Regulation of Angiogenesis in Psoriasis by Dermal Mscs-Derived-EDIL3 via the Integrin Pathway

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Research

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Abstract

One of the earliest events in the development of psoriatic lesion is a vascular network expansion. The abnormal vascular network is associated with increased endothelial cells (ECs) survival, proliferation, adhesion, migration, angiogenesis and permeability in psoriatic lesion. Recently, researchers found that the dermal mesenchymal stem cells (DMSCs) in psoriatic lesion involved in course of psoriasis. Our prior study demonstrated the mRNA and protein expression of the epidermal growth factor (EGF)-like repeats and discoidin I-like domains 3 (EDIL3) which is an important angiogenic and anti-inflammatory mediator in DMSCs was significantly upregulated in psoriasis. So, this study aimed to explore the association between DMSCs-derived-EDIL3 and psoriasis-associated angiogenesis in vivo and in vitro. In the present study, we confirmed that in vitro DMSCs-derived-EDIL3 involved in the adhesion, migration and tube formation of ECs via integrin-FAK/MEK/ERK signaling. In vivo, this research found that after injected recombination EDIL3 protein, the epidermis thickness and microvessel density were both elevated in the IMQ-induced psoriasis-like mouse model. Based on these results, we suggested that the modification of DMSCs, EDIL3 and integrin signal pathway might be a novel therapeutic strategy for psoriasis.

Background

Psoriasis is a common skin disorder that is associated with both a physical and psychological burden [1]. The worldwide prevalence is about 2% [2]. The pathophysiology of psoriasis includes keratinocyte hyperproliferation, infiltration of inflammatory cells, dilatation and tortuous of dermal papillary blood [3]. One of the earliest events in the development of psoriatic lesion is a vascular network expansion, which occurs before epidermal changes and persists after clearance of the clinical lesions [4]. The abnormal vascular network is associated with increased endothelial cells (ECs) survival, proliferation, adhesion, migration, angiogenesis and permeability in psoriatic lesions [5,6]. The abnormal functions of ECs are caused by inflammatory molecules, pro-angiogenic and anti-angiogenic mediators in local microenvironment [7]. Recently, researchers found that the dermal mesenchymal stem cells (DMSCs) in psoriatic lesions produced more angiogenic and proinflammatory mediators and involved in course of psoriasis [8,9,10]. However, whether the upregulated angiogenic and/or proinflammatory factors in DMSCs from psoriasis can influence ECs adhesion, migration and angiogenesis is a lack of understanding.

Our prior study demonstrated the mRNA and protein expression of the epidermal growth factor-like repeats and discoidin □-like domains 3 (EDIL3) in DMSCs was significantly upregulated in psoriasis [8]. EDIL3 is an extracellular matrix protein, also named as developmental endothelial locus-1 (Del-1) and composed of two discoidin □–like domains and three EGF-like repeats, the second of which contains an Arg-Gly-Asp (RGD) motif [11]. The RGD motif can bind with integrin, then effect ECs functions including survival, adhesion, migration and angiogenesis [8,11,12]. During angiogenesis in early embryogenesis [13], tumor [14] and ischemic tissue [15], EDIL3 is an important molecular for mediating ECs functions. In present, whether EDIL3 derived from DMSCs can impact on ECs in psoriasis is unknown. And the

regulation of ECs by EDIL3 is context-dependent [16]. So, this work aimed to explore the association between DMSC-derived-EDIL3 and ECs in psoriasis.

Integrins are heterodimeric transmembrane glycoproteins which are heterodimers constituted of α and β subunits by noncovalent bond [17]. Integrins are also receptors located on cell surface and mediate adhesion between the cells or between cells and extracellular matrix. Besides, they can active the downstream cell signaling transduction pathways and mediate cells survival and migration [18]. EDIL3 was shown to mediate ECs function through binding to integrin αvβ3, αvβ5, α5β1 [13,15,19]. The integrins as the downstream molecular of EDIL3 pathway may mediate the function of ECs in psoriasis. But no study has been reported. The same factor can bind with different integrins in different cell types or even within the same cell and has different biological effects [20]. Moreover, integrin signaling can vary based on the cell type and the integrins involved. It is necessary to further study the downstream signaling pathway of EDIL3 in psoriasis.

Extracellular matrix proteins can activate intracellular signal cascades for various cellular events through activation of their receptors. Interaction of integrins with extracellular matrix proteins generates important intracellular signals for growth, survival, and migration [21]. Intracellular signals from integrins induce the formation of a focal adhesion complex, which is critical for cell adhesion and migration [18, 22] and it is also involved in cellular motility and protection against apoptosis [23]. Focal adhesion kinase (FAK) which is a tyrosine kinase plays a central role during the series of processes [23]. FAK converts inactive Ras-GDP to active Ras-GTP, and the latter acts to mitogen activated protein kinase (MAPK or MEK) and extracellular signal-regulated kinase-1/2 (ERK1/2) [24]. MEK/ERK signal pathway is involved in many cellular functions including cell survival and migration and inhibition of this pathway has been shown to halt the migration of a variety of cell types [25]. In addition, ERK promotes the transcription of a variety of genes, many of which involved in motility [25]. The effects of MEK/ERK in DMSC-derived-EDIL3 on ECs adhesion, migration and angiogenesis is still not confirmed in vivo and in vitro, however, previous results demonstrated that MEK/ERK was well-known pathway associated with ECs functions [26]. In the present study, we hypothesized that DMSC-derived-EDIL3 involved in EC adhesion, migration and angiogenesis and the function acted likely via the integrin-FAK/MEK/ERK signaling pathway.

Materials And Methods

Samples collection

HEK293 cells were kindly donated by Dr. Ruixia Hou (Department of Dermatology, Taiyuan Central Hospital of Shanxi Medical University). DMSCs from five patients with psoriasis and five healthy volunteers were isolated from skin tissues and the isolated and identified methods have been described in previous work [27]. The patients with psoriasis had been diagnosed both clinically and pathologically, and received neither pharmaceutical nor physical therapy within least 12 weeks. Characteristics of psoriatic patients are shown in **table 1**. The normal skin samples were collected from healthy volunteers undergone routine plastic surgery without cutaneous or inflammatory-mediated diseases. HEK293 cells

and DMSCs were incubated in Dulbecco's modified Eagle's media: Nutrient Mixture F-12 (DME/F12, Hyclone, Utah, America) supplemented with 10 % fetal bovine serum (FBS, Hyclone, Utah, America) and 1% penicillin/ streptomycin (Solarbio, Beijing, China) at 37℃ in a humidified atmosphere containing 5% CO_2. Human umbilical cord tissues from five caesarean sections were harvested for isolating primary human umbilical vein endothelial cells (HUVECs). The detail isolation and culture of HUVECs were described below. This study was approved by the Ethics Board of Taiyuan Central Hospital in Shanxi Medical University （No. 2018010）. Written informed consents were obtained from each volunteer prior to the study. The present experiments were performed in accordance with the Helsinki Declaration.

Cells isolation and identification

DMSCs were isolated and cultured as described previously [27]. Briefly, skin tissue was cut into pieces and subcutaneous fat was removed. The dermis was separated from the epidermis by incubating in 0.25% dispase (Hyclone, Utah, USA). Then, dermal pieces were chopped further and filtered. The suspension containing cells was collected. Finally, after centrifugation, cells pellets were seeded into a culture dish, and cultured in DME/F12 supplemented with 10% FBS and 1% antibiotics. The nonadherent cells were removed after 48-72 hours. The medium was refreshed every 5 days and the colonies of DMSCs appeared at 7 to 10 days. The phenotype of DMSCs was identified by flow cytometry at passage 3 using antibodies against human CD105, CD29, CD44, CD73, CD90, CD45, CD34, and CD14 (Becton Dickinson and Company, NY, USA). To further confirm the identification of cells, DMSCs at passage 3 were induced to differentiate into osteoblasts, adipocytes and chondrocytes according to our previously published study [8].

HUVECs were isolated using a standard trypsin enzyme digesting technique. Human umbilical cords were harvested and immediately placed into DME/F12 (Hyclone, Utah, America) supplemented with 10 % FBS (Hyclone, Utah, America) and 1% penicillin/streptomycin (Solarbio, Beijing, China), stored at 4℃, and used within 24 hours. Firstly, to remove blood cells from the umbilical vein, we flushed the vein with warmed phosphate buffer solution (PBS, Solarbio, Beijing, China) until the effluent buffer was transparent or slightly pink. Then, 0.25% trypsin solution was injected into the vein via syringe, and when the solution overflowed from the other end of the umbilical vein with a vessel clamp. Continued to inject the trypsin solution, and tightly clamped it with another vessel clamp until trypsin solution filled with the vein. Finally, the umbilical cord was incubated at 37℃. After 20 minutes, the clamp was opened and the cell suspension was collected. The vein was flushed twice with PBS to collect as many cells as possible. To harvest cells pellets, the cell suspension was centrifuged at 800g for 5 minutes. The cells were resuspended and incubated in T25 cell culture flask with 5ml endothelial basal medium (EBM, Lonza, Basel, Switzerland) supplemented with EGM-2 (Lonza, Basel, Switzerland) at 37℃ in a humidified atmosphere containing 5% CO_2. At 48 to 72 hours, we observed the adherent cell islets under phase-contrast microscopy, then, we removed non-adherent cells by changing the culture medium. At passage 3 to 5, HUVECs were used to conduct subsequent experiments.

The purity of HUVECs was examined by flow cytometry. The surface marker platelet endothelial cell adhesion molecule-1 (PECMA-1, CD31) of ECs was detected. Cells were incubated with fluorescein isothiocyanate (FITC)-conjugated mouse anti-human CD31 (Abcam, London, UK). The expression of CD31 on cells surface was analyzed by flow cytometry (Beckman Coulter, CA, USA).

HUVECs co-culture with DMSCs

Transwell chamber with 0.4μm pore filters (Corning, NY, USA) was selected to create an indirect interaction microenvironment for co-culture. HUVECs were co-cultured with DMSCs in a 12-well transwell plate for 48 hours at 37°C in 5% CO_2. DMSCs (8×10^4 cells/well) were seeded into the upper chambers in the transwell plates followed by 500μL medium (10% v/v FBS in DME/F12). Then HUVECs were seeded into the lower chambers at 1:1 ratio (DMSCs: HUVECs) followed by 1000μL EBM medium supplemented with EGM-2. Before HUVECs adhesion, DMSC-seeded chambers were moved into new 12-well plates for 6 hours to exclude the influence that HUVECs adhered to the well plate. After co-cultured, HUVECs were harvested for real time-quantitative polymerase chain reaction (RT-qPCR), western blot and HUVECs function analysis.

We divided HUVECs into five groups according to DMSCs co-cultured with them. The details of groups were as follows : Control group: HUVECs were cultured in the lower chamber alone. C-DMSCs group: DMSCs from healthy volunteers were co-culture with HUVECs. C-DMSCs$^{EDIL3\text{-}high}$ group: DMSCs from healthy volunteers with over-expressed EDIL3 co-cultured with HUVECs. P-DMSCs group: DMSCs from psoriasis co-cultured with HUVECs. P-DMSCs$^{EDIL3\text{-}low}$ group: DMSCs from psoriasis with low-expressed EDIL3 co-cultured with HUVECs.

Short interference RNA transfection to P-DMSCs

For silencing EDIL3 of DMSCs in psoriasis (P-DMSCs), EDIL3 short interference RNA (si-RNA, GenePharma, Shanghai, China) was transfected using HiperFect transfection reagent (QIAGEN, Dusseldorf, Germany) following the manufacturer's instructions. P-DMSCs were seeded into a 6-well plate until reached about 50% confluence and washed with DMEM/F12 for 3 times prior to transfection. Serum-free DMEM/F12 was mixed with 8μl of HiperFect transfection reagent and 4μg of si-RNA or negative control si-RNA (NC) and incubated for 20 minutes at 37℃. Then the mixture was added into 6-well plate containing of P-DMSCs and cultured at 37 °C for 24 hours. Media containing si-RNA were removed and replaced by DMEM/F12 supplied with 10% FBS after transfected for 24 hours. After 24 hours, the silence efficacy was evaluated by Laser Scanning Confocal Microscopy (LSCM, Olympus FV1200MPE, Japan) and the photographs were gathered. At 48h, 72h and 96h, respectively, cells were harvested for western blot analysis to detect the expression of EDIL3. EDIL3 si-RNA sequences are as follows: forward 5'-GGUGAUAUUUGUGAUCCCATT-3' and reverse 3'-UGGGAUCACAAAUAUCACCTT-5' and negative control si-RNA: forward 5'-UUCUCCGAACGUGUCACGUTT-3' and reverse 3'-ACGUGACACGUUCGGAGAATT-5'.

EDIL3 lentiviral vectors preparation and transduction to DMSCs

Recombinant EDIL3 lentiviral vectors were assembled by an EDIL3 expressed plasmid with green fluorescent protein reporter (pLenti-CMV-EDIL3-Flag-GFP-Puro) and two lentiviral packaging plasmids: psPAX2 and pMD2.G (PPL, Jiangsu, China). EDIL3 expressed plasmid and packaging plasmids transfected HEK293 cells by Lipofectamine 2000 (Signagen, Maryland, USA). After transfection for 48 hours, fluorescence intensity of HEK293 cells was used to evaluate transfection efficiency. And cell supernatant containing recombinant EDIL3 lentiviral vectors were collected and purified using BW-V2001 lentivirus concentration reagent (Biomiga, Beijing, China) according to the manufacturer's instructions.

DMSCs from healthy volunteers (C-DMSCs) were cultured in T25 culture flask with 5ml DME/F12 containing 10% FBS. C-DMSCs grown to 50% confluence were washed for three times by PBS and treated with recombinant EDIL3 lentiviral vectors. Quantification of cell fluorescence was performed using LSCM after 48 hours. Infected cells were harvested for western blot analysis for detecting the expression of EDIL3.

Real time-quantitative polymerase chain reaction (RT-qPCR)

Transfected P-DMSCs were harvested and mRNA was extracted using Trizol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. Then complementary DNA (cDNA) was reversely transcribed with mRNA and PrimeScript™ RT Master Mix kit (Takara, Tokyo, Japan). RT-qPCR was performed in a gradient thermal cycler (Bio-Rad, Cal, USA) using primers and TB Green™ Premix Ex Taq (TaKaRa, Tokyo, Japan). The β-actin was used as an internal control. The EDIL3 specific primers: forward 5'-AGCATACCGAGGGGATACATT-3' and reverse 3'- CAAGGCTCAACTTCGCATTCA-5'. The β-actin primers: forward 5'-CTACAATGAGCTGCGTGTGGC-3' and reverse 3'-CAGGTCCAGACGCAGGATGGC-5'. The expression level of the target gene was calculated according to the formulas: Target gene/ control = $2^{-\Delta\Delta Ct}$, where ΔΔCt = (Ct(target gene)—Ct(reference gene)) treat group—(Ct(target gene)—Ct(reference gene)) control group.

Western blot analysis

Cells were lysed in RIPA buffer (Beyotime, Beijing, China) containing protease inhibitor PMSF (Solarbio, Beijing, China) at ratio (100:1= RIPA : PMSF) and protein phosphatase inhibitor (Solarbio, Beijing, China), then clarified by centrifugation at 13,000g for 10 minutes at 4 °C. Protein concentration was quantified using BCA Protein Assay Kit (Solarbio, Beijing, China). Western blot was performed by automatic protein analyzer (Wes&Jess, NY, USA) according to the manufacturer's instruction. All protein levels were normalized to β-actin. The blots were reacted with rabbit anti-human against β-actin (CST, Boston, USA), phospho-FAK, FAK, phospho-MEK1, MEK1, phospho-ERK1/2, ERK1/2, β3, αv, α5, β1 (all from Abcam, Cambridge, UK).

HUVECs proliferation assay

The proliferative potential of HUVECs was assessed by cell counting kit-8 (CCK8, Boster, Wuhan, China). After co-culture for 48 hours, 100ul HUVECs suspension was added into 96-well plates. According to the

instruction, 10ul CCK-8 solution was added into each well. The plates were incubated at 37°C for 4 hours, followed by the absorbance measurement using an enzyme-marked analysis system (PerlonQ Instruments, Beijing, China). The absorbance measurement of every sample was conducted for three times.

Cell adhesion assay

To evaluate the adherent ability of HUVECs, the assay of attachment of DMSCs to HUVECs was conducted. After co-culture 48 hours, HUVECs monolayer formed and DMSCs-seeded chamber were removed. Then, DMSCs from healthy volunteers were dyed with Calcein-AM (Solarbio, Beijing, China) and resuspended in DME/F12 with 10 % FBS. A total of 500ul cell suspension containing labeled DMSCs ($2Í10^5$ cells/well) was directly added onto the HUVECs monolayers and incubated for 15 minutes at 37°C. Afterwards, the plate was gently washed for three times with warm PBS to remove non-adherent DMSCs. Adherent DMSCs were quantified by counting the average number of cells in five different field of each well using LSCM and analyzed with Image Pro Plus software (NIH, Bethesda, USA).

Cell motility assay

Wound scratch assay was used to assess the motility ability of HUVECs. HUVECs monolayer formed after co-culture 48 hours, and a scratch was gently introduced into the cell monolayer using a 200ul sterile pipette tip. To remove cells debris, the monolayer was gently washed with PBS for 3 times. Afterwards, low serum medium was added into the well. The scratch was visualized using an inverted light microscope (Olympus, Tokyo, Japan). To calculate cell migration, images at different time points (0h and 12h) was taken after scratch. The percentage of wound area recovered was calculated using the formula given below and was analyzed for significance using ANOVA.

$$\text{Percentage of wound area recovered} = \frac{(\text{Initial wound area (0h)} - \text{Final wound area (12h)})}{\text{Initial wound area (0h)}} \times 100\%$$

Transwell migration assay

HUVECs migration assays were performed using 24-well transwell chambers (8μm pore size polyester membrane, Corning, NY, USA). After co-cultured 48 hours, HUVECs were resuspended at a density of $4Í10^4$cells in 100ul serum-free DMEM/F12 and added into the upper chamber. The lower chambers were filled with 600ul EBM medium supplemented with EGM-2 as a chemoattractant. Then the chambers were incubated in 5% CO_2 at 37°C for 24 hours to allow HUVECs to migrate through the polyester membrane. After 24 hours incubation, HUVECs in the upper surficial membrane were removed with a cotton swab, and HUVECs that migrated to the lower membrane were fixed with 4% paraformaldehyde for 30 minutes and stained with 0.1% crystal violet for 20 minutes at room temperature. The migrated cells were visualized and imaged using an inverted microscope (Olympus, Tokyo, Japan). The number of migrated

cells was quantified in three random fields and the data were analyzed using Image J software (NIH, Bethesda, USA).

Tube formation assay

In vitro, the tube formation of ECs was assessed using Matrigel matrix (mimics the natural basement membrane matrix of ECs). All procedures were performed on ice under sterile condition. The 96-well plate and micro pipette tip were pre-cooled. Then, 40μl/well Matrigel (Corning, NY, USA) was coated in 96-well plate and placed at 37° C in a CO_2 incubator for 3 hours. After co-culture, HUVECs were trypsinized and seeded at a density of 2Í10^4 cells/well in 100ul EBM medium containing EGM-2. After 6 hours, at peak of tube formation, the images of tube-like structures were captured using an inverted microscope (Olympus, Tokyo, Japan). The mesh numbers of tube structures were measured using ImageJ software and the results were expressed as the mean ± SEM.

Mice and treatment

All procedures involving in mice experiments were approved by the Animal Care and Use Committee of the Shanxi Medical University in conformity with National Institutes of Health guidelines. BALB/c mice at 6-8-weeks of age were purchased from Changsheng Biological Corporation (Changchun, China). Mice were housed in standard sterile mouse cages. Mice were depilated on the back skin before the treatment using depilatory cream (Veet, Shanghai, China) and randomly assigned to different experimental groups in a blind manner. Grouping and processing are as follows:(1) mice were treated with 62.5mg of vaseline cream on the shaved back for 7 consecutive days (Control, Mingxin, Sichuan, China), (2) mice received a daily topical 62.5mg dose of 5% Imiquimod cream on the back for 7 consecutive days(IMQ, Mingxing, Sichuan, China), (3): mice received a daily topical 62.5mg dose of 5% IMQ cream and subcutaneous injection 50ul NS solution on the back for 7 consecutive days (IMQ+ 0.9% NS),4mice received a daily topical 62.5mg dose of 5% IMQ cream and subcutaneous injection of 50ul EDIL3 protein on the back for 7 consecutive days IMQ+ 20ng/ul EDIL3. Recombinant EDIL3 protein was obtained from R&D Systems (Minnesota, USA). Disease severity of the back lesions was evaluated daily with a semi-quantitative scoring system including scaling and erythema. To evaluate microvessel densities during modeling, at on day 4, half of every group mice were sacrificed by cervical dislocation and skin samples were collected within 2 hours for additional experiments. At the end of the experiment on day 7, remainder mice were sacrificed and skin samples were collected. Skin biopsies were processed for paraffin sections, which were then stained with hematoxylin and eosin (H&E). Moreover, skin biopsies were embedded in tissue-tek OCT compound (Thermo Scientific, Massachusetts, USA) and stored at -80°C.

Immunofluorescent and H&E staining

To measured epidermal thickness and histopathological examinations, paraffin sections were stained with H&E. and images of histopathology were taken by inverted microscopy (Olympus, Tokyo, Japan). Epidermal thickness was measured under microscope.

The immunofluorescent (IF) staining of mice skin biopsies for CD31 was performed to assess the microvessel densities. The 5um thick tissues were sectioned from frozen tissue. To inhibit non-specific antigen–antibody reactions, tissues were blocked with goat serum (Boster Biological Technology, Cal, USA) for 30 minutes and washed for 3 times with PBS buffer. Primary rabbit anti-CD31 (Abcam, London, UK) was added and incubated at 4°C overnight. The next day, recovery temperature at 37℃, slides were washed in PBS for 3 times and incubated with secondary antibody (Goat anti-rabbit IgG, Zhongshanjinqiao, Beijing, China) for 2 hours at room temperature. Finally, slices were washed for three times with PBS, incubated with 4', 6-diamidino-2-phenylindole (DAPI, Solarbio, Beijing, China) for 10 minutes at room temperature. Sections were then imaged using LSCM (Olympus FV1200MPE, Japan).

Statistical analysis

Statistical analyses were performed using 17.0 SPSS software (Chicago, USA). Differences among groups were evaluated using one-way analysis of variance (ANOVA). A two-tailed t-test was used when two groups were compared for statistical significance. All data were expressed as mean ± SEM. $P < 0.05$ considered to be statistically significant (*P <0.05, **P<0.01, ***P <0.001).

Results

Identification and culture of HUVECs

DMSCs were incubated in DMEM/F12 supplied with 10% FBS and 1% antibiotic at 37℃ in a humidified atmosphere containing 5% CO_2. The primary DMSCs attached to the bottom of the plates after 24-72 hours incubation. At day 14 to 17, DMSCs showed typical fibroblast morphology like a spindle with multi-layered flat cell bodies **(Fig.1 A**). The surface markers of DMSCs derived from both psoriasis and healthy individual were positive for CD105, CD29, CD44, CD73, and CD90 and negative for CD45, CD34, and CD14 (**Fig.1B**). In this study, DMSCs were differentiated into adipocytes, osteoblasts, and chondrocytes (**Fig.1 C-E**) in DME/F12 medium with 10% FBS and additional supplementation of respective differentiation medium according to our previous experiments [27].

HUVECs were incubated in EBM supplied with EGM-2 at 37℃ in a humidified atmosphere containing 5% CO_2. The primary HUVECs attached to the bottom of the plates after 24 hours incubation and the blood cells suspended in the medium. Then blood cells were removed through replaced fresh medium. After 3 to 5 days incubation, HUVECs showed cobblestone-like morphology (**Fig.1F**). To further identify HUVECs, the expression of surface marker CD31 was tested by flow cytometry. Consistence with HUVECs identification, the surface marker of the isolated cells was positive CD31 (**Fig.1G**).

Si-RNA successfully reduced EDIL3 level in P-DMSCs

After interference for 24 hours, the transfection efficiency was evaluated with FAM labeled nucleic acid and 90% P-DMSCs were positive with FAM signal by fluorescent microscope analysis (**Fig.2A and 2B**). For 24 hours transfection, mRNA level of EDIL3 was detected by RT-qPCR. Compared with non-transfected P-

DMSCs, expression of EDIL3 in transfected groups was significantly down-regulated by about 76% (**Fig.2C**) (p<0.001). To determine optimize time range of lower expression of EDIL3 induced by si-RNA, at 48h, 72h and 96h, the respective P-DMSCs transfected were collected for western blot. Our data showed that the protein expression of EDIL3 was lower at 48h, however, it recovered at 96h (**Fig.2D**). EDIL3 molecular weight was approximately 53 kDa. Compared with the negative controls, the protein reduction of EDIL3 in P-DMSCs was about 65.4% at 48h, which was significant decreased (**Fig.2E**) (p<0.001). Thus, the subsequent co-cultured incubation was all carried out for 48 hours. These data all confirmed that EDIL3 in P-DMSCs was successfully down-regulated by si-RNA.

Establishment of stable EDIL3 over-expression in DMSCs

As shown in **Fig.3A**, lentiviral vector of EDIL3 with flag tag in which human EDIL3 gene was tagged in frame with green fluorescent protein (GFP) was selected as the cloning and expression vector. EDIL3 fusion expression plasmid was then co-transfected into HEK293 cells along with a lentivirus package mix. After 48 hours, the assessment showed the successful transfection in HEK293 cells (**Fig. 3B and 3C**). After C-DMSCs were transfected using recombinant EDIL3 lentiviral vector, most C-DMSCs expressed GFP showing successful lentiviral vectors packaging and cell transfection (**Fig. 3D and 3E**). Overexpression of EDIL3 was confirmed by western blot compared to with non-transfected cells (**Fig. 3F**). These data demonstrated that the overexpression of EDIL3 in C-DMSCs was successfully manipulated with recombinant EDIL3 lentiviral vectors.

DMSCs-derived-EDIL3 had no effect on HUVECs proliferation

The proliferative capability of HUVECs was assessed using CCK8 assay. Co-culture with DMSCs all showed a positive effect on the proliferation of HUVECs (**Table2**). Compared to the control, all co-cultured groups with DMSCs were significantly increase in cell proliferation (all p<0.05) (**Fig.4**). The proliferation of HUVECs co-cultured with C-DMSCs$^{EDIL3\text{-}high}$ and P-DMSCs was significantly unaltered compared to the C-DMSCs (all p>0.05) (**Fig.4**). The result demonstrated that EDIL3 had no effect on HUVECs proliferation. However, compared with P-DMSCs group, P-DMSCs$^{EDIL3\text{-}low}$ group showed a slight reduction in proliferation of HUVECs (p<0.05) (**Fig.4**). In the overexpressed EDIL3 group, the proliferative capability of HUVECs was not significantly upregulated and the proliferation of HUVECs was decreased after EDIL3 knockdown. The results may be caused by other molecules mediated by EDIL3 but not direct EDIL3.

DMSCs-derived-EDIL3 promoted HUVECs adhesion

Under observation with fluorescence microscope, compared to the control, massive DMSCs that adhered to HUVECs monolayer were observed in the C-DMSCs, C-DMSCs$^{EDIL3\text{-}high}$, P-DMSCs and P-DMSCs$^{EDIL3\text{-}low}$ groups (**Fig.5A-E**). **Table 3** showed the different numbers of adherent cells in five groups. As shown in **Fig.5F**, compared with the control, co-cultured with DMSCs resulted in an increased adherent ability of HUVECs (p < 0.001). Our results showed that C-DMSCs treatment with recombinant EDIL3 lentiviral vector increased HUVECs adhesion by 29.34% compared with the C-DMSCs, and P-DMSCs treatment with si-

EDIL3 inhibited this adhesion by 25.63% compared with the P-DMSCs (**Fig.5F**) (all $p < 0.001$). These findings indicated that DMSCs-derived-EDIL3 promoted HUVECs adhesion.

DMSCs-derived-EDIL3 promoted the motility of HUVECs

Motility is the ability to move spontaneously and actively. After co-culture, to evaluate the motility capacity of HUVECs, we performed wound scratch assay. For each experiment, HUVECs motility was evaluated using a scratch formula. The images at 0h and 12h in all groups were shown in the **Fig.6A**. The recovery percentages in co-cultured groups were all higher than the control (control vs. C-DMSCs vs. C-DMSCs$^{EDIL3\text{-}high}$ vs. P-DMSCs vs. P-DMSCs$^{EDIL3\text{-}Low}$: 13%±5% vs. 31%±2% vs. 47%±3% vs. 64%±4% vs. 41%±3%). The percentage of wound area recovered after 12 hours in C-DMSCs and P-DMSCs were 31% and 64%, respectively ($p< 0.01$) (**Fig.6B**). To evaluate whether the observed increase in migration of HUVECs through treatment with P-DMSCs was mediated by EDIL3, we silenced EDIL3 gene expression in P-DMSCs by transfecting si-RNA and over-expressed EDIL3 in C-DMSCs by stably transfecting EDIL3 lentiviral vectors. Transfection with EDIL3 lentiviral vectors in C-DMSCs significantly promoted the motility of HUVECs ($p< 0.01$) (**Fig.6B**). P-DMSCs silencing EDIL3 specifically inhibited cell migration and motility ($p < 0.001$) (**Fig.6B**). However, compared with the C-DMSCs$^{EDIL3\text{-}high}$, HUVECs motility was significantly increased in P-DMSCs ($p< 0.01$) (**Fig.6B**). Moreover, the HUVECs motility in P-DMSCs$^{EDIL3\text{-}low}$ group was higher than the C-DMSCs group ($p< 0.05$) (**Fig.6B**). Our data suggested that DMSCs-HUVECs co-cultured groups showed faster closure rate than the control group and P-DMSCs obviously promoted HUVEC motility, at least in part, via EDIL3 dependent pathway.

DMSCs-derived-EDIL3 promoted the directional migration of HUVECs

Since cell directional migration is an important event in vascular elongation, we assessed the EDIL3 effect on HUVECs migration using a transwell chamber. Representative photomicrographs of migrated cells towards the lower membranes were taken (**Fig.7A-E**). In co-culture groups, the migration of HUVECs was significantly enhanced (control vs. C-DMSCs vs. C-DMSCs$^{EDIL3\text{-}high}$ vs. P-DMSCs vs. P-DMSCs$^{EDIL3\text{-}low}$: 37.33±5.24 vs. 68.08±5.22 vs. 113.17±3.00 vs. 151.53±3.56 vs. 89.6±2.82). The results demonstrated that DMSCs had a prominent pro-migratory effect on HUVECs. In addition, we observed significant up-regulation of cell migration in co-culture with the P-DMSCs compared to with the C-DMSCs ($p<0.001$) (**Fig. 7F**). Transfected EDIL3 lentiviral vectors in C-DMSCs exhibited a significant increase of HUVECs migratory capacity in comparison with the non-transfected ($p<0.01$) (**Fig.7F**). Moreover, compared with the P-DMSCs, si-RNA of EDIL3 transfected in the C-DMSCs showed a significant reduction of HUVECs migration ($p<0.001$) (**Fig. 7F**). Interestingly, HUVECs also exhibited significantly greater migration in the P-DMSCs group than the C-DMSCs$^{EDIL3\text{-}high}$ under the same conditions ($p<0.01$) (**Fig.7F**), indicating the presence of other pro-angiogenic molecules derived from the P-DMSCs. These results confirmed EDIL3 was the most important pro-angiogenic molecule for directional migration of HUVECs.

DMSCs-derived-EDIL3 induced tube formation of HUVECs in vitro

The process of tube formation is a result of dynamic reorganization of vascular system in vitro and is the characteristic trait of ECs. HUVECs belong to ECs and have the ability of tube formation in vitro. This study further confirmed that our protocols isolating HUVECs was successful and feasible (**Fig. 8A**). The number and stability of tube mesh formation was found to be increased in co-culture groups. After 48 hours, the control no treatment with DMSCs displayed very few tubes, however the prominent branching networks were observed in DMSCs treated groups (**Fig.8 A-E**) and the increase was significant (all $p<0.05$) (**Fig.8F**). Tube-forming ability in C-DMSCs, as measured by the number of meshes assembled, was significantly decreased compared to that in P-DMSCs ($p<0.001$) (**Fig.8F**). In EDIL3 over-expressed group, after co-culture, sprouting HUVECs were numerous and tubes were longer compared to these in C-DMSCs group (**Fig.8 B-F**) ($p<0.01$). Knockdown of EDIL3 in P-DMSCs significantly inhibited the tube formation of HUVECs compared to that in P-DMSCs ($p<0.001$) (**Fig.8F**). A similar effect was observed in EDIL3 over-expressed C-DMSCs and P-DMSCs, however, the increased tube meshes in P-DMSCs were more significant ($p<0.05$) (**Fig.8F**). These indicated that DMSCs-derived-EDIL3 involved in the tube formation of ECs. And EDIL3 involved in psoriasis, and it was an important pro-angiogenic.

EDIL3 promoted angiogenesis in vivo

Although tube formation assay in vitro is widely used to study the ability of ECs angiogenesis, it fails to recapitulate the sprouting behavior of ECs from the preexisting blood vessels and represents the complexities of multicellular interactions that occur in angiogenesis in vivo. Therefore, we intra-dermally injected recombinant EDIL3 protein or 0.9% sodium chloride solution (NS) once per day for 3 and 6 consecutive days in the IMQ-induced psoriasis-like mouse model, and the mice were sacrificed at day 4 and 7 (**Fig.9A**). First, after administrating consecutively IMQ cream or vaseline on the shaved mouse back skin for 6 days, we assessed whether IMQ application induced skin inflammation and hyperproliferation of epidermis accompanied by characteristic structural features of psoriasis and the clinical and pathological phenotypes of the back skin of mice model were shown in **Fig.9B**. Mice treated daily with vaseline did not show any sign of inflammation or psoriasis-like lesion. To further accurately evaluate the clinical phenotype, we applied a semi-quantitative scoring system from 0 to 4 based on their external physical appearance: 0, none, 1, slight, 2, moderate, 3, marked, 4, very marked. The erythema and scaling scores of individual mice in every group were shown in **Fig.9C and D**. In addition, the epidermis thickness of the back lesion was measured in H&E staining tissue slice using a microscope. Histological analysis showed that epidermal thickness was markedly increased in IMQ treated mice compared with that vaseline treated (all $p<0.001$) (**Fig.9E**). And interesting, IMQ+EDIL3-induced psoriasis-like lesion in mouse model resulted in higher epidermis thickness compared with the IMQ+NS treated ($P<0.05$) (**Fig.9E**). These results not only demonstrated that IMQ cream induced psoriasis-like mouse model was accessible but also EDIL3 accelerated the pathological progress of IMQ-induced psoriasis mice.

Study showed that EDIL3 promoted tumors angiogenesis in vivo **[**14]. To determine whether EDIL3 also modulated dermal vascular network expansion in psoriatic mouse models, we conducted immunofluorescence analysis in skin sections and double immunofluorescence staining with DAPI and CD31 was evaluated via two-photon confocal laser microscopy. We confirmed an up-regulation of blood

vessel density in psoriatic mice skin at day 4 or 7 (**Fig.9F and G**) and the result indicated EDIL3 protein significantly increased the blood vessel density. In addition, compared to that at day 4, EDIL3-injected skins exhibited more increased blood vessel density at day 7, and EDIL3 caused a time dependent increase of angiogenesis. Together, these data suggested that the upregulation of EDIL3 can promote microvascular formation in development of psoriasis.

DMSCs-derived-EDIL3 upregulated integrin αvb3 and α5b1 in HUVECs

This study evaluated whether the knockdown of EDIL3 in P-DMSCs could reduce the level of integrin αvb3 and α5b1 for EDIL3 receptors, and whether EDIL3 overexpressed in C-DMSCs could increase the level of integrin αvb3 and α5b1 in HUVECs. After co-culture with DMSCs, the proteins of integrin αv, integrin β3, integrin α5 and integrin β1 in HUVECs were analyzed through western blot. The images of proteins were shown in the **Fig.10 A & B**. The images revealed that both P-DMSCs and EDIL3 overexpressed C-DMSCs upregulated the expression of integrin αvb3 and α5b1 (p<0.05) (**Fig.10B**). These data confirmed that DMSCs-derived-EDIL3 effectively upregulated the expression of integrin αvb3 and α5b1. Moreover, knockdown of EDIL3 in P-DMSCs obviously downregulated them (p<0.05). We also found compared with the controls, the expression of integrin αvb3 and α5b1 was upregulated in all co-cultured groups (p<0.05) (**Fig.10B**). And in P-DMSCs group, the expression of integrin αvb3 and α5b1 was higher than C-DMSCs$^{EDIL3\text{-}High}$ (p<0.05) (**Fig.10B**). Together, these studies confirmed that not only DMSCs-derived-EDIL3 upregulated integrin αvb3 and α5b1 but also the increase of integrin αvb3 and α5b1 in P-DMSCs was more evident compared with that in EDIL3 overexpressed C-DMSCs.

DMSCs-derived-EDIL3 activated the integrin signal pathway in HUVECs

To further investigate the mechanism of EDIL3-associated angiogenesis in ECs, we examined the effects of EDIL3 on integrin signal pathway. A large number of studies have shown that integrin signal pathway involves in FAK, MEK1 and ERK1/2. To verify whether DMSCs-derived-EDIL3 induced changes of FAK, MEK1 and ERK1/2 depended on activation of integrins, after co-culture, we analyzed the protein expressions of FAK/p-FAK, MEK1/p-MEK1, ERK1/2 and p-ERK1/2 in HUVECs. Co-incubation of P-DMSCs and over-expressed EDIL3 C-DMSCs with HUVECs for 48h induced a significant increase of p-FAK, p-MEK1 and p-ERK1/2 proteins in HUVECs (**Fig.11A**) and co-incubation of EDIL3 knockdown P-DMSCs with HUVEC decreased significantly the expression of p-FAK, p-MEK1 and p-ERK1/2 (**Fig.11A**). In addition, we further verified that p-FAK, p-MEK1 and p-ERK1/2 was upregulated in over-expressed EDIL3 group and P-DMSCs (**Fig.11B**). Between the P-DMSCs and over-expressed EDIL3 groups, no significant changes of the protein expression of p-MEK1 and p-ERK1/2 were observed (**Fig.11B**). However, the p-FAK in P-DMSCs group were significantly increased than these in C-DMSCs$^{EDIL3\text{-}High}$ (**Fig.11B**). These suggested the activation of FAK/MEK/ERK signal pathway in HUVECs, which was positively regulated through EDIL3 and integrins which involved in adhesion, migration and tube formation process of HUVECs. After knockdown of EDIL3 in P-DMSCs, we found the phosphorylated proteins of integrin signal pathway were all significantly decreased (**Fig.11B**), demonstrating that DMSCs-derived EDIL3 may be extremely important in integrin signal pathway.

Discussion

Psoriasis is not only an inflammatory-dependent but also an angiogenesis-dependent disease [5,28]. Cytokines produced by immune cells, keratinocytes and ECs perpetuate the inflammatory process via positive feedback loops. Whether it is the inflammatory cell or the angiogenesis that initiates psoriasis is not clear, which like a long standing ‘chicken or egg’ question. Microvascular changes in lesions of psoriasis include vascular enlargement, pronounced tortuosity and elongation, increased permeability and ECs proliferation in the superficial dermis [29]. The expanded and tortuous microvascular in lesions is required for circulation of nutrients, signaling molecules, gas exchange, waste removal and to provide an enlarged endothelial surface area for inflammatory cell trafficking [28]. The initiation or maintenance of the chronic inflammatory state in psoriasis may depend partly on mediators of the angiogenic pathway [30]. It is generally admitted that vascular expansion in psoriasis is secondary to vessel enlargement, elongation and increased tortuosity rather than sprouting angiogenesis from the pre-existing vascular bed [4]. However, both angiogenesis and microvascular tortuosity and elongation make ECs active. So, altered ECs functions are involved in pathological processes of psoriasis and play important role in disease perpetuation and maintenance.

Mesenchymal stem cells (MSCs), that can differentiate into adipocytes, osteoblasts, and chondrocytes, are self-renewing and expandable stem cells [31]. CD73, CD90 and CD105 of MSCs surface are positive, however the markers of the hematopoietic stem cells including CD14, CD45, CD79 and HLA-DR are absent [32]. We isolated DMSCs from skin from psoriatic patients and healthy volunteers and the results showed its surface markers were same as MSCs. And DMSCs also differentiated into adipocytes, osteoblasts and chondrocytes. This study suggested that DMSCs were similar with MSCs derived from other tissues. CD31 is a specific marker of ECs [33] and ECs can form capillary-like structures plated on the Matrigel matrix in vitro, which can be used to define ECs [34]. In present study, we successfully isolated DMSCs and HUVECs and co-cultured them to evaluate the effect of DMSCs on ECs.

Our microarray analysis (data deposited under NCBI GEO GSE42632) showed that DMSCs from psoriatic skin lesions displayed significantly high expression of EDIL3 [27]. We further analyzed the mRNA and protein of EDIL3 using RT-qPCR and western blot. The results showed mRNA and protein expression of EDIL3 in psoriatic DMSCs was markedly higher than these in healthy control [8]. Previous studies have reported that EDIL3 is a recently cloned and characterized unique matrix protein that is expressed during early embryogenesis in ECs [35], and its expression is downregulated in later developmental stages. In healthy adult tissue, EDIL3 becomes quiescent or may no longer be expressed [15, 35, 36]. However, EDIL3 expression can be re-initiated during ischemia and is upregulated in tumor vascular tissues [35,36]. These findings suggested that EDIL3 may have an important role in mediating aberrant vascular remodeling. EDIL3 can stimulate the attachment, migration and tube formation of ECs in vitro and promote angiogenesis in the CAM assay [37]. In addition, EDIL3 also acts as a survival factor of ECs by upregulating the Bcl-2 to prevent EC apoptosis [1]. Studies also demonstrated that EDIL3 prevented apoptosis of endothelial cells through binding integrin when cultured in vitro without any effect on proliferation [38]. Indeed, we also found in psoriasis and overexpressed EDIL3 groups, HUVECs

proliferation was no significant increase compared with that in healthy control. However, compared with the low-expressed EDIL3 group, the proliferation of ECs was slight increase in psoriasis groups. Literatures showed that the anti-apoptotic effect of EDIL3 on HUVECs was in fact additive to the anti-apoptotic role of VEGF and b-FGF [39]. Thereby, we speculated that knockdown of EDIL3 in P-DMSCs weakened the additive anti-apoptotic effect of EDIL3 on other cytokines and the death and the number of HUVECs was decreased. In this study, the OD value in CCK8 assay was significantly decreased, however, this merely represented an increase in the number of HUVECs, not an upregulation of proliferation. Our results suggested that EDIL3 inhibit apoptosis of HUVECs without supporting proliferation.

In this study, we also found that in over-expressed EDIL3 group, adhesion, motility and migration of HUVECs were significantly increased which was consistent with the psoriasis groups. And in EDIL3 knockdown groups, adhesion, motility and migration of HUVECs were significantly decreased. These data suggested that DMSCs-derived-EDIL3 in psoriasis promoted adhesion, motility and migration of HUVECs and involved in the pathology of abnormal vascular network. Some studies have shown EDIL3 can bind different integrins in different cell types or even within the same cell with different effects [20]. Our findings were most consistent with the studies [13] in which DMSCs-derived-EDIL3 promoted the adhesion and migration of ECs. Interestingly, although EDIL3 was overexpressed in C-DMSCs, the migration and motility of HUVECs were still lower than these in P-DMSCs. It was likely that various molecules associated with cells migration and motility were disrupted in P-DMSCs. Prior studies also suggested some cytokines such as VEGF, basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), inducible nitric oxide synthetase (iONS), and NO were up-regulated in P-DMSCs [9,27]. Other studies also found that EDIL3 transcription could be inhibited by other factors secreted by DMSCs, including VEGF and FGF2 [38] and stimulated by TNF-α, IFN-γ, BMP-2 and IL-1α [38,40]. These indicated that cytokines secreted by DMSCs could regulate each other. When we knockdown EDIL3, the migration and motility of HUVECs were significantly decreased in P-DMSCs and C-DMSCs groups. The results may be caused by down-regulated EDIL3 which reduced the release of other cytokines. So, DMSCs-derived-EDIL3 may be not the unique molecule associated with the adhesion, motility and migration of ECs but the important one.

The tube formation ability of ECs involves in the dilatation and tortuous of dermal papillary blood in psoriasis. After co-culture, we analyzed the numbers of the tube meshes of HUVECs in vitro. The numbers of meshes were significantly increased in over-expressed EDIL3 and psoriasis groups. Our data indicated that DMSCs-derived-EDIL3 contributed to activating ECs properties, then promoted tube-structure formation and caused the dilatation and tortuous of microvascular. Compared with the control, the tube formation of HUVECs in EDIL3 knockdown group was decreased without significance. The result may mainly be caused by decreased migration of ECs. The studies of angiogenesis have shown that the process occurs via cell motility, migration and division to form sprouts, and the resulting hollow vascular tubes anastomose to form capillary loops [32]. Cells motility and migration play important roles during elongation of vascular. To further explore the role of EDIL3 in psoriasis in vivo, we detected the epidermis thickness and microvessel density in IMQ-induced mouse model through injecting EDIL3 or NS. Our studies found that the epidermis thickness and microvessel density were both elevated in the IMQ-

induced psoriasis-like mouse models. The increased microvascular in lesions is required for epidermis cells to circulate nutrients, molecules, gas exchange, and waste removal. The hyperplasia of the epidermis and maintenance may depend partly on abnormal elongation of microvascular. The results suggested that EDIL3 accelerated the process of psoriasis through promoting ECs to change into activated phenotype and induce elongation of microvascular.

It is well known that integrins can mediate cell adhesion on matrix through the RGD motif domain [42]. Others showed that EDIL3 regulated cell motility and angiogenesis in vitro or vivo through binding integrin αvβ3, αvβ5 and α5β1 [13, 15, 20, 43, 44]. To clarify the mechanism of the effect of DMSCs-derived-EDIL3 on ECs, we explored the downstream integrin pathway associated with EDIL3 which induced adhesion, migration and angiogenesis of HUVECs. We found that EDIL3 increased expression of integrin αvβ3 and α5β1 in HUVECs. Other studies have also supported this claim that activation of αvβ3 receptor is required for vascular development and may be associated with a decrease in endothelial cell apoptosis and with downstream angiogenic signaling through protein kinases [37]. Thus, it is possible that DMSCs-derived-EDIL3 could enhance ECs adhesion, migration and tube formation by activating the integrin signaling pathway. Multiple integrin signaling pathways have been implicated in cell migration and adhesion, with activation of Src family kinases, FAK, PI3 kinase (PI3K/AKT) and the small GTP binding proteins Rac and Rho [24]. After co-culture with the silenced or over-expressed EDIL3 DMSCs, we detected the protein of FAK/MEK/ERK signal pathway in HUVECs. Our results demonstrated that after the DMSCs-derived-EDIL3 stimulation, FAK-ERK interactions are activated. Analysis of HUVECs following EDIL3 treatment revealed increased integrin-linked kinases such as increased p-FAK, FAK-linked kinase p-MEK and p-ERK, consistent with increased cell adhesion, migration and tube formation. Taken together, these data suggested that DMSCs-derived-EDIL3 mediated ECs attachment, migration and tube formation through RGD motif interaction with the integrin αvβ3 and α5β1 receptors. The integrin family could interact with EDIL3, activating FAK/MEK/ERK signal pathway (**Fig.12**), transferring the extracellular signaling into cytosol, and affecting the expression of nuclei DNA and cytoskeleton.

Conclusions

In conclusion, this study demonstrated DMSCs from healthy and psoriatic skin showed the characteristics of MSCs and all promoted the adhesion, migration and angiogenesis of HUVECs. However, in P-DMSCs, the effects were more obvious. The transwell co-culture system without direct cell-to-cell contact implied DMSCs influenced HUVECs by soluble cytokines. EDIL3 was upregulated in P-DMSCs and was an important pro-angiogenic protein. We demonstrated that DMSCs-derived-EDIL3 induced the adhesion, migration and angiogenesis of HUVECs in vitro and in vivo through enhanced the FAK protein associated with integrin αvβ3 and α5β1 and FAK phosphorylation. And these enhancements correlated with the activation of the MEK/ERK signaling pathway in ECs. The over-expressed EDIL3 in DMSCs induced the activation of the signaling pathway that can be inhibited by knockdown of EDIL3 expression via si-RNA in P-DMSCs, in accordance with the inhibiting the capabilities of adhesion, migration and angiogenesis of ECs. We first examined the effect of DMSCs-derived-EDIL3 in psoriasis on cell adhesion, migration and angiogenesis signaling. Upon binding ligand, the integrins triggered

cytoplasmic signals that modulated cellular functions. Based on these results, we suggested that EDIL3 and integrin signal pathway will provide a valuable therapeutic target to control angiogenesis, the essential step in occurrence and development of psoriasis.

Declarations

Conflicts of interest

All authors indicated no potential conflicts of interest.

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Authors' contributions

NXP and HQX contributed to the conception and design of the study, collection and assembly of the data, data analysis and interpretation, and manuscript writing. LXF, LJ, and LYM performed the animal experiments and were responsible for the animal handling. ZKM, LXH, WY and LY contributed to the experimentation. All authors read and approved the final version of the manuscript.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

All the experimental procedures were performed with the approval of the Experimental Research Institute of the Taiyuan Central Hospital in Shanxi Medical University and followed the guidelines of the Institutional Animal Care and Use Committee.

Consent for publication

Not applicable.

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Tables

Table 1 Characteristics of psoriatic patients

Sample No.	Age, years	Sex	Duration of psoriasis	Plaque location
Y1	23	F	10 years	Back
Y2	14	M	3 months	Arm
Y3	33	F	7 years	Arm
Y4	29	F	20 years	Thigh
Y5	27	M	6 years	Back

Table 2 OD value

group	control	C-DMSC	C-DMSC^{EDIL3high}	P-DMSC	P-DMSCs^{EDIL3Lower}
Mean±SEM	0.30±0.01	0.49±0.04	0.50±0.04	0.53±0.02	0.44±0.03

Table 3 Numbers of adherent cells

group	control	C-DMSC	C-DMSC^{EDIL3high}	P-DMSC	P-DMSCs^{EDIL3Lower}
Mean±SEM	49.27±8.76	136.38±5.38	176.40±6.72	187.02±6.21	139.07±4.30

Figures

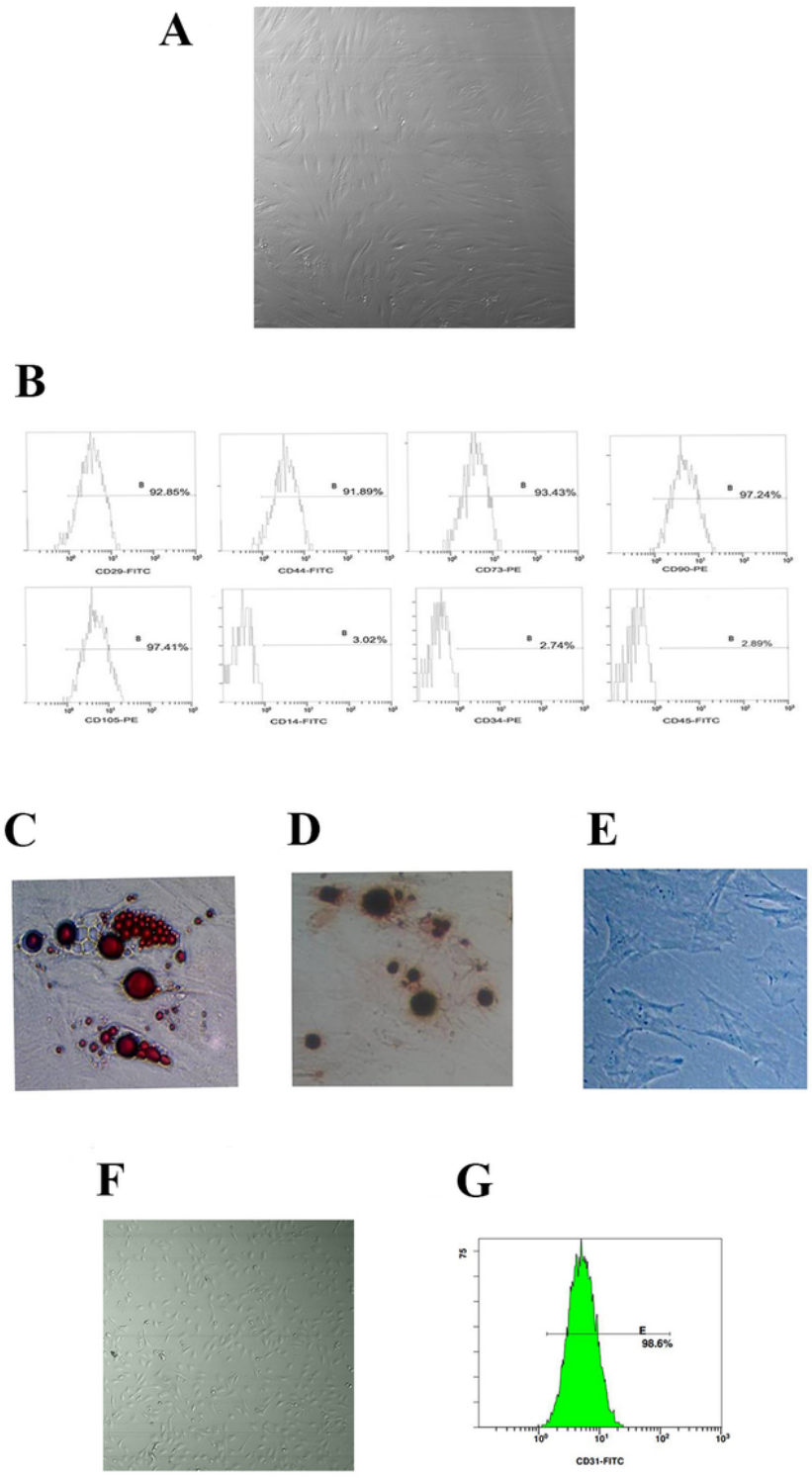

Figure 1

Characteristics of DMSCs and HUVECs. a Fibroblast-like cell morphology of DMSCs. b The surface markers of DMSCs derived from both psoriasis and healthy individual were positive for CD105, CD29, CD44, CD73, and CD90 and negative for CD45, CD34, and CD14 by Flow cytometry. c-e Morphological identification of DMSCs differentiated into lipocytes, osteoblasts and chondrocytes in the respective inductive medium in vitro. c Lipocytes after staining with oil red O. d Osteoblasts after staining with

alizarin red. e Chondrogenic pellet after slicing and staining with toluidine blue. F Cobblestone-like cell morphology of HUVECs. g Flow cytometry revealed that the surface marker CD31 of isolated HUVECs was positive. (40× magnification

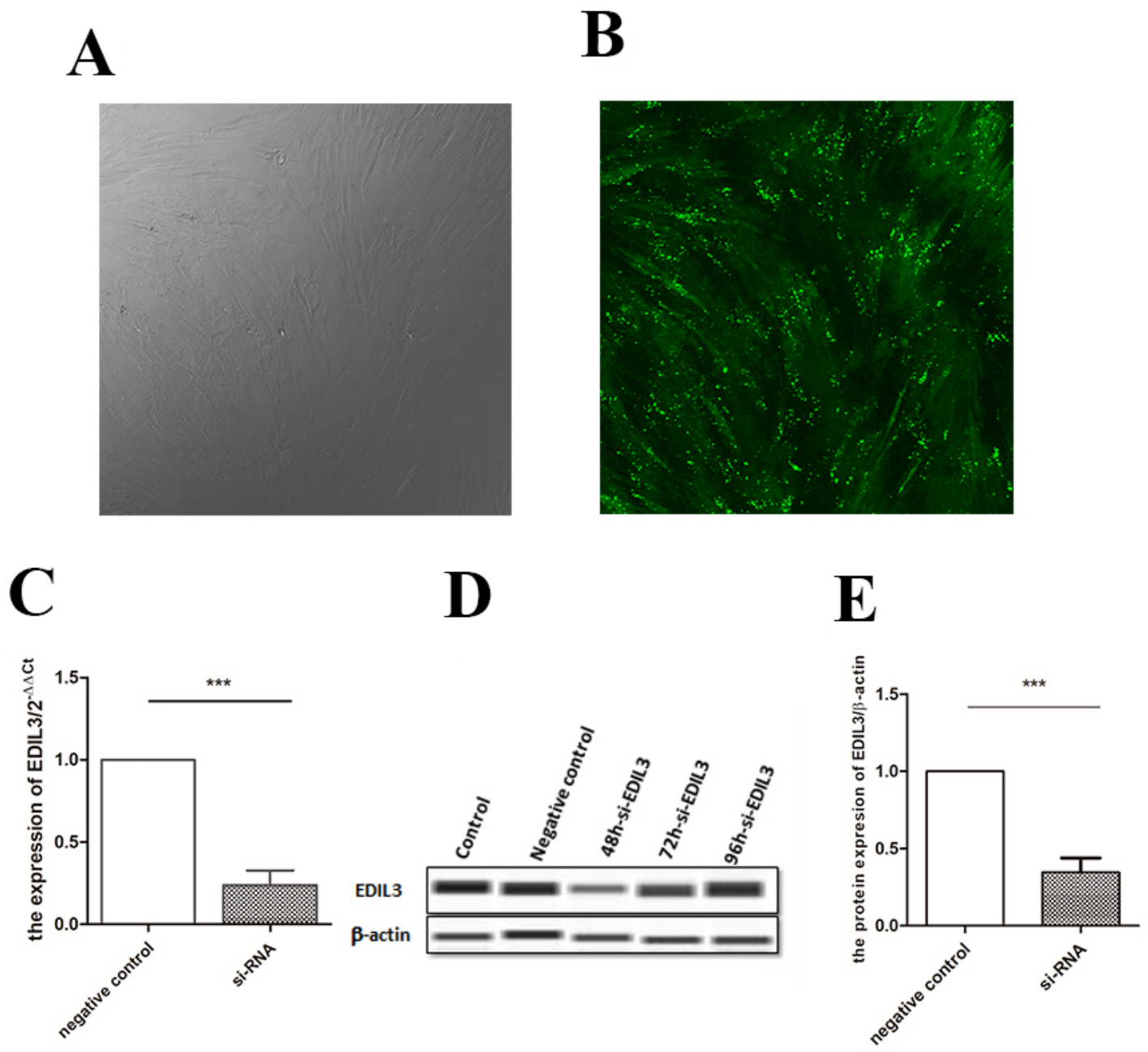

Figure 2

Si-RNA successfully reduced EDIL3 level in P-DMSCs. a P-DMSCs observed via Laser Scanning Confocal Microscopy (LSCM) in bright field. b Transfection efficiency was detected by LSCM in fluorescence field. 90% P-DMSCs were positive for FAM flag. c The mRNA expression of EDIL3 in the si-RNA and negative

control group. The mRNA level of EDIL3 was normalized to β-actin. The relative mRNA expression level of EDIL3 was calculated using the 2−ΔΔCt method. The mRNA expression of EDIL3 in P-DMSCs was reduced through si-RNA compared to the negative control (si-RNA vs. nagetive control, 1 vs. 0.24 ± 0.14, p<0.001, n = 5). d The bands represent β-actin (43 KDa) and EDIL3 (53 KDa) protein. The expression of protein EDIL3 interfered from P-DMSCs was measured at 48h, 72h and 96h, respectively. e The density of each protein band of EDIL3 was normalized to β-actin. The protein level of EDIL3 in the si-RNA and negative control groups was quantitatively calculated at 48h (si-RNA vs. negative control, 1 vs. 0.35 ± 0.09, p<0.001, n = 5). P<0.05 considered to be significant. Graph showed mean±SEM. Asterisks signs represented significant differences relative to the vehicle controls (* p< 0.05, ** p < 0.01, *** p < 0.001).

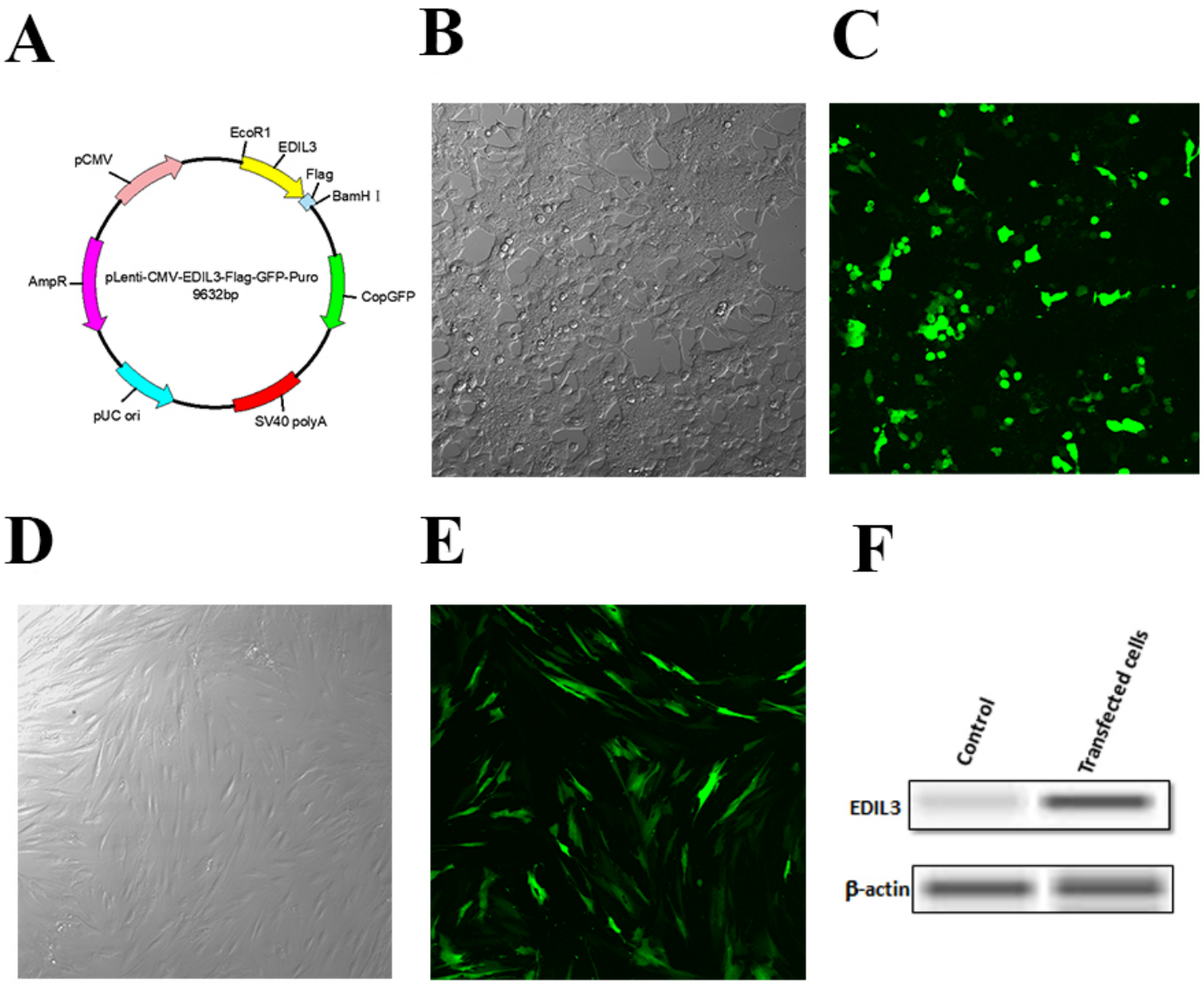

Figure 3

Recombinant EDIL3 lentiviral vectors up-regulated the expression of EDIL3 in C-DMSCs. a pLenti-CMV-EDIL3-Flag-GFP-Puro expression vector map containing GFP-fused EDIL3 gene and AmpR resistance gene. b The EDIL3 fusion expression of plasmid and lentivirus package mix was co-transfected into HEK293 cells. The morphology of HEK293 cells was observed under LSCM in bright field (20× magnification). c Transfection was visualized by GFP reporter. The green fluorescent of HEK293 cells was observed under LSCM in fluorescence field (20×magnification). d Recombinant EDIL3 lentiviral vectors were transfected into C-DMSCs. The morphology of C-DMSCs was observed under LSCM in bright field (10× magnification). e After transfected 48h, green fluorescent of C-DMSCs under LSCM indicated their successful transfection (10× magnification). f After 48h transfection, we tested the transfection efficiency by western blot. Protein expression of EDIL3 was increased in C-DMSCs transduced by recombinant EDIL3 lentiviral vectors compared to non-transfected cells.

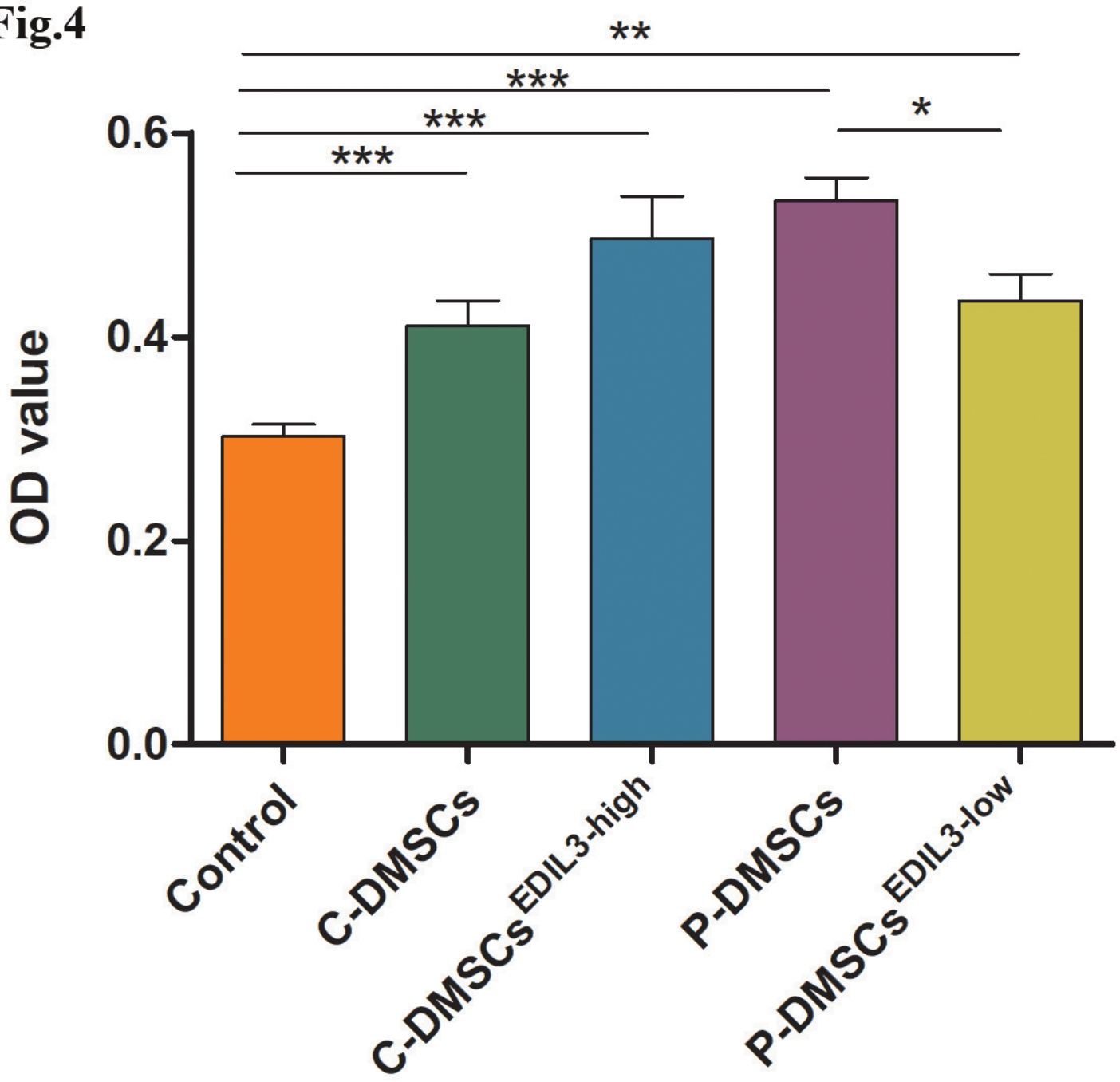

Figure 4

Graphical illustration of the proliferative capability of HUVECs in CCK8 assay. DMSCs significantly promoted the proliferation of HUVECs. However, DMSCs-derived-EDIL3 can't promote the proliferation of HUVECs. Data of OD value are shown as the mean ± SEM (n = 5).

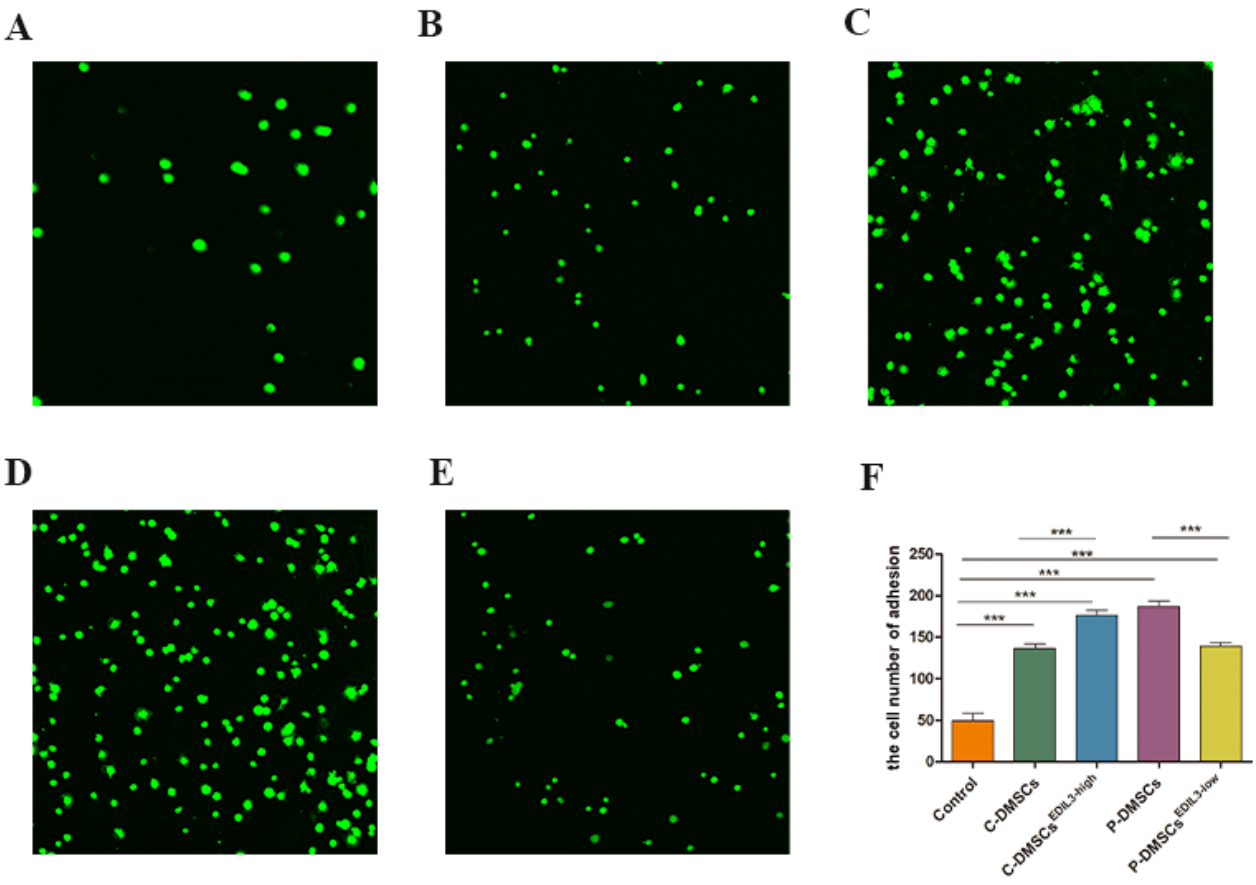

Figure 5

The number of cells adhering to HUVECs monolayer. a-e The cells adhering to HUVECs monolayer with green fluorescence were captured using LSCM. Compared with the control (Fig. 5a), massive DMSCs that adhered to HUVECs were observed in the C-DMSCs, C-DMSCsEDIL3-high, P-DMSCs and P-DMSCsEDIL3-low (Fig. 5b-e). f Statistical analysis of cells adhering to HUVECs monolayer. (10× magnification).

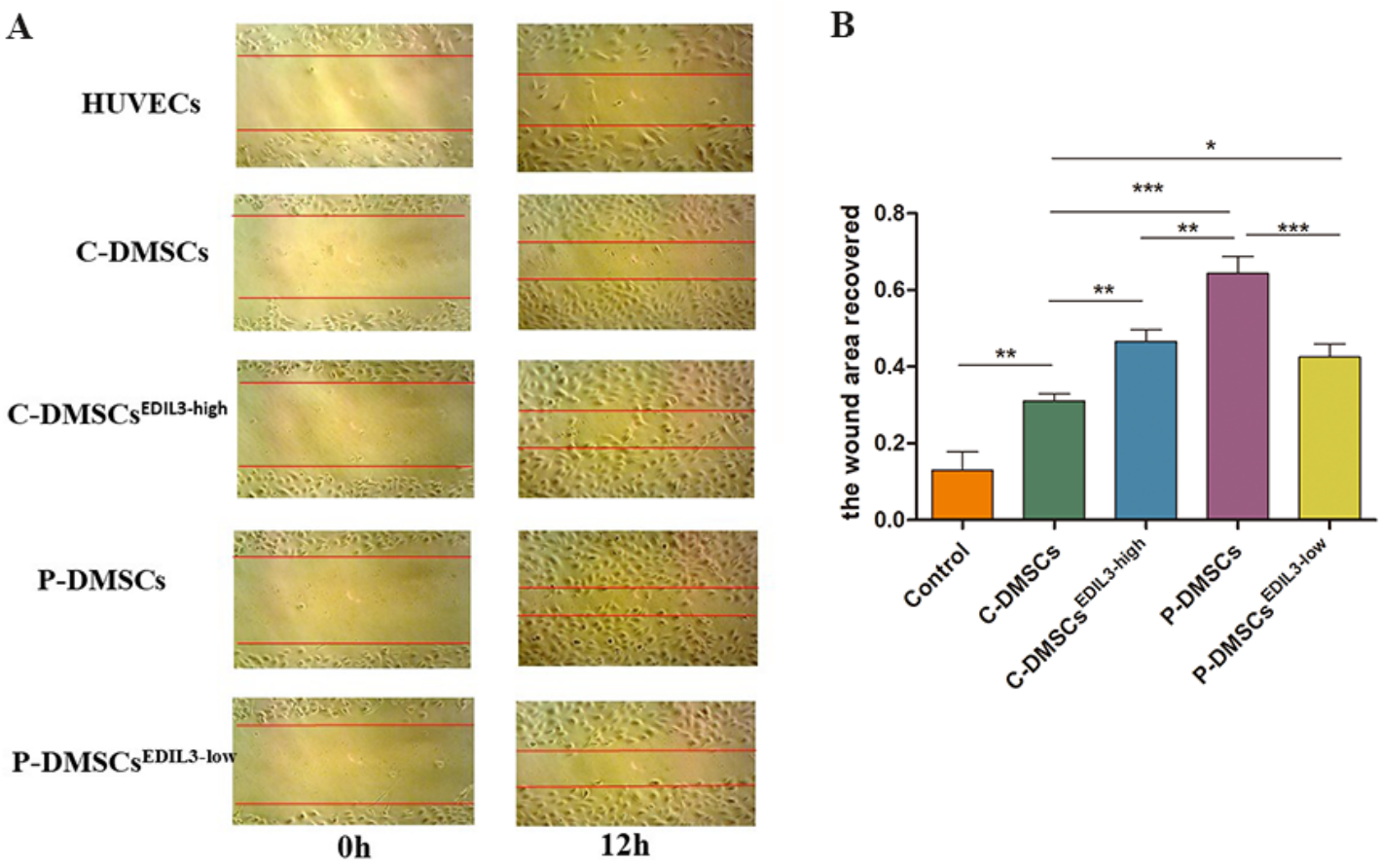

Figure 6

DMSCs-derived-EDIL3 promoted HUVECs motility. a Microscopic images revealed the pattern of HUVECs motility after co-culture with DMSCs at 0h and 12h (10× magnification). b Quantification of scratch closure using the wound healing measurement tool of Image J. The scratch closure was evaluated by scratch formula.

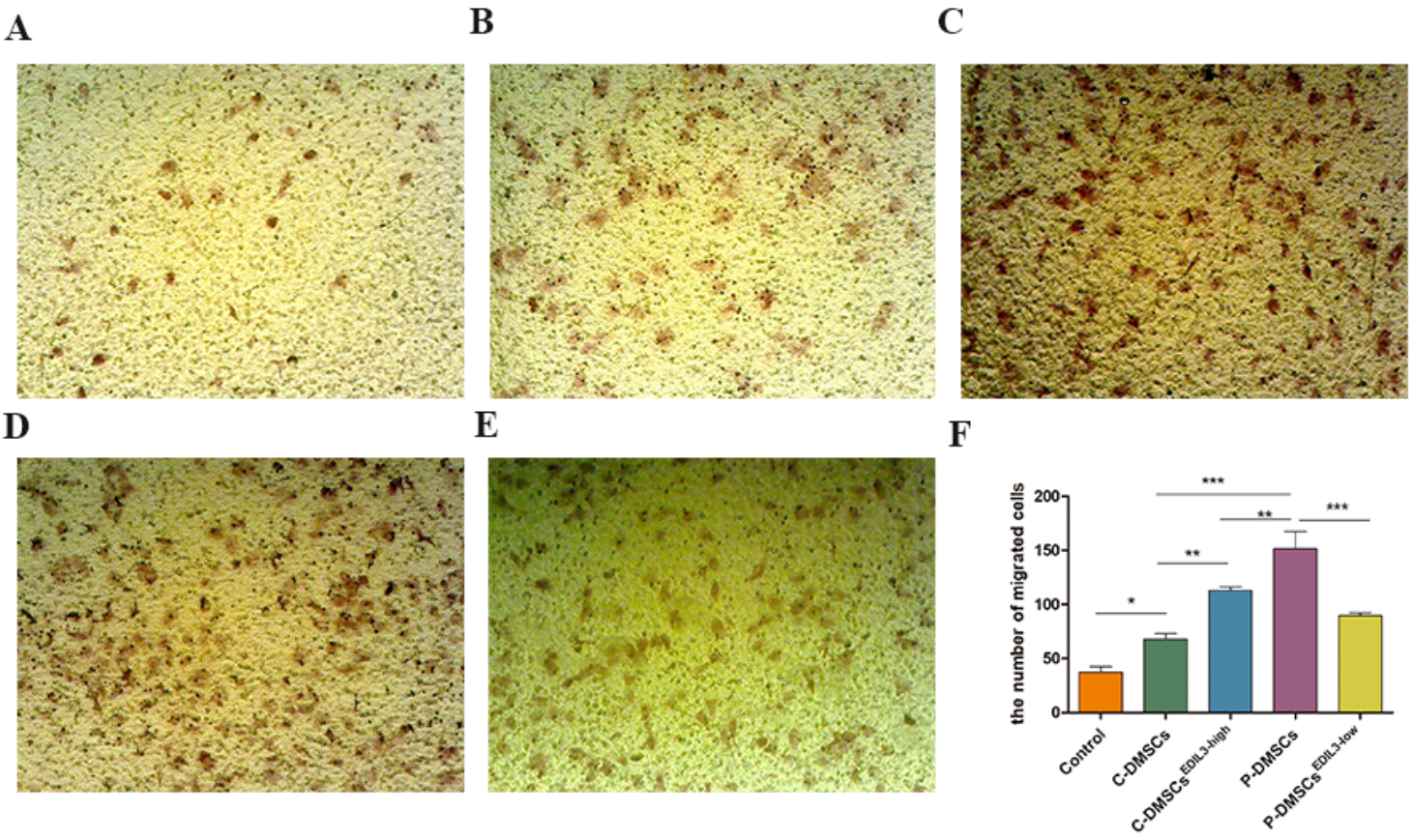

Figure 7

DMSCs-derived-EDIL3 promoted directional migration of HUVECs. a-e The migrated cells toward the lower membranes were stained with 0.1% crystal violet. Representative photomicrographs of migrated cells in (a) control (b) C-DMSCs (c) C-DMSCsEDIL3-high (d) P-DMSCs (e) P-DMSCsEDIL3-low. (magnification 10X). f Quantification of the numbers of migrating HUVECs in different groups was examined using statistical method.

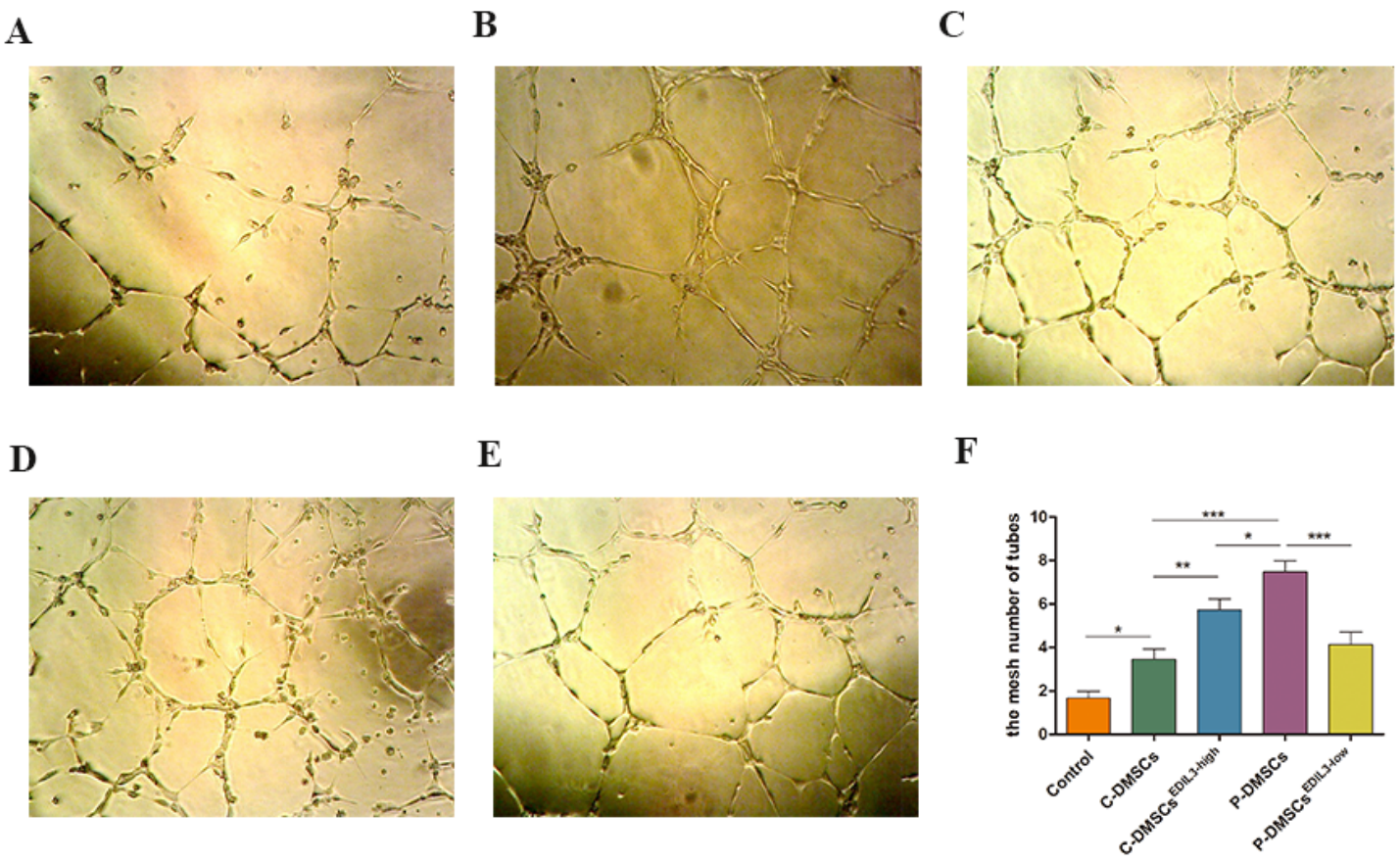

Figure 8

DMSCs-derived-EDIL3 promoted HUVECs tube formation in vitro. a-e After co-culture, HUVECs were seeded onto Matrigel in 96-well plates for 6h and the tubes gradually formed in the (a) control, (b) C-DMSCs, (c) C-DMSCsEDIL3-high, (d) P-DMSCs, and (e) P-DMSCsEDIL3-low groups. Pictures were taken and the numbers of the tube meshes were measured. f Quantitative analysis of the number of tubes meshes.

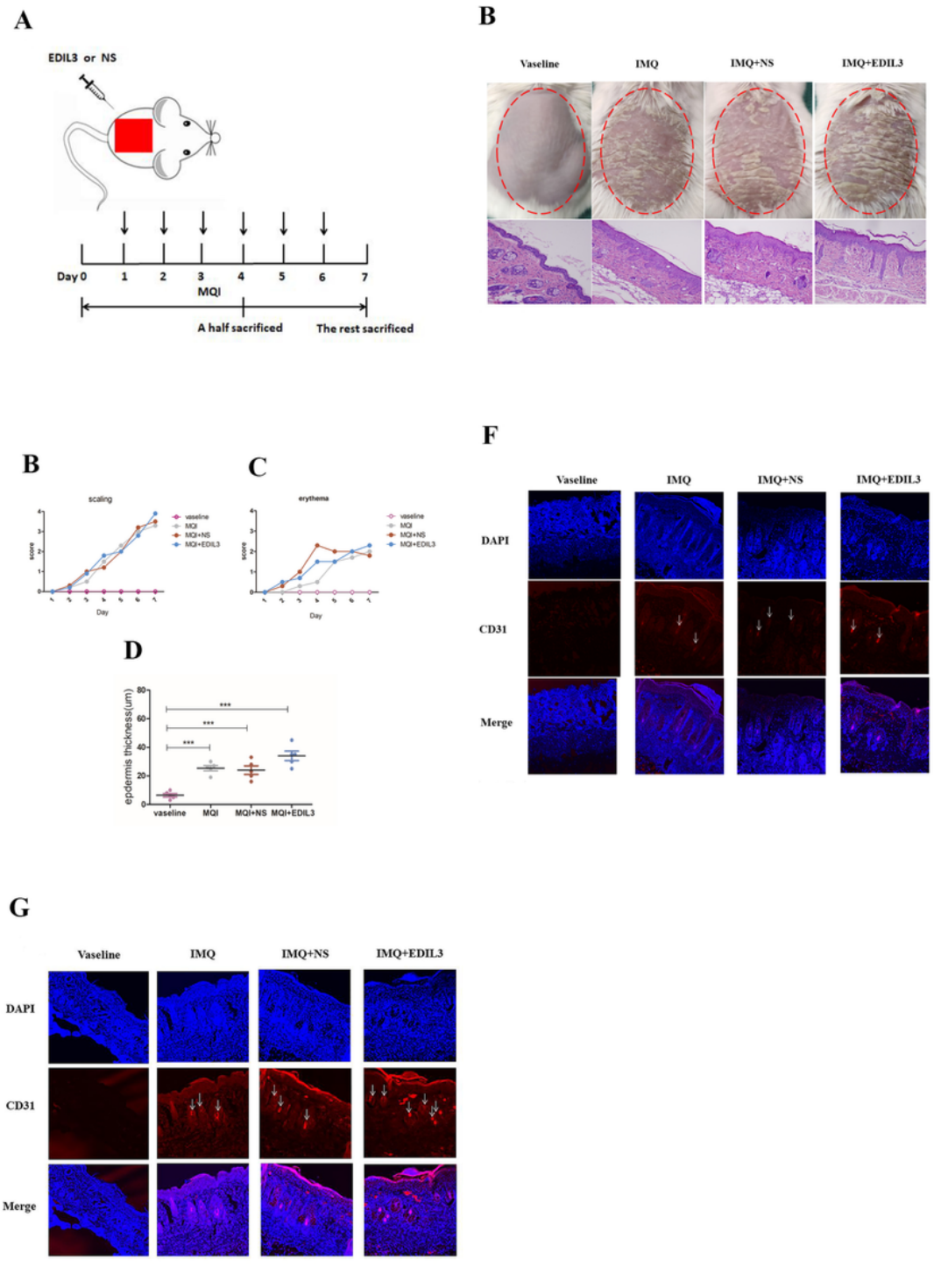

Figure 9

EDIL3 promoted dermal microvascular formation of psoriasis in vivo. a Schematic diagram for intradermal administration of EDIL3 or 0.9% normal saline (NS) on day 1 to 4 or day 1 to 6 during the application of IMQ in BALB/c mice. A half of mice in each group were sacrificed on days 4 and 7 to conduct experiments. b The clinical manifestations and H&E staining of the mice back skin treated for 6 consecutive days with vasaline cream or IMQ and injected with EDIL3 or NS. c Erythema scores of the

back skin were scored daily on a scale from 0 to 4. d Scaling scores of the back skin were scored daily on a scale from 0 to 4. e Epidermis thickness of the back skin was measured on the seventh day under a microscope. f The frozen sections of mice back skin were analyzed using immunofluorescence staining for CD31 (red, ECs surface marker) and DAPI (blue, cell nucleus) on day 4. g The frozen sections of mice back skin were analyzed using immunofluorescence staining for CD31 and DAPI on day 7. The arrows pointed to the microvessels (magnification 10X).

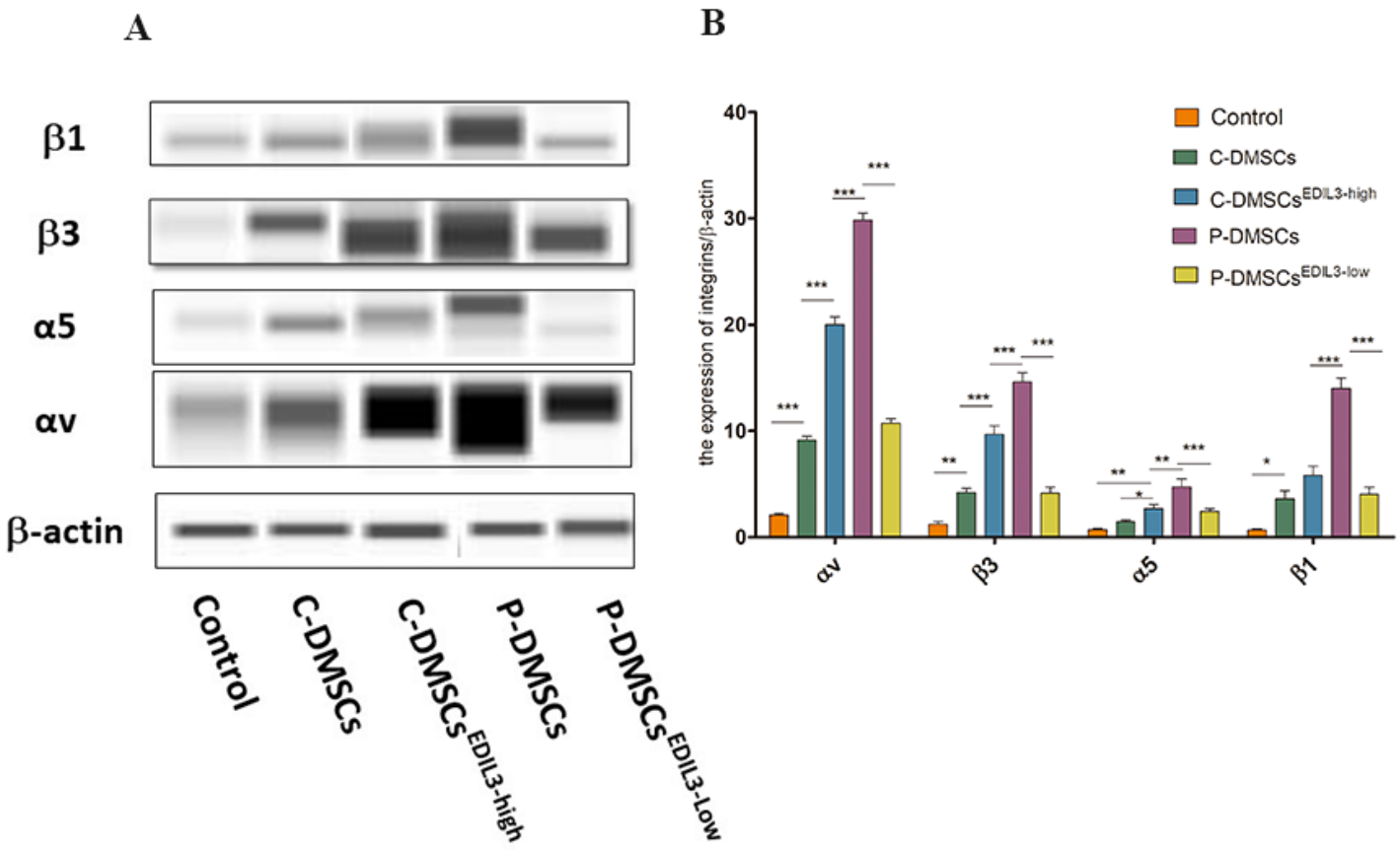

Figure 10

DMSCs-derived-EDIL3 led to increase the expression of integrins αvβ3 and α5β1 in HUVECs. a The molecular weights of integrins αv, β3, α5, β1 and β-actin were respectively 165 kDa, 129 kDa, 165 kDa, 170 kDa and 49 kDa. b Quantified results of integrin proteins.

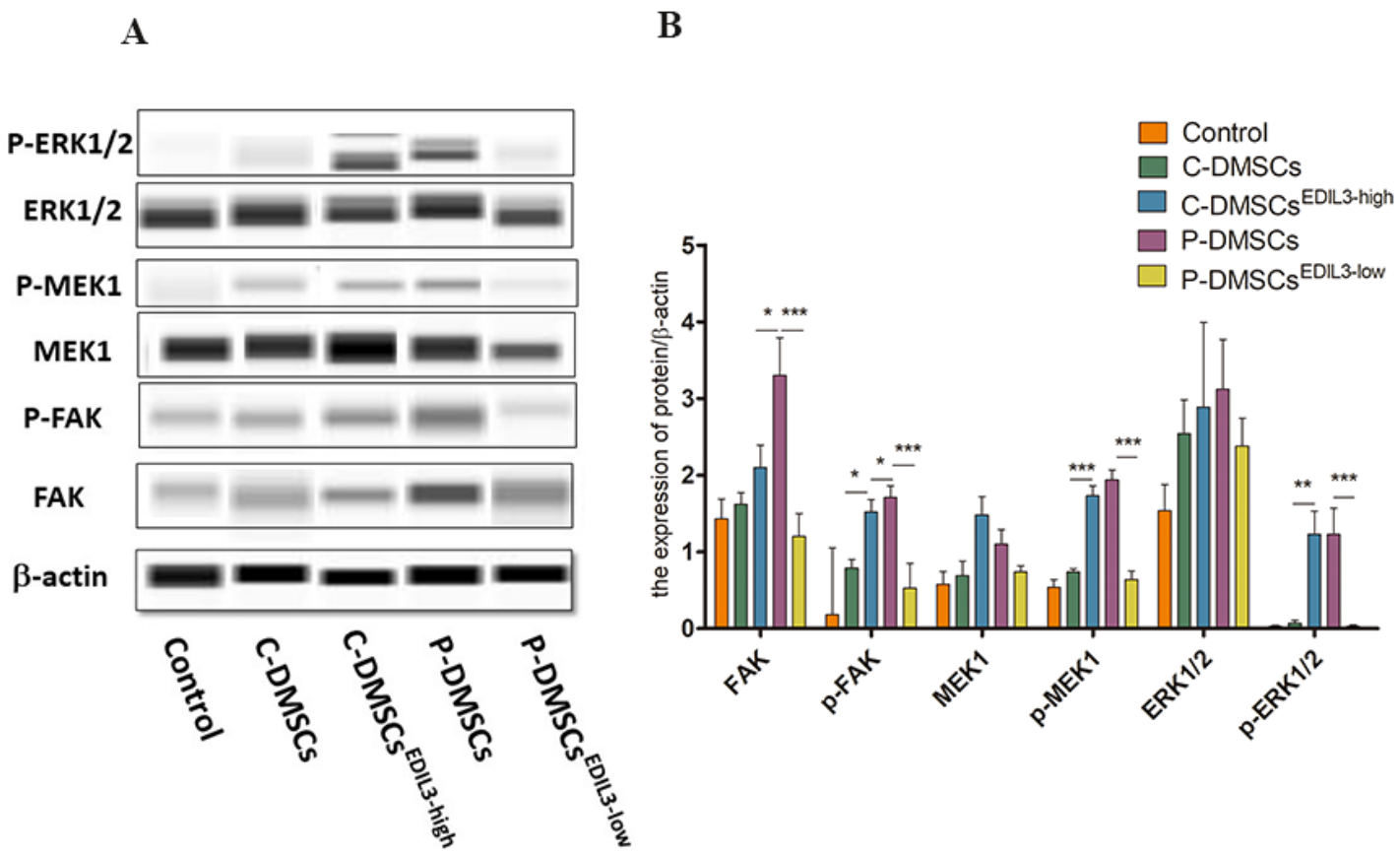

Figure 11

DMSCs-derived-EDIL3 activated FAK/MEK/ERK signal pathway in HUVECs. a The images of protein bands of β-actin, FAK/p-FAK, MEK1/p-MEK1, ERK1/2 and p-ERK1/2. The molecular weights were respectively 49 kDa, 116 kDa, 61 kDa, 50 kDa, 45 kDa, 44 kDa and 45 kDa. b Quantitative analysis of the protein levels.

Fig.12

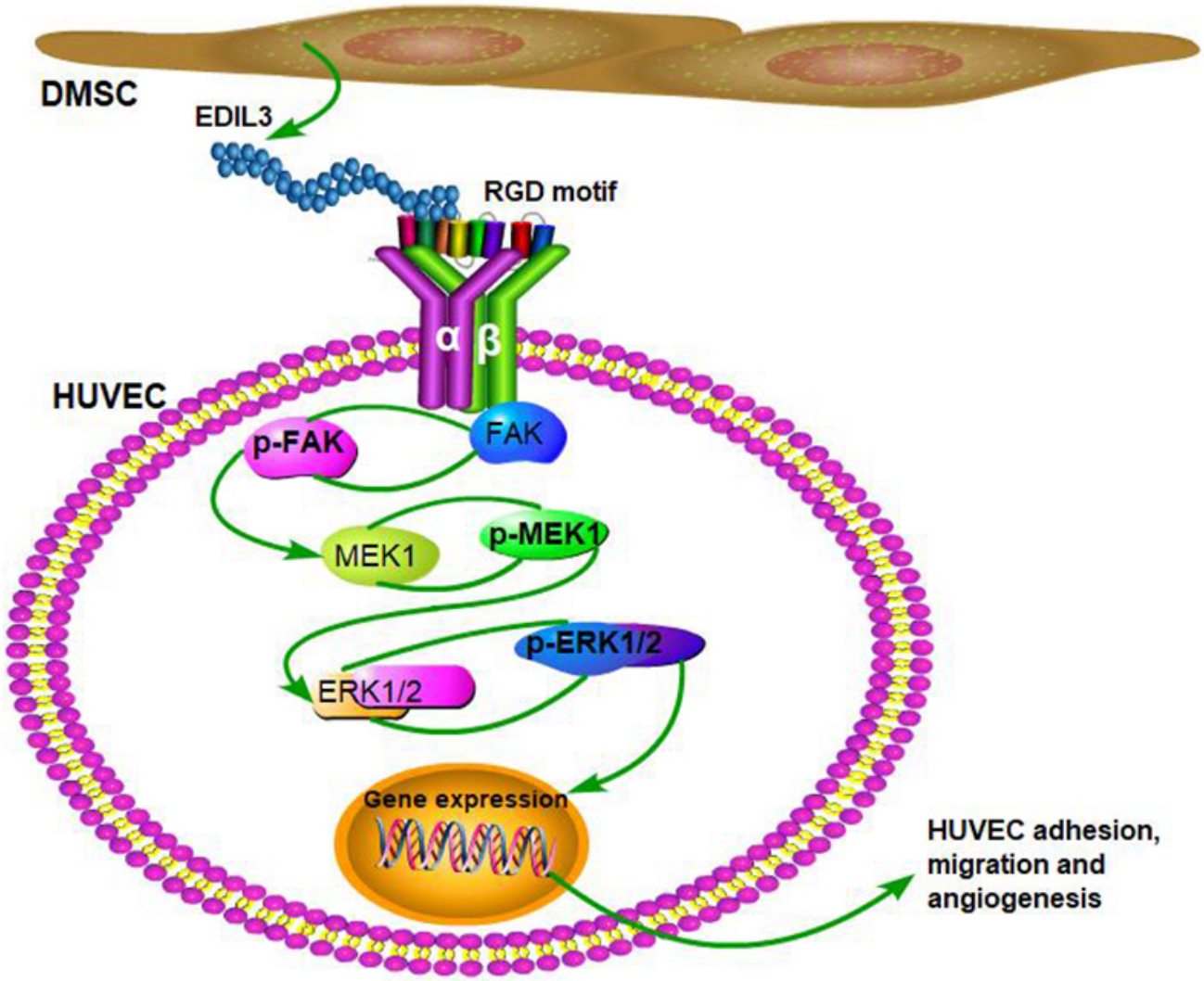

Figure 12

The molecule model of regulating ECs functions by DMSCs-derived-EDIL3. P-DMSCs secreted EDIL3, which upregulated integrin αvβ3 and α5β1 expression through RGD-motif structure in HUVECs. Then, the downstream FAK was activated by integrins and translated into phosphorylated FAK (p-FAK). The p-FAK further activated MEK1 and ERK1/2. The integrin pathway transduced the signal into cell nucleus and regulated genes expression. Ultimately, through EDIL3-integrin pathway, P-DMSCs up-regulated adhesion, migration, tube-formation and angiogenesis of HUVECs.