BMC Genomics

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Screened of long non-coding RNA related to wool development and fineness in Gansu alpine fine-wool sheep

Zhaohua He[1], Fangfang Zhao[1], Hongxian Sun[1], Jiang Hu[1], Jiqing Wang[1], Xiu Liu[1], Mingna Li[1], Zhiyun Hao[1], Zhidong Zhao[1], Bingang Shi[1], Feiyan Liu[2] and Shaobin Li[1*]

Abstract

Wool growth and fineness regulation is influenced by some factors such as genetics and environment. At the same time, lncRNA participates in numerous biological processes in animal production. In this research, we conducted a thorough analysis and characterization of the microstructure of wool, along with long non-coding RNAs (lncRNAs), their target genes, associated pathways, and Gene Ontology terms pertinent to the wool fineness development. The investigation utilized scanning electron microscopy and transcriptomic technology, focusing on two distinct types in Gansu alpine fine-wool sheep: coarse type (group C, MFD = 22.26 ± 0.69 μm, $n = 6$) and fine type (group F, MFD = 16.91 ± 0.29 μm, $n = 6$), which exhibit differing wool fiber diameters. The results showed that fine type wool fiber scales were more regularly distributed in rings with large scale spacing and smooth edges, while coarse type wool fiber scales were more irregularly arranged in tiles with relatively rougher edges, and the density of wool scales was greater than that of fine type wool. Furthermore, a comprehensive analysis revealed 164 differentially expressed lncRNAs along with 146 potential target genes linked to these lncRNAs in the skin tissues from groups C and F. Utilizing functional enrichment analysis on the target genes, we successfully identified a number of target genes might be associated with the improvement of wool fineness, such as *FOXN1*, *LIPK*, *LOC101116068*, *LOC101106296*, *KRTAP5.4*, *KRT71*, *KRT82*, *DNASE1L2*, which are related to hair follicle development, histidine metabolism, epidermal cell differentiation, oxidative phosphorylation and hair cycle process. Additionally, the interoperability network involving lncRNAs-mRNAs indicated lncRNAs (MSTRG.17445.2, XR_006060725.1, MSTRG.871.1, MSTRG.10907.4) might play a significant role in the wool growth development and fineness improvement process. In conclusion, the research enlarges the current lncRNAs database, providing a new insight for the investigation of wool fineness development in fine-wool sheep.

Keywords Long non-coding RNA, Skin, Wool fineness development, Hair follicle, Gansu alpine fine-wool sheep

*Correspondence:
Shaobin Li
lisb@gsau.edu.cn
[1]Gansu Key Laboratory of Herbivorous Animal Biotechnology, College of Animal Science and Technology, Gansu Agricultural University, Lanzhou 730070, China
[2]Animal Husbandry and Veterinary Station, Weiyuan County, Luyuan Township, Dingxi 748200, China

Introduction

As a natural renewable resource [1], wool is widely utilized in textile [2], construction [3, 4], agriculture [5] and cosmetics industries [6]. In the textile industry, wool could mainly be classified into coarse and fine wool based on its fineness [7]. Coarse wool can be employed for the production of carpets, blankets and coarse felts, while fine wool is used for the manufacturing of wool sweaters and wool quilts. The finer the wool, the greater its quality and the higher economic value it could bring [8, 9]. In addition to environmental influences, the regulation of wool characteristics is primarily determined by genetic factors. Hence, identifying the essential genes that influence wool fineness is of paramount importance. Studies have indicated that *PLCB2*, *GNAI3*, *EFNA5* and *PDGFD* genes may contribute to the wool fineness development in fine-wool sheep [10]. Comprehensive analysis via MeRIP-seq and RNA-Seq disclosed that *EDAR*, *FGF5*, *TCHH*, and *KRT2* may be instrumental in the regulation of wool fineness in Chinese Merino sheep [11]. Nevertheless, beyond protein-coding genes, non-coding RNAs are likely to have a regulatory function in numerous biological processes within animal systems [12–14].

LncRNAs represent a category of RNA molecules that are greater than 200 nucleotides in length. These molecules primarily interact with RNA, DNA and proteins to modulate the target genes expression and influence the activity of downstream signaling pathways [15]. They can regulate gene expression through chromatin remodeling, transcriptional or translational regulation, RNA editing, RNA degradation and RNA splicing to participate in mediating various biological processes [16–18]. At present, lncRNAs have been the subject of extensive research across a variety of tissues, including cardiac tissue [19], liver [20], lung [21], skin [22] and others tissues. Previous research has indicated lncRNAs (MSTRG.42054.1, MSTRG.18602.3, and MSTRG.2199.13) may be involved in the cashmere fineness indicators improvement in Jiangnan cashmere goats [23]. LncRNA MSTRG1410 has the potential to influence the traits associated with cashmere fineness through the modulation of the expression of specific target genes such as *TCHH*, *KRT35*, and *JUNB* [24]. In the course of the study, a total of 30 differentially expressed (DE) lncRNAs were identified, which may play a role in the regulatory mechanisms associated with wool fineness in Tibetan sheep [25]. While certain lncRNAs have been identified in particular breeds of sheep and cashmere goats, a significant number of highly tissue-specific lncRNAs remain to be characterized. Additionally, the roles of lncRNAs in the processes of wool development in Gansu alpine fine-wool sheep are not yet well understood. Therefore, we investigated the expression profiles of lncRNAs in skin tissues of Gansu alpine fine-wool sheep.

A selection was conducted based on the mean fiber diameter (MFD) of wool from a cohort of seventy-three Gansu alpine fine-wool sheep belonging to the same group in our research. From this group, 12 individual fine-wool sheep were selected, representing two distinct categories, coarse type and fine type. Utilizing scanning electron microscopy and RNA-Seq technology, the microstructural characteristics of the wool from these two fineness types of fine wool sheep were examined. Subsequently, DE lncRNAs expression profiles were analyzed, with the aim of identifying key lncRNAs associated wool growth and fineness improvement.

Table 1 Measurements of mean fiber diameter in experimental animal

Group	Sample name	Mean fiber diameter (μm)	Mean value (μm)
Fine(F)	F1	16.45	16.91 ± 0.29
	F2	16.83	
	F3	16.87	
	F4	16.93	
	F5	17.01	
	F6	17.34	
Coarse(C)	C1	21.50	22.26 ± 0.69
	C2	21.55	
	C3	21.89	
	C4	22.59	
	C5	22.94	
	C6	23.06	

Materials and methods

Animals and sample collection

In the current study, wool samples were collected from seventy-three one years old foundation ewes during the hair follicle growth phase (July). All samples were sourced from a single flock of Gansu alpine fine-wool sheep. All experimental animals were from the same farm in Tianzhu Tibetan Autonomous County, ensuring a uniform nutritional intake across the subjects. The animals were primarily maintained on a grazing diet, supplemented moderately. Wool specimens were obtained from trailing edge of the left scapula of sheep to evaluate various economic attributes, including wool fineness. The evaluation of these wool characteristics was carried out by the Fiber Quality Monitoring Center located in the Inner Mongolia Autonomous Region of China.

Based on the results of MFD of wool, a total of 12 individuals exhibiting extreme fine wool characteristics were selected from a cohort of 73 fine wool sheep. This cohort included both coarse wool sheep, with an MFD of 22.26 ± 0.69 μm (n = 6), and fine wool sheep, with an MFD of 16.91 ± 0.29 μm (n = 6) (Table 1).

We collected 5 cm^2 of skin specimens from the posterior edge of the left scapula of each ewe. These samples

were subsequently rinsed with phosphate buffered saline (1x PBS). Following this, the skin specimen was rapidly frozen in liquid nitrogen to facilitate transcriptome sequencing and Real-time quantitative polymerase chain reaction (RT-qPCR) analysis (Biological replicates=6). Furthermore, during the process of skin tissue sampling, a 2% lidocaine solution was administered for local anesthesia at the posterior border of the scapula in sheep. After sampling is completed, the wounds were treated with penicillin (Hebei Cheng Sheng Tang Animal Pharmaceutical Co., Ltd., Shijiazhuang, China) to further reduce the risk of infection.

Wool fineness measurement

The evaluation of these wool characteristics was carried out by the Fiber Quality Monitoring Center located in the Inner Mongolia Autonomous Region of China. Wool samples were collated for sampling, washed and dried, and then the wool fineness of 73 fine-wool sheep was measured using an optical fibre diameter analyser (OFDA).

Scanning electron microscopy analysis of wool

Firstly, the wool surface was washed with ultrapure water to get rid of adherents, mucus and impurities. The residual fat was completely removed by soaking in 70% ethanol solution for 2 min, and then dried on filter paper. A layer of conductive adhesive was pasted on the sample holder, and then the washed wool samples were adhered to the conductive adhesive and spray-plated on the wool with an ion sputtering apparatus (E-1045, Japan). A scanning electron microscope (JSM-IT700HR, Japan) was utilized to take images of the coarse type and fine type wool samples, and the area to be observed was selected to take pictures and observe specific alterations.

Total RNA extraction, RNA library construction and sequencing

Total RNA was extracted from tissue samples utilizing the Trizol reagent kit (Invitrogen, Carlsbad, CA, USA). The integrity of the RNA was evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and further validated through RNase-free agarose gel electrophoresis. Following the extraction of total RNA, ribosomal RNAs (rRNAs) were removed to isolate messenger RNAs (mRNAs) and non-coding RNAs (ncRNAs). The resulting enriched mRNAs and ncRNAs were subsequently fragmented into shorter sequences using a fragmentation buffer and reverse transcribed into complementary DNA (cDNA) with random primers. The synthesis of the second-strand cDNA was facilitated by DNA polymerase I, RNase H, deoxynucleotide triphosphates (dNTPs) incorporating dUTP in place of dTTP, and a suitable buffer. The cDNA fragments were then purified using the QiaQuick PCR extraction kit (Qiagen, Venlo, The Netherlands), underwent end repair, had poly(A) tails added, and were ligated to Illumina sequencing adapters. Subsequently, Uracil-N-Glycosylase (UNG) was employed to digest the second-strand cDNA. The resulting digested products were size-selected via agarose gel electrophoresis, amplified through polymerase chain reaction (PCR), and sequenced analysis using the Illumina HiSeq TM 4000 platform (Illumina, CA, USA) in collaboration with Gene Denovo Biotechnology Co. (Guangzhou, China).

Quality assurance, alignment with reference genomes, analysis of differential gene expression

To achieve higher quality clean reads, fastp [26] (version 0.18.0) was utilized to perform quality control on the downstream raw reads, and the screening criteria comprised removal of adapter-containing reads, reads with >10% N, reads with all A bases, and low-quality reads (Q≤20% of bases in the entire read). Bowtie2 [27] (version 2.2.8) was employed to compare the filtered clean reads to the ribosomal database and remove ribosomal RNA (rRNA). Secondly, HISAT2 [28] (v2.1.0) software was employed to align the filtered clean reads with the reference genome of sheep (GCF_016772045.1_ARS-UI_Ramb_v2.0). In combination with the HISAT2 screening results, transcripts were reconstructed for transcript assembly by means of stringtie [29] (version 1.3.4) software, which in turn predicted novel lncRNAs. The screening criteria included eliminating transcripts with uncertain strand orientations, and retaining those with lengths of ≥200 bp and exon number≥2. The coding ability of the novel transcripts was predicted by using three software packages, CPC2 [30], CNCI [31], and FEELNC [32], and the overlap of these transcripts that lack coding potential was considered the novel lncRNA. Gene expression levels measured using fragments per kilobase of transcript sequence per million base pairs sequenced (FPKM) values. The lncRNAs were then statistically analyzed for basic characteristics such as exon number, transcript length, protein coding potential and open reading frame length. The identified lncRNAs were subjected to differential expression analysis by means of DESeq2 [33] (version 1.20.0) with a screening criterion of Fold change>1.5, P-value<0.05.

Prediction of target genes and analysis of functional enrichment for differentially expressed lncRNAs

The target genes of DE lncRNAs identified using both cis and trans approaches. The cis approach involved selecting genes located within 10 kb upstream or downstream of the lncRNA. The trans approach entailed examining the expression correlation between the lncRNAs and the protein-coding genes using Pearson correlation analysis,

which facilitated the prediction of trans target genes. Following the identification of the target genes associated with DE lncRNAs, enrichment analyses were conducted utilizing Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) to further characterize these target genes.

Differentially expressed lncRNAs-mRNAs regulatory networks

In order to investigate the regulation mechanisms by which lncRNAs influence wool growth development of Gansu alpine fine-wool sheep, we identified target genes of prominent functional relevance. This selection was informed by the findings of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses conducted in this study, as well as pertinent literature. The resulting regulatory network of lncRNAs and mRNAs visualized utilizing Cytoscape (version 3.7.1) [34].

RT-qPCR analysis

We randomly selected 11 DE lncRNAs for validation through RT-qPCR to assess the reliability of the RNA-Seqs outcomes. The primers for PCR were designed using Primer 5.0 software. Subsequently, the specificity of these primers evaluated by the NCBI Primer-BLAST tool (Table 2). The RNA specimens employed underwent reverse transcription to cDNA utilizing SuperScriptT-MII reverse transcriptase (Invitrogen, CA, USA). Sheep *β-actin* and *GAPDH* were selected as internal reference genes. In addition, the RT-qPCR reactions were performed using the 2 × ChamQ SYBR qPCR Master system (Vazyme, Nanjing, China) kit. The lncRNAs relative expression were calculated using the $2^{-(\Delta\Delta Ct)}$ methodologies [35].

Table 2 Validation primer of RT-qPCR

lncRNAs	Forward (5′ → 3′)	Reverse (5′ → 3′)
MSTRG.10190.8	AGTGGTGTTATACAAGAAGC TTGA	TGCTGCAATGAAA AGGGCTG
MSTRG.10907.4	GGAAATAAGCGTGGTGCGAC	GCAAAATGAACG GACCCCAC
MSTRG.7452.1	CGCATTCATTGATCTCCCAGC	TACGTCTCGGCAA ATGTGGG
MSTRG.3616.1	GTTTCCAGGTTGTCTCTGGCG	ATACGAGTGTCTG GACTTCCTCA
XR_006057691.1	GTTGTCAAGCAGCGAGTGTG	GTGGTTGTAATTG GGGGCCT
MSTRG.871.1	TTGCCTCCCTGCAAAGCTAA	CAGACAGCAAAG TCCACCCA
MSTRG.7452.3	AGGATCCAACAACCCCACAG	TCTCCGAGTAAGT CAGGCGT
MSTRG.15575.2	CAGACCGGAGCTAAGCAGTT	GCGTACGGCAAA CTACGAAA
MSTRG.17057.1	CTGTGCATGGGTTCCTTTTGC	CAGCACACCTAG AAGATGCCA
MSTRG.17445.2	TCTAGCCACAGACCCATGTC	GCTCTATGCCCAA CTCTGGA
MSTRG.7452.2	CGCCTGACCCCCTATATAAACC	CGGAGGATTTTCT GTTCTCCG
β-actin	AGCCTTCCTTCCTGGGCATGGA	GGACAGCACCGT GTTGGCGTAA
GAPDH	GTCGGAGTGAACGGATTTGG	ACGATGTCCACTT TGCCAGT

Statistical analysis

Independent samples t-test was performed on wool fineness measurements using SPSS 22.0. The results are presented as mean ± standard deviation (SD), with a significance level set at $P<0.05$. Data visualization was conducted using GraphPad Prism version 8.0.1.

Results

Observation of wool fiber microstructure

The results indicated that the scales of fine type wool fiber were more regularly distributed in rings, having large scale spacing and smooth edges, whereas the scales of coarse type wool fiber were more irregularly arrayed in tiles, with relatively rougher edges, and the density of the wool scales was higher than that of group F (Fig. 1A, B).

Data quality control

A total of 75,157,421 raw reads were obtained from the Gansu alpine fine-wool sheep the skin tissues in group C, while group F yielded 71,913,428 raw reads. After getting rid of some low-quality sequences and splice sequences, 66,838,825 and 70,732,108 high-quality sequences (clean reads) were respectively obtained. When these high-quality clean reads were aligned to the sheep reference genome (GCF_016772045.1_ARS-UI_Ramb_v2.0), the mapping rates respectively reached 88.85% and 88.26%. This suggests that the sequencing depth and coverage of the samples satisfy the necessary testing criteria and could be suitable for further sequencing analysis (Table 3).

Identification and characterization of lncRNAs

In accordance with the lncRNA screening prediction criteria, a total of 6669 lncRNAs were recognized within the Gansu alpine fine-wool sheep skin tissues, among which there were 6033 known lncRNAs and 636 novel lncRNAs (Fig. 2A). Additionally, in accordance with the position of lncRNAs in relation to protein-coding genes, these molecules could be categorized into five primary classifications: sense lncRNAs (146), antisense lncRNAs (1267), intronic lncRNAs (109), bidirectional lncRNAs (811) and intergenic lncRNAs (3934), with intergenic lncRNAs being the most numerous (Fig. 2B).

To explore the characteristics of novel lncRNAs in Gansu alpine fine-wool sheep, we made a comparative

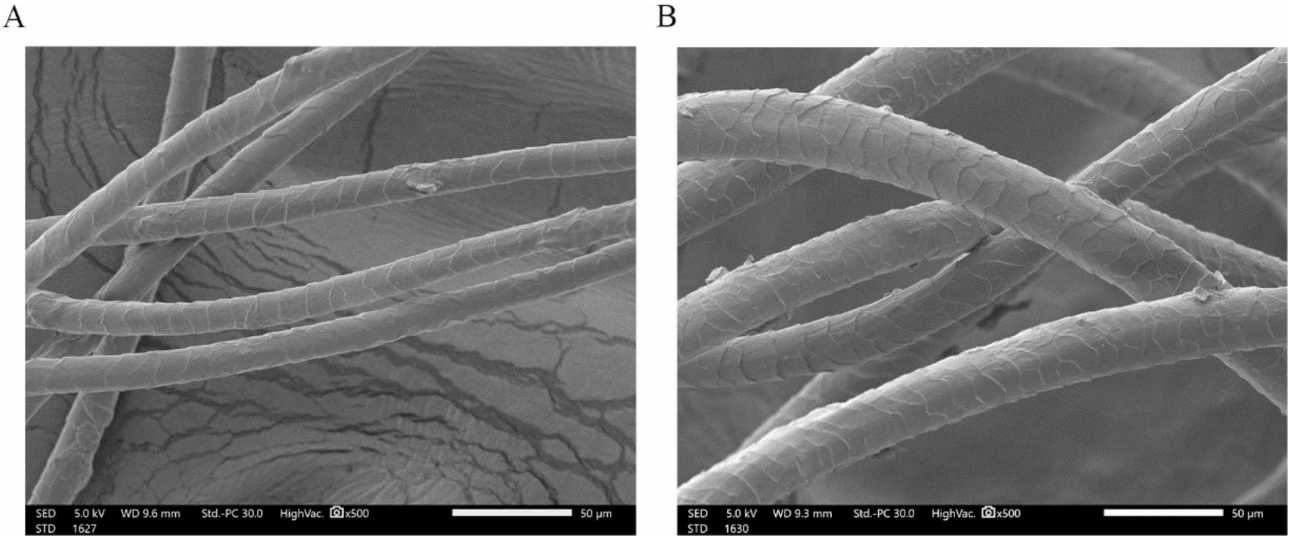

Fig. 1 Microstructure of wool fibers of Gansu alpine fine-wool sheep exhibiting varying degrees of wool fineness. (**A**) Fine type Gansu alpine fine-wool sheep wool microstructure; (**B**) Coarse type Gansu alpine fine-wool sheep wool microstructure

Table 3 Results of clean reads and alignment to the reference genome

Sample	Average raw reads	Average remaining clean reads	Average unmapped reads (%)	Average mapped reads (%)
C	75,157,421	66,838,825	7,433,202 (11.15%)	59,405,622 (88.85%)
F	71,913,428	70,732,108	8,281,271 (11.74%)	62,450,837 (88.26%)

analysis of the transcript lengths, exon numbers, open reading frame lengths and protein coding potentials of lncRNAs and mRNAs. The results demonstrated that the novel lncRNAs exhibited a reduced number of exons, as well as shorter transcript and open reading frame lengths, when compared to mRNAs (Fig. 3A, B, C). LncRNAs exhibited lower expression levels compared to mRNAs ($P<0.05$). Furthermore, the expression levels of novel lncRNAs, as measured by fragments per kilobase of transcript per million mapped reads (FPKM), were found to be significantly greater than those of known lncRNAs ($P<0.001$) (Fig. 3D, E). Additionally, the protein-coding capacity of both novel and established lncRNAs was

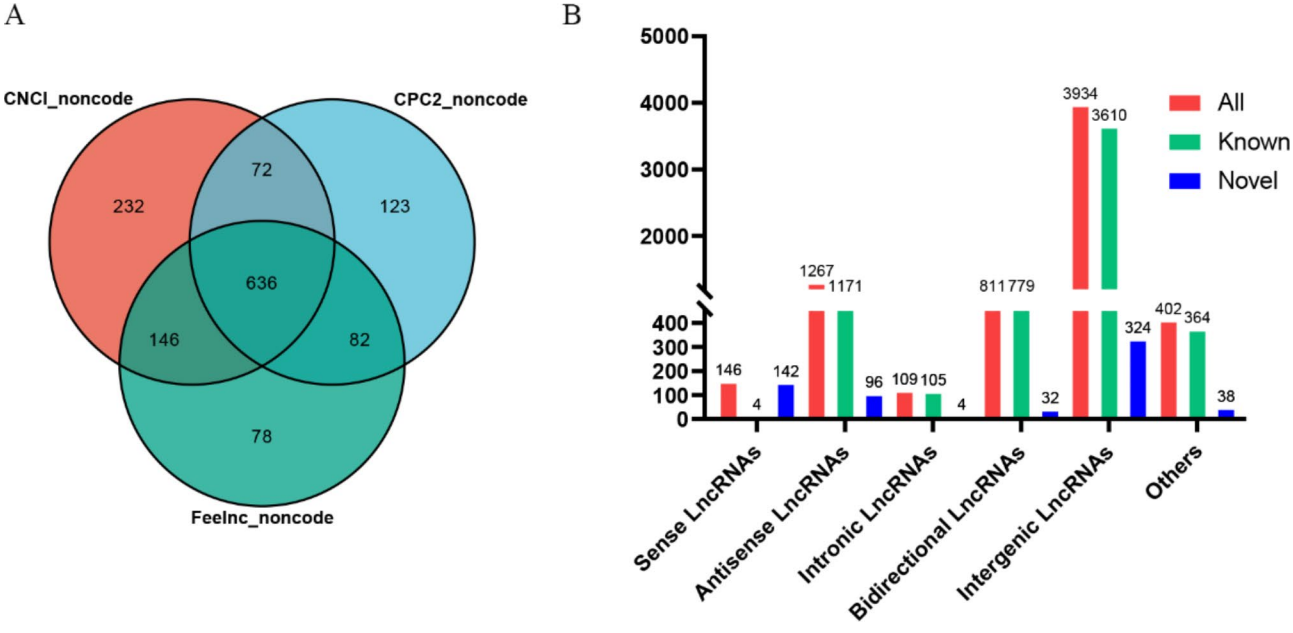

Fig. 2 Identification of lncRNAs in Gansu alpine fine-wool sheep skin tissues. (**A**) Evaluation of novel lncRNAs (**B**) Statistics on lncRNA types

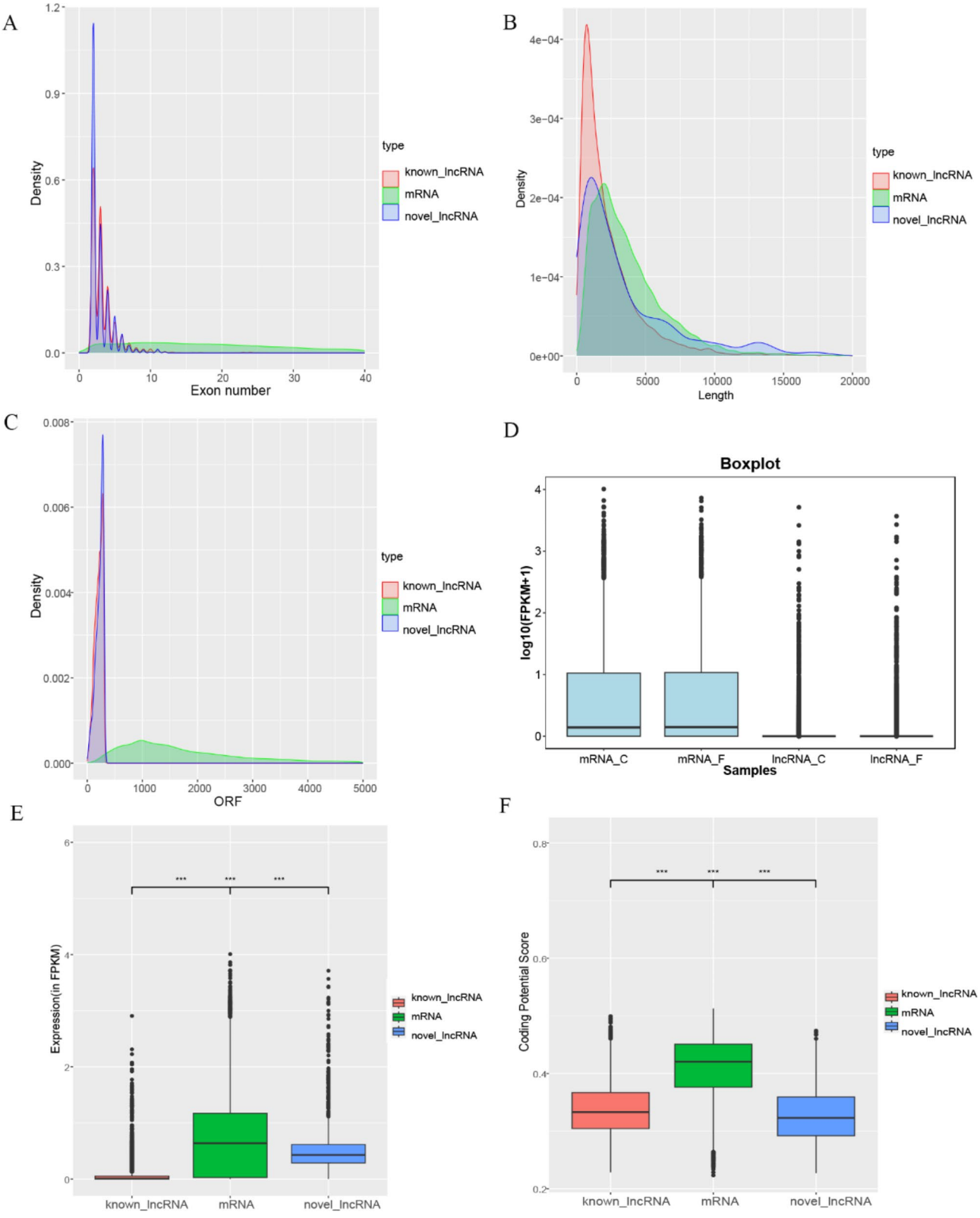

Fig. 3 Analysis of lncRNAs in Gansu alpine fine-wool sheep skin tissues. (**A**) The variation in the number of lncRNAs and mRNAs. (**B**) The variation in lengths of transcripts for lncRNAs and mRNAs. (**C**) The variation in lengths of open reading frames for lncRNAs and mRNAs. (**D**) The expression levels (log10 (FPKM + 1)) of lncRNA and mRNA in Gansu alpine fine-wool sheep. (**E**) The levels of expression among known lncRNAs, mRNA, and novel lncRNAs. (**F**) The analysis of the coding potential scores across known lncRNAs, mRNAs and novel lncRNAs. *** indicates $P < 0.001$

found to be considerably lower in comparison to that of mRNAs ($P<0.001$) (Fig. 3F).

Differential expression lncRNA screening

The present study was undertaken to identify lncRNAs associated with wool development and fineness regulation by analyzing the expression levels of lncRNAs in the skin tissues of fine-wool sheep exhibiting varying degrees of wool fineness. 164 DE lncRNAs were identified in the skin tissues categorized into groups F and C. When comparing to group C, it was observed that 87 lncRNAs were up-regulated, while 77 lncRNAs were down-regulated in the sheep skin tissues of group F (Fig. 4A, B, Table S1).

Analysis of predictions and functional enrichment for target genes of lncRNAs related to wool fineness differences

To investigate the function of lncRNAs in the wool development and fineness improvement in Gansu alpine fine-wool sheep, we carried out corresponding prediction analyses of the lncRNAs target genes, and identified 11 potential target genes from the cis-acting DE lncRNAs and 146 from the trans-acting DE lncRNAs. Then, KEGG and GO enrichment analyses were conducted for the trans-acting DE lncRNAs target genes. The KEGG results indicated that the DE lncRNAs target genes were significantly enriched in 11 pathways ($P<0.05$), including histidine metabolism (ko00340) and oxidative phosphorylation (ko00190) (Fig. 5A). The analysis of GO enrichment identified 358 GO terms that were significantly enriched ($P<0.05$), with 40 related to cellular components, 68 to molecular functions, and 250 to biological processes. Among the GO terms that showed significant enrichment, the highest number was found in biological processes, which also included several significant GO terms associated with the wool development and fineness regulation, including keratinocyte differentiation (GO:0030216), epidermal cell differentiation (GO: 0009913), epidermis development (GO:0008544), hair cycle process (GO:0022405) and hair follicle development (GO:0001942) (Fig. 5B). Among these important KEGG pathways and GO terms, we also found some DE lncRNA target genes overlapping with some DEGs. These specific genes could be essential in the biological processes involved wool quality regulation of Gansu alpine fine-wool sheep (Table 4).

Analysis of regulatory network between lncRNAs and mRNAs associated with wool growth

To investigate the roles of lncRNAs and mRNAs in the regulation of wool growth and fineness improvement in Gansu alpine fine-wool sheep, additional predictive analyses were performed on the potential DE lncRNAs target genes. In this study, we identified 13 crucial DE lncRNAs and established a regulatory network linking lncRNAs and mRNAs by integrating the findings from DE lncRNA target gene prediction (Fig. 6). The findings indicated lncRNAs had target connections with some candidate genes associated with the regulation of wool growth development and fineness (such as *FOXN1*, *LOC101116068*, *KRTAP5.4*, *KRT71*, *KRT82*, etc.). For instance, the down-regulation of XR_006060725.1 and MSTRG.871.1 targeting the *FOXN1* gene and *LOC101116068*, and the up-regulation of *MSTRG.17445.2* targeting the *KRT82* gene and *KRTAP5.4* imply that the regulation of mRNA by lncRNA

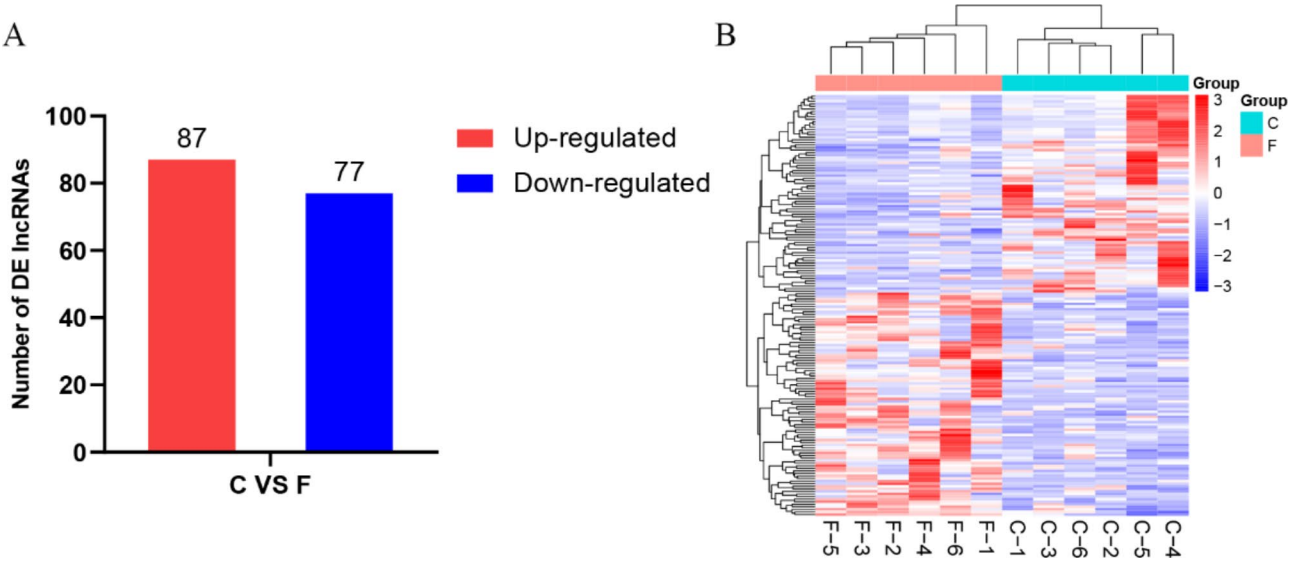

Fig. 4 Differentially expressed lncRNA analysis in skin tissues of Gansu alpine fine-wool sheep with varying wool fineness. (**A**) Differentially expressed lncRNAs histogram corresponding to the various wool fineness levels. (**B**) Differentially expressed lncRNAs heat map associated with differing wool fineness

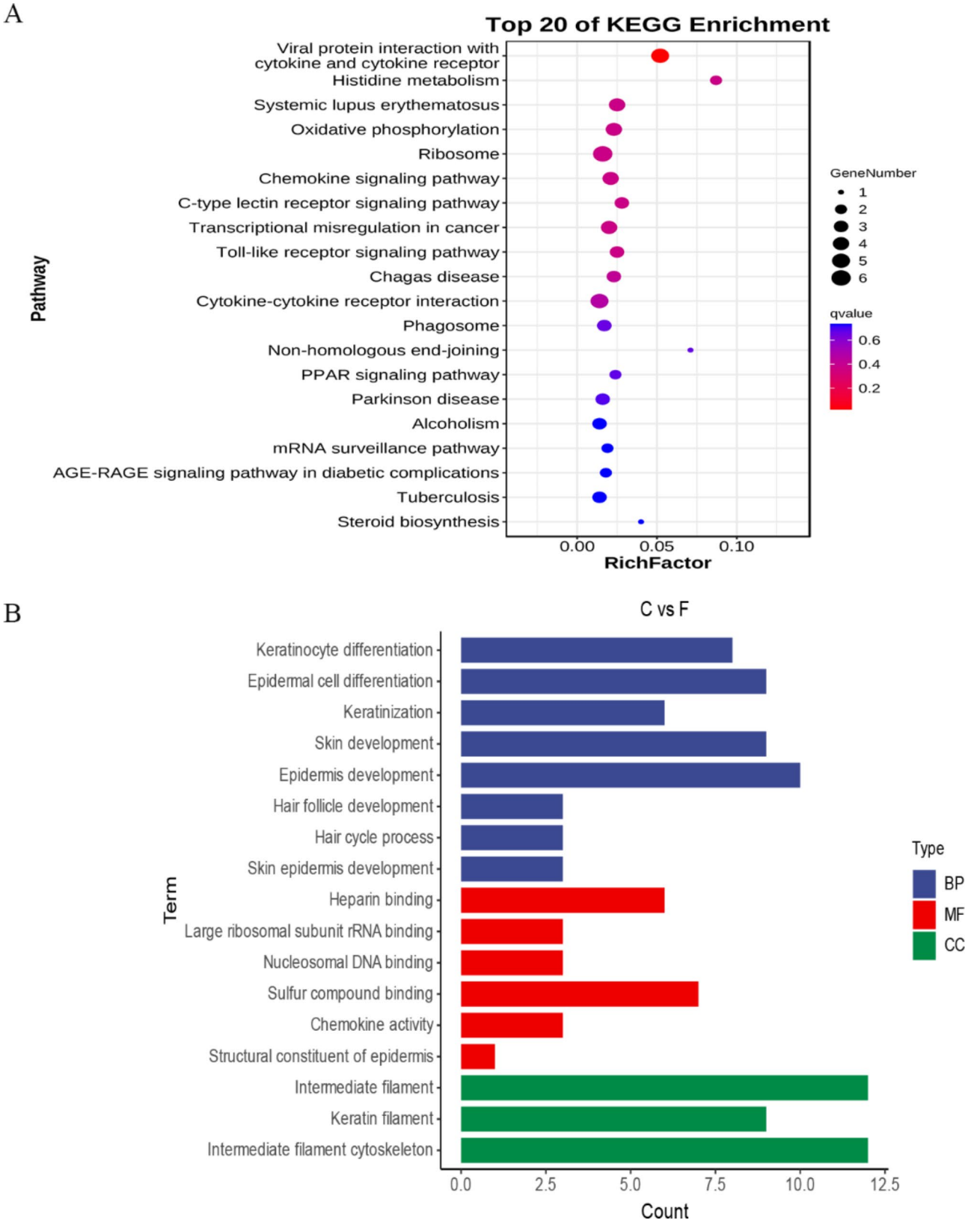

Fig. 5 Analysis of functional enrichment for the target genes of lncRNAs in the skin tissues. (**A**) DE lncRNA target genes Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis; (**B**) DE lncRNA target genes Gene ontology (GO) enrichment analysis

Table 4 The distinction in lncRNA targets and mRNAs related to the wool growth and fineness improvement

KEGG: ko00340 Histidine metabolism	lncRNA targets	*HDC*; *AOC1*
	mRNA	***HDC***; ***AOC1***
KEGG: ko00190 Oxidative phosphorylation	lncRNA targets	***LOC101108663***; ***NDUFA6***; ***ATP12A***; ***ND4L***
	mRNA	*ATP6V0A4*; *LOC101107153*; *ATP6V1B1*; ***LOC101108663***; ***NDUFA6***; ***ATP12A***; ***ND4L***
GO: 0030216 Keratinocyte differentiation	lncRNA targets	***KRT82***; ***LOC101106296***; ***KRT71***; ***LOC101113003***; ***DNASEL2***; ***LIPK***; ***IVL; FOXN1***;
	mRNA	***KRT82***; *PKP3*; ***LOC101106296***; ***KRT71***; *CTSL*; ***FOXN1***; *TGM1*; ***DNASE1L2***; *KRT20*; ***LIPK***; ***IVL***; ***LOC101113003***; *LOC105614079*; *LORICRIN*; *FLG*
GO: 0009913 Epidermal cell differentiation	lncRNA targets	***KRT82***; ***LOC101106296***; ***MCOLN3***; ***FOXN1***; ***KRT71***; ***DNASE1L2***; ***LIPK***; ***IVL; LOC101113003***;
	mRNA	***MCOLN3***; *CDH2*; *CTSL*; ***KRT82***; *PTPRQ*; *PKP3*; ***LOC101106296***; *KRT20*; ***LOC101113003***; *TGM1*; ***DNASE1L2***; ***LIPK***; ***KRT71***; ***IVL***; *LOC105614079*; ***FOXN1***; *LORICRIN*; *FLG*
GO: 0001942 Hair follicle development	lncRNA targets	***FOXN1***; ***KRT71***; ***DNASE1L2***
	mRNA	***FOXN1***; ***KRT71***; ***DNASE1L2***
GO: 0005882 Intermediate filament	lncRNA targets	***KRT82***; *PNN*; *LOC101106296*; ***KRT71***; ***LOC101112657***; ***LOC101116068***; *MNS1*; ***LOC105604748***; ***LOC105610157***; ***LOC105616373***; ***LOC114116855***; ***LOC114116996***
	mRNA	*KRTAP1-1*; *KRTAP1-3*; *KRTAP4.3*; ***KRT82***; *LOC101104203*; *LOC101108536*; *LOC101108798*; ***KRT71***; ***LOC101112657***; *LOC10111315*; *LOC101114287*; *LOC101114537*; *LOC101115634*; ***LOC101116068***; *LOC105604734*; *LOC105604740*; ***LOC105604748***; ***LOC105610157***; ***LOC105616373***; *LOC105616374*; *LOC106991427*; *LOC114110483*; *LOC114110486*; *LOC114112036*; *LOC114116843*; *LOC114116844*; *LOC114116849*; *LOC114116850*; *LOC114116852*; *LOC114116854*; ***LOC114116855***; ***LOC114116996***; *LOC114118844*

The genes highlighted in bold represent potential lncRNA targets involved related to the wool growth and fineness improvement, along with their corresponding mRNAs

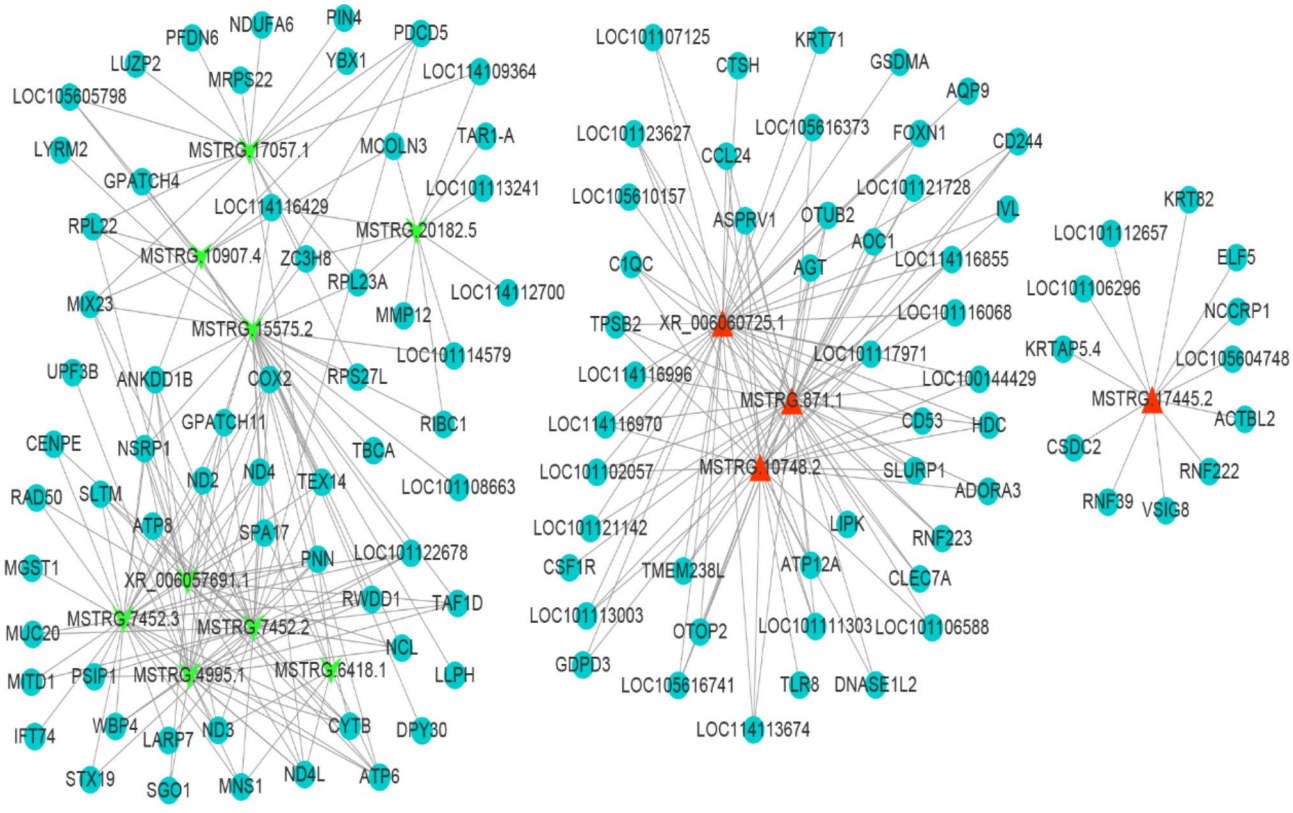

Fig. 6 Construction of the lncRNA-mRNA regulatory network. Red denote lncRNAs that are up-regulated lncRNAs, light green indicates lncRNAs that are down-regulated lncRNAs, and dark green represent the target genes of DE lncRNAs

might also be implicated in the growth development and fineness improvement of wool in Gansu alpine fine-wool sheep. At the same time, the Sankey diagram could more effectively display the relationship between wool development-related DE lncRNAs, target genes and GO terms in which the target genes are significantly enriched (Fig. 7).

Verification of lncRNA expression by RT-qPCR

To confirm the reliability of the lncRNA sequencing results, we randomly selected 11 lncRNAs and assessed their expression levels by means of the RT-qPCR method. The results of all the experiments were in line with the sequencing data (Fig. 8).

Discussion

Hair follicles, as an important skin appendage, can be divided into primary and secondary hair follicles [36]. Primary hair follicles could generate coarse hairs, whereas secondary hair follicles could produce finer hairs. The structure of animal fibers consists of three main parts from outside to inside: the cuticular scale layer, the cortical layer and the medullary layer [37]. However, despite the similarity of the basic structural components of animal fibres, there are some differences in the morphology and chemical composition of these fibres, which in turn may lead to differences in hair fiber fineness, strength and crimp [38]. Depending on the presence or absence of a medullary layer, wool can be classified as medullated or unmedullated. Wool without

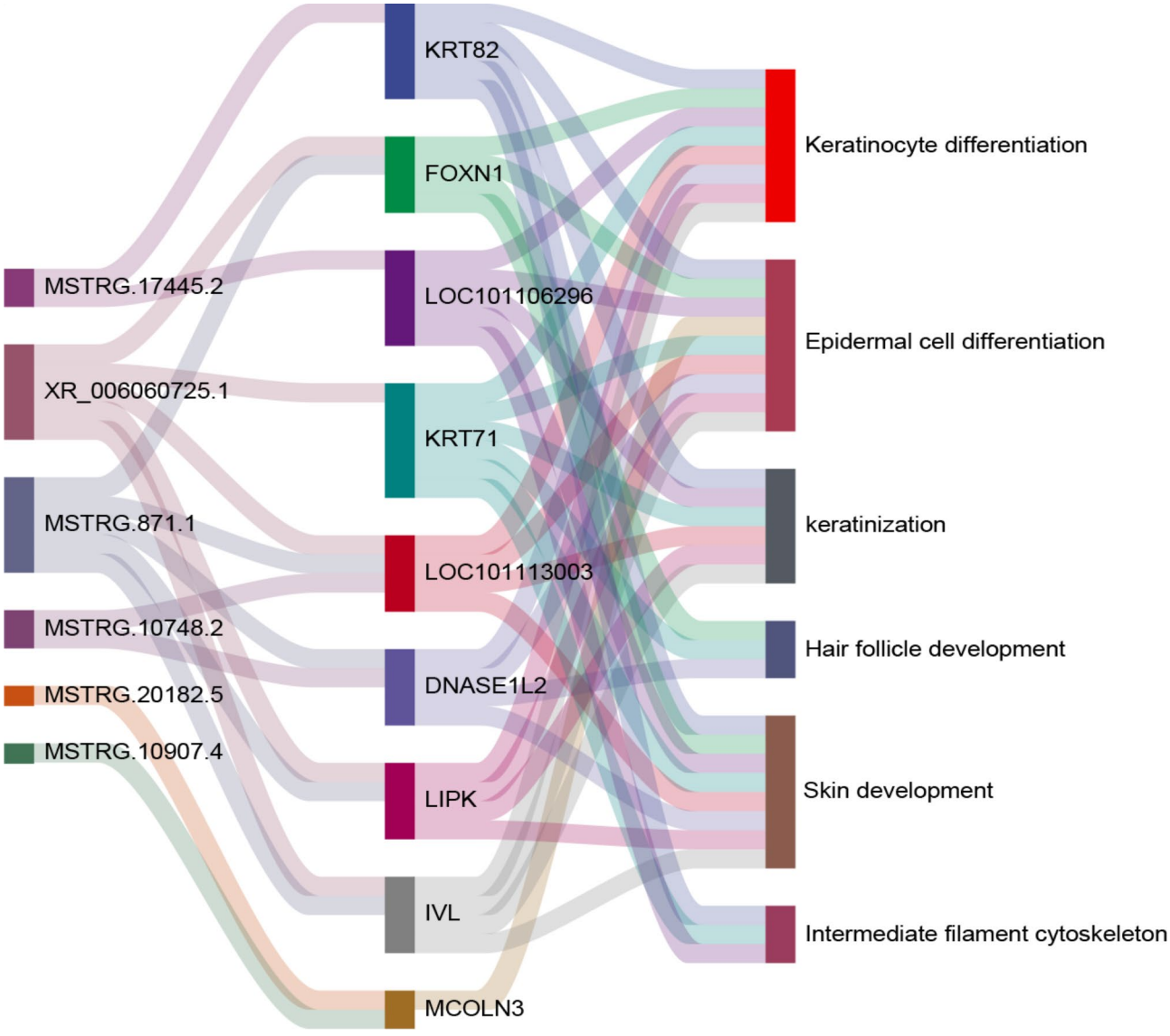

Fig. 7 Sankey diagram illustrating the prominent enrichment GO terms for DE lncRNA target genes related to wool growth development and fineness improvement

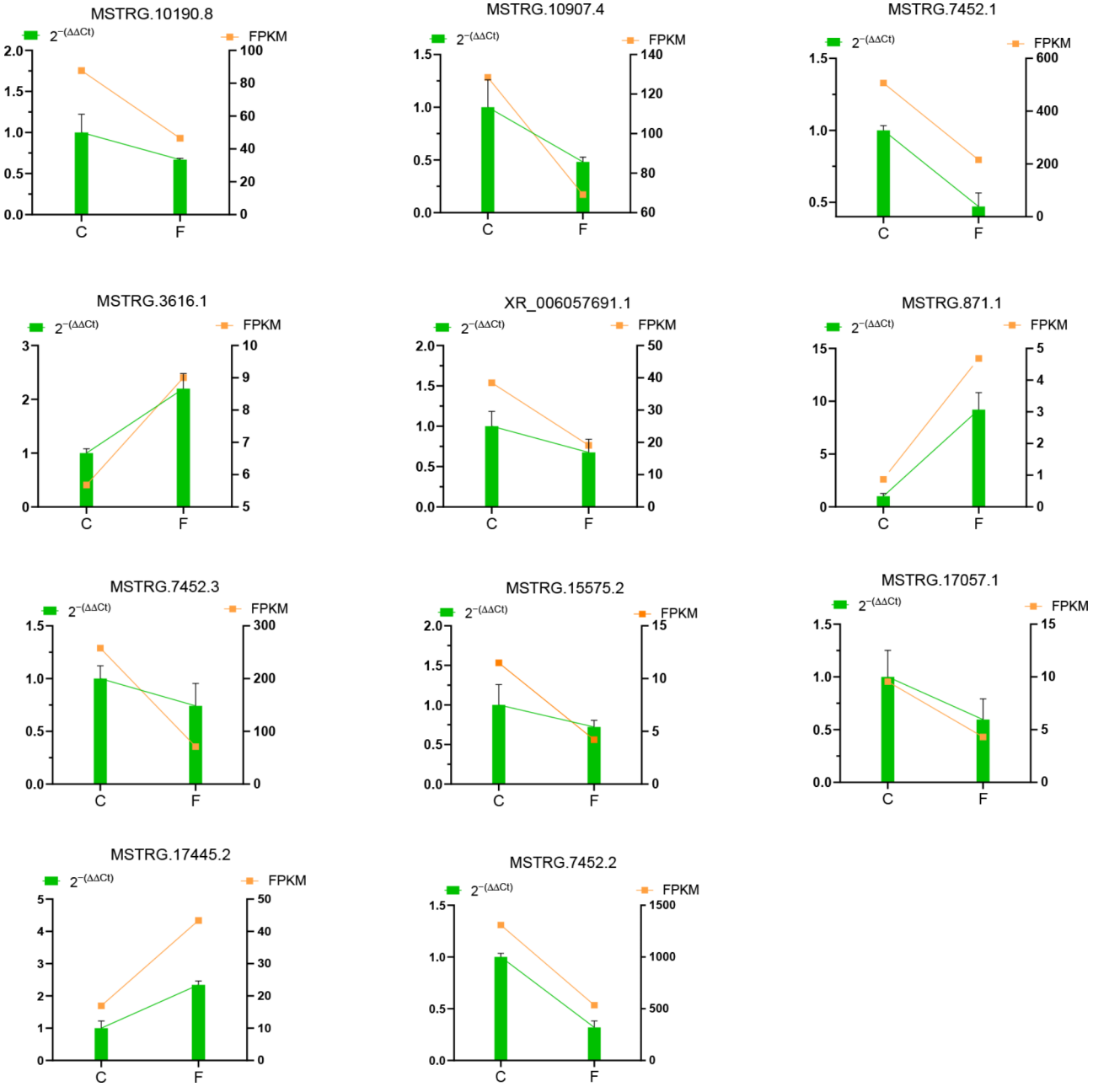

Fig. 8 Comparison of the expression levels of lncRNA as measured by RNA-Seq and RT-qPCR. The RT-qPCR results were shown as mean ± SD, with $2^{-(\Delta\Delta Ct)}$ representing these findings. The RNA-Seq results are indicated by FPKM values

medulla is finer and more economically valuable and is produced only from secondary hair follicles [39]. The cortex is located in the middle part of the hair and it contains cortical fibers, gels, interstitial material, and voids. It has been found that the morphology of the hair fiber cuticle scale layer is not fixed and varies with factors such as nutritional conditions, animal fiber productivity and animal body size [38, 40]. In the present study, we found that fine type wool fiber scales were mostly distributed in more regular rings, with large scale spacing and smooth edges, while coarse type wool fiber scales were mostly arranged in irregular tiles, with relatively rougher edges and relatively narrower spacing of wool scales, further suggesting that the morphological characteristics of the wool fiber scale layer have a significant impact on the fineness traits of wool.

Studies on lncRNAs associated with the growth of hair follicles and skin tissue in sheep and goats have been discussed. It has been demonstrated that lncRNA MSTRG.15931.1 may influence the hair follicle cycle of sheep by interacting with the *PTPRM*, *ELMO1* and *Pip5k1c* genes [41]. lncRNA-599,554 could boost the

inducibility of dermal papilla cells (DPCs) in cashmere goats by positively influencing the expression of *Wnt3a* gene, which in turn promotes secondary hair follicle regeneration, as well as the formation and growth of wool fiber [42]. Additionally, 256 DE lncRNAs were screened during the cyclic growth of cashmere, and they may have an important regulatory role in the growth and cycling of secondary hair follicles in the Jiangnan cashmere goat [43]. In the study involving Liaoning cashmere goat and Inner Mongolia cashmere goat with different fineness, 170 DE lncRNAs were screened and might be involved in the process of cashmere fineness regulation in cashmere goats [24]. The aforementioned studies have established a foundational basis for understanding the mechanisms by which lncRNAs regulate hair follicle development and enhance the economic traits associated with wool production. Nevertheless, there is a paucity of research regarding the regulation of wool fiber diameter by lncRNA in fine wool sheep have scarcely been reported, especially in the context of Gansu alpine fine-wool sheep. In the current research, we identified and characterized 164 DE lncRNAs along with 146 DE lncRNAs target genes, suggesting that these molecular entities may significantly influence the wool fineness improvement in Gansu alpine fine-wool sheep.

Functional enrichment analysis of genes is an effective way to screen pathways that play significant functions in diverse biological activities of animals. Relevant studies have discovered that crucial signaling pathways including WNT signaling pathway [44, 45], TGFβ signaling pathway [46], fibroblast growth factor (FGF) signaling pathway [47], and bone morphogenetic protein (BMP) signaling pathway [48] exert important regulatory roles in the process of cell differentiation and fiber formation. In this research, via the enrichment analysis of target genes, some significant GO terms and KEGG pathways associated with wool growth development and fineness regulation were also sifted out, such hair follicle development, histidine metabolism, epidermal cell differentiation, oxidative phosphorylation and hair cycle process. Histidine is a dietary essential amino acid that is requisite for protein synthesis and cannot be synthesized in animals. Histidine could play a particularly crucial role in the active site of enzymes such as serine proteases (e.g., trypsin), which are members of the catalytic triad [49]. Hence, histidine metabolism also has a role in skin development and wool synthesis. Additionally, oxidative phosphorylation is a biochemical process in which organic matter liberates energy during catabolism, which in turn leads to the synthesis of ATP [50]. As a high-energy phosphate compound, ATP could offer a dependable energy supply for various life activities of plants and animals [51]. Therefore, we surmise that oxidative phosphorylation may also play a major part in energy supply during wool growth development and fineness improvement.

The establishment of lncRNA and mRNA interoperability network is advantageous for identifying critical lncRNAs and corresponding target genes. In the present study, we combined the outcomes of the lncRNA-mRNA interaction network and Sankey diagram (Figs. 6 and 7) to identify certain lncRNAs (such as MSTRG.17445.2, XR_006060725.1, MSTRG.871.1, MSTRG.10907.4) and DE lncRNA target genes (*LIPK*, *LOC101116068*, *LOC101106296*, *FOXN1*, *KRTAP5.4*, *KRT71*, *KRT82*, *DNASE1L2*, etc.) that might play crucial roles in the regulation of wool growth development and fineness improvement in Gansu alpine fine-wool sheep. Researches have demonstrated that Lipase family member K (LIPK), as part of the lipase family, it may play a role in encoding epidermal lipase, which is associated with lipase activity and participates in lipolysis metabolic processes [52, 53]. Epidermal lipase is engaged in epidermal lipid metabolism, and the balance of epidermal lipid metabolism is closely related to epidermal permeability protective function of barriers [54].Moreover, the barrier function of skin is essential and serves as a prerequisite for the hair follicles proper growth and development [55]. Additionally, Recombinant Deoxyribonuclease I Like Protein 2 (DNASE1L2) functions as an endonuclease, which may play a prominent role in the process of nuclear DNA degradation in epidermal keratinocytes [56]. The epidermal stratum corneum could play a vital features in the skin's barrier function, and the degradation of nuclear DNA within keratinocytes is an essential process in the development of a normal stratum corneum [57]. In this study, the *DNASE1L2* and *LIPK* genes exhibited significant enrichment in GO terms such skin development, keratinocyte differentiation, and epidermal cell differentiation, which also further indicates their crucial roles in the normal keratogenesis of animal skin.

Wool is principally constituted by proteins of the keratin family, encompassing keratin and keratin-associated proteins [58]. It has been discovered that polymorphisms and insertions/deletions in keratin and keratin-associated protein genes might have certain impacts on hair production traits [59]. For instance, polymorphisms in the sheep *KRTAP20-2* gene is associated with the crimp rate of wool fiber [60]. Insertions/deletions of bases in the coding region of sheep *KRTAP6-1* are related to alterations in wool fiber diameter [61]. In this research, keratin-related genes like *KRT82*, *KRT71* and *LOC101106296* (*KRTAP 27 – 1*) were screened and significantly enriched in GO terms including hair follicle and skin development. Concurrent research has suggested that the *KRT71* gene are associated with the extent of hair curliness [62–64]. The *KRT82* and *LOC101106296* (*KRTAP 27 – 1*) genes also exert a regulatory function in fineness regulation

and wool development [65, 66]. In addition, based on the results of mRNA skin transcriptome analysis, we found that the mRNA expression levels of *KRTAP6-1*, *LOC101116068*, *KRT82*, *KRT71*, and *LOC101106296* genes in the skin of coarse type wool sheep (expression levels: 641.077, 13.293, 60.995, 576.917, 10.288, respectively) were significantly lower than those in fine type wool sheep (expression levels: 1360.296, 55.776, 94.107, 988.573, 24.394, respectively) ($P<0.05$), indicating that the higher the expression levels of these gene mRNAs, the finer the wool. The results further suggest the significant role of keratin and keratin-associated protein gene family members in the wool growth development and fineness improvement.

Conclusion

In the study, we discovered that the finer the wool, the more regular the circular distribution of wool fiber scales, and the greater the spacing between scales. At the same time, we also screened some DE lncRNAs (MSTRG. 17445.2, XR_006060725.1, MSTRG. 871.1, MSTRG. 10907.4) that may be related to wool development and fineness regulation, target genes of lncRNAs (*FOXN1*, *LIPK*, *LOC101116068*, *LOC101106296*, *KRTAP5.4*, *KRT71*, *KRT82*, *DNASE1L2*), GO terms (such as hair follicle development, hair cycle process and epidermal cell differentiation). The identification of these target genes results indicates that lncRNAs and associated target genes may have a significant character in the wool growth development and wool fineness improvement in Gansu alpine fine-wool sheep.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-11195-0.

Supplementary Material 1

Acknowledgements
Not applicable.

Author contributions
ZHH: Data curation, Writing - Original draft, Software. SBL: Conceptualization, Formal analysis, Writing - Review & Editing, Project administration. FFZ and HXS: Visualization, Investigation. BGS and MNL: Software. ZYH, JQW and XL: Validation, Resources. JH, ZDZ and FYL: Supervision, Visualization.

Funding
This research was funded by the National Natural Science Foundation of China (32060140), the Distinguished Young Scholars Fund of Gansu Province (21JR7RA857), Discipline Team Project of Gansu Agricultural University (GAU-XKTD-2022-21), Fuxi Young Talents Fund of Gansu Agricultural University (Gaufx-03Y04), and Gansu Provincial Key R&D Program Projects (22YF7WA115).

Data availability
Sequence data that support the findings of this study have been deposited in the GenBank Sequence Read Archive (SRA) database under accession number PRJNA1100727.

Declarations

Ethics approval and consent to participate
The experimental animals utilized in this study were sourced from a single farm located in Tianzhu Tibetan Autonomous County. Additionally, informed consent has been secured from the owners of the animals for their participation in the research. Meanwhile, the animal study received approval from the Ethics Committee of Gansu Agricultural University, under protocol code GSAU-ETH-AST-2021-028.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 23 September 2024 / Accepted: 30 December 2024
Published online: 06 January 2025

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