

Screening and Identification of LncRNAs Related to Villus Growth of Liaoning Cashmere Goats and Their Effects on Growth after FGF5 Treatment

Mei Jin (✉ jm6688210@163.com)

Liaoning Normal University <https://orcid.org/0000-0001-7986-5020>

Qin Feng Zhao

Liaoning Normal University

Ping Ni

Liaoning Normal University

Jun Piao

Liaoning Normal University

Ai Jing Piao

Liaoning Normal University

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Abstract

(Background) Liaoning Cashmere Goat cashmere has high economic value FGF5 is an important factor regulating its growth. The role of long non-coding RNA (LncRNA) in the mammalian villus growth cycle has still not been studied in detail.

(Results) We demonstrated that treatment of skin cells with FGF5 inhibited the expression of LncRNA in cells, down-regulated the expression of the target genes CBS and CTH, and promoted the expression of related keratin genes k26, kap11.1. Overexpressing LncRNA reversed the inhibiting effect of FGF5 on the target genes CBS and CTH.

(Conclusions) we believe that FGF5 can regulate the growth and development of Cashmere Goat hair by promoting the expression of related keratin and keratin-associated protein genes. This mechanism is achieved by inhibiting the expression of the LncRNA gene and then down-regulating the expression of the target

genes CBS and CTH.

Background

The Liaoning Cashmere Goat is a unique breed in China whose wool and meat have high functional value. Liaoning Cashmere Goat wool is of high quality and fluff yield. It is a very precious textile fiber and has the reputation of being the “fiber gem” [1-2]. In mammals, hair is a skin derivative that grows and develops from hair follicles: one of the most striking features of hair follicles is that they self-renew throughout the life of an individual and continue to produce new hair [3].

Hair follicles are an important structure for regulating villus growth. There are two main types – primary and secondary hair follicles—and the primary hair follicles can be further differentiated into secondary hair follicles. The primary hair follicle produces hair fibers, and the secondary hair follicle produces velvet fibers [4]. The quality of the fluff is determined by the velvet fibers. The growth and development of hair follicles is a cyclical physiological process that undergoes three regular periods in a year: anagen, catagen, and telogen [5-8]. Many factors— such as endocrine hormones and drug treatment of skin cells— can affect this cycle, which in turn affects the growth of cashmere.

Fibroblast growth factor 5 (FGF5) can regulate the hair follicle from the anagen to the telogen period and change the growth cycle of hair follicle cells. FGF5 is a cell growth factor obtained and purified from the mammalian pituitary gland. It has been found that FGF5 can advance the hair follicle growth cycle into the telogen period and inhibit hair growth [9]. Wichert B et al. found that mutations in the FGF5 gene can cause genetic hair length variation in mice and dogs [10]. Hebert's study found that, if the mouse FGF5 gene is silenced, the mouse hair follicle anagen will be prolonged, and the mouse hair will be longer, which prolongs the telogen of the hair follicle [11]. The FGF5 gene is also a determinant of the long hair phenotype in domestic cats. The long subtype of the FGF5 gene in alpacas prematurely produces a point

mutation in the stop codon (PTC), leading to an increase in alpaca hair length [12]. Steven Kregge found that Sox2 can regenerate hair follicle cells in the skin through FGF5[13-14]. The above results indicate that FGF5 factor plays a very important role in the cyclical changes of hair follicles and the growth and development of villi. Therefore, research on FGF5 in hair follicle development and villus growth is very important.

With the deepening of scientific research, many long non-coding RNAs (LncRNAs) have been shown to have a certain regulatory effect on the metabolism and growth of living organisms[15]. LncRNAs regulation mechanism and participating biological processes mainly include chromosome modification, regulation of transcripts (up- or down-regulation of gene expression), localization of subcellular proteins, cell proliferation and differentiation, immune response mechanisms, tumor cancer, etc.[16-17]. LncRNAs also regulates genes involved in hair follicle development and the postnatal hair cycle[18]. Cai et al. analyzed the regulation of LncRNA AK015322 (LncRNA5322) in hair follicle stem cells (HFT) and the potential mechanism of LncRNA5322-mediated HFT differentiation. The results showed that LncRNA5322 can target miR-21-mediated PI3K-AKT Signaling pathways in HFT promote the proliferation and differentiation of HFT[19]. In 2017, the non-coding RNA database LncRNASNP2

(<http://bioinfo.life.hust.edu.cn/LncRNASNP2>) began providing comprehensive information on LncRNA mutations, as well as the structure and function of LncRNAs. The database contains 141,353 human and 117,405 mouse LncRNAs. Scholars identified LncRNAs associated with growth in cows [20-21].

Even though research on hair follicle development and LncRNAs is increasing, genetic information on Cashmere Goats remains relatively small, and there are few known genes related to the growth and development of villi. We believe that some LncRNAs may play an unknown role in the villi development of Cashmere Goats. This study investigated the effect of LncRNA on the growth and development of villi in skin cells treated with FGF5 and suggests a new way to approach the study of cashmere growth in Cashmere Goats.

Villi formation in Cashmere Goats has been found to require keratin and the keratin-associated protein (KAP) family. Reduction of the keratin K25 gene can cause hair loss [22]; the K17 gene can change the growth cycle of villi [23]; the KAP family is more abundant in mammalian hair follicles, and some keratin genes play a role in the differentiation of hair tissue [24-25]. It has been found that the KAP16.6 and KAP13.1 genes are involved in regulating the size of the villous fiber diameter in sheep [26-27]. The KAP18 gene plays an important role in maintaining flexibility and structural integrity in epithelial cells [28-29]. The keratin-associated protein 13.3 is composed of 156 amino acids, and the Cashmere Goat KAP13.3 gene may affect the structure of this protein and characteristics of cashmere fibers [30-31]. KAP is the main component of wool, and keratin and keratin-associated proteins are composed of amino acids.

The body's complex regulation activities, like cell metabolism, are closely related to the amino acid metabolic pathway; Biolo G et al. found that, when the body's activity is reduced, the stimulation of amino acid synthesis on the body's protein is greatly reduced [32]. Some amino acids have been shown by

scientists to be related to the growth and development of hair follicles. Asparagine plays an important role in the regulation of hair follicle growth and development [33]. By supplementing the cysteine amino acid, cells can resist the decrease in keratin synthesis caused by the loss of iron, which affects the growth and development of hair follicles [34]. Sulfur-containing amino acids (cysteines) are critical for the structural maintenance of keratin and keratin-associated proteins, which are also rich in serine [35]. In summary, we can find that the KAP family and amino acid metabolic pathway play an important role in the regulation of hair follicle development and villus quality. Therefore, it is very important to study the KAP family to regulate the growth of villi.

The villi in the Liaoning Cashmere Goat studied in this experiment are composed of about 90% keratin and keratin-associated proteins. We found that LncRNA is involved in the amino acid metabolic pathway and plays a regulatory role in synthesizing serine and cysteine. For the KAP family, sulfur-containing amino acids (methionine and cysteine, which can be converted into each other) play an important role in their structural maintenance. Therefore, we believe that FGF5 can affect the production of members of the keratin family in Cashmere Goat skin cells by regulating the expression of LncRNA, further affecting the growth and development of Cashmere Goat villi and the quality of villi.

Results

Screening differentially-expressed LncRNA

The total RNA in a sample was subjected to mass detection using an Agilent Technologies 2100 Bioanalyzer and a NanoDrop 1000 spectrophotometer, and coverage analysis of different known gene types of the species samples was performed using HTSeq software. The results of differential expression analysis of LncRNA are shown in Table 1. (screening threshold is Qvalue < 0.05):

Table 1. Screening results of differentially-expressed LncRNA

Sample	Total number of LncRNA of differential expression	Total number of LncRNA of up-regulation expression	Total number of LncRNA of down-regulation expression
F4_24h vs C	164	70	94
F4_72h vs C	189	78	111
F6_24h vs C	123	27	96

The above results showed that F4_24h and C were screened to obtain 164 differentially-expressed LncRNA, of which 70 were up-regulated and 94 were down-regulated; F4_72h was compared to C, and there were 189 differentially-expressed LncRNA, of which 78 were up-regulated and 111 were down-

regulated; F6_24h was compared to C, 123 differentially-expressed LncRNA were obtained, of which 27 were up-regulated and 96 were down-regulated.

Functional enrichment analysis of differentially-expressed LncRNA target genes

After differentially-expressed LncRNA were predicted by cis and trans target genes, GO and KEGG functional enrichment analyses were performed on their target genes, and the screening conditions were corrected ($P < 0.05$). GO analysis showed that the cis and trans target genes of LncRNA differentially-expressed in F4_24h and C groups had no significant enrichment of the GO term; the cis target genes of LncRNA differentially-expressed in F4_72h and C groups had no significant enrichment of the GO term; The trans target gene was mainly enriched in the cellular metabolic process biological_process and cellular macromolecule metabolic process biological_process of BP; cell cellular_component, cell part cellular_component and intracellular cellular_component of CC; and binding molecular_function of MF. The cis and trans target genes of LncRNA differentially-expressed in the F6_24h and C groups also had no significantly enriched GO term. The above results indicated that the GO enrichment results were most significant in the F4_72h treatment group (Fig. 1).

The KEGG pathway enrichment analysis was performed on the target genes of differentially-expressed LncRNA in the three groups (screening conditions: $Qvalue < 0.05$). The results are shown in Table 2.

Table 2. KEGG enrichment analysis results

Sample name	Significantly enriched pathway term	Qvalue	
F4_24h vs C	Cis target gene	-	
	Trans target gene	-	
F4_72h vs C	Cis target gene	-	
	Trans target gene	Ribosome	0.001
		RNA transport	0.018
		Fanconi anemia pathway	0.018
		Huntington's disease	0.018
		Metabolic pathways	0.025
		Aminoacyl-tRNA biosynthesis	0.029
		Citrate cycle (TCA cycle)	0.033
		Alzheimer's disease	0.033
		Ubiquitin mediated proteolysis	0.037
F6_24h vs C	Cis target gene	Taste transduction	0.022
	Trans target gene	-	-

The above results indicate that the pathway enrichment results were most significant in the F4_72h treatment group, which was most closely related to hair follicle development and villus growth. Based on

the above results and by consulting the literature, it was determined that metabolic pathways are involved in the regulation of hair follicle development and villus growth.

Expression verification of target LncRNA

Combined with the screening of LncRNA, prediction of target genes, and bioinformatics analysis of LncRNA, it was finally determined that FGF5 had the most beneficial effects on hair follicle development and villus growth when it treated Liaoning Cashmere Goat skin cells with 10^{-4} g/L for 72 h. Therefore, in the experiment, we screened four LncRNAs related to the action of FGF5 from the 10^{-4} g/L 72 h treatment group: XLOC_011424, XLOC_009522, XLOC_009063, XLOC_011157. To further determine the accuracy of RNA-Seq results, we utilized the qPCR technique to detect the expression of the above four LncRNAs, as shown in Fig. 2 to Fig. 5.

The results showed that, after treating Liaoning Cashmere Goat skin cells with FGF5, the expression of LncRNA XLOC_011424 was down-regulated ($P < 0.05$) to 0.70 times that of the control group; the expression of LncRNA XLOC_009063 was up-regulated ($P < 0.05$) to 1.12 times that of the control group; and the expression of LncRNA XLOC_011157 was down-regulated ($P < 0.05$) to 0.74 times that of the control group. The results of down-regulation of the expression of the four LncRNAs based on RNA-Seq sequencing indicated that LncRNA XLOC_011424 and LncRNA XLOC_011157 were consistent with previous results. We first selected LncRNA XLOC_011424 as the target LncRNA to study its mechanism of action on fluff growth after treating Liaoning Cashmere Goat skin cells with FGF5.

qPCR detection of k26, kap11.1 gene expression in cells after FGF5 drug treatment

After treating Cashmere Goat skin cells with FGF5, the expression levels of k26, kap11.1 genes were detected by qPCR (Fig. 6). The results showed that, compared to untreated cells, the cells with keratin26 genes that were treated with FGF5 were up-regulated at the mRNA level, and the expression level was increased. There was no significant change in the expression of the kap11.1 gene. This indicated that FGF5 played a positive role in the regulation of keratin-related genes k26 but has no clear effect on the keratin-related gene kap11.1 was observed.

FISH immunofluorescence localization

The location of the target LncRNA in the skin cells of Cashmere Goats was studied by the FISH immunofluorescence localization technique (blue staining reagent marks the nucleus, red staining reagent marks the target LncRNA, and overlapped blue and red stain values refer to a mixture of the two). According to the results of this experiment, LncRNA is mainly localized in the nucleus and cytoplasm in the hair cells of Cashmere Goats. After cells were treated with FGF5, it was found that FGF5 induced LncRNA out of the nucleus, where it mainly became localized in the cytoplasm.(Fig. 7)

Detection of the LncRNA target gene and related keratin expression in Liaoning CashmereGoat fibroblasts after LncRNA overexpression

In order to study the effect of LncRNA overexpression on velvet growth-related genes, an LncRNA overexpression vector was constructed by lentiviral-mediated technology and successfully transfected into Cashmere Goat skin fibroblasts. Treating skin cells with FGF5 yielded the following: FGF5 treatment inhibited the expression of LncRNA in cells, down-regulated the expression of the target genes CBS and CTH, and promoted the expression of related keratin genes k26, kap11.1. In order to study the effect of LncRNA on the expression of keratin and keratin-associated proteins—and to construct the relationship between FGF5, LncRNA and KAP family—we overexpressed LncRNA with FGF5 treatment which reversed the inhibiting effect of FGF5 on the target genes CBS and CTH; this further inhibited the expression of k26, kap11.1 in the cells (Fig. 8-9).

Detection of the LncRNA target gene and related keratin expression in Liaoning Cashmere Goat fibroblasts after LncRNA interference

An LncRNA interference vector was constructed by lentiviral-mediated technology and successfully transfected into Cashmere Goat skin fibroblasts. This down-regulated the expression of LncRNA in skin fibroblasts, and the expression of interfering lentiviral vector was detected by qPCR. The results showed that the interference target spots of 31, 131, 231 were significant, and the 131 target spot interference effect reaches 60%, so interference target spot 131 was used for subsequent experiments (Fig. 10). After treatment of skin cells with FGF5, qPCR showed that, when the expression level of LncRNA gene was decreased, the expression levels of the target genes CBS and CTH were inhibited while the expression levels of LncRNA and target genes decreased. The expression levels of the k26, kap11.1 genes all increased (Fig. 11). This indicates that the LncRNA gene plays a negative regulatory role in the function of keratin and keratin-associated protein genes.

Discussion

Liaoning Cashmere Goats are known for their high-quality cashmere and stable genetics. Hair follicle development and villus growth are affected by many factors, one of which is FGF5, a cytokine found to be directly related to hair length [36]. Since the cause of the growth of the Angora mouse hair was discovered, people have been working on the mechanism by which FGF5 influences hair follicle development and villus growth. However, people have been focused on the study of hair follicle development and villus growth related genes, ignoring most effects of non-coding RNA. In recent years, people have begun to pay attention to the regulation of non-coding RNA in various life activities, including hair follicle development and villus growth.

In this study, the FGF5-treated Liaoning Cashmere Goat skin cells were sequenced by RNA-seq sequencing technology, and LncRNA related to hair follicle development and villus growth were screened. We first selected LncRNA XLOC_011424 (LncRNA) for research and established the association between LncRNA and the target genes CBS, CTH, keratin K26 and the keratin-associated proteins KAP11.1. We worked toward explaining the effect of LncRNA XLOC_011424 on regulating hair follicle cycle development and villus growth under FGF5 treatment.

In addition, results of the experiment showed that amino acid metabolism in the cell metabolic pathway is related to hair follicle development and villus growth. It has been reported that amino acid types and contents can affect hair follicle development and villus growth; one of these—cysteine—was found to be involved in the expression of keratin, meaning it affects the development and maturation of hair follicles [37]. Studies have shown that the development of hair follicles and the growth of villi are affected by the type and content of amino acids, that is, the amino acid metabolism pathway can affect the development of hair follicles and the growth of villi.

In this experiment, we found that the target genes corresponding to LncRNA XLOC_011424 are distributed in the amino acid metabolic pathway, which is part of the cellular metabolic pathway, mainly involved in the conversion between serine and cysteine, serine and threonine, indicating indirectly that these amino acids are involved in hair follicle development and regulation of villus growth. We found that cysteine and serine are abundant in keratin and keratin-associated proteins, which are the main components of hair fibers. In the regulation of FGF5 on hair follicle development and villus growth, FGF5 synthesizes cysteine and serine by affecting LncRNA regulation of amino acid metabolism pathway, thereby affecting the expression of keratin and keratin-associated proteins, ultimately affecting hair follicle development and villi growth. In this experiment, interference lentiviral technology and overexpression lentivirus technology were used to clarify the regulation of LncRNA in villus growth and development. The results showed that LncRNA under the action of FGF5 further promotes Cashmere Goat hair follicle growth and development by regulating the expression of target genes and related keratin family genes.

In summary, this study found for the first time that LncRNA is related to the FGF5 regulation of hair follicle development and villus growth; it also demonstrates that the amino acid metabolism pathway is related to hair follicle development and villus growth to some extent. The mechanism by which FGF5 regulates hair follicle development and villus growth by overexpression and interference lentivirus technology further lays a theoretical foundation for molecular-assisted breeding of Liaoning Cashmere Goats.

Conclusion

We demonstrated that treatment of skin cells with FGF5 inhibited the expression of LncRNA in cells, down-regulated the expression of the target genes CBS and CTH, and promoted the expression of related keratin genes k26, kap11.1. Then, overexpressing LncRNA in skin cells reversed the inhibiting effect of FGF5 on the target genes CBS and CTH which further inhibited the expression of k26, kap11.1. Finally, we demonstrated the inhibition of CBS and CTH and elevation k26 and kap11.1 genes when the expression level of LncRNA gene is downregulated using RNA interference. Therefore, we believe that FGF5 can regulate the growth and development of Cashmere Goat hair by promoting the expression of related keratin and keratin-associated protein genes. This mechanism is achieved by inhibiting the expression of the LncRNA gene and then down-regulating the expression of the target genes CBS and CTH.

Methods

Collection of experimental samples and FGF5 drug treatment

The research protocol of this experiment was approved by the Animal Protection and Use Committee of the College of Life Sciences of Liaoning Normal University and the Chinese Agriculture Committee. The animal and experimental operations involved were guided by the animal protection and treatment system.

In mid-September, six adult male Liaoning Cashmere Goats (males have a high cashmere yield) were randomly selected from the Liaoning Cashmere Goat Production Base (Dalian Wafangdian City, Liaoning Province). They were locally anesthetized with 5% procaine (Sangon, Shanghai, China) before skin biopsy. then the subcutaneous adipose tissue was removed, cut into pieces under sterile conditions and cultured with cell tissue adherent method. After the small pieces of tissue adhere to the wall, add 10ml of DMEM medium containing 20% FBS to the bottom of the bottle. After 2-3 days of culture, when the cells grow to 70% -80% confluence, add trypsin to digest. When the cells become round Then, add DMEM medium containing 10% FBS to terminate the digestion. The bottom of the bottle was repeatedly blown with a pipette to make the cells fall off the bottom. The cell mixture was transferred to a centrifuge tube and centrifuged. The supernatant was discarded. 2 ml of DMEM containing 20% FBS party was added to the centrifuge tube, and then the cells were repeatedly blown to divide cells evenly into two flasks, add 7ml of DMEM culture solution containing 20% FBS, and place them in a 37°C 5% CO₂ incubator for cultivation.

Screening of target LncRNA and functional enrichment analysis of target genes

Total RNA was purified from Goat fibroblasts with TRIzol (TaKaRa, Dalian, China). The quality of the total RNA was detected with a NanoPhotometer® spectrophotometer (Implen, Westlake Village, CA, USA), and RNA samples were treated with DNase I (Bao Bioengineering (Dalian) Co. Ltd.). Agarose gel electrophoresis was performed and a NanoDrop1000 micro-UV-visible spectrophotometer and Agilent Technologies 2100 Bioanalyzer was used to determine RNA integrity and a Qubit Fluorometer was used to accurately quantify RNA concentration.

After the RNA was qualified, a library was built; then the library was qualified, and four sets of RNA-Seq library samples were sequenced and evaluated for sequencing data using the Illumina HiSeq™ 2500 (Beijing Novo Zhiyuan Bioinformatics Co., Ltd.) with the PE125 sequencing strategy. The TopHat2 algorithm was used for sequence alignment analysis, and LncRNA was screened and its coding potential analyzed using CPC, CNCI, pfam protein domain and PhyloCSF analyses; the final LncRNA dataset was obtained from the intersection of these methods, Improve the reliability of the screening results. The Cncdiff (<http://cufflinks.cbc.umd.edu/manual.html#cuffdiff>) software was used to quantitatively analyze the LncRNA obtained in the experiment, screen out eligible LncRNA (P-adjust < 0.05, log₂ (Fold change) > 1), and perform cis/trans target gene prediction. We performed GO and KEGG enrichment analyses on target genes to better understand the function of differentially-expressed LncRNA target genes. The expression of the target LncRNA was then verified, the expression data of the target gene were

analyzed by the 2- $\Delta\Delta$ Ct method, and a significant difference analysis was performed using the IBM SPSS Statistics 19 software.

qPCR detection of k26, kap11.1 gene expression levels in cells after FGF5 treatment

qPCR was used to detect the expression levels of k26, kap11.1 genes in cells after FGF5 treatment. TRIzol reagent was used to extract total RNA from samples. Total RNA samples were collected from cells treated with 10⁻⁴ g/L FGF5 for 72 h. RNA samples were treated with DNase I prior to qPCR. Total RNA in the sample was quality tested again. Reverse transcription was carried out according to the instructions of the qPCR kit (Ruisai Biotechnology Co., Ltd., Shanghai), The relative quantification of each gene expression was performed using the $\Delta\Delta$ Ct method. The cDNA was stored at -20 °C. Primers are listed in Table 3.

Table 3. qPCR detection primer information

Primer name	Primer sequence(5'-->3')	Products
goat ACTB-F1	GATGGCTACTGCTGCGTCG	208bp
goat ACTB-R1	GGCATAACAGGTCCTTTTCGG	
goat Kap11.1-F1	CGTACCAGCAGTCCTGCGTG	196bp
goat Kap11.1-R1	GCCAAAGGCGGGCTTATTC	
goat K26-F1	ACAACATGAGGGCTGAGTACGAG	184bp
goat K26-R1	TGAAGTTCTATTTCCAAGGTTTGC	

The total volume of the reaction system was 20 μ L, including 10 μ L of 2*SYBR Green Mix, 1 μ L of primer Mix, 5 μ L of template and 4 μ L of ultrapure water. The PCR conditions applied were pre-formed at 95 °C for 10 s, then subjected to 40 cycles of 95 °C denaturation for 5 s, 60 °C annealing for 30 s, and 72 °C extension for 60 s.

FISH fluorescence localization detection

Experimental materials and instruments are shown in Table 4.

Table 4. FISH fluorescence localization experimental materials and instruments

Name	Manufacturer
Liaoning cashmere goat skin fibroblast	Primary culture of cells in lab,Dalian
FBS	Hyclone, USA
EDTA Na2	Solarbio Co., Ltd., Beijing
SDS	Solarbio Co., Ltd., Beijing
Maleic acid	Solarbio Co., Ltd., Beijing
Tris	Solarbio Co., Ltd., Beijing
LncRNA FISH Probe	Ruibo Biotechnology Co., Ltd., Guangzhou
Laser confocal microscope 1 Leica-SP5	Leica Germany

Liaoning Cashmere Goat skin cell culture and FGF5 treatment was performed as previously described in this study, prepare buffer solution, different concentrations of absolute ethanol solution and DAPI solution, etc.—was used to process the samples and carry out sample hybridization, and finally the samples were DAPI stained, sealed, observed under a confocal microscope, and photographed.

Detection of expression levels of cashmere growth-related genes after LncRNA overexpression

The target gene PCR product and target vector were digested with *ASCI* and *Pme1* (New England Biolabs, USA), respectively. *T4* DNA ligase (New England Biolabs, USA) ligated the digested PCR product and destination vector and 10 μ L of the ligated product was transformed into DH5 α competent cells (Full Golden Biotechnology Co., Ltd., Dalian). The cells were coated onto an LB plate containing ampicillin (Amp) resistance and cultured overnight at 37 °C; then positive clones were identified. Opti-MEM, expression plasmid, packaging plasmid Packaging Mix (Invitrogen, USA), POLO deliverer TM 3000 Transfection Reagent(all from Invitrogen)were added to the culture medium of 293T cells (Jima Biotechnology Co., Ltd., Shanghai), shaken well, and cultured at 37 °C in a 5% CO₂ cell culture incubator (Likang Bios, Shanghai). The cells were collected after transfection for a certain period of time. Finally, LncRNA overexpressing lentivirus was transfected into Cashmere Goat fibroblasts, and the expressions of LncRNA and cashmere growth-related genes were detected by qPCR.

Detection of expression levels of cashmere growth-related genes after LncRNA interference

Three interfering target spots were designed based on the target LncRNA sequence. The interference vector with the target gene was constructed as a template, Lenti-Asc1 -F/Lenti -Pme1 -R were used as primers, and the EGFP-ABCA1-miR fragment was amplified by PCR, forming the enzyme cleavage sites *Ascl* and *Pme1* at both ends of the target fragment. *T4* DNA ligase (New England Biolabs, USA) ligated the digested PCR product and the destination vector, and transformed 10 μ L of the ligated product into DH5 α competent cells (Full Golden Biotechnology Co., Ltd., Dalian), which were coated onto an LB plate containing ampicillin (Amp) resistance, cultured overnight at 37°C, and positive clones were identified. Opti-MEM, expression plasmid, packaging plasmid Packaging Mix (Invitrogen, USA), POLO deliverer TM 3000 Transfection Reagent were added to the culture medium of 293T cells (Jima Biotechnology Co.,

Ltd., Shanghai), shaken well, cultured at 37 °C in a 5% CO₂ cell culture incubator (Likang Bios, Shanghai). The cells were collected after 48h Finally, LncRNA-interfering lentivirus was transfected into Cashmere Goat fibroblasts, and the expressions of LncRNA and cashmere growth-related genes were detected by qPCR.

Abbreviations

FGF5:Fibroblast growth factor 5

LncRNAs:long non-coding RNAs

KAPs: keratin-associated proteins

PHF:primary hair follicles

SHF:secondary hair follicles

HFT:hair follicle stem cells

DP: dermal papilla

cDNA: complementary deoxyribonucleic acid

DAPI: 4'6-diamidino-2-phenylindole

qPCR: Real-time Quantitative PCR Detecting System

DNase I:Deoxyribonuclease I

Amp:ampicillin.

Declarations

Ethics approval and consent to participate:The study has been approved by the Ethics Committee of Liaoning Normal University, and Liaoning Cashmere Goat has obtained permission before the study. The Liaoning Provincial Key Laboratory of Biotechnology and Drug Research is responsible for ensuring the protection and care of vertebrates used in the study. We must ensure that animal research is conducted in accordance with long-term ethical principles and federal, state, and university regulations.

Consent for publication : Not Applicable

Availability of data and material: The datasets generated and/or analysed during the current study are not publicly available due Laboratory data confidentiality but are available from the corresponding author on reasonable request.

Competing interests : The authors declare that they have no competing interests.

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Authors' contributions:

JM and ZF contributed to the work equally and should be regarded as co-first authors.They are Project managers, responsible for the application, implementation, supervision, summary and acceptance of the project and the procurement of experimental consumables and drugs, cell culture, sample preparation.

NP: Responsible for statistical analysis of data.

PJ1: Responsible for collecting the latest research status of similar research topics at home and abroad.

PJ2: Responsible for the acceptance of the results of the phased experiments and the analysis and organization of data.

All authors read and approved the final manuscript.

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Figures

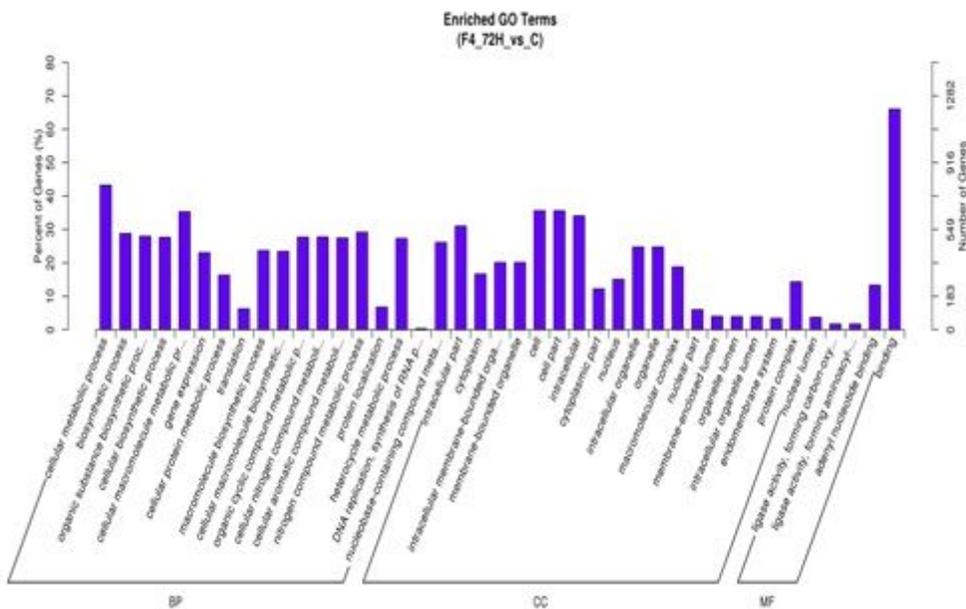


Figure 1

GO term classification of differentially expressed LncRNA target genes between the F4_72h and C group. Abscissa represents enrichment of the GO term, ordinate represents the number of target genes in this term and the percentage of target and annotated genes.

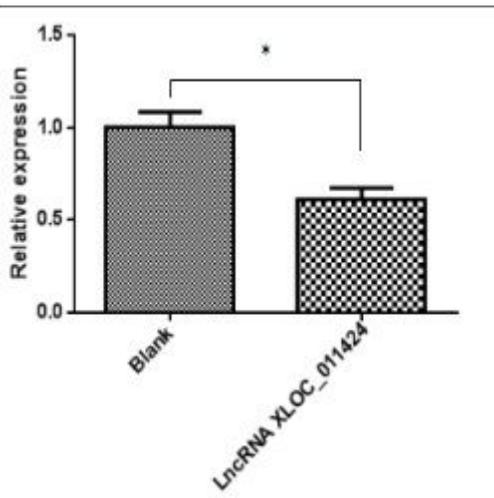


Figure 2

Expression of LncRNA XLOC_011424

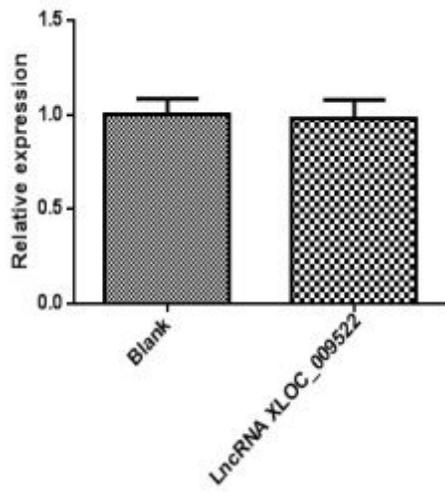


Figure 3

Expression of LncRNA XLOC_009522

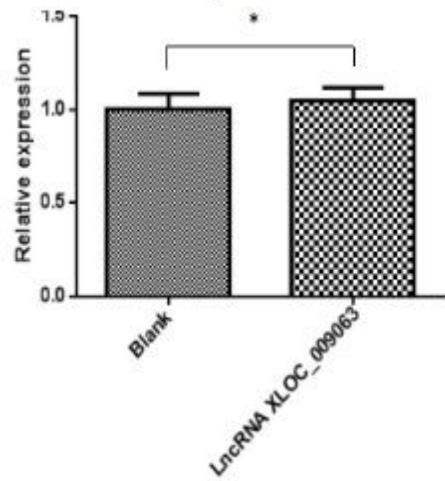


Figure 4

Expression of LncRNA XLOC_009063

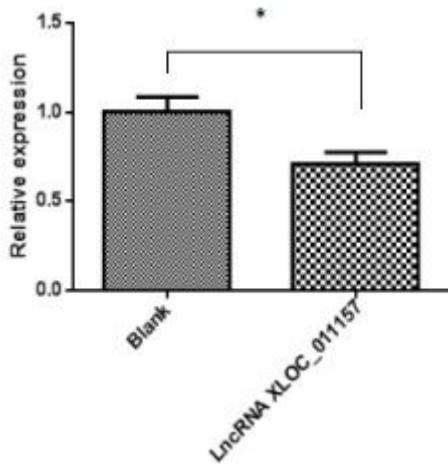


Figure 5

Expression of LncRNA XLOC_011157 *p < 0.05

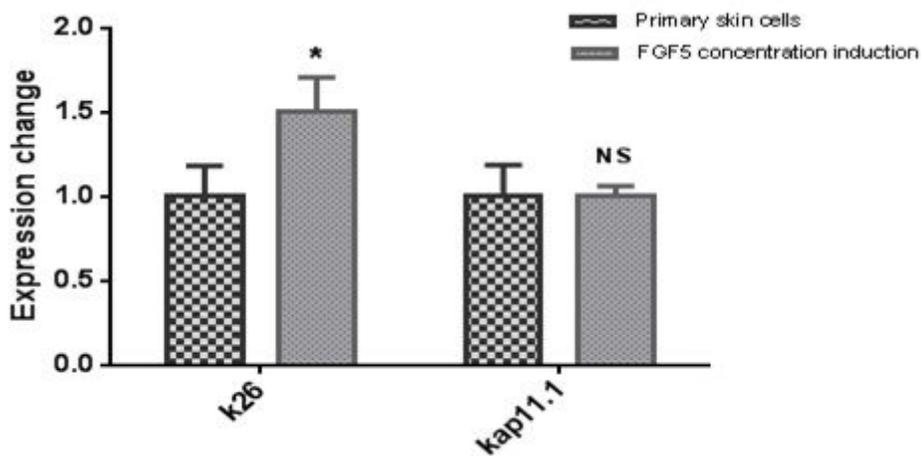


Figure 6

Results of fluorescence quantitative PCR expression of LncRNA keratin gene. Data are the mean \pm SD of three independent experiments, NS (not significant, P > 0.05)

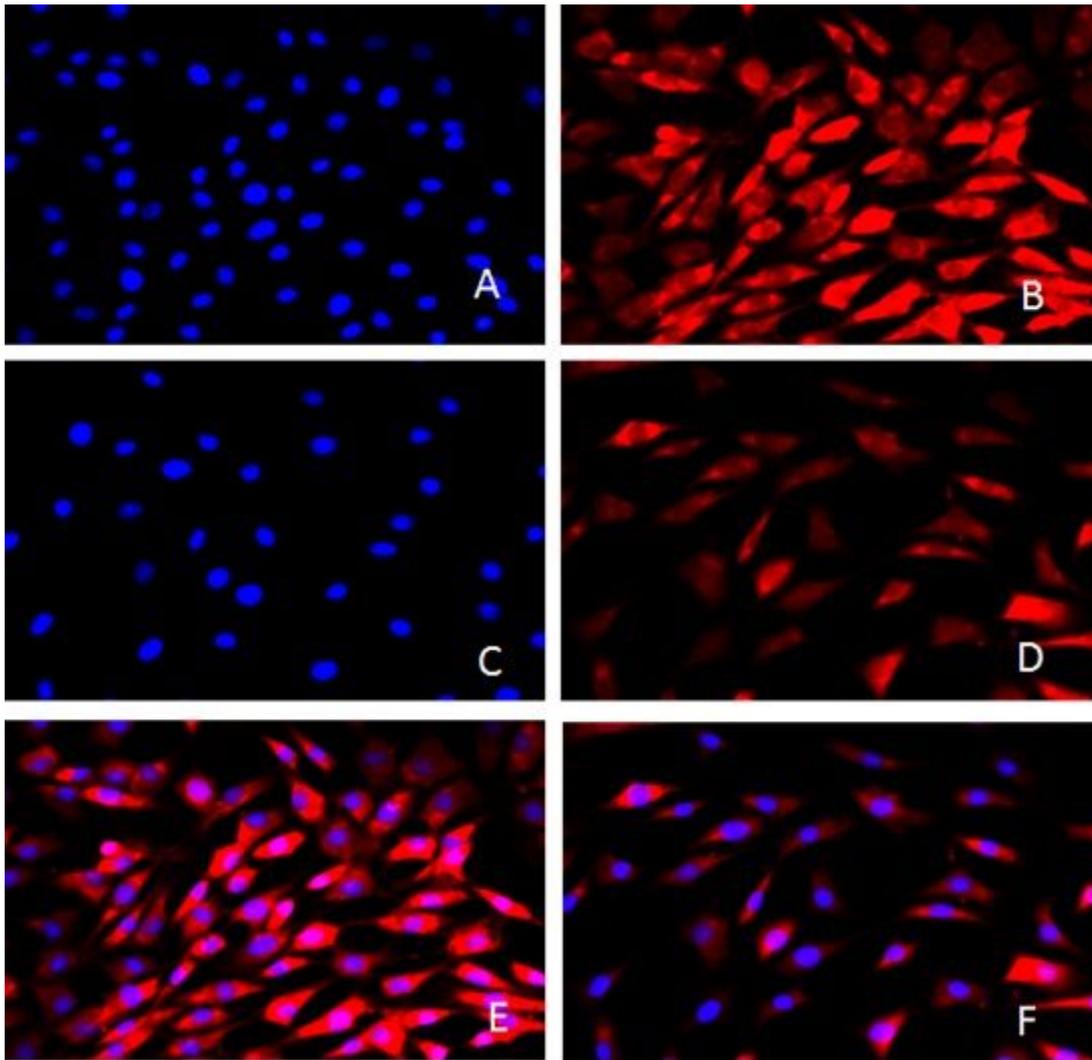


Figure 7

Intracellular mapping of LncRNA before and after treatment with FGF5. Fig. A shows the location of nuclei in untreated sheep skin cells (40x). Fig. B shows the location of the target LncRNA in untreated sheep skin cells (40x). Fig. C shows the location of nuclei in sheep skin cells treated with FGF5 (40x). Fig. D shows the intracellular location of target LncRNA in sheep skin cells treated with FGF5 (40x). Fig. E is a merge diagram of the nucleus and LncRNA sites in untreated sheep skin cells (40x). Fig. F is a merge diagram (40x) of the nucleus and LncRNA sites in sheep skin cells treated with FGF5. The blue fluorescent stain is nuclear staining, and the red fluorescence represents LncRNA staining.

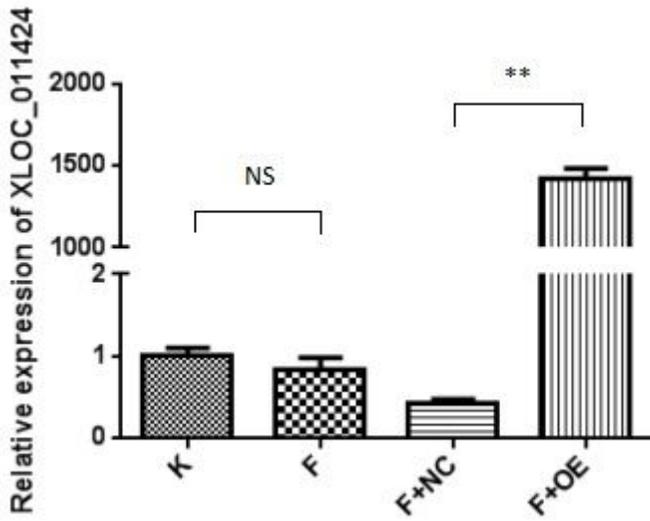


Figure 8

Change in LncRNA expression after LncRNA XLOC_011424 overexpression, as detected by qPCR (**p < 0.01). K indicates blank cells, F indicates cells treated with FGF5, F + NC indicates negative control cells treated with FGF5, and F + OE indicates cells overexpressed with LncRNA after FGF5 treatment. Data are the mean ± SD of three independent experiments, NS (not significant, P > 0.05)

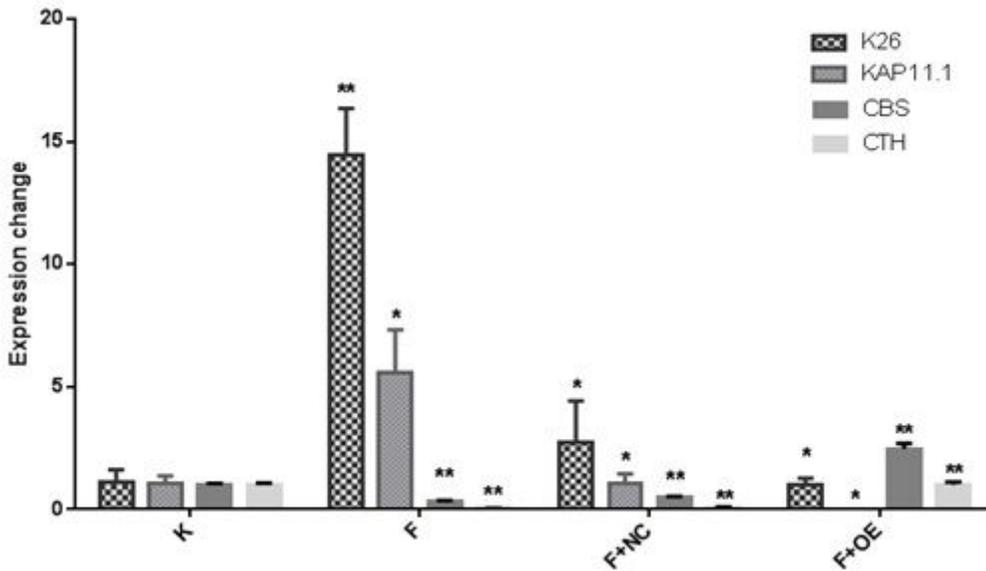


Figure 9

Change in LncRNA keratin gene and target gene expression after LncRNA overexpression, as detected by qPCR (**p < 0.01). K indicates blank cells, F indicates cells treated with FGF5, F + NC indicates negative control cells treated with FGF5, and F + OE indicates cells overexpressed with LncRNA after FGF5 treatment.

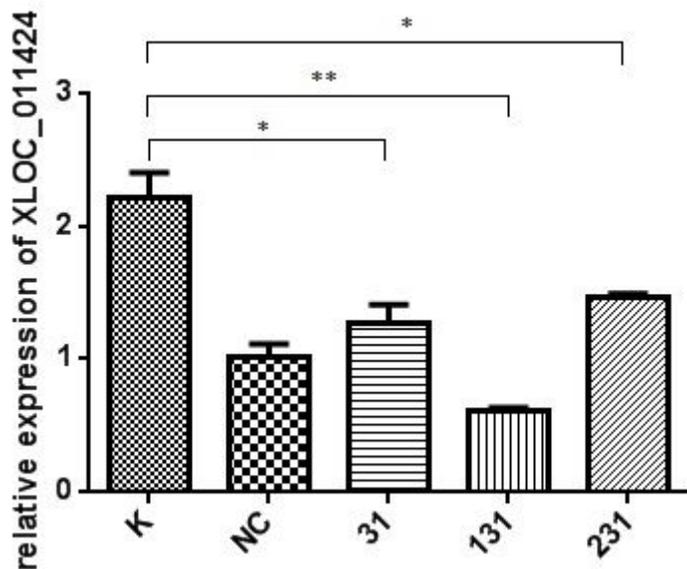


Figure 10

LncRNA gene expression by qPCR. The abscissa is the blank control group, NC is the negative control, and experimental is the group of target spots 31, 131, 231 * $p < 0.05$ ** $p < 0.01$

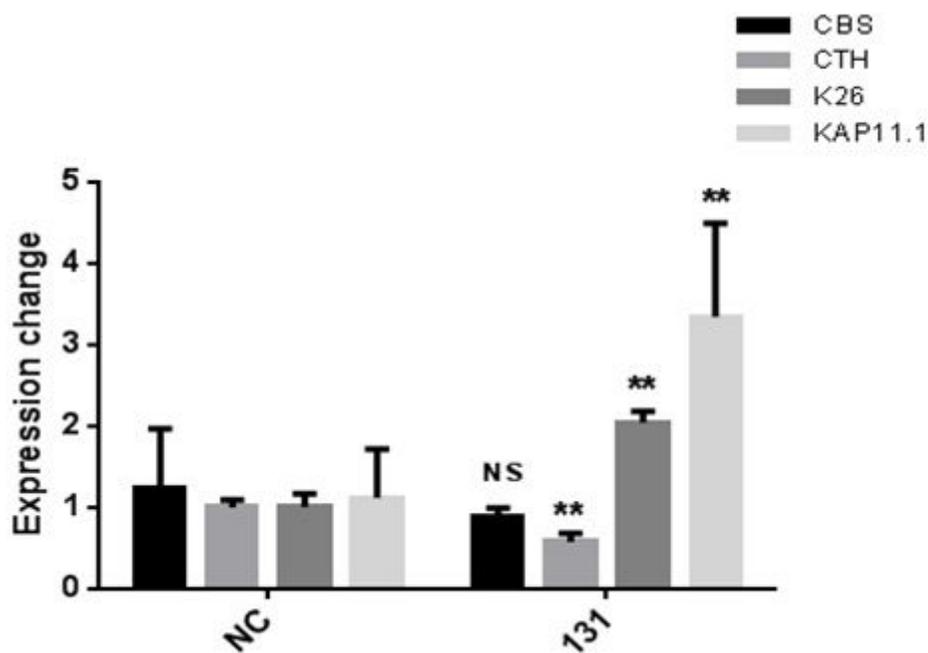


Figure 11

Change in LncRNA keratin gene and target gene expression after LncRNA under-expression was detected by qPCR ** $p < 0.01$. indicates cells overexpressed with LncRNA after FGF5 treatment.