Toremifene Prevents Prostate Cancer in the Transgenic Adenocarcinoma of Mouse Prostate Model

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ABSTRACT

The chemopreventive efficacy of toremifene, an antiestrogen, was evaluated in the transgenic adenocarcinoma of mouse prostate (TRAMP) model. TRAMP mice were segregated into three groups: (a) the low-dose toremifene group (6.6 mg/kg/day); (b) the high-dose toremifene group (33 mg/kg/day); and (c) the control placebo group. Efficacy of treatment was measured by the absence of palpable tumor. To extend these studies using more sensitive techniques, TRAMP mice were then treated with placebo, flutamide (an antiandrogen; 33 mg/kg/day), or toremifene (10 mg/kg/day). Animals from each treatment group were sacrificed at 7, 10, 15, 20, 25, and 30 weeks of age, and prostate tissues and seminal vesicles were harvested. Tissues from animals (n = 5) in each group were evaluated by wholemount dissections of genitourinary tracts, histology, immunohistochemistry, and Western blot analyses. Blood was pooled per group to measure estradiol and testosterone hormonal levels. Tumors formed at week 17 in the placebo group (n = 10), at week 21 in the high-dose toremifene group (n = 12), and at week 29 in the low-dose toremifene group (n = 12). This represents an increased tumor latency of up to 12 weeks. By 33 weeks, all animals in the placebo group had tumors compared with only 35% of the animals treated with toremifene. Although both flutamide and toremifene decreased tumor incidence compared with the placebo, toremifene was more effective than flutamide. High-grade prostate intraepithelial neoplasia was observed in animals in the placebo group, but not in animals treated with toremifene. Moreover, toremifene-treated animals had prolonged survival compared with placebo-treated animals. By 33 weeks of age, 100% of the placebo-treated animals had developed palpable tumors and died, whereas 60% of the toremifene-treated animals were tumor free. T antigen levels in the prostate of toremifene-treated animals were similar to those of placebo-treated, age-matched animals. Whereas serum estradiol levels remained unchanged, the total and free testosterone levels were elevated in the toremifene-treated group. Toremifene treatment did not affect androgen receptor levels. Because toremifene prevented prostate cancer in a milieu of elevated blood free testosterone levels with no change in prostate androgen receptor expression, the mechanism of toremifene’s chemopreventive activity may be through nonandrogenic pathways, such as estrogen receptor signaling.

INTRODUCTION

Prostate cancer is the most frequently diagnosed noncutaneous cancer and the second leading cause of cancer deaths in men (1). Changes in androgen and estrogen levels with age are thought to be involved in prostate cancer because its incidence rises sharply with age (2). The focus of chemoprevention is not on the treatment of the disease (cancer) but rather on the oncogenic process [carcinogenesis (3)]. High-grade PIN 4 is considered a precursor of adenocarcinoma of the prostate because about 60% of men with high-grade PIN develop prostate cancer within 2 years (4, 5). Androgen deprivation by flutamide and LHRH agonists, but not by finasteride, reduced high-grade PIN (5–7). Unfortunately, the serious side effects of testosterone-lowering drugs are not acceptable to men without prostate cancer.

Increasing serum estrogens and decreasing serum androgens and 5α-reductase activity with age lead to stromal hyperproliferation in the prostate (2). Rising estrogens appear to increase sensitivity of the prostate tissue to androgens by up-regulation of the AR (8–10). Estradiol in the presence of androgens has been shown to stimulate carcinoma in situ and adenocarcinoma of the prostate in Noble rats (11–14). Estradiol is also capable of inducing high-grade PIN and prostate cancer in the aging dog (2, 15). Thus, estrogenic stimulation with decreasing androgen levels contributes to the genesis of prostatic dysplasia and subsequent prostate cancer (16–18).

Both the prostatic stroma and epithelium express ERs, and estrogens are important for prostate growth (19, 20). Recently, a new ER, ERβ, was cloned from a rat prostatic cDNA library and is present in murine and human prostates (21–24). Consequently, the previous ER is now designated as ERα. ERα and ERβ are highly homologous, have similar affinity for estradiol, and can hetero- or homodimerize to form a signaling dimeric complex (21, 22). Although estradiol activates both ERα and ERβ, ERα stimulates transcription and cellular proliferation, whereas ERβ quenches ERα activation (25). ERα is localized predominantly in the prostatic stroma (26), whereas ERβ is found in the secretory epithelial cells of the prostate (21, 22).

In the TRAMP model, the PB-Tag transgene is expressed specifically in the epithelial cells of the prostate. The probasin promoter contains an ARE. All TRAMP mice express the transgene in an androgen-dependent manner and eventually develop prostate cancer that mirrors human prostate cancer progression (27, 28). The TRAMP model has several advantages over currently existing models: (a) mice develop progressive forms of PIN as early as 10 weeks and develop invasive adenocarcinoma by 18 weeks of age; (b) metastatic spread of prostate cancer in TRAMP mice to lymph node, lung, kidney, adrenal gland, and bone resembles human disease; (c) development and progression of prostate cancer can be followed within a relatively short period of 10–30 weeks; (d) prostate tumors arise with 100% frequency; and (e) animals may be screened for the presence of the prostate cancer transgene before the onset of clinical prostate cancer. Thus, TRAMP transgenic mice represent a reliable model to directly test the efficacy of chemopreventive agents that may alter prostate carcinogenesis.

SERMs are structurally diverse nonsteroidal compounds that functionally mimic estradiol in their action but also possess cancer-suppressing activity. Tamoxifen, a SERM, has been widely used to treat breast cancer. Toremifene is a chlorinated derivative of tamoxifen that lacks the DNA adduct forming ability of tamoxifen and has lower genotoxicity than tamoxifen (29–31). Toremifene inhibited 7,12-dimethylbenz[a]anthracene-induced rat mammary cancer (32). Toremifene has been used for breast cancer treatment in 27 countries and used for as long as 13 years in Finland (33). Consequently, estrogen receptor modulator; ARE, androgen response element; HRP, horseradish peroxidase; EIA, enzyme immunoassay.
toremifene was selected as the SERM of choice to study its chemopreventive efficacy in the TRAMP model. We report that toremifene suppressed the development of high-grade PIN, decreased prostate cancer incidence, and increased survival.

MATERIALS AND METHODS

The animal experimental protocol was approved by an institutional animal experimentation review board and followed the NIH guidelines for proper and humane use of animals. The TRAMP (C57BL/6 PB-Tag) transgenic mice were cross-bred with FVB wild-type strain; the hybrid litters were screened by PCR for presence of the PB-Tag transgene, and only the males that screened positive were used in this study. Toremifene citrate powder was made into slow-release pellets (Innovative Research of America, Sarasota, FL), and the drug dose was adjusted for growth-related changes in weight. The pellets were implanted s.c. through a 1-cm incision on the flank in PB-Tag mice (4 weeks of age; average weight, 14 g) anesthetized with metofane (Mallinckrodt, Mundelein, IL) as described previously (34).

For palpable tumor study, the number of animals and the doses of toremifene used were based on published animal data (32). Three groups of 10–12 animals each received a 90-day-release drug pellet of either low-dose toremifene (6.6 mg/kg/day) or high-dose toremifene (33 mg/kg/day) or a placebo. Each treated animal received supplemental dosages at 90-day intervals. Starting at the age of 10 weeks, animals were evaluated weekly for the absence or presence of a palpable tumor. The differences between treatment groups were compared by Fisher’s exact test and Wilcoxon’s rank-sum test for statistical analysis. All P values were two-sided.

Because both 6.6 and 33 mg/kg/day of toremifene showed chemopreventive efficacy, we selected an intermediate dose (10 mg/kg/day toremifene) for longitudinal cohort analysis to determine the effects of toremifene on high-grade PIN and tumor incidence. Three cohorts of animals (70 animals/cohort, 10 animals/time point) were treated with placebo, flutamide (33 mg/kg/day), or toremifene (10 mg/kg/day) pellets starting at the age of 10 weeks of age. Animals from each group were sacrificed at 7, 10, 15, 20, 25, 30, and 33 weeks of age. Tissues from mice (n ≥ 5) were evaluated by wholemount dissections of genitourinary tracts, histology, immunohistochemistry, and Western blot. Blood was pooled, and serum was stored at −20°C for assay of hormone levels. Serum testosterone and estradiol levels were assayed using the EIA kits DSL-10–4000AACTIVE and DSL-10–4300AACTIVE, respectively, supplied by Diagnostic Systems Laboratories, Inc. (Houston, TX). Values for the sample analyte were derived by interpolation using standards available with the kit.

Wholemount Analysis and Histology. Wholemounts of ventral prostates (7, 10, 15, 20, and 30 weeks of age) and seminal vesicles (7, 10, 15, and 20 weeks of age) were examined under a dark-field dissection microscope (Olympus SZH stereo-fitted with an Olympus camera). For histological evaluation, prostate tissues were harvested, fixed overnight in 10% buffered formalin, processed in a Shandon-Lipshaw tissue processor, and embedded in paraffin. Tissue sections (4-μm thick) were stained with H&E.

Immunohistochemistry. Paraffinized prostate tissue sections (4 μm) were obtained from 7-, 10-, and 15-week-old animals treated with placebo, flutamide, or toremifene. Antigen retrieval was performed using the Trilogy (Cell Marque, Austin, TX) method according to the manufacturer’s protocol. Tissues were incubated with the anti-Tag primary antibody (Pub101 mouse monoclonal antibody; 1:150; Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h at room temperature. For Tag immunostaining, the M.O.M. kit (Vector Laboratories) was used to eliminate all nonspecific staining attributable to mouse monoclonal primary antibody. Tissue sections were treated with the secondary antibody (goat antimouse IgG; SC2039; Santa Cruz Biotechnology) for 10 min. To quench endogenous peroxidase activity, sections were incubated with 3% hydrogen peroxide in water and then rinsed in PBS for 5 min. ABC reagent (Vectorstain; Vector Laboratories) was applied for 5 min, and the sections were treated with Nova Red substrate (Vector Laboratories) for 10 min and rinsed with running tap water for 5 min. Harris Hematoxylin was used as counterstain. Samples were then dehydrated through a series of alcohol dilutions, cleared through xylene, and mounted on slides using Cytoseal-60 (Stephens Scientific, Kalamazoo, MI).

Western Blot Analyses. Cross-bred Tag-positive male pups (5 pups/group) were treated with either placebo or toremifene (10 mg/kg/day) pellets at 4 weeks of age. Prostate tissues (dorsolateral and ventral lobes) were harvested at 10 and 15 weeks of age, snap-frozen in liquid N2, and stored at −80°C. Western blot analysis of tissue lysates was performed as described previously (34). TRAMP prostate tumor tissue was used as positive control. Blots were blocked overnight at 4°C in BLOTTO and sequentially reacted with the large Tag primary antibody and HRP-conjugated secondary antibody. The AR expression in placebo and toremifene-treated TRAMP mice (15 and 20 weeks of age) was analyzed by Western blot using primary antibody (rabbit polyclonal antibody SC 816; Santa Cruz Biotechnology) and HRP-conjugated secondary antibody. To normalize the results, Tag and AR expression blots were stripped and reacted with antiactin mouse monoclonal primary antibody (Chemicon, Temecula, CA) followed by HRP-conjugated goat antiamouse secondary antibody.

RESULTS

Toremifene Suppresses the Occurrence of Palpable Tumors in TRAMP Mice. Prostate tumors were first palpable in the placebo group (n = 10) by week 17, in the low-dose (6.6 mg/kg/day) toremifene group (n = 12) by week 29, and in the high-dose (33 mg/kg/day) toremifene group (n = 12) by week 21 (Fig. 1). Hence, toremifene increased the latency time of palpable prostate cancer by up to 12 weeks. Tumors were palpable in 25% of the animals by week 18 in the placebo group and by 33–34 weeks in the high- and low-dose toremifene-treated groups. By 34 weeks, 100% of the placebo-treated animals had palpable tumors compared with 35% of the toremifene-treated animals. Differences in the presence of palpable tumors between low- and high-dose toremifene-treated groups versus placebo groups were significant by both log-rank and Wilcoxon’s statistical analysis (P < 0.0003, low-dose toremifene; P < 0.00017, high-dose toremifene). The incidence of palpable tumors was not significantly different in the high- and low-dose toremifene-treated groups. Toremifene-treated animals also had greater survival rates than the placebo-treated group. By 33 weeks, 100% of the placebo-treated animals had developed palpable tumors and died, whereas 60% of the high- and low-dose toremifene-treated animals had no palpable tumors and were still alive. Furthermore, toremifene-treated mice did not exhibit loss of appetite or weight. No skin lesions or preening behavior resulted from the drug. Thus, treatment with either the high or low doses of toremifene significantly decreased the incidence and
increased the latency period of palpable prostate tumors and prolonged survival.

**Toremifene Prevents the Formation and Progression of Prostate Cancer.** The ability of toremifene to suppress prostate carcinogenesis was further investigated by wholemount analysis and histological evaluation as more sensitive measures of tumorigenesis. Genitourinary tracts from placebo- and toremifene-treated TRAMP mice (n ≥ 5) sacrificed at 7, 10, 15, 20, and 30 weeks were examined. Wholemount analysis of murine ventral prostates (Fig. 2) revealed that invasive prostate tumors resulting in fused ducts were detectable as early as 15 weeks of age (Fig. 2C) and that tumors were present in 100% of prostates from the placebo group by 30 weeks of age. In contrast, the toremifene-treated group had no evidence of fused ducts; 100% of prostates from toremifene-treated mice (Fig. 2A) had complete replacement of the normal prostate ductal architecture (Fig. 4A), but not in age-matched toremifene-treated animals (Fig. 4, E and F). Toremifene treatment of TRAMP mice maintained normal prostatic epithelial ductal architecture (Fig. 4A).

Table 1 shows the effects of placebo, flutamide, and toremifene on prostate oncogenesis in the TRAMP model. Placebo-treated mice uniformly developed prostate tumors by 15–20 weeks of age, whereas toremifene-treated animals had a reduction in the development of prostate cancer for up to 33 weeks. By 15 and 20 weeks of age, 50% and 100% of placebo-treated animals had detectable prostate cancer, respectively. In contrast, by 15 and 20 weeks of age, 0% and only 14% of toremifene-treated animals had evidence of prostate cancer, respectively. The time it took for 50% of placebo-treated animals to develop tumors was 15 weeks; only 43% of the toremifene-treated animals had tumors at 33 weeks. Notably, compared with placebo-treated animals, the tumor incidence in TRAMP mice was about 50% lower with flutamide treatment (43%, 50%, and 57%) and about 75% lower with toremifene treatment (14%, 20%, and 28%) at the same ages. By χ² analysis with an overall level of significance of 0.05 and a power of study of 0.80, the data at 30 weeks showed statistical significance (P < 0.031). We did not perform repeated measure ANOVA because the size of the tumor was not measured. These data further confirm that even with a more sensitive assessment of tumorigenicity, toremifene had significant chemopreventive activity. In fact, toremifene is a more potent chemopreventive agent for prostate cancer than flutamide.

**Toremifene Affects Serum Hormonal Levels.** The free and total serum testosterone and serum estradiol levels were measured using the EIAs. Although toremifene did not affect serum estradiol levels, total serum testosterone levels in treated mice were elevated at 10–15 weeks and returned to levels that were comparable with those of placebo-treated animals by 20–30 weeks. In contrast, the level of free serum testosterone remained elevated from 10–30 weeks of age compared with that in placebo-treated animals (Table 2). Thus, chronic use of toremifene in male animals resulted in restoration of total testosterone, but free testosterone levels remained elevated for up to 30 weeks.

**The Large Tag Transgene in the TRAMP Is Not Down-Regulated by Toremifene.** One major concern was that the observed chemopreventive effect of toremifene might be a consequence of direct suppression of the probasin promoter by toremifene, resulting in reduced expression of the large Tag transgene. The probasin promoter has an ARE, and if this chemopreventive effect is mediated by blocking androgen-dependent pathways, then the probasin promoter activity should be inhibited. Consequently, large Tag expression was determined by Western blot analysis, and representative data are shown (Fig. 5). The large Tag oncoprotein was present in TRAMP...
prostate tumor tissue and in tissues obtained from TRAMP mice at 10 and 15 weeks of age treated with and without toremifene (Fig. 5, A and B). Moreover, the level of large Tag was relatively higher in the toremifene-treated prostate than it was in the placebo-treated prostate.

These observations on large Tag expression were confirmed by immunohistochemical staining of 7-, 10-, and 15-week-old prostate tissues from placebo-treated (Fig. 6, A–C) and toremifene-treated animals (Fig. 6, D–F). Prostate tissues from age-matched flutamide-treated animals (Fig. 6, D–F) were used to compare the relative effects of an antiandrogen, flutamide, with the antiestrogen toremifene on large Tag expression. Animals treated with flutamide had no detectable large Tag protein, whereas toremifene and placebo-treated prostate sections had similar amounts of large Tag protein. These studies suggest that flutamide down-regulated the expression of large Tag, which accounted for its prostate chemopreventive activity. In contrast, toremifene did not alter large Tag expression, suggesting that the mechanism of toremifene’s chemopreventive activity against prostate cancer is not related to large Tag expression but rather to direct suppression of carcinogenesis.

**AR Levels in the TRAMP Prostate Are Not Down-Regulated by Toremifene.** AR was present in the TRAMP tumor tissue. Prostatic AR levels were higher in hybrid TRAMP (TRAMP × FVB) transgenic mice compared with nontransgenic mouse with the same genetic background (C57/BL6 × FVB; Fig. 7). By Western blot analysis,
there were no detectable changes in AR levels in prostate tissues in toremifene-treated TRAMP mice compared with placebo-treated TRAMP mice. Thus, toremifene treatment did not alter the expression of AR in the TRAMP prostate.

**DISCUSSION**

The TRAMP model is ideally suited to study chemoprevention because 100% of mice develop prostatic cancer that mirrors the human form of the disease (27, 28). Toremifene treatment significantly reduced the incidence of prostate cancer in TRAMP mice. This is the first report on the chemopreventive potential of a SERM in an autochthonous animal model of primary prostate cancer. The significant increase in the latency period of prostate cancer observed in toremifene-treated animals suggests that toremifene is able to suppress carcinogenesis. In contrast to flutamide, toremifene inhibits prostate carcinogenesis independent of its effect on large Tag.

Toremifene was well tolerated at the doses used in our study, with no obvious signs of toxicity. According to an earlier report (32), toremifene was well tolerated in mice, and the acute LD₅₀ was higher than 2000 mg/kg. No liver tumors were found in toremifene-treated rats (highest dose, 48 mg/kg).

In our study, toremifene treatment of mice resulted in elevated testosterone levels. Elevated testosterone levels with tamoxifen treatment have been reported (35). Toremifene, like tamoxifen, increases circulating testosterone levels by interfering with the hypothalamus-pituitary-gonadal axis (36). Toremifene blunts the pituitary’s ability to suppress LH secretory response to testosterone. However, toremifene does block the local tissue effects of testosterone action, as evidenced by the reduced seminal vesicle size in the face of elevated serum testosterone in our study.

Based on the negative effect of tamoxifen on AR expression, it has been proposed that a SERM exerts antitumor activity by androgen-dependent mechanisms (19, 20, 37). The seminal vesicles, like the prostate, are androgen-dependent, and, predictably, toremifene inhibited androgen-dependent seminal vesicle development in the presence of elevated serum free testosterone. However, the chemopreventive effect of toremifene appears to be independent of its antiandrogen action because (a) toremifene did not suppress the ARE-dependent expression of Tag driven by probasin promoter, (b) the size of the prostate glands was similar for the toremifene- and placebo-treated animals prior to 15 weeks of age, (c) prostatic AR levels were similar in toremifene- and placebo-treated TRAMP mice, and (d) prostate cancer formation was inhibited in a milieu of elevated free testosterone levels. Thus, the mechanism of toremifene chemopreventive activity appears to be through nonandrogenic pathways. In fact, toremifene is a more potent chemopreventive agent than the antiandrogen flutamide.

We propose that toremifene may exert its chemopreventive effects through modulation of ER because prostatic stroma and epithelium both express ERs, and estrogens are clearly implicated in the growth of the prostate (19, 20). In the rodent prostate, ERα is present in the stroma, whereas ERβ is located in the secretory luminal epithelial cell of the prostate (19, 20). Prostate carcinoma development is associated with decreased ERα expression (20).

**Table 1. Effect of placebo, flutamide, or toremifene treatment on the incidence of prostate tumor development in the TRAMP model**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>10 wk</th>
<th>15 wk</th>
<th>20 wk</th>
<th>25 wk</th>
<th>30 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>0% (0/10)</td>
<td>50% (4/8)</td>
<td>100% (5/5)</td>
<td>83% (5/6)</td>
<td>100% (7/7)</td>
</tr>
<tr>
<td>Flutamide (33 mg/kg)</td>
<td>0% (0/6)</td>
<td>0% (0/10)</td>
<td>43% (3/7)</td>
<td>50% (3/6)</td>
<td>57% (4/7)</td>
</tr>
<tr>
<td>Toremifene (10 mg/kg)</td>
<td>0% (0/12)</td>
<td>0% (0/9)</td>
<td>14% (1/7)</td>
<td>20% (1/5)</td>
<td>28% (2/7)</td>
</tr>
<tr>
<td>Toremifene (33 mg/kg)</td>
<td>0% (0/12)</td>
<td>0% (0/9)</td>
<td>14% (1/7)</td>
<td>20% (1/5)</td>
<td>43% (3/7)</td>
</tr>
</tbody>
</table>

* Percentage of animals with tumor is shown. The number of animals with tumor/actual number of animals sacrificed is shown in parentheses.

**Table 2. Effect of placebo or toremifene treatment on serum testosterone and estradiol levels**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>10 wk</th>
<th>15 wk</th>
<th>20 wk</th>
<th>25 wk</th>
<th>30 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>4.14</td>
<td>2.48</td>
<td>1.09</td>
<td>0.15</td>
<td>0.00</td>
</tr>
<tr>
<td>Toremifene</td>
<td>5.41</td>
<td>7.80</td>
<td>13.65</td>
<td>31.94</td>
<td>3.78</td>
</tr>
<tr>
<td><strong>Total testosterone (ng/ml)</strong></td>
<td><strong>4.14</strong></td>
<td><strong>2.48</strong></td>
<td><strong>1.09</strong></td>
<td><strong>0.15</strong></td>
<td><strong>0.00</strong></td>
</tr>
<tr>
<td>Placebo</td>
<td>37.10</td>
<td>17.73</td>
<td>23.78</td>
<td>38.29</td>
<td>30.22</td>
</tr>
<tr>
<td>Toremifene</td>
<td>28.22</td>
<td>13.65</td>
<td>31.94</td>
<td>3.78</td>
<td>9.63</td>
</tr>
<tr>
<td><strong>Estradiol (pg/ml)</strong></td>
<td><strong>37.10</strong></td>
<td><strong>17.73</strong></td>
<td><strong>23.78</strong></td>
<td><strong>38.29</strong></td>
<td><strong>30.22</strong></td>
</tr>
</tbody>
</table>

* No sample.
porting that ER/H9251 (magnification, Pab101 mouse monoclonal antibody; 1:150) followed mice using anti-Tag primary antibody (IgG; secondary antibody) (magnification, ×66).

cells (14, 21–24). Older ERβ knockout (β-ERKO) mice develop prostate hyperplasia, supporting the contention that ERβ normally suppresses prostate epithelial proliferation (38). In contrast, ERα, not ERβ, is the predominant ER expressed in the female reproductive system (21, 22). We believe that earlier data on the role of ER in the prostate must be reevaluated because those studies were unable to distinguish between ERα and ERβ. SERMs can bind to ERα and ERβ and compete with estradiol and other estrogens in breast and prostate tissue (22, 25, 39–41). Signals emanating from SERM-ER interaction result in the inactivation of estrogen-regulated genes, leading to inhibition of cellular proliferation. Recently, Wang et al. (42) have reported that ERα is critical for prostate carcinogenesis because wild-type mice treated with testosterone and estradiol sequentially developed prostate hyperplasia, high-grade PIN, and prostate adenocarcinoma. In contrast, ERα knockout transgenic mice with identical hormone regimen had epithelial hyperplasia but did not exhibit high-grade PIN or prostate cancer. This is reminiscent of our results with TRAMP. The untreated TRAMP mice develop hyperplasia, high-grade PIN, and prostate cancer, and treatment with toremifene reduced high-grade PIN and prostate cancer. We speculate that toremifene may mediate its actions via ERα because a dynamic modulation of ERs is seen in the prostates of toremifene-treated mice.5 The antiproliferative effects of SERMs may also be mediated by other intracellular signaling mechanisms including binding and sequestration of calmodulin (43), inhibition of protein kinase C (44, 45), and induction of p21waf1/cip1 (45). Nonetheless, the exact mechanism of toremifene-mediated chemoprevention of prostate cancer remains to be elucidated.

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