Dietary (n-3) Polyunsaturated Fatty Acids Inhibit HER-2/neu-Induced Breast Cancer in Mice Independently of the PPARγ Ligand Rosiglitazone

Lisa D. Yee,² Donn C. Young,*, Thomas J. Rosol,† Anne M. VanBuskirk, and Steven K. Clinton**

Departments of Surgery, Division of Surgical Oncology, *Biostatistics, †Veterinary Biosciences, and **Internal Medicine, Division of Hematology/Oncology, The Ohio State University, Columbus, OH 43210

ABSTRACT Overexpression of human epidermal growth factor receptor 2 (HER-2/neu) characterizes a molecular subtype of breast cancer associated with poor clinical outcome. Preventive strategies for HER-2/neu-positive breast cancer, which is often estrogen and progesterone receptor negative, remain undefined. Activators of peroxisome proliferator-activated receptor γ (PPARγ), a nuclear hormone receptor also expressed in breast cancer, hold potential as cancer prevention agents. PPARγ ligands include specific fatty acids and synthetic compounds, such as the thiazolidinediones, which appear to inhibit cell proliferation and tumorigenesis. We hypothesized that a thiazolidinedione, rosiglitazone, may serve as a chemopreventive agent for HER-2/neu-associated mammary carcinogenesis, but that efficacy may be influenced by dietary fat content. We studied the effects of diets enriched with corn or fish oil (25% of energy) with and without rosiglitazone (12 g/kg) in a 2 × 2 factorial design on mammary tumorigenesis in murine mammary tumor virus (MMTV)-HER-2/neu transgenic mice. Despite in vitro evidence of antiproliferative effects in an MMTV-HER-2/neu tumor cell line, rosiglitazone did not affect mammary carcinogenesis in vivo. Interestingly, fish oil–based diets markedly suppressed breast tumor incidence (57% of mice vs. 87% of corn oil–fed mice, P = 0.0001) as well as tumor multiplicity (P = 0.001) and mammary gland dysplasia (P = 0.001). These findings demonstrate a potent preventive effect of (n-3) PUFA on HER-2/neu–mediated mammary carcinogenesis, without interaction with a synthetic PPARγ activator. Further studies focusing on the mechanisms by which (n-3) fatty acids suppress HER-2/neu signaling pathways involved in the pathogenesis of breast cancer are warranted. J. Nutr. 135: 983–988, 2005.

KEY WORDS: • breast cancer • HER-2/neu • fatty acids • PPARγ

The role of dietary fat concentration and source in breast carcinogenesis remains speculative. Evidence suggesting that the type of dietary fat, such as the fatty acid profile, may influence breast cancer cell biology or mammary carcinogenesis was derived from in vitro and rodent studies, respectively. In general, cell culture and animal studies point to the stimulatory effect of linoleic acid (LA),³ an (n-6) PUFA, on tumor cell growth in contrast to the inhibitory effects of (n-3) fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (1–3). In vitro experiments demonstrated increased proliferation of breast cancer cells as well as normal human mammary epithelial cells in the presence of LA (4,5). Unlike the tumor-enhancing effects of diets containing elevated amounts of LA, diets enriched with the (n-3) fatty acids EPA and DHA suppress both tumor growth and metastasis in nude mice bearing transplantable human mammary cancers (6–8).

One potential mediator of the effects of specific fatty acids and fatty acid metabolites on mammary tumorigenesis is peroxisome proliferator-activated receptor γ (PPARγ), a ligand-activated transcription factor expressed in normal and malignant mammary epithelial cells (9–13). PPARγ is a member of the steroid nuclear hormone receptor superfamily, with several putative natural ligands, including specific fatty acids and eicosanoids, as well as synthetic activators (14–16). Fatty acid derivatives such as 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) and 13- and 9-hydroxyoctadecadienoic acid were also identified as PPARγ ligands (17,18). Furthermore, PPARγ plays a key role in adipocyte differentiation and fat metabolism (19,20).

Much of what is known about PPARγ was derived from studies with synthetic compounds that activate PPARγ, such as the thiazolidinediones, a widely used class of oral hypoglycemic drugs (21). Rosiglitazone (Avandia®, GlaxoSmithKline and pioglitazone (Actos™, Takeda Chemical Industry) are thiazolidinediones currently utilized for the treatment of insulin-resistant diabetes mellitus. Several studies indicate that activators of PPARγ such as 15d-PGJ2 and thiazolidinediones inhibit the proliferation of breast cancer cells in...
vitro, which appears to involve cell cycle arrest and sensitization to apoptosis (22,23). Thiazolidinedione treatment also inhibited human breast cancer xenograft growth in immunosuppressed mice (11) and impaired the in vitro development of preinvasive mammary lesions in mouse mammary gland organ culture treated with the carcinogen 7,12-dimethylbenz[a]anthracene (24). Administration of a synthetic ligand for PPARγ, GW7845, significantly inhibited nitrosomethyurea-induced mammary cancer in rats (25). These studies suggest that PPARγ ligands, either synthetic or derived from fatty acid metabolites, may inhibit breast tumor development and progression.

Unique patterns of genetic aberration appear to account for heterogeneity in disease progression and response to therapy. One such genetic alteration is the amplification of the human epidermal growth factor receptor 2, HER-2/neu, which occurs in 15–40% of breast cancers (26) and portends a poorer prognosis (27). Because tumors overexpressing HER-2/neu are often resistant to hormone therapy, novel strategies are required for optimally preventing as well as treating this aggressive molecular subtype of breast cancer.

Accordingly, this study explored the effects of 2 potentially interacting variables that could target PPARγ-mediated signaling pathways during HER-2/neu-mediated mammary carcinogenesis. Using a 2 × 2 factorial design, we examined the interactions between dietary fatty acid content [diets enriched with (n-6) PUFA vs. (n-3) PUFA] and a pharmacologic PPARγ ligand (presence or absence of rosiglitazone) on breast carcinogenesis in mouse mammary tumor virus (MMTV)-HER-2/neu transgenic mice.

**MATERIALS AND METHODS**

**Mouse experimental procedure.** Animal care and use were in accord with institution guidelines and approved by the Institutional Animal Care and Use Committee of The Ohio State University. Virgin female FVB/N-TgN(MMTVneu)202Mul transgenic mice (28) were obtained from Jackson Laboratory and housed in groups of up to 5 in plastic shoebox cages with autoclaved bedding and filtered air, with 12 h of darkness daily and an ambient air temperature of 22 ± 2°C. Mice had free access to diet and water. Mice were individually tagged and randomly distributed into 4 (n = 15 per group) diet/treatment groups representing a balanced 2 × 2 factorial design (fish oil vs. corn oil and drug vs. no drug). Mice in groups were balanced for age at the time of initiation of experimental diets at 7–8 wk of age.

Diets were replenished daily, with removal of uneaten pellets and daily monitoring of food intake. The health of the mice was monitored daily, and mice were weighed and inspected for palpable mammary tumors 1–2 times/wk. Two perpendicular diameters were measured weekly once a tumor appeared. Mice were killed by cervical dislocation when tumors reached 1.5–2.0 cm in greatest diameter or measured daily monitoring of food intake. The health of the mice was monitored daily, and mice were weighed and inspected for palpable mammary tumors 1–2 times/wk. Two perpendicular diameters were measured weekly once a tumor appeared. Mice were killed by cervical dislocation when tumors reached 1.5–2.0 cm in greatest diameter or at 15 mo of age. Mammary glands were harvested and fixed in 10% formalin for paraffin embedding. Tumor histopathology was confirmed by evaluation of hematoxylin and eosin (H&E) stained sections.

H&E-stained sections of mammary glands were evaluated without knowledge of the diet groups for mammary gland proliferative lesions based on the consensus report from the 1999 Annapolis meeting on mammary gland pathology of genetically engineered mice (29). Mammary gland atypical ductular hyperplasia was graded on a scale of minimal, mild, mild-moderate, moderate, and marked, based on the percentage of the mammary gland affected, the thickness of the proliferating ductular epithelium, and cellular atypia (high nuclear to cytoplasmic ratio, cytoplasmic basophilia, cytomegaly, karyomegaly, and cellular and nuclear pleomorphism). One representative nontumor-bearing gland was examined for each mouse evaluated.

**Diets.** AIN-93G-based diets (30) were prepared by Research Diets, with either 24.75% of energy as corn oil or 22.50% of energy as menhaden (fish) oil plus 2.25% of energy as corn oil, with or without rosiglitazone maleate (Glaxo Smith Kline) at 12 mg rosiglitazone/kg of diet. This concentration of rosiglitazone was utilized previously in FVB/N mice to achieve ~3 mg/(kg ⋅ d) (31). The 25% of energy as fat was selected for a diet of moderate fat content without consequential imbalance in carbohydrate and protein content relative to nonpurified diets, with corn oil as a source of (n-6) PUFA and fish oil as a source of (n-3) PUFA. To accommodate the modification of fat content of the AIN-93G diet from 16 to 25% of energy, the carbohydrate content was decreased to 55% of energy; t-butylhydroquinone at 0.23 g/kg and vitamin E acetate (Sigma) at 17.4 mg/kg were added for stabilization of the menhaden oil. The diets met the recommended dietary linoleate requirements for mice of 0.68% of energy, and mice were monitored for signs of essential fatty acid deficiency (32). The menhaden oil diets contained 2.1% of energy as LA, and the corn oil diets contained 14.9% of energy as LA. Diets were handled under low light conditions and stored at −20°C.

**Cell culture.** The NT5 cell line (kind gift of Dr. R. Todd Reilly, Johns Hopkins University) was maintained in Iscove’s Modified Dulbecco’s Modified Eagle’s Medium (DMEM) containing 5% FBS, 1% L-glutamine, and 1% antibiotic-antimycotic.

**PPARγ ligands.** Rosiglitazone and ciglitazone were obtained from Sigma Chemical Co. Ciglitazone and the chemical compounds (bispicloic acid) were synthesized using published chemical procedures (33). Rosiglitazone and ciglitazone were dissolved in DMEM at 100 μM and diluted daily in fresh media.

**Western analysis.** Cells were plated in 6-well plates at 2 × 10⁵ cells/well and treated with increasing concentrations (10⁻¹⁰ to 10⁻⁸ M) of rosiglitazone or ciglitazone for 24 h with 0, 0.001, 0.01, 0.1, 1, 5, 10, 50, 100, and 500 nM rosiglitazone. (C) Western blot analysis for phospho-PPARγ expression and tumor cell proliferation. (D) Proliferation of NT5 cells treated for 24 h with vehicle or PPARγ ligands: con = vehicle, cig10 = 10 μM ciglitazone, cig1 = 1 μM ciglitazone, 15dPGJ2 = 1 μM 15dPGJ2, resi10 = 10 μM rosiglitazone, and resi10 = 1 μM resi10. doi:10.1093/jn/984.10.984

**FIGURE 1** PPARγ expression in HER-2/neu transgenic mouse mammary tumor cells and effects of PPARγ ligands on HER-2/neu expression and phosphorylation and tumor cell proliferation. (A) Immunoblot analysis of PPARγ expression and tumor cell proliferation. (B) HER-2/neu expression in NT5 cells treated for 24 h with vehicle or PPARγ ligands: con = vehicle, cig10 = 10 μM ciglitazone, cig1 = 1 μM ciglitazone, 15dPGJ2 = 1 μM 15dPGJ2, resi10 = 10 μM rosiglitazone, and resi10 = 1 μM resi10. (C) Phosphorylation of HER-2/neu in NT5 cells after PPARγ ligand treatment. (D) Proliferation of NT5 cells treated for 24 h with 0, 0.001, 0.01, 0.1, 1, 5, 10, 50, 100, and 500 μM rosiglitazone. Results are expressed as the proportion of control proliferation, with each point representing a mean ± SD, n = 6. *Different from the untreated control (P < 0.05).
becco's Medium (IMDM) (Gibco Invitrogen) with 10% fetal bovine serum (Gibco Invitrogen) in a humidified incubator at 37°C with 5% CO₂. PPARγ ligands included rosiglitazone, ciglitazone, and 15d-PGJ₂ (Cayman Chemical, Alexis Biochemicals) in IMDM with 10% charcoal-stripped fetal bovine serum.

Immunoblotting. Total protein extraction, SDS-PAGE fractionation, transfer, and hybridization procedures were performed as previously described (12). Immobilized proteins were probed using antibodies specific for PPARγ (Santa Cruz Biotechnology), c-erb-2/HER-2 (Upstate), phosphotyrosine (Cell Signaling Technology), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Chemicon). Immunoprecipitation with antibody to c-erb-2/HER-2/neu (Upstate) was performed as previously described (33).

Cell proliferation assays. Methylthiazol tetrazolium in vitro cell proliferation assays (Promega) were performed according to manufacturer’s instructions (12), using varying concentrations of rosiglitazone. Student’s t test was used to determine differences from the control (P < 0.05).

Statistical analysis of diet studies. The analysis of categorical data were performed using χ² and Fisher’s exact tests. Comparisons of the numbers of tumors in treatment groups were made using the nonparametric Kruskal-Wallis test with pairwise comparisons using Bonferroni adjustment. Time to the development of palpable lesions was defined as the period from the initiation of treatment [at 7–8 wk of age] to the development of palpable lesions. Univariate comparisons of time-dependent data were analyzed using Kaplan-Meier plots with logrank tests. In a multivariate setting of drug, diet, and an interaction term of drug × diet, time to detection of palpable tumor was analyzed using Cox proportional hazards regression. The sample size of 15 mice/treatment group was chosen to allow detection of hazard ratios ≥ 4 with a power of 0.80 and 2-sided α of 0.05. Differences were considered significant at P < 0.05.

RESULTS

Antiproliferative effects of PPARγ ligands on HER-2/neu transgenic mammary tumor cells. The NT5 cell line was derived from an FVB/N-TgN(MMTV-neu)202Mul transgenic mouse mammary tumor (34). NT5 cells expressed PPARγ protein (Fig. 1A). By immunostaining, PPARγ expression was also demonstrated in formalin-fixed NT5 cells and MMTV-HER-2/neu mouse mammary tumor specimens (data not shown). Although hormones such as progesterone or dihydrotestosterone can stimulate the MMTV promoter (35), HER-2/neu receptor expression and phosphorylation in NT5 cells were not significantly altered by PPARγ ligand treatment (Fig. 1B, C). However, the PPARγ activator, rosiglitazone, inhibited the proliferation of NT5 cells (Fig. 1D).

Dietary treatment of HER-2/neu transgenic mice. Mice in each of the 4 groups tolerated the diets well, without...
Dysplasia differed between the diet groups (glands available for evaluation, the degree of mammary gland hyperplasia in all cases (Fig. 3). Of the 58 mice with mammary stained sections of mammary glands identified atypical ductal pathologic review of H&E mammary gland histopathology. Oil suppressed mammary tumorigenesis by 30% (tumors. Thus, relative to the corn oil-based diets, dietary fish mice fed corn oil diets developed clinically overt mammary tumors. Five of the 17 mice without clinically detectable tumors were HER-2/neu transgenic mice (logrank \( P = 0.001 \)); in the multivariate Cox model, the drug \( \times \) diet interaction term was not significant (\( P = 0.053 \)).

### DISCUSSION

This study demonstrated a marked effect of dietary fat composition on HER-2/neu-mediated mammary tumorigenesis. Enriched with (n-3) PUFAs, the fish oil significantly suppressed mammary tumor development compared with the corn oil–based diets. Despite prior reports of the preventive benefits of EPA, DHA, and fish oil in carcinogen-induced and transplantable breast tumor development, to our knowledge, this is the first report of breast cancer prevention in a transgenic model of mammary tumorigenesis by solely modulating dietary fat composition. Our data indicate a potent effect of fish oil in prolonging tumor latency and reducing tumor multiplicity in HER-2/neu overexpression–positive, estrogen receptor–negative breast cancer. Most interestingly, the lack of effect of rosiglitazone suggests that the influence of dietary fatty acid composition may be independent of PPAR\( \gamma \) activation.

The mechanisms by which the type of dietary fat influences mammary carcinogenesis are likely complex, with effects at various stages of this multistep process, as summarized in recent reviews (36, 37). PUFAs may modulate diverse biologic processes, including lipid peroxidation, eicosanoid production, growth factor receptor function at the cell membrane level, and signal transduction pathways affecting cell proliferation and survival (2, 38, 39). Whether (n-3) PUFAs specifically target pathways critical to HER-2/neu–mediated signaling, such as those of mitogen-activated protein kinase and phosphoinositide 3-kinase (40, 41), merits further investigation as.

### TABLE 1

<table>
<thead>
<tr>
<th>Diet</th>
<th>Palpable(^2)</th>
<th>Total at necropsy(^3)</th>
<th>Microtumors(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n/\text{mouse} )</td>
<td>( n/\text{mouse} )</td>
<td>( n/\text{gland} )</td>
</tr>
<tr>
<td>Fish oil</td>
<td>0.60 ± 0.63</td>
<td>0.67 ± 0.72</td>
<td>0.16 ± 0.18</td>
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<td>Fish oil + rosiglitazone</td>
<td>0.67 ± 0.62</td>
<td>0.80 ± 0.67</td>
<td>0.09 ± 0.15</td>
</tr>
<tr>
<td>Corn oil</td>
<td>1.33 ± 1.05</td>
<td>1.67 ± 1.29</td>
<td>0.34 ± 0.33</td>
</tr>
<tr>
<td>Corn oil + rosiglitazone</td>
<td>1.60 ± 0.91</td>
<td>2.27 ± 1.71</td>
<td>0.57 ± 0.53</td>
</tr>
<tr>
<td>2-way ANOVA</td>
<td>P = 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Drug</td>
<td>NS(^5)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Interaction</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^{1}\) Values are means ± SD.  
\(^{2}\) Mean number of mammary tumors/mouse for 15 mice.  
\(^{3}\) Mean number of mammary tumors detected at necropsy/mouse for 15 mice.  
\(^{4}\) Mean number of microtumor nodules/mammary gland; 3–8 mammary glands were evaluated per mouse \( n = 108, 97, 96, 90 \) mammary glands from mice fed fish oil, fish oil + rosiglitazone, corn oil, corn oil + rosiglitazone, respectively.  
\(^{5}\) NS, not significant, \( P > 0.05 \).

evidence of toxicity or signs of essential fatty acid deficiency. Neither dietary fat nor drug content significantly altered food consumption or weight gain (\( P > 0.05 \), data not shown). Mice fed the drug-containing diets consumed ~1.2 mg rosiglitazone/ (kg \( \cdot \) d).

**Dietary fish oil inhibits mammary tumor incidence.** Of 60 mice, 72% (43/60) developed palpable mammary tumors. Five of the 17 mice without clinically detectable tumors were euthanized due to illness, and necropsy revealed 2 uterine tumors (fish oil, corn oil + drug), 1 lung tumor (fish oil), 1 anal tumor (fish oil + drug), and lymphoma (corn oil). These extra-mammary tumors were histologically distinct from the mammary tumors and not representative of metastases.

Compared with the corn oil diets, dietary fish oil increased the latency time to mammary gland tumor development in the HER-2/neu transgenic mice (logrank \( P = 0.0001 \)) (Fig. 2A); 57% (17/30) of the mice fed fish oil diets and 87% (26/30) of mice fed corn oil diets developed clinically overt mammary tumors. Thus, relative to the corn oil–based diets, dietary fish oil suppressed mammary tumorigenesis by 30% (\( P = 0.02 \)).

Rosiglitazone did not affect mammary tumor incidence at 15 mo (logrank \( P = 0.22 \)) (Fig. 2B). In the multivariate Cox model, the drug \( \times \) diet interaction term was not significant (\( P = 0.50 \)), whereas diet was significant (\( P = 0.019 \)) and drug treatment was not (\( P = 0.053 \)).

**Dietary fish oil inhibits mammary tumor multiplicity.** Tumor multiplicity differed between diet groups as well, with more palpable mammary tumors per mouse developing in those fed corn oil than in those fed fish oil (\( P = 0.001 \)) (Table 1).

Dietary fish oil also suppressed the number of mammary tumors as determined by gross identification at necropsy, when additional macroscopic tumors were detected (\( P < 0.001 \)), and by microscopic identification of microtumor nodules \( \geq 0.5 \) mm in H&E stained sections of whole mammary glands (\( P < 0.001 \)). Rosiglitazone did not affect tumor multiplicity by palpation (\( P = 0.40 \)), detection at necropsy (\( P = 0.40 \)), or enumeration of microscopic tumor nodules per gland (\( P = 1.0 \)).

**Fish oil consumption is associated with lower-grade mammary gland histopathology.** Pathologic review of H&E stained sections of mammary glands identified atypical ductal hyperplasia in all cases (Fig. 3). Of the 58 mice with mammary glands available for evaluation, the degree of mammary gland dysplasia differed between the diet groups (\( P = 0.001 \)). Mammary glands with less pronounced atypical ductal hyperplasia (i.e., minimal and mild) occurred more frequently in mice fed fish oil, representing 73% (11/15) of fish oil and 87% (13/15) of fish oil + drug groups compared with 62% (8/13) of corn oil and 20% (3/15) of corn oil + drug groups. In order of decreasing frequency, we observed atypia of a higher grade (i.e., mild-moderate, moderate, and marked) in 80% (12/15) of corn oil + drug, 38% (5/13) of corn oil, 27% (4/15) of fish oil and 13% (2/15) of fish oil + drug-fed mice. The only instances of marked atypia occurred in 2 mice consuming the corn oil + drug diet. The degree of atypical ductal hyperplasia in mammary tissue was associated with dietary fat content (\( P = 0.001 \)), although not for drug (\( P = 0.22 \)), with a significant association of corn oil–enriched diets with atypia of increasing severity.

### DISCUSSION

This study demonstrated a marked effect of dietary fat composition on HER-2/neu–mediated mammary tumorigenesis. Enriched with (n-3) PUFAs, the fish oil significantly suppressed mammary tumor development compared with the corn oil–based diets. Despite prior reports of the preventive benefits of EPA, DHA, and fish oil in carcinogen-induced and transplantable breast tumor development, to our knowledge, this is the first report of breast cancer prevention in a transgenic model of mammary tumorigenesis by solely modulating dietary fat composition. Our data indicate a potent effect of fish oil in prolonging tumor latency and reducing tumor multiplicity in HER-2/neu overexpression–positive, estrogen receptor–negative breast cancer. Most interestingly, the lack of effect of rosiglitazone suggests that the influence of dietary fatty acid composition may be independent of PPAR\( \gamma \) activation.

The mechanisms by which the type of dietary fat influences mammary carcinogenesis are likely complex, with effects at various stages of this multistep process, as summarized in recent reviews (36, 37). PUFAs may modulate diverse biologic processes, including lipid peroxidation, eicosanoid production, growth factor receptor function at the cell membrane level, and signal transduction pathways affecting cell proliferation and survival (2, 38, 39). Whether (n-3) PUFAs specifically target pathways critical to HER-2/neu–mediated signaling, such as those of mitogen-activated protein kinase and phosphoinositide 3-kinase (40, 41), merits further investigation as.
well. Indeed, certain molecular, genetic subtypes of breast cancer might exhibit greater susceptibility to the effects of dietary fat content.

Despite the lack of main effects of a synthetic PPARγ ligand or interactions with dietary fat composition, the possibility of a mediating role for PPARγ cannot be eliminated completely. Perhaps additional studies are required with various PPARγ ligands administered over a larger dose range. For example, the inhibitory effects of rosiglitazone in vitro occurred at doses higher than those likely attained under the conditions of our dietary study. Importantly, although rosiglitazone did not affect HER-2/neu-mediated breast cancer, the corn oil + rosiglitazone combination tended to induce greater mammary gland atypia relative to corn oil alone (P = 0.051). Our in vitro findings in MMTV-HER-2/neu breast cancer cells did not indicate that this potential stimulatory effect resulted from activation of the MMTV promoter by the PPARγ ligand. Rosiglitazone possibly elicits a differential response of gene expression patterns at different doses or has nonlinear dose effects. Results of in vitro studies indicated that low, rather than high concentrations of PPARγ ligands could have such differential effects (42,43). Certainly, further studies with the drug given over a larger dose range and increased numbers of mice to enhance statistical power would help resolve these issues. Interestingly, paradoxical stimulation of colon tumorigenesis by treatment with PPARγ ligands was observed in some models of colon cancerogenesis (44,45). A recent study also demonstrated that mammary-specific expression of activated PPARγ in transgenic MMTV-VP-PPARγ mice stimulates tumorigenesis in mammary tissue that is cancer prone (i.e., MMTV-PyV transgenic mice expressing polyoma virus middle T antigen in the mammary gland) but not in tissue from mice with a normal background (46). These reports and our own findings underscore the importance of additional efforts in a variety of models of cancer to understand the molecular, biochemical, and cellular context of PPARγ signaling.

An in vitro study indicated that PPARγ ligands could inhibit neuregulin-mediated activation of HER-2/neu in MCF-7 breast cancer cells (47). However, we did not observe alterations in HER-2/neu expression or phosphorylation in NT5 cells with different PPARγ activators. Whether this lack of suppression relates to this specific model of HER-2/neu overexpression in mammary glands (48) is an important question. This study suggests that additional studies are warranted to investigate the possibility of inhibitory interactions between PPARγ and HER-2/neu signaling pathways.

Taken together, our data demonstrate the strong suppressive effect of (n-3) PUFAs on HER-2/neu-positive breast cancer, suggesting a gene-nutrient interaction of critical importance for mammary cancerogenesis. The addition of rosiglitazone did not alter the main effects of the (n-3) and (n-6) PUFAs, sources of putative natural ligands for PPARγ. Because HER-2/neu overexpression represents an important subtype of human breast cancer with particular aggressiveness, our findings of the protective benefits of diets rich in (n-3) fatty acids in experimental HER-2/neu-mediated breast cancerogenesis establish a foundation for future clinical studies. Indeed, the role of (n-3) fatty acid intake in the etiology of HER-2/neu positive breast cancer as well as in support of established adjuvant therapy in women with HER-2/neu breast cancer should be considered.

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LITERATURE CITED


