Long noncoding RNA LINC01615 promotes keloid development via sponging miR-590-3p to regulate FGF2 expression

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Research

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Abstract

Background A keloid is a benign human skin tumor that resulted by fibrous overgrowths during wound healing. Long noncoding RNAs (lncRNAs) are indicated to involve in the development of keloid. However, the role of lncRNA LINC01615 in regulating keloid development and the underlying mechanism are still unknown.

Methods The expression levels of LINC01615, miR-590-3p and fibroblast growth factor 2 (FGF2) mRNA were detected by quantitative real-time polymerase chain reaction (qRT-PCR). The protein levels of apoptosis-related proteins, α-smooth muscle actin (α-SMA), collagens and FGF2 were measured by western blot. The effect of LINC01615 on keloid development was assessed by cell proliferation, apoptosis and collagen deposition of keloid fibroblasts which were determined by Cell Counting Kit-8 (CCK-8), flow cytometry assay and the protein levels of collagens, respectively. The relationships between LINC01615 and miR-590-3p, miR-590-3p and FGF2 were predicated by online software and confirmed by dual-luciferase reporter assay and RNA pull-down assay.

Results We first found that LINC01615 was upregulated in keloid tissues and fibroblasts, and LINC01615 promoted cell proliferation and collagen deposition and suppressed apoptosis in keloid fibroblasts. LINC01615 targeted miR-590-3p and downregulated miR-590-3p expression, and overexpression of miR-590-3p inhibited the development of keloid. Then, FGF2 was identified as a target of miR-590-3p. LINC01615 facilitated keloid development via regulating FGF2 expression through miR-590-3p.

Conclusion Our study demonstrated that LINC01615 contributed to keloid development via the miR-590-3p/FGF2 axis.

Introduction

Keloid is a fibrous overgrowths disease caused by an exuberant response to wound healing after a skin injury [1, 2]. The main features of keloid are overgrown of keloid fibroblasts and excessive accumulation of extracellular matrix (ECM), especially Collagen. Although keloid is a benign tumor in humans, it is usually accompanied with pain, hyperaesthesia and pruritus that make a burden for patients. Despite the development for the therapies of keloid, such as surgery excision, pressure therapy and radiation therapy, among others, the high recurrence rate is still a big problem [3]. Understanding the molecular mechanism of keloid development is of great significance for the therapy of keloid and prevention of keloid recurrence.

Growing evidence indicates that lncRNAs participate in various cellular processes, such as cell cycle, proliferation, migration, apoptosis and so on [4]. Therefore, LncRNAs function as important regulators in the progression of diseases, including keloid [5]. LncRNA homeobox (HOX) A11 antisense (HOXA11-AS) contributes to cell proliferation, migration and collagen deposition in keloid, thereby facilitating keloid formation [6]. Knockdown of lncRNA H19 suppresses the proliferation of keloid fibroblasts [7]. A previous study reported that LINC01615 was upregulated in keloid tissues, implying that LINC01615 might involve

in the development of keloid [8]. However, the precise function of LINC01615 and the underlying mechanism in keloid is still unknown.

MicroRNAs (miRNAs) have been revealed to play vital roles in multiple diseases. They modulate gene expression through binding to the 3'-untranslated region (UTR) of target genes to degrade messenger RNA (mRNA) or inhibit transcription of mRNA [9]. MiR-590-3p is reported to serve as a tumor suppressor in papillary thyroid carcinoma, nasopharyngeal carcinoma, osteosarcoma and breast cancer [10–13]. On the other hand, it also can play a tumor-promoting action in colorectal cancer and ovarian cancer [14, 15]. Moreover, miR-590-3p represses the progression of cardiac fibroblasts [16]. However, the function of miR-590-3p in keloid has not been elucidated.

Fibroblast growth factor 2 (FGF2), a member of the FGF family, implicates mitosis and cell proliferation [17]. It is revealed that FGF2 plays a crucial role in many physiological and pathological processes [18]. FGF2 facilitated tumor progression in various cancers, including endometrial carcinoma, epithelial ovarian cancer and non-small cell lung cancer [19–21]. In addition, a previous study found that FGF2 was upregulated in keloid fibroblasts and played a promoting role in the progression of keloid [22].

In this study, we investigated the role of LINC01615 in keloid by loss-of-function experiments. Subsequently, the downstream target of LINC01615 and the underlying mechanism in keloid fibroblast were further explored.

Materials And Methods

Clinical samples and cell culture

This study has obtained approval from the Ethics Committee of China-Japan Union Hospital of Jilin University. Human normal skin tissues and keloid tissues were obtained from the healthy sites and keloid of 30 patients by surgery at China-Japan Union Hospital of Jilin University. Each patient signed Informed consent.

Normal fibroblasts and keloid fibroblasts were isolated from normal skin tissues and keloid tissues. After washed with PBS, tissues were cut into pieces of 2-4 mm following digested with trypsin (Thermo Fisher Scientific, Rockville, MD, USA). The suspension was filtered and centrifuged at 1500 rpm for 5 min. Fibroblasts were collected and cultured with Dulbecco's modified Eagles medium (DMEM; Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (Thermo Fisher Scientific), 100 U/mL penicillin and 100 μg/mL streptomycin and at 37℃ and 5% CO_2.

Cell transfection

Small interfering RNA (siRNA) against LINC01615 (si-LINC01615), miR-590-3p mimic (miR-590-3p), miR-590-3p inhibitor (anti-miR-590-3p) and the control fragments (si-NC, miR-NC, anti-miR-NC) were

synthesized from GenePharma (Shanghai, China). For gene overexpression, full sequences of LINC01615 and FGF2 complementary DNA (cDNA) were cloned into pcDNA3.1 vector (Invitrogen), respectively. Cell transfection was performed in light of the instruction of Lipofectamine 3000 (Invitrogen).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from tissues and fibroblasts by TRIzol reagent (Invitrogen). Then cDNA was obtained through reverse transcription using PrimeScript RT Reagent Kit (Takara, Dalian, China) and TaqMan MicroRNA Reverse Transcription Kit (Invitrogen). SYBR Green Master Mix (Takara) was used for qPCR. LINC01615 and FGF2 mRNA were normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and miR-590-3p was normalized by U6. The relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method. The primers were as follow (5'-3'): LINC01615-F: AAGACAGGGGATCCCGAAGA, LINC01615-R: TTTCCTCGGTGCTCACTCAC; FGF2-F: CCGTTACCTGGCTATGAAGG, FGF2-R: ACTGCCCAGTTCGTTTCAGT; miR-590-3p-F: AAAGATTCCAAGAAGCTAAGGGTG, miR-590-3p-R: CCTAACTGGTTTCCTGTGCCTA; GAPDH-F: GGGAAACTGTGGCGTGAT; GAPDH-R: GAGTGGGTGTCGCTGTTGA; U6-F: ATGACGTCTGCCTTGGAGAAC, U6-R: TCAGTGTGCTACGGAGTTCAG.

Cell Counting Kit-8 (CCK-8) assay

CCK-8 (Dojindo, Kumamoto, Japan) was used for the detection of cell proliferation. Transfected cells were seeded into a 96-well plate. After culture for 0 h, 24 h, 48 h and 72 h, CCK-8 reagent was added into per well, and cells were incubated for 2 h. Finally, the absorbance at 450 nm was detected with a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Flow cytometry assay

Apoptosis was detected using an annexin V-fluorescein isothiocyanate (V-FITC)/ propidium iodide (PI) apoptosis kit (BD Bioscience, San Diego, CA, USA) according to the manufacturers' manuals. At 48 h after transfection, Annexin V-FITC and PI were added to incubate the cells for 20 min in the dark condition. The stained cells were examined by a flow cytometer (BD Bioscience).

Western blot

The total proteins were extracted and then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After that, the proteins were shifted onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The membrane was blocked with 5% nonfat milk and then

incubated with primary antibodies against Cleaved-caspase3 (Cleaved-casp3), B-cell lymphoma-2 (Bcl-2), α-SMA, Collagen I, Collagen III, FGF2 or GAPDH (Abcam, Cambridge, UK) overnight at 4℃. Subsequently, the secondary antibody (Abcam) was used to incubate the membrane for 1.5 h at room temperature. Blots were detected with an enhanced chemiluminescence reagent (Millipore).

Target prediction and dual-luciferase reporter assay

The relationship between LINC01615 and miR-590-3p was predicated by DIANA tools, and the relationship between miR-590-3p and FGF2 was predicated by Targetscan. The wild type of LINC01615 and FGF2 3'UTR sequences (LINC01615-WT and FGF2 3'UTR-WT) containing the putative binding sites and the mutant sequence (LINC01615-MUT and FGF2 3'UTR-MUT) were cloned into the luciferase reporter vector pGLO (Promega, Madison, WI, USA), respectively. Each reporter vector and miR-590-3p or miR-NC were co-transfected into keloid fibroblasts. The luciferase activities were detected by a Dual-Luciferase reporter system (Promega) after transfection for 48 h.

RNA pull-down assay

To confirm the binding between LINC01615 and miR-590-3p, RNA pull-down assay was performed. Biotinylated miRNA (Bio-miR-NC or Bio-miR-590-3p) was obtained from Sangon (Shanghai, China). Streptavidin magnetic beads (Thermo Fisher Scientific) were mixed with Bio-miR-NC or Bio-miR-590-3p for 30 min at 4℃, and the mixture was used to incubated cell lysate from keloid fibroblasts for 4 h at 4℃. The beads-binding RNAs were eluted and detected by qRT-PCR.

Statistical analysis

Statistical analysis was conducted by SPSS 22.0. Data were presented as mean ± standard deviation with at least three repeats. The differences between two groups were compared by a two-tailed Student's *t*-test; as for multiple groups, one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) test was used. *P*-value <0.05 was regarded statistically significant.

Results

LINC01615 was upregulated in keloid tissues and fibroblasts

To investigate whether LINC01615 played functions in keloid, normal skin tissues and keloid tissues were firstly collected from patients with keloid. Then the expression level of LINC01615 was detected by qRT-

PCR. The result revealed that LINC01615 was significantly upregulated in keloid tissues (Fig. 1A). Subsequently, we detected the expression of LINC01615 in normal fibroblasts and keloid fibroblasts, and the qRT-PCR result showed that the level of LINC01615 was higher in keloid fibroblasts than that in normal fibroblasts (Fig. 1B). These results indicated that LINC01615 was associated with keloid.

LINC01615 facilitated cell proliferation and collagen deposition and inhibited apoptosis in keloid fibroblasts

In order to explore the role of LINC01615 in keloid development, LINC01615 was silenced or overexpressed in keloid fibroblasts. The expression of LINC01615 was downregulated by si-LINC01615 and upregulated by LINC01615 vector compared with their control groups (Fig. 2A). Proliferation of keloid fibroblasts was suppressed by LINC01615 knockdown and promoted by LINC01615 overexpression (Fig. 2B). Flow cytometry assay manifested that knockdown of LINC01615 induced apoptosis and overexpression of LINC01615 inhibited apoptosis of keloid fibroblasts (Fig. 2C and D). Also, the protein levels of apoptosis-related proteins (Cleaved-casp3 and Bcl-2) were determined. The western blot result showed that Cleaved-casp3 level was increased and Bcl-2 was reduced when LINC01615 was silenced, and overexpression of LINC01615 resulted an inverse effect (Fig. 2E). In addition, the protein levels of α-SMA, Collagen I and Collagen III were downregulated by si-LINC01615 and upregulated by LINC01615 (Fig. 2F). Our data suggested that LINC01615 played a positive role in the development of keloid.

MiR-590-3p was a target of LINC01615

To explore the molecular mechanism of LINC01615 in regulating keloid development, online software DIANA tools was used to predict the downstream target of LINC01615. We predicted miR-590-3p as a potential target of LINC01615, and the putative binding sites were showed in Fig. 3A. Subsequently, dual-luciferase reporter assay was performed. The relative luciferase activity was significantly decreased in keloid fibroblasts co-transfected with LINC01615-WT and miR-590-3p but had no change when co-transfected with LINC01615-MUT and miR-590-3p (Fig. 3B). RNA pull-down assay showed that the enrichment of LINC01615 was greatly higher in Bio-miR-590-3p group than that in Bio-NC group (Fig. 3C). Moreover, the expression of miR-590-3p was upregulated by LINC01615 knockdown and downregulated by LINC01615 overexpression in keloid fibroblasts (Fig. 3D). The expression of miR-590-3p was downregulated in keloid fibroblasts and tissues compared with normal fibroblasts and skin tissues (Fig. 3E and F). Additionally, the expression of miR-590-3p was negatively correlated with LINC01615 expression in keloid tissues (Fig. 3G). These results revealed that LINC01615 targeted miR-590-3p and downregulated its expression.

Overexpression of miR-590-3p repressed cell proliferation and collagen deposition and induced apoptosis in keloid fibroblasts

To investigate the role of miR-590-3p in keloid fibroblasts, miR-590-3p mimic or miR-NC was transfected into keloid fibroblasts. The qRT-PCR result manifested that miR-590-3p was overexpressed in keloid fibroblasts transfected with miR-590-3p (Fig. 4A). CCK-8 assay revealed that overexpression of miR-590-3p inhibited cell proliferation (Fig. 4B); while flow cytometry assay displayed that apoptosis was enhanced by miR-590-3p (Fig. 4C). Besides, the protein level of Cleaved-casp3 was elevated and Bcl-2 was decreased in keloid fibroblasts transfected with miR-590-3p (Fig. 4D and E). Furthermore, α-SMA, Collagen I and Collagen III were downregulated by overexpression of miR-590-3p in keloid fibroblasts (Fig. 4F and G). These results indicated that miR-590-3p suppressed keloid development *in vitro*.

LINC01615 upregulated FGF2 expression through miR-590-3p

To elucidate the mechanism underlying the function of miR-590-3p in keloid, TargetScan was employed to predict the target of miR-590-3p. FGF2 was selected as a possible target of miR-590-3p and the binging sites between miR-411 and the 3'-UTR of FGF2 was exhibited (Fig. 5A). Dual-luciferase reporter assay showed that the luciferase activity was markedly reduced by miR-590-3p in keloid fibroblasts co-transfected with FGF2 3'UTR-WT rather than FGF2 3'UTR-MUT (Fig. 5B). Then, we silenced miR-590-3p by transfecting anti-miR-590-3p in keloid fibroblasts and tested the efficiency by qRT-PCR (Fig. 5C). The mRNA and protein levels were decreased by miR-590-3p and elevated by anti-miR-590-3p (Fig. 5D and E). Besides, knockdown of LINC01615 downregulated the expression of FGF2 and silencing of miR-590-3p reversed this inhibitory effect (Fig. 5F and G). Subsequently, the expression of FGF2 in keloid fibroblasts and tissues was detected. The mRNA and protein levels of FGF2 were significantly increased in keloid fibroblasts compared with normal fibroblasts (Fig. 5H and I). Similarly, FGF2 was upregulated in keloid tissues compared with normal skin tissues (Fig. 5J-L). Also, the mRNA expression of FGF2 was positively correlated with LINC01615 and negatively correlated with miR-590-3p in keloid tissues (Fig. 5M and N). These data indicated that LINC01615 sponged miR-590-3p to regulate FGF2 expression.

LINC01615 regulated cell proliferation, apoptosis and collagen deposition of keloid fibroblasts via the miR-590-3p/FGF2 pathway

To further explore the regulatory mechanism of LINC01615 in keloid, keloid fibroblasts were transfected with si-LINC01615, si-LINC01615+anti-miR-590-3p or si-LINC01615+FGF2. Firstly, the overexpression efficiency of FGF2 vector was verified by qRT-PCR (Fig. 6A). Then, CCK-8 assay indicated that knockdown of LINC01615 inhibited cell proliferation while knockdown of miR-590-3p or overexpression of FGF2 rescued this effect (Fig. 6B). On the contrary, apoptosis was enhanced in keloid fibroblasts transfected with si-LINC01615 and restored when co-transfected with anti-miR-590-3p or FGF2 (Fig. 6C). The protein

level of Cleaved-casp3 was upregulated by LINC01615 knockdown and reversed by miR-590-3p knockdown or FGF2 overexpression; and there was a completely opposite effect on Bcl-2 (Fig. 6D-F). As expected, the inhibitory effects of LINC01615 knockdown on α-SMA, Collagen I and Collagen III were abolished by miR-590-3p knockdown or FGF2 overexpression (Fig. 6G-J). These data suggested that LINC01615 promoted keloid development through the miR-590-3p/FGF2 pathway.

Discussion

In this study, LINC01615 was found to be upregulated in keloid tissues and fibroblasts. Knockdown of LINC01615 inhibited the development of keloid. Additionally, the downstream target of LINC01615 was explored, and the results demonstrated LINC01615 affected keloid development by upregulating FGF2 expression via targeting miR-590-3p.

Previous studies have demonstrated that many lncRNAs were abnormally expressed in keloid compared with normal skin tissues, which indicated that lncRNAs participated in the pathogenesis and progression of the keloid [8, 23]. LINC01615 was upregulated in keloid tissues and fibroblasts. Knockdown of LINC01615 suppressed proliferation and enhanced apoptosis of keloid fibroblasts. Moreover, α-SMA, Collagen I and Collagen III were downregulated by LINC01615 knockdown in keloid fibroblasts. α-SMA was reported to be related to fibrosis, which promoted the differentiation of fibroblasts to myofibroblasts [24, 25]. Besides, the upregulated α-SMA facilitated the deposition of ECM proteins [26]. Collagen I and Collagen III are the main components of ECM. α-SMA, Collagen I and Collagen III were overexpressed in keloid fibroblasts, and α-SMA contributed to collagen gel contraction [27, 28]. LINC01615 knockdown reduced the deposition of ECM, implying the positive function of LINC01615 in keloid development.

Certain miRNAs also have been proved to play crucial roles in the development of keloid. MiR-21 elevated the level of Col1A1 and Col3A1 by inhibiting Smad7 expression in keloid [29]. Overexpression of miR-152-5p suppressed human keloid fibroblasts development by downregulating Smad3 [30]. In our study, miR-590-3p was verified as a target of LINC01615. Further, the effects of miR-590-3p on keloid were investigated. The functional experiments elucidated that overexpression of moR-590-3p restrained cell proliferation and collagen deposition and induced apoptosis in keloid fibroblasts. Moreover, inhibiting miR-590-3p reversed the inhibitory effect of LINC01615 knockdown on keloid fibroblasts. MiR-590-3p can act as a tumor suppressor or tumor promoter in different cancers. However, the role of miR-590-3p in keloid is unclear. A previous study reported that miR-590-3p inhibited cardiac fibroblasts progression [16]. Consistent with this, we also proved the inhibitory effect of miR-590-3p in keloid fibroblasts.

FGF2 is a well-studied cytokine, which plays a vital role in fibrotic response [31]. FGF2 was reported as an antifibrotic that restrained myofibroblast activation [32, 33]. On the other hand, it also played a pro-fibrotic effect on skin wounds. It was found that FGF2 promoted angiogenesis in the wound repair process [34]. In addition, FGF2 expedited fibroblast proliferation during wound repair [35]. Shi et al. demonstrated that FGF2 contributed to the development of keloid [22]. In this study, FGF2 was predicted as a downstream

target of miR-590-3p, and dual-luciferase reporter assay confirmed the relationship between miR-590-3p and FGF2. Moreover, LINC01615 regulated FGF2 expression through miR-590-3p. Overexpression of FGF2 recused LINC01615 knockdown-mediated effects on cell proliferation, apoptosis and collagen deposition of keloid fibroblasts, suggesting that FGF2 promoted keloid development.

Conclusion

In conclusion, we found that LINC01615 was upregulated in keloid, and functional experiments indicated that LINC01615 knockdown suppressed the development of keloid fibroblasts. Subsequently, we proved that LINC01615 targeted miR-590-3p to regulate FGF2 expression. Restoration experiments demonstrated that LINC01615 regulated keloid development via the miR-590-3p/FGF2 axis. Our study might provide a potential therapeutic target for keloid.

Abbreviations

FGF2
fibroblast growth factor 2

Declarations

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None

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Authors' contributions

Xiaoyu Zhang and Yingying Xu participated in the design of the work, methodology, data interpretation, and analysis for the work, carried out the statistical analysis and drafted the manuscript. Kenji Yamaguchi and Jinping Hu participated in methodology, and analysis for the work. Lianbo Zhang and

Jianfeng Wang participated in the methodology, data interpretation, and analysis for the work. Jifeng Tian and Wanying Chen designed the study, participated in data interpretation and methodology. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study has obtained approval from the Ethics Committee of China-Japan Union Hospital of Jilin University

Patient consent for publication

Not applicable

Competing interests

The authors declare that they have no Competing interest.

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Figures

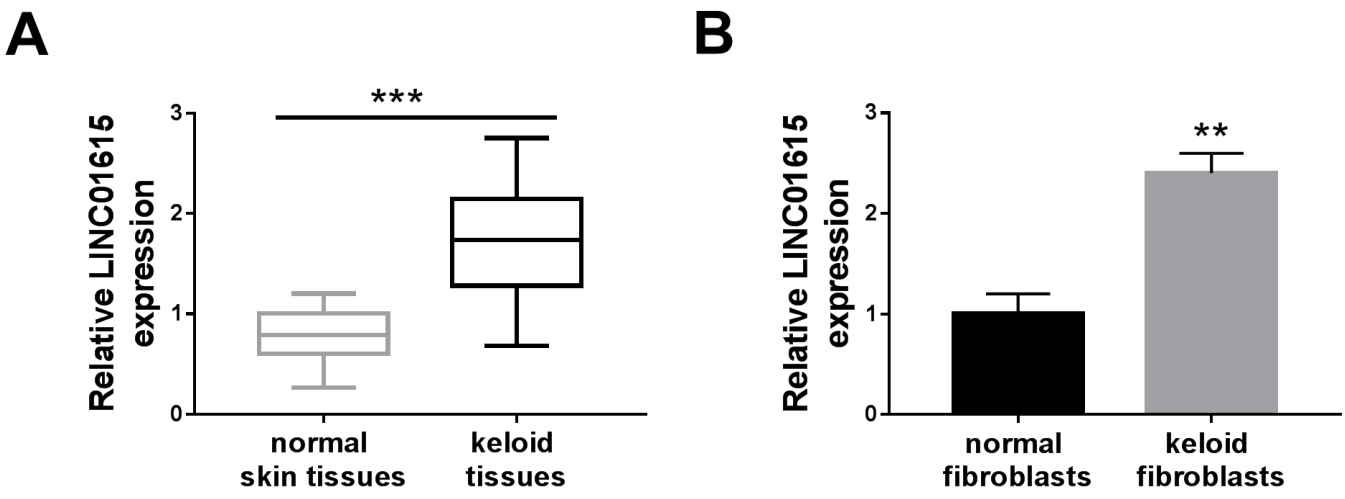

Figure 1

LINC01615 was upregulated in keloid tissues and fibroblasts. (A) The expression of LINC01615 was detected by qRT-PCR in normal skin tissue and keloid tissues. (B) The expression of LINC01615 was detected by qRT-PCR in normal fibroblasts and keloid fibroblasts. **P<0.01, ***P<0.001.

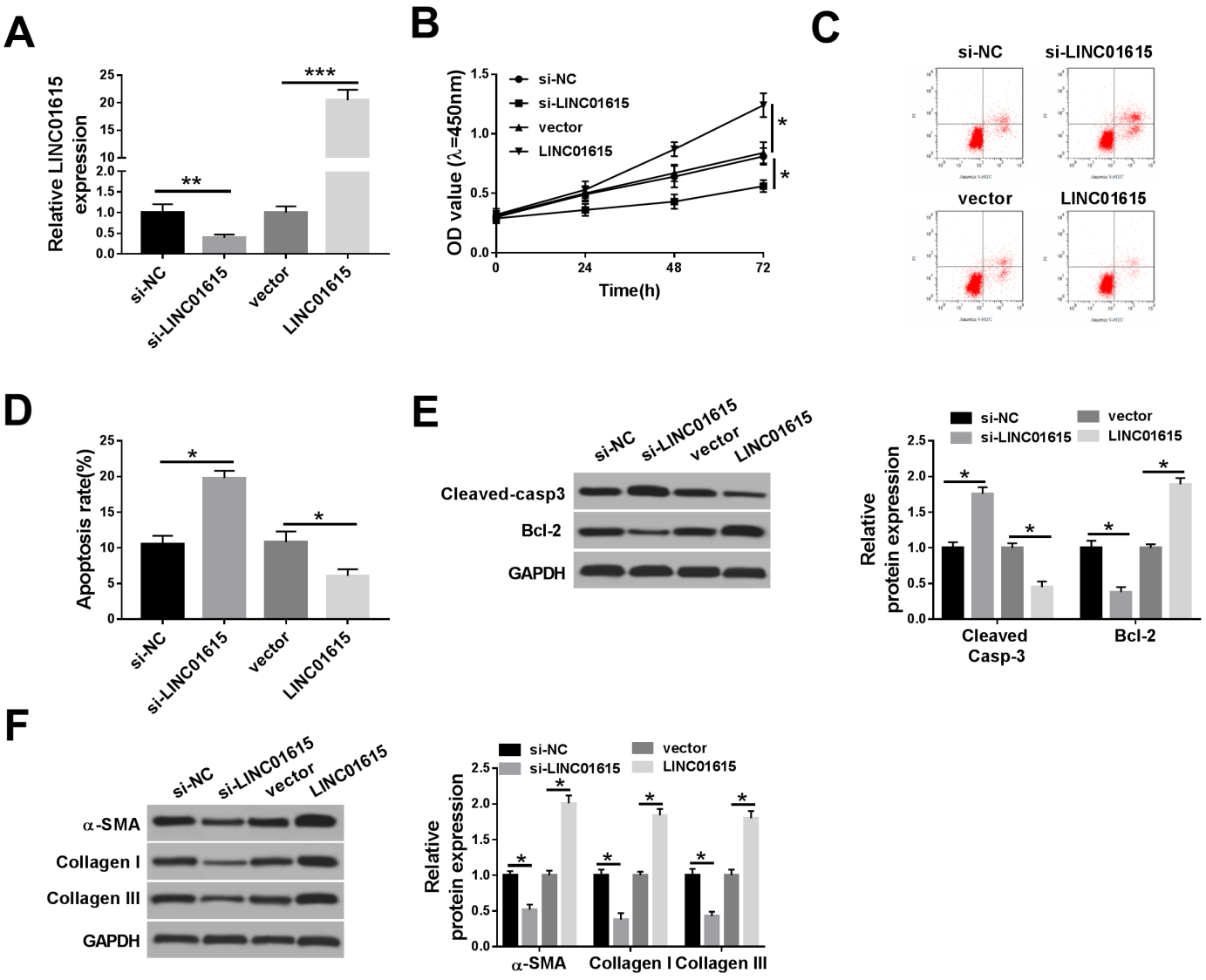

Figure 2

LINC01615 promoted the development of keloid. Keloid fibroblasts were transfected with si-NC, si-LINC01615, vector or LINC01615. (A) The expression of LINC01615 was detected by qRT-PCR. (B) Cell proliferation was assessed by CCK-8 assay. (C and D) Apoptosis was determined by flow cytometry assay. (E) The protein levels of Cleaved-casp3 and Bcl-2 were detected by western blot. (F) The protein levels of α-SMA, Collagen I and Collagen III were detected by western blot. *P<0.05, **P<0.01, ***P<0.001.

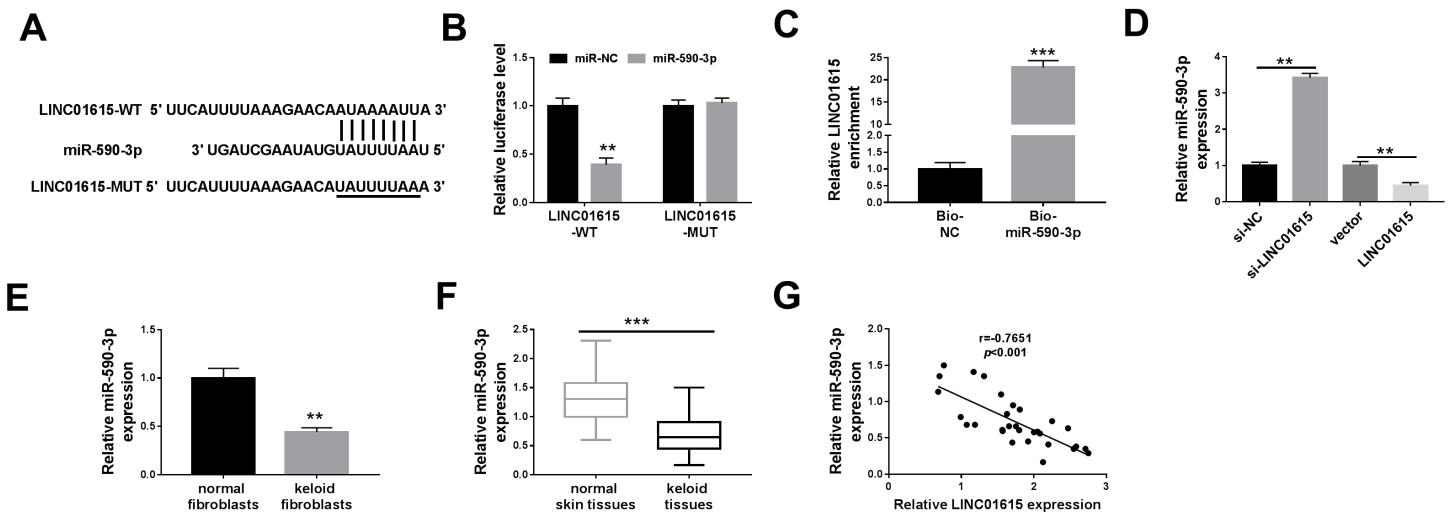

Figure 3

MiR-590-3p was a target of LINC01615. (A) The putative binding sites between LINC01615 and miR-590-3p were predicted by DIANA tools. (B) The luciferase activities of LINC01615-WT and LINC01615-MUT were detected in keloid fibroblasts transfected with miR-NC or miR-590-3p. (C) The binding capacity of LINC01615 and miR-590-3p were confirmed by RNA pull-down assay. (D) The expression of miR-590-3p was detected by qRT-PCR after LINC01615 was silenced or overexpressed. (E) The expression of miR-590-3p was detected by qRT-PCR in normal fibroblasts and keloid fibroblasts. (F) The expression of miR-590-3p was detected by qRT-PCR in normal skin tissue and keloid tissues. (G) The correlation between the expression of LINC01615 and miR-590-3p in keloid tissues was analyzed. **P<0.01, ***P<0.001.

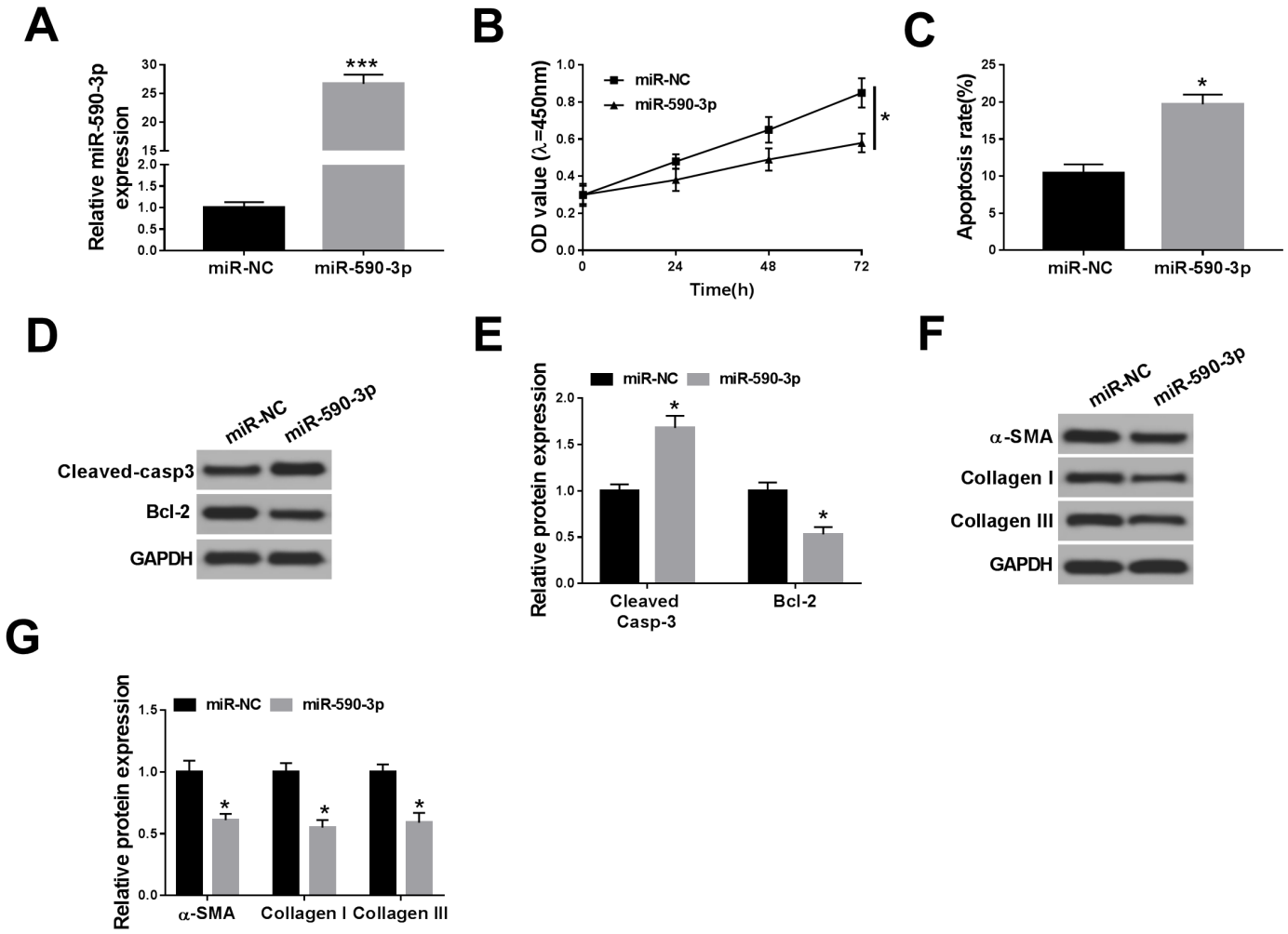

Figure 4

Overexpression of miR-590-3p suppressed the development of keloid. Keloid fibroblasts were transfected with miR-NC or miR-590-3p. (A) The expression of miR-590-3p was detected by qRT-PCR. (B) Cell proliferation was assessed by CCK-8 assay. (C) Apoptosis was determined by flow cytometry assay. (D and E) The protein levels of Cleaved-casp3 and Bcl-2 were detected by western blot. (F and G) The protein levels of α-SMA, Collagen I and Collagen III were detected by western blot. *P<0.05, ***P<0.001.

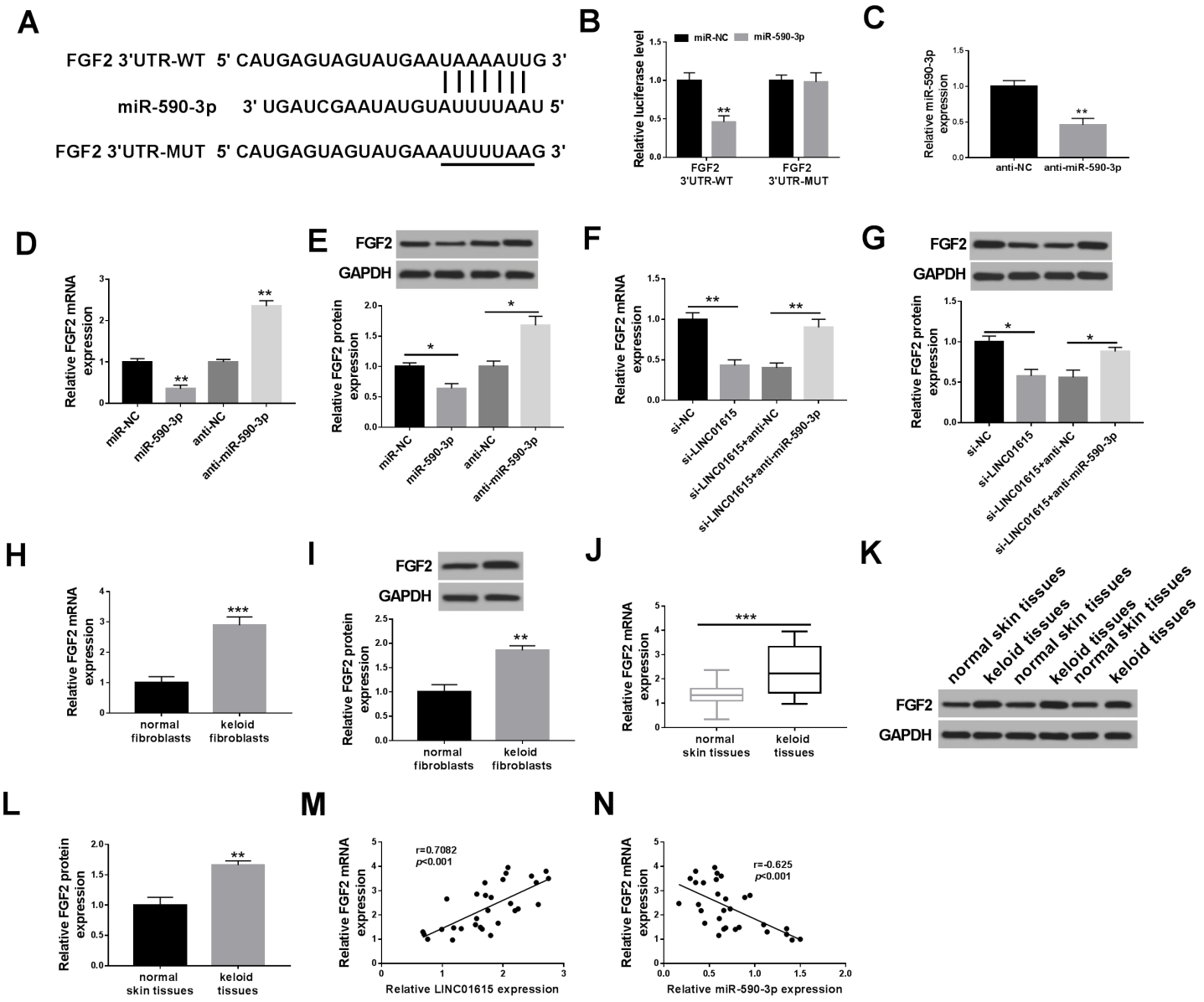

Figure 5

LINC01615 sponged miR-590-3p to regulate FGF2 expression. (A) The putative binding sites between miR-590-3p and FGF2 3'UTR were predicted by Targetscan. (B) The luciferase activities of FGF2 3'UTR-WT and FGF2 3'UTR-MUT were detected in keloid fibroblasts transfected with miR-NC or miR-590-3p. (C) The expression of miR-590-3p was detected by qRT-PCR in keloid fibroblasts transfected with anti-NC or anti-miR-590-3p. (D and E) The mRNA and protein levels of FGF2 were detected in keloid fibroblasts transfected with miR-NC, miR-590-3p, anti-NC or anti-miR-590-3p. (F and G) The mRNA and protein levels of FGF2 were detected in keloid fibroblasts transfected with si-NC, si-LINC01615, si-LINC01615+anti-NC or si-LINC01615+anti-miR-590-3p. (H and I) The mRNA and protein levels of FGF2 were detected in normal fibroblasts and keloid fibroblasts. (J-L) The mRNA and protein levels of FGF2 were detected in normal skin tissues and keloid tissues. (M) The correlation between the expression of FGF2 mRNA and

LINC01615 in keloid tissues was analyzed. (N) The correlation between the expression of FGF2 mRNA and miR-590-3p in keloid tissues was analyzed. *P<0.05, **P<0.01, ***P<0.001.

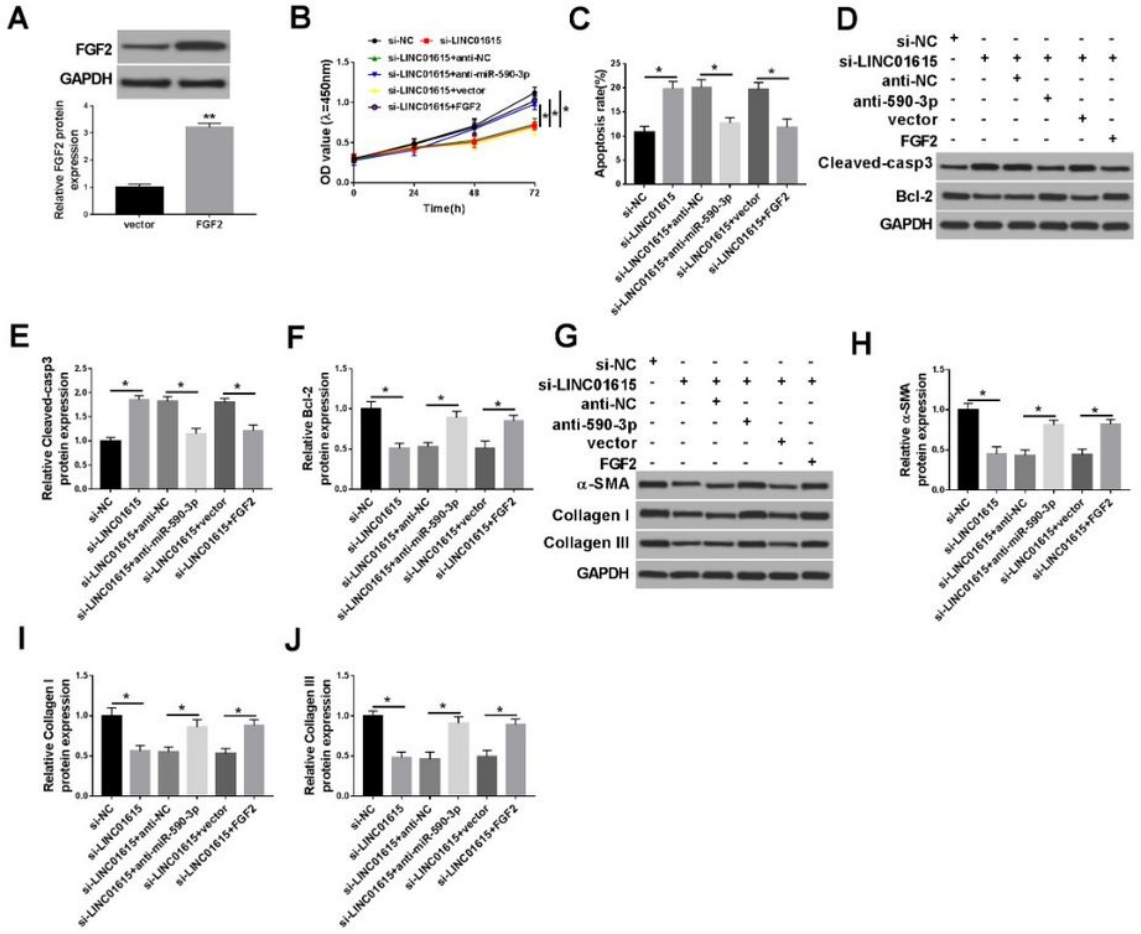

Figure 6

LINC01615 regulated keloid development through the miR-590-3p/FGF2 pathway. FGF2 overexpression vector was used to overexpress FGF2, and then keloid fibroblasts were transfected with si-NC, si-LINC01615, si-LINC01615+anti-NC, si-LINC01615+anti-miR-590-3p, si-LINC01615+vector or si-LINC01615+FGF2. (A) The protein level of FGF2 was detected by western blot in keloid fibroblasts transfected with vector and FGF2. (B) Cell proliferation was assessed by CCK-8 assay. (C) Apoptosis was determined by flow cytometry assay. (D-F) The protein levels of Cleaved-casp3 and Bcl-2 were detected by western blot. (G-J) The protein levels of α-SMA, Collagen I and Collagen III were detected by western blot. *P<0.05, **P<0.01.