinogenesis (16, 50).
These studies indicate that transgenic model systems may be readily amenable to studies of dietary factors, and interactions between diet, tumor formation, and other treatments. In particular, these models provide an opportunity to study the molecular and temporal sequence of tumorigenesis. We have also shown that the interactions we observed in the transgenic model can be recapitulated in both mouse and human cell lines. There have been no human studies to determine if soy/phytoestrogen consumption, or the dose of consumed products or supplements, may modify the preventive or treatment effects of tamoxifen. Our data raise the suspicion that low-dose phytoestrogens may interfere with the prevention or treatment benefits of tamoxifen.

We found that a low-dose isoflavone enriched diet abrogated tamoxifen-associated mammary tumor prevention. Using both human and mouse mammary tumor cell lines, we confirmed that low-dose genistein reversed the growth-inhibitory effects of tamoxifen. These results provide important insights into modulating factors that may influence the efficacy of SERMs for breast cancer prevention or treatment. Whereas we acknowledge that mouse studies may not be directly translatable to the intake of variable or intermittent phytoestrogens by humans, our data raise the concern that deleterious interactions between phytoestrogens and tamoxifen may occur. Given the clinical relevance of this observation, we hope that clinical trials will be designed to explore these interactions. At the very least, physicians and patients should be made aware of these possible interactions and data should be accrued prospectively. It seems likely that many of the over-the-counter diet supplements have biological effects and may interact with prescribed drugs and patient outcomes.

Acknowledgments

Received 6/1/2004; revised 11/10/2004; accepted 11/19/2004.

Grant support: NIH grants RO1 CA82948 and 1P50 CA90108. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

References

Low-Dose Dietary Phytoestrogen Abrogates Tamoxifen-Associated Mammary Tumor Prevention

Bolin Liu, Susan Edgerton, Xiaohe Yang, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/65/3/879

Cited articles
This article cites 48 articles, 11 of which you can access for free at:
http://cancerres.aacrjournals.org/content/65/3/879.full#ref-list-1

Citing articles
This article has been cited by 13 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/65/3/879.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.