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The study of miR-130a expression and its mechanism of action in peripheral blood endothelial progenitor cells (EPCs) in type 2 diabetes mellitus (T2DM)

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TGF-β1 has been reported to suppress miR-130a expression, while being elevated in patients with type 2 diabetes mellitus (T2DM). And IL-18, a potential target of miR-130a, is also up-regulated in T2DM patients. In this study, we aim to investigate the potential involvement of the TGFβ1/miR-130a/IL-18 axis underlying the dysfunction of endothelial progenitor cells (EPCs) in T2DM patients. We performed luciferase assays to confirm the molecular binding between miR-130a, TGF-β1 and IL-18, and real-time PCR and ELISA were performed to observe the changes of TGF-β1, miR-130a, IL-18 and IFN-γ in different cell groups. Tube formation assay, cell adhesion assay and Transwell assay were performed to evaluate effect of TGF-β1/miR-130a/IL-18 axis on the EPCs functions. Accordingly, in EPCs treated with TGF-β1, we found that the levels of miR-130a were reduced, and its expressions were also negatively correlated with the expressions of IL-18 in the EPC groups. Luciferase assays validated IL-18 as a target gene of miR-130a. The over-expression of IL-18, as well as the knockdown of miR-130a, not only increased the expressions of TGF-β1 in EPCs, but also promoted the tube formation, adhesion and migration of EPCs. By contrast, the knockdown of IL-18, as well as the over-regulation of miR-130a, exhibited suppressive effect on the levels of TGF-β1, while inhibiting the tube formation, adhesion and migration of EPCs. In this study, we elucidated the impact of the TGFβ1/miR-130a/IL-18 signaling pathway on the function of EPCs, thus providing theoretical basis for the development of miRNA-targeted therapeutic strategies for patients withT2DM and associated complications.

Keywords Diabetes, Type 2 diabetes mellitus, Endothelial progenitor cells, MiRNA, Peripheral blood

As a prevalent metabolic disorder, diabetes mellitus (DM) has been reported with a clinical incidence of 9.7% in China, among which over 90% of the reported cases are type 2 diabetes mellitus (T2DM)[1]. However, the etiology and pathogenesis of T2DM remain to be further elucidated. Various complications which are related to delayed tissue repair induced by DM could significantly impact the quality of life and prognosis of DM patients[2]. For the process of tissue repair, angiogenesis plays a crucial role[3]. Although angiogenesis is strictly regulated by both positive and negative signaling cues from the adjacent microenvironment, the onset of DM could disrupt this balance, leading to impaired angiogenesis and subsequent delayed tissue repair[4]. Therefore, treatments to restore angiogenic function has emerged as a promising therapeutic strategy for the treatment of complications in DM patients.

Endothelial progenitor cells (EPCs), also known as angioblasts, are precursor cells which can differentiate into mature vascular endothelial cells. First reported in 1997[5], EPCs has been extensively reported to be involved in postnatal neovascularization and play significant roles in the treatment of vascular diseases related to tissue ischemia[6,7]. Research indicates that the number of circulating EPCs are significantly reduced in patients with coronary artery or diabetic peripheral vascular disease[8], and functions of EPCs such as the adhesion, tube formation and migration are impaired in DM patients[9–11], leading to the reduced efficacy of autologous cell transplantation in DM patients compared to non-diabetic patients[12,13]. Thus, the dysfunction of EPCs has become a critical factor limiting the clinical application of autologous cell therapy, and the investigations of the

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molecular mechanisms of EPC dysfunction in DM, especially T2DM, hold significant clinical importance for seeking methods to improve EPC functions.

MicroRNAs (miRNAs) are a class of non-protein-coding small RNA molecules discovered in recent years that regulate gene expression at the post-transcriptional level. miRNAs primarily function to modulate the expression of genes associated with individual growth, development, and disease processes, playing a crucial role in biological development, including early development, cell differentiation, apoptosis, and tumorigenesis[14]. In recent years, research has revealed that miRNAs are also important factors in regulating angiogenesis[15]. Previous studies indicated that the expressions of several miRNAs, such as miR-126, miR-221/222, miR-21, the let-7 family, and miR-17–92, are evidently increased in endothelial cells[16]. For example, let-7f and miR-27b have been found to promote angiogenesis in endothelial cells[17], miR-126 is specifically expressed in endothelial cells and plays a crucial role in maintaining vascular integrity and angiogenesis[18], and the component of the miR-17–92cluster, miR-92a, was also reported to inhibit angiogenesis[13]. Moreover, miR-130a can also regulate angiogenesis of endothelial cells by modulating GAX and HOXA5 expressions[19].

Currently, in the research on the etiology and pathogenesis of T2DM, IL-18 have been extensively reported for its role in the inflammatory cascade. IL-18 not only stimulates the production of cytokines such as interferon-gamma (IFN-γ), interleukin-2 (IL-2), and granulocyte-macrophage colony-stimulating factor (GM-CSF) by T cells and NK cells, but also enhances the expression of Fas ligand by Th1 and NK cells, thereby mediating cytotoxic effects and inducing inflammatory responses. Several previous publications have reported serum IL-18 levels to be increased in patients with T2DM[20–22]. Besides, a multifunctional cytokine which participates in various cellular processes, TGF-β1, has also been confirmed to be closely associated with the onset and progression of DM[23–25]. In our previous study, we found that the level of serum interleukin-18 (IL-18) was evidently higher in T2DM patients (144.9 ± 35.63pg/ml) compared to the healthy participants (98.29 ± 23.83pg/ml), and we also found that TGF-β1 was highly expressed in the serum of T2DM patients (19.9 ± 4.51pg/ml) compared to that in the healthy participants (14.54 ± 3.07 pg/ml). However, the effect of signaling which involves TGF-β1, IL-18, and miR-130a upon EPCs in T2DM remains to be determined. In this study, we suspected that IL-18 is a target gene of miR-130a, and the signaling of TGF-β1/miR-130a/IL-18 may participate in the pathogenesis of T2DM by regulating the functions of EPCs.

Materials and methods

Cell model establishment

To investigate the regulatory relationship between TGF-β1 and miR-130a, cell model I was established as 3 cell groups: a Control group, a TGF-β1 group, and an anti-TGF-β1 group. For the TGF-β1 group and the anti-TGF-β1 group, TGF-β1 small molecule compounds and TGF-β1 inhibitors (Sense: 5'-GGCAUUUGGAGCCUG GACACU-3'; Antisense: 5'-GUGUCCAGGCUCCAA AUGCCU-3') were respectively added to the supernatant of EPCs isolated from the peripheral blood samples of T2DM patients.

To clarify the effect of miR-130a on EPCs function, cell model II was established as a Control group and a pre-miR-130a group, while cell model III was established as a Control group and an anti-miR-130a group. For the pre-miR-130a group and the anti-miR-130a group, pre-miR-130a (5'-GCUUGUGCAAUGUUAAAAG GGCAUUUACACUCC-3') and anti-miR-130a (5'-AUGCCCUUUUAACAUUGCACUG-3') were respectively transfected into EPCs using electroporation.

To further clarify the effect of IL-18 on EPCs functions, cell model IV was established as a Control group and an IL-18 group, while cell model V was established as a Control group and an anti-IL-18 group. For the IL-18 group, IL-18 small molecule compounds were added to the supernatant of EPCs cultured from the peripheral blood samples from healthy controls. For the anti-IL-18 group, IL-18 inhibitors (Sense: 5'-GAAGACAGCUACG ACAA AUGG-3'; Antisense: 5'-CCAUUUGUCGUAGCUGUCUUC-3') were added to the supernatant of EPCs cultured from peripheral blood samples from T2DM patients.

For all cell models, the levels of TGF-β1 and IL-18, as well as the relative expressions of miR-130a and IL-18 mRNA, were evaluated for comparison. For cell model II, III, IV and V, EPCs functions including tube formation ability, adhesion ability and migration ability were assessed.

The ethics committee of Ningbo No.2 Hospital has approved the protocols of this study. All methods were carried out in accordance with the latest version of Declaration of Helsinki. Informed consent was obtained from all subjects before the initiation of this study.

RNA isolation and real-time polymerase chain reaction (PCR)

The EPCs were lysed and centrifugated for the extraction of total RNA by TRIzol reagent (Thermo Fisher Scientific, MA, US) according to the manufacturer's protocols. The isolated RNA was quantified using a spectrophotometer and the purity was assessed by the A260/A280 ratio. RNA was reverse transcribed into cDNA using TaqMan reverse transcription kit (Thermo Fisher Scientific, MA, US) according to kit instructions. And real-time PCR analysis was performed to evaluate the relative expressions of miR-130a, IL-18 mRNA and IFN-γ mRNA using 7500 Real-Time PCR System (Applied Biosystems, Waltham, MA, USA). The forward primer sequence for miR-130a is 5'-CACATTGTGCTACTGTCT-3', and the reverse primer sequence for miR-130a is 5'- GAACATGTC TGCGTATCTC-3'. The forward primer sequence for IL-18 mRNA is 5'-GATAGCCAGCCTAGAGGTATGG-3', and the reverse primer sequence for IL-18 mRNA is 5'-CCTTGATGTTATCAGGAGGATTCA-3'. The forward primer sequence for IFN-γ is 5'-TGAACGCTACACACTGCATCTTGG-3', and the reverse primer sequence for IFN-γ is 5'-CGACTCCTTTTCCGCTTCCTGAG-3'. The relative expression of miR-130a and IL-18 mRNA were respectively normalized to the expression of U6 and GAPDH using the 2ΔΔCt method.

Luciferase assays

Sequence analysis suggested that IL-18 and TGF-β1 could potentially bind to miR-130a via specific binding regions. The binding sequences of IL-18 3'-UTR or TGF-β1 3'UTR were respectively cloned to the downstream of the luciferase reporter gene in the pGL3 firefly luciferase reporter vector (Cat.No. E1751, Promega, WI, US). Meanwhile, miR-130a and scrambled miRNAs were synthesized and respectively used to treat the NIH 3T3 cells. For the validation of bindings between IL-18 and miR-130a, cell groups were established as miR-130a + blank plasmid group, miR-130a + IL-18 3'UTR group, and scramble miRNA + IL-18 3'UTR group. For the validation of bindings between TGF-β1 and miR-130a, cell groups were established as miR-130a + blank plasmid group, miR-130a + TGF-β1 3'UTR group, and scramble miRNA + TGF-β1 3'UTR group. 24 h after the establishment of cell group, dual-luciferase assays were performed to evaluate and compare the luciferase activity of different cell groups.

Tube formation assay

The ability of EPCs to create tubule-like structures was evaluated through a modified tube formation assay as described previously[26]. 24-well plates were coated with a cold Matrigel solution (Sigma-Aldrich, St. Louis, MO, USA) and incubated at 37 °C for a minimum of 30 min to facilitate solidification of the Matrigel. The EPCs were then resuspended in a medium containing 2% reduced fetal bovine serum (FBS) and seeded at the Matrigel-coated plates at a density of 1×10^4 cells/ml. Subsequently, the cells were pre-incubated for 30 min at 37 °C to ensure attachment to the Matrigel substrate. After 24 h of incubation, the development of the tubular structures was observed and captured using a CytoSMART™ Live Cell Imaging System (Lonza Bioscience, Morrisville, NC, USA).

Cell adhesion assay

For the assessment of cell-matrix adhesion, the procedure was carried out as detailed in a previous publication[27]. Briefly, EPCs were seeded at fibronectin-precoated culture plates at a density of 1×10^4 cells/well were plated onto 24-well plates coated with fibronectin and incubated for 30 min at 37 °C. Subsequently, the plates were rinsed with PBS for three times to remove non-adherent cells, and the adherent cells were fixed and stained using Hoechst 33,258 (Beyotime, Shanghai, CN). Fluorescence microscopy (Nikon, Tokyo, JP) was utilized to visualize and enumerate cells in five random fields.

Transwell assay

For the Transwell assay, EPCs were placed in the upper chamber at a density of 1×10^5 cells/ml using a Corning apparatus with an 8-μm pore size. The lower chamber was filled with culture medium containing 1% FBS. EPCs were then incubated for migration for 12 h at 37 °C. Subsequently, the membrane of the upper chamber was carefully extracted, rinsed, and fixed with 4% paraformaldehyde for 30 min at room temperature. The membrane was then excised and stained with crystal violet. Subsequently, the migrated cells were examined using a fluorescence microscope (Nikon, Tokyo, JP) by counting 10 random fields for each condition. Each experiment was performed in triplicates.

Enzyme-linked immunosorbent assay (ELISA) assay

We conducted ELISA assays to assess the levels of inflammatory cytokines in different EPC groups. The cells were initially lysed in a RIPA lysis buffer (Invitrogen, Thermo Fisher Scientific, MA, US) and then subjected to centrifugation at 1070 xg for 10 min to eliminate cellular debris. Subsequently, we employed ELISA kits for IL-18 (Cat No. DL180, R&D systems, MN, US) and TGF-β1 (Cat. No. DB100C, R&D systems, MN, US) to quantify the production of these cytokines according to the instructions provided by manufacturer.

Statistical analysis

All values are expressed as the mean ± SD. The comparisons between cell model I and luciferase assay cell groups were analyzed using one-way ANOVA, corrected by Tukey's post hoc test. And the comparisons between cell model II, III, IV and V were analyzed using Student's t-test. All statistical analysis were performed using SPSS software (Version 26, IBM, Endicott, NY, USA). A value of $p \leq 0.05$ was considered as statistically significant.

Results

The effect of TGF-β1 and anti-TGF-β1 on IL-18 and miR-130a

EPCs isolated from T2DM patients were treated with either TGF-β1 or anti-TGF-β1, and their effects on TGF-β1 and IL-18 levels in the supernatant, as well as miR-130a expression, were assessed. As shown in Fig. 1, ELISA results indicated that EPCs treated with TGF-β1 exhibited significantly higher levels of TGF-β1 (A) and IL-18 (B) in the supernatant compared to cells in the control group. In contrast, treatment with anti-TGF-β1 significantly reduced TGF-β1 levels, indicating effective neutralization of the endogenous TGF-β1 activity by the anti-TGF-β1 (A). Besides, anti-TGF-β1 treatment resulted in a marked decrease in IL-18 levels (B). Furthermore, PCR analysis revealed that the relative expression of miR-130a (C) was evidently downregulated in the TGF-β1 group and upregulated in the anti-TGF-β1 group. These changes TGF-β1 and anti-TGF-β1 potentially suggest that the expressions of TGF-β1 are negatively associated with the expressions of miR-130a.

Establishment of TGF-β1/miR-130a/IL-18 signaling pathway

To establish the regulatory relationship between miR-130a, TGF-β1 and IL-18, luciferase reporter assays were performed following sequence analysis. As shown in Fig. 2, our sequence analysis results confirmed that miR-130a could bind to IL-18 at specific binding sites (A), and the luciferase activity in cells co-transfected with miR-130a and the IL-18 3'UTR was significantly lower than that in cells co-transfected with scramble miRNAs

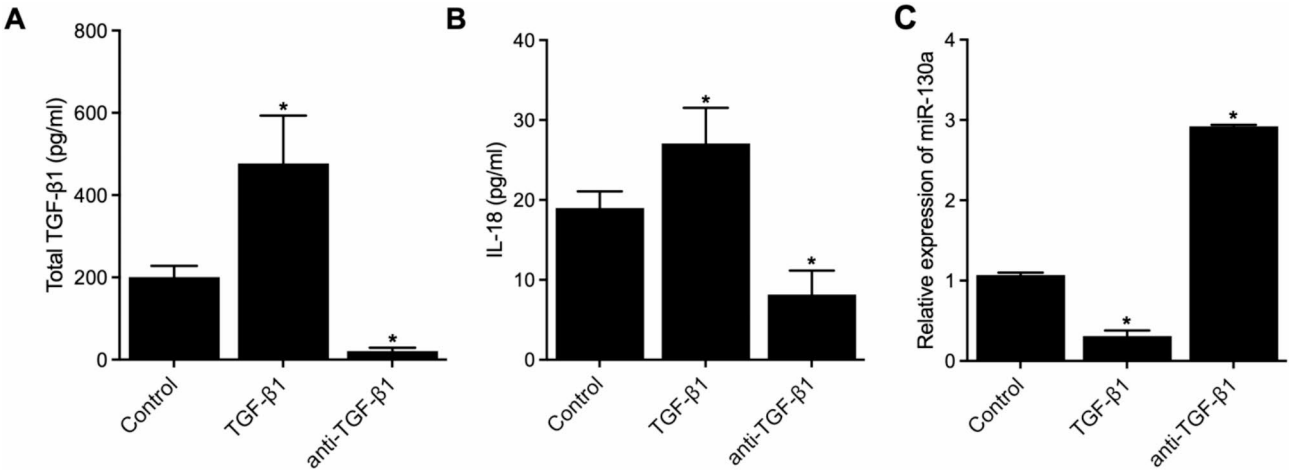

Fig. 1. The effect of TGF-β1 and anti-TGF-β1 on IL-18 and miR-130a expressions. (**A**) The level of total TGF-β1 was evidently up-regulated in the TGF-β1 cell group, while being suppressed in the anti-TGF-β1 cell group; (**B**) The level of IL-18 significantly increased in the TGF-β1 cell group, and was inhibited in the anti-TGF-β1 cell group; (**C**) The relative expression of miR-130a was suppressed in the TGF-β1 cell group, and the administration of anti-TGF-β1 promoted the miR-130a levels.

and IL-18 3'UTR, or cells co-transfected miR-130a and blank plasmids (B). This result indicates that miR-130a directly targets the 3'UTR of IL-18. Moreover, sequence analysis also suggested potential binding sites of miR-130a on the 3'UTR of TGF-β1 mRNA (C), and the corresponding luciferase assay exhibited evidently suppressed luciferase activity in cells co-transfected with miR-130a and TGF-β1 3'UTR (D), validating the direct molecular binding between miR-130a and TGF-β1.

Gain-of-function and loss-of-function analysis of miR-130a in EPCs

To further explore the role of miR-130a in EPCs, cells were transfected with either pre-miR-130a to overexpress miR-130a or anti-miR-130a to inhibit its expression. As shown in Fig. 3, the successful overexpression of miR-130a was confirmed by the evidently increased miR-130a level (A) and the effective knockdown of miR-130a was confirmed by the barely-detected miR-130a relative expression levels in the anti-miR-130a group (H). Moreover, the relative expression of IL-18 mRNA (B), as well as IL-18 production (C), was significantly reduced in the pre-miR-130a group compared to controls. Similar results were observed of the TGF-β1 level (D) in the pre-miR-130a group, suggesting that miR-130a negatively regulates TGF-β1 and IL-18 expressions. By contrast, in the anti-miR-130a group, IL-18 mRNA (I), IL-18 production (J) and TGF-β1 levels (K) were all significantly elevated, further supporting the regulatory role of miR-130a in suppressing IL-18 expression. For the changes in EPCs functions, suppressed tubule formation (E), adhesion (F), and migration (G) were observed in the pre-miR-130a group, while enhanced tubule formation (L), adhesion (M), and migration (N) were observed in the anti-miR-130a group.

Gain-of-function and loss-of-function analysis of IL-18 in EPCs

As shown in Fig. 4, PCR analysis showed that IL-18 treatment in the cells significantly decreased miR-130a expression (A) while elevating IL-18 mRNA expression (B), indicating that IL-18 may suppress miR-130a expression. Conversely, in the anti-IL-18 cell group of which the relative expression of IL-18 mRNA was suppressed (J), miR-130a levels were significantly increased (I), suggesting that inhibition of IL-18 activity can relieve suppression on miR-130a. And ELISA results indicated that TGF-β1 levels were elevated in the IL-18 treated group (D), while anti-IL-18 treatment reduced TGF-β1 levels (L), further confirming the regulatory relationship between IL-18 and TGF-β1. As expected, IL-18 levels were significantly higher in the IL-18 treated group (C) and barely detected in the anti-IL-18 group (K), and IFN-γ mRNA was induced by overexpression of IL-18 (E) while being inhibited by knockdown of IL-18 (M). In respect to EPCs functions, IL-18 treatment promoted EPC tubule formation (F), enhanced EPC adhesion (G) and migration ability (H), while anti-IL-18 treatment exhibited the opposite effects on EPCs functions (N-O).

In summary, our results suggested that the TGFβ1/miR-130a/IL-18 axis plays a pivotal role in the regulation of EPC functions in T2DM. The intricate regulatory network uncovered by our experiments offers novel insights into the molecular mechanisms underlying EPC dysfunction and suggests potential therapeutic targets for T2DM and its associated complications.

Discussion

EPCs are a type of precursor cell originated from bone marrow. They can be mobilized from the bone marrow into peripheral blood under certain physiological or pathological stimuli. Moreover, EPCs can also proliferate and differentiate into vascular endothelial cells in vitro and integrate into ischemic tissues in vivo to form new blood vessels[6,7]. Their main function is to participate in angiogenesis in ischemic tissues and the repair of blood vessels

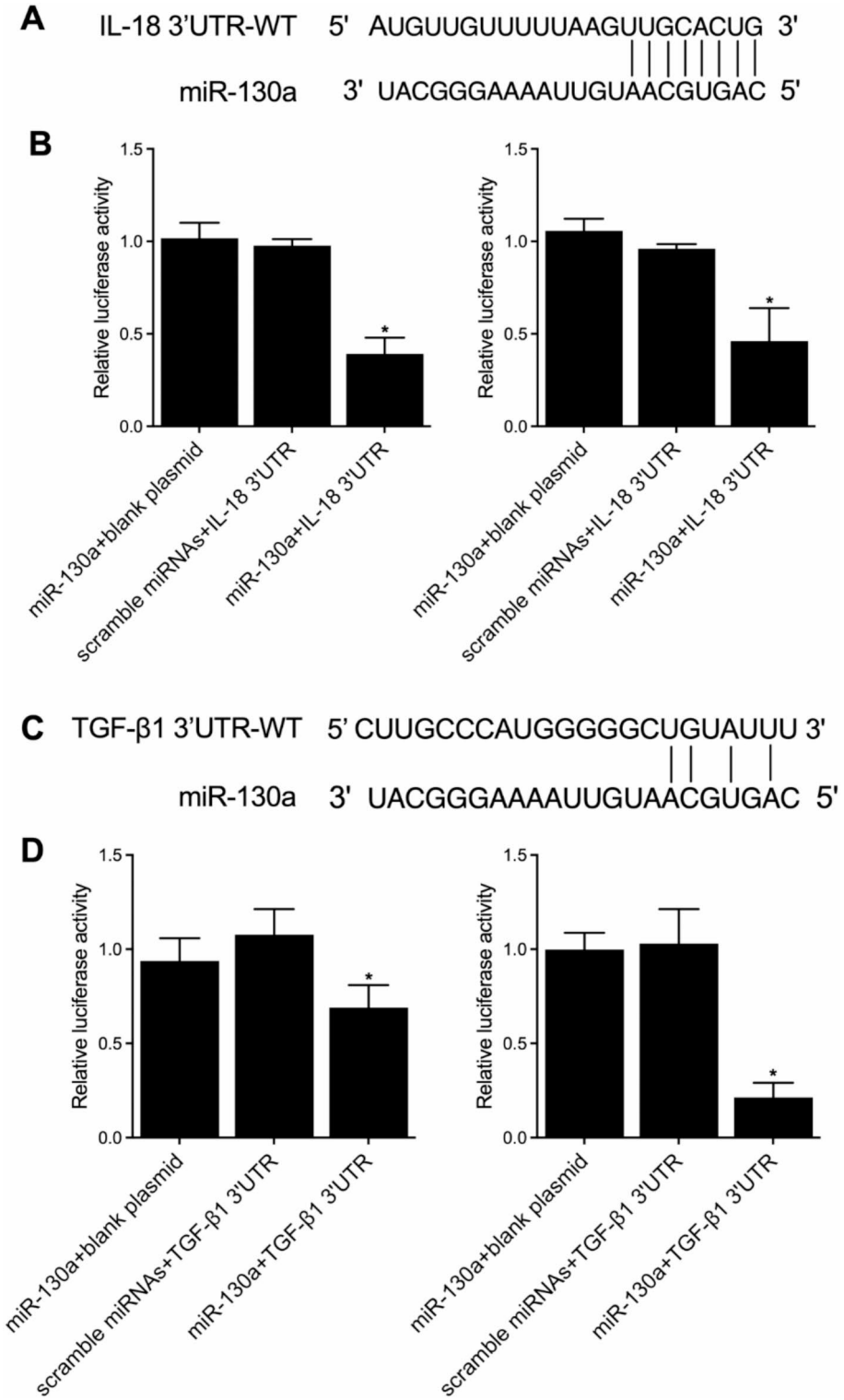

Fig. 2. The establishment of TGF-β1/miR-130a/IL-18 signaling pathway. (**A**)Sequence analysis confirmed that miR-130a could bind to IL-18 at specific binding sites; (**B**) Luciferase activities of cells co-transfected with miR-130a and the IL-18 3'UTR was significantly lower than the miR-130a + blank plasmid group, or scramble miRNAs + IL-18 3'UTR group. (**C**) Sequence analysis confirmed potential binding between miR-130a and TGF-β1 3'UTR; (**D**) Luciferase activities of cells co-transfected with miR-130a and TGF-β1 3'UTR was significantly lower than the miR-130a + blank plasmid group, or scramble miRNAs + TGF-β1 3'UTR group.

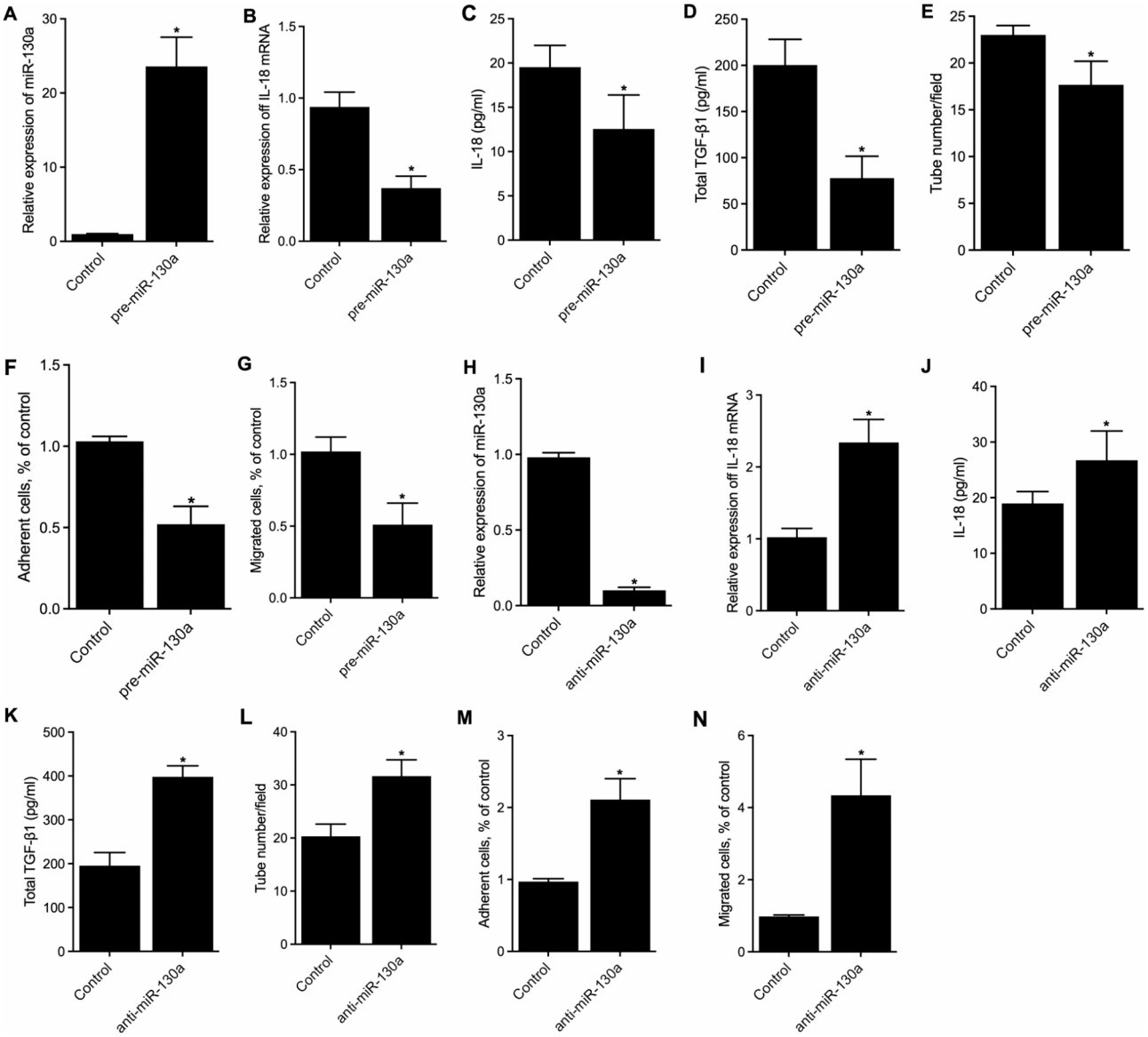

Fig. 3. Gain-of-function and loss-of-function analysis of miR-130a in EPCs. (**A**) Relative expression of miR-130a was sharply elevated in cells treated with pre-miR-130a; (**B**) Relative expression of IL-18 was evidently suppressed in cells treated with pre-miR-130a; (**C**) IL-18 concentration was decreased by the treatment of pre-miR-130a; (**D**) The level of total TGF-β1 was reduced by pre-miR-130a administration; (**E**) Tube number/field was lower in the pre-miR-130a cell group compared to the Control cell group; (**F**) The number of adherent cells was reduced by the administration of pre-miR-130a; (**G**) The number of migrated cells was lower in the pre-miR-130a cell group compared to the Control cell group; (**H**) The successful administration of anti-miR-130a was validated by the reduced levels of miR-130a; (**I**) Relative expression of IL-18 was significantly elevated in cells treated with anti-miR-130a; (**J**) IL-18 production was promoted in the loss-of-function analysis of miR-130a; (**K**) The level of total TGF-β1 was promoted in the loss-of-function analysis of miR-130a; (**L**) Tube number/field was increased in the anti-miR-130a cell group; (**M**) The number of adherent cells was promoted by the administration of anti-miR-130a; (**N**) The number of migrated cells was increased in the anti-miR-130a cell group compared to the Control cell group.

after injury postnatally. EPCs are primarily found in the bone marrow, with very few detected in the peripheral blood, comprising only 0.01% of peripheral cells. The EPCs in peripheral blood are mainly mobilized from the bone marrow and chemotactically migrate to ischemic sites to participate in angiogenesis and endothelial repair. In DM patients, the functions of EPCs including tube formation, adhesion, and migration are crucial for the maintenance of vascular health and the repair of damaged blood vessels. Upon the onset of T2DM, functionality of EPCs is significantly impaired, which can lead to the vascular complications in DM patients. Specially, the tube formation ability of EPCs is significantly reduced, and this impairment is largely attributed to the hyperglycemic environment, which affects the EPCs' proliferative and differentiative capacities[28]. Studies have suggested that

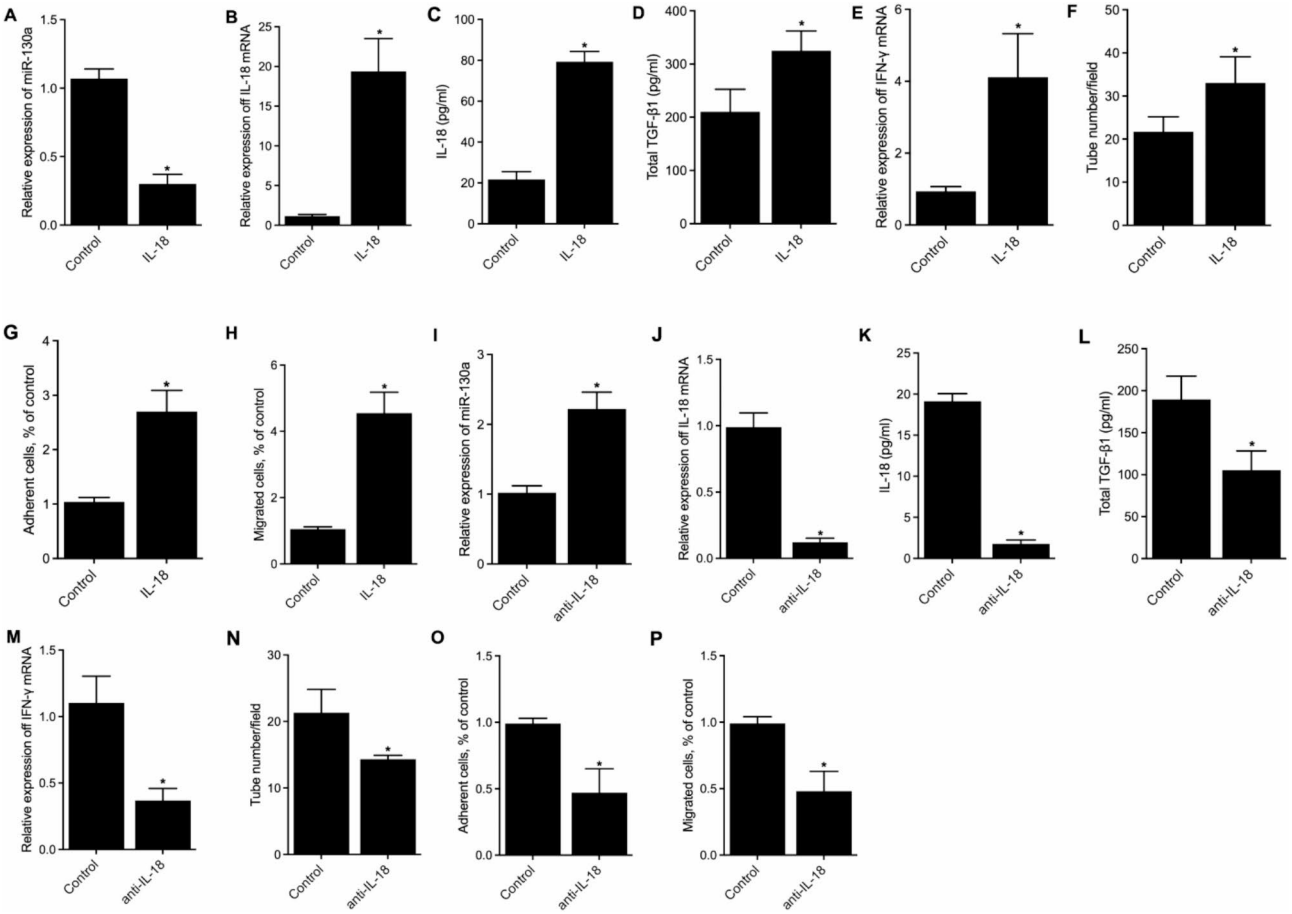

Fig. 4. Gain-of-function and loss-of-function analysis of IL-18 in EPCs (* $P < 0.05$ vs. Control group). (**A**) Relative expression of miR-130a was significantly decreased in the IL-18 treated group; (**B**) IL-18 treatment significantly promoted the relative expression of IL-18 in the IL-18 group. (**C**) IL-18 levels were significantly higher in the IL-18 treated group; (**D**) The level of total TGF-β1 was elevated in the IL-18 treated group; (**E**) Overexpression of IL-18 significantly induced the relative expression of IFN-γ mRNA in the IL-18 group. (**F**) IL-18 treatment promoted EPC tubule formation by increasing tube number/field; (**G**) IL-18 treatment promoted EPC adhesion in the gain-of-function analysis of IL-18; (**H**) IL-18 treatment promoted EPC migration ability in the gain-of-function analysis; (**I**) Relative expression of miR-130a was significantly increased in the anti-IL-18 group; (**J**) Relative expression of IL-18 mRNA was significantly suppressed in the IL-18 treated group; (**K**) IL-18 production was inhibited in the loss-of-function analysis of IL-18; (**L**) The administration of anti-IL-18 decreased the levels of total TGF-β1; (**M**) Knockdown of IL-18 significantly suppressed the relative expression of IFN-γ mRNA; (**N**) Anti-IL-18 treatment impaired EPC tubule formation by decreasing tube number/filed; (**O**) Anti-IL-18 treatment reduced EPC adhesion in the loss-of-function analysis of IL-18; (**P**) Anti-IL-18 treatment decreased EPC migration ability in the loss-of-function analysis.

the increased oxidative stress, inflammatory responses, and high glucose levels in DM patients may disrupt the normal functions of EPCs and thus inhibit tube formation by reducing the expression of angiogenic factors such as VEGF[29]. Besides, the adhesion of EPCs to the extracellular matrix is a fundamental step in vascular repair and regeneration in T2DM. Studies have indicated that EPC adhesion from DM patients is compromised compared to those from healthy individuals[30,31]. This reduction in adhesive capacity could be attributed to hyperglycemia and insulin resistance, which lead to alterations in adhesion molecules such as integrins and selectins[32]. For example, a previous study by Su et al. indicates that reduced adhesion capacity in diabetic EPCs is due to the decreased activation of signaling pathways involved in cell adhesion, such as the PI3K/Akt pathway[33]. Meanwhile, EPC migration to sites of ischemia or vascular injury is vital for effective neovascularization and endothelial repair. However, in T2DM patients, the chemotactic response of EPCs is markedly impaired. This defect in migration can be attributed to the alterations of chemokines and their receptors, such as SDF-1 and CXCR4, under diabetic conditions, and studies have demonstrated that hyperglycemia and advanced glycation end products (AGEs) negatively impact EPC migration by disrupting the actin cytoskeleton and impairing the responsiveness to chemotactic signals[34]. In this study, we isolated EPCs from T2DM patients and treated the cells with TGF-β1 or anti-TGF-β1 to observe the effects of TGF-β1 dysregulation in EPCs. Accordingly, we found that the expressions of TGF-β1 were negatively correlated with the expressions of miR-130a, while being positively

correlated with the expressions of IL-18. Therefore, we further explored the effects of miR-130a or IL-18 on the functions of EPCs.

It is acknowledged that miR-130a plays a pivotal role in the regulation of EPCs. In EPCs derived from DM patients, the downregulation of miR-130a was also observed[35], and the reduced miR-130a expression has been found to be associated with impaired EPC functions, including decreased proliferation, migration, and differentiation, as well as increased apoptosis[35]. One study investigated the underlying molecular mechanisms and suggested that miR-130a negatively regulates Runx3, a gene involved in the promotion of EPC dysfunction in diabetes[36]. Moreover, miR-130a has been shown to modulate the autophagy of EPCs, a process critical for maintaining cellular homeostasis and survival. In a study by Xu et al., it was found that miR-130a regulates autophagy through Runx3, thereby affecting the survival of EPCs[37]. This suggests that miR-130a could be a potential therapeutic target for improving EPC function in diabetic patients. Besides, the high glucose levels in DM patients can affect miR-130a expression, leading to sustained activation of the JNK pathway and enhancing EPC apoptosis and dysfunction[35]. However, different from previous studies, we also observed that in EPCs overexpressing miR-130a, tube formation, cell adhesion and migration were inhibited. We assume that such discrepancies may be due to the fact that the effect of miRNAs can be quite different in different pathological conditions or genetic backgrounds. The regulatory effects of miRNAs are commonly identified via the modulations of its downstream target gene. For this study, we identified a negative regulatory relationship between IL-18 and miR-130a. IL-18 had a positive effect on EPC functions, overexpression of miR-130a exhibited a negative effect on EPC functions.

As for IL-18 in the miR-130a/IL-18 axis, it is a potent pro-inflammatory cytokine that plays a significant role in the regulation of immune responses, particularly in promoting Th1-type reactions. Although its direct relationship with EPCs has not been extensively explored, IL-18 could potentially impact EPCs through its effects on inflammation, which is known to alter EPC mobilization, differentiation, and function. For example, previous studies have suggested that IL-18 might be implicated in the pathogenesis of several vascular diseases, such as atherosclerosis, by influencing the inflammatory processes within the vessel wall[38]. Given that EPCs contribute to the maintenance of vascular integrity and repair, it is suspected that IL-18 could indirectly regulate EPC activity through its impact on vascular inflammation. Moreover, IL-18 has been shown to be involved in the regulation of other cytokines and growth factors that are known to affect EPCs, such as VEGF and GM-CSF[39]. These factors are crucial for EPC mobilization from the bone marrow, survival, and differentiation into mature endothelial cells. In this study, we found that the cell functions including tubule formation, cell adhesion and migration ability were all impaired by the upregulation of IL-18. As IFN-γ has been demonstrated to be regulated IL-18[40], while IFN-γ can regulate the expressions of TGF-β1[41], it can be suggested that IL-18 can regulate the expression of TGF-β1 via the modulation of IFN-γ. Moreover, since the establishment of the miR-130a/TGF-β1 axis has also been reported[42], it can be inferred that the overexpression or knockdown of IL-18 can inhibit or promote the expression of miR-130a. In this study, By examining the relative expression of IFN-γ mRNA, we verified that the upregulation of IL-18 not only suppressed the expression of miR-130a, but also promoted the levels of TGF-β1.

However, there are limitations of this study. Although we evaluated functions of EPCs including tube formation, adhesion and migration, we did not check the expression of VEGF and other angiogenic genes, which may also influence the functions of EPCs. In our future study, we will include the study of angiogenic genes to exclude the potential infllluecnes of confounding factors.

Conclusion

In this study, we elucidated the impact of the TGFβ1/miR-130a/IL- 8 signaling pathway on the function of EPCs, thus providing theoretical basis for the development of miRNA-targeted therapeutic strategies for patients withT2DM and associated complications.

Data availability

Data are available upon reasonable request from the corresponding author.

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Author contributions

Yawei Cai designed and supervised this study, Yawei Cai, Yao Zhu and Ning Xu performed the experiments, Yawei Cai and Tongen Chen collected and analyzed the data, Yawei Cai composed the manuscript and Tongen Chen revised the manuscript. All co-authors approved the final manuscript.

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Declarations

Ethics approval and consent to participate

The ethics committee of Ningbo No.2 Hospital has approved the protocols of this study. All methods were carried out in accordance with the latest version of Declaration of Helsinki. Informed consent was obtained from all subjects before the initiation of this study.

Competing interests

The authors declare no competing interests.

Additional information

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