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Repression of β -catenin signaling by PPAR γ ligands

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Abstract

Aberrant activation of the Wnt/ β -catenin signaling pathway plays a crucial role in oncogenesis of various human malignancies. It has been demonstrated that there is a direct interaction between β -catenin and PPAR γ . Here we examined the effects of fifteen reported PPAR ligands in a reporter gene assay that is dependent on β -catenin activation of TCF/LEF transcription factors; only the thiazolidinedione PPAR γ agonists troglitazone, rosiglitazone and pioglitazone, and a non-thiazolidinedione PPAR γ activator GW1929 inhibited β -catenin-induced transcription in a PPAR γ dependent fashion. The results from mammalian one-hybrid experiments showed that functional PPAR γ was necessary for ligand-dependent inhibition of β -catenin transactivation. However, a PPAR γ activator Fmoc-Leu could not repress β -catenin-mediated signaling and its transactivation activity. These results indicate that activation of PPAR γ is necessary, but not sufficient, for the β -catenin antagonistic activity of a PPAR γ agonist, and that the inhibitory compounds interfere directly with β -catenin transactivation activity.

Keywords

PPAR γ ; β -catenin; troglitazone; rosiglitazone; GW1929

1. Introduction

The Wnt/ β -catenin signaling pathway has a central role in development and tissue homeostasis, and is abnormally regulated in many human diseases, including cancer. The β -catenin molecule plays a crucial function in Wnt signal transduction (Clevers, 2006; Moon et al., 2004; Nusse, 2005; Polakis, 2000). The stability of free cytoplasmic β -catenin is tightly regulated by a destruction complex, which is composed of the adenomatous polyposis coli protein (APC), the glycogen synthetase kinase-3 β enzyme (GSK-3 β), and the scaffolding protein axin. In the canonical Wnt pathway, extracellular Wnt proteins bind to a receptor complex, consisting of a member of the Frizzled (Fzd) family, and the low-density lipoprotein-receptor-related proteins (LRP) 5 or LRP6. Subsequently the cytoplasmic adapter protein disheveled (Dvl) binds to axin in the destruction complex, and prevents GSK-3 β phosphorylation of β -catenin. The unphosphorylated β -catenin accumulates in the cytoplasm and translocates to the nucleus, where it interacts with T cell (TCF) and lymphoid-enhancing (LEF) factors to activate transcription of Wnt target genes including c-myc, cyclin D1 and many others (Crawford et al., 1999; He et al., 1998; Shtutman et al., 1999).

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The peroxisome proliferator activated receptors (PPARs) belong to the superfamily of nuclear receptor transcription factors, and consist of three members, PPAR α , PPAR β/δ and PPAR γ (Kliwer et al., 2001). Upon activation by specific ligands, PPAR molecules form heterodimers with the retinoid-X receptor (RXR), to form a complex that regulates the transcriptional activity of target genes by binding to specific PPAR response element sequences (PPRE) within promoter regions. PPAR γ influences diverse biological processes, including glucose homeostasis, lipid metabolism, cellular proliferation, and differentiation (Debril et al., 2001; Lehrke and Lazar, 2005). PPAR γ ligands encompass a wide range of structurally diverse compounds, including naturally occurring and synthetic long chain polyunsaturated fatty acids, thiazolidinediones and some nonsteroidal anti-inflammatory drugs (NSAIDs) (Forman et al., 1995; Kliwer et al., 1995; Kliwer et al., 1997; Lehmann et al., 1995). In addition to its ligand-dependent activator function, PPAR γ can repress other signaling pathways, such as AP-1, NFAT, NF κ B, and STAT1, by direct interaction with other transcription factors, competition for coregulators, and blockade of corepressor clearance (Pascual et al., 2005; Welch et al., 2003).

Previous studies have demonstrated crosstalk between the β -catenin and PPAR γ signaling pathways. Girnun et al. reported that PPAR γ could suppress β -catenin levels and colon carcinogenesis, but only before mutational damage to the APC/ β -catenin pathway was evident (Girnun et al., 2002). Other studies showed that β -catenin could directly interact with PPAR γ and RXR α (Jansson et al., 2005; Liu et al., 2006; Lu et al., 2005; Xiao et al., 2003). The functional interaction between β -catenin and PPAR γ involved the TCF/LEF binding domain of β -catenin and a catenin binding domain (CBD) within PPAR γ itself (Liu et al., 2006). Earlier experiments from our laboratory indicated that repression of β -catenin signaling by NSAIDs required high-level expression of PPAR γ and RXR α (Lu et al., 2005). These studies indicated that anti-cancer activity of PPAR γ agonist may relate to its ability to inhibit β -catenin signaling. The PPAR γ ligands with the potential to inhibit β -catenin signaling should be candidate agents for chemoprevention and chemotherapy. Thus, it is important to determine the effects of PPAR γ ligands with diverse chemical structures on the β -catenin signaling pathway.

In this study, we examined the effects of a series of PPAR ligands on β -catenin signaling. The thiazolidinedione PPAR γ agonists, including troglitazone, rosiglitazone and pioglitazone, and a non-thiazolidinedione PPAR γ activator, GW1929, all inhibited β -catenin-mediated signaling. These PPAR γ ligands also inhibited the transactivation activity of β -catenin. Both effects required expression of a functional PPAR γ protein. However, other compounds that activated a PPAR γ dependent reporter gene, as well as a PPAR α agonist, had no β -catenin antagonistic activity. The results indicate that activation of PPAR γ is necessary but not sufficient for PPAR γ ligand-mediated repression of β -catenin signaling.

2. Materials and Methods

2.1. Chemical reagents

GW1929 (N-(2-Benzoylphenyl)-O-[2-(methyl-2-pyridinylamino)ethyl]-L-tyrosine hydrochloride), GW9662 (2-Chloro-5-nitro-N-phenylbenzamide), tetradecylthioacetic acid, Fmoc-Leu, L-165.041 (4-[3-(4-Acetyl-3-hydroxy-2-propylphenoxy)propoxy]phenoxyacetic acid) and Azelaoyl PAF were purchased from Sigma-Aldrich (St. Louis, MO). T0070907 (2-chloro-5-nitro-N-4-pyridinyl-benzamide) and LY171883 (1-[2-hydroxy-3-propyl-4-[4-(1H-tetrazol-5-yl)butoxy]phenyl]-ethanone) were purchased from Biomol Research Laboratories (Plymouth Meeting, PA). Rosiglitazone, pioglitazone, MCC-555 (5-[[6-[(2-fluorophenyl)methoxy]-2-naphthalenyl]methyl]-2,4-thiazolidinedione), and GW501516 ([2-methyl-4-[[4-methyl-2-[4-(trifluoromethyl)phenyl]-5-thiazolyl]methyl]thio]phenoxy]-acetic acid) were obtained from Axxora (San Diego, CA). Pseudolaric acid B and PPAR γ antagonist III (G3335,

dipeptide H-Trp-Glu-OH) were obtained from Calbiochem (La Jolla, CA). Troglitazone was a gift from Dr. David W. Rose (University of California San Diego).

2.2. Plasmids

The reporter plasmids TOPflash, (AOX)₃-TK-Luc and UAS-TK-Luc, and the expression vectors for disheveled (Dvl), β -catenin, PPAR γ , PPAR γ EA469, RXR α , VP16-PPAR γ and Gal4-PBP were previously described (Lu et al., 2005; Lu et al., 2004). The Gal4- β -catenin expression vector was prepared by ligating β -catenin cDNA into the SalI and BamHI sites of a Gal4 expression vector. The construct was sequenced to confirm its identity.

2.3. Cell culture and Transfection

The human embryonic kidney cell line HEK293 (American Type Culture Collection, Rockville, MD) was transfected using the FuGene transfection reagent (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions.

HEK293 cells were grown for at least 24 h in 12-well plates prior to transfection. At about 50% confluence, cells were transfected with 0.5 μ g of reporter plasmid, 0.1 μ g of control plasmid pCMX β gal, 0.1–0.2 μ g expression plasmids, and carrier DNA pcDNA3 plasmid for a total of 1 μ g/well. After overnight incubation, the cells were washed and given fresh medium that contained 0.5% FBS supplemented with the different PPAR γ ligands or solvent (DMSO). After 24 h, the cells were lysed in isotonic potassium phosphate buffer, pH 7.8, containing 1% Triton X-100, and luciferase activities were assayed in the presence of substrate using a microtiter plate luminometer (MicroBeta TriLux, Gaithersburg, MD). The luciferase values were normalized for variations in transfection efficiency using the β -galactosidase internal control. In the Results section, data are expressed as percentage of control luciferase or fold stimulation of luciferase activity compared to the basal level. All the transfection results represent means of a minimum of three independent transfections assayed in duplicate, $\pm$ the standard error of the mean (S.E.M.).

3. Results

3.1. Repression of β -catenin signaling by PPAR γ ligands

Our previous study demonstrated that NSAIDs could inhibit β -catenin signaling in a PPAR γ -dependent manner. The presence of RXR α strongly enhanced the β -catenin antagonistic activity of the NSAIDs (Lu et al., 2005). In order to determine if other PPAR ligands could modulate β -catenin signaling, 15 reported PPAR ligands were tested in a cell-based TOPflash reporter assay as described previously (Lu et al., 2005). The TOPflash reporter construct was transfected into HEK293 cells with expression plasmids for Dvl, PPAR γ and RXR α . As noted earlier, Dvl is an upstream activator of the β -catenin pathway and activates the TCF/LEF response elements by elevating β -catenin levels. Cells were treated with increasing concentrations of PPAR ligands (0.625–10 μ M) for 24 h. As shown in Table 1, the thiazolidinedione PPAR γ agonists, including troglitazone, rosiglitazone and pioglitazone, and a non-thiazolidinedione PPAR γ activator, GW1929, significantly inhibited Dvl-stimulated transcription. In contrast, PPAR γ antagonists and selective agonists for PPAR α and PPAR δ had little or no effect on Dvl-mediated TOPflash reporter activity (Table 1); nor did the PPAR γ activators azelaoyl PAF, MCC-555, pseudolaric acid B or Fmoc-Leu repress β -catenin-mediated signaling. Since troglitazone, rosiglitazone and GW1929 displayed the most potent inhibitory effects on β -catenin signaling, they were chosen for further experimentation. Fmoc-Leu was also chosen for further studies because of its opposite effect on β -catenin-mediated signaling.

The activation function 2 (AF-2) domain of nuclear receptors is necessary for ligand-dependent transactivation. To determine the role of AF-2 in PPAR γ ligand-mediated inhibition of β -catenin signaling, a mutant PPAR γ , which contains a point mutation in the AF2 domain that destroys its transactivation, transrepression, and coactivator activity, was analyzed in cotransfection assays (Li et al., 2000). The PPAR γ ligands were tested over a wide range of concentrations (5 nM–5 μ M). As shown in Figure 1, in the presence of wild-type PPAR γ and RXR α , three PPAR γ agonists (Troglitazone, Rosiglitazone and GW1929) inhibited β -catenin signaling in a concentration dependent manner. In contrast, their inhibitory effects were completely blocked by the mutant PPAR γ (Fig. 1B, 1C and 1D). This result reveals that PPAR γ agonist-induced repression of β -catenin signaling requires a functional activated PPAR γ . Interestingly, the PPAR γ activator Fmoc-Leu had the opposite effect on β -catenin-mediated transcription in the presence of wild-type PPAR γ or mutant PPAR γ (Fig. 1E).

3.2. Transcriptional activity of PPAR γ and β -catenin inhibition

To determine whether PPAR γ agonist-induced repression of β -catenin signaling correlated with the capacity to activate PPAR γ dependent transcription, we compared the effects of troglitazone, rosiglitazone, GW1929 and Fmoc-Leu on PPAR γ activation using a peroxisome proliferator response element (PPRE)-driven promoter reporter, (AOX) $_3$ -TK-Luc. All four PPAR γ activators increased the transcriptional activity of the PPAR γ reporter (Fig. 2). Fmoc-Leu was the most potent inducer in the PPRE-luciferase reporter system, but had no repressive effect on β -catenin-mediated signaling (Table 1 & Fig. 1E). Thus, activation of PPAR γ dependent transcription is necessary but not sufficient for the β -catenin antagonistic activity of PPAR γ agonists.

3.3. PPAR γ agonist-induced interaction of PPAR γ with PBP

The PPAR-binding protein (PBP) act as an anchor for the thyroid hormone receptor-associated protein/vitamin D $_3$ receptor-interacting protein (DRIP)/activator-recruited cofactor complex. To examine the effects of PPAR γ agonists on the interaction of PPAR γ with PBP, a mammalian two-hybrid assay comprised of Gal4-PBP and VP16-PPAR γ was used. In this assay, the reporter plasmid UAS-TK-Luc contains five copies of the Gal4 binding element. As shown in Figure 3, treatment with troglitazone, rosiglitazone and GW1929 induced an interaction between PPAR γ and PBP that was 3.8-7.6 fold above the activity observed in the absence of ligands. No signal was detected in the presence of the VP16-PPAR γ fusion factor alone. The PPAR γ activator Fmoc-Leu, however, had no effect in this reporter assay (Fig. 3), suggesting that its binding to PPAR γ could not induce its interaction with PBP.

3.4. PPAR γ agonist-induced repression of the transactivation activity of β -catenin

To determine directly the effects of PPAR γ agonists on β -catenin transactivation activity, a plasmid encoding a Gal4 DNA-binding domain (DBD) fused to β -catenin (Gal4- β -catenin) was constructed. In this system, the Gal4- β -catenin fusion protein-induced activation of a UAS-TK-Luc reporter was dependent on the activation domain of β -catenin. Transfection of an expression plasmid for Gal4- β -catenin led to more than 10-fold increase in expression of reporter gene (Fig. 4). Treatment with PPAR γ agonists had little or no direct effect on reporter gene activity (Fig. 4). However, in the presence of PPAR γ and RXR α , troglitazone, rosiglitazone and GW1929, but not Fmoc-Leu, suppressed the transactivation activity of β -catenin. Mutant PPAR γ completely abolished PPAR γ agonist-mediated repression of β -catenin transactivation (Fig. 4). These results confirm that PPAR γ ligand-mediated repression of β -catenin transactivation depends on a functional PPAR γ .

4. Discussion

Our previous studies demonstrated that NSAIDs inhibited Wnt/ β -catenin signaling in a PPAR γ and RXR α dependent manner (Lu et al., 2005). However, the mechanisms involved in PPAR γ -mediated repression of β -catenin signaling are still largely unknown. Although PPAR γ activation was reported to induce β -catenin degradation, we did not observe significant PPAR γ -induced changes in β -catenin expression at the tested concentrations (data not shown). Instead, results from mammalian one-hybrid experiments showed that a functional PPAR γ confers ligand-specific inhibition of β -catenin transactivation.

The PPAR γ ligands have chemopreventative and/or chemotherapeutic activity against a wide variety of cancer cells (Grommes et al., 2004; Koeffler, 2003). There may be multiple mechanisms by which the PPAR γ ligands exert their anti-cancer effect, such as by increasing expression of the cyclin dependent kinase inhibitors p21^{waf1} and p27^{kip1}, and by decreasing levels of protein phosphatase 2A, cyclins D₁ and E, inflammatory cytokines, and nuclear factor κ B (Altioek et al., 1997; Koeffler, 2003; Koga et al., 2001; Wang et al., 2001). Here we showed that three thiazolidinedione PPAR γ activators, troglitazone, rosiglitazone and pioglitazone, as well as a non-thiazolidinedione PPAR γ activator, GW1929, all repressed β -catenin dependent transcription. The inhibition of the Wnt/ β -catenin signaling mediated by PPAR γ ligands, may contribute to the anti-cancer activity of these drugs. Importantly, the PPAR γ ligand-induced repression of β -catenin signaling may be cell type-specific and depends on cellular levels of β -catenin, PPAR γ and RXR. Further studies are needed to test the effects of PPAR γ ligands on the Wnt/ β -catenin signaling pathway in cancer cells with high levels of β -catenin, PPAR γ and RXR.

Our studies reveal that PPAR γ activation and β -catenin inhibition are not equivalent. Some PPAR γ agonists such as Fmoc-Leu, azelaoyl PAF, MCC-555 and pseudolaric acid B had little or no repressive effects on β -catenin signaling. These results suggest that PPAR γ ligand-induced inhibition of β -catenin signaling is not simply a consequence of the nuclear hormone receptor's transcriptional activity. Therefore, it would be useful to examine the repressive effect of all known PPAR γ ligands on β -catenin signaling, in order to determine the structure activity relationship required for Wnt/ β -catenin antagonism.

The PPAR γ activator Fmoc-Leu is a N-protected leucine analogue, structurally different from the glitazones, which binds to and induces a change of PPAR γ confirmation, resulting in the differential recruitment of co-regulators (Rocchi et al., 2001). Consistent with these previous findings, Fmoc-leu was incapable of inducing the interaction of PPAR γ with PBP, unlike troglitazone, rosiglitazone and GW1929. Fmoc-Leu strongly activated the transcriptional activity of a PPRE reporter, but had no inhibitory effects on both β -catenin-mediated signaling and its transactivation activity. It is noteworthy that Fmoc-Leu actually enhanced β -catenin-mediated activation of TCF/LEF and its transactivation activity. This enhancing effect did not seem to depend on PPAR γ binding because a similar effect was observed in the absence of PPAR γ or in the presence of mutant PPAR γ . Like Fmoc-Leu, a PPAR γ antagonist GW9662 also exhibited an enhancing effect on β -catenin signaling (Table 1). Our preliminary result suggests that the enhancing effect of GW9662 is not dependent on PPAR γ (data not shown).

Collectively, these results confirm that activation of PPAR γ is not sufficient for ligand-induced repression of β -catenin signaling. One potential explanation for the data is that ligand-induced conformational changes of PPAR γ lead to altered cofactor recruitment. The cofactors required for repression of β -catenin signaling may be different from those that regulate PPAR γ transcriptional activity.

In summary, our studies provide insight but raise new questions regarding the mechanisms involved in PPAR γ ligand-induced repression of β -catenin signaling. Some PPAR γ agonists

may bind to a β -catenin/PPAR γ /RXR α complex and induce conformational changes that alter the pattern of cofactor recruitment. Further studies are required to characterize the roles of specific cofactors in PPAR γ ligand-induced inhibition of the Wnt/ β -catenin signal transduction pathway.

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References

- Altiock S, Xu M, Spiegelman BM. PPAR γ induces cell cycle withdrawal: inhibition of E2F/DP DNA-binding activity via down-regulation of PP2A. *Genes Dev* 1997;11:1987–1998. [PubMed: 9271121]
- Clevers H. Wnt/beta-catenin signaling in development and disease. *Cell* 2006;127:469–480. [PubMed: 17081971]
- Crawford HC, Fingleton BM, Rudolph-Owen LA, Goss KJ, Rubinfeld B, Polakis P, Matrisian LM. The metalloproteinase matrilysin is a target of beta-catenin transactivation in intestinal tumors. *Oncogene* 1999;18:2883–2891. [PubMed: 10362259]
- Debril MB, Renaud JP, Fajas L, Auwerx J. The pleiotropic functions of peroxisome proliferator-activated receptor gamma. *Journal of molecular medicine (Berlin, Germany)* 2001;79:30–47.
- Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM. 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. *Cell* 1995;83:803–812. [PubMed: 8521497]
- Girnun GD, Smith WM, Drori S, Sarraf P, Mueller E, Eng C, Nambiar P, Rosenberg DW, Bronson RT, Edelmann W, Kucherlapati R, Gonzalez FJ, Spiegelman BM. APC-dependent suppression of colon carcinogenesis by PPAR γ . *Proc Natl Acad Sci U S A* 2002;99:13771–13776. [PubMed: 12370429]
- Grommes C, Landreth GE, Heneka MT. Antineoplastic effects of peroxisome proliferator-activated receptor gamma agonists. *The lancet oncology* 2004;5:419–429. [PubMed: 15231248]
- He TC, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, Morin PJ, Vogelstein B, Kinzler KW. Identification of c-MYC as a target of the APC pathway. *Science New York, NY* 1998;281:1509–1512.
- Jansson EA, Are A, Greicius G, Kuo IC, Kelly D, Arulampalam V, Pettersson S. The Wnt/beta-catenin signaling pathway targets PPAR γ activity in colon cancer cells. *Proc Natl Acad Sci U S A* 2005;102:1460–1465. [PubMed: 15665104]
- Kliwer SA, Lenhard JM, Willson TM, Patel I, Morris DC, Lehmann JM. A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. *Cell* 1995;83:813–819. [PubMed: 8521498]
- Kliwer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahli W, Willson TM, Lenhard JM, Lehmann JM. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. *Proc Natl Acad Sci U S A* 1997;94:4318–4323. [PubMed: 9113987]
- Kliwer SA, Xu HE, Lambert MH, Willson TM. Peroxisome proliferator-activated receptors: from genes to physiology. *Recent progress in hormone research* 2001;56:239–263. [PubMed: 11237216]
- Koeffler HP. Peroxisome proliferator-activated receptor gamma and cancers. *Clin Cancer Res* 2003;9:1–9. [PubMed: 12538445]
- Koga H, Sakisaka S, Harada M, Takagi T, Hanada S, Taniguchi E, Kawaguchi T, Sasatomi K, Kimura R, Hashimoto O, Ueno T, Yano H, Kojiro M, Sata M. Involvement of p21(WAF1/Cip1), p27(Kip1), and p18(INK4c) in troglitazone-induced cell-cycle arrest in human hepatoma cell lines. *Hepatology Baltimore, Md* 2001;33:1087–1097.

- Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J Biol Chem* 1995;270:12953–12956. [PubMed: 7768881]
- Lehrke M, Lazar MA. The many faces of PPARgamma. *Cell* 2005;123:993–999. [PubMed: 16360030]
- Li M, Pascual G, Glass CK. Peroxisome proliferator-activated receptor gamma-dependent repression of the inducible nitric oxide synthase gene. *Molecular and cellular biology* 2000;20:4699–4707. [PubMed: 10848596]
- Liu J, Wang H, Zuo Y, Farmer SR. Functional interaction between peroxisome proliferator-activated receptor gamma and beta-catenin. *Molecular and cellular biology* 2006;26:5827–5837. [PubMed: 16847334]
- Lu D, Cottam HB, Corr M, Carson DA. Repression of beta-catenin function in malignant cells by nonsteroidal antiinflammatory drugs. *Proc Natl Acad Sci U S A* 2005;102:18567–18571. [PubMed: 16352713]
- Lu D, Zhao Y, Tawatao R, Cottam HB, Sen M, Leoni LM, Kipps TJ, Corr M, Carson DA. Activation of the Wnt signaling pathway in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A* 2004;101:3118–3123. [PubMed: 14973184]
- Moon RT, Kohn AD, De Ferrari GV, Kaykas A. WNT and beta-catenin signalling: diseases and therapies. *Nat Rev Genet* 2004;5:691–701. [PubMed: 15372092]
- Nusse R. Wnt signaling in disease and in development. *Cell Res* 2005;15:28–32. [PubMed: 15686623]
- Pascual G, Fong AL, Ogawa S, Gamliel A, Li AC, Perissi V, Rose DW, Willson TM, Rosenfeld MG, Glass CK. A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. *Nature* 2005;437:759–763. [PubMed: 16127449]
- Polakis P. Wnt signaling and cancer. *Genes Dev* 2000;14:1837–1851. [PubMed: 10921899]
- Rocchi S, Picard F, Vamecq J, Gelman L, Potier N, Zeyer D, Dubuquoy L, Bac P, Champy MF, Plunket KD, Leesnitzer LM, Blanchard SG, Desreumaux P, Moras D, Renaud JP, Auwerx J. A unique PPARgamma ligand with potent insulin-sensitizing yet weak adipogenic activity. *Molecular cell* 2001;8:737–747. [PubMed: 11684010]
- Shtutman M, Zhurinsky J, Simcha I, Albanese C, D'Amico M, Pestell R, Ben-Ze'ev A. The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway. *Proc Natl Acad Sci U S A* 1999;96:5522–5527. [PubMed: 10318916]
- Wang C, Fu M, D'Amico M, Albanese C, Zhou JN, Brownlee M, Lisanti MP, Chatterjee VK, Lazar MA, Pestell RG. Inhibition of cellular proliferation through IkappaB kinase-independent and peroxisome proliferator-activated receptor gamma-dependent repression of cyclin D1. *Molecular and cellular biology* 2001;21:3057–3070. [PubMed: 11287611]
- Welch JS, Ricote M, Akiyama TE, Gonzalez FJ, Glass CK. PPARgamma and PPARdelta negatively regulate specific subsets of lipopolysaccharide and IFN-gamma target genes in macrophages. *Proc Natl Acad Sci U S A* 2003;100:6712–6717. [PubMed: 12740443]
- Xiao JH, Ghosn C, Hinchman C, Forbes C, Wang J, Snider N, Cordrey A, Zhao Y, Chandraratna RA. Adenomatous polyposis coli (APC)-independent regulation of beta-catenin degradation via a retinoid X receptor-mediated pathway. *J Biol Chem* 2003;278:29954–29962. [PubMed: 12771132]

Fig. 1.

PPAR γ agonists repress β -catenin-mediated signaling in a PPAR γ dependent manner. (A) A schematic representation of the TOPflash reporter used in this study. TOPflash reporter was transfected into HEK293 cells with expression plasmids for Dvl, RXR α and PPAR γ or PPAR γ EA469 as indicated. After overnight incubation, transfected cells were treated for 24 h with increasing concentrations of troglitazone (B), rosiglitazone (C), GW1929 (D) and Fmoc-Leu (E), after which the reporter gene activity was measured. The results are expressed as % control TOPflash reporter activity $\pm$ S.E.M.

Fig. 2.

PPAR γ agonists stimulate PPRE reporter gene transcription. (A) A schematic representation of the PPRE reporter (AOX)₃-TK-Luc. (B) HEK293 cells were transfected with (AOX)₃-TK-Luc reporter with or without PPAR γ or PPAR γ EA469, and then were incubated with 5 μ M of different PPAR γ ligands, as indicated. The fold increase in luciferase activities $\pm$ S.E.M. was determined.

Fig. 3.

PPAR γ agonists induce the interaction of PPAR γ with its binding protein PBP. (A) Schematic representation of the mammalian two-hybrid system used in this study. (B) The reporter plasmid UAS-TK-Luc was transfected into HEK293 cells with a VP16-PPAR γ expression plasmid, either alone or with VP16-PPAR γ and Gal4-PBP. Transfected cells were treated with different PPAR γ activators (5 μ M each) as indicated. Cells were harvested 24 h after treatment, and then luciferase activities were determined.

Fig. 4.

PPAR γ agonists repress the transactivation activity of β -catenin in the presence of functional PPAR γ . (A) The reporter gene UAS-TK-Luc was transfected into HEK293 cells, together with a Gal4- β -catenin expression plasmid. Then cells were exposed to 5 μ M troglitazone, 5 μ M rosiglitazone, 5 μ M GW1929, 5 μ M Fmoc-Leu or vehicle alone for 24 h, harvested, and extracted for determination of luciferase activity. The results are expressed as fold induction of luciferase activity compared to the basal level $\pm$ S.E.M. (B) HEK293 cells were transfected with UAS-TK-Luc, the reporter construct, along with expression plasmids for Gal4- β -catenin, RXR α , PPAR γ or PPAR γ EA469, as indicated. After transfection, cells were grown with the PPAR γ agonists troglitazone, rosiglitazone, GW1929 and Fmoc-Leu (5 μ M each), and assayed for luciferase activity 24 h later. The results are expressed as % control reporter activity $\pm$ S.E.M.

Table 1

Inhibition of β -catenin signaling by PPAR ligands

Drugs	Functions	Percent control (%)					
		0.625 μ M	1.250 μ M	2.500 μ M	5.000 μ M	10.00 μ M	
Troglitazone	PPAR γ agonist	47.5 $\pm$ 3.5	35.9 $\pm$ 0.1	32.7 $\pm$ 0.1	29.3 $\pm$ 0.6	28.1 $\pm$ 0.3	
Rosiglitazone	PPAR γ agonist	29.9 $\pm$ 1.6	27.8 $\pm$ 1.4	27.6 $\pm$ 0.4	27.1 $\pm$ 1.1	27.8 $\pm$ 0.9	
Proglitazone	PPAR γ agonist	40.7 $\pm$ 1.6	35.0 $\pm$ 1.3	34.2 $\pm$ 0.7	35.0 $\pm$ 1.8	40.7 $\pm$ 1.0	
GW1929	PPAR γ agonist	30.2 $\pm$ 1.6	29.1 $\pm$ 1.1	28.7 $\pm$ 1.6	27.4 $\pm$ 2.1	27.4 $\pm$ 1.5	
Azelaoyl PAF	PPAR γ agonist	107.6 $\pm$ 6.6	102.0 $\pm$ 6.3	95.6 $\pm$ 6.4	97.5 $\pm$ 9.8	93.2 $\pm$ 4.6	
Fmoc-Leu	PPAR γ agonist	126.0 $\pm$ 1.4	139.9 $\pm$ 1.1	166.9 $\pm$ 2.6	183.7 $\pm$ 11.8	192.6 $\pm$ 6.3	
MCC-555	PPAR γ agonist	164.6 $\pm$ 3.4	132.5 $\pm$ 4.0	108.6 $\pm$ 0.8	109.0 $\pm$ 3.0	123.7 $\pm$ 3.3	
Pseudolaric acid B	PPAR γ & PPAR δ agonist	114.1 $\pm$ 5.0	82.2 $\pm$ 5.5	63.3 $\pm$ 3.2	61.2 $\pm$ 7.0	60.7 $\pm$ 5.2	
Tetradecylthioacetic acid	PPAR agonist	90.6 $\pm$ 5.9	92.4 $\pm$ 2.4	95.2 $\pm$ 3.2	97.8 $\pm$ 4.8	94.9 $\pm$ 3.3	
L165.041	PPAR δ agonist	95.7 $\pm$ 4.5	83.6 $\pm$ 2.2	74.8 $\pm$ 4.3	64.4 $\pm$ 1.9	53.7 $\pm$ 0.6	
GW501516	PPAR δ agonist	90.6 $\pm$ 6.2	87.2 $\pm$ 3.6	84.9 $\pm$ 4.9	80.0 $\pm$ 2.9	75.5 $\pm$ 4.5	
LY171883	PPAR α agonist	82.6 $\pm$ 14.7	88.7 $\pm$ 3.2	88.7 $\pm$ 2.4	78.3 $\pm$ 4.0	70.9 $\pm$ 3.5	
GW9662	PPAR γ antagonist	97.7 $\pm$ 0.1	110.8 $\pm$ 3.4	136.5 $\pm$ 7.7	167.5 $\pm$ 2.2	212.8 $\pm$ 1.5	
T0070907	PPAR γ antagonist	108.8 $\pm$ 6.3	106.6 $\pm$ 10.5	115.0 $\pm$ 4.3	118.1 $\pm$ 3.8	120.1 $\pm$ 7.5	
PPAR antagonist III (G3335)	PPAR γ antagonist	108.5 $\pm$ 3.0	95.4 $\pm$ 3.3	94.5 $\pm$ 5.3	91.2 $\pm$ 4.7	93.7 $\pm$ 3.1	

HEK293 cells were transfected with TOPflash reporter and expression plasmids encoding Dvl, PPAR γ , and RXR α . After overnight incubation, the cells were treated for 24 h with increasing concentrations of PPAR ligands. The reported data are the mean percentages of TOPflash activity relative to cells treated with the vehicle alone (vehicle treatment = 100%) $\pm$ S.E.M.