Ectopic expression of porcine peroxisome-proliferator-activated receptor delta regulates adipogenesis in myoblasts

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ABSTRACT

It is well known that peroxisome-proliferator-activated receptor $\gamma$ (PPAR $\gamma$) plays a critical role in regulating adipogenesis. In rodents, PPAR $\delta$ is expressed before PPAR $\gamma$ during adipocyte differentiation. Thus, the interaction between PPAR $\delta$ and PPAR $\gamma$ during adipogenesis needs to be elucidated. The current experiment was designed to study the interaction of porcine PPAR $\delta$ and PPAR $\gamma$ in mouse myoblast cells. Inhibition of myogenesis was observed in myoblasts expressing porcine PPAR $\delta$, similar to myoblast expressing PPAR $\gamma$. Treatment of myoblasts expressing PPAR $\delta$ with ligands for both PPAR $\delta$ and $\gamma$ enhanced lipogenesis to a greater extent than treatment with a PPAR $\gamma$ ligand alone. The ability to transdifferentiate myoblasts into adipocytes was decreased in myoblasts co-expressing PPAR $\delta$ with either wild-type or mutated PPAR $\gamma$ (serine 112 was mutated to alanine) compared to myoblasts expressing either type of PPAR $\delta$ alone. Adipose transdifferentiation in myoblasts co-expressing PPAR $\delta$ and mutated PPAR $\gamma$ was greater than in myoblasts co-expressing PPAR $\delta$ and wild-type PPAR $\gamma$. Our results suggest that PPAR $\delta$ has two different roles in regulating adipogenesis, i.e., suppression of myogenesis to enhance transdifferentiation of myoblasts into adipocytes and interaction with PPAR $\gamma$ to modify adipogenesis. Therefore, PPAR $\delta$ may have a significant role in adipogenesis.

Key Words: Adipocyte differentiation, Peroxisome proliferator-activated receptor $\delta$, Peroxisome proliferator-activated receptor $\gamma$.

INTRODUCTION

In rodent, peroxisome-proliferator-activated receptor $\delta$ (PPAR $\delta$) is wildly expressed in several tissues, including adipose tissue, intestine, skeletal muscle, lung and heart. The expression of PPAR $\delta$ in proliferating preadipocytes is undetectable and increases gradually during adipocyte differentiation (Amri et al., 1995). Preadipocyte overexpressing PPAR $\delta$ with long chain fatty acids promotes adipogenesis (Bastie et al., 2000). Ectopic expression of PPAR $\delta$ in fibroblasts with long chain fatty acids alone do not induce adipogenesis but stimulation in the presence
of PPAR γ ligand (Bastie et al., 1999). Therefore, PPAR δ seems to have a facilitating role in adipogenesis.

The information of porcine PPAR δ is still poorly understood, especially in functional study. In previous studies, we have demonstrated that ectopic expression of porcine PPAR γ induces adipogenesis in myoblasts (Yu et al., 2006). The expression of PPAR δ is earlier than PPAR γ during adipocyte differentiation in rodent adipocytes (Amri et al., 1995). We hypothesize that a relationship between PPAR δ and PPAR γ in regulating adipocyte differentiation. In this study, we created C2C12 myoblasts expressing porcine PPAR δ, or co-expressing PPAR δ with either wild-type or mutated PPAR γ (serine 112 was mutated to alanine). Transfected myoblasts with porcine PPAR δ stimulated adipogenesis after addition of both PPAR δ and PPAR γ ligands, whereas a decreased lipid accumulation was observed in myoblasts co-expressing PPARs compared with expressing PPAR γ alone.

MATERIALS AND METHODS
Stably transformed cells with PPAR δ or PPAR γ and induction of myoblast transdifferentiation

The porcine PPAR δ cDNA was cloned from porcine adipose tissue. The PCR products were cloned into a mammalian expression vector and transfected into C2C12 myoblasts by lipofection. To establish expression of both porcine PPAR δ and PPAR γ cell models, C2C12 myoblasts containing either wild-type PPAR γ or mutated PPAR γ were also transfected with porcine PPAR δ. Myoblasts stably expressing PPAR δ were established by puromycin selection. After drug selection, the cells were cultured without selection medium and allowed to propagate to 80% confluence in DMEM with 10% FBS. Confluent cells were then cultured in adipogenic differentiation medium [DMEM containing 10% fetal bovine serum, 1 mM dexamethasone, and 5 μg/mL insulin] and with or without 1 μM rosiglitazone, a PPAR γ ligand and 1 μM L165041, a PPAR δ ligand. After 10 days of culture, total RNA was purified to determine gene expression.

Northern blot and statistical analysis

The RNA was separated by electrophoresis and blotted to nylon membranes. The membrane was prehybridized at 42 °C and then hybridized with isotope labeled complementary DNA probes. Hybridization results were quantified by phosphor-image analysis. The densitometric value for an individual transcript in a sample lane was normalized to the densitometric value for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA in the same lane. The treatment effects were analyzed using an ANOVA procedure to determine the main effects of the form of PPAR δ and PPAR γ in presence or absence of its ligands.
Duncan’s new multiple range test was used to evaluate differences among means (SAS Inst. Inc., Cary, NC). A significant difference indicates that P value is not greater than 0.05.

RESULTS AND DISCUSSION
The presence or absence of rosiglitazone and L165041 in adipogenic medium had no effect on myogenesis (Figure 1B, C and D). The myotube formation was inhibited when myoblast expressing PPAR δ compared with transfection of empty vector cells (Figure 1A vs. E). Similar results were observed in our previous studies, myoblasts containing PPAR γ had an ability to interfere in myocyte differentiation (Yu et al., 2006). After exposure of rosiglitazone to the adipogenic differentiation medium for 10 days, lipid-droplets were visualized in myoblasts expressing PPAR δ but absence in addition L165041 in adipogenic differentiation medium (Figure 1). It was well known that ligands for PPARs can activate more than one receptor isoform, hence adipocyte differentiation was increased in medium containing rosiglitazone even if absence of L165041 (Figure 1F). The maximum of lipid accumulation was observed in addition of both PPAR ligands (Figure 1H). This result suggests indirectly that PPAR γ with its ligand has crucial potential in modulating adipocyte differentiation. In loss of function study, it has been demonstrated that lipid accumulation and adipogenic marker genes are decreased in PPAR δ-null adipocytes (Matsusue et al., 2004). In our results, myoblasts expressing PPAR δ with PPAR γ and PPAR δ ligands enhanced adipogenesis. It implies that PPAR δ appeared to accelerate adipogenesis. The downstream gene of PPAR γ, adipocyte fatty acid binding protein (aP2) mRNA was highly expressed in myoblasts containing PPAR δ in the presence of rosiglitazone. However, addition of PPAR δ and PPAR γ ligands in adipogenic medium had a greater stimulation of aP2 expression compared with presence of single PPAR ligand. For lipoprotein lipase (LPL) mRNA, it was also increased in the same condition. It has been known that expression of aP2 and LPL are regulated by PPAR γ. Deducing from our results, we hypothesize that high aP2 and LPL transcripts were attributed to PPAR γ function and ectopic PPAR δ modulated PPAR γ expression by binding its peroxisome proliferator response element. A late myogenic marker gene, myogenin was decreased in cells transfected with PPAR δ and both of ligands had no effect on myogenesis (Figure 3). Similar results were observed in another myogenic marker gene, myogenic regulatory factor 4 (MRF4). The suppression of myogenic marker genes in myoblasts expressing PPAR δ was consistent with ectopic expression of PPAR γ in myocytes. These results demonstrated that adipogenesis related transcription factors have the capability of impairing myogenesis. Furthermore, mRNA for aP2 and LPL were expressed at a low level in myoblasts containing either
wild-type PPAR γ or mutated PPAR γ and PPAR δ compared with expressing either wild-type PPAR γ or mutated PPAR γ alone (Figure 4). This phenomenon was also found in preadipocyte expressing PPAR δ and PPAR γ. The over-expression of PPAR δ indeed can suppress PPAR γ-mediated adipogenesis (Shi et al., 2002). However, reduction of myogenic genes expression was enhanced in C2C12 myoblasts containing both PPARs (Figure 5). Thus, PPAR δ and PPAR appeared to have a synergic effect in the inhibition of myogenesis.

CONCLUSION
In the current study, we demonstrated that PPAR δ has the ability to promote transdifferentiation of myoblasts into adipocytes and interact with PPAR γ to modify adipogenesis. Therefore, PPAR δ may have a significant role in adipogenesis.

REFERENCES
Figure 1. Ligand-induced morphological alterations and accumulation of lipid droplets. Microscographs of C2C12 myocyte with empty vector (A-D), C2C12 expressing porcine peroxisome proliferator-activated receptor δ (PPAR δ; E-H) are shown. Cells were maintained in adipogenic medium (Dulbecco’s modified Eagle medium/dexamethasone/insulin/10% fetal bovine serum) ± 1 μM rosiglitazone (B and F), 1 μM L165041 (C and G) and both of ligands (D and H) to d 10 postconfluence. Magnification was 60.

Figure 2. Expression of adipogenic marker genes. After confluence, C2C12 myocytes with empty vector (Empty vector) and C2C12 expressing porcine peroxisome proliferator-activated receptor δ (PPAR δ) were cultured for 10 d. The expression of adipocyte-specific genes [adipocyte fatty acid-binding protein (aP2) and lipoprotein lipase (LPL)] was determined and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The bars indicate the means ± SE for cells from 3 independent replicates (n = 3). ND = not detected. \(^{a-c}\)Means without a common letter differ, \(P < 0.05\).
Figure 3. Expression of myogenic marker genes. After confluence, C2C12 myocyte with empty vector (Empty vector) and C2C12 expressing porcine peroxisome proliferator-activated receptor δ (PPAR δ) were cultured for 10 d. The expression of myogenic genes [Myogenin and myogenic regulatory factor -4 (MRF4)] was determined and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The bars indicate the means ± SE for cells from 3 independent replicates (n = 3). a–c Means without a common letter differ, \( P < 0.05 \).

Figure 4. Expression of adipogenic marker genes. After confluence, C2C12 expressing wild-type porcine PPAR γ (WT-PPAR γ), C2C12 expressing mutated porcine PPAR γ (MU-PPAR γ), C2C12 expressing wild-type porcine PPAR γ and PPAR δ (WT-PPAR γ PPAR δ) and C2C12 expressing mutated porcine PPAR γ and PPAR δ (MU-PPAR γ PPAR δ) were cultured for 10 d. The expression of adipocyte-specific genes [adipocyte fatty acid-binding protein (aP2) and lipoprotein lipase (LPL)] was determined and normalized to GAPDH. The bars indicate the means ± SE for cells from 3 independent replicates (n = 3). a–g Means without a common letter differ, \( P < 0.05 \).
lipase (LPL)] was determined and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The bars indicate the means ± SE for cells from 3 independent replicates (n = 3). \(^{a-c}\) Means without a common letter differ, \(P < 0.05\).

Figure 5. Expression of myogenic marker genes. After confluence, C2C12 expressing wild-type porcine PPAR \(\gamma\) (WT-PPAR \(\gamma\)), C2C12 expressing mutated porcine PPAR \(\gamma\) (MU-PPAR \(\gamma\)), C2C12 expressing wild-type porcine PPAR \(\gamma\) and PPAR \(\delta\) (WT-PPAR \(\gamma\) PPAR \(\delta\)) and C2C12 expressing mutated porcine PPAR \(\gamma\) and PPAR \(\delta\) (MU-PPAR \(\gamma\) PPAR \(\delta\)) were cultured for 10 d. The expression of myogenic genes [Myogenin and myogenic regulatory factor-4 (MRF4)] was determined and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The bars indicate the means ± SE for cells from 3 independent replicates (n = 3). ND = not detected. \(^{a-c}\) Means without a common letter differ, \(P < 0.05\).