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Regulation of peroxisome proliferator-activated receptor gamma on milk fat synthesis in dairy cow mammary epithelial cells

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Abstract Peroxisome proliferator-activated receptor gamma (PPARγ) participates in lipogenesis in rats, goats, and humans. However, the exact mechanism of PPARγ regulation on milk fat synthesis in dairy cow mammary epithelial cells (DCMECs) remains largely unexplored. The aim of this study was to investigate the role of PPARγ regarding milk fat synthesis in DCMECs and to ascertain whether milk fat precursor acetic acid and palmitic acid could interact with PPARγ signaling to regulate milk fat synthesis. For this study, we examined the effects of PPARγ overexpression and gene silencing on cell growth, triacylglycerol synthesis, and the messenger RNA (mRNA) and protein expression levels of genes involved in milk fat synthesis in DCMECs. In addition, we investigated the influences of acetic acid and palmitic acid on the mRNA and protein levels of milk lipogenic genes and triacylglycerol synthesis in DCMECs transfected with PPARγ small interfering RNA (siRNA) and PPARγ expression vector. The results showed that when PPARγ was silenced, cell viability, proliferation, and triacylglycerol secretion were obviously reduced. Gene silencing of PPARγ significantly downregulated the expression levels of milk fat synthesis-related genes in DCMECs. PPARγ overexpression improved cell viability, proliferation, and triacylglycerol secretion. The expression levels of milk lipogenic genes were significantly increased when PPARγ was overexpressed. Acetic acid and palmitic acid could markedly improve triacylglycerol synthesis and upregulate the expression levels of PPARγ and other lipogenic genes in DCMECs. These results suggest that PPARγ is a positive regulator of milk fat synthesis in DCMECs and that acetic acid and palmitic acid could partly regulate milk fat synthesis in DCMECs via PPARγ signaling.

Keywords PPARγ · Dairy cow mammary epithelial cells · Acetic acid · Palmitic acid · Milk fat synthesis

Introduction

Peroxisome proliferator-activated receptors (PPARs) belong to members of the nuclear receptor superfamily that can serve as intracellular biosensors of fatty acid (FA) levels and alter lipid metabolism. As a member of the PPAR family, peroxisome proliferator-activated receptor gamma (PPARγ) is expressed in a wide variety of cells (Kang *et al.* 2015). Increasing investigations have reported that PPARγ participated in lipogenesis and differentiation of cells in adipose tissue (Park *et al.* 2014; Yang *et al.* 2014; Nie *et al.* 2015; Yamaguchi *et al.* 2015). Genes related to de novo FA synthesis, triacylglycerol (TAG) synthesis, and other genes including fatty acid binding protein 3 (FABP3) were upregulated in adipose tissue of rats (Way *et al.* 2001) and humans (Kolak *et al.* 2007) by rosiglitazone (ROSI), a specific PPARγ agonist. Bionaz and Loor identified a gene network with key roles in coordinating milk fat synthesis and highlighted the synergistic action of PPARγ, PPAR gamma coactivator 1 alpha (PPARGC1A), and insulin-induced gene 1 (INSIG1), which control the function and expression of another important transcription factor, sterol regulatory element-binding protein 1

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(SREBP1), in dairy cow (Bionaz and Loor 2008b). PPARγ is thought to play a role in regulating milk fat synthesis because of the significant increase in PPARγ expression between bovine pregnancy and lactation (Bionaz and Loor 2008a). Kadegowda et al. (2013) found that the inhibition of PPARγ signaling was involved in decrease of lipid synthesis in murine mammary tissue. ROSI-activated PPARγ is also known to result in a marked increase in the expression of genes associated with TAG synthesis and secretion in goat mammary epithelial cells (Shi *et al*. 2013). Although there were some relevant reports about the role of PPARγ regarding lipogenesis in rats, goats, and humans, the exact mechanism of PPARγ regulation on lipid synthesis in dairy cow mammary epithelial cells (DCMECs) remains largely unexplored.

Recently, a number of studies demonstrated that PPARγ could affect the growth of various cells. Meshkani et al. (2014) found that ROSI augmented the cell viability and ameliorated palmitate-induced apoptosis in skeletal muscle cells. Pang et al. (2014) also declared that the pretreatment with PPARγ agonist pioglitazone effectively protected cerebellar granule cells (CGCs) against nutrient deprivation-induced apoptosis. The ameliorative role of PPARγ agonist on cell growth has also been reported in other cells such as cardiomyocytes (Kim *et al*. 2012) and sebocytes (Schuster *et al*. 2011). However, whether PPARγ could improve DCMECs growth has not been studied so far.

Acetic acid and β-hydroxybutyric acid are the main precursors for milk lipogenesis in the bovine mammary gland (Bernard *et al*. 2008). Maxin et al. (2011) reported that ruminal infusion of acetate to dairy cows could change milk fat composition and increase milk fat content by 6.5%. Acetic acid enhances de novo synthesis and desaturation of FAs in the bovine mammary gland (Jacobs *et al*. 2013). Hong et al. (2005) demonstrated that acetate stimulated fat accumulation in 3T3-L1 adipocytes with upregulation of PPARγ2. In mammary gland, preformed long-chain fatty acids (greater than 16 carbons) and a portion of palmitate for comprising milk fat are derived from blood circulation (Bauman and Griinari 2003). Kadegowda et al. (2009) found that palmitate promoted TAG synthesis and upregulated the messenger RNA (mRNA) expression of some genes associated with milk fat in DCMECs. Qi et al. (2014) found that palmitate inhibited de novo synthesis of milk FAs through regulating related gene expression. However, the exact regulatory mechanisms of milk fat precursors acetic acid and palmitic acid on milk lipogenesis in DCMECs are poorly understood. Moreover, whether milk fat synthesis regulated by acetic acid and palmitic acid occurs via a PPARγ pathway has not been reported.

In the present study, we designed experiments to detect cell growth, TAG synthesis, and the expression of milk fat synthesis-related genes in DCMECs after PPARγ gene silencing and PPARγ overexpression. In addition, we also examined the effects of acetic acid and palmitic acid on TAG synthesis and the expression of milk fat synthesis-related genes in DCMECs with PPARγ gene silencing and PPARγ overexpression. The aim of this investigation was to illuminate the role of PPARγ on milk fat synthesis in DCMECs and to determine whether acetic acid and palmitic acid could interact with PPARγ signaling to regulate milk fat synthesis in the dairy cow mammary gland.

Materials and Methods

Cell preparation and treatments Reagents were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada) unless otherwise stated. Fetal bovine serum (FBS) and Dulbecco's modified Eagle medium: F12 (DMEM/F12) base were obtained from GIBCO BRL (Life Technologies, Carlsbad, CA). All animals received humane care as outlined in the Guide for the Care and Use of Experimental Animals of the National Institutes of Health. The animal procedures were approved by the Animal Care Committee of the Northeast Agricultural University. The healthy multiparous Holstein cows (5 yr old) in a midlactation period (100 d postpartum) averaging 609 ± 9.08 kg (mean ± SE) live weight, 30 ± 0.5 kg/d milk production, and a parity of 3.1 ± 0.19 over three generations were obtained from the Holstein Cattle Association of Australia, as previously published (Wang *et al*. 2014). Cows were slaughtered by exsanguination, and then, mammary gland parenchymal tissues were individually isolated and sheared into 1-mm^3 pieces. These pieces were moved into the bottom of the cell culture bottles. After approximately 3~4 h, 2 mL of culture medium was gently added into the culture bottles. The medium was changed once after Day 3, and the cells were observed with an inverted microscope (DFC280, Leica, Wetzlar, Germany). For 15~30 d, the cell culture bottle was covered with fibroblasts, myoepithelial cells, and mammary epithelial cells. DCMECs were purified according to previous reports (Cui *et al*. 2011; Tong *et al*. 2011; Tong *et al*. 2012). Due to different sensitivity of several cells to digestion with 0.25% trypsin plus 0.02% EDTA, a nearly pure sample of DCMECs was isolated after three to four passages of separation and culture. Therefore, DCMECs passaged three to four times were used for experimental assays. The purified cells were cultured in basic culture medium (DMEM/F12 base with 10% FBS added, 5 μg/mL insulin, 1 μg/mL hydrocortisone, 5 μg/mL prolactin, 100 U/mL penicillin, and 0.1 mg/mL streptomycin). We have tested and authenticated the cell lines utilized in the research by immunofluorescence for the epithelial cell marker cytokeratin 18 (CK18). For experimental assays, DCMECs in the logarithmic growth phase were plated at 3×10^4 cells/cm^2 in 6-well plates and the medium was replaced with DMEM/F12 base containing insulin, hydrocortisone, and prolactin (concentrations as above).

Immunofluorescence DCMECs were seeded on glass coverslips to 30~50% confluency in 6-well plates. The cells were rinsed twice with PBS and fixed in 4% (*w*/*v*) ice-cold formaldehyde at 4°C for 10 min. The slides were rinsed three times with Tris-buffered saline, 0.1% Tween 20 (TBS/T) for 5 min. To detect endogenous CK18 or PPARγ, fixed DCMECs were incubated in blocking buffer (Tris-buffered saline with 5% BSA and 0.1% Triton X-100) for 1 h at 37°C and then incubated with anti-CK18 primary antibody or anti-PPARγ primary antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) at a 1:100 dilution for 1.5 h at 37°C. After rinsing three times in TBS/T, specimens were incubated in the dark with fluorescein isothiocyanate (FITC)-conjugated secondary antibodies at a 1:200 dilution for 1 h at 37°C and incubated with PI for 15 min at 37°C. Finally, after rinsing three times in TBS/T, the coverslips were visualized using a Leica TCS-SP2 AOBS confocal laser scanning microscope.

Gene silencing of PPARγ DCMECs were transfected with PPARγ small interfering RNA (siRNA) or a negative control siRNA (Gene Pharma Co., Ltd., Shanghai, China) using Lipofectamine TM 2000 (LF2000, Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The sequences of siRNA were PPARγ-specific siRNA: sense 5′-GCCCA UUGAGGACAUACAATT-3′, antisense 5′-UUGUAUGUC CUCAAUGGGCTT-3′. The negative scrambled control siRNA (sense 5′-UUCUCCGAACGUGUCACGUTT-3′, antisense 5′-ACGUGACACGUUCGGAGAATT-3′) had no significant homology to any DCMEC gene. For silencing of PPARγ gene expression, transfected cells were cultivated for 48 h. The cells were then collected for further experiments.

Generation of pGCMV-IRES-EGFP-PPARγ and transfection Total RNA from cultured DCMECs was extracted using TRIzol reagent (Invitrogen). The complementary DNA (cDNA) was synthesized, and the desired sequence of the PCR product was inserted into the pMD18-T plasmid (TaKaRa Company, Dalian, China), followed by identification with restriction enzymes Xho I and EcoR I (TaKaRa Company) and DNA sequencing. The PPARγ gene was subcloned into the pGCMV-IRES-EGFP vector (GenePharma Co. Ltd.). Therefore, recombinant plasmids were obtained and identified by digestion with Xho I and EcoR I. The following primers were designed with particular restriction enzyme sites to clone the complete coding region of PPARγ. The forward primer was 5′-CCCTCGAGATG\ GGTGAAACTCTGGG-3′ (Xho I), and the reverse primer was 5′-CGGAATTCCTAATACAAGTCCTTGTAG-3′ (EcoR I). The optimized amplification conditions were annealing at 56.6°C and extension at 72.2°C for 35 cycles.

Transient transfection was performed according to a previous report of Lu (Lu *et al*. 2012). Briefly, DCMECs were transfected with pGCMV-IRES-EGFP-PPARγ or empty vector, which was added to balance the total amounts of transfected DNA samples using Lipofectamine TM 2000 (LF2000, Invitrogen) according to the manufacturer's protocol. Nontransfected cells were prepared as controls in the same manner as the transfected cells. Cells were cultivated for 48 h and then collected for further experiments.

Cell viability and cell proliferation assay Cell viability and cell proliferation were determined by using the CASY-TT Analyzer System (Schärfe System GmbH, Reutlingen, Germany) according to the manufacturer's instructions. After calibration with live and dead DCMECs, cursor positions were set to 11.75 to 50.00 μm (evaluation cursor) and 7.63 to 50.00 μm (standardization cursor). DCMECs were trypsinized and then diluted (1:100) with CASY electrolyte solution prior to examination. All experiments were performed in triplicate.

Quantitation of secreted triacylglycerol in the culture medium The amount of triacylglycerol secreted into the culture medium was determined using commercially available assay kits (triacylglycerol detection kit, ApplyGEN, Beijing, China) according to the manufacturer's recommended protocol.

RNA extraction and quantitative real-time PCR Total RNA extraction of DCMECs was performed using TRIzol reagent (Invitrogen). The integrity of the RNA samples was assessed by analyzing the ribosomal RNA (rRNA) bands of the 28S rRNA band and 18S rRNA band with 5 μL of each sample using 1% agarose gel electrophoresis. The brightness of the 28S rRNA band of all RNA samples is approximately twice that of the 18S rRNA band. The purity of RNA samples was verified by using the OD260/280 ratio obtained with an ultraviolet spectrophotometer (Beckman DU800, Beckman Coulter Inc, Fullerton, CA), and only samples with a ratio greater than 1.8 were used. Subsequently, RNA was reversely transcribed into cDNA using Thermoscript reverse transcriptase (TaKaRa) according to the manufacturer's protocol. Quantitative real-time PCR (qRT-PCR) reactions were performed using the real-time PCR Sensimix^{TM} SYBR and Fluorescein Kit, and the analysis was performed by an ABI PRISM 7300 RT-PCR System (Applied Biosystems, Foster City, CA) in a total volume of 20 μL using 96-well microwell plates. β-Actin was used as a reference gene. The primers for amplification are shown in Table 1. Primers were designed with Primer primier 5.0 (PREMIER Biosoft, Palo Alto, CA). The quality of the primer was confirmed according to the method by Shi et al. (2013). The efficiency of each primer pair was tested using the standard curve method (Rutledge and Cote 2003). qRT-PCR analysis was performed using the ΔΔCt method (Huang *et al*. 2012).

Table 1 Genes and primers for qRT-PCR

Gene	Accession number	Primer (5′ → 3′)	Efficiency
PPARγ	NM_181024.2	F: TCAAAGTGGAGCCTGTATC R: CATAGTGGAACCCTGACG	2.12
SREBP1	NM_001113302.1	F: AGTAGCAGCGGTGGAAGT R: GCAGCGGCTCTGGATT	2.08
INSIG1	NM_001077909.1	F: AAAGTTAGCAGTCGCGTCGTC R: TTGTGTGGCTCTCCAAGGTGA	2.18
SCAP	NM_001101889.1	F: CCATGTGCACTTCAAGGAGGA R: ATGTCGATCTTGCGTGTGGAG	2.10
CD36	NM_001046239.1	F:CCTATAACTGGATTTACTTTACGGTTTG R: GGCAGGTGGGAGGGAGTG	2.15
FABP3	NM_174313.2	F: GAACTCGACTCCCAGCTTGAA R: AAGCCTACCACAATCATCGAAG	2.16
ACSL1	NM_001076085.1	F: GTGGGCTCCTTTGAAGAACTGT R: ATAGATGCCTTTGACCTGTTCAAAT	2.09
ACSS2	NM_001105339.1	F: GGCGAATGCCTCTACTGCTT R: GGCCAATCTTTTCTCTAATCTGCTT	2.11
ACC	NM_174224.2	F: AGACAAACAGGGACCATT R: AGGGACTGCCGAAACAT	2.16
FAS	NM_001012669.1	F: CCACGGCTGTCGGTAAT R: CGCTCCCACTCATCCTG	2.13
SCD	NM_173959.4	F: CTGTGGAGTCACCGAACC R: TAGCGTGGAACCCTTTT	2.20
GPAT	NM_001012282.1	F: GCAGGTTTATCCAGTATGGCATT R: GGACTGATATCTTCCTGATCATCTTG	2.17
AGPAT6	NM_001083669.1	F: AAGCAAGTTGCCCATCCTCA R: AAACTGTGGCTCCAATTTCGA	2.12
DGAT1	NM_174693.2	F: CCACTGGGACCTGAGGTGTC R: GCATCACCACACACCAATTCA	2.15
β-Actin	NM_173979	F: AAGGACCTCTACGCCAACACG R: TTTGCGGTGGACGATGGAG	2.10

Western blot analysis Western blot analysis was performed using standard techniques reported by Lu (Lu *et al*. 2012). Total cell lysate containing approximately 30 μg protein was separated on 10% SDS-PAGE gel and transferred onto nitrocellulose membranes (Bio-RAD, Shanghai, China). Membranes were blocked in 5% skim milk (in Tris-buffered saline with 5% skim milk and 0.1% Tween-20). Membranes were probed with primary antibodies against PPARγ, sterol regulatory element-binding protein 1 (SREBP1), insulin-induced gene 1 (INSIG1), SREBP cleavage-activating protein (SCAP), CD36 molecule (CD36), FABP3, acyl-CoA synthetase long-chain family member isoform 1 (ACSL1), acyl-CoA synthetase short-chain family member 2 (ACSS2), acetyl-coenzyme A carboxylase (ACC), fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD), glycerol-3-phosphate acyltransferase (GPAT), 1-aryl glycerol-3-phosphate O-acyltransferase 6 (AGPAT6), β-actin (Santa Cruz Biotechnology), and diacylglycerol acyl transferase 1 (DGAT1) (Abcam Technology, Cambridge, MA), followed by a second incubation with secondary antibodies (1:1000) conjugated to HRP (ZSGB-BIO, Beijing, China). The chemiluminescence detection of HRP-conjugated secondary antibodies was performed using Super ECL plus (ApplyGEN).

Statistical analysis All data were expressed as the mean ± standard deviation ($n = 3$). Statistical analysis of all data was performed using SPSS for Windows (version 16; SPSS Inc., Chicago, IL). When a significant value ($p < 0.05$) was obtained by a one-way analysis of variance, further analysis was carried out. All data were normally distributed and passed equal variance testing. Differences between means were assessed using Tukey's honestly significant difference test for post hoc multiple comparisons. Statistical significance was declared at $p < 0.05$.

Results

Culture and identification of DCMECs and cytolocalization of PPARγ The primary cells derived from seeded bovine mammary tissues were cultured in vitro for 5 to 10 d to develop a mixed growth of fibroblasts and a relatively

small number of mammary epithelial cells (Fig. 1*A*a). DCMECs in culture flasks usually were purified by enzyme digestion with 0.25% trypsin three to five times to remove fibroblasts. Under contrast phase microscopy, these purified DCMECs all displayed a round or oval shape with a beehive-shaped arrangement, as expected (Fig. 1*A*b). These cells were also stained positively by immunofluorescence for the epithelial cell marker CK18 (Fig. 1*A*c). This result showed that we obtained purified DCMECs for subsequent experiments. In the cytolocalization experiment, the PPARγ protein was stained green and the cell nucleus was stained red. The results indicated that PPARγ expression was mainly localized in the cell nucleus and only a little was found in the cytoplasm of the DCMECs (Fig. 1*B*).

PPARγ gene silencing decreased cell growth, triacylglycerol synthesis, and milk lipogenic gene expression in DCMECs To investigate the effect of PPARγ knockdown on milk fat synthesis in DCMECs, PPARγ RNA silencing was conducted by RNAi, and the expression of the PPARγ gene and other related genes in milk lipogenesis was examined using qRT-PCR and western blotting. Cell viability and proliferation were detected by CASY. In addition, triacylglycerol content was also determined using a triacylglycerol detection kit. The PPARγ mRNA level was markedly decreased in the PPARγ knockdown group (Fig. 2*A*). The PPARγ protein expression in the PPARγ knockdown group was also significantly lower than that in the negative control group (Fig. 2*B*, *C*). PPARγ expression was observably inhibited in DCMECs transfected with PPARγ siRNA. PPARγ gene silencing markedly reduced the mRNA and protein expression levels of SREBP1, INSIG1, SCAP, CD36, FABP3, ACSL1, ACC, FAS, SCD, GPAT, AGPAT6, and DGAT1 compared with the negative control group, whereas ACSS2 displayed no significant change (Fig. 2*A*–*C*). Additionally, PPARγ knockdown significantly decreased cell viability and proliferation in cells transfected with PPARγ siRNA compared to the cells transfected with scramble siRNA (Fig. 2*D*, *E*). Moreover, triacylglycerol content in the cell medium of the PPARγ knockdown group was also observably lower than that of the negative control group (Fig. 2*C*).

PPARγ overexpression improved cell growth, triacylglycerol synthesis, and milk lipogenic gene expression in DCMECs To investigate the influence of PPARγ gene overexpression on milk lipogenesis in DCMECs, we generated pGCMV-IRES-EGFP-PPARγ. Then, the expression of the PPARγ gene and other related genes for milk fat synthesis in DCMECs after PPARγ overexpression for 48 h was determined using qRT-PCR and western blotting. Cell viability and proliferation were detected by CASY. The triacylglycerol content in culture medium was also examined using a triacylglycerol detection kit. Significant increases of the mRNA and protein levels of PPARγ in pGCMV-IRES-EGFP-PPARγ

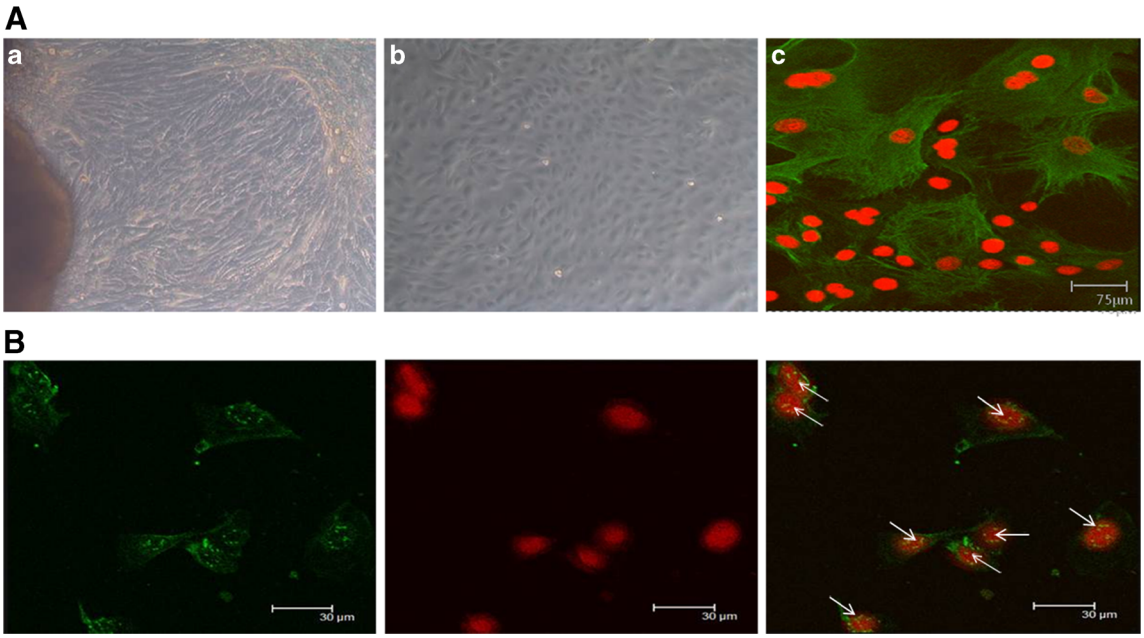

Figure 1. Culture and identification of DCMECs and cytolocalization of PPARγ. (*A*) Culture, purification, and identification of DCMECs. (a) A mixed growth of a large number of fibroblasts and a small amount of mammary epithelial cells was observed by contrast phase microscopy (200×); (b) cellular morphology of purified DCMECs was observed by contrast phase microscopy (200×); (c) purified DCMECs expressed CK18. CK18 was counterstained with FITC, and nuclei were counterstained with propidium iodide (PI). *Scale bar*: 75 μm. (*B*) Localization of PPARγ in lactating DCMECs. PPARγ was counterstained with FITC, and nuclei were counterstained with PI. *Scale bar*: 30 μm. The *arrows* indicated the positive signals of PPARγ. Representative images were from one of three independent experiments.

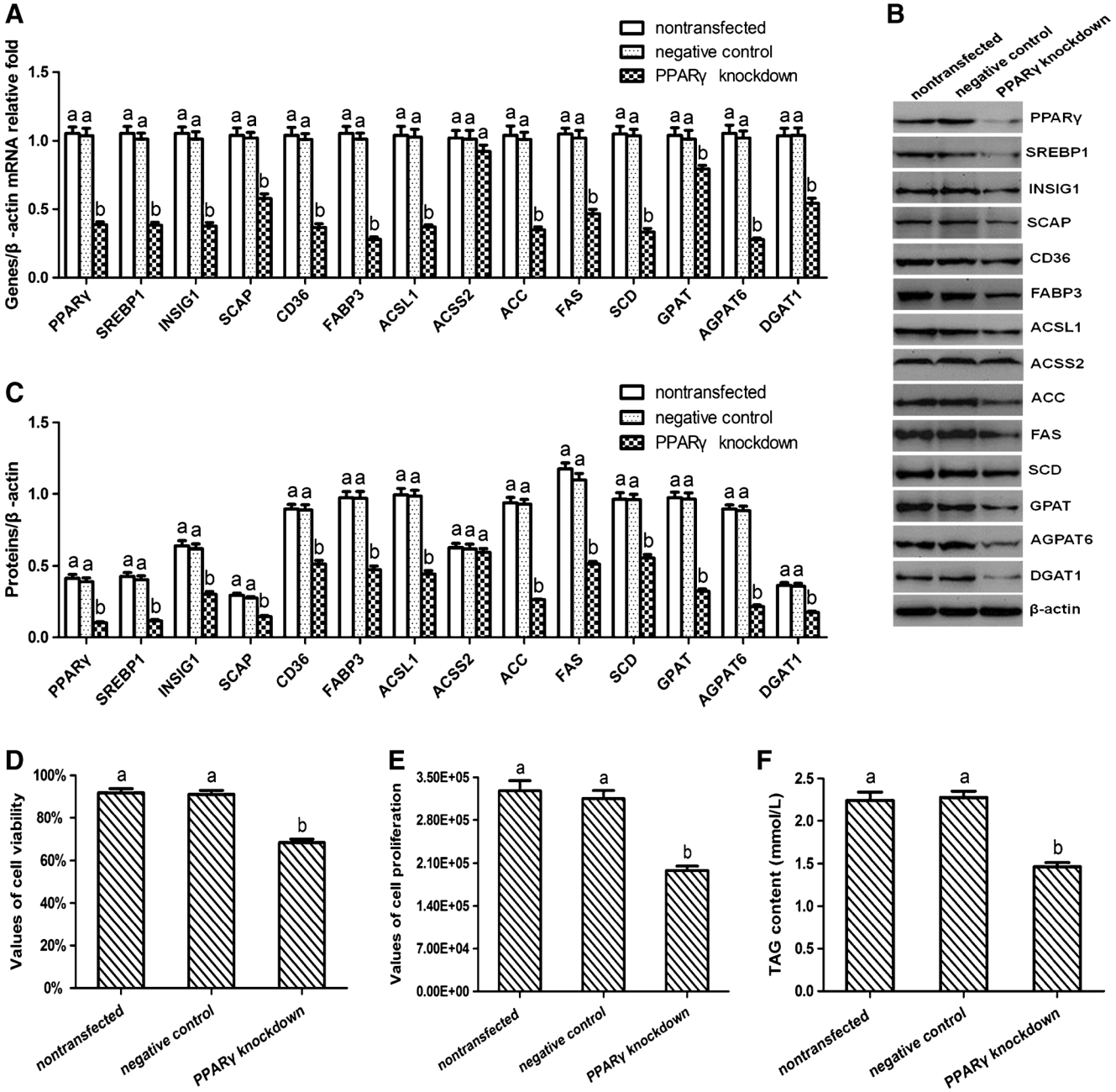

Figure 2. The effect of PPARγ knockdown on milk fat synthesis and cell growth in DCMECs. DCMECs were divided into three groups: a nontransfected group without siRNA, a negative control group with a negative siRNA, and a transfected group with a PPARγ-specific siRNA. All DCMECs grew for 48 h. (*A*) Relative mRNA expression of milk fat synthesis-related genes in DCMECs after PPARγ knockdown for 48 h was measured by qRT-PCR. (*B*) Protein expression of milk fat synthesis-related proteins in DCMECs after PPARγ knockdown for 48 h was assessed by western blot analysis. (*C*) Protein expression levels of milk fat synthesis-related proteins were quantified (proteins/β-actin relative fold) by grayscale scan. (*D*, *E*) Cell viability and cell proliferation of DCMECs growing for 48 h were measured using CASY-TT after PPARγ knockdown. (*F*) TAG content in the culture medium of DCMECs in response to PPARγ knockdown for 48 h was detected using a TAG detection kit. Each value is the mean ± SD, $n = 3$. *Means with different lowercase letters* are significantly different ($p < 0.05$), and *means with common lowercase letters* are not significantly different ($p > 0.05$).

groups were observed compared to the empty vector group (Fig. 3*A–C*). The results indicated that PPARγ was highly and stably expressed in DCMECs after pGCMV-IRES-EGFP-PPARγ transfection. Moreover, PPARγ gene overexpression conspicuously upregulated the mRNA and protein expression levels of SREBP1, INSIG1, SCAP, CD36, FABP3, ACSL1, ACC, FAS, SCD, GPAT, AGPAT6, and DGAT1, but not ACSS2, compared to those of the empty vector group (Fig. 3*A–C*). Compared with the empty vector group, the cell viability and proliferation of DCMECs were

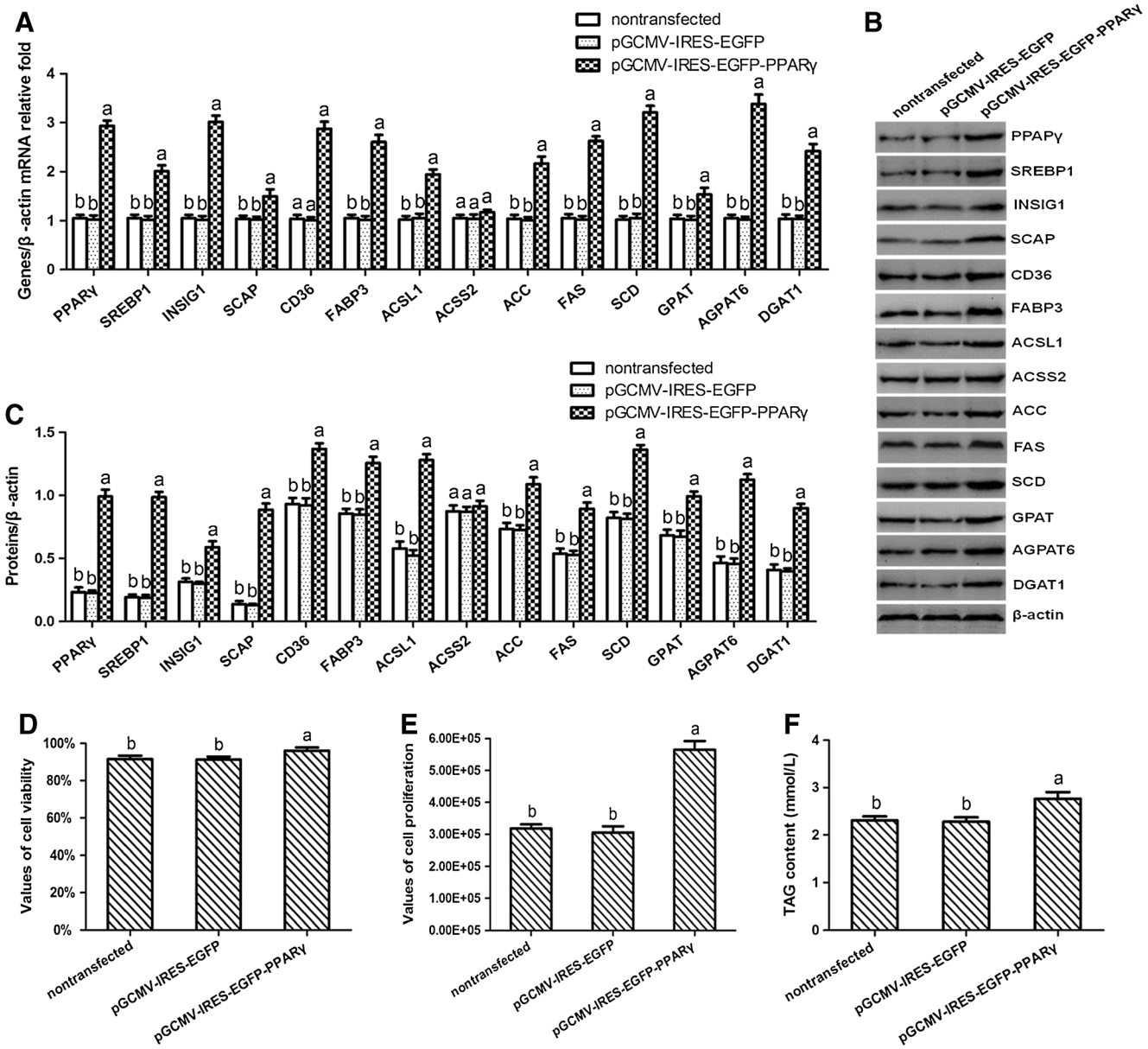

Figure 3. The effect of PPARγ overexpression on milk fat synthesis and cell growth in DCMECs. DCMECs were divided into three groups: a nontransfected group without vector, a pGCMV-IRES-EGFP empty vector group, and a pGCMV-IRES-EGFP-PPARγ group. All DCMECs grew for 48 h. (*A*) Relative mRNA expression levels of milk fat synthesis-related genes in DCMECs after PPARγ overexpression for 48 h were determined by qRT-PCR. (*B*) Protein expression of milk fat synthesis-related proteins in DCMECs growing for 48 h after PPARγ overexpression was detected by western blot analysis. (*C*) Protein expression levels of milk fat synthesis-related proteins were quantified (proteins/β-actin relative fold) by grayscale scan. (*D*, *E*) Cell viability and cell proliferation of DCMECs growing for 48 h were assessed using CASY-TT after overexpression of PPARγ. (*F*) TAG content in the culture medium of DCMECs in response to PPARγ overexpression for 48 h was measured using a TAG detection kit. Each value is the mean ± SD, $n = 3$. *Means with different lowercase letters* are significantly different ($p < 0.05$), and *means with common lowercase letters* are not significantly different ($p > 0.05$).

evidently enhanced by transfection with pGCMV-IRES-EGFP-PPARγ (Fig. 3*D*, *E*). In addition, overexpression of PPARγ notably increased the triacylglycerol secretion of DCMECs (Fig. 3*F*).

Acetic acid enhanced milk lipogenic gene expression and triacylglycerol synthesis in DCMECs To evaluate whether acetic acid could regulate milk fat synthesis in DCMECs, the cells were administered sodium acetate (12 mmol/L) for 48 h. Then, the mRNA and protein expression levels of milk lipogenic genes were determined using qRT-PCR and western blotting. The triacylglycerol content in the culture medium of DCMECs was also assessed using a triacylglycerol detection kit. Our results demonstrated that sodium acetate treatment significantly increased the mRNA and protein expression levels of PPARγ in DCMECs compared to those in the control

group (Fig. 4*A–C*). In addition, sodium acetate supplement resulted in the mRNA and protein expression upregulation of other milk lipogenic genes such as SREBP1, ACC, FAS, SCD, GPAT, AGPAT6, and DGAT1 in DCMECs compared with that of the control group (Fig. 4*A–C*). Moreover, compared to the control group, the secretion of triacylglycerol increased significantly after sodium acetate treatment (Fig. 4*D*). These findings indicated that acetic acid promotes milk lipogenic gene expression and triacylglycerol synthesis in DCMECs.

Acetic acid-regulated milk fat synthesis is related to PPARγ in DCMECs To clarify whether acetic acid regulates milk fat synthesis in DCMECs through the PPARγ pathway, we utilized the technology of PPARγ gene silencing and PPARγ overexpression to alter PPARγ expression of DCMECs and simultaneously treated DCMECs with sodium acetate (12 mmol/L) for 48 h. The mRNA expression levels of milk lipogenic genes were detected by qRT-PCR. The protein expression levels of PPARγ and SREBP1 were determined by western blotting. In addition, the triacylglycerol content was examined using a triacylglycerol detection kit. Our results showed that sodium acetate markedly increased the mRNA levels of PPARγ, SREBP1, ACC, FAS, SCD, GPAT, AGPAT6, and DGAT1 in DCMECs transfected with PPARγ siRNA compared to the levels of the PPARγ gene silencing group not treated with sodium acetate but did not restore the mRNA levels to those of milk lipogenic genes observed in the negative control group simultaneously treated with sodium acetate (Fig. 5*A*). Similarly, the protein levels of PPARγ and SREBP1 in DCMECs with PPARγ gene silencing were significantly elevated by sodium acetate, but this increase did not reach the levels of the negative control group treated with sodium acetate (Fig. 5*B*, *C*). In addition, sodium acetate also evidently increased the triacylglycerol content in the cell culture medium of DCMECs transfected with PPARγ siRNA (Fig. 5*D*).

Moreover, the effects of sodium acetate on milk fat synthesis in DCMECs transfected with pGCMV-IRES-EGFP-PPARγ are shown in Fig. 6. Sodium acetate addition evidently upregulated the mRNA expression levels of PPARγ, SREBP1, ACC, FAS, SCD, GPAT, AGPAT6, and DGAT1

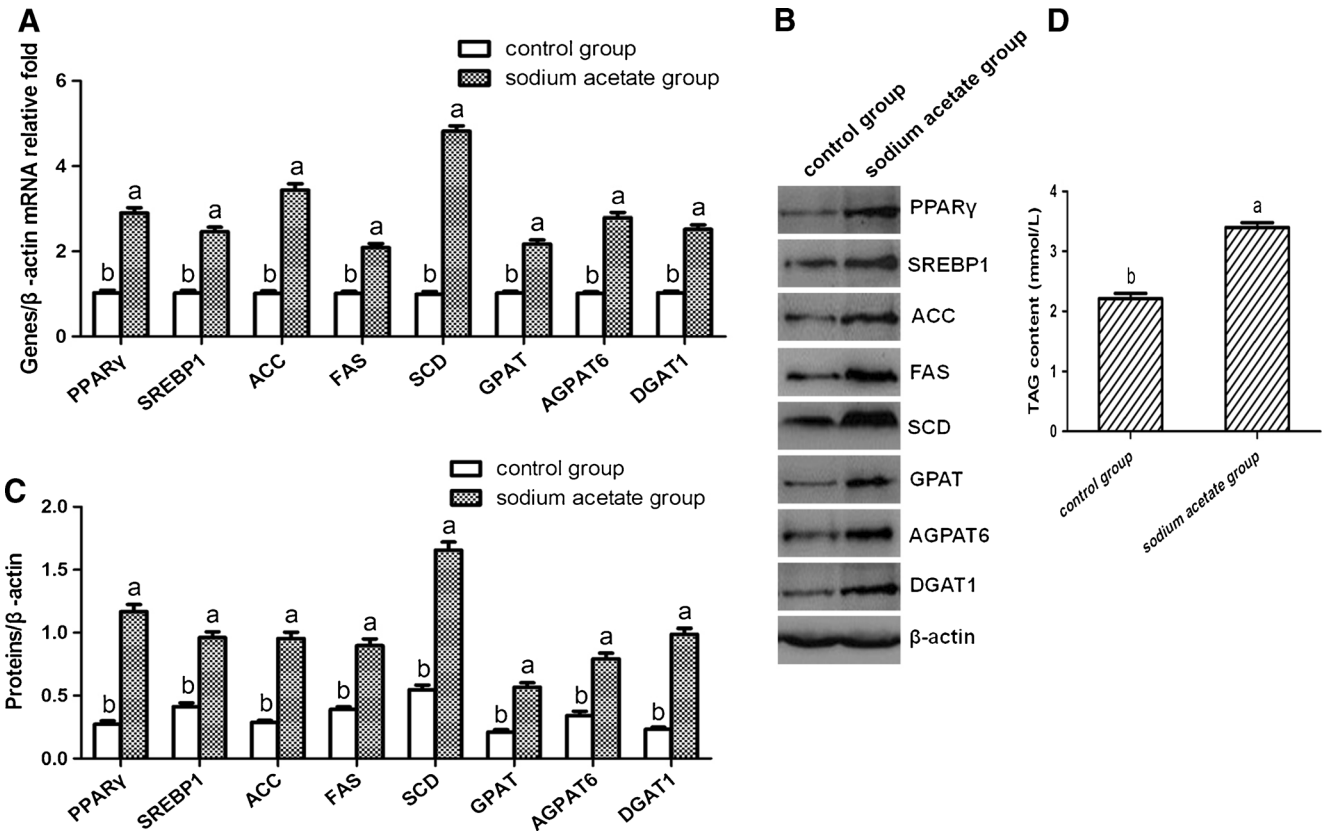

Figure 4. The effect of acetic acid on milk fat synthesis in DCMECs. DCMECs were treated with sodium acetate (12 mmol/L) for 48 h or not treated (control). (*A*) Relative mRNA expression levels of genes related to milk fat synthesis in DCMECs after acetic acid treatment for 48 h were measured using qRT-PCR. (*B*) Protein expression of milk fat synthesis-related proteins in DCMECs in response to sodium acetate for 48 h was assessed using western blotting. (*C*) Protein expression levels of milk fat synthesis-related proteins were quantified (proteins/β-actin relative fold) by grayscale scan. (*D*) TAG content in the culture medium of DCMECs after sodium acetate administration for 48 h was detected using a TAG detection kit. Each value is the mean ± SD, $n = 3$. *Means with different lowercase* letters are significantly different ($p < 0.05$), and *means with common lowercase letters* are not significantly different ($p > 0.05$).

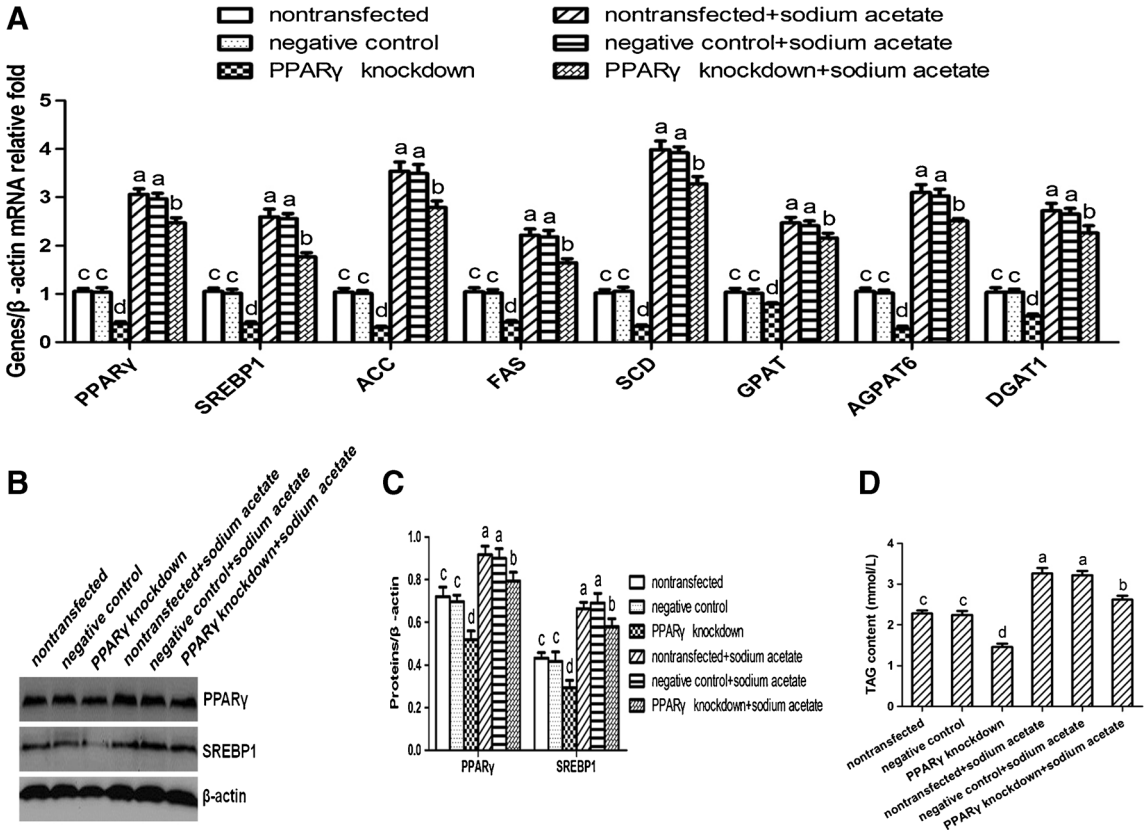

Figure 5. The effect of acetic acid on milk fat synthesis in DCMECs with PPARγ knockdown. DCMECs were divided into six groups: a nontransfected group, a negative control group, a PPARγ siRNA-transfected group, and three similar groups treated with sodium acetate (12 mmol/L). DCMECs of the six groups grew for 48 h. (*A*) Relative mRNA expression levels of milk fat synthesis-related genes in DCMECs were assessed using qRT-PCR. (*B*) Protein expression of PPARγ and SREBP1 in DCMECs was measured using western blotting. (*C*) Protein expression levels of PPARγ and SREBP1 were quantified (proteins/β-actin relative fold) by grayscale scan. (*D*) TAG content in the culture medium of DCMECs was determined using a TAG detection kit. Each value is the mean ± SD, $n = 3$. *Means with different lowercase letters* are significantly different ($p < 0.05$), and *means with common lowercase letters* are not significantly different ($p > 0.05$).

in DCMECs with pGCMV-IRES-EGFP-PPARγ compared with the pGCMV-IRES-EGFP-PPARγ group and the empty vector group simultaneously treated with sodium acetate (Fig. 6*A*). In addition, compared to the DCMECs transfected only with pGCMV-IRES-EGFP-PPARγ and the DCMECs transfected with the empty vector and simultaneously treated with sodium acetate, the protein expression of PPARγ and SREBP1 in DCMECs with pGCMV-IRES-EGFP-PPARγ was enhanced by sodium acetate administration (Fig. 6*B*, *C*). Moreover, sodium acetate also significantly improved the secretion of triacylglycerol in DCMECs transfected with pGCMV-IRES-EGFP-PPARγ compared to the other groups (Fig. 6*D*). These findings suggest that sodium acetate partly regulates milk fat synthesis in DCMECs via PPARγ.

Palmitic acid affected milk lipogenic gene expression and triacylglycerol synthesis in DCMECs To determine whether palmitic acid could mediate milk fat synthesis in DCMECs, the cells were treated with palmitic acid (150 μmol/L) for 48 h. Then, the mRNA and protein expression levels of milk lipogenic genes were examined using qRT-PCR and western blotting. The triacylglycerol content in the culture medium of DCMECs was tested using a triacylglycerol detection kit. The results of this investigation indicated that in comparison to the control group, palmitic acid significantly improved mRNA and protein levels of PPARγ in DCMECs (Fig. 7*A–C*). Additionally, the mRNA and protein expression of milk fat synthesis-related genes, such as SREBP1, SCD, GPAT, AGPAT6, and DGAT1, were evidently increased in DCMECs treated with palmitic acid compared to that of the control group, whereas the mRNA and protein levels of ACC and FAS were downregulated (Fig. 7*A–C*). Simultaneously, palmitic acid treatment was also found to result in an observable increase in triacylglycerol secretion in DCMECs (Fig. 7*D*). These results suggest that palmitic acid regulates the expression of milk fat synthesis-related genes and triacylglycerol synthesis in DCMECs.

Palmitic acid modulation of milk fat synthesis is associated with PPARγ in DCMECs To ascertain whether palmitic acid modulates milk fat synthesis via the PPARγ pathway in DCMECs, we adjusted PPARγ expression in DCMECs using

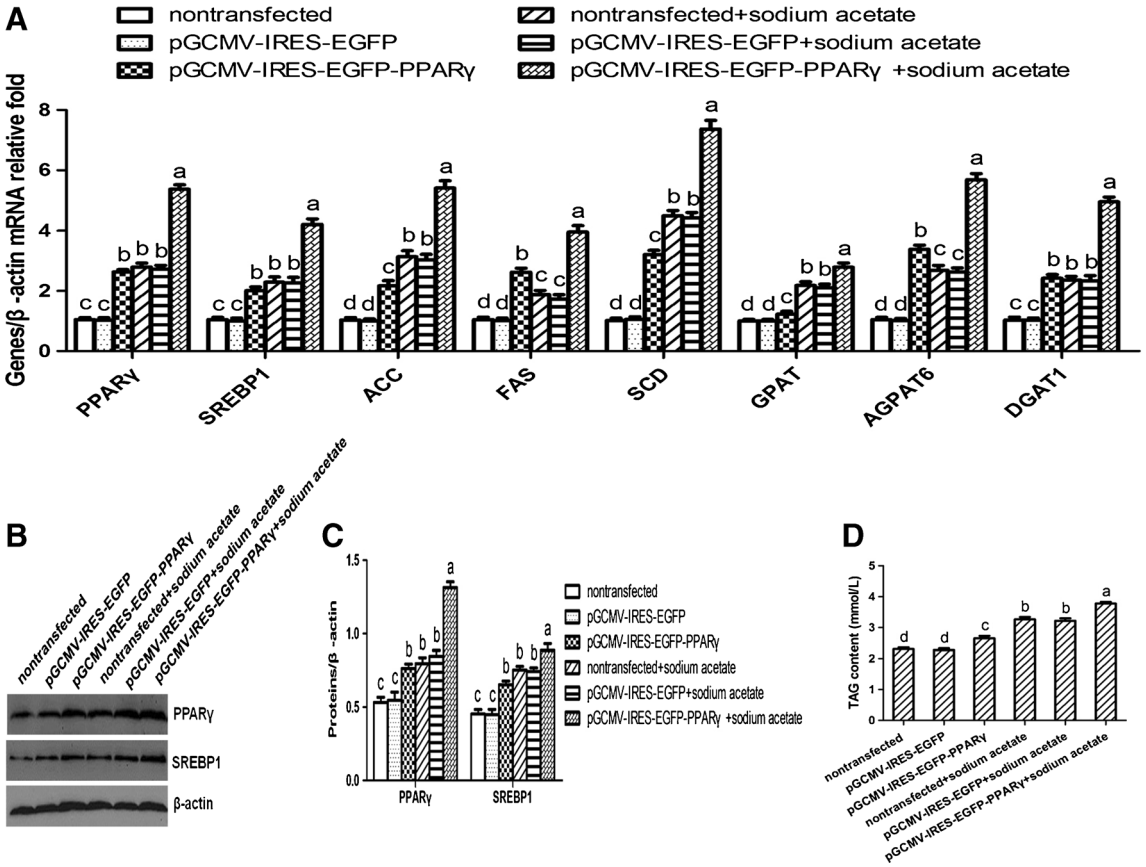

Figure 6. The effect of acetic acid on milk fat synthesis in DCMECs with PPARγ overexpression. DCMECs were divided into six groups: a nontransfected group, a pGCMV-IRES-EGFP empty vector group, a pGCMV-IRES-EGFP-PPARγ group, and three similar groups treated with sodium acetate (12 mmol/L). DCMECs of the six groups grew for 48 h. (*A*) Relative mRNA expression levels of milk fat synthesis-related genes in DCMECs were assessed using qRT-PCR. (*B*) Protein expression of PPARγ and SREBP1 in DCMECs was measured by western blot analysis. (*C*) Protein expression levels of PPARγ and SREBP1 were quantified (proteins/β-actin relative fold) by grayscale scan. (*D*) TAG content in the culture medium of DCMECs was determined using a TAG detection kit. Each value is the mean ± SD, $n = 3$. *Means with different lowercase letters* are significantly different ($p < 0.05$), and *means with common lowercase letters* are not significantly different ($p > 0.05$).

PPARγ gene silencing and PPARγ overexpression and concurrently administered DCMECs with palmitic acid (150 μmol/L) for 48 h. Then, the mRNA expression levels of milk lipogenic genes were measured by qRT-PCR, and the protein expression levels of PPARγ and SREBP1 were evaluated by western blot analysis. In addition, triacylglycerol content was also determined using a triacylglycerol detection kit. Our results indicated that with the exception of the ACC and FAS genes, palmitic acid observably upregulated the mRNA levels of PPARγ, SREBP1, SCD, GPAT, AGPAT6, and DGAT1 genes in DCMECs with PPARγ knockdown compared to the PPARγ knockdown group not treated with palmitic acid but did not restore these to the levels of the negative control group concurrently treated with palmitic acid (Fig. 8*A*). Analogously, the PPARγ and SREBP1 protein levels in DCMECs transfected with PPARγ siRNA were markedly increased by palmitic acid but did not reach the levels observed in the negative control group simultaneously treated with palmitic acid (Fig. 8*B*, *C*). Moreover, in comparison to the untreated PPARγ knockdown group, palmitic acid also improved triacylglycerol secretion of DCMECs transfected with PPARγ siRNA (Fig. 8*D*).

The influences of palmitic acid on milk fat synthesis in DCMECs with PPARγ overexpression are shown in Fig. 9. In addition to enhancing ACC and FAS genes, palmitic acid apparently enhanced the mRNA levels of PPARγ, SREBP1, SCD, GPAT, AGPAT6, and DGAT1 in DCMECs transfected with pGCMV-IRES-EGFP-PPARγ compared to the empty vector group that was simultaneously treated with sodium acetate and the pGCMV-IRES-EGFP-PPARγ group (Fig. 9*A*). Moreover, palmitic acid also increased PPARγ and SREBP1 protein levels in DCMECs with pGCMV-IRES-EGFP-PPARγ compared to the DCMECs transfected only with pGCMV-IRES-EGFP-PPARγ and the DCMECs transfected with empty vector and simultaneously treated with palmitic acid (Fig. 9*B*, *C*). Additionally, in contrast to the other groups, the triacylglycerol content in the cell medium of DCMECs transfected with pGCMV-IRES-EGFP-PPARγ

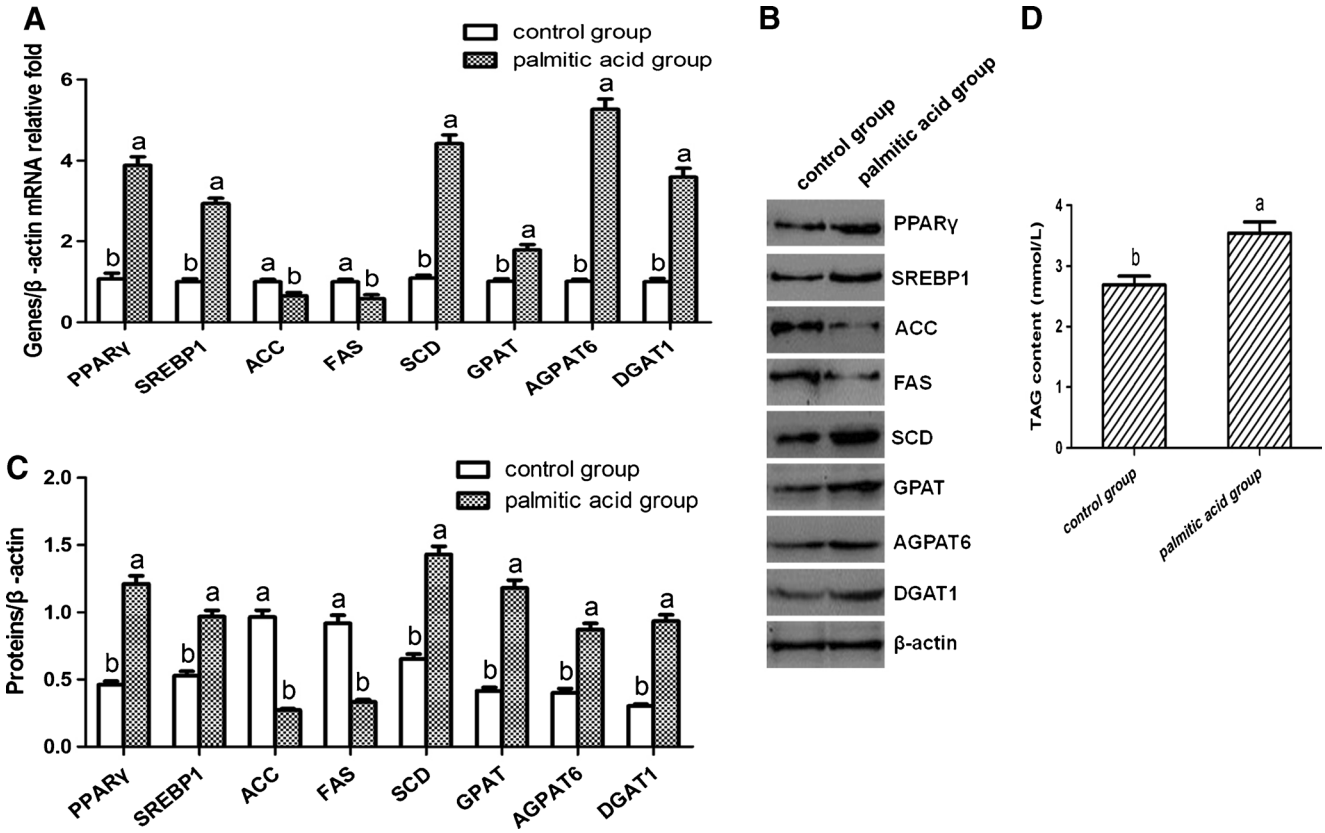

Figure 7. The effect of palmitic acid on milk fat synthesis in DCMECs. DCMECs were treated with palmitic acid (150 μmol/L) for 48 h or not treated (control). (*A*) Relative mRNA expression levels of genes related to milk fat synthesis in DCMECs after palmitic acid treatment for 48 h were measured by qRT-PCR. (*B*) Protein expression of milk fat synthesis-related proteins in DCMECs in response to palmitic acid for 48 h was assessed using western blotting. (*C*) Protein expression levels of milk fat synthesis-related proteins were quantified (proteins/β-actin relative fold) by grayscale scan. (*D*) TAG content in the culture medium of DCMECs after palmitic acid administration for 48 h was detected using a TAG detection kit. Each value is the mean ± SD, $n = 3$. *Means with different lowercase letters* are significantly different ($p < 0.05$), and *means with common lowercase letters* are not significantly different ($p > 0.05$).

was apparently improved by palmitic acid (Fig. 9*D*). These results showed that palmitic acid partly modulates milk fat synthesis through PPARγ activity in DCMECs.

Discussion

Milk fat synthesis is a complicated process that requires many enzymes and proteins. PPARγ has been demonstrated to play key roles in cell growth, apoptosis, and lipogenesis (Kim *et al.* 2012; Lee *et al.* 2012; Li *et al.* 2014). In this study, we explored the roles of PPARγ in the lipid synthesis of DCMECs. We first observed that PPARγ is mainly distributed in the nucleus of DCMECs. PPARγ belongs to a nuclear receptor and works as a critical transcription factor in regulating the expression of genes involved in lipid storage and metabolism (Liu *et al.* 2014; Thomas *et al.* 2012; Zhang *et al.* 2014). We also found that PPARγ largely functions in the nucleus of DCMECs.

The quantity and viability of DCMECs are determining factors for milk production (Boutinaud *et al.* 2004). Our CASY-TT results showed that PPARγ knockdown decreased the viability and proliferation of DCMECs. In contrast, PPARγ overexpression improved DCMEC viability and proliferation. Our results suggested that PPARγ could promote DCMEC growth. This finding was similar to previous studies carried out in skeletal muscle cells (Meshkani *et al.* 2014) and CGCs (Pang *et al.* 2014), where the activation of PPARγ enhanced cell viability. In addition, many reports indicated that PPARγ agonist protected cardiomyocyte and central neurons from apoptosis (Kim *et al.* 2012; Pang *et al.* 2014). These findings are in accordance with our results in the cell viability and proliferation studies. Therefore, we speculated that the changes of PPARγ expression through overexpression or siRNA inhibition can alter the sensitivity of cells to apoptosis and affect the growth of DCMECs.

In cow milk, the most predominant lipid class of milk fat is TAG (Bauman and Griinari 2000). PPARγ activation by ROSI increased TAG content and elevated the number and size of lipid droplets in the mouse liver (Rull *et al.* 2014). In addition, the downregulation of PPARγ was associated with a significant decrease in lipid accumulation in 3T3-L1

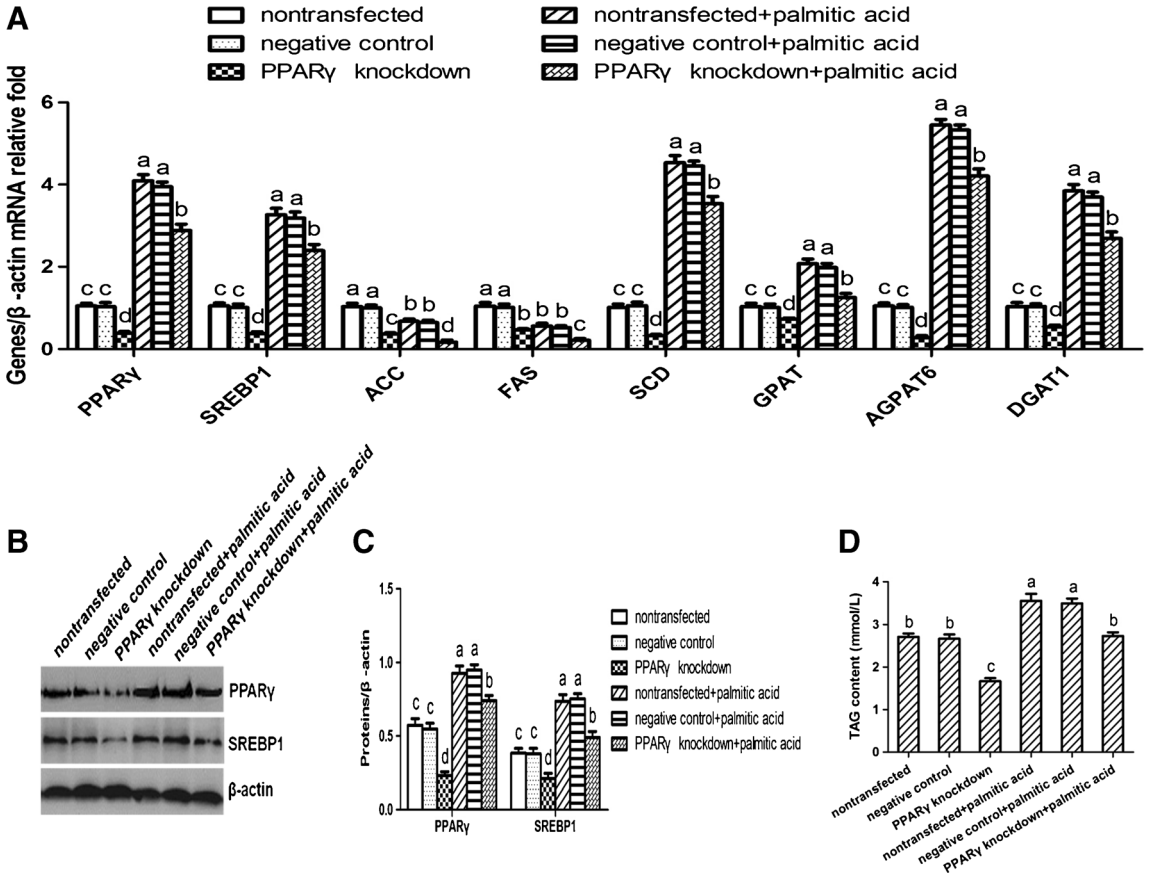

Figure 8. The effect of palmitic acid on milk fat synthesis in DCMECs with PPARγ knockdown. DCMECs were divided into six groups: a nontransfected group, a negative control group, a PPARγ siRNA-transfected group, and three similar groups treated with palmitic acid (150 μmol/L). DCMECs of the six groups grew for 48 h. (*A*) Relative mRNA expression levels of milk fat synthesis-related genes in DCMECs were assessed by qRT-PCR. (*B*) Protein expression of PPARγ and SREBP1 in DCMECs was measured by western blot analysis. (*C*) Protein expression levels of PPARγ and SREBP1 were quantified (proteins/β-actin relative fold) by grayscale scan. (*D*) TAG content in the culture medium of DCMECs was determined using a TAG detection kit. Each value is the mean ± SD, $n = 3$. *Means with different lowercase letters* are significantly different ($p < 0.05$), and *means with common lowercase letters* are not significantly different ($p > 0.05$).

adipocytes (Park *et al.* 2014). In this study, PPARγ overexpression induced DCMECs to synthesize abundant TAG. However, PPARγ knockdown resulted in an observable decrease in the level of TAG. These results indicated that PPARγ could influence TAG synthesis and secretion in DCMECs. Thus, we speculated that PPARγ, as a major transcription factor, possibly played its regulatory effects through participating in some molecular events associated with milk fat synthesis in bovine mammary gland.

Ma and Corl (2012) reported that SREBP1 has an important role in the integrated regulation of key enzymes for lipid synthesis in DCMECs. However, SREBP1 is first synthesized as an inactive precursor. INSIG1 and SCAP are involved in the activation of SREBP1 (Espenshade and Hughes 2007). Using PPARγ overexpression and RNA interference experiments, we verified that PPARγ enhanced the mRNA and protein levels of SREBP1, INSIG1, and SCAP. Kast-Woelbern et al. (Kast-Woelbern *et al.* 2004) found that PPARγ could indirectly regulate the activity of SREBP1 by inducing the expression of INSIG1 in white adipose tissue. Shi et al. (2013) observed that the expression of SREBP1 and SCAP decreased by 50 and 43% after PPARγ knockdown in goat mammary cells. We inferred that PPARγ may be a positive mediator of SREBP1 and could regulate the activity of SREBP1 by altering the expression of INSIG1 and SCAP in DCMECs. CD36 takes part in FA import in DCMECs, and a key function of FABP3 in the mammary gland is to provide FAs for SCD. ACSL1 and ACSS2 are mainly responsible for the activation of long-chain FAs and short-chain FAs in bovine mammary tissue, respectively (Bionaz and Loor 2008b). CD36, FABP4, and ACSL1 are PPARγ target genes in non-ruminants (Berger and Moller 2002). In our study, we observed that overexpression of PPARγ resulted in an increase in the mRNA and protein levels of CD36, FABP3, and ACSL1 and a slight decrease in ACSS2 mRNA and protein levels, while PPARγ gene silencing had contrary effects. Luo et al. (2010) found that PPARγ knockout reduced the transcription and protein levels of CD36 and FABP in cardiomyocytes. Treating rats with a potent PPARγ-specific ligand could increase the mRNA expression of ACSL1 in

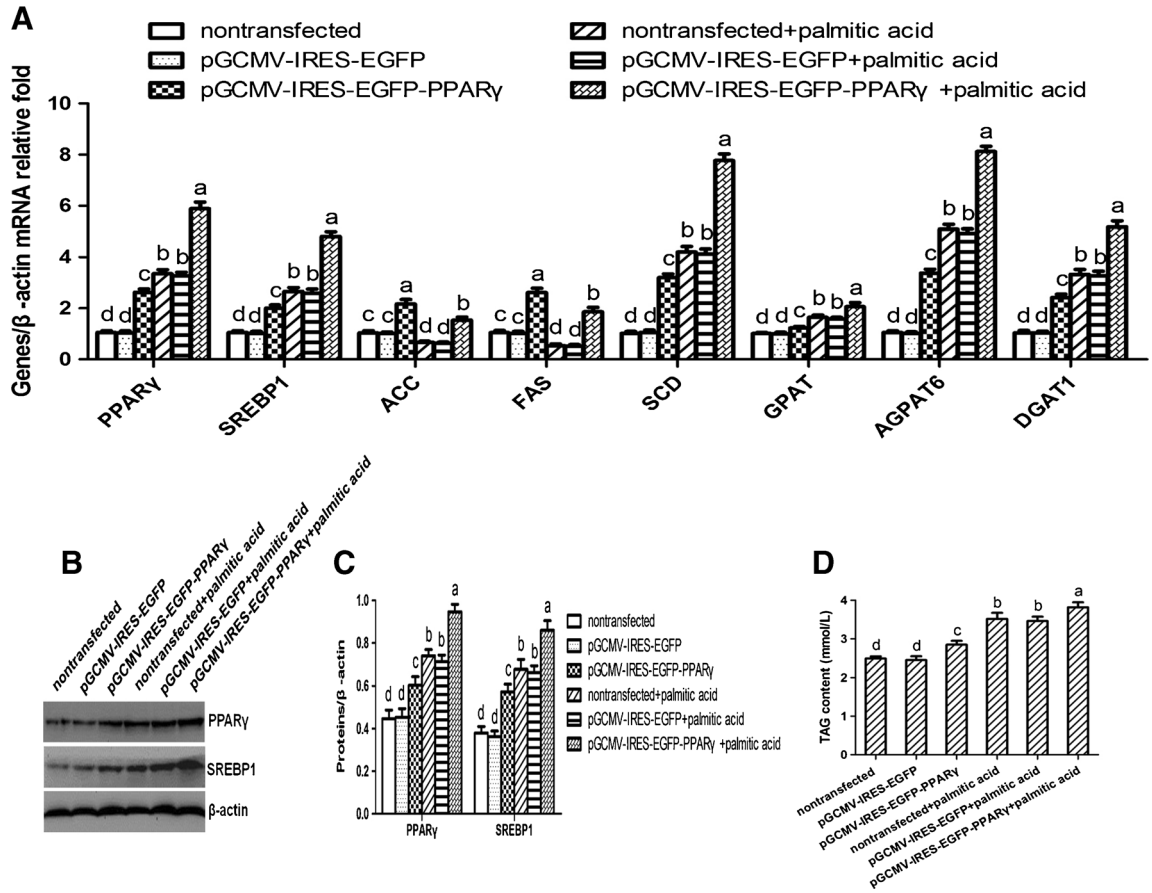

Figure 9. The effect of palmitic acid on milk fat synthesis in DCMECs with PPARγ overexpression. DCMECs were divided into six groups: a nontransfected group, a pGCMV-IRES-EGFP empty vector group, a pGCMV-IRES-EGFP-PPARγ group, and three similar groups treated with palmitic acid (150 μmol/L). DCMECs of the six groups grew for 48 h. (*A*) Relative mRNA expression levels of milk fat synthesis-related genes in DCMECs were assessed by qRT-PCR. (*B*) Protein expression of PPARγ and SREBP1 in the DCMECs was measured by western blot analysis. (*C*) Protein expression levels of PPARγ and SREBP1 were quantified (proteins/β-actin relative fold) by grayscale scan. (*D*) TAG content in the culture medium of DCMECs was determined using a TAG detection kit. Each value is the mean ± SD, $n = 3$. *Means with different lowercase letters* are significantly different ($p < 0.05$), and *means with common lowercase letters* are not significantly different ($p > 0.05$).

muscle adipose tissue (Gerhold *et al.* 2002). Our results also indicated that PPARγ could promote the expression of genes associated with fatty acid uptake, intracellular transport, and activation that facilitate the supply of FAs for TAG synthesis in DCMECs. ACC and FAS are two key enzymes responsible for de novo synthesis of FAs in DCMECs. SCD helps to introduce a double bond in the Δ9 position of myristoyl-, palmitoyl-, and stearoyl-CoA to produce monounsaturated FAs (Ntambi and Miyazaki 2003). PPARγ knockout in mice hepatocytes downregulated the expression of genes involved in lipogenesis (SREBP1c, SCD1, and ACC) (Moran-Salvador *et al.* 2011). Inhibition of PPARγ could decrease the expressions of CD36 and FAS in 3T3-L1 adipocytes (Jin *et al.* 2012). In our study, PPARγ also affected the mRNA and protein levels of ACC, FAS, and SCD in DCMECs. These findings suggested that ACC, FAS, and SCD are the target genes of PPARγ, and PPARγ exerts a positive role in de novo synthesis and desaturation of FAs. In DCMECs, GPAT, AGPAT6, and DGAT1 are implicated in catalyzing TAG synthesis (Bionaz and Loor 2008b). ROSI-induced PPARγ activation increased TAG accumulation by enhancing GPAT and DGAT activities in rat brown adipose tissue (Festuccia *et al.* 2009). PPARγ siRNA transfection dramatically reduced the mRNA levels of TAG synthesis-related genes DGAT1 and AGPAT6 by 52 and 67%, respectively (Shi *et al.* 2013). In the current study, PPARγ promoted the mRNA and protein expression of GPAT, AGPAT6, and DGAT1, whereas gene silencing of PPARγ resulted in the opposite effects. Thus, we also deduced that PPARγ enhanced TAG synthesis in DCMECs by stimulating the expression of GPAT, AGPAT6, and DGAT1. In general, we proposed that PPARγ plays a pivotal role in milk fat synthesis by regulating the expression of lipogenic genes related to FA uptake, FA activation, FA transport, de novo FA synthesis, FA desaturation, TAG synthesis, and transcriptional regulation factor SREBP1 in the bovine mammary gland.

Acetic acid is a primary precursor of milk fat synthesis in the mammary gland (Purdie *et al*. 2008). Previous studies reported that acetic acid could act as a signaling molecule that regulated the expression of lipid metabolism genes in hepatocytes (Fushimi and Sato 2005; Fushimi *et al*. 2006). Acetic acid exposure for 3 to 5 d of DCMECs resulted in a significant increase of TAG accumulation (Yonezawa *et al*. 2004). Jacobs et al. (2013) showed that acetate upregulated the mRNA expression of SCD1 and ACC in a cultured bovine mammary cell line, which suggested that acetate had a stimulatory role in mammary FA formation. In this study, we found that sodium acetate promoted TAG synthesis and led to significantly increased PPARγ expression at both mRNA and protein levels in DCMECs. Moreover, the mRNA and protein levels of other lipogenic genes, including SREBP1, ACC, FAS, SCD, GPAT, AGPAT6, and DGAT1, were also upregulated by sodium acetate. Given these results, we speculated that the regulation by sodium acetate on milk fat synthesis may be associated with PPARγ.

Acetic acid is reported to increase PPARγ2 mRNA levels and reduce leptin mRNA expression in DCMECs (Yonezawa *et al*. 2004). Alex et al. (2013) found that short-chain FAs could transactivate and bind to PPARγ and then mediate the expression of other genes in the colon. In this study, to clarify whether milk fat synthesis promoted by acetic acid is via PPARγ signaling, we detected the influences of acetic acid on the expression of milk lipogenetic genes and TAG synthesis in DCMECs transfected with PPARγ siRNA or PPARγ expression vector. We found that sodium acetate significantly induced TAG synthesis in DCMECs with PPARγ knockdown or PPARγ overexpression. Moreover, sodium acetate treatment significantly increased the mRNA levels of PPARγ, SREBP1, ACC, FAS, SCD, GPAT, AGPAT6, and DGAT1 and the protein expression levels of PPARγ and SREBP1 in DCMECs with PPARγ gene silencing or PPARγ overexpression. These results further demonstrated that the modulation by acetic acid on milk lipogenesis is related to PPARγ in DCMECs. Yonezawa et al. (2009) found that GPR41 and GPR43 were expressed in DCMECs and that short-chain FAs are involved in the cell signaling pathway through binding to and activation of GPR41 and GPR43. Acetate could stimulate fat synthesis via GPR43, and GPR43 inhibition reduced the mRNA expression of PPARγ2 and lipid accumulation in 3T3-L1 cells (Hong *et al*. 2005). In the current study, we suggested that acetic acid may promote the expression and activation of PPARγ via a certain signal molecule such as GPR43 and then induce the mRNA and protein expression of other lipogenic genes, which contributed to improve TAG synthesis in DCMECs. Thus, acetic acid played a positive role in milk fat synthesis partly through the PPARγ pathway.

Palmitic acid is the first FA generated from de novo synthesis of the FA pathway in the mammary gland and can also be derived from blood circulation for fat synthesis. Palmitic acid induced a significant increase in intracellular TAG content in pancreatic beta cells (Wang *et al*. 2015) and primary hepatocytes (Pan *et al*. 2011). Zhao et al. (2014) showed that palmitic acid could increase SREBP-1c mRNA expression in NIT-1 pancreatic beta cells. Kadegowda et al. (2009) found that the mRNA levels of AGPAT6 and DGAT1 genes in DCMECs were also upregulated by the addition of palmitic acid. In the current study, we observed that palmitic acid enhanced TAG accumulation and simultaneously increased the mRNA and protein levels of PPARγ and other lipogenic genes, including SREBP1, SCD, GPAT, AGPAT6, and DGAT1, in DCMECs. However, palmitic acid treatment led to an obvious decrease in ACC and FAS expression levels in DCMECs, which was in line with a previous study in which several long-chain FAs, including palmitic acid, significantly suppressed de novo synthesis of FAs and inhibited ACC and FAS mRNA expression (Kadegowda *et al*. 2009). Our results suggested that palmitic acid promoted TAG synthesis and the expression of some milk lipogenic genes probably through the PPARγ pathway. Moreover, we also inferred that palmitic acid, as the first product from de novo FA synthesis, inhibited de novo synthesis of milk FA in DCMECs.

Xie et al. (2012) found that palmitic acid significantly increased CD36 and PPARγ mRNA levels in jejunum tissue. The treatment of cultured hepatocytes with palmitic acid upregulated the expression of PPARγ (Allman *et al*. 2010). In nonruminants, most long-chain FAs are natural ligands of PPARγ, which bind to PPARγ to elicit changes in gene expression and lipogenesis (Desvergne *et al*. 2006; Bensinger and Tontonoz 2008). In our study, we also further analyzed the influences of palmitic acid on TAG synthesis and the expression of milk lipogenetic genes in DCMECs transfected with PPARγ siRNA or PPARγ expression vector. We observed that the addition of palmitic acid promoted TAG synthesis in DCMECs with PPARγ knockdown or PPARγ overexpression. Moreover, palmitic acid robustly upregulated the mRNA expression levels of PPARγ, SREBP1, SCD, GPAT, AGPAT6, and DGAT1 genes and the protein abundances of PPARγ and SREBP1 in DCMECs with PPARγ knockdown or PPARγ overexpression. Therefore, we speculated that palmitic acid probably serves as a ligand that binds and activates PPARγ, which then regulates the expression of other milk lipogenic genes and the synthesis of milk fat in DCMECs.

Conclusion

In summary, the present study showed that PPARγ was a positive regulator of milk fat synthesis in DCMECs by improving cell viability, proliferation ability, and triacylglycerol secretion and by regulating the mRNA and protein expression

levels of genes involved in milk fat synthesis in DCMECs. Moreover, acetic acid and palmitic acid could regulate milk fat synthesis in DCMECs via PPARγ signaling.

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