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Peroxisome proliferator-activated receptor γ1 and γ2 isoforms alter lipogenic gene networks in goat mammary epithelial cells to different extents

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ABSTRACT

In nonruminants, the alternative splicing of peroxisome proliferator-activated receptor γ (PPARG) generates *PPARG1* and *PPARG2* isoforms. Although transcriptional control differences between isoforms have been reported in human adipose tissue, their roles in ruminant mammary cells are not well known. To assess which of these isoforms is more closely associated with the regulation of mammary lipogenic pathways, their tissue distribution was analyzed and the expression of key genes regulating lipogenic gene networks was measured after overexpression of the 2 isoforms in goat mammary epithelial cells (GMEC). The expression of *PPARG2* was markedly greater in adipose tissue, whereas *PPARG1* is the main isoform in goat mammary tissue (ratio of *PPARG1*:*PPARG2* was close to 37:1). As was reported in previous work, *PPARG1* upregulated the transcription regulators *SREBF1* and *PPARG* and the lipogenic genes *FASN*, *ACACA*, and *SCD*. Along with a tendency for greater expression of *AGPAT6*, *DGAT1*, and *PLIN2*, these data suggest that *PPARG1* is the isoform controlling lipogenesis in mammary cells. Addition of the PPARG ligand rosiglitazone (ROSI) to GMEC overexpressing both isoforms upregulated the expression of *LPL* and *CD36*, which help control uptake of long-chain fatty acids into mammary cells. Other responses to ROSI addition to GMEC overexpressing *PPARG1* and *PPARG2* included upregulation of *AGPAT6*, *DGAT1*, *INSIG1*, *SREBF1*, and *NR1H3*. Although the data suggest that both *PPARG1* and *PPARG2* could affect mammary lipogenesis via control of gene expression when stimulated (e.g., by ROSI), the fact that *PPARG1* is more abundant in mammary tissue and that its overexpression alone upregulated key lipogenic gene networks suggest that it is the more important isoform in goat mammary cells.

Key words: fatty acid metabolism, mammary gland, lactation, gene network, nuclear receptor

INTRODUCTION

Peroxisome proliferator-activated receptor gamma (**PPARG**) is a ligand-dependent member of the nuclear hormone receptor superfamily with a key role in fat cell differentiation and lipid filling (Rosen and Spiegelman, 2001). Various long-chain fatty acids (**LCFA**; Kadegowda et al., 2009) and chemical compounds (e.g., thiazolidinediones; Khandoudi et al., 2002) can bind to and activate PPARG, leading to changes in mRNA expression of target genes (Lee et al., 2002). To date, 2 isoforms of PPARG have been identified, *PPARG1* and *PPARG2*, which are generated by alternative use of 2 different promoters (Elbrecht et al., 1996). Compared with *PPARG1*, *PPARG2* has an additional 30 AA in the N terminus sequence in goat (Shi et al., 2013a). In nonruminants, the expression of *PPARG2* is specific to adipose tissue, whereas *PPARG1* is expressed to different extents in several tissues (Fajas et al., 1997).

The specific roles of PPARG isoforms have been extensively studied in humans, leading to the conclusion that *PPARG2* activates adipogenesis in adipose tissue (Ren et al., 2002). The A/B-domain in the N terminus of *PPARG2* plays a gene-specific role for transactivation and co-factor recruitment (Bugge et al., 2009). However, both PPARG1 and PPARG2 have the intrinsic ability to stimulate robust adipogenesis in cultured U2OS cells (a human osteosarcoma cell line). In response to low concentrations of rosiglitazone (**ROSI**), *PPARG2* has a greater ability to induce adipogenesis compared with *PPARG1* (Mueller et al., 2002).

Compared with humans and rodents, the role of PPARG isoforms in coordinating gene network expression in ruminant mammary gland is not well described. Kadegowda et al. (2009), working with bovine mammary cells, were the first to demonstrate that ROSI or saturated LCFA could upregulate lipogenic gene network expression (Kadegowda et al., 2009). Previous work with goat mammary epithelial cells (**GMEC**) demonstrated that after incubation with ROSI, several

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key enzymes related to triacylglycerol (**TAG**) synthesis and secretion were upregulated (Shi et al., 2013b). Conversely, after the knockdown of *PPARG*, key enzymes related to TAG synthesis and secretion were downregulated (Shi et al., 2013b). An important limitation of these earlier studies with ruminant mammary cells is that potential differences of each PPARG isoform could not be distinguished.

Although a recent study revealed that both PPARG isoforms could affect expression of genes related to desaturation and de novo fatty acid synthesis (Shi et al., 2013a), the role of each PPARG isoform in coordinating lipogenic gene networks in mammary cells has not been addressed. Thus, the main objective of this study was to overexpress *PPARG1* and *PPARG2* isoforms in GMEC and measure the mRNA expression of several potential downstream targets of PPARG, representing different aspects of lipid metabolism, to evaluate which of these isoforms is more closely associated with the regulation of the lipogenic pathways.

MATERIALS AND METHODS

Tissue Collection

The Animal Care and Use Committee of the Northwest A&F University approved all procedures involving the use of live goats. Three-year-old Xinong Saanen dairy goats at peak lactation and nonlactating (n = 3 for each physiological state) from the experimental farm at the Northwest A&F University, Shaanxi, China, were used in the study. Nonlactating goats (n = 3) were killed by captive bolt stunning followed by exsanguination. Within 20 min of exsanguination, samples of subcutaneous adipose, skeletal muscle, rumen, mammary gland, liver, spleen, lung, heart, and kidney tissues were collected. Mammary gland tissue was harvested via biopsy from lactating goats (n = 3). Percutaneous biopsies from each goat were obtained from the right or left udder as previously described (Farr et al., 1996). Briefly, after making the skin incision, blunt dissection of the mammary capsule was performed to ensure that the tissue obtained was mammary parenchyma during the biopsy. All tissue samples were obtained under sterile conditions and immediately frozen in liquid nitrogen until RNA extraction.

Adenovirus Generation

The adenovirus vectors were generated as described previously (Shi et al., 2013a). Briefly, the cDNA sequences of dairy goat *PPARG1* [National Center for Biotechnology Information (NCBI) no. HQ589347.1] and *PPARG2* (NCBI no. JN854246) were subcloned into the pAdTrack-CMV plasmid vector between the *Xho*I and *Sca*I (New England BioLabs Inc., Ipswich, MA) restriction sites to generate pAdTrack-CMV-*PPARG1* and pAdTrack-CMV-*PPARG2* vectors. These 2 vectors were inserted into an adenoviral vector (pAdEasy-1) to generate adenoviral plasmids in BJ5183 cells. The adenoviral plasmids linearized by *Pac*I (New England BioLabs Inc.) were transfected into 293A cells to generate the adenovirus pAd-*PPARG1* and pAd-*PPARG2*. The adenovirus (**Ad-GFP**) used as a positive control was a gift from Zhijie Chang (Tsinghua University, Beijing, China).

Cell Culture and Treatments

Mammary epithelial cells were isolated from peak-lactation Xinong Saanen goats as described previously (Wang et al., 2010). Details of cell culture were described recently (Lin et al., 2013; Shi et al., 2013b). Cultures of GMEC at approximately 80% confluence were transfected with 1 of the 3 adenovirus supernatants (Ad-PPARG1, Ad-PPARG2, or Ad-GFP). Transfected GMEC were cultured with the PPARG-specific ligand ROSI (Biovision, Milpitas, CA) or control (dimethyl sulfoxide, DMSO, Sigma, St. Louis, MO) at 50 μM after 24 h of initial culture and then harvested at 48 h (24 h later) for RNA extraction. The 293A cells for adenovirus generation and amplification were cultured in the basal Dulbecco's modified Eagle's medium (Gibco Products International Inc., Langley, OK) containing 10% fetal bovine serum (Gibco Products International Inc.).

Total RNA Extraction, Purification, and Quantitative Real-Time PCR

The procedures for total RNA extraction, purification and quantitative real-time PCR (**qPCR**) were recently described (Lin et al., 2013; Shi et al., 2013b). Total RNA from goat tissues was extracted with Trizol reagent (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's directions. Total RNA from GMEC was extracted using the RNA Prep Pure cell kit (Tiangen Biotech Co. Ltd., Beijing, China) according to the manufacturer's protocol. The RNA used in the qPCR was treated with DNAase (Tiangen Biotech Co. Ltd., Beijing, China) to remove genomic DNA contamination. Synthesis of cDNA was conducted using the PrimeScript RT kit (Takara Bio Inc., Otsu, Japan) according to the manufacturer's instructions. The qPCR was performed according to manufacturer's instructions using SYBR Green (SYBR Premix Ex Taq II, Perfect Real Time, Takara Bio Inc.).

Genes studied include those related to FA uptake [lipoprotein lipase (***LPL***) or CD36 molecule (thrombo-