

Tamoxifen Prevents Premalignant Changes of Breast, but not Ovarian, Cancer in Rats at High Risk for Both Diseases

Alison Y. Ting,^{1,2} Bruce F. Kimler,^{1,4} Carol J. Fabian^{1,3} and Brian K. Petroff^{1,3,5}

Abstract

Women at increased risk for breast cancer are at increased risk for ovarian cancer as well, reflecting common risk factors and intertwined etiology of the two diseases. We previously developed a rat model of elevated breast and ovarian cancer risk, allowing evaluation of dual-target cancer prevention strategies. Tamoxifen, a Food and Drug Administration–approved breast cancer chemoprevention drug, has been shown to promote ovarian cysts in premenopausal women; however, the effect of tamoxifen on ovarian cancer risk is still controversial. In the current experiment, Fischer 344 rats ($n = 8$ per treatment group) received tamoxifen (TAM) or vehicle (control) in factorial combination with combined breast and ovarian carcinogen (17 β -estradiol and 7,12 dimethylbenza[a]anthracene, respectively). Mammary and ovarian morphologies were normal in the control and TAM groups. Carcinogen (CARC) treatment induced mammary dysplasia with elevated cell proliferation and reduced estrogen receptor- α expression and promoted preneoplastic changes in the ovary. In the CARC + TAM group, tamoxifen reduced preneoplastic changes and proliferation rate in the mammary gland, but not in the ovary, compared with rats treated with carcinogen alone. Putative stem cell markers (Oct-4 and aldehyde dehydrogenase 1) were also elevated in the mammary tissue by carcinogen and this expansion of the stem cell population was not reversed by tamoxifen. Our study suggests that tamoxifen prevents early progression to mammary cancer but has no effect on ovarian cancer progression in this rat model.

The development of promising breast cancer chemoprevention agents [i.e., selective estrogen receptor modulators (SERMs), aromatase inhibitors, and retinoids; refs. 1–3] has been permitted by minimally invasive techniques to access tissue, availability of surrogate biomarkers, and relatively high incidence of the disease (4, 5). In contrast, ovarian cancer prevention trials are seldom attempted due to low disease incidence, the absence of accepted disease-specific biomarkers, and the invasiveness of sampling for ovarian tissue. Consequently, although most ovarian cancers are diagnosed at advanced stages resulting in high mortality rates, prevention of ovarian cancer remains elusive (6). One practical approach for successful prevention of ovarian cancer may be the development of chemoprevention agents acting simultaneously against both ovarian and breast cancers.

Authors' Affiliations: ¹Breast Cancer Prevention Center, Departments of ²Molecular and Integrative Physiology, ³Internal Medicine, and ⁴Radiation Oncology, and ⁵Center for Reproductive Sciences, University of Kansas Medical Center, Kansas City, Kansas

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Requests for reprints: Brian Petroff, Department of Internal Medicine, The University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160. Phone: 913-588-3992; Fax: 913-588-3821; E-mail: bpetroff@kumc.edu.

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Breast and ovarian adenocarcinomas share numerous risk factors (e.g., estrogen exposure, ovulation, nulliparity, obesity, family history, and *BRCA1/2* mutations) and women at increased risk for one of these cancers are often also at risk for the other, suggesting intertwined disease pathways (7, 8). Recent studies have shown that women receiving hormone replacement therapy are at increased risk for both cancers (9–11). Alternatively, drugs that decrease ovarian cancer risk may actually increase the incidence of breast cancer (e.g., progesterone; refs. 12, 13). To date, no human chemoprevention trials have been designed simultaneously targeting both breast and ovarian cancers despite the promise of such an approach. Indeed, successful human ovarian cancer chemoprevention has only been shown incidentally during the course of breast cancer prevention trials (i.e., fenretinide; ref. 14). To investigate common chemoprevention strategies, our laboratory has developed a preclinical model that exhibits early changes of mammary and ovarian carcinogenesis in the rat (15). This model allows observation of synergistic and antagonistic drug actions against breast and ovarian cancers that are ignored when each cancer is examined in isolation.

Tamoxifen, the most commonly used breast cancer chemoprevention drug, blocks cell proliferation in the breast and has been shown to cause tumor regression and inhibit tumor formation, especially in ER⁺ breast tumors (16). In the ovary, especially in premenopausal women, tamoxifen has been suggested to promote abnormal ovarian function and cyst

formation, a putative ovarian preneoplastic change (17, 18). Tamoxifen and other (SERMs) have also been used to stimulate ovarian function in subfertile women with some questions about the effect on ovarian cancer risk (19, 20).

Tamoxifen prevents 70% of ER⁺ breast cancers in high-risk women but fails to prevent ER⁻ and some ER⁺ tumors (3). One possibility for the lack of tamoxifen efficacy on 30% of ER⁺ cancers may be the presence of an estrogen-independent breast stem cell population (21). The existence of self-renewing, pluripotent stem cells has been shown both in human breast and in rodent mammary glands (22, 23). Following recurrent carcinogen exposure, these long-lived breast stem cells are thought to accumulate mutations leading to tumor formation. The size of the breast stem cell pool has therefore been hypothesized to serve as a determinant of the likelihood for breast cancer incidence. Indeed, several studies have suggested a strong correlation between increased number of breast stem cells and elevated breast cancer risk, as well as a possible intervention that targets stem cell for cancer treatment and prevention (24–27). In the current study, we use a combined breast and ovarian cancer model to examine the effect of tamoxifen on markers of cancer risk, stemness, and progression in the ovary and mammary gland during carcinogenesis.

Materials and Methods

Animals and treatments

Female Fischer 344 rats (Harlan; $n = 8$ per treatment group), weighing 50 to 55 g, were housed in a climate and light (12 h light:12 h dark) controlled environment and received food and water *ad libitum*. All experimental protocols were approved by the University of Kansas Medical Center Animal Care and Use Committee. Animals were randomly assigned into four different treatment groups as shown in Table 1. Rats were anesthetized using ketamine hydrochloride and xylazine (80 and 8 mg/kg, respectively). Hemiovariectomy was done aseptically to concentrate ovulation on the remaining ovary and hasten a senescent hormonal milieu (28, 29) as these are risk factors of human ovarian cancer (15, 30, 31). The remaining ovary was treated by passing a 7,12 dimethylbenza[*a*]anthracene (DMBA)-impregnated (2.5-mm region dipped in melted DMBA) or vehicle 5-0 silk suture through the ovary twice such that the DMBA or vehicle region was apposed directly and gently secured to the ovarian surface epithelium. Rats receiving ovarian DMBA were subsequently treated with E₂ (1.5 mg, pellet implant, Hormone Pellet Press; ref. 32). Our laboratory has previously shown that this treatment combination promotes progression to simultaneous mammary and ovarian cancers in the rat after 6 mo of treatment (15). Rats were further treated with tamoxifen (5 mg, pellet implant, Hormone Pellet Press) or vehicle to test the effect of tamoxifen in early mammary and ovarian cancers (33).

Tissue preparation

Rats were killed by decapitation at 6 mo posttreatment and the right thoracic mammary glands were fixed in 4% paraformaldehyde and embedded in paraffin. Right abdominal mammary glands were spread onto a glass slide, fixed in 4% paraformaldehyde overnight, hydrated, infused with alum carmine (4 d), dehydrated, cleared in xylene, and stored in methyl salicylate. Left thoracic mammary glands were snap frozen and stored at -80°C . The ovary was bisected through the site of DMBA application. One half was fixed in 4% paraformaldehyde and embedded in paraffin and the remainder was snap frozen and stored at -80°C .

Immunohistochemistry

Six-micron sections of mammary glands and ovaries were deparaffinized, rehydrated, and stained with H&E. Mammary and ovarian sections (midsagittal, at three different equidistant levels per tissue) were evaluated for morphologic changes associated with early progression to mammary adenocarcinoma and epithelial ovarian cancer by an observer blinded to treatment group identity (34, 35). Adjacent sections were prepared for immunostaining by antigen retrieval (93°C, 10 mmol/L citrate buffer, 25 min) and incubation with 0.3% hydrogen peroxide (Lab Vision). Nonimmune serum or primary antibodies against Ki-67 (1:100; clone Ki-S5; rabbit monoclonal antibody, Lab Vision), ER α (1:200; MC-20; mouse monoclonal antibody, Santa Cruz), aldehyde dehydrogenase-1 (ALDH-1) family member A1 (1:150; rabbit polyclonal; Abcam), or Oct-3/Oct-4 (1:50, mouse monoclonal, Santa Cruz) were applied and visualized with biotinylated secondary antibodies (Lab Vision) and diaminobenzidine chromogen. All incubations were carried out using a DAKO LV-1 autostainer.

Protein isolation and immunoblotting

Samples of mammary gland ($n = 4$) and ovary ($n = 4$) from all treatment groups were homogenized in lysis buffer (Cell Signaling Technology) with 1 mmol/L phenylmethylsulfonyl fluoride. The lysates were centrifuged at $10,000 \times g$ for 15 min at 4°C and supernatant was collected. Protein concentrations were measured with a bicinchoninic acid protein assay kit (Pierce). Following boiling for 5 min in Laemmli sample buffer (Bio-Rad), samples (25 μg protein) and ladders (Kaleidoscope prestained standards, Bio-Rad) were run on 10% Tris-HCl Criterion Precast gels (Bio-Rad) under reducing conditions and transferred onto nitrocellulose membrane. Membranes were blocked with 10% milk in TBS with Tween 20 (TBST) for 1 h at room temperature and incubated with antibodies against ALDH-1 family member A1 (1 $\mu\text{g}/\text{mL}$), cyclooxygenase-2 (COX-2; 2 $\mu\text{g}/\text{mL}$, rabbit polyclonal, Lab Vision), or Oct-3/Oct-4 (1:200) at 4°C overnight. Following washing in TBST, blots were incubated in peroxidase-conjugated donkey anti-mouse, anti-rabbit, or anti-goat antibodies (Jackson ImmunoResearch Laboratories) for 2 h at room temperature and washed. Heart tissue lysates were used as positive control for ALDH1A1 immunoreactivity (36) and primary antibody omission was used as negative control. Protein signals were visualized with chemiluminescent substrate (Pierce) and protein bands were quantified using GelPro. Equal protein loading

Table 1. Experimental groups to examine the effect of tamoxifen on the progression toward concurrent mammary and ovarian cancers

Treatment group	CONT	TAM	CARC	CARC + TAM
Ovarian treatment	Vehicle	Vehicle	DMBA	DMBA
Systemic treatment	Vehicle	Vehicle	E ₂	E ₂
Tamoxifen treatment	Vehicle	Tamoxifen	Vehicle	Tamoxifen

NOTE: CONT, vehicle-treated animals; TAM, tamoxifen-treated animals; CARC, carcinogen-treated animals.

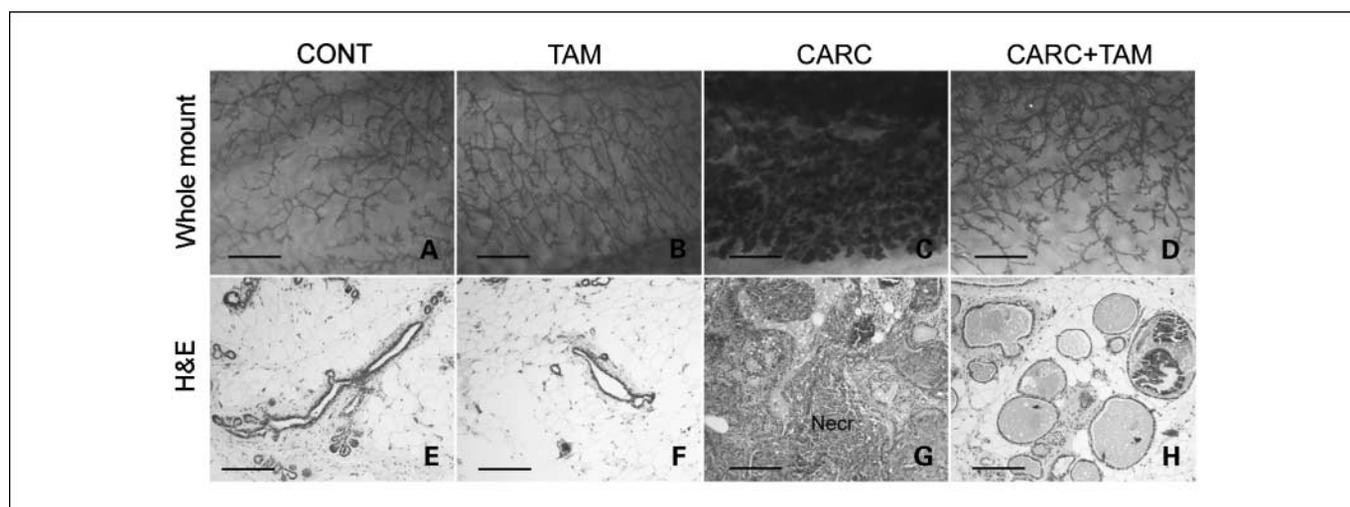


Fig. 1. Whole mounts (A-D; $\times 20$), and H&E sections (E-H, $\times 100$) of the mammary gland. Controls (CONT) and tamoxifen-treated (TAM) animals showed normal mammary histology (A-B, E-F). Carcinogen treatment (CARC) caused preneoplasia and neoplasia (C and G), and this effect was blocked by carcinogen + tamoxifen (CARC + TAM; D and H). Secreting mammary glands were observed in CARC + TAM rats. Bar, 5 mm (A-D), 200 μ m (E-H).

was confirmed by stripping (BlotFresh, SignaGen) and reprobing the membranes with β -actin antibody (1:20,000; goat polyclonal; Santa Cruz). Data are presented as integrated absorbance.

Quantitative analysis of preneoplastic lesions

Mammary sections stained with H&E were evaluated and each section was assigned a dysplasia score according to the presence of preneoplastic and neoplastic lesions associated with breast cancer progression (15, 35). A score of 0 represented normal mammary histology. Preneoplastic changes included mild (score = 1) or severe (score = 2) ductal hyperplasia and/or hyperplasia with atypia (score = 3). Neoplastic changes included ductal carcinoma *in situ* (score = 4) and invasive cancer (score = 5). The sum of scores from all three sections from each animal was used as the total dysplasia score, a value ranging from 0 to 15.

Preneoplastic changes of the ovary were defined as surface hyperplasia, inclusion cysts, stromal hyperplasia, and papilloma, each being a separate histologic parameter (15, 34, 37). For each ovarian section, each parameter was given a score of 0, 1, or 2 based on the severity or prevalence of each preneoplastic category (i.e., a score of 0 represented normal histology; a score of 1 corresponded to a moderate prevalence or degree of change; and a score of 2 indicated a high incidence or degree of abnormality). Scores for all four histologic parameters were added up to give a dysplasia score for each ovarian section. The sum

of all three dysplasia scores for each animal gave rise to the total dysplasia score, a value ranging from 0 to 24. These preneoplastic criteria are the same as those used by Stewart et al. (34) with this rat model of ovarian adenocarcinoma.

Ki-67 and ER α expression in the mammary ductal epithelia cells and ovarian surface epithelium was quantified by counting immunoreactive cells and total cells (at least 1,000 cells were evaluated per section) and presented as percent immunoreactive epithelial cells. The location and distribution of ALDH-1 and Oct-3/Oct-4 expression were documented. All data are presented as mean \pm SE. Protein levels of Ki-67, ER α , COX-2, ALDH-1, and Oct-3/Oct-4 expression as determined by immunohistochemistry and Western blot were analyzed using one-way ANOVA with treatment type as main effect. Dysplasia scores were analyzed using a nonparametric test (Mann Whitney test). Differences were considered significant when $P \leq 0.05$.

Results

Tamoxifen blocks mammary carcinogenesis

Mammary gland whole mounts. Control and tamoxifen-treated (TAM) rats had normal mammary morphology (Fig. 1A and B). Carcinogen (E_2 + DMBA) treatment increased area occupied by alveoli (Fig. 1C). This effect was markedly reduced by tamoxifen in the carcinogen and tamoxifen-treated (CARC + TAM) group (Fig. 1D).

Mammary tissue histology. Controls showed a normal appearance of lobular/acinar units surrounded by abundant adipose tissue (Fig. 1E). These units constituted a single layer of myoepithelium and inner mammary epithelial cells. TAM animals also showed normal mammary histology (Table 2; Fig. 1F). All CARC animals exhibited pathologic mammary histology ranging from hyperplasia to disseminated ductal carcinoma *in situ* and had higher dysplasia scores when compared with controls (Fig. 1G; Table 2; $P < 0.0001$). In CARC + TAM rats, the number and morphology of lobular units were restored to near normality (Fig. 1H). These rats also showed mildly increased ductal branching and enlarged intraductal lumen as compared with controls, but no dysplastic foci were present in any of these animals (Table 2).

Table 2. Mammary and ovarian dysplasia scores

Animal group	Mammary gland	Ovaries
CONT	0	0.44 \pm 0.24
TAM	0	1.38 \pm 0.32
CARC	7.88 \pm 0.79*	7.29 \pm 1.09*
CARC + TAM	0	5.83 \pm 0.54*

NOTE: Dysplasia scores are presented as mean \pm SE for each treatment group.

*Differs significantly from control ($P < 0.05$). In the ovary, the differences between CONT and TAM as well as CARC and CARC + TAM were not significant ($P = 0.202$ and $P = 0.085$, respectively).

Tamoxifen neither blocks nor accelerates the progression to ovarian cancer in a rat model

Controls showed normal ovarian morphology with mild inflammatory reaction to suture materials and rare inclusion cysts (Fig. 2A and E; Table 2). Compared with controls, TAM rats showed a slight increase in dysplasia score mostly due to the occasional presence of inclusion cysts; however, this difference was not significant ($P = 0.202$; Fig. 2B and F; Table 2). Consistent with our previous findings, CARC rats received higher dysplasia score when compared with controls ($P < 0.0001$) and showed markedly abnormal ovarian morphology with disorganized granulosa clusters, stromal hyperplasia, epithelial hyperplasia, papilloma, and glandular cystic changes resembling inclusion cysts (Fig. 2C and G; Table 2). In the CARC + TAM group, tamoxifen did not reduce the degree of ovarian preneoplasia following carcinogen treatment ($P = 0.0851$; Fig. 2D and H; Table 2). Interestingly, tamoxifen treatment seemed to increase the number of ovarian follicles when compared with those of the CARC group (data not shown).

Expression of Ki-67, ER α , and COX-2 in mammary gland and ovary under normal or dysplastic conditions

Ki-67 expression was localized in the nucleus of ductal epithelial cells in the mammary gland. The average numbers of Ki-67 positive cells per 100 ductal epithelia were 5.17 ± 2.11 , 4.58 ± 1.93 , 22.94 ± 3.57 , and 8.75 ± 0.79 in control, TAM, CARC, and CARC + TAM animals, respectively. These data showed that cellular proliferation was elevated in the mammary gland of CARC-treated animals when compared with control ($P < 0.0001$) and TAM ($P < 0.0001$) animals. Tamoxifen inhibited carcinogen-induced Ki-67 elevation in CARC + TAM rats when compared with CARC rats ($P = 0.0003$; Fig. 3). Very few ovarian surface epithelial cells expressed Ki-67 ($< 0.2\%$ immunoreactivity) and there was no difference in expression among the treatment groups ($P > 0.05$).

ER α expression. Carcinogen treatment depleted ER α expression (immunoreactivity, $0.25 \pm 0.06\%$; Fig. 3) in the mammary gland when compared with control ($10.30 \pm 1.855\%$; $P < 0.0001$)

and TAM-treated animals ($11.87 \pm 0.88\%$; $P < 0.0001$). While no significant differences were detected, there was a trend for ER α expression to increase in response to tamoxifen treatment in CARC + TAM animals ($3.61 \pm 0.25\%$; $P = 0.063$) when compared with the CARC group. In the ovary, percentages of ER α immunoreactivity in ovarian surface epithelium were 40.97 ± 5.00 , 47.45 ± 1.57 , 41.69 ± 5.98 , and 40.68 ± 6.66 for control, TAM, CARC, and CARC + TAM animals, respectively. No change in ER α expression was found in the ovary among the treatment groups.

Inflammation biomarker. COX-2 protein level was elevated in the mammary gland of CARC rats (integrated absorbance, 517.49 ± 197.27) when compared with control (11.47 ± 0.56 ; $P = 0.0067$) and TAM (37.61 ± 8.28 ; $P = 0.0089$) animals. Tamoxifen treatment reduced COX expression in CARC + TAM rats when compared with the CARC group (7.60 ± 1.00 ; $P = 0.0065$; Fig. 4A and B). In the ovary, COX-2 expression was not altered by carcinogen treatment ($P > 0.05$).

Levels of putative stem cell markers in the mammary gland and ovary

In the mammary gland, immunoblot analysis showed that Oct-3/Oct-4 and ALDH-1 expression were increased in CARC rats compared with controls (Fig. 4A and B; $P = 0.014$ and 0.012 , respectively). Surprisingly, while tamoxifen drastically reduced histologic progression to breast cancer, tamoxifen had no effect on the induction of stem cell markers by sustained exposure to estrogen. Our results showed that ALDH-1 and Oct-3/Oct-4 levels between CARC and CARC + TAM animals and between control and TAM animals do not differ ($P > 0.05$, Fig. 4). Immunohistochemistry revealed that while no immunoreactivity was observed in ductal epithelial cells of control and TAM animals, ALDH-1-positive cells were present in the cytoplasm of a few lobules in CARC and CARC + TAM animals (Fig. 4C). However, immunoreactivity of Oct-3/Oct-4 was not observed in the selected mammary gland sections. While the stem cell hypothesis has been explored in breast carcinogenesis, no putative stem cell markers have been suggested to be associated

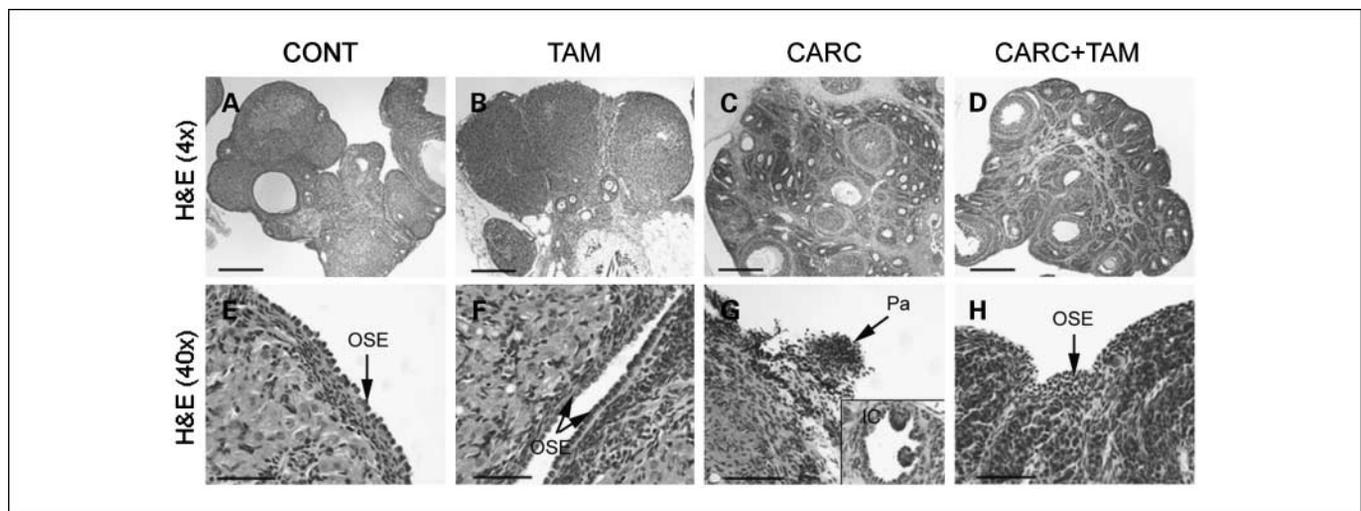


Fig. 2. H&E sections of the ovary. Bar, 600 μm (A-D), 50 μm (E-H and inset). CONT, controls; TAM, tamoxifen-treated rats; CARC, E₂/DMBA-treated rats; OSE, ovarian surface epithelia; Pa, papilloma; IC, inclusion cyst.

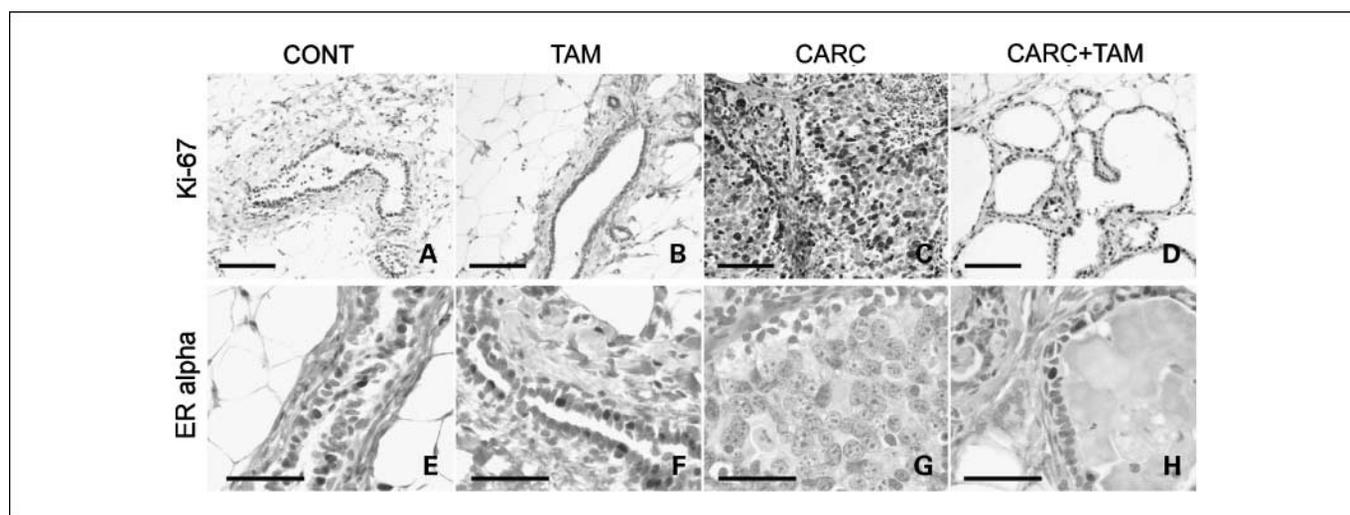


Fig. 3. Ki-67 and ER α immunostaining in the mammary gland. Mammary epithelial proliferation (% Ki-67) was increased above controls in CARC animals (A and C; $P < 0.05$). Carcinogen + tamoxifen treatment reduced this elevation (C and D; $P < 0.05$). Tamoxifen alone did not affect percent Ki-67 compared with controls (A and B). Carcinogen treatment depleted ER expression in the mammary gland when compared with control and TAM-treated animals (E-G; $P < 0.05$). There was a trend for ER α expression to increase in response to tamoxifen treatment in CARC + TAM animals when compared with the CARC group; however, this difference was not significant (G and H; $P = 0.063$). Bar, 100 μ m (A-D), 50 μ m (E-H).

with ovarian carcinogenesis. In the current study, immunohistochemistry data suggest that ALDH-1 and Oct-3/Oct-4 are not expressed in ovarian surface epithelia.

Discussion

Mammary gland

Tamoxifen inhibited mammary cancer progression in our preclinical model of breast and ovarian carcinogenesis, consistent with previous data from clinical trials and animal studies (3, 38). Ki-67, a proliferation marker, and COX-2, an inflammation marker, are potential markers of breast cancer risk and have been used as surrogate markers of response in human phase II chemoprevention trials (39). In our rat model, Ki-67 and COX-2 also correlated with progression of mammary carcinoma. Mammary ER α expression is down-regulated in CARC animals, consistent with previous studies showing the loss of ER α following E $_2$ -initiated cell proliferation (15, 40); however, it is also possible that the loss of ER α is temporary and is caused by ligand-induced receptor degradation (41).

Ovary

The current study is the most detailed experiment investigating the effect of tamoxifen on ovarian physiology and cancer progression. While our study showed that tamoxifen does not retard ovarian cancer, this negative finding is very important and in agreement with the human literature with more intensive biomarker and histopathology data than in human study. Although there seemed to be a slight increase in dysplasia in the ovary of animals treated with tamoxifen alone when compared with controls, this elevation was not significant. While there is a possibility of cancer incidence with longer tamoxifen administration, 6 months of treatment (one quarter of life span in rats) in the current experiment far exceeds the recommended treatment time for women taking tamoxifen (≤ 5 years). Women taking tamoxifen have an increased risk for developing follicular cysts in the ovary (17, 18), and it has

been suggested that tamoxifen-induced ovarian cysts may contribute to increased risk of ovarian cancer (42). However, in the current experiment, tamoxifen neither augments nor diminishes preneoplastic lesions induced by carcinogen treatment in the ovary in our high-risk model. Our results therefore suggest that tamoxifen, as a common prevention therapy for breast cancer, does not affect ovarian cancer risk in animals at high risk for both mammary and ovarian cancers. While COX-2 levels remained unchanged in the different treatment groups, recent studies revealed the relevance of COX-1, but not COX-2, expression in ovarian tumor development (43). The role of COX-1 in mammary and ovarian carcinogenesis should be further investigated using this model. In addition, no endometrial neoplasia was observed in our model following 6 months of sustained tamoxifen administration.

Stem cell biomarkers

Oct-4 is a transcriptional factor expressed by early embryonic and germ cells and has been used to identify pluripotent cell populations (44). ALDH-1, an enzyme that is required for the conversion of retinol to retinoic acids, is highly enriched in hematopoietic stem cells, and recently, researchers have suggested its presence in breast stem cells as well (45). Our data showed increased expression of both markers in the mammary gland of rats treated with carcinogens. This finding suggests that stem cell populations are expanded during mammary carcinogenesis in our model.

Estrogen is used to induce mammary carcinogenesis in the current experiment. The mechanism by which estrogen acts on stem cell number is still unclear because most studies agree that breast stem cells are ER $^-$ (26). However, studies have also shown that dysregulation of breast stem cells, or an increased stem cell pool size, can be induced by exposure to elevated breast epithelial mitogens such as insulin-related growth factor-1 and steroid hormones including estrogens (46, 47). One rationale for this effect of estrogen is via an indirect mechanism or stem cell niche; thus, estrogen acts on ER $^+$ cells

surrounding the stem cell and promotes paracrine signaling (26, 48). Interestingly, rats treated with carcinogen + tamoxifen were rescued from progression toward mammary cancer but still exhibited elevated mammary stem cell markers. This observation may suggest that tamoxifen, while retarding breast cancer progression, does not act on the stem cell population but rather has its effects on the differentiated epithelia. This, in turn, is consistent with the absence of ER in the breast stem cell (26); however, our understanding of mammary stem cell markers and biology will need to improve to fully answer this question.

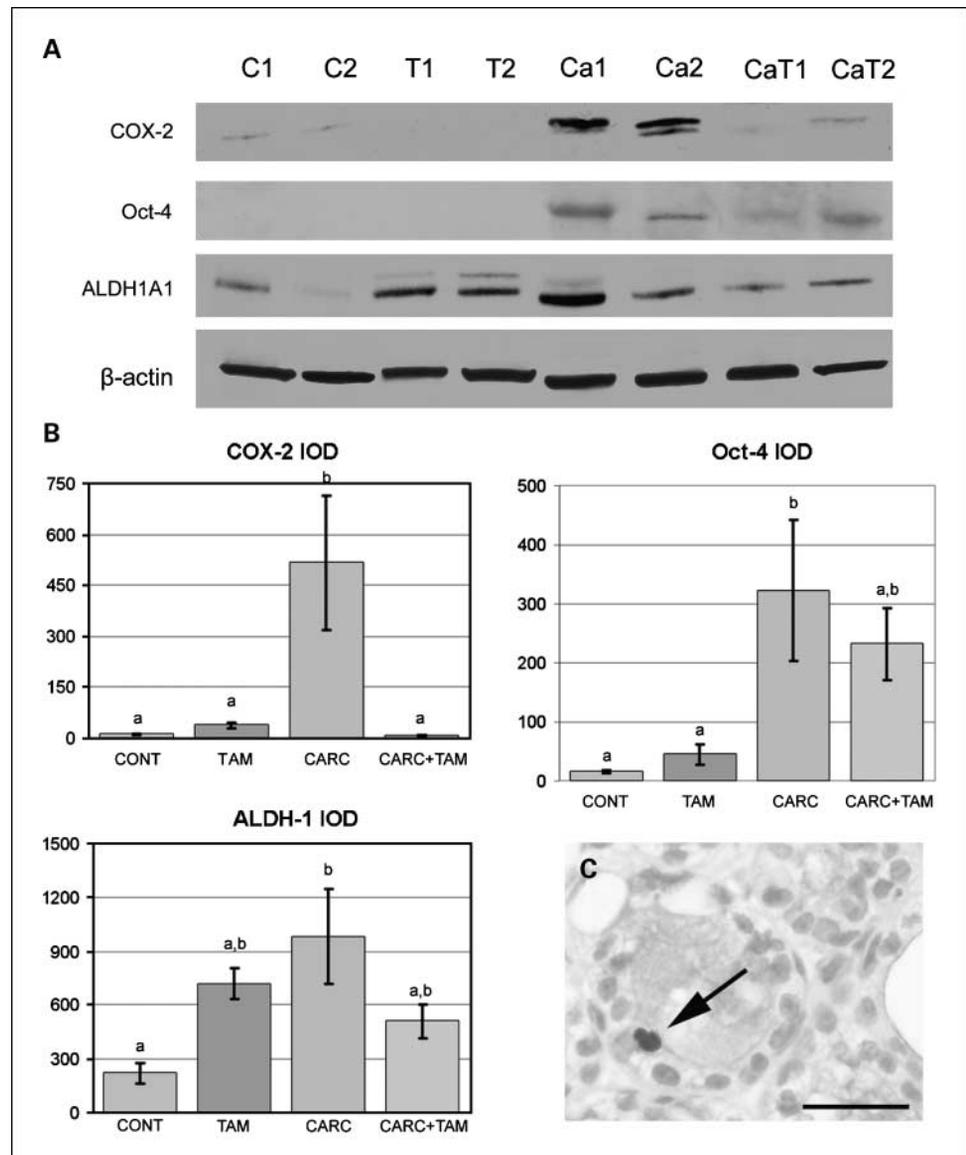
Combined model of breast and ovarian cancer prevention

The rat model of breast and ovarian carcinogenesis used here, while allowing us to observe synergistic and antagonistic drug action in our search for a dual-target prevention strategy, has some inherent limitations. The human population best modeled by these experiments is probably meno-

pausal women on hormone replacement therapy, and the results may be less relevant to other populations. This model is also focused on early changes of breast and ovarian cancers because these are the intended targets for cancer chemoprevention, rather than following animals to tumor incidence. While this shortens the trials and parallels our human chemoprevention studies (39, 49), it does entail the use of surrogate end point biomarkers for cancer with their inherent uncertainties.

Breast and ovarian cancers share similar etiology (endocrine background, risk factors, epithelial origin, etc.) reflecting common disease pathways; however, these cancers show discrepancy in terms of development and cancer cell type. This difference in pathology may be due to differences in the cells of origin or the hormonal milieu surrounding them. Despite these differences in the later stages of disease, the initiation factors for breast and ovarian cancers are similar, and therefore, it is plausible to target both cancers simultaneously for prevention.

Fig. 4. COX-2, Oct-4, and ALDH-1 expression in the mammary gland. **A**, representative Western blots of mammary glands from rats treated with vehicle (C1 and C2), tamoxifen (T1 and T2), carcinogen (Ca1 and Ca2), and carcinogen + tamoxifen (CaT1 and CaT2). β -Actin was used as a loading control. **B**, quantitative analysis of Western blots showing that COX-2, Oct-4, and ALDH-1 levels (y axis, integrated absorbance (IOD) were elevated following carcinogen treatment (CARC) compared with controls (CONT). COX-2 expression was reduced by tamoxifen in the CARC + TAM group. Letters indicate significant differences among the treatment groups. **C**, cytoplasmic ALDH-1 immunostaining was observed in a few luminal epithelial cells in the mammary gland of CARC animals (arrow). No immunoreactivity was observed in control and TAM animals. Bar, 50 μ m.



In the present study, we have shown that although tamoxifen is an effective breast cancer prevention drug for ER⁺ disease, it does not retard the development of ovarian preneoplasia and therefore is not ideal for simultaneous prevention of breast and ovarian cancers. Our results also suggest that although tamoxifen has been shown to induce ovarian cyst formation, it does not increase ovarian cancer risk in this model. Mechanistically, hormonal mammary carcinogenesis in this model is accompanied with elevated expression of ALDH-1 and Oct-4, and this putative expansion of the ALDH-1- or Oct-4-positive stem cell population is not reversed by tamoxifen cancer chemoprevention. These data also confirm that our combined breast and ovarian cancer model allows the observation of synergistic and antagonistic drug actions on

the breast and ovary. Simultaneous breast and ovarian cancer prevention is biologically feasible and may offer the best possibility for ovarian cancer prevention. Future studies will include investigation of common mechanism/disease pathways and evaluation of other candidate drugs for simultaneous chemoprevention of both breast and ovarian cancers using this model.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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