

miR-124-3p targeting of TGF- β 1 inhibits the proliferation of hypertrophic scar fibroblasts

Suwen Zhang^{B-D,F}, Simeng Pan^{A,C,E,F}

Department of Plastic Surgery, Tongde Hospital of Zhejiang Province, Hangzhou, China

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online)

Adv Clin Exp Med. 2021;30(3):263–271

Address for correspondence

Simeng Pan

E-mail: simeng_pan@163.com

Funding sources

None declared

Conflict of interest

None declared

Received on May 21, 2020

Reviewed on October 24, 2020

Accepted on December 17, 2020

Published online on March 30, 2021

Abstract

Background. microRNAs are involved in a variety of physiological and pathophysiological processes, but their role in the pathogenesis of hypertrophic scars (HS) is not fully understood. Transforming growth factor β 1 (TGF- β 1) plays an important role in the genesis and development of HS.

Objectives. In this study, we hypothesized that a post-translational miRNA mechanism regulates the expression of TGF- β 1 in HS fibroblasts (HSFBs) and participates in the development of HS.

Materials and methods. Predictions from EBCORl, PicTar and miRBase databases showed that miR-124-3p can target and regulate the expression of TGF- β 1. We collected HS tissue and corresponding normal tissue from 25 patients with HS who had been operated on for the first time.

Results. The expression level of miR-124-3p in HS tissue was significantly lower than in normal tissue, while the expression level of TGF- β 1 mRNA was significantly higher than in normal tissue ($p < 0.05$), showing a negative correlation between them. Results from a luciferase reporter assay showed that miR-124-3p targets the 3'-UTR of TGF- β 1 and inhibits its expression. After miR-124-3p mimics were transfected into HSFBs, the expression of TGF- β 1, α -smooth muscle actin (α -SMA), collagen I, survivin, and Bcl-2 were reduced and the expression of Bax was increased, with significant decreases in DNA synthesis, proliferation and survival. However, after a miR-124-3p inhibitor was transfected into HSFBs, these effects were reversed as the expression of TGF- β 1, α -SMA, collagen I, survivin, and Bcl-2 increased, expression of Bax decreased, and DNA synthesis, proliferation and survival cells increased significantly.

Conclusions. miR-124-3p can inhibit the proliferation of HSFBs by targeting TGF- β 1, and miR-124-3p may thus be a potential therapeutic target in HS.

Key words: TGF- β 1, proliferation, fibroblasts, hypertrophic scarring, miR-124-3p

Cite as

Zhang S, Pan S. miR-124-3p targeting of TGF- β 1 inhibits the proliferation of hypertrophic scar fibroblasts.

Adv Clin Exp Med. 2021;30(3):263–271.

doi:10.17219/acem/131753

DOI

10.17219/acem/131753

Copyright

Copyright by Author(s)

This is an article distributed under the terms of the Creative Commons Attribution 3.0 Unported (CC BY 3.0) (<https://creativecommons.org/licenses/by/3.0/>)

Background

Hypertrophic scar (HS) is a type of pathological scar, which is the result of local over-healing after dermal injury of human skin. The main pathological characteristics are excessive proliferation and differentiation of fibroblasts and enhanced deposition of collagen-based extracellular matrix (ECM) components.¹ Histologically, HS contains myofibroblasts, which participate in the contraction of scars.² These cells cause contraction of the surrounding ECM, resulting in an increase in the density of scar tissue, which not only affects the appearance and function of patients' skin, but may also cause psychological trauma. Hypertrophic scar is one of the most difficult problems to solve in burn, plastic surgery and in the general field of wound repair. Because of the lack of effective treatment methods, HS treatment is very difficult and often requires repair surgery, which leads to the formation of HS again.³ At present, the mechanism of HS formation is not fully understood. Elucidating the molecular mechanism of HS will help contribute to the discovery of new therapeutic targets.

Hypertrophic scar is characterized by excessive proliferation of fibroblasts and aberrant ECM formation. It consists of collagen, fibronectin (FN) and aminoglycan. In normal fibroblasts, the synthesis and decomposition of ECM are in a dynamic equilibrium, which maintains the relative stability of ECM. In HS, this balance is impaired due to increased ECM synthesis, and deficiencies in its degradation and turnover. It has been shown that the amount of FN synthesized by HS fibroblasts *in vitro* is 4 times that of normal fibroblasts, and the amount of collagen synthesized is 3 times that of normal fibroblasts.⁴ The expression level and activity of collagenase in HS fibroblasts were significantly lower than that in normal fibroblasts.⁵

Scar formation can be divided into 3 different stages: inflammation, hyperplasia and remodeling.⁶ In the inflammatory stage, a large number of chemokines and inflammatory factors are produced, which can induce angiogenesis, re-epithelialization, fibroblast recruitment, proliferation, and ECM deposition. The balance between the recruitment and proliferation of fibroblasts and the production and degradation of ECM is mainly regulated by fibroblast growth factors, including insulin-like growth factor, platelet-derived growth factor and transforming growth factor β (TGF- β). Among these growth factors, TGF- β 1 is widely reported to be related to the formation of HS, and mediates fibroblast proliferation, collagen production, ECM deposition, and myofibroblast differentiation during wound healing.^{6–10} In HS fibroblasts, TGF- β 1 has been shown to increase the phosphorylation of Smad2/3 and Smad4 and decrease Smad7 phosphorylation, resulting in the over-accumulation of Collagen-1 (Col-1).¹¹ In addition, TGF- β 1 can induce collagen production and contraction of human skin fibroblasts (HSFBs) derived from HS.¹² This suggests that TGF- β 1 may be a key target for the development of new strategies for HS treatment. The TGF- β 1 can induce macrophage M2 polarization

in the process of damage repair, making M2 macrophages a “double-edged sword.” On the one hand, they are essential for tissue repair, but on the other hand, they are also potential mediators of fibrosis and scar formation.¹³ Studies show that inhibition of macrophage M2 polarization can inhibit fibroblast activation and the formation of scars.^{14,15}

MicroRNAs (miRNA) are endogenous non-coding RNAs with regulatory function found in eukaryotes. They are involved in the regulation of cellular processes such as cell proliferation, differentiation and apoptosis. They can also aggravate or inhibit the onset and development of various diseases, including inflammation, cancer and fibrosis.^{16–18} Through bioinformatic approach, we predicted that miR-124-3p may target the 3'-UTR of TGF- β 1 to inhibit its expression. Recent reports show that miR-124-3p is considered to be a tumor suppressor in some cancers, including hepatocellular carcinoma,¹⁹ cervical cancer²⁰ and gastric cancer.²¹ Therefore, it is speculated that miRNAs may post-transcriptionally regulate the expression of TGF- β 1 in HS.

Objectives

In this study, we evaluated the expression levels of miR-124-3p in HS and normal skin tissues and found that miR-124-3p was downregulated in HS and was negatively correlated with TGF- β 1 expression. We found that modulating expression of miR-124-3p in HSFBs could affect cell proliferation.

Materials and methods

Samples

From January 2018 to January 2020, tissue samples from HS patients and corresponding normal tissues (NC) were collected from the plastic surgery department. Fresh tissue was stored directly in liquid nitrogen. Written informed consent was obtained from all patients. This study was approved by the ethics committee of Zhejiang Tongde Hospital (Hangzhou, China).

Cell culture and transfection

Human skin fibroblasts were prepared according to a previously reported method.²² The human skin fibroblast cell line HSF2 was purchased from National Biomedical Experimental Cell Resource Bank (Beijing, China). They were cultured with high sugar Dulbecco's modified Eagle's medium DMEM (Gibco, Carlsbad, USA) containing 10% fetal bovine serum (FBS; Gibco), 100 U/mL penicillin G, and 100 U/mL streptomycin sulfate at 37°C and 5% CO₂.

The TGF- β 1 expression vector pEX-3-TGF- β 1, pGPU6-TGF- β 1, miR-124-3p mimic, and miR-124-3p inhibitor were purchased from Gene Pharma (Shanghai, China).

They were transfected into HSFs and HSF2 cells. All transfections were performed with Lipofectamine 3000 Reagent (Life Technologies, Carlsbad, USA) according to the manufacturer's protocol. pEX3 and pGPU6 empty vectors were used as negative controls.

EdU incorporation assay

EdU (5-ethynyl-2'-deoxyuridine) is a thymidine analogue, which can get incorporated into synthetic DNA molecules instead of thymine (T) during DNA replication. The ethynyl on EdU can covalently react with fluorescently labeled small molecule azide probes (such as Azide Alexa Fluor 488, Azide Alexa Fluor 555, Azide Alexa Fluor 594, and Azide Alexa Fluor 647) through the catalysis of a univalent copper ion, forming a stable triazole ring so that the newly synthesized DNA can be labeled by the corresponding fluorescent probe, allowing for the detection of proliferating cells using appropriate fluorescence detection equipment.

In order to detect DNA synthesis in different groups of cells, 2×10^4 cells were added into each well of a 24-well plate to which EdU was added at a final concentration of 20 μ M; the cells were cultured for 24 h. The cells were washed twice with pre-chilled PBS and fixed for 20 min with pre-chilled 4% paraformaldehyde. They were treated with 0.5% Triton X-100 and stained with Cell-Light™ EdU In Vitro Imaging kit (Shanghai Beyotime Biotechnology Co., Ltd., Shanghai, China) according to the manufacturer's protocol. Nuclei of cells were stained with DAPI (Shanghai Beyotime Biotechnology), and EdU-positive cells were observed with an inverted immunofluorescence microscope.

Cell viability assay

Cells were harvested (from tissue), suspended in serum-free medium and plated into 96-well plates (1×10^4 /well, 5 wells in each group). The cells were cultured in an incubator at 37°C and 5% CO₂ for 12 h. The MTT (10 μ L, 5 mg/mL; Sigma-Aldrich, St. Louis, USA) was added into each well at 0 h, 24 h, 48 h, and 72 h respectively, and cultured for 4 h. After incubation, the culture media was discarded and dimethyl sulfoxide (DMSO; 200 μ L/well) was added to the wells. The plates were placed on a shaker for 5–10 min at room temperature in the dark. Absorbance values at 570 nm were measured with a Microplate Reader (Multiskan MK3; Thermo Fisher Scientific, Waltham, USA). The experiment was repeated 3 times.

Cell apoptosis detection by flow cytometry

Apoptosis analysis was performed using the Annexin V-FITC Analysis Kit (Shanghai Beyotime Biotechnology) according to the manufacturer's protocol. Cells were collected, digested with trypsin without EDTA and washed

3 times with ice-cold phosphate-buffered saline (PBS) and centrifugation. Cells (1×10^6) were collected and resuspended in 300 μ L of 1X binding buffer (Thermo Fisher Scientific). Annexin V-FITC (195 μ L) and PI-PE (5 μ L) were added to the cells according to the manufacturer's protocol. After 10 min of incubation at 4°C in the dark, pre-chilled 1X binding buffer (200 μ L) was added. Apoptosis was detected using flow cytometry. The results were analyzed using CELLQUEST software (BD Biosciences, Franklin Lakes, USA). The experiment was repeated 3 times.

Double luciferase reporter gene analysis

In order to examine the potential interaction between miR-124-3p and the 3'-UTR of TGF- β 1, we constructed wild-type (wt-pGL3-TGF- β 1) and mutant (mut-pGL3-TGF- β 1) luciferase reporter genes based on the predicted binding site. The constructs were co-transfected with miR-124-3p and Renilla luciferase into HEK293 cells. The cells were lysed using the Dual-Luciferase Reporter Assay System (Promega, Madison, USA) according to the instructions after culture for 48 h. The results were detected using Panomics Luminometer (Affymetrix, Santa Clara, USA) after the luminescence was added. The sea renin fluorescence was used as an internal reference.

RNA extraction and qRT-PCR

Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. RNA concentration and purity were determined using a NanoDrop™ 1000 spectrophotometer (Thermo Fisher Scientific). Total RNA (1 μ g) was subjected to reverse transcription using miScript II RT Kit (Qiagen, Hilden, Germany). Real-time polymerase chain reaction (RT-PCR) was performed using Taqman Universal Mix II No UNG (Thermo Fisher Scientific), PCR primers, miScript SYBR® Green PCR Kit (Qiagen), and the ABI StepOne Plus system (Applied Biosystems, Waltham, USA). At the end of each reaction, a melting curve analysis was performed to confirm the absence of primer dimers. The glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene was used as an internal control for normalization of RNA quantity and quality differences in the samples. Quantification of target gene expression was performed using the 2^{- $\Delta\Delta$ Ct} method. Primer sequences are listed in Table 1.

Table 1. Primers used

Primer name	Sequence (5'-3')
TGF- β 1 F	GGCCAGATCCTGTCCAAGC
TGF- β 1 R	GTGGGTTTCCACCATTAGCAC
GAPDH F	TGGGTGTGAACCACGAGAA
GAPDH R	GGCATGGACTGTGGTCATGA

Western blotting detection

Cells were harvested and lysed with Cell Lysis Solution (Sigma-Aldrich). The supernatant was collected after centrifugation at 4°C (10,000 rpm) for 5 min. Total proteins were extracted and protein concentration was determined using the bicinchoninic acid assay (BCA) assay. Proteins (50 µg per lane) were separated using 12% SDS-PAGE. Proteins were then electrotransferred to a polyvinylidene fluoride (PVDF) membrane (Amersham Biosciences, Piscataway, USA). The PVDF membrane was rinsed with TBS for 10–15 min and placed in TBS/T blocking buffer containing 5% (w/v) skimmed milk powder. Then, it was incubated at 4°C overnight following the addition of an appropriate dilution of primary antibodies (1:2000 α -smooth muscle actin (α -SMA); 1:1000 collagen I; 1:2000 survivin; 1:2000 Bcl-2; 1:2000 Bax; 1:5000 GAPDH; all from Abcam, Cambridge, UK). The membrane was then rinsed with TBS-Tween 20 (TBST) 3 times and incubated with a horseradish peroxidase (HRP)-labeled goat anti-mouse IgG secondary antibody (1:50,000; Abcam) at room temperature for 1 h. After incubation, the membrane was rinsed 3 times with TBST. Protein bands were detected using an enhanced chemiluminescence kit (Perkin-Elmer, Waltham, USA) and quantified as a ratio to GAPDH. Quantification was performed using Imagequant LAS4000 (GE Healthcare, Tokyo, Japan).

Statistical analysis

Statistical analysis was performed using SPSS v. 17.0 software (SPSS Inc., Chicago, USA). The data are expressed as mean \pm standard deviation (SD). The 2 groups of variables, which were comparable with F-test, were compared with unpaired Student's t-test. The mean values of multiple groups were compared using one-way analysis of variance (ANOVA). The correlation of the 2 genes was examined with Spearman correlation test. A p-value <0.05 was considered to be statistically significant.

Results

miR-124-3p expression was downregulated and negatively correlated with TGF- β 1 mRNA expression in HS

The RT-PCR results showed that the expression levels of miR-124-3p in HS tissues were significantly lower than that in NC tissue (Fig. 1A), while the expression levels of TGF- β 1 mRNA in HS tissues were significantly higher than in NC tissue (Fig. 1B), and there was a negative correlation between them (Fig. 1C). The miR-124-3p expression in HSFBs was significantly lower than in HSF2 cells (Fig. 1D), while TGF- β 1 mRNA expression in HSFBs was significantly higher than in HSF2 (Fig. 1E).

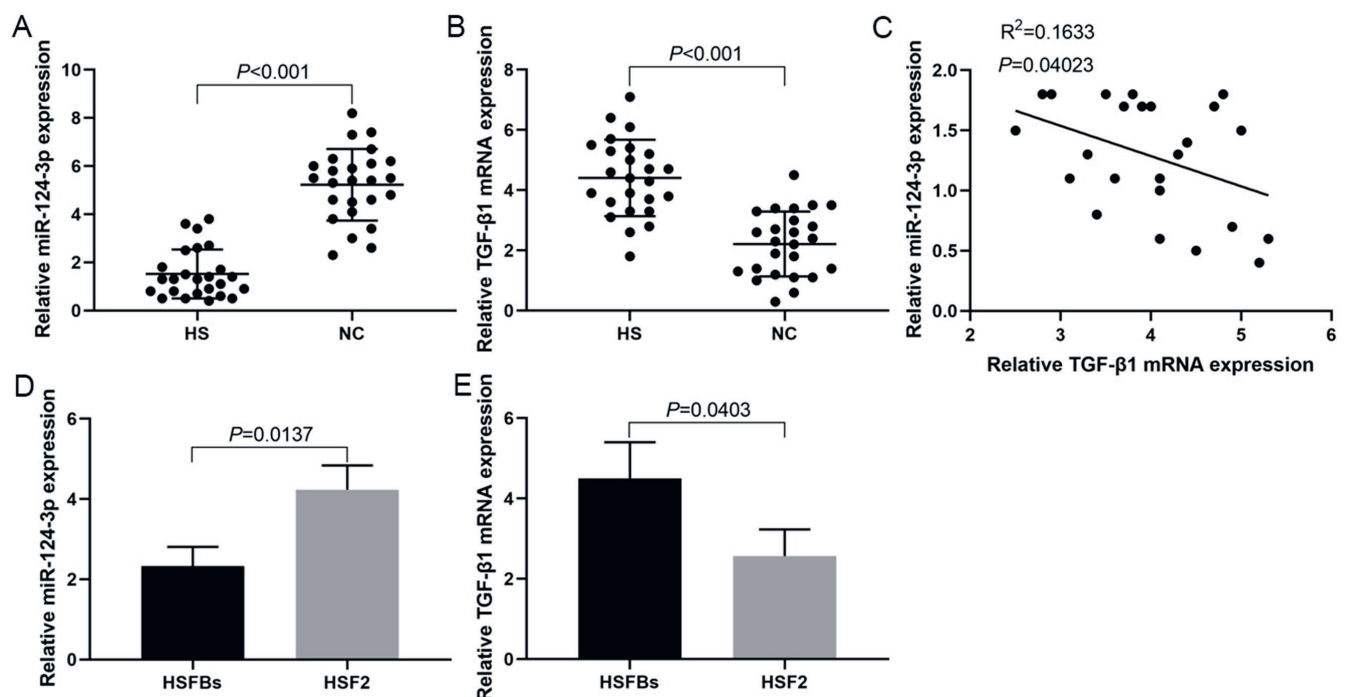


Fig. 1. miR-124-3p expression was downregulated, and is negatively correlated with TGF- β 1 expression in HS. A. miR-124-3p expression levels in HS tissues and NC tissues; B. TGF- β 1 expression in HS tissues and NC tissues; C. Correlation analysis of miR-124-3p and TGF- β 1 mRNA expression in HS tissue; D. miR-124-3p expression in HSF2 and HSFBs; E. TGF- β 1 mRNA expression in HSF2 and HSFBs.

miR-124-3p inhibits HSF2 cell proliferation

In HSF2s with low expression of miR-124-3p, DNA synthesis, cell proliferation and cell survival significantly decreased after being transfected with miR-124-3p

mimics (Fig. 2A,C,E). However, in HSF2 cells with high expression of miR-124-3p, DNA synthesis, cell proliferation and cell survival significantly increased after transfection transfected with a miR-124-3p inhibitor (Fig. 2B,D,F).

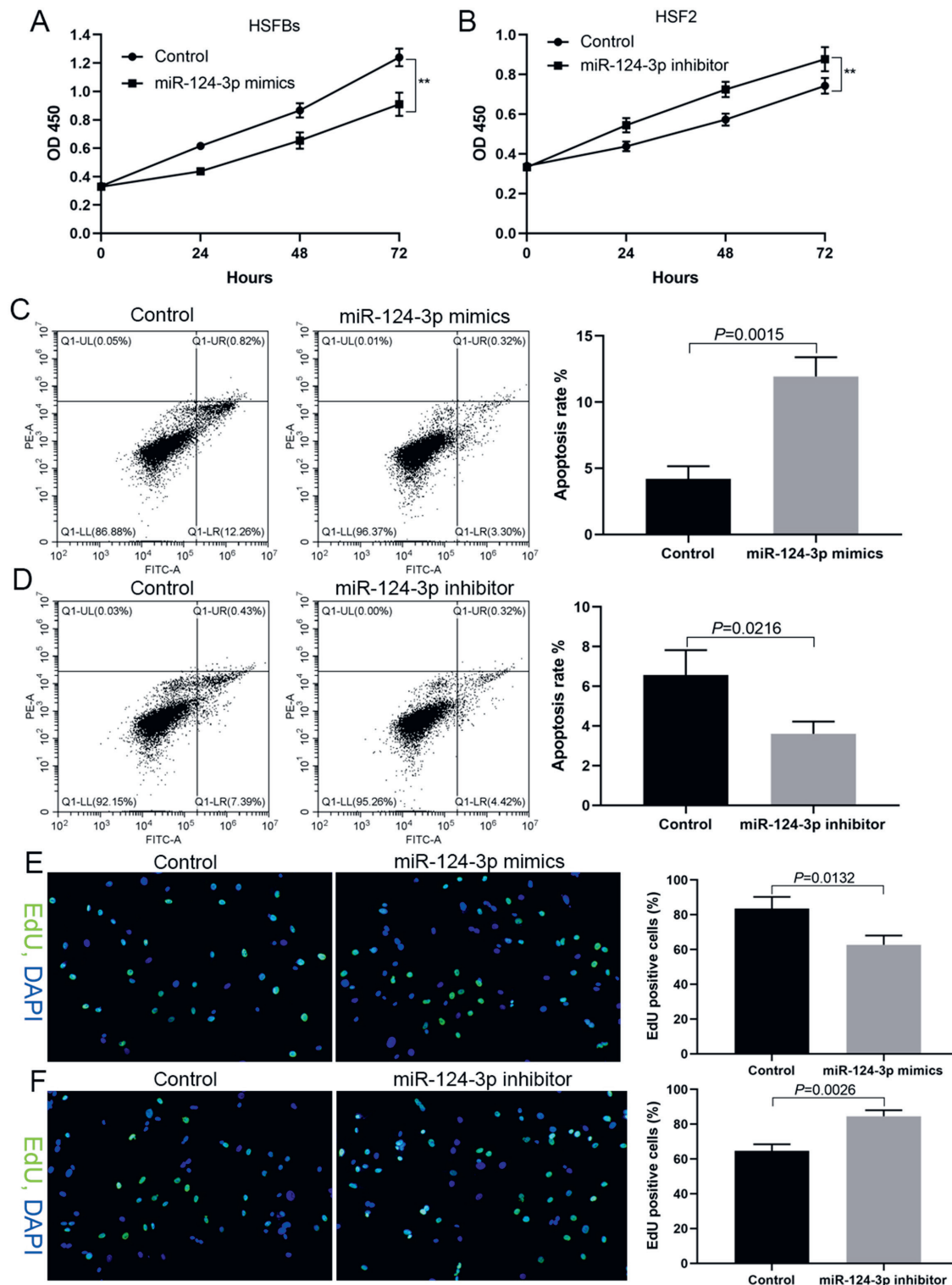


Fig. 2. miR-124-3p inhibits HSF2 proliferation. A. Evaluation of cell proliferation after miR-124-3p mimics were transfected into HSF2s cells using the CCK-8 assay; B. Cell proliferation after miR-124-3p inhibitor was transfected into HSF2 cells using the CCK-8 assay; C. Detection of apoptosis using flow cytometry after miR-124-3p mimics were transfected into HSF2s cells; D. Detection of apoptosis using flow cytometry after miR-124-3p inhibitor was transfected into HSF2 cells; E. Measurement of DNA synthesis after miR-124-3p mimics were transfected into HSF2s cells; F. Measurement of DNA synthesis after miR-124-3p inhibitor was transfected into HSF2 cells

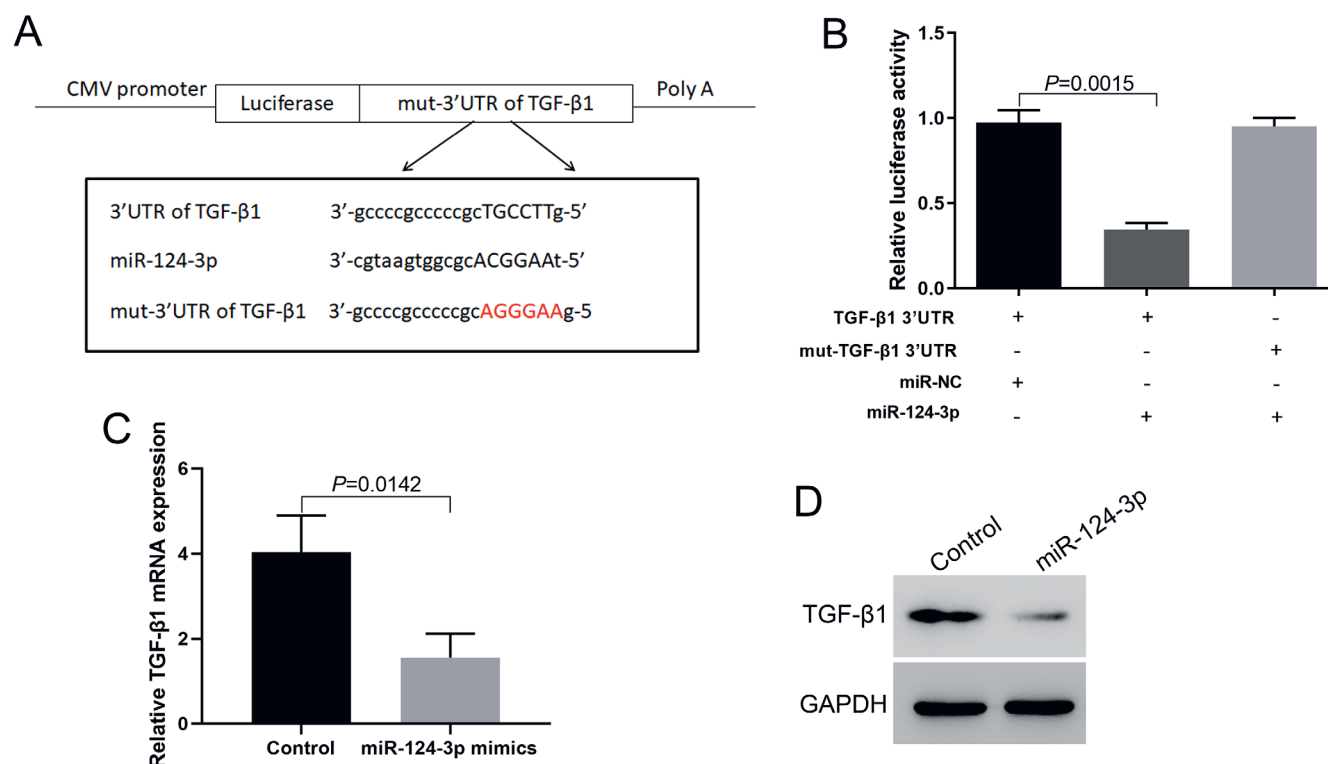


Fig. 3. miR-124-3p targets the 3'-UTR of TGF-β1 and inhibit TGF-β1 expression. A. Bioinformatics prediction shows that there is a binding site for miR-124-3p in the 3'-UTR of TGF-β1; B. Double-luciferase reporter gene analysis showed that miR-124-3p targets the 3'-UTR of TGF-β1 to inhibit the expression of luciferase; C. The effect of miR-124-3p on TGF-β1 mRNA expression was detected with RT-PCR; D. The effect of miR-124-3p on TGF-β1 protein expression was detected with western blot

miR-124-3p can target the 3'-UTR of TGF-β1 and inhibit TGF-β1 expression

Bioinformatics prediction showed that miR-124-3p can target the 3'-UTR of TGF-β1 (Fig. 3A). The double-luciferase reporter gene assay showed that miR-124-3p could target the 3'-UTR of TGF-β1 to inhibit the expression of luciferase. When the 3'-UTR of TGF-β1 was mutated, the inhibition of miR-124-3p was eliminated (Fig. 3B). RT-PCR and western blotting results showed that miR-124-3p can directly downregulate TGF-β1 expression (Fig. 3C,D).

miR-124-3p affected the proliferation of HSFs by regulating TGF-β1 expression

In HSFs transfected with miR-124-3p mimics or shRNA against TGF-β1, cell proliferation was inhibited, while in HSFs co-transfected with miR-124-3p mimics and TGF-β1, cell proliferation was restored (Fig. 4A,C,E). In HSF2 transfected with miR-124-3p inhibitor or TGF-β1, cell proliferation was promoted, while in HSF2 co-transfected with miR-124-3p inhibitor and TGF-β1 shRNA, cell proliferation was inhibited (Fig. 4B,D,F).

miR-124-3p affects the expression of downstream effectors related to HS formation

The expression of α-SMA, collagen I, survivin, and Bcl-2 was inhibited, and Bax expression increased after transfection of miR-124-3p mimics into HSFs (Fig. 5A). However, the expression of α-SMA, collagen I, survivin, and Bcl-2 was promoted, and Bax expression was inhibited with a miR-124-3p inhibitor (Fig. 5B).

Discussion

miRNA is a type of endogenous, non-coding, small molecule RNA that has generally 19–22 nucleotides in length and is involved in the regulation of gene expression at the post-transcriptional level. It has been shown that miRNA can regulate the development of HS in many ways, including through the TGF-β/Smad signaling pathway,²³ ECM synthesis and degradation,²⁴ proliferation and differentiation of HSFs, and epithelial–mesenchymal transition (EMT).²⁵ miR-140-5p, miR-23b, miR-let-7b, and miR-153 can all regulate the TGF-β/Smad signaling pathway including TGF-β type I and type II receptors (RI and RII, respectively): miR-140-5p can negatively regulate the expression of TGF-βRII, while

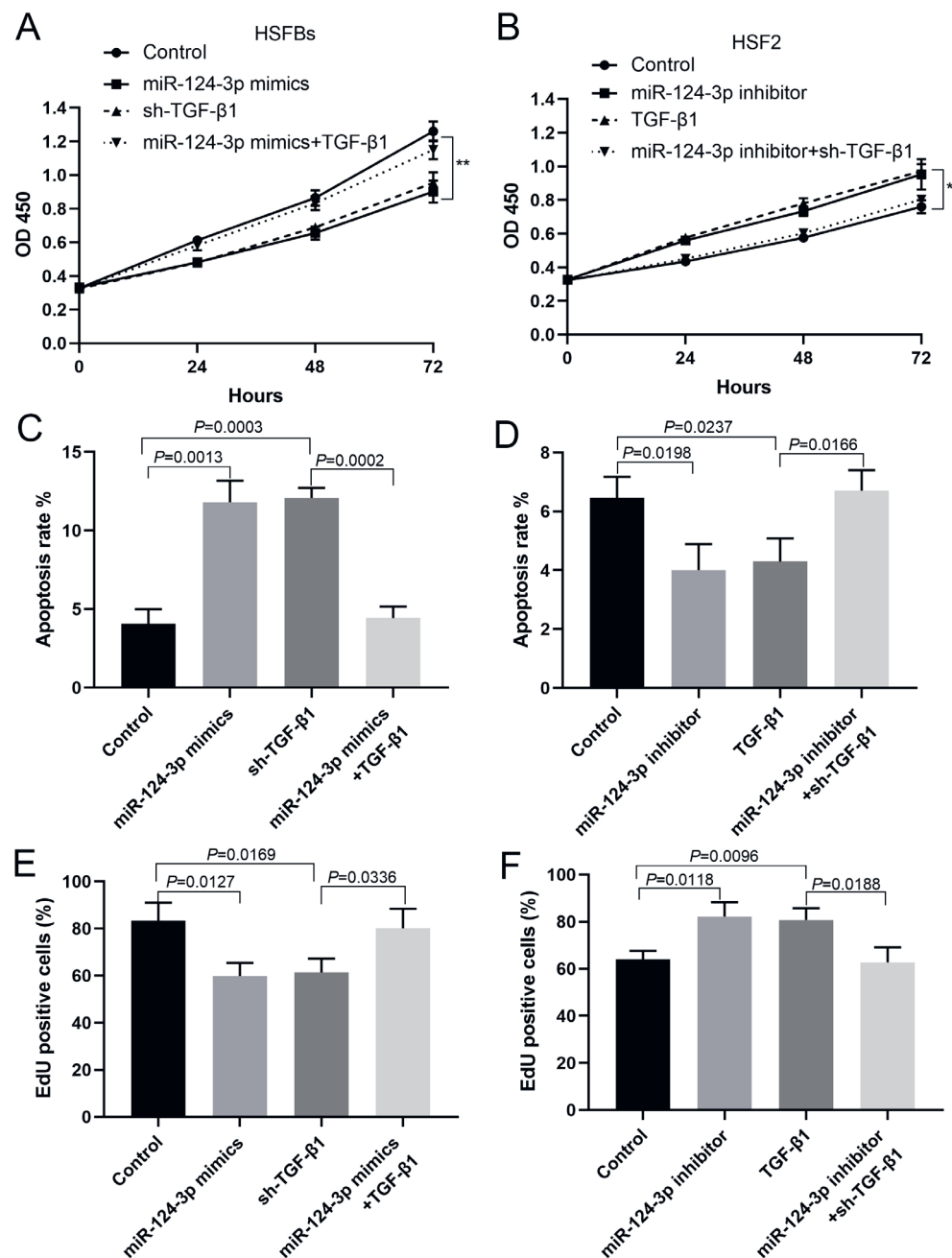


Fig. 4. miR-124-3p affects the proliferation of HSFs by regulating TGF-β1 expression. A. Detection of HSFs proliferation with CCK-8 assay; B. Detection of HSF2 proliferation using CCK-8 assay; C. Detection of HSFs apoptosis using flow cytometry; D. Detection of HSF2 apoptosis using flow cytometry. E. Detection of apoptosis in HSFs using the EdU assay; F. Detection of apoptosis in HSF2 cells using the EdU assay

miR-23b and miR-let-7b can upregulate TGF-βRII expression. miR-17-5p and miR-20 are also closely related to HS through their regulation of TGF-βRII expression.²⁶ miR-21 and miR-503 can also act on Smad7, a negative regulator of TGF-β/Smad signaling.²⁶ miR-29b can also act on the 3'-UTR of collagen I and inhibit its expression directly.²⁷ miR-10a and miR-181 can target PAI-1 and UPA to regulate the expression of collagen I in HS.²⁸ In HSFs, the expression of miR-196a is significantly decreased, resulting in increased expression of collagen I and III and promotion of scar formation.²⁹ miR-146a inhibits the differentiation of HSFs by acting on Smad4.³⁰ The miR-200 family can affect EMT in HSFs by regulating the expression of ZEB1 and ZEB2.³¹

The miR-124 family of miRNAs is highly expressed in differentiated and mature neurons. They were first found in large numbers in the mouse brain, accounting for 5–48% of total miRNAs in the organ.³² It was found that miR-124-3p belonged to the miR-124 family, members of which not only participate in brain development and nerve function, but also as tumor suppressors in some cancers. For example, miR-124-3p can target BCL2L1 to inhibit cell proliferation in angiomyolipoma cells,³³ and in gastric cancer, miR-124-3p can target Rac1 and SP1 to inhibit cell proliferation and could be used as an independent indicator of survival and treatment strategies.²⁰ In hepatocellular carcinoma, the long non-coding RNA (lncRNA) lncRNA MALAT1 can act as a molecular sponge

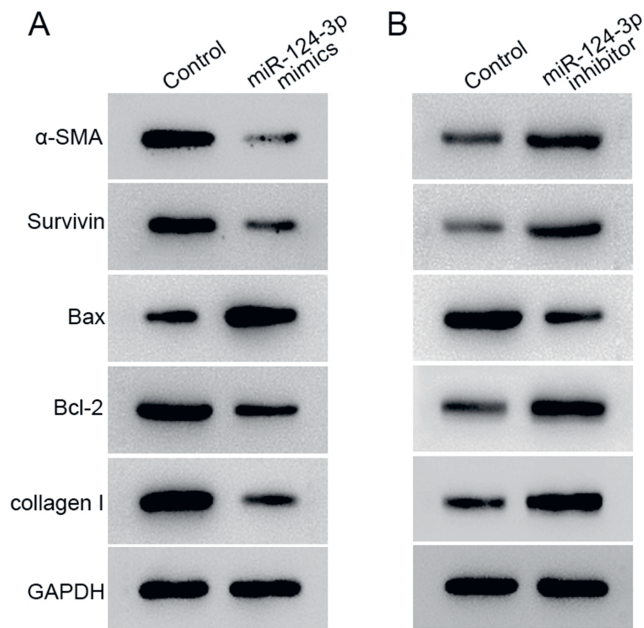


Fig. 5. miR-124-3p can affect the expression of downstream effectors associated with HS formation. A. The expression of α -SMA, collagen I, survivin, Bcl-2, and Bax after transfection of miR-124-3p mimics into HSFs; B. The expression of α -SMA, collagen I, survivin, Bcl-2, and Bax after transfection of miR-124-3p mimics into HSF2

to absorb miR-124-3p to inhibit its effects and upregulate expression of the EMT marker Slug to promote tumor metastasis.³⁴ Aberrant cell proliferation similar to tumors is observed in HS. Whether miR-124-3p is involved in regulating HSF cell proliferation, has not yet been reported.

In this study, we found that expression levels of miR-124-3p in HS tissue was significantly lower than that in corresponding normal skin tissue, and miR-124-3p in HSFs was also significantly lower than that in HSF2 cells. miR-124-3p targeted the 3'-UTR of TGF- β 1 to inhibit its expression. The expression of TGF- β 1 in HS tissue was significantly higher than that in corresponding normal skin tissue, and the expression levels of miR-124-3p was negatively correlated with TGF- β 1 expression.

The TGF- β 1 has a variety of biological functions. After skin injury, high amounts of TGF- β 1 are released to increase collagen and ECM deposition.³⁵ The TGF- β 1 can induce collagen production and contraction of fibroblasts in HS. It can also upregulate the expression of α -SMA and promote the differentiation of normal fibroblasts into myofibroblasts.³⁶ In a study by Shah et al., it was found that neutralization of TGF- β 1 could reduce scar formation in rats, which provided direct evidence of a role for TGF- β 1 in the formation of scars.³⁷ Loiselle et al. found that regulation of the TGF- β signaling pathway prevents scar formation during flexor tendon repair.³⁸ Fibroblasts are the main participants in wound repair, being involved in the formation of granulation tissue, collagen synthesis and interaction with the ECM to promote scar hyperplasia. In the process of HS formation,

the proliferation of fibroblasts promotes the secretion of collagen, resulting in a large amount of collagen deposition. At the same time, fibroblasts also release a lot of growth factors to promote scar hyperplasia. Therefore, the uncontrolled proliferation of fibroblasts is the basis of scar formation.

Limitations

The main limitation of this study is the small sample size. More clinical samples need to be analyzed to clarify the clinical significance of miR-124-3p in hypertrophic scarring.

Conclusions

In this study, we found that transfection of miR-124-3p mimics in HSFs inhibit the expression of TGF- β 1, α -SMA, collagen I, survivin, and Bcl-2, while promoting Bax expression. However, inhibition of miR-124-3p in normal human skin fibroblast cells (HSF2) promotes the expression of TGF- β 1, α -SMA, collagen I, survivin, and Bcl-2, and inhibits Bax expression. These results suggested that in HS, miR-124-3p may target TGF- β 1 to inhibit the expression of anti-apoptotic proteins such as survivin and Bcl-2, and promote the expression of the pro-apoptotic protein Bax. The transformation of fibroblasts into myofibroblasts is one of the important mechanisms of scar formation. There are a large number of myofibroblasts in HS tissue; α -SMA is a myofibroblast marker.³⁹ Myofibroblasts increase the deposition of ECM. The number and function of myofibroblasts determine the speed and degree of scar formation. Inhibition of fibroblast to myofibroblast transformation is an important strategy in preventing scar tissue development. This study showed that miR-124-3p inhibits the expression of α -SMA and collagen I, suggesting that miR-124-3p plays an important role in TGF- β 1-induced differentiation of HS fibroblasts. In summary, we found that miR-124-3p inhibited the proliferation of HSFs by inhibiting TGF- β 1 expression, which suggests that miR-124-3p may be a potential therapeutic target in HS.

ORCID iDs

Suwen Zhang <https://orcid.org/0000-0002-1862-436X>
Simeng Pan <https://orcid.org/0000-0003-2717-5049>

References

- Zhang J, Li Y, Bai X, Li Y, Shi J, Hu D. Recent advances in hypertrophic scar. *Histol Histopathol*. 2018;33(1):27–39. doi:10.14670/HH-11-908
- Hinz B. The role of myofibroblasts in wound healing. *Curr Res Transl Med*. 2016;64(4):171–177. doi:10.1016/j.retram.2016.09.003
- Lee HJ, Jang YJ. Recent understandings of biology, prophylaxis and treatment strategies for hypertrophic scars and keloids. *Int J Mol Sci*. 2018;19(3):711. doi:10.3390/ijms19030711
- Ishise H, Larson B, Hirata Y, et al. Hypertrophic scar contracture is mediated by the TRPC3 mechanical force transducer via NF κ B activation. *Sci Rep*. 2015;5(1):11620. doi:10.1038/srep11620

5. Trisliana Perdanasari A, Torresetti M, Grasseti L, et al. Intralesional injection treatment of hypertrophic scars and keloids: A systematic review regarding outcomes. *Burns Trauma*. 2015;3:14. doi:10.1186/s41038-015-0015-7
6. Kwan PO, Tredget EE. Biological principles of scar and contracture. *Hand Clin*. 2017;33(2):277–292. doi:10.1016/j.hcl.2016.12.004
7. Wei G, Xu Q, Liu L, et al. LY2109761 reduces TGF-beta1-induced collagen production and contraction in hypertrophic scar fibroblasts. *Arch Dermatol Res*. 2018;310(8):615–623. doi:10.1007/s00403-018-1849-1
8. Nong X, Rajbanshi G, Chen L, et al. Effect of artesunate and relation with TGF-beta1 and SMAD3 signaling on experimental hypertrophic scar model in rabbit ear. *Arch Dermatol Res*. 2019;311(10):761–772. doi:10.1007/s00403-019-01960-7
9. Yang JH, Yoon JY, Moon J, Min S, Kwon HH, Suh DH. Expression of inflammatory and fibrogenic markers in acne hypertrophic scar formation: Focusing on role of TGF-beta and IGF-1R. *Arch Dermatol Res*. 2018;310(8):665–673. doi:10.1007/s00403-018-1856-2
10. Sun Q, Guo S, Wang CC, et al. Cross-talk between TGF-beta/Smad pathway and Wnt/beta-catenin pathway in pathological scar formation. *Int J Clin Exp Pathol*. 2015;8(6):7631–7639. PMID:26261683
11. Zhang YF, Zhou SZ, Cheng XY, et al. Baicalein attenuates hypertrophic scar formation via inhibition of the transforming growth factor-beta/Smad2/3 signaling pathway. *Br J Dermatol*. 2016;174(1):120–130. doi:10.1111/bjd.14108
12. Fang X, Hu X, Zheng Z, et al. Smad interacting protein 1 influences transforming growth factor-beta1/Smad signaling in extracellular matrix protein production and hypertrophic scar formation. *J Mol Histol*. 2019;50(6):503–514. doi:10.1007/s10735-019-09844-w
13. Eming SA, Wynn TA, Martin P. Inflammation and metabolism in tissue repair and regeneration. *Science*. 2017;356(6342):1026–1030. doi:10.1126/science.aam7928
14. Chen J, Zhou R, Liang Y, Fu X, Wang D, Wang C. Blockade of lncRNA-ASLNC55088-enriched exosome generation in M2 macrophages by GW4869 dampens the effect of M2 macrophages on orchestrating fibroblast activation. *FASEB J*. 2019;33(11):12200–12212. doi:10.1096/fj.201901610
15. Sun X, Phan TN, Jung SH, et al. LCB 03-0110, a novel pan-discoidin domain receptor/c-Src family tyrosine kinase inhibitor, suppresses scar formation by inhibiting fibroblast and macrophage activation. *J Pharmacol Exp Ther*. 2012;340(3):510–519. doi:10.1124/jpet.111.187328
16. Nejad C, Stunden HJ, Gantier MP. A guide to miRNAs in inflammation and innate immune responses. *FEBS J*. 2018;285(20):3695–3716. doi:10.1111/febs.14482
17. Liu B, Shyr Y, Cai J, Liu Q. Interplay between miRNAs and host genes and their role in cancer. *Brief Funct Genomics*. 2018;18(4):255–266. doi:10.1093/bfpg/ely002
18. O'Reilly S. MicroRNAs in fibrosis: Opportunities and challenges. *Arthritis Res Ther*. 2016;18:11. doi:10.1186/s13075-016-0929-x
19. Long HD, Ma YS, Yang HQ, et al. Reduced hsa-miR-124-3p levels are associated with the poor survival of patients with hepatocellular carcinoma. *Mol Biol Rep*. 2018;45(6):2615–2623. doi:10.1007/s11033-018-4431-1
20. Liu F, Hu H, Zhao J, et al. miR-124-3p acts as a potential marker and suppresses tumor growth in gastric cancer. *Biomed Rep*. 2018;9(2):147–155. doi:10.3892/br.2018.1113
21. Salazar-Ruales C, Arguello JV, Lopez-Cortes A, et al. Salivary MicroRNAs for early detection of head and neck squamous cell carcinoma: A case-control study in the high altitude Mestizo Ecuadorian population. *Biomed Res Int*. 2018;2018:9792730. doi:10.1155/2018/9792730
22. Kashiwama K, Mitsutake N, Matsuse M, et al. miR-196a downregulation increases the expression of type I and III collagens in keloid fibroblasts. *J Invest Dermatol*. 2012;132(6):1597–1604. <https://doi.org/10.1038/jid.2012.22>
23. Guo J, Lin Q, Shao Y, Rong L, Zhang D. miR-29b promotes skin wound healing and reduces excessive scar formation by inhibition of the TGF-beta1/Smad/CTGF signaling pathway. *Can J Physiol Pharmacol*. 2017;95(4):437–442. doi:10.1139/cjpp-2016-0248
24. Bi S, Cao C, Chai LL, Li SR, Yang DY. Regulatory mechanism of miR-29 over TGF-beta1 and COL1 in scar cells. *Eur Rev Med Pharmacol Sci*. 2017;21(10):2512–2517. PMID:28617563
25. Guo B, Hui Q, Xu Z, Chang P, Tao K. miR-495 inhibits the growth of fibroblasts in hypertrophic scars. *Aging (Albany NY)*. 2019;11(9):2898–2910. doi:10.18632/aging.101965
26. Li H, Yang R, Fan X, et al. MicroRNA array analysis of microRNAs related to systemic scleroderma. *Rheumatol Int*. 2012;32(2):307–313. doi:10.1007/s00296-010-1615-y
27. Tsitoura E, Wells AU, Karagiannis K, et al. MiR-185/AKT and miR-29a/collagen 1a pathways are activated in IPF BAL cells. *Oncotarget*. 2016;7(46):74569–74581. doi:10.18632/oncotarget.12740
28. Li C, Zhu HY, Bai WD, et al. MiR-10a and miR-181c regulate collagen type I generation in hypertrophic scars by targeting PAI-1 and uPA. *FEBS Lett*. 2015;589(3):380–389. doi:10.1016/j.febslet.2014.12.024
29. Lu Q, Guo Z, Xie W, et al. The lncRNA H19 mediates pulmonary fibrosis by regulating the miR-196a/COL1A1 axis. *Inflammation*. 2018;41:896–903. doi:10.1016/j.febslet.2014.12.024
30. Qiu M, Li T, Wang B, Gong H, Huang T. miR-146a-5p Regulated cell proliferation and apoptosis by targeting SMAD3 and SMAD4. *Protein Pept Lett*. 2020;27(5):411–418. doi:10.2174/0929866526666190911142926
31. Xiong M, Jiang L, Zhou Y, et al. The miR-200 family regulates TGF-beta1-induced renal tubular epithelial to mesenchymal transition through Smad pathway by targeting ZEB1 and ZEB2 expression. *Am J Physiol Renal Physiol*. 2012;302(3):F369–F379. doi:10.1152/ajprenal.00268.2011
32. Krichevsky AM, Sonntag KC, Isacson O, Kosik KS. Specific microRNAs modulate embryonic stem cell-derived neurogenesis. *Stem Cells*. 2006;24(4):857–864. doi:10.1634/stemcells.2005-0441
33. Cai Y, Wang W, Guo H, Li H, Xiao Y, Zhang Y. Correction: miR-9-5p, miR-124-3p, and miR-132-3p regulate BCL2L1 in tuberous sclerosis complex angiomyolipoma. *Lab Invest*. 2019;99(3):443–444. doi:10.1038/s41374-018-0131-7
34. Cui RJ, Fan JL, Lin YC, et al. miR-124-3p availability is antagonized by lncRNA-MALAT1 for Slug-induced tumor metastasis in hepatocellular carcinoma. *Cancer Med*. 2019;8(14):6358–6369. doi:10.1002/cam4.2482
35. Pan SC, Lee CH, Chen CL, Fang WY, Wu LW. Angiogenin attenuates scar formation in burn patients by reducing fibroblast proliferation and transforming growth factor beta1 secretion. *Ann Plast Surg*. 2018;80(2S Suppl 1):S79–S83. doi:10.1097/SAP.0000000000001306
36. Goldberg MT, Han YP, Yan C, Shaw MC, Garner WL. TNF-alpha suppresses alpha-smooth muscle actin expression in human dermal fibroblasts: An implication for abnormal wound healing. *J Invest Dermatol*. 2007;127(11):2645–2655. doi:10.1038/sj.jid.5700890
37. Shah M, Foreman DM, Ferguson MW. Neutralisation of TGF-beta 1 and TGF-beta 2 or exogenous addition of TGF-beta 3 to cutaneous rat wounds reduces scarring. *J Cell Sci*. 1995;108(Pt 3):985–1002. PMID:7542672
38. Loiselle AE, Yukata K, Geary MB, et al. Development of antisense oligonucleotide (ASO) technology against Tgf-beta signaling to prevent scarring during flexor tendon repair. *J Orthop Res*. 2015;33(6):859–866. doi:10.1002/jor.22890
39. Muchaneta-Kubara EC, el Nahas AM. Myofibroblast phenotypes expression in experimental renal scarring. *Nephrol Dial Transplant*. 1997;12(5):904–915. doi:10.1093/ndt/12.5.904