Sterol regulatory element binding protein-1 (SREBP-1)c promoter: Characterization and transcriptional regulation by mature SREBP-1 and liver X receptor α in goat mammary epithelial cells

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ABSTRACT

Sterol regulatory element binding protein-1 (SREBP-1) is a key transcription factor that regulates lipogenesis in rodent liver. Two isoforms (SREBP-1a and SREBP-1c) of SREBP-1 are transcribed by an alternative promoter on the same gene (*SREBF1*), and the isoforms differ only in their first exon. Although the regulatory effects of SREBP-1 on lipid and milk fat synthesis have received much attention in ruminants, *SREBP-1c* promoter and its regulatory mechanisms have not been characterized in the goat. In the present study, we cloned and sequenced a 2,012-bp fragment of the *SREBP-1c* 5'-flanking region from goat genomic DNA. A luciferase reporter assay revealed that SREBP-1c is transcriptionally activated by the liver X receptor α (LXR α) agonist T0901317, and is decreased by SREBP-1 small interfering (si)RNA. A 5' deletion analysis revealed a core promoter region located –395 to +1 bp upstream of the transcriptional start site (TSS). Site-directed mutagenesis of LXR α binding elements (LXRE1 and LXRE2) and sterol regulatory elements (SRE1 and SRE2) revealed that the full effects of T0901317 require the presence of both LXRE and SRE. We also characterized a new SRE (SRE1) and demonstrated a direct role of SREBP-1 (auto-loop regulation) in maintaining its basal transcription activity. Results suggest that goat *SREBP-1c* gene is transcriptionally regulated by mature SREBP-1 (auto-loop circuit regulation) and LXR α in goat mammary epithelial cells.

Key words: sterol regulatory element binding protein-1c (SREBP-1c), liver X receptor α (LXR α), milk fat, transcription regulation

INTRODUCTION

The sterol regulatory element binding proteins (SREBP) are basic helix-loop-helix leucine zipper transcription factors synthesized as inactive precursors anchored in the membranes of the endoplasmic reticulum (Brown and Goldstein, 1997). Upon activation, the precursor protein undergoes a sequential 2 steps of proteolytic processing in Golgi apparatus, resulting in the release of N-terminal active fragment (mature SREBP protein). Mature SREBP then translocates to the nucleus and binds to the sterol response element (SRE) in the promoter of target genes and to itself to regulate lipid metabolism (Eberlé et al., 2004; Espenshade, 2006). The identification of SRE in the promoter of SREBP-1c implies that mature SREBP-1 protein can bind to its own promoter and positively regulate its own expression through a characteristic “auto-loop circuit” regulation (Amemiya-Kudo et al., 2000; Lengi and Corl, 2010; Takeuchi et al., 2010).

Three SREBP members have been characterized; SREBP-1a and SREBP-1c are transcribed from a single gene, *SREBF1* (human chromosome 17, mouse chromosome 11) but using different promoters, and they differ only in their first exons, whereas SREBP-2 is encoded by *SREBF2* and mainly regulates cholesterol synthesis (Horton et al., 2002). The use of different promoters leads to a longer NH₂-terminal transactivation domain in SREBP-1a than in SREBP-1c (Shimano et al., 1997), and it is highly expressed in cell lines and tissues with high capacity for cell proliferation, such as spleen and intestine. The SREBP-1c isoform is the predominant form expressed in liver, adipose tissue, and skeletal muscle in mice and humans (Shimomura et al., 1997).

Studies in rodents and dairy cows have provided strong evidence for SREBP-1 as a central player in the regulation of mammary fatty acid synthesis (Bionaz and Looor, 2008; Harvatine et al., 2009). Another important upstream regulator modulating SREBP-1c ex-

Received September 5, 2015.

Accepted October 30, 2015.

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pression transcriptionally is liver X receptor α (**LXR α** ; Yoshikawa et al., 2001). The LXR α form heterodimers with retinoid X receptors (**RXR**) and then bind to specific DNA sequences (known as **LXRE**) in the promoter of target genes. Studies to date have identified LXRE in the promoter of cow, mouse, and human *SREBP-1c* gene, and these LXRE elements are demonstrated to be required for the full activation of SREBP-1 expression upon treatment with LXR α agonist (T0901317), indicating a direct regulation of SREBP-1c by LXR α (Repa et al., 2000; Yoshikawa et al., 2001; Lengi and Corl, 2010).

Goat milk represents an important source of nutrients for humans, but few studies have examined the role of goat SREBP-1c transcription regulation on milk fatty acid synthesis and there is no molecular information on the SREBP-1c promoter. Thus, the present study was designed to characterize the goat SREBP-1c promoter and to investigate its transcriptional regulation mechanism via LXR α and SREBP-1 in goat mammary epithelial cells (**GMEC**).

MATERIALS AND METHODS

The Animal Care and Use Committee of the Northwest A&F University (China) approved all procedures involved in the use of live goats.

Cloning of *SREBP-1c* Promoter Region and Bioinformatics Analysis

The goat *SREBP-1c* 5'-flanking sequence was amplified from goat blood genomic DNA using PrimeSTAR HS DNA Polymerase (Takara Bio Inc., Otsu, Japan) according to a previous publication (Lengi and Corl, 2010). Primers used for PCR amplification are listed in Table 1 (−2,012 to +149 bp; 2,161 bp). The amplified product was cloned into the pMD19-T vector (Takara Bio Inc.) and sequenced (Sangon Biotech., Shanghai, China). Transcription factor binding sites of the SREBP-1c promoter were predicted using PATCH public 1.0 (<http://www.gene-regulation.com/cgi-bin/pub/programs/patch/bin/patch.cgi>) and MatInspector (<http://www.genomatix.de/>).

Deletion Analysis and Plasmid Construction

The PCR primers were designed to hybridize at −2,012, −1,549, −1,105, −395, and −86 bp to generate the corresponding 5'-flanking deletion plasmid derivatives using the common downstream primer at +149 bp (5' deletion primers are listed in Table 1). The resulting amplicon was cloned into the pGL3-Basic vector (Pro-

mega, Madison, WI) by using *KpnI*/*HindIII* (Takara Bio Inc.) enzyme sites. All the plasmids were confirmed by DNA sequencing.

Site-Directed Mutagenesis

Overlap extension PCR technology was used to generate site-directed mutants of the SREBP-1c promoter. Primers were designed to destroy the transcription factor binding sites by altering 3 nucleotides within each site (primer sequences are listed in Table 1). For the site mutant promoter, PCR reactions were conducted to generate 2 DNA fragments containing the designated mutations in the overlapping regions. Subsequently, 2 DNA fragments were pooled together as a PCR template to generate a full-length DNA fragment. The resulting amplicons were ligated to pGL3-basic vector after digestion with *KpnI*/*HindIII* (Takara Bio Inc.) to create mutant constructs. Each mutant construct was confirmed by DNA sequencing.

Cell Culture and Transfection

The GMEC were isolated and cultured as previously described (Wang et al., 2010; Lin et al., 2013; Shi et al., 2013). Before experiments, cells were seeded in a 48-well plate (Corning Inc., Corning, NY). Once grown to 80 to 90% confluence, GMEC were transiently transfected with 200 ng of total DNA per well following the manufacturer's instructions for the X-treme GENE HP DNA Transfection Reagent [Roche (China) Ltd., Shanghai, China]. Renilla luciferase vector (pRL-TK) was used as an internal control to normalize transfection efficiency at a 25:1 ratio of pGL-SREBP1c and pRL-TK vector. For treatment with T0901317 after DNA transfection, medium supplemented with 1 μ M T0901317 (dissolved in DMSO) was used, and the control group was treated with DMSO only. Each treatment was repeated in triplicate.

Small Interfering RNA Transfection

A gene-specific small interfering (**si**)RNA targeted for SREBP-1 was designed and synthesized commercially (Invitrogen, Carlsbad, CA). The siRNA sequence for SREBP-1 and its interfering efficiency have been published previously (Li et al., 2015). A scrambled siRNA was designed and provided by the same company and used as a negative control. After a 12-h transfection with DNA plasmid, siRNA were delivered to cells using Lipofectamine RNAiMAX (Invitrogen) at a final concentration of 100 nM according to the manufacturer's instructions.

Table 1. Primers used for cloning, deletion, and site-directed mutagenesis of sterol regulatory element binding protein-1c (SREBP-1c)

Primer name	Primer ¹	Sequence ² (5' to 3')	Binding region ³
Cloning primers	Forward	GGGAGATTGGAAGGTAGAGC	−2,012
	Reverse	GGCACCGAGGATGGAAC	+149
5' deletion primers	F-2012	GGGAGATTGGAAGGTAGAGC	−2,012
	F-1549	AGTCAGACCGTGAGTTCGCC	−1,549
	F-1105	ACAAAGAAGGGTGGATATGTG	−1,105
	F-395	AACTGAGGCTGAGCGTGGTCGA	−395
	F-86	CCGCTACGCGGGGCGGGG	−86
	R+149	GGCACCGAGGATGGAAC	+149
	SRE1	GAGCCTGTCAACCCAGGACTTCCT	−823
Site-directed mutagenesis primers	SRE1-mut	GAGCCTGTATCTCAGGACTTCCT	−823
	SRE1-anti-mut	AGGAAGTCCTGAGATAACAGGCTC	−823
	SRE2	GCGCGCTCACCCCATGCCCGGCCCG	−111
	SRE2-mut	GCGCGCTTATCTCATGCCCGGCCCG	−111
	SRE2-anti-mut	CGGGCCGGGCATGAGATAAGCGCGC	−111
	LXRE1	ACTTGACCGCCAGTAACCCCTGCAGACG	−289
	LXRE1-mut	ACTTGACCGCCAGTGATACCTGCAGACG	−289
	LXRE1-anti-mut	CGTCTGCAGGTATCACTGGCGGTCAAGT	−289
	LXRE2	AGCGTCCGCTAGTAACCCCGGCCCG	−237
	LXRE2-mut	AGCGTCCGCTAGTGATACCGGCCCG	−237
	LXRE2-anti-mut	GGGGCCGGTATCACTAGCGGACGCT	−237

¹SRE = sterol response element; LXRE = LXR (liver X receptor) response elements.

²Mutation letters are shown in bold font.

³Plus (+) and minus (−) indicate the number of nucleotides upstream (−) and downstream (+) from the transcriptional start site.

Luciferase Assays

After a 48-h incubation, cells were harvest and lysed for luciferase activity measurement. The relative luciferase activity was a ratio of firefly to Renilla luciferase activities assayed in a microplate reader by using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions.

Data Analysis

All the treatments were conducted in triplicate and the results are presented as means $\pm$ standard error of the mean. Statistical analyses were performed with the Student's *t*-test (paired and 2-tailed) and one-way ANOVA using SPSS 17.0 (SPSS Inc./IBM Corp., Chicago, IL). Differences were considered statistically significant when $P < 0.05$.

RESULTS

Cloning, Sequencing, and Characterization of the Goat SREBP-1c Promoter

A 2,161-bp fragment of 5'-flanking sequence containing 2,012 bp upstream of the transcription start site (+1) was amplified by PCR. An initiation codon ATG was located in exon 1, and a putative TATA box-like sequence was located at −37 bp. Bioinformatics analysis showed transcription factor binding sites for SREBP-1

(SRE, −97 and −808 bp), LXR (LXRE, −202 and −271 bp), nuclear factor Y (**NF-Y**, −120 and −766 bp), and transcription factor Sp1 (−69, −87, and −835 bp) (Figure 1).

Detection of SREBP-1c Relative Luciferase Activity

The GMEC transfected with pGL3-SREBP1c had an approximately 110-fold higher level of relative luciferase activity compared with pGL3-basic-transfected groups (Figure 2A), demonstrating strong activation of SREBP1c. By treating the pGL3-SREBP1c transfected group with SREBP-1-specific siRNA and T0901317 (agonist of LXR α), we observed that siSREBP1 significantly reduced the relative luciferase activity of SREBP-1c (Figure 2B), whereas T0901317 treatment resulted in a dramatic increase of SREBP-1c promoter activity (Figure 2C).

Luciferase Assay for Confirmation of the SREBP-1c Core Promoter Region

To determine the core promoter region and characterize its essential *cis*-regulatory elements, we generated a series of 5'-flanking deletion constructs (−2,012 bp/+149 bp, −1,549 bp/+149 bp, −1,105 bp/+149 bp, −395 bp/+149 bp, and −86 bp/+149 bp) of SREBP-1c promoter and transfected them into GMEC. Results revealed no reduction in SREBP-1c promoter activity due to deletion of −2,012 bp/+149 bp, −1,549 bp/+149 bp,

Figure 1. Schematic representation and characterization of goat sterol regulatory element binding protein-1c (SREBP-1c) promoter. The +1 indicates the transcriptional start site (TSS). Nucleotides of putative transcription factor binding sites and TATA box-like sequence are in boldface, italic, and underlined. SRE = sterol response element; LXRE = LXR (liver X receptor) response elements; NF-Y = nuclear factor Y; Sp1 = transcription factor Sp1.

–1,105 bp/+149 bp, and –395 bp/+149 bp, whereas a dramatic decrease in SREBP-1c promoter activity was observed when deleting up to –86 bp/+149 bp (Figure 3A). Except for the –86 bp/+149 bp fragment, the LXR α agonist T0901317 induced the SREBP-1c promoter reporter gene significantly (Figure 3B). Taken together, these results suggest that the sequences between –395 bp and +149 bp contain the core promoter region of SREBP-1c and it bears the *cis*-functional elements required for its transcriptional activation in response to T0901317.

SRE Are Responsible for Maintaining SREBP-1c Basal Transcription Activity

Bioinformatics analysis revealed 2 SRE and 2 LXRE in the cloned fragment. One SRE (SRE2) and 2 LXRE (LXRE1 and LXRE2) are located in the core promoter region of SREBP-1c (Figure 1). To identify functional elements for the expression of SREBP-1c, we constructed a series of site-mutated expression vectors and transiently transfected them into GEMC for luciferase reporter assays. Results indicated that a single mutation of LXRE (mL1 or mL2) and a simultaneous mutation of both LXRE (mL1 mL2) did not reduce SREBP-1c promoter activity significantly. Mutations of SRE2 (mS2) decreased its promoter activity dramati-

cally. And mutations of all sites (mL1 mL2 mS2) nearly abolished promoter activity (Figure 4A).

To determine the role of SRE1 (located at –808 bp) on SREBP-1c expression, we also generated a series of mutant reporter vectors containing –1,105 to +149 bp. No significant reductions of SREBP-1c promoter activity were detected after LXRE mutation (Figure 4B). However, single mutations of SRE (mS1 or mS2) and both mutations of SRE (mS1 mS2) all reduced promoter activity significantly. Mutations of the 2 LXRE and 2 SRE (mL1 mL2 mS1 mS2) resulted in very low activity (Figure 4B). Taken together, these results indicate that SRE are critical for maintaining SREBP-1c basal transcription activity, and LXRE play only a permissive role.

LXRE Are Responsible for Full Activation of SREBP-1c in Response to T0901317

To investigate the role of LXRE and SRE on the effects of T0901317 treatment on SREBP-1c promoter activity, we transiently transfected these site-directed mutant expression vectors into GMEC followed by treatment with DMSO and 1 μ M T0901317 before luciferase reporter assays. The SRE mutations decreased but did not abolish the activation effects of T0901317 (Figure 5A and 5B). Similarly, mutations of

Figure 2. Measurement of sterol regulatory element binding protein-1c (SREBP-1c) luciferase activity. (A) Relative luciferase activity of goat mammary epithelial cells transfected with plasmid pGL3-SREBP1c compared with pGL3-basic; (B) effects of SREBP-1 small interfering (si)RNA transfection on SREBP-1c promoter activity; (C) effects of 1 μ M T0901317 (liver X receptor α agonist) or dimethyl sulfoxide (DMSO) on SREBP-1c promoter activity. Values are presented as LSM $\pm$ SEM; ** P < 0.01; * P < 0.05.

either LXRE suppressed the transcriptional activation of SREBP-1c in response to T0901317. Mutations of both LXRE resulted in an abolishment of the T0901317 activation effect. In addition, T0901317 had no effect on SREBP-1c promoter activity of GMEC transfected with mutations of both LXRE and SRE constructs. These results indicated that LXRE are critical for full activation of SREBP-1c by T0901317. Furthermore, the SRE only enhance this effect and are not indispensable for this regulation.

DISCUSSION

Since the first purification and characterization of SREBP from nuclear extracts of human HeLa cells (Wang, 1993), their functions in fatty acid and cholesterol synthesis in various cellular systems have been widely investigated (Horton et al., 2003; Chen et al., 2004; Zhao, 2014). Data from a recent study indicated that SREBP-1 regulates the synthesis of glycogen and gluconeogenic genes (Ruiz et al., 2014). Studies in ruminants also provide strong evidence for SREBP-1 as a central player in the pathways regulating mammary fatty acid synthesis (Bionaz and Looor, 2008; Harvatine et al., 2009).

The importance of LXR α in regulating SREBP-1 expression has already been characterized in previous studies (DeBose-Boyd et al., 2000; Repa et al., 2000; Lengi and Corl, 2010). Our results are in accordance with published results and confirmed that full activation of SREBP-1c by T0901317, a specific LXR α agonist, requires the presence of both LXRE and SRE. Although activation of both LXR α and LXR β upregulated the expression of SREBP-1c (Repa et al., 2000), the LXR β isoform seems mainly involved in ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1)- and ATP-binding cassette, sub-family G (WHITE), member 1 (ABCG1)-mediated increase of high-density lipoprotein and cholesterol (Quinet et al., 2006), whereas LXR α is the main subtype controlling liver lipogenesis through the activation of SREBP-1c transcription (Peet et al., 1998).

The novel LXRE sequences are composed of 2 inverted DR4-type LXRE at an approximately 30-bp interval, and both binding sites contribute to the activation of SREBP-1c (Yoshikawa et al., 2001). As shown in Figure 1, the 2 identified LXRE complexes can induce a strong response to LXR activation. By transiently transfecting the $-2,012$ bp/+149 bp fragment into GMEC for luciferase assay, we determined that the promoter activity in the pGL3-SREBP1c-transfected versus pGL3-basic group was significantly higher (Figure 2A). Treatment with T0901317 and SREBP-1 siRNA showed direct regulation of SREBP-1c by LXR and SREBP-1 (Figure

Figure 3. Deletion analysis of goat sterol regulatory element binding protein-1c (SREBP-1c) promoter in goat mammary epithelial cells (GMEC). (A) Relative luciferase activity (firefly:Renilla) after 48-h transfection with different 5'-flanking deletion constructs (–2,012 bp/+149 bp, –1,549 bp/+149 bp, –1,105 bp/+149 bp, –395 bp/+149 bp, –86 bp/+149 bp); (B) effects of 1 μ M T0901317 (liver X receptor α agonist) or dimethyl sulfoxide (DMSO) on SREBP-1c promoter activity in GMEC transfected with different 5'-flanking deletion constructs. Values are presented as LSM $\pm$ SEM; ** P < 0.01.

2B and 2C). These results indicate that the cloned fragment was the SREBP-1c promoter.

It was previously reported that insulin activates the human SREBP-1c promoter through SRE motifs (Dif et al., 2006). In fact, a full stimulatory response of SREBP-1c to insulin requires the presence of all sites, with mutations at any sites (SRE or LXRE) in the proximal SREBP-1c promoter reducing the stimulatory effect of insulin by 50% (Cagen et al., 2005). In our study, the same phenomenon was observed only with the site-directed mutations of the –395 to +149 bp fragment. The single mutation of LXRE or SRE reduced the activation effect of SREBP-1c by T0901317, and the double mutations of LXRE almost abolished the stimulatory effect of T0901317. However, the reduction effect when each SRE was mutated was not as strong with the –1,105 to +149 bp fragment mutations as with –395 to +149 bp. These results further confirmed the hypothesis that the proximal elements are essential for gene expression.

By transiently transfecting the SREBP-1c 5'-flanking truncated luciferase reporter vector into GMEC, we identified a core region that is critically important for SREBP-1c promoter activity (Figure 3A). The changes in stimulatory effects by T0901317 with the different 5'-flanking truncated fragments further demonstrated that only LXRE in the core region are responsible for the T0901317-induced activation of SREBP-1c (Figure 3B). The relative promoter activity of SREBP-1c with the simultaneous mutations of both LXRE was not stimulated by T0901317 treatment. In contrast, the mutations in the SRE element still responded to T0901317, with its promoter activity being significantly higher than that of the control group. Taken together, these results demonstrated that full activation of SREBP-1c by LXR α , through T0901317, requires the presence of both LXRE located in the –395 to –86 bp region of the SREBP-1c promoter.

The results of the T0901317 treatment after site-directed mutations further confirmed that LXRE sites

Figure 4. Effects of site-directed mutagenesis on sterol regulatory element binding protein-1c (SREBP-1c) promoter activity. (A) Relative luciferase activity changes after 48 h of transfection with a -395 bp/+149 bp mutated construct of liver X receptor response elements (LXRE)1, LXRE2, and sterol response element (SRE)2; (B) relative luciferase activity changes after a 48-h transfection with a -1,105 bp/+149 bp mutated construct of LXRE1, LXRE2, SRE1, and SRE2. WT = wild type; mL1 = LXRE1 mutant; mL2 = LXRE2 mutant; mS1 = SRE1 mutant; mS2 = SRE2 mutant. Values are presented as LSM $\pm$ SEM; ** P < 0.01; * P < 0.05.

are necessary for the T0901317-mediated activation of SREBP-1c promoter in GMEC (Figure 5A and 5B). A single mutation of either LXRE or double mutations of both LXRE did not significantly reduce SREBP-1c promoter activity, which is in agreement with similar studies (Repa et al., 2000; Yoshikawa et al., 2001; Lengi and Corl, 2010). However, our results are in contrast to those of other studies in which mutations of LXRE sites resulted in a remarkable decrease of human SREBP-1c promoter activity (Dif et al., 2006). We attributed this inconsistency in responses to inherent species differences; for example, to goat and cow being ruminants.

In addition to regulation by LXR α , the presence of SRE elements in the goat SREBP-1c promoter implies that mature SREBP-1 protein may bind to SRE to regulate its own expression. Such an “auto-loop” regulatory mechanism has been demonstrated in previous studies (Amemiya-Kudo et al., 2000; Yoshikawa et al., 2001; Takeuchi et al., 2010). Our present study supports this regulatory mechanism. Furthermore, we observed

that mutations in either SRE significantly decreased the basal transcription activity of SREBP-1c promoter (Figure 4A and 4B). In fact, promoter activity was even lower with the SRE double mutations (Figure 4B). Those results are in accordance with research into the human SREBP-1c promoter (Dif et al., 2006). In contrast, the present data are inconsistent with published research from bovine mammary cells (Lengi and Corl, 2010). This contradiction may be due to the fragment length used for the site-directed mutagenesis vector construction, because most of the functional binding sites are enriched in the proximal promoter regions (Seo et al., 2009).

However, by transfecting the same luciferase reporter construct of bovine SREBP-1c promoter into both MAC-T cells and bovine mammary epithelial (BME-UV) cells, results from Lengi and Corl (2010) showed that the relative luciferase activity of SREBP-1c was significantly lower in BME-UV cells than in MAC-T cells. Thus, it is possible that the inconsistency in

response is a result of the use of immortalized cells (MAC-T, BME-UV) compared with primary cells. In fact, previous work demonstrated marked transcriptional differences between MAC-T cells and bovine mammary tissue (Hosseini et al., 2013). Further studies are needed to clarify whether the response we detected for SREBP-1c is unique to goat or also occurs in primary bovine mammary cells.

Previous studies performed with bovine identified 1 SRE site in the proximal region (located in -100 to +1 bp region) of the SREBP-1c promoter (Lengi and Corl, 2010). Although in the human SREBP-1c promoter, 2 SRE sites are located in the -257 to +1 bp region (Dif et al., 2006), the current experiment identified a new SRE site, which is located at -808 bp. Results of site-directed mutagenesis further confirmed that the newly characterized SRE site and the SRE site located in the core promoter region are both important for

maintaining SREBP-1c basal transcriptional activity. These results may imply that, for goat SREBP-1c gene regulation, the positive auto-loop circuit regulation is even more important than the LXR-mediated regulation.

We also noted that each mutation of SRE or LXRE site in the proximal region impaired the stimulatory effect of T0901317, indicating the requirement of combinatorial actions of both SRE and LXRE. Cagen et al. (2005) analyzed the rat SREBP-1c promoter and identified NF-Y, Sp1 transcription factor, LXRE, and SRE binding sites, and argued that each of these sites is required for the full stimulatory response of the SREBP-1c promoter to insulin. In the promoter of the human lipin 1 (*LPIN1*) gene (target gene of SREBP-1c), the SRE and NF-Y sites were shown to be essential for the binding of nSREBP-1c protein to its promoter, thus positively activating its expression (Ishimoto et al.,

Figure 5. Effects of T0901317 (liver X receptor α agonist) treatment on site-directed mutagenesis promoter activity. (A) Effects of 1 μ M T0901317 or dimethyl sulfoxide (DMSO) treatment on sterol regulatory element binding protein-1c (SREBP-1c) promoter activity in goat mammary epithelial cells (GMEC) transfected with a -395 bp/+149 bp fragment plasmid bearing mutations in liver X receptor response elements (LXRE)1, LXRE2, and sterol response element (SRE)2; (B) effects of 1 μ M T0901317 or DMSO treatment on SREBP-1c promoter activity in GMEC transfected with a -1,105 bp/+149 bp fragment plasmid bearing mutations in LXRE1, LXRE2, SRE1, and SRE2. WT = wild type; mL1 = LXRE1 mutant; mL2 = LXRE2 mutant; mS1 = SRE1 mutant; mS2 = SRE2 mutant. Values are presented as LSM $\pm$ SEM; ** P < 0.01; * P < 0.05.

2009). The present study revealed that NF-Y and Sp1 are located in the region near the SRE sites. Although we did not test promoter activity after site-directed mutations of these sites, our results with the T0901317 treatment after mutations of SRE or LXRE, together with the rodent and human data, strongly support the idea that combined actions of SRE, LXRE, Sp1, and NF-Y elements constitute a complex. Such a complex seems essential for the full activation of SREBP-1c and its target genes.

CONCLUSIONS

We isolated and characterized a 2.0-kbp fragment of SREBP-1c 5'-flanking region, and identified a core promoter region that is essential for the maintenance of its basal transcription activity. Site-directed mutagenesis of LXRE and SRE revealed that the full effects of T0901317 require the presence of both LXRE and SRE. A new SRE element (SRE1) was characterized and a direct role (auto-loop regulation) in maintaining basal transcription activity of SREBP-1c was demonstrated. Overall, our results provide information that could aid in designing studies (in vitro or in vivo) to test the potency of specific nutrients (e.g., long-chain fatty acids) for activating transcriptional mechanisms that could alter goat milk quality.

ACKNOWLEDGMENTS

This research was jointly supported by the Transgenic New Species Breeding Program of China (Beijing, China; 2014ZX08009-051B) and Special Fund for Agro-scientific Research in the Public Interest (Beijing, China; 201103038).

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