Neu-Induced Retroviral Rat Mammary Carcinogenesis: A Novel Chemoprevention Model for Both Hormonally Responsive and Nonresponsive Mammary Carcinomas


Abstract
 Clinically relevant animal models of mammary carcinogenesis are crucial for the development and evaluation of new breast cancer chemopreventive agents. The neu-induced retroviral rat mammary carcinogenesis model is based on the direct in situ transfer of the activated neu oncogene into the mammary epithelium using a replication-defective retroviral vector. The resulting mammary carcinomas in intact Wistar-Furth rats exhibit a mixed hormonal response in the same proportion as has been observed in women. In intact rats, ~50% of mammary carcinomas can be prevented by tamoxifen treatment. In ovariectomized animals, the mammary carcinomas are hormonally nonresponsive and cannot be prevented by tamoxifen. We evaluated the efficacy of retinoic X receptor–selective retinoids (retinoids) in this novel model of mammary carcinogenesis. The retinoids LG100268 and bexarotene (LG1069, Targretin) were highly efficacious in the prevention of neu-induced mammary carcinomas. Dietary LG100268 at 100 mg/kg diet decreased tumor multiplicity by 32% (P = 0.0114) in intact rats and 50% (P < 0.0001) in ovariectomized rats. Bexarotene treatment at a dose of 250 mg/kg diet was associated with reductions in tumor multiplicity of 84% (P < 0.0001) and 86% (P < 0.0001) in intact and ovariectomized animals, respectively. In addition to tumor multiplicity, proliferation and apoptosis were modulated by bexarotene treatment independently of estrogen signaling. The neu-induced retroviral rat mammary carcinogenesis model represents a valuable addition to existing rodent chemoprevention models. The model is useful for assessing the efficacy of chemopreventive agents, specifically those compounds that target hormonally nonresponsive tumors. (Cancer Res 2006; 66(13): 6884-91)

Introduction
 Despite recent advances in breast cancer chemoprevention (1–3), the American Cancer Society estimates that in 2005 ~211,240 women will be diagnosed with breast cancer, accounting for close to one third of all new cancer cases among women this year. This statistic shows the need for nontoxic chemoprevention agents that can be broadly applied to target the mammary epithelium. Clinically relevant breast cancer animal models are important for the development and validation of such compounds.

Currently, the most frequently used breast cancer chemoprevention models are chemically induced rat models and transgenic mouse models. In the rat, the polycyclic hydrocarbon 7,12-dimethylbenz(a)anthracene (DMBA; refs. 4, 5) and the direct acting N-methyl-N-nitrosourea (NMU; refs. 6, 7) induce mammary cancer at high frequency. However, these tumors are distinct from those seen in humans, as they hardly ever progress into invasive malignancies and are for the most part hormone responsive. Ovariectomy or treatment with selective estrogen response modulators (SERM) results in >80% reduction in tumor multiplicity in both DMBA- and NMU-induced rat carcinogenesis models (8, 9). In addition, the relevance of DMBA- and NMU-driven models to human breast cancer etiology is uncertain, as neither of these classes of carcinogens is associated with the human disease. Among transgenic rodent models used to study the chemoprevention of breast cancer, the C3(1)-SV40 T-antigen (10, 11) and mouse mammary tumor virus (MMTV)–erbB2 transgenic mice (12, 13) are most frequently used. Tumors of these transgenic mice are generally estrogen receptor (ER) negative and therefore tend to be uniformly hormonally nonresponsive. In C3(1)-SV40 T-antigen transgenic mice for instance, there is no significant difference in tumor multiplicity between mice ovariectomized before sexual maturation or after puberty, tamoxifen-treated mice, and intact control animals (14).

Our laboratory has developed a rat breast cancer model, in which carcinogenesis is induced by the in situ transfer of the activated neu oncogene into the mammary epithelium, using a replication defective retroviral vector (15). The neu-induced retroviral rat mammary carcinogenesis model has a variety of unique attributes, which allow for the adaptation of this technology into a novel model of breast cancer chemoprevention.

As is often observed in human breast cancers, the tumors of the neu-induced retroviral rat mammary carcinogenesis model arise in a monoclonal manner, resulting from single cell transformations (15). The tumors therefore develop in an unperturbed cellular milieu.

The development, progression, and morphologies of neu-induced mammary carcinomas closely resemble those of human mammary tumors (15, 16). Within 2 to 7 weeks after infusion, in situ cribiform-comedo carcinomas appear, some of which later progress into locally invasive mammary carcinomas. These lesions are the histopathologic equivalent of the comedo-type ductal carcinomas in situ (DCIS) in early human breast cancer (17). Therefore, the model offers multiple clinically relevant end points.

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(DCIS and frank mammary carcinomas) for evaluation of chemoprevention agents.

In the neu-induced retroviral rat mammary carcinogenesis model, the activated neu oncogene is driven by the Moloney murine leukemia virus long terminal repeat in a hormonally independent manner. This is critical for assessing the efficacy of potential chemopreventive agents, especially those that might alter hormone metabolism, such as SERMs or aromatase inhibitors.

The most important aspect of the neu-induced retroviral rat mammary carcinogenesis model is its ability to generate both hormonally responsive and nonresponsive mammary carcinomas in the same animal model. In intact animals of this model, ~60% of mammary carcinomas respond to hormone ablation via bilateral ovariectomy after tumors have arisen (18). These tumors represent potential targets for chemoprevention by a strong antiestrogenic agent. The National Surgical Adjuvant Breast and Bowel Project (NSABP) P-1 Study found that the antiestrogenic drug tamoxifen prevents ~50% of invasive breast cancer (1) in women. Intact animals are therefore useful to study chemopreventive effects on a specific proportion of hormonally responsive and nonresponsive mammary carcinomas, which is similar to that in women. Rats ovariectomized before histopathologic lesions are present develop hormonally nonresponsive mammary carcinomas (18). This model is particularly useful for assessing the efficacy of chemopreventive agents on hormonally nonresponsive carcinomas. It is for this subset of breast cancers that new chemoprevention strategies are urgently needed.

We evaluated the efficacy of tamoxifen (a SERM) and the retinoic X receptor (RXR)–selective retinoid (rexinoid) class of chemopreventive agents on mammary carcinomas of both intact and ovariectomized configurations of the neu-induced retroviral rat mammary carcinogenesis model.

Tamoxifen is a nonsteroidal antiestrogenic compound, which prevents signaling through the ER. Tamoxifen binding causes a conformational change of the ER, preventing the transcriptional activation of estrogen response element containing gene targets (19). Based on these observations, we hypothesized that tamoxifen should be able to prevent the hormonally responsive portion of mammary carcinomas in intact animals but should have no effect on hormonally nonresponsive carcinomas in intact or ovariectomized rats. It could therefore serve as an internal control for defining the specific subset of hormonally responsive mammary carcinomas in the intact model.

Retinoids, naturally occurring or synthesized vitamin A analogues, present important prospects for breast cancer chemoprevention (20, 21). They regulate a variety of cellular processes, including cell growth, differentiation, and apoptosis (22). Retinoids exert their regulatory signals primarily through the retinoic acid receptor (RAR) and RXR. They can control such diverse processes because both RAR and RXR can heterodimerize with a variety of other nuclear receptors (23). Here, we will discuss the efficacy of the RXR-specific retinoids LG100268 and bexarotene (LG1069, Targretin) to modulate tumor multiplicity, tumor volume, proliferation, and apoptosis in the neu-induced retroviral rat mammary carcinogenesis model.

Materials and Methods

Neu-induced retroviral rat model. All animal experiments were done at our facility under protocols approved by the University of Wisconsin Medical School Animal Care and Use Committee (Madison, WI). Virgin Wistar-Furth (WF) female rats were obtained from Harlan Sprague-Dawley (Madison, WI) at an age of 6 weeks. All rats were group housed in suspended wire cages and maintained at a light/dark cycle of 12 hours, receiving Teklad lab meal (8604) and acidified water ad libitum. After 1 to 2 weeks of acclimation, at an age of ~50 to 60 days, all rats underwent retroviral infusion with the pJR-neu vector, which induces mammary carcinogenesis by expressing the activated HER-2/neu oncogene. The construction and generation of the pJR-neu retroviral vector have been described previously (15, 24). Details on retroviral gene transfer into the mammary epithelium of the laboratory rat have also been published (25). In brief, a suspension of replication-defective amphotropic retrovirus containing the activated neu oncogene was infused into the central ducts of the 12 rat mammary glands. The rats of the intact model were infused with viral titers between 1 × 10^6 and 5 × 10^6 colony-forming units (CFU)/mL and were left intact. The animals of the ovariectomized model received viral titers between 5 × 10^5 and 1 × 10^6 CFU/mL and underwent a bilateral ovariectomy 2 days after the infusion. At 4 days after infusion, the rats were randomly assigned to the treatment groups, and the experimental diets were started. All animals were weighed and palpated for mammary tumors weekly. Rats in the restricted diet group were single housed, weighed daily, and fed sufficient control diet to parallel the body weight gain of the tamoxifen-fed (2 mg/kg diet) group. The studies were terminated 12 weeks after infusion for intact animals and 18 weeks after infusion for ovariectomized rats. At necropsy, the total number, mammary gland locations, and size of each mammary carcinoma were recorded. A minimal size criterion of 3 mm in the largest two dimensions of each mammary carcinoma was applied to be included in the analysis. Tumor volumes were calculated from three-dimensional measurements using the formula volume = 1/2 × length × width × height.

NMU treatment in WF rats. The WF rats used in the NMU-induced rat mammary carcinogenesis model received a single dose of 50 mg NMU/kg rat body weight by i.p. injection at an age of 8 weeks. Four days after NMU treatment, the administration of chemopreventive agents was started. This experiment was terminated at 18 weeks after NMU treatment.

Chemopreventive agents. Tamoxifen was purchased from Sigma (St. Louis, MO). LG100268 (Ligand Pharmaceuticals, San Diego, CA) and bexarotene (Onyx Scientific, Sunderland, United Kingdom) were obtained through the Division of Cancer Prevention Repository, National Cancer Institute (Rockville, MD). All experimental diets were dry mixed in Teklad 4% fat rodent meal (Teklad, Madison WI), which was also used as control diet. Dietary tamoxifen was prepared as 2 mg/kg diet, LG100268 as 100 mg/kg diet, and bexarotene as 250 mg/kg diet. All diets were prepared fresh weekly and stored at ~20°C.

Proliferation and apoptosis. Animals were infused in accordance with the procedure for neu-induced retroviral rat mammary carcinogenesis. Eight weeks after infusion, the experimental diets (control, tamoxifen at 2 mg/kg diet, and bexarotene at 250 mg/kg diet) were started. The experimental diets were given for 13 consecutive days. Three hours before necropsy, the animals underwent an i.p. injection of 5-bromo-2′-deoxyuridine (BrdUrd) at a dose of 50 mg/kg body weight. The rats were sacrificed, and mammary carcinomas were collected, fixed, and paraffin embedded. Consecutive slices were stained with H&E for histologic evaluation or used for proliferation and apoptosis assays.

Proliferating cells were evaluated by BrdUrd staining (26) using In Situ Detection kit (550803, BD Biosciences, San Jose, CA). Apoptotic cells were identified by terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) (27). The In situ Cell Death Detection kit (1684817, Roche Diagnostics, Penzberg, Germany) was used. For both assays, slides were stained following the manufacturer's recommendations. BrdUrd incorporation and TUNEL staining were evaluated by light microscopy. Approximately 1500 cells per tumor were scored for proliferating or apoptotic cells.

Statistical analysis. The number of carcinomas per animal at necropsy was modeled using generalized linear models assuming Poisson and binomial variability, respectively. Differences due to diet were assessed by comparing the change scaled deviance due to diet with the χ^2 reference distribution. If a significant effect of diet was found, each diet was compared with control individually using a similar procedure.
Tumor volumes were log_{10} adjusted to transform the variable into a normal distribution. A single-sided \( t \) test was done to obtain the \( P \) s for differences in tumor volume. Statistical analysis for proliferation and apoptosis assays were done by Wilcoxon rank-sum (Mann-Whitney) test.

Results

The neu-induced retroviral rat mammary carcinogenesis models. The neu-induced retroviral rat mammary carcinogenesis model (Fig. 1) reproducibly induces mammary carcinomas using viral titers in the range from \( 1 \times 10^5 \) to \( 1 \times 10^6 \) CFU/mL. The numbers of carcinomas developing per rat is dependent on the multiplicity of infection, which can be regulated by adjusting the retroviral titers. Animals in the ovariectomized model were given 2- to 10-fold higher retroviral titers and were kept on the diet study >6 weeks than the intact rats to account for the effects of ovariectomies in reducing tumor multiplicity and extending latency.

Effects of tamoxifen in the neu-induced retroviral rat mammary carcinogenesis models. Approximately 50% of mammary carcinomas in the intact model are hormonally responsive and can therefore be prevented by tamoxifen (2 mg/kg diet) treatment. In two independent experiments using the intact model, the average number of mammary carcinomas developing per rat following tamoxifen treatment was decreased by 57% (\( P < 0.0001 \); Fig. 2A) and 49% (\( P < 0.0001 \); Fig. 2C). In a third experiment, dietary tamoxifen treatment reduced tumor multiplicity in intact rats by only 17% (not significant; Fig. 2B) at time of necropsy; however, palpation data showed consistently reduced tumor multiplicity compared with the control group through week 11 after infusion (\( P = 0.03 \); Table 1, experiment 2a). The mammary carcinomas developing in this intact model therefore display a mixed hormonal response.

Tamoxifen treatment also reduces tumor volume in the intact model. In two independent experiments in intact rats, tumor volume was reduced by 52% (\( P = 0.0017 \); Table 2, experiment 1a) and 50% (\( P = 0.0266 \); Table 2, experiment 3a).

In the ovariectomized model, tamoxifen treatment had no significant effect on tumor multiplicity compared with the control groups (Table 1; Fig. 3A-C). Similarly to tumor multiplicity, tamoxifen was not able to reduce tumor volume in ovariectomized rats (Table 2, experiments 1b and 3b).

Effects of LG100268 in the neu-induced retroviral rat mammary carcinogenesis models. In both intact and ovariectomized rats, dietary administration of the RXR-selective retinoid (rexinoid) LG100268 caused a significant reduction in tumor multiplicity. In intact animals, LG100268 reduced tumor number by 32% (\( P = 0.0035 \); Fig. 2B). In ovariectomized rats, LG100268 decreased tumor multiplicity by 51% (\( P < 0.0001 \); Fig. 3B).

Effects of bexarotene in the neu-induced retroviral rat mammary carcinogenesis models. The retinoid bexarotene was able to significantly reduce tumor multiplicity in both intact and ovariectomized rats. In intact animals, bexarotene reduced tumor number by 84% (\( P < 0.0001 \); Fig. 2C). In addition, bexarotene was able to significantly reduce tumor volume (\( P = 0.0015 \); Table 2) in intact rats. In ovariectomized rats, bexarotene decreased tumor multiplicity by 86% (\( P < 0.0001 \); Fig. 3C). Interestingly, bexarotene was unable to reduce tumor volume in ovariectomized rats.

Effects of dietary tamoxifen and rexinoids LG100268 and bexarotene on body weight gain. Dietary administration of tamoxifen, LG100268, and bexarotene was associated with small to moderate reductions in final body weight (5-15% compared with control) in intact rats (Table 1). These same compounds caused moderate reductions in final body weight (10-25% compared with control) in ovariectomized rats. Tamoxifen treatment caused a significant reduction in final body weight in intact rats of 9%.

![Figure 1. The neu-induced retroviral rat mammary carcinogenesis model.](imageURL)
fewer tumors. In contrast, rats on the restricted diet weighed on an average of 9% ($P < 0.0001$) less than the LG100268-treated animals; however, they developed 53% ($P = 0.0007$) more mammary carcinomas.

**Effects of tamoxifen and bexarotene on tumor multiplicity in NMU-treated WF rats.** In the NMU-treated WF rats, the dietary administration of either tamoxifen or bexarotene led to a complete prevention of mammary tumor development. At 18 weeks after NMU treatment, the control rats had developed an average of 3.3 mammary carcinomas per rat (Fig. 4). In contrast, tamoxifen-treated animals had a tumor multiplicity of 0.1 ($P < 0.0001$) mammary carcinomas per rat. No mammary tumors ($P < 0.0001$) were detected in the bexarotene-treated rats. As observed in the neu-infused rats, the administration of the chemopreventive compounds was associated with moderate reductions in final body weight of 12% ($P < 0.0001$) with tamoxifen and 10% ($P < 0.0001$) with bexarotene (Table 1, experiment 4).

**Histopathologic analysis.** Histopathologic evaluation of H&E-stained tumor sections classified all tumors as mammary carcinomas. No systematic morphologic differences were associated with chemoprevention or short-term treatment with tamoxifen or rexinoids.

**Effects of tamoxifen and bexarotene on proliferation and apoptosis in neu-induced mammary carcinomas.** In short-term treatment experiments, tamoxifen treatment did not result in statistically significant reductions of proliferation rates for mammary carcinomas in either intact or ovariectomized rats. There was, however, a clear trend toward a reduced proliferation index of tumors in intact rats, in which tamoxifen lowered the mean proliferation rate from 6.7% to 5.4% and the median proliferation rate from 6.7% to 4.2% (Fig. 5A). Such trend was not observed for tumors from ovariectomized animals (Fig. 5B). Tamoxifen treatment increased the apoptotic index of the neu-induced mammary carcinomas from 0.6% to 3.3% ($P < 0.0001$; Fig. 5C) in intact rats. In ovariectomized animals, there was no measurable difference in the rate of apoptosis between control- and tamoxifen-treated rats (Fig. 5D).

The rexinoid bexarotene reduced the rate of proliferation of neu-induced mammary carcinomas in both intact and ovariectomized rats. It decreased the mean proliferation index from 6.7% to 3.5% ($P = 0.01$; Fig. 5A) in intact rats and from 9% to 2.4% ($P < 0.0001$; Fig. 5B) in ovariectomized animals. Bexarotene also increased the rate of apoptosis of neu-induced mammary carcinomas in both intact and ovariectomized rats. It increased the mean apoptotic index from 0.6% to 2.6% ($P < 0.0001$; Fig. 5C) in intact rats and from 0.8% to 3.8% ($P < 0.0001$; Fig. 5D) in ovariectomized animals.

**Discussion**

The neu-induced retroviral rat mammary carcinogenesis model recapitulates the pattern of hormonal responsiveness of human breast carcinomas. In the intact configuration of this model, tamoxifen administration leads to an ~50% reduction in tumor multiplicity. This number is similar to the 49% reduction seen in overall breast cancer occurrence in women treated with tamoxifen as observed in the NSABP Breast Cancer Prevention P-1 Study (1). In contrast, the administration of dietary tamoxifen was unable to prevent the development of mammary cancers in ovariectomized animals, in which tumors are hormone nonresponsive. Because tamoxifen exerts its effects by blocking ER signaling, it has no effect on ER-negative breast cancer in women, which was also confirmed.

Figure 2. Tumor multiplicity in the intact neu-induced retroviral rat model. The efficacies of (A) tamoxifen, (B) tamoxifen and LG100268, and (C) tamoxifen and bexarotene to prevent the development of mammary carcinomas with mixed hormonal response were evaluated. Tumor multiplicity curves are labeled with average number of mammary carcinomas per rat at the date of necropsy.
in the NSABP Breast Cancer Prevention P-1 Trial (1). Tumor size is a second variable that can be evaluated in the neu-induced retroviral rat mammary carcinogenesis model. We observed that tamoxifen treatment is associated with a 50% reduction in tumor volume in intact rats but has no effect in ovariectomized animals. These results suggest that tamoxifen administration in the neu-induced retroviral rat mammary carcinogenesis model may be used as an internal control, clearly defining the subsets of hormonally nonresponsive. Furthermore, the results from the tamoxifen experiments lead us to believe that the division between

Table 1. Chemopreventive effects of tamoxifen and retinoids on tumor multiplicity and final body weights

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Model</th>
<th>INT/OVX</th>
<th>Length</th>
<th>n, rats</th>
<th>Agents (dosage)</th>
<th>Tumor multiplicity* (last palpation date, Ca/rat), P</th>
<th>Tumor multiplicity* (necropsy, Ca/rat), P</th>
<th>Final body weights (g), P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a Neu INT</td>
<td>12 weeks</td>
<td>24</td>
<td>Control diet</td>
<td>8.1</td>
<td>9.1</td>
<td>204</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2a Neu INT</td>
<td>12 weeks</td>
<td>15</td>
<td>Control diet</td>
<td>3.0, &lt;0.0001</td>
<td>3.9, &lt;0.0001</td>
<td>186, &lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3a Neu INT</td>
<td>12 weeks</td>
<td>25</td>
<td>Tamoxifen (2 mg/kg diet)</td>
<td>5.1, 0.0341</td>
<td>7.9, NS</td>
<td>176, &lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b Neu OVX</td>
<td>18 weeks</td>
<td>23</td>
<td>Tamoxifen (2 mg/kg diet)</td>
<td>2.3, &lt;0.0001</td>
<td>3.6, &lt;0.0001</td>
<td>184, &lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tumor multiplicities are represented as average number of mammary carcinomas per rat. P values are indicated for the difference between control and agent results.

Table 2. Chemopreventive effects of tamoxifen and retinoids on tumor volume

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Model</th>
<th>INT/OVX</th>
<th>Length</th>
<th>n, rats</th>
<th>n, tumors</th>
<th>Agents (dosage)</th>
<th>Tumor volume*, volume (log10 volume), P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a Neu INT</td>
<td>12 weeks</td>
<td>24</td>
<td>153</td>
<td>Control diet</td>
<td>383 mm³ (2.15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2a Neu INT</td>
<td>12 weeks</td>
<td>15</td>
<td>75</td>
<td>Tamoxifen (2 mg/kg diet)</td>
<td>381 mm³ (1.92), 0.0017</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3a Neu INT</td>
<td>12 weeks</td>
<td>15</td>
<td>54</td>
<td>Control diet</td>
<td>201 mm³ (1.86)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1b Neu OVX</td>
<td>18 weeks</td>
<td>24</td>
<td>63</td>
<td>Tamoxifen (2 mg/kg diet)</td>
<td>101 mm³ (1.68), 0.0266</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3b Neu OVX</td>
<td>18 weeks</td>
<td>24</td>
<td>73</td>
<td>Bexarotene (250 mg/kg diet)</td>
<td>48.5 mm³ (1.42), 0.0015</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tumor volumes were calculated from three-dimensional measurements using the formula \( \text{volume} = \frac{1}{2} \times \text{length} \times \text{width} \times \text{height} \). Data were normalized by log10 transformation. P values are indicated for the difference between control and agent results.
hormonally responsive and nonresponsive mammary carcinoma as seen in intact rats has clinical relevance and may share important aspects of human breast cancer etiology. The importance of this observation lies in the fact that several potent chemopreventive agents exist for the prevention of hormonally responsive breast cancer (e.g., SERMs and aromatase inhibitors) but that prevention strategies for hormonally nonresponsive breast cancer are urgently needed.

One promising group of chemoprevention agents that might fulfill this need is RXR-selective retinoids (rexinoids). The rexinoid LG100268 has proven to be powerful compounds for the prevention and treatment of mammary tumors in a variety of rodent models. In the NMU-induced rat mammary carcinogenesis model, dietary LG100268 at a dose of 100 mg/kg diet is able to prevent 76% of mammary tumors (28). In the neu-induced retroviral rat mammary carcinogenesis model, we observed a reduction in tumor multiplicity of 32% and 50% in intact and ovariectomized animals, respectively. We therefore conclude that the rexinoid LG100268 is highly efficacious for the prevention of hormonally nonresponsive mammary carcinomas.

Another rexinoid, bexarotene (LG1069, Targretin), has been shown previously to be effective against ER-negative mammary tumors of transgenic mice. In MMTV-erbB2 transgenic mice, bexarotene is able to reduce tumor multiplicity by 85% (29) and in C3(1)-SV40 T-antigen transgenic mice by 50% (30). It has been reported previously that in NMU-treated Sprague-Dawley rats, gavage treatment of bexarotene was able to reduce tumor multiplicity by 90% (8), and dietary doses of 275 mg/kg diet were able to completely prevent mammary tumor development (31). These data are consistent with our own observations, in which dietary bexarotene at 250 mg/kg diet was able to completely prevent mammary tumors in NMU-treated WF animals. Similarly, dietary bexarotene treatment at 250 mg/kg diet in the neu-induced retroviral rat mammary carcinogenesis model resulted in tumor multiplicity reductions of 84% and 86% in intact and ovariectomized animals, respectively. The mechanism underlying the chemopreventive effects of bexarotene therefore seems to be independent of hormonal responsiveness and estrogen signaling, resulting in significant reductions in tumor formation in transgenic mice, in addition to having similar chemopreventive effects on intact and ovariectomized rats.

Histopathologic analysis of neu-induced mammary carcinomas did not reveal any consistent morphologic signs of differentiation in response to tamoxifen or rexinoid treatment; however, both proliferation and apoptosis were modulated by these compounds. In general, the results of the proliferation and apoptosis assays paralleled those of tumor multiplicities in terms of hormonal responsiveness. Tamoxifen treatment showed a clear trend, albeit not at statistically significant level, toward lowering the proliferation index in carcinomas of intact rats but not those from ovariectomized animals. Correspondingly, tamoxifen-treated tumors showed an increased rate of apoptosis only in the intact rat model. Such an effect was not observed in the hormonally nonresponsive mammary carcinomas of the ovariectomized model. These data agree with previously published in vivo data, in which tamoxifen treatment decreased proliferation rates while increasing apoptosis of hormonally responsive mammary tumors in an NMU rat model (32).

Short-term administration of bexarotene lead to reductions in proliferation rates of mammary carcinomas in both intact and ovariectomized rats. In addition, bexarotene treatment increased apoptosis significantly tumors from both hormonal states. It has been reported previously that bexarotene is capable of modulating both proliferation and apoptosis of hormonally responsive mammary tumors in the NMU rat model (31, 33), and we report here that these effects extend to hormonally nonresponsive neu-induced mammary carcinomas in ovariectomized rats.

**Figure 3.** Tumor multiplicity in the ovariectomized neu-induced retroviral rat model. The efficacies of (A) tamoxifen, (B) tamoxifen, LG100268, and a restricted diet of 10 to 12 g daily, and (C) tamoxifen and bexarotene to prevent the development of hormonally nonresponsive mammary carcinomas were evaluated. Tumor multiplicity curves are labeled with average number of mammary carcinomas per rat at the date of necropsy.

**Figure 4.** Tumor multiplicity in intact NMU-treated WF rats. The efficacy of tamoxifen and bexarotene to prevent the development of hormonally responsive mammary carcinomas was evaluated. Tumor multiplicity curves are labeled with the average number of mammary carcinomas per rat at the date of necropsy.
therefore conclude that short-term modulation of proliferation and apoptosis in response to bexarotene treatment occurs independently of estrogen signaling as do the chemopreventive properties of bexarotene. This correlation of both chemotherapeutic and chemopreventive effects suggests that the depression of proliferation and induction of apoptosis are rexinoid-induced mechanisms by which the chemoprevention of neu-induced mammary carcinomas occurs.

Interestingly, when comparing the tumor multiplicity of the tamoxifen-treated group with its body weight–matched control group, it seems that tamoxifen-fed animals have in fact higher tumor multiplicities. There are partial agonistic effects associated with tamoxifen binding at the ER, especially at low doses of estradiol (34), which may be able to substitute for missing estrogen (35). In addition, there was a slight, although not statistically significant, overrepresentation of ER-negative breast cancer incidence in the tamoxifen group of the NSABP Breast Cancer Prevention P-1 Trial (1).

In summary, the neu-induced retroviral rat mammary carcinogenesis model represents a powerful tool for investigating the efficacy of chemoprevention compounds. The intact model exhibits a mixed hormonal response in much the same proportions as the hormonal response seen in women, and ovariectomized animals produce hormonal nonresponsive tumors, which cannot be prevented by routine SERM treatment. The intact configuration should therefore prove useful to evaluate the effects of combination therapy, especially the combination of a SERM or aromatase inhibitor along with nonendocrine target-based agents, such as a retinoids. We believe that the neu-induced retroviral rat mammary carcinogenesis model will also find application in identifying molecular changes in response to tumorigenesis and drug treatment. This in turn will be instrumental for the development of surrogate biomarkers for the efficacy of chemoprevention agents, helping to bring much needed, new, and specific compounds into clinical trials.

Acknowledgments

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References


Figure 5. Proliferation and apoptotic indices of neu-induced mammary carcinomas. The proliferation index was measured by BrdUrd incorporation in neu-induced mammary carcinomas from intact (A) and ovariectomized (B) rats. TUNEL was used to assess the apoptotic index in intact (C) and ovariectomized (D) rats. Nine mammary carcinomas were evaluated per treatment group. Approximately 1,500 cells were scored in each tumor. Labeled bar indicates means. *, P = 0.01; **, P < 0.0001.
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Bouchard L, Lamarre L, Tremblay PJ, Jolicoeur P.


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