Transcriptome analysis of messenger RNA and long noncoding RNA related to different developmental stages of tail adipose tissues of sunite sheep

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Research

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Abstract

Background: Sunite sheep are a fat-tailed sheep species with a low percentage of intramuscular fat and good quality lean meat, and their tail fat can be used as a source of dietary fat by humans. To understand the potential regulatory mechanism of different growth stages of tail fat in Sunite sheep, we performed high-throughput RNA sequencing to characterize the long noncoding RNA (lncRNA) and messenger RNA (mRNA) expression profiles of the sheep tail fat at the age of 6 months, 18 months, and 30 months.

Results: A total of 223 differentially expressed genes (DEGs) and 148 differentially expressed lncRNAs were found in the tail fat of 6-, 18-, and 30-month-old sheep (false discovery rate < 0.05, |Fold Change| ≥ 2). Based on the Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analysis, we found that fat-related DEGs were mainly expressed at 6 months of age, and gradually decreased at 18 and 30 months of age. The target gene prediction analysis shows that most of the lncRNAs target more than 20 mRNAs as their trans-regulators (53 mRNAs at most). Further, we obtained several fat-related differentially-expressed target genes; these target genes interact with different differentially expressed lncRNAs at various ages and play an important role in the development of tail fat. Based on the DEGs and differentially expressed lncRNAs, we established three co-expression networks for each comparison group.

Conclusions: Finally, we conclude that the development of the sheep tail fat is more active during the early stage of growth and gradually decreases with the increase in age. The mutual regulation of lncRNAs and mRNAs may play a key role in this complex biological process, and our findings will provide some basic theoretical data for future studies on tail fat development of fat-tailed sheep.

Background

Adipose tissue is found in various parts of the sheep body, some of which are the subcutaneous layer under the skin, around the kidneys, within the abdominal cavity, and buttocks, especially in the fat-tailed sheep. There are several fat-tailed sheep species in China such as Sunite sheep (SS), Wuzgumuqin sheep, Wuranke Sheep, and Kazakh sheep. SS, a Mongolian sheep breed, is mainly raised in the Xilingol grassland of Inner Mongolia. These sheep are accustomed to voluntary movement and typically free-feed (or naturally graze) throughout their lives. SS is a meat breed, and the important phenotype of SS is the fat tail. As the age of SS increases, the tail fat increases continuously and reaches the weight of about 3–4.5 kg at 30 months of age (30M). Tail fat can be used by humans as an important source of dietary fat [1, 2] and provides the energy needed by the human body. As a by-product of mutton, it can also be used as a raw material for daily-use products, such as soap, cosmetics, and medicinal materials.

Adipose tissue plays a vital role in maintaining the balance of homeostatic metabolic processes in domestic animals. During severe conditions, such as food scarcity resulting from migration, drought, and winter, the tail fat can provide energy [3]. According to a previous study [1], fat-tailed sheep have a low percentage of intramuscular fat and provide good quality lean meat. In contrast, short-tailed sheep have higher intramuscular fat storage. Thus, the mechanism of tail fat deposition is worth studying. Many studies have employed RNA sequencing (RNA-seq) to explore differentially expressed genes (DEGs) in the adipose tissues in fat-tailed sheep recently. To gain a better understanding of fat deposition, Li et al. [4] performed RNA-seq of
perirenal, subcutaneous, and tail fat tissues from Guangling Large-Tailed and Small-Tailed Han (STH) sheep to determine their transcriptome profiles. The result showed that a total of 4,131 DEGs were identified in tail fat tissue, and 49 genes were shown to be involved in the peroxisome proliferator-activated receptor (PPAR) signaling pathway, which is the key pathway to balance fat metabolism [5]. Wang et al. [6] used transcriptome sequencing to compare the transcriptome profiles of tail fat tissue between Kazak and Tibetan sheep. This study identified 646 DEGs between the two breeds, and the top two genes with the largest fold change (NELL1 and FMO3), which may be relevant to fat metabolism in adipose tissues. The adipose tissue of STH sheep and Dorset sheep were analyzed by RNA-seq technology [7]; 602 DEGs were identified in two breeds of sheep, and some of these genes were significantly enriched in the triglyceride biosynthetic process. Kang et al. [8] performed DEG analysis in three types of adipose tissue (subcutaneous, visceral, and tail) in Tan sheep and identified 1,058 DEGs. Among which, HOTAIR_2, HOTAIR_3, and SP9 were highlighted in the tail region, and could be associated with tail fat development in sheep. Further, the IncRNAs and mRNAs associated with tail fat deposition and development in Lanzhou fat-tailed sheep (long fat-tailed sheep), STH sheep (thin-tailed sheep), and Tibetan sheep (short thin-tailed sheep) were analyzed; 407 DEGs and 68 differentially expressed (DE) IncRNAs were identified [9]. It was shown that the DEGs and target genes of DE IncRNAs were enriched in fatty acid metabolism and fatty acid elongation-related pathways through gene ontology (GO) analysis and Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis, which contribute to fat deposition. Network contribution based on DE mRNA and IncRNAs shows that some DE IncRNAs (TCONS_00372767, TCONS_00171926, TCONS_00054953, and TCONS_00373007) may play an important role in tail fat deposition processes. Bakhtiarizadeh & Salami [10] have performed the transcriptome analysis in fat-tailed (Lori-Bakhtiari) and thin-tailed (Zel) Iranian sheep breeds and identified 7 DE IncRNAs and 311 DEGs between the two breeds. Further, the target prediction analysis shows that the novel IncRNAs can regulate the expression of genes involved in lipid metabolism through cis- or trans-regulation. In addition, the animal quantitative trait loci database suggested 1 intronic and 6 intergenic IncRNAs as candidates of sheep fat-tail development. Transcriptome analyses were performed in specific sheep tissues to reveal the potential regulatory roles of IncRNAs, such as in the skeletal muscle [11, 12, 13], pituitary [14, 15, 16], testis [17, 18], and ovaries [19, 20, 21]. However, related research on IncRNA in sheep tail fat is still lacking, including the regulation mechanism of fat deposition and related molecular pathways of tail fat development. To better understand the potential role of mRNAs and IncRNAs in fat-tailed sheep, we explored the transcriptomic differences in SS’s tail fat at three different growth stages, 6 months of age (6M), 18 months of age (18M), and 30M. This facilitated the characterization of the mRNA and IncRNA expression profiles in the fat tail of SS, and elucidate the molecular mechanism of fat deposition. Our findings may lay a foundation for further studies in fat-tailed sheep. In particular, our study provides some information on the mechanism of fat development in fat-tailed sheep during different growth processes, which is of great significance for the development and utilization of by-products of meat breeds of sheep.

Materials And Methods

Animal and tail fat tissue collection

Nine castrated Sunite rams were selected from three different growth stages, 6M (n = 3), 18M (n = 3), and 30M (n = 3), respectively. All sheep were raised under the same conditions, including food, water source, and
environment. After slaughtering, adipose tissue was sampled from the tail fat (top 1/3) and cut into small pieces of 2 mm × 2 mm × 2 mm [7]. These small pieces were immediately placed into cryotube (sterile without enzyme), frozen in liquid nitrogen, and transferred to −80 °C until RNA extraction.

All experimental procedures were approved by the Animal Ethics Committee of the Inner Mongolia Agricultural University’s Animal Experimentation Area and followed the Chinese Animal Protection Law.

RNA extraction and RNA-seq
Total RNA from the nine adipose tissue samples was extracted using Trizol reagent (Invitrogen, CA, USA) according to the manufacturer’s procedure. Quantity and purity of the total RNA were analyzed with Bioanalyzer 2100 and RNA 6000 Nano LabChip Kit (Agilent, CA, USA), respectively, with RNA integrity number > 7.0. Approximately 10 µg of total RNA was used to deplete ribosomal RNA by following the manufacturer’s instructions of the Epicentre Ribo-Zero Gold Kit (Illumina, San Diego, USA). After purification, divalent cations were applied to fragment poly (A) and tail poly (A) + RNA fractions into small pieces under high temperature. Then, the cleaved RNA fragments were reverse transcribed to create the final complementary DNA (cDNA) library according to the protocol for the mRNA-Seq sample preparation kit (Illumina, San Diego, USA), and the average insert size for the paired-end libraries was 300 bp (± 50 bp). Eventually, the paired-end sequencing was performed following the vendor’s recommended protocol of the Illumina Hiseq 4000.

Transcripts assembly
The FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) software was used to verify the sequence quality, and adaptor contamination, low-quality bases, and undetermined bases in the raw data were removed by the Cutadapt software [22]. The clean reads were mapped into the genome of sheep (Ovis aries v3.1) using Bowtie2 [23] and Tophat2 [24], and mapped reads were assembled using the StringTie software [25]. To reconstruct a comprehensive transcriptome, all transcriptomes from sheep samples were merged using Perl scripts. After the generation of the final transcriptome, the expression levels of all transcripts were estimated using the StringTie and R package Ballgown [26].

IncRNA Identification and different expression analysis
First, transcripts that overlapped with known mRNAs and transcripts smaller than 200 bp were excluded. Subsequently, the Coding Potential Calculator (CPC) [27] and Coding-Non-Coding Index (CNCI) software tools [28] along with Pfam database [29] were utilized to predict transcripts with coding potential. Transcripts that scored CPC < −1 and CNCI < 0 were discarded. The remaining transcripts with class code (i, j, o, u, x, =) were considered as IncRNAs. The definition of class code is as follows: (i) a transcript falling entirely within a reference intron (intronic); (j) potentially novel isoform or fragment at least one splice junction is shared with a reference transcript; (o) generic exonic overlap with a reference transcript; (u) unknown, intergenic transcript (intergenic); (x) exonic overlap with reference on the opposite strand (antisense); (=) complete match, considered as known IncRNA. Expression levels of IncRNAs and mRNAs were calculated as fragments per kilobase of transcript per million mapped reads using the StringTie. The DE mRNAs and IncRNAs were determined with an absolute value of log₂ (Fold Change) ≥ 1 and false detection rate (FDR) < 0.05 using the Ballgown [26].

Target gene prediction and functional analysis of IncRNAs
In order to explore the functions of IncRNAs, the DE IncRNAs were analyzed for target prediction. In this study, coding genes 100,000 bp upstream and downstream of the target gene were considered as the cis-target genes. The targets in trans were defined by calculating the expressed correlation with IncRNAs. Then, we performed GO and KEGG analysis of the DE IncRNA targets and mRNAs, respectively, using the in-house scripts. The significance was expressed as FDR < 0.05.

**Construction of the co-expression network**
To gain a better understanding of interactions between the DEGs and DE IncRNAs, the Pearson correlation coefficient (COR) of mRNA-IncRNA co-expression network was calculated. Finally, the mRNA-IncRNA co-expression network was constructed using Cytoscape (version 3.7.2) with an absolute value of COR ≥ 0.7.

**Results And Discussion**

**RNA-Seq analysis**
The results of the RNA-Seq reads mapping is shown in Table 1. To identify the potential function of IncRNAs in tail fat tissues, the nine cDNA libraries were sequenced using the Illumina Hiseq 4000 platform. A total of 139.82 G raw data were generated from the nine adipose tissues. After filtering out low-quality reads, 131.61G valid data were obtained, and the average valid ratio (reads) was 94%. In detail, the valid reads obtained were: (1) 94,550,880; 98,994,810; and 99,167,036 per fat tail tissue sample from 6M (A1, A2, and A3), (2) 96,734,688; 97,915,792; and 95,384,562 per fat tail tissue sample from 18M (B1, B2, and B3), and (3) 98,530,172; 95,442,058; and 100,597,240 per fat tail tissue sample from 30M (C1, C2, and C3), respectively. The average percentage of Q20 and Q30 base was more than 99% and 98%, respectively, and the percentage of the Guanine-Cytosine (GC) content of each sample on an average was 48%. Above all, we indicate that results of the RNA sequencing were highly reliable, and follow-up analysis can be carried out.
Table 1
Summary of the reads mapped to the tail adipose tissue transcriptomes

| Sample | Raw Data | Valid Data | Valid Ratio(reads) | Q20% | Q30% | GC content% |
|--------|----------|------------|--------------------|------|------|-------------|
|        | Read     | Base       | Read              | Base |      |             |
| A1     | 101449274| 15.22G     | 94550880          | 14.18G | 93.20 | 99.74       |
| A2     | 103736482| 15.56G     | 98994810          | 14.85G | 95.43 | 99.81       |
| A3     | 105051744| 15.76G     | 99167036          | 14.88G | 94.40 | 99.77       |
| B1     | 102382092| 15.36G     | 96734688          | 14.51G | 94.48 | 99.76       |
| B2     | 103267850| 15.49G     | 97915792          | 14.69G | 94.82 | 99.79       |
| B3     | 102376260| 15.36G     | 95384562          | 14.31G | 93.17 | 99.70       |
| C1     | 105462958| 15.82G     | 98530172          | 14.78G | 93.43 | 99.70       |
| C2     | 101700360| 15.26G     | 95442058          | 14.32G | 93.85 | 99.71       |
| C3     | 106595358| 15.99G     | 100597240         | 15.09G | 94.37 | 99.67       |

A1, A2, and A3 are 6 months of age; B1, B2, B3 are 18 months of age; C1, C2, and C3 are 30 months of age; valid ratio (reads) = (valid reads/raw reads).

Summary of IncRNA and mRNA expression

To understand the expression profile of the IncRNAs in the tail fat tissue of SS, we identified the expression levels of the IncRNAs and compared them with the expression levels of mRNA. First, a total of 20,670 mRNAs and 6,794 IncRNAs were identified. Here, 5,722 IncRNAs were identified as novel, and the remaining 1,702 IncRNAs were identified as known, which is more than the number of IncRNAs present in chicken [30] and cattle [31] adipose tissue. According to the classification rules, we classify novel IncRNAs as 1,395 (21%) class i, 354 (5%) class j, 288 (4%) class o, 3,174 (47%) class u, 511 (7%) class x, and 1,702 (16%) class = as known IncRNA (Fig. 1a). The chromosome distribution of IncRNAs is shown in circos figure (Fig. 1b). We found that most of the IncRNAs were mainly enhanced in chromosomes 1, 2, and 3. Then IncRNAs and mRNAs were compared with exon number, open reading frames (ORF) length, transcript length, and expression levels. The mRNAs and IncRNAs had 9.7 and 1.8 exons on an average; 86% of IncRNAs contained 1–2 exons, and 38% of mRNAs contained more than 9 exons (Fig. 2a). The size of the ORF of IncRNAs and mRNAs are mainly concentrated in the range of 0–200 and 0–600 amino acids, respectively (Fig. 2b). The majority of IncRNAs and mRNAs were > 1000 bp in size, and short-range (≤ 300 bp–600 bp) IncRNAs were more than mRNAs. The average length of IncRNAs and mRNAs was 3184 bp and 1903 bp, respectively. This significant difference might be due to the quantity gap of IncRNA and mRNA under similar distribution patterns (Fig. 3a). The expression levels of IncRNA were higher than the expression levels of mRNAs (Fig. 3b), which suggests that the IncRNAs may play an important role in the development of sheep tail fat tissue.

Different expression analysis
We compared the expression profiles between any two stages (30M vs 18M, 18M vs 6M, and 30M vs 6M) using $|\log_2(\text{Fold Change})| \geq 1$ and FDR < 0.05 to identify DEGs and DE IncRNAs. In the comparison between 30M vs 6M, we found 377 DEGs (167 up-regulated and 210 downregulated genes). In the 30M vs 18M group, 125 DEGs (56 upregulated and 69 downregulated genes) were obtained. In the comparison of 18M vs 6M, 75 DEGs (38 upregulated and 37 downregulated) were found (Fig. 4). Furthermore, 4 DEGs were commonly expressed in the comparison groups of 30M vs 18M and 18M vs 6M, including IFIT5, THBS1, ENSOARG00000004030, and ENSOARG00000018868. Sixty-eight DEGs were commonly expressed in 30M vs 6M and 30M vs 18M, and 35 DEGs were commonly expressed between 30M vs 6M and 18M vs 6M. On the other hand, 151 DE IncRNAs were identified in 30M vs 6M, 30M vs 18M, and 18M vs 6M. Among them, 78 DE IncRNAs including 38 upregulated (36 novel, 2 known) and 40 downregulated (39 novel, 1 known), 71 DE IncRNAs including 30 upregulated (30 novel, 0 known) and 41 downregulated (38 novel, 3 known), 61 DE IncRNAs including 34 upregulated (33 novel, 1 known) and 27 downregulated (25 novel, 2 known), respectively (Fig. 5). Fifteen DE IncRNAs were commonly expressed in the comparison groups of 30M vs 18M and 18M vs 6M, 25 DE IncRNAs were commonly expressed in 30M vs 6M and 18M vs 6M, and 19 DE IncRNAs were commonly expressed in 30M vs 6M and 30M vs 18M.

**Functional analysis of DEGs**

The top 15 GO terms and KEGG Pathway analysis performed in DEGs of the fat tail tissue at three different growth stages are shown in the scatterplot (Fig. 6). GO terms were determined by three functions, including cellular component, biological process, and molecular function. We found that more than half of the GO terms were enriched in the biological process in the comparison groups, and the cellular component obtained the most number of genes in the three comparison groups. In the three stages, the DEGs were significantly enriched in 80 GO terms, and several fat related functions were obtained, including fatty acid beta-oxidation, triglyceride biosynthetic process, triglyceride homeostasis, lipid homeostasis, lipid biosynthetic process, regulation of fat cell differentiation, suggesting that these functions might contribute to the development of the sheep tail fat. We found five highly expressed DEGs, namely EHHADH, LPIN1, ACACA, THRSP, and GPAT4, which were related to these functions. The previous study shows that LPIN1 deficiency will lead to a significant decrease in adipose tissue and abnormal expression of adipogenic genes. Conversely, increased expression of LPIN1 in skeletal muscle or adipose tissue will promote obesity in mice [32]. EHHADH is associated with the expression of genes involved in the tricarboxylic acid cycle, mitochondrial and peroxisome fatty acid oxidation, and is indispensable for the production of medium-chain dicarboxylic acids in mice during fasting [33]. ACACA is considered to be a key regulator of fat production and a limiting factor in the synthesis of long-chain fatty acids. Acetyl-CoA can be converted to malonyl-CoA [34], which may play a key role in energy metabolism and homeostasis in sheep tail fat cells. THRSP is involved in the process of adipogenesis in rodents, and it may be a potential marker gene for bovine intramuscular fat. Studies have shown that THRSP is mainly expressed in adipocyte nuclei, intramuscular adipocytes, and related cells and expressed in mature adipocytes rather than in the early stages of adipogenesis [35]. In our study, the expression of the THRSP gene was higher in the tail adipose tissue during 6M and 18M, and significantly lower at 30M. We can speculate that in the early fat tissue of sheep's tail, fat hypertrophy is mainly manifested by the increase in the number of fat cells, and as the age increases, fat hypertrophy is reflected by the increase in the volume of fat cells. In addition, there were also some highly expressed genes related to fat metabolism, such as GPAT4, ACSM1, ACSM3, ACAT1, TKT, and ECHS1. GPAT4 was reported to be responsible
for maintaining triacylglycerol stores [36], and ACSM1, ACSM3, and ACAT1 were related to fat deposition and fatty acid metabolism [37, 38, 39]. ECHS1 was shown to be associated with the fatty acid beta-oxidation [40]. Studies have shown that TKT expression affects fatty acid oxidation and mitochondrial function [41]. On the other hand, a total of 8 KEGG pathways were significantly enriched in three different stages. They were mainly focused on metabolism processes, including Carbon metabolism, Mineral absorption, Glutathione metabolism, Butanoate metabolism, and some related amino acid metabolism. Based on the KEGG pathway analysis, those highly expressed DEGs were related to Butanoate metabolism, Fatty acid metabolism, Glycerolipid metabolism, PPAR signaling pathway, which may contribute to the fat deposition in sheep tail fat.

Based on the above analysis and further screening, we obtained several DEGs that may be related to fat tail development. We performed a hierarchical clustering analysis to show the expression patterns of these DEGs (Fig. 7). It is not difficult to find that most of the genes are active in the early months of age, especially during the 6M, and the expression of DEGs decreased gradually with the increase in age. Therefore, we indicate that the vitality of fat development weakens with the increase of age, that is to say, the development of tail fat will be more active at the age of 6M, but will gradually decrease at the age of 18M and 30M. There is a significant difference between 6M and 30M of age. Further, the expression pattern at 18M, as the middle month, plays the role of transitioning from high metabolic activity to low metabolic activity. However, our findings are only possible in theory, and the mechanism needs to be further identified.

**Target Gene Prediction and Functional Analysis**

In order to explore how lncRNA participates in regulation, we predicted DE lncRNAs based on cis- and trans-regulation in three different stages of the fat tail development. In our study, 148 DE lncRNAs (68 upregulated and 80 downregulated) were obtained and the target genes prediction analysis was performed in these DE lncRNAs. A total of 186, 113, and 150 GO terms were significantly enriched in target genes of 30M vs 6M, 30M vs 18M, and 18M vs 6M (FDR < 0.05), respectively. The top 15 GO terms and KEGG pathway of target genes of DE lncRNAs in the three comparison groups are shown in the scatterplot (Fig. 8). There were 5 common GO terms enriched in the three comparison groups, namely plasma membrane, extracellular exosome, membrane, extracellular, and cytoplasm. The target genes of DE lncRNAs in 30M vs 6M were significantly enriched in 4 KEGG pathways, including calcium signaling pathway, cell adhesion molecules, oxytocin signaling pathway, and tight junction. Among these DE lncRNA, only one cis-regulated target gene was obtained: MSTRG.13384.1 targets CLDN4. We found that most of the lncRNAs were targets to more than 20 mRNAs as their trans-regulators, MSTRG.20969.1 targets to 53 mRNAs, as the largest number in 30M vs 6M. The most commonly enriched top 5 target genes were SLC7A6 (38 DE lncRNA), CDS2 (32 DE lncRNA), CA3 (31 DE lncRNA), SLC6A2 (31 DE lncRNA), and PRTG (30 DE lncRNA). These target genes were mainly enriched in cellular components, such as membrane and integral component of membrane. Previous studies have indicated that with obesity, the concentration and activity of CA3 in rat adipose tissue decreased [42]. The complement and coagulation cascades are the only KEGG pathway that is significantly enriched in 30M vs 18M. In this comparison group, MSTRG.12899.1 and ENSOART00000028120 were connected to 38 mRNAs as the largest number in 30M vs 18M, respectively. The most commonly enriched top 5 target genes were SNORA23 (29 DE lncRNA), ERICH6B (27 DE lncRNA), ENSOARG0000018868 (22 DE lncRNA), FBP2 (22 DE lncRNA), and ENSOARG0000014791 (16 DE lncRNA), among which ENSOARG0000018868 is related to
lipid binding. In 18M vs 6M, MSTRG.14210.1 targets to 48 mRNAs as the largest number. The most commonly enriched top 5 targets genes were HECW1 (23 DE lncRNA), CRHR2 (19 DE lncRNA), FRK (19 DE lncRNA), IFIT5 (19 DE lncRNA), and PTPRZ1 (19 DE lncRNA). Based on the GO analysis, these target genes were mainly enriched in molecular function, including ATP binding and Hippo signaling pathway, and Steroid hormone biosynthesis were obtained in KEGG analysis.

Further, we obtained several fat related DE target genes in these three comparison groups. Among these targets DEGs, some of them were regarded as common DE target genes in two comparison groups, including TRIB3, ACSM1, ACSM3, TKT, SPTB, and ASGR in 30M vs 6M and 30M vs 18M, and CYP1A1 and LBP in 30M vs 6M and 18M vs 6M. Based on the GO analysis, these DEGs were mainly enriched in fatty acid ligase activity, fatty acid biosynthetic process, glyceraldehyde-3-phosphate biosynthetic process, negative regulation of fat cell differentiation, and some lipid related functions, such as lipid binding and lipid homeostasis. It has been reported that TRIB3 was might inhibit subcutaneous fat deposition in Large White pig, and IncRNA XLOC_064871 trans-regulates TRIB3, so XLOC_064871 might play an important role in adipocyte differentiation and fatty acid metabolism in pig [43]. CYP1A1 is only expressed at 6M in our study, and it was reported to be expressed in brown adipose tissue [44]. The study showed that using a specific anti-LBP antibody to inhibit LBP activity can improve the adipogenic status of fully differentiated adipocytes, which makes LBP is a novel adipokine that might display an essential role in inflammation and obesity-associated adipose tissue dysfunction [45]. In addition, we found that the same target gene was affected by different amounts and types of DE lncRNAs at different ages. For example, in 30M vs 6M and 30M vs 18M, 13 and 11 DE lncRNAs were connected to ACSM1, and there were four common IncRNA targets to ACSM1 between two comparison groups; 13 and 15 IncRNAs were connected to ACSM3; 22 and 19 IncRNAs were connected to TKT between two comparison groups, however MSTRG.3410.1 is the only one IncRNA that acts as a target to ACSM3 and TKT, which suggest that MSTRG.3410.1 may be related to the fat deposition. It could indicate that different IncRNAs with different regulation patterns may impact the target gene expression pattern and play its role in different growth stages of sheep tail fat.

**Co-expression network construction**

We constructed three co-expression networks based on DEGs and DE lncRNA in sheep fat tail using Cytoscape (version 3.7.2) (Fig. 9). A total of 538, 158, and 184 pairs of co-expression pairs were obtained in 30M vs 6M, 30M vs 18M, and 18M vs 6M, respectively. In the comparison of 30M vs 6M, 78 DE lncRNAs connected to the 26 mRNAs, and 538 pairs of co-expression pairs were obtained (403 positively and 135 negatively correlated). There were 20 IncRNAs connected to more than 10 mRNAs. MSTRG.20969.1 and MSTRG.12518.1 were connected to mRNA 21 and 20, respectively. In the 30M vs 18M, 52 DE lncRNAs connected to the 9 mRNAs, and 149 pairs (118 positively correlated and 31 negatively correlated) were obtained. ENSOART00000028120 and MSTRG.19382.4 were co-expressed with 7 mRNAs. In 18M vs 6M, 60 DE lncRNAs connected to the 13 mRNAs, and 184 pairs (139 positively correlated and 45 negatively correlated) of co-expression pairs were obtained. MSTRG.15348.1, MSTRG.14210.1, and MSTRG.14211.1 were co-expressed with 8 mRNAs. In these two comparison groups, there were only 7 (30M vs 18M), and 8 (18M vs 6M) mRNAs connected to single lncRNA, at most. This indicate that these co-expression pairs might play a crucial role, and IncRNA may regulate the development of sheep tail fat mainly through positive correlation with multiple mRNAs.
Conclusions

In our study, we used transcriptome analysis to explore the underlying molecular mechanism of different growth stages of SS tail fat. We identified 377 DEGs and 78 DE IncRNAs between the 30M vs 6M, 125 DEGs and 71 DE IncRNAs were found between the 30M vs 18M, and 75 DEGs and 61 DE IncRNAs were found between the 18M vs 6M (FDR < 0.05 and |Fold Change| ≥ 2), respectively. According to the GO and KEGG analysis of DEGs, we conclude that the fat deposition in the sheep tail may be active in the early stages of growth and gradually decrease with the increase of age, and 18M may be a transitional period in this process. On the other hand, IncRNA participates in the regulation of the growth and development of tail fat by targeting the mRNA. These findings could provide a better understanding of the regulatory mechanism of sheep tail fat development and provide basic theoretical data for further research.

Abbreviations

mRNA: Messenger RNA
LncRNA: Long Noncoding RNA
DEGs: Differentially Expressed Genes
DE IncRNA: Differentially Expressed IncRNA
SS: Sunite Sheep
RNA-seq: RNA Sequencing
GO: Gene Ontology
KEGG: Kyoto Encyclopedia of Genes and Genomes
6M: 6 Months of Age
18M: 18 Months of Age
30M: 30 Months of Age
FDR: False Detection Rate

Declarations

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**Declarations of interest**

None.

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**Contributions**

XGH and GB designed the study. XGH, YYY, XQ, LC and YFH analyzed the data. JDW, LNS and GB provided ideas and suggestions. XGH wrote the paper. All authors read and approved the final manuscript.

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**Ethics declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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