RETRACTED ARTICLE: Up-regulated lncRNA5322 elevates MAPK1 to enhance proliferation of hair follicle stem cells as a ceRNA of microRNA-19b-3p

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ABSTRACT
Hair follicle stem cells (HFSCs), located in the bulge region of the follicle, maintain hair follicle growth and cycling. Long non-coding RNAs (lncRNAs), non-protein coding transcripts, are widely known to play critical roles in differentiation and proliferation of stem cells. Therefore, the current study aimed to explore the regulatory roles of lncRNA5322 in HFSCs proliferation and the underlying regulatory mechanisms. Initially, the expression patterns of lncRNA5322 and microRNA-19b-3p (miR-19b-3p) in HFSCs were detected. Subsequently, gain-and loss-of-functions analyses were conducted to explore the roles of lncRNA5322, miR-19b-3p and mitogen-activated protein kinase 1 (MAPK1) in cell proliferation, colony formation and apoptosis of HFSCs, with the expression of cyclin-dependent kinase (CDK)1 and CDK2 examined. Also, the interaction relationships among lncRNA5322, miR-19b-3p and MAPK1 were explored. Furthermore, a mouse model was established to detect the roles of lncRNA5322, miR-19b-3p, and MAPK1 in wound contraction and epidermal regeneration. Over-expressed lncRNA5322 was found to promote proliferation, colony formation ability but inhibit apoptosis of HFSCs, in addition to up-regulation of the expression of CDK1 and CDK2. lncRNA5322 was found to act as a ceRNA of miR-19b-3p which directly targeted MAPK1. Furthermore, up-regulation of lncRNA5322 enhanced wound contraction and epidermal regeneration in vivo by increasing the expression of MAPK1 through functioning as a ceRNA of miR-19b-3p. In summary, the results in this study suggested that lncRNA5322 serves as a ceRNA of miR-19b-3p to elevate the expression of MAPK1, ultimately promoting HFSCs proliferation, wound contraction and epidermal regeneration of mouse model.

Introduction
Adult stem cells are known to substitute dying cells and repair injured tissues as soon as possible, thus playing a critical role in the maintenance of tissue homeostasis. Adult stem cells can be obtained from various tissues, such as the system of hair follicle (HF) [1]. This system primarily comprises epithelial cells forming a temporary area accommodating transit-amplifying cells and terminally differentiated lineages, and a permanent area containing the hair follicle stem cells (HFSCs) [2]. HFSCs are essential for the generation, maintenance and renewal of the continuously cycling hair follicle. Apart from the regular physiological functions in vivo, HFSCs are also known to serve as stem cells essential for wound tissue repair after deep skin injury, tissue engineering and regenerative medicine [3]. HFSCs have been widely accepted as an intriguing source for blood vessel engineering and valuable stem cell-based therapy [4]. More importantly, HFSCs possess the features of strong correlation with proliferation, colony formation, and regeneration [5]. HFSCs have garnered great attention, due to the fact that they are easily accessible and less invasive with lower morbidity rate in the donor site and high successful rates. Thereby, it is reasonable to suggest that elucidation of their molecular mechanism could facilitate the application of HFSCs in therapeutic conditions.

Long non-coding RNAs (lncRNAs), noncoding transcripts with a length of 200 nucleotides, have been extensively reported to play essential roles in the regulation of stem cell proliferation and differentiation [6,7]. For instance, lncRNA5322 (also lncRNA AK015322) was demonstrated to enhance the
proliferation and differentiation of HFSCs [8]. In addition, Hu et al. reported that IncRNA AK015322 is indispensable for male reproduction and could serve as a competing endogenous RNA (ceRNA) of microRNA-19b-3p (miR-19b-3p) [9]. MicroRNAs (miRs) are a family of short non-coding RNAs with the length of 21–24 nucleotides, which exert crucial roles in multiple cellular processes such as cell progression, proliferation and differentiation [10]. In particular, miR-214 and miR-125b have been found to operate as key mediators of HF maintenance in the telogen stage through their suppressive roles in HFSCs proliferation and differentiation [11]. Similarly, miR-19b-3p was confirmed to be associated with an enhanced proliferation of goat male germline stem cells [12]. Furthermore, miR-19b-3p was demonstrated to target mitogen-activated protein kinase 1 (MAPK1) in embryonic fibroblasts from the great tit under hypoxic conditions [13]. MAPKs represent the key components of the signal transduction system with essential functions in regulating diverse cellular development, such as the differentiation of stem cells [14]. Notable, it was proven that the MAPK signaling pathway plays an important role in the hair cycle and quiescence of HFSCs [15]. In the current study, we aim to investigate the effect of IncRNA5322 on HFSCs proliferation and further evaluate the underlying mechanisms.

Materials and methods

Ethics statement

This study protocol was approved by the Experimental Animal Ethics Committee of Zhengzhou University. The animal experiments were strictly conducted following the principles of the Ethics Committee. All efforts were made to minimize the pain and suffering of the included animals.

Cell culture

HFSCs purchased from Beijing Jing-Meng Stem Cell Technology Co., Ltd. (Beijing, China) were cultured in minimum essential medium (MEM; Sigma Aldrich; Merck KGaA, Darmstadt, Germany) supplemented with 10% fetal bovine serum (FBS; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). While the human embryonic kidney (HEK)-293T cells (American Type Culture Collection, Manassas, VA, USA) were cultured in Dulbecco’s modified eagle’s medium (DMEM; Life Technologies, Carlsbad, CA, USA) supplemented with 10% FBS (Life Technologies Inc., Grand Island, New York, USA), 100 μg/mL streptomycin, and 100 U/mL penicillin (Life Technologies Inc., Grand Island, New York, USA). All cells were maintained in a humidified environment at 37°C with 5% CO2 in air [9].

Lentiviral infection

Cells were seeded into a 24-well plate and allowed to reach 70–80% confluence, then infection was conducted with the addition of 10 μL (10^7 U/mL) lentivirus containing (LV)-negative control (NC) (cells treated with NC of over-expression of IncRNA5322 lentivirus), IncRNA5322 (cells treated with over-expression of IncRNA5322 lentivirus), sponge (cells treated with NC of silencing of IncRNA5322 lentivirus), IncRNA5322 sponge (cells treated with silencing of IncRNA5322 lentivirus), miR-19b-3p mimic NC (cells treated with miR-19b-3p mimic NC), miR-19b-3p mimic (cells treated with miR-19b-3p mimic), miR-19b-3p inhibitor NC (cells treated with miR-19b-3p inhibitor NC), miR-19b-3p inhibitor (cells treated with miR-19b-3p inhibitor), LncRNA5322S sponge + inhibitor-NC (cells treated with NC of silencing of IncRNA5322 lentivirus and miR-19b-3p inhibitor NC), LncRNA5322 sponge + miR-19b-3p inhibitor (cells treated with silencing of IncRNA5322 lentivirus and miR-19b-3p inhibitor), LncRNA5322 sponge + oe-NC (cells treated with silencing of IncRNA5322 lentivirus and over-expression of MAPK1 lentivirus) or LncRNA5322 sponge + oe-MAPK1 (cells treated with silencing of IncRNA5322 lentivirus and over-expression of MAPK1 lentivirus). All aforementioned lentivirus (10^7 U/mL), miR-19b-3p mimic/inhibitor and NC were purchased from Shanghai GenePharma Co, Ltd. (Shanghai, China). Cell transfection was carried out according to the instructions of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) using 100 nM miR-19b-3p mimic/inhibitor or NC. The medium was replaced with complete medium 6–8 h after transfection at 37°C with 5% CO2 in air. After another period of 24–48 h incubation, the cells were collected for subsequent experimentation.
Reverse transcription quantitative polymerase chain reaction (Rt-qPCR)

Total RNA content was extracted from the cells according to the instructions of the Trizol™ reagent (15,596,018, Invitrogen, Carlsbad, CA, USA). One μL RNA sample was reverse transcribed into complementary DNA (cDNA) template using the PrimeScript RT reagent kit (TaKaRa Bio, Inc., Otsu, Japan). Real-time fluorescence quantitative detection was performed using a miScript kit (Qiagen, Valencia, CA, USA) on the 7500-fluorescence quantitative instrument (ABI Company, Oyster Bay, NY, USA). The employed primers (Table 1) were synthesized by the Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 were regarded as an internal control for lncRNA5322 and miR-19b-3p, respectively, and the fold changes of genes between the experimental group and the control group were calculated using relative quantification (the 2−ΔΔC_{t} method) [16]. The experiment was independently repeated three times to obtain the mean value.

Cell counting kit-8 (CCK-8) assay

The cell proliferation was evaluated using the colorimetric cell proliferation assay CCK-8. After 48 h of transfection, 10 μL CCK-8 reagent was added to each well and incubated for 1–4 h. The optical density (OD) value at an excitation wavelength of 450 nm was measured using the CCK-8 kit (Dojindo Laboratories, Kumamoto, Japan) and the microplate reader (ELx800 Universal Microplate Reader, BioTek Inc., Highland Park, IL) [9]. The experiment was independently repeated three times to obtain the mean value.

Soft agar colony formation assay

In order to perform the soft agar colony formation assay, experiments were carried out in 6-well plates coated with a base layer of culturing medium containing agar, and a total of 2000–4000 cells were seeded in the 6-well plates and incubated for 12–14 days at 37°C with 5% CO₂ in air, with the culture medium being changed every 2 days. When the colonies were large enough to be identified and quantified, the experiment was terminated. The cell colonies were then fixed with 4% polyformaldehyde for 10 min, and stained with 1% crystal violet. The number and size of colonies were quantified using the Fiji software [17,18]. The experiment was independently repeated three times to obtain the mean value.

Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) assay

Apoptosis was assessed using the TUNEL assay. In brief, 48 h after transfection, the cells were rinsed with phosphate-buffered saline (PBS) three times, fixed with 4% polyformaldehyde for 30 min, and permeabilized with 0.5% TritonX-100 for 10 min. Subsequently, the cells were incubated with 50 μL TUNEL probe (C10618, Invitrogen, Carlsbad, CA, USA) at room temperature for 4 h, counter-stained using 4ʹ,6-Diamidino-2-Phenylindole (DAPI) at room temperature for 3 min, and observed under an inverted microscope (BX51, Olympus Corporation, Tokyo, Japan), with the nucleus exhibiting green fluorescence being considered as positive [16]. A total of four visual fields were randomly selected to count the number of total cells and apoptotic cells. The experiment was independently repeated three times to obtain the mean value.

Western blot analysis

HFSCs were lysed with radioimmunoprecipitation assay (RIPA) lysis buffer (Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA) and homogenized at 4°C for 10 min. The protein concentration was determined using a bicinchoninic acid (BCA)
Protein Assay Kit (53,225, Invitrogen, Carlsbad, CA, USA). After separation on 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the proteins (20 µg/lane) were transferred onto the polyvinylidene difluoride (PVDF) membrane, which was then blocked with 5% skimmed milk overnight at 4°C and washed with Tris-buffered saline Tween-20 (TBST). Then, the membrane was incubated at 4°C overnight with the following primary monoclonal antibodies: mouse antibody against cyclin-dependent kinase 1 (CDK1; ab18; dilution ratio of 1: 1000), rabbit antibody against CDK2 (ab32147; dilution ratio of 1: 1000), mouse antibody against GAPDH (ab8245; dilution ratio of 1: 10,000), and mouse antibody against MAPK1 (dilution ratio of 1: 200, MAB1230-SP). CDK1, CDK2, and GAPDH were purchased from Abc a mI n c .( C a m b r i d g e ,M A ,U S A ) a n d M A P K 1 w a s p u r c h a s e d f r o m R & D S y s t e m s ( M i n e a p o l i s, MN, USA). After three washes with TBST, the membrane was incubated with horseradish peroxidase (HRP)-labeled secondary antibody rabbit anti-mouse IgG (ab6728; dilution ratio of 1: 2000, Abcam Inc., Cambridge, MA, USA) or goat anti-rabbit IgG (ab6721; dilution ratio of 1: 2000, Abcam Inc., Cambridge, MA, USA) at 37°C for 1 h. After incubation, the blots were detected using an electrochemiluminescence (ECL) kit (Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA). The gray value of the target band was analyzed using the Image J software. The experiment was independently repeated three times to obtain the mean value.

RNA immunoprecipitation (RIP) assay

The HFSCs were lysed with lysis buffer (25 mM Tris-HCl [pH = 7.4]; 150 mM NaCl; 0.5% NP-40; 2 mM ethylenediaminetetraacetic acid [EDTA]; 1 mM NaF, and 0.5 mM dithiothreitol) containing RNase (Life Technologies Inc., Grand Island, New York, USA) and protease inhibitor cocktail (Roche, Basel, Switzerland). The lysate was then centrifuged at 12,000 g for 30 min with the supernatant collected. Subsequently, the protein G-agarose beads, Argonaute 2 (Ago2; P10502500, Otwo Biotech, Shenzhen, Guangdong, China), and IgG (Sigma-Aldrich, St. Louis, MO, USA) were incubated at 4°C for 2 h, followed by incubation with cell lysate overnight at 4°C. The beads were washed three times with wash buffer (50 mM Tris-HCl; 300 mM NaCl [pH = 7.4]; 1 mM MgCl 2; 0.1% NP-40), and RNA was then extracted from the beads using the Trizol™ reagent (Invitrogen, Carlsbad, CA, USA). The expression patterns of lncRNA5322 and miR-19b-3p were detected using RT-qPCR.

Dual-luciferase reporter gene assay

Full-length wild type (wt) or mutant type (mut) sequence of lncRNA5322 was cloned into the Pmel/NaeI site of the pMIR-reporter. Next, HEK293T cells were co-transfected with miR-19b-3p mimic (100 nM) and scramble NC (Shanghai GenePharma Co. Ltd., Shanghai, China) together with lncRNA5322-wt or lncRNA5322-mut. After 48 h of transfection, the cells were collected and lysed, and then the luciferase activity was detected. Similarly, the potential relationship between miR-19b-3p and MAPK1 was determined using a dual-luciferase reporter gene assay. For this, the 3’ untranslated region (3’UTR) of MAPK1-3’UTR (NM_001357115) containing miR-19b-3p-wt or the mutant binding site was cloned into the downstream of psiCHECKTM-2r vector (Promega Corporation, Madison, WI, USA) [19]. Transfection was performed using the Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions.

Establishment of wound mice models

A total of 60 mice (weighing [24 ± 3 g]) were purchased from the Experimental Animal Center of Zhengzhou University (Zhengzhou, Henan, China). The mice were anesthetized by intraperitoneal inoculation with 3% pentobarbital sodium. After removal of dorsal hair, routine disinfection using iodophor and deiodination were conducted, and circular wounds without damaging the muscles were created 1 cm near the dorsal spines, and the wounds were not bandaged or administered any drug treatment. The mice were then individually reared in sterile laboratories which were disinfected every day. The mice were inoculated only once with 10 μL lentivirus (107 U/mL) and 100 nM miR-19b-3p antagonim/antagomir-NC on the wound surface. The mice were treated with sponge + antagonim
NC, lncRNA5322 sponge + antagonir NC, lncRNA5322 sponge + miR-19b-3p antagonir, lncRNA5322 sponge + oe-NC, or lncRNA5322 sponge + oe-MAPK1. The wounds were monitored and photographed on the 6th day, the 12th day, and the 18th day after inoculation. After inoculation, all mice were euthanized by injection with an overdose of anesthetic, and then skin tissues were extracted from the wound surface of mice and stored at −20°C for hematoxylin-eosin (HE) staining [20,21].

**Statistical analysis**

All experimental data were analyzed using the SPSS 21.0 software (IBM Corp. Armonk, NY, USA). Measurement data were presented as mean ± standard deviation. Data between two groups were analyzed using the independent sample $t$-test and corrected using the Welch test. Normal distribution of data among multiple groups was conducted using the Shapiro-Wilk Test. The measurement data under normal distribution were analyzed using one-way analysis of variance (ANOVA). Among multiple groups, the pairwise comparisons were analyzed using the least significant difference (LSD) test, and the data at different time points were analyzed using repeated measures ANOVA. The data in skew distribution were compared using the non-parametric Kruskal-Wallis test. A value of $p < 0.05$ was considered to be of statistical significance.

**Results**

**lncRNA5322 enhances HFSCs proliferation**

The expression patterns of lncRNA5322 in HFSCs were detected using RT-qPCR after 48 h of transfection. The results (Figure 1(a)) showed that the HFSCs transfected with lncRNA5322 exhibited higher expression of lncRNA5322 compared to the HFSCs transfected with LV-NC ($p < 0.05$). Compared to the sponge treatment, lncRNA5322 sponge was found to reduce the expression of lncRNA5322 ($p < 0.05$). These results confirmed successful transfection, and that the obtained HFSCs could be used for subsequent experimentation. In order to explore the effects of lncRNA5322 on HFSCs proliferation, HFSCs were transfected with LV-NC, lncRNA5322, sponge, and lncRNA5322 sponge, followed by detection using the CCK-8 assay (Figure 1(b)) and soft agar colony formation assay (Figure 1(c)). Up-regulation of lncRNA5322 significantly enhanced cell viability and

**Figure 1.** lncRNA5322 promotes the proliferation of HFSCs. (a) relative expression patterns of lncRNA5322 in HFSCs detected by RT-qPCR. (b) cell viability of HFSCs measured by CCK-8 assay. (c) colony formation of HFSCs measured by soft agar colony formation assay. (d) protein expression patterns of CDK1 and CDK2 in HFSCs determined by Western blot analysis. (e) mRNA expression patterns of CDK1 and CDK2 in HFSCs detected by RT-qPCR. (f) apoptosis of HFSCs measured by TUNEL assay (200 ×). * $p < 0.05$ vs. the HFSCs treated with LV-NC; # $p < 0.05$ vs. the HFSCs treated with sponge. The data were measurement data and presented as mean ± standard deviation. Data in Panel A, C, D, and E were analyzed using one-way ANOVA and data in Panel B were analyzed using repeated measures ANOVA. The experiment was independently repeated three times to obtain the mean value. LncRNA, long non-coding RNA; HFSCs, hair follicle stem cells; LV, lentiviral vectors; RT-qPCR, reverse transcription quantitative polymerase chain reaction; NC, negative control; CDK-8 cell counting kit-8; CDK, cyclin-dependent kinase; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling; ANOVA, analysis of variance.
colony formation ability compared to the LV-NC treatment ($p < 0.05$). In contrast, lncRNA5322 sponge-treated HFSCs exhibited attenuated cell viability and colony formation ability compared to the sponge-treated HFSCs ($p < 0.05$). Besides, the mRNA and protein expression patterns of CDK1 and CDK2 were detected by RT-qPCR and Western blot analysis, the results revealed that the mRNA and protein expression of CDK1 and CDK2 in HFSCs was significantly increased after transfection with lncRNA5322, while those were decreased in HFSCs transfected with lncRNA5322 sponge compared with LV-NC and sponge, respectively (all $p < 0.05$) (Figure 1(d,e)). Furthermore, TUNEL assay was performed to examine the apoptosis of HFSCs after various transfections. As shown in Figure 1(f), compared with LV-NC transfection, over-expression of lncRNA5322 reduced the number of TUNEL-positive HFSCs ($p < 0.05$), while lncRNA5322 sponge treatment elevated the number of TUNEL-positive HFSCs in comparison with the HFSCs delivered with sponge ($p < 0.05$). All the aforementioned results demonstrated that lncRNA5322 could promote proliferation of HFSCs.

**lncRNA5322 functions as a competing endogenous RNA (ceRNA) of miR-19b-3p**

LncRNA has previously been confirmed to regulate the expression of miRNA by acting as a ceRNA [9], and thereby, the current study further aimed to investigate the relationship between lncRNA5322 and miR-19b-3p. RIP assay was initially employed to detect whether lncRNA5322 interacted with RNA-induced silencing complex mouse Ago2 antibody in HFSCs. The RIP results showed that lncRNA5322 was markedly enriched in Ago2 antibody-treated HFSCs compared with the IgG antibody-treated HFSCs (Figure 2(a)). In addition, the RegRNA website predicted that there was a potential binding site between miR-19b-3p and lncRNA5322 (Figure 2(b)). Additionally, RT-qPCR was conducted to determine the expression patterns of lncRNA5322 in HFSCs with transfection of miR-19b-3p, and the results revealed that miR-19b-3p overexpression downregulated the expression of lncRNA5322 in HFSCs (Figure 2(c)). Further, dual-luciferase reporter gene assay indicated that over-expression of miR-19b-3p repressed the luciferase activity of the reporter plasmid containing a full-

![Image](image-url)
length lncRNA5322 sequence, but did not influence that of lncRNA5322 with mutated binding site of miR-19b-3p (Figure 2(d)). All these results suggested that lncRNA5322 was a decoy of miR-19b-3p.

**Up-regulation of miR-19b-3p suppresses HFSCs proliferation**

In order to explore the effects of miR-19b-3p on HFSCs proliferation, the cells were transfected with miR-19b-3p inhibitor, miR-19b-3p mimic and corresponding controls. After transfection, CCK-8 assay and soft agar colony formation assay were performed to examine the viability and colony formation ability of HFSCs. As depicted in Figure 3(a,b) compared with the miR-19b-3p inhibitor NC, miR-19b-3p inhibitor-treated HFSCs exhibited increased cell viability and colony formation ability (p < 0.05), while miR-19b-3p mimic-treated HFSCs presented with decreased cell viability and colony formation ability in contrast to the treatment of miR-19b-3p mimic-NC (p < 0.05). Western blot analysis and RT-qPCR results showed that miR-19b-3p inhibitor led to the up-regulation of CDK1 and CDK2 (p < 0.05), while miR-19b-3p mimic down-regulated the protein and mRNA expression of CDK1 and CDK2 (p < 0.05) compared with their controls, respectively (Figure 3(c)). Furthermore, TUNEL assay was performed to detect the role of miR-19b-3p in HFSCs apoptosis, which revealed that the number of TUNEL-positive HFSCs was reduced after transfection with miR-19b-3p inhibitor (p < 0.05), but elevated after transfection with miR-19b-3p mimic in comparison with their corresponding controls (p < 0.05) (Figure 3(d)). Based on these results, a conclusion could be drawn that the inhibition of miR-19b-3p promoted HFSCs proliferation.

**Over-expression of lncRNA5322 elevates MAPK1 expression by competitively binding to miR-19b-3p**

The Targetscan website was used to predict the potential binding site of miR-19b-3p and MAPK1, and it revealed one putative miR-19b-3p-binding site in the MAPK1-3'UTR (Figure 4(a)). Subsequently, a dual-luciferase reporter gene assay was conducted to verify the prediction (Figure 4(b)). It was observed that after co-transfection with miR-19b-3p mimic and MAPK1-3'UTR-wt, the luciferase activity of HFSCs was markedly reduced compared with the transfection with the mimic-NC and MAPK1-3'UTR-wt, however, there were no significant differences in the luciferase activity after co-transfection with miR-19b-3p mimic and MAPK1-3'UTR-mut. Additionally, RT-qPCR and Western blot analysis

![Figure 3. Inhibition of miR-19b-3p induced the proliferation of HFSCs.](image)

(a) cell viability of HFSCs after transfection with miR-19b-3p mimic or inhibitor. (b) colony formation ability of HFSCs detected by soft agar colony formation assay. (c) protein and mRNA expression patterns of CDK1 and CDK2 in HFSCs determined by Western blot analysis and RT-qPCR. (d) apoptosis of HFSCs measured by TUNEL assay (200 ×). * p < 0.05 vs. the HFSCs transfected with miR-19b-3p inhibitor NC; # p < 0.05 vs. the HFSCs transfected with miR-19b-3p mimic NC. The data were measurement data and expressed as mean ± standard deviation. Data in Panel A were analyzed using the repeated measures ANOVA and data among multiple groups in Panel B, C, and D were analyzed using one-way ANOVA. The experiment was independently repeated three times to obtain the mean value. miR-19b-3p, microRNA-19b-3p; HFSCs, hair follicle stem cells; CCK-8, cell counting kit-8; CDK, cyclin-dependent kinase; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling; NC, negative control; ANOVA, analysis of variance.
were conducted to measure the mRNA and protein expression patterns of MAPK1 in HFSCs transfected with miR-19b-3p mimic or inhibitor. The results indicated that knockdown of miR-19b-3p upregulated the mRNA and protein expression of MAPK1, while over-expression of miR-19b-3p led to the opposite results in comparison with the treatment of inhibitor-NC and mimic-NC, respectively (all \( p < 0.05 \)) (Figure 4(c,d)). From the above-mentioned results and the known interactions of lncRNA5322 with miR-19b-3p by acting as the ceRNA of miR-19b-3p, we applied RT-qPCR and

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**Figure 4.** Up-regulation of lncRNA5322 increases MAPK1 by competitively binding to miR-19b-3p. (a) the binding site of miR-19b-3p in the 3'UTR of MAPK1 predicted in the Targetscan website. (b) interaction between miR-19b-3p and MAPK1 confirmed by dual-luciferase reporter gene assay. (c) relative mRNA expression of MAPK1 in HFSCs transfected with miR-19b-3p mimic or inhibitor examined by RT-qPCR. (d) protein expression patterns of MAPK1 in HFSCs transfected with miR-19b-3p mimic or inhibitor detected by Western blot analysis. (e) mRNA expression patterns of MAPK1 in HFSCs treated with lncRNA5322 mimic or inhibitor detected by RT-qPCR. F, protein expression patterns of MAPK1 in HFSCs delivered with lncRNA5322 mimic or inhibitor determined by Western blot analysis. * \( p < 0.05 \) vs. the miR-19b-3p inhibitor NC-treated HFSCs; # \( p < 0.05 \) vs. the miR-19b-3p mimic NC-treated HFSCs. The data were measurement data and expressed as mean ± standard deviation. Data among multiple groups in Panel C and D were analyzed using one-way ANOVA and data in Panel B were analyzed using independent sample t-test. The experiment was independently repeated three times to obtain the mean value. miR-19b-3p, microRNA-19b-3p; MAPK1, mitogen-activated protein kinase 1; HFSCs, hair follicle stem cells; RT-qPCR, reverse transcription quantitative polymerase chain reaction; NC, negative control; ANOVA, analysis of variance.
Western blot analysis to further identify whether lncRNA5322 affected the expression patterns of MAPK1 in HFSCs transfected with lncRNA5322 mimic/inhibitor or NC. The results revealed that the mRNA and protein expression of MAPK1 was significantly increased by transduction with lncRNA5322 compared with transduction with LV-NC, while an opposite effect was induced by lncRNA5322 sponge in contrast to the sponge treatment (all \( p < 0.05 \)) (Figure 4(e,f)). Collectively, these results suggested that MAPK1 was a target gene of miR-19b-3p, and lncRNA5322 affected the MAPK1 expression by competitively binding to miR-19b-3p.

**miR-19b-3p/MAPK1 mediates the effects of lncRNA5322 on HFSCs proliferation**

Our results indicated that miR-19b-3p directly targeted MAPK1, and next, we evaluated whether the miR-19b-3p/MAPK1 pathway was involved in the effect of lncRNA5322 on HFSCs proliferation and colony formation ability. As detected by CCK-8 assay (Figure 5(a)) and soft agar colony formation assay (Figure 5(b)), the cell viability and colony formation ability of HFSCs were weakened after co-treatment with lncRNA5322 sponge and miR-19b-3p inhibitor NC relative to combined treatment with sponge and miR-19b-3p inhibitor NC (\( p < 0.05 \)), while the treatment with both lncRNA5322 sponge and miR-19b-3p inhibitor augmented cell viability and colony formation ability of HFSCs in contrast to co-treatment with lncRNA5322 sponge and miR-19b-3p inhibitor NC (\( p < 0.05 \)). In comparison with treatment with both lncRNA5322 sponge and oe-NC, lncRNA5322 sponge together with oe-MAPK1 enhanced cell viability and colony formation ability of HFSCs (\( p < 0.05 \)). Besides, the results of western blot analysis and RT-qPCR (Figure 5(c)) suggested that compared with HFSCs treated with both sponge and miR-19b-3p inhibitor NC, HFSCs treated with both lncRNA5322 sponge and inhibitor-NC exhibited dramatically decreased protein and mRNA expression of MAPK1, CDK1 and CDK2 (\( p < 0.05 \)). In contrast to

**Figure 5.** miR-19b-3p/MAPK1 mediates the effects of lncRNA5322 on HFSCs proliferation. (a) cell viability of HFSCs after transfection detected by CCK-8 assay. (b) colony formation ability of HFSCs after transfection measured by soft agar colony formation assay. (c) protein and mRNA expression patterns of CDK1 and CDK2 in HFSCs determined by Western blot analysis and RT-qPCR. (d) apoptosis of HFSCs detected by TUNEL assay (200 ×). *\( p < 0.05 \) vs. the HFSCs treated with both sponge and inhibitor NC; #\( p < 0.05 \) vs. the HFSCs treated with both lncRNA5322 sponge and inhibitor-NC; &\( p < 0.05 \) vs. the HFSCs treated with both lncRNA5322 sponge and oe-NC. The data were measured data and expressed using mean ± standard deviation. Data in Panel A were analyzed using repeated measures ANOVA and data among multiple groups in Panel B, C, and D were analyzed using one-way ANOVA. The experiment was independently repeated three times to obtain the mean value. miR-19b-3p, microRNA-19b-3p; lncRNA, long non-coding RNA; MAPK1, mitogen-activated protein kinase 1; HFSCs, hair follicle stem cells; CCK-8, cell counting kit-8; CDK, cyclin-dependent kinase; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling; NC, negative control; ANOVA, analysis of variance; ceRNA, competing endogenous RNA.
the treatment with both lncRNA5322 sponge and inhibitor-NC, co-treatment with lncRNA5322 sponge and miR-19b-3p inhibitor significantly increased the protein and mRNA expression of MAPK1, CDK1 and CDK2 ($p < 0.01$). In comparison with the treatment with both lncRNA5322 sponge and oe-NC, the protein and mRNA expression of MAPK1, CDK1 and CDK2 was obviously increased by co-treatment with lncRNA5322 sponge and oe-MAPK1 ($p < 0.05$). Further, the results of TUNEL staining (Figure 5(d)) showed that transfection with both lncRNA5322 sponge and miR-19b-3p inhibitor or oe-MAPK1 caused a decreased number of TUNEL-positive HFSCs compared with co-transfection of lncRNA5322 sponge with miR-19b-3p inhibitor NC or oe-NC ($p < 0.05$). These results verified that miR-19b-3p/MAPK1 pathway is involved in the effect of lncRNA5322 on HFSCs proliferation.

**LncRNA5322 mediates miR-19b-3p/MAPK1 to promote wound contraction and epidermal regeneration in vivo**

Wound mice models were established to investigate the effects of lncRNA5322 on wound contraction and epidermal regeneration of the mice. As shown in Figure 6(a,b) the wound contraction area was found

**Figure 6.** LncRNA5322 improves wound contraction and epidermal regeneration in vivo by mediating miR-19b-3p/MAPK1. (a) wound contraction area of the mice with different treatments. (b) statistical diagram of wound contraction area. (c) epidermal regeneration of the mice with different treatment examined by HE staining (200 ×). * $p < 0.05$ vs. the mice treated with both sponge and antagonir NC; # $p < 0.05$ vs. the mice treated with both lncRNA5322 sponge and antagonir NC; & $p < 0.05$ vs. the mice treated with both lncRNA5322 sponge and oe-NC. The data were measurement data and expressed as mean ± standard deviation (n = 12 mice in each group). Data in Panel B were analyzed using one-way ANOVA. LncRNA, long non-coding RNA; miR-19b-3p, microRNA-19b-3p; MAPK1, mitogen-activated protein kinase 1; NC, negative control; ANOVA, analysis of variance; ceRNA, competing endogenous RNA.
to be reduced and healing time was increased following inoculation of lncRNA5322 sponge along with miR-19b-3p antagomir NC compared with sponge along with miR-19b-3p antagomir NC (p < 0.05), however, the combination of lncRNA5322 sponge and miR-19b-3p antagomir or oe-MAPK1 led to increased wound contraction area and reduced healing time compared with the co-treatment with lncRNA5322 sponge and antagonir-NC or oe-NC (p < 0.05). The results of HE staining (Figure 6(c)), which was performed to observe epidermal regeneration, revealed that at the 18th day of wound healing, there was no new and complete epidermis observed in the mice treated with lncRNA5322 sponge and miR-19b-3p antagomir NC relative to the mice treated with sponge and miR-19b-3p antagomir NC (p < 0.05). Treatment of both lncRNA5322 sponge and miR-19b-3p antagomir or oe-MAPK1 led to epidermal regeneration (p < 0.05) in comparison with the co-treatment with lncRNA5322 sponge and oe-NC (p < 0.05). Taken together, it was proven that lncRNA5322 enhanced wound contraction and epidermal regeneration in vivo by mediating miR-19b-3p/MAPK1.

Discussion

HFSCs are characterized by their ability of self-renewal and withhold the important potentials for mammalian hair growth and skin wound repair, and thus HFSCs are considered to be promising therapeutic targets for human disease and skin injuries [22,23]. However, the underlying regulatory functions of HFSCs which may possess therapeutic value remain to be elucidated [24]. Therefore, the current study aimed to investigate how lncRNA5322 affected the proliferation of HFSCs. Our results indicated that overexpressed lncRNA5322 increased the expression of MAPK1 to promote proliferation, wound contraction and epidermal regeneration of HFSCs by serving as a ceRNA of miR-19b-3p.

It is widely known that numerous lncRNAs serve as ceRNAs or regulatory sponges of miRs to regulate miR expression in human diseases [25,26]. For instance, lncRNA5322 was discovered to regulate the proliferation of spermatogonial stem cell by serving as a ceRNA of miR-19b-3p, which directly targeted MAPK1 [9]. Similarly, lncRNA-H19 was demonstrated to contribute to enhanced survival and angiogenic ability of mesenchymal stem cells (MSCs) by acting as a ceRNA of miR-199a-5p [27]. Our results also suggested that lncRNA5322 regulates the proliferation of HFSCs by acting as a ceRNA of miR-19b-3p.

MAPK1 was revealed to be a direct target gene of miR-19b-3p in embryonic fibroblasts under hypoxia [13]. Furthermore, it was previously demonstrated that miR-23a-5p could specifically bind to MAPK13 to regulate the osteogenic differentiation of human mesenchymal stem cells [28]. Moreover, miR-19b expression was found to be negatively associated with MAPK14 expression in hepatocellular carcinoma (HCC), and elevated miR-19b was suggested to be a predictor of positive prognosis for HCC patients [29]. Interestingly, both miR-128 and miR-19b have been demonstrated to target SMAD2 to participate in the differentiation process of human HF-MSCs into smooth muscle cells (SMCs), and thus serve as potential targets for tissue engineering and regenerative medicine for SMCs [30,31]. Similarly, lncRNA X inactive-specific transcript (XIST) positively regulated the expression of MAPK1 to participate in the progression of HCC by serving as a ceRNA of miR-194-5p [32]. It was revealed that lncRNA PlncRNA-1 could activate the transforming growth factor-β-regulated Wnt/β-catenin signaling pathway, thus promoting HFSCs proliferation and differentiation [33]. Another study found that lncRNA5322 targets the PI3K/AKT signaling pathway, which is regulated by miR-21, in order to enhance the proliferation of HFSCs [8]. In the current study, we uncovered that lncRNA5322 serves as a ceRNA of miR-19b-3p, which directly targets MAPK1 in HFSCs. LncRNA5322 over-expression, MAPK1 elevation, or miR-19b-3p depletion all led to the elevated HFSCs proliferation, wound healing and epidermal regeneration. Consistently, lncRNA AK015322 was demonstrated to function as a ceRNA of miR-19b-3p to induce proliferation of spermatogonial stem cells [9]. Additionally, Wu et al. previously demonstrated that miR-19b was closely involved in the progression of gastric cancer as a result of the maintenance of self-renewal and proliferative functions of gastric cancer stem cells [34].

Apart from proliferation, both lncRNAs and miRs have also been highlighted to play essential roles in the regulation of the process of wound healing
Depletion of miR-204 and miR-205 was previously demonstrated to lead to enhanced transient cell proliferation and migration, which are critical processes for successful wound healing [37]. Apoptosis signal-regulating kinase 1 (ASK1), a member belonging to the MAP3K family, was further revealed to be essential for epithelial wound healing and depleted ASK1 could retard hair regrowth induced by wounding [38], which was in line with our finding that elevated MAPK1 could improve wound healing, wound contraction and epidermal regeneration in vivo. Moreover, Lhx2 transcriptional factor expressed in HF bud, with critical functions in regulating activities of stem cells, was confirmed to contribute to enhanced epidermal regeneration in HFSCs following injury [39]. These findings demonstrate that HFSCs proliferation, wound healing and epidermal regeneration were enhanced with up-regulated LncRNA5322, elevated MAPK1, or inhibited miR-19b-3p.

Taken together, the results in the current study evidence that up-regulation of LncRNA5322 contributes to enhanced HFSCs proliferation by positively regulating the expression of MAPK1 through functioning as a ceRNA of miR-19b-3p (Figure 7), which provides new insight in understanding the regulatory mechanism in HFSCs functions. However, the detailed molecular mechanism by which LncRNA5322 regulated HFSCs in specific human diseases required further research to bear positive therapeutic results.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**Figure 7.** The molecular mechanism involved LncRNA5322/miR-19b-3p/MAPK1 affecting HFSCs and wound contraction and epidermal regeneration of mice. LncRNA5322 acted as a ceRNA of miR-19b-3p, and over-expression of LncRNA5322 improved cell proliferation and colony formation ability while inhibited apoptosis of HFSCs and enhanced wound healing and epidermal regeneration of mice by up-regulating MAPK1 through sponging miR-19b-3p. LncRNA, long non-coding RNA; MAPK1, mitogen-activated protein kinase 1; miR-19b-3p, microRNA-19b-3p; HFSCs, hair follicle stem cells; ceRNA, competing endogenous RNA.
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