Screening and Identification of The Key LncRNAs Associated With Fat Deposition in Ovine Tail

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Research Article

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Abstract

Background: Sheep is an important meat-producing animal, and low tail fat deposition level has become one of the main targets of meat production. LncRNAs have recently received special attention due to their critical role in many important biological processes. There are few reports on its regulatory function in ovine fat deposition. In this study, two sheep populations with different tail types in Xinjiang, Bashby sheep (fat-tailed) and the hybrid population of Bashby sheep and wild argali (small-tailed), were selected for whole transcriptome resequencing from their tail tissues.

Results: First, 728 differentially expressed lncRNA (DElncRNAs) of tail fat between Bashby and F2 sheep were identified by RNA-seq. Second, the tissue expression profile and relative expression difference between Bashby and F2 sheep of 7 of 728 DE lncRNAs were analyzed by RT-PCR. We conclude that MSTRG.37980, MSTRG.38164, MSTRG.36912 and MSTRG.36913 positively regulate fat deposition, while MSTRG.8169, MSTRG.24995 and MSTRG.31389 inhibit fat deposition. Third, GO and KEGG analysis revealed that lncRNA targets were mainly participated in energy metabolism, growth and development and immunity, such as viral carcinogenesis, Chemokine signaling pathway, B cell receptor signaling pathway. And the expression pattern of target genes in each tissue was similar to that of the corresponding lncRNAs, which required us to conduct further research on target genes.

Conclusions: This study was the first to systematically identify fat deposition-associated lncRNAs in ovine tail fat and construct DElncRNAs profiles. Our findings will help understand the molecular mechanism of fat deposition from transcriptomic perspectives.

Background

Long noncoding RNA (lncRNA) is a class of noncoding RNA molecules with a length of more than 200 nucleotides, which can regulate a variety of biological processes such as cell proliferation, differentiation, apoptosis and autophagy. Unlike mRNAs, the primary structure of lncRNAs is not highly conserved, and a considerable number of lncRNAs are transcribed from introns, exons, intergenic regions and overlapping regions [1]. At the beginning of the 21st century, scientists around the world were still focusing their research on the properties and biological functions of RNA, which encodes proteins, and messenger RNA (mRNA). LncRNAs are the scientific frontier of the genome era and may reveal new RNA-mediated genetic expression regulatory networks, which could clearer clarify function of genome from the perspective of non-coding RNAs. With the application of high-throughput sequencing, lots of biologically functional lncRNAs have been discovered.

The argali is considered one of the ancestors of domestic sheep living in a wide area of central Asia, and its tail followed by natural and artificial selection is small. At present, a large amount of fat is deposited in the tail of domestic sheep. For example, the fat tail of Bashby sheep is the result of artificial selection to meet the current demand of the fatty food industry. Fat is not only an important energy storage substance, but also plays an important role in the regulation of animal energy balance, and is closely related to meat production, meat quality, meat flavor, and other meat production traits. However, nowadays, "fat surplus" means that people no longer pursue quantity but quality, meanwhile, the disorder of fat function can lead to a series of

cardiovascular diseases, such as hyperlipidemia and diabetes. Therefore, the molecular regulation mechanism of animal fat deposition has attracted much attention.

Animal fat deposition is a complex biological process, which is regulated by a cascade of genetic factors such as functional genes, noncoding RNAs, and adipogenic related signaling pathways, among which lncRNAs play an important role in regulation. In recent years, increasing studies have found that lncRNA plays an important role in animal fat deposition. Several lncRNAs have been found to regulate adipogenesis, such as lncRNA NEAT1 in 3T3-L1 cells [2] and BLNC1 in mice [3]. In humans, the lncRNA ADINR promotes adipogenesis by activating transcription of CCAAT enhancer binding protein α (C /EBPα) [4]. LncRNA H19 inhibits adipocyte differentiation during BMSCs entry into adipocytes [5]. LncRNA MEG3 is involved in the balance between adipogenic and osteogenic differentiation of human adipose stem cells [6]. However, there are few reports about the regulatory function of lncRNA in ovine fat deposition and how to regulate mRNA expression. How the fat tail of sheep is formed in the evolutionary process is still a scientific question.

This study chooses Bashby sheep and the second generation of Bashby and argali sheep (F2) cross as an object in Xinjiang the collection and two groups of fat tail, respectively, and small tail of the organization, using the whole transcriptome technology, choose the fat tail high expressing the differences of lncRNA, forecast its target genes, the mRNA and lncRNA conjoint analysis, identification of key genes affecting fat deposition in the tail. To provide a basis for the identification of functional genes related to the regulation mechanism of sheep fat metabolism. It is of great significance to reveal the molecular mechanism of fat deposition in the evolutionary process of tail fat and to excavate the key genes for the cultivation of healthy and efficient new sheep varieties and the improvement of human health.

Results

Transcriptome sequencing data

A total of 1,438,500,450 raw reads were obtained by RNA-seq high-throughput sequencing of tail fat from Bashby sheep and F2 sheep. A total of 1,338,966,230 clean reads were obtained after filtering the original data obtained by sequencing, it accounts for 90.64~95.61% of high-quality sequence data, and Q20% is more than 99% in all samples, and GC% is between 47~49.50%, as shown in Table 1. The reliability of the sequencing results can be used for subsequent data analysis.

Sequence alignment results with reference genomes

The comparison between the transcriptome data and the reference gene sequence was shown in Table 2. The comparison efficiency between the original sequence data and the reference genome of each sample ranged from 85.28 to 91.06%.

Screening and clustering analysis of differentially expressed lncRNAs

Analyze the differential expression of samples by Deseq2 software and determine the differentially expressed lncRNAs in tail fat of different breeds of sheep by Volcano map, and calculate whether the difference was significant (Figure 1). The results showed that 270 lncRNAs were downregulated and 458 lncRNAs were

upregulated. Cluster analysis was then performed on the differentially expressed lncRNAs screened out from the tail fat of different breeds of sheep (Figure 2) to determine the expression patterns of DElncRNAs in the tail fat of different tail types of sheep.

Prediction of lncRNA target genes

We used the co-localization of lncRNA and protein-coding genes to predict the target genes of lncRNA, to further study the function of the lncRNA obtained from ovine tail fat, in which the upstream and downstream 100 kb of lncRNA was used as the threshold value of co-localization. The results showed that 728 DElncRNAs targeted 17,850 target genes. In these predictions, one lncRNA targeted multiple mRNAs, and simultaneously, multiple lncRNAs targeted the same mRNA. MSTRG.36913, MSTRG.24995, MSTRG.37980, MSTRG.38164, MSTRG.36912, MSTRG.8169 and MSTRG.31389 target genes related to fat deposition and fat metabolism, such as THRSP, FASN, SCD and GPAM, etc. Partial results of lncRNA targeting genes involved in fat deposition and fat metabolism are shown in Table 3.

Functional and pathway analysis of DElncRNAs targets

GO enrichment was performed by DElncRNAs (Figure 3). The integral component of the membrane, positive regulation of GTPase activity, inflammatory response, immune response, lateral plasma membrane, defense response to the virus were significantly enriched in several terms, and the integral component of the membrane was most significantly enriched. DElncRNAs were analyzed by KEGG pathway analysis (Figure 4). It is mainly enriched in Viral carcinogenesis, Type I diabetes mellitus, T cell receptor signaling pathway, Systemic lupus erythematosus, Staphylococcus aureus infection, Primary immunodeficiency, Osteoclast differentiation, Natural killer cell mediated cytotoxicity, Measles, Leishmaniosis, Intestinal immune network for IgA production, Hematopoietic cell lineage, Graft-versus-host disease, Fc gamma R-mediated phagocytosis, Cytokine-cytokine receptor interaction, Chemokine signaling pathway, B cell receptor signaling pathway, Autoimmune thyroid disease, Allograft rejection, Alcoholism. In addition, a total of 7 of 728 DElncRNAs (MSTRG.37980, MSTRG.38164, MSTRG.36912, MSTRG.36913, MSTRG.8169, MSTRG.24995 and MSTRG.31389) and the target genes (*SCD*, *GPAM*, *THRSP*, *FASN*, *NDUFC2*, *WDTC1* and *PIK3R1*) related to fat metabolism were screened by investigating literatures.

Quantitative analysis verified sequencing accuracy

To verify the accuracy of RNA-seq data, 7 significantly DElncRNAs (MSTRG.37980, MSTRG.38164, MSTRG.36912, MSTRG.36913, MSTRG.8169, MSTRG.24995 and MSTRG.31389) related to fat deposition were screened to be analyzed by qRT-PCR. The results (Figure 5) showed that the quantitative and RNA-seq results of 7 lncRNA in the tail adipose tissue of Bashby and F2 were the same, indicating that the RNA-seq results were more accurate and reliable, indicating that expressed assessment and identification of lncRNAs were persuasive and could be used for subsequent experiments.

Differential expression of lncRNAs in different tissues

In order to explore the DElncRNAs in different tissues of Bashby sheep (CG) and F2 (TG), we conducted relative quantitative analysis of 7 DElncRNAs by qPCR (Figure 6). We found that there were differences and

similarities in the expression patterns of DElncRNAs in tissues of Bashby and F2. The significant expression level of tail fat in F2 was MSTRG.37980, MSTRG.38161, MSTRG.24995, while that in Bashby was only MSTRG.37980 and MSTRG.24995. MSTRG.37980 and MSTRG.38164 were highly expressed of the large intestine in F2, but lower in Bashby. This indicated that the expression of DElncRNAs in different tissues was different among species.

Difference analysis of DElncRNAs in the same tissue of different tail fat type sheep

The expression levels of 7 DElncRNAs were determined in 11 parts of Bashby (CG) and F2 (TG) (Figure 7.). We can know that these 7 DElncRNAs are extremely significant or significantly different in the tail fat tissues of Bashby and F2, and the expression of MSTRG.37980, MSTRG.38166, MSTRG.36913 and MSTRG.24995 in F2 is lower than that of Bashby, while the expression of MSTRG.36912, MSTRG.8169 and MSTRG.31389 is higher in F2 (P<0.05). For subcutaneous fat tissue, the expression of MSTRG.37980, MSTRG.38164, MSTRG.36913, and MSTRG.36912 in F2 was significantly lower than that in Bashby, while the expression of MSTRG.8169 was opposite (P<0.05). Meanwhile, the expression abundance of MSTRG.37980 in longissimus dorsi muscle, MSTRG.37980 and MSTRG.24995 in small intestine, MSTRG.38164 and MSTRG.36913 in liver of F2 sheep was significantly lower than that of Bashby (P<0.05). And the expression abundance of MSTRG.36913 in quadriceps femoris, MSTRG.24995 in rumen, MSTRG.36912 and MSTRG.36389 in arm triceps of F2 sheep was significantly higher than that of Bashby (P<0.05). These DElncRNAs not only differ in tail fat tissues, but also indirectly regulate fat deposition in other tissues.

Expression maps of DElncRNAs target genes in different tissues of sheep with different tail types

The target gene expression levels determined at 11 sites in Bashby sheep (CG) and F2 (TG) are basically the same as lncRNAs, except for the *PIK3R1* in the longissimus dorsi muscle (Figure 8). We could see that the expression levels of *SCD*, *THRSP* and *FSAN* in F2 tail fat tissue were significantly lower than those of Bashby, while *NDUFC2*, *WDTC1* and *PIK3R1* were significantly higher in Bashby (P<0.05). Meanwhile, the expression abundance of *GPAM* in liver, *THRSP* in subcutaneous fat and liver, *FSAN* in small intestine, *NDUFC2* in subcutaneous fat and duodenum of F2 sheep was significantly lower than that of Bashby (P<0.05). And the expression abundance of *SCD* in cecum, *THRSP* in longissimus dorsi muscle, *FASN* in rumen, *DOUFC2* in arm triceps, liver, rumen, and small intestine, *PIK3R1* in longissimus dorsi muscle, arm triceps, quadriceps femoris, liver, rumen, large and small intestine of F2 sheep was significantly higher than that of Bashby (P<0.05). This indicates that the target genes are differentially expressed in different tissues of sheep with different tail types, thereby indirectly or directly participating in ovine tail fat deposition.

Discussion

Fat deposition is a complex process that is affected by genetic and environmental factors, but the former is the most important. According to the current market needs, the large tail of sheep no longer meets the favorite of modern consumers, and the large amount of fat deposition in the tail of the sheep causes feed and so many wastes, therefore, small-tailed sheep is the direction of future breeding. Nowadays, there are many methods for the study of a scientific problem, and it is also very important to find a way to study the evolution of sheep tail fat. As high-throughput sequencing technology continues to improve, fat deposition-related

lncRNAs have been found in pigs, chicken and goats. Researchers found that there are 55 differential lncRNAs in the longissimus dorsi fat of Laiwu pigs and Large White pigs. Among them, XLOC_046142, XLOC_004398 and XLOC_015408 may activate protein kinase 2 (MAPKAPK2), nuclear receptor subfamily 1D group member 2 (NR1D2), and aldone reductase family 1 member C4 (AKR1C4), play key regulatory roles in the process of intramuscular fat formation , which leads to differences in fat deposition between the two pig breeds[8]. Wei found that in preadipocytes lncRNA PU.1 can combine with *PU.1* mRNA to form an antisense RNA dimer, the dimer blocks the translation of PU.1 and down-regulates PU.1 protein expression, and promote the differentiation of pig pre-adipocytes into fat [9]. Zhou found that some lncRNA genes overlap with the differential methylation region (DMR). Among them, the long intergenic noncoding RNA (linc-sscg3623) in large white pigs and the adipose tissue of Rongchang pigs showed differential methylation, suggesting that fat deposition may be related to linc-sscg3623 gene related [10]. Zou found that the high expression of lincRNA-00061360 in Wannanhua pig can positively regulate the expression of acetyl-coenzyme a carboxylase β (ACACB), an essential regulator of fatty acid oxidation pathway, thereby promoting fat synthesis or deposition and forming a higher back Intramuscular fat content of long muscles [11]. Miao studied the expression level of lncRNA in the muscle adipose tissue of Jinhua pigs and Landrace pigs, and found that there were 119 differentially expressed lncRNAs, of which 6 co-expressed lncRNAs were involved in fat deposition and lipid metabolism pathways [12]. Li studied the expression of lncRNA in the liver, abdominal fat and breast muscle of Landes geese during different feeding periods. The expression of LOC106047490 in the three tissues was significantly reduced, which may be induced by high glucose, insulin and unsaturated fatty acids. The lncRNA gene is in adult goose has the highest expression in abdominal fat, indicating that LOC106047490 of this gene is related to fat metabolism [13]. Zhang investigated chicken lncRNA abdominal preadipocytes, and found that there are 1,336 lncRNAs in preadipocytes that are differentially expressed in different stages of differentiation, and the number of differential expressions of lncRNAs is reduced, which is related to the process of adipocyte differentiation [14]. Zheng predicted the expression of lncRNA in adipose cells of 1472 goats through CPC (Coding potential calculator) and CPAT (Coding potential Assessment Tool), 29 of which were significantly expressed in intramuscular adipocytes before and after maturation in Inner Mongolia cashmere goats [15].

These studies on lncRNA reveal many mechanisms for fat deposition; however, few researches have been done on fatty deposits-related lncRNAs in sheep. Mohammad [16] conducted a comparative transcriptome analysis of Iranian large-tailed sheep and small-tailed sheep, studied the expression of sheep fat-tail lncRNA and its possible role in fat deposition, and identified 7 differentially expressed genes. Miao [17] have determined the transcriptome profile of heart, skin, muscle, mammary gland and adipose tissue through deep sequencing in sheep, and analyzed the transcriptome information of subcutaneous adipose tissue between small-tailed Han sheep and Dorset sheep. Ma [18] conducted high-throughput RNA sequencing on the three individuals in order to evaluate the lncRNAs and mRNAs related to tail fat deposition and development in Lanzhou large-tailed sheep, small-tailed Han sheep and Tibetan sheep. In our research, we through sequencing the tail tissues of Bashby sheep and F2 to construct differ-ential expression profiles of fat deposition-related lncRNAs in ovine tail fat. 728 DElncRNAs were identified and a total of 17,850 target genes were mapped from those. GO and KEGG analysis of DElncRNAs targets indicated that targets were mainly related to energy metabolism, growth and development and immunity, such as Viral carcinogenesis,

Chemokine signaling pathway, B cell receptor signaling pathway, which required us to conduct further research on target genes.

We selected 7 significantly DElncRNAs and their target genes to perform expression profiles in different tissues of Bashby and F2. In our study, the target gene expression levels determined at 11 sites are basically the same as lncRNAs. It is worth noting that the tails of MSTRG.37980, MSTRG.38164, MSTRG.36913 and MSTRG.24995 and their target genes in F2 sheep are significantly lower than those in Bashby. *SCD*, the target gene of MSTRG.37980, encodes stearoyl-coenzyme A desaturase and plays a key role in PPAR signaling pathway to activate adipogenic differentiation of adipocytes [19], the main role of *GPAM* (mitochondrial glycerol-3-phosphate acyltransferase) is to regulate the fat deposition, energy consumption, carcass quality and overall metabolism of mammals by catalyzing the biosynthesis of triacylglycerol and phospholipids[20, 21]. Thyroid hormone respond-sive Spot14 protein (*THRSP*), also known as SPOT 14 or S14, is a small molecular weight acidic intraconuclear protein that is highly expressed in liver, breast and adipose tissue of animals [22, 23]. Fatty acid synthase (*FASN*) is a key enzyme in the process of fatty acid synthesis in mammals, and plays an extremely important role in the complex metabolic regulation of the body [24]. Genome-wide association analysis shows that *FASN* plays an important role in the body's substance metabolism and the occurrence and development of diseases [25]. Similarly, from our experiments, we know that in addition to the differential expression of these target genes in the tail fat, *CSD* in the large intestine, *GPAM* in the liver, *THRSP* in the subcutaneous fat, liver and longissimus dorsi, and *FSAN* in the rumen. This suggests that lncRNAs also indirectly inhibits sheep tail fat deposition by regulating target genes. Of course, there are also lncRNAs that promote fat deposition, while MSTRG.36912, MSTRG.8169 and MSTRG.31389 and their target genes are high expression of F2. Studies have shown that the ubiquinone oxidoreductase subunit C2 (*NDUFC2*) is highly related to *THRSP*, and is highly expressed in buffalo adipose tissue and mature adipocytes. And functional assays demonstrated that *NDUFC2* promotes adipogenic differentiation by upregulating and CCAAT enhancer binding protein alpha (C/EBPα) in buffalo and promotes fat deposition in buffalo [23, 26], our experiment has also achieved similar results, MSTRG.36912 promotes fat deposition by up-regulating its target gene *NDUFC2*. WD and tetratricopeptide repeats 1 (*WDTC1*) encodes an evolutionarily conserved suppressor of lipid accumulation. At present, it is more recognized that the reduction of *WDTC1* leads to obesity in mice and humans [27, 28]. Our research also supplements this point MSTRG.8169 is highly expressed in F2 tail fat, which in turn regulates the expression of wdtc1 and inhibits ovine fat deposition. Phosphoinositide-3-kinase regulatory subunit 1(*PIK3R1*) is closely related to the role of insulin, the increase in *PIK3R1* can reduce the risk of obesity caused by the decrease in insulin. Studies have shown that the decrease in obesity may not be due to malnutrition alone, but the increase in *PIK3R1* leads to an increase in energy expenditure [29]. In our study, the high expression of MSTRG.31389 in F2 led to the expression of its target gene *PIK3R1* and thereby inhibited fat deposition. Combined with previous reports, it suggested that this may be related to the metabolism of sheep.

Conclusion

In conclusion, this study was the first to systematically identify fat deposition-associated lncRNAs in ovine tail fat and construct DElncRNAs profiles. LncRNAs played crucial roles in energy metabolism, growth and development and immunity in sheep by regulating under target gene. The MSTRG.37980, MSTRG.38164,

MSTRG.36912, MSTRG.36913, MSTRG.8169, MSTRG.24995 and MSTRG.31389 were verified to be key lncRNA of fat deposition, regulating protein-coding genes *SCD*, *GPAM*, *THRSP*, *FASN*, *NDUFC2*, *WDTC1* and *PIK3R1*. However, the detailed mechanism of fat deposition-associated lncRNAs still requires further experimental verification.

Methods

Sample Collection

The animals needed came from Yumin County of Tacheng, Xinjiang. Bashby sheep (fat-tailed) and the second generation of wild argali × Bashby sheep (F2) (small-tailed) under the same breeding conditions performance were randomly selected, which consisted 6 each extreme tail type with an age of 10 months old. A total of 11 kinds of tissues were immediately collected after slaughtered, which include tail fat, subcutaneous fat, longissimus dorsi, arm triceps, quadriceps femoris, liver, rumen, duodenum, caecum, the large intestine, small intestine, and then the collection good fresh tissue into 2 ml RNase-Free cryopreserved tubes, staging in liquid nitrogen, back to the lab after all samples will move quickly to -80 ℃ refrigerator until they were used for the extraction of total RNA.

Total RNA Extraction

Tissue samples were taken out of -80 ℃ refrigerator and put into a mortar (the sample was about 100 mg) for grinding. Total RNA was extracted by Trizol (Invitrogen, Carlsbad, CA, USA), and 2 μL of RNA was extracted by 1% agarose gel for integrity inspection. And then, 1μL RNA stock solution was added into the well to be detected by Nanodrop spectrophotometer (Implen, Weslake Village, USA), and the RNA concentration was detected. The concentration of different samples and the value of OD260/280 were recorded, respectively.

Construction of cDNA Libraries and Illumina Sequencing

Reverse transcription was performed according to the instructions of PrimeScript RT Reagent Kit with gDNA Eraser Kit, and the removed cDNA was cryopreserved at -20℃. After quality control, the cDNA libraries were constructed according to the instructions of the Illumina kit (Illumina, San Diego, CA, USA). Next, we used Qubit 2.0 for the preliminary quantification of the constructed libraries and diluted the library to 1 ng/uL, and then used Agilent 2100 to determine the insert size and qRT-PCR to determine the effective concentrations. After the libraries were qualified, they were pooled and sequenced using an Illumina HiSeq 2500 platform according to the effective concentrations and the requirement of target data quantity. Paired-end sequencing with the 150 bp read length was performed by Lianchaun Bioinformatics Technology Co. Ltd (Hangzhou, China) [7].

Sequencing Data Analyses

The original image data files from the sequencing platform were transformed into raw sequenced reads by Consensus Assessment of Sequence and Variation (CASA V A pipeline v2.0, Illumina Inc., San Diego, CA, USA) based calling analyses. Sequence alignment software HISAT v2.0.4 (Johns Hopkins University,

Baltimore, MD, USA) was used to align the sequences of each sample with the reference genome (https://www.ncbi.nlm.nih.gov/bioproject/645786). The expression level of lncRNAs was estimated by FPKM (fragments per Kilobase of transcript per million fragments mapped). In the process of differential expression of lncRNAs (DElncRNAs), Fold Change ≥2 and FDR < 0.01 were used as screening criteria. Finally, functional enrichment analysis on the lists of DEGs was carried out using GOseq software (Illumina, San Diego, California, USA) for GO terms and KOBAS (KO Based Annotation System) for KEGG pathway [1, 7] .

Target gene prediction and Functional annotations

The target genes of differentially expressed lncRNAs were predicted by cis or trans methods. For the target gene prediction of cis method, genes with a distance of 10 kb upstream or downstream of lncRNA were selected as the target genes of cis. The Miranda method was used to predict the trans-target genes.

Quantitative Real Time PCR

We selected 7 significantly DElncRNAs (MSTRG.37980, MSTRG.38164, MSTRG.36912, MSTRG.36913, MSTRG.8169, MSTRG.24995 and MSTRG.31389) from 11 tissues of Bashby sheep and F2 sheep, respectively, to verify the accuracy of sequencing results by qRT-PCR. And the target genes sequences were obtained from GenBank and the primers were designed using Allele ID 6.0 software. The β-actin was used as the internal control gene. The information about the primers was listed in Additional file 1. Based on Ct values of the quantitative expression results, the $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression.

Statistical Analysis

All data were analyzed using ANOVA (One-way analysis of variance) and LSD (least significant difference method) of SPSS 21.0 software. Statistical significance was presented at an alpha level of $P<0.05$.

Abbreviations

F2: second generation hybrid of wild Argali sheep; CG: Bashby sheep; TG: F2 sheep; DElncRNAs: differentially expressed lncRNA; KEGG: kyoto encyclopedia of genes and genomes; GO: Gene Ontology; KOBAS, KO Based Annotation System; qRT-PCR: quantitative reverse transcriptase PCR; SCD: Stearoyl-coenzyme A desaturase; GPAM: glycerol-3-phosphate acyltransferase; THRSP: Thyroid hormone respond-sive Spot14 protein; FASN: Fatty acid synthase; NDUFC2: the ubiquinone oxidoreductase subunit C2; WDTC1: WD and tetratricopeptide repeats 1; PIK3R1: Phosphoinositide-3-kinase regulatory subunit 1.

Declarations

Ethics approval

All experimental procedures including animal care and tissue sample collection were approved and carried out in accordance with the relevant guidelines set by the Ethics of Xinjiang Agriculture University (Approval number: 2017010).

Statement of euthanasia

In our experiment, the sheep were put to death in accordance with the requirements of euthanasia, and no any euthanizing agent was used. And no lethal reagents (such as chloral hydrate, ether, chloroform) were used.

In this experiment, 3 males and 3 females of Bashby sheep and F2 generation sheep were slaughtered in strict accordance with ethical methods, and the method used was bloodletting (accordance with ARRIVE guidelines (https://arriveguidelines.org)).

Consent for publication

Not applicable

Availability of data and materials

The full RNA-seq data sets have been submitted to NCBI BioProject under Accession: PRJNA766844. The original data of RNA sequencing has been uploaded to SRA database, the number is SUB10440742. Additional data can be found in Additional files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

XHS conducted the experiments and prepared the materials involved in this study. XHS and HYH performed the bioinformatics analysis. HYH and CF designed and coordinated the research. XHS and HYH drafted the manuscript. CF, LLL, and WJL helped revise the manuscript. All authors read and approved the final version of the manuscript.

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Tables

Table 1 Statistical table of sequencing data

Sample	Raw Data		Valid Data		Valid Ratio(reads)	Q20%	Q30%	GC content%
	Read	Base	Read	Base				
ST1	122233026	18.33G	112682976	16.90G	92.19	99.73	97.57	48
ST2	117305806	17.60G	112153604	16.82G	95.61	99.94	98.61	48
ST3	120483426	18.07G	113832564	17.07G	94.48	99.93	98.43	49
ST4	120182016	18.03G	108938308	16.34G	90.64	99.67	97.38	49.50
ST5	116963622	17.54G	110193226	16.53G	94.21	99.80	97.66	48
ST6	124013636	18.60G	117480482	17.62G	94.73	99.93	98.52	47
BT1	124048270	18.61G	116055054	17.41G	93.56	99.95	98.68	48
BT2	118306172	17.75G	104418552	15.66G	88.26	99.53	96.88	48
BT3	106063180	15.91G	98731006	14.81G	93.09	99.76	97.48	48
BT4	125305924	18.80G	118011172	17.70G	94.18	99.94	98.62	48
BT5	119443014	17.92G	110743918	16.61G	92.72	99.93	98.48	49.50
BT6	124152358	18.62G	115725368	17.36G	93.21	99.89	98.24	49

Notes: ST is for Bashby sheep and BT is for F2. The same below.

Table2 The list of genome mapping

Sample	Valid reads	Mapped reads	Unique Mapped reads	Multi Mapped reads
ST1	1E+08	96092919(85.28%)	74541267(66.15%)	21551652(19.13%)
ST2	1E+08	99681766(88.88%)	77763857(69.34%)	21917909(19.54%)
ST3	1E+08	100620286(88.39%)	78853222(69.27%)	21767064(19.12%)
ST4	1E+08	93187477(85.54%)	69841149(64.11%)	23346328(21.43%)
ST5	1E+08	96204661(87.31%)	73594924(66.79%)	22609737(20.52%)
ST6	1E+08	106452186(90.61%)	83258160(70.87%)	23194026(19.74%)
BT1	1E+08	105683469(91.06%)	83063566(71.57%)	22619903(19.49%)
BT2	1E+08	92291065(88.39%)	71053370(68.05%)	21237695(20.34%)
BT3	1E+08	87117428(88.24%)	67178773(68.04%)	19938655(20.19%)
BT4	1E+08	107455811(91.06%)	83679060(70.91%)	23776751(20.15%)
BT5	1E+08	99989415(90.29%)	76931070(69.47%)	23058345(20.82%)
BT6	1E+08	102463492(88.54%)	78681052(67.99%)	23782440(20.55%)

Table 3 Some lncRNAs and their target genes related to fat deposition

Target gene	Description	lncRNA
THRSP	thyroid hormone responsive	MSTRG.36913
FASN	fatty acid synthase	MSTRG.24995
SCD	stearoyl-CoA desaturase	MSTRG.37980
GPAM	glycerol-3-phosphate acyltransferase, mitochondrial	MSTRG.38164
NDUFC2	NADH: ubiquinone oxidoreductase subunit C2	MSTRG.36912
WDTC1	WD and tetratricopeptide repeats 1	MSTRG.8169
PIK3R1	phosphoinositide-3-kinase regulatory subunit 1	MSTRG.31389

Figures

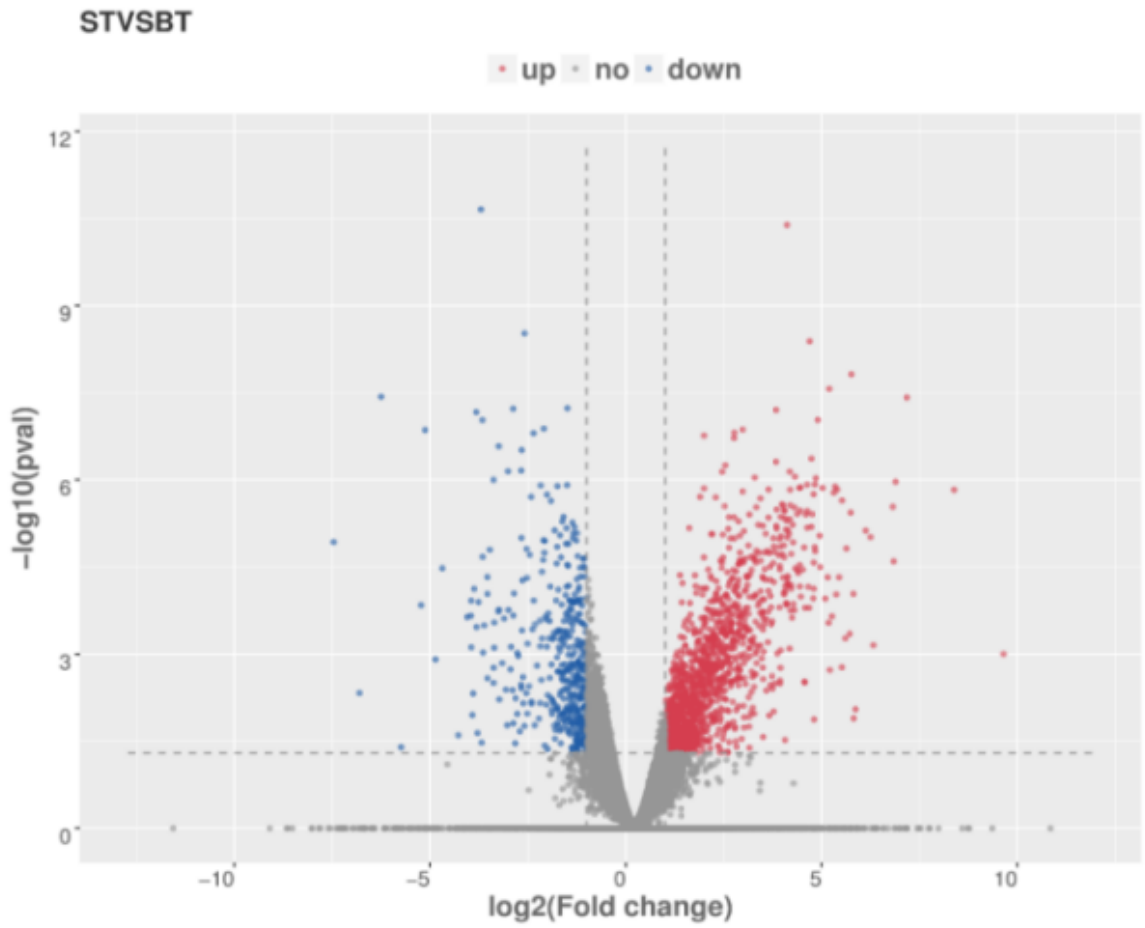

Figure 1

Volcanic map of differentially expressed lncRNAs

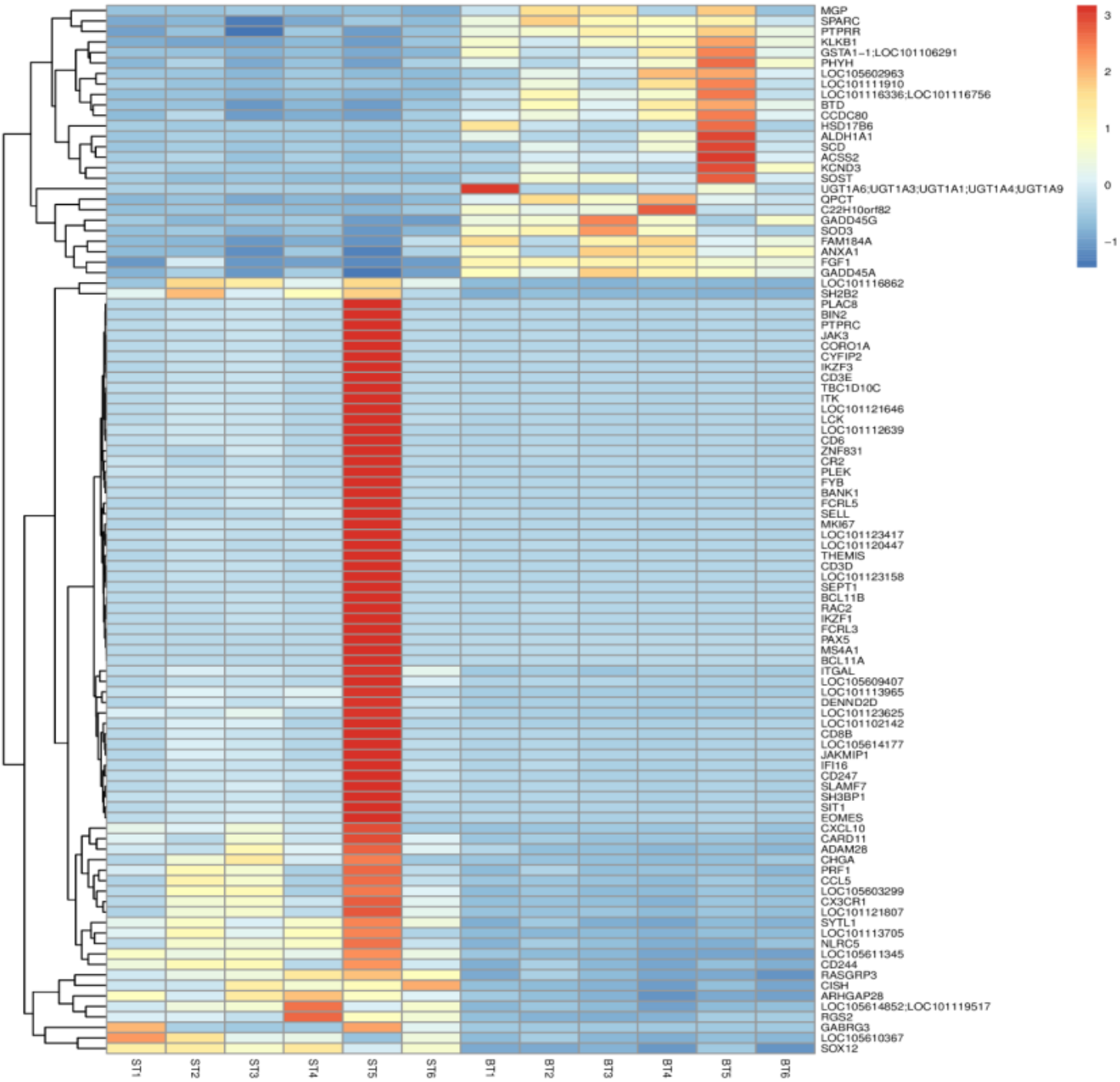

Figure 2

Cluster analyses of differentially expressed lncRNAs

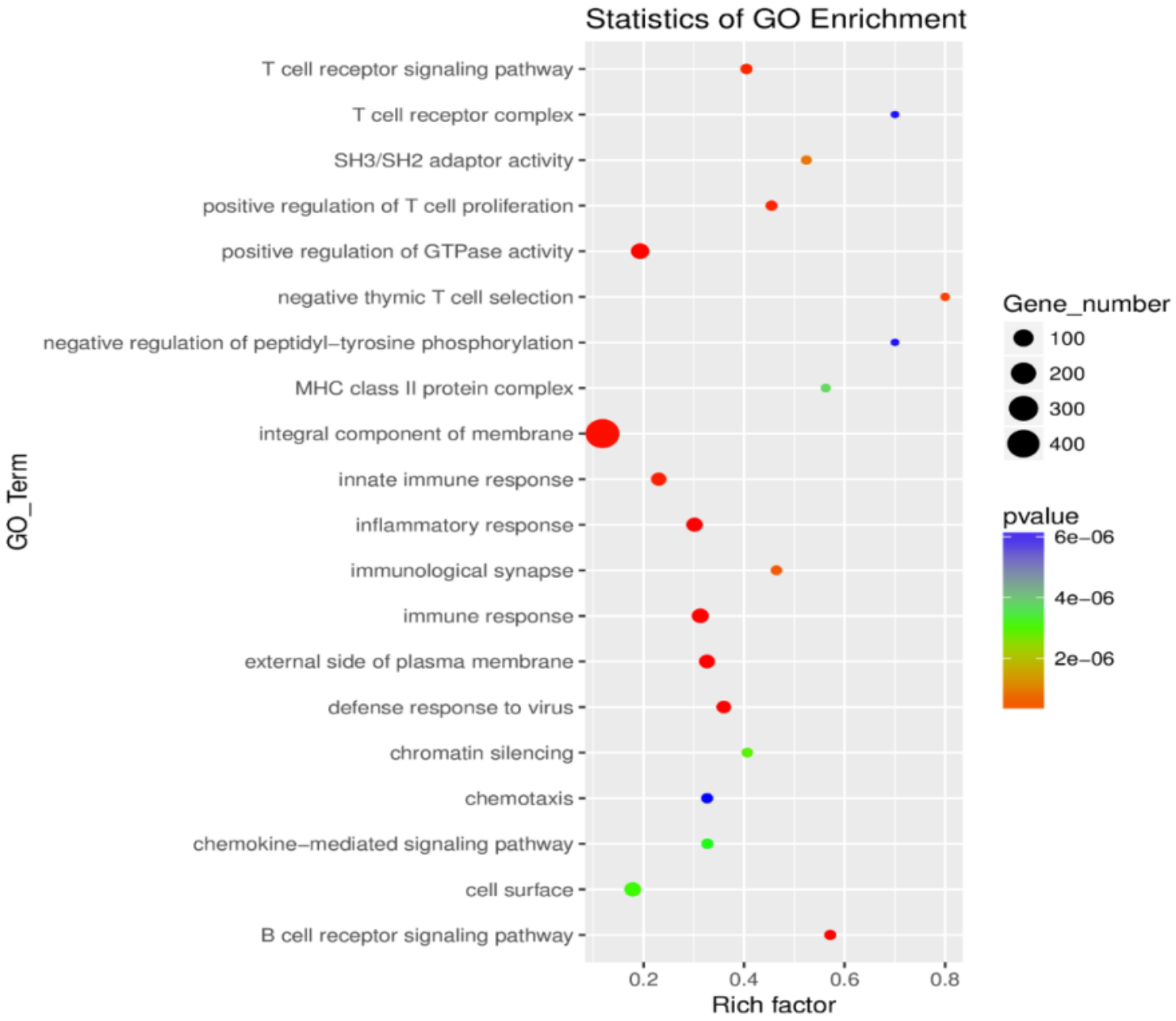

Figure 3

GO Enrichment of tail fat with different lncRNAs between fat-tailed sheep and small-tailed

Figure 4

KEGG analyses of the tail fat with different lncRNAs between fat-tailed sheep and small-tailed

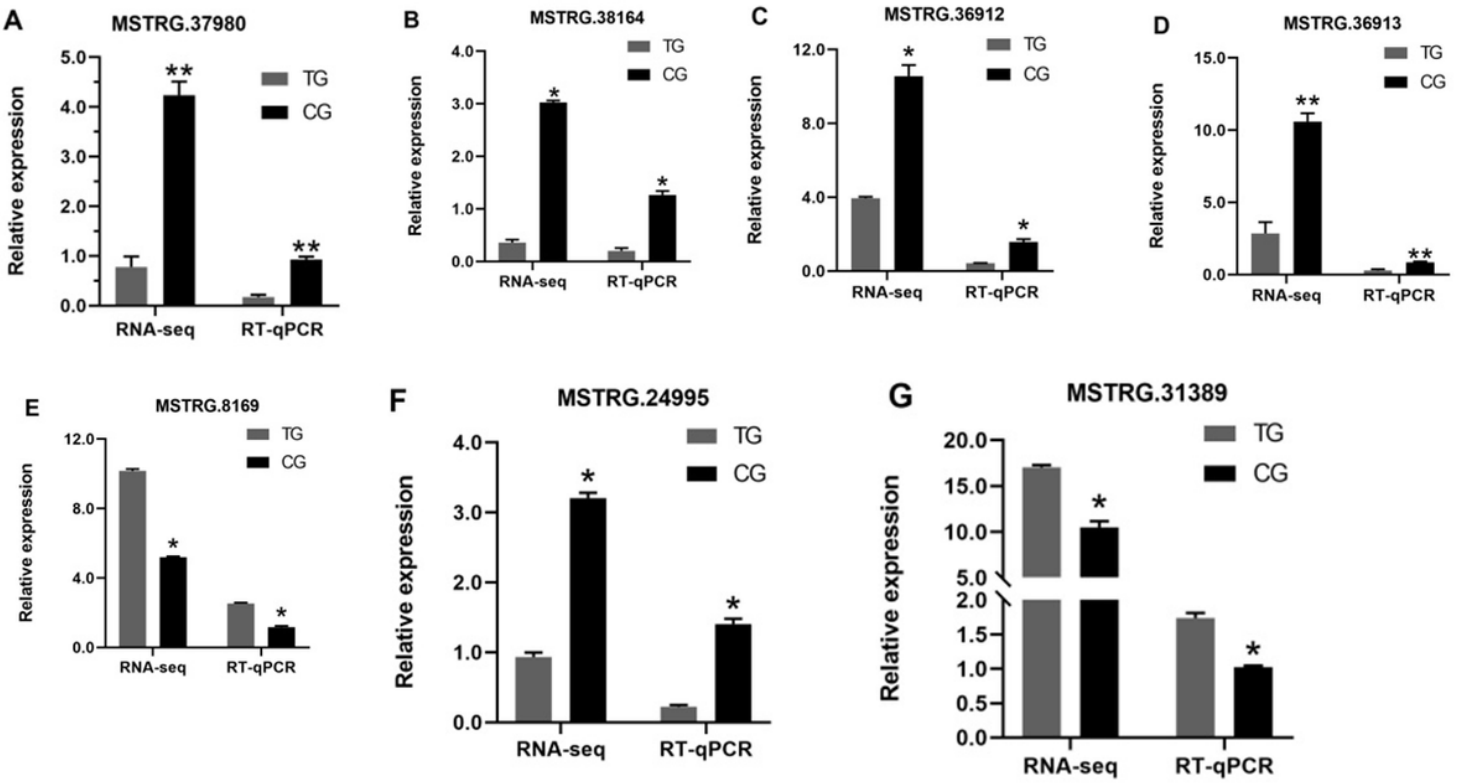

Figure 5

Comparison of expression levels of lncRNAs and RNA-Seq in Bashby and F2.*P < 0.05

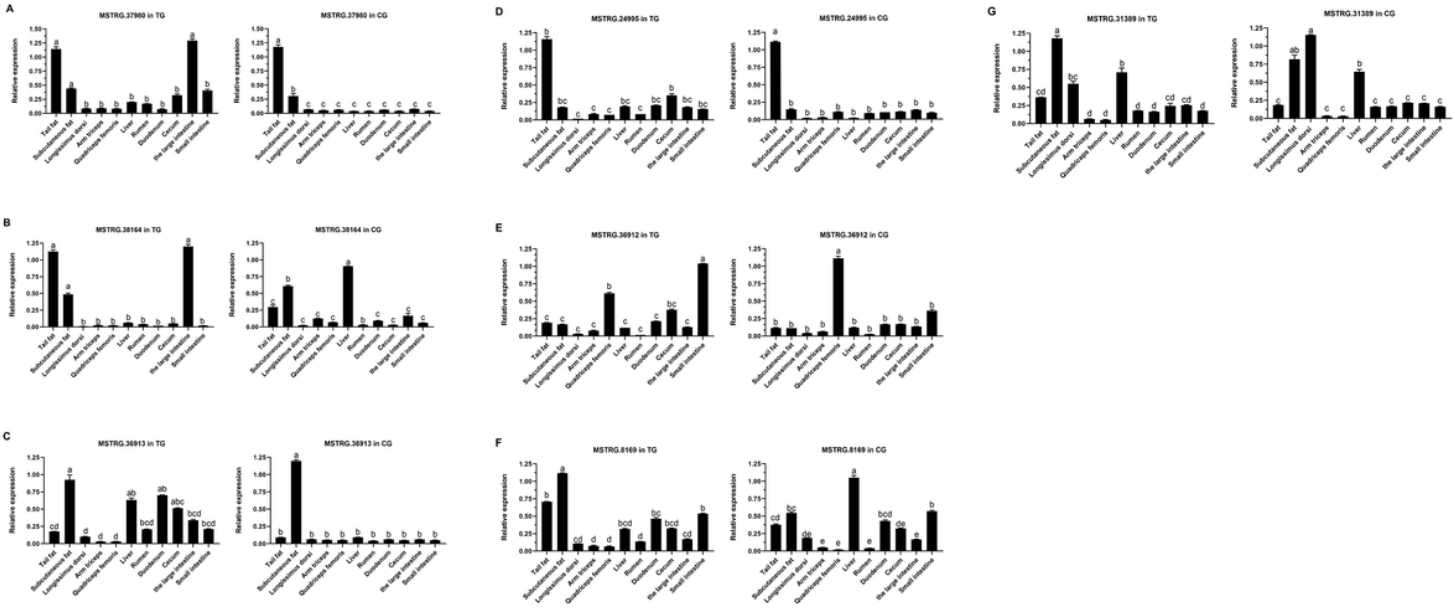

Figure 6

The relative expression levels of DElncRNAs in the tissues of the second generation (TG) and Bashby sheep (CG). Data were analyzed using the one-way measures analysis of variance (ANOVA) and least significant difference method (LSD). Data are expressed as means ± SE. The different small letter superscripts mean

significant difference (P<0.05); while with the same letter superscripts mean no significant difference (P>0.05)

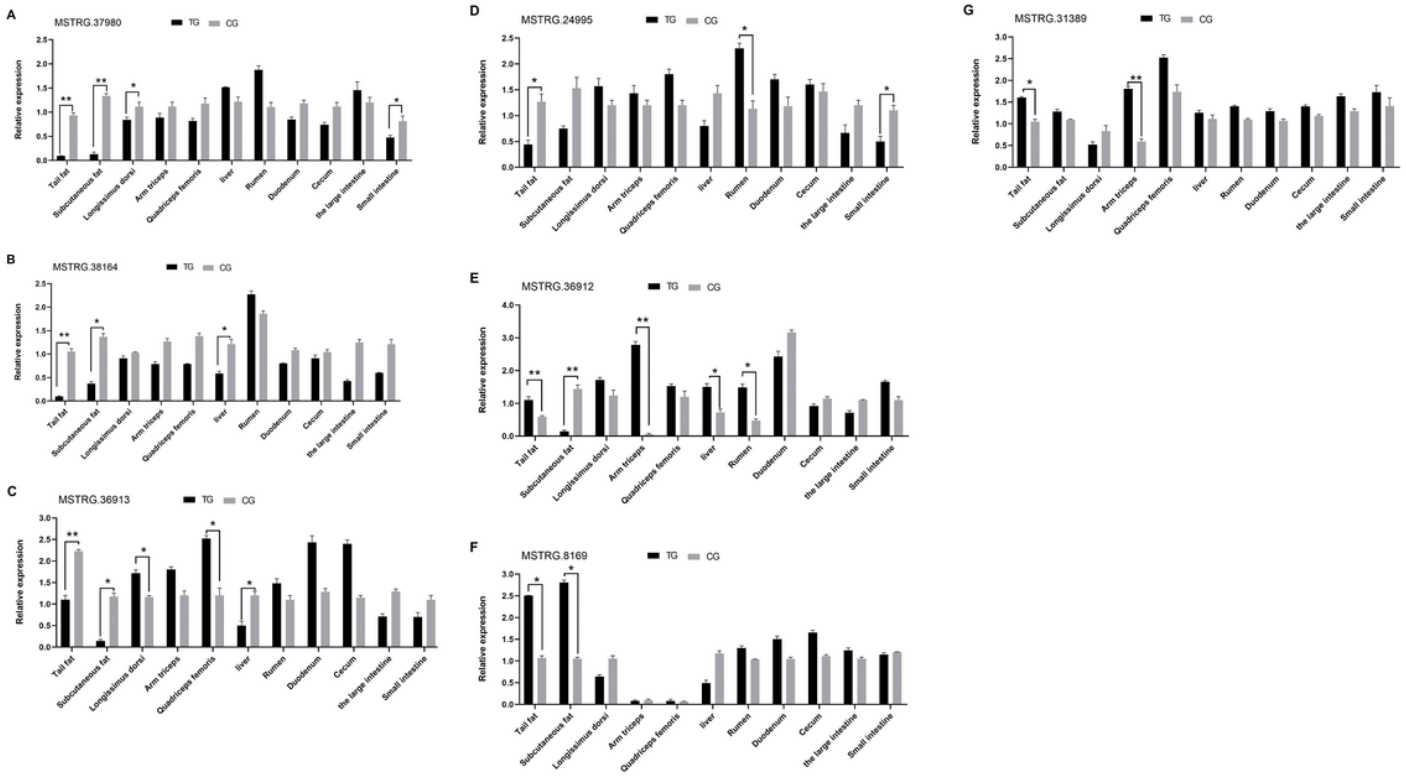

Figure 7

Relative expression differences of DElncRNAs in Bashby sheep (CG) and F2 (TG). Data were analyzed using the one-way measures analysis of variance (ANOVA) and least significant difference method (LSD). Data are expressed as means ± SE. *P<0.05, **P<0.01

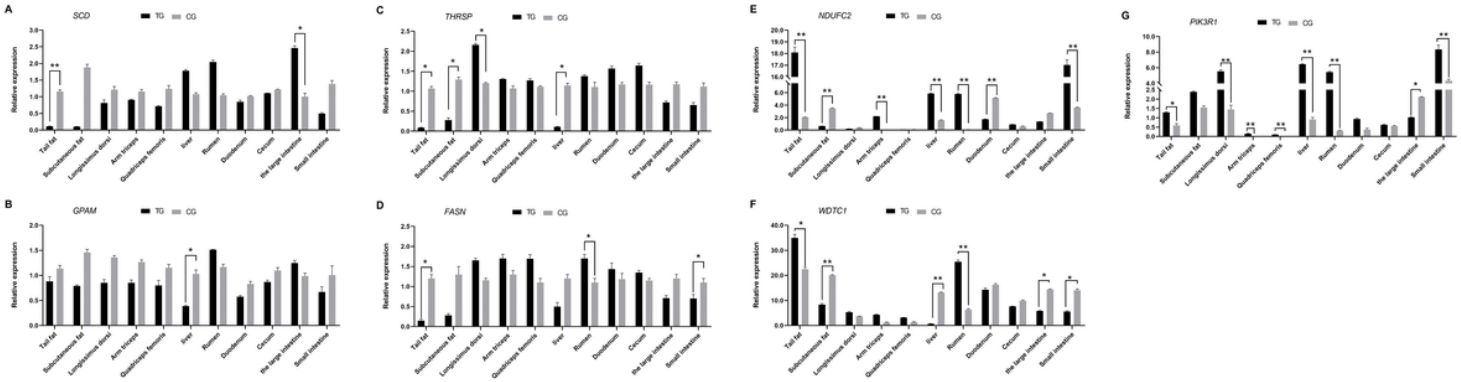

Figure 8

Relative expression differences of target genes in Bashby sheep (CG) and F2 (TG). Data were analyzed using the one-way measures analysis of variance (ANOVA) and least significant difference method (LSD). Data are expressed as means ± SE. *P<0.05, **P<0.01

Supplementary Files

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