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

Received October 28, 2017.

Accepted December 29, 2017.

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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, knock-down of *Elovl5* decreased elongation of C16:1n-7, whereas *Elovl5* 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 *PacI* (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% CO₂ 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 nM 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,

TAG assays, and fatty acid extraction. Sequences of the siRNA oligonucleotides for *ELOVL5* (accession: NM_001285628.1; **siELOVL5**) were designed and synthesized by Jima Biotechnology Co. Ltd. (Shanghai, China). The sequences for the siELOVL5 are described in Supplemental Table S1 (<https://doi.org/10.3168/jds.2017-14061>). Scrambled siRNA is a functional nontargeting siRNA that is used as a negative control (**siNC**).

Total RNA Extraction and Quantitative Real-Time PCR

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 (<http://www.tiangen.com/asset/imsupload/up0203129001467351047.pdf>). Genomic DNA contamination was removed using DNase provided with the kit. Synthesis of cDNA was conducted using the PrimeScript RT reagent Kit with gDNA Eraser (Takara Bio Inc., Otsu, Japan) according to the manufacturer's instructions (<http://www.takarabiomed.com.cn/Product.aspx?m=20141215102916640154#p>). The quantitative real-time PCR (qPCR) was performed according to the manufacturer's instructions using SYBR Green (SYBR Premix Ex Taq II, Perfect Real Time, Takara Bio Inc.).

Several genes related to TAG synthesis [diacylglycerolacyltransferase1 (*DGAT1*), *DGAT2*, perilipin2 (*PLIN2*)] or *SCD1*, fatty acid desaturase 1 (*FADS1*), and *FADS2* were selected to evaluate functional outcomes after alteration of *ELOVL5* expression. All the qPCR reactions were performed in a Bio-Rad CFX96 (Bio-Rad Laboratories Inc., Hercules, CA) sequence detector, and data were normalized to ubiquitously expressed transcript (*UXT*), mitochondrial ribosomal protein L39 (*MRPL39*), and ribosomal protein S9 (*RPS9*; Bionaz and Loo, 2008). The primer sequences of the genes are described in Supplemental Table S2 (<https://doi.org/10.3168/jds.2017-14061>).

Fatty Acid Analysis

After washing 3 times with PBS, treated GMEC were scraped off the culture dish with a 2-mL aliquot of 2.5% (vol/vol) vitriol:methanol. Total lipid extraction and methylation were performed according to Shi et al. (2013a). The methyl ester of margaric acid (17:0) was used as the internal standard. Methylated lipid samples were analyzed using a GC-MS (Agilent Technologies, Santa Clara, CA) installed with a DB-23 column following a published procedure (Shi et al., 2017b). The relative proportions of fatty acids were determined as the ratio of each fatty acid peak relative to the total fatty acid peaks identified.

Cellular TAG Assays

The protocol for cellular TAG assay was described previously (Kang et al., 2015; Shi et al., 2015b). Briefly, total cellular TAG was extracted using the GPO-Trinder triglyceride assay kit (<http://www.applygen.com/a/meixueyushenghuaceding/276.html>; Applygen Technologies, Beijing, China). The concentration of TAG was determined according to the manufacturer's instructions on a plate reader (Thermo Fisher Scientific). The concentrations were calculated using the equation obtained from a linear regression of the standard curve, which was established according to the manufacturer's procedure (<http://www.applygen.com/a/meixueyushenghuaceding/276.html>). The quantification of total cellular TAG was normalized to the cellular protein concentration. Protein concentration of each well was determined using a BCA protein assay kit (Pierce, Thermo Fisher Scientific) according to the manufacturer's instructions (<https://www.thermofisher.com/order/catalog/product/23225>).

Statistical Analysis

Treatments were replicated at least 3 times in culture wells, and the PCR was performed in triplicate. Results are expressed as mean $\pm$ standard error of the mean. Data of qPCR were analyzed using the $2^{-\Delta\Delta C_t}$ method. Significance of RNA expression of *ELOVL5* in mammary tissue across different stages of lactation was determined by 1-way ANOVA. The data for qPCR, TAG content, and fatty acid ratio after altering *ELOVL5* expression were determined via Student's *t*-test. Significance was declared at $P < 0.05$.

RESULTS

ELOVL5 Expression in Dairy Goat Mammary Gland Tissue

As shown in Figure 1, the nonlactating mammary gland had the highest reads per kilobase of exon model per million mapped reads value for *ELOVL5*, followed by the late-lactation samples ($P < 0.05$). Among the 3 stages, we detected lowest expression of *ELOVL5* at peak lactation ($P < 0.05$).

Overexpression of *ELOVL5* Altered Genes Related to TAG Synthesis and Fatty Acid Desaturation

Compared with controls (Ad-GFP), the expression of *ELOVL5* after 48 h increased markedly in cells infected with Ad-*ELOVL5* ($P < 0.01$, Figure 2A). Compared with the control (Ad-GFP), overexpression of *ELOVL5*

significantly upregulated the expression of *PLIN2* ($P < 0.05$) and downregulated the expression of *DGAT2* ($P < 0.05$), whereas it had no effect on the *DGAT1* ($P > 0.05$) mRNA level (Figure 2B).

Regarding the genes related to fatty acid desaturation, compared with control (Ad-GFP), overexpression of *ELOVL5* had no significant effect on *SCD1* and *FADS1* mRNA level. However, it significantly decreased the mRNA level of *FADS2* ($P < 0.05$; Figure 2C).

Overexpression of *ELOVL5* Decreased the Concentration of C16:1

As shown in Figure 3, overexpression of *ELOVL5* significantly decreased the concentration of C16:1n-7 ($P < 0.05$). However, overexpression of *ELOVL5* had no effect on the concentration of C16:0, C18:0, C18:1n-7, C18:1n-9, and C18:2.

Knockdown of *ELOVL5* Altered Genes Related to TAG Synthesis and Fatty Acid Desaturation

As shown in Figure 4A, treatment of GMEC with siRNA significantly reduced *ELOVL5* mRNA abundance by 92% ($P < 0.01$). Compared with control (Ad-GFP), knockdown of *ELOVL5* downregulated the mRNA level of *PLIN2* ($P < 0.05$; Figure 4B). However, no significant changes were observed for the mRNA ex-

Figure 1. The mRNA abundance of elongation of very long chain fatty acid-like fatty acid elongase 5 (*ELOVL5*) at 3 stages of lactation in goat mammary tissues. The transcriptome data set is from mammary tissue harvested 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) by a veterinarian after slaughter. The DESeq software (<http://www.huber.embl.de/users/anders/DESeq>) was used for abundance analysis of gene expression. Reads per kilobase of exon model per million mapped reads (RPKM) were used for assessing gene expression. Values are means $\pm$ SEM for 3 individual goats. Significance of *ELOVL5* expression in mammary tissue across different stages of lactation was determined by 1-way ANOVA. The different letters denote significant ($P < 0.05$) differences.

Figure 2. Overexpression of elongation of very long chain fatty acid-like fatty acid elongase 5 (*ELOVL5*) altered genes related to triacylglycerol synthesis and desaturation. The goat mammary epithelial cells (GMEC) were transfected with recombinant adenovirus expressing *ELOVL5* (Ad-ELOVL5) or green fluorescent protein (Ad-GFP) for 48 h and then collected for mRNA extraction. Panel A: mRNA expression of *ELOVL5*. Panel B: mRNA expression of genes related to triacylglycerol synthesis (*DGAT1*, *DGAT2*, and *PLIN2*). Panel C: mRNA expression of genes related to desaturation (*SCD1*, *FADS1*, and *FADS2*). Values are means $\pm$ SEM from 3 individual cultures. ** $P < 0.01$ compared with control (Ad-GFP). * $P < 0.05$ compared with control (Ad-GFP). NS represents no significant change compared with control (Ad-GFP). The data were analyzed via Student's *t*-test. Significance was declared at $P < 0.05$.

Figure 3. Overexpression of *ELOVL5* altered the concentration of C16 to C18 carbon fatty acids in goat mammary epithelial cells (GMEC). The GMEC were transfected with recombinant adenovirus expressing *ELOVL5* (Ad-ELOVL5) or green fluorescent protein (Ad-GFP) for 48 h and then collected for fatty analysis. Data are reported as ratio of total fatty acids. Values are means $\pm$ SEM from 3 individual cultures. The data were analyzed via Student's *t*-test. * $P < 0.05$ compared with control (Ad-GFP).

pression level of *DGAT1*, *DGAT2*, *SCD1*, *FADS1*, and *FADS2* in the cells incubated with siELOVL5 (Figures 4B and 4C).

Knockdown of *ELOVL5* Altered the Concentration of LCFA

Compared with the control group incubated with siNC, the relative concentration of C18:1n-7 in GMEC incubated with siELOVL5 decreased significantly ($P < 0.05$). In contrast, knockdown of *ELOVL5* resulted in a marked increase of C16:1 ($P < 0.05$; Figure 5). No significant changes were observed in the concentration of C16:0, C18:0, C18:1n-7, and C18:2 after knockdown of *ELOVL5*.

Alteration of *ELOVL5* mRNA Expression Had No Effect on Cellular TAG in GMEC

As shown in Figure 6A and 6B, the concentration of cellular TAG increased did not change significantly ($P > 0.05$) after overexpression or knockdown of *ELOVL5* in GMEC.

DISCUSSION

The LCPUFA are bioactive cellular components of membrane phospholipids and can act as direct modulators of gene expression (Mashek and Coleman, 2006).

In humans and rats, desaturases and elongases control the endogenous synthesis of LCPUFA (Zhang et al., 2016). A critical role of *ELOVL5* in elongating fatty acid substrates ranging from 16 to 20 carbons was reported in humans and rodents (Tripathy et al., 2014). However, whether this enzyme plays the same role in ruminant mammary cells has not been addressed. The present study is novel in that we studied the selective regulation of gene expression and LCFA composition by *ELOVL5* in GMEC through altering *ELOVL5* expression. Thus, the present findings underscore an important role for *ELOVL5* in altering the elongation of 16-carbon to 18-carbon UFA.

The markedly lower expression of *ELOVL5* at peak lactation compared with the nonlactating and late-lactating period suggests a different role for *ELOVL5* during lactation. In the physiological context of milk fat synthesis in the lactating mammary gland, the present data agree with the observation in mouse liver that a consequence of decreased *Elovl5* activity is the activation of sterol regulatory element binding protein 1c (*SREBP1c*) and its target lipogenic genes (Moon et al., 2009). This negative relationship between *ELOVL5* and lipogenesis is supported by the high expression of *SREBP-1c* and its target genes in lactating bovine (Bionaz and Looor, 2008) and goat mammary tissue (Shi et al., 2015a). The low expression of *ELOVL5* at peak of lactation agrees with the fact that long-chain fatty acids in ruminant milk are mainly taken up from the

Figure 4. Knockdown of elongation of very long chain fatty acid-like fatty acid elongase 5 (*ELOVL5*) altered genes related to triacylglycerol synthesis and desaturation. The goat mammary epithelial cells (GMEC) were incubated with small interfering RNA target *ELOVL5* (siELOVL5) or negative control (siNC) for 48 h and then collected for mRNA extraction. Panel A: mRNA expression of *ELOVL5*. Panel B: mRNA expression of genes related to triacylglycerol synthesis (*DGAT1*, *DGAT2*, and *PLIN2*). Panel C: mRNA expression of genes related to desaturation (*SCD1*, *FADS1*, and *FADS2*). Values are means $\pm$ SEM from 3 individual cultures. The data were analyzed via Student's *t*-test. ** $P < 0.01$ compared with control (siNC). * $P < 0.05$ compared with control (siNC). NS represents no significant change compared with control (siNC).

circulating plasma pool (Harvatine et al., 2009). Together with the fact that *ELOVL5* catalyzes the elongation of C18:3n-6 to C20:3n-6 and C18:4n-3 to C20:4n-3, which in turn negatively affects SREBP-1 activity (Moon et al., 2009), the higher expression of *ELOVL5* in dry and late-lactating stages suggest that *ELOVL5* may participate in the development of goat mammary tissue through its endogenous products.

The *Elovl5* substrates include C16:1n-7 and PUFA such as C18:2n-6 and C18:4n-3 (Zhang et al., 2016). Elongation of fatty acids by *Elovl5* is essential for hepatic lipid metabolism in mice, as overexpression of *Elovl5* in liver decreases TAG content (Wang et al., 2008) and knockdown of *Elovl5* leads to fatty liver and increased lipogenic gene expression (Moon et al., 2009). In the present study, reduction of *ELOVL5* expression in GMEC decreased the elongation of C16:1n-7 to C18:1n-7 and overexpression of *ELOVL5* decreased the concentration of C16:1n-7, illustrating a role for *ELOVL5* in the elongation of C16:1n-7. This finding agrees with data in insulinoma-1 cells (Wang et al., 2008). The fact that overexpression of *ELOVL5* in GMEC had no effect on C18:1n-7 was likely due to limited substrate availability as GMEC cultured in vitro have very low concentrations of C16:1n-7 relative to C16:0. However, despite the marked change in expression of *ELOVL5* due to its overexpression, only modest changes in cellular LCUFA composition were detected. Such responses could be partly explained by the lack of exogenous availability of 16:1 for the enzyme reaction and also lack of an increase in *ELOVL5* protein. The lack of a goat-specific antibody for *ELOVL5* clearly is a limiting factor in the present study. Despite being unable to measure *ELOVL5* protein, the present data suggest that the alteration in 16-carbon to 18-carbon UFA composition was a primary result of changes in *ELOVL5* mRNA.

The desaturase and elongase work in concert during the biosynthesis of LCUFA (Wang et al., 2006). The lack of effect of *ELOVL5* expression on *SCD1* in the present study is consistent with the observation in mice with a knockdown of *Elovl5*. The lack of change in the concentration of C18:1n-9 agreed with the lack of change of *SCD1* expression when *ELOVL5* was knocked down or upregulated. The finding that in cells overexpressing *ELOVL5*, expression of *FADS2* was downregulated agrees with the data in mouse liver. The downregulation of *FADS2* may have been caused by the alteration of fatty acids (such as C16:1 or C18:1n-7), which are natural agonists for nuclear transcription factors [e.g., peroxisome proliferator activated receptor (PPAR) or SREBP1]. This idea is supported by data from mice showing that hepatic *Elovl5* activity affects multiple

Figure 5. Knockdown of *ELOVL5* altered the concentration of C16 to C18 carbon fatty acids in goat mammary epithelial cells (GMEC). The GMEC were incubated with small interfering RNA target *ELOVL5* (siELOVL5) or negative control (siNC) for 48 h and then collected for fatty analysis. Data are reported as ratio of total fatty acids. Values are means $\pm$ SEM from 3 individual cultures. The data were analyzed via Student's *t*-test. **P* < 0.05 compared with control (siNC).

pathways controlling lipid and carbohydrate composition (Wang et al., 2008). The products of *ELOVL5* can serve to activate various signaling pathways related to lipid and carbohydrate metabolism (Tripathy et al., 2010, 2014). The lack of change for *FADS1* after overexpression or knockdown of *ELOVL5* indicated a potential lack of interaction between the 2 genes in vitro. Collectively, the evidence suggests *ELOVL5* may play a role in the cellular homeostasis via controlling cellular concentrations of LCUFA (Green et al., 2010), which serve as cell-signaling molecules during lactation.

In ruminants, the enzymes involved in synthesis of TAG including *DGAT1* and *DGAT2* are upregulated during lactation (Bionaz and Looor, 2008). The decrease in *DGAT2* after overexpression of *ELOVL5* is consistent with data in mice demonstrating that *Elov5* activity resulted in lower hepatic TAG content (Tripathy et al., 2014). However, overexpression or knockdown of *ELOVL5* in GMEC did not alter the content of TAG. The lack of change in TAG might have been due to insufficient substrate in vitro for GMEC. The lack of change in *DGAT1* after altering *ELOVL5* expression confirmed our previous hypothesis that *DGAT1* protein is prone to respond to the availability of endogenous fatty acids for TAG synthesis (Shi et al., 2017a).

The *PLIN2* protein is essential for the formation and secretion of lipid droplets during lactation (Shi et al., 2015b) and is a target of PPAR (Kang et al., 2015; Shi et al., 2017b). In the present study, *PLIN2* expression had a similar pattern as *ELOVL5*. This result did not agree with the lack of change in TAG and the fact that *Elov5* activity is associated with a

decrease in liver TAG content in mice (Tripathy et al., 2014). However, the response is consistent with evidence that *Elov5* could affect the PPAR or SREBP1 pathway via changes in the concentration of its fatty acid products (Tripathy et al., 2014). It is also worth noting that *PLIN2* expression was significantly upregulated when concentration of C16:1 decreased, and was downregulated when concentration of C16:1 increased. This suggests that C16:1 might be the key product or substrate of *ELOVL5* that can signal through the PPAR pathway. This idea is supported by data showing that *ELOVL5* functions to keep cellular concentrations of C16:1n-7 low, thereby preventing its accumulation and any potential downstream signaling event (Green et al., 2010). The potential mechanistic role of C16:1n-7 in ruminant mammary cells is unclear; thus, more experiments appear warranted. Collectively, the data from the present study suggest that *ELOVL5* plays an important regulatory role in controlling LCUFA elongation in goat mammary cells.

CONCLUSIONS

The upregulation of *ELOVL5* decreased the concentration of C16:1n-7, whereas knockdown decreased the concentration of C18:1n-7 and increased that of C16:1n-7. Activation of *ELOVL5* altered the expression of genes associated with fatty acid desaturation and TAG synthesis, and the accumulation of C16:1n-7 seems to be involved. In conclusion, at least in vitro, these data demonstrate a direct role for *ELOVL5* in modulating LCUFA synthesis in GMEC.

Figure 6. Elongation of very long chain fatty acid-like fatty acid elongase 5 (*ELOVL5*) did not significantly alter the accumulation of cellular triacylglycerol. The goat mammary epithelial cells (GMEC) were transfected with recombinant adenovirus expressing *ELOVL5* (Ad-ELOVL5) or green fluorescent protein (Ad-GFP) or incubated with small interfering RNA target *ELOVL5* (siELOVL5) or negative control (siNC), and collected at 48 h for cellular triacylglycerol analysis. Values are means $\pm$ SEM from 3 individual cultures. The data were analyzed via Student's *t*-test. No significant change compared with control (Ad-ELOVL5 vs. Ad-GFP or siELOVL5 vs. siNC) is denoted by NS.

ACKNOWLEDGMENTS

This research was supported by National Natural Science Foundation of China (31702090 and 31460585, Beijing, China).

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