https://doi.org/10.17221/72/2024-CJAS

LGALS3 promotes the proliferation of Liaoning Cashmere goat skin fibroblasts by regulating the expression of *PIEZO1*

Mei Jin, Weiyu Fan, Jinxu Xu*

Liaoning Provincial Key Laboratory of Biotechnology and Drug Discovery, College of Life Sciences, Liaoning Normal University, Dalian, P.R. China

**Corresponding author: jm6688210@163.com*

Citation: Jin M., Fan W., Xu J. (2025): LGALS3 promotes the proliferation of Liaoning Cashmere goat skin fibroblasts by regulating the expression of *PIEZO1*. Czech J. Anim. Sci., 70: 102–112.

Abstract: Long non-coding RNA (lncRNA) plays a role in the reconstruction of hair follicles and in the formation and growth of cashmere fibres. One cashmere growth-associated lncRNA was successfully screened out at the early stage in the laboratory, which was named LncRNA MTC. In this study, the binding of galectin 3 (LGALS3) to LncRNA MTC was examined through RNA immunoprecipitation (RIP) in skin fibroblasts of Liaoning Cashmere goats. The pLenti-LGALS3-His-IRES-EGFP lentiviral overexpression vector was built, and the result of CCK8 indicated that the overexpression of LGALS3 significantly enhanced the viability of skin fibroblasts ($P < 0.05$). A total of 15 proteins interacting with LGALS3 were identified through co-immunoprecipitation (CO-IP) combined mass spectrometry, which were primarily rich in ECM-receptor interaction, focal adhesion, PI3K-AKT, and other signalling pathways. Moreover, PIEZO1 refers to a mechanically sensitive ion channel protein that is capable of regulating the cell proliferation. As revealed by qPCR results, overexpression of *LGALS3* significantly upregulated the expression of *PIEZO1*, which was effectively interfered by siRNA. The result of CCK8 indicated that siPIEZO1 significantly inhibited cell proliferation, whereas overexpression of LGALS3 protected cells from the suppression of siPIEZO1 ($P < 0.01$). This study revealed that LGALS3 is capable of stimulating the proliferation of skin fibroblasts by regulating *PIEZO1*. The effect of LGALS3 on the proliferation of skin fibroblasts from the protein interaction was explored, and this study is expected to lay a certain scientific basis for the research on the hair follicle development mechanism of Liaoning Cashmere goats.

Keywords: co-immunoprecipitation; lentiviral overexpression vector; LncRNA MTC; RNA immunoprecipitation; siRNA

The Liaoning Cashmere goat refers to a precious resource variety prohibited from gene outflow in China. The villi produced by Liaoning Cashmere goat have a high economic value, and the Liaoning Cashmere goat ranks first in China in the yield of single cashmere. The secondary hair follicle (HF) is a complex small organ on the skin, which is an important structure to produce villi, and its growth cycle is a complex biological process (Zhang et al. 2021). Ren et al. (2016) suggested that lncRNAs are capable of regulating hair follicle development and pigmentation in goats. Jin et al. (2018) showed that a differentially expressed lncRNA (XLOC_005914) was successfully selected

Supported by the National Natural Science Foundation of China (Project No. 31772557) and by the Dalian Science and Technology Innovation Fund Project (Project No. 2019J12SN65).

https://doi.org/10.17221/72/2024-CJAS

after 0.2 g/l melatonin (MT) treatment for 72 h and named LncRNA MTC. The result suggested that LncRNA MTC is capable of facilitating goat skin fibroblast proliferation and regulating hair follicle development and cashmere growth by activating NF-κB protein kinase. RNA pull-down/MS was used to demonstrate that LncRNA MTC could bind to different proteins before and after MT treatment. Specifically, the LGALS3 protein did not bind to LncRNA MTC before MT treatment of skin fibroblasts, while the binding amount between the LGALS3 protein and LncRNA MTC increased after MT treatment of skin fibroblasts (Jin et al. 2024).

A major way of lncRNA functioning is to combine with other proteins to form the RNA-protein complex (RNP) (Moore 2005; Mitchell and Parker 2014). Existing research has suggested that lncRNA is capable of interacting with members of the galectin family to regulate cell proliferation. For instance, Wang et al. (2022a) noted that paeoniflorin is capable of inhibiting the proliferation and migration of keratinocytes via the lncRNA NEAT1/miR-3194-5p/galectin 7 axis. In addition, lncRNA MEG3-210 interacts with galectin 1 to facilitate endometrial stromal cell proliferation via the p38 MAPK and PKA/SERCA2 signalling pathways (Liu et al. 2020). Given the vital role played by galectin in cell proliferation, the regulation of galectin in proliferation should be studied in depth.

LGALS3, a member of the lectin family, refers to a protein with a relative molecular mass of 29–34 kDa, which can exert biological functions by combining with other proteins. Studies have proved that knocking out LGALS3 can reduce the human cardiomyocyte apoptosis induced by ischemia/reperfusion (I/R). Moreover, the CO-IP results indicated that there was a significant binding effect between LGALS3 and Bcl-2 protein, thus reducing the anti-apoptotic effect of BCL2 and accelerating cardiomyocyte apoptosis (Zhang et al. 2020). In addition, LGALS3 can interact with the transforming growth factor beta 1 (TGFB1) to facilitate the proliferation and migration of human pulmonary artery smooth muscle cells by activating the Smad2/3 signalling pathway (Cao et al. 2021). This suggests that LGALS3 is capable of interacting with other proteins and has certain effects on the proliferation of Liaoning Cashmere goat skin fibroblasts.

Piezo type mechanosensitive ion channel component 1 (Er blood group) (PIEZO1), i.e., FAM38A, is an ion channel protein with a molecular weight of nearly 233–286 kDa and 38 transmembrane helical domains. The activation of different cells exerts different effects on cell proliferation. In mouse osteoblasts and odontoblasts, PIEZO1 activation using channel-specific agonists or mechanical stimulation can inhibit cell proliferation (Miyazaki et al. 2019; Yoneda et al. 2019). *In vitro*, cyclic mechanical stretch (CMS) upregulates the expression of PIEZO1 in human dermal fibroblasts (HDFs) and improves the proliferation, motility, and differentiation of HDFs, whereas the biological functions of PIEZO1 in goat skin fibroblasts remain unclear.

In this study, a new pathway was clarified, in which LGALS3 regulates the proliferation of Liaoning Cashmere goat skin fibroblasts through protein interaction. The results suggested that LGALS3, a binding protein of LncRNA MTC, is capable of interacting with PIEZO1 and facilitating skin fibroblast proliferation in Liaoning Cashmere goat by upregulating the expression of *PIEZO1*. In brief, the data of this study provide new mechanistic insights into the regulation of hair follicle development and villus growth in Liaoning Cashmere goats.

MATERIAL AND METHODS

Ethics statement

The research has been approved by the Liaoning Normal University Ethics Committee, Ethics Review No. LL2022039.

Cell culture

Liaoning Cashmere goat skin fibroblasts are the existing primary cells in our laboratory. They were obtained from the skin of the scapular region of 3-month-old Liaoning Cashmere goats and were primarily cultured by the trypsin digestion method. 293T cells were purchased from the Chinese Academy of Sciences Cell Resource Library (Beijing, P.R. China). The complete cell medium was mixed with foetal bovine serum (Gemini, Woodland, USA) and DMEM high-glucose medium (KeyGen BioTECH, Jiangsu, P.R. China) at 1 : 9. The cells were cultured in an incubator at a temperature of 37 °C and CO_2 concentration of 5% (Heal

https://doi.org/10.17221/72/2024-CJAS

Force, Shanghai, P.R. China). After 24 h, the old culture medium was discarded and replaced with fresh complete cell culture medium, and then subsequent cell experiments were carried out.

RNA extraction and reverse transcription – quantitative PCR (RT-qPCR)

Reverse transcription of 1 μg RNA to cDNA was performed using a Thermo Scientific™ RevertAid First Strand cDNA Synthesis Kit (Vazyme, Jiangsu, P.R. China) under standard conditions. Quantitative real-time polymerase chain reaction (qPCR) was performed to determine the expression levels of specific genes using SYBR Premix Ex Taq Kit (Takara, Dalian, P.R. China), and actin beta was used as an internal control to normalise the data. All data were calculated using the $2^{-\Delta\Delta Ct}$ method, and each sample was detected in triplicate. Results data are expressed as mean ± standard deviation. ANOVA (one-way analysis of variance) was used to check the significance of the mean using Graphpad Prism v7.0. The detailed information about primers is shown in Electronic Supplementary Material (ESM) Table S1.

Protein extraction and Western blotting

The cell culture dish was removed from the incubator and the culture medium was discarded. An amount of 120 μl of pre-cooled RIPA neutral lysis buffer (Beyotime, Shanghai, P.R. China) was added to extract the total protein in the cells. Protein lysates of skin fibroblast cells were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to 0.45-mm PVDF membrane (Vazyme, Jiangsu, P.R. China). The membrane was sealed at room temperature with blocking buffer (Beyotime, Shanghai, P.R. China) for 1 h, and incubated with the primary 6x His Rabbit polyclonal antibody (10001-0-AP; Proteintech, Wuhan, P.R. China). The dilution ratio of the primary antibody was 1 : 5 000, and it was incubated at 4 °C overnight. On the second day, after the membrane was washed three times with TBST (Tris Buffered Saline with Tween 20), it was incubated with the secondary antibody: goat anti-rabbit IgG (AS014; Wuhan, P.R. China). The dilution ratio of the secondary antibody was 1 : 10 000, and it was incubated at room temperature for 1 hour. Protein bands were observed using the SuperSignal chemiluminescent substrate (Fdbio, Shanghai, P.R. China).

Construction, packaging, and target cell infection of the overexpressed lentiviral vector pLenti-LGALS3-His-IRES-EGFP

The *Asc*1 and *Pme*1 restriction enzyme sites were added to the 5' and 3' ends of the coding sequence fragment of the *LGALS3* gene (XM_018054119.1), respectively, and a 6x His tag was added to the 5' end. The target sequence was synthesised by Sangon Biotech (Shanghai, P.R. China). Then, the target sequence was ligated to the pLenti6.3-IRES2-EGFP/V5 DEST vector using T4 ligase (Sangon Biotech, Shanghai, P.R. China). The positive clones were sequenced. The *LGALS3* gene lentiviral expression vector (pLenti-LGALS3-His) was co-transfected with packaging plasmids (Invitrogen, California, USA) into 293T cells. After culturing for 8 h, the medium was replaced with complete culture medium and continued to be cultured for 48 h to collect the virus-containing culture medium. The lentiviral titre was 1.0×10^8 TU/ml, and the optimal multiplicity of infection (MOI) was 100. Polybrene (Sigma, Shanghai, P.R. China) with a final concentration of 8 μg/ml was added to enhance the infection effect. After 6 h of infection, the medium was replaced with fresh medium and continued to be cultured. The overexpression efficiency was examined by RT-qPCR 48 h after infection.

RNA immunoprecipitation (RIP) assay

RNA immunoprecipitation (RIP) assay is a technique that enriches RNAs bound to specific proteins using antibodies to study the interaction between RNA and proteins. Skin fibroblasts were collected and RIP lysis was added to prepare a cell suspension which was centrifuged at 14 000 rpm for 10 min at 4 °C, and the supernatant was retained. Subsequently, 100 μl RIP Wash Buffer was added to resuspend the magnetic beads, and Anti-His and IgG antibodies were introduced to form

https://doi.org/10.17221/72/2024-CJAS

a bead-antibody complex. The cell supernatant was added into the complex to prepare a magnetic bead-antibody complex. The complex was mixed with protease and incubated at 55 °C for 30 minutes. RNA was extracted and then reversely transcribed into cDNA for qPCR detection.

Co-immunoprecipitation (CO-IP) assay

Co-immunoprecipitation (CO-IP) assay is used to detect and verify the interaction between proteins. The IgG group is a negative control group with non-specific antibodies, used to rule out non-specific binding interference, while the IP group uses specific antibodies to precipitate the target protein and its interacting protein complexes. The LGALS3 lentivirus vector with His label was transfected into skin fibroblasts, and cell lysates were extracted after transfection for immunoprecipitation. A 50% protein A/G working solution prepared using lysate and protein A/G agarose magnetic beads was added after the protein lysate was incubated with His antibody at 4 °C overnight. The samples were centrifuged at 4 °C for 2 h, the supernatant was discarded, boiled at 100 °C for 5 min, separated by SDS-PAGE and detected through mass spectrometry.

siRNA transfection

siCY3 was adopted to study the optimal transfection concentration of siRNA for skin fibroblasts. The three siRNAs of PIEZO1 (XM_018061851.1) were synthesised by JTSBIO (Wuhan, P.R. China), and the sequences are as follows: siRNA1: AGGACAACTGCATCATACA; siRNA2: GCCTCAAGTACTATGTGAA; siRNA3: CTGCCAATCTCAAGACCAT. According to the operating instructions, siRNA (100 nM) was transfected into cells for 24 h, 48 h and 72 h with the transfection reagent POLO3000 (R&S, Shanghai, P.R. China), and the transfection efficiency was detected through RT-qPCR.

CCK8 assay

CCK8 assay is based on the principle that the WST-8 reagent is reduced by dehydrogenases in living cells to form a formazan dye, and the absorbance is measured to reflect the number of living cells, which is used to detect cell proliferation activity.

Skin fibroblasts were counted, and cell suspensions were inoculated into 96-well plates with a density of nearly 8 000 cells/well, and placed in the cell incubator for culture overnight. Transfection treatment was performed according to the experimental requirements, and incubated in the incubator for 1–5 days. After 10 µl CCK8 (Solarbio, Beijing, P.R. China) was added to the respective well and mixed, then incubated in the incubator for 4 hours. Furthermore, the absorbance value at 450 nm was examined.

Statistical analysis

The data were analysed using GraphPad Prism v8.0.2 (GraphPad Software, USA). 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.

RESULTS

RIP experiment verified that the LGALS3 protein binds to LncRNA MTC

PLenti-LGALS3-His-IRES-EGFP lentiviral overexpression vector was constructed to further demonstrate that LGALS3 and LncRNA MTC can bind to each other, and the results are presented in Figure 1A. The overexpression efficiency was nearly the 8-fold (8 ± 0.25) value of the control, which can be employed for subsequent RIP experiments.

The RIP results (Figure 1B) indicated that the enrichment of LncRNA MTC in the pLenti-LGALS3-His group reached the 6-fold (6 ± 1.3) level compared with the control group ($P < 0.01$), consistent with the results of RNA pull-down experiments, and there was a combination between LncRNA MTC and LGALS3.

https://doi.org/10.17221/72/2024-CJAS

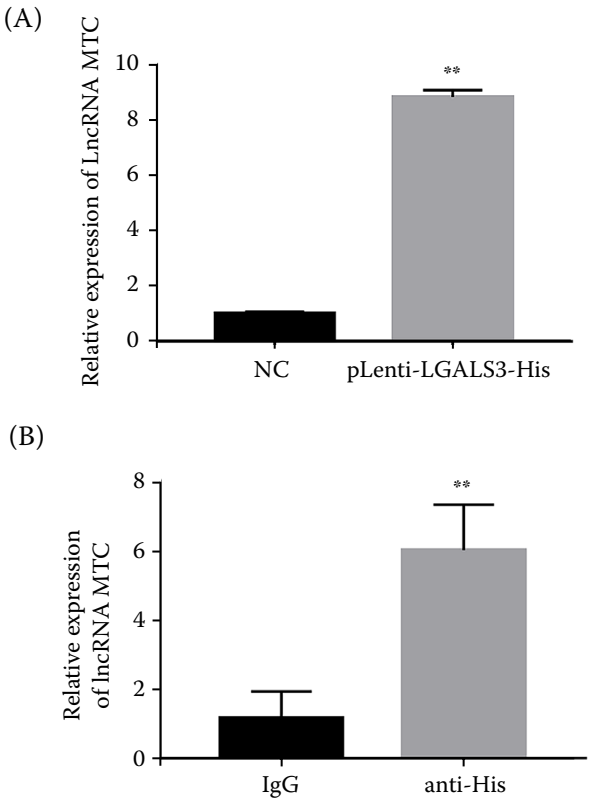

Figure 1. RIP experiment verified that the LGALS3 protein binds to LncRNA MTC

(A) Overexpression efficiency of LGALS3. (B) Expression of the target gene LncRNA MTC

Data are presented as the mean ± SD; $n = 3$; $^{**}P < 0.01$

NC = negative control group

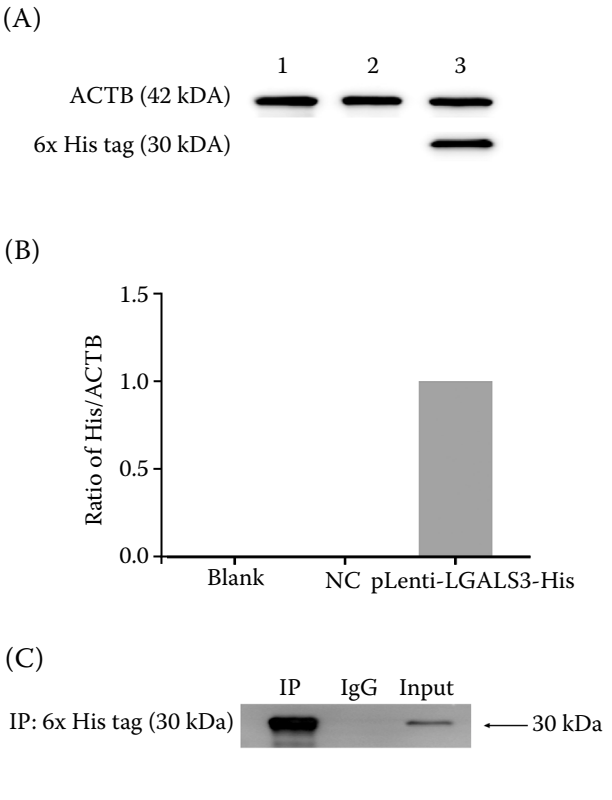

Figure 2. Expression of His tag and interaction protein enrichment

(A) Western blot detects the expression of 6x His tag protein; Lane 1: Empty cell group, Lane 2: lv-NC group, Lane 3: lv-LGALS3-His group. (B) Western blot grey value statistics. (C) IP enrichment of LGALS3 interaction protein

The IgG group is a negative control group containing non-specific antibodies, while the IP group is the 6x His antibody group

Screening and identification of proteins interacting with LGALS3 by CO-IP mass spectrometry

LGALS3 served as the bait protein. Western blotting results are presented in Figure 2A, the expression of His in cells infected by pLenti-LGALS3-His was significantly increased, indicating that the His tag was labelled and applicable to subsequent enrichment detection. The IP results are presented in Figure 2B, with clear bands of IP group lanes, suggesting that the LGALS3 interacting protein was enriched.

The IgG group and IP group protein glue strips were identified through mass spectrometry, and the result information statistics are shown in ESM Table S2: a total of 6 558 spectra was obtained in the IgG group, and the number of matched spectra was 106 after identification by search engine, with 84 peptides and 29 proteins that were identified; 7 514 spectra were obtained in the IP group, and the number of matched spectra was 159, with 131 peptides and 29 proteins that were identified. The analysis was performed in five aspects, including "unique spectrum number", "unique peptide number", "protein mass", "protein coverage" and "peptide length", and the statistical charts were drawn (Figure 3).

Bioinformatics analysis of LGALS3 interacting proteins

The data of IgG and IP groups were collated. On that basis, 15 proteins that interacted with LGALS3BP were screened, as listed in ESM Table S3. The identified proteins were analysed by GO, KOG and KEGG, and the results of GO analysis

https://doi.org/10.17221/72/2024-CJAS

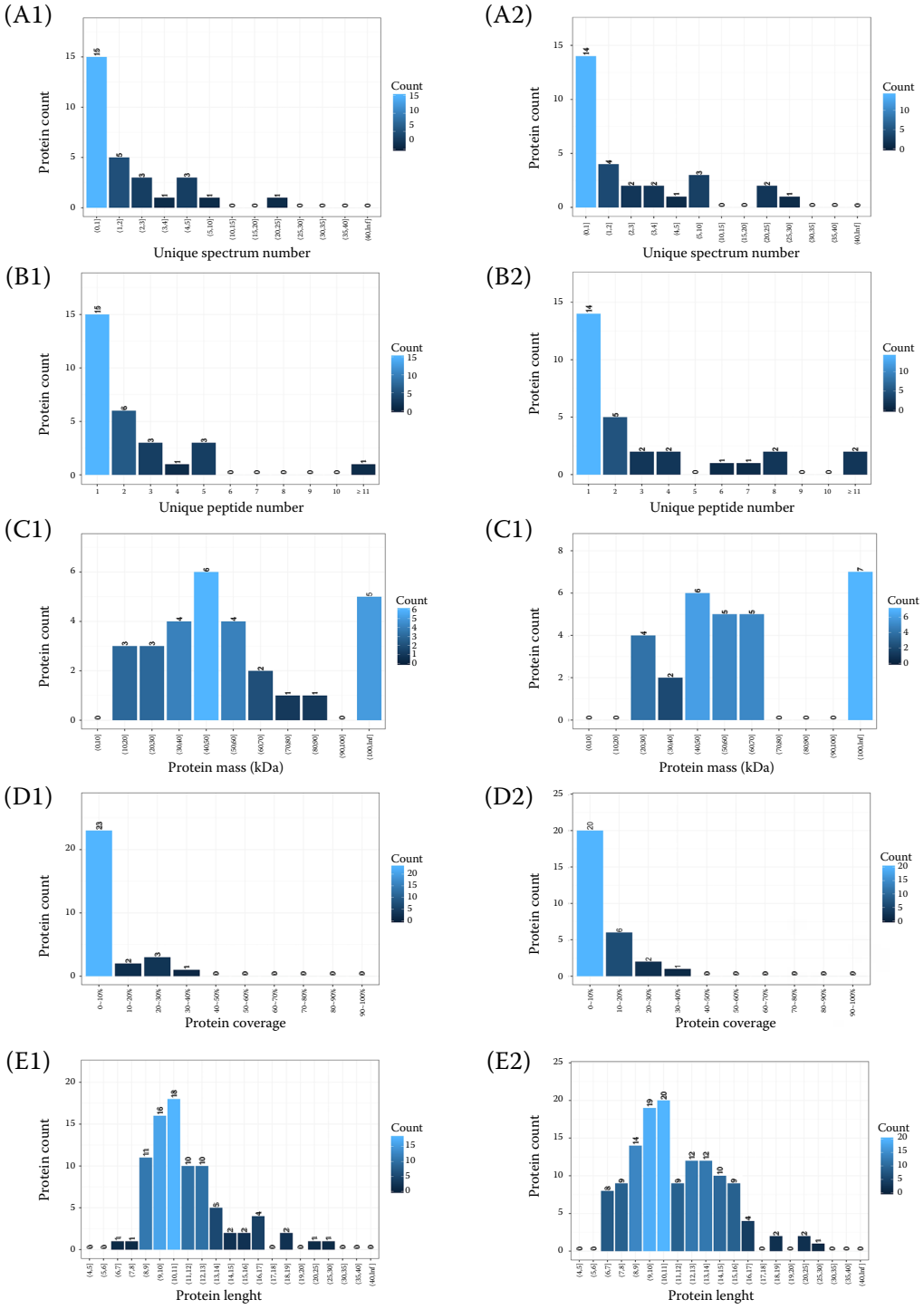

Figure 3. Basic statistics of protein identification results in IgG and IP groups

(A1–E1) is the IgG group, and (A2–E2) is the IP group. The lighter the colour, the more the protein. (A1,2) Unique spectrum distribution map, the horizontal axis is the number of unique matching maps for each protein, and the vertical axis is the number of proteins. (B1,2) Unique peptide distribution map, the horizontal axis is the number of unique matching peptides for each protein, and the vertical axis is the number of proteins. (C1,2) Protein mass distribution diagram, the horizontal axis is the protein mass interval, and the vertical axis is the corresponding protein number. (D1,2) Protein coverage distribution map, the horizontal axis is the percentage interval of protein coverage, and the vertical axis is the number of proteins. (E1,2) Peptide length distribution diagram, the horizontal axis is the length interval of each peptide, and the vertical axis is the number of corresponding peptides

https://doi.org/10.17221/72/2024-CJAS

are presented in Figure 4A. The interacting proteins were mainly enriched in components (e.g., cells and organelles) in the cell composition (CC) category, involved in 5 molecular functions (e.g., binding and structural molecular activity), as well as 19 entries (e.g., cell proliferation, cell growth, and regulation of biological processes) in the biological process (BP) category. Figure 4B presents the results of KOG analysis, the functions of interacting proteins are mainly closely related to cellular processes and signals (e.g., nuclear structure, cell cycle, cell division, chromosome partitioning, intracellular trafficking, secretion, as well as signal transduction mechanisms). KEGG results are listed in ESM Table S4, the interacting proteins were enriched in 10 entries (e.g., focal adhesion, PI3K-AKT signalling pathway, oestrogen signalling pathway, cholesterol metabolism, and ECM-receptor interaction). The results of the clustering analysis are illustrated in Figure 4C, interacting proteins are mainly involved in signal transduction, cellular community – eukaryotes, etc.

Lastly, PIEZO1, which was correlated with hair follicle development, was selected for subsequent experiments. qPCR results indicated that PIEZO1 was significantly upregulated after infection with pLenti6.3-LGALS3 for 48 h ($P < 0.05$) (Figure 4D).

LGALS3 promotes cell proliferation through upregulation of PIEZO1

Different concentrations of CY3-labelled siRNA(siCY3) were transfected into Liaoning Cashmere goat skin fibroblasts. The fluorescence intensity increased as the siRNA concentration increased. The highest fluorescence intensity was observed at a concentration of 100 nM and

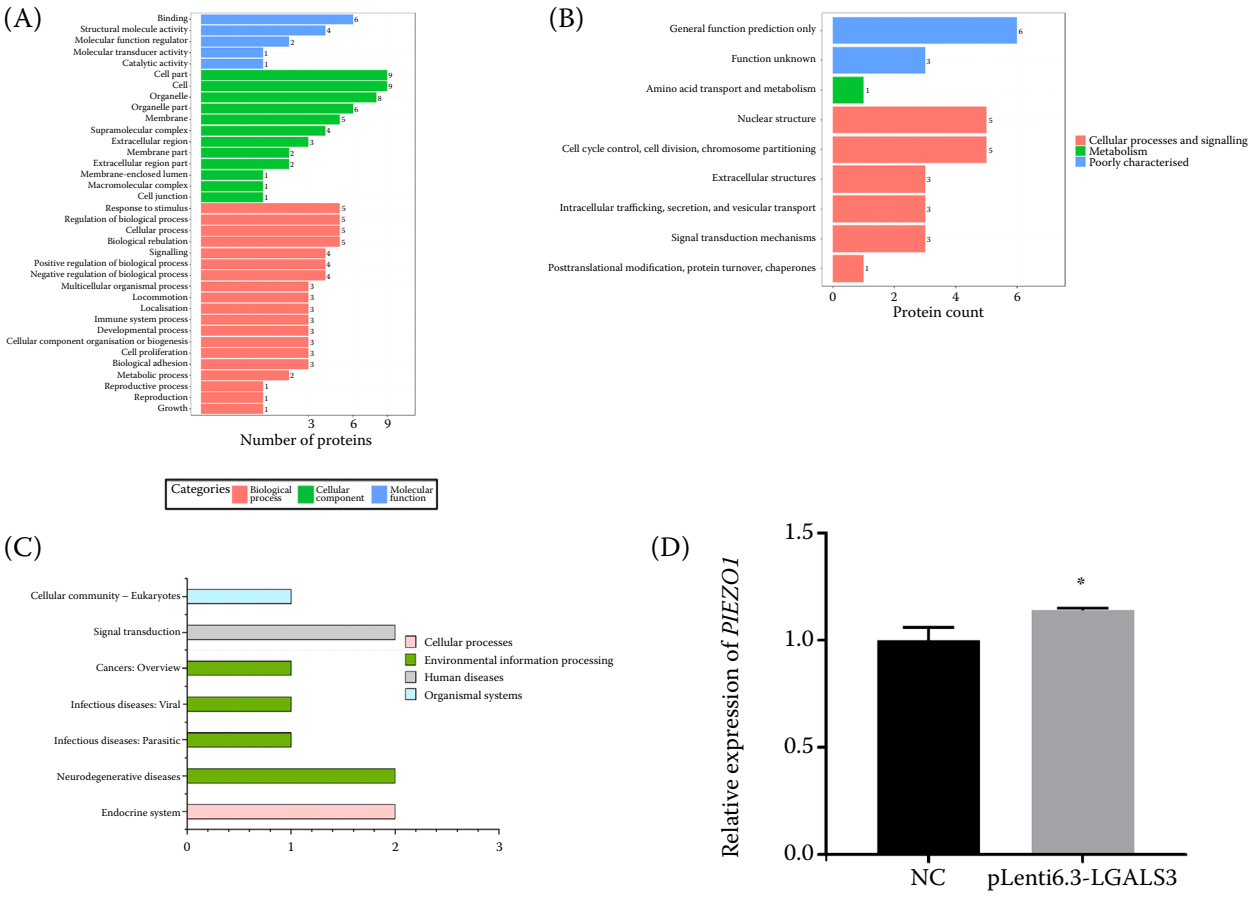

Figure 4. Bioinformatics analysis of interacting proteins

(A) GO functional annotation analysis of LGALS3 interacting proteins. (B) KOG functional annotation analysis of LGALS3 interacting proteins. (C) Cluster analysis of the KEGG pathway of LGALS3 interacting proteins. (D) Expression of the *PIEZO1* after pLenti6.3-LGALS3 treatment

Data are presented as the mean ± SD; $n = 3$; $^*P < 0.05$

https://doi.org/10.17221/72/2024-CJAS

the cells were in good growth status (Figure 5A). Consequently, the transfection concentration of 100 nM for siPIEZO1 was chosen for subsequent experiments. PIEZO1 was knocked down using three siRNAs, and the knockdown efficiency of the three siRNAs transfected at different times was examined to deeply explore the function of PIEZO1 in the proliferation of Liaoning Cashmere goat skin fibroblasts. The results are presented in Figure 5B. Compared with siNC, the knockout efficiency of siPIEZO1 reached 90% after transfection for 24 hours. The knockout efficiency reached 70% after transfection for 48 hours. Compared with siRNA2 and siRNA3, the transfection efficiency of siRNA1 was more stable. Accordingly, PIEZO1 siRNA1 (0.27 ± 0.2) was selected for subsequent experiments.

CCK8 technology was adopted to detect cell proliferation for 1–5 days to study the effect of LGALS3 and PIEZO1 on cell proliferation. The results showed that compared with the control, siPIEZO1 treated cells showed a significant inhibi-

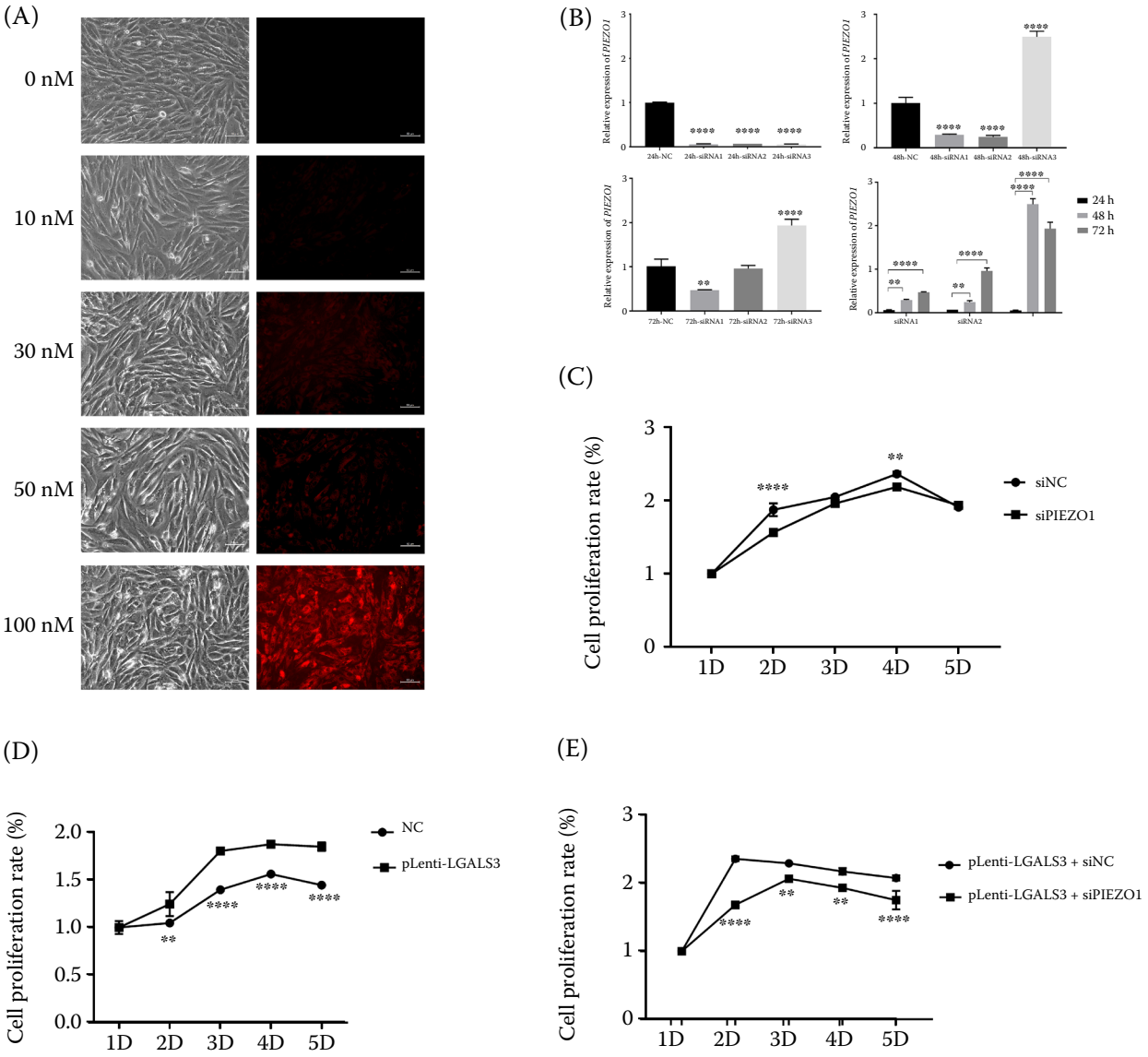

Figure 5. LGALS3 promotes cell proliferation by upregulating the expression of siPIEZO1

(A) Liaoning Cashmere goat skin fibroblasts transfected with siCY3 at different concentrations. (B) Knockdown efficiency of siRNA-PIEZO1 was performed by RT-qPCR. (C–D) CCK8 detected proliferation of skin fibroblasts transfected with siPIEZO1 and pLenti-LGALS3, respectively. (E) CCK8 detected proliferation of skin fibroblasts co-transfected with pLenti-LGALS3 and siRNA-PIEZO1

Data are presented as the mean ± SD; $n = 3$; $^{**}P < 0.01$, $^{****}P < 0.000\ 1$

https://doi.org/10.17221/72/2024-CJAS

tory effect on cell viability after 48 h (Figure 5C), overexpression of LGALS3 significantly promoted cell proliferation after 48 h (Figure 5D); while this inhibitory effect was constricted by overexpression of LGALS3 when cells were co-transfected with pLenti6.3-LGALS3 and siRNA1 (Figure 5E). It was shown that LGALS3 could promote the proliferation of skin fibroblasts by upregulating PIEZO1.

DISCUSSION

The pLenti-LGALS3-His-IRES-EGFP lentiviral overexpression vector was constructed. In addition, RNA pull-down combined with mass spectrometry was adopted. In accordance with the following screening criteria, the intensity was increased, following an order of Peptides > 2, Unique > 2, and Coverage > 8. The target protein LGALS3, with significantly increased expression after MT treatment, was correlated with cell proliferation, hair follicle development and other functions and then screened out.

The binding relationship was further verified using RIP; the results confirmed LGALS3 as a binding protein of villus growth-associated LncRNA MTC, suggesting that LGALS3 takes on a critical significance to the investigation of cell proliferation and hair follicle development. A total of 15 proteins interacting with LGALS3 were identified through CO-IP combined mass spectrometry, and bioinformatics analysis was conducted. Among the 10 items mainly enriched in interaction proteins by KEGG analysis, ECM receptor interaction, focal adhesion, and PI3K-AKT signalling were all presented in the transition from telogen to anagen in the hair follicle growth cycle. They served as candidate biomarkers for hair follicle regeneration and affected hair follicle development by regulating cell proliferation and migration, thus affecting the villus growth (Zhao et al. 2021; Li et al. 2022). The ECM receptors combined to their ligands, thus causing melanoblasts to proliferate and migrate, which filled the hair follicles (Cao et al. 2022). The focal adhesion can bind to the surrounding extracellular matrix and facilitate the migration of hair follicle stem cells to hair follicles (Haage et al. 2020). Moreover, the activation of the PI3K/AKT signalling pathway can upregulate cyclinD1, facilitate cell cycle progression, and induce the proliferation of hair follicle-derived mesenchymal stem cells, thus promoting hair follicle development (Wang et al. 2022b). This study also provides novel ideas for further exploration of the molecular mechanism of cell proliferation and hair follicle development.

The above 15 interacting proteins were evaluated, and the result indicated that the PI3K-AKT signalling pathway was inhibited after the knockout of PIEZO1 in osteoblasts (Bai et al. 2017). In addition, the Wnt signalling pathway served as a classical pathway of hair follicle proliferation, and the addition of Wnt signalling factors to the dermal papilla cells took on a critical significance in stimulating the hair growth.

It has been reported that PIEZO1 is capable of inducing the expression of the related gene *WNT16* (Gentile and Garcovich 2019; Chen et al. 2021), indicating that PIEZO1 plays a role in cell proliferation-associated signalling pathways. Galectin 3 binding protein (LGALS3BP) refers to a ubiquitous and multifunctional secreted glycoprotein. Natarajamurthy et al. (2019) confirmed the interaction of galectin 3 and LGALS3BP through Surface Plasmon Resonance (SPR). Choi et al. (2022) also suggested that LGALS3BP facilitates cell proliferation by promoting galectin 3-mediated epidermal growth factor receptor (EGFR) signalling. Existing research has demonstrated that keratin 33A, with a molecular weight of nearly 47 kDa, is a vital structural protein of goat cashmere fibre, which can serve as a marker protein to predict cashmere yield. It has been revealed keratin 33A is closely correlated with the formation of villi (Seki et al. 2011). The above scientific research results further confirm the credibility of the CO-IP results in this study.

PIEZO1 was screened out, which was a protein that can participate in the PI3K-AKT signalling pathway for further research. qPCR, CCK8 were adopted to deeply explore the mode of action of LGALS3 and its interacting protein PIEZO1, as well as the vital role of the interaction with them in the proliferation of skin fibroblasts in cashmere goats in Liaoning Cashmere goat skin fibroblasts. The results indicated that *PIEZO1* increased significantly after 48 h of the treatment with pLenti6.3-LGALS3-His; siRNA PIEZO1 significantly reduced the cell proliferation rate, which could be constricted by overexpression of LGALS3. As revealed by the above results, LGALS3 can facilitate the proliferation of skin fibroblasts by upregulating the expression of *PIEZO1*.

https://doi.org/10.17221/72/2024-CJAS

CONCLUSIONS

In brief, there is an interaction between LGALS3 and PIEZO1 in Liaoning Cashmere goat skin fibroblasts, and LGALS3 facilitates cell proliferation by upregulating *PIEZO1*.

This study lays a theoretical foundation for further research on whether the interaction between them mediates the activation of downstream signalling pathway and regulates cell proliferation through other ways (e.g., protein kinase/phosphorylation).

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Bai T, Liu F, Zou F, Zhao G, Jiang Y, Liu L, Shi J, Hao D, Zhang Q, Zheng T, Zhang Y, Liu M, Li S, Qi L, Liu JY. Epidermal growth factor induces proliferation of hair follicle-derived mesenchymal stem cells through epidermal growth factor receptor-mediated activation of ERK and AKT signaling pathways associated with upregulation of cyclin D1 and downregulation of p16. Stem Cells Dev. 2017 Jan 15;26(2):113-22.

Cao J, Wang J, Zhou H, Hu J, Liu X, Li S, Luo Y, Hickford JGH. Sequence variation in caprine KRTAP6-2 affects cashmere fiber diameter. Animals. 2022 Aug 11;12(16): eXXX.

Cao N, Tang X, Gao R, Kong L, Zhang J, Qin W, Hu N, Zhang A, Ma K, Li L, Si JQ. Galectin-3 participates in PASMC migration and proliferation by interacting with TGF-β1. Life Sci. 2021 Jun 1;274:119347.

Chen P, Zhang G, Jiang S, Ning Y, Deng B, Pan X, Liu S, He Y, Zhang L, Wan R, Wu Z, He Q, Yin J, Wang H, Li J. Mechanosensitive PIEZO1 in endothelial cells promotes angiogenesis to support bone fracture repair. Cell Calcium. 2021 Jun 7;97:102431.

Choi YS, Kim MJ, Choi EA, Kim S, Lee EJ, Park MJ, Kim MJ, Kim YW, Ahn HS, Jung JY, Jang G, Kim Y, Kim H, Kim K, Kim JY, Hong SM, Kim SC, Chang S. Antibody-mediated blockade for galectin-3 binding protein in tumor secretome abrogates PDAC metastasis. Proc Natl Acad Sci U S A. 2022 Jul 26;119(30):e2119048119.

Gentile P, Garcovich S. Advances in regenerative stem cell therapy in androgenic alopecia and hair loss: Wnt pathway, growth factor, and mesenchymal stem cell signaling impact analysis on cell growth and hair follicle development. Cells. 2019 May 16;8(5):eXXX.

Haage A, Wagner K, Deng W, Venkatesh B, Mitchell C, Goodwin K, Bogutz A, Lefebvre L, Van Raamsdonk CD, Tanentzapf G. Precise coordination of cell-ECM adhesion is essential for efficient melanoblast migration during development. Development. 2020 Jul 17;147(14): dev190223.

Jin M, Cao M, Cao Q, Piao J, Zhao F, Piao J. Long noncoding RNA and gene expression analysis of melatonin-exposed Liaoning cashmere goat fibroblasts indicating cashmere growth. Naturwissenschaften. 2018 Sep 26; 105(9-10):60.

Jin M, Han X, Fan W, Zhang Y, Qiu X. LncRNA MTC enhances the proliferation of Liaoning cashmere goat skin fibroblasts by modulating GSTM1-ASK1 interaction. Cytologia (Tokyo). 2024 Jan;89(1):29-37.

Li C, Feng C, Ma G, Fu S, Chen M, Zhang W, Li J. Time-course RNA-seq analysis reveals stage-specific and melatonin-triggered gene expression patterns during the hair follicle growth cycle in Capra hircus. BMC Genomics. 2022 Feb 16;23(1):140.

Liu Y, Ma J, Cui D, Fei X, Lv Y, Lin J. LncRNA MEG3-210 regulates endometrial stromal cell migration, invasion, and apoptosis through p38 MAPK and PKA/SERCA2 signaling via interaction with galectin-1 in endometriosis. Mol Cell Endocrinol. 2020 Aug 1;513:110870.

Mitchell SF, Parker R. Principles and properties of eukaryotic mRNPs. Mol Cell. 2014 May 22;54(4):547-58.

Miyazaki A, Sugimoto A, Yoshizaki K, Kawarabayashi K, Iwata K, Kurogoushi R, Kitamura T, Otsuka K, Hasegawa T, Akazawa Y, Fukumoto S, Ishimaru N, Iwamoto T. Coordination of WNT signaling and ciliogenesis during odontogenesis by piezo-type mechanosensitive ion channel component 1. Sci Rep. 2019 Oct 14;9(1):14762.

Moore MJ. From birth to death: The complex lives of eukaryotic mRNAs. Science. 2005 Sep 2;309(5740):1514-8.

Natarajamurthy SH, Sistla S, Dharmesh SM. Disruption of galectin-3 and galectin-3 binding protein (G3BP) interaction by dietary pectic polysaccharides (DPP) – Arrest of metastasis, inhibition of proliferation, and induction of apoptosis. Int J Biol Macromol. 2019 Oct 15; 139:486-99.

Ren H, Wang G, Chen L, Jiang J, Liu L, Li N, Zhao J, Sun X, Zhou P. Genome-wide analysis of long non-coding RNAs at early stage of skin pigmentation in goats (Capra hircus). BMC Genomics. 2016 Jan 19;17:67.

Seki Y, Yokohama M, Wada K, Fujita M, Kotani M, Nagura Y, Kanno M, Nomura K, Amano T, Kikkawa Y. Expression analysis of the type I keratin protein keratin 33A in goat coat hair. Anim Sci J. 2011 Dec;82(6):773-81.

https://doi.org/10.17221/72/2024-CJAS

Wang D, Cheng S, Zou G, Ding X. Paeoniflorin inhibits proliferation and migration of psoriatic keratinocytes via the lncRNA NEAT1/miR-3194-5p/galectin-7 axis. Anticancer Drugs. 2022a Jan 1;33(1):e423-33.

Wang J, Wu X, Kang Y, Zhang L, Niu H, Qu J, Wang Y, Ji D, Li Y. Integrative analysis of circRNAs from Yangtze River Delta white goat neck skin tissue by high-throughput sequencing (circRNA-seq). Anim Genet. 2022b Jun; 53(3):405-15.

Yoneda M, Suzuki H, Hatano N, Nakano S, Muraki Y, Miyazawa K, Goto S, Muraki K. PIEZO1 and TRPV4, which are distinct mechano-sensors in the osteoblastic MC3T3-E1 cells, modify cell proliferation. Int J Mol Sci. 2019 Oct 8;20(19):eXXX.

Zhang M, Cheng K, Chen H, Tu J, Shen Y, Pang L, Wu W. Galectin-3 knockdown inhibits cardiac ischemia-reperfusion injury through interacting with Bcl-2 and modulating cell apoptosis. Arch Biochem Biophys. 2020 Nov 15; 694:108602.

Zhang Y, Zhang D, Xu Y, Qin Y, Gu M, Cai W, Bai Z, Zhang X, Chen R, Sun Y, Wu Y, Wang Z. Selection of cashmere fineness functional genes by translatomics. Front Genet. 2021;12:775499.

Zhao M, Zhou H, Luo Y, Wang J, Hu J, Liu X, Li S, Zhang K, Zhen H, Hickford JGH. Variation in a newly identified caprine KRTAP gene is associated with raw cashmere fiber weight in Longdong cashmere goats. Genes. 2021 Apr 22;12(5):eXXX.

Received: May 16, 2024
Accepted: February 26, 2025
Published online: March 24, 2025