Hindawi Publishing Corporation
PPAR Research
Volume 2013, Article ID 701017, 8 pages
http://dx.doi.org/10.1155/2013/701017

Research Article

Pseudoginsenoside F11, a Novel Partial PPARγ Agonist, Promotes Adiponectin Oligomerization and Secretion in 3T3-L1 Adipocytes

Guoyu Wu,[1] Junyang Yi,[1] Ling Liu,[1] Pengcheng Wang,[1] Zhijie Zhang,[2] and Zhen Li[1]

[1] *MOE Key Laboratory of Bioinformatics, School of Life Sciences, Tsinghua University, Beijing 100084, China*
[2] *Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China*

Correspondence should be addressed to Zhen Li; lizhen@tsinghua.edu.cn

Received 3 August 2013; Revised 30 September 2013; Accepted 2 October 2013

Academic Editor: Richard P. Phipps

PPARγ is a nuclear hormone receptor that functions as a master regulator of adipocyte differentiation and development. Full PPARγ agonists, such as the thiazolidinediones (TZDs), have been widely used to treat type 2 diabetes. However, they are characterized by undesirable side effects due to their strong agonist activities. Pseudoginsenoside F11 (p-F11) is an ocotillol-type ginsenoside isolated from *Panax quinquefolium L.* (American ginseng). In this study, we found that p-F11 activates PPARγ with modest adipogenic activity. In addition, p-F11 promotes adiponectin oligomerization and secretion in 3T3-L1 adipocytes. We also found that p-F11 inhibits obesity-linked phosphorylation of PPARγ at Ser-273 by Cdk5. Therefore, p-F11 is a novel partial PPARγ agonist, which might have the potential to be developed as a new PPARγ-targeted therapeutics for type 2 diabetes.

1. Introduction

The nuclear hormone receptor PPARγ (peroxisome proliferator-activated receptor γ) is a ligand-activated transcription factor highly expressed in the adipose tissues [1]. By binding to PPARγ-responsive regulatory elements as heterodimers with retinoid X receptor (RXR), PPARγ regulates the expression of networks of genes involved in adipogenesis, lipid metabolism, inflammation, and maintenance of metabolic homeostasis [2]. PPARγ consists of an amino terminal activation domain (AF-1), a highly conserved DNA-binding domain (DBD), and a C-terminal ligand-binding domain (LBD) containing a ligand-dependent transactivation domain (AF-2) [3]. Ligand binding promotes a conformational change which allows for differential recruitment of cofactors and subsequent modulation of PPARγ activity [4, 5].

PPARγ is the pharmacological target of the insulin-sensitizing thiazolidinediones (TZDs) that have been widely used in the treatment of type 2 diabetes. TZDs function as selective PPARγ ligands and induce transcription of PPARγ-targeted genes [6]. Derivatives of TZD, such as rosiglitazone (Avandia) and pioglitazone (Actos), are highly effective in treating type 2 diabetes and are well tolerated by the majority of patients [1]. However, they are associated with various undesirable side effects, including weight gain, fluid retention, edema, congestive heart failure, and bone fracture [7, 8]. Long-term use of TZDs may be associated with increased risk of bladder cancer [9]. These limitations have raised substantial concerns and significantly impaired their future in many countries [10]. Therefore, it is critical to develop TZD substitutes for improved therapies of type 2 diabetes. Studies in animal models and in clinical trials have shown that the side effects of TZD can be minimized without loss of insulin sensitization by partial PPARγ agonists [8, 11–16].

Adiponectin is an insulin-sensitizing adipokine secreted specifically by the adipose tissues as high, medium, and low molecular weight forms (HMW, MMW, and LMW) [17, 18]. The HMW adiponectin is more metabolically active and has a more relevant role in insulin sensitivity and in protecting against diabetes [19–21]. The serum level of adiponectin inversely correlates with obesity and directly correlates with insulin sensitivity [22, 23]. Additionally, serum adiponectin levels increase with weight loss, caloric restriction, or TZD treatment [24–27]. The PPARγ agonists increase serum levels of adiponectin by upregulating the transcription of adiponectin through a PPARγ-responsive element (PPRE) present in the promoter of adiponectin [26, 28]. In addition, PPARγ agonists regulate adiponectin oligomerization and secretion by increasing the expression of Ero1-Lα and DsbA-L, which have been reported to promote adiponectin oligomerization and secretion [29, 30].

Pseudoginsenoside F11 (p-F11) is an ocotillol-type ginsenoside isolated from the roots and leaves of *Panax quinquefolium L.* (American ginseng) [31–33]. p-F11 has been demonstrated to antagonize the learning and memory deficits induced by morphine, scopolamine, and methamphetamine [34–37], suggesting that p-F11 might be a candidate for the treatment of drug abuse. With its antiamnesic effect, p-F11 might also serve as a potential therapeutic target for Alzheimer's disease [38].

p-F11 was identified as one of the natural compounds that can promote preadipocyte differentiation in our screen for partial PPARγ agonists. In the present study, we further examined the effect of p-F11 on adipogenesis and the transcriptional activity of PPARγ. We demonstrate that p-F11 is a novel partial PPARγ agonist. In addition, we found that p-F11 inhibits Cdk5-mediated phosphorylation of PPARγ and promotes the oligomerization and secretion of adiponectin. Thus, p-F11 is a potential PPARγ-targeted drug for the treatment of type 2 diabetes.

2. Materials and Methods

2.1. Materials. Pseudoginsenoside F11 (p-F11), rosiglitazone, GW9662, 3-isobutyl-1-methylxanthine, dexamethasone, insulin, Oil Red O, and antibody against β-actin were purchased from Sigma. PPARγ antibody and phospho-(Ser) CDKs substrate rabbit monoclonal antibody were purchased from Santa Cruz or Cell Signaling, respectively. TNF-α was purchased from Sino Biological.

2.2. RNA Isolation and Real-Time PCR. Total RNA was isolated from 3T3-L1 adipocytes and quantitative real-time PCR was used as described previously [39]. PCR reactions were carried out in an ABI PRISM 7500 real-time PCR system. The expression levels of adiponectin and PPARγ were normalized using β-actin as an internal control.

2.3. Cell Culture, Cell Differentiation, and Treatment. 3T3-L1 preadipocytes (ATCC) and 293T cells were grown in DMEM (Invitrogen) supplemented with 10% FBS (Hyclone) at 37°C in 5% CO_2 and 3T3-L1 preadipocytes were induced to differentiate into mature adipocytes by the standard protocol as previously described [39, 40]. In order to examine the effect on differentiation, p-F11 or rosiglitazone was added to the culture medium in the presence or absence of GW9662 during differentiation. On Day 8 after differentiation, the lipid droplets in the cells were stained and quantified as previously described [40].

To examine the effect of p-F11 on adiponectin oligomerization and secretion, mature 3T3-L1 adipocytes were starved in a serum-free medium containing 0.05% BSA for 24 h, followed by treatment with p-F11 for another 24 h.

To examine the effect on PPARγ phosphorylation at Ser-273, 3T3-L1 adipocytes were pretreated with TNF-α, followed by treatment with p-F11 or rosiglitazone for 1 hour, as described previously [11].

2.4. SDS-PAGE and Western Blotting Analysis. Cell lysates or culture medium of 3T3-L1 adipocytes were subjected to 2–15% gradient gel electrophoresis under nonreducing and nonheat-denaturing conditions as described previously [17, 39, 40]. Adiponectin oligomers and the total amount of adiponectin were detected using antibodies against the globular domain or the N-terminal peptide of adiponectin. PPARγ was detected using antibodies specific for PPARγ, and phosphorylated PPARγ at Ser-273 was detected using anti-CDK substrate antibody after immunoprecipitation with anti-PPARγ antibody [11]. The amount of p-PPARγ, total PPARγ, total adiponectin, and adiponectin oligomers was quantified by analyzing the bands on the western blots using NIH ImageJ software. All experiments were performed at least three times and representative results were presented. Results are expressed as the means ± SD. Student's t-test was used for statistical analyses; P value < 0.05 was considered to be statistically significant.

2.5. Luciferase Reporter Assay. 293T cells were transfected with PPRE-TK-Luciferase reporter along with PPARγ and RXRα expression vectors. 24 hours after transfection, the cells were treated with p-F11 or rosiglitazone in the presence or absence of GW9662. The cells were harvested after treatment for 24 hours. A reporter luciferase assay kit (Promega) was used to measure luciferase activity according to the manufacturer's instructions. Luciferase activities were normalized to Renilla activities cotransfected as an internal control [40].

3. Results

3.1. p-F11 Is a Partial PPARγ Agonist with Modest Adipogenic Activity. To examine the effect of p-F11 on differentiation, 3T3-L1 preadipocytes were induced to differentiate in the presence of p-F11. Rosiglitazone (Rosi), which was reported to activate PPARγ and promote preadipocyte differentiation [6], was used as a positive control in all of our experiments.

We found that p-F11 promoted the differentiation of 3T3-L1 preadipocytes. The number of lipid droplets, evaluated by Oil Red O staining, was increased by p-F11 in a dose-dependent way (Figure 1(a)). However, 40 μM of p-F11 induced adipogenesis to a lesser extent than 0.5 μM Rosi (Figure 1(b)), indicating that p-F11 is less potent than Rosi in promoting adipogenesis.

PPARγ is a dominant regulator of adipocyte differentiation [41]. To determine whether PPARγ is involved in p-F11-promoted differentiation, 3T3-L1 preadipocytes were induced to differentiate in the presence of both p-F11 and GW9662, a specific PPARγ antagonist. We found that the effect of p-F11 on differentiation was completely abolished by GW9662 (Figures 1(a) and 1(b)). This result suggested that p-F11 promotes differentiation by activating PPARγ.

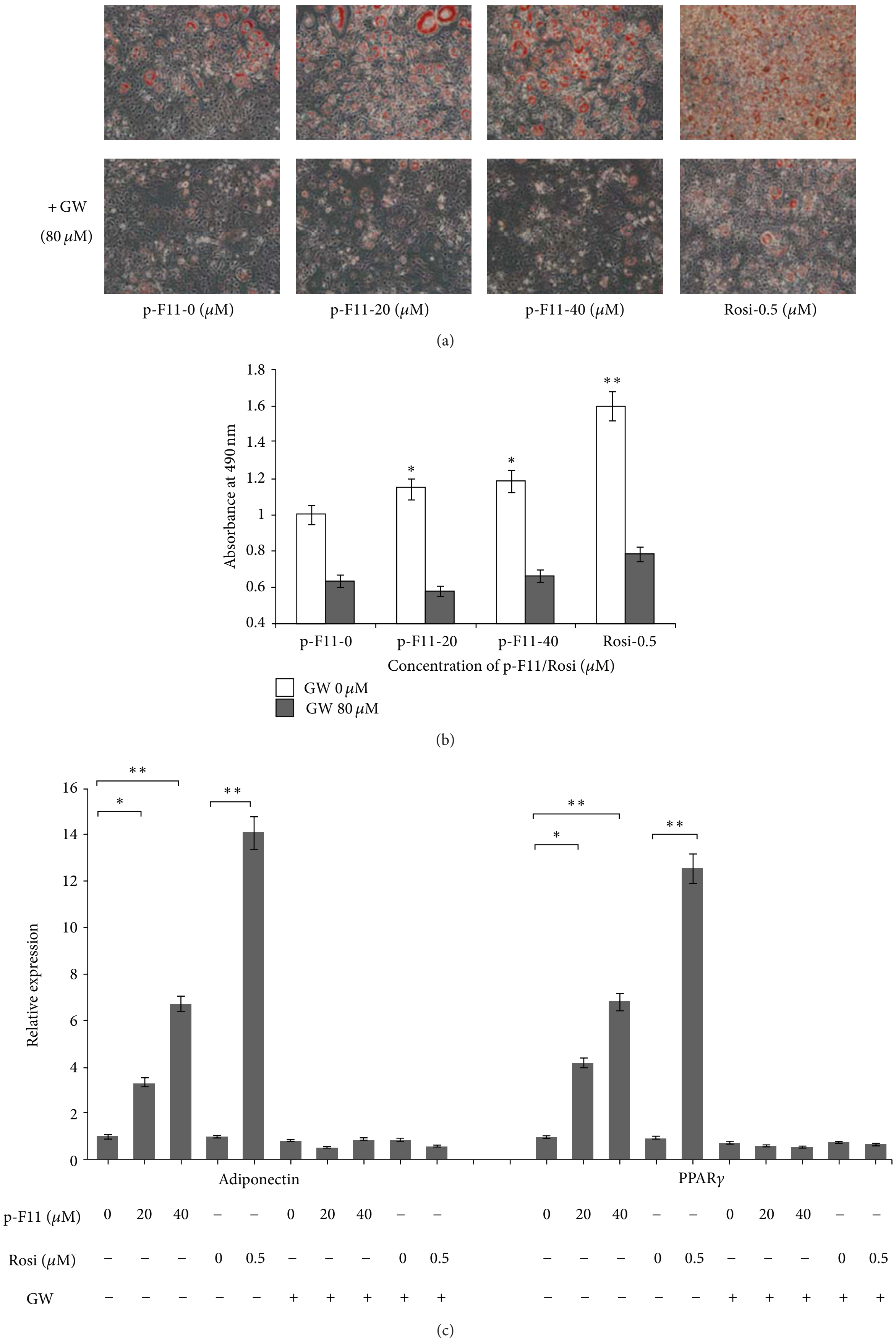
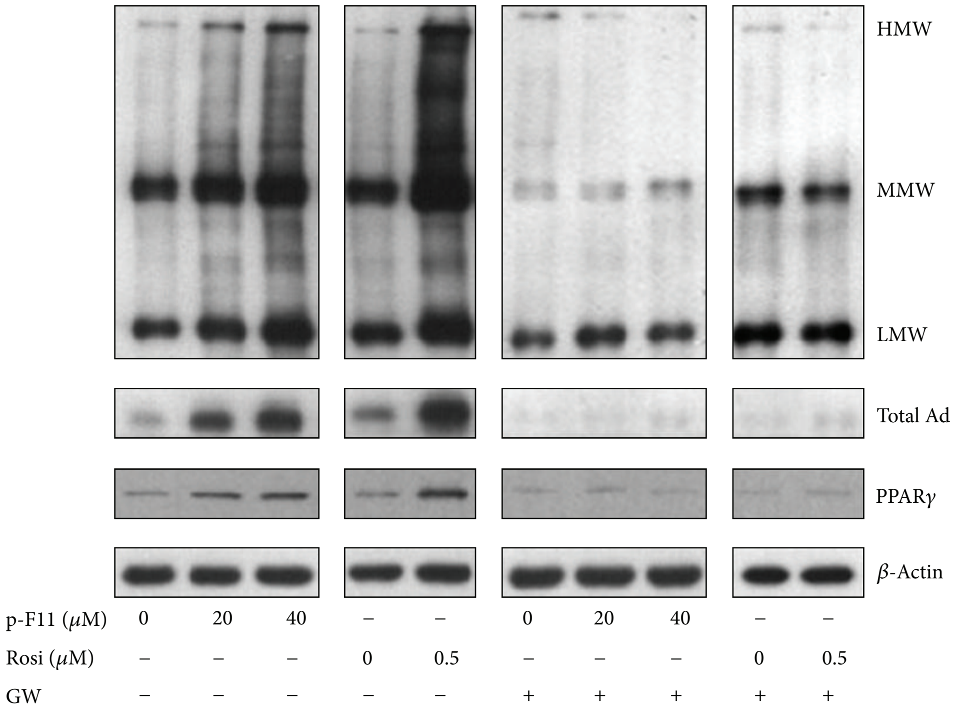


Figure 1: Continued.



(d)

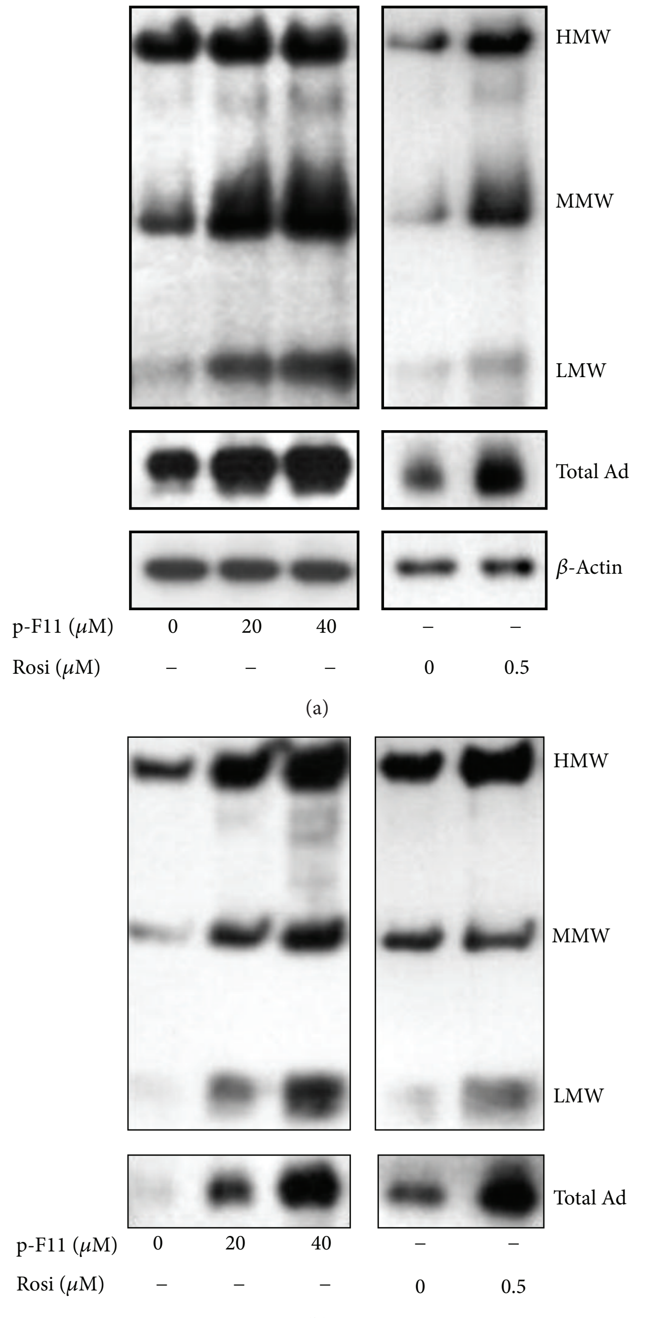
Figure 1: p-F11 promotes preadipocyte differentiation by activating PPARγ. 3T3-L1 preadipocytes were induced to differentiate with 0, 20, 40 μM Pseudoginsenoside F11 (p-F11) or 0.5 μM rosiglitazone (Rosi) in the absence or presence of 20 μM GW9662 for 8 days. (a) The cells were stained with Oil Red O. Quantification of the staining results was presented in (b). Results (mean ± SD, $n = 3$) were expressed as a percentage of the control (p-F11 0 μM). $^{*}P < 0.05$; $^{**}P < 0.01$. (c) The mRNA level of adiponectin and PPARγ was examined by quantitative real-time PCR. The results were expressed relative to β-actin and presented as a percentage of the control. (d) The cell lysates were subjected to 2%–15% gradient gel electrophoresis under nonreducing and non-heat-denaturing conditions to detect the three oligomeric forms of adiponectin (LMW, MMW, and HMW) (top panel). The amount of total adiponectin (Total Ad), PPARγ, or actin was detected with antibodies against the N-terminal peptide of adiponectin, PPARγ, or β-actin, respectively.

To further investigate the effect of p-F11 on differentiation, we examined the expression of PPARγ as well as adiponectin, which is a PPARγ-responsive gene. Similar to Rosi, p-F11 increased the mRNA and protein level of both PPARγ and adiponectin (Figures 1(c) and 1(d)). In addition, the level of different adiponectin oligomers (LMW, MMW, and HMW) was increased by p-F11 dose dependently (Figure 1(d)). GW9662 blocked the effects of p-F11 or Rosi (Figures 1(c) and 1(d)). Therefore, p-F11 upregulates the expression and oligomerization of adiponectin by activating PPARγ during the differentiation of 3T3-L1 preadipocytes.

3.2. p-F11 Promotes Adiponectin Oligomerization and Secretion in 3T3-L1 Adipocytes. To examine the effect of p-F11 on adiponectin in mature adipocytes, we treated 3T3-L1 adipocytes with p-F11 or rosiglitazone for 24 hours. The cellular level of adiponectin oligomers was increased by p-F11 in a dose-dependent way (Figure 2(a)). Furthermore, p-F11 increased the secretion of different adiponectin oligomers, particularly the HMW adiponectin, in a way similar to rosiglitazone (Figure 2(b)). These results suggested that p-F11 promotes the oligomerization and secretion of adiponectin in mature adipocytes.

3.3. p-F11 Exhibits PPARγ-Activating Activity in 293T Cells. To further demonstrate the PPARγ-activating activity of p-F11, we examined the effect of p-F11 on PPARγ transcriptional activity by luciferase reporter assays. 293T cells were transfected with PPRE-TK-Luciferase reporter along with PPARγ and RXRα expression vectors, followed by treatment with p-F11 for 24 hours. p-F11 dose dependently increased the transcriptional activity of PPARγ, which was abrogated by GW9662 (Figure 3). However, p-F11 exhibited lower transcriptional activity than Rosi. These results further demonstrated that p-F11 is a partial PPARγ agonist.

3.4. p-F11 Inhibits Obesity-Linked Phosphorylation of PPARγ at Ser-273 in 3T3-L1 Adipocytes. In addition to their capacity to enhance the transcriptional activity of PPARγ, PPARγ agonists have a separable biochemical activity, blocking the obesity-linked phosphorylation of PPARγ at Ser-273 by Cdk5. This phosphorylation results in the dysregulation of a subset of beneficial PPARγ-regulated genes, such as adiponectin, that are known to be associated with insulin sensitization [11]. The insulin-sensitizing effects of PPARγ agonists are more closely correlated with their ability to inhibit phosphorylation of PPARγ at Ser-273 [12–14].



(a)

(b)

Figure 2: p-F11 promotes adiponectin oligomerization and secretion in 3T3-L1 adipocytes. 3T3-L1 adipocytes were treated with p-F11 or Rosi for 24 hours. The amount of each oligomer and total adiponectin in the cell lysates (a) or in the culture medium (b) were detected as described in Figure 1(d).

To examine the effect of p-F11 on Ser-273 phosphorylation of PPARγ, 3T3-L1 adipocytes were first treated with TNF-α to induce the phosphorylation, followed by treatment with p-F11 or Rosi. We found that p-F11 dose dependently decreased Ser-273 phosphorylation of PPARγ (Figure 4(a)). Approximately 50% inhibition was seen with 80 μM p-F11 (Figure 4(b)). Therefore, p-F11 inhibits phosphorylation of PPARγ at Ser-273.

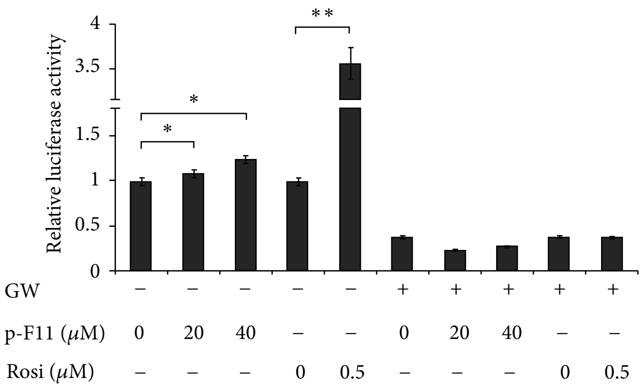


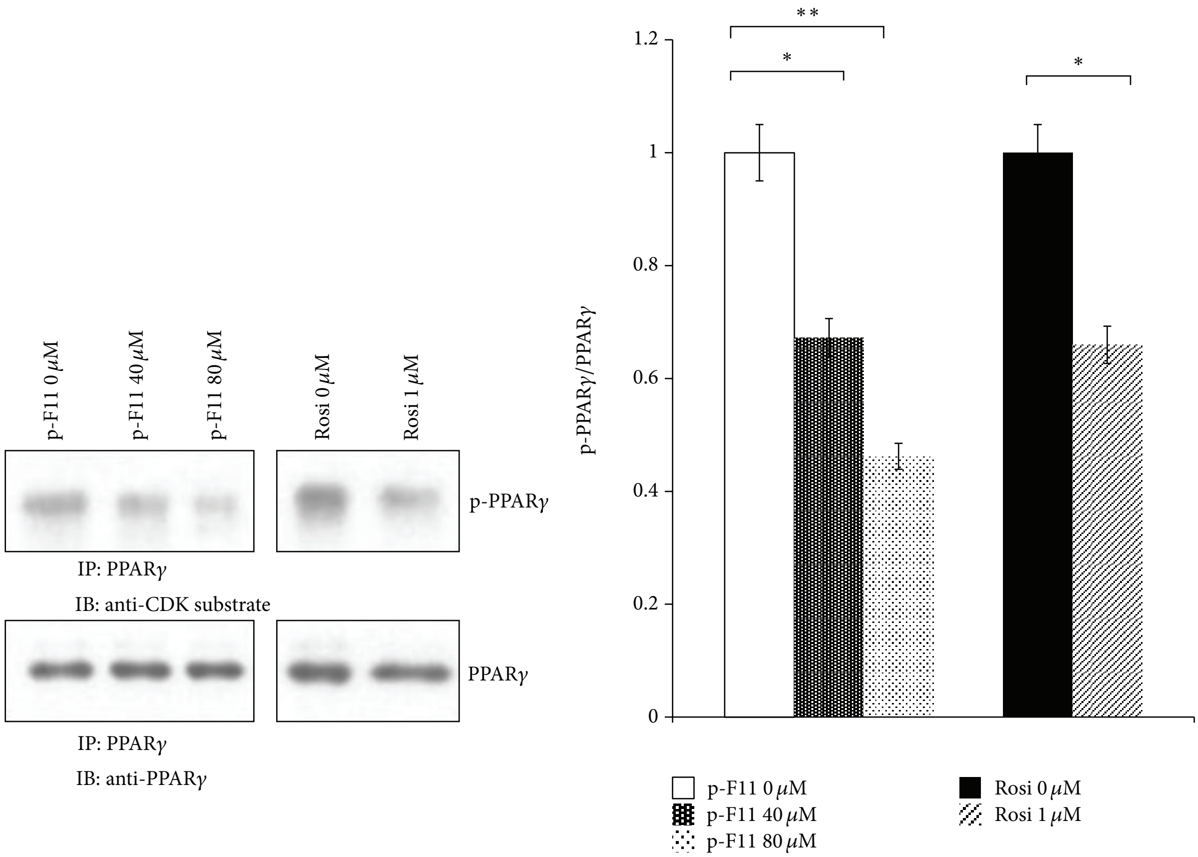
Figure 3: p-F11 exhibits PPARγ-activating activities in 293T cells. 293T cells were transfected with PPRE-TK-Luciferase reporter along with PPARγ and RXRα expression vectors for 24 hours. The cells were treated with p-F11 or Rosi in the presence or absence of GW9662 for another 24 hours. The cell extracts were subjected to luciferase assay. The results were presented as a percentage of the control (p-F11 0 μM).

4. Discussion

In this study, we found that p-F11 promoted the differentiation of 3T3-L1 preadipocytes, which was completely inhibited by GW9662 (Figure 1). This result suggested that p-F11 promoted adipogenesis by activating PPARγ. We also found that p-F11 promoted adiponectin oligomerization and secretion in 3T3-L1 adipocytes (Figure 2). Furthermore, p-F11 activated the transcriptional activity of PPARγ in the reporter assay (Figure 3). Therefore, PPARγ is a novel PPARγ agonist. However, 40 μM of p-F11 elicited weaker adipogenic and transcriptional activity than 0.5 μM of Rosi (Figures 1 and 3). Therefore, p-F11 is a partial PPARγ agonist.

Like other PPARs, PPARγ has a large ligand binding pocket which allows it to accommodate a wide range of ligands, including endogenous ligands such as native and modified fatty acids and prostaglandins [42]. Ligand binding induces a large conformational change in helix 12 of LBD of PPARγ, which creates a hydrophobic cleft on the surface of the proteins that serves as a high affinity docking site to recruit transcriptional coactivators [4]. The partial agonist activity of GQ-16 results from its weak ability to stabilize helix 12 of PPARγ, which is different from the binding mode of TZDs [12]. INT131, another partial PPARγ agonist with robust glucose-lowering activity and reduced side effects, forms hydrophobic contacts with the ligand-binding pocket without direct hydrogen-bonding interactions to key residues in helix 12 that are characteristic of full agonists [43]. With its unique structure, it remains to be determined how p-F11 interacts with PPARγ.

Ser-273 is situated immediately adjacent to the first β-sheet of PPARγ, which has been shown to mediate contacts between PPARγ and RXRα. Phosphorylation of Ser-273 by Cdk5 disrupts the contacts, leading to decreased expression of a subset of PPARγ-regulated genes which are known to be associated with insulin sensitization [11]. Therefore, Ser-273 phosphorylation is a key determinant of whole body



(a)

(b)

Figure 4: p-F11 inhibits PPARγ phosphorylation at Ser-273. 3T3-L1 adipocytes were pretreated with TNF-α, followed by treatment with p-F11 or Rosi for 1 hour. Phosphorylated PPARγ at Ser-273 (p-PPARγ) was detected using anti-CDK substrate antibody after immunoprecipitation with anti-PPARγ antibody. The amounts of p-PPARγ and total PPARγ were quantified and p-PPARγ/PPARγ ratio was presented as a percentage of the control (p-F11 0 μM or Rosi 0 μM).

insulin sensitivity [11, 44, 45]. TZDs, such as rosiglitazone and pioglitazone, are synthetic ligands that function as strong agonists on PPARγ and potent insulin sensitizers. However, they have several undesirable side effects, such as weight gain and edema. New classes of antidiabetic drugs can be developed by specifically targeting the Cdk5-mediated phosphorylation of PPARγ at Ser-273. Several PPARγ ligands, with or without classical agonist properties, were identified so far. GQ-16, MRL24, and amorfrutins are partial PPARγ agonists, whereas SR1664 lacks classical transcriptional agonism. They all inhibit Ser-273 phosphorylation of PPARγ [11–14]. GQ-16 has been shown to do so by stabilizing the helix 3 and β-sheet region of PPARγ, shielding Ser-273 from phosphorylation by Cdk5 [12]. All these agonists promote insulin sensitization without weight gain and other some unwanted side effects, demonstrating that the insulin-sensitizing effects of these PPARγ ligands are derived from their capacity to inhibit phosphorylation. In other words, the undesirable side effects of TZD drugs might be due to their strong agonist actions. Therefore, moderate activation of PPARγ might be better to uncouple the insulin-sensitizing effects from the adverse side effects. p-F11 exhibits weak transcriptional activity compared to rosiglitazone (Figures 1 and 3). On the other hand, p-F11 is very potent in blocking Cdk5-mediated phosphorylation of PPARγ, with higher concentrations being as efficacious as Rosi (Figure 4). p-F11 also promotes adiponectin oligomerization and secretion (Figure 2). We are currently examining the effect of p-F11 on the insulin sensitivity of diabetic mice.

Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolium L.*) are perennial aromatic herbs that are widely used in oriental medicine and have been demonstrated to have various health benefits including diabetes treatment. For example, American ginseng has been shown to be effective in improving glycemic control in type 2 diabetes [46]. Only present in American ginseng, p-F11 has been used for the unambiguous identification of Asian ginseng and American ginseng [32, 33]. p-F11 has been reported to exhibit neuroprotective and antiamnesic effects [34–37]. In this paper, we demonstrated that p-F11 is a partial PPARγ agonist, which is the first natural compound in American ginseng to have this activity. p-F11 does not seem to have any toxic effects on cell viability during differentiation or in mature adipocytes (data not shown). The fact that p-F11 exhibits partial efficacy in activating PPARγ, but increases adiponectin secretion and inhibits obesity-linked phosphorylation of PPARγ, makes it a potential therapeutic agent for the treatment of type 2 diabetes.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors thank Professor Jang Hyun Choi and Professor Bruce Spiegelman for their valuable advice on detecting Cdk5-mediated phosphorylation of PPARγ. This work was supported by the National Science Foundation for Fostering Talents in Basic Research of the NSFC (no. J1030622), the NSFC (nos. 30470354 and 30570356), and the Program for New Century Excellent Talents in University (NCET).

References

[1] M. Ahmadian, J. M. Suh, N. Hah et al., "PPARγ signaling and metabolism: the good, the bad and the future," *Nature Medicine*, vol. 19, no. 5, pp. 557–566, 2013.

[2] M. Lehrke and M. A. Lazar, "The many faces of PPARgamma," *Cell*, vol. 123, no. 6, pp. 993–999, 2005.

[3] T. M. Willson, M. H. Lambert, and S. A. Kliewer, "Peroxisome proliferator-activated receptor γ and metabolic disease," *Annual Review of Biochemistry*, vol. 70, pp. 341–367, 2001.

[4] L. Nagy and J. Schwabe, "Mechanism of the nuclear receptor molecular switch," *Trends in Biochemical Sciences*, vol. 29, no. 6, pp. 317–324, 2004.

[5] C. K. Glass and M. G. Rosenfeld, "The coregulator exchange in transcriptional functions of nuclear receptors," *Genes & Development*, vol. 14, no. 2, pp. 121–141, 2000.

[6] J. M. Lehmann, L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer, "An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ)," *The Journal of Biological Chemistry*, vol. 270, no. 22, pp. 12953–12956, 1995.

[7] R. A. Defronzo, "Banting Lecture. from the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus," *Diabetes*, vol. 58, no. 4, pp. 773–795, 2009.

[8] B. L. Blazer-Yost, "PPARgamma agonists: blood pressure and edema," *PPAR Research*, vol. 2010, Article ID 785369, 5 pages, 2010.

[9] R. Mamtani, K. Haynes, W. B. Bilker et al., "Association between longer therapy with thiazolidinediones and risk of bladder cancer: a cohort study," *Journal of the National Cancer Institute*, vol. 104, no. 18, pp. 1411–1421, 2012.

[10] D. Jones, "Potential remains for PPAR-targeted drugs," *Nature Reviews Drug Discovery*, vol. 9, no. 9, pp. 668–669, 2010.

[11] J. H. Choi, A. S. Banks, J. L. Estall et al., "Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPARγ by Cdk5," *Nature*, vol. 466, no. 7305, pp. 451–456, 2010.

[12] A. A. Amato, S. Rajagopalan, J. Z. Lin et al., "GQ-16, a novel peroxisome proliferator-activated receptor γ (PPARγ) ligand, promotes insulin sensitization without weight gain," *The Journal of Biological Chemistry*, vol. 287, no. 33, pp. 28169–28179, 2012.

[13] J. H. Choi, A. S. Banks, T. M. Kamenecka et al., "Antidiabetic actions of a non-agonist PPARγ ligand blocking Cdk5-mediated phosphorylation," *Nature*, vol. 477, no. 7365, pp. 477–481, 2011.

[14] C. Weidner, J. C. de Groot, A. Prasad et al., "Amorfrutins are potent antidiabetic dietary natural products," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 19, pp. 7257–7262, 2012.

[15] D. H. Lee, H. Huang, K. Choi, C. Mantzoros, and Y.-B. Kim, "Selective PPARγ modulator INT131 normalizes insulin signaling defects and improves bone mass in diet-induced obese mice," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 302, no. 5, pp. E552–E560, 2012.

[16] A. Chandalia, H. J. Clarke, L. E. Clemens et al., "MBX-102/JNJ39659100, a novel non-TZD selective partial PPAR-γ agonist lowers triglyceride independently of PPAR-α activation," *PPAR Research*, vol. 2009, Article ID 706852, 12 pages, 2009.

[17] H. Waki, T. Yamauchi, J. Kamon et al., "Impaired multimerization of human adiponectin mutants associated with diabetes—molecular structure and multimer formation of adiponectin," *The Journal of Biological Chemistry*, vol. 278, no. 41, pp. 40352–40363, 2003.

[18] T.-S. Tsao, E. Tomas, H. E. Murrey et al., "Role of disulfide bonds in Acrp30/adiponectin structure and signaling specificity—different oligomers activate different signal transduction pathways," *The Journal of Biological Chemistry*, vol. 278, no. 50, pp. 50810–50817, 2003.

[19] U. B. Pajvani, M. Hawkins, T. P. Combs et al., "Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity," *The Journal of Biological Chemistry*, vol. 279, no. 13, pp. 12152–12162, 2004.

[20] Y. Wang, M.-H. Yau, A. M. Xu, and K. S. L. Lam, "Post-translational modifications of adiponectin: mechanisms and functional implications," *Biochemical Journal*, vol. 409, no. 3, pp. 623–633, 2008.

[21] T. Yamauchi and T. Kadowaki, "Adiponectin receptor as a key player in healthy longevity and obesity-related diseases," *Cell Metabolism*, vol. 17, no. 2, pp. 185–196, 2013.

[22] Y. Arita, S. Kihara, N. Ouchi et al., "Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity," *Biochemical and Biophysical Research Communications*, vol. 257, no. 1, pp. 79–83, 1999.

[23] K. Hotta, T. Funahashi, Y. Arita et al., "Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 20, no. 6, pp. 1595–1599, 2000.

[24] T. Bobbert, H. Rochlitz, U. Wegewitz et al., "Changes of adiponectin oligomer composition by moderate weight reduction," *Diabetes*, vol. 54, no. 9, pp. 2712–2719, 2005.

[25] T. P. Combs, A. H. Berg, M. W. Rajala et al., "Sexual differentiation, pregnancy, calorie restriction, and aging affect the adipocyte-specific secretory protein adiponectin," *Diabetes*, vol. 52, no. 2, pp. 268–276, 2003.

[26] N. Maeda, M. Takahashi, T. Funahashi et al., "PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein," *Diabetes*, vol. 50, no. 9, pp. 2094–2099, 2001.

[27] J. M. Tishinsky, D. J. Dyck, and L. E. Robinson, "Lifestyle factors increasing adiponectin synthesis and secretion," *Vitamins and Hormones*, vol. 90, pp. 1–30, 2012.

[28] M. Iwaki, M. Matsuda, N. Maeda et al., "Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors," *Diabetes*, vol. 52, no. 7, pp. 1655–1663, 2003.

[29] M. Liu, L. Zhou, A. Xu et al., "A disulfide-bond A oxidoreductase-like protein (DsbA-L) regulates adiponectin multimerization," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 47, pp. 18302–18307, 2008.

[30] L. Qiang, H. Wang, and S. R. Farmer, "Adiponectin secretion is regulated by SIRT1 and the endoplasmic reticulum oxidoreductase ero1-l alpha," *Molecular and Cellular Biology*, vol. 27, no. 13, pp. 4698–4707, 2007.

[31] R.-M. Yu, Q.-X. Jin, H. Sun, W.-C. Ye, and Y. Zhao, "The growth characteristics and ginsenosides isolation of suspension-cultured crown gall of Panax quinquefolium," *Chinese Journal of Biotechnology*, vol. 21, no. 5, pp. 754–758, 2005.

[32] T. W. Chan, P. P. But, S. W. Cheng, I. M. Y. Kwok, F. W. Lau, and H. X. Xu, "Differentiation and authentication of Panax ginseng, Panax quinquefolius, and ginseng products by using HPLC/MS," *Analytical Chemistry*, vol. 72, no. 6, pp. 1281–1287, 2000.

[33] W. Li, C. Gu, H. Zhang et al., "Use of high-performance liquid chromatography-tandem mass spectrometry to distinguish Panax ginseng C. A. Meyer (Asian ginseng) and Panax quinquefolius L. (North American ginseng)," *Analytical Chemistry*, vol. 72, no. 21, pp. 5417–5422, 2000.

[34] Z. Li, C. F. Wu, G. Pei, Y. Y. Guo, and X. Li, "Antagonistic effect of pseudoginsenoside-F11 on the behavioral actions of morphine in mice," *Pharmacology Biochemistry and Behavior*, vol. 66, no. 3, pp. 595–601, 2000.

[35] Y. Hao, J. Y. Yang, C. F. Wu, and M. F. Wu, "Pseudoginsenoside-F11 decreases morphine-induced behavioral sensitization and extracellular glutamate levels in the medial prefrontal cortex in mice," *Pharmacology Biochemistry and Behavior*, vol. 86, no. 4, pp. 660–666, 2007.

[36] Z. Li, Y. Y. Guo, C. F. Wu, X. Li, and J. H. Wang, "Protective effects of pseudoginsenoside-F11 on scopolamine-induced memory impairment in mice and rats," *Journal of Pharmacy and Pharmacology*, vol. 51, no. 4, pp. 435–440, 1999.

[37] C. F. Wu, Y. L. Liu, M. Song et al., "Protective effects of pseudoginsenoside-F11 on methamphetamine-induced neurotoxicity in mice," *Pharmacology Biochemistry and Behavior*, vol. 76, no. 1, pp. 103–109, 2003.

[38] C. M. Wang, M. Y. Liu, F. Wang et al., "Anti-amnesic effect of pseudoginsenoside-F11 in two mouse models of Alzheimer's disease," *Pharmacology Biochemistry and Behavior*, vol. 106, pp. 57–67, 2013.

[39] Y. Li, P. C. Wang, Y. Zhuang et al., "Activation of AMPK by berberine promotes adiponectin multimerization in 3T3-L1 adipocytes," *FEBS Letters*, vol. 585, no. 12, pp. 1735–1740, 2011.

[40] Z. F. Chen, L. Zhang, J. Y. Yi et al., "Promotion of adiponectin multimerization by emodin: a novel AMPK activator with PPARγ-agonist activity," *Journal of Cellular Biochemistry*, vol. 113, no. 11, pp. 3547–3558, 2012.

[41] P. Tontonoz, E. D. Hu, and B. M. Spiegelman, "Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor," *Cell*, vol. 79, no. 7, pp. 1147–1156, 1994.

[42] R. T. Gampe Jr., V. G. Montana, M. H. Lambert et al., "Asymmetry in the PPARgamma/RXRalpha crystal structure reveals the molecular basis of heterodimerization among nuclear receptors," *Molecular Cell*, vol. 5, no. 3, pp. 545–555, 2000.

[43] A. Motani, Z. Wang, J. Weiszmann et al., "INT131: a selective modulator of PPAR gamma," *Journal of Molecular Biology*, vol. 386, no. 5, pp. 1301–1311, 2009.

[44] Z. E. Floyd and J. M. Stephens, "Controlling a master switch of adipocyte development and insulin sensitivity: covalent modifications of PPARγ," *Biochimica et Biophysica Acta*, vol. 1822, no. 7, pp. 1090–1095, 2012.

[45] J. P. Whitehead, "Diabetes: new conductors for the peroxisome proliferator-activated receptor γ (PPARγ) orchestra," *The International Journal of Biochemistry & Cell Biology*, vol. 43, no. 8, pp. 1071–1074, 2011.

[46] I. Mucalo, D. Rahelic, E. Jovanovski et al., "Effect of American ginseng (Panax quinquefolius L.) on glycemic control in type 2 diabetes," *Collegium Antropologicum*, vol. 36, no. 4, pp. 1435–1440, 2012.