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Fatty acid elongase 5 (ELOVL5) alters the synthesis of long-chain unsaturated fatty acids in goat mammary epithelial cells

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ABSTRACT

Increased production of long-chain unsaturated fatty acids (LCUFA) can have a positive effect on the nutritional value of ruminant milk for human consumption. In nonruminant species, fatty acid elongase 5 (ELOVL5) is a key enzyme for endogenous synthesis of long-chain unsaturated fatty acids. However, whether ELOVL5 protein plays a role (if any) in ruminant mammary tissue remains unclear. In the present study, we assessed the mRNA abundance of *ELOVL5* at 3 stages of lactation in goat mammary tissue. Results revealed that *ELOVL5* had the lowest expression at peak lactation compared with the nonlactating and late-lactating periods. The *ELOVL5* was overexpressed or knocked down to assess its role in goat mammary epithelial cells. Results revealed that *ELOVL5* overexpression increased the expression of perilipin2 (*PLIN2*) and decreased diacylglycerolacyltransferase 2 (*DGAT2*) and fatty acid desaturase 2 (*FADS2*) mRNA, but had no effect on the expression of *DGAT1*, *FADS1*, and stearoyl-CoA desaturase 1 (*SCD1*). Overexpression of *ELOVL5* decreased the concentration of C16:1n-7, whereas no significant change in C18:1n-7 and C18:1n-9 was observed. Knockdown of *ELOVL5* decreased the expression of *PLIN2* but had no effect on *DGAT1*, *DGAT2*, *FADS1*, *FADS2*, and *SCD1* mRNA expression. Knockdown of *ELOVL5* increased the concentration of C16:1n-7 and decreased that of C18:1n-7. The alterations of expression of genes related to lipid metabolism after overexpression or knockdown of *ELOVL5* suggested a negative feedback regulation by the products of ELOVL5 activation. However, the content of triacylglycerol was not altered by knockdown or overexpression of *ELOVL5* in goat mammary epithelial cells, which might have been due to the insufficient availability of substrate in vitro. Collectively, these are the first in vitro results highlighting an important role of ELOVL5 in the elongation of 16-carbon to 18-carbon unsaturated fatty acids in ruminant mammary cells.

Key words: elongase, unsaturated fatty acid, ruminant, milk fat

INTRODUCTION

Fatty acids are one of the most valuable components in dairy production (Shingfield et al., 2008), especially the MUFA. These MUFA are substrates for the synthesis of PUFA and, thus, contribute to human health (Han et al., 2017). The activity of fatty acid desaturases and elongases determines the synthesis of end products of MUFA or PUFA synthesis (Lee et al., 2016). In ruminant mammary tissue, the functional roles of fatty acid desaturases [e.g., stearoyl-CoA desaturase 1 (**SCD1**)] have been investigated (Cecchinato et al., 2012; Lee et al., 2016; Yao et al., 2017).

The fatty acid elongase or elongation of very long chain fatty acid-like fatty acid elongase (**ELOVL**) includes 7 isotypes (i.e., ELOVL1 to ELOVL7). The RNA sequence revealed that at least 3 isoforms of fatty acid elongase are expressed in goat mammary gland tissue (Shi et al., 2015a). The isoforms are *ELOVL5*, *ELOVL6*, and *ELOVL7*. Recent data in goat mammary cells indicated that ELOVL6 plays a role in the elongation of long-chain SFA (C16:0 to C18:0; Shi et al., 2017a). The ELOVL5 is involved in the elongation of various PUFA containing 18 and 20 carbons (Green et al., 2010). These data indicated that ELOVL family may play an important role in mammary fatty acid metabolism. However, the role of ELOVL in the ruminant mammary gland remains unclear.

At least in nonruminants, the ELOVL5 protein is sensitive to diet and hormones (Zhang et al., 2016). In mice, elevating Elovl5 activity increased hepatic and plasma levels of dihomo-γ-linolenic acid (C20:3n

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-6), decreased hepatic arachidonic acid (C20:4n-6) and docosahexaenoic acid (C22:6n-3) content (Wang et al., 2008), and reduced hepatic triacylglycerol (**TAG**; Tripathy et al., 2014). In rat insulinoma-1 cells, knockdown of Elovl5 decreased elongation of C16:1n-7, whereas Elovl-5 overexpression increased synthesis of 18:1n-7 (Green et al., 2010). It is evident that ELOVL5 changed the hepatic fatty acid content through affecting multiple pathways related to lipid metabolism [e.g., sterol-regulatory element binding protein-1 (SREBP-1) signaling pathway (Wang et al., 2008; Moon et al., 2009) or Akt pathway (Tripathy et al., 2010)]. Collectively, the data from rodents underscore an important role for Elovl5 in the overall process of long-chain unsaturated fatty acid (**LCUFA**) synthesis.

Despite evidence in mice supporting an essential role of Elovl5 in PUFA synthesis, no functional data are available on ELOVL5 in ruminant mammary cells. Whether ELOVL5 plays a role in the synthesis and alteration of LCUFA composition in ruminant mammary cells remains unknown. We hypothesized that ELOVL5 has a role in the process of LCUFA synthesis in ruminant mammary cells. To assess the hypothesis, both RNA interference and overexpression were performed in goat mammary epithelial cells (**GMEC**).

MATERIALS AND METHODS

mRNA Abundance of ELOVL5

The transcriptome data set from goat mammary tissue is deposited at the National Center for Biotechnology Information (BioProject ID: PRJNA243005). The details about these samples were described previously (Shi et al., 2015a). Briefly, 3- to 4-yr-old Xinong Saanen dairy goats from the experimental farm of Northwest Agricultural University, Shaanxi, China, were used. Mammary tissue at peak lactation (3 goats, 100 d postpartum), late lactation (cessation of milking, 3 goats, 310 d postpartum), and the nonlactating period (3 goats, nonlactation and nonpregnant period) were collected by a veterinarian after slaughter. Upon RNA sequencing, Bowtie 0.12.8 was used to align reads to the goat genome (Dong et al., 2013). The DESeq (a software for differential gene expression analysis based on negative binomial distribution of data; http://www.huber.embl.de/users/anders/DESeq) was used for analysis of gene expression in response to stage of lactation. 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 were used for measuring gene expression of *ELOVL5*.

Adenovirus Generation

The whole process for generation and proliferation of recombinant adenovirus expressing ELOVL5 (**Ad-ELOVL5**) was carried out as previously described (Shi et al., 2016). Briefly, the *ELOVL5* cDNA (GenBank No.: NM_001285628.1) was subcloned into the pAdTrack-CMV plasmid vector to generate pAdTrack-CMV-ELOVL5 vectors. 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 adenovirus pAd-ELOVL5.

Cell Culture

The GMEC were isolated from peak-lactation Xinong Saanen goats as described previously (Wang et al., 2010; Shi et al., 2014). Details of the cell culture were described recently (Lin et al., 2013; Shi et al., 2013b). Briefly, cells were incubated at 37°C in 5% CO_2 and air. Culture medium was composed of Dulbecco's modified Eagle medium/F12 (Hyclone, Beijing, China) containing insulin (5 mg/L, Sigma-Aldrich, St. Louis, MO), hydrocortisone (5 mg/L, Sigma-Aldrich), penicillin/streptomycin (10k U/L, Harbin Pharmaceutical Group, China), epidermal growth factor (1 mg/L, Sigma-Aldrich), and fetal bovine serum (10%, Gibco, Waltham, MA). To promote lactogenesis, GMEC were cultured in a lactogenic medium for 24 h before initial experiments. Lactogenic medium contained the culture medium plus prolactin (2 μg/mL, Sigma-Aldrich). The 293A cells for adenovirus generation were cultured in basal DMEM medium (Gibco) containing 10% fetal bovine serum.

The GMEC at about 80% confluence were transfected with adenovirus supernatant (Ad-ELOVL5 or Ad-GFP). The transfected GMEC were collected after 48 h of culture for lipid extraction, total RNA extraction, and TAG assay.

RNA Interference

For *ELOVL5* mRNA interference, cells cultured in 6-well plates were transfected with 60 n*M* small interfering RNA (**siRNA**) using transfection reagent (Lipofectamine RNAiMAX, Thermo Fisher Scientific Inc., Waltham, MA) in lactogenic medium without antibiotic. The transfection was performed according to manufacturer's instructions. Transfected GMEC were collected after 24 h of initial culture, and then harvested at 48 h (24 h later) for RNA extraction,