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Peroxisome proliferator-activated receptor delta facilitates lipid secretion and catabolism of fatty acids in dairy goat mammary epithelial cells

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ABSTRACT

In rodents, peroxisome proliferator-activated receptor delta (PPARD) is associated primarily with catabolism of fatty acids. However, the role of PPARD in regulating lipid metabolism in ruminant mammary gland remains unknown. In the present study, we assessed the mRNA abundance of *PPARD* at 3 stages of lactation in goat mammary tissue. Results revealed that *PPARD* had lower expression at peak lactation than in the nonlactating period. Luciferase assays revealed that GW0742 (GW), a specific PPARD ligand, enhanced the activity of the *PPARD* response element in goat mammary epithelial cells. Activation of *PPARD* by GW selectively upregulated the expression of genes related to fatty acid activation (*ACSL1*), lipid droplet formation (*PLIN2*), and transport (*FABP4*), and had no effect on genes involved in de novo fatty acid synthesis (*ACACA* and *FASN*), desaturation (*SCD*), hydrolysis and oxidation (*PNPLA2* and *CPT1A*), transport and uptake (*FABP3* and *CD36*), or triacylglycerol synthesis (*DGAT1* and *AGPAT6*) in goat mammary epithelial cells. In contrast, knockdown of *PPARD* using small interfering RNA dramatically decreased the expression of genes related to fatty acid activation (*ACSL1*) and lipid formation (*PLIN2*) and increased the expression of genes related to fatty acid transport (*FABP3*) and triacylglycerol synthesis (*AGPAT6* and *DGAT1*). The expression of genes related to fatty acid synthesis (*FASN*), hydrolysis (*PNPLA2*), and fatty acid oxidation (*CPT1A*) was downregulated significantly only after knockdown of *PPARD* in cells incubated with GW. We observed no significant change in fatty acid profiles. However, the total cellular triacylglycerol increased after knockdown of *PPARD* in goat mammary epithelial cells plus GW. Collectively, these results highlight an important role for PPARD in the homeostasis of ruminant mammary cells by facilitating fatty acid activation and lipid droplet formation and secretion.

Key words: goat, peroxisome proliferator-activated receptor (PPAR), milk fat, gene expression

INTRODUCTION

Peroxisome proliferator-activated receptors (**PPAR**) are ligand-activated transcription factors that belong to the nuclear receptor superfamily and include 3 closely related members: PPAR alpha (**PPARA**), gamma (**PPARG**), and delta (**PPARD**). Each member displays a tissue-selective expression pattern in ruminants, with PPARG and PPARA predominantly expressed in adipose tissue and liver, respectively, and PPARD broadly expressed in various tissues, including adipose, rumen, liver, and lung (Bionaz et al., 2013). In nonruminants, the role of PPARG in determining adipocyte differentiation and promoting lipid storage has been well established (Kawai and Rosen, 2010). Recent studies have also underscored a key role for PPARG in controlling lipid metabolism in goat mammary gland (Shi et al., 2013a; Kang et al., 2015). Similarly, a large body of evidence also supports the function of PPARA as a controller of the catabolism of fatty acids (Roberts et al., 2011; Pol et al., 2015). However, compared with PPARG and PPARA, knowledge about PPARD in the context of ruminant mammary lipid metabolism remains unknown.

In rodents, the primary role of PPARD is the control of catabolism of fatty acids in muscle (Barak et al., 2002). The expression of *PPARD* in skeletal muscle is increased upon fasting and upon exercise (Giordano Attianese and Desvergne, 2015), suggesting a role for PPARD in the adaptive response of skeletal muscle to increased demand for catabolism of fatty acids. Conversely, when *PPARD* is deleted in skeletal muscle, the muscle fibers exhibit lower oxidative activity, and body fat mass increases (Schuler et al., 2006).

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A biologic role for PPARD in the catabolism of fatty acids in white and brown adipose tissue is supported by evidence from *PPARD*-transgenic mice. In line with an increase in catabolism of fatty acids, transgenic mice expressing *PPARD* are resistant to lipid accumulation induced by high-fat diets and exhibit diminished lipidemia (Wang et al., 2003). However, the fact that mice carrying an adipose-specific deletion of *PPARD* did not have alterations in fat mass content was indicative of an indirect effect of PPARD in adipose tissue (Lee et al., 2006). Interestingly, the exploration of PPARD-mediated response in the liver suggested that PPARD also promotes liver lipogenesis (Liu et al., 2011).

Unlike in rodents, the function of PPARD in ruminants remains largely unknown. A similar expression of *PPARD* has been observed in various cow tissues, and *PPARD* was more abundant than *PPARG* in cow mammary gland (Bionaz et al., 2013). Contrasting roles for PPARG and PPARD in primary bovine mammary cells has been reported, where several PPARG ligands reduced the expression of *PPARD* (Lutzow et al., 2008). In contrast, the fact that activation of PPARD increased the activity of glyceraldehyde-3-phosphate dehydrogenase indicated a potential role for PPARD in adipogenesis in sheep (Soret et al., 1999). The opposite responses for catabolism of fatty acids and lipogenesis in these published experiments indicate that PPARD may play different roles in different tissues. Thus, it was hypothesized that activation or knockdown of *PPARD* would alter the mRNA expression of genes related to lipid metabolism.

To our knowledge, few available data directly support a role for PPARD in lipid metabolism in ruminants. To investigate the effect of PPARD on lipid metabolism in ruminant mammary gland in the present study, PPARD was activated by use of a chemical ligand or was knockdown via small interfering RNA (**siRNA**) in goat mammary epithelial cells (**GMEC**).

MATERIALS AND METHODS

mRNA Abundance of PPARD

The transcriptome data set from 3 lactating goat mammary tissue samples were deposited at the National Center for Biotechnology Information (GEO Series GSE87089). Details of these samples have been described previously (Shi et al., 2015). Briefly, we used 3- to 4-yr-old Xinong Saanen dairy goats from the experimental farm of Northwest Agricultural University, Shaanxi, China. Mammary tissue at peak lactation (3 goats, 100 d postpartum), late lactation (cessation of milking, 3 goats, 310 d postpartum), and non-lactation (3 goats, nonlactating and nonpregnant) was collected by a veterinarian after slaughter. All goats were managed in a similar fashion, and were fed a mixed diet consisting of corn, soybean meal, bran, rapeseed meal, and a mineral-vitamin mixture. We used Bowtie 0.12.8 (http://bowtie-bio.sourceforge.net/index.shtml) to align reads to the goat genome (Dong et al., 2013). We used DESeq (a software for differential gene expression analysis based on negative binomial distribution of data; http://www-huber.embl.de/users/anders/DESeq) to analyze the abundance of gene expression. The single-end mapping method was used for read analysis; one read was compared with multiple genes. Reads per kilobase of exon model per million mapped reads (**RPKM**) were used for measuring gene expression of *PPARD.*

Cell Culture and Treatments

The GMEC were isolated from Xinong Saanen goats at peak lactation, as described previously (Wang et al., 2010; Shi et al., 2014). Details of cell culture were described recently (Lin et al., 2013; Shi et al., 2013b). To promote lactogenesis, GMEC were cultured in a lactogenic medium for 24 h before initial experiments. Cells were cultured in a 6-well culture plate and subcultured to 90% confluence; they were then treated with PPARD-specific ligand GW0742 (**GW**, Sigma-Aldrich, St. Louis, MO) at 1 μM in lactogenesis medium. Then the cells were collected at 0, 12, and 24 h for total RNA extraction or triacylglycerol (**TAG**) assays. The lactogenesis medium was composed of DMEM/F12 (Hyclone, Beijing, China), insulin (5 mg/L, Sigma-Aldrich), hydrocortisone (5 mg/L, Sigma-Aldrich), penicillin/streptomycin (10k unit/L, Harbin Pharmaceutical Group, Harbin, China), prolactin (2 μg/mL, Sigma-Aldrich) and BSA (1 g/L, Sigma-Aldrich).

RNA Interference

To determine *PPARD* mRNA interference, cells cultured in 6-well plates were transfected with 100 nM siRNA and 7.5 μL of transfection reagent (Lipofectamine RNAiMAX; Thermo Fisher Scientific Inc., Waltham, MA) in lactogenesis medium without antibiotic. The transfection was performed according to the manufacturer's instructions (http://tools.thermofisher.com/content/sfs/manuals/Lipofectamine_RNAiMAX_Reag_protocol.pdf). Transfected GMEC were cultured with 1 μM GW (Sigma-Aldrich) or control (dimethyl sulfoxide, **DMSO**; Sigma-Aldrich) after 24 h of initial culture, and then harvested at 48 h (24 h later) for RNA extraction, TAG assays, and fatty acid extraction. The sequences of the siRNA oligonucleotides for *PPARD* (accession XM_004018768.3) were 5′-GCUG-