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Research article

Identification of lncRNAs expression and their regulatory networks associated with development and growth of skeletal muscle in sheep using RNA-Seq

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Abstract

Importance of the work: Sheep are a major source of mutton production. Therefore, recognizing the genes that influence muscle growth rates in sheep is useful in breeding programs.

Objectives: To identify the genes regulated by long non-coding RNAs (lncRNAs) that are effective in sheep skeletal muscle growth and development.

Materials & Methods: In total, 141,079,400 short reads for 12 muscle tissue (longissimus dorsal) samples of 6 young animals and 6 adults from domestic sheep were used as input for the FastQC and Trimmomatic software packages. Expression analysis was investigated using Cuffdiff. The ClueGO plug-in in Cytoscape was used for gene ontology. Novel lncRNAs were distinguished using Cuffcompare.

Results: Of the 82 lncRNA genes, 15 genes were up-regulated and 67 genes were down-regulated in adults compared with young animals and there was a higher expression of the lncRNA genes in the young than in the adult individuals based on cluster analysis. There were 26 genes of Gene Ontology classified as Biological Processes that significantly enriched the regulation of muscle development and differentiation. The findings showed that lncRNAs regulated both miRNAs and mRNAs that chiefly participate in the muscle growth and development in Texel sheep. Novel lncRNA transcripts were identified, such as TCONS_00063730, TCONS_00063387, TCONS_00063388 and TCONS_00033181, that had a strong correlation with mRNAs, while other novel lncRNA transcripts, such as TCONS_00040327, TCONS_00061518, TCONS_00064968 and TCONS_00033645, had greater correlation with miRNAs.

Main finding: New candidate regulator genes were identified for future molecular and genetic studies on sheep muscle growth and development. This might be helpful in selection programs to improve mutton production.

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Introduction

The domestic sheep (*Ovis aries*) is one of the most important domestic animals that is used mainly for meat (Hamadalahmad et al., 2020). Currently, researchers concentrate on the molecular mechanisms to improve the skeletal muscle in sheep, which is of vital importance to enhance the meat production of existing sheep breeds (Betti et al., 2022). It is necessary to recognize genes that influence muscle growth rates in sheep because sheep are an important species especially for mutton production and they are distributed broadly as domestic animals (Li et al., 2020; Mohammadi et al., 2018). The homeland of Texel sheep is in the Netherlands on Texel Island. The production systems of this breed have transferred from an initial concentration on wool to now being mainly for meat production in Australia and New Zealand (Kinka and Young, 2019; Huang et al., 2020).

Transcriptome studies in mammalian species in the large majority have resulted in the identification of various types of non-coding RNAs of which long non-coding RNAs (lncRNAs) are the more abundant (approximately 75% of the genome) and are also a functionally versatile category; they participate in essential functions by regulating differentiation, maintenance development in cell identification and disease (Neguembor et al., 2014). The most plentiful ncRNA families which are more than 200 nucleotides in length include: PIWI-interacting RNA (piRNA) (Itou et al., 2015; Lim et al., 2015), microRNA (miRNA) (Bhin et al., 2015; Grossman and Shalgi, 2016), small interfering RNA (siRNA)(Hilz et al., 2016). Studies have shown lncRNAs as a novel regulatory molecule are shared in mammalian evolution (Ulitsky and Bartel, 2013).

Progress in RNA sequencing technologies has exposed the complication of genomes. Non-coding RNAs form the majority (98%) of the transcriptome, and some significant functions of regulatory RNA have been discovered (Zhu et al., 2017). Understanding the mechanism of the non-coding RNAs action is one of the most important challenges facing biology today. LncRNAs can be categorized into diverse subtypes (Antisense, Intergenic, Overlapping, Intronic, Bidirectional and Processed) according to the direction and location of transcription to other genes (Peschansky and Wahlestedt, 2014; Mattick and Rinn, 2015). Many lncRNAs are expressed in a tissue-specific pattern, with more variation between tissues than protein-coding genes (Derrien et al., 2012). It was believed that the lncRNAs had no ability for coding because of their deficiency in optimal open reading frames. Still, more recent researches have revealed that lncRNAs could encode a few peptides because the lncRNAs had small open reading frames; therefore, encoding a few peptides can have crucially vital functions (Anderson et al., 2016; Nelson et al., 2016).

More recently, lncRNAs have been identified as being involved in muscle differentiation as crucial regulators. For example, Linc-RAM (long intergenic non-protein coding RNA activator of myogenesis), Lnc-mg (myogenesis-associated lncRNA), and Linc-MD1 (long intergenic non-protein coding RNA, muscle differentiation 1) are involved in myogenesis (Zhu et al., 2017). LncRNA-Six1 can stimulate cell proliferation, which contributes to muscle growth (Cai et al., 2017). Other study revealed the lncRNA transcripts expressed in the skeletal muscle of sheep (Chao et al., 2016). Sun et al. (2016) demonstrated that lncMD (muscle-specific lncRNA) competes with endogenous RNA (ceRNA) to boost muscle differentiation.

LncRNA targeting genes participate in the signaling paths associated with the development and growth of muscle in sheep based on Gene Ontology (GO) and KEGG enrichment analysis (Li et al., 2019; Yuan et al., 2020). The sheep is one of the most important livestock animals used mainly for mutton production (Aljubouri et al., 2020), with growing biological evidence referring to lncRNA roles in muscle development and differentiation. However, to date, only some novel lncRNAs with their targeted genes have been distinguished in detail, such as miRNAs and mRNAs. Therefore, the current study aimed to identify the role of novel lncRNAs and the target genes (miRNAs and mRNAs) regulated by lncRNAs that are effective in the growth and development of skeletal muscle tissue in Texel sheep. The knowledge created from such identification could be useful in future research exploring the lncRNA functions in the longissimus dorsal muscle of sheep. It may also inform the regulation of miRNA and mRNA genes, which participate in critical roles in muscle growth and development by binding their sites. Such work would lay the basis for animal breeding and genetic policies to enhance the growth and development of meat production in sheep.

Materials and Methods

Data collection and samples

The RNA sequences data (paired-end) used in the analysis were retrieved from the Ensemble database (Clark et al., 2017) with accession numbers ERR4891 and ERR4892. The sheep reference genome (Oar_v3.1) and gene model annotation GTF (Oar_v3.1.96) files were downloaded from the Ensemble database. The sample consisted of muscle tissues (longissimus

dorsal) from six juveniles (aged 8–9 mth) and six adult Texel sheep (Clark et al., 2017). Tissues were preserved in RNAlater and kept at -80°C, with the RNA extraction based on the TRizol protocol explained in detail by Clark et al. (2017).

RNA sequences data analysis

The total RNA raw reads produced 141,079,400 pieces of data. Sequencing used the Illumina HiSeq 2500 platform with (2 × 151 bp) paired-end reads. The RNA raw reads were submitted to quality control using the FastQC version 0.11.5 software (Andrews, 2010). Reads containing adapters, bases below quality and low-quality reads were trimmed from the raw data using the Trimmomatic version 0.36 software (Bolger et al., 2014) Finally, reads were kept with 20 as a minimum Phred quality score and a minimum length of 36 bp.

LncRNA identification pipeline

The pipeline in Fig. S1 was used to identify the lncRNAs. The first step generated the index sheep reference genome (Oar_v3.1) using the command hisat2-build and the paired-end clean reads aligned to the sheep reference genome (Oar_v3.1) using the Hisat2 software (Kim et al., 2015). The mapping reads were from the per library assembly with the Cufflinks software (v2.2.1.OSX_x86_64; http://cufflinks.cbcb.umd.edu; Trapnell et al., 2010). All assemblies for samples were merged to generate an assembly GTF file using the Cuffmerge software (Trapnell et al., 2012). Expression analysis used the Cuffdiff version 2.2.1 software (Trapnell et al., 2010) to identify differentially expressed genes (DEGs) between the two groups (six samples of adult sheep and six samples of young sheep).

Genes with a false discovery rate (FDR) ≤ 0.05 were considered as DEGs. GO analysis was performed using the Cytoscape version 3.7.2 software with the ClueGO plug-in (Bindea et al., 2009) for determining the function of the lncRNA genes that might participate in common biological responses or possess related functions. Enriched GO terms were tested for significance at $p < 0.05$ (Saedi et al., 2022). Heatmap analysis (Babicki et al., 2016) was performed to visualize the differences in the expression level of the lncRNA genes in the longissimus dorsal muscle tissue at different developmental stages. A volcano plot using the ggplot2 package in the R program (R Core team, 2021) was used for the visual identification of up-regulated and down-regulated DE lncRNA genes (based on log2 fold-change ≥ 1.0; $p < 0.05$). The second step used the Cuffcompare version 2.2.1 software (Trapnell et al., 2010) to select transcripts with "i" (intronic transcripts), "u" (intergenic transcripts), and "x" (antisense transcripts) classes, depending on the reference transcriptome. Filtering the pipeline to identify the potential candidate transcripts from all assembling transcripts used the criteria: 1) the transcripts with one exon number and length ≥ 200 bp were kept (Ji et al., 2020); and 2) the three tools applied to predict the coding potential for all transcripts were: Coding Potential Calculator 2 (CPC2; score ≥ 0.5; Kang et al., 2017), Coding-Potential Assessment Tool (CPAT; score ≥ 0.36; Wang et al., 2013) and Coding-Non-Coding Identifying Tool (CNIT; score > 0 indicates coding RNA, score < 0 indicates non-coding RNA; Guo et al., 2019). The predicted transcripts that were potentially coding, were removed using any of the three tools above, and those predicted as non-coding potential were considered as candidate lncRNAs.

3. A novel and known lncRNA was distinguished from candidate lncRNAs using the NONCODE version 5 databases (http://www.noncode.org/; Fang et al., 2018) for an integrated knowledge database dedicated to ncRNAs, especially lncRNAs.

Target prediction of candidate lncRNAs

The lncRNA structures (secondary and tertiary) also play an important role in the mechanism of their actions by making binding locations for other bio-molecules such as DNA/RNA/proteins. Additionally, lncRNA can interact with miRNA, which participates in the post-translational process by acting as a decoy or sponge and thus maintains the stability of the mRNA (Ramakrishnaiah et al., 2020). Interactions were performed between the lncRNAs–miRNAs and lncRNAs–mRNAs that are present only in the skeletal muscle of sheep, to explore the target genes that may be regulated by the candidate lncRNAs. The lncRNAs can interact with miRNAs to control gene expression by the miRNA sponge mechanism (Johnsson et al., 2013). Therefore, the determination of miRNA target locations on lncRNAs would provide evidence for lncRNA functional research. Interactions between lncRNAs–miRNAs were executed using the database of lncRNASNP2 (Miao et al., 2018), which produced miRNA genes intersected using the Pita, TargetScan and Miranda tools and a free base binding energy < -20, with a score ≥ 150. To reduce the occurrence of positive false results, the final targeted miRNAs in the lncRNASNP2 database were intersected with the results obtained from tools such as the miRanda, TargetScan, and Pita (Miao et al., 2018).

Interaction between the lncRNAs-mRNAs using the RIsearch software (Wenzel et al., 2012) was used to predict the binding locations between lncRNAs-mRNAs with free energy values no more than -50 (Yuan et al., 2020).

Construction of regulatory networks

The regulatory networks (lncRNAs-regulated) for interaction between lncRNAs-mRNAs and lncRNAs-miRNAs were constructed for visualizing and integrating the networks using the Cytoscape software version 3.7.2 (Shannon et al., 2003).

Results

RNA sequencing and mapping

For identification of the lncRNAs in addition to probable functional lncRNAs, which may be involved in the sheep skeletal muscle tissue growth and development, 12 samples from young and adult muscle tissue (longissimus dorsal) were sequenced using Illumina HiSeq 2500. To recognize the samples from the various sequencings, the six young Texel sheep were identified from ERR489116_1_fastqc to ERR489121_2_fastqc as C1, C3, C5, C7, C9 and C11, while the six adults were identified as two adult Texel sheep from ERR489188_1_fastqc to ERR489189_2_fastqc as C13 and C15 and four adult Texel sheep from ERR489242_1_fastqc to ERR489245_2_fastqc as C17, C19, C21 and C23.

Six samples from the longissimus dorsal muscle tissue of juveniles (8–9 mth), namely C1, C3, C5, C7, C9 and C11 and six samples (C13, C15, C17, C19, C21 and C23) for the adult individuals were used to identify the DE lncRNAs between the two different physiological stages. Before trimming, the total RNA sequencing obtained 141,079,400 short reads with the young and adult stages averaging 7,1664,033 and 69,415,367 short reads, respectively. After trimming the total RNA, the raw data consisted of 103,922,274 and then removing low-quality reads was reduced to 37,157,126. On average, 51% of the clean reads were aligned exactly to the sheep reference genome, while on average, overall alignment rate was 91.5% for clean reads. The GC percentage was more than 44% (Table S1).

Differentially expressed lncRNAs in sheep muscle tissue

In total, 260 lncRNA genes were significantly differentially expressed between the young and adult individuals. All genes with FDR < 0.05 were considered as DEGs. Among them, there were 82 lncRNA genes (15 genes up-regulated and 67 genes down-regulated in adults compared with young, based on log2 fold-change≥1.0; $p < 0.05$), as shown in Fig .1A and Table S3. Cluster analysis was performed using Heatmap (Babicki et al., 2016) to visualize the differences in the expression level in the lncRNA genes in the longissimus dorsal muscle at different developmental stages.

The results demonstrated that the styles of different lncRNA expression were correlated with the young and adult stages and in Fig. 1B, there was higher expression of the lncRNA genes in the young than in the adult individuals using cluster analysis (based on FPKM for 82 of the expressed genes). Among the top 10, the expressed lncRNAs were most abundant in the longissimus dorsal muscle tissue (Table 1). These findings may assist in identifying DE lncRNAs for particular periods in the growth and development of the skeletal muscle in sheep.

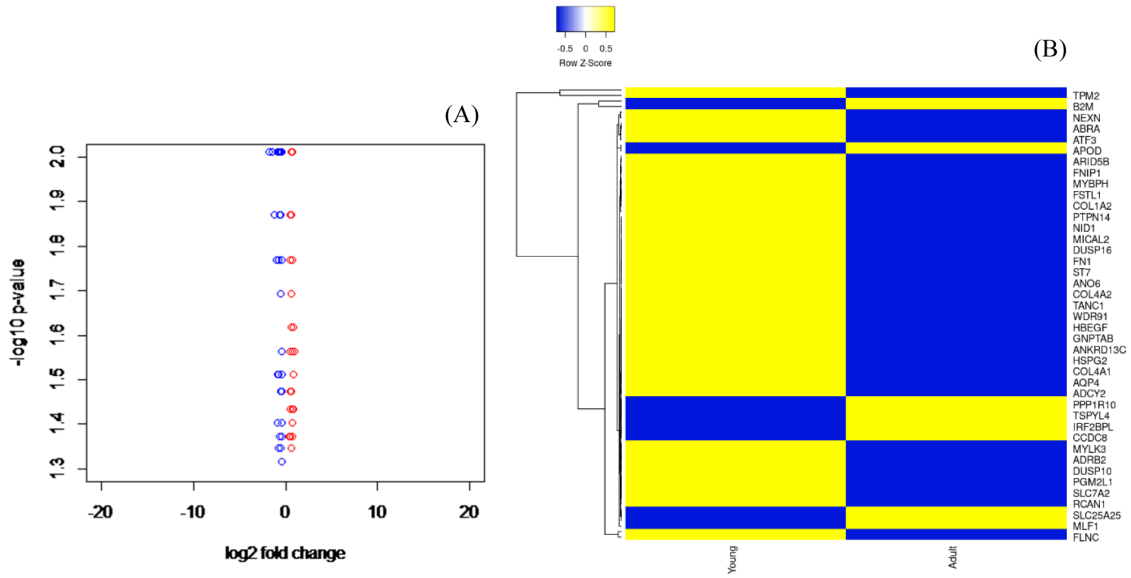

Fig. 1 Analysis of differentially expressed (DE) lncRNA genes: (A) volcano plot of 82 DE lncRNA genes in young and adult individuals; (B) heatmap for clustering analysis of 82 DE lncRNA genes in young and adult individuals, where blue and red circles denote down- and up-regulated expression, respectively and yellow in the heatmap refers to the higher expression of genes in young compared to adult individuals

Table 1 Top 10 expressed lncRNAs most abundant in skeletal muscle from adult and young Texel sheep

No.	LncRNA gene	Gene_id	Chromosome locus	Log2 (fold-change ≥1.0)	Adjusted p ($p < 0.05$)
1	APOD	XLOC_000970	1:190322030-190335306	1.3656	0.00518531
2	C7	XLOC_012175	16:33481055-33542068	1.63463	0.00518531
3	DDIT4	XLOC_023295	25:28192240-28194468	1.69527	0.00518531
4	TSPYL4	XLOC_033990	8:21477848-21482100	1.70352	0.00518531
5	PVALB	XLOC_026714	3:180006100-180067626	2.36872	0.005185
6	ZC3H12C	XLOC_010487	15:19647455-19734973	-1.0246	0.00518531
7	SIK3	XLOC_011063	15:27001572-27122508	-1.01641	0.00518531
8	FLNC	XLOC_028293	4:92858801-92887441	-1.01271	0.00518531
9	MMP2	XLOC_008916	14:23102468-23341508	-1.00391	0.016986755
10	SPARC	XLOC_030802	5:60404914-60427915	-1.00261	0.00518531

GO enrichment analysis

GO analysis of the DE lncRNA genes was performed to explore their functions using the ClueGO plug-in in the Cytoscape software version 2.7.2 software (Bindea et al., 2009). In total, 25 GO terms significantly enriched, with 11 of these terms associated with biological processes (BPs), such as regulation of the muscle system process, muscle organ development, striated muscle cell differentiation, striated muscle cell development and muscle tissue development, while others were associated with cellular components (CC), such as contractile fiber, sarcomere and basement membrane.

RNA polymerase II regulatory region DNA binding, transcription regulatory region sequence-specific DNA binding and RNA polymerase II regulatory region sequence-specific DNA binding were associated with molecular functions (MFs). In total, 26 genes of the GOs were classified as BP, of which 2 genes (FOS in GO:0007517 and GO:0060537) were up-regulated in muscle organ and tissue development. In comparison, 24 genes were down-regulated, 9 genes as CC were down-regulated and 18 genes as molecular functions (MFs) among them 6 genes (FOS, HES1 in each of GO:0001012, GO:0000976 and GO:0000977) were up-regulated, while the remaining 12 genes were down-regulated. The significantly enriched GO terms are shown in Table 2.

Table 2 GO enrichment analysis of differentially expressed lncRNAs

GO term		Number of genes	Gene name	Regulated direction	p-Value ($p < 0.05$)
Biological process					
GO:0090257	regulation of muscle system process	2	TMEM38B	Down	0.04
			ADRB2	Down	
GO:0007517	muscle organ development	8	ARID5B	Down	
			ANKRD1	Down	0.01
			COL3A1	Down	
			FOS	Up	
			PI16	Down	
			RCAN1	Down	
			ATF3	Down	
			XIRP2	Down	
GO:0051146	striated muscle cell differentiation	5	FLNC	Down	0.01
			MYLK3	Down	
			PI16	Down	
			RCAN1	Down	
			TANC1	Down	
GO:0055002	striated muscle cell development	4	ALPK2	Down	0.01
			MYLK3	Down	
			PI16	Down	
			RCAN1	Down	

Table 2 Continued

GO term		Number of genes	Gene name	Regulated direction	*p*-Value ($p < 0.05$)
GO:0060537	muscle tissue development	7	ALPK2	Down	0.04
			ATF3	Down	
			COL3A1	Down	
			FOS	Up	
			PI16	Down	
			RCAN1	Down	
			XIRP2	Down	
Cellular component					
GO:0043292	contractile fiber	3	ABRA	Down	0.01
			MMP2	Down	
			XIRP2	Down	
GO:0030017	sarcomere	2	XIRP2	Down	0.04
			MMP2	Down	
GO:0005604	basement membrane	4	ANXA2	Down	0.02
			COL4A1	Down	
			FN1	Down	
			NID1	Down	
Molecular function					
GO:0001012	RNA polymerase II regulatory region DNA binding	6	ARID5B	Down	0.03
			ATF3	Down	
			FOS	Up	
			HES1	Up	
			KLF4	Down	
			KLF6	Down	
GO:0000976	transcription regulatory region sequence-specific DNA binding	6	ARID5B	Down	0.03
			ATF3	Down	
			FOS	Up	
			HES1	Up	
			KLF4	Down	
			KLF6	Down	
GO:0000977	RNA polymerase II regulatory region sequence-specific DNA binding	6	ATF3	Down	0.03
			ARID5B	Down	
			FOS	Up	
			HES1	Up	
			KLF4	Down	
			KLF6	Down	

Identification of lncRNAs in sheep skeletal muscle

To identify the candidate lncRNA transcripts in sheep skeletal muscle tissue in the young and adult samples, first, 69,790 transcripts were filtered with only classes u, i, x using the Cuffcompare software. Then 15,841 of the remaining transcripts were categorized with 14,203 transcripts as class code u (intergenic), 72 transcripts as class code i (intronic) and 1,566 transcripts as class code x (antisense).

Around 75% of the assembled transcripts were removed by the selection classes u, i and x. The remaining 15,841 transcripts were filtered to a length ≥ 200bp and exon number ≥1 that resulted in the removal of 1,310 transcripts (Ji et al., 2020). After completing the length filter, 1,4531transcripts were submitted for filter coding potential using CPC2, CPAT and CNIT (Table 3). This produced 994 non-coding transcripts. Then, these transcripts were distinguished between the novel and known lncRNAs using the NONCODE version 5 databases (Fang et al., 2018). Finally, 466 known and 528 novel transcripts were obtained.

Table 3 Coding potential using Coding Potential Calculator 2 (CPC2), Coding-Potential Assessment Tool (CPAT) and Coding-Non-Coding Identifying Tool (CNIT)

Tools	Total coding/ non-coding transcripts	Noncoding	Coding	Cutoff	Class-code
CPAT	1,312	1,190	122	Coding ≥ 0.364 Noncoding < 0.364	Intergenic = 942 (75 coding +867 noncoding) Antisense = 353 (47 coding + 306 noncoding) Intronic = 17 (0 coding +17 non-coding)
CPC2	1,318	1,149	169	Coding ≥ 0.5 Noncoding < 0.5	Intergenic = 944 (116 coding +828 noncoding) Antisense = 355 (53 coding +302 noncoding) Intronic = 19 (0 coding +19 noncoding)
CNIT	1,293	1,105	188	Coding > 0 Noncoding < 0	Intergenic = 924 (133 coding +791 noncoding) Antisense = 351 (53 coding +298 noncoding) Intronic = 18 (2 coding +16 noncoding)

LncRNAs features

The LncRNA transcripts features were screened for the number of chromosomes, the number of exons and the length of transcripts. (Fig. 2). The results showed that most of the lncRNA transcripts were distributed on chromosomes 3 and 1. The lncRNA transcripts were divided into all Texel sheep chromosomes but did not appear on the Y chromosome (Fig. 2C). Furthermore, most lncRNA transcripts had one and two exons per transcript. The transcripts with only one exon were more frequent than the transcripts that with two exons (Fig. 2B). The lengths of lncRNA transcripts were in the range 200–20,00 bp (Fig. 2A). The results demonstrated that the number of intergenic lncRNA transcripts was more than the antisense and intronic transcripts. Furthermore, the number of novel lncRNA transcripts was more than the number of known lncRNA transcripts (Fig. 2D).

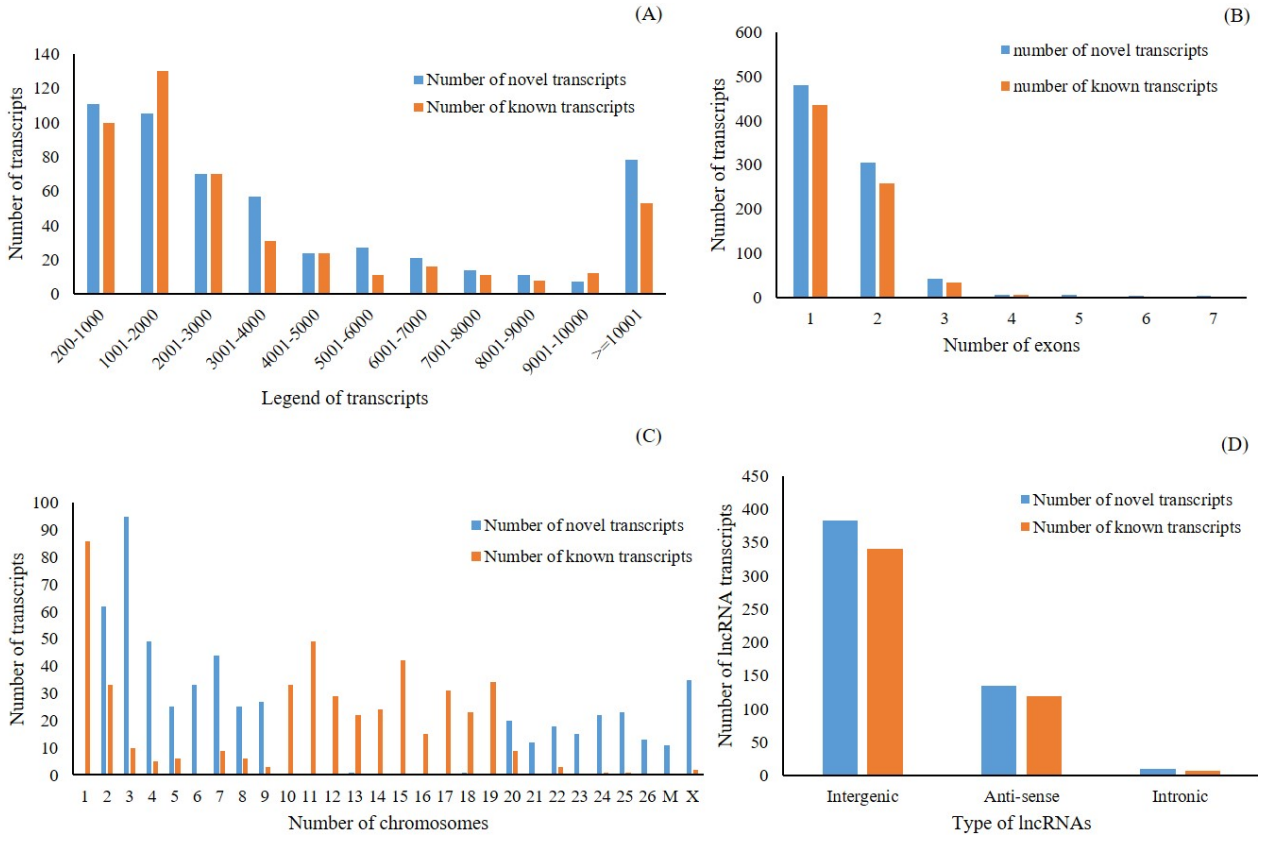

Fig. 2 LncRNA transcripts features: (A) lengths for novel and known transcripts; (B) number of exons for novel and known transcripts; (C) chromosomes for novel and known transcripts; (D) type of lncRNAs for novel and known transcripts

Target prediction of candidate lncRNAs

LncRNAs-mRNAs interaction prediction

To predict the binding locations between the lncRNAs and protein-coding genes, 994 sequences of known and novel lncRNA transcripts (query) were interacted with 1,0921 mRNAs detected in sheep skeletal muscle (target) using the RIsearch software (RNA interaction search; Wenzel et al., 2012) under a free energy of no more than -50. The results showed that only 474 from 994 lncRNAs had 1,537 targets of mRNA. Among them, 262 and 212 lncRNA sequences were novel and known lncRNAs, respectively. TCONS_00063730, TCONS_00063387 and TCONS_00063388 for novel intergenic lncRNAs and TCONS_00033181 for novel antisense lncRNAs had strong correlations with mRNAs, while only TCONS_00060832 and TCONS_00060833 for known antisense lncRNAs had strong correlations with mRNAs.

Interaction prediction of LncRNAs-miRNAs

To predict miRNA targets on lncRNA sequences, 994 known and novel lncRNA transcripts were submitted to the lncRNASNP2 database to explore the potential function of the lncRNA transcripts on miRNA binding.

The results indicated that only 116 lncRNA transcripts were bound with 125 miRNAs under a cutoff energy < -20 (score ≥ 150). Among them, 55 and 61 lncRNA transcripts were novel and known lncRNAs, respectively. Furthermore, the results were filtered using the miRNAs data detected in sheep skeletal muscle by Zhang et al., (2013). All 116 lncRNA transcripts had a relationship with 125 miRNAs, but TCONS_00040327, TCONS_00061518 and TCONS_00064968, and TCONS_00012728, TCONS_00036070 and TCONS_00043102 for novel and known intergenic lncRNA transcripts, respectively, had greater correlation with miRNAs detected in sheep skeletal muscle. In addition, found TCONS_00033645 and TCONS_00052875 for novel and known antisense lncRNA transcripts, respectively, had greater correlation with miRNAs detected in sheep skeletal muscle. Furthermore, the lncRNA transcripts targeted miRNAs, such as oar-miR-22, oar-miR-29, oar-miR-493, oar-miR-432 and oar-miR-433, that were mentioned in other studies, were differentially expressed after birth and during the embryonic stages, respectively (Yuan et al., 2020).

LncRNAs-mRNAs interaction network construction

Regulatory interaction networks were constructed between 200 novel lncRNA transcripts and their target genes (mRNAs) using the Cytoscape version 3.7.2 software (Shannon et al., 2003), as shown in Fig. 3. The network results showed that among the 200 novel lncRNA transcripts, TCONS_00048535, TCONS_00061466, TCONS_00047958 and TCONS_00053067 had more relationships with 8, 7, 7 and 7 protein-coding genes, respectively, compared to the other lncRNA transcripts (Table S6). Protein-coding genes, such as the MIS18BP1 and TAF9B genes, were strongly associated with 38 and 37 novel lncRNA transcripts, respectively (Table S6).

Network construction of lncRNAs-miRNAs interactions

Only 55 novel lncRNA transcripts (intergenic, antisense and intronic) targeting 59 miRNAs were selected. The regulatory interaction networks were constructed between 55 novel lncRNA transcripts and their target genes (miRNAs) using the Cytoscape version 3.7.2 software (Shannon et al., 2003), as shown in Fig. 4. The network results showed that among the 55 novel lncRNA transcripts, TCONS_00040327, TCONS_00061518, TCONS_00064968 and TCONS_00033645 had more associations with 2, 2, 2 and 2 miRNAs, respectively, detected in sheep skeletal muscle compared to other novel lncRNA transcripts (Tables S7 and S8). In addition, miRNAs detected in sheep skeletal muscle, such as oar-miR-127 and oar-miR-432, had a strong association with 7 and 7 novel lncRNA transcripts, respectively, compared to other miRNAs (Tables S7 and S8).

Discussion

Many studies have shown that lncRNAs could be critical as regulatory factors in the growth of muscle (Legnini et al., 2014; Ballarino et al., 2015; Mueller et al., 2015; Wang et al., 2015). The critical roles of lncRNAs in numerous vital biological processes and diseases, such as cancer (Weikard et al., 2017; Saliani et al., 2021), are the subject of current research. Rapid advances in modern techniques for RNA sequencing have helped to discover many lncRNAs in organisms such as humans and mice; however, studies on lncRNAs in sheep, especially, are very few (Bakhtiarizadeh et al., 2016). Therefore, databases are available for most organisms except sheep. A search for lncRNAs in the NONCODE database, identified 17 species, excluding sheep (Zhao et al., 2016). In the current study, RNA-Seq technology was applied to detect novel lncRNAs and their regulatory functions associated with the growth and development of skeletal muscle in Texel sheep.

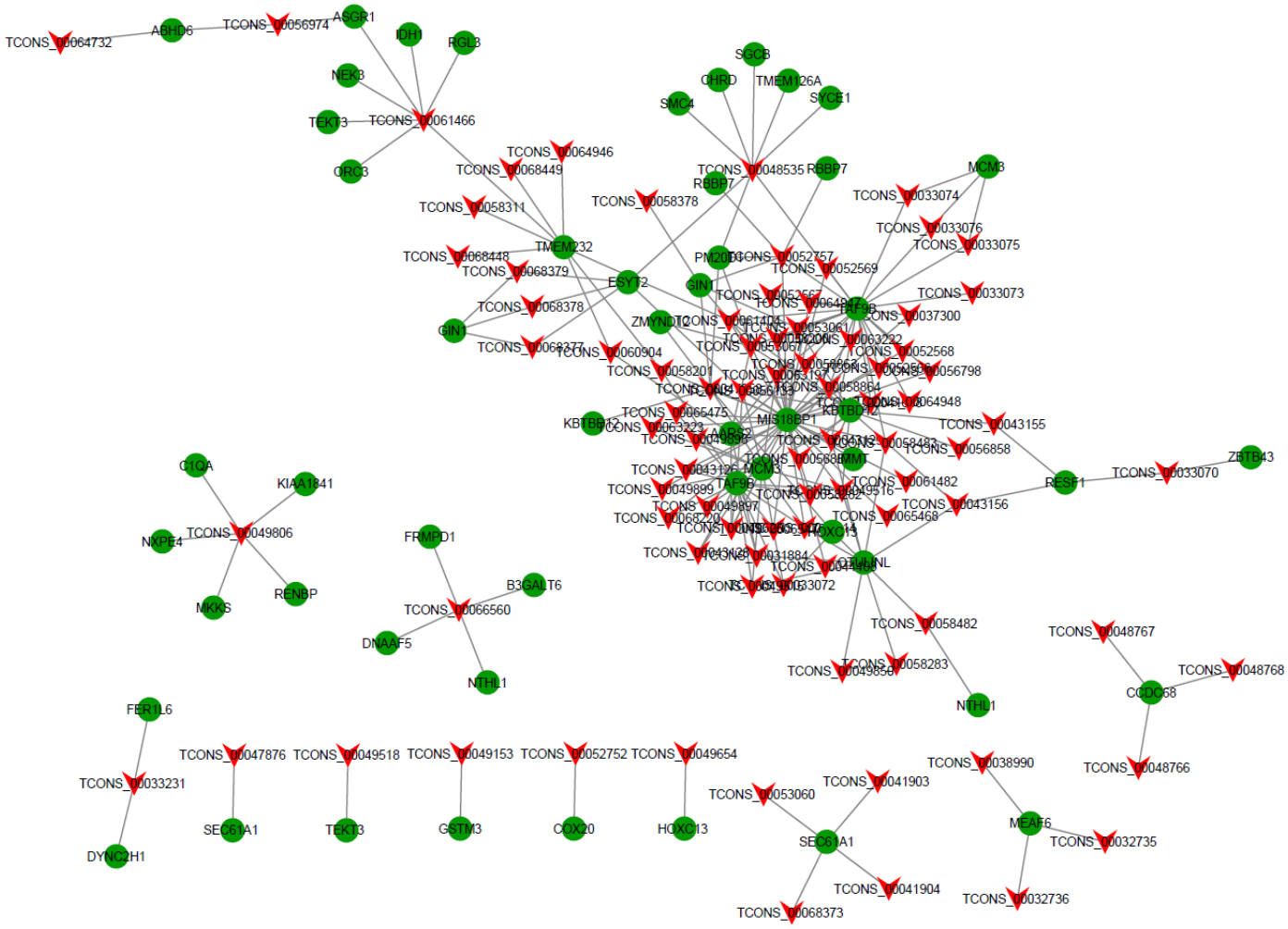

Fig. 3 Interaction network of lncRNA-mRNA in sheep skeletal muscle tissue, where red triangles and green circles represent lncRNA and mRNAs, respectively and relationships between two target nodes are shown as solid lines

Based on the current literature, this was the first study identifying the expression of lncRNAs and their regulatory networks associated with the development and growth of skeletal muscle at different stages in Texel sheep using the RNA-Seq method. After merging all transcript files using the Cuffmerege software, a merged output file was produced as the input for the Cuffdiff software to identify DEGs between the two groups of adult and young skeletal muscle. From the 260 significantly differentially expressed lncRNA genes, 82 lncRNA genes were identified, with 15 being up-regulated in adults compared to young sheep and 67 being down-regulated (log2 fold-change ≥1.0; $p < 0.05$), with a higher expression of the lncRNA genes in the young than in the adult individuals (Table S2). The GO analysis revealed that 25 GO terms were significantly enriched ($p < 0.05$); among them, 5 were associated with biological processes (BPs), such as regulation of muscle system process, muscle organ development, striated muscle cell differentiation, striated muscle cell development and muscle tissue development. These findings supported the vital role of DE lncRNAs in the growth and development of Texel sheep skeletal muscle. In total, 528 novel lncRNA transcripts were identified, consisting of 383 intergenic lncRNAs, 135 antisense lncRNAs and 10 intronic lncRNAs, using acceptable filtering criteria. The number of novel lncRNA transcripts was consistent with another study (Bakhtiarizadeh and Salami, 2019). The novel lncRNA transcripts in the current study shared many features with other mammalian lncRNAs, such as having few exon numbers and a large exon size (Bakhtiarizadeh et al., 2016; Palmieri et al., 2017). These results showed that the pipeline used in the current study to identify the lncRNA transcripts was dependable, and the findings could be used as a reasonable basis for more analysis.

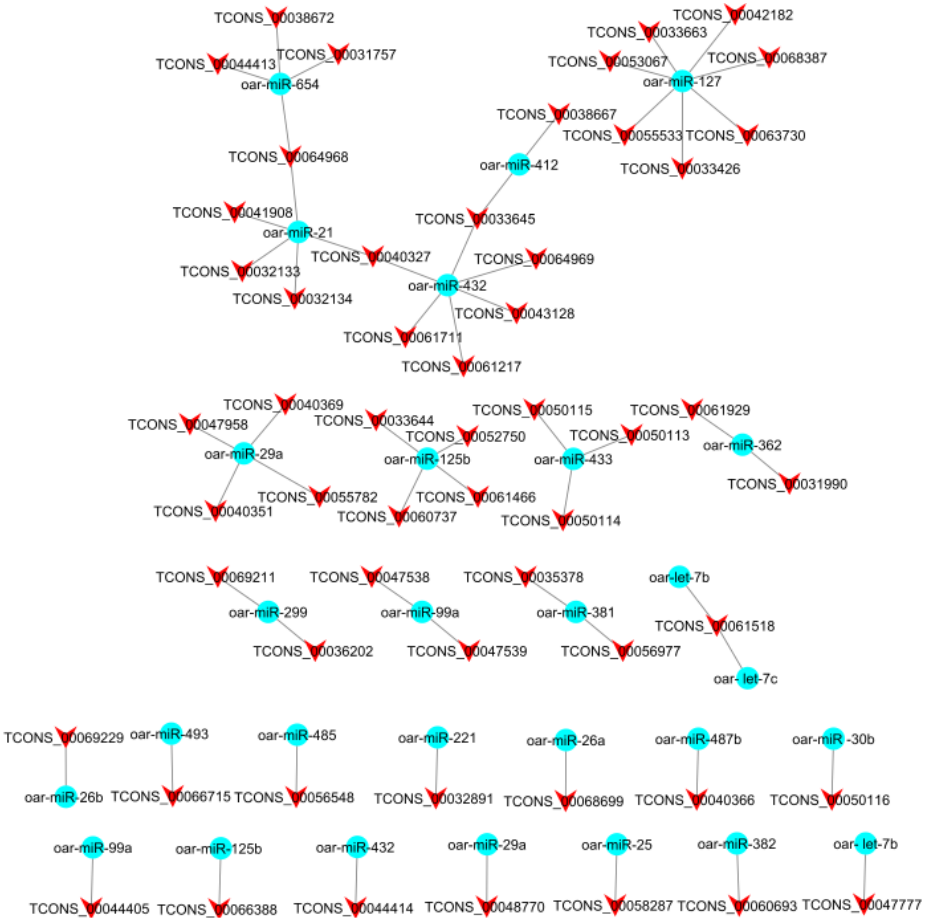

Fig. 4 Interaction network of lncRNA-miRNA in sheep skeletal muscle tissue, where red triangles and aquamarine circles represent lncRNAs and miRNAs, respectively and relationships between two target nodes are shown as solid lines

The increasing weight of published evidence suggests that lncRNAs, are crucial regulators of gene expression in differentiation, development and human illnesses. The regulatory mechanisms of lncRNAs can be classified into four main models: signals, decoys, scaffolds and guides. The cumulative evidence suggests that lncRNAs are capable of modulating almost every cellular process by their binding to proteins, mRNAs, miRNAs and/or DNAs (Li et al., 2019). Consequently, to predict the bounds of lncRNAs with sheep skeletal muscle mRNAs, the RIsearch software (Wenzel et al., 2012) was applied with a free energy of base binding of no more than -50, consistent with (Yuan et al., 2020). The findings revealed that 474 lncRNA transcripts were bound with 1,537 targets of sheep skeletal muscle mRNA. Among them, 262 lncRNA transcripts were novel lncRNAs, such as TCONS_00063730, TCONS_00063387 and TCONS_00063388 for novel intergenic lncRNAs, and TCONS_00033181 for novel antisense lncRNA, with all these having strong correlations with sheep skeletal muscle mRNAs. In addition, novel antisense lncRNA TCONS_00033181 targeted seven sheep skeletal muscle mRNAs (LRRFIP1, UBQLN4, ITSN1, CEP63, TINAGL1, SERPINE1, ECSIT), while the novel intergenic lncRNAs (TCONS_00063730, TCONS_00063387, TCONS_00063388) targeted 18 sheep skeletal muscle mRNAs (PPM1J, CHODL, EBPL, DPEP3, TNPO1, EDEM1, RBM15B, UBE2R2, SERINC2, TAF4B, GPATCH11, IMPDH1, ENSOARG00000018777, MCL1, ENSOARG00000009490, SIAE, ENSOARG00000004465, RIC8A) All these mRNAs were reported by Clark et al. (2017) as differentially expressed genes between the purebred Texel and hybrid T × BF (Texel × Scottish Blackface) in skeletal muscle. The complete information on the interaction between the novel lncRNAs and mRNAs is provided in Table S4.

The competitive endogenous RNA (ceRNA) hypothesis suggests that RNA transcripts, involving non-coding and coding RNAs, compete for post-translational regulation with shared miRNA binding positions (Salmena et al., 2011). Therefore, lncRNA can competitively bind some miRNAs,

which play critical roles in muscle growth and decrease the impact of regulation of miRNAs on their target genes and hence act in vital roles themselves in the growth of muscle (Wu et al., 2020). To forecast miRNA targets on lncRNAs sequences, the lncRNASNP2 database (Miao et al., 2018) was sued with a cutoff (energy < -20 ، score ≥ 150) consistent with (Jiang et al., 2020) to discover the potential function of the lncRNA transcripts on miRNA binding. The results demonstrated that 116 lncRNA transcripts had an association with 125 miRNA targets. Among them, 55 lncRNA transcripts were novel lncRNAs. Additionally, the results were filtered with miRNA data detected in sheep skeletal muscle (Zhang et al., 2013) and this identified novel intergenic lncRNAs (TCONS_00040327, TCONS_00061518, TCONS_00064968) and a novel antisense lncRNA (TCONS_00033645) that had additional relationships with miRNAs. For example, TCONS_00040327 was associated with oar-miR-432 and oar-miR-21.

Yuan et al. (2020) reported high expression was abundant in miR-432 during the embryo stage of skeletal muscle in fetal sheep. Another study reported that oar-miR-432 inhibited myoblast differentiation and proliferation and could inhibit myogenesis by targeted P55PIK and E2F3 genes using the signaling pathway PI3K/AKT/mTOR (Ma et al., 2017). These miRNAs (miR-21, miR-155, miR-143, miR-221, miR-23a) are involved in the proliferation, development and/or differentiation of skeletal muscles (Fatima and Morris, 2013; Zhang et al., 2017). Kaur et al. (2020) revealed that miR-21 was highly differentially expressed (log2 FC = 4) in the Bandur breed and was associated with sheep muscle growth and development. They reported that TCONS_00061518 was associated with oar-let-7b and oar- let-7c. The family of let-7 miRNAs includes one of the vital regulatory factors in the developmental process (Zhang et al., 2013). TCONS_00064968 is associated with oar-miR-21 and oar-miR-654, while miRNA-21 is a new myogenic involved in the skeletal muscle development of pigs and targeting the TGFβI gene can modulate PI3K/Akt/mTOR signaling (Bai et al., 2015). Zhang et al. (2021) noticed that miR-21-5p stimulated the proliferation and differentiation of SMSCs in chicken by targeting KLF3.

Notably, miRNA-21 targeted mRNA (MEF2C) associated with sheep skeletal muscle (Liu et al., 2019). TCONS_00033645 is associated with oar-miR-412 and oar-miR-432. The current results identified novel intergenic TCONS_00035378 and TCONS_00056977 binding with oar-miR-381, as reported in another study that targeted multiple muscle-related mRNAs, such as MEF2C, IGF2 and MBNL1 (Liu et al., 2019), with oar-miR-381-3p being one of the most abundant miRNAs found in sheep skeletal muscle at different developmental stages (Zhao et al., 2016). The current study identified that novel intergenic TCONS_00033426, TCONS_00042182 and TCONS_00063730, and novel antisense TCONS_00033663, TCONS_00053067 and TCONS_00055533 targeted oar-miR-127. Another study showed that miR-127 had a higher expression level in the before-birth stage compared with the afterbirth stage, suggesting that miR-127 may play a vital role in embryonic myogenesis (Liu et al., 2019). Yuan et al. (2020) detected important upregulations of highly differentially expressed miRNAs, such as miR-136 and miR-127, in the skeletal muscle of embryonic sheep. Zhai et al. (2017) found that miR-127 was mainly expressed in the tissue of skeletal muscle and that it was upregulated during the differentiation of satellite cell (SC) and C2C12. The current study detected different novel lncRNA that targeted the oar-miR-29a, such as TCONS_00047958, TCONS_00040369, TCONS_00048770, TCONS_00055782, TCONS_00040351. Other studies reported that miR-29a was involved in the development and growth of skeletal muscle (Galimov et al., 2016; Muluhngwi et al., 2017; Zhou et al., 2016).

Wu et al. (2020) showed that miR-29a might inhibit proliferation and differentiation in Hu sheep skeletal muscle satellite cells (SMSCs). In addition, the novel lncRNAs TCONS_00052750, TCONS_00060737, TCONS_00066388, TCONS_00033644 and TCONS_00061466 targeted oar-miR-125b, as mentioned by Kaur et al. (2020) were differentially expressed in Bandur sheep and was correlated with their muscle growth and development. Liu et al. (2019) demonstrated that miR-125b targeted many mRNAs associated with sheep skeletal muscle, such as MYEF2, MYBL2 and Sirt1. TCONS_00050114, TCONS_00050115 and TCONS_00050113 bound with oar-miR-433. Zhao et al. (2016) reported that oar-miR-433-3p was most abundant at different developmental stages in sheep skeletal muscle, whereas Yuan et al. (2020) discovered that miR-433 appeared as broad expression in the skeletal muscle of embryonic sheep. The detailed information of the interaction between novel lncRNAs and miRNAs is provided in Table S5. These results suggested that novel lncRNAs may play a vital role in the growth and development of skeletal muscle in Texel sheep.

Conclusion

In total, 82 DE lncRNA genes were identified with 15 up-regulated in adults compared with 67 down-regulated

(log2 fold-change≥1.0, P< 0.05) in young sheep, with a higher expression of the lncRNA genes in the young than in the adult individuals. The 82 DE lncRNA genes were submitted for GO analysis and among them, 26 were classified as biological processes (BPs) that were related to the regulation of muscle organs, cell, tissue development and differentiation.

The study detected novel lncRNA transcripts, such as TCONS_00063730, TCONS_00063387, TCONS_00063388 and TCONS_00033181, that had strong correlations with mRNAs, while other novel lncRNA transcripts, such as TCONS_00040327, TCONS_00061518, TCONS_00064968 and TCONS_00033645, had greater correlations with miRNAs detected in sheep skeletal muscle. This study should provide additional information on novel lncRNAs that can be applied in modulating both miRNAs and mRNAs, which play crucial roles in muscle growth and development, by interacting with their binding sites to help progress research into meat production processes.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Aljubouri, T.R.S., Al-Shuhaib, M.B.S., Javadmanesh, A. 2020. HMGA2 gene polymorphisms and their effects on main growth traits indices in Awassi and Karakul sheep. Agr. Nat. Resour. 54: 587-594. doi.org/10.34044/j.anres.2020.54.6.03

Anderson, D.M., Makarewich, C.A., Anderson, K.M., Shelton, J.M., Bezprozvannaya, S., Bassel-Duby, R., Olson, E.N. 2016. Widespread control of calcium signaling by a family of SERCA-inhibiting micropeptides. Sci. Signal. 9: ra119. doi.10.1126/scisignal.aaj1460

Andrews, S. 2010. FastQC: A quality control tool for high throughput sequence data. Cambridge, UK. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/, 10 April 2021.

Babicki, S., Arndt, D., Marcu, A., Liang, Y., Grant, J.R., Maciejewski, A., Wishart, D.S. 2016. Heatmapper: web-enabled heat mapping for all. Nucleic Acids Res. 44: W147–W153. doi.org/10.1093/nar/gkw419

Bai, L., Liang, R., Yang, Y., et al. 2015. MicroRNA-21 regulates PI3K/Akt/mTOR signaling by targeting TGFβI during skeletal muscle development in pigs. PloS One. 10: e0119396. doi.org/10.1371/journal.pone.0119396

Bakhtiarizadeh, M.R., Hosseinpour, B., Arefnezhad, B., Shamabadi, N., Salami, S.A. 2016. *In silico* prediction of long intergenic non-coding RNAs in sheep. Genome 59: 263–275. doi.org/10.1139/gen-2015-0141

Bakhtiarizadeh, M.R., Salami, S.A. 2019. Identification and expression analysis of long noncoding RNAs in fat-tail of sheep breeds. G3-Genes Genom. Genet. 9: 1263–1276. doi.org/10.1534/g3.118.201014

Ballarino, M., Cazzella, V., D'Andrea, D. et al. 2015. Novel long noncoding RNAs (lncRNAs) in myogenesis: A *miR-31* overlapping lncRNA transcript controls myoblast differentiation. Mol. Cell. Biol. 35: 728–736. doi.org/10.1128/MCB.01394-14

Betti, S., Tahmoorepur, M., Javadmanesh, A. 2022. Alternatively spliced novel lncRNAs and their target genes in ovine skeletal muscle, J Cell Mol Res, 14: -. doi:10.22067/jcmr.2022.74635.1027

Bhin, J., Jeong, H.-S., Kim, J.S., et al. 2015. PGC-enriched miRNAs control germ cell development. Mol. Cells. 38: 895–903. DOI:10.14348/molcells.2015.0146

Bindea, G., Mlecnik, B., Hackl, H., et al. 2009. ClueGO: A Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics 25: 1091–1093. doi.org/10.1093/bioinformatics/btp101

Bolger, A.M., Lohse, M., Usadel, B. 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30: 2114–2120. doi.org/10.1093/bioinformatics/btu170

Cai, B., Li, Z., Ma, M., Wang, Z., Han, P., Abdalla, B.A., Nie, Q., Zhang, X. 2017. LncRNA-Six1 encodes a micropeptide to activate *Six1* in *Cis* and is involved in cell proliferation and muscle growth. Front. Physiol. 8: 230. doi.org/10.3389/fphys.2017.00230

Chao, T., Wang, G., Wang, J., Liu, Z., Ji, Z., Hou, L., Zhang, C. 2016. Identification and classification of new transcripts in dorper and small-tailed Han sheep skeletal muscle transcriptomes. PloS One 11: e0159638. doi.org/10.1371/journal.pone.0159638

Clark, E.L., Bush, S.J., McCulloch, M.E., et al. 2017. A high resolution atlas of gene expression in the domestic sheep (*Ovis aries*). PLoS Genet. 13: e1006997. doi.org/10.1371/journal.pgen.1006997

Derrien, T., Johnson, R., Bussotti, G., et al. 2012. The GENCODE v7 catalog of human long noncoding RNAs: Analysis of their gene structure, evolution, and expression. Genome Res. 22: 1775–1789. doi.org/10.1101/gr.132159.111

Fang, S., Zhang, L., Guo, J., et al. 2018. NONCODEV5: A comprehensive annotation database for long non-coding RNAs. Nucleic Acids Res. 46: D308–D314. doi.org/10.1093/nar/gkx1107

Fatima, A., Morris, D.G. 2013. MicroRNAs in domestic livestock. Physiol. Genomics 45: 685–696. doi.org/10.1152/physiolgenomics.00009.2013

Galimov, A., Merry, T.L., Luca, E., et al. 2016. MicroRNA-29a in adult muscle stem cells controls skeletal muscle regeneration during injury and exercise downstream of fibroblast growth factor-2. Stem Cells 34: 768–780. doi.org/10.1002/stem.2281

Grossman, H., Shalgi, R. 2016. A role of microRNAs in cell differentiation during gonad development. In: Piprek, R. (Ed.). Molecular Mechanisms of Cell Differentiation in Gonad Development. Results and Problems in Cell Differentiation, Vol. 58. Springer. Cham, Switzerland, pp. 309–336. doi.org/10.1007/978-3-319-31973-5_12

Guo, J.-C., Fang, S.-S., Wu, Y., et al. 2019. CNIT: A fast and accurate web tool for identifying protein-coding and long non-coding transcripts based on intrinsic sequence composition. Nucleic Acids Res. 47: W516–W522. doi.org/10.1093/nar/gkz400

Hamadalahmad, A., Almeziad, M., Javadmanesh, A. 2020. Genetic similarity comparison between some Iranian and Middle Eastern sheep breeds using mitochondrial control region sequencing. Dysona Life Science 1: 20–24. doi: 10.30493/dls.2020.105085

Hilz, S., Modzelewski, A.J., Cohen, P.E., Grimson, A. 2016. The roles of microRNAs and siRNAs in mammalian spermatogenesis. Development 143: 3061–3073. doi.org/10.1242/dev.136721

Huang, Y., Wang, Y., Zhang, C., Sun, X. 2020. Biological functions of circRNAs and their progress in livestock and poultry. Reprod. Domest. Anim. 55: 1667–1677. doi.org/10.1111/rda.13816

Itou, D., Shiromoto, Y., Shin-ya, Y., et al. 2015. Induction of DNA methylation by artificial piRNA production in male germ cells. Curr. Biol. 25: 901–906. doi.org/10.1016/j.cub.2015.01.060

Ji, H., Niu, C., Zhan, X., et al. 2020. Identification, functional prediction, and key lncRNA verification of cold stress-related lncRNAs in rats liver. Sci. Rep. 10: 521. doi.org/10.1038/s41598-020-57451-7

Jiang, R., Wang, T., Zhou, F., Yao, Y., He, J., Xu, D. 2020. Bioinformatics-basedidentificationofmiRNA-,lncRNA-,andmRNA-associatedceRNA networks and potential biomarkers for preeclampsia. Medicine 99: e22985| dx.doi.org/10.1097/MD.0000000000022985

Johnsson, P., Ackley, A., Vidarsdottir, L., Lui, W.O., Corcoran, M., Grandér, D., Morris, K.V. 2013. A pseudogene long-noncoding-RNA network regulates PTEN transcription and translation in human cells. Nat. Struct. Mol. Biol. 20: 440–446. doi.org/10.1038/nsmb.2516

Kang, Y.-J., Yang, D.-C., Kong, L., Hou, M., Meng, Y.-Q., Wei, L., Gao, G. 2017. CPC2: A fast and accurate coding potential calculator based on sequence intrinsic features. Nucleic Acids Res. 45: W12–W16. doi.org/10.1093/nar/gkx428

Kaur, M., Kumar, A., Siddaraju, N.K., et al. 2020. Differential expression of miRNAs in skeletal muscles of Indian sheep with diverse carcass and muscle traits. Sci. Rep. 10: 16332. doi.org/10.1038/s41598-020-73071-7

Kim, D., Langmead, B., Salzberg, S.L. 2015. HISAT: A fast spliced aligner with low memory requirements. Nat. Methods. 12: 357–360. doi.org/10.1038/nmeth.3317

Kinka, D., Young, J.K. 2019. Evaluating domestic sheep survival with different breeds of livestock guardian dogs. Rangeland Ecol. Manag, 72: 923–932. doi.org/10.1016/j.rama.2019.07.002

Legnini, I., Morlando, M., Mangiavacchi, A., Fatica, A., Bozzoni, I. 2014. A feedforward regulatory loop between HuR and the long noncoding RNA linc-MD1 controls early phases of myogenesis. Mol. Cells. 53: 506–514. doi.org/10.1016/j.molcel.2013.12.012

Li, J., Jian, Y., Jia, L., et al. 2020. Molecular characterization of tick-borne bacteria and protozoans in yaks (*Bos grunniens*), Tibetan sheep (*Ovis aries*) and Bactrian camels (*Camelus bactrianus*) in the Qinghai-Tibetan Plateau Area, China. Ticks Tick-Borne Dis. 11: 101466. doi.org/10.1016/j.ttbdis.2020.101466

Li, Z., Zhao, W., Wang, M., Zhou, X. 2019. The role of long noncoding RNAs in gene expression regulation. In: Vlachakis, D. (Ed.). Gene Expression Profiling in Cancer. IntechOpen. London, UK, pp. 1–17.

Lim, S.L., Qu, Z.P., Kortschak, R.D., et al. 2015. HENMT1 and piRNA stability are required for adult male germ cell transposon repression and to define the spermatogenic program in the mouse. PLoS Genet. 11: e1005782. doi.org/10.1371/journal.pgen.1005620

Liu, Z., Li, C., Li, X., et al. 2019. Expression profiles of microRNAs in skeletal muscle of sheep by deep sequencing. Asian Austral. J. Anim. 32: 757–766. doi.org/10.5713/ajas.18.0473

Ma, M., Wang, X., Chen, X., Cai, R., Chen, F., Dong, W., Yang, G., Pang, W. 2017. MicroRNA-432 targeting *E2F3* and *P55PIK* inhibits myogenesis through PI3K/AKT/mTOR signaling pathway. RNA Biol. 14: 347–360. doi.org/10.1080/15476286.2017.1279786

Mattick, J.S., Rinn, J.L. 2015. Discovery and annotation of long noncoding RNAs. Nat. Structur. Mol. Biol. 22: 5–7. doi.org/10.1038/nsmb.2942

Miao, Y.-R., Liu, W., Zhang, Q., Guo, A.-Y. 2018. lncRNASNP2: An updated database of functional SNPs and mutations in human and mouse lncRNAs. Nucleic Acids Res. 46: D276–D280. doi.org/10.1093/nar/gkx1004

Mohammadi, F., Tahmoorespur, M., Javadmanesh, A. 2018. Study of differentially expressed genes, related pathways and gene networks in sheep fetal muscle tissue in thin- and fat-tailed breeds. Anim. Sci. J. (Pajouhesh & Sazandegi) 32: 301–312. doi.org/10.22092/asj.2018.122913.1749

Mueller, A.C., Cichewicz, M.A., Dey, B.K., Layer, R., Reon, B.J., Gagan, J.R., Dutta, A. 2015. MUNC, a long noncoding RNA that facilitates the function of MyoD in skeletal myogenesis. Mol. Cell. Biol. 35: 498–513. doi.org/10.1128/MCB.01079-14

Muluhngwi, P., Krishna, A., Vittitow, S.L., Napier, J.T., Richardson, K.M., Ellis, M., Mott, J.L., Klinge, C.M. 2017. Tamoxifen differentially regulates miR-29b-1 and miR-29a expression depending on endocrine-sensitivity in breast cancer cells. Cancer Lett. 388: 230–238. doi.org/10.1016/j.canlet.2016.12.007

Neguembor, M.V., Jothi, M., Gabellini, D. 2014. Long noncoding RNAs, emerging players in muscle differentiation and disease. Skeletal Muscle 4: 8. doi.org/10.1186/2044-5040-4-8

Nelson, B.R., Makarewich, C.A., Anderson, D.M., et al. 2016. A peptide encoded by a transcript annotated as long noncoding RNA enhances SERCA activity in muscle. Science 351: 271–275. doi.org/10.1126/science.aad4076

Palmieri, G., Paliogiannis, P., Sini, M.C., et al. 2017. Long non-coding RNA CASC2 in human cancer. Crit. Rev. Oncol. Hemat. 111: 31–38. doi.org/10.1016/j.critrevonc.2017.01.003

Peschansky, V.J., Wahlestedt, C. 2014. Non-coding RNAs as direct and indirect modulators of epigenetic regulation. Epigenetics 9: 3–12. doi.org/10.4161/epi.27473

Ramakrishnaiah, Y., Kuhlmann, L., Tyagi, S. 2020. Towards a comprehensive pipeline to identify and functionally annotate long noncoding RNA (lncRNA). Comput. Biol. Med. 127: 104028. doi.org/10.1016/j.compbiomed.2020.104028

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Saedi,N.,Aminafshar,M.,Chamani,M.,Honarvar,M.,Javadmanesh,A.2022. Expression pattern and network visualization of genes involved in milk persistency in bovine mammary tissue. Agr. Nat. Resour. 56: 23–34. doi.org/10.34044/j.anres.2021.56.1.03

Salmena, L., Poliseno, L., Tay, Y., Kats, L., Pandolfi, P.P. 2011. A ceRNA hypothesis: The Rosetta Stone of a hidden RNA language? Cell 146: 353–358. doi.org/10.1016/j.cell.2011.07.014

Saliani, M., Mirzaiebadizi, A., Javadmanesh, A., Siavoshi, A., Ahmadian, M.R. 2021. KRAS-related long noncoding RNAs in human cancers. Cancer Gene Ther. 2021. doi.org/10.1038/s41417-021-00381-x

Shannon, P., Markiel, A., Ozier, O., et al. 2003. Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res. 13: 2498–2504. doi.org/10.1101/gr.1239303

Sun, X., Li, M., Sun, Y., et al. 2016. The developmental transcriptome sequencing of bovine skeletal muscle reveals a long noncoding RNA, lncMD, promotes muscle differentiation by sponging miR-125b. BBA Mol. Cell. Res. 1863: 2835–2845. doi.org/10.1016/j.bbamcr.2016.08.014

Trapnell, C., Roberts, A., Goff, L., et al. 2012. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat. Protoc. 7: 562–578. doi.org/10.1038/nprot.2012.016

Trapnell, C., Williams, B.A., Pertea, G., et al. 2010. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat. Biotechnol. 28: 511–515. doi.org/10.1038/nbt.1621

Ulitsky, I., Bartel, D.P. 2013. lincRNAs: Genomics, evolution, and mechanisms. Cell 154: 26–46. doi.org/10.1016/j.cell.2013.06.020

Wang, L., Park, H.J., Dasari, S., Wang, S., Kocher, J.-P., Li, W. 2013. CPAT: Coding-Potential Assessment Tool using an alignment-free logistic regression model. Nucleic Acids Res. 41: e74. doi.org/10.1093/nar/gkt006

Wang, L., Zhao, Y., Bao, X., et al. 2015. LncRNA Dum interacts with Dnmts to regulate Dppa2 expression during myogenic differentiation and muscle regeneration. Cell Res. 25: 335–350. doi.org/10.1038/cr.2015.21

Weikard, R., Demasius, W., Kuehn, C. 2017. Mining long noncoding RNA in livestock. Anim. Genet. 48: 3–18. doi.org/10.1111/age.12493

Wenzel, A., Akbaşli, E., Gorodkin, J. 2012. RIsearch: Fast RNA-RNA interaction search using a simplified nearest-neighbor energy model. Bioinformatics 28: 2738–2746. doi.org/10.1093/bioinformatics/bts519

Wu, T., Wang, S., Wang, L., et al. 2020. Long noncoding RNA (lncRNA) CTTN-IT1 elevates skeletal muscle satellite cell proliferation and differentiation by acting as ceRNA for *YAP1* through absorbing miR-29a in Hu sheep. Front. Genet. 11: 843. doi.org/10.3389/fgene.2020.00843

Yuan, C., Zhang, K., Yue, Y., et al. 2020. Analysis of dynamic and widespread lncRNA and miRNA expression in fetal sheep skeletal muscle. PeerJ 8: e9957. doi.org/10.7717/peerj.9957

Zhai, L., Wu, R., Han, W., Zhang, Y., Zhu, D. 2017. miR-127 enhances myogenic cell differentiation by targeting S1PR3. Cell Death Dis. 8: e2707. doi.org/10.1038/cddis.2017.128

Zhang, D., Ran, J., Li, J., et al. 2021. miR-21-5p Regulates the proliferation and differentiation of skeletal muscle satellite cells by targeting KLF3 in chicken. Genes 12: 814. doi.org/10.3390/genes12060814

Zhang, S., Zhao, F., Wei, C. et al. 2013. Identification and characterization of the miRNA transcriptome of *Ovis aries*. PloS One 8: e58905. ***doi.*** org/10.1371/journal.pone.0058905

Zhang, W.R., Zhang, H.N., Wang, Y.M., Dai, Y., Feng, L., Li, X., Ding, X.B., Guo, H. 2017. miR-143 regulates proliferation and differentiation of bovine skeletal muscle satellite cells by targeting IGFBP5. In Vitro Cell. Dev. Biol. Animal 53: 265–271. doi.org/10.1007/s11626-016-0109-y

Zhao, Q., Kang, Y., Wang, H.-Y., et al. 2016. Expression profiling and functional characterization of miR-192 throughout sheep skeletal muscle development. Sci. Rep. 6: 30281. doi.org/10.1038/srep30281

Zhou, Y., Gu, P., Shi, W., et al. 2016. MicroRNA-29a induces insulin resistance by targeting PPARδ in skeletal muscle cells. Int. J. Mol. Med. 37: 931–938. doi.org/10.3892/ijmm.2016.2499

Zhu, M., Liu, J., Xiao, J., et al. 2017. Lnc-mg is a long non-coding RNA that promotes myogenesis. Nat. Commun. 8: 14718. doi.org/10.1038/ncomms14718