British Journal of Pharmacology (2004) 143, 933–937
www.nature.com/bjp

SPECIAL REPORT

GW9662, a potent antagonist of PPARγ, inhibits growth of breast tumour cells and promotes the anticancer effects of the PPARγ agonist rosiglitazone, independently of PPARγ activation

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Peroxisome proliferator-activated receptor gamma (PPARγ), a member of the nuclear receptor superfamily, is activated by several compounds, including the thiazolidinediones. In addition to being a therapeutic target for obesity, hypolipidaemia and diabetes, perturbation of PPARγ signalling is now believed to be a strategy for treatment of several cancers, including breast. Although differential expression of PPARγ is observed in tumours compared to normal tissues and PPARγ agonists have been shown to inhibit tumour cell growth and survival, the interdependence of these observations is unclear. This study demonstrated that the potent, irreversible and selective PPARγ antagonist GW9662 prevented activation of PPARγ and inhibited growth of human mammary tumour cell lines. Controversially, GW9662 prevented rosiglitazone-mediated PPARγ activation, but enhanced rather than reversed rosiglitazone-induced growth inhibition. As such, these data support the existence of PPARγ-independent pathways and question the central belief that PPARγ ligands mediate their anticancer effects *via* activation of PPARγ.
British Journal of Pharmacology (2004) **143,** 933–937. doi:10.1038/sj.bjp.0705973

Keywords: Breast cancer; GW9662; peroxisome proliferator-activated receptor (PPAR); PPARγ; rosiglitazone

Abbreviations: BADGE, bisphenol A diglycidyl ether; ER, oestrogen receptor; GW9662, 2-chloro-5-nitrobenzanilide; PPAR, peroxisome proliferator-activated receptor; PPRE, PPAR response element; RXR, retinoid X receptor

Introduction Activation of peroxisome proliferator-activated receptor (PPAR)γ and regulation of gene transcription is a multistep process that involves ligand binding, heterodimerisation with retinoid X receptor (RXR), interaction with sequence-specific gene promoter elements (termed PPAR response elements (PPREs)), and recruitment of coactivators and other nuclear coregulatory proteins (Kliewer *et al*., 2001; Gill & Roberts, 2003). As well as being central to the regulation of lipid metabolism, PPARγ is now believed to have a role in tumorigenesis (Roberts-Thomson, 2000; Gill & Roberts, 2003). In breast tissue, agonists of PPARγ have been shown to inhibit cell growth, reduce oestrogen production by adipose tissue, inhibit oestrogen receptor (ER) activity and play a role in tumour regression (Agarwal *et al*., 2000; Rubin *et al*., 2000; Pighetti *et al*., 2001; Wang & Kilgore, 2002; Qin *et al*., 2003). Similarly, in cultured breast cells PPARγ agonists can induce extensive lipid accumulation and reduce clonogenic survival (Elstner *et al*., 1998). As such, PPARγ agonists have been proposed as anticancer therapeutics for breast cancer, a theory currently being tested in clinical trials (Burstein *et al*., 2003).

Although the therapeutic potential of PPARγ agonists as anticancer agents is clearly evident, the role of PPARγ activation in the process and thus their mechanism of action remain unclear. For instance, no clear correlation is observed between the sensitivity of the MT breast cell line panel to PPARγ ligands and their PPARγ expression levels (Mueller *et al*., 1998). Similarly, studies using murine embryonic stem cells and fibroblasts demonstrated the presence of PPARγ-independent pathways of growth inhibition by PPARγ agonists (Palakurthi *et al*., 2001). This study sought to investigate whether PPARγ activation was central to the inhibition of human breast cancer cell growth induced by PPARγ ligands. Breast tumour cell growth and survival were assessed using the potent irreversible PPARγ selective antagonist 2-chloro-5-nitrobenzanilide (GW9662) in the presence and absence of the PPARγ agonist rosiglitazone. Furthermore, since PPARγ agonists have already shown clinical worth for other human diseases and are currently in clinical trials as anticancer agents (Burstein *et al*., 2003), this study may have important implications for breast cancer therapy.

Methods *Cell culture* Human breast cancer cell lines (MCF-7, MDA-MB-231, MDA-MB-468) were obtained from the European Cell Culture Collection (ECACC). All cell lines were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine and 1 mM sodium pyruvate in a humidified 5% CO_2 atmosphere at 37°C. The media were changed every 3 days and the cells passaged *via* trypsinisation before reaching confluency.

Western blotting Cells grown in monolayer were harvested at subconfluency, lysed and total protein isolated. Briefly, cells were scraped from the flask, lysed in RIPA buffer (150 mM NaCl, 50 mM Tris–HCl, pH 7.4, 5 mM EDTA, 1% NP40, 0.1% SDS, 1 mM phenylmethylsulphonyl fluoride (PMSF) and proteinase inhibitor cocktail (1×; Sigma, Dorset, U.K.)),

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Advance online publication: 8 November 2004

sonicated and centrifuged. Protein concentrations of the resultant supernatants were determined using the BioRad protein assay (BioRad, U.K.). Cell lysates (30 μg protein per lane) were separated on 10% SDS–polyacrylamide gels and transferred to polyvinylidenedifluoride (PVDF) membranes (Hybond-P; Amersham, U.K.). Nonspecific protein binding was blocked using 2% ECL advanced blocking reagent (Amersham, U.K.). Blots were incubated overnight with anti-PPARγ antibody (Santa Cruz; diluted 1 : 1000) or anti-PPARα antibody (Santa Cruz; diluted 1 : 1000), followed by horseradish-peroxidase-conjugated goat anti-rabbit total IgG (Dako; diluted 1 : 2000) for 2 h. Immunoreactive bands were visualised using an enhanced chemiluminescence (ECL) detection kit (Amersham) according to the manufacturer's instructions and images captured using a BioRad FX-Imaging system.

MTT cell survival studies Rosiglitazone and GW9662 were purchased from Cayman Chemicals (St Louis, MO, U.S.A.). Cells (MCF7, MDA-MB-231, MDA-MB-468) were plated in 96-well plates at a density of 1×10^3 cells per well in RPMI medium. After overnight incubation to allow for cell attachment, the medium was removed and replaced with fresh medium containing varying concentrations of rosiglitazone (1–100 μM), GW9662 (100 nM-50 μM) or solvent (dimethyl sulphoxide (DMSO)) alone. MDA-MB-231 cells were also subjected to combinations of rosiglitazone (10, 50 μM) and GW9662 (1, 10 μM) added simultaneously. The final concentration of DMSO in all cases did not exceed 0.1% and was not found to be cytotoxic in any of the cell lines tested at this concentration. Chemosensitivity was assessed following a continuous 72 h exposure using a standard 3-[4, 5-dimethylthiazolyl]-2,5-diphenyltetrazolium bromide (MTT) assay.

Cell growth assay MDA-MB-231 cells were seeded at a density of 1×10^5 cells per 25 cm^3 tissue culture flask. After 24 h (day 0), the growth medium was replaced with fresh medium containing rosiglitazone (50 μM), GW9662 (10 μM) or both together. Control flasks received 0.1% DMSO. Cells were harvested on days 0, 3, 5, 7, 10 for each treatment condition by trypsinisation, stained using trypan blue, and the total and viable number of cells per flask calculated using a haemocytometer.

Preparation of nuclear extracts of treated cells for measurement of PPARγ and PPARα activity Levels of PPARγ and α activity following rosiglitazone (50 μM), GW9662 (10 μM) or combination treatment were measured in the nuclear fraction of MDA-MB-231 and MCF7 cells using the PPAR transfactor kit (BD Biosciences Clontech, U.K.) as described below. Cells were seeded at a density of 1×10^6 cells per 75 cm^3 tissue culture flask. After 24 h, culture medium was removed and replaced with fresh medium containing the appropriate treatment. Following 2, 4, 8, 24 h of treatment, nuclear extracts were isolated (five flasks per treatment condition) using a transfactor extraction kit (BD Biosciences Clontech, U.K.), as per the manufacturer's instructions. The protein concentrations of the resultant supernatants (nuclear extract) were determined using the BioRad protein assay (BioRad, U.K.).

Quantitative measurement of PPARγ and PPARα activity PPARγ and PPARα activity was assayed in nuclear extracts using the PPAR transfactor assay (BD Biosciences Clontech, U.K.), following the manufacturer's instructions. Briefly, following blocking of nonspecific binding to the PPRE-coated wells, 50 μg of nuclear extract or positive control extract (supplied with the kit) was added to the appropriate wells and the plate incubated for 60 min at room temperature. Primary anti-PPARγ antibody or anti-PPARα antibody was added to each well and the plate incubated for 60 min. Secondary antibody (anti-rabbit IgG-HRP) was added to each well and incubated for 30 min. Colorimetric detection of bound antibody was performed by addition of TMB substrate, incubation at room temperature for 20 min, addition of sodium azide stop buffer and measurement of absorbance at 655 nm using a microplate reader.

Statistical analyses Statistical analyses were undertaken using the SPSS software package, version 11.0 (SPSS Inc., Chicago, IL, U.S.A.). Values represent mean $\pm$ standard error (s.e.) of replicate experiments. Data were analysed by Student's *t*-test for and the threshold for statistical significance was assigned at $P = 0.05$.

Results *Effect of the PPARγ agonist rosiglitazone and PPARγ antagonist GW9662 on cell survival* All of the cell lines utilised in this study were found to express PPARγ protein (Figure 1), in agreement with previous studies (Jiang *et al*., 2003). In contrast, PPARα protein was detectable in both MDA-MB-231 and MDA-MB-468 cells, but not the MCF7 cell line (Figure 1), a previously unreported observation. This expression profile was supportive of the use of these cells for this study. To investigate the consequence of activation or inhibition of PPARγ upon survival of breast cell lines, MCF7, MDA-MB-231 and MDA-MB-468 cells were subjected to various doses of rosiglitazone or GW9662 for 72 h and cell viability analysed by MTT assay. Response to rosiglitazone was similar for all the three cell lines, with an IC_{50} of approximately 50 μM. Although this concentration appears relatively high, it is in line with values observed with other PPARγ agonists in breast cell lines (Clay *et al*., 2001; Yin *et al*., 2001). GW9662 acts as a potent antagonist of PPARγ in both cell-free and cell-based assays by covalently modifying a cysteine residue in the ligand-binding site of PPARγ (Leesnitzer *et al*., 2002). Since GW9662 does not induce PPARγ-mediated transcription (Leesnitzer *et al*., 2002) and exogenously added PPARγ agonists were not added to the culture

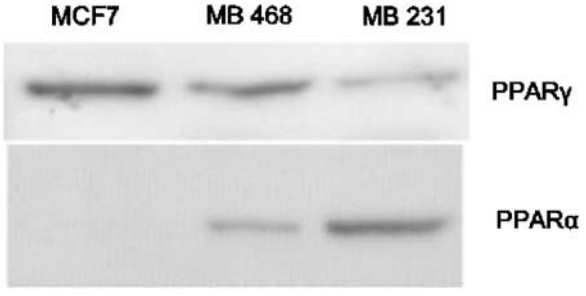

Figure 1 Expression of PPARγ and PPARα protein in human breast cell lines. Each lane was loaded with 30 μg cell lysate. MB468: MDA-MB-468; MB231: MDA-MB-231.

media in this study, no response to GW9662 was expected as measured by MTT assay. Surprisingly, all the three cell lines demonstrated comparable loss of viability in response to GW9662 (Figure 2; IC_{50} 20–30 μM), suggesting either the existence of PPARγ agonistic properties of GW9662 or growth-inhibitory mechanisms independent of PPARγ. ER alpha and beta (ERα, β) have been shown to inhibit PPAR transactivation and exhibit signal crosstalk with PPARγ (Wang & Kilgore, 2002). Therefore, to reduce complicating factors when evaluating the 'true' involvement of PPARγ activation in the growth-inhibitory response of rosiglitazone and GW9662, our subsequent studies focused primarily on the ERα-negative and oestrogen nonresponsive MDA-MB-231 cell line.

GW9662 does not prevent rosiglitazone-mediated growth suppression The observation that GW9662 itself has growth-inhibitory properties prompted us to investigate the effect of GW9662 upon rosiglitazone-induced cell growth suppression (Figure 3). As a consequence of GW9662 being a high-affinity selective PPARγ inhibitor capable of fully abrogating PPARγ activation and signalling (Leesnitzer *et al*., 2002), GW9662 was expected to prevent rosiglitazone-mediated growth inhibition. Surprisingly, co-treatment of MDA-MB-231 cells with both rosiglitazone and GW9662 resulted in an additive effect upon cell survival rather than the predicted subtractive effects, as measured by MTT assay (Figure 3). Analysis of the cellular growth kinetics of MDA-MB-231 cells in the presence of rosiglitazone and GW9662 further confirmed this observation, in that GW9662 did not prevent rosiglitazone-induced growth inhibition, but instead acted to enhance the effect of rosiglitazone (Figure 4). Co-treatment with both 50 μM rosiglitazone and 10 μM GW9662 resulted in statistically lower viable cell numbers after 7 days when compared to treatment with either 50 μM rosiglitazone ($P=0.001$) or 10 μM GW9662 ($P=0.01$) alone. Our data also indicated that cells in the presence of rosiglitazone and GW9662 were reaching the plateau phase of growth earlier than that observed in control conditions (Figure 4). Although the reason for this observation is currently unclear, it may suggest that the induction of cellular differentiation by PPARγ ligands, as reported previously (Mueller *et al*., 1998), can occur independently of PPARγ.

PPARγ activation is not involved in growth suppression induced by rosiglitazone and GW9662 Since both rosiglitazone and GW9662 had been shown to inhibit cell survival and retard cell growth, it was important to determine whether these effects were dependent upon activation of the PPARγ receptor. Using a modified ELISA assay (Shen *et al*., 2002), the degree of PPARγ activation was determined in parallel to the cell survival and growth study. As predicted, rosiglitazone (50 μM) and GW9662 (10 μM) treatment resulted in an induction and inhibition of PPARγ activation, respectively (Figure 5). In contrast, no activation or inhibition of PPARα activity was detected with either rosiglitazone or GW9662 (data not shown), demonstrating that the observed effects were not due to perturbation of PPARα activation. Interestingly, levels of PPARγ activation in cells exposed to GW9662 alone were significantly lower than that observed in either the solvent or untreated controls (Figure 5), suggesting a degree of background PPARγ activation. This background PPARγ activity is likely to be as a consequence of endogenous PPARγ agonists within the cell growth media, which also contains 10% fetal

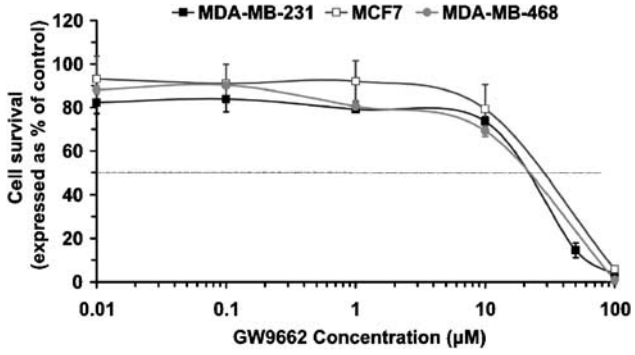

Figure 2 Effect of the PPARγ antagonist GW9662 on viability of human breast cell lines. MCF7, MDA-MB-468, MDA-MB-231 were treated with GW9662 (0.1–50 μM) for 72 h, and cell viability measured by MTT assay. Results, expressed as % of solvent control, are the mean $\pm$ s.e. from three separate experiments.

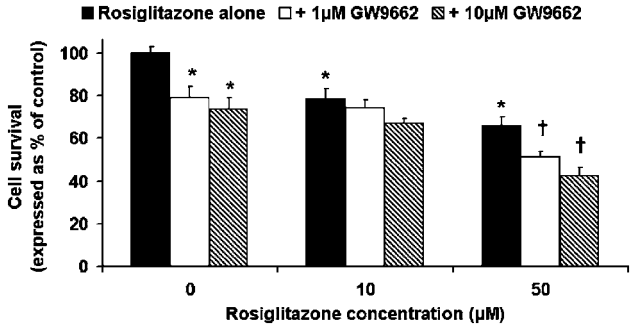

Figure 3 Effect of co-treatment of the PPARγ antagonist GW9662 and the PPARγ agonist rosiglitazone on viability of MDA-MB-231 cells. Cells were treated with rosiglitazone (0, 10, 50 μM) in the presence or absence of GW9662 (0, 1, 10 μM) for 72 h and cell viability measured by MTT assay. Results, expressed as % of solvent control, are the mean $\pm$ s.e. of three independent experiments. $^{*}P<0.01$ vs solvent control; $^{\dagger}P<0.01$ vs 50 μM rosiglitazone.

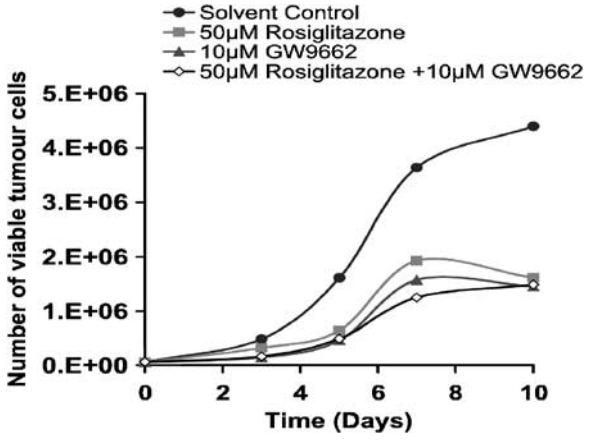

Figure 4 Effect of rosiglitazone and GW9662 on cell growth kinetics of MDA-MB-231 cells. Cells were treated with either 50 μM rosiglitazone, 10 μM GW9662, combination of rosiglitazone and GW9662 or solvent alone, and the total number of viable cells calculated by trypan blue exclusion over a 10-day period. Error bars are omitted for clarity.

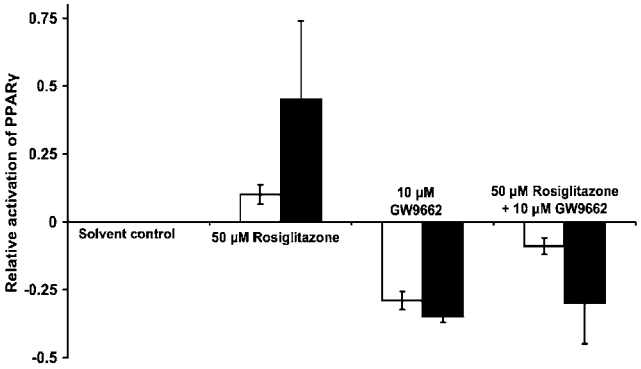

Figure 5 Levels of PPARγ activation following treatment with rosiglitazone and/or GW9662. MDA-MB-231 (white bars) and MCF7 (black bars) cells were treated with rosiglitazone (50 μM), GW9662 (10 μM) or both for 4 h and the level of PPARγ activity measured by the PPARγ transfactor modified ELISA assay. Results are expressed as fold change in activity compared to solvent control. Results are the mean $\pm$ s.e. of three experiments.

calf serum. Treatment of MDA-MB-231 cells with both rosiglitazone (50 μM) and GW9662 (10 μM) simultaneously did not result in activation of PPARγ (Figure 5; $P \leqslant 0.01$ compared to either treatment alone). In addition to the 4 h timepoint (Figure 5), similar activity responses were observed at 2, 8 and 24 h post-treatment (data not shown) and in the MCF7 cell line (Figure 5). Taken together, these data strongly suggest that PPARγ activation may not be involved in the inhibition of cell survival or cell growth.

Discussion PPARγ is central to cellular lipid metabolism and is activated by a diverse range of compounds including fatty acids, eicosinoids and insulin sensitisers (Kliewer *et al*., 2001; Gill & Roberts, 2003). As such, PPARγ has been identified as a therapeutic target for obesity, hypolipidaemia and diabetes (Kliewer *et al*., 2001). More recently, based on both *in vitro* and *in vivo* studies, perturbation of PPARγ expression and activity has been suggested as a therapeutic strategy for several epithelial tumour types, including breast (Elstner *et al*., 1998; Roberts-Thomson, 2000; Rubin *et al*., 2000; Pighetti *et al*., 2001; Wang & Kilgore, 2002). Although this is an interesting concept and is supported by a large number of primarily *in vitro* studies, the involvement of PPARγ activation and its subsequent signalling pathway in such cases is purely implied based on parallel observation in other cell systems, such as lipid metabolism, and molecular studies demonstrating drug–receptor interaction. Understanding the involvement and role of PPARγ in breast tumour growth inhibition has important implications for both interpreting the results of current clinical trials (Burstein *et al*., 2003) and the pharmacology of such compounds.

In our study, the PPARγ ligand rosiglitazone activated PPARγ (Figure 5) and inhibited growth and survival of breast cell lines *in vitro*, independently of their reported oestrogen receptor-α status, supporting the proposed therapeutic potential of these compounds in breast cancer and suggesting the direct involvement of PPARγ in the process. Surprisingly, the selective and irreversible PPARγ antagonist GW9662 also inhibited cell growth and survival of all cell lines (Figure 2), but in this case PPARγ activity was strongly inhibited (Figure 5). These data demonstrated that GW9662, in addition to inhibition of PPARγ activity, prevented cell growth and survival independently of PPARγ. Controversially, treatment of cells with GW9662 did not prevent rosiglitazone-mediated growth inhibition as expected (Figures 3 and 4), but instead acted to further reduce cell survival and growth (Figure 3). The level of PPARγ activity in this situation was between that observed for rosiglitazone and GW9662 alone, at a level similar to that of the controls (Figure 5). This suggests that either growth inhibition induced by rosiglitazone occurs independently of PPARγ activation or that GW9662 inhibits a pathway secondary to that of rosiglitazone.

The suggestion that PPARγ activation is not involved in inhibition of cell survival and growth induced by known PPARγ agonists is supported by a study using xenografted PPARγ-null embryonic stem cells in which inhibition of tumour growth by TZDs occurred independently of PPARγ (Palakurthi *et al*., 2001). Furthermore, apoptosis induced by the PPARγ ligand 15-deoxy $\Delta^{12,14}$-prostaglandin J2 has also been shown to occur independently of PPARγ activation (Clay *et al*., 2002). In glioma cells, inhibition of cell survival by PPARγ agonists occurred independently of PPARγ activity, and was suggested to be as a result of loss of mitochondrial membrane potential and production of reactive oxygen species (Perez-Ortiz *et al*., 2004). This mechanism of PPARγ-independent inhibition of cell survival is further supported by a study addressing growth inhibition by the PPARγ antagonist bisphenol A diglycidyl ether (BADGE), which induced apoptosis mediated by Bax-dependent release of cytochrome *c* (Fehlberg *et al*., 2003). Although further studies are required, our results in breast cell lines and those in other cell types (Palakurthi *et al*., 2001; Clay *et al*., 2002; Fehlberg *et al*., 2003; Perez-Ortiz *et al*., 2004) suggest that the central belief that PPARγ ligands, especially the thiazolidinediones, mediate their anticancer effects *via* activation of the PPARγ receptor is questionable. The recent observations that PPARγ ligands can perturb mitochondrial membrane potential (Perez-Ortiz *et al*., 2004), alter intracellular calcium storage (Palakurthi *et al*., 2001) and effect release of apoptotic factors from the mitochondria (Clay *et al*., 2002; Fehlberg *et al*., 2003) suggest the existence of an alternative pathway for the anticancer effects of PPARγ ligands and a potential novel target for the development of anticancer therapeutics.

We thank members of the Tom Connors Cancer Research Centre for technical help with this study. This work was supported by Cancer Research UK (grant number C459/A2579) and The Breast Cancer Campaign (grant number 2001:231).

References

AGARWAL, V.R., BISCHOFF, E.D., HERMANN, T. & LAMPH, W.W. (2000). Induction of adipocyte-specific gene expression is correlated with mammary tumor regression by the retinoid X receptor-ligand LGD1069 (targretin). *Cancer Res.,* **60,** 6033–6038.

BURSTEIN, H.J., DEMETRI, G.D., MUELLER, E., SARRAF, P., SPIEGELMAN, B.M. & WINER, E.P. (2003). Use of the peroxisome proliferator-activated receptor (PPAR) gamma ligand troglitazone as treatment for refractory breast cancer: a phase II study. *Breast Cancer Res. Treat.,* **79,** 391–397.

CLAY, C.E., MONJAZEB, A., THORBURN, J., CHILTON, F.H. & HIGH, K.P. (2002). 15-Deoxy-delta12,14-prostaglandin J2-induced apoptosis does not require PPARgamma in breast cancer cells. *J. Lipid Res.,* **43,** 1818–1828.

CLAY, C.E., NAMEN, A.M., ATSUMI, G., TRIMBOLI, A.J., FONTEH, A.N., HIGH, K.P. & CHILTON, F.H. (2001). Magnitude of peroxisome proliferator-activated receptor-gamma activation is associated with important and seemingly opposite biological responses in breast cancer cells. *J. Investig. Med.,* **49,** 413–420.

ELSTNER, E., MULLER, C., KOSHIZUKA, K., WILLIAMSON, E., PARK, D., ASOU, H., SHINTAKU, P., SAID, J., HEBER, D. & HP, K. (1998). Ligands for peroxisome proliferator-activated receptorgamma and retinoic acid receptor inhibit growth and induce apoptosis of human breast cancer cells *in vitro* and in BNX mice. *Proc. Natl. Acad. Sci. U.S.A.,* **95,** 8806–8811.

FEHLBERG, S., GREGEL, C.M., GOKE, A. & GOKE, R. (2003). Bisphenol A diglycidyl ether-induced apoptosis involves Bax/Bid-dependent mitochondrial release of apoptosis-inducing factor (AIF), cytochrome *c* and Smac/DIABLO. *Br. J. Pharmacol.,* **139,** 495–500.

GILL, J. & ROBERTS, R. (2003). PPARs and cancer. In: *Protein–Fatty Acid Interactions in the Mammalian Cell,* ed. Dutta-Roy, A. & Spener, F. New York: VCH-Wiley.

JIANG, W.G., DOUGLAS-JONES, A. & MANSEL, R.E. (2003). Expression of peroxisome-proliferator activated receptor-gamma (PPARgamma) and the PPARgamma co-activator, PGC-1, in human breast cancer correlates with clinical outcomes. *Int. J. Cancer,* **106,** 752–757.

KLIEWER, S.A., XU, H.E., LAMBERT, M.H. & WILLSON, T.M. (2001). Peroxisome proliferator-activated receptors: from genes to physiology. *Recent Progr. Hormone Res.,* **56,** 239–263.

LEESNITZER, L.M., PARKS, D.J., BLEDSOE, R.K., COBB, J.E., COLLINS, J.L., CONSLER, T.G., DAVIS, R.G., HULL-RYDE, E.A., LENHARD, J.M., PATEL, L., PLUNKET, K.D., SHENK, J.L., STIMMEL, J.B., THERAPONTOS, C., WILLSON, T.M. & BLANCHARD, S.G. (2002). Functional consequences of cysteine modification in the ligand binding sites of peroxisome proliferator activated receptors by GW9662. *Biochemistry,* **41,** 6640–6650.

MUELLER, E., SARRAF, P., TONTONOZ, P., EVANS, R.M., MARTIN, K.J., ZHANG, M., FLETCHER, C., SINGER, S. & SPIEGELMAN, B.M. (1998). Terminal differentiation of human breast cancer through PPAR gamma. *Mol. Cell,* **1,** 465–470.

PALAKURTHI, S., AKTAS, H., GRUBISSICH, L., MORTENSEN, R. & HALPERIN, J. (2001). Anticancer effects of thiazolidinediones are independent of peroxisome proliferator-activated receptor gamma and mediated by inhibition of translation initiation. *Cancer Res.,* **61,** 6213–6218.

PEREZ-ORTIZ, J.M., TRANQUE, P., VAQUERO, C.F., DOMINGO, B., MOLINA, F., CALVO, S., JORDAN, J., CENA, V. & LLOPIS, J. (2004). Glitazones differentially regulate primary astrocyte and glioma cell survival: involvement of ROS and PPARgamma. *J. Biol. Chem.,* **279,** 8976–8985.

PIGHETTI, G.M., NOVOSAD, W., NICHOLSON, C., HITT, D.C., HANSENS, C., HOLLINGSWORTH, A.B., LERNER, M.L., BRACKETT, D., LIGHTFOOT, S.A. & GIMBLE, J.M. (2001). Therapeutic treatment of DMBA-induced mammary tumors with PPAR ligands. *Anticancer Res.,* **21,** 825–829.

QIN, C., BURGHARDT, R., SMITH, R., WORMKE, M., STEWART, J. & SAFE, S. (2003). Peroxisome proliferator-activated receptor gamma agonists induce proteasome-dependent degradation of cyclin D1 and estrogen receptor alpha in MCF-7 breast cancer cells. *Cancer Res.,* **63,** 958–964.

ROBERTS-THOMSON, S.J. (2000). Peroxisome proliferator-activated receptors in tumorigenesis: targets of tumour promotion and treatment. *Immunol. Cell Biol.,* **78,** 436–441.

RUBIN, G.L., ZHAO, Y., KALUS, A.M. & SIMPSON, E.R. (2000). Peroxisome proliferator-activated receptor gamma ligands inhibit estrogen biosynthesis in human breast adipose tissue: possible implications for breast cancer therapy. *Cancer Res.,* **60,** 1604–1608.

SHEN, Z., PEEDIKAYIL, J., OLSON, G.K., SIEBERT, P.D. & FANG, Y. (2002). Multiple transcription factor profiling by enzyme-linked immunoassay. *Biotechniques,* **32,** 1168 1170–1172, 1174 passim.

WANG, X. & KILGORE, M.W. (2002). Signal cross-talk between estrogen receptor alpha and beta and the peroxisome proliferator-activated receptor gamma1 in MDA-MB-231 and MCF-7 breast cancer cells. *Mol. Cell. Endocrinol.,* **194,** 123–133.

YIN, F., WAKINO, S., LIU, Z., KIM, S., HSUEH, W.A., COLLINS, A.R., VAN HERLE, A.J. & LAW, R.E. (2001). Troglitazone inhibits growth of MCF-7 breast carcinoma cells by targeting G1 cell cycle regulators. *Biochem. Biophys. Res. Commun.,* **286,** 916–922.

(Received May 6, 2004
Revised July 27, 2004
Accepted August 11, 2004)