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Total glucosides of paeony improves the immunomodulatory capacity of MSCs partially via the miR-124/STAT3 pathway in oral lichen planus

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ABSTRACT

Mesenchymal stem cells (MSCs) have been used clinically and experimentally to relieve severe immune-related diseases due to their immunomodulatory properties, but these are impaired by inflammation. Oral lichen planus (OLP) is a T cell-mediated chronic inflammatory mucosal disease. In the present study, we found MSCs from OLP with higher expression of interleukin (IL)-6, tumour necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β) and IL-10 compared with control. Total glucosides of paeony (TGP) significantly improves the immunomodulatory function of MSCs by inhibiting IL-6 and TNF-α expression and increasing TGF-β and IL-10 expression. Moreover, TGP can downregulate *p*-STAT3 expression through upregulation of miR-124. The changes of IL-6, TGF-β and *p*-STAT3 were further confirmed by overexpression and knockdown of miR-124 in MSCs. Taken together, the immune-regulating function of MSCs can be improved by TGP via the miR-124/STAT3 pathway.

1. Introduction

Mesenchymal stem cells (MSCs) are adult stem cells, which were first isolated and identified in the bone marrow (BM). MSCs have now been found in many other tissues, such as adipose, umbilical cord blood, dental pulp and oral mucosa [1–4].

MSCs have been reported to be fibroblast-like cells that positively express CD105, CD73 and CD90, while negatively expressing CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR [5]. For their self-renewal, multipotency and immunomodulatory properties, MSCs are an attractive tool for regenerative medicine and immune-related diseases. To date, MSCs have been used to treat several immune-related diseases in clinical and experimental trials, such as graft-versus-host disease (GVHD), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and inflammatory bowel disease (IBD) [6–9]. Most cases have obtained favourable outcomes. However, some studies have shown that the inflammatory microenvironment affects the immunomodulatory function of MSCs [10–13]. This might be due to a poor survival rate of MSCs, leading to the weakened immunoregulatory properties and treatment efficacy.

Oral lichen planus (OLP) is recognised as a chronic inflammatory disease that involves oral mucosa and may be accompanied by skin lesions, which has no obvious cause [14]. The prevalence of OLP in the general adult population is 0.5–3% and often found in middle-aged female patients [15]. Moreover, it has a frequency of malignant transformation between 0.4% and 5% [16–18]. In terms of the pathogenesis of OLP, some studies have shown that uncertain stimulations can activate dendritic cells. They produced interleukin IL-12, IL-18 and tumour necrosis factor alpha (TNF-α) to home T cells to lesions and activate T cell differentiation into Th1 and Th17 cells. These cells secrete many inflammatory mediators, including IL-6, IL-8 and TNF-α, finally leading to OLP lesions [14]. MSCs in OLP tissues were first isolated in our previous study [1]. However, whether the functions of OLP-derived MSCs (OLP-MSCs) are impaired and the relationship between the function of OLP-MSCs and the progression of OLP remain largely unknown.

Total glucosides of paeony (TGP) is an effective constituent purified and extracted from the dried root of Paeonia. Paeonia lactiflora, which

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is called “Shao Yao” in Chinese, has been widely used in China as a therapeutic drug for pain, congestion and inflammation [19]. Studies show that TGP dramatically impacts immune-related diseases, such as RA, psoriasis and SLE [20–22]. TGP can increase synoviocyte proliferation and reduce IL-1β, TNF-α, IL-2 and IL-6 expression and increase IL-4, IL-10 and TGF-β expression through the cAMP [23,24] and NF-κB pathways [25]. In clinical therapy, TGP has been proven as a safe and effective drug for OLP with rare side effects [26]. However, the effect of TGP on OLP-MSCs has not yet been investigated.

Several studies have shown that microRNAs (miRs) play a very important role in regulating inflammation-related diseases [27–29]. miRs are non-coding transcripts of 18–25 nucleotides. miR-124 is a member of miRNAs. In traumatic brain injury (TBI), miR-124-3p in microglial exosomes after TBI can inhibit neuronal inflammation and contribute to neurite outgrowth via inhibiting mTOR signalling [28]. In addition, phosphorylated signal transducer and activator of transcription 3 (*p*-STAT3) would be decreased if increased miR-124-3p expression in IBD [30]. Similar reports show that overexpression of miR-124-3p downregulates inflammatory cytokine by blocking nuclear factor (NF)-κB signalling [31]. Moreover, miR124-3p expression the affects the self-renewal of mouse embryonic stem cells by inhibiting the MEK/ERK pathway [32].However, the effect of miR-124-3p on the immunomodulatory function of MSCs has not been reported so far.

In this study, the effect of TGP on OLP-MSCs will be investigated to further explore its possible mechanisms. We also investigated whether function of MSCs primed with TGP is enhanced via the miR-124-3p/STAT3 pathway and whether TGP may recover the damaged immunomodulatory function of OLP-MSCs.

2. Materials and methods

2.1. OLP clinical specimen collection

OLP tissues biopsied from the buccal mucosa of OLP patients. OLP diagnoses agreed with the modified World Health Organisation diagnostic criteria for OLP [31]. Normal oral mucosal tissues were obtained from patients who underwent the crown lengthening surgery or mucous retention cyst resection. Samples were obtained between September 2016 and March 2017 and stored at a biobank at the Peking University School of Stomatology. The following inclusion criteria were used: OLP patients confirmed by clinical features and pathology; available MSCs of OLP; aged 30 - 50 years old; not receiving steroids and immunomodulatory drugs for at least 1 month; normal results of routine blood, liver, and kidney function tests; absence of autoimmune disease, severe infection, diabetes, and other related diseases; and signed informed consent. The exclusion criteria were as follows: either clinical features or pathology are OLP; received other therapeutics before biopsy.

This study was approved by the Ethics Committee of the Peking University School of Stomatology. The clinical characteristics of OLP and normal MSCs donors are listed in Table 1.

2.2. Immunohistochemistry

Thirty OLP tissue lesions and 20 normal oral mucosal paraffin-embedded tissues were from the Department of Pathology of the Peking University School of Stomatology. The tissues were sectioned at 4-μm intervals and placed on coated slides. After antigen retrieval using the high-pressure method, the slides were then incubated with antibody against TNF-α (1:200; Santa Cruz, USA), IL-10 (1:100; Santa Cruz, USA), IL-6 (1:200; Proteintech, USA), and TGF-β (1:200; Proteintech, USA) overnight at 4 °C. Next, slides were washed three times with PDS and incubated with secondary antibodies (ZSGB-BIO, CHINA) for 40 min, developed with 3, 3′-Diaminobenzidine (ZSGB-BIO, China) substrates and counter-stained with haematoxylin. After dehydration, slides were visualised under a microscope. In order to estimate the positive cell intensity, we used Image-Pro-plus software to analyse.

Table 1
The clinical characteristics of OLP and normal MSC donors.

Control no.	Gender	Age	Patient no.	Gender	Age
Control 1	Female	34	OLP 1	Male	42
Control 2	Male	40	OLP 2	Male	32
Control 3	Female	48	OLP 3	Female	45
Control 4	Male	50	OLP 4	Female	34
Control 5	Male	30	OLP 5	Female	52
Mean ± SD		40.4 ± 8.65			41 ± 8.19

2.3. Isolation, identification, and culture of OLP-MSCs and N-MSCs

Human OLP-MSCs were isolated by enzyme digestion and tissue culture. Briefly, the collected tissues were treated aseptically and incubated with dispase (2 mg/mL; Sigma–Aldrich, St. Louis, MO, USA) in sterile phosphate-buffered saline (PBS) to separate the epithelial and sub-epithelial lamina propria. Sub-epithelial lamina propria were minced into 1-mm^3 fragments and digested into a single-cell suspension with 3 mg/mL collagenase type I and 4 mg/mL dispase mixture for 1 h. The suspension was filtered with cell strainer (Falcon, BD Lab-ware, Franklin Lakes, NJ, USA) and cultured in α-modified Eagle’s minimum essential medium (α-MEM, Gibco, USA) containing 10% foetal bovine serum (FBS; Gibco, USA) and 1% penicillin–streptomycin. MSCs of the second to sixth generations were harvested for further identification and the following experiments.

According to the protocol described in the previous studies, MSCs were characterised by flow cytometry for the expression of CD29, CD90, CD34, CD45. Adipo-, osteo-, and neuro-genesis of OLP-MSCs and N-MSCs were evaluated according to previous studies [1,3]

2.4. Effects of TGP on MSCs proliferation

To investigate the effect and optimal concentrations of TGP on MSCs, Cell Counting Kit-8 (CCK-8, Dojindo, Japan) assay was applied to assess cell viability and proliferation. OLP-MSCs and N-MSCs were seeded into a 96-well plate at 2000 cells per well in triplicate (Corning, USA) for 24 h. Then, the cells were treated with various concentrations of TGP (0, 10, 100, 1,000, and 2000 μg/mL) (Lansen, China) in α-MEM for 12 h, 24 h, 36 h, and 72 h. At the indicated time, 10 μl of CCK-8 reagent were added to each well and incubated for 1 h. Organisational development (OD) values were measured at 450 nm to determine the effects of TGP on MSCs proliferation.

2.5. Effect of TGP on cytokine production of OLP-MSCs and N-MSCs at mRNA level

OLP-MSCs and N-MSCs were cultured in six-well plates (Corning, USA) at 600,000 cells/well with 2 ml of MSCs culture medium respectively, after 24 h, the medium was removed, and free-foetal bovine serum α-MEM medium without TGP and with 10 μg/ml TGP was added to the wells for 3 h and 6 h. Total RNA was extracted using Trizol (Invitrogen, USA). Equivalent amounts of RNA were reverse-transcribed using a Revert-Aid First Strand cDNA Synthesis Kit (Takara, China). Then, cDNA was used as template for each PCR using SYBR Green PCR Master Mix (Roche, USA) and running the 7500 Real-time PCR system (Applied Biosystems, USA). All experiments were conducted in triplicate. To quantify relative gene expression, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as internal control, and the expression level of IL-6, IL-10, TNF-α and TGF-β were normalised to internal control. The primer sequences are listed in Table 2.

Table 2
Primer sequences.

Gene	Forward primer	Reverse primer
IL-6	AGCCCACCGGGAACGAAAGA	AAGGCAACTGGACCGAAGGC
TNF-a	AATAGGCTGTTCCCATGTAGC	AGAGGCTCAGCAATGAGTGA
IL-10	TCTCACCCAGGGAATTCAAA	AAGTGATGCCCCAGGCA
TGF-β	ATGTCACCGGAGTTGTGCGG	CGGTAGTGAACCCGTTGATGTCC
GAPDH	CGACAGTCAGCCGCATCTT	CCAATACGACCAAATCCGTTG
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCG

2.6. Effect of TGP on cytokine production released from OLP-MSCs and N-MSCs using enzyme-linked immunosorbent assay (ELISA) kits

MSCs were seeded and treated with TGP as above described. At 3 h and 6 h after treatment, supernatants were collected, and the production of IL-6, TNF-α, IL-10 and TGF-β by MSCs was detected using ELISA kits (Proteintech, USA).

2.7. Detection of the expression of miR-124-3p in OLP-MSCs and N-MSCs and the changes of miR-124-3p after TGP treatment

MSCs were seeded and treated with TGP or without TGP as above described. After 6 h treatment, total RNA was extracted using Trizol, then a professional microRNA Revert-Aid First Strand cDNA Synthesis Kit (GeneCopoeia, USA) was used. Lastly, the relative expression of miR-124-3p (Ribobio, Guangzhou, China) was examined using a 7500 Real-time PCR system.

2.8. The effect of miR-124-3p mimic and inhibitor on the immunomodulatory function of OLP-MSCs

miR-124-3p mimic and inhibitor(miR20000422-1-5, Ribobio, China) were transfected into OLP-MSCs when cell confluence reached 60–70%. In brief, the miR-124-3p mimic was diluted to a final concentration of 20 mM. Next, 5 μl of miR-124-3p mimic was combined with an equal volume of Lipofectamine-3000 (Invitrogen, USA) in 500 μL of serum-free α-MEM and incubated for 15 min at room temperature. The transfection mixture was added to the 6-well culture plates, to which 1500 μL of serum-free α-MEM was added. The same method used to transfect miR-124-3p mimic except for miR-124-3p inhibitor volume is 10 μL. Instead of the medium with complete medium after 24 h post-transfection, at 48 h post-transfection, the OLP-MSCs were harvested for quantitative polymerase chain reaction (qPCR) and western blot.

2.9. Detection of t-STAT3 and p-STAT3 expression by western blot

MSCs were lysated by radioimmunoprecipitation assay (RIPA) lysis buffer with protease inhibitors. The protein concentration was determined by BCA method. Forty micrograms of protein was run in 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to polyvinylidene difluoride membranes. After incubation with *t*-STAT3 and *p*-STAT3 primary antibodies (1:1000, CST, USA) overnight at 4 ℃, the membranes were washed and incubated with secondary antibodies (1;5000, ZSGB-BIO, CHINA). The bands were detected using ECL Plus Western Blotting Substrate (Pierces, USA). The relative expression of proteins evaluated with Image J. After stripping, the membrane was probed with anti-GAPDH (1:1000, CST, USA) as a control.

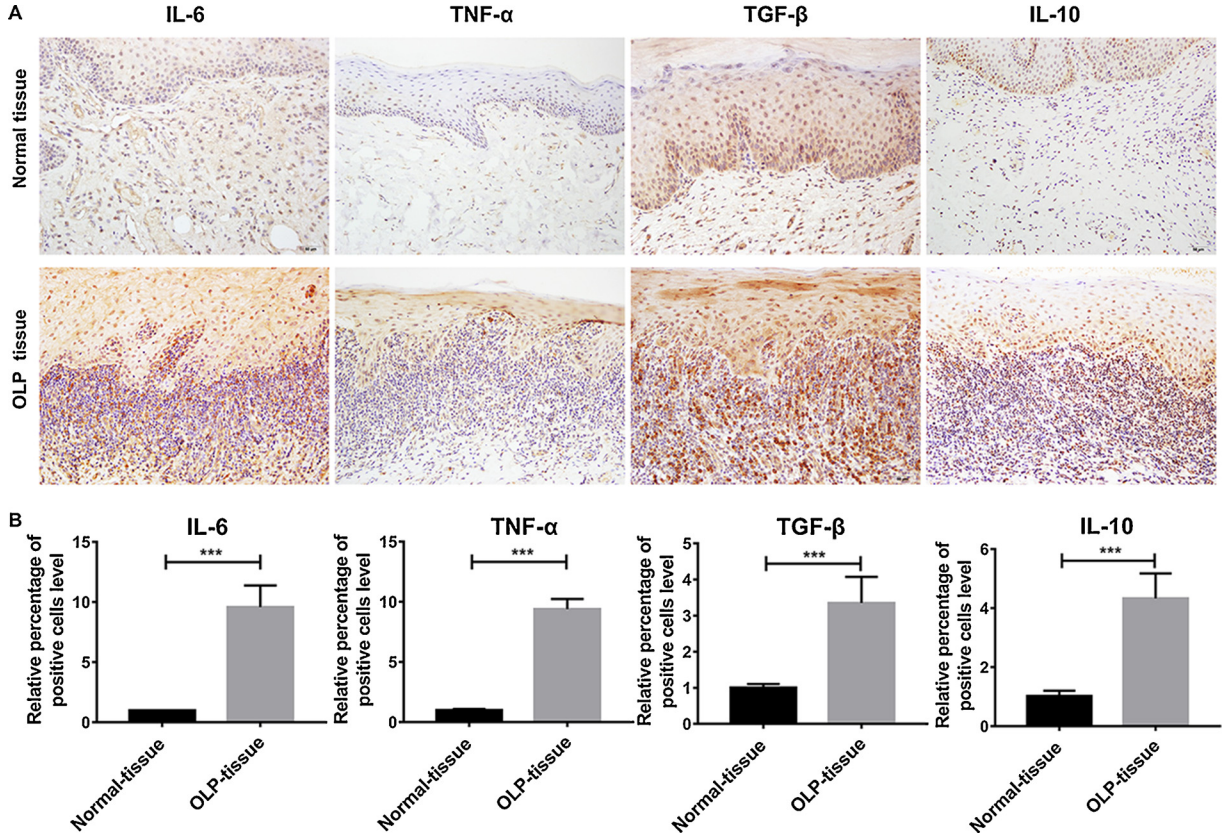

Fig. 1. Immunohistochemical staining for IL-6, TNF-α, TGF-β and IL-10 in OLP and normal sub-epithelial lamina tissues.
(A) Comparison with normal tissue, OLP represents higher expression of IL-6, TNF-α, TGF-β and IL-10. Original magnification × 200 (scale bars 50 μm). (B) Statistical analysis of the immunohistochemistry images, statistical significance was showed as ***, $P < 0.001$.

2.10. Statistical analysis

All data are expressed as mean ± SEM from at least three independent experiments. Data analyses were performed using SPSS 13.0 software. Differences among three or more groups were analysed using one-way analysis of variance (ANOVA) and the post hoc Bonferroni tests for pairwise comparisons. For comparison between two groups, independent two-tailed Student's t-tests were performed. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Expression of IL-6, TNF-α, TGF-β and IL-10 in OLP and normal sub-epithelial lamina tissues

To verify the expression of IL-6, TNF-α, TGF-β and IL-10 in OLP and normal sub-epithelial lamina tissues, immunohistochemical staining was performed. As shown in Fig. 1, compared with normal sub-epithelial lamina, four cytokines all expressed significantly higher in OLP tissues, which confirmed that the OLP microenvironment co-exists between pro-inflammatory and anti-inflammatory cytokines.

3.2. Production of IL-6, TNF-α, TGF-β and IL-10 in N-MSCs and OLP-MSCs

To explore the effect of the OLP microenvironment on MSCs, we performed reverse transcription (RT)-PCR and ELISA to examine the production of IL-6, TNF-α, TGF-β and IL-10 in OLP-MSCs and N-MSCs as shown in Fig. 2, and the results were in line with immunohistochemistry results, which indicated that MSCs with immunomodulatory function could have been changed in the OLP environment.

3.3. TGP decreased IL-6, TNF-α while increasing TGF-β and IL-10 in OLP-MSCs and N-MSCs both at RNA and protein levels

By exploring the effect of TGP on OLP-MSCs and N-MSCs proliferation, TGP was found to have no effect on MSCs proliferation. Therefore, TGP may affects the immunomodulatory function of MSCs. To investigate whether TGP treatment can improve the production of the immunomodulatory cytokines secreted by MSCs, 10 μg/mL of TGP was used to treat OLP-MSCs and N-MSCs for 3 h and 6 h, real-time PCR results are shown in Fig. 3A, when MSCs were treated with TGP for 3 h and 6 h, N-MSCs and OLP-MSCs showed a similar inhibitory trend of IL-6 and TNF-α expression. Moreover, the expression levels of IL-6 and TNF-α in the OLP group at 6 h post-TGP treatment returned to less than their normal level in the N-MSCs group without TGP treatment. However regarding TGF-β and IL-10 expression trends, OLP-MSCs showed different pattern from N-MSCs after TGP treatment at indicated times. TGF-β expression was increased in OLP-MSCs ($P < 0.01$) while it hardly changed in the N-MSCs group. IL-10 mRNA expression was markedly increased in N-MSCs at both 3 h and 6 h after treatment, but significantly decreased at 3 h after treatment, then clearly upregulated at 6 h after TGP treatment in OLP-MSCs. Meanwhile, ELISA results showed that the protein level of IL-6, TGF-β and IL-10 had the same expression pattern as their RNA levels (Fig. 3B). The ELISA results of TNF-α were undetectable with the present method.

3.4. Expression of miR-124-3p/STAT3 and the relationship with inflammatory cytokines in OLP-MSCs and N-MSCs

The above study indicates that TGP can regulate the inflammatory cytokine expression of MSCs. However, the way in which TGP affects MSCs was unclear. To solve this problem and determine whether miR-124-3p/STAT3 plays a vital role in regulating the immunomodulation of OLP-MSCs and N-MSCs, the expression of miR-124-3p was tested using real-time PCR, as well as the expression of *t*-STAT3 and *p*-STAT3 by western blot in OLP-MSCs and N-MSCs. The results showed that N-

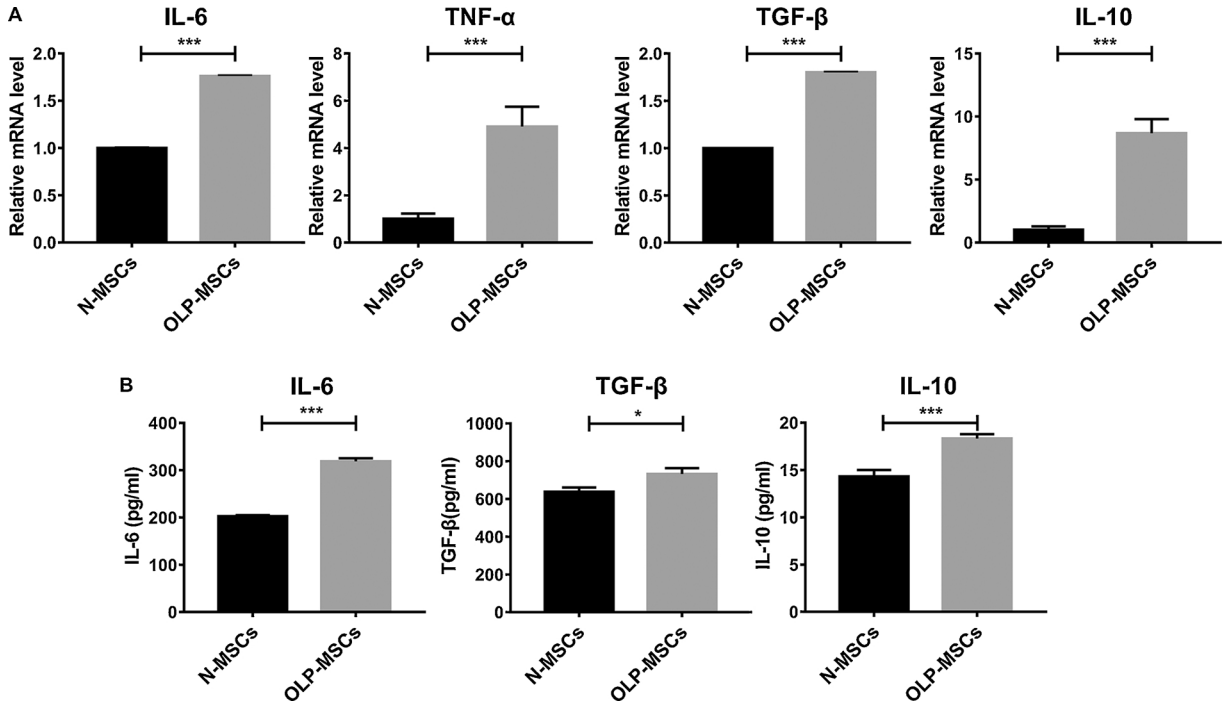

Fig. 2. Production of IL-6, TNF-α, TGF-β and IL-10 in OLP-MSCs and N-MSCs with RT-PCR and ELISA.
(A) The relative mRNA expression of IL-6, TNF-α, TGF-β and IL-10 in OLP-MSCs and N-MSCs with RT-PCR. (B) The protein expression of IL-6, TGF-β and IL-10 in OLP-MSCs and N-MSCs with ELISA. Results were expressed as mean ± standard deviation (SD) and statistical significance was showed as *, $P < 0.05$; ***, $P < 0.001$.

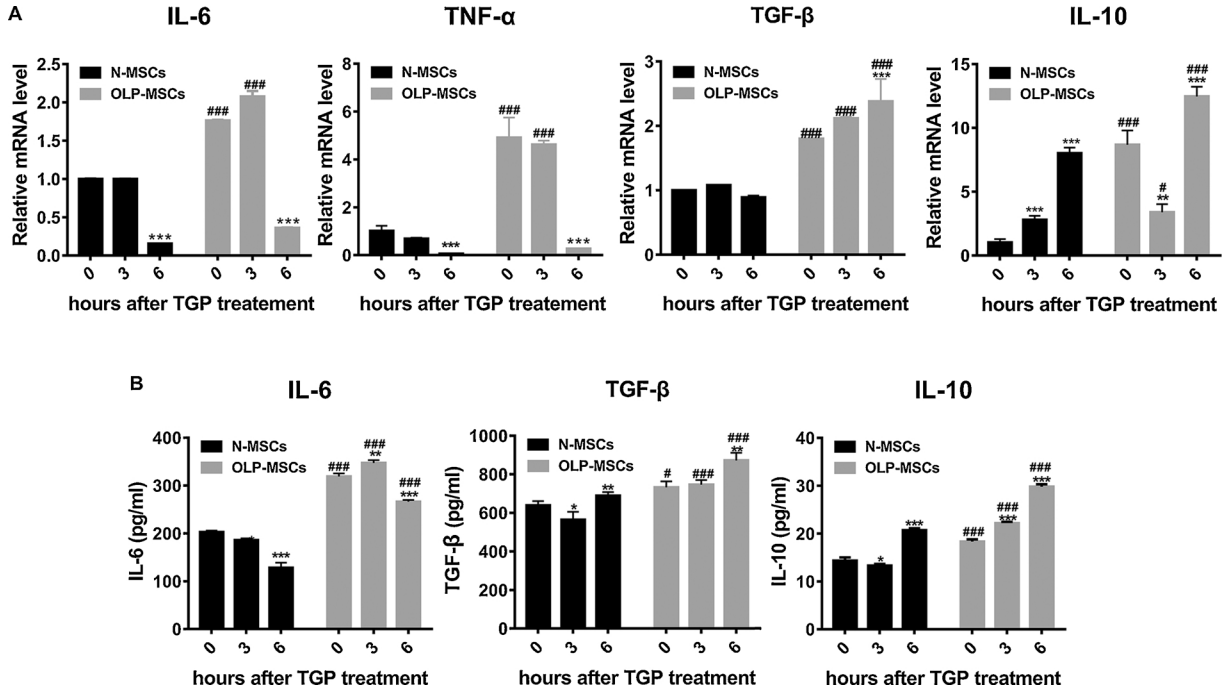

Fig. 3. Production of IL-6, TNF-α, TGF-β and IL-10 in primed OLP-MSCs and N-MSCs with TGP using RT-PCR and ELISA.
(A) The relative mRNA expression of IL-6, TNF-α, TGF-β and IL-10 in primed OLP-MSCs and N-MSCs with TGP using Real-time PCR. (B) The protein expression of IL-6, TGF-β and IL-10 in priming OLP-MSCs and N-MSCs with TGP using ELISA. Results were expressed as mean ± standard deviation (SD) and statistical significance was showed as *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$. Representative difference among different time of TGP treatment in OLP-MSCs and N-MSCs group, # ($P < 0.05$), ## ($P < 0.01$), ###($P < 0.001$), Representative difference between OLP-MSCs and N-MSCs group in the same time after treatment of TGP.

MSCs expressed higher miR-124-3p but *t*-STAT3 and *p*-STAT3 expression was lower compared with OLP-MSCs (Fig. 4A).

Next, miR-124-3p mimics were transfected into MSCs. Decrease IL-6, *t*-STAT3, and *p*-STAT3 expression was discovered, and increase of TGF-β expression was investigated (Fig. 4B).

However, miR-124-3p inhibitor was introduced into MSCs. Expected results were found. IL-6, *t*-STAT3 and *p*-STAT3 were significantly increased, and TGF-β expression was decreased in the knockdown of miR-124-3p group (Fig. 4C).

3.5. TGP restores the expression of miR-124-3p in OLP-MSCs

To understand whether TGP restores miR-124-3p expression in OLP-MSCs, TGP (10 μg/mL) was used to treat OLP-MSCs for 6 h. The results showed that TGP enhanced miR-124-3p expression and decreased *t*-STAT3 and *p*-STAT3 expression (Fig. 5A). This effect also regardless of whether miR-124-3p was overexpressed (Fig. 5B)or inhibited (Fig. 5C).

4. Discussion

OLP is a chronic inflammatory disease characterised by dense subepithelial lymphocytic infiltration, increased number of intra-epithelial lymphocytes and degeneration of basal keratinocytes. Additionally, it prevails among 40-60-year-old female patients [16,33]. In this study, OLP tissues expressed more IL-6, TNF-α, and IL-10 than control. Given that OLP has a high morbidity in people more than 40 years old and has complex inflammation conditions. It is unknown whether anything of OLP-MSCs has been altered. To address this issue, we firstly compared OLP-MSCs with N-MSCs in growth, cytokines expression. Our results show that MSCs express more inflammatory cytokines, such as IL-6 and TNF-α. Therefore, we deduce that the fate of MSCs is decided by their surrounding niche microenvironment.

MSCs held a promise for autoimmune diseases and tissue regeneration and have been used in the treatment of GVHD, RA, SLE, and other inflammatory diseases [5,6,11,32]. However, MSCs functions were damaged in inflammatory environments. This led to a decrease in or loss of their therapeutic effect [9,10,34]. Moreover, age-related changes also impact MSCs functions. Studies show that young donor MSCs have a more potent immunomodulatory effect than aged donor MSCs [35–38]. In this experiment, MSCs isolated from 30-50-year-old OLP patients and normal volunteers. Their effects are similar to those reported in the above studies. Compared with N-MSCs, OLP-MSCs not only have lower proliferation [1,18], but also express more inflammatory cytokines. These results are consistent with the characteristics of OLP disease. Thus, we deduce that the immunomodulatory property of OLP-MSCs has been changed. It may be a good method for us to alleviate OLP by modulating the immune function of OLP-MSCs.

A Chinese herb-derived TGP has been approved by the China State Food and Drug Administration for treating a variety of clinical applications to relieve cramping and congestion, and reduce inflammation for many years [19]. It was also found to be a safe and effective drug for OLP with rare side effects, but the mechanisms of TGP remain largely unknown [39].

Based on above issues, we examined the effect of different doses of TGP on N-MSCs and OLP-MSCsproliferation. Results showed that TGP had no effect on MSCs proliferation. Thus, we hypothesised that TGP may primarily affect immunomodulatory properties. To verify this hypothesis, we treated N-MSCs and OLP-MSCs with TGP at different times to study relative cytokine changes. The pro-inflammatory cytokines IL-6 and TNF-α expression were significantly decreased by TGP, while anti-inflammatory cytokines TGF-β and IL-10 expression were increased. One study also found that TGP also had no effect on HaCaT cell proliferation, but could help to reduce the cytokines secreted from LPS-stimulated HaCaT cells [39]. Therefore, we believe that TGP can enhance the immunomodulatory function of MSCs but not proliferation. However, the underlying mechanism by which TGP affects MSCs has not been illustrated.

Several studies have shown that microRNA (miRs) have an important role in inflammatory disease [31,40,41]. Thus, we firstly examined the expression of miRs in N-MSCs and OLP-MSCs. and

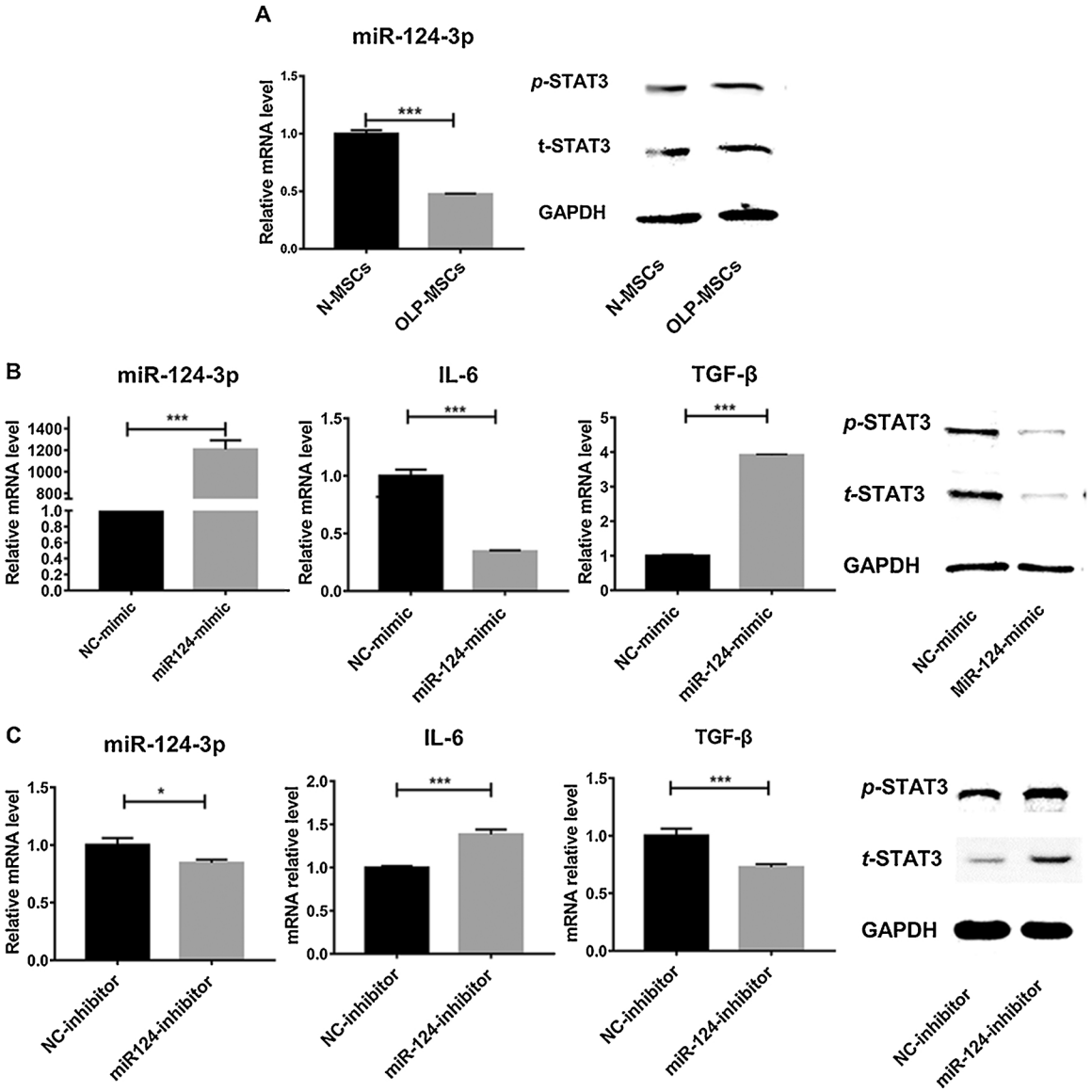

Fig. 4. Expression of miR-124-3p/STAT3 and the relationship with inflammation cytokines in OLP-MSCs and N-MSCs using real-time PCR and western blot.
(A) Expression of miR-124-3p, *t*-STAT3, *p*-STAT3 in OLP-MSCs and N-MSCs (B) Expression of IL-6, TGF-β, *t*-STAT3 and *p*-STAT3 in OLP-MSCs after overexpression of miR-124-3p.(C) Expression of IL-6, TGF-β, *t*-STAT3, *p*-STAT3 in OLP-MSCs after knockdown of miR-124-3p. Results were expressed as mean ± standard deviation (SD), *, $P < 0.05$; ***, $P < 0.001$.

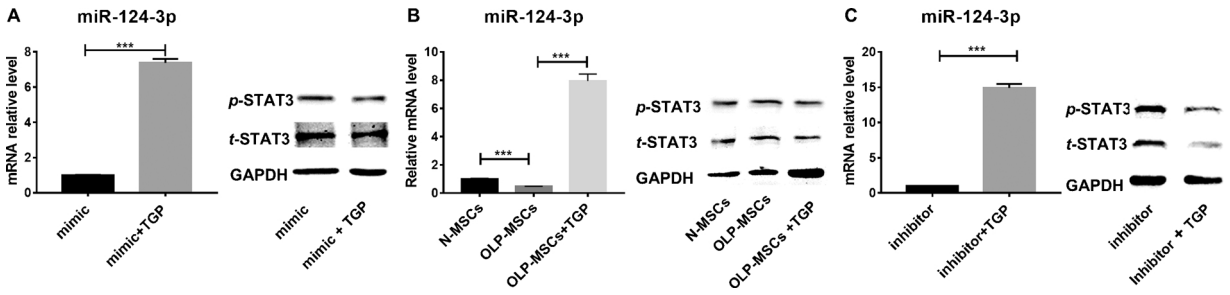

Fig. 5. TGP restores the expression of miR-124-3p in OLP-MSCs.
(A) Expression of miR-124-3p, *t*-STAT3, *p*-STAT3 in N-MSCs, OLP-MSCs and TGP treatment OLP-MSCs with real-time PCR and western blot. (B) Expression of miR-124-3p, *t*-STAT3, *p*-STAT3 in overexpression miR-124-3p and after TGP treatment. (C) Expression of miR-124-3p, *t*-STAT3, *p*-STAT3 in knockdown miR-124-3p and after TGP treatment. Results were expressed as mean ± SD. ***, $P < 0.001$.

discovered that miR-124-3p had lower expression in OLP-MSCs compared with N-MSCs. This result showed that miR-124-3p may be a key molecule in regulating immunomodulatory function of MSCs.

MicroRNAs are small 18 to 22 base pair-long oligonucleotides that regulate gene expression. They suppress the translation by binding to the 3′- and 5′-untranslated regions of the targeted mRNAs [42]. A previous study showed that miR-124-3p is expressed less in inflamed mucosa than non-inflamed mucosa in Crohn's disease (CD) [29]. Similar results were also seen in other inflammatory diseases, such as sepsis [41], chronic skin inflammation [31], and neuronal injury [43]. It should be noted that overexpression of miR-124-3p can significantly decrease pro-inflammatory cytokines by targeting STAT3 [40,44]. In our study, in accordance with the above studies, after treating MSCs with TGP for 6 h, miR-124-3p expression significantly increased. When we overexpressed miR-124-3p mimics in MSCs from OLP, IL-6 and STAT3 were decreased and TGF-β was increased. The opposite results were got after knockdown of miR-124-3p expression. Previous studies have shown that the STAT3 pathway not only affects IL-6 expression, but also regulates TGF-β expression [45,46]. Thus, we suppose that TGP can increase the expression of miR-124-3p to enhance MSCs immune-regulatory function via the STAT3 pathway.

In summary, our study presents new methods which can improve the immunomodulatory effect of OLP-MSCs. However, in our study, OLP-MSCs express more inflammatory cytokines than N-MSCs, which is in accordance with its surrounding microenvironment.TGP has a significant effect on decreasing pro-inflammatory cytokines and increasing anti-inflammatory mediators via the miR-124-3p/STAT3 pathway. We suggest that TGP may be a new and safe drug for improving the function of MSCs.

5. Conflict of interest

All authors have read the journal's policy on the disclosure of potential conflicts of interest and have none to declare.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2018.05.076.

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