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Original Article

Functional analysis of the PIP5K1A gene in Liaoning Cashmere goats: an investigation based on bioinformatics, tissue localization, and biological functions

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Abstract

Liaoning cashmere goat is an outstanding breed in China primarily for cashmere production, with strict controls against genetic outflow. Melatonin(MT) is a key factor affecting cashmere growth, and preliminary transcriptome sequencing indicated that melatonin upregulates the expression of the *PIP5K1A* gene in skin fibroblasts. To predict the physicochemical properties of PIP5K1A in Liaoning cashmere goats, ascertain the tissue localization of PIP5K1A in their skin, and explore the role and mechanism of PIP5K1A in the proliferation of skin fibroblasts. This aims to provide new insights into improving the yield and quality of cashmere. Bioinformatics software was used to predict the physicochemical properties, hydrophobicity, signal peptides, transmembrane regions, secondary structure, subcellular localization, and conserved sites of PIP5K1A, and to construct a phylogenetic tree for the *PIP5K1A* gene across different species. siRNA technology was employed to interfere with PIP5K1A in skin fibroblasts; lentiviral vector construction techniques were used to overexpress PIP5K1A in skin fibroblasts; Western blot analysis detected changes in protein expression in cells; RT-qPCR was used to detect changes in gene expression; cell viability was assessed using the CCK-8 method; hair follicle structure was observed by HE staining; and immunohistochemistry was used to detect the distribution of PIP5K1A in skin hair follicles. The *PIP5K1A* gene in Liaoning cashmere goats encodes 573 amino acids, classified as a soluble unstable protein without signal peptides or transmembrane regions; its secondary structure is primarily random coil; it contains one conserved domain - PIPKc superfamily. Phylogenetic analysis of the *PIP5K1A* gene from different species shows that goats have the closest kinship with sheep. Immunohistochemistry confirmed that PIP5K1A is specifically expressed in the outer root sheath of hair follicles; melatonin promotes the expression of the PIP5K1A gene and protein level; overexpression of PIP5K1A can promote cell proliferation, whereas interference with PIP5K1A significantly reverses melatonin-induced cell proliferation; after overexpressing PIP5K1A, the expression levels of chi-miR-34c-5p and chi-miR-novel-86 are downregulated. Conclusion: PIP5K1A is located in the outer root sheath of skin hair follicles in Liaoning Cashmere Goats. Overexpression of PIP5K1A can promote the proliferation of skin fibroblasts, interference with PIP5K1A can significantly reverse cell proliferation induced by melatonin, and PIP5K1A can regulate the expression of chi-miR-34c-5p and chi-miR-novel-86 in skin fibroblasts of Liaoning cashmere goats.

Keywords: Liaoning Cashmere Goat, Skin Fibroblasts, Immunohistochemistry, PIP5K1A, Cell Proliferation, Melatonin

1. Introduction

The Liaoning Cashmere Goat is known for its excellent wool production performance, with all production performance indicators meeting the standards for cashmere goat breeding in China, significantly impacting the development of goat farming in Liaoning Province and the entire country. Its genetic performance is stable, contributing significantly to the improvement and breeding of cashmere goat varieties in China. The growth of cashmere is a complex physiological process closely related to various signaling pathways, gene expression patterns, and the cyclical development of hair follicles[1]. Hair follicles, miniature organs embedded in the animal's skin[2], are formed through the interaction between epidermal cells and dermal cells[3]. Dermal cells, including dermal papilla cells and fibroblasts, express genes related to cashmere growth specifically in skin fibroblasts, promoting the growth of cashmere fibers. Dermal cells do not directly participate in the development of hair follicles but are often considered the "signal center" of hair follicles[4, 5]. Skin fibroblasts, covering the outer connective tissue sheath of hair follicles, can initiate hair follicle morphogenesis and coordinate hair growth[6]. Initially, mesenchymal cells signal the epidermis to form a hair germ, then the hair germ releases factors inducing the formation of dermal fibroblasts and dermal papillae; finally, the dermal papilla releases a "second signal" promoting the proliferation and differentiation of epithelial cells, thereby forming a structurally

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complete hair follicle[7].

Preliminary studies have shown that 200 ng/L melatonin promotes the proliferation of Liaoning Cashmere Goat skin fibroblasts. Transcriptome sequencing results revealed that miR-34c-5p targets the regulation of PIP5K1A expression, playing a critical role in the melatonin-regulated ceRNA network[8]. PIP5K1A is a Type I phosphatidylinositol 4-phosphate 5-kinase, with three isoforms: PIP5K1A, PIP5K1B, and PIP5K1C. Among them, PIP5K1A is a major member of the lipid kinase family, with the first human and mouse PIP5K1A and PIP5K1B cloned by two different research groups, who chose opposite names for them, meaning the human PIP5K1B and the mouse PIP5K1A encode the same protein[9-11]. PIP5K1A acts as a critical lipid kinase upstream of the PI3K/AKT pathway and is a major regulator in many biological processes, primarily through phosphorylation and dephosphorylation to regulate biosynthesis and signaling pathways in cells[9, 12-14]. PIP2 (phosphatidylinositol-4,5-bisphosphate) is mainly produced by Type I phosphatidylinositol 4-phosphate 5-kinases (PIP5KIs) within the cell[15]. PIP5K1A promotes myocyte differentiation by activating the AKT pathway mediated by PIP2 and cytoplasmic calcium release[16]. β2-microglobulin (B2M) activates the PI3K/AKT/mTOR signaling pathway by interacting with PIP5K1A[17]. The PI3K/AKT pathway is a core pathway controlling cell survival and metabolism[18], playing a crucial role in hair follicle regeneration[19], the cyclical development of cashmere goat hair follicles[20], the formation of different hair types in cashmere goats[21], cashmere growth[19]. PIP5K1A plays a key role in the biological development of skeletal muscle[22], keratinocyte differentiation[23], epidermal differentiation[24], the process of cell-directed migration[25] autophagic lysosomal regeneration (ALR) [26], and the development of breast[12] and cervical cancer[27]. However, there is limited research on the structure and function of the *PIP5K1A* gene in cashmere goats.

This study focuses on skin fibroblasts and skin tissues of Liaoning Cashmere Goats. Based on the existing goat PIP5K1A protein sequence, it predicts the physicochemical properties, hydrophobicity, signal peptides, secondary structure, subcellular localization, functional sites, and functions of the PIP5K1A protein. It identifies the localization of PIP5K1A in skin tissues, explores its role in cell proliferation, and further examines the effect of PIP5K1A on miRNA levels, aiming to provide a basis for research into the regulation of cashmere growth and development by PIP5K1A.

2. Materials and methods

2.1. Melatonin Treatment and Cell Culture

MT Treatment: Initially, 0.02 g of MT powder (Sigma, Japan) was fully dissolved in 100 μL of cell culture-grade DMSO (Solarbio, China) to prepare a 200 g/L MT stock solution. Subsequently, a concentration gradient dilution method was used to dilute the stock solution with complete culture medium to 200 ng/L MT cell treatment solutions, prepared fresh for use. The complete cell culture medium was composed of fetal bovine serum (Gemini, USA): DMEM high glucose medium (KeyGen BioTECH, China) at a ratio of 2:8, with 1% penicillin-streptomycin solution (Solarbio, China). Cells were cultured in a 37°C, 5% CO_2 incubator (Heal Force, China).

2.2. RT-qPCR

Under standard conditions, RNA from Liaoning Cashmere Goat skin fibroblasts was extracted using Trizol (Vazyme, Japan), and 1 μg of RNA was reverse-transcribed to cDNA using Thermo Scientific™ RevertAid First Strand cDNA Synthesis Kit (Thermo, USA). Real-time quantitative polymerase chain reaction (RT-PCR) was performed to detect specific miRNA expression levels using TB Green® Premix Ex Taq™ (Takara, Japan), with U6 serving as an internal reference for data normalization. All data were calculated using the 2^-ΔΔCt method, with three replicates per sample. Primer information is as follows, PIP5K1A-F: CCGGGCAGCATATCTGAGAG; PIP5K1A-R: TGCCGTGGGTCTCTTGATTC; primers for chi-miR-novel-86 and chi-miR-34c-5p were purchased from RiboBio (RiboBio, China).

2.3. WB

Skin fibroblast lysates were run on a 10% SDS-PAGE gel and transferred to a 0.45 μm PVDF membrane (Millipore, Germany) for detection with specific antibodies: anti-PIP5K1A (Proteintech, USA) and anti-GAPDH (ABclonal, China) antibodies. Antibodies were diluted with antibody diluent (Abbkine, China). The dilution ratio for anti-PIP5K1A antibody was 1:5000, and for anti-GAPDH antibody was 1:10000. Secondary antibodies were anti-IgG, diluted at 1:10000. Protein bands were observed using a super signal chemiluminescent substrate (Fdbio, China), with GAPDH as a control.

2.4. CCK-8

The Cell Counting Kit-8 (Solarbio, China) was used to assess dermal fibroblast growth. Approximately 3.0×10^3 cells were seeded in each well of a 96-well plate, with all cells adhering to the surface within 12 hours. Cell proliferation was determined by measuring the absorbance at 450 nm using a MuLTiSKAN MK3 device (Thermo, USA) after incubating with 10 μL of CCK-8 solution at 37°C for 2 hours.

2.5. Lentiviral Gene Overexpression Vector Construction, Packaging, and Transfection

Enzyme cutting sites NheI/AscI were added upstream and downstream of the *PIP5K1A* (XM_005677614.3) CDS sequence fragment, and the target fragment was connected to Plenti6.3-IRES-EGFP (Research Science, China) using T4 DNA ligase (Thermo, USA). Positive clones were sequenced. The *PIP5K1A* gene lentiviral expression vector(OE-PIP5K1A) was co-transfected with packaging plasmids (Invitrogen, USA) into 293T cells. After culturing for 8 hours, the medium was replaced with complete culture medium and continued to be cultured for 48 hours to collect the virus-containing culture medium. Based on preliminary studies, the lentiviral titer was determined to be 1.0×10^8 TU/mL, with the optimal MOI being 20. Polybrene was added at a final concentration of 8 μg/ml to enhance infection. After 6 hours of infection, the medium was replaced with virus-free complete culture medium and continued to be cultured. GFP expression by the lentivirus reporter gene was observed and recorded 48 hours post-infection. The vector map for Plenti6.3-IRES-EGFP is shown in Supplementary Material 2.

2.6. Hematoxylin and Eosin (H&E) Staining

Paraffin-embedded Liaoning Cashmere Goat skin tissues were sectioned. The skin sections were deparaffinized in xylene and rehydrated in graded ethanol. Subsequently, the sections were stained with hematoxylin for 15 minutes, differentiated with 1% hydrochloric acid in ethanol for 3 seconds, blued in 0.2% ammonia water for 5 minutes, and stained with 0.5% eosin (alcohol solution) for 1 minute. After gradual dehydration in ethanol and clearance in xylene, the sections were mounted with neutral balsam and observed under a microscope.

2.7. Immunohistochemistry (IHC)

Deparaffinized skin tissue sections were immersed in Tris-EDTA antigen retrieval solution and heated in a microwave on medium power for 15 minutes before cooling to room temperature. The sections were then submerged in 3% H_2O_2 solution at 37°C for 15 minutes, washed three times with TBS for 5 minutes each time, and incubated overnight at 4°C with anti-PIP5K1A antibody at a dilution of 1:50. A blank control was set by incubating sections in TBS buffer instead of the primary antibody. The next day, sections were incubated with a secondary anti-IgG antibody at a dilution of 1:50. After DAB coloring, sections were counterstained with hematoxylin solution for 5 minutes, dehydrated, mounted, and observed under a microscope.

2.8. Statistical Analysis

Data were analyzed using GraphPad Prism version 8.0.2. Continuous variables with a normal distribution were expressed as mean ± standard deviation (SD); non-normally distributed variables were reported as median (interquartile range). The means of two continuous normally distributed variables were compared using the independent samples t-test. P-value < 0.05 was considered statistically significant.

3. Results

3.1. Bioinformatics Analysis of the *PIP5K1A* Gene

Based on bioinformatics databases, a bioinformatics analysis was conducted on the goat *PIP5K1A* gene to provide a foundation for studying its function (see Supplementary Material 1 for biological analysis methods). According to the goat PIP5K1A protein sequence encoded in GenBank (Accession No.: XP_005677671.2), the physicochemical properties of the goat *PIP5K1A* gene-encoded protein were analyzed using the ProtParam tool. The amino acid composition (Figure 1A) reveals that the goat *PIP5K1A* gene encodes 573 amino acids, with serine (Ser) being the most abundant, constituting 11.0% of all amino acids. The number of negatively charged amino acids (Asp+Glu) is 59, while the number of positively charged amino acids (Arg+Lys) is 70. The molecular formula is $C_{2824}H_{4450}N_{778}O_{851}S_{25}$, with a total atomic count of 8928. The extinction coefficient when all cysteines form cystines is 40520 L/(mol·cm), corresponding to an absorbance (Abs) of 0.636; the extinction coefficient when all cysteines do not form cystines is 39770 L/(mol·cm), with an absorbance of 0.624. The estimated half-life is 30 hours (mammalian reticulocytes, in vitro), with an instability index of 49.70, aliphatic index of 74.36, and an average hydrophilicity of -0.410. The ProtScale tool was used to analyze the hydrophobicity/hydrophilicity of the goat *PIP5K1A* gene-encoded protein (Figure 1B), with the highest score of 2.267 at position 401 for isoleucine, indicating the strongest hydrophobicity; the lowest score of -3.033 at position 269 for serine indicates the strongest hydrophilicity. The entire peptide chain is hydrophilic, predicting the goat *PIP5K1A* gene-encoded protein to be soluble. SignalP 4.1 Server software analyzed the signal peptide of the goat *PIP5K1A* gene-encoded protein (Figure 1C). The results show that the raw cleavage site score (C value), signal peptide score (S value), and combined cleavage site score (Y value) do not meet the criteria of a typical signal peptide (C, Y values tend toward +1; S value is high before the cleavage site and decreases after it), indicating the absence of a signal peptide in the goat *PIP5K1A* gene. The TMHMM Server v.2.0 software analyzed the transmembrane region of the goat *PIP5K1A* gene-encoded protein (Figure 1D). The results show that the goat *PIP5K1A* gene-encoded protein lacks a transmembrane region and is located extracellularly. PSORT II Prediction software predicted the subcellular localization of the goat PIP5K1A protein. The results show that the protein is present in the nucleus, mitochondria, peroxisomes, cytoplasm, and extracellular (including the cell wall) with probabilities of 65.2%, 8.7%, 8.7%, 8.7%, and 8.7%, respectively. Predict Protein software predicted the secondary structure of the goat *PIP5K1A* gene-encoded protein. The results show that H (α-helix):E (β-sheet):L (random coil) = 20.94%:12.57%:66.49%, with a large proportion of random coils in the entire secondary structure, giving the protein diverse conformations. Figure 1E shows that PIP5K1A contains a conserved structural domain, Phosphatidylinositol phosphate kinase (PIPK) catalytic domain family, located at amino acids 79-454. A phylogenetic tree constructed using the Neighbor-Joining Method (Figure 1F) reveals that goats have the closest kinship with sheep (*Ovis aries*) and a close relationship with *Bos taurus* and *Bison bison bison*, but a more distant relationship with members of the Artiodactyla order such as *Vicugna pacos* and *Camelus dromedarius*.

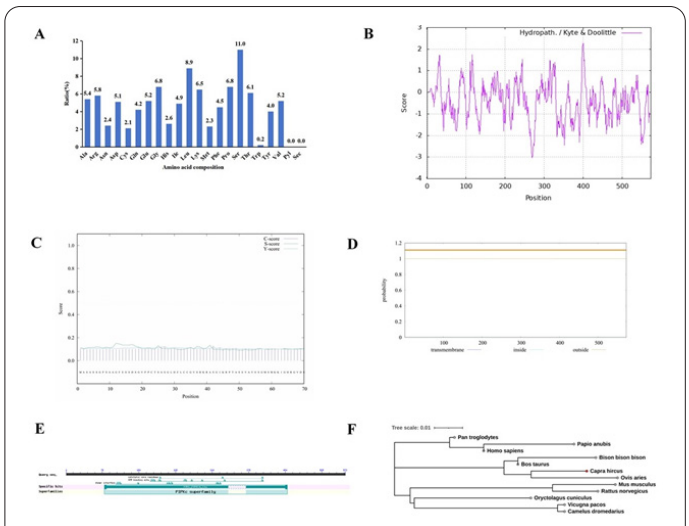

Fig. 1. Bioinformatics analysis of the *PIP5K1A* gene. A: Amino acid composition of the goat *PIP5K1A* gene-encoded protein. B: Hydrophobicity/Hydrophilicity analysis results of the goat *PIP5K1A* gene-encoded protein. C: Signal peptide prediction results for the goat *PIP5K1A* gene-encoded protein. D: Transmembrane region prediction results for the goat *PIP5K1A* gene-encoded protein. E: Conservation domain prediction for the goat *PIP5K1A* gene-encoded protein. F: Phylogenetic tree of the *PIP5K1A* gene across different species.

3.2. Localization of PIP5K1A in Liaoning Cashmere Goat Skin Hair Follicles

Cross-sectional images reveal the presence of primary follicles (PF) and secondary follicles (SF) (Figure 2A). The tissue structure of Liaoning cashmere goat skin hair follicles includes the medulla of the shaft (Med), the cortex of the shaft (CTX), the inner root sheath (IRS), and the outer root sheath (ORS), along with a connective tissue sheath (Figure 2B). Immunohistochemistry was performed on cross-sections of Liaoning Cashmere Goat skin to detect PIP5K1A protein. IHC staining shows that the negative control group (Figure 2C) had a background staining of blue or light blue, while the PIP5K1A antibody test group (Figure 2D) exhibited notable brown-yellow or brownish staining, indicating specific immunoreactive expression of PIP5K1A protein, mainly localized in the outer root sheath of the hair follicles.

3.3. Melatonin Promotes Proliferation of Liaoning Cashmere Goat Skin Fibroblasts via PIP5K1A

After treating Liaoning Cashmere Goat skin fibroblasts with 200 ng/μL melatonin for 48 hours, the expression levels of PIP5K1A in the cells were assessed. Results showed that melatonin significantly upregulated *PIP5K1A* gene expression (Figure 3A) and increased PIP5K1A protein levels (Figure 3B). Successful overexpression of PIP5K1A in skin fibroblasts (fluorescent images in Supplementary file 3) promoted cell proliferation (Figure 3C). Using siRNA technology to interfere with the expression of PIP5K1A in skin fibroblasts, WB results indicated that PIP5K1A siRNA significantly reduced PIP5K1A protein levels (Figure 3D), and CCK8 results demonstrated that interfering with PIP5K1A significantly reversed the promotive effect of MT on cell proliferation (Figure 3E).

3.4. PIP5K1A Regulates miRNA Expression

After overexpressing PIP5K1A, chi-miR-34c-5p and chi-miR-novel-86 expression levels were downregulated, with no significant change in the expression of chi-30f-3p (Figure 4A). Aligning the sequences of chi-miR-34c-5p and chi-miR-novel-86 with miRNAs from other mammals revealed that both miRNAs have their seed regions at the 5' end 2-8 bases identical to those in humans, with chi-miR-34c-5p showing the highest conservation; chi-miR-novel-86 is completely identical to hsa-miR-1264 (Figures 4B-C). Figures 4D-E predicted that hsa-miR-34c-5p has 51 target genes, and hsa-miR-1264 has 65 target genes. Figures 4F-G indicate that has-miR-34c-5p target genes are primarily involved in the Phospholipase D signaling pathway and Autophagy. hsa-miR-1264 is mainly involved in Circadian entrainment, cAMP signaling pathway, AMPK signaling pathway, and other signaling pathways.

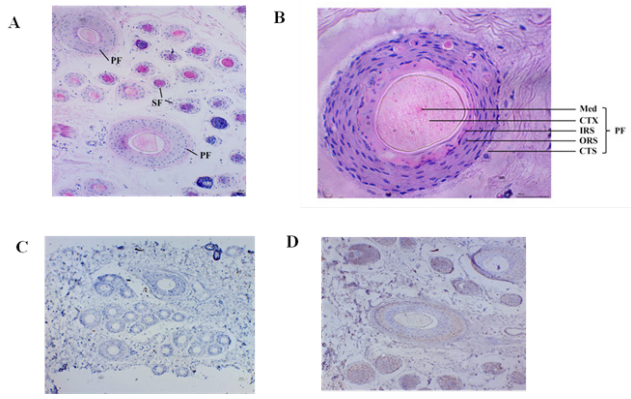

Fig. 2. Localization of PIP5K1A in Liaoning cashmere goat skin hair follicles. A-B: Cross-sectional paraffin sections of Liaoning Cashmere Goat skin hair follicles. C: Immunohistochemistry results of the PBS-negative control group. D: Cross-sectional localization of PIP5K1A in Liaoning cashmere goat skin hair follicles.

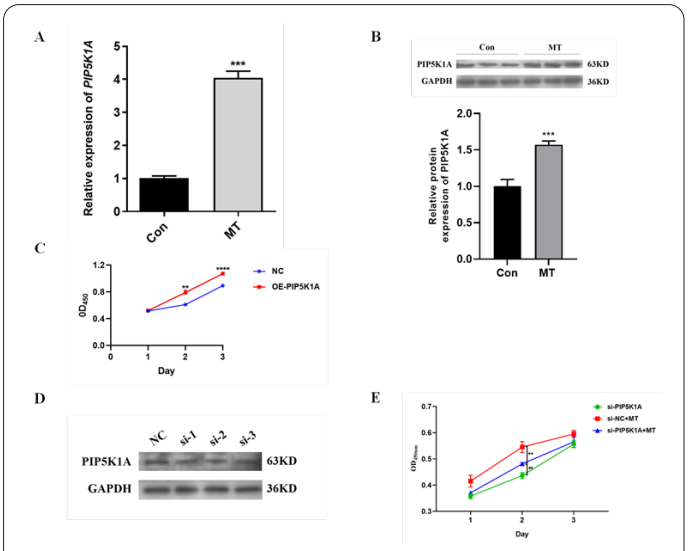

Fig. 3. Melatonin promotes skin fibroblast proliferation via PIP5K1A. A: RT-qPCR detection of *PIP5K1A* gene expression levels. B: WB detection of PIP5K1A protein expression levels. C: Cell proliferation assessed by CCK-8. D: WB validation of siRNA interference effect on PIP5K1A. E: Cell proliferation assessed by CCK-8. RT-qPCR, WB, and CCK-8 results were repeated at least three times. Results are presented as mean±SD. Significant outcomes are denoted as *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

4. Discussion

In this study, we predicted through bioinformatics that the PIP5K1A protein is composed of 573 amino acids, with serine being the most abundant. The entire peptide chain is hydrophilic, lacks signal peptides and transmembrane regions, and contains a conserved domain of the PIPKc superfamily (Phosphatidylinositol phosphate kinase (PIPK) catalytic domain family). The PIPKc domain is a conserved area in the kinase core of the PIP5Ks family, present in Type I, II, and III PIP5Ks enzymes. PIP5K catalyzes the phosphorylation of PI4P to form PIP2[28]. The secondary structure of this protein is predominantly random coils, giving it a variety of conformations. Phylogenetic analysis of the *PIP5K1A* gene found close kinship between goats, sheep, cattle, and bison.

Xie et al.'s immunohistochemical analysis of adult skin sections revealed that PIP5K1A is present in all layers of the epidermis[23]. Our study discovered positive expression of PIP5K1A protein in the skin hair follicle's outer root sheath[29], suggesting PIP5K1A plays a role in hair follicle development since activation of hair follicle growth relies on the proliferative activity of the hair matrix and outer root sheath near the dermis.

Previous research reported that 200 ng/L melatonin promotes skin fibroblast proliferation and inhibits apoptosis. This study found that melatonin can upregulate the gene and protein expression levels of PIP5K1A in skin fibro-

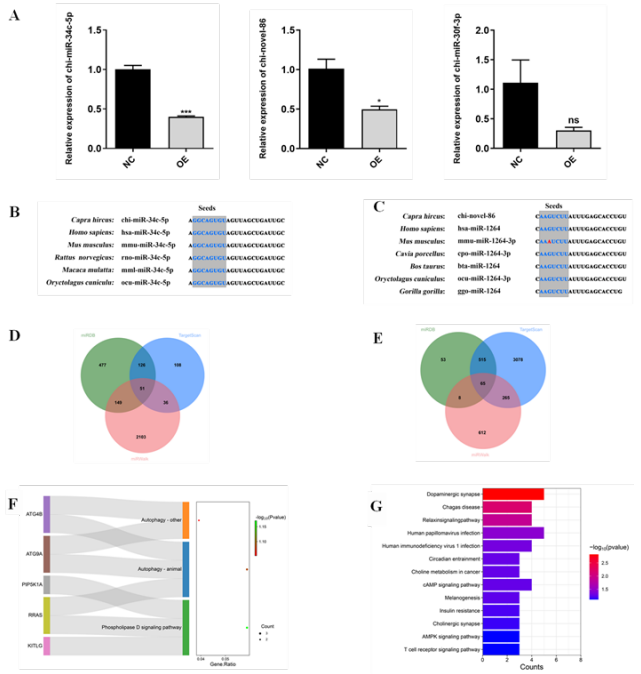

Fig. 4. PIP5K1A regulates miRNA expression. A: RT-qPCR detection of miRNA expression levels. B-C: Comparison charts of mature miRNA sequences across different species. D-E: Venn diagrams of target genes predicted by miRwalk, TargetScan, and miRDB; F-G: KEGG enrichment analysis.

blasts, suggesting that PIP5K1A is involved in the process of melatonin-induced cell proliferation. PIP5K1A is a key regulatory molecule in the metabolism of PIP2, regulating biological processes such as cell proliferation and apoptosis[12, 30]. PIP5K1A participates in liver regeneration after partial hepatectomy and hepatocyte proliferation. The knockout of PIP5K1A in HepG2 cells (human liver cancer cells) inhibited the proliferation stimulated by Hepatocyte Growth Factor (HGF) [13]. During apoptosis, PIP5KA is cleaved by caspase3, and overexpression of PIP5KA protects cells from apoptosis by generating PIP2, inhibiting caspase activity[31]. Overexpression of PIP5KA maintaining PIP2 levels can make cells resistant to H_2O_2-induced apoptosis[32]. Our results are consistent with these previous reports, highlighting the important role of PIP5K1A in promoting cell growth. Our study demonstrates that overexpression of PIP5K1A in Liaoning Cashmere Goat skin fibroblasts promotes cell proliferation, and PIP5K1A is a key molecule in melatonin-induced proliferation of goat skin fibroblasts. Interference with PIP5K1A reduced the effectiveness of melatonin in promoting cell proliferation.

This study is the first to demonstrate that PIP5K1A can regulate miRNA levels in cashmere goat skin fibroblasts. Our previous research showed that miR-34c-5p can target the regulation of PIP5K1A[8]. After overexpressing PIP5K1A, RT-qPCR detection found that the expressions of chi-miR-34c-5p and chi-miR-novel-86 were reduced, indicating that PIP5K1A can regulate the biogenesis of miRNAs. There is a feedback loop between chi-miR-34c-5p and *PIP5K1A*, but the mechanisms of this feedback require further analysis. Li et al. initially revealed that PPK-1/PIP5K1A regulates let-7 miRNA levels by interacting with the nuclear export protein XPO5 in the nucleus, regulating the level of mature miRNA by blocking the binding of XPO5 to pre-let-7 miRNA, and this action is independent of PIP5K1A's kinase activity[33]. Our findings in Liaoning Cashmere Goat skin fibroblasts that PIP5K1A can regulate the expression levels of chi-miR-34c-5p and chi-miR-novel-86 add to the understanding of the relationship between PIP5K1A and miRNA biogenesis. However, the reasons PIP5K1A participates in miRNA biogenesis are unclear and require further experimental validation.

Due to the absence of a goat database in miRNA target gene prediction software, chi-miR-34c-5p is completely consistent with human miR-34c-5p; chi-miR-novel-86 is completely identical to human miR-1264. Using miWalk, TargetScan, and miRDB software to predict the target genes of miR-34c-5p and miR-1264 and analyzing the intersection, KEGG enrichment analysis of the 51 target genes of miR-34c-5p and 65 target genes of miR-1264 showed that chi-miR-34c-5 and chi-miR-novel-86 might promote skin fibroblast proliferation through signaling pathways such as Phospholipase D signaling pathway, Autophagy, Circadian entrainment, cAMP signaling pathway, and AMPK signaling pathway.

5. Conclusion

In summary, this study found that PIP5K1A is a soluble unstable protein without signal peptides or transmembrane regions, containing a conserved PIPKc domain, and is closely related to sheep. It further confirms that melatonin-mediated PIP5K1A promotes proliferation in Liaoning Cashmere Goat skin fibroblasts and that PIP5K1A can regulate miRNA expression levels. Although no single gene or miRNA can be said to "control" the growth of cashmere, these and other studies show a feedback loop between *PIP5K1A* and miR-34c-5p plays an important role in regulating the growth of skin fibroblasts. This study provides useful information for understanding the mechanism by which melatonin promotes cashmere goat hair growth and lays a foundation for revealing the biological function of the *PIP5K1A* gene and the mechanism of cashmere goat hair growth and development.

Conflict of Interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Liaoning Normal University. The Liaoning Cashmere Goat skin fibroblasts and paraffin-embedded skin tissue blocks used were preserved from previous studies, and this study did not involve human or animal experiments.

Informed Consent

The authors declare that no patients were used in this study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Mei Jin: Project administration and supervision; Weiyu

Fan: Validation, Methodology, Writing – review & editing; Tianwei Xue: Writing – original draft; Linlin Cong: Data curation.

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