Stromal adipocyte PPARγ protects against breast tumorigenesis

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Introduction

Currently, one out of every eight North American women is diagnosed with breast cancer during her lifetime, and it is estimated that 1 in 35 will ultimately succumb to the disease (1,2). In 2011, the American Cancer Society estimates that 230,480 American women will be diagnosed with breast cancer and 39,520 will die from disease progression, with equally poor prognosis among the smaller number of men who are also susceptible (2). Improving our understanding of the interactions between genetic and environmental risk factors will enhance the therapeutic options and outcomes for breast cancer patients.

Several genetic factors are known to contribute to breast cancer including the functional loss of tumour suppressor genes, such as BRCA1 (3) and PTEN (4), as well as the over-expression of oncoproteins, such as HER2/neu (5). The candidate tumour suppressor gene

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susceptibility of obese individuals to breast tumour development (26). Given the prevalence of PPARγ in adipose tissue and its demonstrated role in adipokine secretion, the role of adipocyte-specific PPARγ signalling in breast tumorigenesis was evaluated.

DMBA is a polycyclic aromatic hydrocarbon (PAH) widely used in mammary gland carcinogenesis models, including animal models of hormone-dependent breast cancer (27,28). DMBA is probably the most extensively characterized procarcinogen representative of other PAHs found in the environment. It requires metabolic activation to electrophiles that can lead to DNA adducts, gene mutations and oxidative damage (27,29). Due to the presence of stromal–epithelial crosstalk mediated by PPARγ, it is hypothesized that adipocytes and stromal cells work in concert to regulate breast cancer development.

In vivo studies using adipocyte-specific PPARγ knockout (PPARγ-A KO) mice, where PPARγ expression and signalling is disrupted in mammary stromal adipocytes, show enhanced susceptibility to DMBA-mediated breast tumorigenesis. This suggests the presence of a stromal–epithelial crosstalk mediated by PPARγ, and is supported by findings of an increased survival advantage conveyed by loss of PPARγ in mammary stromal adipocytes associated with decreased BRCA1 levels, and enhanced circulating leptin.

Materials and methods

Chemicals and reagents
DMBA, 10% phosphate-buffered formalin, glyceral and ammonium persulphate were purchased from Sigma (St Louis, MO). Tris and SDS were purchased from Fisher (Whitby, ON). iScript cDNA synthesis kit, iQ SYBR Green Supermix and acrylamide were purchased from Bio-Rad (Hercules, CA). Trizol and RNase Away were obtained from Invitrogen (Carlsbad, CA). Rosiglitazone (Avandia) was purchased from GlaxoSmithKline (Mississauga, ON). Primary and secondary antibodies to detect PPARγ, BRCA1 and β-actin were obtained from Santa Cruz Biotechnology (San Diego, CA).

Animals
All mice were treated in accordance with protocols approved by the Queens University Animal Care Committee, and housed in microisolator cages to ensure pathogen-free status. The cages were maintained on a 12 h light/dark cycle, with food and water provided ad libitum. PPARγ-WT and PPARγ-A KO females were generated from crosses of previously described PPARγ−/− mice (30) with transgenic mice expressing Cre recombinase (Cre) under the control of the adipocyte lipid-binding promoter 2 (Ap2) (31) generously provided by Dr Barbara Kahn. The mice used in this study are of mixed C57Bl/6N;Sv129/N;FVB/NCr background and were bred using sibling matings for at least 15 generations prior to the start of the study.

In vivo tumorigenesis studies
In vivo mammary tumorigenesis was generated as described previously (22): Briefly, 8- to 12-week-old female mice were gavaged p.o. with 0.1 ml (total 1 mg) DMBA (dissolved in corn oil) once a week for 6 weeks. On week 7, mice were randomized to continue on a normal chow diet or one supplemented with the gold standard PPARγ activator, ROSI (4 mg/kg/day). Gross/clinical examinations for at least 15 generations prior to the start of the study.

Immunofluorescent staining
Blood collected from mice pre-treatment, at week 13 and upon necropsy was spun down to obtain serum,flash frozen in liquid nitrogen and stored at −80°C. Plates and standards to detect mouse serum leptin were set up according to manufacturer’s (Cayman Chemical) instructions and read at 450 nm.

Immunofluorescent staining
Formalin fixed, paraffin-embedded tissue blocks were sectioned into 5 μm slices, mounted on slides, and incubated at 55°C overnight. Samples were deparaffinized and rehydrated by washing in: xylene, 4 min; xylene, 4 min; xylene, 4 min; 100% ethanol, 3 min; 85% ethanol, 3 min; 70% ethanol, 3 min; ddH2O, 4 min. Slides were placed in 1:10 sodium citrate buffer solution at 95°C for 20 min, then washed in 0.3% Triton X-100. Samples were then incubated in blocking solution for 30 min and then incubated in primary antibody overnight at 4°C. The primary antibodies used were: anti-PPARγ (sc-166520; 1:500) were applied in a 5% BSA solution for 60 min at room temperature. Slides were rinsed briefly; ddH2O, 4 min. Slides were placed in 1:10 sodium citrate buffer solution at 95°C for 20 min, then trypsinized with 1× trypsin (Sigma) for 20 min at 37°C. After washing, the slides were placed in Triton X-100 buffer solution, followed by a 30 min incubation period in 5% BSA block in TBS. After washing, primary antibodies (Santa Cruz) for PPARγ (sc-7196; 1:500) and β-casein (sc-166520; 1:500) were applied in a 5% BSA solution for 60 min at room temperature. Slides were rinsed with Tris-buffered saline (TBS), and then incubated in fluorescein isothiocyanate (Santa Cruz; 1:500) and Alexa Fluor 594 (Invitrogen; 1:500) fluorescent-conjugated secondary antibodies in 5% BSA for 15 min at room temperature. After a final rinsing regime, tissues were cover-slipped with mounting media containing 4',6-diamidino-2-phenylindole (DAPI) stain (Vectorshield). Fluorescence was detected and quantitated using an Olympus BX51 fluorescence microscope, and QCapture Pro (Version 5) and ImagePro Plus (Version 6) software.

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G.Skelhorne-Gross et al.

Results

Confirmation of PPARγ recombination

The PPARγ-A KO mice generated here showed some lipodystrophy at a young age, reflected in lower adipose tissue and body weights compared with PPARγ-WT mice (Supplementary Figure 1 available at Carcinogenesis Online), consistent with other adipocyte-targeted PPARγ KO models (34,35). PPARγ-A KO mice had decreased WAT and BAT weights as a percentage of total body weight, as well as some hepatomegaly although other tissues examined, such as spleen and skeletal muscle, were unaffected (Supplementary Figure 1 is available at Carcinogenesis Online). To confirm the efficacy of Ap2Cre transgene in this floxed mouse model, a Southern blot was performed. About ~70-90% PPAR recombination occurred in all adipose tissue stores examined, with the highest levels in brown adipose, in both sexes (Figure 1A). Western analysis of untreated whole mammary gland tissue from 8-week-old virgin females from each genotype revealed that <2% relative PPARγ protein expression remained in female PPARγ-A KO mice compared with congenic PPARγ-WT controls (Figure 1B). Untreated PPARγ-A KO mice had no significant spontaneous increase in lethality or tumorigenesis when followed for up to 15 months (data not shown).

PPARγ deletion in stromal adipocytes enhances breast tumorigenesis

Tumorigenic studies were initiated in female virgin mice from each genotype by treating with 1 mg DMBA once a week for 6 weeks. At week 7, mice were randomized into continuing on a normal chow diet (DMBA Only; PPARγ-WT, n = 25 and PPARγ-A KO, n = 29) or one supplemented with ROSI (DMBA + ROSI; PPARγ-WT, n = 34 and PPARγ-A KO, n = 35) and followed for 25 weeks for tumour outcomes. Two PPARγ-A KO mice being treated with DMBA were found dead in their cage on weeks 10 and 12, respectively, due to unrelated causes, and were excluded from further analyses. Disruption of both PPARγ alleles in adipocytes did not significantly affect overall survival of mice treated with DMBA alone or DMBA + ROSI compared with PPARγ-WT controls. Mice from both genotypes treated with DMBA reached 50% survival at week 16 (Figure 2). There was a trend towards increased overall survival in PPARγ-WT mice treated with DMBA + ROSI (median survival week 21) compared with similarly treated PPARγ-A KO mice (median survival week 18), although this difference was not statistically significant.

Total tumour incidence was not significantly different for DMBA-treated PPARγ-WTs (84%) compared with similarly treated PPARγ-A KO (100%) mice. The pattern of DMBA-mediated tumour types were consistent with those previously associated with administration of this chemical carcinogen, and comprised mainly mammary tumours, as well as ovarian, uterine, skin tumours and lymphomas. Co-treatment with ROSI did not significantly alter the types or total tumour incidences observed at necropsy among DMBA + ROSI-treated PPARγ-WT (91%) or PPARγ-A KO (100%) mice. In contrast, PPARγ expression in adipocytes showed a trend towards reduced overall mammary tumour incidence with DMBA-treated PPARγ-WT controls compared with PPARγ-A KO mice (respective mean: 32% versus 48%, P < 0.08). More interestingly, PPARγ-A KO mice had a 2-fold significantly increased incidence of malignant mammary tumours versus PPARγ-WT mice (mean ± SD: 44 ±10% versus 20 ±9%, P < 0.01). In DMBA + ROSI co-treatment studies, activation of PPARγ signalling significantly reduced DMBA-mediated mammary tumour incidence among PPARγ-WTs more so than in PPARγ-A KO mice (mean ± SD: 29±8% versus 46±8%, P < 0.05). In addition, DMBA + ROSI-treated PPARγ-A KO mice had significantly more malignant mammary tumours than similarly treated PPARγ-WT mice (mean ± SD: 40±8% versus 26±8%, P < 0.01) (Figure 2C).

We next evaluated the role of adipocyte-specific PPARγ expression on mammary tumour multiplicity (Figure 2D). Endogenous PPARγ expression significantly decreased total mammary tumours per mouse by ~60% among DMBA Only-treated PPARγ-WTs compared with PPARγ-A KO mice (respective mean ± SD: 0.44 ±0.15 versus 0.70 ±0.17 tumours per mouse, P < 0.05). Interestingly, compared with DMBA-treated PPARγ-WT mice, mean malignant mammary multiplicity was significantly increased 2.5-fold among PPARγ-A KO mice, (mean ± SD: 0.59 ±0.14 versus 0.24 ±0.10 tumours per mouse, P < 0.05). This pattern was consistent in mice co-treated with ROSI as mean malignant multiplicity was also significantly increased nearly 2-fold in PPARγ-A KO mice compared with PPARγ-WT mice (mean ± SD: 0.65 ±0.15 versus 0.38 ±0.13 tumours per mouse, P < 0.05).

Beyond reductions in mammary tumour incidence, PPARγ signalling delayed mammary tumour onset (Figure 3A and B). Latency was not affected in mice treated with DMBA regardless of genotype. In contrast,
there was significantly increased latency among DMBA + ROSI-treated PPARγ-WT compared with PPARγ-A KO mice ($P < 0.05$). Notably, adipocyte PPARγ signalling did not affect mammary tumour volume (Figure 3C). Co-treatment studies with DMBA + ROSI reduced mammary tumour volume. DMBA-treated PPARγ-WT mice developed confirmed mammary tumours with an average volume of 3-fold larger than the average mammary tumours in PPARγ-WT mice treated with DMBA + ROSI (respective volume ± SD, 1.84 ± 1.75 cm$^3$ versus 0.77 ± 0.74 cm$^3$, $P < 0.05$). PPARγ-A KO mice also benefited from ROSI treatment, with average necropsy mammary tumours of nearly half the size of the average mammary tumours in DMBA-treated PPARγ-A KO mice (0.56 ± 0.71 cm$^3$ versus 0.95 ± 1.25 cm$^3$, $P < 0.05$).

Mammary tumours were classified by our collaborating pathologists as benign or malignant, and subtyped as adenocarcinomas, squamous cell carcinomas, spindle cell carcinomas or small cell carcinomas (Figure 4). PPARγ-A KO mice presented with significantly more adenocarcinomas ($P < 0.05$), the most common type of mammary tumour found in the clinic, compared with PPARγ-WT mice treated with or without ROSI co-treatment (Figure 4E).

**Stromal adipocyte-specific PPARγ upregulates BRCA1**

Quantitative real-time polymerase chain reaction was performed to evaluate breast tumour relevant targets (PPARγ1, PPARγ2, BRCA1, PTEN) using RNA isolated from (n = 3) untreated normal mammary gland samples from PPARγ-WT and PPARγ-A KO mice (Figure 5A). GAPDH was used as an internal standard with which to compare changes in target transcription levels and all data are presented relative to GAPDH expression. Relative PPARγ1 and PPARγ2 transcript levels were significantly decreased in untreated PPARγ-A KO compared with PPARγ-WT mice by 5- and 20-fold (mean target gene transcriptional expression ± SE: 22.2 ± 11% for PPARγ1, and 4.4 ± 2% for PPARγ2, $P < 0.05$). BRCA1 expression was also significantly reduced by 20-fold to 5.8 ± 2% in PPARγ-A KO mammary glands compared with PPARγ-WT control mammary glands ($P < 0.05$). Comparably, PTEN transcription was not affected by PPARγ signalling in adipocytes.

In order to evaluate BRCA1 expression changes resulting from loss of adipocyte-specific PPARγ, immunofluorescent (IF) imaging for PPARγ and BRCA1 expression was performed and quantitated (Figure 5B) on (n = 3) untreated mammary glands from PPARγ-WT (Figure 5C and E, respectively) and PPARγ-A KO (Figure 5D and F, respectively) mice. IF analysis indicated that loss of PPARγ expression was specific to mammary stromal adipocytes in PPARγ-A KO mice (Figure 5C and D). Images from PPARγ-WT glands indicate that BRCA1 is expressed in epithelial (pan-cytokeratin positive) cells as well as stromal (pan-cytokeratin negative) cells. Bright field images confirm that these stromal cells are predominantly adipocytes (data not shown). Images from PPARγ-A KO mammary glands show that BRCA1 expression is no longer present in adipocytes although it is retained in epithelial cells.

**Stromal adipocyte-specific PPARγ expression decreases leptin secretion**

Adipocyte-specific PPARγ signalling decreased pre-study serum leptin secretion in untreated PPARγ-WT mice compared with PPARγ-A KO mice (respective mean ± SE: 22.3 ± 2 ng/ml versus 27.9 ± 5 ng/ml, $P < 0.05$) (Figure 6A). Similarly, serum leptin levels measured throughout the tumorigenic study followed the same pattern with DMBA Only-treated PPARγ-A KO mice having higher serum leptin levels than PPARγ-WT mice at mid-study (26.9 ± 4.4 versus 15.6 ± 3.3 ng/ml, $P < 0.05$) and necropsy (10.0 ± 0.8 versus 8.0 ± 0.6 ng/ml, $P < 0.05$). Co-treatment with DMBA + ROSI did not significantly alter serum leptin levels among any of the groups examined.

**PPARγ activity is dysregulated in tumorigenesis**

Representative tumours from PPARγ-WT and PPARγ-A KO mice treated with DMBA or DMBA + ROSI were evaluated for expression changes in genes described previously (Figure 6B–E) as well as leptin receptor and estrogen receptor (ER)α (Figure 7A and B). A trend was observed towards increased PPARγ1, PPARγ2 and PTEN transcription in tumours from PPARγ-WT, but not from PPARγ-A KO.
mice treated with DMBA + ROSI compared with respective DMBA alone controls, although this pattern was not statistically significant. BRCA1 mRNA expression varied between tumours collected from mice in the same group as well as between groups, but favoured reduced expression in all but one sample from PPARγ-A KO mice compared with PPARγ-WT controls. Leptin receptor RNA levels within mammary tumours were similarly evaluated, and expressed to the same extent irrespective of genotype or treatment group (Figure 7A). More interestingly, mammary tumours from PPARγ-A KO mice in either treatment group expressed ~3-fold more ERα levels compared with their, respectively, treated PPARγ-WT controls (P < 0.05) (Figure 7B). DMBA + ROSI treatment significantly reduced ERα levels in both genotypes (P < 0.05) (Figure 7B).

Discussion

We generated PPARγ-A KO mice that are as viable as their congenic PPARγ-WT littermates, and had no significant spontaneous increase in lethality or breast tumorigenesis by 15 months of age (data not shown). This study provides the first direct in vivo evidence that PPARγ signalling in stromal adipocytes attenuates DMBA-mediated breast tumorigenesis. This is consistent with other studies suggesting that PPARγ plays a protective role in the progression of tumorigenesis (36). Analysis of the PPARγ-A KO mouse model revealed the Ap2Cre+ transgene produced between 70 and 90% PPARγ recombination depending on the adipose store examined, with BAT representing highest recombination levels. This translated into significant reduction in PPARγ protein expression in whole mammary glands including their fat pads, which was subsequently revealed by IF analysis as specific to mammary stromal adipocytes. Gross body and adipose store weights were decreased in PPARγ-A KO mice that is consistent with other adipocyte-targeted PPARγ KO mouse models (34). However, disruption of PPARγ in the present model, subsequent to adipogenesis, did not alter serum glucose levels (data not shown), which may have otherwise produced a diabetic phenotype and confounded the tumorigenic findings.

We and others have reported on the role of PPARγ expression within mammary epithelial cells in DMBA-mediated breast tumorigenesis (37) (J.Roche and C.Nicol, in preparation). Here, the role of PPARγ in mammary stromal adipocytes, as well as the effect of...
PPARγ activation, during breast tumorigenesis was also clarified. PPARγ-A KO mice were twice as likely to develop DMBA-mediated malignant mammary tumours and there was a trend towards increased overall mammary tumour incidence compared with identically treated PPARγ-WT control mice. In light of the other statistically significant data between these groups, this trend is probably a real biologic effect and would be significant with greater numbers of treated mice. These data strongly suggest that PPARγ signalling within adipocytes inhibits the onset of mammary tumours. While changes in tumour incidence did not correlate with an effect on overall survival during the course of this study, a longer duration of observation may have revealed that other measures of breast tumorigenesis were affected. PPARγ-A KO
would seem appropriate. The important role played by BRCA1 in familial and sporadic breast transcription in the tumours, since each tumour was subdivided into DNA damage (41). This may explain the varying levels of BRCA1 expression including autoregulation and response to DNA damage repair, adipocyte-specific BRCA1 expression within these cells. Even though BRCA1 is most well known for its role in DNA damage repair, adipocyte-specific BRCA1 expression is also capable of autoregulation (10). However, to date, no one has examined the cell-specific transcription and expression of these PPARγ targets in breast tumours.

This study revealed that normal PPARγ activity in adipocytes upregulates expression of PPARγ1, PPARγ2 and BRCA1 expression in these cells. Even though BRCA1 is most well known for its role in DNA damage repair, adipocyte-specific BRCA1 expression is also capable of autoregulation (40). In addition to providing a molecular rationale for the protective effects of PPARγ in adipocytes, the association with BRCA1 also suggests another predictive biomarker for PPARγ-activating therapy. If, in fact, the protective effects of PPARγ are dependent on functional BRCA1, clinicians could use BRCA1 to predict response. Other mechanisms have been proposed to increase BRCA1 expression including autoregulation and response to DNA damage (41). This may explain the varying levels of BRCA1 expression observed in the tumours, since each tumour was subdivided into fixed and frozen samples. However, the role of tumour heterogeneity as a contributing factor cannot be eliminated. Nevertheless, given the important role played by BRCA1 in familial and sporadic breast cancer, continued clarification of BRCA1 regulatory mechanisms would seem appropriate.

He et al. (35) previously reported that their adipocyte-targeted PPARγ KO mice have reduced circulating leptin. However, their mice were not stressed with chemical carcinogens, and serum analysis was done with 14-month-old mice, nearly 8 months after our mice completed tumorigenic study. These differences in study parameters may explain the suggested discrepancy between leptin levels observed in our respective untreated mice. Nevertheless, in support of our findings, Catalano et al. (42) published in vivo and in vitro evidence that PPARγ ligands inhibited leptin-induced upregulation of leptin and the leptin receptor, while our manuscript was in preparation. Leptin enhances mammary tumorigenesis by stimulating cell growth and survival in addition to increasing the effects of estrogen on mammary epithelial cells (43). Here we refine the model by showing that PPARγ within adipocytes affects leptin secretion in vivo. The present data also suggest that PPARγ in adipocytes does not directly regulate PTEN transcription, however, other factors are involved in PTEN regulation including autoregulation and expression through the stabilization of p53 (44). This is the first direct in vivo evidence that endogenous PPARγ signalling within adipocytes prevents against DMBA-mediated breast tumour progression.

In addition to suggesting that PPARγ signalling within adipocytes confers a protective in vivo role in mammary tumorigenesis, this study indicates that PPARγ activation with ROSI is also protective. Others have shown that activating PPARγ with various natural and synthetic ligands protects against tumorigenesis (45). A short pilot study was performed using ROSI on human patients diagnosed with breast cancer. After a few weeks, the ROSI treatment did not affect Ki67 levels, a marker of proliferation, however it did increase adiponectin (46). In order to justify further clinical trials using PPARγ-activating mono- or combination-therapy, over longer timelines, more pre-clinical work needs to be done to elucidate the protective mechanisms and identify which patients and breast tumour subtypes are most likely to respond to therapy.

Here strong evidence is provided that activation of PPARγ with ROSI protects against DMBA-mediated breast tumorigenesis, and that this protection is dependent on the presence of PPARγ in adipocytes. For more than a decade, ROSI has been used clinically to treat and prevent type II diabetes (12), a disease that is associated with several cancers including breast (47). ROSI co-treatment significantly increases mammary tumour latency in PPARγ-WT, but not PPARγ-A KO, mice. This implies that PPARγ activation slows or attenuates mammary tumour growth in an adipocyte-specific manner. In contrast, ROSI co-treatment decreases mammary tumour volume, evaluated at necropsy, in both mouse genotypes. Given all mammary tumours irrespective of genotype are of epithelial cell origin, which are not affected by the targeted PPARγ deletion approach used here, activation of PPARγ signalling would be expected to exert similar effects on the mammary tumours in both genotypes. Taken together, ROSI co-treatment provides significant direct and indirect protective effects on DMBA-mediated mammary tumour growth in our model.

PPARγ activation also increases PPARγ1, PPARγ2 and PTEN transcription in tumours from PPARγ-WTs but not PPARγ-A KO mice. Importantly, this suggests that the protective effect of PPARγ activation requires the presence of PPARγ in adipocytes. The variability between samples is probably due to the inherent heterogeneity within tumours. One mammary tumour from a PPARγ-WT mouse treated with DMBA + ROSI exhibited unusually high levels of BRCA1 transcription. Interestingly, this tumour was confirmed to be more undifferentiated and aggressive compared with the others in its matched group. It also cannot be excluded that there may be tumour-specific translational inhibition preventing expression of BRCA1 protein, thus causing mRNA accumulation.

Mammary tumours from all groups expressed the leptin receptor suggesting that those from PPARγ-A KO mice would be responsive to the significantly elevated serum leptin levels observed in these animals. As described above, it was independently reported that PPARγ activation counteracted leptin stimulatory effects on breast tumorigenesis in vitro and in vivo (42). Here we show more directly that loss of PPARγ increases mammary stromal adipocyte-specific secretion of leptin, and PPARγ activation with ROSI decreases serum leptin levels contributing to the protective effect against mammary tumorigenesis. Leptin has multiple biological actions including the regulation of endothelial cell proliferation and apoptosis through
which it can promote angiogenesis (48–50). It was also reported that high levels of circulating leptin, similar to those found in obese individuals, can drive breast tumorigenesis (51). The data here suggest that serum leptin levels may be useful as a biomarker for at least a subset of breast cancer patients who may warrant combination therapies, including PPARγ activators, to aid in the management of the growth and spread of breast tumours.

More strikingly, ERR levels were almost 3-fold higher among mammary tumours from PPARγ-A KO mice compared with their, respectively, treated PPARγ-A WT controls, and while significantly reduced by co-treatment with ROSI, this fold difference was maintained between genotypes. This suggests that mammary tumours from PPARγ-A KO mice are especially capable of responding to estrogen signalling. Ghosh et al. (2007) (39) reported that BRCA1 signalling in adipocytes reduces expression of aromatase that limits its estrogen synthesis. Taken together, in our PPARγ-A KO model with reduced BRCA1 expression, aromatase is probably increased, enhancing estrogen secretion which, alone or in combination with increased leptin secretion, may drive DMBA-mediated growth and progression of ERRt expressing mammary tumours. Ongoing studies are further examining the enhanced susceptibility of PPARγ-A KO mice to DMBA-mediated breast tumorigenesis, and how the expression and activated signalling of PPARγ within other mammary-associated cell types interact in vivo, but is beyond the scope of this article.

Ultimately, the long-term aims of these studies are to reduce breast cancer-related deaths by not only elucidating how genetic and environmental risk factors interact in vivo, but also discerning the genetic signature of patients who may benefit from PPARγ-targeted therapy. The data here provide the first direct evidence that mammary stromal adipocyte-specific PPARγ signalling locally maintains BRCA1 expression and systemically reduces leptin levels. It also provides further evidence that PPARγ-activating drugs, alone or in combination therapy, may be efficacious in treating at least a subset of breast cancer patients.

Supplementary material
Supplementary Figure 1 can be found at http://carcin.oxfordjournals.org/

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References

1419

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