Activation of Peroxisome Proliferator-Activated Receptor-β/δ (PPAR-β/δ) Inhibits Human Breast Cancer Cell Line Tumorigenicity

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Abstract
The effect of activation and overexpression of the nuclear receptor PPAR-β/δ in human MDA-MB-231 (estrogen receptor–negative; ER–) and MCF7 (estrogen receptor–positive; ER+) breast cancer cell lines was examined. Target gene induction by ligand activation of PPAR-β/δ was increased by overexpression of PPAR-β/δ compared with controls. Overexpression of PPAR-β/δ caused a decrease in cell proliferation in MCF7 and MDA-MB-231 cells compared with controls, whereas ligand activation of PPAR-β/δ further inhibited proliferation of MCF7 but not MDA-MB-231 cells. Overexpression and/or ligand activation of PPAR-β/δ in MDA-MB-231 or MCF7 cells had no effect on experimental apoptosis. Decreased clonogenicity was observed in both MDA-MB-231 and MCF7 overexpressing PPAR-β/δ in response to ligand activation of PPAR-β/δ as compared with controls. Ectopic xenografts developed from MDA-MB-231 and MCF7 cells overexpressing PPAR-β/δ were significantly smaller, and ligand activation of PPAR-β/δ caused an even greater reduction in tumor volume as compared with controls. Interestingly, the decrease in MDA-MB-231 tumor size after overexpression of PPAR-β/δ and ligand activation of PPAR-β/δ correlated with increased necrosis. These data show that ligand activation and/or overexpression of PPAR-β/δ in two human breast cancer cell lines inhibits relative breast cancer tumorigenicity and provide further support for the development of ligands for PPAR-β/δ to specifically inhibit breast carcinogenesis. These new cell-based models will be invaluable tools for delineating the role of PPAR-β/δ in breast cancer and evaluating the effects of PPAR-β/δ agonists. Mol Cancer Ther; 13(4); 1008–17. ©2014 AACR.

Introduction
PPAR-β/δ belongs to the PPAR family of nuclear hormone receptors, which regulates a variety of biologic processes, including cell differentiation, cell proliferation, lipid accumulation, and glucose and fatty acid metabolism (1–4). Thus, PPARs also influence the development of human diseases, such as diabetes and cancers. PPAR-β/δ primarily acts as a transcription factor through dynamic interactions with chromatin with multiple levels of regulation, including the expression level of the receptor, the expression level of cofactor proteins, the presence of endogenous/exogenous ligands, and the availability of binding sites on chromatin containing target genes and regulatory regions that are modulated through these interactions (5). However, PPAR-β/δ can also directly interact with other transcription factors such as the p65 subunit of NF-kB and repress gene expression; an event associated with its known anti-inflammatory functions (reviewed in refs. 1, 4, 6).

Despite the large body of evidence that PPAR-β/δ is a protein expressed at very high levels in the skin, intestine, and liver (7), has known potent anti-inflammatory activities (8), and is required for many important physiologic processes (9), the role of PPAR-β/δ in carcinogenesis remains controversial. For example, some studies indicate that activating PPAR-β/δ promotes tumorigenesis, whereas antagonizing PPAR-β/δ inhibits tumorigenesis, for multiple types of cancer (reviewed in refs. 1, 4, 6). In contrast, other studies show that activating PPAR-β/δ inhibits tumorigenesis, whereas antagonizing PPAR-β/δ promotes or has no effect on tumorigenesis, for multiple types of cancer (reviewed in refs. 1, 4, 6).

Thus, controversy exists in the literature indicating that expression and/or activating PPAR-β/δ either promotes or inhibits breast cancer based on in vitro and
in vivo models. For example, the estrogen receptor-positive (ER+), MCF7 cell line cultured in vitro with a PPAR-β/δ ligand exhibited enhanced cell proliferation and when PPAR-β/δ was overexpressed in the same cell line, ligand activation of PPAR-β/δ caused an even greater increase in average cell proliferation (10). Interestingly, the estrogen receptor-negative (ER−), MDA-MB-231 cell line did not exhibit any change in cell proliferation following treatment with PPAR-β/δ ligands. This may suggest that this effect was, in part, dependent on the presence of a functional ER (10). In contrast, another study showed that increased expression of PPAR-β/δ was associated with terminal differentiation of MCF7 cells (11), an effect that is known to coincide with their withdrawal from the cell cycle (12). The latter study is also consistent with another study that found inhibition of cell proliferation following ligand activation of PPAR-β/δ in MCF7 cells (13). Interestingly, the difference between results from the above studies and those observed by Stephen and colleagues (10) was not due to differences in the ligands used or the presence or lack of serum. In addition, antagonizing PPAR-β/δ with GSK3787 had no effect on MCF7 cell proliferation (14). In vivo analysis examining the effect of PPAR-β/δ activation has also led to results that are difficult to interpret. For example, cyclooxygenase-2 (COX2)-null crossed with Ppar-β/δ-null mice exhibits reduced tumorigenicity as compared with controls, but tumor multiplicity was not measured (15). This study reduced tumorigenicity as compared with controls, but when PPAR-β/δ was overexpressed in the same cell line, ligand activation of PPAR-β/δ did not exhibit any change in cell proliferation, cell cycle, apoptosis, clonogenicity, and tumorigenicity using both in vitro, ex vivo, and in vivo approaches.

Materials and Methods

Cell lines

The human breast cancer cell lines MDA-MD-231 and MCF7 were obtained from American Type Culture Collection. The cell lines were tested for authenticity in October 2010 using Short Tandem Repeat Analysis (IDEXX-RADIL). The alleles for nine different markers were determined and confirmed to be of human origin and nonmammalian interspecies contamination was detected. The MCF7 cells matched the genetic profile reported for that cell line. The MDA-MB-231 cells matched the genetic profile reported for this cell line for seven of the nine alleles, but were missing an allele at TPOX and had an extra allele at vWA when compared with the profile reported for cell line HTB-26 (MDA-MB-231). Cells were cultured in Dulbecco’s Modified Eagle Media supplemented with 10% FBS, 1% nonessential amino acid, and 1% penicillin-streptomycin (Invitrogen) at 37°C with 5% carbon dioxide. The human breast cancer cell lines were cultured with or without different concentrations of the highly specific PPAR-β/δ agonist GW0742 (kindly provided by Drs. Andrew Billin and Timothy Willson, GlaxoSmithKline) at concentrations ranging from 0.01 to 10.0 μmol/L for 24 to 96 hours.

Stable human MDA-MD-231 and MCF7 breast cancer cell lines overexpressing PPAR-β/δ

Stable human MDA-MD-231 and MCF7 breast cancer cell lines overexpressing PPAR-β/δ were generated using the MigR1 retroviral vector that uses a mouse stem cell virus promoter to drive expression of PPAR-β/δ, followed by an internal ribosome entry site and a sequence encoding enhanced GFP (eGFP; ref. 19) as previously described (20, 21). This vector allows for expression of a protein of interest and/or eGFP (the latter served as a control), which facilitates identification and sorting of cells that have stably integrated the MigR1 retroviral vector as previously described (20, 21).

Western blotting

Soluble protein lysates were prepared as previously described (20). Thirty micrograms of protein per independent sample was resolved using SDS-PAGE (10% or 12%) and transferred to polyvinylidene difluoride membranes using an electroblotting method. The membranes were incubated with blocking buffer (5% dried milk in TBS with Tween-20, TBST) at room temperature, washed with TBST, and then incubated with primary antibodies against either, human PPAR-β/δ, cellular retinoic acid-binding protein II (CRABP-II; Abcam), p65, proliferating cellular nuclear antigen (PCNA), β-actin (Santa Cruz Biotechnologies), fatty acid-binding protein 5 (FABP5; Biovendor), PARP (Cell Signaling Technology), or lactate dehydrogenase (LDH, Rockland) at 4°C overnight. After washing three times with TBST at room temperature for 10 minutes each, the membranes were incubated with biotin-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories) at room temperature and then with
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Quantitative real-time PCR

Quantitative PCR (qPCR) was used to measure the PPAR-β/δ target gene angiopoietin-like protein 4 (ANGPTL4) mRNA expression as previously described (22). Each assay included a standard curve and a non-template control performed in triplicate. The relative mRNA level of ANGPTL4 was normalized to the relative mRNA level of glyceraldehydes-3-phosphate dehydrogenase (GAPDH).

Cell proliferation assay

To determine the effect of overexpression and/or ligand activation of PPAR-β/δ on cell proliferation, the xCELLigence system (Roche) was used as previously described (14). Briefly, MDA-MB-231, MDA-MB-231-MigR1 (control cells containing the MigR1 vector-expressing eGFP), and MDA-MB-231-hPPAR-β/δ cells (cells overexpressing human PPAR-β/δ and eGFP) or MCF7, MCF7-MigR1, and MCF7-hPPAR-β/δ cells were seeded on 16-well E-plates. Twenty-four hours after plating, the cells were cultured with medium with or without GW0742 for another 72 hours. Cell proliferation was monitored in real-time and growth curves recorded using the Real-Time Cell Analyzer (RTCA) DP Analyzer and RTCA Software (Roche).

Clonogenicity assay

Cells were plated on 60 mm culture dishes (800 cells/plate) and cultured in medium with or without GW0742 for 12 days to assess clonogenicity as previously described (18). The plating efficiency and surviving fraction were calculated as previously described (18).

Ectopic xenografts

Six-week-old female immunodeficient athymic nude (nu/nu) mice (Frederick National Laboratory for Cancer Research, Frederick, MD) were injected subcutaneously with 5 × 10^6 cells. The MDA-MB-231-MigR1 cells were injected in the left rear flank and the MDA-MB-231-hPPAR-β/δ cells were injected in the right rear flank. For analysis of the MCF7 cells, mice were implanted with a pellet containing 17β-estradiol (dose = 0.72 mg/kg) one day before injecting cells. The MCF7-MigR1 cells were injected in the left rear flank and the MCF7-hPPAR-β/δ cells were injected in the right rear flank. Groups of mice were then treated with or without GW0742 (2.5 mg/kg/d) for up to 36 days. The GW0742 was provided by daily dosing with a pellet made with Bacon-flavored Transgenic Dough Diet (Bioserv, Inc.) mixed with either vehicle control (0.02% dimethylsulfoxide) or GW0742. Body weight and tumor volumes were measured three times a week. Mice were euthanized by overexposure to carbon dioxide, and tumors harvested. Half of each tumor was fixed in 10% formalin, and the other half was snap frozen in liquid nitrogen for subsequent analysis of proteins by Western blot analysis or mRNA expression by qPCR as described above.

Statistical analysis

All in vitro experimental groups were performed in triplicate and repeated using three independent samples of cells. The xenograft studies were performed twice using a total of 10 mice per treatment group. The data were analyzed for statistical significance using analysis of variance followed by the post hoc Tukey test. Statistical significance was considered when P ≤ 0.05. Values are presented as the mean ± SEM.

Results

Confirmation of functional overexpression of PPAR-β/δ in MDA-MD-231 and MCF7 breast cancer cell lines

Fluorescent microscopic examination of control cells confirmed the lack of eGFP expression in both MDA-MB-231 and MCF7 cells, whereas both cell lines containing the MigR1 vector expressed eGFP (Fig. 1A). Similarly, eGFP was expressed in both MDA-MB-231 and MCF7 cells overexpressing hPPAR-β/δ (Fig. 1A). Increased expression of PPAR-β/δ was confirmed by Western blot analysis in both MDA-MB-231-hPPAR-β/δ and MCF7-hPPAR-β/δ cells by 5-fold and approximately 8-fold, respectively (Fig. 1A and B). Ligand activation of PPAR-β/δ increased expression of the PPAR-β/δ target gene ANGPTL4 in MDA-MB-231 cells and MDA-MB-231-MigR1 cells compared with controls, and the extent of induction was markedly higher in MDA-MB-231-hPPAR-β/δ cells (Fig. 1C). In contrast, ligand activation of PPAR-β/δ did not influence the expression of ANGPTL4 mRNA in normal MCF7 and MCF7-MigR1 cells compared with controls, but did markedly increase the expression of this PPAR-β/δ target gene ANGPTL4 in MCF7-hPPAR-β/δ cells (Fig. 1C). The lack of a statistically significant increase in ANGPTL4 mRNA in MCF7 and MCF7-MigR1 cells by ligand activation of PPAR-β/δ could be due to the fact that expression of PPAR-β/δ was not detectable in MCF7 cells compared with low but measureable expression of MDA-MB-231 cells (Fig. 1B).

Influence of overexpressed PPAR-β/δ in MDA-MD-231 and MCF7 breast cancer cell line proliferation

Overexpression of PPAR-β/δ in MDA-MD-231 and MCF7 breast cancer cell lines inhibited cell proliferation after 24 to 96 hours of culture as compared with controls (Fig. 2A and E). Ligand activation of PPAR-β/δ in MDA-MD-231, MDA-MD-231-MigR1, or MDA-MD-231-hPPAR-β/δ cells did not further influence this effect (Fig. 2B–D), whereas ligand activation of PPAR-β/δ in MCF7-hPPAR-β/δ did inhibit cell proliferation as compared with controls, but this effect was only observed with the highest

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dose of 10 µmol/L GW0742 (Fig. 2F–H). None of these changes in cell proliferation resulting from overexpression and/or ligand activation of PPAR-β/δ in MDA-MD-231 and MCF7 breast cancer cell lines were associated with alterations in cell-cycle progression (Supplementary Fig. S1).

**Overexpression and/or ligand activation of PPAR-β/δ in MDA-MD-231 and MCF7 breast cancer cell lines has no effect on inducible apoptosis**

As previous studies proposed a link between ligand activation of PPAR-β/δ and inhibition of apoptosis (reviewed in ref. 4), the effect of overexpression and/or ligand activation of PPAR-β/δ was examined using two different approaches to induce apoptosis: staurosporine and UV treatment. Staurosporine induced apoptosis in MDA-MD-231, MDA-MD-231-MigR1, and MDA-MD-231-hPPAR-β/δ cells but no differences in the concentration of staurosporine required for this effect, or the timing of PARP cleavage following staurosporine were observed between the MDA-MD-231 cell lines (Supplementary Fig. S2A and S2B). Furthermore, the ligand activation did not influence staurosporine-induced PARP cleavage between any of the MDA-MD-231 cell lines (Supplementary Fig. S2C). A similar lack of effect was observed in MCF7, MCF7-MigR1, or MCF7-hPPAR-β/δ cell lines (Supplementary Fig. S2A–S2C). Overexpression of PPAR-β/δ did not influence UVB-induced PARP cleavage in either MDA-MD-231, MDA-MD-231-MigR1, and MDA-MD-231-hPPAR-β/δ cells or MCF7, MCF7-MigR1, or MCF7-hPPAR-β/δ cells (Supplementary Fig. S3A). Furthermore, ligand activation of PPAR-β/δ did not alter PARP cleavage in any of the MDA-MD-231 or MCF7 cell lines as compared with controls (Supplementary Fig. S3B).

**Ligand activation of PPAR-β/δ inhibits clonogenicity**

To begin to determine whether overexpression and/or ligand activation of PPAR-β/δ influenced relative tumorigenicity of these cells, anchorage-dependent clonogenicity was examined. No difference in plating efficiency was observed between MDA-MD-231, MDA-MD-231-MigR1, and MDA-MD-231-hPPAR-β/δ cells or MCF7, MCF7-MigR1, and MCF7-hPPAR-β/δ cells (Fig. 3A and B). Although ligand activation of PPAR-β/δ had no effect on the relative clonogenicity of either MDA-MB-231 or MCF7 cells, inhibition of clonogenicity was observed in MDA-MD-231-MigR1 cells treated with 10 µmol/L GW0742 as compared with controls (Fig. 3C). Moreover, ligand activation of PPAR-β/δ more markedly inhibited clonogenicity in MDA-MD-231-hPPAR-β/δ between 0.1 and 10 µmol/L GW0742 as compared with controls (Fig. 3C). Ligand activation of PPAR-β/δ had no effect on the relative clonogenicity of MCF7-MigR1 cells but was
inhibited with 1 µmol/L GW0742 in MCF7-hPPAR-β/δ cells as compared with controls (Fig. 3D).

Overexpression and/or ligand activation of PPAR-β/δ in MDA-MD-231 and MCF7 breast cancer cell lines inhibits ectopic xenografts in part by enhancing necrosis

Overexpression of PPAR-β/δ markedly reduced average tumor volume in both MDA-MD-231 (60%) and MCF7 (20%) xenografts as compared with control MDA-MD-231-MigR1 or MCF7-MigR1 xenografts (Fig. 4A–D and 4E–H). Overexpression of PPAR-β/δ markedly reduced average tumor volume in both MDA-MD-231 (60%) and MCF7 (20%) xenografts as compared with control MDA-MD-231-MigR1 or MCF7-MigR1 xenografts (Fig. 4A–D and 4E–H).
was higher in ligand-treated MCF7-hPPAR-β/δ xenografts (Fig. 5A and B). In contrast, expression of p65 effect not found in similarly treated MDA-MB-231-hPPAR-β/δ xenografts from MDA-MB-231-MigR1 cells compared with control (Supplementary Fig. S4C), and interestingly, ligand activation of PPAR-β/δ caused an increase in the total necrotic region of xenografts from control MDA-MB-231-MigR1 cells compared with control (Supplementary Fig. S4D). In addition, the percentage of necrotic area was increased by ligand activation of PPAR-β/δ in control MDA-MD-231-MigR1 xenografts as compared with control MDA-MD-231-MigR1 xenografts. This increase in the percentage of necrotic area was higher in both control and ligand-treated xenografts found in similarly treated MDA-MB-231-hPPAR-β/δ xenografts (Fig. 5A and B). Increased expression of ANGPTL4 mRNA confirmed effective activation of PPAR-β/δ in the xenografts following treatment with GW0742 (Fig. 4G and H). Histopathological analysis revealed that the necrotic index was greatest in xenografts from ligand-treated MDA-MB-231-hPPAR-β/δ xenografts (Supplementary Fig. S4C), and interestingly, ligand activation of PPAR-β/δ caused an increase in the total necrotic region of xenografts from control MDA-MB-231-MigR1 cells compared with control (Supplementary Fig. S4D). In addition, the percentage of necrotic area was increased by ligand activation of PPAR-β/δ in control MDA-MB-231-MigR1 xenografts as compared with control MDA-MD-231-MigR1 xenografts. This increase in the percentage of necrotic area was higher in both control and ligand-treated xenografts

Supplementary Fig. S4A and S4B). Ligand activation of PPAR-β/δ caused even further reduction in average tumor volume of MDA-MD-231-MigR1 (50%), MDA-MD-231-hPPAR-β/δ (90%), MCF7-MigR1 (60%), and MCF7-hPPAR-β/δ (80%) xenografts as compared with controls, and this effect was more pronounced in the MDA-MD-231-hPPAR-β/δ and MCF7-hPPAR-β/δ xenografts compared with their respective controls (Fig. 4A–D and Supplementary Fig. S4A and S4B). These changes were reflected by similar changes in relative tumor weight (Fig. 4E and F). Expression of PCNA was also decreased by ligand activation of PPAR-β/δ in MDA-MD-231- hPPAR-β/δ xenografts as compared with controls; an effect not found in similarly treated MCF7-hPPAR-β/δ xenografts (Fig. 5A and B). In contrast, expression of p65 was higher in ligand-treated MCF7-hPPAR-β/δ xenografts compared with controls and this effect was not

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from MDA-MD-231-hPPAR-β/δ cells (Supplementary Fig. S4E). These changes in necrosis were not observed in xenografts from MCF7 cells. Thus, despite the increased expression of PPAR-β/δ in two human breast cancer cell line-derived tumors, the ratio of FABPs:CRABP-II (Fig. 5A and B) had no influence on relative tumorigenicity in vivo as suggested by others (23).

Discussion

Overexpression and/or ligand activation of PPAR-β/δ in both an ER+ and ER- human breast cancer cell line has marked effects on cell growth, anchorage-dependent clonogenicity, and relative tumorigenicity in an ectopic xenograft model. The fact that ligand activation of PPAR-β/δ inhibited ER+ MCF7 cell proliferation is consistent with a previous study (13) as this effect was only observed with 10 μmol/L GW0742 in MCF7 cells cultured in medium with fetal calf serum. The inhibitory effect of ligand activation on cell proliferation was not observed in the ER-, MDA-MB-231 cells. Interestingly, when PPAR-β/δ was overexpressed in both MDA-MB-231 and MCF7 cells, inhibition of cell proliferation was observed in both breast cancer cell lines, but the only cells that exhibited inhibition of cell proliferation following ligand activation of PPAR-β/δ were the ER+, MCF7 cells. These results are in direct contrast with a previous study showing that ligand activation of PPAR-β/δ increased proliferation of ER+ human breast cancer cell lines (MCF7 and T47-D) but not ER-, MDA-MB-231 cells, and that inducible expression of PPAR-β/δ enhanced the increase in cell proliferation following ligand activation of PPAR-β/δ (10). The reason for these opposing results cannot be clearly established from the present studies. However, it is important to note that the present study confirmed that the increase in expression of PPAR-β/δ ranged from 5- to approximately 8-fold using quantitative Western blotting, included a positive control for PPAR-β/δ protein, and also validated a functional increase in expression of the PPAR-β/δ target gene ANGPTL4, whereas the former study relied on expression of mRNA and qualitative immunofluorescence to demonstrate relative expression of PPAR-β/δ. Furthermore, of the two studies showing that ligand activation of PPAR-β/δ inhibited human breast cancer cell lines, one study used Coulter counting and examined cell growth over time (13), whereas the present study used real-time assessment of cell proliferation. In contrast, Stephen and colleagues used an enzyme-linked assay that could be influenced by the induction of mitochondrial dehydrogenase enzymes, which are known to be induced by PPAR-β/δ ligands (24). Thus, it remains possible that differences in the relative level of PPAR-β/δ expression and/or the approach used to assess cell proliferation could explain the differences observed between these studies.

Results from the present studies are the first to demonstrate that ligand activation of PPAR-β/δ inhibited anchorage-dependent clonogenicity in human breast cancer cell lines. It is also important to note that this effect was greater in ER+, MDA-MB-231 cells that overexpressed PPAR-β/δ as compared with controls, and that this effect was not as marked in the ER+, MCF7 cells that overexpressed PPAR-β/δ. These data are consistent with a previous study showing that anchorage-dependent clonogenicity was dose dependently decreased by ligand activation of PPAR-β/δ in a mouse mammary gland cancer cell line (18). Results from the present studies are also consistent with the observed inhibition of ectopic xenograft growth in vivo. For example, ligand activation of PPAR-β/δ inhibited tumor growth in xenografts developing from injected ER+, MDA-MB-231 cells and ER+, MCF7 cells. Although others have suggested that GW0742 can weakly interact with other nuclear receptors other than PPAR-β/δ (25), the dose of GW0742 used for the in vivo studies in the present experiments likely resulted in average serum concentrations less than 1 μmol/L as shown by others (26). Because this concentration is markedly lower than that required to weakly interact with other nuclear receptors in vitro, and the in vitro models...
overestimate the concentration required to interact with a nuclear receptor in vivo, it is unlikely that the effects mediated by GW0742 are mediated by interactions with other receptors other than PPAR-β/δ given the high affinity for GW0742 for this receptor. In addition, overexpression of PPAR-β/δ had a greater inhibitory effect on xenograft tumor growth in ER−/−, MDA-MB-231 cells as compared with ER+/+, MCF7 cells. The findings that overexpression of PPAR-β/δ inhibited both anchorage-dependent clonogenicity and ectopic xenograft growth are of particular interest because expression of PPAR-β/δ mRNA has been reported to be significantly lower in ductal breast carcinomas as compared with normal ductal epithelium in humans (27–29).

The mechanisms that underlie the observed inhibitory effect on xenograft tumor growth seem to be qualitatively different between ER−/− and ER+/+ human breast cancer cells. For example, decreased expression of PCNA was observed in ER−/−, MDA-MB-231 xenografts following ligand activation of PPAR-β/δ and this effect was not found in similarly treated xenografts developing from ER+/+, MCF7 cells. There was also a marked increase in necrosis found in ER−/−, MDA-MB-231 xenografts that was enhanced by ligand activation of PPAR-β/δ. This effect was not found in ER+/+, MCF7 xenografts. Although the mechanisms that induce apoptosis and necrosis are not divergent, there is evidence suggesting that drugs that promote necrosis in tumors may be more efficacious for chemoprevention/chemotherapy as compared with drugs that induce apoptosis (30). This hypothesis deserves further evaluation with GW0742 and other PPAR-β/δ agonists as this phenotype has never been observed to date. Combined, these data suggest that ER−/− breast cancer cells could be effectively targeted by developing an approach to increase expression of PPAR-β/δ and by treatment with PPAR-β/δ agonists. This hypothesis deserves further experimentation. In contrast, increased expression of p65 was noted in PPAR-β/δ ligand-treated ER−/−, MCF7 xenografts. This effect was not observed in other treatment groups of ER+/+, MCF7 xenografts or in the ER−/−, MDA-MB-231 xenografts. The reason why p65 expression was increased in PPAR-β/δ ligand-treated ER−/−, MCF7 xenografts cannot be determined by the current studies. However, it is worth noting that there are many reports showing that PPAR-β/δ can bind p65 and interfere with NF-kB–dependent proinflammatory signaling (reviewed in ref. 4, 6). This suggests that the higher expression of p65 could be the result of its stabilization through its increased binding with PPAR-β/δ and the inhibition of xenograft growth may be due to reduced p65-dependent proinflammatory signaling. Further studies are needed to examine this hypothesis.

Overexpression and/or ligand activation of PPAR-β/δ had no influence on either staurosporine or UVB-induced apoptosis. Previous studies by others have suggested that PPAR-β/δ promotes antiapoptotic signaling through a variety of mechanisms (reviewed in refs. 4, 6). One hypothesis is that FABP5 can deliver ligands directly to PPAR-β/δ and promote antiapoptotic activity; this has been suggested in a breast cancer model (23). However, results from the present studies and other work have shown that despite increasing the intracellular concentration of PPAR-β/δ and in the presence of FABP5, FABP5 does not function to deliver ligands to PPAR-β/δ, and activating PPAR-β/δ does not result in antiapoptotic activities (20–22, 31). These findings re-emphasize the need for further studies examining the role of PPAR-β/δ in apoptosis to clarify these disparities.

The results from the present studies can also not explain why other studies using mouse models suggest that PPAR-β/δ promotes breast cancer. This has been suggested by studies showing that mammary tumorigenesis is mitigated in Cox2-null mice crossed with Ppdr-β/δ-null mice (15), studies showing that DMBA-induced mammary tumorigenesis is enhanced by ligand activation of PPAR-β/δ (16), and studies using a transgenic mouse model showing enhanced mammary tumorigenesis by overexpression of mouse PPAR-β/δ (17). One possible explanation is that there are species differences between the mouse and human PPAR-β/δ, whereby the mouse PPAR-β/δ promotes mammary tumorigenesis while the human PPAR-β/δ inhibits mammary tumorigenesis. However, there are also many examples from other models suggesting that PPAR-β/δ promotes cancer in mice and other examples suggesting that PPAR-β/δ inhibits cancer in mice (reviewed in ref. 1, 2, 4, 6). For example, ligand activation of PPAR-β/δ inhibits proliferation of a mouse mammary gland cancer cell line by increasing apoptosis and inhibits anchorage-dependent clonogenicity (18). Furthermore, there is recent evidence from human studies suggesting that PPAR-β/δ is a tumor suppressor. For example, others have shown that expression of PPAR-β/δ mRNA is lower in ductal breast carcinomas as compared with nontransformed tissue (27–29, 32). Furthermore, analysis of protein expression of human breast cancer as compared with control nontransformed tissue (The Human Protein Atlas, version 12, www.proteinatlas.org) indicates that expression of PPAR-β/δ is low or not detectable in 11 of 12 samples (33). This is similar to analysis of PPAR-β/δ protein expression of 195 tumors compared with control nontransformed tissue where expression of PPAR-β/δ is markedly lower in tumors, including colon, prostate, ovarian, cervical, endometrial, head and neck, thymus, glioma, lymphoma, lung, melanoma, skin, testis, urothelial, renal, stomach, pancreatic, and liver (33). This suggests that the idea of a species difference is unlikely and the confusion in the literature is more likely due to differences in experimental approaches and conditions used to study the role of PPAR-β/δ in cancer. Whether the immunocompromised nature of the mice used for the present studies influenced the outcome is also uncertain. Thus, the new stable ER−/−, MDA-MB-231 and ER+/+, MCF7 human breast cancer cell lines characterized in the present study represent invaluable tools for future studies to more definitively determine the role of PPAR-β/δ in breast
cancer; an area of research that clearly requires more investigation.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: P.-L. Yao, J.M. Peters
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): P.-L. Yao, J.L. Morales
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): P.-L. Yao, J.L. Morales, B.-H. Kang, J.M. Peters
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Activation of Peroxisome Proliferator-Activated Receptor-\(\beta/\delta\) (PPAR- \(\beta/\delta\)) Inhibits Human Breast Cancer Cell Line Tumorigenicity

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