MiR-488 facilitates wound healing through CYP1B1-mediated Wnt/β-catenin signaling pathway by targeting MeCP2

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Research Article

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Abstract

**Background:** Diabetic wounds are difficult to heal, but its pathogenesis has not been elucidated. MicroRNAs (miRNAs) are considered to act as key roles in wound healing. In this paper, the role of miR-488 in wound healing was investigated.

**Methods:** The mRNA and protein expressions were assessed using RT-qPCR and western blot. The gene methylation was measured by MSP assay. Cell proliferation, apoptosis and migration were assessed using BrdU, flow cytometer and wound healing assay, respectively. Additionally, the angiogenesis ability of HUVEC cells was analyzed using *in vitro* angiogenesis assay. Dual-luciferase reporter assay was adopted to analyze the interaction between miR-488 and MeCP2.

**Results:** Here our results displayed that miR-488 and CYP1B1 expressions were markedly reduced in wound tissues of diabetic with skin defect, while MeCP2 was significantly upregulated. Function assays displayed that miR-488 promoted cell proliferation and migration as well as HUVEC cell angiogenesis through regulation of MeCP2, while inhibited the apoptosis. MiR-488 overexpression could also accelerate wound healing *in vivo*. MeCP2 functioned as the target of miR-488, and suppressed wound healing *in vitro*. We subsequently confirmed MeCP2 suppressed CYP1B1 expression via promoting its methylation status. In addition, CYP1B1 knockdown inhibited wound healing. Furthermore, MeCP2 overexpression abolished the promoting effect of miR-488 on wound healing. It was also turned out that Wnt4/β-catenin pathway was the downstream pathway of miR-488/MeCP2/CYP1B1 in regulating wound healing.

**Conclusion:** MiR-488 is a potential therapeutic target for diabetic wound healing, since miR-488 overexpression promoted wound healing through activating CYP1B1-mediated Wnt4/β-catenin signaling pathway by targeting MeCP2.

1 Introduction

Skin refers to the tissue covering the surface of the human body and in direct contact with the external environment with the functions of anti-friction and anti-infection. Skin injury is a common event after accidental trauma [1]. Wound healing consists of four processes: inflammation, cell proliferation, scar formation and tissue regeneration, involving a variety of skin cells, including vascular endothelial cells, keratinocytes and fibroblasts [2, 3]. Delayed wound healing has always been an important health problem worldwide, especially among diabetic patients [4]. Approximately 15% of diabetic patients are at risk of developing non-healing ulcers [5]. According to reports, the cost of treating diabetic wounds is approximately $10 billion per year in USA [6], imposing a huge burden on patients’ lives. However, wound healing is a complicated process, and its mechanism involved is still unclear.

MicroRNAs (MiRNAs) refer to single-stranded non-coding RNAs with a length 20–22 nts, which participate in regulating many biological processes including wound healing [5, 7]. As proof, miR-497 expression in the skin wounds of diabetic mice was significantly reduced compared with normal mice,
and its upregulation accelerated wound healing [4]. In addition, miR-152-3p inhibition could enhance diabetic wound repair [8]. MiR-488 was previous confirmed as a target for treatment of multiple diseases, consisting of colorectal cancer [9] and panic disorder [10]. Notably, miR-488 overexpression could enhance the viability of osteoblasts and inhibit cell apoptosis, thereby promoting fracture healing [11]. Nevertheless, the function of miR-488 in regulating diabetic wound healing is largely unknown, which deserves to be further probed.

Methyl-CpG-binding protein 2 (MeCP2), functions in inhibiting the expression of downstream targets by binding to methylated DNA [12]. The role of MeCP2 in skin biology and diseases has been studied [13]. As proof, MeCP2 overexpression in fibroblasts could fibroblast proliferation and migration [14]. In addition, MeCP2 silencing was reported to promote myofibroblast transdifferentiation to enhance wound healing generated at sites of injury [13]. However, the mechanism of MeCP2 regulating wound healing hasn’t been elucidated. Herein, it was predicted that miR-488 had potential binding sites to MeCP2 through TargetScan, StarBase and miRDB.

Cytochrome P450 1B1 (CYP1B1) is a monooxygenase belonging to the cytochrome P450 family. As previously reported, upregulation of CYP1B1 was observed in previously injured and dexamethasone (a drug widely used in clinical practice to improve wound healing)-treated skin [15], indicating the beneficial effects of CYP1B1 in wound healing. Notably, CYP1B1 was the target of MeCP2 in regulating lung epithelial cell injury [16]; however, whether the similar regulatory relationship exists in wound healing remains to be further probed.

Herein, the function of miR-488 in regulating wound healing was probed. MiR-488 promoted wound healing through CYP1B1-mediated Wnt4/β-catenin signaling pathway by targeting MeCP2. Our research provided a theoretical basis for the treatment of diabetic wound healing.

2 Materials and Methods

Clinical samples collection

Wound tissues of diabetic patients with long-term skin defects were collected from the area around the lesion of the skin ulcer in diabetic patients in conventional surgeries. The control group was composed of 30 patients who did not have diabetes but who had undergone an orthopedic operation for a non-traumatic disease. All samples were stored at -80 °C for further detection. This study was passed the review of Ethics Committee of Xiangya Hospital of Central South University. All patients signed informed consent forms.

Cell culture

Human skin fibroblasts (HSF), keratinocytes (HaCaT) and human umbilical vein endothelial cells (HUVEC), purchased from ATCC (VA, USA), were cultured in DMEM (Gibco, MD, USA) containing 10% FBS (Gibco) at 37°C with 5% CO₂.
Cell transfection

The overexpression plasmid of MeCP2 (oe-MeCP2), the short hairpin RNA of CYP1B1 (sh-CYP1B1) and mimics/inhibitor of miR-488 and the negative controls, obtained from GenePharma (Shanghai, China), were transfected into cells with Lipofectamine™ 3000 (Invitrogen, CA, USA).

Wound healing assay

Cells were cultured in 6-well plates (5 × 10^5 cells/well, Corning, NY, USA) for 12h. After removing the medium, an artificial wound was created using a sterile pipette tip. Cells were washed, cultured, and the images were taken at 0 h and 24 h.

5-bromo-2'-deoxyuridine (BrdU) assay

Cells were seeded into 24-well plates and incubated with BrdU solution (10 μM, RiboBio, Guangzhou, China) for 4 h. Cells were washed 3 times with PBS and fixed with 4% paraformaldehyde for 10 min, and the DNA was denatured. The unspecific antigens were blocked using 1% bovine serum albumin. Then cells were incubated with BrdU monoclonal antibody (ab6326, 1: 300, Abcam, Cambridge, UK) overnight followed by incubation with secondary antibody (Beyotime) for 2 h. The nuclei were stained with DAPI (RiboBio), and cells were observed by a fluorescent microscope.

Cell apoptosis assay

Cells were re-suspended in Annexin-binding buffer (500 μL, Beyotime) and subsequently stained with Annexin V-FITC (10 μL) and PI stain for 10 min and subjected to flow cytometry (BD, NJ, USA).

Angiogenesis assay of HUVECs

HUVEC Cells were plated into 96-well plates (Corning) coated with Matrigel (1:1 dilution, BD, NJ, USA). After cell attachment, cells were cultured in complete medium for 24 h. Angiogenesis was observed under a microscope (Nikon).

Quantitative real-time polymerase chain reaction (RT-qPCR)

After total RNA was extracted with TRIzol (ThermoFisher Scientific, MA, USA), miRNAs were collected by the miRNA isolation kit (Takara, Osaka, Japan) and detected using Taqman miRNA assay kit (Takara). The cDNA was synthesized with reverse transcriptase kits (Toyobo, Tokyo, Japan) and subjected to RT-qPCR assay using SYBR (Thermo Fisher Scientific). GAPDH and U6 were used as the reference genes. The data was analyzed with 2^-ΔΔCT method. The primers used in the study were listed as follows (5’-3’):

miR-488 (F): CGGGGCAGCUCAGUACAG

miR-488 (R): CAGTGCGTGTCGTGGAGT
Western blot

The proteins were isolated with RIPA, which further transferred to a PVDF membrane (Millipore, MA, USA). Then, membranes were incubated with antibodies against MeCP2 (Abcam, 1:1000, ab253197), CYP1B1 (Abcam, 1:1000, ab185954), Wnt4 (Abcam, 1:1000, ab262696), β-catenin (Abcam, 1:1000, ab32572), and β-actin antibody (Abcam, 1:10000, ab8226) overnight, then hybridized with secondary antibody (Abcam, 1:10000, ab7090) for 60 min. Blots were visualized by GEL imaging system (Bio-Rad, CA, USA) and subsequently analyzed with ImageJ software.

Dual luciferase reporter assay

MeCP2 3′-UTR fragment containing miR-488 binding site were amplified by PCR, and inserted into the pGL3 vector (GenePharma). Then, cells were co-transfected with wt-MeCP2 or mut-MeCP2 plasmids and miR-488 mimics/inhibitor or mimics/inhibitor NC. The luciferase activity was subsequently assessed.

Methylation specific PCR (MSP)

Genomic DNA was isolated using the DNA mini kit (Qiagen, Dusseldorf, Germany), and subjected to bisulfite. MSP of bisulfite-transformed DNA was performed with a nested, two-stage PCR method as previous described [17]. PCR products were analyzed by gel electrophoresis.

Construction of in vivo wound model

A total 24 male SD rats (8 weeks of age) were purchased from SJA Laboratory Animal Co., Ltd (Hunan, China). This study was passed the review of Ethics Committee of Xiangya Hospital of Central South University. Rats were intraperitoneal injected with 50 mg/kg streptozotocin (STZ, Sigma-Aldrich, MO, USA) to induce diabetes. After 48 h, if the blood glucose concentration of the rat was greater than or equal to 16.7 mmol/L, it was considered to have diabetes. A full-thickness skin wound in the diabetic rats was
created as previously reported [18]. The diabetic rats were randomly divided into three groups: control group, ago-miR-NC (mimics NC) mimics group and ago-miR-488 (miR-488 mimics) group (8 rats for each). Lentivirus (2 × 10^8 IFU/mL) harboring mimics NC or miR-488 mimics (purchased from Hanbio, Shanghai, China) were injected intradermally into the wound edges of rats. Wound images were acquired on days 0, 3, 7 and 11. Wound samples were collected 14 days after injury.

**Hematoxylin-eosin (HE) staining**

The skin tissue was fixed with 4% paraformaldehyde overnight and cut into 4 μm thick. Then sections were dehydrated with gradient alcohol, washed and embedded in paraffin. The sections were subsequently stained with HE (Sigma-Aldrich). Sections were observed under a microscope (Nikon).

**Masson staining of skin tissues**

The skin tissue sections were stained with Weigert's hematoxylin (Solarbio, Beijing, China) and Masson's trichrome solution for 5 min, respectively. Sections were observed under a microscope (Nikon).

**Data analysis**

All the data were obtained from three independent experiments, analyzed by SPSS 19.0 (IBM, Armonk, NY) and presented as means ± SD. Between-group differences and multi-group comparisons were determined using Student's *t*-test and one-way ANOVA, respectively. The *p* value lower than 0.05 was referred as significant.

**3 Results**

### 3.1 miR-488, MeCP2 and CYP1B1 expressions in wound tissues of diabetic with skin defect

We firstly observed that miR-488 expression was markedly reduced in wound tissues of diabetic with skin defect compared to control skin tissues (Fig. 1A). In addition, results from RT-qPCR revealed that MeCP2 was highly expressed in wound tissues of diabetic with skin defect, while CYP1B1 was low expressed (Fig. 1B-C). All these results suggested that miR-488 and CYP1B1 expressions were markedly reduced in wound tissues of diabetic with skin defect compared to control skin tissues, while MeCP2 was significantly upregulated.

### 3.2 miR-488 facilitated cell proliferation and migration as well as angiogenesis *in vitro*

As reported, endothelial cells, keratinocytes and fibroblasts are all involved in the wound healing process [19]. To probe the potential role of miR-488 in wound healing, miR-488 overexpression and inhibition were induced in HSF, HaCaT and HUVEC cells, and cell behaviors and the angiogenic response of endothelial cells were evaluated. Firstly, miR-488 expression in HSF, HaCaT and HUVEC cells was markedly reduced by miR-488 inhibitor, while increased by miR-488 mimics (Fig. 2A), revealing the transfection was successful. As displayed in Fig. 2B-D, cell proliferation and migration of HSF and HaCaT
cells were markedly inhibited by miR-488 inhibitor, while promoted by miR-488 mimics. Besides, cell apoptosis showed the opposite trend. We then probed whether miR-488 could influence the angiogenesis of endothelial cells, and the result displayed that miR-488 mimics transfection resulted in enhanced angiogenesis, while miR-488 inhibitor transfection led to reduced angiogenesis (Fig. 2E). Taken together, miR-488 could promote HUVEC, HaCaT and HSF cell proliferation and migration as well as HUVEC cell angiogenesis in vitro, while suppressed the apoptosis.

3.3 MeCP2 functioned as the target of miR-488

As well known, miRNAs mediate post-transcriptional genes expressions through binding with its targets [20]. Through bioinformatics databases (TargetScan, StarBase and miRDB), it was predicted that miR-488 had a binding site to MeCP2 (Fig. 3A). As displayed in Fig. 3B, the luciferase activity presented by wt-MeCP2 reporter was significantly reduced by miR-488 mimics and elevated by miR-488 inhibitor but didn't affect mut-MeCP2 reporter activity, suggesting that miR-488 directly targeted MeCP2. In conclusion, we identified MeCP2 as the target of miR-488.

3.4 MeCP2 overexpression suppressed cell proliferation and migration as well as angiogenesis

Firstly, we observed that both MeCP2 expression in HSF and HUVEC cells was decreased by miR-488 mimics transfection and elevated by miR-488 inhibitor transfection (Fig. 4A-B). To further probe the function of MeCP2 in wound healing, MeCP2 overexpression was induced in HSF and HUVEC cells. As demonstrated in Fig. 4C-D, MeCP2 overexpression markedly increased MeCP2 expression in HSF and HUVEC. Functional experiments subsequently displayed that MeCP2 overexpression reduced HSF and HUVEC cell proliferation and increased the apoptosis (Fig. 4E-F). In addition, MeCP2 overexpression resulted in reduced HSF cell migration and HUVEC cell angiogenesis (Fig. 4G-H). In total, MeCP2 repressed wound healing.

3.5 MeCP2 suppressed cell proliferation and migration as well as angiogenesis through boosting CYP1B1 methylation

MeCP2 was previously reported to promote CYP1B1 methylation in lung epithelial cell injury [16], however the regulatory relationship between MeCP2 and CYP1B1 in wound healing remained unknown. As shown in Fig. 5A, CYP1B1 level in HSF and HUVEC cells was significantly suppressed by MeCP2 overexpression. Interestingly, we observed the methylation existed in the CYP1B1 promoter region, and 5-Aza reduced the methylation in the CYP1B1 promoter region (Fig. 5B). We also found that MeCP2 overexpression could promote CYP1B1 promoter methylation in HSF and HUVEC cells (Fig. 5C). To investigate the function of CYP1B1 in wound healing, sh-CYP1B1 was transfected into HSF and HUVEC cells, and we observed that CYP1B1 silencing resulted in the decreased protein level of CYP1B1 as well as reduced Wnt4 and β-catenin levels (Fig. 5D). Results from flow cytometry assay and BrdU assay showed that cell proliferation were obviously repressed following sh-CYP1B1 transfection, while cell apoptosis was promoted (Fig. 5E-F). Additionally, migratory ability of HSF cells was obviously inhibited following sh-CYP1B1 transfection (Fig. 5G), and the angiogenic responses of HUVEC cells was markedly suppressed by CYP1B1
knockdown (Fig. 5H). In summary, MeCP2 regulated cell proliferation, and apoptosis, HSF cell migration as well as HUVEC cell angiogenesis via regulation of CYP1B1 methylation.

3.6 miR-488 promoted cell proliferation and migration as well as angiogenesis through MeCP2-CYP1B1 axis-mediated Wnt4/β-catenin signaling pathway

To explore the potential function of miR-488/MeCP2/CYP1B1 axis in wound healing, miR-488 mimics and/or MeCP2-overexpression vector were transfected into HSF and HUVEC cells. Firstly, we observed that miR-488 was markedly upregulated in miR-488 mimics + sh-NC group and miR-488 mimics + MeCP2 group compared to other groups (Fig. 6A). It was also turned out that miR-488 overexpression resulted in reduced MeCP2 levels and elevated CYP1B1, Wnt4 and β-catenin levels in HSF and HUVEC cells, while MeCP2 overexpression eliminated the effects of miR-488 mimics on these proteins (Fig. 6B). In addition, cell apoptosis of HSF and HUVEC cells was inhibited after miR-488 upregulation, while MeCP2 overexpression abolished this effect of miR-488 mimics (Fig. 6C). Additionally, miR-488 overexpression significantly promoted cell proliferation, which were abolished by MeCP2 overexpression (Fig. 6D). Moreover, the migration of HSF cells and angiogenesis of HUVEC cells was markedly promoted by miR-488 overexpression, while MeCP2 overexpression reversed the effect of miR-488 mimics (Fig. 6E-F). In summary, miR-488 facilitates cell proliferation and migration as well as angiogenesis through CYP1B1-mediated Wnt4/β-catenin signaling pathway by targeting MeCP2.

3.7 miR-488 accelerated wound healing in vivo

We observed decreased MeCP2 expression and increased miR-488, CYP1B1 expressions in the skin of the rats injected with miR-488 mimics compared to controls (Fig. 7A). Results of western blot revealed that miR-488 mimics injection resulted in decreased MeCP2 level and increased CYP1B1, Wnt4, β-catenin levels in the skin of the rats (Fig. 7B). It was observed that miR-488 upregulation accelerated wound healing in rats (Fig. 7C). HE staining results demonstrated that the epidermis and dermis of the control rats were markedly thinner than those of the rats injected with miR-488 mimics (Fig. 7D). In addition, we observed more wavy collagen bers in skin of the rats injected with miR-488 mimics compared to controls (Fig. 7E). Taken together, miR-488 overexpression accelerated wound closure in vivo.

4 Discussion

As widely illustrated, miRNAs are expressed abnormally during wound healing [3]. In addition, miRNAs are considered as potential therapeutic targets in diabetic wound healing [21, 22]. Herein, we found that miR-488 was significantly downregulated in wound tissues of diabetic with skin defect compared to control skin tissues, which was never reported before. Functionally, miR-488 overexpression accelerated wound healing in vitro and in vivo. Mechanistically, miR-488 promoted CYP1B1-mediated Wnt4/β-catenin signaling pathway by targeting MeCP2. Therefore, our findings indicated that miR-488 had the potential to be a therapeutic target for diabetic wound healing.
Accumulating studies show that miRNAs are involved in regulating wound healing processes. For instance, Wang et al. revealed the topical administration of miR-129 and miR-335 upregulation accelerated wound healing in diabetic animals [5]. Besides, miR-497 overexpression around the wounds accelerated wound closure in diabetic mice [4]. MiR-488 was previously reported as a tumor suppressor in multiple cancers [23, 24]. The role of miR-488 in wound healing is largely unknown. In the current paper, we firstly observed that miR-488 was significantly downregulated in wound tissues of diabetic with skin defect compared to control skin tissues. We subsequently confirmed that miR-488 upregulation induced HUVEC, HaCaT and HSF cells proliferation and migration as well as HUVEC cell angiogenesis in vitro, while inhibited the apoptosis; and miR-488 inhibition showed the opposite effects. In addition, miR-488 overexpression accelerated wound closure in diabetic mice. All our above results suggested that miR-488 presented beneficial effects in wound healing processes.

Next, we focused on the downstream of miR-488. By using bioinformatics databases (TargetScan, StarBase and miRDB), we found that miR-488 had a binding site to MeCP2. By using dual-luciferase reporter assay, we confirmed their direct interaction. It was also observed that MeCP2 expression was enhanced by miR-488 inhibition, while was suppressed by miR-488 overexpression. MeCP2, the Rett syndrome factor, is the prototypic methylCpG-binding protein [25]. Recent studies have confirmed the important roles in wound healing. As proof, MeCP2 overexpression in hepatic stellate cells could suppress key genes regulating the myofibroblast phenotype [13]. Considering that myofibroblasts play an important role in wound healing, it’s suggested that MeCP2 is an inhibitor of wound healing [13]. It was also reported that MeCP2 overexpression inhibited a series of cellular events during wound healing, such as myofibroblast differentiation, fibroblast proliferation and fibroblast migration [14]. Nevertheless, the interaction between miR-488 and MeCP2 in wound healing hasn’t been elucidated. In the current study, function assays displayed that MeCP2 overexpression inhibited cell proliferation and HSF cell migration as well as HUVEC cell angiogenesis in vitro, while promoted the apoptosis. Taken together, miR-488 promoted wound healing via directly interacting with MeCP2. It was observed that CYP1B1 expression was markedly reduced in HSF and HUVEC cells following MeCP2 overexpression. It was previously reported that MeCP2 promoted CYP1B1 promoter methylation in epithelial cell injury [16]. However, the role of CYP1B1 in wound healing has never been reported before. Herein, we confirmed MeCP2 suppressed CYP1B1 expression via promoting its methylation status in HSF and HUVEC cells. In addition, CYP1B1 knockdown inhibited cell proliferation and HSF cell migration as well as HUVEC cell angiogenesis in vitro, while promoted the apoptosis. Moreover, MeCP2 overexpression abolished the promotion effects of miR-488 overexpression on cell proliferation and migration as well as angiogenesis in vitro. In conclusion, miR-488 might facilitate wound healing through CYP1B1 by targeting MeCP2.

Some studies have emphasized the role of Wnt/β-catenin signaling pathway in wound healing [26, 27]. For instance, Ma et al. demonstrated that mesenchymal stem cell-derived exosomes enhanced cell proliferation, migration, and inhibit cell apoptosis via activating Wnt/β-catenin signaling in cutaneous wound healing [28]. In the current study, we found that miR-488 overexpression increased Wnt4 and β-catenin levels in HSF and HUVEC cells, which was eliminated by MeCP2 overexpression. Collectively, miR-
488 promoted wound healing through activating CYP1B1-mediated Wnt4/β-catenin signaling pathway by targeting MeCP2.

Taken together, we verified the beneficial effect of miR-488 by overexpressing miR-488 to promote wound healing. Finally, we revealed a novel mechanism that miR-488/MeCP2/ CYP1B1/Wnt4/β-catenin axis was involved in wound healing processes.

**Abbreviation**

MicroRNA, (miR); Methyl-CpG-binding protein 2, (MeCP2); Cytochrome P450 1B1, (CYP1B1); 5-bromo-2’-deoxyuridine, (BrdU); Methylation specific PCR, (MSP); Quantitative real-time polymerase chain reaction, (RT-qPCR); Hematoxylin-eosin, (HE); Sodium dodecyl sulfate-polyacrylamide gel electrophoresis, (SDS-PAGE); Standard deviation, (SD); Analysis of variance, (ANOVA)

**Declarations**

**Conflicts of interest**

All authors agree with the presented findings, have contributed to the work, and declare no conflict of interest.

**Data Availability**

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

**References**


Figures

Figure 1

miR-488, MeCP2 and CYP1B1 expressions in wound tissues of diabetic with skin defect.

(A) miR-488 expression in wound tissues of diabetic with skin defect and control skin tissues was determined using RT-qPCR. (B-C) MeCP2 and CYP1B1 levels in tissues were assessed by RT-qPCR. The data were expressed as mean ± SD. n = 30. ** P < 0.01, *** P < 0.001.
**Figure 2**

**miR-488 facilitated cell proliferation and migration as well as angiogenesis *in vitro***.

MiR-488 mimics and inhibitor were transfected into HSF, HaCaT and HUVEC cells. (A) miR-488 expression was detected using RT-qPCR. (B) Cell apoptosis was measured by flow cytometry assay. (C) BrdU assay was employed to analyze cell proliferation (scale bar = 100 µm). (D) Wound healing assay was
performed to determine cell migration. (E) *In vitro* angiogenesis assay was conducted to evaluate the angiogenesis ability of HUVEC cells (scale bar = 100 µm). The data were expressed as mean ± SD. All data was obtained from at least three replicate experiments. *P < 0.05, ** P < 0.01, *** P < 0.001.

**Figure 3**

MeCP2 was identified as the target of miR-488.

(A) Bioinformatics databases (TargetScan, StarBase and miRDB) were adopted to predict the binding site between miR-488 and MeCP2. (B) Dual-luciferase reporter assay was performed to analyze the interaction between miR-488 and MeCP2 in HSF and HUVEC cells. The data were expressed as mean ± SD. All data was obtained from at least three replicate experiments. ** P < 0.01.
MeCP2 overexpression suppressed cell proliferation and migration as well as angiogenesis

(A-B) RT-qPCR and western blot were adopted to examine MeCP2 expression in HSF and HUVEC cells transfected with miR-488 mimics or inhibitor, respectively. MeCP2 overexpression was induced in HSF and HUVEC cells. (C-D) MeCP2 expression in cells was detected using RT-qPCR and western blot.
respectively. (E) Flow cytometry assay was adopted to analyze cell apoptosis. (F) Cell proliferation was evaluated by BrdU assay (scale bar = 100 µm). (G) Wound healing assay was adopted to measure HSF cell migration. (H) The angiogenesis ability of HUVEC cells was determined using in vitro angiogenesis assay (scale bar = 100 µm). The data were expressed as mean ± SD. All data was obtained from at least three replicate experiments. *P < 0.05, ** P < 0.01, *** P < 0.001.
MeCP2 suppressed cell proliferation, migration as well as angiogenesis through boosting CYP1B1 methylation.

(A) CYP1B1 level in HSF and HUVEC cells after MeCP2 overexpression was assessed by western blot. (B) HSF and HUVEC cells were treated with 5Aza or DMSO, and CYP1B1 promoter methylation was assessed by MSP. (C) CYP1B1 promoter methylation was assessed by MSP in HSF and HUVEC cells with MeCP2 overexpression. HSF and HUVEC cells were transfected with sh-NC or sh-CYP1B1. (D) CYP1B1, Wnt4 and β-catenin levels were determined using western blot. (E) Flow cytometry was adopted to examine cell apoptosis. (F) BrdU assay was employed to evaluate cell proliferation (scale bar = 100 µm). (G) Wound healing assay was adopted to analyze HSF cell migration. (H) The angiogenesis ability of HUVEC cells was determined using *in vitro* angiogenesis assay (scale bar = 100 µm). The data were expressed as mean ± SD. All data was obtained from at least three replicate experiments. *P < 0.05, **P < 0.01.
Figure 6

miR-488 promoted cell proliferation and migration as well as angiogenesis through MeCP2-CYP1B1 axis-mediated Wnt4/β-catenin signaling pathway

MiR-488 mimics and/or MeCP2-overexpression vector were transfected into HSF and HUVEC cells. (A) miR-488 expression was evaluated by RT-qPCR. (B) MeCP2, CYP1B1, Wnt4 and β-catenin levels were
measured by western blot. (C) Flow cytometry was performed to assess cell apoptosis. (D) Cell proliferation was evaluated by BrdU assay (scale bar = 100 µm). (E) Wound healing assay was adopted to analyze migratory ability of HSF cells. (F) The angiogenesis ability of HUVEC cells was determined using in vitro angiogenesis assay (scale bar = 100 µm). The data were expressed as mean ± SD. All data was obtained from at least three replicate experiments. *P < 0.05, ** P < 0.01, *** P < 0.001.

Figure 7
miR-488 accelerated wound healing *in vivo.*

We injected miR-488 mimics or mimics NC intradermally into the wound edges of diabetic rats after the skin was injured. (A) miR-488, MeCP2 and CYP1B1 expressions in the skin of the rats were measured using RT-qPCR. (B) Western blot was employed to evaluate MeCP2, CYP1B1, Wnt4 and β-catenin levels in skin tissues. (C) Representative wound healing images, and the wound closures were quantified. (D-E) HE and Masson staining of skin tissues (scale bar = 50 μm). The data were expressed as mean ± SD. n = 8. *P* < 0.05, **P** < 0.01, ***P** < 0.001.