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Original Article

Circ_0057452 functions as a ceRNA in hypertrophic scar fibroblast proliferation and VEGF expression by regulating TGF-β2 expression and adsorbing miR-145-5p

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Abstract: Objective: To explore the mechanism by which circ_0057452/miR-145-5p/TGF-β2 axis regulates fibroblast proliferation as well as VEGF expression in hypertrophic scars (HS). Methods: The expression of circ_0057452, miR-145-5p and TGF-β2 in HS tissues and fibroblasts was measured by quantitative real-time Polymerase Chain Reaction (qRT-PCR). The targeting relations between circ_0057452 and miR-145-5p, miR-145-5p and TGF-β2 were identified using dual-luciferase reporter assay. The expression of circ_0057452, miR-145-5p and TGF-β2 in fibroblasts was interfered with and cells were grouped. In each group, changes in cell proliferation were detected using CCK8 assay, apoptosis was measured by flow cytometry, and VEGF secreted in cell culture supernatant was tested by ELISA kit. Results: Compared with normal tissues and fibroblasts, the expressions of circ_0057452 and TGF-β2 were increased and miR-145-5p decreased in HS tissues and cells (all $P<0.05$). Compared with the si-NC group, cell proliferation and VEGF expressions were decreased and the apoptotic rate increased in the si_circ_0057452 group (all $P<0.05$). Compared with the oe-NC group, cell proliferation and VEGF expression were increased and the apoptotic rate decreased in the oe-circ_0057452 group (all $P<0.05$). Compared with the oe-circ_0057452 + miR-NC group, the number of apoptotic cells was increased, and cell proliferation, as well as VEGF expression were decreased in the oe-circ_0057452 + miR-145-5p mimic group (all $P<0.05$). Compared with the miR-NC group, cell proliferation and VEGF expression were reduced and the apoptotic rate was increased in the miR-145-5p mimic group (all $P<0.05$). Compared with the miR-145-5p mimic + vector group, cell proliferation and VEGF expression were elevated, and apoptosis was inhibited in the miR-145-5p mimic + TGF-β2 group (all $P<0.05$). Conclusion: circ_0057452 can competitively bind miR-145-5p to induce the expression of TGF-β2, and then promote the proliferation of HS fibroblasts and secretion of VEGF, which is expected to be effective in the treatment of HS.

Keywords: Hypertrophic scar, fibroblast, proliferation, VEGF

Introduction

Hypertrophic scar (HS) is a fibrotic disease that results from excessive tissue regeneration after injuries or burns and is characterized by excessive collagen production and deposition in the extracellular matrix, and abnormal proliferation of fibroblasts [1-4]. Currently, treatments for HS include skin grafting, steroid injection, pressure therapy, silicone dressings, and surgical excision [5, 6]. However, these treatments are of limited effects, so it is very important and necessary to elucidate the pathogenesis of HS and explore new treatments for this disease.

Circular RNAs (circRNAs), as a novel class of non-coding RNAs without the influences of RNA exonucleases, whose molecules are characterized by closed-loop structures, are one of the hotspots in RNA research [7, 8]. Previous bioinformatic analyses have shown that circ_0057452 expressions are significantly enhanced in keloid tissues, but how it is involved in the development of keloids, and its specific mechanism have not yet been clarified [9]. Therefore, in this study, we investigated whether circ_0057452 plays a role in the pathogenesis of HS from the perspective that the biological behavior of fibroblasts affects the progression of HS, and analyzed the effect of inter-

actions between circ_0057452 and other molecules on HS.

MicroRNAs (miRNAs) are a type of non-coding RNAs of 18-23 nucleotides that negatively regulate target genes through translational repression or RNA degradation [10, 11]. Many studies have found that miRNAs play a vital role in regulating fibroblast proliferation, apoptosis, fibrosis, and transdifferentiation. It has been reported that miR-1-3p/miR-214-5p inhibits the proliferation and migration of keloid fibroblasts and induces apoptosis by inhibiting the expression of TM4SF1 [12]. Also, relevant reports have illustrated that transforming growth factor beta 1 (TGF-β1) promotes keloid fibroblast proliferation and transdifferentiation by upregulating miR-21 [13]. Previous studies have shown that miR-145-5p can reduce hypertrophic scars and inhibit the formation of fibrosis by suppressing the expression of Smad2/Smad3, suggesting that miR-145-5p can improve HS [14]. However, whether there is a connection between circ_0057452 and miR-145-5p requires further validation.

Some studies have found that the isoform of transforming growth factor β (TGF-β) is key in hypertrophic scar formation [15]. Polo et al found that elevated TGF-β2 expression is an important factor in the formation of hypertrophic scar [16]. Through bioinformatical analysis, we investigated whether TGF-β2 is a downstream target of miR-145-5p, and further studied its specific mechanism.

In this study, we aimed to investigate the specific mechanism of circ_0057452 in the formation of HS and further elucidate the effect of circ_0057452 targeting miR-145-5p/TGF-β2 axis on the proliferation and apoptosis of fibroblasts in HS.

Materials and methods

Bioinformatic tools

We searched the sequence of circ_0057452 on the circbase website (http://circbase.org), conducted sequence prediction of miR-145-5p on miRbase database (http://mirbase.org), and analyzed the binding site between these two genes using RNA22 web tool (http://cm.jefferson.edu/rna22/). Prediction of target genes of miRNAs was carried out on Targetscan (http://www.targetscan.org/vert_72/), and KEGG analysis was performed on Database for annotation, visualization, and integrated discovery (DAVID) (https://david.ncifcrf.gov/).

Sample collection

The scar tissues and adjacent normal tissues of 20 HS patients were collected during plastic surgery for burns in our hospital. The patients were at the proliferative phase of wound healing with obvious pain, redness, itching, and other symptoms around the scar tissue. Patients were included if they: (1) had a scar affecting less than 10% of body surface area; (2) were healed from burn injuries no more than 6 months. Patients were excluded if they: (1) suffered from other connective tissue diseases and local or systemic diseases affecting the test results; (2) did not complete the prescribed treatment regimen or lost to follow-up; (3) were involved in other research projects at the same time [17, 18]. Collected skin samples were immediately frozen in liquid nitrogen after excision for future use. The study was approved by the Ethics Committee of our hospital and all patients gave informed consent.

Cell culture

Hypertrophic scar fibroblasts (HSFB) and normal skin fibroblasts (NSFB) were obtained from the American type Culture Collection (ATCC) cell bank were cultured in DMEM medium (11995, Beijing Solarbio Science & Technology Co., Ltd., China) containing 10% fetal bovine serum at 37°C with 5% CO_2. The medium was changed every 3 days, and cells of 3 to 4 passages were selected for the experiment.

Cell grouping and transfection

The expression of circ_005745, miR-145-5p and TGF-β2 in HS cells was interfered with and the cells were divided into the blank group (cells + blank vector), oe-NC group (cells + transfected oe-NC sequence), si-NC group (cells + transfected si-NC sequence), miR-NC group (cells + transfected miR-NC sequence), oe-circ_0057452 group (cells + transfected circ_0057452 mimic), si-circ_0057452 group (cells + transfected circ_0057452 siRNA vector), miR-145-5p mimic group (cells + transfected miR-145-5p mimic), oe-circ_0057452 + miR-NC group (cells + transfected circ_0057452

Table 1. qRT-PCR sequences

Gene		Sequence (5'-3')
circ_0057452	Forward	TAATTGACTCGCCATACTTG
	Reverse	ATCTTCTCTGTTATCTGTGGA
miR-145-5p	Forward	TTGTAATTATGTGCTTAGCATT
	Reverse	TGGATGTGGCCTGAACTTG
TGF-β2	Forward	CGTTCAGTTCGTTCCCAAT
	Reverse	GCTACACGCCTGTTTCCA
GAPDH	Forward	GACTCATGACCACAGTCCATGC
	Reverse	AGAGGCAGGGATGATGTTCTG
U6	Forward	CTCGCTTCGGCAGCACA
	Reverse	AACGCTTCACGAATTTGCGT

mimic + miR-NC), oe-circ_0057452 + miR-145-5p mimic group (cells + transfected circ_0057452 mimic + miR-145-5p mimic), miR-145-5p mimic + vector group (cells + transfected miR-145-5p mimic + blank vector), miR-145-5p mimic + TGF-β2 (cells + transfected with miR-145-5p mimic + TGF-β2). Transfections were performed according to the instructions of Lipofectamine™ 3000 kit (L3000150, Thermo Fisher, USA). Transfection vectors and sequences were synthesized by Shanghai GenePharma Co., Ltd.

qRT-PCR

All RNA was extracted from tissues and cells using TRIzol reagent (15596018, Beijing Solarbio Science & Technology Co., Ltd., China), and total RNA concentration was determined by UV spectrophotometer (Multiskan Sky, Thermo Fisher, USA). Then the RNA was reverse transcribed into cDNA following the manufacturer's instructions of the GoScript™ Reverse Transcriptase System (A5001, Promega, USA). The reaction system was constructed and conditions were prepared following the instructions of qRT-PCR kit (12594025, Thermo Fisher, USA). U6 snRNA was used as the reference gene for miRNA and GAPDH for the remaining factors. Relative gene expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method. See **Table 1** for primer sequences.

Fluorescence in situ hybridization (FISH)

The distribution of circ_0057452 in the cells was detected by fluorescence in situ hybridization (FISH) kits (KA2375, AmyJet Scientific Inc, China), and circ_0057452 was captured using a Cy5-labeled probe. After prehybridization, cells were hybridized overnight in hybridization buffer using circ_0057452 probe overnight at 37°C. Nuclei were labeled by DAPI (4',6-diamidino-2-phenylindole) staining solution (C0060, Beijing Solarbio Science & Technology Co., Ltd., China), and experimental images were obtained under confocal microscope (LEXT OLS4500, OLYMPUS, Japan).

Dual-luciferase reporter assay

Wild-type and mutant sequences containing binding sites of circ_0057452 and miR-145-5p were inserted into pmirGLO vector, and the wild-type (or mutant) sequence and miR-145-5p mimic or control vector was transfected into HS fibroblasts using Lipofectamine™ 3000 kit (L3000150, Thermo Fisher, USA). After 48 hours, luciferase activity in the cells was measured using a dual-luciferase reporter assay kit (RG088S, Shanghai Beyotime Biotechnology Co., Ltd., China), in which the activity of Renilla luciferase was chosen as an internal reference. Validation of the miR-145-5p targeting TGF-β2 was performed by constructing wild-type and mutant sequences containing miR-145-5p and TGF-β2 binding sites and following experimental procedures described above.

CCK8

Cell proliferation in each group was assessed with Cell Counting Kit-8 (CCK8) assay. Transfected cells in each group were seeded in 96-well plates at a density of $2*10^3$ per well, and 10 μL of CCK8 solution (C0040, Shanghai Beyotime Biotechnology Co., Ltd., China) was added after 24 h, 48 h, and 72 h of incubation, respectively. The absorbance of the cells at 450 nm was determined by a microplate reader (HBS-1101, Nanjing DeTie Laboratory Equipment Co., Ltd., China).

Flow cytometry

HS fibroblasts in each group were collected and seeded in 96-well plates at a density of $1*10^6$ per well, and the staining solution was prepared using Annexin V-FITC/PI Apoptosis Detection Kit (C1062L, Shanghai Beyotime Biotechnology Co., Ltd., China), after which the cells were stained, and apoptosis was tested by flow cytometry (Attune NxT, Thermo Fisher, USA).

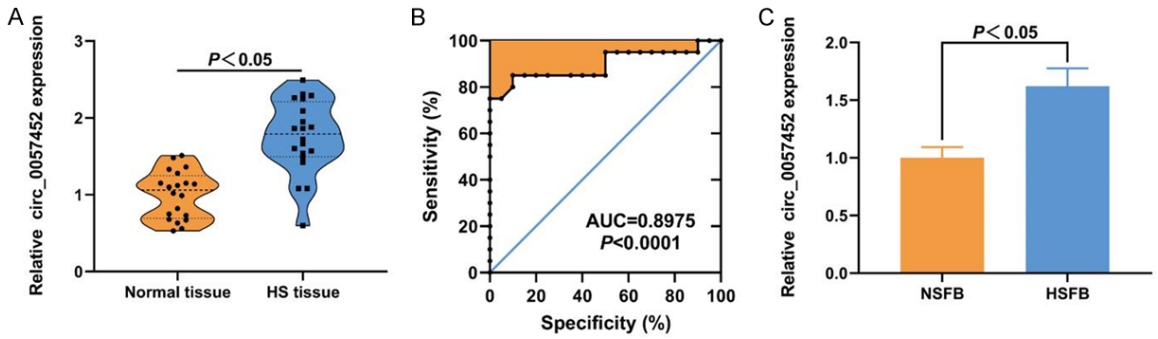

Figure 1. Upregulation of circ_0057452 in HS tissues and fibroblasts. A: Expression of circ_0057452 in normal tissues and HS tissues; B: Analysis of diagnostic value of circ_0057452 in HS; C: Expression of circ_0057452 in normal fibroblasts and HS fibroblasts.

ELISA tests

Cells in the logarithmic growth phase were washed in phosphate-buffered saline (PBS) (CO221A, Shanghai Beyotime Biotechnology Co., Ltd., China), and cultured in serum-free Dulbecco's modified Eagle Medium (DMEM) (11965, Beijing Solarbio Science & Technology Co., Ltd., China) for 48 h. After that, the supernatant was collected for the determination of VEGF concentration in each group using an ELISA test kit (PV963, Shanghai Beyotime Biotechnology Co., Ltd., China). All procedures were performed in accordance to the manufacturer's instructions.

Statistical analysis

Data were analyzed using SPSS 22.0 software (IBM, USA), and the software GraphPad Prism 9.0 was used to draw experimental graphs. Enumeration data were presented as n and analyzed by chi-square test or Fisher's exact probability test. Measurement data were examined by Levene's test and Shapiro test to determine whether they conformed to the normal distribution and homogeneity of variance. Normally distributed data and homogeneity of variance were described as mean ± standard deviation ($\bar{x}$ ± sd). A t-test was adopted for comparison between two groups. One-way analysis of variance was used for comparison of more than two groups, and SNK-q method was used for further pairwise comparison between groups. For data violating normal distribution or homogeneity of variance, Mann-Whitney-Wilcoxon test was used for comparison between two groups. Bonferroni method was applied for pairwise comparison of multiple groups. P less than 0.05 was considered significant.

Results

Upregulation of circ_0057452 in HS tissues and fibroblasts

We measured circ_0057452 in HS tissues and adjacent normal tissues, in order to explore the changes in circ_0057452 expression in HS. Quantitative real-time Polymerase Chain Reaction (qRT-PCR) revealed that circ_0057452 was more highly expressed in HS tissues than in normal tissues (P<0.05) (**Figure 1A**). This could be used as an effective diagnostic indicator of HS (AUC = 0.8975, P<0.0001) (**Figure 1B**). Additionally, the expression of circ_0057452 was also elevated in HS fibroblasts compared with normal fibroblasts (P<0.05) (**Figure 1C**).

Inhibition of HS fibroblast proliferation and VEGF secretion, and apoptosis acceleration through silencing circ_0057452

Previous studies have confirmed that circ_0057452 is up-regulated in HS tissues and fibroblasts, while its specific mechanism has not been clarified. Therefore, with the purpose of evaluating the effect of circ_0057452 on the proliferation of HS fibroblasts, we determined the proliferation of the cells by CCK8 assay. As shown in **Figure 2A**, cell proliferation was decreased after silencing circ_0057452 (P< 0.05). Afterwards, we examined the expression of VEGF and found that VEGF expression in si-circ_0057452 group was decreased compared with the si-NC group (P<0.05) (**Figure 2B**). Additionally, flow cytometry revealed (**Figure**

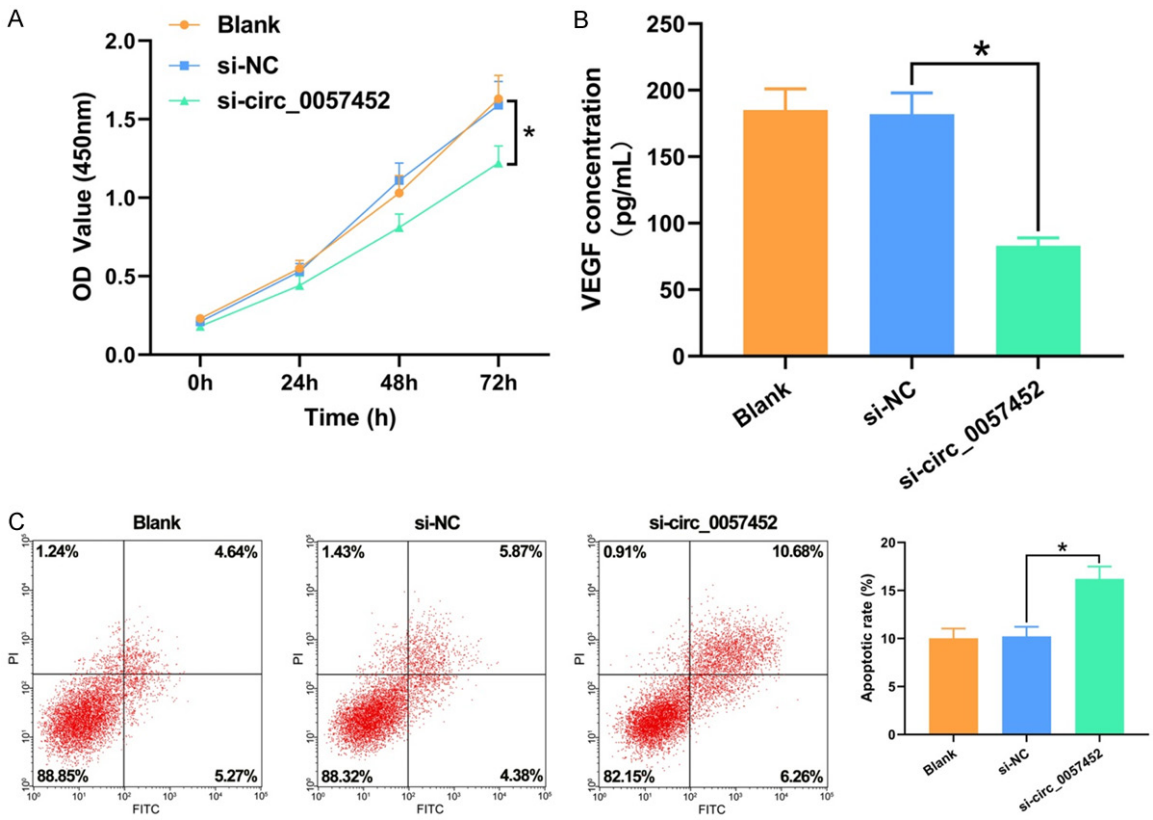

Figure 2. Inhibition of HS fibroblast proliferation, and VEGF secretion and apoptosis promotion by downregulation of circ_0057452. A: CCK8 test results; B: ELISA test results; C: Flow cytometry test results. Compared with the si-NC group, *P<0.05.

2C) that silencing circ_0057452 could promote apoptosis (P<0.05). However, there was no significant difference in various indicators between the blank group and the si-NC group (P>0.05). These results suggest that downregulation of circ_0057452 can inhibit the proliferation of HS fibroblasts and VEGF secretion and accelerate apoptosis.

Targeted regulation of miR-145-5p by circ_0057452 acting as ceRNA

Through RNA22 target discovery tool, it was found that there was a specific binding site between circ_0057452 and miR-145-5p (**Figure 3A**). In addition, qRT-PCR detection assay revealed that miR-145-5p expression was downregulated in HS tissues and fibroblasts compared with normal tissues and fibroblasts (**Figure 3B** and **3C**) (all P<0.05). Afterwards, FISH analysis confirmed the presence of circ_0057452 in the cytoplasm (**Figure 3D**), and the results of dual-luciferase reporter assay showed that transfection of miR-145-5p significantly reduced luciferase activity of HS fibroblasts in transfected circ_0057452-wt (P< 0.05), but it did not affect luciferase activity of HS fibroblasts in transfected circ_0057452-mut (**Figure 3E**). Overall, there is a targeting relation between circ_0057452 and miR-145-5p.

Partial reversion of the effect of circ_0057452 overexpression on HS through overexpression of miR-145-5p

Previous studies have confirmed that miR-145-5p is a downstream miRNA of circ_0057452, but their role in the development of HS needs further exploration. In this study, the expression of circ_0057452 or miR-145-5p was upregulated in HS fibroblasts. Compared with the oe-NC group, CCK8 assay showed that overexpression of circ_0057452 could promote cell proliferation (**Figure 4A**) (P<0.05). ELISA revealed that the expression of VEGF was significantly increased in the oe-circ_0057452 group (**Figure 4B**) (P<0.05), and flow cytometry results

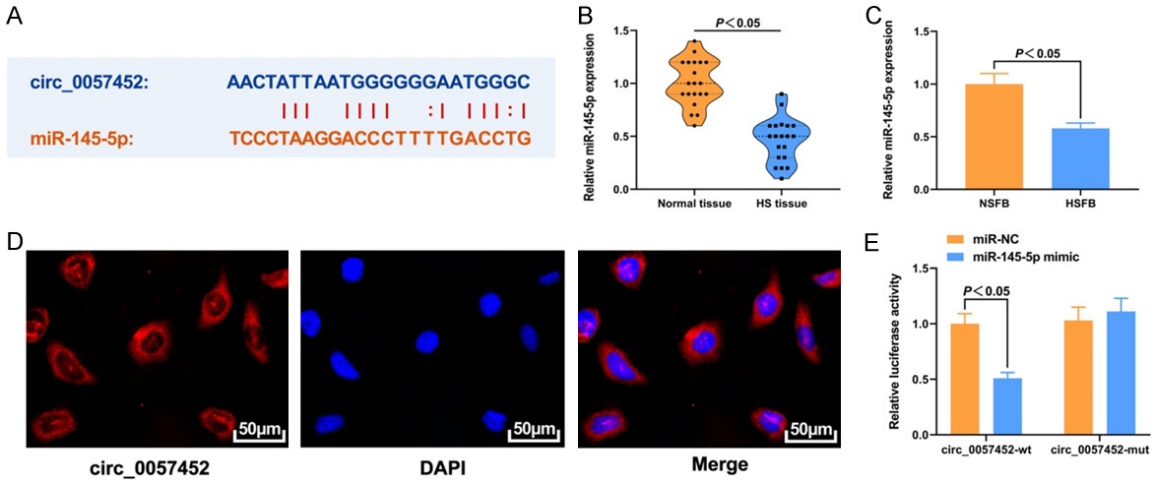

Figure 3. Validation of interaction between circ_0057452 and miR-145-5p. A: Prediction results from RNA22 target discovery tool; B: miR-145-5p expression in tissues; C: miR-145-5p expression in cells; D: Fluorescence in situ hybridization (FISH) results (200×); E: Dual-luciferase reporter assay results.

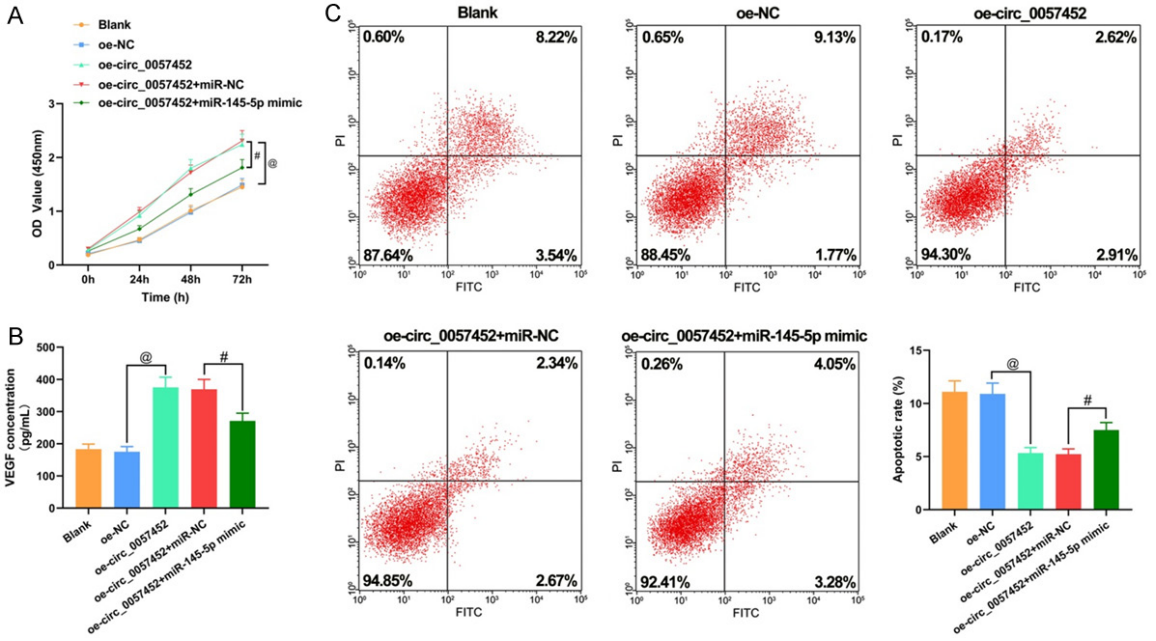

Figure 4. Partial reversion of the effect of circ_0057452 overexpression on cells by upregulation of miR-145-5p. A: CCK8 test results; B: ELISA test results; C: Flow cytometry results. Compared with the oe-NC group, @P<0.05; compared with the oe-circ_0057452 + miR-NC group, #P<0.05.

illustrated that the apoptotic rate in the oe-circ_0057452 group was reduced (**Figure 4C**) (P<0.05). However, upregulation of miR-145-5p could partially reverse the effect of overexpression of circ_0057452 on cells, inhibit cell proliferation, VEGF expression, and promote apoptosis. No significant difference was noted between the blank group and the oe-NC group (P>0.05).

Targeted regulation of TGF-β2 by miR-145-5p

To find the targets of circ_0057452/5miR-145-5p, the downstream targets of miR-145-5p were predicted on Targetscan website, and the top 200 genes were used for KEGG analysis. Through KEGG analysis, it was found (**Figure 5A**) that genes were most enriched in the MAPK signaling pathway, which has been con-

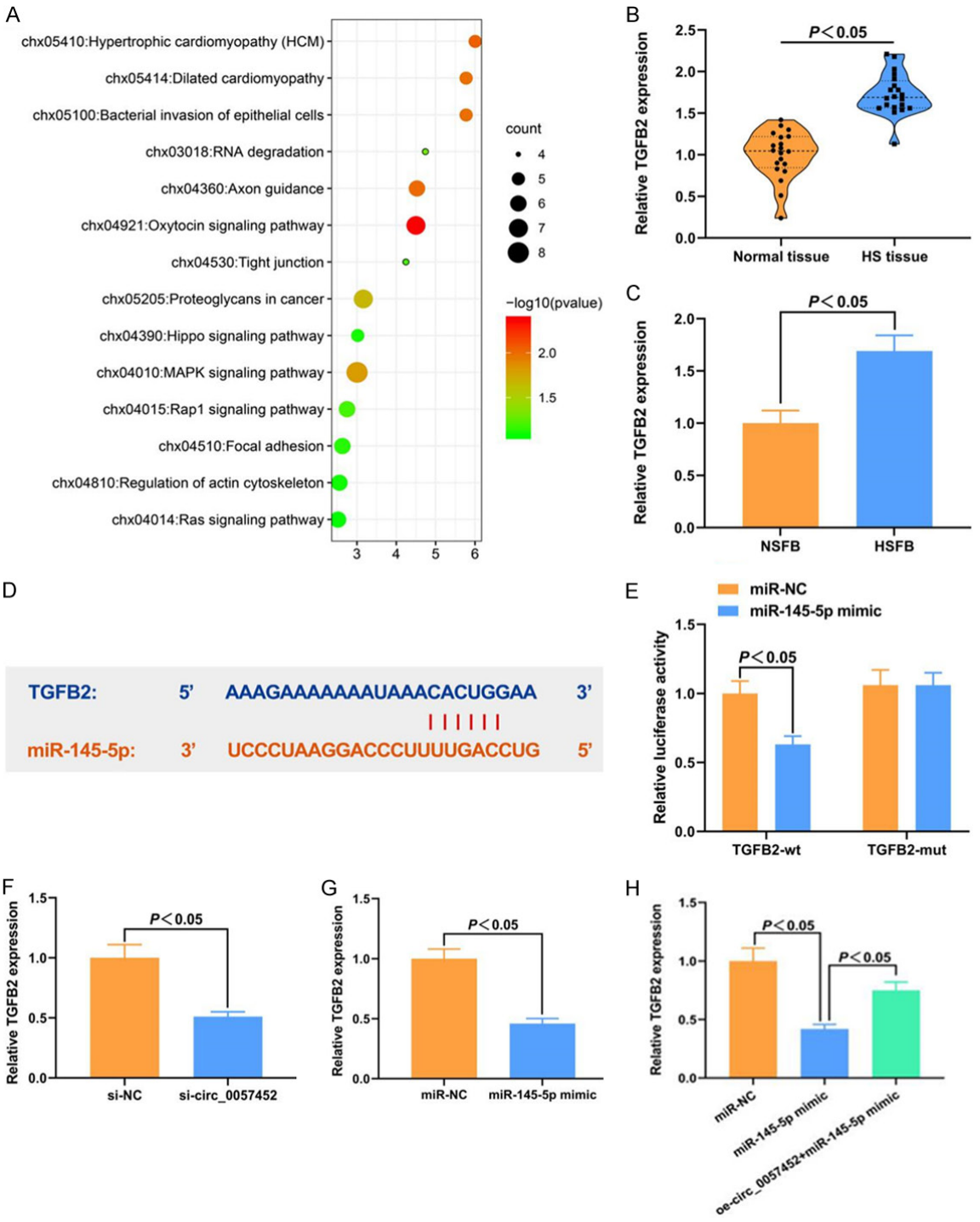

Figure 5. Validation of interaction between circ_0057452, miR-145-5p, and TGF-β2. A: KEGG analysis results; B: TGF-β2 expression in tissues; C: TGF-β2 expression in cells; D: Prediction results from Targetscan website; E: Dual-luciferase reporter assay results; F: Effect of silencing circ_0057452 on TGF-β2; G: Effect of overexpression of miR-145-5p on TGF-β2; H: Effect of simultaneous overexpression of circ_0057452 and miR-145-5p on TGF-β2.

firmed to be one of the important pathways in the pathogenesis of HS. Thus, we focused on MAPK signaling pathway, and studied enriched genes, mainly including FGF5, PPP3CA, TGF-β2, MAP3K1, RASA2, PLA2G4A, DUSP6, and CRKL. It was previously confirmed that TGF-β2 plays

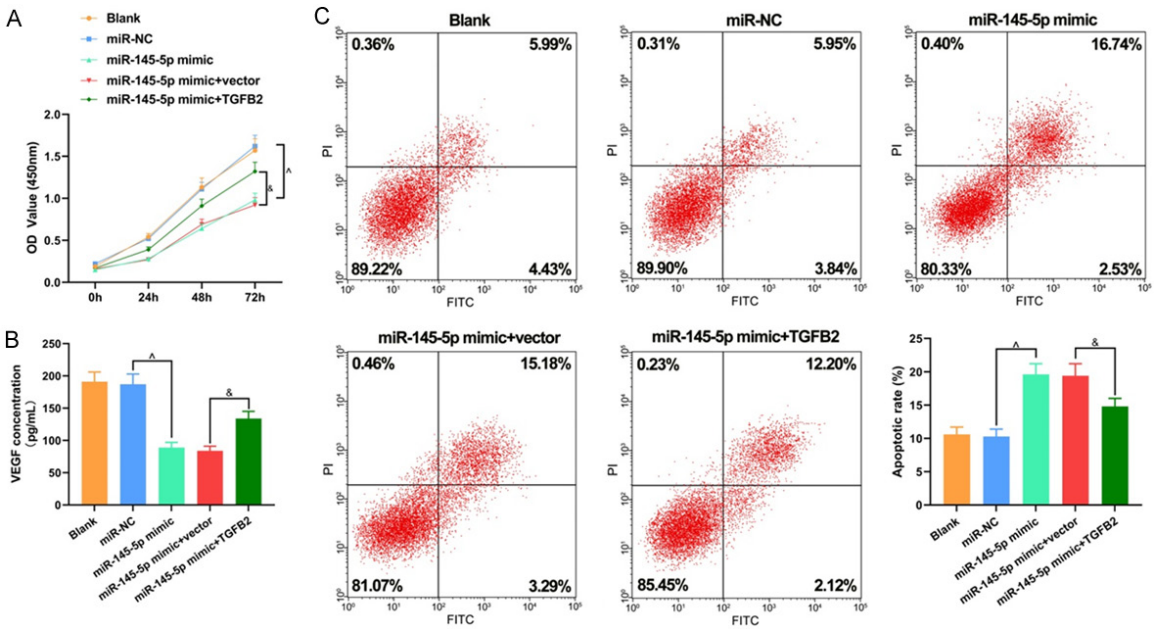

Figure 6. Upregulation of miR-145-5p reversed by TGF-β2 overexpression. A: CCK8 test results; B: ELISA test results; C: Flow cytometry results. Compared with the miR-NC group, ^P<0.05; compared with the miR-145-5p mimic + vector group, &P<0.05.

an important role in the pathogenesis of HS, so we selected TGF-β2 for qRT-PCR testing and found that TGF-β2 expression was elevated in HS tissues and fibroblasts compared with normal tissues and fibroblasts (**Figure 5B** and **5C**) (all P<0.05). A targeted binding site between miR-145-5p and TGF-β2 was found by target prediction on Targetscan (**Figure 5D**). Dual-luciferase reporter assay confirmed that transfected miR-145-5p significantly suppressed luciferase activity in HS fibroblasts transfected with TGF-β2-wt (P<0.05), but did not significantly affect the luciferase activity in HS fibroblasts transfected with TGF-β2-mut (P>0.05) (**Figure 5E**). Then regulation of circ_0057452/miR-145-5p/TGF-β2 expression in HS fibroblasts revealed (**Figure 5F-H**) that silencing of circ_0057452 could decrease TGF-β2 expression parallel to the effect after overexpression of miR-145-5p (all P<0.05). TGF-β2 expression was downregulated after overexpression of miR-145-5p, while simultaneous overexpression of circ_0057452 and miR-145-5p could promote TGF-β2 expression (P<0.05). These experiments confirmed that TGF-β2 is a target of circ_0057452/5miR-145-5p.

Partial reversion of the effect of overexpression of miR-145-5p on HS by TGF-β2

Afterwards, we detected cell proliferation by the use of CCK8, VEGF expression by ELISA, and apoptosis by flow cytometry after regulating miR-145-5p and TGF-β2 in HS fibroblasts. We found that overexpression of miR-145-5p could markedly inhibit the proliferation of HS fibroblasts, suppress VEGF expression, and promote apoptosis. However, when miR-145-5p and TGF-β2 were both overexpressed, it was found that miR-145-5p upregulation could be reversed (**Figure 6**) (all P<0.05). No significant difference was observed between the blank group and the miR-NC group (P>0.05), suggesting that TGF-β2 can promote HS.

The suggested mechanism of circ_0057452 as a ceRNA adsorbing miR-145-5p to regulate TGF-β2 in hypertrophic scars is shown in **Figure 7**.

Discussion

The results of this study confirmed that circ_0057452 was upregulated in hypertrophic scar (HS), and could regulate the proliferation, apoptosis, and other biologic behaviors of HS fibroblasts. Such function was achieved by affecting the miR-145-5p/TGF-β2 axis, which provides a novel pathway for the diagnosis and treatment of HS, and further clarifies its pathogenesis.

First, we explored the role of circ_0057452 in HS. Using qRT-PCR, it was revealed that

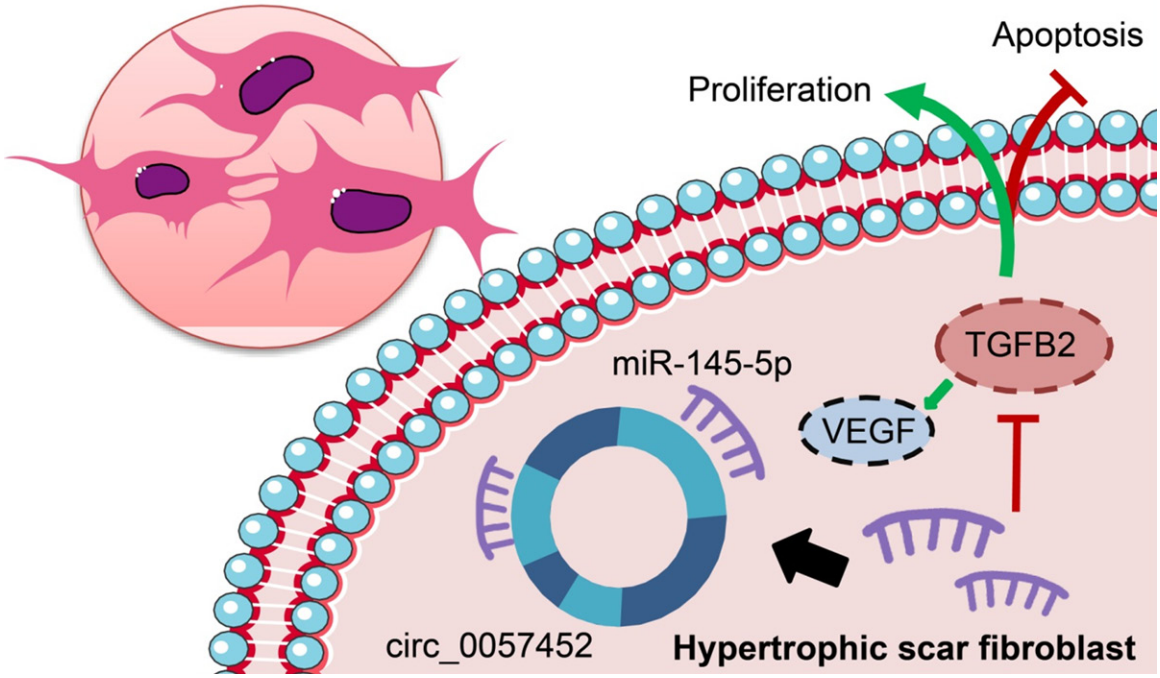

Figure 7. circ_0057452 acting as a ceRNA for miR-145-5p to regulate TGF-β2 expression and promote HS fibroblast proliferation and VEGF expression.

circ_0057452 was upregulated in HS tissues and fibroblasts. Studies have shown that circRNAs are involved in the regulation of a variety of cellular processes and disease development through gene expression [19, 20]. Downregulation of circ_0057452 expression in HS fibroblasts revealed that the proliferation of fibroblasts and VEGF secretion were significantly inhibited, and apoptotic rate was increased. A previous study found that some circRNAs are strongly associated with scars and that circ_0057452, circ_0007482, circ_0020792, circ_0057342, and hsa_circ_0043688 are significantly upregulated in keloid tissues [9]; however, that study only investigated the expression of these circRNAs, and did not shed much light on how circ_0057452 affects HS. With circbase database, it was found that circ_0057452 was transcribed from COL5A2 collagen genes, and the synthesis of collagen was also vital for HS formation, which further justified our exploration of the role of circ_0057452 in HS. Fibroblasts play a major part in collagen synthesis and scar hyperplasia, and the massive proliferation of fibroblasts is the underlying cause of HS formation [21, 22]. Vascular endothelial growth factor (VEGF), a pro-angiogenic cytokine in current studies, is essential in promoting tissue repair, wound healing, and growth of blood vessels. It can enhance capillary permeability and exacerbate the deposition of extracellular matrix components [23, 24]. Scar hyperplasia is accompanied by visible capillary hyperplasia. Several studies have shown that high levels of VEGF are closely related to scar formation in normal scars, hypertrophic scars, and keloids [25]. Our study confirmed that circ_0057452 promotes HS by regulating HS fibroblast proliferation and VEGF secretion.

The target of circ_0057452 was further retrieved and miR-145-5p was included in the study. Our results showed that circ_0057452 could influence HS as a ceRNA for miR-145-5p. After simultaneous overexpression of miR-145-5p and circ_0057452, the effect of upregulating miR-145-5p was partially reversed by circ_0057452 overexpression. Previous studies have found that the expression of miR-145-5p was significantly decreased in HS tissues and fibroblasts [14]. Through CCK8, ELISA, and flow cytometry, we found that the proliferation of HS fibroblasts and the expression of VEGF decreased and the number of apoptotic cells increased after upregulation of miR-145-5p. Analysis of circRNA-miRNA networks showed that circRNAs interact with miRNAs as ceRNAs

to form a complex network of mutual regulation [26, 27]. These findings further validated the important role of circ_0057452 and miR-145-5p in HS.

Typically, miRNA function is exerted by directing miRNA-induced silencing complexes to target mRNAs, which represses gene expression post-transcriptionally, so we predicted the downstream mRNA target of miR-145-5p on TargetScan. TGF-β2 is an important profibrotic growth factor and has been found to promote HS formation after upregulation [16]. We further confirmed through detection that TGF-β2 expression was much higher in normal tissues and fibroblasts than in HS tissues and fibroblasts. KEGG analysis revealed that TGF-β2 signaling through the MARK pathway is important in the pathogenesis of HS, and dual luciferase reporter assay confirmed TGF-β2 as a downstream target gene of miR-145-5p. Subsequently, it was found by regulating the expression of circ_0057452/miR-145-5p/TGF-β2 that there is an interaction network of circRNA/miRNA/mRNA. Then we found by regulating the expression of miR-145-5p/TGF-β2 in HS fibroblasts that the impact of miR-145-5p overexpression could be alleviated by upregulation of TGF-β2, partly increasing the proliferation of HS fibroblasts, promoting VEGF expression, and decreasing apoptosis. Also, our experiments further confirmed that TGF-β2 promotes the proliferation of HS fibroblasts. However, we only conducted in vitro experiments and did not explore the effect of circ_0057452-miR-145-5p-TGF-β2 in vivo. Thus, further study and discussion are necessary to build a foundation for the clinical application of targeted therapy for HS. This study will provide some scientific basis for understanding the occurrence and development of HS, identifying intervention targets, and determining clinical diagnostic markers.

In summary, the circ_0057452-miR-145-5p-TGF-β2 axis is involved in the development of HS. Circ_0057452 can promote the proliferation of HS fibroblasts, increase VEGF expression, and inhibit apoptosis by interacting with miR-145-5p/TGF-β2.

Disclosure of conflict of interest

None.

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