MicroRNA-185 inhibits the proliferation and migration of HaCaT keratinocytes by targeting peroxisome proliferator-activated receptor β

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Abstract. Proliferation and migration of keratinocytes are major processes of skin wound repair after injury. It has been indicated that microRNAs (miRNAs/miRs) are associated with the proliferation and migration of keratinocytes. However, the mechanism by which miR-185 affects these processes in keratinocytes remains unclear. In the present study, the expression level of miR-185 and peroxisome proliferator-activated receptor β (PPARβ) was examined by reverse transcription-quantitative PCR in HaCaT keratinocytes. Cell proliferation was evaluated using Cell Counting Kit-8 and colony formation assays. Western blot analysis was used to detect the levels of cell proliferation, migration and PI3K/AKT signaling pathway-associated proteins. In addition, the migratory capacity of the cells was determined using Transwell assay. The target gene of miR-185 was verified using dual-luciferase reporter assay. The results indicated that overexpression of miR-185 inhibited proliferation, migration and activation of the PI3K/AKT signaling pathway in HaCaT keratinocytes. PPARβ was indicated to be a target of miR-185 and its overexpression promoted the proliferation and migration of HaCaT keratinocytes, while its knockdown exhibited the adverse effects. Furthermore, PI3K inhibitor LY294002 inhibited activation of the PI3K/AKT signaling pathway and decreased the proliferation and migration of HaCaT keratinocytes. In addition, overexpressed PPARβ reversed the suppressive effects of miR-185 overexpression on proliferation, migration and activation of the PI3K/AKT signaling pathway. In conclusion, the results of the present study demonstrated that miR-185 suppressed activation of the PI3K/AKT signaling pathway via targeting PPARβ, thereby regulating proliferation and migration in HaCaT keratinocytes. The present study provided a novel theoretical basis for the use of miR-185 as a target in wound repair.

Introduction

Wound repair is the main form of adult skin wound healing and is a complex, multistep process, which includes the interaction of various cells, growth factors and cytokines (1,2). Proliferation and migration of keratinocytes are considered to be important processes of wound repair (3). Connective tissue growth factor cellular communication network factor 2 promotes the re-epithelialization at the wound site, thereby completing the process of wound repair (4). Therefore, the better understanding of the factors that affect keratinocyte proliferation and migration may provide novel therapeutic strategies for wound repair.

Previous studies have indicated that the process of skin wound healing was associated with the expression of microRNAs (miRNAs/miRs), including miR-105, miR-29a and miR-125b (5-7). miRNAs are small non-coding RNAs, which bind to mRNA resulting in its transcriptional inhibition or degradation, thereby regulating gene expression (8,9). The abnormal regulation of certain miRNAs has been indicated to serve a vital role in the aberrant healing of wounds, such as miR-140 and miR-126 (5,7). Yang and Yee (10) demonstrated that miR-185 could bind to the 3'-untranslated region (3'-UTR) of versican and participate in wound healing in transgenic mice. However, the specific mechanism of miR-185 function in wound healing remains unclear.

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated receptors that belong to the nuclear hormone receptor family, including three different subtypes: PPARα, PPARβ/δ and PPARγ (11). Previous studies have suggested that PPARβ is associated with inflammation and tumor progression (12-14). In addition, PPARβ has also been demonstrated to participate in wound healing. For instance, it has been reported that the anti-apoptotic function of PPARβ was important in maintaining the proliferation and migration of keratinocytes (15). Moreover, PPARβ has been indicated to alleviate the inflammatory response of macrophages and inhibit the apoptosis of keratinocytes, therefore indicating that it may be used as a target in the development of wound healing drugs (16). Tan *et al* (17) revealed that the upregulation of PPARβ, which was induced by the inflammatory response, was critical for skin wound healing. The aforementioned studies have suggested that PPARβ may be a key molecule involved in wound healing.

The present study investigated the effects of miR-185 and PPARβ on the proliferation and migration of HaCaT keratinocytes. In addition, the mechanism by which miR-185 regulated these processes in HaCaT keratinocytes was explored. The current study aimed to provide a novel basis for elucidating the mechanism of wound healing.

Materials and methods

Cell culture. HaCaT keratinocytes were donated by Dr Petra Boukamp (German Cancer Research Center, Heidelberg, Germany). The cells were cultured in DMEM (Beijing Solarbio Science & Technology Co., Ltd.) containing 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and 1% antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) at 37℃ in an incubator with 5% CO_2. HaCaT keratinocytes were treated with 50 μmol/l PI3K inhibitor LY294002 (Genomeditech Biotechnology) for 24 h at 37℃. According to the amount of LY294002 added, the equal amounts of DMSO (Genomeditech Biotechnology) were added to the cells as control.

Cell transfection. miR-185 agomir (5'-UGGAGAGAAAGG CAGUUCCUGA-3') or antagomir (5'-UCAGGAACUGCC UUUCUCUCCA-3') and their negative controls (NCs), miR-NC (5'-CUAGUCAUCGAUGUCGUAGCA-3') or anti-miR-NC (5'-CAGUACAUUGGUUCUGCAA-3'), were synthesized by Vigene Biosciences. PPARβ overexpression plasmid (PPARβ forward, 5'-GCTCTAGAGCGGAGCGTGTGACGCTGC G-3' and reverse, 5'-GGGGTACCTTAAATATTTAATTCC CATT-3') or small interfering (si)RNA (si-PPARβ forward, 5'-GCAAGCCCUUCAGUGACAUTT-3' and reverse, 5'-AUG UCACUGAAGGGCUUGCTT-3') and their negative controls (vector forward, 5'-CTAGAGAACCCACTGCTTAC-3' and reverse 5'-TAGAAGGCACAGTCGAGG-3'; or si-NC forward 5'-UUCUCCGAACGUGUCACGUTT-3' and reverse 5'-ACG UGACACGUUCGGAGAATT-3') were purchased from Shanghai GeneChem Co., Ltd. Transfection was performed using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37℃ for 48 h. HaCaT keratinocytes were seeded into six-well-plates at a density of 1x10⁵ cells/m and incubated at 37℃ for 48 h. A total of 50 nM oligonucleotides and 2 μg plasmid were transfected into HaCaT keratinocytes at

a confluence of 60%. After transfection for 48 h, the cells were collected for subsequent experiments.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from HaCaT keratinocytes using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. PrimeScript™ RT Reagent kit (Takara Biotechnology Co., Ltd.) was used to synthesize cDNA according to the manufacturer's instructions with the following temperature protocol: 37℃ for 30 min and 95℃ for 10 min. mirVana qRT-PCR miRNA Detection kit (Thermo Fisher Scientific, Inc.) was used to quantify the expression of miR-185 using the following thermocycling conditions: 95℃ for 3 min, followed by 40 cycles at 95℃ for 15 sec and 60℃ for 30 sec. SYBR Premix Ex Taq™ (Tli RNaseH plus; Takara Biotechnology Co., Ltd.) was used for qPCR. The thermocycling conditions were as follows: 95℃ for 5 min, followed by denaturation at 94℃ for 30 sec, annealing at 60℃ for 30 sec, for 45 cycles. The primer sequences used were as follows: miR-185 forward, 5'-GTGCAGGGTCCGAGGTATG-3' and reverse, 5'-TGGAGAGAAAGGCAGTTCCTGA-3'; U6 forward, 5'-GCAGGAGGTCTTCACAGAGT-3' and reverse, 5'-TCTAGAGGAGAAGCTGGGGT-3'; PPARβ forward, 5'-TGAGCCTAAGTTTGAATTTGC-3' and reverse, 5'-TCT CGGTTTCGGTCTTCTTG-3'; GAPDH forward, 5'-GCA CCGTCAAGCTGAGAAC-3' and reverse, 5'-TGGTGAAGA CGCCAGTGGA-3'. Relative expression was determined with the $2^{-\Delta\Delta Cq}$ method (18) using U6 or GAPDH as internal controls.

Cell Counting Kit-8 (CCK-8) assay. HaCaT keratinocytes (~5,000 cells) were seeded into 96-well plates. Following transfection, HaCaT keratinocytes were cultured for 24, 48 or 72 h at 37℃. A CCK-8 kit (Beijing Solarbio Science & Technology Co., Ltd.) was used to detect the proliferative ability of HaCaT keratinocytes. In brief, 10 μl CCK-8 reagent was added to each well at a certain time point (24, 48 or 72 h) and cultured for 4 h at 37℃. The absorbance at 450 nm was measured using an immunoassay analyzer (Bio-Rad Laboratories, Inc.).

Colony formation assay. Following transfection for 48 h, HaCaT keratinocytes were seeded into six-well plates (~200 cells/well). After 2 weeks, HaCaT keratinocytes were washed with PBS, fixed with 4% paraformaldehyde for 20 min and stained with 0.5% crystal violet for 15 min at room temperature. The number of cell colonies (>50 cells) was measured using ImageJ software (Version 1.8.0; National Institutes of Health), and at least three independent repeats were conducted for each treatment.

Western blotting. Total protein was obtained from HaCaT keratinocytes using RIPA lysis buffer (Wuhan Boster Biological Technology, Ltd.) and quantified using BCA Protein Assay kit (Pierce; Thermo Fisher Scientific, Inc.). Subsequently, proteins were separated with 10% SDS-PAGE and transferred onto PVDF membranes (Bio-Rad Laboratories, Inc.). After blocking with 5% non-fat milk at room temperature for 2 h, the membranes were incubated with the following primary antibodies: Cyclin D1 (1:200; cat. no. ab16663), CDK6 (1:3,000; cat. no. ab151247), CDK4 (1:1,000; cat. no. ab95255), p-AKT-T308 (1:1,000; cat. no. ab8933), AKT

(1:500; cat. no. ab8805) (all from Abcam), MMP-2 (1:2,000; cat. no. sc-10736), MMP-9 (1:2,000; cat. no. sc-10737), PPARβ (1:5,000; cat. no. sc-74440), integrin-linked kinase (ILK; 1:2,000; cat. no. sc-20019) (all from Santa Cruz Biotechnology, Inc.), phosphoinositide-dependent protein kinase 1 (PDK1, 1:2,000; cat. no. BA4499), p-AKT-S473 (1:1,000; cat. no. P00024-6), (all from Boster Biological Technology Co., Ltd.) or GAPDH (1:2,500; cat. no. ab9485; Abcam) at 4°C overnight. Subsequently, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody Goat Anti-Rabbit IgG H&L HRP (1:50,000; cat. no. ab205718; Abcam) or Goat Anti-Mouse IgG H&L HRP (1:20,000; cat. no. ab205719; Abcam) at room temperature for 1 h. The protein signals were visualized using a Western Blotting Luminol Reagent (cat. no. sc-2048; Santa Cruz Biotechnology, Inc.). Image-Pro Plus software v6.0 (Media Cybernetics, Inc.) was used to analyze the protein signals.

Transwell assay. HaCaT keratinocytes were digested with trypsin at 37°C for 2 min and resuspended in DMEM after transfection for 48 h. Subsequently, cell suspensions ($2x10^5$ cells/ml) were added to the upper chambers of Transwell equipment with 8-μm polycarbonate membrane filter (Corning, Inc.). The lower chambers were filled with DMEM containing 10% FBS. After incubation for 24 h at 37°C, the migrated HaCaT keratinocytes were stained with 0.5% crystal violet for 15 min at room temperature and counted using an optical inverted microscope (magnification, x100; Leica Microsystems GmbH).

Dual-luciferase reporter assay. The binding sites between the PPARβ 3'-UTR and miR-185 was predicted using the DIANA tools website (v5.0; http://diana.imis.athena-innovation.gr/DianaTools/index.php). PPARβ 3'-UTRs containing wild-type (WT) and mutant (Mut) miR-185 binding sites were cloned into pMIR-REPORT miRNA expression reporter vector (Thermo Fisher Scientific, Inc.) to generate PPARβ-WT and PPARβ-Mut reporter vectors. HaCaT keratinocytes were co-transfected with the aforementioned reporter vectors and miR-185, anti-miR-185, miR-NC or anti-miR-NC using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). A Dual-Luciferase Reporter Assay kit (Genomeditech Biotechnology) was used to detect the relative luciferase activity, which was normalized to *Renilla* luciferase activity, after transfection for 48 h.

Statistical analysis. Data are presented as the mean ± SD of three independent experimental repeats, and were analyzed using an unpaired Student's t-test or one-way ANOVA followed by Tukey's post hoc test. GraphPad Prism v5 software (GraphPad Software, Inc.) was used to perform statistical analysis. $P<0.05$ was considered to indicate a statistically significant difference.

Results

miR-185 inhibits the proliferation of HaCaT keratinocytes. To investigate the effect of miR-185 on HaCaT keratinocytes, miR-185 or anti-miR-185 were transfected into HaCaT keratinocytes. RT-qPCR results indicated that miR-185 significantly upregulated, while anti-miR-185 inhibited the expression level of miR-185, validating their transfection efficiency (Fig. 1A). Subsequently, the effect of miR-185 on the proliferative ability of HaCaT keratinocytes was investigated. CCK-8 assay results demonstrated that miR-185 overexpression significantly suppressed cell proliferation, while miR-185 inhibition exhibited the opposite effect compared with their respective controls (Fig. 1B). Furthermore, the colony formation assay revealed that overexpression of miR-185 decreased, while knockdown of miR-185 increased the number of colonies in HaCaT keratinocytes (Fig. 1C). Moreover, detection of the expression level of proliferation-related proteins demonstrated that overexpression of miR-185 reduced, while knockdown of miR-185 increased the protein expression level of cyclin D1, CDK6 and CDK4 (Fig. 1D). Taken together, the results indicated that miR-185 suppressed proliferation in HaCaT keratinocytes.

miR-185 suppresses migration in HaCaT keratinocytes. The migratory ability of HaCaT keratinocytes was examined using Transwell and western blot assays. The results indicated that miR-185 significantly inhibited the number of migrated HaCaT keratinocytes. By contrast, anti-miR-185 markedly increased the number of migrated HaCaT keratinocytes (Fig. 2A). Moreover, detection of the protein expression levels of MMP-2 and MMP-9, which have been associated with cell migration (19), revealed that miR-185 significantly reduced the expression level of MMP-2 and MMP-9, while anti-miR-185 exhibited the adverse effect (Fig. 2B). Therefore, the results indicated that miR-185 reduced the migratory ability of HaCaT keratinocytes.

miR-185 directly targets PPARβ in HaCaT keratinocytes. The main role of miRNAs is the recognition of target mRNAs via base complementary pairing, thereby degrading or inhibiting the translation of the target mRNAs (20). To determine the molecular mechanism by which miR-185 affects HaCaT keratinocyte proliferation and migration, the DIANA tools website (v5.0; http://diana.imis.athena-innovation.gr/DianaTools/index.php) was used to predict the potential target genes of miR-185, the results of which indicated that PPARβ 3'-UTR presented a complementary binding sequence with miR-185 (Fig. 3A). To further validate this association, a dual-luciferase reporter assay was performed. The results indicated that miR-185 significantly inhibited, and anti-miR-185 enhanced the luciferase activity of PPARβ-WT 3'-UTR, but neither exhibited any effect on the luciferase activity of PPARβ-Mut 3'-UTR (Fig. 3B and C). In addition, the effect of miR-185 on PPARβ expression level was investigated, the results of which indicated that miR-185 overexpression suppressed the mRNA and protein level of PPARβ, whereas miR-185 knockdown resulted in the adverse effect (Fig. 3D and E). Moreover, the levels of PI3K/AKT signaling pathway-related proteins were examined, and it was revealed that overexpression of miR-185 reduced the protein level of ILK and PDK1, the phosphorylation level of AKT at S473 and T308 and the p-AKT/AKT ratio. Conversely, inhibition of miR-185 increased the protein level of ILK and PDK1, the phosphorylation level of AKT at S473 and T308 and the p-AKT/AKT ratio (Fig. 3E). These data indicated that miR-185 regulated PPARβ expression and inhibited activation of the PI3K/AKT signaling pathway.
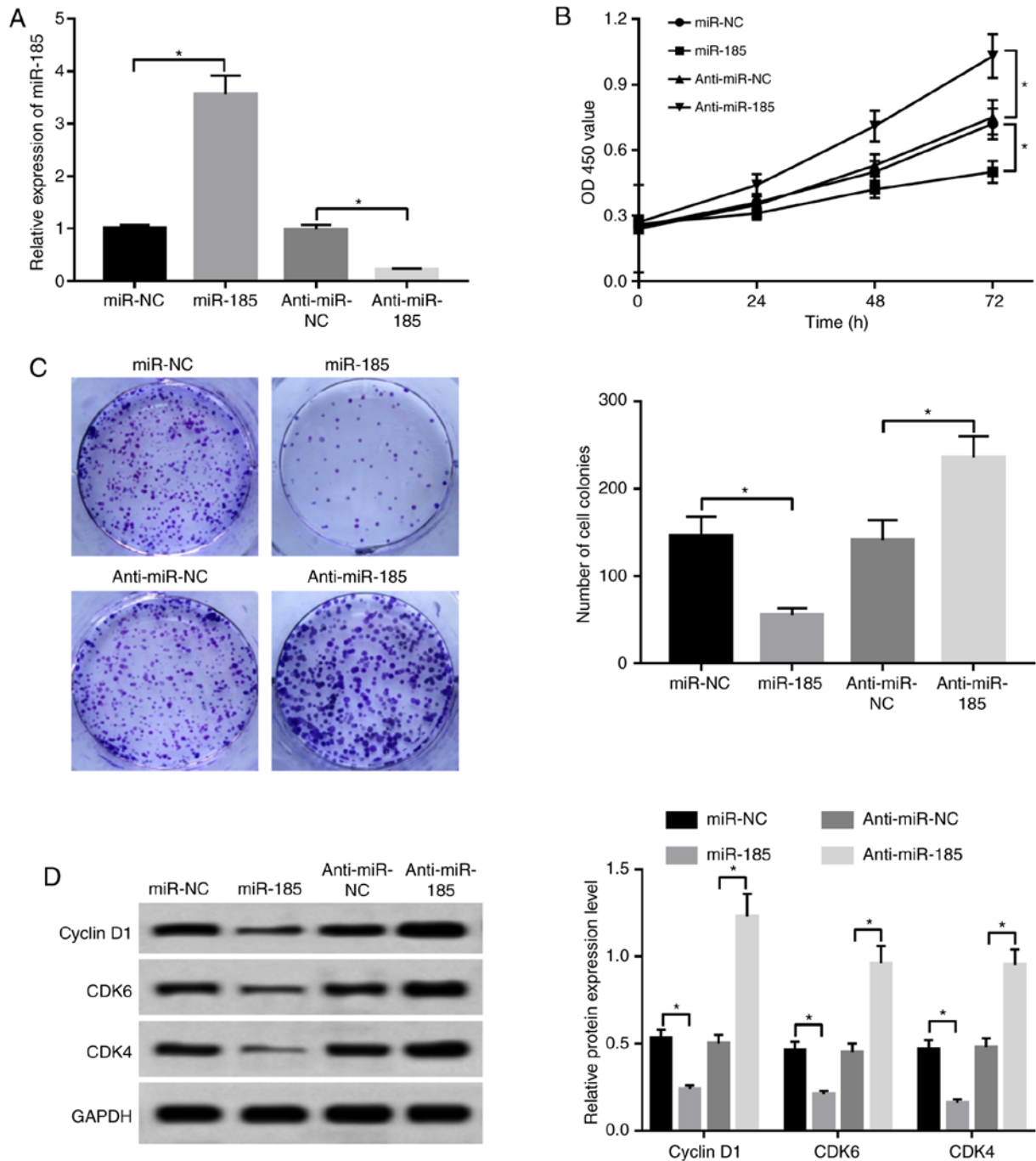
Figure 1. Effect of miR-185 expression on the proliferation of HaCaT keratinocytes. HaCaT keratinocytes were transfected with miR-185 and anti-miR-185 or their NCs. (A) Reverse transcription-quantitative PCR was performed to examine the expression of miR-185 and evaluate the transfection efficiency. (B) Cell Counting Kit-8 assay was conducted to assess the proliferation of HaCaT keratinocytes. (C) Colony formation assay was performed to examine the colony forming ability of HaCaT keratinocytes. (D) Western blot analysis was performed to evaluate the protein level of cyclin D1, CDK6 and CDK4 in HaCaT keratinocytes. *P<0.05. miR, microRNA; NC, negative control; OD, optical density.

PPARβ promotes the proliferation and migration of HaCaT keratinocytes. To investigate the effect of PPARβ on proliferation and migration in HaCaT keratinocytes, si-PPARβ or PPARβ overexpression plasmids were transfected into HaCaT keratinocytes. Detection of the protein level of PPARβ via western blotting demonstrated that si-PPARβ significantly decreased, and the PPARβ overexpression plasmid increased the expression level of PPARβ compared with their respective controls, indicating that transfection was successful (Fig. 4A). CCK-8 and clone formation assays indicated that PPARβ

silencing inhibited proliferation in HaCaT keratinocytes, whereas its overexpression exhibited the opposite effect (Fig. 4B and C). Moreover, PPARβ knockdown significantly suppressed, while its overexpression promoted the migration of HaCaT keratinocytes (Fig. 4D). In addition, western blot analysis revealed that knockdown of PPARβ decreased the protein level of cyclin D1, CDK6, CDK4, MMP-2 and MMP-9, while PPARβ overexpression resulted in the opposite effect (Fig. 4E). The results indicated that PPARβ promoted proliferation and migration in HaCaT keratinocytes.
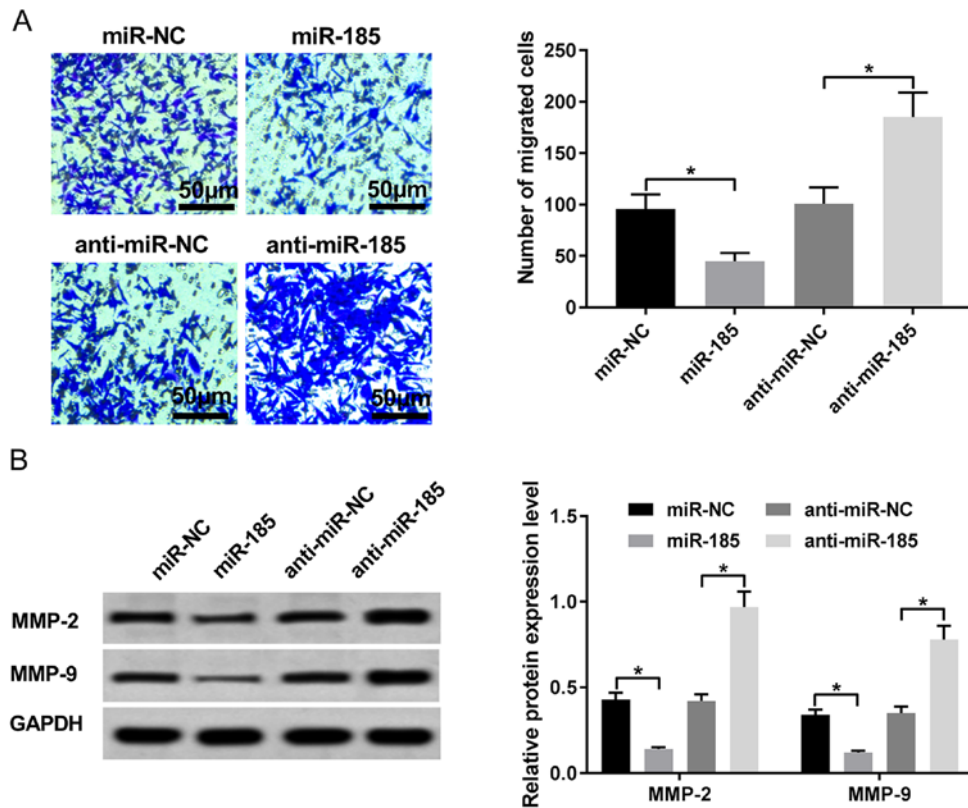
Figure 2. Effect of miR-185 expression on the migration of HaCaT keratinocytes. HaCaT keratinocytes were transfected with miR-185 and anti-miR-185 or their NCs. (A) The number of migrated HaCaT keratinocytes was determined using Transwell assay. (B) The protein level of MMP-2 and MMP-9 in HaCaT keratinocytes was detected via western blot analysis. $^*P<0.05$. miR, microRNA; NC, negative control.
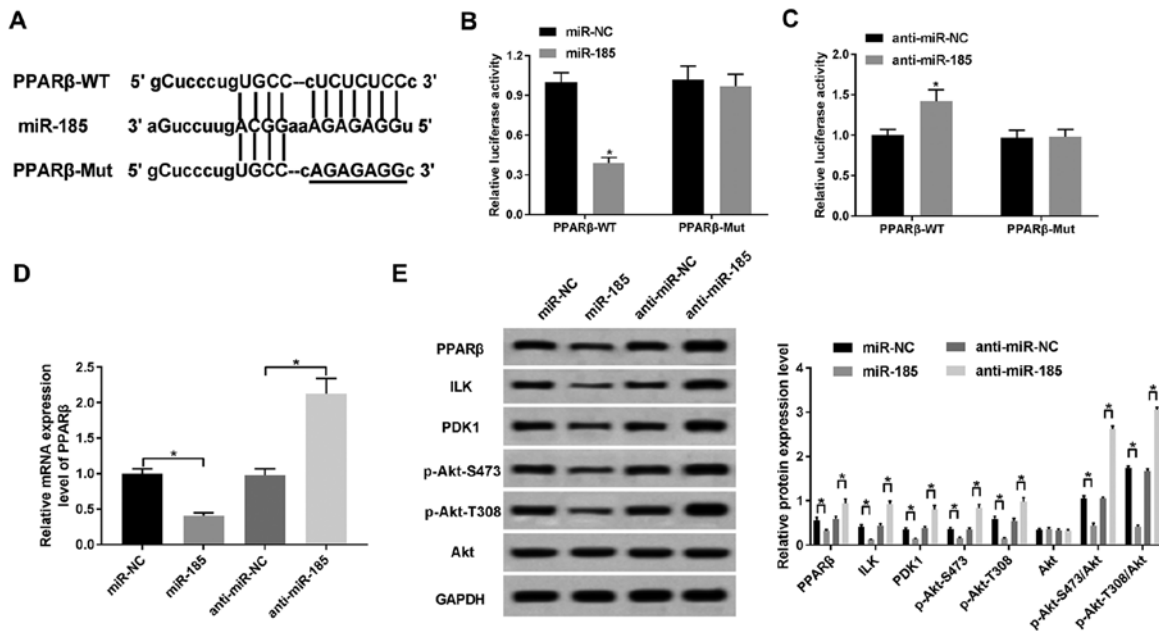

Figure 3. miR-185 directly targets PPARβ in HaCaT keratinocytes. (A) The WT and mutant binding sites between miR-185 and PPARβ are presented. The matched sequences of miR-185 and PPARβ 3'-UTR are expressed in uppercase, and those that cannot be matched are expressed in lowercase. (B and C) A dual-luciferase reporter assay was performed to verify the binding of miR-185 to PPARβ 3'-UTR. (D) The expression level of PPARβ was examined by reverse transcription-quantitative PCR. (E) Western blot analysis was performed to determine the protein level of PPARβ, ILK, PDK1, p-AKT and AKT. $^*P<0.05$. PPARβ, peroxisome proliferator-activated receptor β; ILK, integrin-linked kinase; PDK1, phosphoinositide-dependent protein kinase 1; miR, microRNA; NC, negative control; WT, wild-type; Mut, mutant; p, phosphorylated.

Inhibition of the PI3K/AKT pathway represses proliferation and migration in HaCaT keratinocytes. To verify the role of the PI3K/AKT signaling pathway in HaCaT keratinocytes, cells were treated with 50 μmol/l PI3K inhibitor
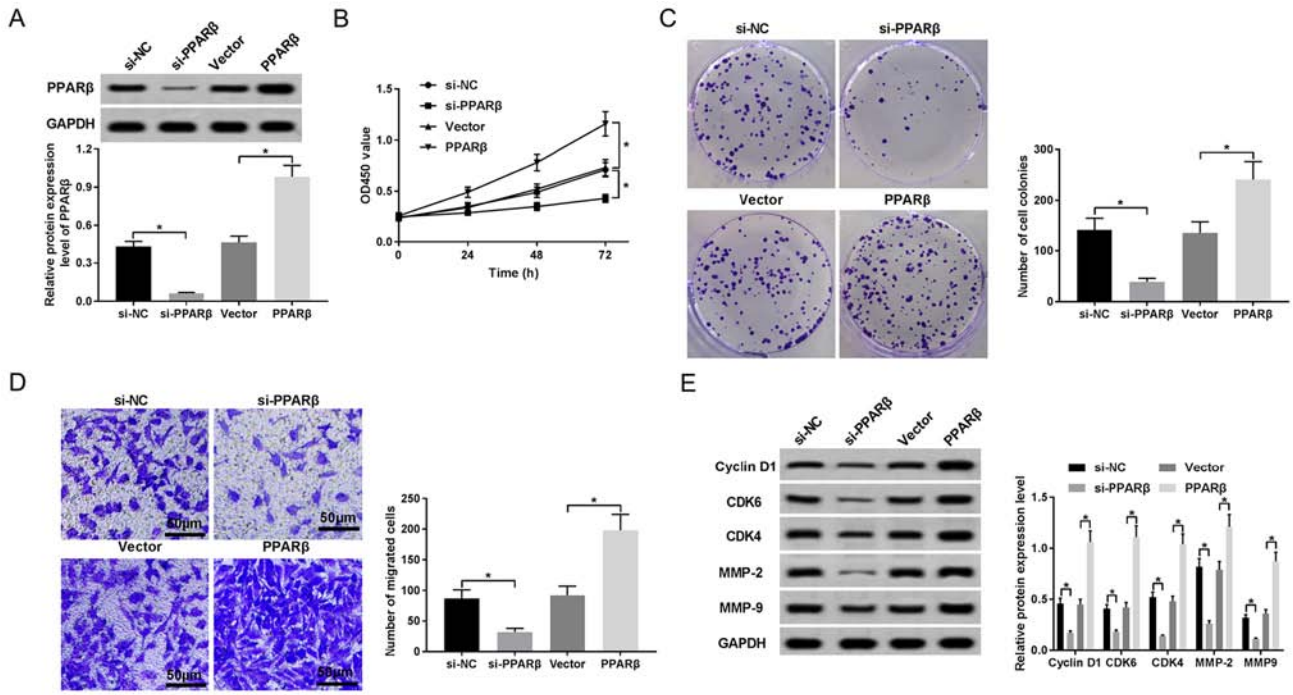
Figure 4. Effect of PPARβ expression level on the proliferation and migration of HaCaT keratinocytes. HaCaT keratinocytes were transfected with si-PPARβ and PPARβ overexpression plasmid or their NCs. (A) Western blot analysis was performed to examine the protein level of PPARβ and evaluate the transfection efficiency. (B) Cell Counting Kit-8 assay was performed to determine the proliferation of HaCaT keratinocytes. (C) The number of colonies in HaCaT keratinocytes was assessed using colony formation assay. (D) Transwell assay was conducted to assess the migration of HaCaT keratinocytes. (E) The protein level of cyclin D1, CDK6, CDK4, MMP-2 and MMP-9 in HaCaT keratinocytes was determined by western blot analysis. *P<0.05. PPARβ, peroxisome proliferator-activated receptor β; NC, negative control; si, small interfering.

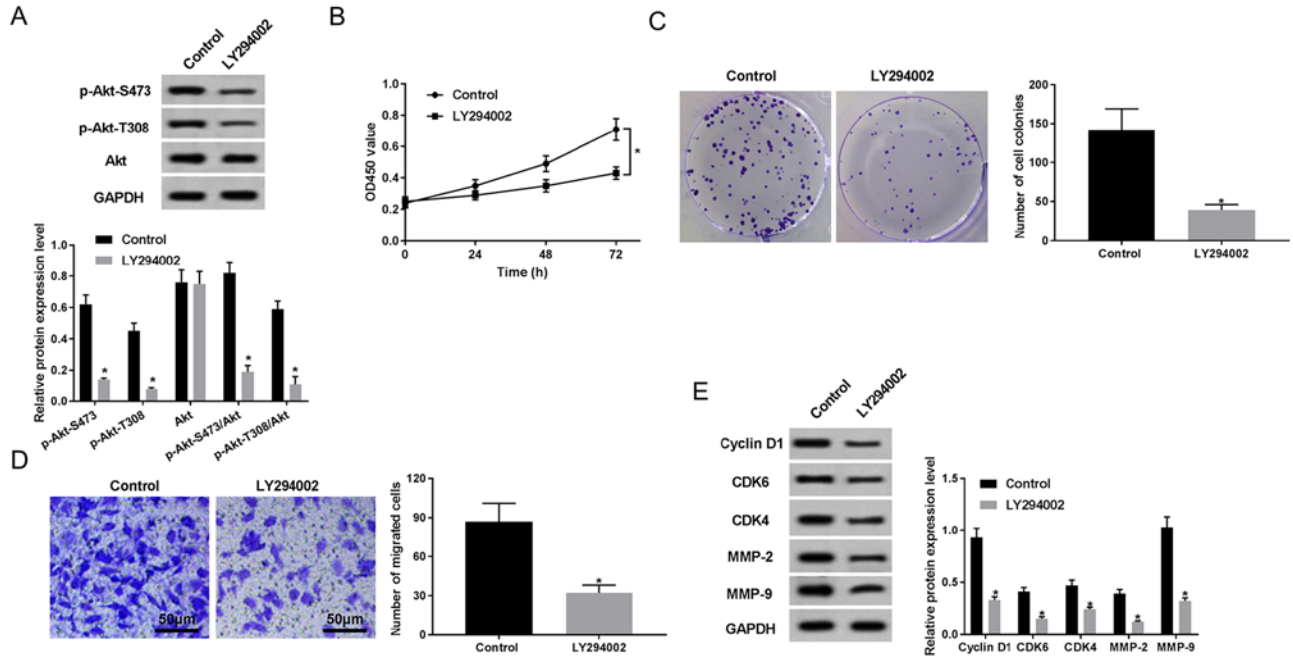
Figure 5. Effect of PI3K inhibitor LY294002 on the proliferation and migration of HaCaT keratinocytes. HaCaT keratinocytes were treated with 50 µmol/l PI3K inhibitor LY294002 for 48 h. (A) The phosphorylation level of p-AKT at S473 and T308 and the p-AKT/AKT ratio was determined via western blot analysis. (B) Proliferation of HaCaT keratinocytes was assessed using Cell Counting Kit-8 assay. (C) Colony formation assay was employed to assess the number of colonies in HaCaT keratinocytes. (D) The number of migrated HaCaT keratinocytes was examined using Transwell assay. (E) The protein level of cyclin D1, CDK6, CDK4, MMP-2 and MMP-9 in HaCaT keratinocytes was determined via western blot analysis. *P<0.05 vs. control. p, phosphorylated.

LY294002, and the results revealed that LY294002 significantly inhibited the phosphorylation level of AKT at S473 and T308 and the p-AKT/AKT ratio (Fig. 5A). CCK-8 and colony formation assays indicated that inhibition of PI3K significantly reduced cell proliferation (Fig. 5B and C), and Transwell assay demonstrated that LY294002 suppressed
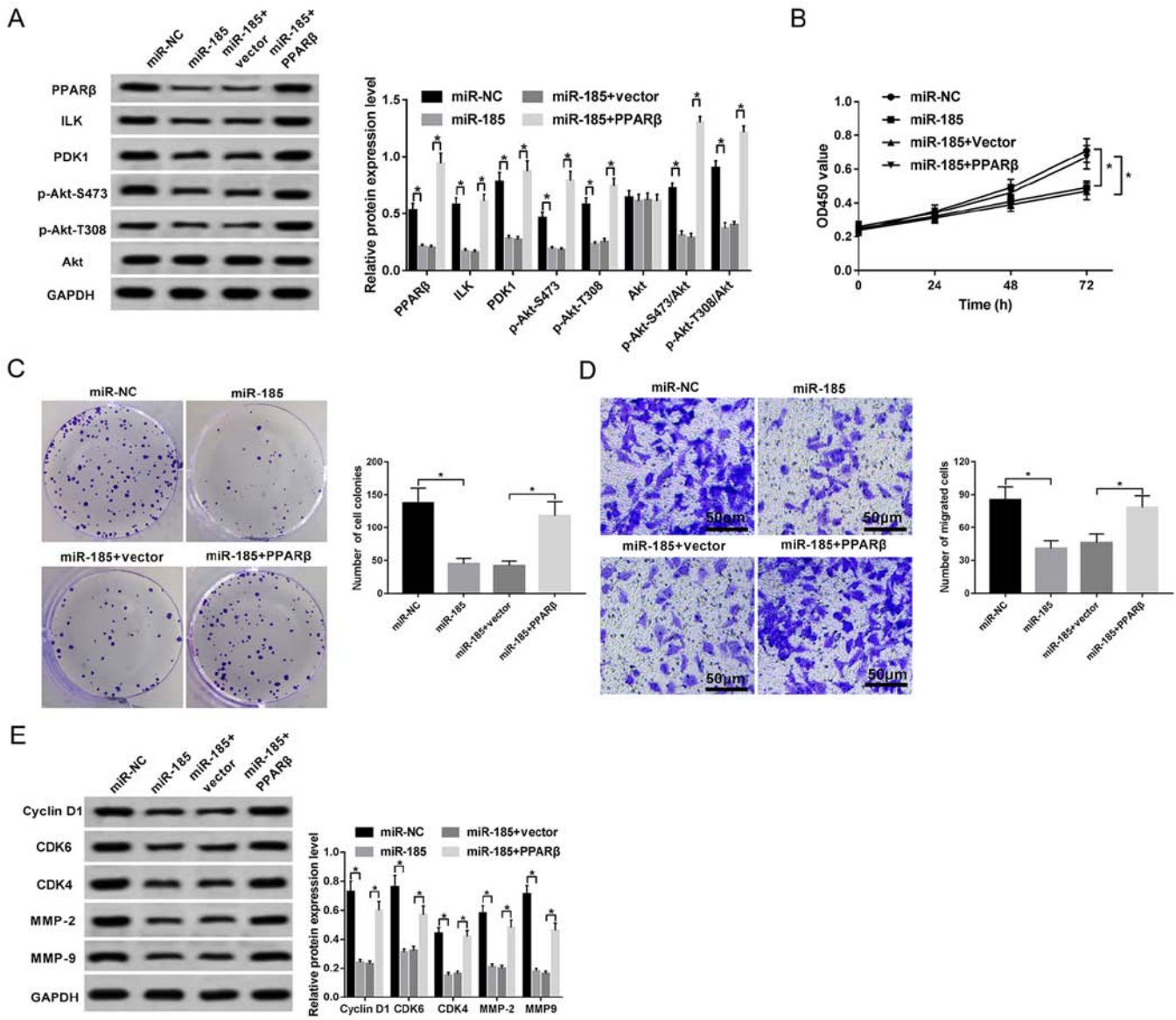
Figure 6. Effect of miR-185 and PPARβ overexpression on the proliferation and migration of HaCaT keratinocytes. HaCaT keratinocytes were transfected with miR-185 and PPARβ overexpression plasmid or their NCs. (A) The protein level of PPARβ, ILK, PDK1, p-AKT-S473, p-AKT-T308 and AKT in HaCaT keratinocytes was determined via western blot analysis. (B) Proliferation of HaCaT keratinocytes was assessed using Cell Counting Kit-8 assay. (C) The number of colonies in HaCaT keratinocytes was determined using colony formation assay. (D) Transwell assay was used to assess the number of migrated HaCaT keratinocytes. (E) The protein level of cyclin D1, CDK6, CDK4, MMP-2 and MMP-9 in HaCaT keratinocytes was assessed via western blot analysis. *P<0.05. PPARβ, peroxisome proliferator-activated receptor β; ILK, integrin-linked kinase; PDK1, phosphoinositide-dependent protein kinase 1; miR, microRNA; NC, negative control; p, phosphorylated.

the migration of HaCaT keratinocytes (Fig. 5D). In addition, protein detection results revealed that PI3K inhibitor LY294002 significantly reduced the expression level of proliferation and migration-related proteins (Fig. 5E). The results suggested that activation of the PI3K/AKT signaling pathway was required to maintain the normal functions of HaCaT keratinocytes.

miR-185 suppresses the proliferation and migration of HaCaT keratinocytes by targeting PPARβ to inhibit the PI3K/AKT signaling pathway. In order to confirm whether the effects of miR-185 and PPARβ on HaCaT keratinocytes were mediated by the PI3K/AKT signaling pathway, miR-185 and PPARβ overexpression plasmid were co-transfected into HaCaT keratinocytes. The results indicated that PPARβ overexpression

restored the suppressive effect of miR-185 overexpression on the protein level of ILK and PDK1, the phosphorylation level of AKT at S473 and T308 and the p-AKT/AKT ratio, indicating that PPARβ promoted activation of the PI3K/AKT signaling pathway (Fig. 6A). In addition, PPARβ overexpression reversed the inhibitory effect of miR-185 overexpression on the proliferation and migration of HaCaT keratinocytes (Fig. 6B-D). Moreover, PPARβ overexpression reversed the suppressive effect of miR-185 overexpression on the protein expression level of cyclin D1, CDK6, CDK4, MMP-2 and MMP-9, further suggesting that PPARβ and miR-185 were implicated in regulating proliferation and migration in HaCaT keratinocytes (Fig. 6E). The results indicated that miR-185 inhibited the activation of the PI3K/AKT signaling pathway by targeting PPARβ in HaCaT keratinocytes.

Discussion

Skin damage initiates wound healing, which is a complex process involving numerous cell types, growth factors, cytokines and extracellular matrix components (21,22). Wound repair comprises three main stages, inflammation, new tissue formation and remodeling, where the formation of new tissue involves the proliferation and migration of various cell types, including keratinocytes (3). HaCaT is the first normal differentiated permanent epithelial cell line from adult skin, and its function is similar to that of normal keratinocytes. Its discovery provides a convenient cell model for the study of human keratinocytes (23,24).

miR-185 has been revealed to be a cancer-related miRNA participating in various cellular processes, including proliferation, invasion and migration (25,26). In the present study, the function of miR-185 in keratinocytes was explored, and it was demonstrated that miR-185 suppressed the proliferation and migration of HaCaT keratinocytes, indicating that miR-185 may be an effective target for wound repair.

Previous studies have revealed that the level of PPARβ was increased rapidly in the early phase after skin injury, and it was expressed at a high level during the wound healing process (27,28). Lack of PPARβ has been reported to promote the apoptosis of keratinocytes and reduce the migratory ability of cells in wound healing (29). In the present study, PPARβ was indicated to be a target gene of miR-185, and its expression was revealed to be regulated by miR-185. In subsequent experiments, knockdown of PPARβ was demonstrated to decrease keratinocyte proliferation and migration, and it was indicated that overexpression of PPARβ may present a positive effect on wound repair.

The PI3K/AKT signaling pathway is a classical signaling pathway regulating cell proliferation, differentiation and apoptosis (30,31). AKT has been indicated to be recruited to the cell membrane along with PDK1, and amino acid residues T308 and S473 of AKT have been demonstrated to be phosphorylated by PDK1, thereby activating AKT (32). Activated AKT can phosphorylate multiple target proteins, including E-cadherin, β-catenin and Vimentin, serving an important regulatory role in tumor aggressiveness (33,34). Decreased phosphorylation of AKT has been reported to reduce the proliferation and migration of human primitive skin keratinocytes, leading to impaired wound healing (35). The present study revealed that miR-185 decreased the protein level of ILK, PDK1, p-AKT-S473 and p-AKT-T308, therefore it was hypothesized that the PI3K/AKT signaling pathway participated in the regulation of keratinocyte functions. To further verify this hypothesis, LY294002 was used to suppress the activation of PI3K/AKT signaling pathway. The results indicated that inhibition of the PI3K/AKT signaling pathway significantly decreased the proliferation and migration of HaCaT keratinocytes. Moreover, western blot results demonstrated that inhibition of the PI3K/AKT signaling pathway reduced the protein level of cyclin D1, CDK6, CDK4, MMP-2 and MMP-9. Previous studies have revealed that PPARβ served a vital role in regulating the PI3K/AKT signaling pathway (15,36). In the present study, the overexpression results of PPARβ indicated that PPARβ restored the inhibitory effect of miR-185 overexpression on the PI3K/AKT signaling pathway. These results verified that the PI3K/AKT signaling pathway was associated with the proliferation and migration of keratinocytes.

The present study presents certain limitations. Firstly, the effect of miR-185 was only examined on HaCaT keratinocytes, but it is unclear whether it exhibits the same effect on other types of keratinocytes, therefore additional confirmation is required. Secondly, only the proliferation and migration of keratinocytes were examined, without considering the density, stratification and differentiation of keratinocytes. On the other hand, in addition to affecting the proliferation and migration of keratinocytes, whether miR-185 exhibits the same effect on fibroblasts, endothelial, perivascular and inflammatory cells remains unknown. Lastly, only *in vitro* experiments were conducted without additional *in vivo* experimental data. Therefore, these issues should be further explored in future studies.

In conclusion, the results of the present study demonstrated that miR-185 inhibited proliferation and migration in HaCaT keratinocytes by targeting PPARβ to modulate the PI3K/AKT signaling pathway. The elucidation of the effect of miR-185 on keratinocyte proliferation and migration may provide a theoretical basis for the study of factors affecting wound repair. In addition, the understanding of miR-185 function also provides novel insights for improving the process of wound repair.

Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

JY and FC conceived and designed the present study. JY, PD, YQ, XF, HW and FC performed the experiments and acquired the data. JY performed data analysis and interpretation. JY and PD were involved in the preparation of the manuscript. JY and PD confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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