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Peroxisome proliferator activated receptor delta regulates lipid droplet formation and transport in goat mammary epithelial cells

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ABSTRACT

Even though recent evidence in goat mammary epithelial cells (GMEC) suggest a role of peroxisome proliferator-activated receptor delta (PPARD) in regulating lipid homeostasis, its role is not fully understood. Our hypothesis was that PPARD regulates lipid transport processes in GMEC and, thus, plays a crucial role in regulating fat formation. The *PPARD* was overexpressed using an adenovirus system (Ad-PPARD) with recombinant green fluorescent protein (Ad-GFP) as the control. Results revealed that overexpression of *PPARD* markedly upregulated the mRNA abundance of *PPARD*. Compared with the control (Ad-GFP+dimethyl sulfoxide), overexpression of *PPARD* alone had no effect on mRNA expression of *CD36*, *SCD1*, *FABP4*, *ACSL1*, and *ADRP*. The cultures overexpressing *PPARD* with the PPARD ligand GW0742 (GW) upregulated the expression of *CD36*, *FABP3*, *FABP4*, *ACSL1*, and *ADRP*. Overexpression of *PPARD* in GMEC plus GW increased the concentration of 16:1 and 18:1-*trans* and was associated with upregulation of *SCD1*. Compared with the control (Ad-GFP+dimethyl sulfoxide), the decrease of triacylglycerol concentration coupled with upregulation of genes related to lipid droplet secretion (e.g., *ADRP* and *ACSL1*) induced by *PPARD* overexpression suggests a role in lipid droplet (LD) secretion. Luciferase assay revealed that GW increased the ADRP promoter activity in a dose-dependent manner. Knockdown of *PPARD* impaired the increase of ADRP promoter activity induced by GW, whereas GW enhanced the activity of ADRP promoter in GMEC overexpressing *PPARD*. Data with the *ADRP* 5′-flanking truncated luciferase reporter suggest a core region (−1,444 to −990 bp) response element for the induction of GW. This core region contains a known PPARG response element (PPRE) at −1,003 to −990 bp. When the PPRE was mutated, the overexpression of *PPARD* had no effect on *ADRP* promoter activity. Collectively, these results reveal a novel role for PPARD in lipid homeostasis via promoting fatty acid transport and LD formation through a mechanism of direct binding to the promoter of key genes. Hence, PPARD activity may contribute to fatty acid transport and LD formation during lactation.

Key words: perilipin, promoter, peroxisome proliferator-activated receptor response element

INTRODUCTION

Transcription factors play important roles regulating fatty acid metabolism in ruminant mammary tissue (Harvatine et al., 2009; Oppi-Williams et al., 2013). Peroxisome proliferator-activated receptors (**PPAR**) are nuclear proteins that regulate the synthesis of lipid or lipid oxidation upon activation by endogenous or synthetic agonists (Bionaz et al., 2013). In the ruminant, the role of PPAR α (**PPARA**) (Bionaz et al., 2013) and PPAR γ (**PPARG**; Shi et al., 2013b, 2014b) in controlling lipid metabolism in mammary tissue has been well studied. Despite data indicating that its function seems to overlap with PPARA in terms of fatty acid (**FA**) oxidation (Giordano Attianese and Desvergne, 2015), compared with PPARG and PPARA, the biologic role of PPAR delta (**PPARD**) remains unclear. The finding in mice that PPARD activates an array of genes required for FA degradation and uncoupling suggested an opposite role of PPARD compared with PPARG (Wang et al., 2003). Thus, exploring and understanding the regulatory role of PPARD in lipid metabolism in mammary cells may contribute to improving efficiency and quality of ruminant milk production.

The lactating mammary gland is one of the most activate in terms of synthesis, utilization, and transport of FA. The FA transport process is related to the func-

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tion of several key proteins (Haunerland and Spener, 2004; Wang et al., 2015) such as fatty acid binding proteins (**FABP**). The integral membrane protein coded by the CD36 molecule (***CD36***) is believed to physically transport the FA across the plasma membrane (Pepino et al., 2014). A growing body of data suggests that FA uptake also requires concomitant FA activation to acyl-CoA (Mashek and Coleman, 2006). The protein long-chain FA acyl-CoA synthetase (**ACSL**) plays a central role in the FA uptake and also is associated with lipid droplet secretion (**LD**; Grevengoed et al., 2014). Once activated to acyl-CoA, FA have diverse cellular fates, but the 2 predominant routes of metabolism are degradation via oxidation or incorporation into complex lipids (e.g., triacylglycerol, **TAG**; Bionaz and Loor, 2008a). In the lactating mammary gland, the FA are mainly incorporated into lipid droplets and secreted out of the epithelial cells. Recent data underscored the role of adipocyte differentiation-related protein (**ADRP**) in promoting LD formation and secretion in dairy goat mammary epithelial cells (**GMEC**; Shi et al., 2015b). A growing body of data supports the view that the process of FA transport is regulated by multiple transcription factors, including PPAR (Bionaz et al., 2013; Shi et al., 2014b).

Data in GMEC demonstrating that PPARD activation by the synthetic ligand GW0742 (**GW**) upregulated the expression of genes containing *FABP4* and *ADRP* suggested a role of PPARD in promoting FA transport (Shi et al., 2017). Furthermore, the knockdown of PPARD selectively downregulated *ACSL1* and *ADRP* mRNA abundance in GMEC, underscoring a role for PPARD in lipid transport (Shi et al., 2017). It is hypothesized that overexpression of *PPARD* would upregulate the mRNA expression of genes related to FA transport and lipid droplet formation in ruminant mammary cells at least in part through binding the promoter region.

To investigate the role of PPARD in regulating FA transport and lipid droplet formation in ruminant mammary cells, in this study, goat *PPARD* was overexpressed in GMEC. The activity of the *ADRP* promoter was also measured to assess the effect of PPARD on *ADRP* transcription.

MATERIALS AND METHODS

Adenovirus Generation

The *PPARD* (GenBank no. XM_004018768.3) cDNA was cloned from GMEC, and then subcloned into the pAdTrack-CMV plasmid vector to generate pAdTrack-CMV-PPARD vector. The vector was inserted into an adenoviral vector (pAdEasy-1) to generate adenoviral plasmids in BJ5183 cells. The adenoviral plasmids linearized by *Pac*I (New England Biolabs, Ipswich, MA) were transfected into 293A cells to generate the adenoviral Ad-PPARD. The generation and proliferation of adenovirus (Ad-PPARD) was performed as previously described (Shi et al., 2013a). The 293A cell line for adenovirus generation was cultured in basal Dulbecco's modified Eagle medium (Gibco, Waltham, MA) containing 10% fetal bovine serum. A recombinant adenovirus of green fluorescent protein (Ad-GFP) was used as a control.

Cell Culture and Treatments

The GMEC were isolated from peak lactation Xinong Saanen goats as described previously (Shi et al., 2014a). The GMEC were isolated from 3 individual goats. Details of cell culture are described elsewhere (Shi et al., 2013b, 2014a). Briefly, the collected mammary tissues were pooled together and cut into small pieces using scissors before plating on cell culture dishes. The GMEC were isolated from this mixture of tissue. To promote lactogenesis, GMEC were cultured in a lactogenic medium for 24 h before initial experiments. Lactogenic medium was composed of Dulbecco's modified Eagle medium/F12 (Hyclone, Beijing, China), insulin (5 mg/L, Sigma-Aldrich, St. Louis, MO), hydrocortisone (5 mg/L, Sigma-Aldrich), penicillin/streptomycin (10,000 units/L, Harbin Pharmaceutical Group, Harbin, China), prolactin (2 μg/mL, Sigma-Aldrich), and BSA (1 g/L, Sigma-Aldrich).

For the adenovirus infection, the GMEC cultured in 6-well plates were incubated with adenovirus medium Ad-GFP or Ad-PPARD, respectively. Treated GMEC were cultured with 1 μM GW [final concentration, diluted in dimethyl sulfoxide (**DMSO**), Sigma-Aldrich] or control (DMSO, Sigma-Aldrich) after 24 h of initial culture, and then harvested at 48 h (24 h later) for RNA extraction, cellular TAG assay, or lipid extraction. The adenovirus Ad-GFP was used as a control.

Total RNA Extraction and Quantitative PCR

Total RNA was extracted using the RNA Prep pure cell Kit (Tiangen Biotech Co. Ltd., Beijing, China) and quantitative PCR (**qPCR**) was performed according to the manufacturer's instructions using SYBR Green (SYBR Premix Ex Taq II, Perfect Real Time, Takara Bio Inc., Dalian, China; Shi et al., 2014b). Genes studied included *PPARD*, *ACSL1*, *ADRP*, *CD36*, *FABP3*, *FABP4*, and *SCD1*. All the qPCR reactions were performed in a Bio-Rad CFX96 (Bio-Rad Laborato-