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Research Article

Effects of FGF5-mediated LncRNA on the skin fibroblast growth of Liaoning Cashmere goats

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Abstract

In the present study, long non-coding RNA associated with hair growth in the goats was screened and identified using RNA sequencing technology, and its impact on the growth of Cashmere goats treated with fibroblast growth factor 5 was evaluated. GO and KEGG analysis confirmed that the best treatment condition of FGF-5 was 10^{-4} g/L treatment 72H (F4_72H). We studied cystathionine at high and low expression of LncRNA β synthase (CBS), cystathionineγ, the expression changes of cleaving enzyme (CTH), keratin 26 (k26) and keratin associated protein 11-1 (kap11.1). Results show that FGF-5 can suppress the showing of LncRNA in skin cells, lower the showing of target genes CBS and CTH, and promote the showing of related keratin genes k26 and kap11.1. FGF-5 may regulate Cashmere hair growth and development by promoting the showing of related keratin and keratin-associated protein genes (KAPs). This mechanism is achieved by suppressing the showing of the LncRNA gene and also by lowering the showing of the target genes CBS and CTH.

Keywords: Cashmere, FGF-5, Long non-coding RNA, Protein

Highlights

- This paper determined that LncRNA regulates the expansion of villi by regulating target genes and keratin genes in the amino acid metabolism pathway.
- This paper first found that FGF-5 can have an impact on how keratin family members in Cashmere goat skin cells by regulating the expression of LncRNA.

INTRODUCTION

The Liaoning Cashmere goat is a breed unique to China, whose hair and meat have high functional value. The wool of Liaoning Cashmere goats (hereinafter referred to as "the Goat") is of excellent quality and has a high wool content. It is a very precious textile fiber and has the reputation of being the "fiber gem" (Hu *et al*., 2013; Zhu *et al*., 2018). In mammals, hairs are skin derivatives that grow and develop from hair follicles; one of the most remarkable features of hair follicles is that they renew themselves and continue to produce new hairs throughout the life of the individual (Welle and Wiener, 2016).

Hair follicles are an important structure for regulating villus growth. There are two main categories: primary and secondary hair follicles, and primary hair follicles (PF) can be further differentiated into secondary hair follicles (SF). The primary hair follicle produces hair fibers, and the secondary hair follicle produces velvet fibers (Dong *et al*., 2013). 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 (FGF-5) regulates the hair follicle from the anagen phase to the resting phase, altering the growth cycle of the hair-follicle cells. FGF-5 is a cell growth factor obtained and purified from the mammalian pituitary gland. It has been found that FGF-5 can advance the hair-follicle growth cycle into the telogen period and inhibit hair growth (Rogers *et al*., 2002). Drögemüller *et al*. (2010) found that mutations in the FGF-5 gene can cause genetic hair length variation in mice and dogs. Hébert *et al*. (1994) reported that if the FGF-5 gene was silenced in mice, the hair-follicle anagen phase would be prolonged, and mice would have longer hairs, thus extending the resting phase of the hair follicle. The FGF-5 gene is also a determinant of the long hair phenotype in domestic cats. According to Pallotti *et al*. (2018), the long isoform of the alpaca FGF-5 gene prematurely causes a point mutation in the stop codon (PTC), which lengthens the alpacas' hair. Sox2 uses FGF-5 to repair hair-follicle cells in the skin (Johnston *et al*., 2013; Kregel *et al*., 2013). The aforementioned findings imply that the FGF-5 factor is crucial for the villi's growth and development as well as the hair follicle's cyclical alterations. Therefore, it is

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crucial to conduct a study on the part of FGF-5 in the rise of villus and hair follicles.

The development of RNA sequencing (RNA-seq) technologies and mapping of expressed transcripts revealed that while the human genome is pervasively transcribed, only a small fraction of RNAs (~ 2%) code for proteins (Birney *et al*., 2007). A great deal of expressed transcripts lacks the code for proteins; those longer than 200 nt are generally referred to as long non-coding RNAs (LncRNAs) (Hangauer *et al*., 2013). 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 (Klattenhoff *et al*., 2013). In addition, LncRNAs control genes involved in postnatal hair cycle and hair follicle development (Lin *et al*., 2014). LncRNA AK015322 (LncRNA5322) regulation in hair-follicle stem cells (HFT) and a putative mechanism for IncRNA5322-mediated HFT differentiation were both examined by Cai *et al*. (2018). The results showed that LncRNA5322 can target miR-21-mediated PI3K-AKT Signaling pathways in HFT and encourage the development and proliferation of HFT (Cai *et al*., 2018). In 2017, the non-coding RNA database LncRNASNP2 began providing comprehensive information on LncRNA mutations, also, the structure and function of LncRNAs. There are 117,405 mice and 141,353 human LncRNAs in the database. Researchers have discovered LncRNAs as a result of cattle growth (Huang *et al*., 2012; Weikard *et al*., 2013).

It has been discovered that keratin and the keratin-associated protein (KAP) family are essential for villi production in Cashmere goats. The KAP family is more prevalent in mammalian hair follicles, some keratin genes are involved in the differentiation of hair tissue, and reduction of the keratin K25 gene can result in hair loss (Nanashima *et al*., 2008). Additionally, the K17 gene can alter the growth cycle of villi (Tong and Coulombe, 2006). It has been learned that the KAP16.6 and KAP13.1 genes regulate the size of the villous fiber diameter in sheep (Meng *et al*., 2009; Liffers *et al*., 2011). To be able to keep epithelial cells flexible and structurally sound, the KAP18 gene is crucial (Eriksson *et al*., 2009; Duncan *et al*., 2012). The Cashmere goat KAP13.3 gene may affect the architecture of keratin-associated protein 13.3, which has 156 amino acids, as well as the properties of Cashmere fibers (Andrews *et al*., 2017). Keratin and keratin-associated proteins (KAP), which make up the majority of wool, are made of amino acids. Therefore, understanding the KAP family is crucial to controlling villi growth.

About 90% of the villi in the goat used in this experiment are made of keratin and proteins related to keratin. We discovered that LncRNA participates in the metabolic route for amino acids and controls the creation of 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. As a result, we think that FGF-5 can influence the showing of LncRNA in Cashmere goat skin cells, which can further influence the growth and development of Cashmere goat villi as well as the quality of villi.

MATERIALS AND METHODS

FGF-5 medication therapy and sample collection for research: The Chinese Agriculture Committee and the College of Life Sciences at Liaoning Normal University both gave their approval to the research protocol for this investigation. The animal and experimental operations involved were guided by the animal protection and treatment system.

In mid-September, six adult male goats (males have a high Cashmere yield) were randomly selected from the 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 the cell tissue adherent method. After the small pieces of tissue adhered to the wall, 10 mL of Dulbecco's modified eagle medium (DMEM) containing 20% fetal bovine serum (FBS) was added to the bottom of the bottle. After 2-3 days of culture, when the cells grew to 70% -80% confluence, trypsin was added to digest. When the cells became round, DMEM medium containing 10% FBS was included to terminate the digestion. The portion of the bottle's base was repeatedly blown with a pipette to make the cells fall off to the bottom. The cell mixture was transferred to a centrifuge tube and centrifuged. The supernatant was discarded. 2 mL of DMEM containing 20% FBS was added to the centrifuge tube. Then the cells were repeatedly blown, distributed evenly into two flasks, added 7 mL of DMEM culture solution containing 20% FBS, and placed in a 37°C, 5% Carbon dioxide (CO_2) 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 Deoxyribonuclease I (DNase I) [Bao Bioengineering (Dalian) Co. Ltd.]. Agarose gel electrophoresis was performed, and a NanoDrop1000 micro-UV-visible spectrophotometer and Agilent Technologies 2100 Bioanalyzer accustomed to determine RNA integrity and a Qubit Fluorometer accustomed to accurately quantifying RNA concentration.

After the RNA was qualified, a library was built. The library was qualified, and four sets of RNA-Seq library samples were sequenced and evaluated for sequencing data using the Illumina Hiseq TM 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 data set was obtained from the intersection of these methods, improve the reliability of the screening results. The Cncdiff (http://cufflinks.cbcb.umd.edu/manual.html#cuffdiff) software accustomed 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 showing 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 FGF-5 treatment: Quantitative Real-time PCR (qPCR) was used to detect k26, kap11.1 genes' expression levels after FGF-5 treatment. TRIzol reagent was accustomed to extract total RNA from samples. Total RNA samples were collected from cells treated with 10^{-4} g/L FGF-5 for 72 h. RNA samples were treated with DNase I prior to qPCR. The 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), and the relative quantification of each gene expression was performed using the ΔΔ Ct method. The cDNA was stored at -20°C. Primers are listed in Table 1.

The total volume of the reaction system was 20 μL, including 10 μL of 2*SYBR Green Mix, 1 μL of primer Mix, 5 μL of template and 4 μL 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.

Materials and equipment for conducting experiments on FISH fluorescence localization are presented in Table 2.

The goat skin cell culture and FGF-5 treatment were carried out in this investigation as previously reported,

Table 1. qPCR detection primer information

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

Table 2. 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 (Leica-SP5)	Leica, Germany

preparing buffer solution, different concentrations of absolute ethanol solution and 4′6-diamidino-2-phenylindole (DAPI) solution, etc.—accustomed to process the samples and carry out sample hybridization, and finally, the samples were DAPI stained, sealed, observed under a confocal microscope, and photographed.

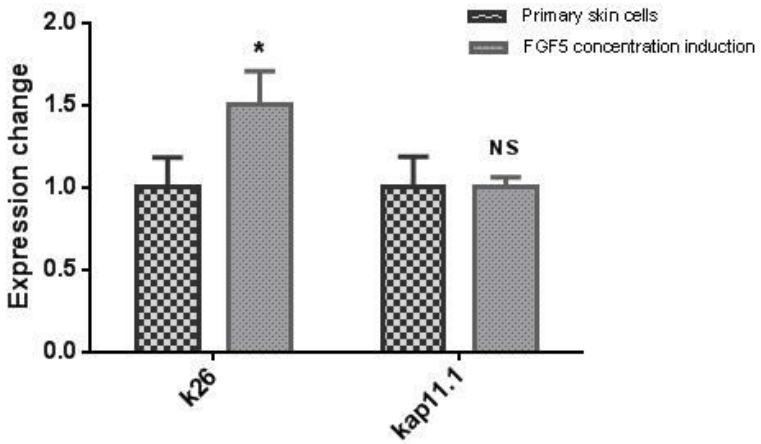
Cashmere growth-related gene expression levels after lncRNA overexpression: Asci and Pme1 (New England Biolabs, USA) were used to digest the target gene PCR product and the target vector, respectively. T4 DNA ligase (New England Biolabs, USA) ligated the digested PCR product and destination vector, and 10 μL of the ligated product was transformed into DH5α 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% CO_2 cell culture incubator (Likang Bios, Shanghai). After transfection, the cells were collected for a predetermined amount of time. Finally, Cashmere goat fibroblasts were transfected with a LncRNA overexpressing lentivirus, and qPCR was accustomed to identify the showing of LncRNA and genes relevant to cashmere growth.

Genes involved in cashmere growth are detected 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 was used as primers, and the EGFP-ABCA1-miR fragment was amplified by PCR, forming the enzyme cleavage sites AscI and PmeI at both ends of the target fragment. T4 DNA ligase (New England Biolabs, USA) ligated the digested result of PCR and the destination vector, and transformed 10 μL of the ligated product into DH5α 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 supplemented the culture medium of 293T cells (Jima Biotechnology Co., Ltd., Shanghai), shaken well, cultured at 37°C in a 5% CO_2 cell culture incubator (Likang Bios, Shanghai). After 48 hours, the cells were harvested. Finally, Cashmere goat fibroblasts were transfected with LncRNA-interfering lentivirus, and qPCR was accustomed to identify the showing of LncRNA and genes relevant to Cashmere growth.

RESULTS

qPCR detection of k26, kap11.1 gene expression in cells after FGF-5 drug treatment: K26 and kap11.1 gene expression levels were found by qPCR following FGF-5 treatment of Cashmere goat skin cells (Fig. 1). The outcomes demonstrated that the cells with keratin26 genes that were treated with FGF-5 had up-regulated mRNA levels and higher expression levels when compared to untreated cells. The kap11.1 gene's expression did not change significantly. This indicated that FGF-5 played a positive role in the regulation of keratin-related gene k26 but had no clear effect on the keratin-related gene kap11.1 was observed.

Analysis of differentially expressed LncRNA target



[Data are the mean ± SD of three independent experiments, NS (not significant, P > 0.05)]

Fig. 1. Results of fluorescence quantitative PCR expression of LncRNA keratin gene

Table 3. 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

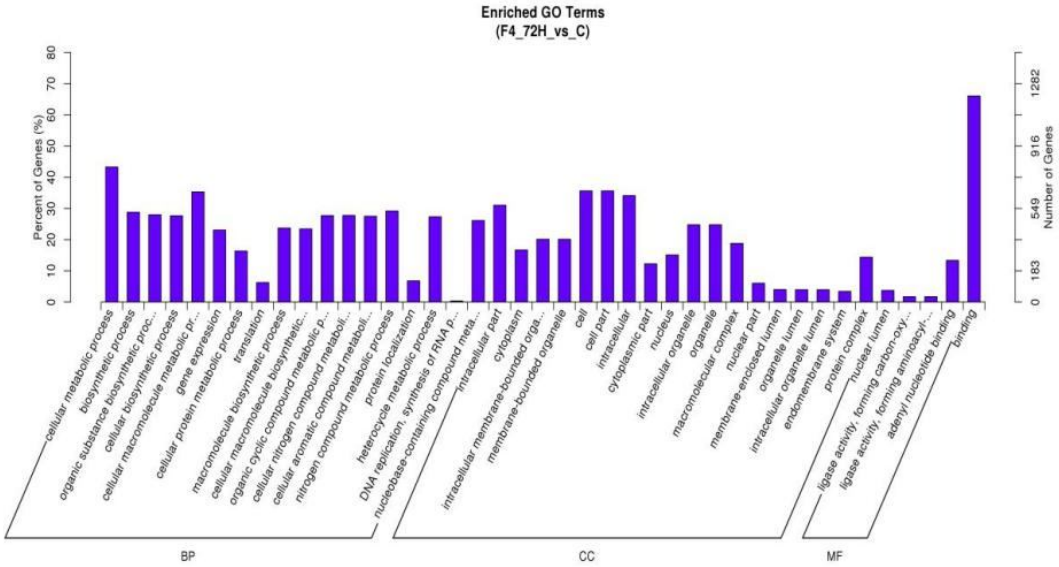


Fig. 2. GO term classification of differentially expressed LncRNA target genes between the F4_72h and C group. Abscissa represents enrichment of the GO term, ordinate represents the number of target genes in this term and the percentage of target and annotated genes

genes' functional enrichment: 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 study of differences in expression that LncRNA is shown in Table 3 (screening threshold is Q-value <0.05).

The outcomes above demonstrated 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 was obtained, of which 27 were up-regulated, and 96 were down-regulated.

After differentially-expressed LncRNA was 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 GO enrichment results were most significant in the F4_72h treatment group (Fig. 2).

The KEGG pathway enrichment analysis was performed on the target genes of differentially-expressed LncRNA in the three groups (Fiscreening conditions: Q-value< 0.05). The results are shown in Table 4.

Expression verification of target LncRNA and FISH immunofluorescence localization: Combined with the screening of LncRNA, prediction of target genes, and bioinformatics analysis of LncRNA, it was finally determined that FGF-5 had the most beneficial effects on hair-follicle development and villus growth when it treated the goat skin cells with 10^{-4} g/L for 72 h. The target genes enriched in the Metabolic pathways were analyzed, and the corresponding LncRNAs were found to be XLOC_011424, XLOC_009522, XLOC_009063, and XLOC_011157. Therefore, in the experiment, we screened four LncRNAs from the 10^{-4} g/L 72 h treatment group that were associated with the activity of FGF-5. We used the qPCR method to find the showing of the

Table 4. KEGG enrichment analysis results

	Sample name	Significantly enriched pathway term	Q value
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	-	-

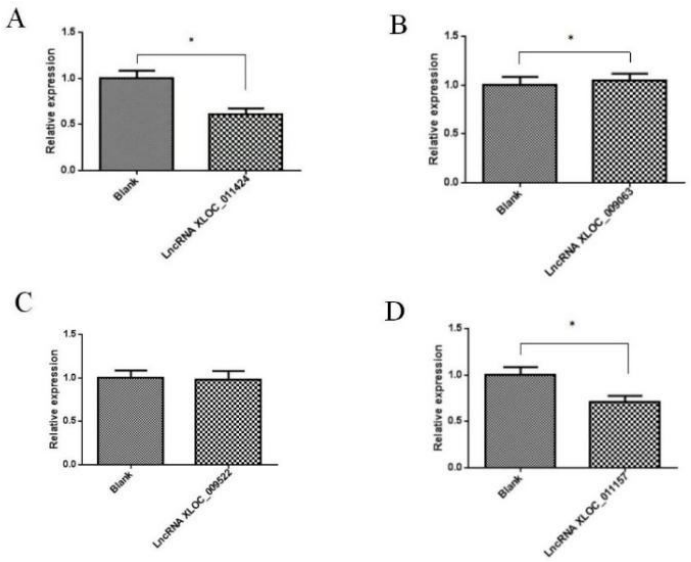


Fig. 3. A. Expression of LncRNAs XLOC_011424 (*: P<0.05); B. Expression of LncRNAs XLOC_009522; C. Expression of LncRNAs XLOC_009063 (*: P<0.05); D. Expression of LncRNAs XLOC_011157 (*:<0.05)

aforementioned four LncRNAs in order to further assess the correctness of the RNA-Seq data, as shown in Fig. 3.

The findings demonstrated that FGF-5 treatment decreased the showing of LncRNA XLOC_011424 (P<0.05) to 0.70 times that of the control group, increased the showing of LncRNA XLOC_009063 (P 0.05) to 1.12 times that of the control group, and decreased the showing of LncRNA XLOC_011157 (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. After giving the goat skin cells FGF-5, we first chose LncRNA XLOC_011424 as the target LncRNA to investigate its mechanism of action on fluff growth.

Using the FISH immunofluorescence localization approach, the target LncRNA's location in the skin cells of Cashmere goats was investigated. The nucleus is marked by blue staining reagent, and the target LncRNA is marked by red staining reagent, and overlapped blue and red stain values denote a mixture of the two. The findings of this study indicate that LncRNA is primarily found in the nucleus and cytoplasm of Cashmere goat

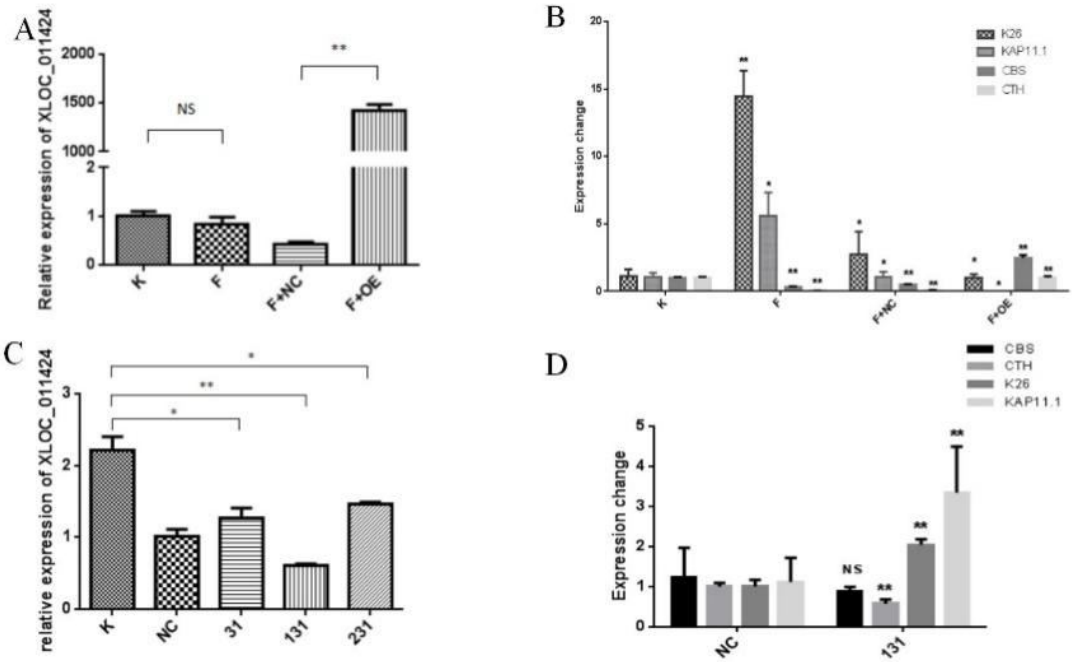


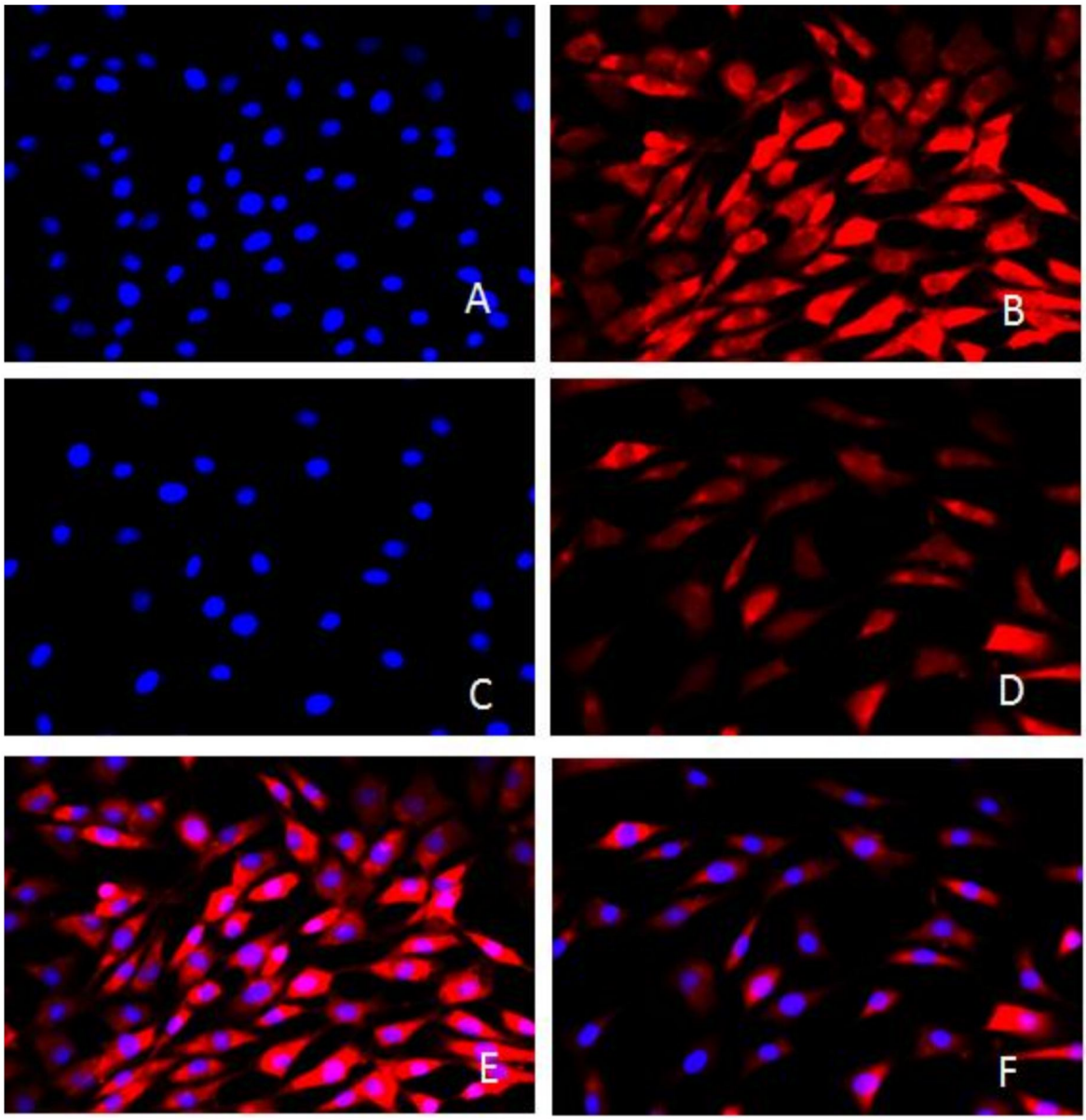
Fig. 5 A-B: K indicates blank cells, F indicates cells treated with FGF5, F + NC indicates negative control cells treated with FGF5, and F + OE indicates cells overexpressed with LncRNA after FGF5 treatment: A. Change in LncRNA expression after LncRNA XLOC_011424 overexpression, as detected by qPCR (p<0.01). Data are the mean ± SD of three independent experiments, NS (not significant, P>0.05); B. Change in LncRNA keratin gene and target gene expression after LncRNA overexpression, as detected by qPCR(**p<0.01); C. 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); D. Change in LncRNA keratin gene and target gene expression after LncRNA under-expression was detected by qPCR(**p<0.01), indicating cells overexpressed with LncRNA after FGF5 treatment.**

hair cells. After cells were treated with FGF-5, it was found that FGF-5 induced LncRNA out of the nucleus, where it mainly became localized in the cytoplasm (Fig. 4).

LncRNA overexpression/interference results in the identification of the LncRNA target gene and associated keratin expression in the goat fibroblasts: Using lentiviral-mediated technology, a LncRNA overexpression vector was created and successfully transfected into Cashmere goat skin fibroblasts to better understand the impact of LncRNA overexpression on velvet growth-related genes. FGF-5 treatment of skin cells led to the following results: FGF-5 therapy reduced the cellular expression of LncRNA, down regulated the showing of the target genes CBS and CTH, and heightened expression of related keratin genes k26 and kap11.1 in addition to promoting the production of these target genes. We overexpressed LncRNA with FGF-5 treatment, which reversed FGF-5's inhibitory effect on the target genes CBS and CTH and further inhibited the showing of kap11.1 in the cells, studying how LncRNA affects the production of keratin 5 and proteins linked with keratin and constructing the relationship between FGF-5, LncRNA and KAP family (Fig. 5A-B).

Lentiviral-mediated technology was accustomed to create a LncRNA interference vector that was effectively transfected into Cashmere goat skin fibroblasts. In skin fibroblasts, this reduced the showing of LncRNA, and qPCR is a method to determine the showing of the interfering lentiviral vector. The interference target spot 131 was chosen for the following trials (Fig. 5C) because the outcomes showed that the interference target spots of 31, 131, and 231 were substantial, with the 131 target spot interference effect reaching 60%. The target genes' levels of expression CBS and CTH were repressed whereas the manifestation levels of LncRNA and the target genes dropped after FGF-5 treatment of skin cells. This was demonstrated by qPCR. K26 and kap11.1 gene expression levels all increased (Fig. 5D). This suggests that the LncRNA gene negatively regulates the keratin and keratin-associated protein genes.

Effect of LncRNA on Liaoning Cashmere goat skin fibroblasts



[A: Shows the location of nuclei in untreated sheep skin cells (40x); B: Shows the location of the target LncRNA in untreated sheep skin cells (40x); C: Shows the location of nuclei in sheep skin cells treated with FGF5 (40x); D: Shows the intracellular location of target LncRNA in sheep skin cells treated with FGF5 (40x); E: It is a merge diagram of the nucleus and LncRNA sites in untreated sheep skin cells (40x); F: It 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]

Fig. 4. Intracellular mapping of LncRNA before and after treatment with FGF5

DISCUSSION

In this study, RNA-seq sequencing technology accustomed to sequence the FGF-5-treated goat skin cells and LncRNA linked to hair-follicle development and villus growth were screened. In order to conduct study, we first chose LncRNA XLOC_011424 (LncRNA) and determined its relationships to the target genes CBS, CTH, keratin K26, and the keratin-associated proteins KAP11. We tried to explain how LncRNA XLOC_011424 affected the growth of villus cells and the development of the hair-follicle cycle when FGF-5 was present.

In addition, the results of the experiment showed that amino acid metabolism in the cell metabolic pathway is related to hair-follicle development and villus growth. 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 discovered that the primary building blocks of hair fibers, keratin and proteins related to keratin are rich in cysteine and serine. By influencing the LncRNA regulation of the amino acid metabolism pathway, FGF-5 affects the synthesis of cysteine and serine, which in turn affects the showing of keratin and keratin-associated proteins, which in turn affects hair-follicle development and villi growth. This investigation utilized interference lentiviral technology and overexpression lentivirus technology to better understand how LncRNA is regulated in villus growth and development. The findings demonstrated that LncRNA, acting in conjunction with FGF-5, further enhances the growth and the creation of Cashmere goat hair follicles by controlling the showing of target genes and associated keratin family genes.

Additionally, to show that the amino acid metabolism route is somewhat connected to hair-follicle development and villus growth, this study, for the first time, discovered that LncRNA is related to the control of hair-follicle development and villus growth by FGF-5. The theory behind how molecularly aided breeding of the goats governs hair-follicle development and villus growth is furthered by the method by which FGF-5 regulates these processes using overexpression and interference lentivirus technology. The Cashmere output and quality of the goats were found to be improved by LncRNA XLOC_ 011424, XLOC_ 011157, XLOC_ 005914, and XLOC_ 018763. Whereas, amino acids are the fundamental components that makeup protein molecules, the protein is the primary carrier of life processes. L-cysteine, which is a crucial part of keratin and can stimulate various cellular pathways, is currently regarded as a conditionally essential thioamino acid. Melatonin, a nuclear factor corresponding to the LncRNA MTC effect-B signal transduction that is involved in hair-follicle development, is most beneficial for Cashmere growth at an intensity of 0.2 g/L for 72 hours, according to preliminary Gene ontology (GO) and pathway analysis findings from the lab. We discovered that the lncRNA XLOC_ 005914 (lncRNA MTC) was increased by melatonin. Cell proliferation rose in cells that express a lot of LncRNA MTC under 72-hour 0.2 g/L conditions, whereas it decreased in fibroblasts that express less LncRNA MTC. This shows that FGF-5 governs the creation of Cashmere and hair follicles through signal transduction and encourages fibroblast proliferation.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: MJ: Project managers, responsible for the application, implementation, supervision, summary and acceptance of the project and the procurement of experimental consumables and drugs, cell culture, and sample preparation; YPL, XYL: Responsible for statistical analysis of data; PN: Responsible for integration of experimental results; JP: Responsible for collecting the latest research status of similar research topics at home and abroad; JAP: 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.

Ethical Statement: All applicable international, national, and/or institutional principles for the care and use of animals have been observed.

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