Overexpression of Hepatocyte growth factor in dental pulp stem cells ameliorates the severity of psoriasis by downregulating Th1 and Th17 cells and upregulating Treg cells

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Research

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Abstract

Background

Psoriasis is a kind of autoimmune disease still lacking standard treatment. Recently, it was demonstrated that mesenchymal stem cells (MSCs) are capable of immunoregulation. The underlying mechanism might involve the secretion of soluble cytokines, such as hepatocyte growth factor (HGF). This study aims to investigate the therapeutic effect of dental pulp stem cells (DPSCs), and hepatocyte growth factor (HGF) overexpressed DPSCs (HGF-DPSCs) on psoriatic mice and the underlying mechanisms.

Methods

DPSCs were isolated and transfected by adenovirus vector carrying human HGF cDNA (Ad-HGF). DPSCs and HGF-DPSCs were transplanted into the psoriatic mice model. On 7th day of post-transplantation, the mice were euthanized and sacrificed. The psoriatic skin lesions were analyzed by hematoxylin-eosin (H&E) and immunohistochemical staining for histopathological changes, and quantitative real time-polymerase chain reaction (Q-PCR) was applied to detect the expression levels of T-bet, IFN-γ, GATA3, IL-4, RORγt, IL-17A, IL-17F, IL-23, Foxp3 and IL-10. The concentrations of IFN-γ, TNF-α, and IL-17A in the mice blood serum were measured by MILLIPLEX analysis. Human peripheral blood mononuclear cells (PBMCs) were cocultured with DPSCs or HGF-DPSCs under appropriate stimulations. The proliferation index and quantity of T helper 1 (Th1) cells, T helper 2 (Th2) cells, interleukin 17 (IL-17)-secreting helper T (Th17) cells, and regulatory T (Treg) cells were analyzed by flow cytometry. The concentrations of IFN-γ, IL-4, TGF-β1, and IL-6 in the coculture supernatants were quantitated by enzyme linked immunosorbent assay (ELISA) analysis.

Results

In psoriatic mice, HGF overexpression enhanced the amelioration of epidermal thickening, inflammation infiltration and keratinocyte differentiation by DPSCs treatment. In the psoriatic skin lesions, HGF overexpression enhanced the downregulation of T-bet, IFN-γ, RORγt, IL-17A, IL-17F, IL-23, and upregulation of Foxp3 and IL-10 by DPSCs treatment. When cocultured with PBMCs, HGF overexpression significantly enhanced the downregulation of Th1 and Th17 cells and upregulation of Treg cells by DPSCs, but did not affect their suppression effect on lymphocyte proliferation.

Conclusion

HGF overexpression enhanced the treatment effect of DPSCs on psoriasis by downregulating Th1 and Th17 cells' activity and upregulating Treg cells' activity.

Background

Psoriasis is an immune-mediated chronic skin disease, involving local and systemic inflammation responses. The main pathological features of psoriasis are skin thickening, scales, erythema (1), and overexpressing of inflammatory cytokines like interferon-gamma (IFN-γ), tumor necrosis factor-α (TNF-α), and interleukin 17A (IL-17) (2). Psoriasis was initially regarded as a T helper 1 (Th1) cell-mediated skin disease (3). In psoriatic plaques and peripheral blood of psoriasis patients, type 1 inflammatory cells and cytokines were overexpressed (4). While following the identification of interleukin 17 (IL-17)-secreting helper T (Th17) cell, numerous studies revealed that Th17 cells and their related cytokines like IL-17 and IL-23 play essential roles in psoriatic pathological processes (5). Therefore, new promising treatment strategies for psoriasis, such as biologics, start emerging in the last two decades (6). Biologics are a kind of antibody medicine targeting the elevated inflammatory cytokines in psoriasis such as TNF-α, IL-23, and IL-17 (7). However, severe adverse events had been reported in clinical trials using biologics, like severe infections (such as sepsis and tuberculosis), non-melanoma skin cancers, multiple sclerosis, lupus, and congestive heart failure (8–10). The possible reason might be that the biologics neutralize all the targets they accessed, while the targeted cytokines participate not only in psoriasis pathological changes but also in other physiological processes, which might be affected by the neutralization (11, 12). Hence, new immunoregulation strategies for psoriasis treatment were needed.

Mesenchymal stem cells (MSCs) are self-renewal and multipotent adult stem cells, which possess immunoregulation abilities (13). MSCs could sense the status of the immune system and regulate it into a homeostatic state (14, 15). When the immune system is under activated, the MSCs promote inflammation, and when the immune system is over-activated, they restrain inflammation (16). Therefore, MSCs are promising candidates for the treatment of immune-related diseases, like psoriasis. It has been proved that umbilical cord blood-derived MSCs, umbilical cord derived MSCs and embryonic stem cell-derived MSCs could ameliorate psoriasis' pathological changes in imiquimod (IMQ) induced psoriatic mice (17–19). Moreover, the psoriatic symptoms were alleviated by the intravenous injection of umbilical cord-derived or adipose derived MSCs in clinical trials (20, 21). The major mechanism underlying the immunoregulation by MSCs involves the secretion of soluble factors, such as hepatocyte growth factor (HGF) (22, 23). HGF is a kind of scatter factor, which is primarily secreted by mesenchymal cells (24). It was found that HGF had therapeutic effects in various inflammatory diseases, including collagen induced arthritis (25), graft-versus-host disease (26), and experimental allergic encephalitis (EAE) (27). It was reported that the treatment effect of MSCs conditioned medium on EAE was blocked by HGF neutralization (28). Therefore, overexpression of HGF is expected to further enhance MSCs’ treatment effect on psoriasis.

Dental pulp stem cells (DPSCs) are a kind of MSCs like postnatal stem cells derived from ectoderm (29), which also exhibit immunomodulatory abilities (30–33). In this study, HGF overexpressed DPSCs (HGF-DPSCs) were fabricated by transfection with adenovirus vector carrying human HGF cDNA (Ad-HGF), then applied on a psoriasis mouse model. Our findings suggested that HGF overexpression enhanced the

treatment effect of DPSCs on the pathological changes and inflammation conditions in psoriasis, which mainly by downregulating Th1 and Th17 cell response and upregulating Treg cell activity.

Materials And Methods

Human DPSCs isolation and culture

Dental pulp tissues were obtained from healthy human orthodontic molars of adult patients (19-29 years old) at the Dental Clinic of Beijing Stomatological Hospital. This study was conducted under approved guidelines set by the Research Ethics Committee of Capital Medical University, China. All patients gave their written informed consent to participate in the study. The pulp tissues were cut into about 1 mm^3 and digested in a solution of 3 mg/ml collagenase type I enzyme (Worthington Biochem, USA) and 4 mg/ml dispase type enzyme (Boehringer Mannheim, German) for 1 h at 37℃ with vigorous shaking. Afterward, the digested tissue precipitations by centrifugation were seeded into culture flasks (NEST, USA) with α-MEM supplemented with 20% fetal bovine serum (Every Green, China), 100 units/ml of penicillin and streptomycin (Sigma, USA), and then incubated in 5% CO_2 atmosphere at 37℃. Upon reaching 80% confluence, hDPSCs were detached with 0.05 % trypsin (Sigma, USA), and passed with α-MEM supplemented with 10% fetal bovine serum (Every Green, China). The cells at passage 6 (P6) were used in this study, while extra cells were cryopreserved in liquid nitrogen for further use (-196 ℃).

Adipogenic differentiation of DPSCs

Human DPSCs (P6) were seeded in six-well plates (NEST, USA) at a density of 1×10^5 cells/well. Then, cultured for 14 days with adipogenic induction medium, supplemented with 0.5mM 3-isobuty-1-1-Methylxanthine, 1μM dexamethasone, and 0.1mM indomethacin (Sigma, USA). Then the cells were fixed in 4% (v/v) phosphate-buffered paraformaldehyde and stained with 0.5% Oil Red O (Sigma, USA) solution. To examine the adipogenic differentiation, the stained cells were observed and photographed under a phase-contrast inverted microscope (Olympus, Japan).

Osteogenic differentiation of DPSCs

Human DPSCs (P6) were seeded in six-well plates (NEST, USA) at a density of 1×10^5 cells/well. Next, cells were cultured for 21 days with osteogenic induction medium, supplemented with 100nM dexamethasone, 50nM ascorbic acid, and 10 mM β-glycerophosphate (Sigma, USA). Then the cells were fixed in 4% (v/v) phosphate-buffered paraformaldehyde and stained with 1% alizarin red solution. To examine the osteogenic differentiation, the stained cells were observed and photographed under a phase-contrast inverted microscope (Olympus, Japan).

Transfection of DPSCs by Ad-HGF

Adenovirus vector lack of exogenous genes (Ad-Null) and Ad-HGF were used in this study. hDPSCs were infected with 150 multiplicities of infection (MOI) of Ad-Null or Ad-HGF. The Ad-Null transfected DPSCs

(Null-DPSCs) and HGF-DPSCs were collected 48 hours post-transfection for use. HGF expression was analyzed by quantitative real time-polymerase chain reaction (Q-PCR) and enzyme linked immunosorbent assay (ELISA).

Immunophenotype analysis of DPSCs and HGF-DPSCs

To characterize the immunophenotype of DPSCs and HGF-DPSCs, their expression of surface markers was analyzed by flow cytometry. Briefly, the tested cells were trypsinized and washed with phosphate buffer saline (PBS). 2×10^5 cells/tube were harvested and incubated with various antibodies, including human CD73, CD90, CD105, CD45, CD19, CD34, CD11b, HLA-DR in the dark for 30min at room temperature, mouse IgG1 FITC and mouse IgG1 PE were used as isotype controls (BD Bioscience, USA). The analysis was performed with a FACS Calibur flow cytometer (BD Bioscience, USA) and FlowJo software (FlowJo, Ashland, USA).

Imiquimod induced psoriasis like skin inflammation in mice and treatment

All animal experiments were approved by the ethics committee of the Beijing Institute of Radiation Medicine, and all procedures were carried out in accordance with the relevant guidelines and regulations. Female BALB/C mice (18-22 g, 8 weeks old) were held in a specific pathogen free (SPF) animal laboratory house for one week before the experiment. Then, 62.5 mg of imiquimod cream (Med Shine pharmaceutical, China) was applied on the shaved back skin of the mice for 6 consecutive days with or without intravenous tail injection of DPSCs or HGF-DPSCs on day −1. The Blank group was tail intravenous injected of normal saline (Shijiazhuang No.4 pharmaceutical, China). While the Control group was applied with IMQ treatment and tail intravenous injected of normal saline (Shijiazhuang No.4 pharmaceutical, China). The mice were euthanized on day 6, psoriasis like back skin lesions were collected for histological and immunohistochemical analysis and Q-PCR analysis; the blood serums were collected for MILLIPLEX analysis.

Histological and immunohistochemical analysis of the back skins

Sections from the mice back skins were stained with hematoxylin-eosin (H&E) stain for histological evaluation as previously described (34). Briefly, the skin samples were fixed and embedded in paraffin. Tissue sections of 5 μm thick were deparaffinized with xylene and rehydration with a graded ethanol solution, then stained with H&E. All sections were observed and photographed under a phase-contrast inverted microscope (Olympus, Japan), the epidermal thickness of psoriasis like back skin lesions were measured by Image J software (National Institutes of Health, USA)

The above skin sections were analyzed by immunohistochemical stains. Briefly, the sections were incubated with the following primary antibodies at 4 °C overnight: rabbit anti-mouse CD8, CD83, CK6, and CK17 (Bioss, China). The biotinylated goat anti-rabbit secondary antibodies were conjugated to streptavidin-peroxidase, and DAB solution were used for the visualization of immunoreactivity (Bioss, China). All the sections were observed and photographed with a light microscope (Olympus, Japan).

Q-PCR analysis

Q-PCR analysis was performed to measure gene expression of HGF in DPSCs, Null-DPSCs or HGF-DPSCs; T-box transcription factor 21 (T-bet), Interferon-gamma (IFN-γ), GATA binding protein 3 (GATA3), Interleukin-4 (IL-4), Retinoic acid-related orphan receptor-γt (RORγt), Interleukin 17A (IL-17A), Interleukin 17F (IL-17F), Interleukin 23 (IL-23), Forkhead box protein 3 (FOXP3), Interleukin 10 (IL-10) in the skin lesions of all mice. Briefly, total ribose nucleic acid (RNA) from all samples was extracted by using TRIzol Reagent (Invitrogen, USA), according to the manufacturer's instructions. First-strand complementary deoxyribonucleic acid (cDNA) was synthesized, and the quantification of messenger RNA (mRNA) expression was performed on ABI PRISM 7500 FAST sequence detector (Applied Biosystems). The expression of the above genes was evaluated by Q-PCR using the human primers listing in Table 1 and mouse primers listing in Table 2, with GAPDH as internal control, respectively. The relative gene expression levels were calculated by the $2^{-\Delta\Delta CT}$ method.

MILLIPLEX analysis

The concentrations of inflammatory cytokines IFN-γ, TNF-α, and IL-17A in the mice blood serum were measured by using a MILLIPLEX kit, according to the manufacturer's instructions (Mouse Th17 Magnetic Bead Panel, Merck Millipore, USA). Briefly, the background, standard, or sample were mixed with chemically dyed antibody bound beads in the plate wells, incubate with agitation overnight at 4°C, and then incubated with biotinylated detection antibodies for 1 hour and Streptavidin-Phycoerythrin reporter molecules for 30 minutes at room temperature. The data was collected by MAGPI and analyzed with Milliplex Analyst software (Merck Millipore, USA).

Peripheral blood mononuclear cells (PBMCs) isolation

Human PBMCs were isolated from the buffy coats of healthy volunteers by a protocol approved by the Beijing Red Cross Blood Center at Beijing. Briefly, the buffy coat samples were diluted with an equal volume of PBS, then slowly layered upon human lymphocyte separation medium (specific gravity 1.077, TBD sciences, China), and centrifuged at 600 × g for 20 min. PBMCs were collected from the interphase and washed with PBS supplemented with 5% fetal bovine serum (Every Green, China). The isolated PBMCs were cultured in RPMI-1640 medium (Gibco, Germany) supplemented with 10% fetal bovine serum (Every Green, China), 100 units/ml of penicillin, and streptomycin (Sigma, USA).

CD4+ T cell subpopulations detection

To investigate the immunomodulatory effects of DPSCs or HGF-DPSCs on CD4+ T cell differentiation, Mitomycin C (Selleck Chemicals, USA) treated DPSCs or HGF-DPSCs were cocultured with PBMCs and stimulated with CD3/CD28 Streptamers (IBA Lifesciences, Germany) and recombinant human interleukin 2 (IL-2, Roche, USA). Briefly, 2×10^5 DPSCs or HGF-DPSCs were plated in six-well plates (NEST, USA), and treated with 25 μg/ml Mitomycin C for 30 min at 37°C. PBMCs were suspended in RPMI-1640 medium (Gibco, Germany) supplemented with 10% fetal bovine serum (Every Green, China), 15 μl/ml CD3/CD28

Fab-Strep, 200 units/ml interleukin 2, 100 units/ml penicillin and streptomycin (Sigma, USA) at a concentration of 1×10^5 cells/ml. The PBMCs were added to six-well plates with or without DPSCs or HGF-DPSCs at a ratio of 1:1, then incubated for 5 days.

The supernatants were collected to measure the production of CD4+ T cell-related cytokines IFN-γ, IL-4, TGF-β1, and IL-6 by ELISA. The PBMCs were harvested and the percentages of CD4+IFN-γ+ Th1 cells, CD4+IL-4+ Th2 cells, CD4+IL-17A+ Th17 cells and CD4+CD25+CD127- Treg cells were analyzed by flow cytometry. Briefly, to detect Th1, Th2, and Th17 cells, the PBMCs were stimulated for 4h with a leukocyte activation cocktail in the presence of GolgiStop (BD Bioscience, USA). Then, the PBMCs were incubated with BB700 conjugated anti-CD4 antibody (BD Bioscience) for 30 min at 4℃ in the dark. Afterward, the cells were fixed and permeabilized, and stained with FITC conjugated anti-IFN-γ antibody, APC conjugated anti-IL-4 antibody, PE conjugated anti-IL-17A antibody (BD Bioscience, USA) for 30 min in the dark at 4℃; to detect Treg cells, the PBMCs were stained with BB700 conjugated anti-CD4 antibody, BB515 conjugated anti-CD25 antibody, and Alexa Fluor 647 conjugated anti-CD125 (BD Bioscience, USA) for 30 min in the dark at 4℃. The stained cells were analyzed by FACS Calibur flow cytometer (BD Bioscience, USA) and FlowJo software (FlowJo, Ashland, USA).

PBMCs proliferation assay

To investigate the immunomodulatory effects of DPSCs or HGF-DPSCs on lymphocyte proliferation, Mitomycin C (Selleck Chemicals, USA) treated DPSCs or HGF-DPSCs were cocultured with Dye 670 (Thermo Fisher Scientific, USA) labeled PBMCs under the stimulation of phytohemagglutinin (PHA, Sigma, USA). Briefly, 2×10^5 DPSCs or HGF-DPSCs were plated in six-well plates (NEST, USA), and treated with 25 μg/ml Mitomycin C for 30 min at 37°C. PBMCs were labeled with 5μM Dye 670 for 10 min at 37°C, and suspended in RPMI-1640 medium (Gibco, Germany) supplemented with 10% fetal bovine serum (Every Green, China), 10μg/ml PHA (Sigma, USA), 100 units/ml of penicillin and streptomycin (Sigma, USA) at a concentration of 1×10^5 cells/ml. The PBMCs were added to six-well plates with or without DPSCs or HGF-DPSCs at a ratio of 1:1, then incubated for 3 days. The proliferation index of PBMCs was analyzed by FACS Calibur flow cytometer (BD Bioscience, USA) and ModFit software (Verity Software House, USA).

ELISA analysis

The concentrations of HGF in the culture supernatants of DPSCs or Null-DPSCs or HGF-DPSCs; IFN-γ, IL-4, TGF-β1, and IL-6 in the coculture supernatants of PBMCs with or without DPSCs or HGF-DPSCs were quantitated by anti-human ELISA kits according to the manufacturer's instructions (Neobioscience Biotech, China). Briefly, all samples were centrifuged at 400 × g for 10 min, then diluted or stimulated respectively. The pretreated samples were added to the test wells and incubated for 90 min at 37℃ to capture the antibodies. Afterward, the test wells were incubated with biotinylated antibodies and avidin HRP successively. The color was developed and stopped by using tetramethylbenzidine substrate solution and stop solution. The plates were read by a microplate manager (Bio-Rad, USA) at 450 nm.

Statistical analysis

All data were expressed as the mean ± SD. The significant difference was assessed by unpaired t-test ($p<0.05$).

Results

Characterization of DPSCs

DPSCs were obtained from human dental pulp. Spindle like cells were observed around the digested pulp tissue on day 10 (Fig. 1A). The cells were digested with trypsin and passaged. At P6, the cells were spirally arranged and had the same typically spindle shape as MSCs (Fig. 1B). After 14 days of adipogenic induction and oil red stain, lipid droplets were clearly observed at the internal side of the cells (Fig. 1C). After 21 days of osteogenic induction, mineralized nodules were formed and verified by alizarin red staining (Fig. 1D). The results of the flow-cytometric analysis demonstrated that the DPSCs positively expressed CD73, CD90 and CD105, and negatively expressed CD45, CD19, CD34, CD11b, and HLA-DR (Fig. 1E). These results indicate that the isolated cells were MSCs.

Characterization of HGF-DPSCs

To evaluate the transduction effect, the mRNA and protein expression levels of HGF in DPSCs, Null-DPSCs and HGF-DPSCs were tested. The Q-PCR analysis showed that the HGF gene expression was significantly higher in HGF-DPSCs than in DPSCs and Null-DPSCs, and there is no difference between the DPSC group and Null-DPSC group ($p>0.05$, Fig. 2A). The concentration of HGF was 1475 ± 326.1pg/mL in DPSCs culture supernatants, 1420 ± 18.78pg/mL in Null-DPSCs, and 18611 ± 187.9pg/mL in HGF-DPSCs culture supernatants (Fig. 2B), indicating that HGF was successfully overexpressed by Ad-HGF transfection in HGF-DPSCs. The results of the flow-cytometric analysis demonstrated that the HGF-DPSCs positively expressed CD73, CD90 and CD105, and negatively expressed CD45, CD19, CD34, CD11b, and HLA-DR (Fig. 2C), suggesting that HGF-DPSCs still exhibited MSC characteristics.

HGF overexpression enhanced the treatment effect of DPSCs on the pathological changes and inflammation condition in psoriatic mice

To investigate the effect of HGF-DPSCs on psoriasis, a mouse model of psoriasis was established by IMQ induction (35), and treated with DPSCs or HGF-DPSCs. The concentration of HGF in the mice blood serum was higher in the DPSC group and HGF-DPSC group compared with the Control group, and significantly higher in the HGF-DPSC group ($p<0.001$, Fig. 3C), indicating that HGF was overexpressed by HGF-DPSCs transplant in the treated mice. H&E staining results showed that compared to the Blank group, IMQ application induced psoriasis like pathological changes in the back skin of the control mice, such as inflammatory infiltration, thickened epidermally, parakeratosis and hyperkeratosis. Moreover, both DPSCs and HGF-DPSCs treatment ameliorated these psoriatic like pathological changes. Compared with the DPSC group, the inflammatory infiltration and thickened epidermal were more alleviated in the HGF-

DPSC group (Fig. 3A). As shown in the calculation results (Fig. 3B), the epidermal thickness was lower in the DPSC group and HGF-DPSC group compared with the Control group and more lower in the HGF-DPSC group. To further investigate the pathological changes, the expression of inflammatory infiltration-related cytokines CD8 and CD83, and keratinocyte differentiation-related cytokines CK6 and CK17 in the psoriatic skin lesions were tested. As shown in the immunohistochemical results (Fig. 4), in the Control group, the expression of CD8, CD83, CK6, and CK17 were upregulated compared with the Blank group. While compared with the Control group, the expression of CD8, CD83, CK6, CK17 were downregulated in DPSC group and HGF-DPSC group. Between the DPSC group and the HGF-DPSC group, the expression levels of CK6, and CK17 were more lower in the HGF-DPSC group. The above results indicate that HGF overexpression enhanced the treatment effect of DPSCs on the pathological changes in psoriatic mice.

It was demonstrated that serum IFN-γ, TNF-α, and IL-17A levels are significantly higher in psoriatic patients (2). In this study, as shown by the MILLIPLEX analysis, the serum expression levels of IFN-γ, TNF-α and IL-17A were higher in the Control group compared with the Blank group, and lower in both DPSC group and HGF-DPSC group compared with the Control group, with a significantly more lower rate in HGF-DPSC group (Fig. 3D, E, F). These results indicate that HGF overexpression enhanced the inhibition effect of DPSCs on the inflammation in psoriatic mice.

HGF overexpression reduced Th1 and Th17 differentiation and promoted Treg differentiation in psoriatic mice

To investigate the mechanism of the treatment effect of HGF overexpression on psoriasis, the expression levels of CD4+ T cell-related key transcription factors and cytokines in the mice skin lesions were analyzed by Q-PCR. The results showed that compared to Blank group, IMQ application upregulated the expression levels of Th1 transcription factor T-bet and cytokine IFN-γ and Th17 transcription factor RORγt and cytokines IL-17A, IL-17F, and IL-23; downregulated the expression levels of Th2 transcription factor GATA3 and cytokine IL-4, Treg transcription factor Foxp3 and cytokine IL-10 in the Control group. While compared to the Control group, DPSCs and HGF-DPSCs treatment significantly downregulated the expression levels of T-bet, IFN-γ, RORγt, IL-17A, IL-17F, IL-23, and upregulated the expression levels of GATA3, Foxp3, and IL-10. Moreover, compared to the DPSC group, HGF overexpression enhanced the downregulation of T-bet, IFN-γ, RORγt, IL-17A, IL-17F, and IL-23, and upregulation of Foxp3, IL-10 in the HGF-DPSC group (Fig. 5). These results indicate that HGF overexpression ameliorated the severity of psoriasis by reducing Th1 and Th17 differentiation and promoting Treg differentiation.

HGF overexpression enhanced the downregulation of Th1, Th17 cells and upregulation of Treg cells by DPSCs

To verify the effect of HGF overexpression on Th1, Th17, and Treg cells, DPSCs or HGF-DPSCs were cocultured with PBMCs under appropriate polarizing condition. The flow cytometric analysis results showed that in the DPSC group and HGF-DPSC group, the percentages of Th1, Th17 cells were downregulated, and the percentages of Treg cells were upregulated compared to Control group; however, the percentages of Th2 cells show no difference between all the three groups. Compared to the DPSC

group, the downregulation of Th1, Th17 cells and the upregulation of Treg cells in the HGF-DPSC group were enhanced (Fig. 6A,6B). The concentrations of IFN-γ (related to Th1 cell differentiation), IL-4 (related to Th2 cell differentiation), IL-6 (related to Th17 and Treg cell differentiation), TGF-β (related to Th17 and Treg cell differentiation) in the coculture supernatants were measured. As shown in the ELIA results (Fig. 6C), in the DPSC group and HGF-DPSC group, the concentration of IFN-γ was significantly downregulated, the concentration of IL-6, TGF-β were significantly upregulated compared to the Control group. Compared to the DPSC group, the concentrations of IFN-γ and IL-6 were lower and the concentration of TGF-β was higher in the HGF-DPSC group. However, there was no difference in the concentration of IL-4 between all the three groups. These results indicate that HGF overexpression suppressed the secretion of Th1, Th17 cell-related cytokines, and promoted Treg cell-related cytokines.

To investigate whether HGF overexpression also suppressed lymphocytes activity, the PHA stimulated PBMCs proliferation was tested. As shown in the results (Fig. 6D, 6E), compared to the Control group the proliferation rate of PBMCs were suppressed in both the DPSC group and HGF-DPSC group, while there was no difference between these two groups ($p>0.05$). These results indicate that the cell activity of PBMCs was not affected by HGF overexpression.

Discussion

Psoriasis is a Th1 and Th17 cells overacting-related chronic inflammation disease, in this study, we firstly demonstrated that DPSCs were capable of alleviating the pathological severity and inflammation condition of psoriasis. DPSCs are a kind of MSC like postnatal stem cells, which possess self-renewal, multiple differentiation and immune regulation potentials (36–38). MSCs can be easily isolated from a variety of tissues, including bone marrow, adipose tissue, umbilical cord, dental pulp, and so on (39–42). It was demonstrated that MSCs derived from different tissues have different characteristics and potentials. For instance, MSCs derived from adipose tissue have a more promising ability to suppress the stimulated PBMCs proliferation and inhibiting the immature dendritic cells differentiation than those derived from bone marrow (43). While MSCs derived from the umbilical cord are more immune privileged than the MSCs derived from adipose tissue (44). Therefore, an appropriate donor source of MSCs is critical for clinical application. Previously we found that DPSCs showed a stronger downregulation effect on Th1 and Th17 cells and stronger upregulation effect on Treg cells than umbilical cord derived MSCs (UC-MSCs) (Fig. S1), suggesting that DPSCs are appropriate candidates for psoriasis treatment. In this study, we demonstrated that DPSCs alleviated the severity of psoriasis mainly by downregulating Th1 and Th17 cells and upregulating Treg cells. Th1 and Th17 cells are inflammation reaction-related T helper cells. Treg cells are a kind of immune regulatory cells, are capable of suppressing autoimmunity (45). It has been demonstrated that Th17 and Treg cells share the same precursor cell, and the combination of IL-6 and TGF-β leads to the activation of RORγt and differentiation of Th17, while TGF-β alone induces Foxp3 expression and Treg differentiation (46). In this study, when cocultured with PBMCs, DPSCs upregulated the expression levels of both IL-6 and TGF-β, meanwhile downregulated Th17 cells and upregulated Treg cells (Fig. 6). The reason might be IL-6 and TGF-β regulate the activity of Th17 and Treg cells in a concentration-dependent manner. In the presence of IL-6, a low concentration of TGF-β favors

Th17 cell differentiation, while the high concentration of TGF-β promotes the differentiation of Treg cells (47). Therefore, DPSCs suppressed Th17 cells and promoted Treg cells mainly by upregulating the expression of TGF-β. Moreover, it was reported that TGF-β could inhibit the expression of Th1 transcription factor T-bet and also suppress Th1 cells (48, 49). Hence, the underlying mechanism of the treatment effect of DPSCs on psoriasis might be their strong ability to promote the TGF-β secretion.

HGF is primarily secreted by mesenchymal cells, plays an essential role in tissue protection and regeneration, and is useful for attenuating inflammatory response (50, 51). In this study, HGF overexpression enhanced the immune regulatory ability of DPSCs, and their treatment effect on psoriasis. As a pleiotropic growth factor, HGF is related to both regeneration and immune regulation, and can act synergistically or antagonistically with TGF-β. During fibrosis, HGF frequently down-regulates TGF-β to alleviate fibrotic degree by reducing the excessive deposition of collagen and extracellular matrix (52–54). However, in organ transplantation conditions, HGF usually elevates TGF-β expression to prolong the transplants' survival by decreasing allograft rejection (55–57). In an experimental autoimmune encephalitis mouse model, HGF treatment upregulates the population of IL-10 secreted Tregs (44). While it was demonstrated that IL-10 secreted Tregs can produce TGF-β (58). In this study, HGF overexpression up-regulated the expression of TGF-β in the psoriatic mice model (Fig. S2). These results indicate that in inflammation conditions, HGF might suppress the severity of inflammation by acting synergistically with TGF-β. Consequently, HGF overexpressing enhanced the treatment effect of DPSCs might through upregulating the expression of TGF-β.

This study still has several limitations. First, the psoriasis mouse model adopted in this study was induced by IMQ. It is a prevalent model. However, the skin inflammation decreases during the seventh or eighth day when IMQ is applied to the mice. Therefore, the longtime treatment effect cannot be evaluated in this study. Second, further investigations are needed to confirm the role of TGF-β in the psoriasis severity, as well as the correlations between HGF overexpression and TGF-β under inflammation conditions.

Conclusion

Our study firstly demonstrated that the administration of DPSCs ameliorated the pathological severity and inflammatory condition of psoriasis. And HGF overexpression enhanced the treatment effect of DPSCs on psoriasis, which by downregulating Th1 and Th17 cells and upregulating Treg cells. The possible mechanism might be that under inflammation conditions, HGF enhanced DPSCs' upregulation effect on TGF-β expression.

Abbreviations

Ad-HGF: Adenovirus vector carrying human HGF cDNA; Ad-Null: Adenovirus vector not carrying exogenous genes; cDNA: Complementary deoxyribonucleic acid; DPSCs: Dental pulp stem cell; ELISA: Enzyme linked immunosorbent assay; FOXP3: Forkhead box protein 3; GAPDH: Glyceraldehyde -3-

phosphate dehydrogenase; GATA3: GATA binding protein 3; HGF: Hepatocyte growth factor; HGF-DPSCs: HGF enhanced DPSCs; IFN-γ: Interferon-gamma; IFN-γ: Interferon-gamma; IL-10: Interleukin 10; IL-17: Interleukin 17; IL-17A: Interleukin 17A; IL-17F: Interleukin 17F; IL-2: Interleukin 2; IL-23: Interleukin 23; IL-4: Interleukin 4; IMQ: Imiquimod; MOI: Multiplicities of infection; mRNA: Messenger RNA; MSCs: Mesenchymal stem cells; Null-DPSCs: Ad-Null transfected DPSCs; P6: Passage 6; PBMCs: Peripheral blood mononuclear cells; PBS: Phosphate buffer saline; Q-PCR: Quantitative real time-polymerase chain reaction; RNA: Ribose Nucleic Acid; RORγt: Retinoic acid-related orphan receptor-γt; SPF: Specific pathogen free; T-bet: T-box transcription factor 21; Th1: T helper 1; Th17: Interleukin 17 (IL-17)-secreting helper T; Th2: T helper 2; TNF-α: Tumor necrosis factor-α; Treg: Regulatory T.

Declarations

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Author contributions

Hongfang Meng: Design of the study, Data curation, Formal analysis, Writing - original draft. Fen Wei: Performed ELISA and Q-PCR experiments. Ying Zhou: Assisted with the isolation, culture, and characterization of stem cells. Zhiqiang Ge: Writing - review & editing. Jide Jin: Data curation, Writing - review & editing. Hua Wang: Data curation, Writing - review & editing. Chu-tse Wu: Funding acquisition, Supervision. All authors approved the final version of the manuscript to be published.

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Availability of data and materials

All the data and materials were included in this published article.

Ethics approval and consent to participate

The teeth we used for DPSC isolation were obtained from Dental Clinic of Beijing Stomatological Hospital. All donors were gaven their written informed consent to participate. The procedure was approved by guidelines set by the Research Ethics Committee of Capital Medical University, China. Human PBMCs were isolated from the buffy coats of healthy volunteers by a protocol approved by the Beijing Red Cross Blood Center at Beijing. All the animal experiments were approved by the ethics committee of Beijing Institute of Radiation Medicine, and all procedures were carried out in accordance

with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (USA).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 The human primer for Quantitative Real time-PCR

Genes	Forward primer	Reverse primer
GADPH	5´-ACAGTCAGCCGCATCTTCTT-3´	5´-AAATGAGCCCCAGCCTTCTC-3´
HGF	5´-CCGACTGGCTCTTTTAGGCAC-3´	5´-CTCTGCATAGGGGATGGCGA-3´

GAPDH: glyceraldehyde -3-phosphate dehydrogenase; HGF: hepatocyte growth factor.

Table 2 The mouse primer for Quantitative Real time-PCR

Genes	Forward primer	Reverse primer
GADPH	5´-TCCACTGGCGTCTTCAC-3´	5´-GGCAGAGATGATGACCCTTTT-3´
T-bet	5´-ACAACCCCTTTGCCAAAGGA-3´	5´-TCCCCCAAGCAGTTGACAGTT-3´
IFN-γ	5´-TCAAGTGGCATAGATGTGGAAGAA-3´	5´-TGGCTCTGCAGGATTTTCATG-3´
GATA3	5´-TTATCAAGCCCAAGCGAAGG-3´	5´-CATTAGCGTTCCTCCTCCAGAG-3´
IL-4	5´-CATCGGCATTTTGAACGAG-3´	5´-TTGGAAGCCCTACAGACGAG-3´
RORγt	5´-AGCATCTATAGCACTGACGG-3´	5´-CAGAAACTGGGAATGCAGTG-3´
IL-17A	5´-TTTTCAGCAAGGAATGTGGA-3´	5´-TTCATTGTGGAGGGCAGAC-3´
IL-17F	5´-CAAGAAATCCTGGTC TTG-3´	5´-GAGCATCTTCTCCAACCTGAA-3´
IL-23	5´-ACTCCCCATTCCTACTTCTCCCT-3´	5´-CACTTGCTGCATGAGGAATTGTA-3´
FOXP3	5´-TGCAGGGCAGCTAGGTACTTGTA-3´	5´-TCTCGGAGATCCCCTTTGTCT-3´
IL-10	5´-GCTCTTACTGACTGGCATGAG-3´	5´-CGCAGCTCTAGGAGCATGTG-3´

GAPDH: glyceraldehyde -3-phosphate dehydrogenase; T-bet: T-box transcription factor 21; IFN-γ: Interferon-gamma; GATA3: GATA binding protein 3; IL-4: Interleukin-4; RORγt: Retinoic acid-related orphan receptor-γt; IL-17A: Interleukin 17A; IL-17F: Interleukin 17F; IL-23: Interleukin 23; FOXP3: Forkhead box protein 3; IL-10: Interleukin 10.

Figures

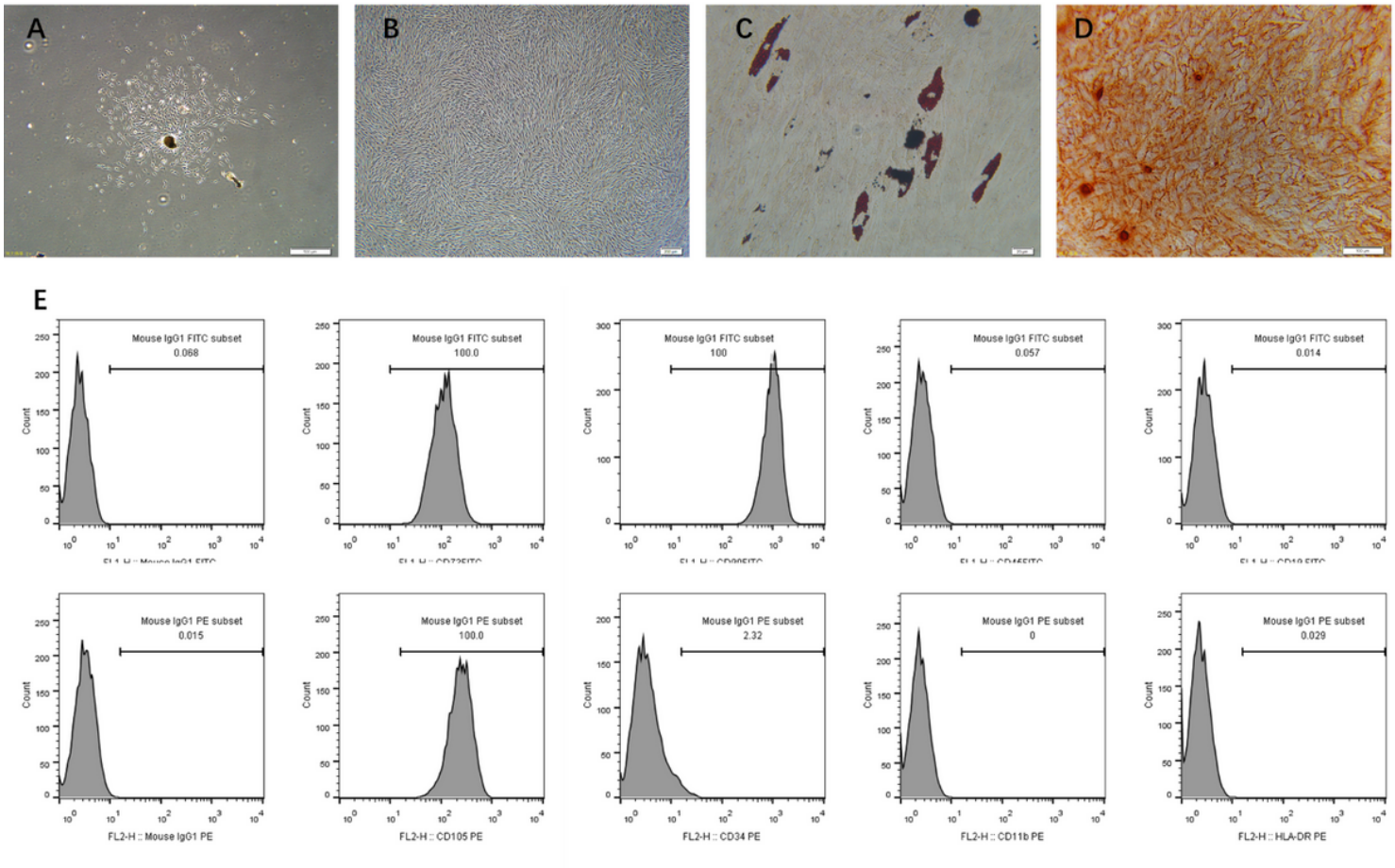

Figure 1

Isolation and characterization of dental pulp stem cells (DPSCs). (A) The digested human dental pulp block adhered to the culture flask, the cell colony was formed and observed on day 10. (B) Human DPSCs were spirally arranged with spindle shape at the sixth passage. (C) Red oil droplets were observed in hDPSCs after adipogenic induction for 14 days. (D) Red mineralized nodules were observed in hDPSCs after osteogenic induction for 21 days. (E) Flow-cytometric analysis showed that hDPSCs positively expressed CD73, CD90, and CD105, and negatively expressed CD45, CD19, CD34, CD11b, HLA-DR.

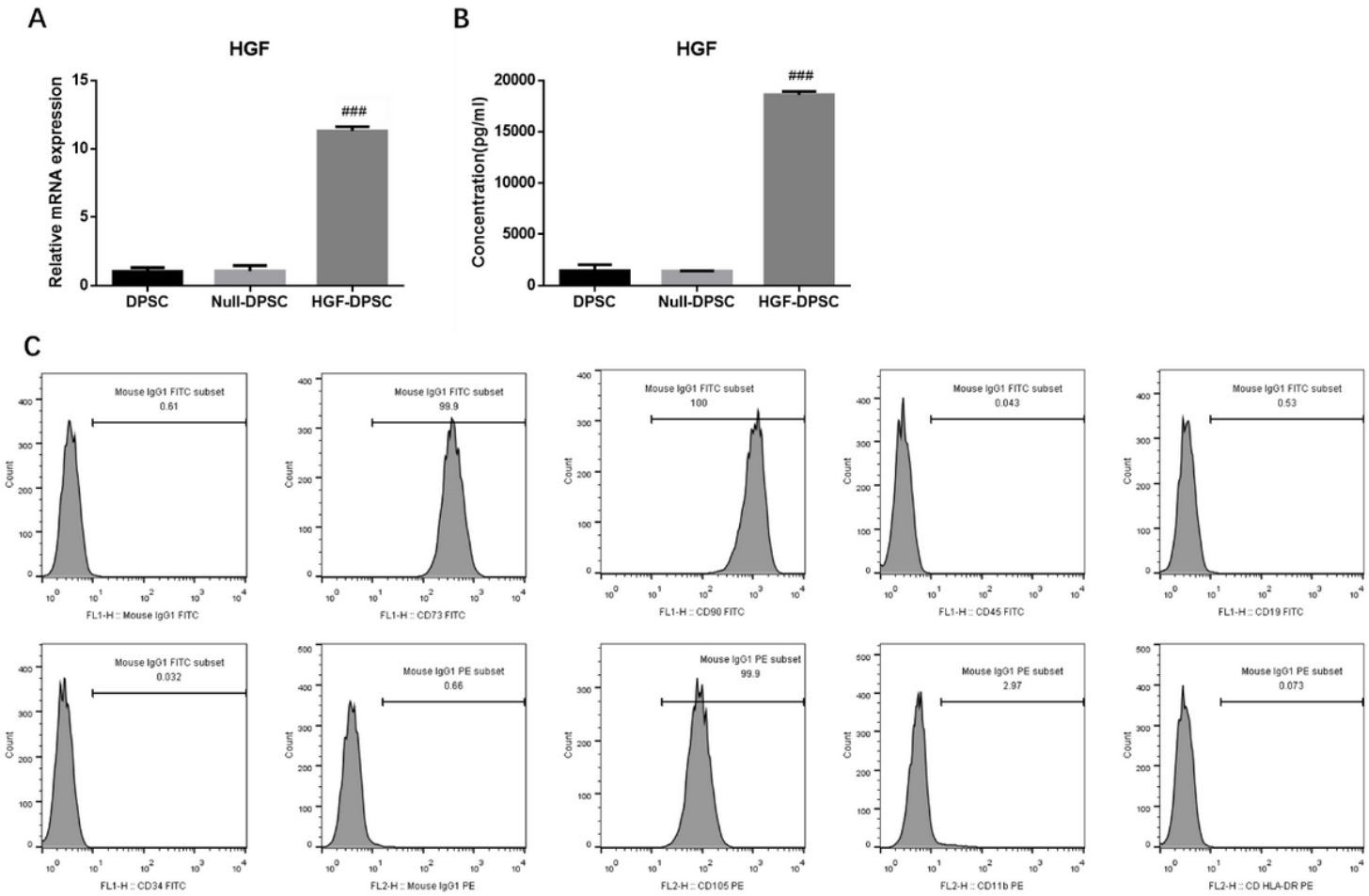

Figure 2

Measurement of HGF expression level in HGF-DPSCs. DPSCs were transfected by Ad-Null or Ad-HGF, 48 hours later, (A) the relative HGF mRNA expression level of HGF-DPSCs was tested by Q-PCR and calculated by the 2-ΔΔCT method. (B) The concentration of HGF protein in the culture supernatant was tested by ELSA. (C) The immunophenotype of HGF-DPSCs was analyzed by flow-cytometric, HGF-DPSCs positively expressed CD73, CD90, CD105, and negatively expressed CD45, CD19, CD34, CD11b, and HLA-DR. ### means $p < 0.001$, compared with DPSC group.

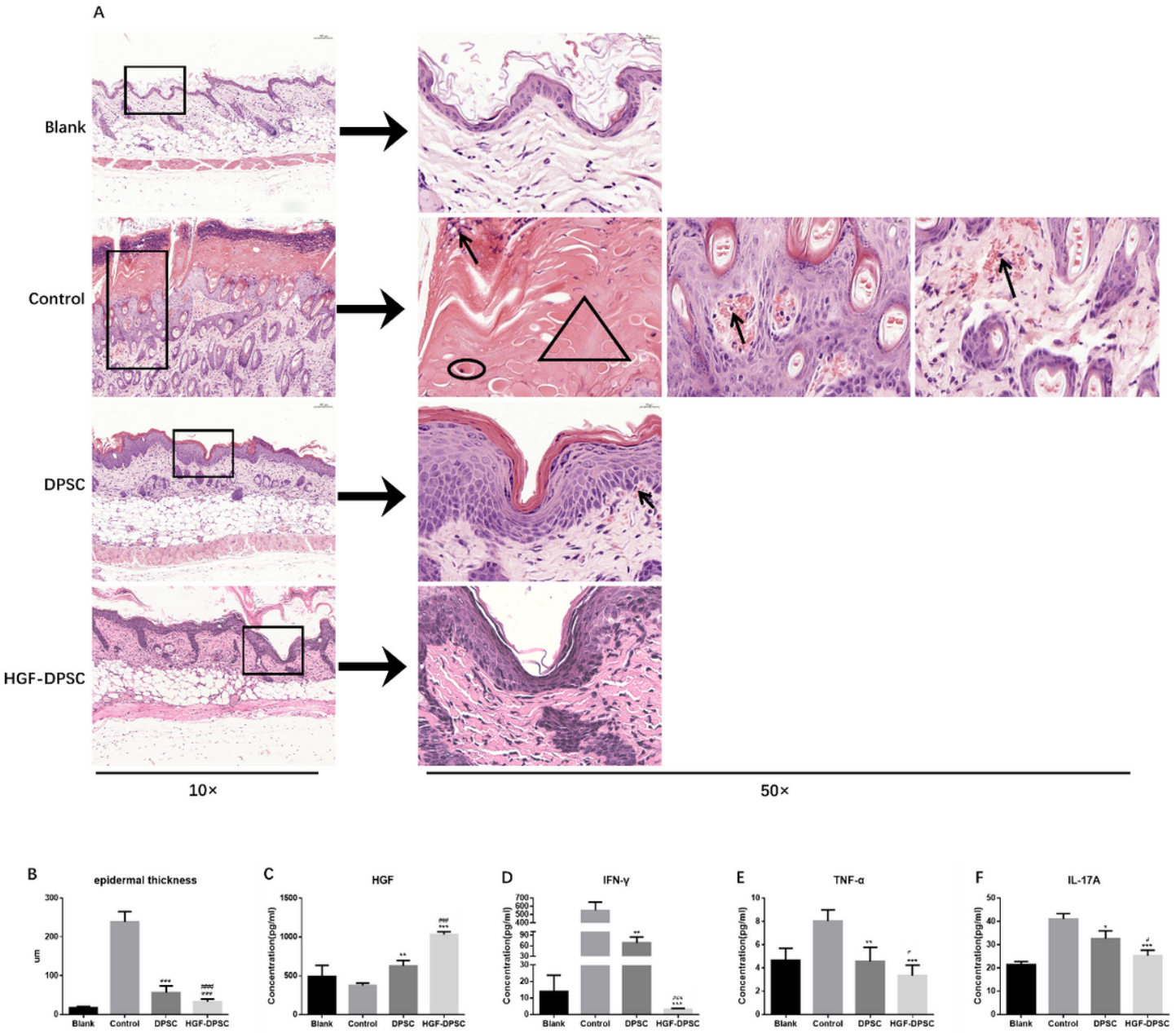

Figure 3

The treatment effect of HGF overexpression on IMQ induced psoriasis mice. (A) H&E staining of the mice back skins in each group, inflammatory infiltration (arrow), parakeratosis (circle), hyperkeratosis (triangle). (B) The epidermal thickness of the back skins were measured and calculated by Image J software. (C) The concentrations of HGF in blood serums of all mice were tested by ELISA. The concentrations of IFN-γ (D), TNF-α (E) and IL-17A (F) in blood serums of all mice were tested by MILLIPLEX analysis. Blank group: no topical application of IMQ, intravenous injection of normal saline; Control group: topical application of IMQ, intravenous injection of normal saline; DPSC group: topical application of IMQ, intravenous injection of 2×106 DPSCs; HGF-DPSC group: topical application of IMQ, intravenous injection of 2×106 HGF-DPSCs. * means P<0.05, compared with Control; ** means P<0.01,

compared with Control group; *** means p < 0.001, compared with Control group; # means P<0.05, compared with DPSC group; ## means P<0.01, compared with DPSC group.

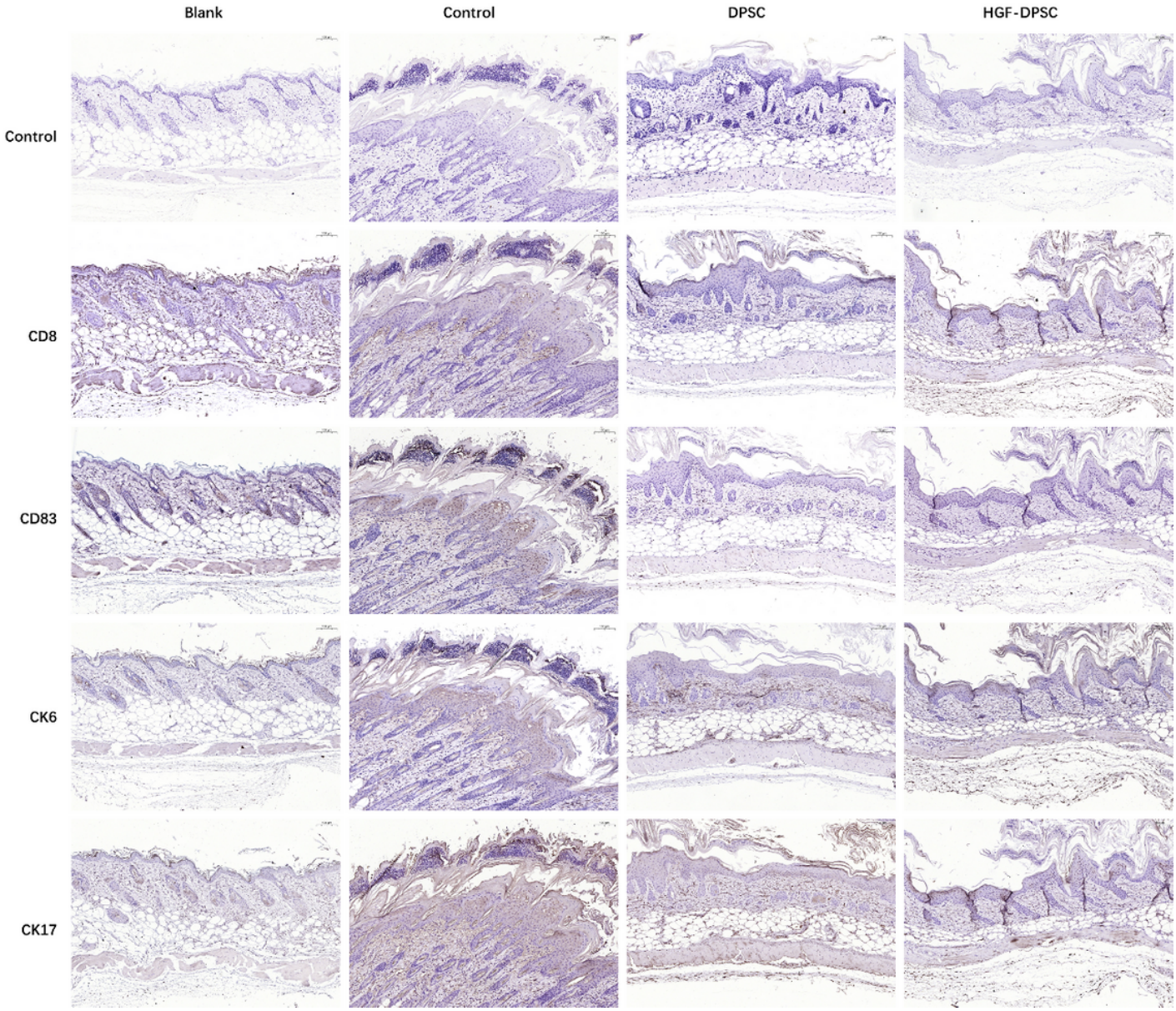

Figure 4

Immunohistochemical examination of the psoriatic back skin lesions. The skin sections were analyzed by immunohistochemical staining with anti-mouse CD8, CD83, CK6, and CK17.

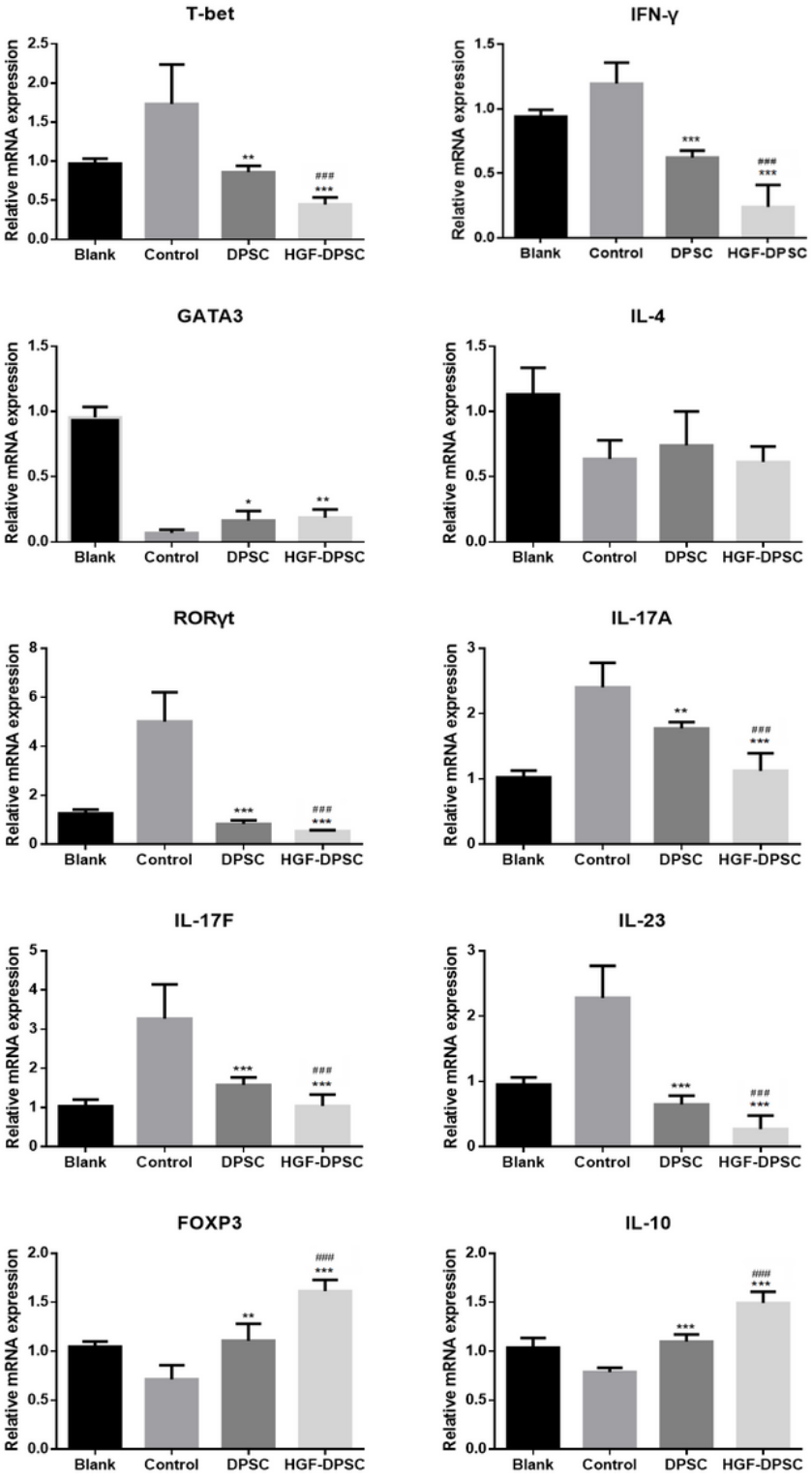

Figure 5

The expression levels of T helper cells' transcription factors and cytokines in the skin lesions of all mice. The expression levels of T-bet, IFN-γ, GATA3, IL-4, RORγt, IL-17A, IL-17F, IL-23, FOXP3 and IL-10 in the skin lesions of all mice were tested by Q-PCR and calculated by the 2-ΔΔCT method. * means P<0.05, compared with Control group; ** means P<0.01, compared with Control group; *** means p < 0.001, compared with Control group; ### means p < 0.001, compared with DPSC group.

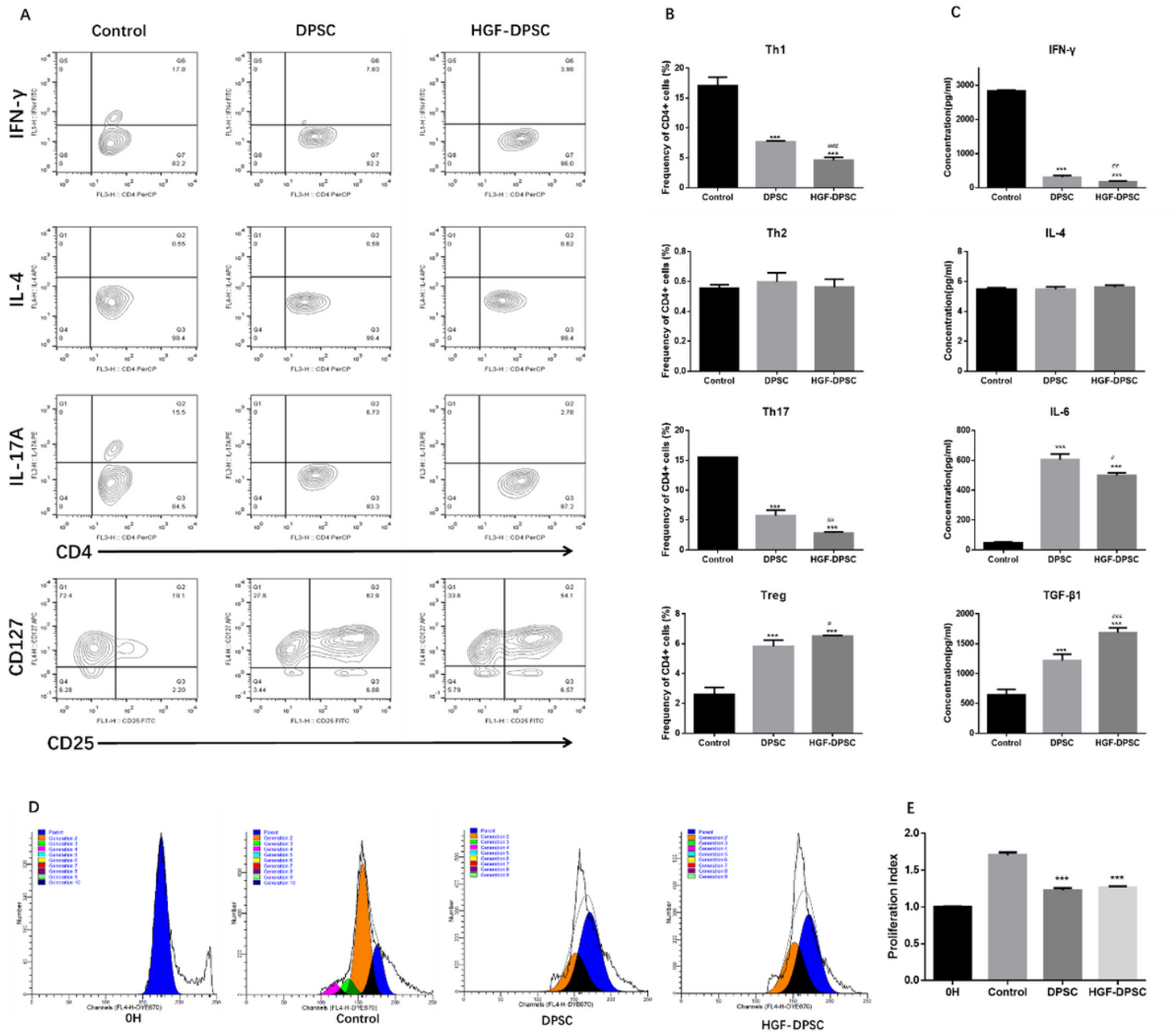

Figure 6

The effects of HGF overexpression on the differentiation and proliferation of stimulated PBMCs. PBMCs were cocultured with or without mitomycin C treated DPSCs or HGF-DPSCs under the stimulation of CD3/CD28 Streptamers and recombinant human IL-2. (A) The PBMCs were harvested, and the percentages of CD4+IFN-γ+ Th1, CD4+IL-4+ Th2, CD4+IL-17A+ Th17, and CD4+CD25+CD127- Treg were tested by flow cytometry and analyzed by FlowJo software; (B) The percentages of Th1, Th2, Th17, and Treg in CD4+ cells were calculated in each group; (C) The concentrations of CD4+ T cells differentiation-related cytokines IFN-γ, IL-4, TGF-β1, and IL-6 were tested by ELISA. Dye 670 labeled PBMCs were cocultured with or without mitomycin C treated DPSCs or HGF-DPSCs under the stimulation of PHA. (D) The fluorescences of Dye 670 in PBMCs were tested by flow cytometer and analyzed by ModFit software; (E) The proliferation index was calculated in all groups. Control group: PBMCs cultured alone under

appropriate stimulation; DPSC group: PBMCs cocultured with DPSCs under appropriate stimulation; HGF-DPSC group: PBMCs were cocultured with HGF-DPSCs under appropriate stimulation; 0H: PBMCs labeled with Dye 670 at zero hour. *** means $p < 0.001$, compared with Control group; # means $P<0.05$, compared with DPSC group; ## means $P<0.01$, compared with DPSC group; ### means $p < 0.001$, compared with DPSC group.

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