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ORIGINAL RESEARCH

Inhibition of microRNA-139-5p Improves Fibroblasts Viability and Enhances Wound Repair in Diabetic Rats Through AP-1 (c-Fos/c-Jun)

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Introductions: Diabetic foot ulcers (DFU) are notoriously difficult to heal, however, its underlying molecular mechanisms are unknown. MicroRNA-139-5p participates in various biological processes, including cancer and vascular endothelial injury, while its role in diabetic wound healing has not been reported.

Methods: Sprague-Dawley (SD) rats were intraperitoneally injected with streptozotocin and a 1.0 cm full-layer dorsal skin wound was made to establish a diabetic wound model. On days 1, 4, 7, and 10 after the wound was made, a solution containing microRNA-139-5p antagomir or control was injected along the dorsal edge of the wound. Wound healing was analyzed using Image J, histological analysis and molecular analysis. Skin tissues from 4 diabetic and 4 matched non-diabetic ulcer patients were obtained to detect microRNA-139-5p expression. In vitro, human skin fibroblasts were transfected with microRNA-139-5p inhibitors/mimics, the function of the fibroblasts was evaluated by CCK-8 assay and scratch assay, and AP-1 (c-Fos/c-Jun) was detected.

Results: Obviously elevated microRNA-139-5p expression was detected in the wound tissue of the rats with diabetes and patients with DFUs, and the microRNA-139-5p antagonist-treated diabetic wounds had faster healing rates. The pace of diabetic wound re-epithelialization and angiogenesis was accelerated, and the expression of AP-1 family members (c-Fos/c-Jun), and VEGF, PDGF was upregulated in the wound tissue of diabetic rats treated with topical microRNA-139-5p antagomir. In vitro, the expression of microRNA-139-5p was up-regulated in human skin fibroblasts induced by high glucose treatment, while the function of the cell proliferation and migration was promoted and the level of AP-1 (c-Fos/c-Jun) was increased after transfected with the microRNA-139-5p inhibitor, and vice versa. Our study further verified that microRNA-139-5p regulated the migration of human skin fibroblasts by modulating c-Fos.

Conclusion: Inhibiting microRNA-139-5p improves fibroblasts viability and promotes diabetic wound healing, suggesting that this may be a therapeutic strategy for diabetic foot ulcer.

Keywords: miR-139-5p, diabetes, wound healing, AP-1, fibroblasts

Introduction

Diabetic foot ulcer (DFU), a paradigmatic example of chronic non-healing wounds, constitutes one of the most severe and debilitating complications of diabetes.[1] About 25% of individuals with diabetes will experience foot ulceration at some point during their lifetime, with a staggering rate of reoccurrence (40% in 1 year and 65% in 5 years),[2–4] and limited options to improve DFU healing.[5] Persistent hyperglycemia,[6] chronic inflammation,[7] peripheral neuropathy, impaired angiogenesis[8] and decreased fibroblasts activity,[9] in diabetes patients are factors influencing the wound healing

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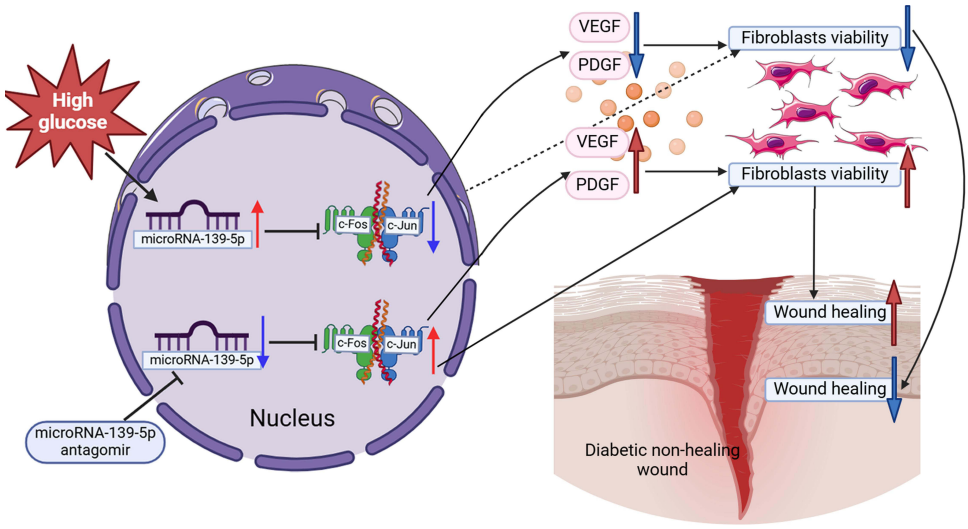
Graphical Abstract



process.[10] However, since the pathophysiology of diabetic wounds is complex, the molecular mechanism underlying the wound-healing process in diabetes remains largely obscure.

MicroRNAs (miRNAs) have been shown to directly bind to target mRNA to regulate post-transcriptional gene expression.[11] Many researchers have corroborated the involvement of miRNAs in the pathogenesis of wound healing processes. For instance, miR-155 inhibition could accelerate diabetic wound healing by decreasing inflammatory cells accumulation and enhancing vascularization and collagen formation.[12] The downregulation of the miR-99 family boosted the proliferation and migration of keratinocytes, promoting wound closure.[13] Recently, microRNA-139-5p (miR-139-5p) was considered as a potential genomic driver of diabetic pathogenesis in bone marrow-derived Lin-/VEGF-R2+ endothelial progenitor cells, as evidenced by microarray experiment.[14] Our previous research found that miR-139-5p upregulation is related to vascular endothelium dysfunction in diabetes, and downregulation of miR-139-5p promotes Endothelial Colony Forming Cells (ECFCs)-mediated angiogenesis.[15] In the Gene Ontology analysis of human retinal microvascular endothelial cells induced by VEGF mediated angiogenesis, miR-139-5p has also been linked to angiogenesis and cell migration.[16] Moreover, the dysregulated expression of miR-139-5p is frequently demonstrated in diverse cancers,[17,18] and diseases, including neurodegeneration[19] and obesity.[20] Overall, miR-139-5p could serve as a significant biological marker for human diseases, including diabetes. Therefore, we tried to determine whether miR-139-5p was associated with poor wound healing in diabetes.

The transcription factor complex, activating protein-1 (AP-1), is a transcription factor complex composing c-Jun and c-Fos proteins and has been identified as a target gene subject to modulation by miR-139-5p.[15,21] Research has indicated that AP-1 exerts a pivotal regulatory influence on the transcription of a multitude of genes during the wound healing process.[22,23] Fibroblasts are essential cells involved in the process of wound repair. The dysfunction of fibroblasts during wound healing can exert a profound impact on multiple aspects, encompassing angiogenic progression, immune response, and extracellular matrix deposition, thereby hindering the normal transition from the inflammatory phase to the proliferative phase. This dysfunction is a significant factor in the development of refractory wounds, especially in the context of diabetic foot ulcers.[24–26] However, it remains unclear whether the inhibition of miR-139-5p can activate human skin fibroblasts through AP-1 and promote wound healing.

This paper confirmed that miR-139-5p was highly expressed in the wound tissue of diabetic patients with foot ulcers and diabetic rats. We further explored the roles of miR-139-5p/AP-1 in regulating fibroblast function and the affection of miR-139-5p antagomir on wound healing in diabetic rats. These findings may provide a new way to promote diabetic wound healing.

Materials and Methods

Animals Model for Diabetic Wound Healing

A total of 24 Sprague-Dawley (SD) rats (8–10 weeks, 200–300g) were purchased from Hunan SJA Laboratory Animal Co. Ltd. located in Changsha, China. At the Department of Laboratory Animals of Central South University (CSU), water and food were sourced from a pathogen-free facility. The rats were then housed in an environment with a temperature range of 22–24°C, and maintained in accordance with the Guide for Care and Use of Laboratory Animals and the Animal Welfare Act. Experiments were performed with the approval of the Institutional Animal Care Committee of Central South University. (Ethical Approval Number: CSU-2024-0155). After the adaptive feeding period (a two-week period), the rats were subjected to intraperitoneal injection of 40 mg/kg streptozotocin (STZ) (from Sigma, USA) (100 mm citrate-buffered saline solution, pH 4.5). The induction of diabetes was confirmed through weekly blood glucose measurements exceeding 16.7 mmol/L and the presence of polyuria and polydipsia symptoms. Six months later, diabetic animals were used for wound-healing experiments.

The dorsal hair of diabetic and normal rats is shaved off with a razor and hair removal cream, and rats are anaesthetized with isoflurane for gas. A 1.0 cm full-thickness dorsal skin wound is created using a biopsy punch. Then, the rats wound, which are randomized into three groups (n=8 each group) with different interventions.

MiR-139-5p Antagomir Administration at the Wound Site

SD rats were randomly assigned into 3 groups, including a normal control group (NC), a model control group (DM), and a miR-139-5p antagomir group (DM+miR-139-5p antagomir). On the second day after the wound was induced, each wound was subjected to a subcutaneous injection of a total of 200 μL of a solution at four different sites along the dorsal wound edge. This injection was administered on days 1, 4, 7, and 10 to ensure consistent treatment. To silence the expression of miR-139-5p, a chemically synthesized antagonist called miR-139-5p antagomir (RIBOBIO, Guangzhou, China), at a concentration of 20μg/100μL was used. Additionally, the negative control of miR-139-5p (called mir-NC) was injected subcutaneously, which is a random miRNA sequence and has been confirmed to have no recognizable effect on miR-139-5p. For the normal control group wounds, an equal amount of an injection containing saline was administered.

Estimation of Wound Closure Rate

The wound was photographed immediately after the surgery was performed. To document the progress of wound healing, high-resolution images were captured using a Canon G9 camera every two days after wound establishment. These images were then analyzed using Image J version 1.46 (American NIH Image). The wound healing rate was calculated using the formula: wound healing rate (%) = (Day 0 wound area - Day N wound area) / Day 0 wound area × 100%.

Histologic and Immunofluorescence Analysis

On the 8th day of wound treatment, wounds and surrounding healthy tissues from the experimental and control groups were collected immediately after euthanizing the rats with isoflurane in an airtight chamber. Specimens riveted with 4% paraformaldehyde (Sigma Aldrich, St. Louts, USA), and formed paraffin-embedded wound tissue. Sectioned at 5 μm thickness the tissue samples were stained using hematoxylin and eosin. This staining technique allowed for the visualization of the tissue structure under an inverted microscope. The healing process of the skin tissue, known as epithelization, was measured and documented.

Sections from the central region of the wound were prepared for immunofluorescence analysis. The sections underwent deparaffinized, rehydration, and twice PBS (Gibco, USA) washes, followed by a 15-minute incubation with H_2O_2. After washing twice again with PBST (Gibco, USA), the slices were incubated overnight at 4°C with primary antibodies against CD31, which is a marker for endothelial cells. Subsequently, the specimens were applied HRP-polymer goat anti-rabbit secondary antibodies after washes and followed by counterstaining with DAPI. All antibodies were obtained from Proteintech (Wuhan, China). Finally, 3 fields of view were randomly taken from each group of sections under 200x microscope. The number of positive staining in each field was counted, and the results were expressed as number/mm^2.

Cell Culture, Treatment, and Transfection

Human skin fibroblasts (from Central Laboratory of the Third Xiangya Hospital, Changsha, China) were used for the experiments. These fibroblasts were cultured in a high-glucose DMEM medium with 10% fetal bovine serum (Gibco, USA) incubating at 37 °C, with 5% CO_2 and 95% humidity. To detach the cells, 0.25% trypsin digestion was performed. 6-well plates were seeded 1×10^5 cells/well and incubated for 24 hours. After the initial incubation, the cells were exposed to either high glucose (33.0 mM) or a high osmotic control group, which supplemented with 27.5 mM mannitol while adding 5.5 mM glucose, for a duration of 72 hours. The next step involved transfection of the human skin fibroblasts. Human skin fibroblasts were cultivated in 6-well plates (3×10^4 cells/well) until achieving 30–50% confluence. Then, these cells were transfected using either miR-139-5p mimic or inhibitor (RIBOBIO, Guangzhou, China), as well as their respective negative controls, all at a concentration of 50 nM. The transfection was performed using Lipofectamine 3000 from Invitrogen (Invitrogen, Thermo Fisher Scientific, USA). Forty-eight hours after the transfection, TRIzol (Invitrogen, USA) was added to the cells for RNA extraction. The extracted RNA was then used for qRT-PCR detection. At 72 hours after transfection, protein lysate was collected to extract the protein and perform Western blot analysis.

Cell Counting Kit-8 (CCK-8) Test

Following the above transfection methods, miR-139-5p mimics or inhibitors (RIBOBIO, Guangzhou, China) were introduced into human skin fibroblasts after 48 hours. To carry out this process, the intervened cells were plated onto 96-well plates with a proper density of 3000 cells/well and allowed to adhere for 24 hours. Next, 10μL CCK-8 reagent (Dojindo, Japan) was added to serum-free medium and followed by a 1–4 hour incubation period, and the absorbance value will be set to 450nm.

Cell Migration Test

Human skin fibroblasts were cultivated and then transfected with either a 50 nM miR-139-5p inhibitor or miR-139-5p mimic for 48h. Once the cell layers had reached confluence, a sterile monolayer was created and a wound was made by scratching the surface using a 10-μL pipette. The degree of cell migration was examined under an inverted microscope, and photographs of the cell migration were taken at 0 and 24 hours. Calculating the migration area was conducted by Image J.

Tissue Collection and RNA Extraction

In order to establish a wound model, skin wound tissue was obtained from diabetes rats and normal rats and stored in −80°C refrigerator. Foot ulcer tissues from four diabetic patients (n=4; age range: 36–73 years; 3 males, 1 female; HbA1c levels: 7.1% −10.4%) and tissues of damaged skin wounds from four patients without diabetes (n=4; age range: 21–60 years; all males) were collected. All procedures of this work were performed in compliance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and has been approved by Ethics Committee of the Third Xiangya Hospital. (Ethical Approval Number: fast-24461) Informed consent was obtained for experimentation with human subjects.

On the 8th day after the wound intervention, the wound tissue was obtained and divided into three parts to facilitate subsequent analysis. One part of the tissue (50 to 100mg) was homogenized using a polytron homogenizer and 1 mL TRIzol (Sigma Aldrich, USA). Afterwards, the RNA in the homogenized tissue was purified in accordance with the instructions provided by the manufacturer. The extracted RNA pellets would be dissolved in 20 μL of DEPC water (phygene, Wuhan, China). Then the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA), which was employed to determine RNA concentration and purity. The extracted RNA specimens were kept at −80°C for further tests.

Western Blot Analysis

The wound tissues of both the experimental and control groups were lysed with RIPA lysis buffer (Sigma Aldrich, St. Louts, USA), including PMSF (Sigma Aldrich, St. Louts, USA). The lysate was centrifuged at 4°C for 10 min at 12000 g, and the resulting supernatant was then boiled for 10 min. The tissue protein was transferred to the PVDF membrane (Millipore, USA) after being subjected to SDS-PAGE for 1.5 h. The membrane was blocked with 5% skim milk in TBST for approximately 1 h, followed by an overnight incubation with initial antibodies against AP-1 (c-Jun/c-Fos), VEGF, and PDGFA. All initial antibodies were from Santa Cruz Biotechnology Inc. (United States). Then the

Table 1 RT-PCR Primer Sequences

Primer	Sequence
miR-139-5p	Forward: 5′-ACACTCCAGCTGGGTCTACAGTGCACGTGTC-3′ Reverse: 5′-TGGTGTCGTGGAGTCG-3′
U6	Forward: 5-CTCGCTTCGGCAGCACA-3′ Reverse: 5-AACGCTTCACGAATTTGCGT-3′

membrane and the secondary antibodies corresponding to different antibodies were incubated again for 1 h and then soaked in ECL luminous solution (Advansta, USA) completely, the chemiluminescence instrument examined the protein bands, and the software calculated the grey value.

Real-Time Quantitative Reverse Transcription PCR (qRT-PCR)

In this work, we extracted the total RNA by TRIzol reagent (Invitrogen, USA) and reverse transcribed that into c-DNA of miR-139-5p and U6 by RT reverse transcription kit (Thermo Fisher Scientific, USA). qRT-PCR has been conducted using SYBR green real-time PCR master mix kit (Toyobo, Japan) under 95°C for 5s, 60°C for 15s, 72°C for 15s for 45 cycles, and collecting data at 72°C. The $2^{-\Delta\Delta Ct}$ design was adopted to calculate miR-139-5p against U6 gene expression. The miR-139-5p and U6 primers used in PCR were extracted with Ribobio (RIBOBIO, Guangzhou, China) (Table 1).

Statistical Analysis

The differences between the two groups were analyzed using a two-tailed Student's *t*-test, while a single-factor analysis of variance (ANOVA) was employed for comparing three or more groups. Data are presented as mean±SD, with statistical significance set at P<0.05.

Results

The Expression of miR-139-5p Was Upregulated in Diabetes Wound Tissues, as Well as Fibroblasts Under High Glucose (HG) Concentration

To elucidate the putative association between miR-139-5p and impaired wound healing in individuals with diabetes, the expression profiles of miR-139-5p in wound tissues from diabetic patients and diabetes-induced rats. The results revealed a significant upregulation of miR-139-5p expression, with 1.6- and 4.8-fold increases relative to the control group, respectively (Figure 1A and B). Furthermore, our experiments demonstrated a remarkable elevation in miR-139-5p expression when fibroblasts were exposed to a high glucose (HG) concentration of 33.0 mM (Figure 1C). Those results indicated that the miR-139-5p up-regulation may contribute to the poor healing of DFUs.

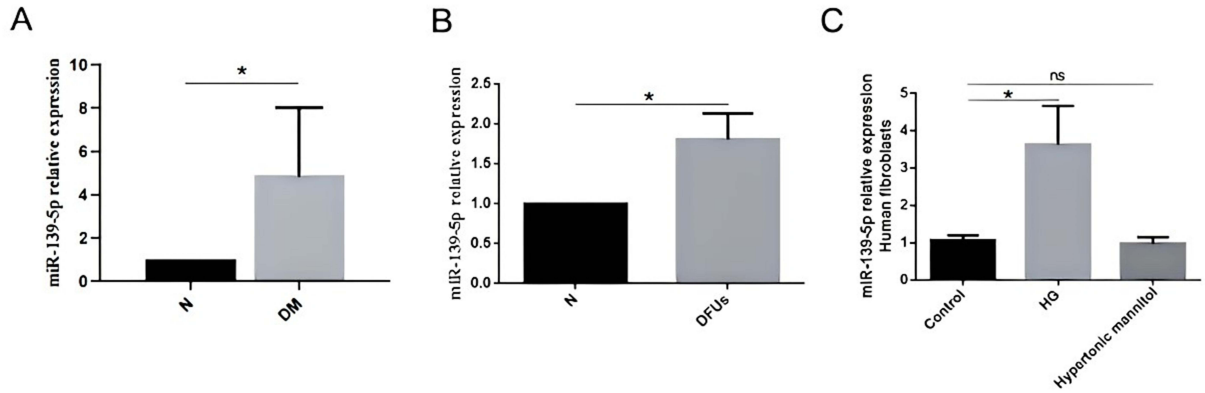


Figure 1 Relative expression levels of miR-139-5p in wound tissues and fibroblasts. (**A**) qRT-PCR analysis revealed that the relative expression levels of miR-139-5p in wound tissue from diabetic rats (n=4) versus normal rats (n=4), *$P < 0.05$; (**B**) MiR-139-5p levels were distinctly raised in the DFUs patients (n=4) compared to controls (n=4), *$P < 0.05$. (**C**) Increased expression levels of miR-139-5p in HG compared to NC (n = 3), *$P < 0.05$; ns $P < 0.05$. Values are normalized to U6 mRNA and reported as means ± SD.

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MiR-139-5p Antagomir Promotes Diabetic Wound Healing

To assess the therapeutic potential of miR-139-5p inhibition on wound healing, we locally injected the miR-139-5p antagomir or negative control (NC) around 1 cm diameter wounds on the backs of diabetic rats. This injection was performed on days 1, 4, 7, and 10 after the wounds were created. Interestingly, we found that the wounds treated with the miR-139-5p antagomir exhibited a significantly accelerated healing rate compared to NC-treated wounds (Figure 2A and B). Further histologic analysis revealed

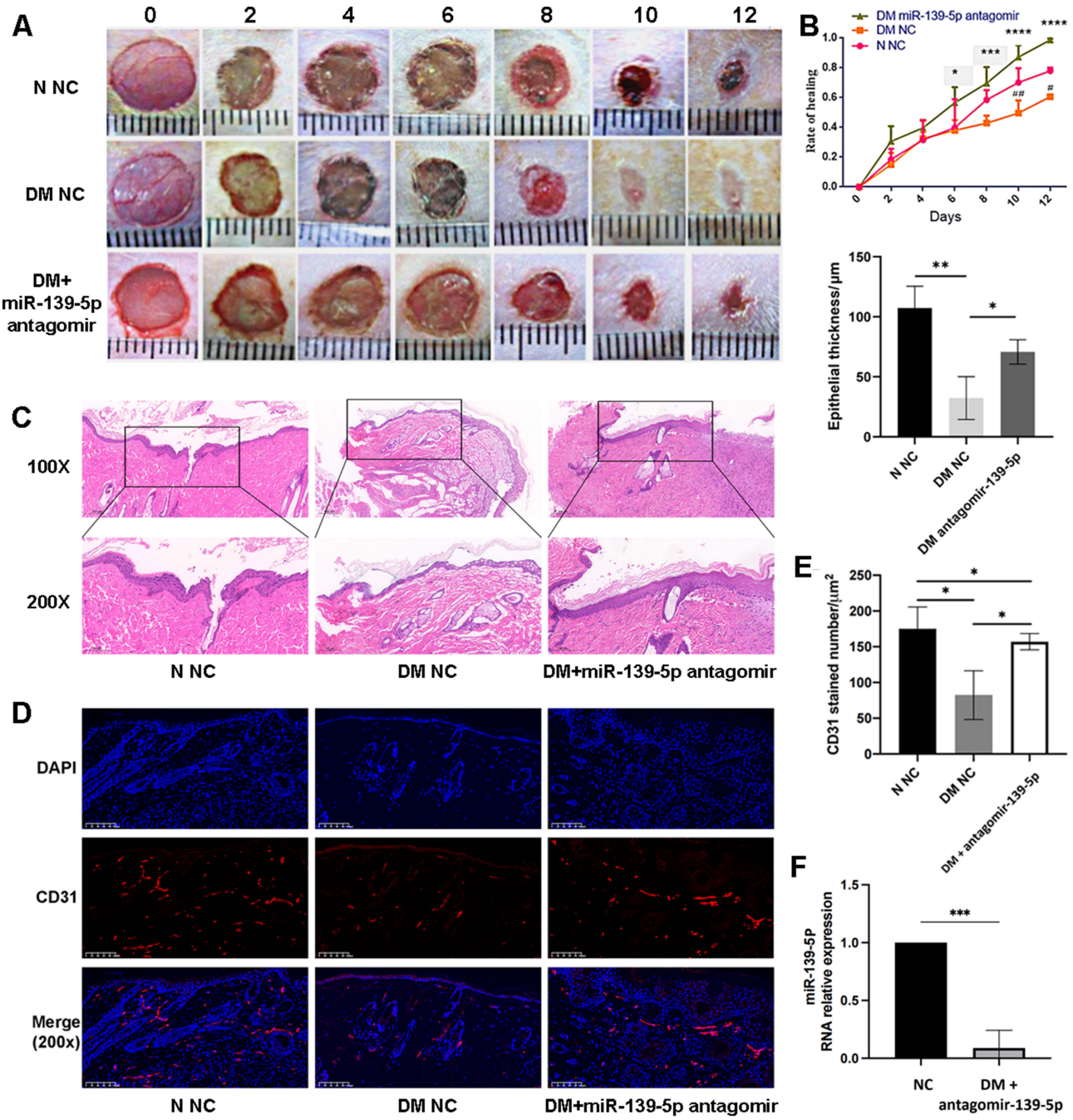

Figure 2 MiR-139-5p antagomir promotes diabetic wound healing. (**A** and **B**) Representative images showing the healing progression of wounds, and the rate of wound closure for corresponding days as indicated. (n=8/group, day0-8) (n=5/group, day10-12); $^{*}P < 0.05$, $^{***}P < 0.001$, $^{****}P < 0.0001$, DM miR-139-5p antagomir vs DM NC; $^{\#\#}P < 0.01$, $^{\#}P < 0.05$, DM NC vs N NC. (**C**) Representative HE stained histological image showing changes in epidermal and dermal thickness in wound sections (100x and 200x), and thickness of the epithelial tissue measured in micrometers (μm) (n=3), $^{**}P < 0.01$, $^{*}P < 0.05$. (**D** and **E**) Representative immunofluorescence images of CD31 in wound sections (scale bar:100μm). Neovascular density was expressed by the number of CD31 immunofluorescence staining. (n=3), $^{*}P < 0.05$. (**F**) Significant decrease in miR-139-5p levels was confirmed by qRT-PCR, following direct injection of miR-139-5p antagomir into wounds area of diabetic rats. (n=3), $^{***}P < 0.001$.

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that the epidermis and dermis were noticeably thicker in miR-139-5p antagomir-treated wounds, as observed via HE staining (Figure 2C). Additionally, the number of blood vessels, assessed through CD31 staining, was significantly higher in the miR-139-5p antagonist-treated wounds compared to those treated with the NC. (Figure 2D and E). Significant reduction in miR-139-5p expression were exhibited in tissues administered with miR-139-5p antagomir compared to the NC group, as verified by qRT-PCR of wound tissues obtained after intervention (Figure 2F). Overall, our findings suggest that the miR-139-5p antagonist can enhance both angiogenesis and epithelialization in diabetic rats' wounds, leading to an accelerated healing rate.

MiR-139-5p Regulates the Function of Human Skin Fibroblast in Vitro

Since fibroblasts were fundamental to wound healing, we investigated whether miR-139-5p regulated the function of fibroblasts. Human skin fibroblasts were transfected with miR-139-5p inhibitor/mimic (Figure 3A). The CCK-8 test demonstrated that miR-139-5p inhibitor enhanced the proliferation of human skin fibroblasts, while miR-139-5p mimic showed the opposite effect (Figure 3B). Scratch assay also showed miR-139-5p inhibitor promoted the migration of fibroblasts, conversely, miR-139-5p mimic inhibited the migration of human skin fibroblasts (Figure 3C and D). In short, these results suggested that the miR-139-5p down-regulation could enhance the viability of human skin fibroblast.

Analysis of Downstream Target Gene of miR-139-5p

AP-1 (c-Jun/c-Fos) has been identified as a target gene of miR-139-5p based on bioinformatics analysis and previous literature.[22,23] To further validate it as a target gene for miR-139-5p in fibroblasts, we transfected human skin fibroblasts with either miR-139-5p inhibitor or mimic. We observed that the expression of AP-1 (c-Jun/c-Fos) was significantly increased when miR-139-5p was downregulated in these fibroblasts (Figure 4B). Conversely, the upregulation of miR-

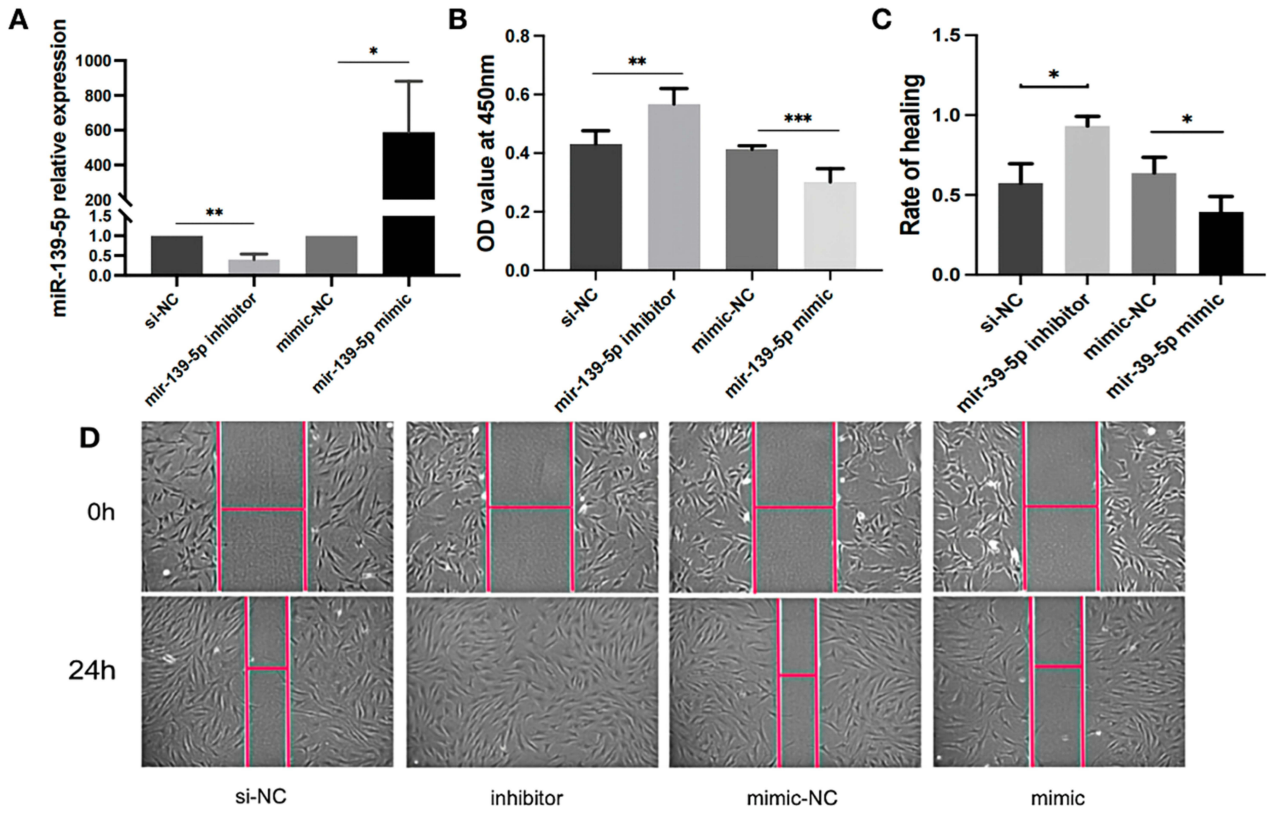

Figure 3 miR-139-5p inhibited migration and proliferation of human skin fibroblasts in vitro. The human skin fibroblast at passage 4 was transduced with si-NC, mimic-NC, miR-139-5p inhibitor, and miR-139-5p mimic, respectively. (**A**) qRT-PCR was used to determine the expression levels of miR-139-5p. (n=3), **P* < 0.05, ***P* < 0.01. (**B**) CCK8 assays were used to assess the proliferation of fibroblasts. (n=3), ***P* < 0.01, ****P* < 0.001. (**C, D**) Fibroblast migration was evaluated using scratch assays. (n=3), **P* < 0.05.

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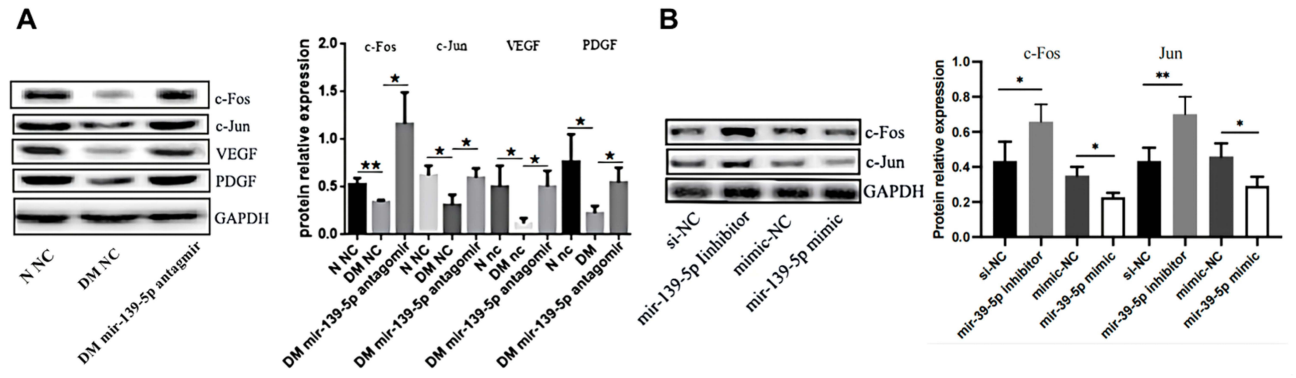

Figure 4 MiR-139-5p antagomir upregulates AP-1(c-Jun/c-Fos) levels and growth factor levels. (**A**) Protein levels of c-Jun and c-Fos, VEGF, and PDGF in the wound tissues were assessed using Western blot analysis. (n=3), $^{**}P < 0.01$,$^{*}P < 0.05$. (**B**) Western blot analysis confirmed the expression of c-Jun/c-Fos in human skin fibroblasts after transfection with miR-139-5p mimics or inhibitors (n=3). $^{**}P < 0.01$,$^{*}P < 0.05$.

139-5p led to a decrease in AP-1 (c-Jun/c-Fos) expression. These results provide evidence that AP-1 (c-Jun/c-Fos) is a target gene of miR-139-5p in fibroblasts.

Furthermore, we also investigated the levels of AP-1 (c-Jun/c-Fos), VEGF, and PDGF in diabetic wounds. Compared to normal wound tissues, the levels of AP-1 (c-Jun/c-Fos), VEGF, and PDGF were significantly lower in diabetic wounds (Figure 4A). However, when treated with miR-139-5p antagomir, the levels of AP-1 (c-Jun/c-Fos), VEGF, and PDGF expression in diabetic wound tissues almost completely recovered. Taken together, our findings demonstrate that miR-139-5p plays a regulatory role in the expression of AP-1 (c-Jun/c-Fos), VEGF, and PDGF in both skin fibroblasts and wound tissues of diabetic rats.

MiR-139-5p Mediates the Migration of Fibroblasts by Regulating AP-1

To further determine the functional activity of miR-139-5p mediated by AP-1 in human skin fibroblasts, miR-139-5p mimics and/or c-Fos plasmids were used to treat human skin fibroblasts and migration ability was estimated through scratch assays. The results showed that the upregulation of c-Fos could rescue the inhibition of cell migration caused by the miR-139-5p mimics (Figure 5A–5D). On the contrary, we transfected human skin fibroblasts with miR-139-5p inhibitors and c-Fos siRNA, and observed that downregulation of c-Fos can counteract the promotion of cell migration by miR-139-5p inhibitors (Figure 5A–5D). These results suggest that miR-139-5p is capable of regulating the functional activity of skin fibroblasts by modulating the AP-1 pathway.

Discussion

Wound healing is a complicated and finely orchestrated process that involves a multitude of molecular events, including the recruitment of cells, cellular proliferation[24] and the deposition of the extracellular matrix,[25] as well as various inflammatory components and growth factors[26] that require appropriate and precise response. Fibroblasts serve as the primary effector cells in the repair of soft tissue wounds, playing an essential role in wound contraction, collagen production, and the overall remodeling of tissue, and as a signaling cell, it secrets the growth factors crucial for intercellular communication during reparative process.[27] However, in diabetic conditions, persistent hyperglycemia and associated issues such as neuropathy, vascular disease, and oxidative stress can impede angiogenesis and disrupt normal fibroblast function.[27] These factors culminate in hindered epithelial regeneration and delayed wound healing. DFU wounds expose patients to the risk of amputation and death. A comprehensive understanding of the DFU pathophysiology and the targeted pathways involved in fibroblast dysregulation is a necessary condition for developing innovative new treatment methods urgently needed by these patients.

MicroRNAs are essential for various cellular processes, such as growth, development, and differentiation.[28] In recent years, there has been a growing interest among scientists and clinicians in the application of miRNA for tissue engineering.[28,29] Specifically, researchers have found that the use of porcine acellular dermal matrix leads to a reduction in

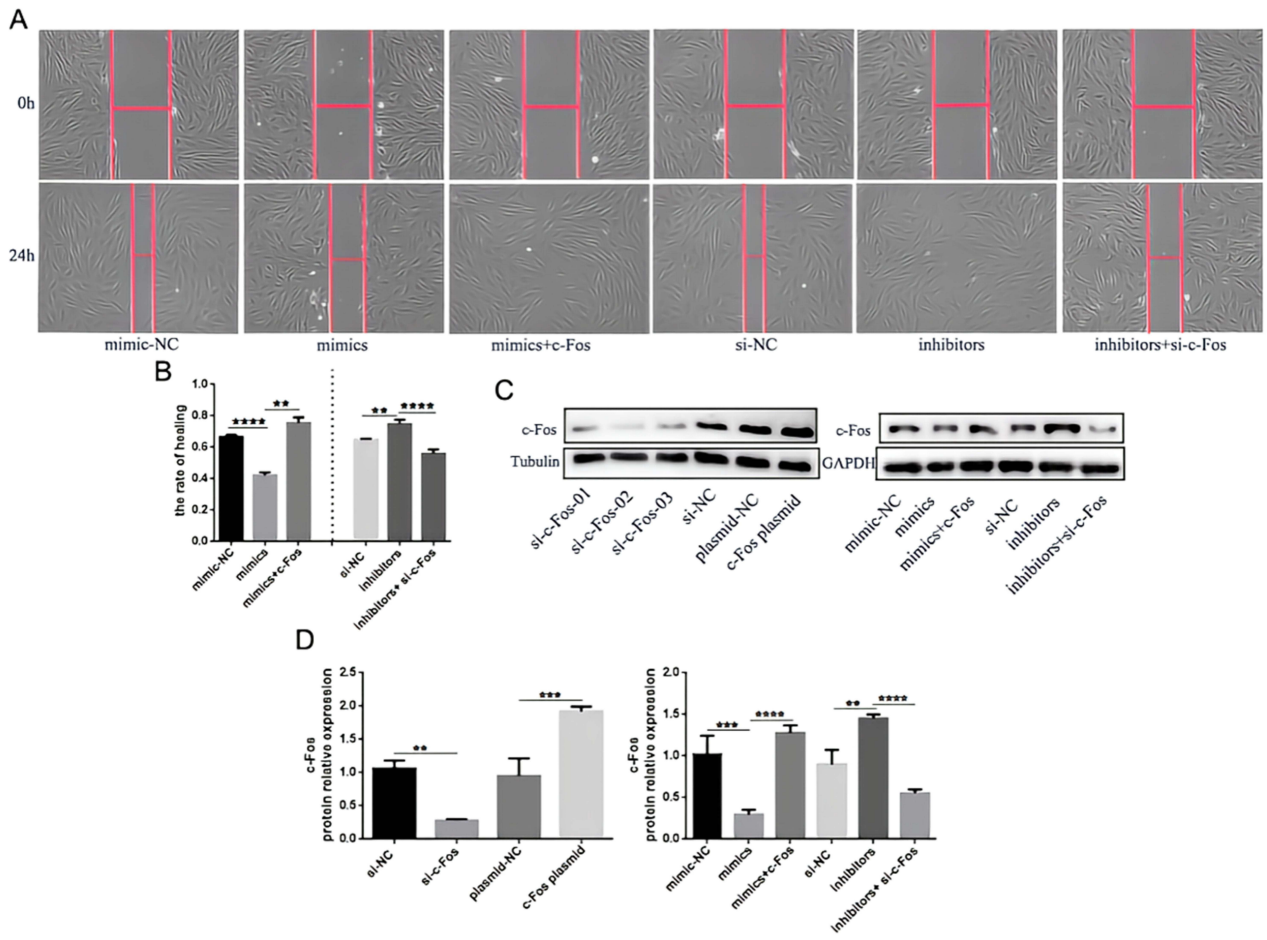

Figure 5 MiR-139-5p/c-Fos regulated the migration of human skin fibroblasts. All groups' migration (**A** and **B**) were evaluated by scratch experiments (n=3) in human skin fibroblasts transfected with miR-139-5p mimics and/or c-Fos plasmids as well as miR-139-5p inhibitors and/or c-Fos siRNA. $^{**}P < 0.01$, $^{****}P < 0.0001$. (**C** and **D**) The expression of c-Fos was validated by Western blot after respective transfection in each group (n=3). $^{****}P < 0.0001$, $^{***}P < 0.001$, $^{**}P < 0.01$.

the expression of miR-124-3p.1/139-5p in wounds, ultimately enhancing the healing of cutaneous wounds.[30] This discovery has sparked interest in using miR-139-5p inhibitors for soft tissue repair in tissue engineering. MiR-139-5p has been found to be abnormally expressed in multiple solid tumors, involving cell proliferation and apoptosis, and is associated with tumor growth, invasion, and metastasis.[17,31] Furthermore, recent studies have found that miR-139-5p is involved in regulating angiogenesis. For example, the miR-139-5p-CXCR4 axis exerts pivotal effects in vascular development,[32] and the activation of the CD44/miR-139-5p pathway is involved in hyaluronic acid promoting angiogenesis of ECFCs combined with mesenchymal stem cells (MSCs).[33] A recent study by Mohammad R. Irhimeh et al reported that miR-139-5p may serve as a potential genomic driver of diabetic pathogenesis in Lin-/VEGF-R2+EPCs.[14] Our previous research demonstrated that the upregulation of miR-139-5p is linked to diabetic endothelial cell dysfunction.[15] These findings indicate that miR-139-5p may be a crucial effector of diabetic angiogenesis impairment. Besides, it was found that the miR-139-5p expression was increased in the diabetic rats' pancreas and the inhibition of miR-139-5p facilitated the anti-apoptotic effect of liraglutide on the pancreas tissues and INS-1 cells of diabetic rats by targeting IRS1.[34] Despite these discoveries, it remains unclear whether miR-139-5p is naturally present in epithelial tissue and whether its dysregulation is associated with impaired diabetic epithelial tissue repair and regeneration. Therefore, in this study, we collected wound tissue from diabetic foot ulcers and diabetic rats and observing a notable upregulation of miR-139-5p in both types of wound samples, the topical application of miR-139-5p antagomir to skin wounds in diabetic rats resulted in prominent regenerative effects at the wound sites. These effects included faster wound closure, higher re-epithelialization rates, and the formation of new blood vessels. These findings suggest its potential as a novel therapeutic approach for enhancing wound healing.

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Furthermore, in vitro studies, upregulation of miR-139-5p expression was observed in human skin fibroblasts induced by high glucose treatment, which inhibited the function of fibroblasts. The inhibition of miR-139-5p expression enhanced the functional characteristics of fibroblasts, including cell proliferation and migration ability, these findings indicate that miR-139-5p upregulation may contribute to the pathogenesis of impaired fibroblasts in diabetic wounds, and might act as an important mediator of poor healing outcomes in diabetic wounds. As mentioned above, the interaction between cells in the dermis, matrix, vascular endothelium, and immune system makes wound healing possible, and activated fibroblasts are the "masters" of this phenomenon.[24–27] Therefore, in addition to improving endothelial function, the activation of fibroblasts may be the main mechanism by which miR-139-5p antagonists stimulate wound healing.

In our previous study, we verified that miR-139-5p regulates the function of vascular endothelial cells or MSCs by specifically targeting c-Jun or c-Fos/AP-1.[15,21] Additionally, another study found that miR-139-5p expression was markedly decreased in the hearts of patients with hypertrophic cardiomyopathy (HCM), and it was shown to inhibit cardiac hypertrophy by targeting c-Jun.[35] These findings highlight the importance of miR-139-5p in regulating cellular functions through the modulation of AP-1 activity. In this study, our results showed that miR-139-5p could regulate the function of skin fibroblasts by AP-1(c-Fos/c-Jun). By investigating the effects of wound healing-related factors on the application of miR-139-5p antagomir, we found that miR-139-5p antagomir increased the expression of AP-1 (both c-Fos and c-Jun), as well as VEGF and PDGF in diabetic wound healing. These factors are known to be pluripotent cytokines involved in maintaining skin homeostasis and promoting various wound healing processes, such as fibroblast proliferation, migration, angiogenesis, and re-epithelialization.[25,26,36] It is worth mentioning that AP-1, as a transcription factor, is pivotal in mediating the transcription of genes associated with key cellular processes, such as cell growth, differentiation, and cell-cell interactions.[37,38] Previous studies have shown that AP-1/c-Jun specifically promotes epidermal keratinocyte proliferation, and knocking out the AP-1 subunit in mouse fibroblasts play an essential role in the development of epithelial structures.[39] Additionally, both c-Fos and c-Jun have been associated with the regulation of the inflammatory phase and angiogenesis during wound healing.[40] In a study of primary biliary cholangitis, it was found that miR-139-5p inhibits inflammation by targeting c-Fos.[22] Furthermore, previous research has indicated that VEGF stimulates endothelial cell migration and proliferation by activating the AP-1.[41] AP-1 activation has been recognized as one of the main transcriptional mechanisms that drive VEGF expression in response to various stimuli in different tumor cell lines and endothelial cells as well.[42] Similarly, PDGF-B can also be regulated by AP-1 and plays a critical role in promoting the wound-healing process. We recently found that overexpression of c-Jun in human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) enhanced the paracrine secretion of VEGF and PDGF, resulting in accelerated wound closure, increased re-epithelialization, and angiogenesis at the wound bed.[43] Based on these findings, we propose that miR-139-5p antagomir can promote the wound healing process by targeting and activating AP-1, which subsequently leads to the upregulation of VEGF and PDGF expression.

Overall, upregulation of miR-139-5p could be induced by hyperglycemic toxicity in the diabetic wound tissue and fibroblasts, while inhibiting miR-139-5p improves fibroblasts viability and promotes diabetic wound healing by upregulation of AP-1, suggesting that inhibition of miR-139-5p holds promise as a novel therapeutic approach for treating chronic wounds associated with diabetes, such as diabetic foot ulcers. Upregulation of miR-139-5p may serve as a valuable indicator for identifying diabetic patients with a higher risk of poor wound healing outcomes.

Data Sharing Statement

The original data presented in the study are included in the article, and further inquiries can be directed to the corresponding authors.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Third Xiangya Hospital (protocol code: fast-24461). Animals were maintained in accordance with the Guide for Care and Use of Laboratory Animals and the Animal Welfare Act. Experiments were performed with the approval of the Institutional Animal Care Committee of Central South University. (Ethical Approval Number: CSU-2024-0155)

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in the study.

References

1. RCW M. Epidemiology of diabetes and diabetic complications in China. *Diabetologia*. 2018;61(6):1249–1260. doi:10.1007/s00125-018-4557-7
2. Senneville É, Albalawi Z, van Asten SA, et al. IWGDF/IDSA guidelines on the diagnosis and treatment of diabetes-related foot infections (IWGDF/IDSA 2023). *Diabetes Metab Res Rev*. 2024;40(3):e3687. doi:10.1002/dmrr.3687
3. Lim JZ, Ng NS, Thomas C. Prevention and treatment of diabetic foot ulcers. *J R Soc Med*. 2017;110(3):104–109. doi:10.1177/0141076816688346
4. Li Y, Teng D, Shi X, et al. Prevalence of diabetes recorded in mainland China using 2018 diagnostic criteria from the American Diabetes Association: national cross sectional study. *BMJ*. 2020;369:m997. doi:10.1136/bmj.m997
5. Wolcott R. Economic aspects of biofilm-based wound care in diabetic foot ulcers. *J Wound Care*. 2015;24(5):189–190,192–184. doi:10.12968/jowc.2015.24.5.189
6. Patel S, Srivastava S, Singh MR, Singh D. Mechanistic insight into diabetic wounds: pathogenesis, molecular targets and treatment strategies to pace wound healing. *Biomed Pharmacother*. 2019;112:108615. doi:10.1016/j.biopha.2019.108615
7. Louiselle AE, Niemiec SM, Zgheib C, Liechty KW. Macrophage polarization and diabetic wound healing. *Transl Res*. 2021;236:109–116. doi:10.1016/j.trsl.2021.05.006
8. Zhu Y, Wang Y, Jia Y, Xu J, Chai Y. Roxadustat promotes angiogenesis through HIF-1alpha/VEGF/VEGFR2 signaling and accelerates cutaneous wound healing in diabetic rats. *Wound Repair Regen*. 2019;27(4):324–334. doi:10.1111/wrr.12708
9. Wan R, JP W, Grundman K, Lang L, DJ G, Galiano RD. Diabetic wound healing: the impact of diabetes on myofibroblast activity and its potential therapeutic treatments. *Wound Repair Regen*. 2021;29(4):573–581. doi:10.1111/wrr.12954
10. Chang M, Nguyen TT. Strategy for Treatment of Infected Diabetic Foot Ulcers. *Acc Chem Res*. 2021;54(5):1080–1093. doi:10.1021/acs.accounts.0c00864
11. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet*. 2010;11(9):597–610. doi:10.1038/nrg2843
12. Ye J, Kang Y, Sun X, Ni P, Wu M, Lu S. MicroRNA-155 Inhibition Promoted Wound Healing in Diabetic Rats. *Int J Low Extrem Wounds*. 2017;16(2):74–84. doi:10.1177/1534734617706636
13. Jin Y, Tymen SD, Chen D, et al. MicroRNA-99 family targets AKT/mTOR signaling pathway in dermal wound healing. *PLoS One*. 2013;8(5):e64434. doi:10.1371/journal.pone.0064434
14. Irhimeh MR, Hamed M, Barthelmes D, et al. Identification of novel diabetes impaired miRNA-transcription factor co-regulatory networks in bone marrow-derived Lin-/VEGF-R2+ endothelial progenitor cells. *PLoS One*. 2018;13(7):e0200194. doi:10.1371/journal.pone.0200194
15. Luo YF, XX W, Zhao LL, et al. MicroRNA-139-5p upregulation is associated with diabetic endothelial cell dysfunction by targeting c-jun. *Aging*. 2020;13(1):1186–1211. doi:10.18632/aging.202257
16. Walz JM, Wecker T, Zhang PP, et al. Impact of angiogenic activation and inhibition on miRNA profiles of human retinal endothelial cells. *Exp Eye Res*. 2019;181:98–104. doi:10.1016/j.exer.2019.01.006
17. Hou J, Zhuo H, Chen X, et al. MiR-139-5p negatively regulates PMP22 to repress cell proliferation by targeting the NF-κB signaling pathway in gastric cancer. *Int J Biol Sci*. 2020;16(7):1218–1229. doi:10.7150/ijbs.40338
18. Sun H, Dai J, Chen M, et al. miR-139-5p Was Identified as Biomarker of Different Molecular Subtypes of Breast Carcinoma. *Front Oncol*. 2022;12:857714. doi:10.3389/fonc.2022.857714
19. Saba R, Goodman CD, Huzarewich RLCH, Robertson C, SA B. A miRNA signature of prion induced neurodegeneration. *PLoS One*. 2008;3(11):e3652. doi:10.1371/journal.pone.0003652
20. Kim NH, Ahn J, Choi YM, et al. Differential circulating and visceral fat microRNA expression of non-obese and obese subjects. *Clin Nutr*. 2020;39(3):910–916. doi:10.1016/j.clnu.2019.03.033
21. Liang F, Luo YF, Guo Z, Qian Q, Meng XB, Mo ZH. MicroRNA-139-5p mediates BMSCs impairment in diabetes by targeting HOXA9/c-Fos. *FASEB J*. 2023;37(1):e22697. doi:10.1096/fj.202201059R
22. Katsumi T, Ninomiya M, Nishina T, et al. MiR-139-5p is associated with inflammatory regulation through c-FOS suppression, and contributes to the progression of primary biliary cholangitis. *Lab Invest*. 2016;96(11):1165–1177. doi:10.1038/labinvest.2016.95

23. Cao DN, Shi JJ, Wu N, Li J. Modulation of miR-139-5p on chronic morphine-induced, naloxone-precipitated cAMP overshoot in vitro. *Metab Brain Dis*. 2018;33(5):1501–1508. doi:10.1007/s11011-018-0257-8
24. Sawaya AP, Stone RC, Brooks SR, et al. Deregulated immune cell recruitment orchestrated by FOXM1 impairs human diabetic wound healing. *Nat Commun*. 2020;11(1):4678. doi:10.1038/s41467-020-18276-0
25. Kunkemoeller B, Kyriakides TR. Redox Signaling in Diabetic Wound Healing Regulates Extracellular Matrix Deposition. *Antioxid Redox Signal*. 2017;27(12):823–838. doi:10.1089/ars.2017.7263
26. White MJV, Briquez PS, White DAV, Hubbell JA. VEGF-A, PDGF-BB and HB-EGF engineered for promiscuous super affinity to the extracellular matrix improve wound healing in a model of type 1 diabetes. *NPJ Regen Med*. 2021;6(1):76. doi:10.1038/s41536-021-00189-1
27. Voza FA, Huerta CT, Le N, et al. Fibroblasts in Diabetic Foot Ulcers. *Int J mol Sci*. 2024;25(4):2172. doi:10.3390/ijms25042172
28. Creugny A, Fender A, Pfeffer S. Regulation of primary microRNA processing. *FEBS Lett*. 2018;592(12):1980–1996. doi:10.1002/1873-3468.13067
29. Davis FM, Kimball A, Boniakowski A, Gallagher K. Dysfunctional Wound Healing in Diabetic Foot Ulcers: new Crossroads. *Curr Diab Rep*. 2018;18(1):2. doi:10.1007/s11892-018-0970-z
30. Chen X, Yang R, Wang J, et al. Porcine acellular dermal matrix accelerates wound healing through miR-124-3p.1 and miR-139-5p. *Cytotherapy*. 2020;22(9):494–502. doi:10.1016/j.jcyt.2020.04.042
31. Yang C, Xia Z, Zhu L, et al. MicroRNA-139-5p modulates the growth and metastasis of malignant melanoma cells via the PI3K/AKT signaling pathway by binding to IGF1R. *Cell Cycle*. 2019;18(24):3513–3524. doi:10.1080/15384101.2019.1690881
32. Papangeli I, Kim J, Maier I, et al. MicroRNA 139-5p coordinates APLNR-CXCR4 crosstalk during vascular maturation. *Nat Commun*. 2016;7(1):11268. doi:10.1038/ncomms11268
33. Luo Y, Liang F, Wan X, et al. Hyaluronic Acid Facilitates Angiogenesis of Endothelial Colony Forming Cell Combining With Mesenchymal Stem Cell via CD44/ MicroRNA-139-5p Pathway. *Front Bioeng Biotechnol*. 2022;10:794037. doi:10.3389/fbioe.2022.794037
34. Li J, Su L, Gong YY, et al. Downregulation of miR-139-5p contributes to the antiapoptotic effect of liraglutide on the diabetic rat pancreas and INS-1 cells by targeting IRS1. *PLoS One*. 2017;12(3):e0173576. doi:10.1371/journal.pone.0173576
35. Ming S, Shui-Yun W, Wei Q, et al. miR-139-5p inhibits isoproterenol-induced cardiac hypertrophy by targetting c-Jun. *Biosci Rep*. 2018;38(2):BSR20171430. doi:10.1042/BSR20171430
36. Yates S, Rayner TE. Transcription factor activation in response to cutaneous injury: role of AP-1 in reepithelialization. *Wound Repair Regen*. 2002;10(1):5–15. doi:10.1046/j.1524-475X.2002.10902.x
37. Bejjani F, Evanno E, Zibara K, Piechaczyk M, Jariel-Encontre I. The AP-1 transcriptional complex: local switch or remote command? *Biochim Biophys Acta Rev Cancer*. 2019;1872(1):11–23. doi:10.1016/j.bbcan.2019.04.003
38. Shaulian E, Karin M. AP-1 as a regulator of cell life and death. *Nat Cell Biol*. 2002;4(5):E131–6. doi:10.1038/ncb0502-e131
39. Schumacher M, Schuster C, Rogon ZM, et al. Efficient keratinocyte differentiation strictly depends on JNK-induced soluble factors in fibroblasts. *J Invest Dermatol*. 2014;134(5):1332–1341. doi:10.1038/jid.2013.535
40. Ye F, Zeng Q, Dan G, et al. Nitrogen mustard prevents transport of Fra-1 into the nucleus to promote c-Fos- and FosB-dependent IL-8 induction in injured mouse epidermis. *Toxicol Lett*. 2020;319:256–263. doi:10.1016/j.toxlet.2019.10.006
41. Chang HJ, Park JS, Kim MH, et al. Extracellular signal-regulated kinases and AP-1 mediate the up-regulation of vascular endothelial growth factor by PDGF in human vascular smooth muscle cells. *Int J Oncol*. 2006;28(1):135–141.
42. Jia J, Ye T, Cui P, Hua Q, Zeng H, Zhao D. AP-1 transcription factor mediates VEGF-induced endothelial cell migration and proliferation. *Microvasc Res*. 2016;105(103–108):103–108. doi:10.1016/j.mvr.2016.02.004
43. Yue C, Guo Z, Luo Y, Yuan J, Wan X, Mo Z. c-Jun Overexpression Accelerates Wound Healing in Diabetic Rats by Human Umbilical Cord-Derived Mesenchymal Stem Cells. *Stem Cells Int*. 2020;2020:7430968. doi:10.1155/2020/7430968