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Inhibition of Proliferation, Migration, and Invasion of Keloid Fibroblasts By miR-183-5p Through Downregulating EGR1

Inhibición de la Proliferación, Migración e Invasión de Fibroblastos Queloides por miR-183-5p Mediante la Regulación Negativa de EGR1

Mingxi Li[1]; Haiyan Qin[1]; Lingfeng Pan[1]; Guang Zhang[2]; Liehao Yang[1]; Changcai Zhou[1]; Caihong Li[1]; Zichao Wang[1] & Lianbo Zhang[1]

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SUMMARY: Keloid scar is a unique benign fibroproliferative tumor of the human skin. Previously, it was reported that early growth response 1 (EGR1), a transcription factor, promotes keloid fibrosis; however, the mechanism by which EGR1 modulates keloid formation was not elaborated. In this research, the specific function and the microRNA (miRNA) regulatory network of EGR1 in keloids was examined. Keloid fibroblasts (KFs) were transfected with EGR1-small interfering RNA (siEGR1), EGR1-overexpression plasmid (pcDNA3.1-EGR1), and microRNA (miR-183-5p)-mimics to regulate the expression of EGR1 and miR-183-5p. The study employed dual-luciferase reporter assays to explore the targeting regulation of miR-183-5p on EGR1. Additionally, Western blotting, flow cytometry, qRT-PCR, cell count kit-8 (CCK-8), transwell, and wound healing assays, and RNA sequencing were conducted. EGR1 was upregulated in KFs, and EGR1 silencing diminished proliferation, fibrosis, migration, invasion, and apoptosis of cells. In KFs, the expression of miR-183-5p was reduced, leading to the inhibition of cell proliferation, migration, and invasion. Conversely, it enhanced apoptosis. By targeting EGR1, miR-183-5p partially counteracted the impact of EGR1 on migration, invasion, and fibrosis in KFs. The findings imply that miR-183-5p suppresses keloid formation by targeting EGR1. As a result, EGR1 holds promise as a potential therapeutic target for preventing and treating keloids.

KEY WORDS: miR-183-5p; Keloid fibroblast; EGR1; Fibrosis.

INTRODUCTION

Keloid scars are benign fibroproliferative tumors of the human skin. These are refractory scars commonly observed in dermatology practice or after plastic surgery. Scars are distinguished by the excessive accumulation of fibrous tissue in the skin and subcutaneous layers. Keloids, in contrast to true neoplasms, do not exhibit distant metastasis or spontaneous occurrence. However, keloids have many tumor-like features, such as lacking spontaneous regression, invasiveness into their normal surrounding skin, and uncontrolled proliferation, which are not present in hypertrophic scars (Tan *et al*., 2019). The precise mechanism underlying this phenomenon remains unclear. After a skin injury, some prerequisite factors or a key regulator may modulate multiple signaling pathways in dermal fibroblasts, resulting in the formation of a keloid scar. The altered phenotype of keloid fibroblasts (KFs) is at the core of keloid formation (Hunasgi *et al*., 2013). Therefore, investigating the molecular mechanisms of fibrosis, proliferation, and invasion of KFs is crucial to improving treatmentstrategies.

The early growth response 1 (EGR1) gene encodes a zinc-finger transcription factor and is related to the pathogenesis of many human diseases. Its expression is regulated by several signals including cytokines, growth factors, stress signals, and environmental factors such as ultraviolet rays, ionizing radiation, and injury. EGR1 exhibits high expression levels in diverse neoplasms, including prostate and gastric cancers (Luo *et al*., 2021). Moreover, it plays a crucial role in regulating various

[1] Department of Plastic Surgery, China-Japan Union Hospital of Jilin University, Changchun, Jilin 130033, China.
[2] Department of Thyroid Surgery, China-Japan Union Hospital of Jilin University, Changchun, Jilin 130033, China.
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aspects of tumor cell behavior, such as proliferation, migration, invasion, and apoptosis. It can also serve as an oncogene or a tumor suppressor gene and additional functions in various types of cancer. In addition, EGR1 is closely associated with fibrotic disorders. EGR1 promotes TGF-β-induced liver fibrogenesis by binding to the BrD4 promoter and enhancing its expression (Tian *et al*., 2022). Previously, it was reported that EGR1 contributes to the progression of keloids by targeting NOX4, thereby promoting oxidative stress and fibrosis (Qin *et al*., 2022). Therefore, EGR1 can be a possible drug target for keloid. However, the detailed mechanism by which EGR1 affects keloid formation is not known.

MicroRNAs (miRNAs) are noncoding RNAs that can downregulate the expression of target genes by inhibiting translation. miRNAs are involved in keloid pathogenesis, and miR-188-5p and miR-661 slow down the progression of KFs (Wu *et al*., 2022; Zhou *et al*., 2022). By comprehending the involvement of miRNA regulation in the pathogenesis of keloids and identifying pertinent biomarkers, there is potential for the development of miRNA-based therapeutics specifically targeted for keloid treatment. MiR-183-5p found on chromosome 7q32 is involved in many processes that drive tumor progression. It is dysregulated in several cancer types, such as colorectal cancer and pancreatic adenocarcinoma (Miao *et al*., 2016). Furthermore, miR-183-5p is an effective inhibitor of EGR1 in synovial sarcoma by targeting its mRNA (Sarver *et al*., 2010). However, its specific impact on keloids remains ambiguous.

In this investigation, the significance of miR-183-5p and EGR1 in the KFs was explored, which are the main cells involved in keloid formation. This research provides the baseline for a novel EGR1-targeted therapeutic strategy for keloids.

MATERIAL AND METHOD

Tissue Samples: Keloid tissue samples were collected from nine individuals who underwent keloidectomy at the Plastic Surgery Department of China-Japan Union Hospital of Jilin University between July 2020 and September 2021. Before undergoing surgery, the individuals had not received any medication or radiotherapy as part of their treatment scheme. Additionally, normal skin tissue (Nskin) samples were collected from seven individuals undergoing cosmetic surgery and free skin grafting. Table I provides comprehensive patient information. After collection, the tissue samples were stored in DMEM complete medium at 4 °C for subsequent processing. It is important to note that all samples were acquired following written informed consent from the individuals, and the research was approved by the Ethics Committee of China-Japan Union Hospital.

Table I. Profiles of the keloid samples and normal skin samples.

A. Profiles of the keloid samples.

NO.	Sex	Age(years)	Ethnicity	Body region	Duration (months)
1	Male	43	Han	Chest	21
2	Male	31	Han	Earlobe	9
3	Male	36	Han	Earlobe	18
4	Male	13	Korean	Neck	9
5	Female	31	Han	Chest	40
6	Female	19	Han	Earlobe	12
7	Female	13	Mongolian	Earlobe	24
8	Female	35	Han	Shoulder	16
9	Female	18	Han	Back	48

B. Profiles of the normal skin samples.

NO.	Sex	Age(years)	Ethnicity	Body region	Duration (months)
1	Male	29	Mongolian	Palpebra	/
2	Male	35	Han	Arm	/
3	Female	22	Han	Palpebra	/
4	Female	40	Han	Palpebra	/
5	Female	59	Han	Eyebrow	/
6	Female	24	Han	Palpebra	/
7	Female	33	Han	Back	/

Cell Culture and Transfection: Primary human KFs and normal skin fibroblasts (NFs) were separated from keloid and Nskin specimens, respectively, utilizing the tissue block method (Qin *et al*., 2021). Further experiments were conducted using NFs and KFs at 2–4 passages. The fibroblasts were cultivated in DMEM (Corning, NY, USA) supplemented with 1 % streptomycin/penicillin and 10 % fetal bovine serum (BI, Kibbutz, Israel). The incubation was carried out in a 5 % CO_2 incubator at 37°C.

The miR-183-5p mimic (S: UAUGGCACUGGU AGAAUUCACU, AS: UGAAUUCUACCAGUGCC AUAUU), small interfering RNA targeting human EGR1 mRNA [siEGR1; S: GCCUAGUGAGCAUGACCAATT and AS: dTdT (UUGGUCAUGCUCACUAGGCTT)] were procured from GenePharma Co. (Suzhou, China). The corresponding negative control [NC; S: UUCUU CGAACGUGUCACGUTT, AS: ACGUGACA CGUUC GGAGAATT], miR-183-5p inhibitor (AGUGA AUUCU ACCAGUGCCAUA), and inhibitor NC (CAGUACU UUUGUGUAGUACAA) were also procured from GenePharma. The pcDNA3.1-EGR1 and pcDNA3.1 plasmid vectors were obtained from Cell Signaling Technology (Shanghai, China). Lipoplus reagent (Sagecreation, Beijing, China) was used for transfection. The transfection efficiency was measured utilizing qRT-PCR after 24 h of transfection and western blotting after 48 h of transfection.

Dual-luciferase Reporter Assay: Both the wild-type EGR1 3'UTR and the mutated EGR1 3'UTR were cloned with miR-183-5p binding sites for the psiCHECK-2 vector. Additionally, 293T cells were cultured overnight onto a 96-well plate and grown to 50%–60% confluency. Subsequently, psiCHECK-EGR1 WT and psiCHECK-EGR1 were co-transfected into the 293T cells using miR-183-5p mimics/NC for 36 h. The relative luciferase and renilla signals of each experimental category were assessed by means of the Dual-Luciferase® Reporter assay, with firefly luciferase serving as the internal reference.

Proliferation Assay: The proliferative ability of the cells was detected utilizing the cell count kit-8 (CCK-8) assay. Initially, KFs were seeded onto a 96-well plate at a density of 3 × 10^3 cells per well and subjected to overnight incubation. Afterward, 110 μL CCK8 working solution (100 μL DMEM complete medium) was added to each well at 0, 24, 48, and 72 h, and the plate was then incubated in the darkness for 4 h. Finally, the absorbance (OD) at 450 nm was quantified utilizing a Bio-Rad plate reader.

Wound Healing Assay: Lines were gently and slowly scratched when approximately 100 % confluency was achieved after transfection in a 6-well plate. Further, the cell monolayer was gently scratched utilizing a 1-ml pipette tip. The plates were rinsed thrice with PBS to eliminate detached cells, and fresh serum-free DMEM medium was introduced into each well for 36 h. The wound-healing status was checked under an inverted microscope, and the wound-healing percentage was assessed and calculated employing Image J.

Transwell Assay: To evaluate KF migration and invasion, transwell chambers with 8-mm pore size (Biofil, Guangzhou, China) were employed. In the transwell upper chamber, matrix gels (Corning) were introduced. The KFs that were previously transfected were then suspended in serum-free DMEM medium at a density of 1.5×10^5 cells/ml. A cell suspension of 0.2 ml was carefully added to the upper compartment of the chamber, while 0.7 ml of complete medium was simultaneously incorporated into the lower compartment. The transwell chamber was subsequently incubated for 24 h at 37°C. Following incubation, the cells were fixed using 4 % paraformaldehyde and then stained with 1 % crystal violet for 15 min. After the removal of the cells from the upper surface of the transwell with a cotton pellet, the number of cells that migrated and invaded the lower surface was counted using a microscope.

Apoptosis Assay: Apoptosis of different cell groups was confirmed using an Annexin V/propidium iodide kit and flow cytometry. EDTA-free pancreatin was used to harvest the transfected cells. Following a wash with cold PBS, the cells were suspended in 100 μL of binding buffer. Subsequently, 5 μL of Annexin V-FITC and 10 μL of propidium iodide were incorporated, and the cells were incubated for 15 min at room temperature in the darkness. After that, an additional 400 μL of binding buffer was combined, and the signal was detected using flow cytometry.

RNA extraction and qRT-PCR: Total RNA and miRNA were isolated utilizing Trizol (Invitrogen, Carlsbad, USA). For the reverse transcription of total RNA, the reagents obtained from Takara (Dalian, China) were used. The cDNA synthesis from total RNA was conducted utilizing the miRNA First Strand cDNA Synthesis Tailing Reaction Kit (Sangon Biotech, Shanghai, China) for miRNA. The reverse transcription of miR-183-5p was performed using stem-loop primers and the miRNA reverse transcription kit from GenePharma (Suzhou, China). The qRT-PCR was conducted utilizing a CFX96 Real-Time PCR system from Bio-Rad. The qRT-PCR was monitored using SYBR Green from Promega (Beijing, China). The primer sequences employed in this research are presented in Table II.

Western Blot Analysis: Complete extraction of protein from cells was achieved utilizing RIPA buffer. The protein concentration was assessed by employing a BCA protein assay. Subsequently, 20 μg of protein was isolated on a 10 % SDS-PAGE gel, at 80 V for 30 min followed by 1 h at 120 V. After the separation, the proteins were transferred onto a PVDF membrane for 90 min at 90 V. Following that, 5 % skim milk was used for blocking the membranes for 2 h at room temperature. Subsequently, the membranes were incubated with the following primary antibodies at 4°C: EGR1 (1:500; Abmart, China), caspase3 (1:500; Abmart), collagen 1 (1:500; Abmart), collagen 3 (1:1000; Abmart), α-SMA (1:1000; Immunoway, USA), and vimentin (1:1000;

Wanlei, China). Anti-GAPDH (1:5000; Immunoway) and anti-β-actin (1:5000; Immunoway) were used as internal controls. Afterward, TBST (Tris-buffered saline with Tween 20) was utilized for washing the membranes and incubated with secondary antibodies at room temperature for 2 h. Eventually, an ECL (enhanced chemiluminescence) detection kit was employed for the visualization of the protein bands, and densitometric analysis was carried out using Image J.

Statistical Analyses: The data were presented as mean (± S.D.), and statistical analysis was executed employing GraphPad Prism 8.0. T-test was utilized to compare data conforming to normal distribution between both categories. Wilcoxon test was used for non-conformable normal distribution data. Comparative analysis of the differences across three or more groups of data was carried out utilizing one-way ANOVA. $P < 0.05$ was determined statistically significant.

Table II. All primer sequences used for qRT-PCR.

Gene	Sequences(5'-3')	
Collagen I	Sense:	GAGGGCAACAGCAGGTTCACTTA
	Antisense:	TCAGCACCACCGATGTCCA
Collagen III	Sense:	CCACGGAAACACTGGTGGAC
	Antisense:	GCCAGCTGCACATCAAGGAC
EGR1	Sense:	TTGCCACTCAGTCGGGCT
	Antisense:	TGGGTTTGATGAGCTGGGAC
GAPDH	Sense:	GTGAAGGTCGGAGTCAACG
	Antisense:	TGAGGTCAATGAAGGGGTC
miR-29c-5p	Sense:	TGACCGATTTCTCCTGGTGTT
	Antisense:	universal reverse primer (Sangon Biotech, Shanghai, China)
miR-133a-3p	Sense:	GGTCCCCTTCAACCAGCTG
	Antisense:	universal reverse primer (Sangon Biotech, Shanghai, China)
miR-133a-5p	Sense:	CAGCTGGTAAAATGGAACCAAAT
	Antisense:	universal reverse primer (Sangon Biotech, Shanghai, China)
miR-133b	Sense:	TGGTCCCCTTCAACCAGCTA
	Antisense:	universal reverse primer (Sangon Biotech, Shanghai, China)
miR-183-5p	Sense:	ACTCGCTTGCTTCCTTTCAG
	Antisense:	CAGAGCAGGGTCCGAGGTA
U6	Sense:	CGCTTCGGCAGCACATATAC
	Antisense:	TTCACGAATTTGCGTGTCATC

RESULTS

Suppression of proliferation, migration, invasion, extracellular matrix (ECM) deposition, and apoptosis of KFs by EGR1 knockdown: The expression level of EGR1 in KFs was examined to evaluate its functional role in keloid formation. Figures 1A and B show the schematic presentation of the findings. EGR1 was upregulated in KFs compared with NFs. Following this, siEGR1 was used to examine the effect of EGR1 downregulation on the cellular phenotypes. CCK8 assay was employed for the measurement of the KF proliferation after successful transfection. EGR1 downregulation significantly reduced the proliferative ability of KFs (Fig. 1C). Moreover, the results of wound healing assay suggested that EGR1 downregulation suppressed KF migration after 36 h of transfection (Fig. 1D). The findings of the Transwell assay demonstrated a considerable decrease in the counts of migrated and invaded cells in the EGR1-downregulation groups (Fig. 1E). Further, the role of EGR1 in apoptosis was determined. The outcomes of the flow cytometry indicated that the rate of apoptosis was reduced in KFs after transfection with siEGR1 (Fig. 1F). Additionally, outcomes of the Western blotting implied that the levels of protein expression of cleaved-caspase3 were low in the same group (Fig. 1G). Additionally, transfection of KFs with siEGR1 resulted in the reduction of ECM-associated genes (Fig. 1H). Taken together, EGR1 was upregulated in KFs, and downregulation of EGR1 suppressed proliferation, migration, invasion, ECM deposition, and apoptosis of KFs.

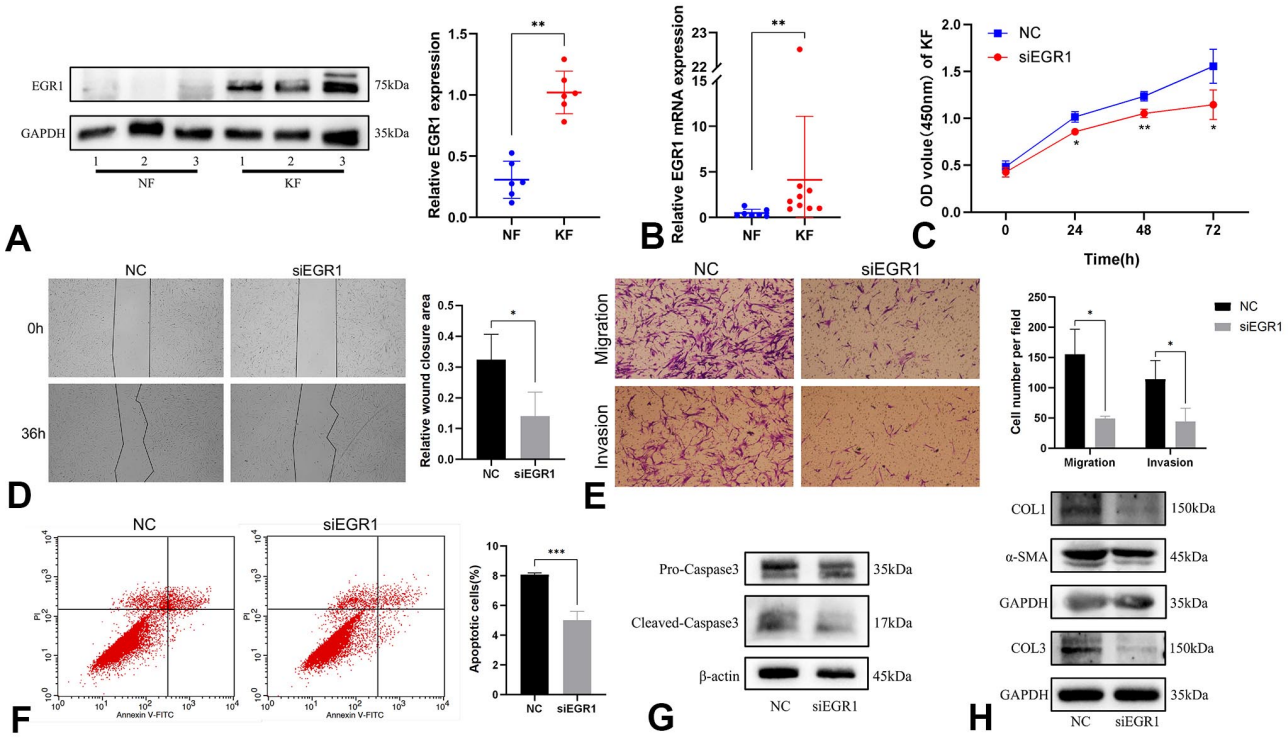

Fig. 1. Suppression of proliferation, migration, invasion, ECM deposition, and apoptosis of KFs by the EGR1 knockdown. (A) Western blotting and (B) qRT-PCR analysis of EGR1 expression in KFs and NFs. (C) Proliferation of KFs transfected with siEGR1 or NC (detected utilizing the CCK-8 assay). (D) Measurement of cell migration using the wound-healing assay. (E) Assessment of cell migration and invasion using the transwell assay. (F) Assessment of apoptosis using flow cytometry. (G, H) Western blots show the expression of cleaved-caspase3, pro-caspase3, collagen1 (COL 1), collagen3 (COL 3), and α-SMA proteins. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

Downregulation of miR-183-5p in KFs or tissues: The miRNA expression in keloid and Nskin was analyzed using microarray analysis (Fig. 2A). In total, 247 known differentially expressed miRNAs were identified, and 113 and 134 were downregulated and upregulated, respectively ($P < 0.05$ and fold change < 0.5; Fig. 2B). In total 4 miRNAs were selected. These results were validated using qRT-PCR to verify the reliability of the microarray data. Consistent with the microarray data, the expressions of miR-29C-5p, miR-133a-3p, miR-133a-5p, and miR-133b were considerably downregulated in the keloid tissue (Fig. 2C). MiR-183-5p was expressed at low levels in the keloid tissues (Fig. 2B). The total RNA was subsequently isolated from KFs (n = 9) and NFs (n = 7) to assess the miR-183-5p expression utilizing qRT-PCR. Compared with NFs, miR-183-5p expression was considerably downregulated in KFs, suggesting that miR-183-5p may play a significant role in keloid progression (Fig. 2D).

Suppression of proliferation, migration, and invasion of KFs and promotion of apoptosis by overexpression of miR-183-5p: Referring to a previously conducted study of siEGR1, the targeting relationship of miR-183-5p and EGR1 was evaluated using bioinformatics software. A hypothesis was made stating that the impact of the overexpression of miR-183-5p on the cellular phenotypes may be similar to those of siEGR1. The overexpression of miR-183-5p by the transfection of miRNA mimics suppressed KF proliferation (Fig. 3A). Furthermore, the findings of wound healing assay confirmed that the migration capacity of KF was decreased by the miR-183-5p overexpression after 36 h of transfection (Fig. 3B). The outcome of transwell assay revealed that cellular migratory and invasive abilities were decreased in the miR-183-5p mimics group (Figs. 3C, D). In contrast, the rate of apoptosis (quantified using flow cytometry) elevated after transfection with miR-183-5p mimics in KFs (Fig. 3E). The expression level of cleaved-caspase3 protein was also elevated in the miR-183-5p mimics group (Fig. 3F). Taken together, miR-183-5p suppressed the proliferation, migration, and invasion of KFs and promoted their apoptosis.

Suppression of the EGR1 expression by miR-183-5p through binding directly to 3'UTR: A hypothesis stating that miR-183-5p participates in keloid formation by targeting EGR1 was made. Hence, the regulatory correlation of miR-

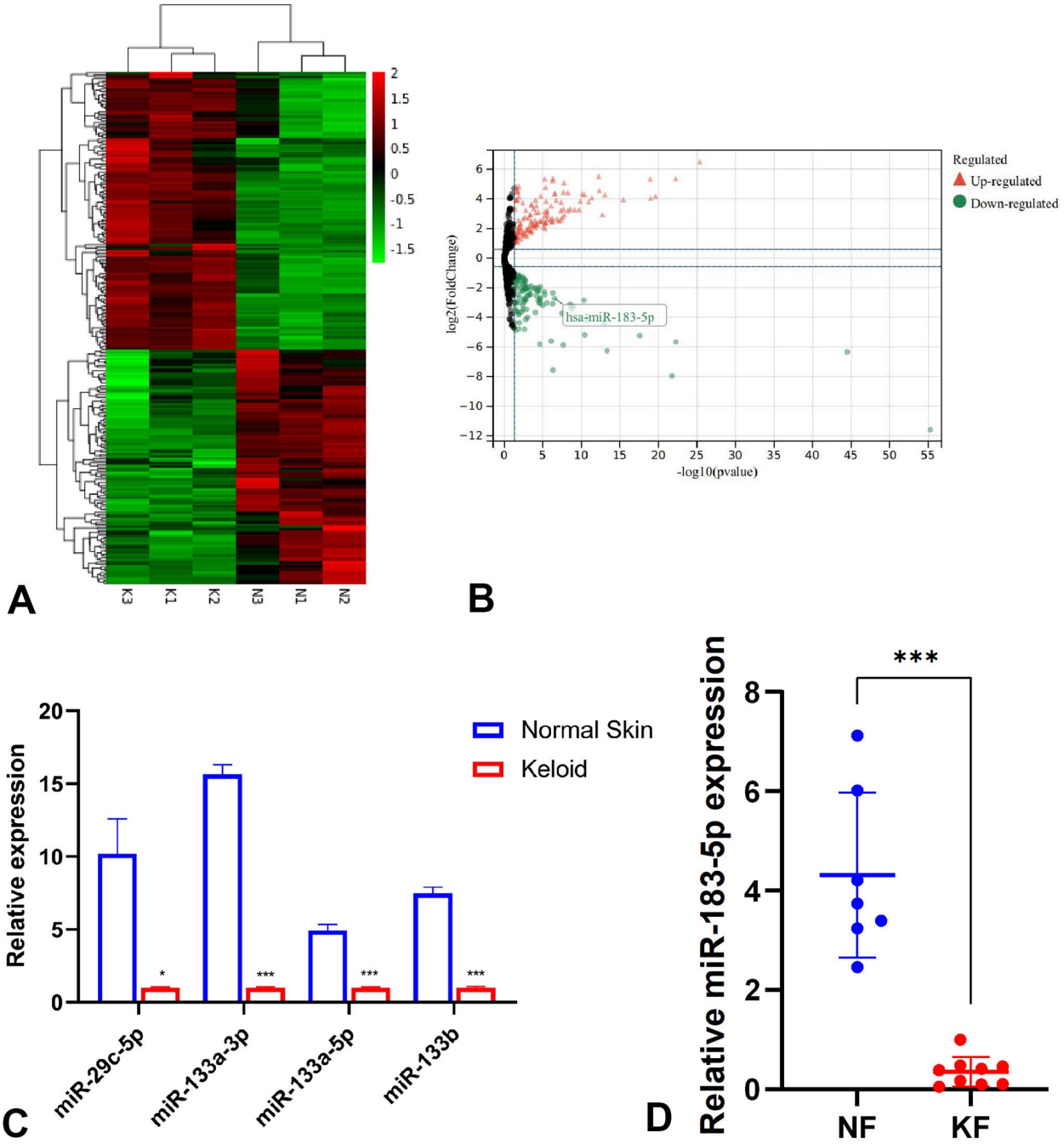

Fig. 2. Downregulation of miR-183-5p in KFs or tissues. (A) Heat map. (B) Volcano plots of microarray analysis for the microRNA expression in Keloid vs. Nskin. (C) qRT-PCR analysis of four differentially expressed miRNAs in the keloid and Nskin. (D) qRT-PCR analysis of the miR-183-5p expression in KFs (n = 9) and NFs (n = 7). *$P < 0.05$ and ***$P < 0.001$.

183-5p with EGR1 was verified. The findings of the dual-luciferase reporter assay implied that miR-183-5p mimics considerably reduced the activity of luciferase in the vectors with the forecasted binding site in the 3' UTR of EGR1. However, it did not affect the luciferase activity of vectors with mutant sequences of the predicted binding site (Fig. 4A). The findings of western blotting and qRT-PCR, performed after transfecting miR-183-5p mimics in KFs, indicated the negative impact of miR-183-5p on EGR1, indicating that miR-183-5p overexpression reduced both protein and mRNA expressions of EGR1 (Figs. 4B, C). Therefore, the resulting data implied that miR-183-5p regulated the EGR1 expression by directly binding to 3'UTR.

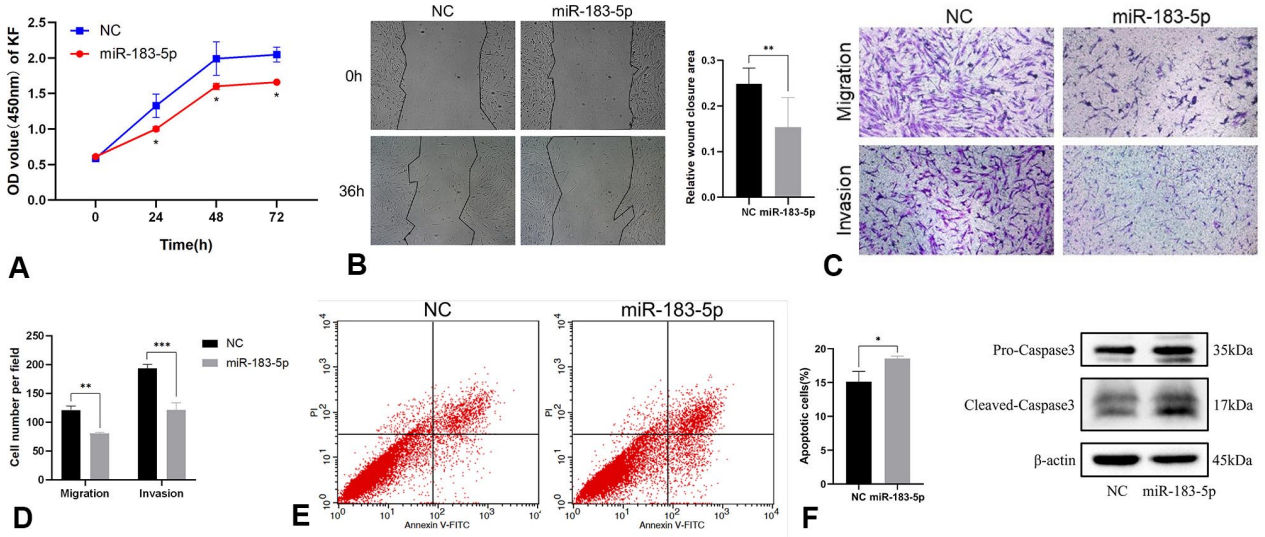

Fig. 3. Suppression of proliferation, migration, and invasion of KFs and promotion of their apoptosis by overexpression of miR-183-5p. (A) Proliferation of KFs after transfection (detected using the CCK-8 assay). (B) Assessment of cell migration using the wound healing assay. (C, D) Measurement of cell migration and invasion using the transwell assay. (E) Assessment of apoptosis using flow cytometry. (F) Western blots show the changes in the expression of cleaved-caspase3 and pro-caspase3 proteins. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

A rescue experiment was conducted for further validation of this conclusion. KFs were treated with both miR-183-5p mimics and pcDNA3.1-EGR1 to determine if overexpression of miR-183-5p could eliminate the fibrosis-promoting effect of EGR1. The expression levels of ECM biomarkers were determined utilizing western blotting and qRT-PCR. In comparison to the cells transfected with pcDNA3.1-EGR1 alone, the expression levels of ECM biomarkers were decreased in KFs following co-transfection with both pcDNA3.1-EGR1 and miR-183-5p mimics (Fig. 4D, E). Subsequently, miR-183-5p inhibitors and siEGR1 were utilized to transfect the KFs, and the combination partly abrogated the suppression of the EGR1 protein levels compared with siEGR1 alone (Fig. 4F). In the transwell assay, miR-183-5p inhibitors enhanced cell migration and invasion capacities in comparison with the above effects of miR-183-5p mimics. In addition, the inhibitory effects of siEGR1 on the migratory ability of KFs were partly reversed by the miR-183-5p inhibitors (Fig. 4G). Hence, EGR1 can mediate the inhibitory effect of miR-183-5p on KF function.

DISCUSSION

Keloids are a consequence of excessive wound healing. The incidence of keloids is high in people with Hispanic, Asian, Afro-American, Afro-Caribbean, or African ancestry (Tan *et al.*, 2019), and the negative effects on the quality of life of affected patients are well-documented. The main treatments for keloids include operative and non-operative methods. The recurrence rate of keloids is high with surgery alone; therefore, surgery can be combined with radiotherapy, intralesional corticoid injection, or laser therapy in the clinic. The clinical guidelines for diagnosis and treatment have not been developed because of the poor understanding of the pathologic mechanisms of keloid scar formation and the absence of consensus on the gold standard for treating pathologic scars (Ogawa *et al.*, 2019). Hence, exploring the mechanism of keloid progression is crucial for a better understanding of the underlying biological processes that lead to keloid formation and growth along with screening of potential therapeutic targets.

EGR1 is predicted as a potential target for preventing hypertrophic and keloid scars (Zhang *et al.*, 2018), and its abnormal expression in different diseases, including tumors, atherosclerosis, inflammation, and fibrotic diseases, has been widely reported. Notably, EGR1 shows “duality” in the pathogenesis of different tumors (Li *et al.*, 2019). As an oncogene, EGR1 can activate the p38 MAPK pathway and act directly on the cyclin D1 promoter to shorten the G1 phase and promote tumor proliferation (Park *et al.*, 2016). In addition, EGR1 helps in promoting the invasiveness and migration of esophagus squamous cancer cells by activating LCN2 transcription (Zhao *et al.*, 2019). Loss-of-function experiments were conducted for determining the functional effect of EGR1 on KFs. EGR1

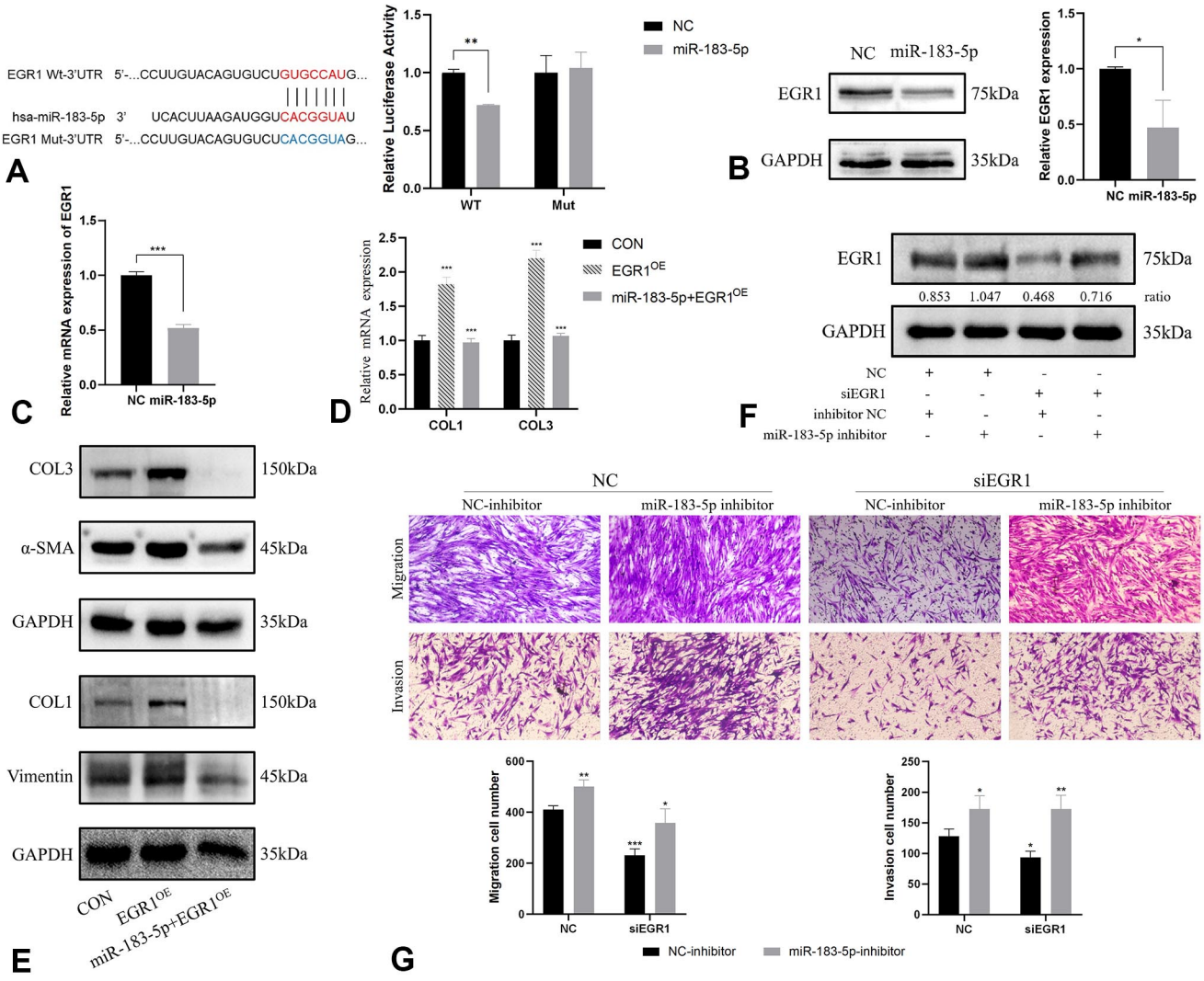

Fig. 4. Suppression of proliferation, migration, invasion, ECM deposition, and apoptosis of KFs through the EGR1 knockdown. (A) Target site is between EGR1 and miR-183-5p, as indicated by the outcomes of the dual-luciferase reporter assay. The expression level of EGR1 in KFs transfected with miR-183-5p mimics was evaluated using (B) western blotting and (C) qRT-PCR. Expression levels of ECM-related genes in KFs transfected with mimics-NC+vector, mimics-NC+pcDNA3.1-EGR1, and miR-183-5p mimics+pcDNA3.1-EGR1 evaluated using (D) qRT-PCR and (E) western blotting. (F) Western blots show the protein expression of EGR1 in KFs transfected with NC+inhibitor-NC, NC+miR-183-5p-inhibitor, siEGR1+ inhibitor-NC, and siEGR1+ miR-183-5p-inhibitor. (G) Measurement of the migration and invasion abilities of co-transfected KFs utilizing the transwell assay. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

silencing inhibited the proliferation, migration, and invasion of KFs. Furthermore, an inhibitory effect of EGR1 silencing was observed on apoptosis in KFs, reflecting the tumor suppressor function of EGR1. EGR1 may directly regulate the transcriptional expression of apoptosis-inducing factors (such as BAX, NAG1, and PTEN) by binding to their promoter (Wang *et al.*, 2015). Moreover, EGR1 is a novel target for antifibrotic treatment because it is a key mediator of fibrosis (Bhattacharyya *et al.*, 2011). TGF-β signaling pathway has a pivotal role in skin fibrosis. At least two EGR1-binding sites occur in the TGF-β1 promoter region, suggesting that EGR1 participates in keloid formation and fibrosis (Liu *et al.*, 1996). Interestingly, EGR1 silencing decreased the expression levels of ECM-associated genes.

The functional part of miR-183-5p in keloid formation has not been extensively researched. Several authors have elaborated that miR-183-5p is a tumor suppressor miRNA. Zou *et al.* (2021) showed that miR-

183-5p was expressed at low levels in cervical cancer, and miR-183-5p expression targeted TMED5 to inhibit tumor progression. In addition, miR-183 inhibited viability, migration, and invasion of epithelium on adenomyosis through downregulation of MMP-9 (Wang & Chen, 2022). It was observed that miR-183-5p affected the KF phenotypes in the same way as reported by prior studies. miR-183-5p overexpression inhibited the proliferation, migration, and invasion of KFs. Moreover, miR-183-5p is a pro-apoptotic factor in breast cancer cells as it targets the 3'UTR of PLK1, thereby suppressing PLK1, increasing p-p53 expression levels, and further promoting apoptosis (Kudo *et al*., 2022). It was also found that miR-183-5p can promote KF apoptosis. Therefore, it can be a potential therapeutic target for treating keloids. The EGR1 gene is highly expressed in KFs; therefore, targeting EGR1 may be a potential therapy against keloid formation.

Previously, the similarity between the impact of miR-183-5p mimics and siEGR1 in KFs was observed. A dual-luciferase reporter assay was carried out to find out the targeting relation among miR-183-5p and EGR1. However, each miRNA can regulate numerous genes. The target genes of miR-183-5p include ZEB2, FOXO1, RGS2, and ABAT (Wang *et al*., 2021; Mo *et al*., 2022). Moreover, the function of miRNA is also associated with the relative abundance of target genes. Therefore, rescue experiments were designed to further show that the miR-183-5p/EGR1 axis existed in KFs and miR-183-5p functioned by targeting EGR1.

However, this research was limited in several respects. Firstly, contradictory results were obtained on the role of miR-183-5p mimics and siEGR1 in KF apoptosis, suggesting that miR-183-5p may help in promoting apoptosis by some other pathways. Secondly, the downstream signaling molecules of the miR-183-5p/EGR1 axis were left unexplored. Finally, animal experiments were not performed. Therefore, further research is needed to address these questions.

CONCLUSION

The expression of EGR1 is upregulated in KFs, and the downregulation of EGR1 suppresses the proliferation, migration, invasion, apoptosis, and fibrosis of KFs. In addition, EGR1 is targeted and inhibited by miR-183-5p, and it mediates the inhibitory function of miR-183-5p. Hence, the miR-183-5p/EGR1 axis affects keloid cell phenotypes and can be a therapeutic target for treating keloid.

LI, M.; QIN, H.; PAN, L.; ZHANG, G.; YANG, L.; ZHOU, C.; LI, C.; WANG, Z. & ZHANG, L. Inhibición de la proliferación, migración e invasión de fibroblastos queloides por miR-183-5p mediante la regulación negativa de EGR1. *Int. J. Morphol., 41(6)*:1610-1619, 2023.

RESUMEN: La cicatriz queloide es un tumor fibroproliferativo benigno único de la piel humana. Anteriormente, se informó que la respuesta de crecimiento temprano 1 (EGR1), un factor de transcripción, promueve la fibrosis queloide; sin embargo, no se explicó el mecanismo por el cual EGR1 modula la formación de queloides. En esta investigación, se examinó la función específica y la red reguladora de microARN (miARN) de EGR1 en queloides. Se transfectaron fibroblastos queloides (KF) con ARN de interferencia pequeño de EGR1 (siEGR1), plásmido de sobreexpresión de EGR1 (pcDNA3.1-EGR1) y miméticos de microARN (miR-183-5p) para regular la expresión de EGR1 y miR-183. -5p. El estudio empleó ensayos de indicador de luciferasa dual para explorar la regulación dirigida de miR-183-5p en EGR1. Además, se realizaron pruebas de transferencia Western, citometría de flujo, qRT-PCR, kit de recuento celular-8 (CCK-8), transwell y curación de heridas, y secuenciación de ARN. EGR1 estaba regulado positivamente en KF, y el silenciamiento de EGR1 disminuyó la proliferación, fibrosis, migración, invasión y apoptosis de las células. En KF, la expresión de miR-183-5p se redujo, lo que llevó a la inhibición de la proliferación, migración e invasión celular. Por el contrario, mejoró la apoptosis. Al apuntar a EGR1, miR-183-5p contrarrestó parcialmente el impacto de EGR1 en la migración, invasión y fibrosis en KF. Los hallazgos implican que miR-183-5p suprime la formación de queloides al apuntar a EGR1. Como resultado, EGR1 es prometedor como objetivo terapéutico potencial para prevenir y tratar los queloides.

PALABRAS CLAVE: miR-183-5p; Fibroblasto queloide; EGR1; Fibrosis.

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Corresponding author:
Lianbo Zhang
Department of Plastic surgery
China-Japan Union Hospital of Jilin University
No.126 Sendai Street
Changchun, Jilin, 130033
CHINA

E-mail: lianbo@jlu.edu.cn