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LINC00937 suppresses keloid fibroblast proliferation and extracellular matrix deposition by targeting the miR-28-5p/MC1R axis

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Summary. Long noncoding RNAs (lncRNAs) are the most recently discovered class of noncoding RNAs. LncRNAs play a crucial role in multiple disorders. However, the role and mechanism of action of lncRNAs in keloids remain unclear. Here, qRT-PCR and western blotting assays were performed to determine the expression of genes and proteins, respectively. MTT assays were carried out to measure the proliferation of keloid fibroblasts. In addition, a luciferase activity assay was conducted to investigate the relationships between LINC00937 and miR-28-5p and between miR-28-5p and MC1R. The results showed that LINC00937 and MC1R were decreased, whereas miR-28-5p was increased in keloid tissues. LINC00937 overexpression in keloid fibroblasts could repress the extracellular matrix (ECM) deposition and cell proliferation and promote MC1R expression. Moreover, high expression of miR-28-5p and low expression of LINC00937 were detected in keloid fibroblasts. We further showed that LINC00937 promoted MC1R expression by sponging miR-28-5p. Finally, our data indicated that LINC00937 inhibited the ECM deposition and proliferation of keloid fibroblasts by inhibiting miR-28-5p and facilitating MC1R expression. Overall, LINC00937 suppressed the ECM deposition and proliferation of keloid fibroblasts by acting as an miR-28-5p sponge and promoting MC1R expression. Our data suggested that LINC00937 is a potential target for keloid treatment.

Key words: Keloid, Long noncoding RNA, LINC00937, Keloid fibroblasts, Extracellular matrix deposition

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Introduction

Keloid is a dermal fibroproliferative disease accompanied by pain and itching in involved regions and primarily occurs in the ear, jaw, shoulder deltoid and sternum handle (He et al., 2017). Keloids are characterized by fibrosis caused by overabundant deposition of extracellular matrix (ECM) and abnormal proliferation of keloid fibroblasts (KD-FBs) (Andrews et al., 2016). Currently, surgical excision, medical treatment (such as corticosteroid drugs, bleomycin and interferon) and laser or light-based therapies are still the major strategies for keloid treatment (Mamalis et al., 2014). However, recurrence after surgical excision and treatment is common, even with combination therapies. Hence, it is necessary to explore the pathogenesis of keloids and provide a novel target for disease treatment.

In recent years, an increasing number of studies have revealed the crucial role of long noncoding RNAs (lncRNAs) in keloids. Chip analysis showed that multiple lncRNAs are aberrantly expressed in keloid tissues, such as ENST00000419703 and uc003jox.1 (Yuan et al., 2017). Huang et al. proved that numerous hedgehog signaling pathway-related lncRNAs, such as HNF1A-AS1 and AC073257.2, are abnormally expressed in keloid tissues and pointed out that these two lncRNAs affect the proliferation and growth of keloid cells (Huang et al., 2018). In addition, Wang et al. demonstrated that lncRNA H19 is increased in both keloid tissues and fibroblasts, and increasing the expression of H19 could enhance the viability and proliferation of KD-FBs by promoting COL1A1 expression through miR-29a inhibition (Wang et al., 2020). These studies proved the potential of lncRNAs to act as treatment targets in keloids. Wang et al. found that H19 acted as a sponge for miR-29a and exerted its regulatory effect via a competing endogenous RNA (ceRNA) mechanism.

Melanocortin-1 receptor (MC1R) is a well-known

seven-transmembrane G protein-coupled receptor that is expressed in skin and other tissues. MC1R plays an important role in multiple biological functions, such as the immune response, oxidative stress response and sebaceous gland secretion (Chen et al., 2019; Jackson et al., 2019). Moreover, MC1R was also indicated to be involved in the regulation of fibroblasts by affecting collagen deposition (Stanisz et al., 2011). It was demonstrated that MC1R has a high affinity for proopiomelanocortin-derived α-melanocyte-stimulating hormone (α-MSH). *In vitro*, α-MSH was proven to inhibit the expression of collagen I, collagen III and collagen V in TGF-β1-induced human dermal fibroblasts by binding to MC1R (Böhm and Luger, 2004; Böhm et al., 2004). Our previous study revealed that MC1R is decreased in keloid tissues and KD-FBs. Downregulated MC1R limited the interaction of MC1R with α-MSH, which resulted in the deposition of collagen and subsequent excessive deposition of ECM and keloids (Luo et al., 2013).

Recently, Yuan et al. analyzed the differentially expressed lncRNAs between keloid tissues and normal tissues, as well as between KD-FBs and normal fibroblasts (NL-FBs). According to the results of their work, LINC00937 was significantly downregulated in both keloid tissues and KD-FBs (Yuan et al., 2017). However, the role and mechanism of action of LINC00937 in keloids remain unknown. In the present study, we verified the low expression of LINC00937 in keloid tissues and proved that increasing LINC00937 expression could significantly suppress the ECM deposition of KD-FBs. Then, we further indicated that LINC00937 inhibited ECM deposition of KD-FBs by promoting the expression of MC1R by acting as a miR-28-5p sponge. Our data provide reliable evidence to support the potential of LINC00937 as a treatment target for keloids.

Table 1. Clinical and demographic characteristics of patients included in this study.

Number	Sex	Age (years)	Etiology	Anatomic site	Duration (years)
K1	Female	42	Acne	Chest	4
K2	Female	36	Acne	Chest	3
K3	Male	37	Acne	Upper back	7
K4	Female	20	Acne	Chest	8
K5	Male	43	Infection	Upper back	5
K6	Male	20	Acne	Upper back	5
K7	Female	40	Acne	Chest	10
K8	Female	62	Infection	Upper back	6
K9	Female	52	Infection	Upper back	8
K10	Male	30	Acne	Chest	2
K11	Female	28	Acne	Upper back	3
K12	Male	40	Infection	Upper back	3
K13	Female	16	Acne	Chest	5
K14	Male	58	Infection	Upper back	6
K15	Female	25	Infection	Chest	1

K, Keloid patients.

Materials and methods

Source of samples

This study was approved by the Ethics Committee of Renmin Hospital of Wuhan University. All works were accomplished in light of the principles of the Declaration of Helsinki. Informed consent was obtained from all patients before the operation. None of the patients had been pretreated for keloids before the operation. Keloid patients were diagnosed according to a previous study (Yuan et al., 2017). The clinical and demographic characteristics of each subject are shown in Table 1. Keloid tissues and matched normal tissues were isolated from 15 patients (nine females and six males; 16 to 62 years old) who underwent therapy at Renmin Hospital of Wuhan University. Keloid tissues were isolated from the upper back or chest, and the lesion and normal tissues from individual patients served as the tested samples and the controls, respectively. All tissues were immediately frozen in liquid nitrogen following excision from patients and then stored at -80°C until analysis. Next, the difference in the expression of LINC00937 between keloid tissues and normal tissues was verified by qRT-PCR assays.

QRT-PCR analysis

Total RNA was extracted from tissues and cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Next, cDNA was synthesized using a SuperScript® II Reverse Transcriptase kit (Invitrogen) according to the protocol. After that, real-time PCR was carried out to measure the relative gene expression of LINC00937, miR-28-5p and MC1R on a ViiA 7 Real-time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) using SYBR Green MasterMix (Invitrogen). The reaction conditions were 95°C for 10 min, 95°C for 10 sec with 40 cycles, and finally 60°C for 1 min. Finally, the relative expression of genes was calculated in accordance with the $2^{-\Delta\Delta Ct}$ method. *GAPDH* served as the internal reference for LINC00937 and MC1R mRNA, and the relative expression of miR-28-5p was normalized to that of *U6*. The primer sequences are shown in table 2.

Cell culture

KD-FBs and NL-FBs were isolated from keloid tissues and matched normal tissues, respectively. Fresh removed specimens were washed with sterilized PBS three times. Subsequently, the tissues were cut into blocks 3×8×5 mm in size and then maintained with 5 mg/ml dispase II (Sigma-Aldrich, St. Louis, MO, USA) at 37°C for 4 hours. After the epidermis was removed, the tissues were cut into smaller blocks 2×2×2 mm in size and then maintained with 3 mg/ml collagenase type I (Thermo Fisher Scientific, Waltham, MA, USA) at 37°C for 2 hours. Next, excessive tissue blocks were

removed, and the suspension was filtered using a 200 mesh cell strainer. After that, the cells were incubated with Dulbecco's modified Eagle's medium (DMEM; Gibco, Invitrogen) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 mg/ml streptomycin in an incubator (37°C, 5% CO_2). Glucose concentration is 1000 mg/L. The medium was replaced every three days, and finally, passage 3 cells were used for the next study.

Cell transfection

Full-length LINC00937 was subcloned into the pcDNA 3.1 plasmid (HANBIO, Shanghai, China) to obtain the LINC00937 overexpression plasmid (pcDNA-LINC00937). For our study, pcDNA-LINC00937, empty vector, miR-28-5p mimic, mimic negative control (mimic NC), miR-28-5p inhibitor, inhibitor NC, MC1R siRNA and siRNA-NC were transfected into KD-FBs using Lipofectamine® 3000 (Thermo Fisher Scientific, Inc.). These mimics and inhibitors were purchased from Suzhou GenePharma Co., Ltd. (Suzhou, China). Twenty-four hours later, the expression of genes and proteins in KD-FBs and the proliferation of KD-FBs were examined.

MC1R siRNA: sense: 5'-GUGCUGGAGACUA CUAUCATT-3', and anti-sense: 5'-UGAUAGUAGUC UCCAGCACTT-3'; siRNA NC: sense: 5'-GUGAUGA AGCCUGCUACCATT-3', and anti-sense: 5'-UGGU AGCAGGCUUCAUCACTT-3'; miR-28-5p inhibitor: 5'-CUCAAUAGACUGUGAGCUCCUU-3'; inhibitor NC: 5'-UUGUACUACACAAAAGUACUG-3'.

MTT assay

An MTT assay was performed to measure the proliferation of KD-FBs. After 24 hours of transfection, the cells were plated into 96-well plates (1×10^5 cells per well). Next, 20 μl MTT (5 mg/ml, Solarbio, Beijing, China) solution was added to each well and then incubated with the cells for 48 hours. After that, the MTT solution was replaced by 150 μl dimethyl sulfoxide. Finally, the OD value was measured at 490 nm using a microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Western blotting assay

Total protein was isolated from KD-FBs using RIPA lysis buffer (Beyotime Institute of Biotechnology, Beijing, China), and then the concentration was determined using NanoDrop 2000 software. Equal amounts of protein (25 μg) from each group were loaded onto and separated by 12% SDS-PAGE. Then, the proteins were transferred to PVDF membranes, which were then incubated with 5% nonfat milk for 1 hour at room temperature. Subsequently, the membranes were incubated with primary antibodies against α-smooth muscle actin (α-SMA, 1:2000, Abcam, Cambridge, UK), collagen I (1:2000, Abcam), collagen III (1:2000, Abcam), fibronectin (1:2000, Abcam), MC1R (1:1000, Abcam), tubulin (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and β-actin (Santa Cruz) at 4°C overnight. Next, the membranes were incubated with HRP-labeled secondary antibodies (1:4000, Abcam) for 1 hour at room temperature. Finally, the membranes were immersed in an ECL developer (Merck Millipore, Billerica, MA, USA), and then the relative expression of proteins was analyzed using ImageJ software. Tubulin served as the internal reference for α-SMA, collagen I, collagen III and fibronectin, and β-actin served as the reference for MC1R.

Luciferase activity assay

LINC00937-wild type (WT) and mutant (Mut), MC1R 3'UTR-WT and Mut were obtained from Sangon (Shanghai, China). To investigate the interaction between LINC00937 and miR-28-5p, LINC00937-WT or LINC00937-Mut together with miR-28-5p mimic or mimic NC were cotransfected into 293 cells. To explore the relationship between miR-28-5p and MC1R mRNA, miR-28-5p mimic or mimic NC together with MC1R 3'UTR-WT or MC1R 3'-UTR-Mut were transfected into 293 cells. Forty-eight hours later, the luciferase activity of the cells was measured using a dual-luciferase reporter gene kit (Beyotime, Shanghai, China).

Statistical analysis

All data were analyzed by SPSS 22.0 statistical software (IBM Corp., Armonk, NY), and the data are shown as the mean ± standard deviation (SD). Significant differences between two independent groups were ensured by Student's t-test, and comparisons among multiple groups were verified by one-way analysis of variance. P values lower than 0.05 indicated statistically significant differences.

Table 2. The primer sequences.

Gene name	forward	reverse
LINC00937	5'-CGGGTCCTTCCTCTTCCCCA-3'	5'-CGCAGCCTCTTCTCTTCGGG-3'
MC1R	5'-CTTCTGCCTCAAGGGTGCTG-3'	5'-TCAACAGTGGAGCTGAGGACG-3'
GAPDH	5'-GAAGGTGAAGGTCGGAGTC-3'	5'-GAAGATGGTGATGGGATTTC-3'
miR-28-5p	5'-GGTCCTTGCCCTCAAGGAGCTCACA-3'	5'-AGTGCCTGCCCTCCAGGAGCTCACA-3'
U6	5'-GCTTCGGCAGCACATATACTAAAT-3'	5'-CGCTTCACGAATTTGCGTGTCAT-3'

Results

Increased LINC00937 expression repressed the proliferation and ECM deposition of KD-FBs

First, we measured the expression level of LINC00937 in both keloid tissues and normal tissues. Our data showed that LINC00937 was significantly downregulated in keloid tissues (Fig. 1A). Then, to perform our study at the cellular level, we isolated KD-FBs and NL-FBs from keloid tissues and matched normal tissues, and images of the cells are shown in Fig.

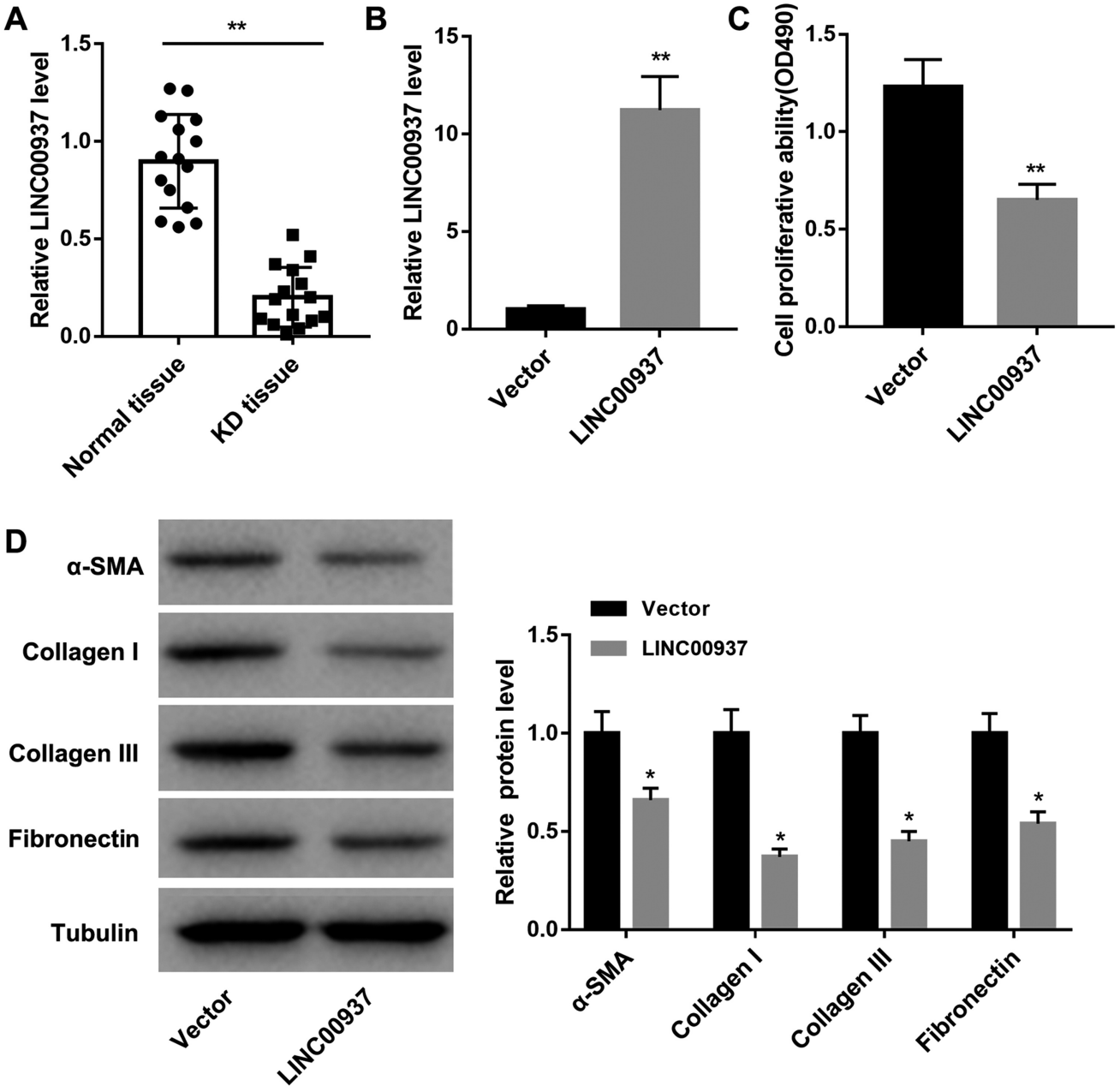

Fig. 1. Overexpression of LINC00937 inhibited KD-FB proliferation and ECM deposition. **A.** The expression of LINC00937 in 15 normal tissues and 15 keloid tissues was measured using qRT-PCR. n=15. **P<0.01 contrasted with normal tissue. **B.** The transfection efficiency of empty vector and pcDNA-LINC00937 was examined using qRT-PCR. n=3. **P<0.01 vs. Vector. **C.** Twenty-four hours after transfection, an MTT assay was performed to detect the proliferation of KD-FBs. n=3. **P<0.01 compared with vector alone. **D.** western blotting assays were performed to measure the expression of α-SMA, collagen I, collagen III and fibronectin in KD-FBs. n=3. *P<0.05 vs. Vector.

2. To investigate the role of LINC00937 in the proliferation and ECM deposition of KD-FBs, an LINC00937 overexpression plasmid (pcDNA-LINC00937) was constructed. The expression of LINC00937 in KD-FBs was remarkably induced by pcDNA-LINC009937 (Fig. 1B). Subsequently, an MTT

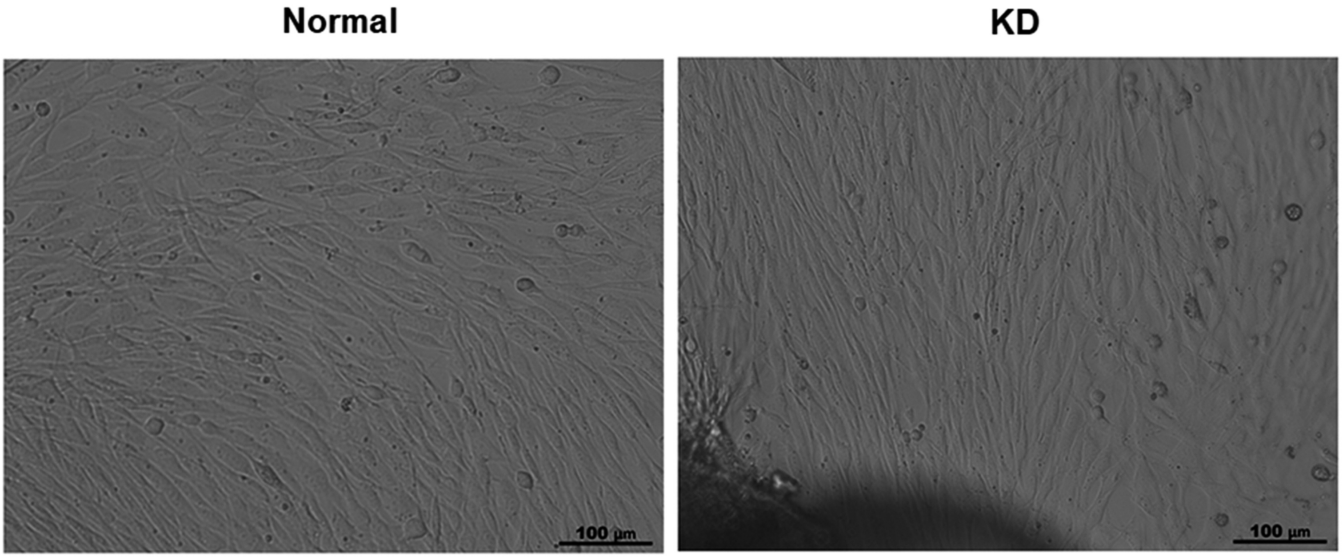

Fig. 2. Images of fibroblast cells isolated from keloid tissues and matched normal tissues.

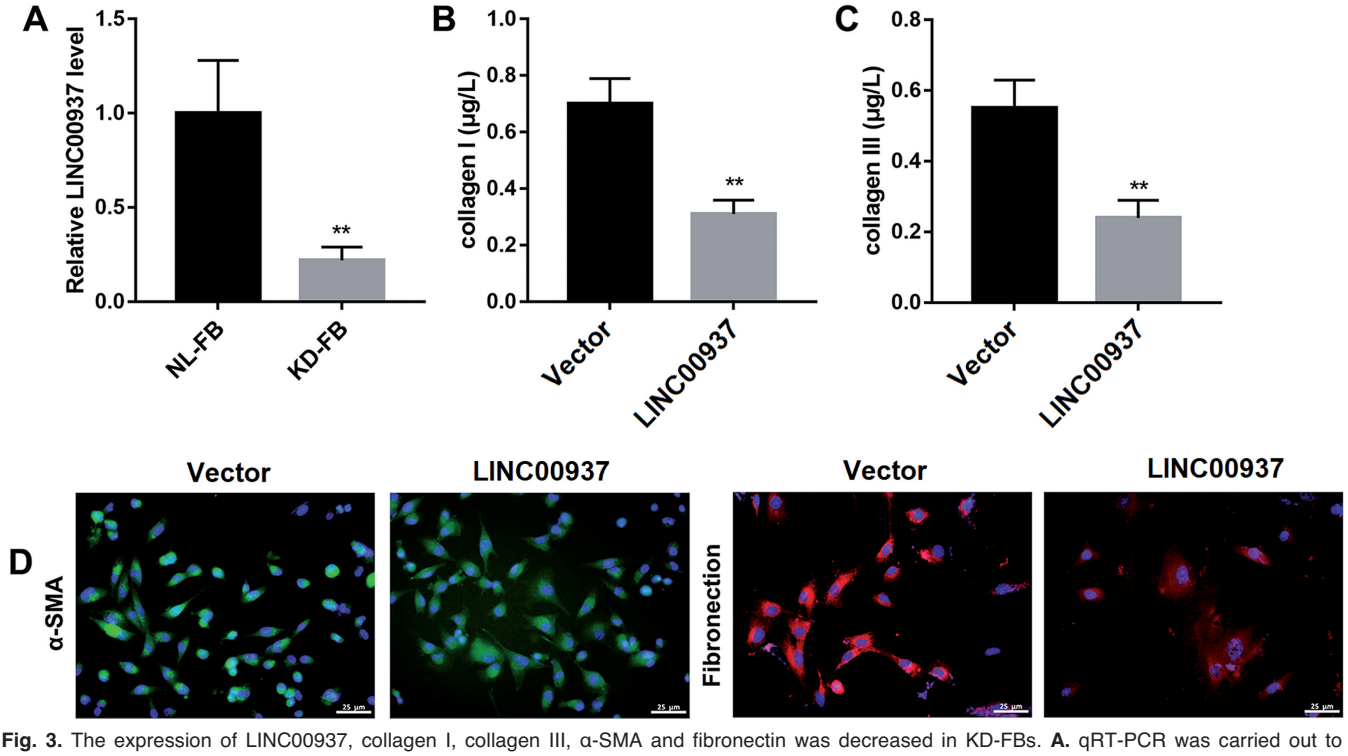

Fig. 3. The expression of LINC00937, collagen I, collagen III, α-SMA and fibronectin was decreased in KD-FBs. **A.** qRT-PCR was carried out to examine the expression of LINC00937 in both NL-FBs and KD-FBs. n=3. **P<0.01 compared with NL-FB. Twenty-four hours later, LINC00937 overexpression plasmid and empty vector transfection. **B, C.** ELISA was performed to analyze the concentration of collagen I and collagen III in the cell culture supernatants of KD-FBs. n=3. **P<0.01 compared with Vector. **D.** Immunofluorescence was utilized to detect the expression of α-SMA and fibronectin in KD-FBs. n=3.

assay was carried out to detect the proliferation of KD-FBs. As shown in Fig. 1C, the proliferation of KD-FBs was repressed after LINC00937 was overexpressed. It is well known that α-SMA, collagen I, collagen III and fibronectin are important components of the ECM, and the excessive upregulation of these factors is a crucial characteristic of ECM deposition and fibrosis (Frangogiannis, 2017; Li et al., 2017). Here, we found that upregulating LINC00937 could reduce the expression of α-SMA, collagen I, collagen III and fibronectin in KD-FBs (Fig. 1D). In addition, we further found that the concentrations of collagen I and collagen III were downregulated in the cell supernatant of KD-FBs when LINC00937 was increased (Fig. 3B,C). Immunofluorescence assays also proved that the expression levels of α-SMA and fibronectin were decreased in KD-FBs after increasing LINC00937 (Fig. 3D). In summary, LINC00937 was decreased in keloid tissues, and its overexpression repressed the proliferation and ECM deposition of KD-FBs. These data indicated

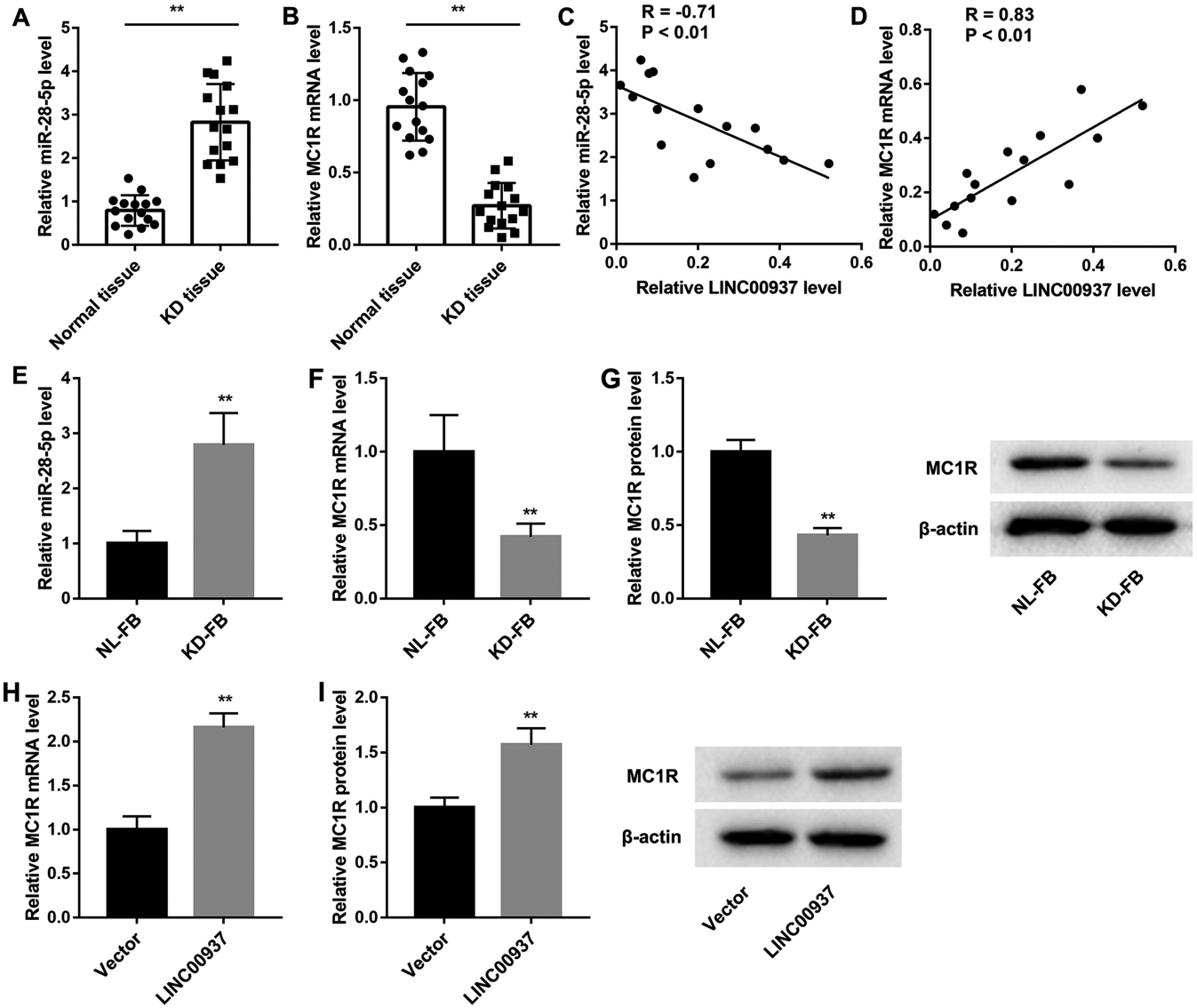

Fig. 4. Increased LINC00937 promoted MC1R expression. **A.** The expression of miR-28-5p in both keloid tissues and normal tissues was measured by a qRT-PCR assay. n=15. **P<0.01 vs. normal tissue. **B.** The expression of MC1R mRNA in tissues was measured by qRT-PCR. n=15. **P<0.01 vs. normal tissue. **C.** The relationship between LINC00937 levels and miR-28-5p levels in keloid tissues was analyzed. **D.** The relationship between LINC00937 levels and MC1R mRNA levels in keloid tissues was also analyzed. **E.** The expression of miR-28-5p in both NL-FBs and KD-FBs was measured by a qRT-PCR assay. n=3. **P<0.01 vs. NL-FB. **F, G.** qRT-PCR and western blotting were performed to determine the expression of MC1R at both the gene and protein levels, respectively. n=3. **P<0.01 vs. NL-FB. **H, I.** After 24 hours of pcDNA-LINC00937 transfection, the expression levels of MC1R mRNA and protein were measured by qRT-PCR and western blot assays, respectively. n=3. **P<0.01 vs. vector.

the potential of LINC00937 to act as an inhibitory regulator of keloids.

LINC00937 overexpression facilitated the expression of MC1R

Furthermore, our results showed that miR-28-5p was increased in keloid tissues (Fig. 4A), while MC1R mRNA was decreased (Fig. 4B). The level of LINC00937 was negatively correlated with miR-28-5p in keloid tissues (Fig. 4C), but the level of LINC00937 was positively correlated with MC1R mRNA in keloid tissues (Fig. 4D). Next, we determined the expression difference of miR-28-5p in NL-FBs and KD-FBs, and the expression of MC1R and LINC00937 was also measured. In the present study, we found that miR-28-5p was upregulated in KD-FBs (Fig. 4E). However, the expression of MC1R at both the gene (Fig. 4F) and protein (Fig. 4G) levels was significantly downregulated in KD-FBs. Immunofluorescence staining for MC1R also showed that it was decreased in KD-FBs (Fig. 5). The expression of LINC00937 was also downregulated in KD-FBs (Fig. 3A). Importantly, we further revealed that overexpression of LINC00937 promoted MC1R mRNA expression (Fig. 4H), and the expression of MC1R at the protein level was also enhanced following overexpression of LINC00937 (Fig. 4I). These data indicated that LINC00937 suppressed the proliferation and ECM deposition of KD-FBs, possibly by promoting MC1R expression.

LINC00937 promoted MC1R expression by acting as a miR-28-5p sponge

To investigate the relationship between LINC00937, miR-28-5p and MC1R, we predicted the binding sites between LINC00937 and miR-28-5p and between miR-28-5p and the MC1R 3'-UTR. Then, a luciferase activity assay demonstrated the interaction between LINC00937 and miR-28-5p and between miR-28-5p and MC1R mRNA (Fig. 6A). We further showed that the expression of LINC00937 in KD-FBs was inhibited following an increase in miR-28-5p, and decreasing the levels of miR-28-5p could facilitate the expression of LINC00937 (Fig. 6B). In addition, we also indicated that increasing miR-28-5p suppressed MC1R expression at both the gene and protein levels and that decreasing miR-28-5p promoted MC1R expression (Fig. 6C,D). Then, rescue experiments were conducted to determine whether LINC00937 promoted MC1R expression by acting as a miR-28-3p sponge. Here, our data showed that the increase in MC1R mRNA and protein expression by LINC00937 was partly reversed by increasing miR-28-5p (Fig. 6E,F). Overall, LINC00937 acted as a sponge for miR-28-5p and promoted MC1R expression by targeting miR-28-5p.

LINC00937 repressed the proliferation and ECM deposition of KD-FBs by targeting miR-28-5p/MC1R signaling

Based on the above experiments, we further

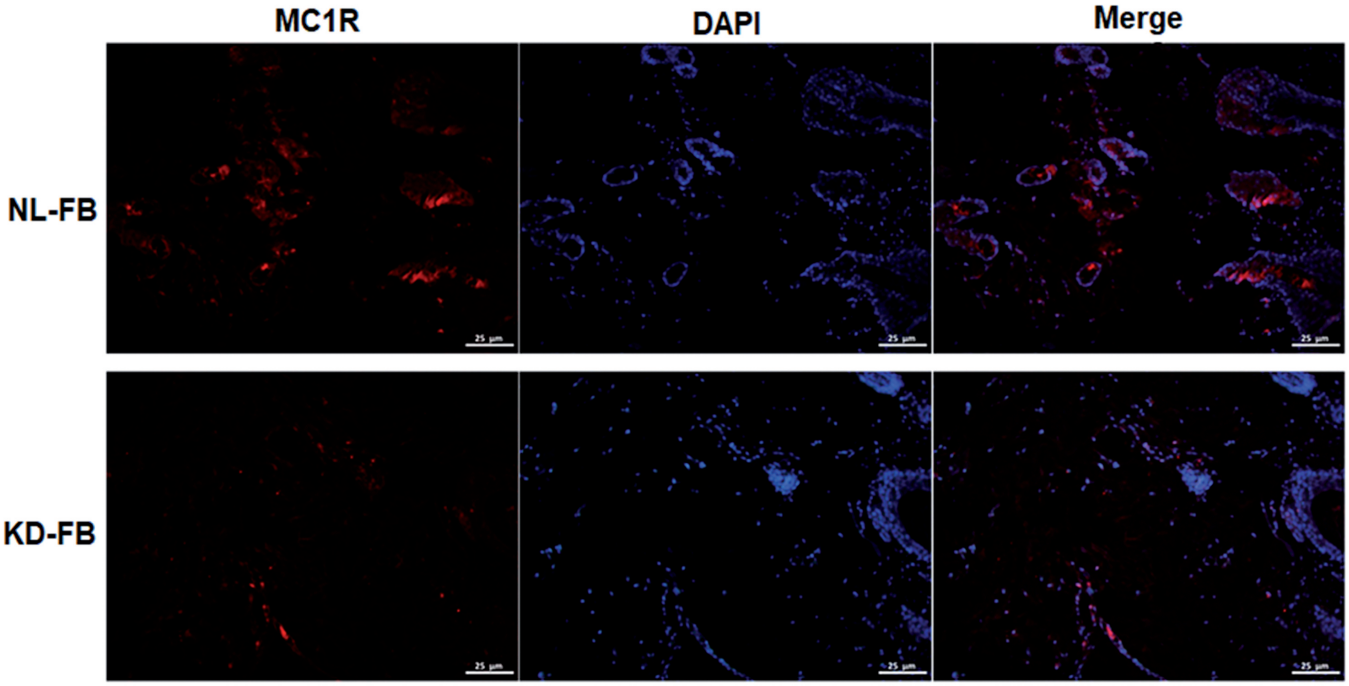

Fig. 5. MC1R was decreased in KD-FBs. The expression of MC1R in both NL-FBs and KD-FBs was measured by immunofluorescence assay. n=3.

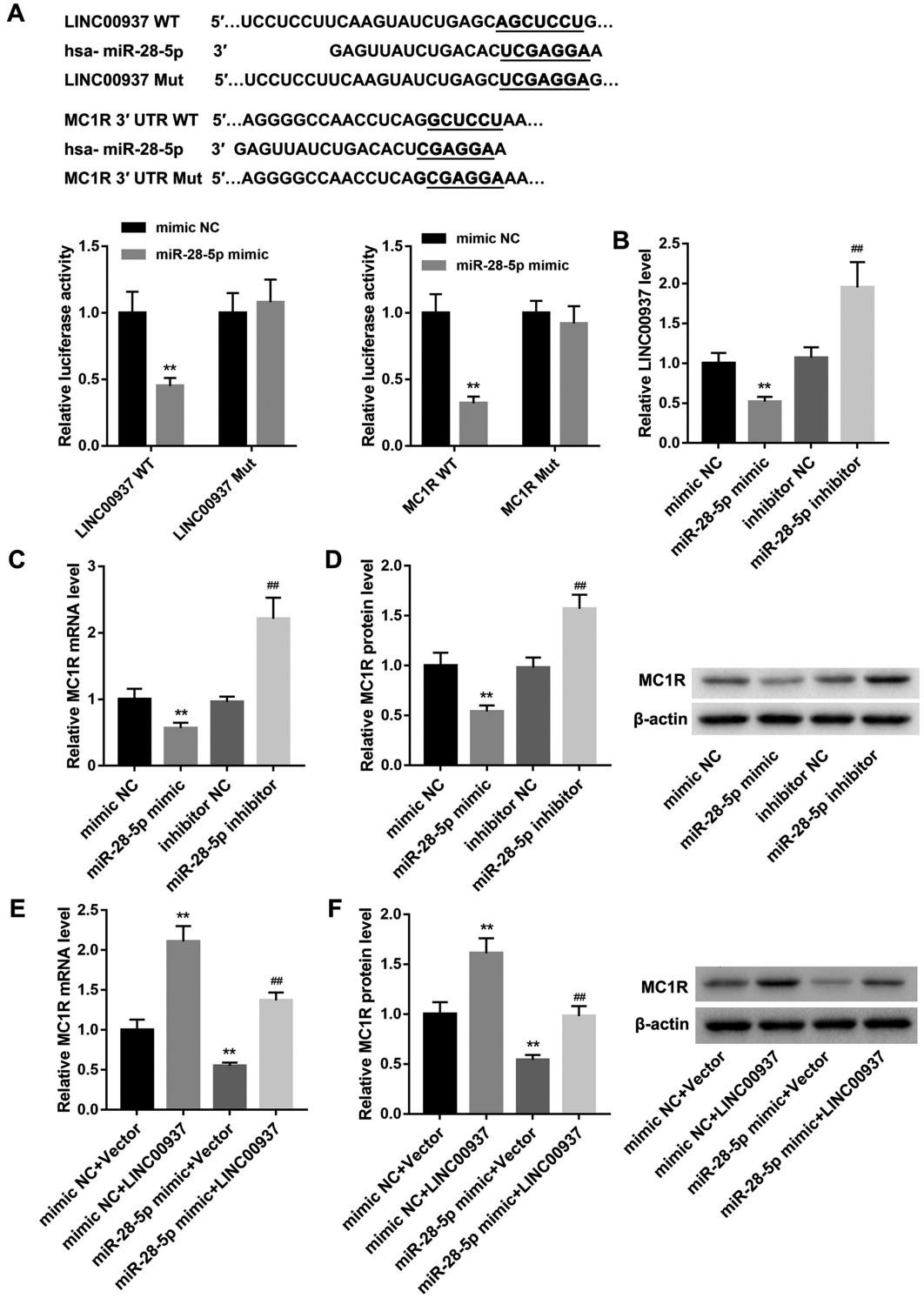

Fig. 6. LINC00937 facilitated MC1R expression by acting as a miR-28-5p sponge. **A.** The relationship between LINC00937 and miR-28-5p and between miR-28-5p and MC1R mRNA were investigated using a luciferase activity assay. The underlined region represents the mutant binding sites between miR-28-5p and LINC00937 or the MC1R 3'UTR. n=3. Then, KD-FBs were transfected with miR-28-5p mimic, miR-28-5p inhibitor or its control 24 hours later. **B.** The expression of LINC00937 in cells was measured by a qRT-PCR assay. n=3. **P<0.01 vs. mimic NC, and ##P<0.01 vs. inhibitor NC. **C.** qRT-PCR was performed to examine the expression of MC1R mRNA. n=3. **P<0.01 vs. mimic NC, and ##P<0.01 vs. inhibitor NC. **D.** Western blotting was carried out to measure the expression of MC1R protein. n=3. **P<0.01 vs. mimic NC, and ##P<0.01 vs. inhibitor NC. Finally, KD-FBs were co-transfected with miR-28-5p mimic and pcDNA-LINC00937. After 24 hours **(E)**, the expression of MC1R mRNA in the cells was detected by qRT-PCR. n=3. **P<0.01 vs. mimic NC + Vector, and ##P<0.01 vs. miR-28-5p mimic + Vector. **F.** The expression of MC1R protein was measured by western blot assay. **P<0.01 vs. mimic NC + Vector, and ##P<0.01 vs. miR-28-5p mimic + Vector.

explored the regulatory mechanism of LINC00937 in keloid fibrosis. Our results indicated that the inhibition of LINC00937 on KD-FB proliferation was partly reversed by increasing miR-28-5p (Fig. 7A). Moreover, the inhibitory effect of LINC00937 on α-SMA, collagen I, collagen III and fibronectin expression in KD-FBs was partly reversed by increasing miR-28-5p (Fig. 7B). Furthermore, KD-FBs were co-transfected with pcDNA-LINC00937 and MC1R siRNA, and the qRT-PCR results showed that LINC00937-induced downregulation of KD-FB proliferation could be blocked by MC1R knockdown (Fig. 7C). Consistently, the inhibition of α-SMA, collagen I, collagen III and fibronectin expression by LINC00937 was partly limited by a decrease in MC1R (Fig. 7D). Taken together, our data demonstrated that LINC00937 repressed the proliferation and ECM deposition of KD-FBs by inhibiting miR-28-3p and promoting MC1R expression.

Discussion

LncRNAs are a recently discovered type of noncoding RNA. Increasing evidence has demonstrated that lncRNAs are important regulators of gene expression and are involved in multiple biological processes, including proliferation, apoptosis, cell viability, DNA damage and repair (He et al., 2020). LncRNAs are considered potential treatment targets in many diseases. Mechanistically, in addition to interacting with RNA binding proteins, lncRNAs were

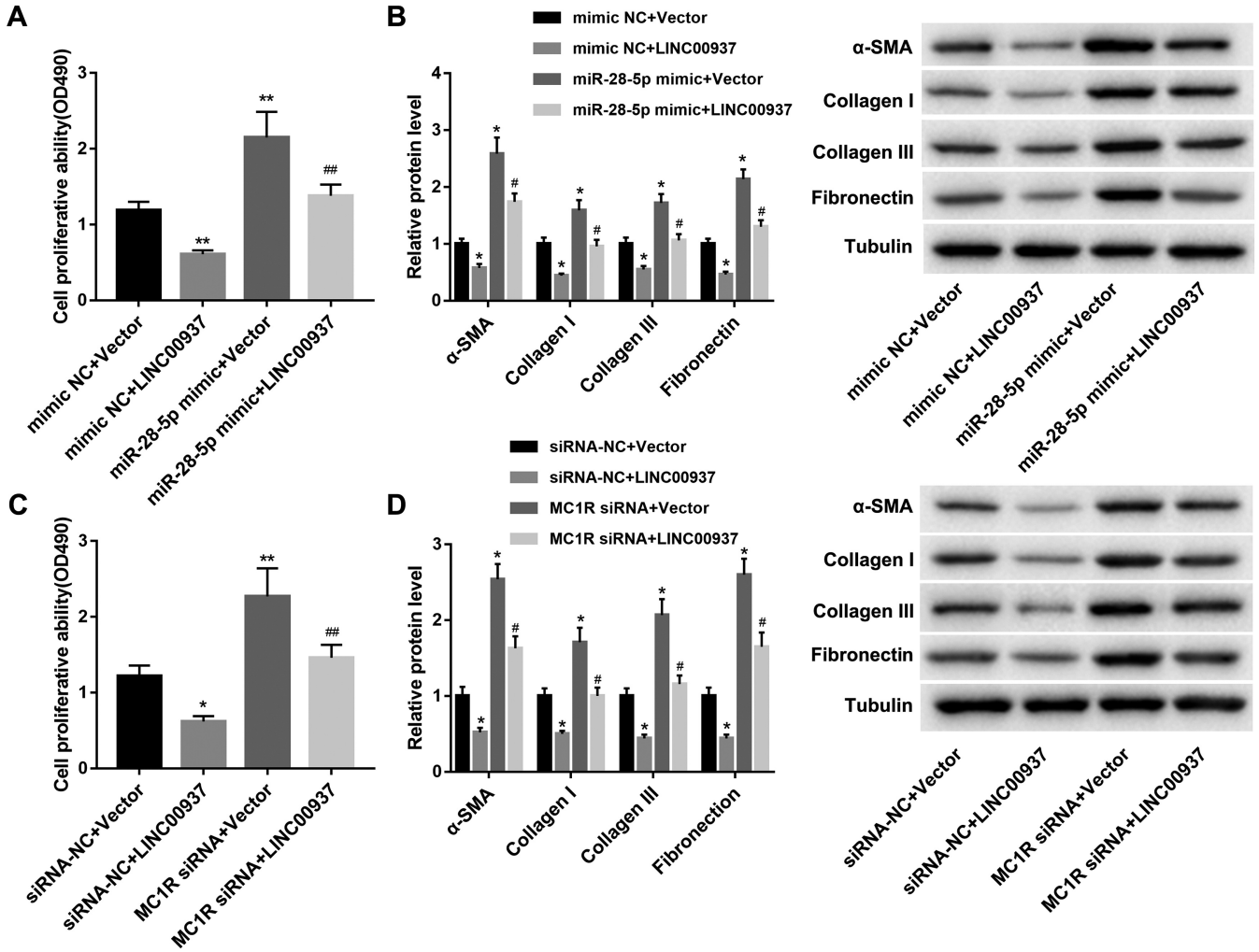

Fig. 7. LINC00937 inhibited the proliferation and ECM deposition of KD-FBs by targeting miR-28-5p and MC1R. **A.** The proliferation of KD-FBs was measured by using the MTT assay. **B.** The expression of α-SMA, collagen I, collagen III and fibronectin was measured by western blot. n=3. **P<0.01 compared with the mimic NC + Vector group; ##P<0.01 compared with the mimic NC + LINC00937 group. **C.** An MTT assay was carried out to examine KD-FB proliferation. **D.** Western blotting was performed to measure the expression of α-SMA, collagen I, collagen III and fibronectin in KD-FBs. n=3. *P<0.05 and **P<0.01 compared with the siRNA-NC + Vector group; #P<0.05 and ##P<0.01 compared with the siRNA-NC + LINC00937 group.

also found to bind with RNA and DNA. Most notably, lncRNAs prevent miRNA-mediated repression of gene expression by binding to miRNAs and functioning as ceRNAs (Kopp and Mendell, 2018). In recent years, numerous studies have demonstrated that lncRNAs are also involved in the deposition of ECM. For instance, Li et al. indicated that lncRNA TM1P3 promotes ECM degradation of chondrocytes by serving as a miR-22 sponge, thus enhancing the development of osteoarthritis (Li et al., 2019). In addition, Duan et al. reported that in diabetic nephropathy, TUG1 could repress the expression of TGF-β1, fibronectin, and collagen IV by functioning as a miR-377 sponge in high glucose-induced mesangial cells, thus impeding ECM accumulation in the cells (Duan et al., 2017).

Excessive ECM deposition is a major pathological alteration in keloids. During the development of keloids, ECM composition displays increased collagen I, collagen III, fibronectin, biglycan and periostin and decreased elastin and decorin (Limandjaja et al., 2020). Recently, Jin et al. showed that lncRNA HOXA11-AS is increased in keloid tissues, and downregulation of HOXA11-AS could restrain collagen I synthesis and KD-FB proliferation. They further indicated that HOXA11-AS promotes the development of keloids by acting as a miR-124-3p sponge (Jin et al., 2019). Zhu et al. demonstrated that lncRNA-ATB is highly expressed in keloid tissues and KD-FBs, and knock down of lncRNA-ATB notably represses the autocrine secretion of TGF-β2, which is closely associated with the formation of scars and fibrosis, thus obstructing the development of keloids (Zhu et al., 2016). These studies suggested the important role of lncRNAs in keloid development. In the present study, we proved that LINC00937 was decreased in keloid tissues and KD-FBs. Upregulation of LINC00937 could reduce the deposition of ECM and the proliferation of KD-FBs. MiRNAs, a group of short noncoding RNAs, are closely associated with the expression of genes at the posttranscriptional level. It was indicated that miRNAs play a crucial role in ECM production and degradation, as well as aberrant cell proliferation (Yu et al., 2015). Here, we found that miR-28-5p was increased in KD-FBs. The expression level of LNC00937 was negatively correlated with the level of miR-28-5p in keloid tissues. Importantly, LINC00937 acted as a sponge for miR-28-5p and facilitated the expression of MC1R by repressing miR-28-5p.

In humans, MC1R was identified on chromosome 16 and belongs to the family of five seven-transmembrane G-protein coupled receptors. Compared with NL-FBs, the expression of the *MC1R* gene was significantly downregulated in KD-FBs (Moscowitz et al., 2019). The activation of α-MSH and MC1R not only promotes the production of pigment but also exerts antagonistic action on cutaneous inflammatory and fibrogenic responses (Böhm and Stegemann, 2014; Carney et al., 2019). A previous study demonstrated that insufficient expression of MC1R by human dermal fibroblasts contributes to excess collagen synthesis in keloid scars (Luo et al., 2013). In addition, it was proven that activation of MC1R could inhibit the proliferation and metabolic programming of synovial fibroblasts (Montero-Melendez and Nagano, 2020). In the present study, we found that MC1R was decreased in keloid tissues and KD-FBs, and its expression was promoted by LINC00937. We further proved that MC1R was a downstream target of miR-28-5p and that LINC00937 inhibited the deposition of ECM and the proliferation of KD-FBs by upregulating MC1R and decreasing miR-28-5p.

Conclusion

In summary, our data indicated that LINC00937 reduced the deposition of ECM and proliferation of KD-FBs by acting as a miR-28-5p sponge and inhibiting MC1R expression. Our data revealed the regulatory mechanism of LINC00937 in keloids for the first time and provided evidence to support the potential of LINC00937 as a treatment target of keloids. However, additional studies are needed to explore the relationship between LINC00937 and other lncRNAs that affect the development of keloids and to further elucidate the pathogenesis of keloids.

Conflict of interest. All the authors declare no conflict of interest.

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