Porcine peroxisome proliferator-activated receptor \(\gamma\) induces transdifferentiation of myocytes into adipocytes


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ABSTRACT: Peroxisome proliferator-activated receptor \(\gamma\) (PPAR\(\gamma\)) is a nuclear transcription factor that regulates adipocyte differentiation and lipogenic genes during adipogenesis. The activity of rodent PPAR\(\gamma\) is regulated by phosphorylation of serine 112. The current experiment was designed to study the ability of porcine PPAR\(\gamma\) to stimulate transdifferentiation of myoblasts to adipocytes by overexpressing wild-type PPAR\(\gamma\) or mutated PPAR\(\gamma\) (serine 112 was mutated to alanine) in mouse myoblast cells. The expression of adipogenic marker genes (adipocyte fatty acid binding protein, lipoprotein lipase, and glycerol-3 phosphate dehydrogenase) in cells stably expressing mutated porcine PPAR\(\gamma\) was greater than in cells with wild-type PPAR\(\gamma\), indicating that the mutated PPAR\(\gamma\) has greater adipogenic capability than the wild-type PPAR\(\gamma\). Under treatment with a ligand, both wild-type and mutant porcine PPAR\(\gamma\)-expressing C2C12 myoblasts differentiated into adipocytes in 10 d. The expression of myogenic marker genes (myogenin, myogenic regulatory factor-4) was suppressed in cells transfected with the mutated PPAR\(\gamma\) or wild-type PPAR\(\gamma\). Moreover, wild-type and mutant PPAR\(\gamma\) were able to inhibit myogenesis without addition of a ligand. Our results suggest that porcine wild-type PPAR\(\gamma\) and mutated PPAR\(\gamma\) can both convert myoblast cells into adipocytes, and also that the ability to transdifferentiate was greater in cells containing the mutated PPAR\(\gamma\) than in cells containing the wild-type PPAR\(\gamma\). Therefore, the existence of serine 112 in PPAR\(\gamma\) may have a role in regulating adipocyte differentiation.

Key words: adipocyte differentiation, adipocyte gene, myoblast transdifferentiation, myocyte gene, peroxisome proliferator-activated receptor \(\gamma\)

INTRODUCTION

The peroxisome proliferator-activated receptor \(\gamma\) (PPAR\(\gamma\)) is a critical transcription factor in the regulation of adipogenesis. Ectopic expression of rodent PPAR\(\gamma\) in fibroblasts stimulates adipocyte development (Tontonoz et al., 1994). In addition, expression of rodent PPAR\(\gamma\) and the CCAAT/enhancer-binding protein \(\alpha\) (C/EBP\(\alpha\)) in myoblasts activates an adipogenic program in this cell line (Hu et al., 1995). The regulation of PPAR\(\gamma\) activity is posttranslationally modified. Phosphorylation of rodent PPAR\(\gamma\) serine 112 by mitogen-activated protein (MAP) kinase decreases the ability of PPAR\(\gamma\) to regulate adipogenic genes and decreases differentiation (Hu et al., 1996). In NIH 3T3 cells, replacement of rodent PPAR\(\gamma\) serine 112 with alanine promotes the function of PPAR\(\gamma\) during adipogenesis and enhances the ligand sensitivity through prevention of MAP kinase phosphorylation (Hu et al., 1996).

Thiazolidinediones (TZD), compounds used to treat type 2 diabetes, are ligands for PPAR\(\gamma\) (Kletzein et al., 1992). The TZD act as adipogenic agents in porcine preadipocytes, suggesting they are ligands for porcine PPAR\(\gamma\) (Tchoukalova et al., 2000). Although the expression of PPAR\(\gamma\) is positively associated with porcine adipocyte differentiation (Ding et al., 1999), there is no direct evidence to demonstrate the function of porcine PPAR\(\gamma\) on adipocyte differentiation. Intramuscular fat is not abundant in porcine muscle. Thus, it is of interest to increase the number of intramuscular adipocytes. In this study, we transfected the myogenic C2C12 myoblast cell line with wild-type or mutated porcine PPAR\(\gamma\) (ser 112→ala). Upon addition of a PPAR\(\gamma\) ligand, transfected cells with wild-type PPAR\(\gamma\) or mutated PPAR\(\gamma\) were transdifferentiated from a myogenic to an adipogenic type.

MATERIALS AND METHODS

Full Length cDNA Cloning and Gene Construction

The animal protocol was approved by the Animal Care and Use Committee of the National Taiwan Uni-

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Table 1. Characteristics of the probe primer sequences for northern analysis

<table>
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<th>Gene1</th>
<th>Primer2</th>
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<td>730</td>
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<tr>
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<td>756</td>
</tr>
<tr>
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<tr>
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<td>A 5′-AACCTGGTCCTCAGGTGAG-3′</td>
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1 GenBank accession No. is indicated parenthetically.  
2 S = sense; A = antisense.

versity. Two 2-mo-old crossbred pigs were killed by electrocution combined with exsanguinuation for gene cloning and gene expression studies (Liu et al., 2005). Longissimus muscle and s.c. adipose tissue were obtained, and RNA was extracted with guanidinium-phenol-chloroform (Chomczynski and Sacchi, 1987) using modifications by Hsu and Ding (2003). Total RNA was reverse transcribed using a kit, SuperScript II First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). First strand cDNA from pig adipose tissue was used for cloning PPARγ2 (Accession No. AF103946). The point-mutated PPARγ (ser 112→ala), which resulted in a nonfunctional phosphorylation site, was created by primer design. The PCR products were cloned into a mammalian expression vector with a CMV promoter (pIRES-EGFP, Clontech, Mountain View, CA) to drive the expression of wild-type PPARγ or mutated PPARγ. Sequences of these recombinant molecules were determined and confirmed.

Establishment of Cell Lines Stably Expressing Pig PPARγ

The C2C12 myoblasts (CRL-1772, ATCC, Manassas, VA) were cultured in Dulbecco’s modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS) at 37°C in an atmosphere of 5% CO2 in air. After confluence, C2C12 myoblast cells differentiated into mature myotubes when the serum concentration was lowered by replacing the 10% FBS with 2% horse serum.

The empty mammalian expression vector (CV) or vector containing wild-type PPARγ or mutated PPARγ was transfected into C2C12 myoblasts by lipofection (Fugene 6, Roche Applied Science, Indianapolis, IN). Cell lines stably expressing CV or PPARγ were established by antibiotic selection using G418. In this system, antibiotic resistance is an indication of successful integration of foreign genes into the genome of the cells. After transfection with individual plasmids, cells were maintained in nonselective medium for 2 d, after which the nonselective medium was replaced with selective medium containing the antibiotic. The selection

Figure 1. Analysis of exogenous wild-type porcine peroxisome proliferator-activated receptor γ2 (PPARγ) and mutated porcine PPARγ in C2C12 myoblasts. The C2C12 control cells (C), C2C12 cells expressing wild-type PPARγ (WT), and C2C12 cells expressing mutated PPARγ (MU) were cultured in Dulbecco’s modified Eagle medium+10% fetal bovine serum until confluence was reached. Total RNA (20 μg) was analyzed for porcine PPARγ and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by northern blot. Tissue total RNA from pig adipose tissue (F) and muscle (M) was used for positive and negative comparison. Two crossbred pigs were used for the study. The GAPDH was an indicator of equivalent RNA loading.
Figure 2. Expression of adipogenic and myogenic marker genes. After confluence, normal C2C12 myocytes (C), C2C12 expressing empty vector (CV1 and CV2), C2C12 expressing wild-type porcine peroxisome proliferator-activated receptor γ2 (PPARγ; WT), and C2C12 expressing mutated porcine PPARγ (MU) were cultured for 10 d. The expression of adipocyte-specific genes [lipoprotein lipase (LPL) and adipocyte fatty acid-binding protein (aP2)] and myogenic genes [myoblast determination protein-1 (MyoD) and myogenin] was determined and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The bars indicate the means ± SE from cells for 3 independent replicates (n = 3). ND = not detected. Means without a common letter differ, *P* < 0.05. Rosi = 1 μM of rosiglitazone treatment.

was for at least 1 mo. Dead cells were eliminated through frequent replacement of the selective medium, until distinct colonies could be visualized under the selective-medium environment. Individual colonies were isolated to culture cells for further propagation.

**Induction of Myocyte Transdifferentiation**

After the cell lines were established, 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% FBS, 1 μM dexamethasone, and 5 μg/mL insulin with or without 1 μM rosiglitazone, a PPARγ ligand]. For myogenic differentiation, cells were cultured in DMEM plus 2% horse serum, with or without 1 μM PPARγ ligand. Cells were cultured in myogenic or adipogenic medium for 10 d, with a medium change every 2 d. The adipogenic and myogenic media ± rosiglitazone, were used to assess the capacity of C2C12 myoblasts containing wild-type or mutated PPARγ to modify adipogenesis and myogenesis. Each experiment was repeated 3 times.

After 10 d of culture, cells on the plates were stained with Oil Red O to measure the degree of adipocyte differentiation (Ramirez-Zacarias et al., 1992). Cellular RNA was extracted to determine the mRNA concentrations for several genes whose expression increases during adipocyte differentiation: PPARγ, lipoprotein lipase (LPL; an early marker), adipocyte fatty acid-binding protein (aP2; a late marker), and glyceral-3 phosphate dehydrogenase (GPDH; a marker for triacylglycerol synthesis activity). The mRNA for genes representing myoblast differentiation were also
Figure 3. Ligand-induced morphological alterations and accumulation of lipid droplets. Microscographs of normal C2C12 myotubes (A and D), C2C12 expressing wild-type porcine peroxisome proliferator-activated receptor γ2 (PPARγ; B and E), and C2C12 expressing mutated porcine PPARγ (C and F) are shown. Cells were maintained in adipogenic medium (Dulbecco’s modified Eagle medium/dexamethasone/insulin/10% fetal bovine serum) ± 1 μM rosiglitazone (D–F) to d 10 postconfluence. Lipid deposition for normal C2C12 myoblasts (G), C2C12 expressing wild-type PPARγ (H), and C2C12 expressing mutated PPARγ (I) is shown for cells that were grown in adipogenic medium for 10 d and stained with Oil Red O. Cells stained with Oil Red O appear as dark areas on the plates. Rosiglitazone (1 μM) was absent (G–I) or present (J–L). Magnification was 60×. Bars indicate a length of 100 μm.

measured: myoblast determination protein-1 (MyoD; an early marker), myogenic factor-5 (Myf5; an early marker), myogenin (a late marker), and myogenic regulatory factor-4 (MRF4; a late marker).

Extraction of RNA

Total RNA was extracted for northern analysis. The quality of the RNA was monitored by examination of the 18S and 28S ribosomal RNA bands after electrophoresis. The RNA was quantified by spectrophotometry at 260 nm and stored at −70°C.

Northern Analysis

Total RNA (20 μg of each sample) was electrophoresed and transferred to nylon membranes. The probes for the genes measured were generated by PCR using the primer pairs listed in Table 1. The membrane was prehybridized at 42°C in UltraHyb (Ambion, Austin, TX) for 1 h, and then the denatured cDNA probe (95°C for 5 min) was added at a concentration of 1 pM and allowed to hybridize with the targeted gene transcripts overnight at 42°C. Hybridization was quantified by phosphor-image analysis as previously described (Hsu...
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Figure 4. Expression of adipogenic marker genes. After confluence, normal C2C12 myocytes (C), C2C12 expressing wild-type porcine peroxisome proliferator-activated receptor \( \gamma \)2 (PPAR\( \gamma \); WT), and C2C12 expressing mutated porcine PPAR\( \gamma \) (MU) were cultured for 10 d. The expression of adipocyte-specific genes [PPAR\( \gamma \), lipoprotein lipase (LPL), adipocyte fatty acid-binding protein (aP2), and glycerol-3 phosphate dehydrogenase (GPDH)] was determined and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The bars indicate the means ± SE from cells for 3 independent replicates (n = 3). ND = not detected. Means without a common letter differ, \( P < 0.05 \). Rosi = 1 \( \mu \)M of rosiglitazone treatment.

et al., 2004; Wang et al., 2006). 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.

Statistical Analysis

The treatment effects were analyzed using an ANOVA procedure to determine the main effects of the form of PPAR\( \gamma \) and presence or absence of a ligand. 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

Ectopic Expression of Wild-Type PPAR\( \gamma \) and Mutated PPAR\( \gamma \) in C2C12 Myoblasts

Expression of pig PPAR\( \gamma \) in adipose tissue was robust, whereas expression in muscle was weak (Figure 1). No PPAR\( \gamma \) was detected in the RNA from untransfected C2C12 myoblasts (Figure 1). However, C2C12 myoblasts transfected with wild-type porcine PPAR\( \gamma \) or mutated porcine PPAR\( \gamma \) expressed a high level of PPAR\( \gamma \) mRNA (Figure 1).

Wild-Type PPAR\( \gamma \) and Mutated PPAR\( \gamma \) Trigger Transdifferentiation in Adipogenic Medium

The stably transformed porcine PPAR\( \gamma \) or mutated PPAR\( \gamma \) increased the expression of aP2 and LPL, 2
Figure 5. Expression of adipogenic marker genes under different concentrations of rosiglitazone. After confluence, C2C12 myocytes expressing wild-type porcine peroxisome proliferator-activated receptor γ2 (PPARγ WT) and C2C12 expressing mutated porcine PPARγ (MU) were cultured for 10 d. The average mRNA concentration of the cells expressing the wildtype PPARγ (WT) with 0.0625 μM rosiglitazone was designated as 1, and the other data were expressed relative to the WT value. The expression of adipocyte-specific genes [(adipocyte fatty acid-binding protein (aP2) and lipoprotein lipase (LPL)] was increased as the rosiglitazone concentration increased, with the expression level in MU greater than that in the WT. The bars indicate the means ± SE from cells for 3 independent replicates (n = 3).

PPARγ targeting genes (Figure 2). Two empty vector containing C2C12 clones (CV1 and CV2) expressed aP2, LPL, MyoD, and myogenin (myogenic markers) in the same manner as in the normal C2C12 cells, indicating that the insertion of the vector does not have an effect on the expression of adipogenic and myogenic genes in these cells. The addition of TZD did not affect the expression of adipogenic or myogenic genes in the CV1 and CV2 (Figure 2). Normal C2C12 cells differentiate to multinucleated myotubes in adipogenic medium (Figure 3, panel A). The presence or absence of rosiglitazone in adipogenic medium had no effects on myogenesis (Figure 3, panel A vs. D). Rosiglitazone at 1 μM did not trigger myoblast transdifferentiation into adipocytes. In contrast, myoblasts expressing native PPARγ or mutated PPARγ were maintained but did not progress to myotubes (Figure 3, panels B and C). This observation demonstrated that porcine PPARγ was able to block the myogenic program without exogenous ligand activation. However, after addition of rosiglitazone to the adipogenic differentiation medium for 10 d, lipid droplets were visualized in myoblasts expressing wild-type PPARγ and mutated PPARγ (Figure 3, panels E and F). To determine the degree of accumulation of intracellular triacylglycerol, cells were stained with Oil-Red-O and photographed on d 10 (Figure 3, panels G to L). After Oil-Red-O staining, myoblasts expressing wild-type PPARγ or mutated PPARγ displayed a low degree of triacylglycerol accumulation when no ligand was added to the adipogenic medium (Figure 3, panels H and I). However, after addition of the ligand to adipogenic medium, high levels of triacylglycerol were observed in wild-type PPARγ and mutated PPARγ C2C12 myoblasts (Figure 3, panels K and L).

Normal C2C12 myoblasts in adipogenic medium had a low level of PPARγ mRNA expression (Figure 4, panel A). The PPARγ mRNA expression may result from the medium insulin and dexamethasone, but the expression was not great enough to trigger the myoblast transdifferentiation even in the presence of rosiglitazone. The PPARγ mRNA expression in myoblasts containing wild-type PPARγ or mutated PPARγ was the same and at a high level (Figure 4, panel A). These levels were enough to cause the expression of downstream genes for adipogenesis, especially in the presence of the PPARγ ligand, rosiglitazone. Lipoprotein lipase could not be detected in normal C2C12 (Figure 4, panel B). However, high levels of LPL expression were obtained in cells ectopically expressing PPARγ under rosiglitazone stimulation (Figure 4, panel B). Similar results (Figure 4, panels C and D) were obtained for other PPARγ activated genes, aP2 and GPDH. Further experiments demonstrated that the effectiveness of increasing the expression of PPARγ targeting genes (aP2 and LPL) was greater by mutated
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**Figure 6.** Expression of myogenic marker genes. After confluence, normal C2C12 myoblasts (C), C2C12 expressing wild-type porcine peroxisome proliferator-activated receptor γ2 (PPARγ; WT), and C2C12 expressing mutated porcine PPARγ (MU) were grown for 10 d in adipogenic medium. The expression of myogenic genes [myoblast determination protein-1 (MyoD), myogenic factor-5 (Myf5), 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–cMeans without a common letter differ, *P* < 0.05. Rosi = 1 μM of rosiglitazone treatment.

PPARγ compared with the wild-type PPARγ under several concentrations of its ligand (Figure 5). These results demonstrated that exogenous porcine PPARγ could cause expression of adipogenesis-related genes to trigger the adipogenic program in C2C12 myoblasts.

Myogenic gene, MyoD, was expressed in all cell types at an equivalent level in the presence or absence of ligand (Figure 6, panel A). However, another early expressed myogenic marker, Myf5, was expressed at a greater level in cells transfected with wild-type PPARγ or mutated PPARγ compared with control cells; addition of rosiglitazone had no effect (Figure 6, panel B). The mRNA for myogenin and MRF4, genes representing later stages of myoblast differentiation, were expressed at a lower level in C2C12 cells expressing the wild-type PPARγ or the mutated PPARγ compared with normal C2C12 (Figure 6, panels C and D). Our experiments demonstrated that PPARγ could downregulate myogenic differentiation genes and inhibit myogenesis in adipogenic medium whether rosiglitazone was present or not.

**Wild-Type PPARγ and Mutated PPARγ Suppressed Myogenesis**

Normal C2C12 myoblasts differentiate very well to form myotubes under a 2% horse serum treatment (Figure 7, panel A). Addition of the PPARγ-ligand to the myogenic medium had no effect on myogenic differentiation (Figure 7, panel D). In contrast, cells expressing wild-type PPARγ or mutated PPARγ maintained an undifferentiated state, and myotubes were rarely visualized in myogenic medium (Figure 7, panels B and C). Addition of rosiglitazone to myogenic medium trig-
Inhibition of myotube formation by overexpression of porcine peroxisome proliferator-activated receptor γ (PPARγ). Microscopic observation of normal C2C12 myoblasts (A and D), C2C12 expressing wild-type porcine PPARγ (B and E), and C2C12 mutated porcine PPARγ (C and F) is indicated. Cells were maintained in myogenic medium (Dulbecco’s modified Eagle medium with 2% horse serum) to d 10 postconfluence; rosiglitazone (1 μM) was added to cells indicated in panel D–F. Arrows indicate lipid droplets (Oil Red O-stained). Magnification was 60×. Bars indicate a length of 100 μm.

DISCUSSION

Preadipocyte and myoblast lineages are derived from the same multipotent mesodermal progenitor (Grigoriadis et al., 1988). After determination, preadipocytes and myoblasts undergo terminal differentiation and develop into functional cells. Differentiation of preadipocytes into adipocytes is regulated by several transcription factors, including PPARγ, C/EBPα, and sterol regulatory element binding protein-1c (SREBP-1c or ADD1). During rodent adipogenesis, PPARγ is an early expressed transcription factor that stimulates the process of adipocyte differentiation in vitro and in vivo (Rosen et al., 1999). Several adipogenesis-related genes are modulated by binding the ligand activated PPARγ to their PPARγ-response elements. For example, aP2 (Tontonoz et al., 1994) and phosphoenolpyruvate carboxykinase (Tontonoz et al., 1995) are regulated by PPARγ. Therefore, the primary transcription factor to stimulate rodent adipogenesis is PPARγ (Rosen et al., 1999).

The differentiation of myoblasts and preadipocytes involves different transcription factor programs. In myogenesis, this process is regulated by a family of basic helix-loop-helix transcription factors, including MyoD, Myf5, myogenin, and MRF4. The MyoD and Myf5 are expressed at early stages and participate in myoblast determination, whereas myogenin and MRF4 are expressed at later stages and promote myotube formation in terminal differentiation (Weintraub et al., 1989; Emerson, 1993). In recent years, mouse C2C12 and G8 myoblast cell lines have been used to
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Figure 8. Expression of adipogenic marker genes in cells maintained in myogenic medium. Normal C2C12 myoblasts (C), C2C12 expressing wild-type porcine peroxisome proliferator-activated receptor γ2 (PPARγ; WT), and C2C12 expressing mutated porcine PPARγ (MU) were grown in myogenic medium ± 1 μM rosiglitazone to d 10 postconfluence. The expression of adipocyte-specific genes [PPARγ, lipoprotein lipase (LPL), adipocyte fatty acid-binding protein (aP2), and glycerol-3 phosphate dehydrogenase (GPDH)] 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. Means without a common letter differ, P < 0.05. Rosi = 1 μM rosiglitazone treatment.

In rodents, the PPARγ serine 112 can be phosphorylated by MAP kinase during preadipocyte proliferation, and the phosphorylated PPARγ has reduced transcriptional regulatory activity (Hu et al., 1996; Shao et al., 1998). Furthermore, ectopic expression of rodent PPARγ and C/EBPα in G8 myoblasts induces myoblast conversion to adipocytes (Hu et al., 1995).

In rodents, the PPARγ serine 112 can be phosphorylated by MAP kinase during preadipocyte proliferation, and the phosphorylated PPARγ has reduced transcriptional regulatory activity (Hu et al., 1996; Shao et al., 1998). In the current study, the mutation of serine 112 in the porcine PPARγ seems to enhance the ability of PPARγ to regulate transcription of its targeting genes. However, a lack of phosphorylation information for the porcine PPARγ limits the ability to speculate the precise mechanism. Regardless of the precise mechanism, the mutated PPARγ has greater ability to promote adipogenesis in our C2C12 myoblasts than does the wild-type PPARγ. The results also support a model in which decreased phosphorylation of mutated PPARγ enhances transcriptional activity to drive adipogenesis.

The TZD enhances the sensitivity of tissues to insulin and are widely used to treat type-2 diabetes. Preadipocytes treated with TZD have increased expression of adipogenesis-related genes and differentiation (Kleitzien et al., 1992). It has been suggested that 5 μM rosiglitazone activates adipogenic genes in mouse C2C12 myoblasts to trigger the conversion to adipocytes (Teboul et al., 1995), although the rat L6 myoblast cell line did not transdifferentiate into adipocytes in the presence of TZD (Hammarstedt and Smith, 2003). In our studies, addition of 1 μM rosiglitazone to C2C12 myoblasts without the transfected PPARγ had no effect on transdifferentiation in adipogenic or
Figure 9. Expression of myogenic marker genes. Normal C2C12 myoblasts (C), C2C12 expressing wild-type porcine peroxisome proliferator-activated receptor γ2 (PPARγ; WT), and C2C12 expressing mutated porcine PPARγ (MU) were cultured for 10 d postconfluence in myogenic medium as indicated in Figure 7. The expression of myocyte-specific genes [myoblast determination protein-1 (MyoD), myogenic factor-5 (Myf5), Myogenin, and myogenic regulatory factor-4 (MRF4)] was determined and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The bars indicate means ± SE for cells from 3 independent replicates (n = 3). Means without a common letter differ, P < 0.05. Rosi = 1 μM of rosiglitazone treatment.

myogenic medium, suggesting that 1 μM rosiglitazone was not enough to trigger the adipogenic program of myoblasts without the expression of exogenous PPARγ.

Expression of PPARγ and C/EBPα in G8 myoblasts causes transdifferentiation into adipocytes (Hu et al., 1995). In addition, C2C12 myoblasts transfected with PPARδ had increased endogenous PPARγ and conversion to adipocytes under TZD stimulation (Holst et al., 2003). These results imply that ectopic expression of PPARγ with its ligand is able to enhance myocyte transdifferentiation to adipocytes. In the current studies, the addition of rosiglitazone to cells expressing porcine PPARγ increased the expression of adipogenic genes, i.e., LPL, aP2, and GPDH. In adipogenic medium, the mutated PPARγ had an increased capacity to enhance adipogenesis compared with the wild-type PPARγ only in the presence of the ligand. The strength of the adipogenic outcome in cells transfected with either PPARγ is indicated by the presence of a few lipid containing cells and increased LPL mRNA even when the myoblasts were cultured in myogenic medium.

The terminal myogenic differentiation genes, myogenin and MRF4, were reduced in cells containing either PPARγ type. During myogenesis, the MyoD expression is present at a steady state level, whereas the expression of Myf5 increased and peaked at d 4 then reduced until d 10 (Dedieu et al., 2002). We observed the same expression of MyoD in all cells. However, the reason for the increased Myf5 expression in genetically modified cells compared with non-transfected myoblasts is unclear.

Taken together, porcine PPARγ was able to trigger myoblasts transdifferentiation into adipocytes in the presence of a PPARγ-ligand. The current finding is
consistent with the hypothesis that decreased phosphorylation of mutated PPARγ has enhanced activity on stimulating adipocyte differentiation. Treatment with a PPARγ-ligand enhanced the adipogenic effect. Future targeting of the wild-type or mutated PPARγ to myoblasts in vivo may provide a mechanism to enhance marbling in pigs.

LITERATURE CITED


