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Taxifolin attenuates IMQ-induced murine psoriasis-like dermatitis by regulating T helper cell responses via Notch1 and JAK2/STAT3 signal pathways

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ABSTRACT

Psoriasis is a T cells mediated chronic skin inflammation in which helper T (Th) cells play in critical roles in its pathogenesis. Taxifolin (TXL) has been discovered to exert various pharmacological activities. In this study, we wished to observe whether TXL had potential activities on psoriasis, and how it works. We found that TXL can inhibit LPS-induced abnormal proliferation in Hacat cell line, ant also significantly alleviate the IMQ-induced psoriasis in BALB/c mice, comparing with the control group. Although TXL has no significant effects on the ratio of total T cells in skin draining lymph nodes (SDLN), it decreases the ratio of pro-inflammatory Th1 and Th17 cells, both in skin lesions and SDLN. Our results also disclosed that TXL may regulate Th cells differentiation by inhibiting the transcript factors, including T-bet, GATA-3 and RORγt. Further data show that TXL can inhibit Notch1 and Jak2/Stat3 signal pathways. In summary, TXL may be able to treat psoriasis by regulating Th cells differentiation via inhibiting Notch1 and Jak2/Stat3 pathways.

1. Introduction

Psoriasis is an immune-mediated chronic inflammatory skin disease and characterized by excessive proliferation and aberrant differentiation of keratinocytes, infiltration of immune cells in the epidermis and dermis [1]. These series of events induce the development of psoriatic lesions, which manifests as sharply demarcated red scales and erythematous on the skin with itching and pain [2]. Though the etiology of psoriasis still has not been determined, activated T cells appear to be crucial in promoting both disease onset and maintenance.

The activation of keratinocytes also seems to play a major role in the disease, which in part is mediated by cytokines secreted by T cells [3]. Meanwhile, psoriasis is a CD4 + T cell-mediated autoimmune and inflammatory cutaneous disorder, and anti-CD4 antibodies improve psoriasis [4]. CD4 + T cells can be further divided into several subtypes, such as type 1 T helper (Th1) cells, type 2 T helper (Th2) cells, and type 17 T helper (Th17) cells, et.al. At the earliest, psoriasis is thought to be induced by self-reactive interferon γ (IFN-γ) producing Th1 lymphocytes. IFN-γ and its transcription factor T-bet are increased in the peripheral circulation and skin lesions of patients with psoriasis [5]. T-bet not only plays a key role in Th1 cell differentiation, but also inhibits the production of Th2-specific cytokine IL-4 and induces the transfer of Th2 cell dominance to the opposite direction [6–8]. Conversely, the Th2-specific transcription factor GATA3 promotes Th2 cell differentiation [9] and induces the production of Th2 cytokines [10]. The increasing IL-4 not only improves psoriasis via modification of dermal Th2 cells, but also has an anti-inflammatory effects and anti-regenerative effects on the psoriatic lesions [11]. Moreover, among the

Abbreviations: TXL, taxifolin; Th cell, helper T cell; IFN-γ, interferon γ; IL-4, interleukin 4; IL-17a, interleukin 17a; REG3A, human regenerating islet-derived protein 3-alpha; RORγt, retinoid-related orphan receptor γt; FBS, fetal bovine serum; PBS, phosphate buffer saline; DMSO, dimethyl sulfoxide; LPS, lipopolysaccharide; DMEM, Dulbecco's modified eagle medium; MTT, methyl thiazolyl tetrazolium; IMQ, imiquimod; CsA, cyclosporin; HE, hematoxylin and eosin; PCNA, proliferating cell nuclear antigen; EDTA, ethylene diamine tetraacetic acid; BCA, bicinchoninic acid; BSA, albumin from bovine serum; SD, standard deviation

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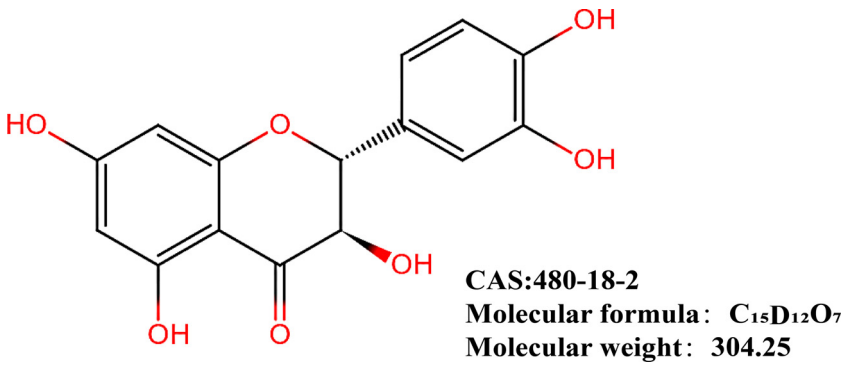

Fig. 1. Chemical information and structure of Taxifolin (TXL).

Table 1
Primers used in this study.

Genes	Sequences
GAPDH	F: GAATGACCCCTTCATTGACC
	R: GACAAGCTTCCCGTTCTCAG
IFNγ	F: TCTGGGCTTCTCCTCCTGCGG
	R: GGCGCTGGACCTGTGGGTTG
IL-4	F: TTGTCATCCTGCTCTTCTTTCT
	R: TTGTCATCCTGCTCTTCTTTCT
IL-17a	F: ATCCCTCAAAGCTCAGCGTGTC
	R: GGGTCTTCATTGCGGTGGAGAG
T-bet	F: AGCAAGGACGGCGAATGTT
	R: AGCAAGGACGGCGAATGTT
GATA3	F: CCCTTCTCCAAGACGTCCAT
	R: CTTTCTCATCTTGCCTGGCC
RORγt	F: GGATGAGATTGCCCTCTA
	R: GGGTGGACATATAAGCGGTTC

cytokines which are critical to psoriasis, Th2 cytokine IL-4 is the only one that can significantly and effectively induce the expression of GATA3 in the epidermis [12].

During recent years, a significant amount of both clinical and experimental data have established Th17 cells as key players in the pathogenesis of psoriasis [13,14]. Th17 cytokines, especially IL-17a, stimulate the production of antimicrobial peptides by keratinocytes, which in turn promote the recruitment of inflammatory cells. It can enhance keratinocyte proliferation and inhibit keratinocyte differentiation [15]. Two closely related transcription factors, retinoid-related orphan receptor (ROR)γt and RORα, play an important role in Th17 cell differentiation [16,17]. RORγt deficient T cells are defective in Th17 cell differentiation. Mice with RORγt-deficient T cells lack tissue-infiltrating Th17 cells and have attenuated autoimmune diseases [18].

Taxifolin (TXL), also named as dihydroquercetin (Fig. 1), is a common flavonoid usually found in Pinaceae plants, such as Pseudotsuga taxifolia, Taxus chinensis, Cedrus deodara and Pinus roxburghii [19,20] and food. It also can be isolated from other plants such as Rhododendron mucronulatum, Rhizoma smilacis glabrae and Silybum marianum [21]. TXL has been discovered to exert various pharmacological activities, including anticancer, antioxidant, anti-inflammatory, anti-proliferative [22]and antibacterial activities [23–25]. TXL has been utilized clinically for the treatment of cardiovascular and cerebrovascular diseases [26], and also strongly inhibited amyloidogenesis in a synergistic manner by suppressing P-JAK2/P-Stat3 sinal pathaway [27]. However, it's unknown whether TXL can inhibit the IMQ-induced psoriasis-like dermatitis in mice yet.

In this study, we firstly evaluated the anti-proliferation effects for TXL in vitro and in vivo. And then tested whether TXL alleviate mouse psoriasis, as well as the mechanisms.

2. Materials and methods

2.1. Cell line and cell culture

2.1.1. Cell culture and LPS induced proliferation model

Human immortal keratinocytes (Hacat) cell line was derived from the China Center for Type Culture Collection (CCTCC, Wuhan, China), and grown in DMEM (Gibico, Life Technologies, USA) medium containing 10 % fetal bovine serum (FBS, Gibico, Life Technologies, USA) in a humidified atmosphere(5 % CO_2, 37℃).

The cell was cultured to adhere to the wall 70 %–80 %, after wash twice with PBS, cells were completely digested with 0.25 % Trypsin (Gibico, Life Technologies, USA) and then resuspended in DMEM medium which contains 10 % FBS and planted in 96-well plate (Corning, USA) at 3500 cells/100 μl, incubated at 37℃ for 24 h. LPS were dissolved in DEME medium containing 2 % serum and 0.5 % DMSO (Sigma-Aldrich, USA) was added to the cultured cells, and further cultured for 24 h.

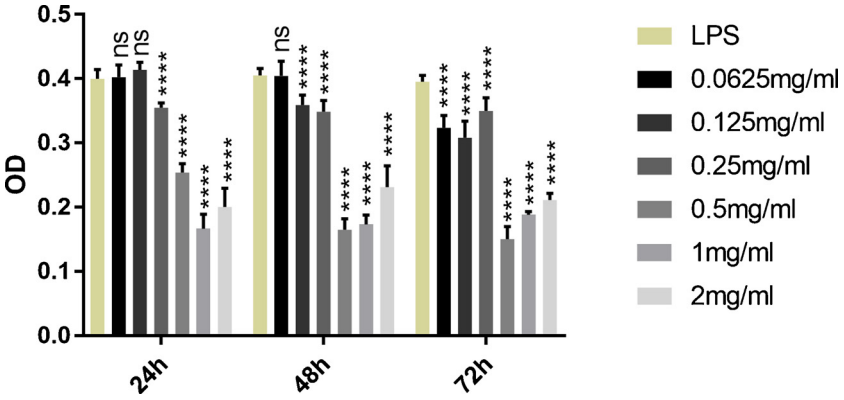

Fig. 2. Taxifolin (TXL) inhibits the LPS-induced abnormal proliferation of inflammatory HaCat cells. The proliferation of HaCat cells in vitro was measured by MTT assays in 24 h, 48 h and 72 h, after reseeded and treated by LPS and Taxifolin. Data are shown as mean ± SD (n = 6, **** $p < 0.0001$ vs. the control group: LPS treated group). The results indicate that TXL inhibits LPS induced keratinocytes proliferation.

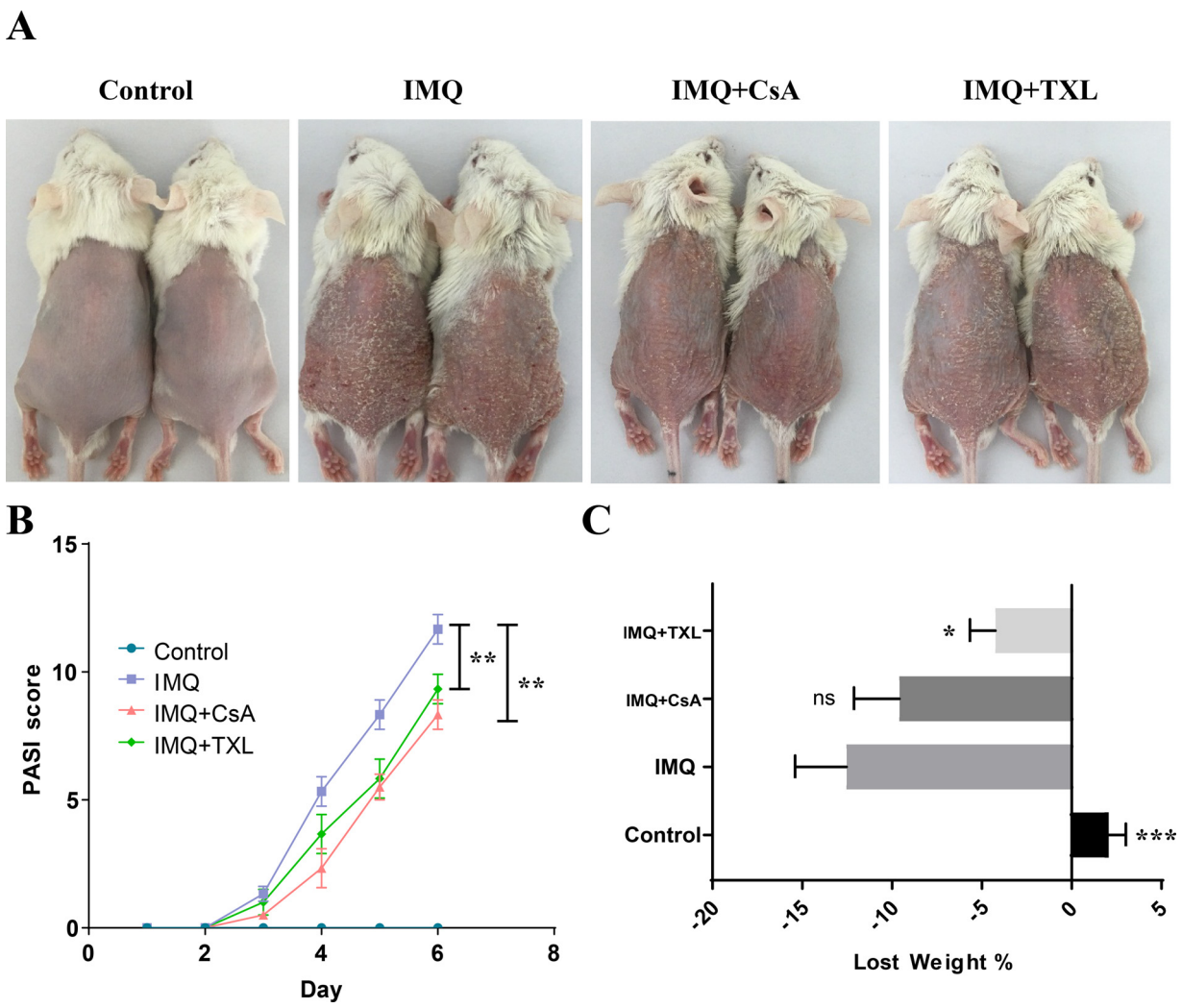

Fig. 3. Taxifolin (TXL) alleviates IMQ-induced psoriasis-like symptoms in mice. A: TXL alleviates mice back skin lesions; B: PASI scores (erythema, scaling and induration) of the back dermatitis were scored daily; C: Lost weight (%) in day6; Symbols indicate mean of PASI score or lost weight (%) ± SD; Comparing to IMQ group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; n = 3, the data shown are representative of three experiments.

2.1.2. HaCat cell proliferation assays in vitro

In vitro HaCat cell proliferation was measured by MTT assays. TXL (Chengdu Herbpurify Co. LTD, China) was prepared into 2 mg/ml,1 mg/ml,0.5 mg/ml,0.25 mg/ml,0.125 mg/ml and 0.0625 mg/ml with DMEM medium containing 2 % FBS and 0.1 % dimethyl sulfoxide (DMSO). Briefly, after LPS-induced for 24 h, TXL was added to each well in different concentrations with 6 replicate wells per concentration. After 24 h, 48 h and 72 h, the supernatant was removed, and then 100 μl MTT solution which containing 0.5 %MTT was added into each well, and placed in the incubator for 4 h. After further 4 h, the supernatant was removed, and 150 μl DMSO was added into each well. The plate was placed on a shaking table to oscillate at a low speed for 10 min to fully dissolve. The absorbance was measured at the wavelength 490 nm. The cell proliferation was presented as OD value.

2.2. Mice and treatment

All animal experiments were approved by the Institutional of Animal Care and Use Committee of Guangdong provincial hospital of Chinese Medicine and performed in accordance with the National Institutes of Health Guidelines on Laboratory Research. The BALB/C mice (male, 7–9 weeks old) used in this study were purchased from Guangdong experimental animal center (Guangzhou, China). They were housed under specific pathogen-free conditions at the animal center with free access to food and water.

Mice were randomly divided into 4 groups (n = 3 per group, repeat 3 times): control group, Imiquimod (IMQ) group, IMQ + Cyclosporin (CsA) group, and IMQ + Taxifolin (TXL) group. All the mice had been oral treated for 7 days. Among them, oral treated group were given 25 mg/kg CSA or 40 mg/kg TXL, which were dissolved in distilled water, while control group and IMQ group were only given distilled water.

Imiquimod is a ligand of TLR7 (and TLR8 in human), which is widely topical used to build the psoriasis-like mouse model [28]. From day 3rd, All the IMQ treated mice were topically treated daily with 50 mg 5 % Imiquimod cream (Sichuan Mingxin Pharmaceutical Co., Ltd. Sichuan, China) on shaved back skin for five consecutive days. And the control group was given the base material.

2.3. The measure of skin inflammation

The skin inflammation was evaluated by three indexes: erythema, thickness, and scale. These indexes were evaluated independently using a defined rating system (0: none, 1: mild, 2: moderate, 3: marked, 4: severe) [29]. PASI score was the sum of these three numerical values.

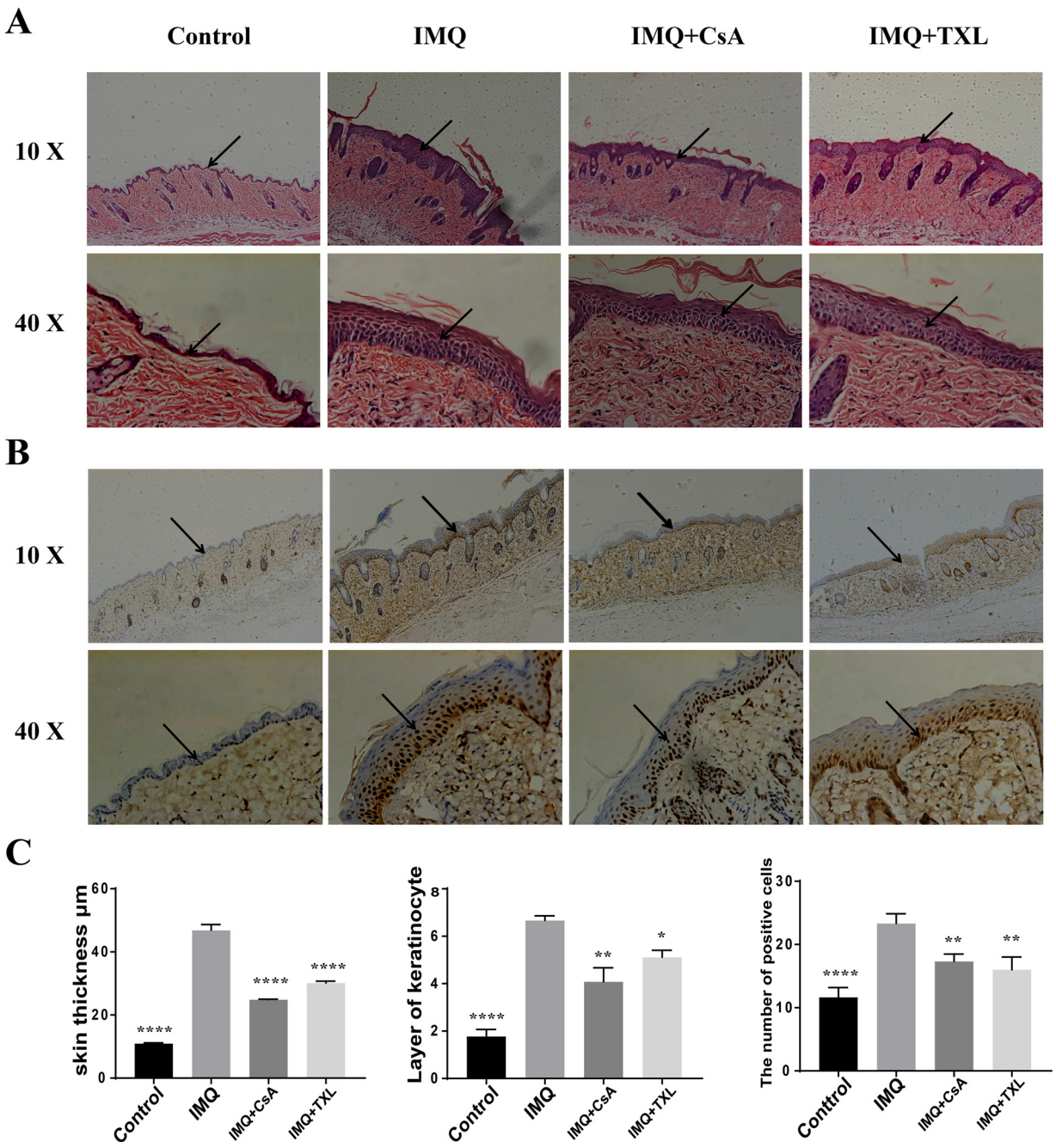

Fig. 4. Taxifolin (TXL)suppresses IMQ-induced psoriasis-like inflammation and the thickening of epidermis. A: H&E staining of skin lesions; B: PNCA staining of skin lesions; C: Thickness of epidermis (μm), the cell layers of the epidermis, the number of PNCA staining positive cells in the epidermis. Symbols indicate mean ± SD comparing to IMQ group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $n = 6$.

2.4. Histopathology

Skin samples of mice had been stored in 4 % Polyformaldehyde fixing solution for 48 h and embedded in paraffin. And then they were stained with HE (Hematoxylin and Eosin, HE) and PCNA (Proliferating Cell Nuclear Antigen, PCNA) according to the standard method. The epidermal thickness and the number of cell layers of epidermis in HE sections and the number of positive cells in PCNA sections were observed and counted in three fields of view from 40*40 of the microscope for each section

2.5. Flow cytometer analysis

Skin draining lymph nodes were collected from the arms and legs of each mouse and put into the precooled wash buffer (2 % FBS and 0.2 % 0.5 M EDTA in 1*PBS). All lymph nodes were ground with the frosted surface of glass slides and passed through 70 μm strainers to generate the single-cell suspensions. Then, $1*10^6$ single cells were seeded into each well in 24 well plates with 1 ml culture medium (10 % FBS in RPMI1640), and then had been stimulated by Leukocyte Activation Cocktail (2.5 μl/ml PMA and 1 μl/ml Ionomysin, BD Pharmingen) for 6 h.

Surface staining was applied with anti-FcγR II/III(Clone 2.4G2) for five minutes to block non-antigen-specific binding of immunoglobulins. Then cells were stained with the following conjugated monoclonal antibodies: PE-Cy 7 Hamster TCR β chain (Clone: H57597), FITC Rat Anti-Mouse CD4 (Clone: RM4-5), PE Rat Anti-Mouse CD4 (Clone: RM4-5).

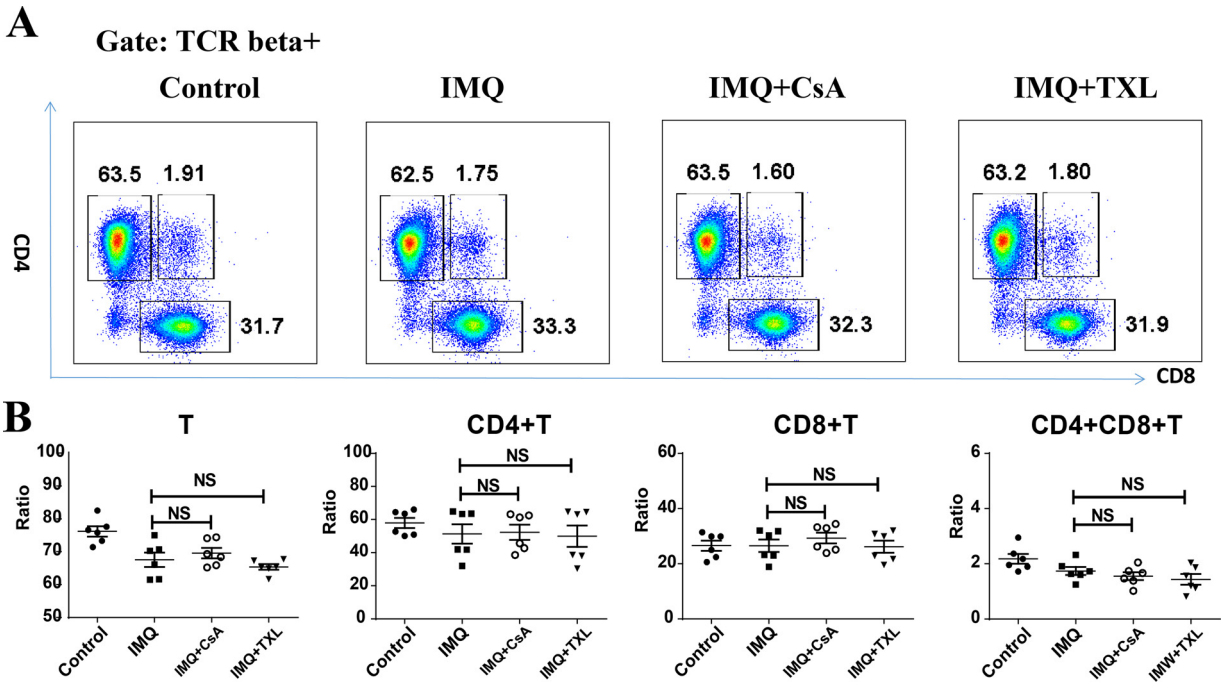

Fig. 5. Taxifolin (TXL) has no significant effect on the ratio of total TCRβ + T cells, as well as the ratios of TCRβ + CD4 + T cells, TCRβ + CD8 + T cells and TCRβ + CD4 + CD8 + T cells in the mice skin draining lymph nodes. A: Gating strategy and flow cytometry scatter plot; B: Frequency of T cells, CD4 + T cells, CD8 + T cells and double positive T cells. Symbols indicate mean ± SD comparing to IMQ treated only group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; n = 6, the experiment shown is representative of three experiments.

For intracellular cytokines staining, cells were fixed and permeabilized with pre-cooled IC/Foxp3 fixed buffer and staining with antibodies: PE Mouse Anti-T-bet (Clone: O4-46), Alexa Fluor 647 Rat Anti-Mouse RORγt (Clone: Q31-378), FITC Rat Anti-Mouse IFN-γ (Clone: XMG1.2), PE-CF594 Rat Anti-Mouse IL-17a (Clone: TC11-18H10). Stained cells were acquired using the FACS Aria III (BD Biosciences, San Jose, CA, USA). All flow cytometry data were analyzed with FlowJo software version 7.6.1 for Microsoft (TreeStar, Sam Carlos, CA, USA).

2.6. Quantitative real-time RT-PCR

Skin tissues were homogenized and total RNA was extracted using RNA prep Pure Tissue Kit (TianGEN, China). The quality of RNA samples was assessed by the 260/280 absorbance ratio, which ranged from 1.9-2.1. The EvoScript Universal cDNA Master (Roche, Germany)was used to synthesize the first-strand cDNA and the relative expression levels IFNγ, IL-17a, T-bet, and RORγt mRNA were quantified by FastStart Universal SYBR Green Master (ROX)with given primers (Table 1) on a ViiA 7 Dx (Applied Biosystems, USA) and analyzed by relative standard curve method. The relative expression of mRNAs were normalized to that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and were calculated using $2^{-\Delta\Delta Ct}$ method, where $\Delta Ct = Ct_{\text{target gene}} - Ct_{\text{GAPDH}}$, and $\Delta\Delta Ct = \Delta Ct - \Delta Ct_{\text{Blank}}$.

2.7. Western blot analysis

Protein was extract by 400 μl RIPA Lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1 % Triton X-100, and 5 mM ethylenediaminetetraacetic acid) from Skin lesions. Protein concentrations were measured by BCA protein assay kit (Beyotime, P0010, china) and each sample is quantified to 40 μg. Equal amounts of protein are separated by 10 % sodium dodecyl sulfate (SDS) PAGE and then transferred to a polyvinylidene difluoride membrane. The membranes were labeled with the primary antibody: GAPDH (D16H11) XP Rabbit mAb (1:1000 dilution, CST, USA), JAK2(D2E12) XP Rabbit mAb (1:1000 dilution, CST, USA), Stat3(D182 J) Rabbit mAb (1:1000 dilution, CST, USA), Phospho-Stat3(Tyr705) (D3A7) (1:2000 dilution, CST, USA), Jagged1(D4Y1R) XP Rabbit mAb (1:1000 dilution, CST, USA), Notch1(D1E11) XP Rabbit mAb (1:1000 dilution, CST, USA), RBPJK (ERP13479) (1:2000 dilution, abcam, UK). And then the membranes were incubated with the Anti-rabbit IgG HRP-linked Antibody (1:5000 dilution, CST, USA). Membranes were treated with SuperSignal ® West Pico chemiluminescence substrate and protein bands were visualized by detecting the enhanced chemiluminescence by Bio-Rad Gel imaging system.

2.8. Statistical analysis

The data were represented as mean ± standard deviation(SD), and one-way analysis of variance (ANOVA) was used for statistical analysis. All data were processed by GraphPad prism 5 systems. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Taxifolin reduces LPS-induced abnormal proliferation on HaCaT cell line

HaCaT cell line is the human immortalized epidermal cells. It was usually used to test the effects of dermatological drugs on human keratinocytes. The preventive effect of TXL on the LPS-induced abnormal proliferation of human keratinocytes was examined. Cells were treated with TXL ranging from 0.0625 mg/ml to 2 mg/ml. TXL observably inhibited the LPS-induced abnormal proliferation, especially at the concentration of 0.25 mg/ml after 24 h ($p < 0.0001$). The maximal inhibition ratio was approximately 80 % (Fig. 2).

3.2. Taxifolin alleviates IMQ-induced psoriasis-like clinical symptoms in mice

We further evaluated the protective effects of TXL on the skin inflammation in IMQ-induced mouse model. As shown in Fig. 3A, TXL significantly alleviates the IMQ-induced skin inflammation, including

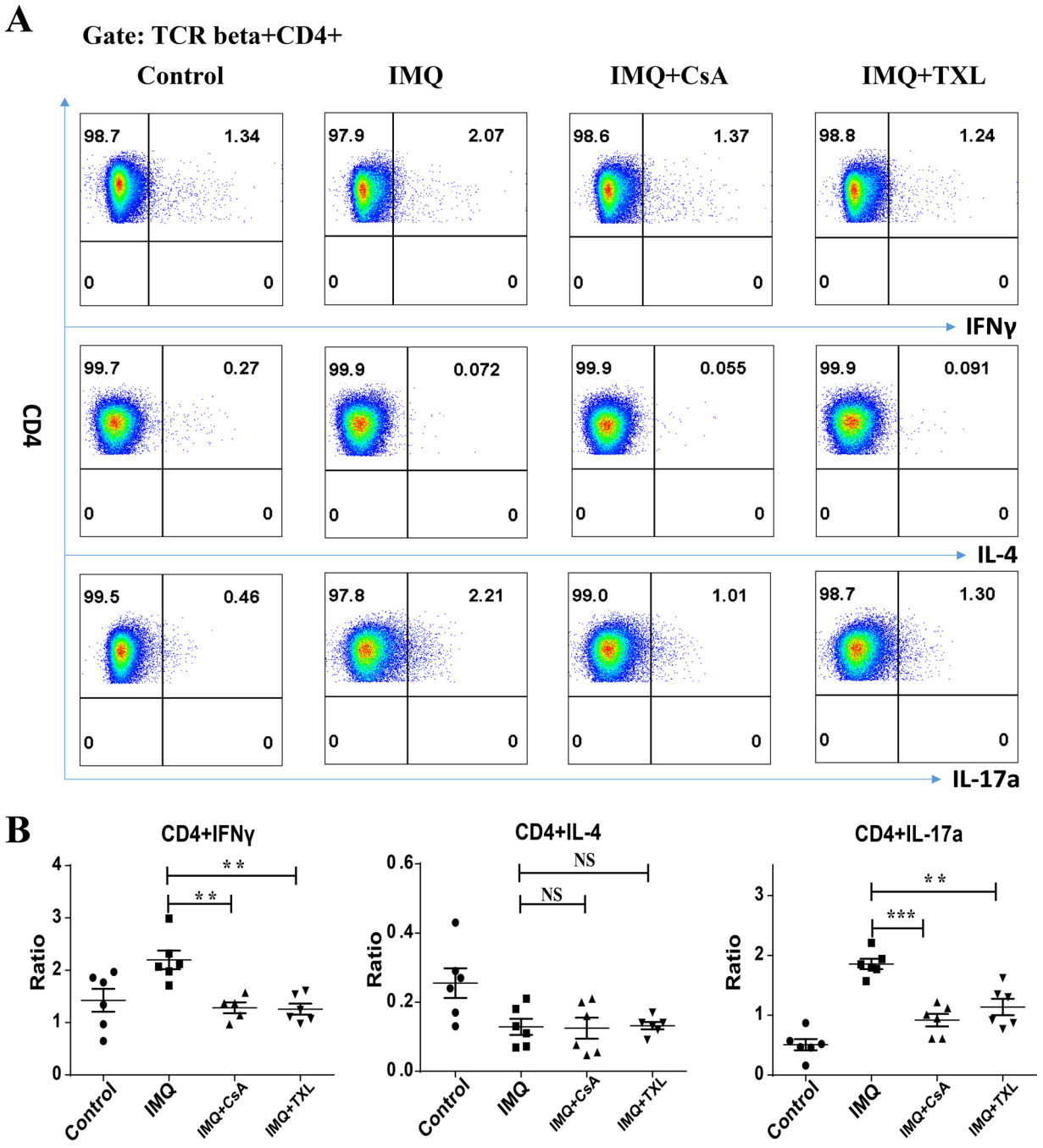

Fig. 6. Taxifolin (TXL) differently regulates the expressions of TCRβ + CD4 + IFNγ + Th1 cells, TCRβ + CD4 + IL-4 + Th2 cells and TCRβ + CD4 + IL-17 + Th17 cells in the mice skin draining lymph nodes. A: Gating strategy and flow cytometry scatter plot; B: Frequency of TCRβ + CD4 + IFNγ + Th1 cells, TCRβ + CD4 + IL-4 + Th2 cells, TCRβ + CD4 + IL-17a + Th17 cells. Symbols indicate mean ± SD comparing to IMQ treated only group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; n = 6, the experiment shown is representative of three experiments.

scales, erythema, and thickness of epidermis. The PASI scores is decreased sharply in TXL group on 6th day ($p < 0.01$), comparing to those in the IMQ group, Fig. 3B. Moreover, the body weight is an important indicator of systemic inflammation. As shown in Fig. 3C, TXL obviously rescues the weight loss of mice after continuous IMQ painting on day6 ($p < 0.05$), comparing to the IMQ group, as well as the CSA group.

3.3. Taxifolin inhibits IMQ-induced proliferation of keratinocytes and skin inflammation in vivo

Since TXL inhibited keratinocytes proliferation in vitro and alleviated IMQ-induced skin psoriasis-like syndromes in vivo, H&E and PCNA staining were performed to evaluate the skin lesions after treatment with TXL. The results show that TXL decreases the hyperkeratosis of epidermal and the infiltration of immune cells in derm (Fig. 4A). In addition, TXL also decreases the number of PCNA positive keratinocytes in vivo ($p < 0.01$) (Fig. 4B, C). The number of epidermal layers ($p < 0.05$) and the thickness ($p < 0.01$) in TXL group are significantly reduce in epidermal, comparing to IMQ group (Fig. 4C).

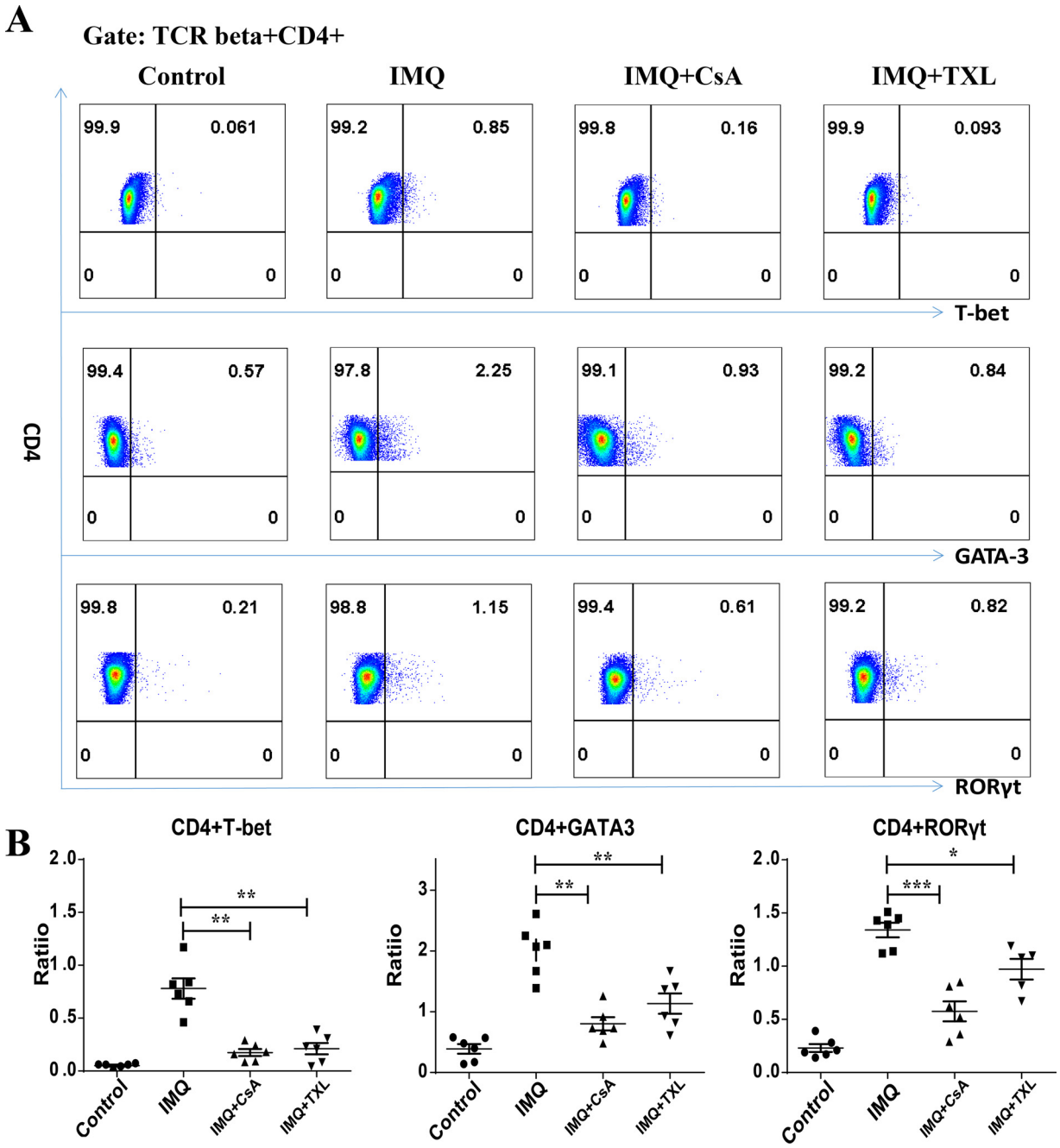

Fig. 7. Taxifolin (TXL) inhibits The expressions of Th1, Th2 and Th17 transcription factors, T-bet, GATA3, and RORγt in the mice skin draining lymph nodes. A: Gating strategy and flow cytometry scatter plot; B: Frequency of TCRβ + CD4 + T-bet + Th1 cells, TCRβ + CD4 + GATA3 + Th2 cells, TCRβ + CD4 + RORγt + Th17 cells. Symbols indicate mean ± SD comparing to IMQ treated only group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; n = 6, the experiment shown is representative of three experiments.

3.4. Taxifolin has no significant effects on the ratios of total T cells and CD4 + T cells and TCRβ + CD4 + IL-4 + T helper(Th) 2 cells, but it deregulates the ratios of TCRβ + CD4 + IFNγ + Th 1 cell and TCRβ + CD4 + IL17 + Th17 cells in skin draining lymph nodes (DLN)

Given the Pro-inflammatory Th1 and Th17 cells and anti-inflammatory Th2 cells playing essential roles in the pathogenesis of psoriasis, we firstly investigated the effects of TXL on total T cells, CD4 + T cells, CD8 + T cells, Th1, Th2, and Th17 cells subsets by flow cytometry. Accordingly, TXL has no obvious effects on the ratios of total T cells, $CD4^+$ T cells, CD8 + T cells and double-positive T cells ($p > 0.05$) in DLN (Fig. 5). As shown in Fig. 6, the proportion of T helper cell subtypes were observed in skin DLN. TXL decreases the ratio of TCRβ + CD4 + IFNγ + Th 1 cells ($p < 0.01$)and TCRβ + CD4 + IL17 + Th17 cells ($p < 0.01$). But there is no obviously effect on TCRβ + CD4 + IL-4 + Th 2 cells ($p > 0.05$).

3.5. Taxifolin suppresses the ratios of Th1, Th2 and Th17 cell by downregulating the expression of transcription factors, T-bet, GATA3 and RORγt in skin DLN

As well known, T-bet and GATA3 are the specific transcript factor for Th1 and Th2 cells, while RORγt is the transcript factor for Th17 cells. Therefore, we further determined the expressions of these three transcript factors in TCRβ + CD4 + T cell population in DLN. As expected, the ratios of T-bet, GATA3and RORγt are all down-regulated in

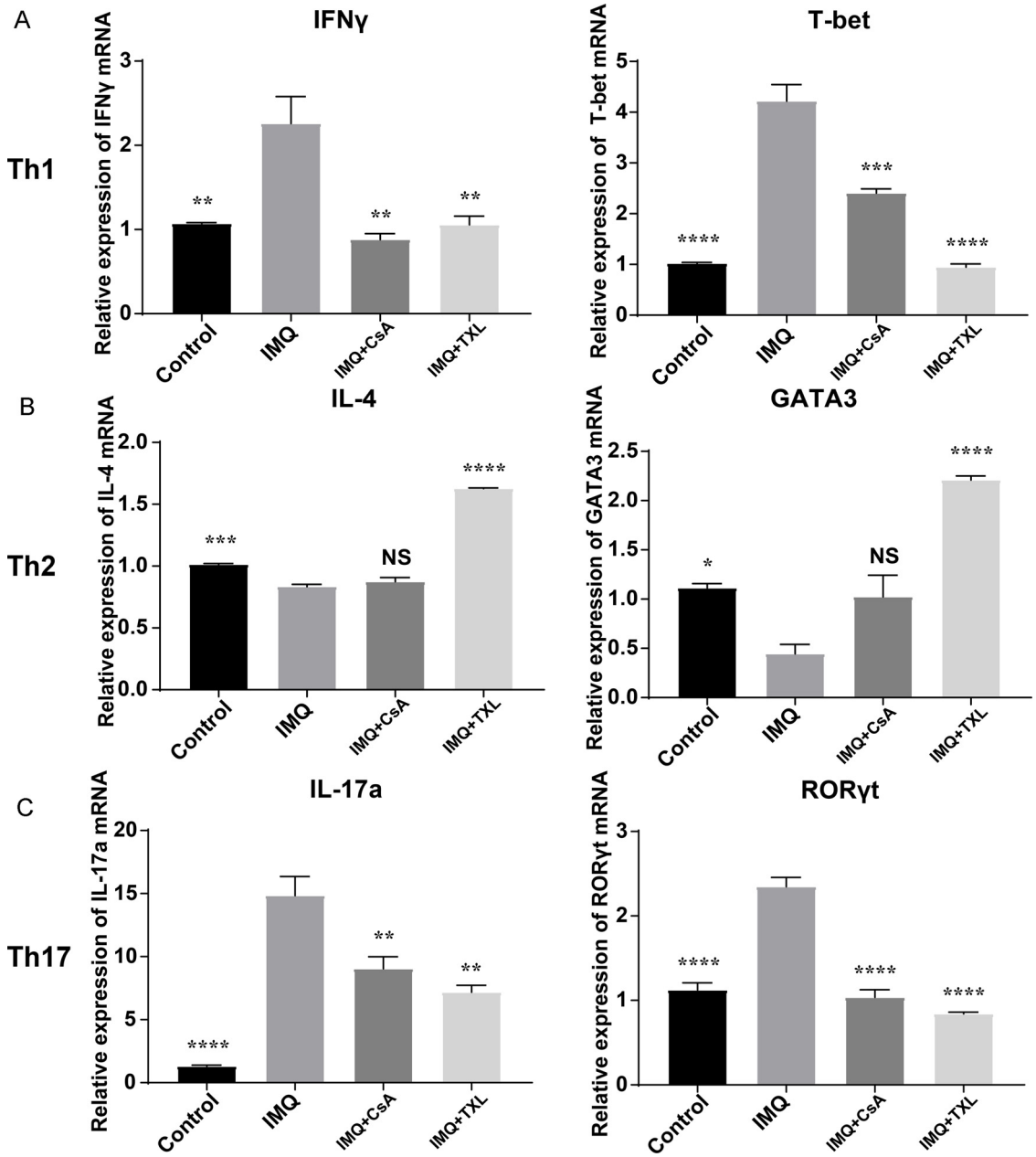

Fig. 8. Taxifolin (TXL) inhibits the relative expressions of IFNγ mRNA, IL-4 mRNA, IL-17mRNA, and the corresponding transcript factors T-bet mRNA, GATA3mRNA, RORγt mRNA in mice skin lesions. A: The expressions of IFN-γ and transcription factor T-bet in mice skin lesions; B: The expressions of IL-4 and transcription factor GATA3 in mice skin lesions; C: The expressions of IL-17 and transcription factor RORγt in mice skin lesions; Symbols indicate mean ± SD comparing to IMQ group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; n = 6.

TXL group ($p < 0.05$) and CSA group ($p < 0.05$), comparing to IMQ group (Fig. 7).

3.6. Taxfolin also inhibits the relative expressions of INF-γ and IL-17 mRNA, as well as their transcription factors, T-bet and RORγt in mice skin lesions, but promotes the relative expression of IL-4 and its transcription factor GATA3

Next, we also assayed the relative expressions of INF-γ mRNA, IL-4 mRNA and IL-17 mRNA and their transcription factors T-bet, GATA3 and RORγt in the mouse's skin lesions by real-time quantitative PCR. As shown in Fig. 8, TXL significantly attenuats the expressions of IL-17 mRNA ($p < 0.01$), RORγt mRNA ($p < 0.0001$), IFN-γmRNA ($p < 0.01$), and T-bet mRNA ($p < 0.0001$), simultaneously promotes the expressions of IL-4 mRNA ($p < 0.0001$) and GATA3 mRNA ($p < 0.0001$).

3.7. Taxifolin inhibits the signaling pathway of STAT3/JAK2 in mice skin lesions

Western blotting analysis of STAT3 and JAK2 was performed on skin tissues of the IMQ-induced mice skin lesions. The expressions of STAT3 and JAK2 both are increased following IMQ painting ($p < 0.001$), although TXL had no significant effects on the expression of total STAT3 protein ($p > 0.05$), it can obviously inhibit the expression of Pstat3 ($p < 0.05$) and JAK2 ($p < 0.05$) (Fig. 9A).

3.8. Taxifolin suppresses the Notch1 pathway in psoriasis skin lesions

Finally we used Western blotting to investigate the expression of

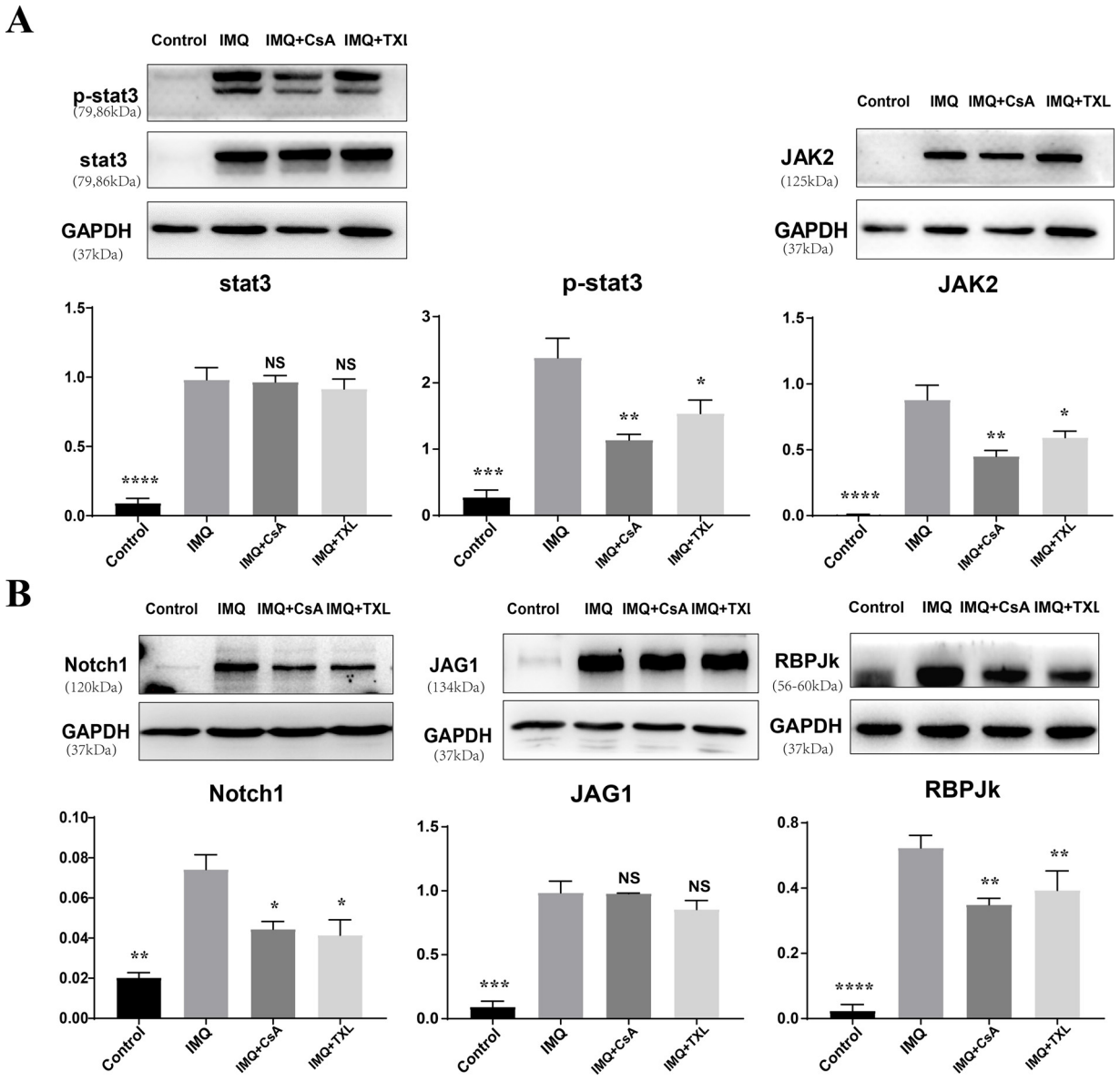

Fig. 9. Taxifolin (TXL) may regulate T helper cell differentiations via Jak2/STAT3 and Notch1 signaling pathways. A: Relative expressions of protein STAT3, pSTAT3 and JAK2. B: Related expressions of protein Notch1, JAG1 and RBPJK. Symbols indicate mean ± SD comparing to IMQ group, * $p < 0.05$, **$p < 0.01$, ***$p < 0.001$; $n = 6$.

proteins in Notch1 pathway in mice skin lesions. The results reveal that expressions of Notch1, its transcription factor RBP-Jk and ligand JAG1 were higher than that of the IMQ group ($p < 0.01$). TXL significantly down-regulates the expressions of notch1 ($p < 0.05$) and RBP-Jk ($p < 0.01$), but has no effect on the expression of JAG1 ($p > 0.05$) (Fig. 9B).

4. Discussion

In recent years, TXL has been attracted particular interest as a health-promoting agent because of its pharmacological activities in the management of inflammation, tumors, microbial infections, oxidative stress, cardiovascular, and liver disorders [30,31]. In this study, we demonstrate the anti-psoriasis effects for TXL on Hacat cell line and IMQ-induced psoriasis-like BALB/C mouse model. TXL shows significant inhibition of LPS-induced abnormal proliferation of human keratinocytes and alleviation of clinic syndromes in psoriasis–like mice. Further results show that TXL may regulate Th cell differentiation via regulating transcript factors, as well as Notch1 signal pathway and Jak2/ Stat3 signal pathway.

Hacat cell line is widely used for studies of skin disease and preliminarily screening the potential therapeutic drugs [32,33]. It was reported that TXL inhibited IFNγ-induced ICAM-1 expression in Hacat cells via Jak1/stat1 signal pathway [34]. In this study, we evaluated that TXL inhibited the LPS-induced abnormal proliferation in Hacat cells. This result indicates that TXL may have an effect on keratinocytes in the inflammatory condition, and reminds that it may have anti-psoriasis activity.

Psoriasis is considered to be a T cell-driven skin disease [13]. Therapeutic strategies targeting T cells and the relative cytokines were confirmed to be efficacy and widely used in clinic for the psoriasis patients [35,36]. TXL had been reported to a relatively active inhibitor of cytotoxic T lymphocytes generation, but was essentially unable to inhibit CTL effector function [37]. Our results demonstrated that although TXL had on effects on the total ratios of T cells, CD4 + T cells, and CD8 + T cells in the skin draining lymph nodes. But the followed results showed that TXL regulated the imbalance by regulating the differentiation of the most known CD4 + Th cell subsets, IFNγ + Th1

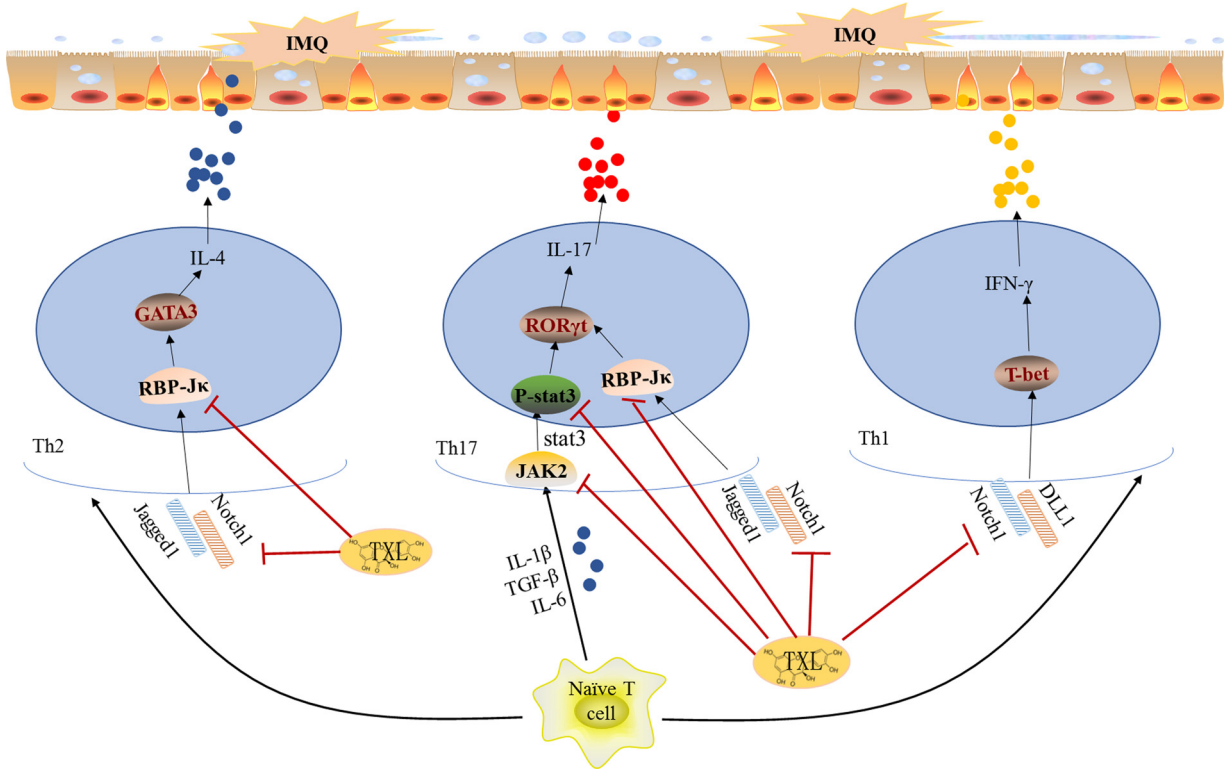

Fig. 10. The schematic depicts the probable mechanisms of Taxifolin on improving the psoriasis-like skin inflammation in BALB/C mice.

cells, IL-4 + Th2 cells and IL-17 + Th17 cells. TXL sharply decreased the ratios of Pro-inflammatory Th1 cells and Th17 cells both in draining lymph nodes and skin lesions, while it significantly increased the ratio of anti-inflammatory Th2 cells in skin lesions. These results suggest therapeutic potential of TXL in psoriasis.

In order to further reveal the CD4 + T helper cell modulatory mechanisms of TXL, we determined the specific transcript factors for Th cells. The higher expressions of Th1 specific transcript factor T-bet, Th2 specific transcript factor GATA3, and Th17 specific transcript factor RORγ**t**, were found in the psoriasis patients and mouse model [38,39]. Our data show that TXL decreases the ratios of T-bet and RORγt in DLN, and also the relative expression of mRNA of them in skin lesions. However there are more complex in the expression of GATA3. The GATA3 serves as a key switch in both epidermal and T helper cell differentiation, and significantly down regulates in lesional psoriatic skin, so it plays key role in the psoriatic inflammation, keratinocytes proliferation and skin barrier dysfunction [11,40]. Out data also show decreasing expression of GATA3 mRNA in IMQ induced mouse skin lesions, while TXL increases the expression of it. But we observed the higher expression of GATA3 in the CD4 + T cells in mouse DLN. That may due to the GATA3 especially lower expression in the regenerating epidermis [40], since the psoriatic lesions have drastically thickness of epidermis.

Notch signaling has been shown to play a key role in cell activities, such as proliferation, differentiation, and regulation of cellular functions [41–44], and specifically in the differentiation of CD4 + T cells [45]. Upregulated expression of Notch1 has been also demonstrated in psoriatic lesions, which can mediate the abnormal differentiation of epidermal keratinocytes and implies the possible role of Notch1 in the pathogenesis of psoriasis [46,47]. In this study, we find that the expression of Notch1 and its transcription factor RBPJK, notch ligands JAG1 are all increased in mouse skin lesions. Oral treatment of TXL inhibits the expressions of Notch1, RBP-Jk in skin lesions.

Notch can also mediate IFN-γ producing Th1 cell differentiation independently of RBP-Jk [45]. Inhibiting Notch signal prevents Th1 differentiation in vitro and in vivo, possibly by blocking T-bet expression [41]. In addition, T-bet is also inhibited in RBPJK defective cells. So TXL may inhibit Th1 cell response by downregualting Notch1 signaling. Furthermore, Notch acted in parallel with GATA-3 to synergistically activate IL-4 expression, and GATA-3 as a direct transcriptional Notch target that acts in concert with Notch signaling to generate optimal Th2 cell responses [48]. Previous experiments have shown that Notch1 may also directly regulates the development of Th17 subsets of cells by binding to RORγt and IL-17 promoters, at least in part by regulating these two promoters [49]. Therefore, TXL may inhibit psoriasis-like dermatitis by down regulating Notch1/ RORγt / IL-17a axis.

As a central regulator of the IL-23/Th17 axis, Overexpression of STAT3 results in greatly increased numbers of IL-17 producing cells and enhances the expression of RORγt [50]. Thus STAT3 plays a central role in the development and pathogenesis of psoriasis [51,52]. STAT3 is activated via phosphorylation on a conserved Tyrosine residue operated by receptor associated JAK kinase, such as mainly JAK1 and JAK2. Although JAK2/STAT3 doesn't act on Th17 cells directly, it blocks the upstream IL-23 to Biological decrease the Th17 cell differentiation and then the cytokines [51]. In this study, we found that the expression of p-stat3 and JAK2 protein in psoriatic lesions was higher than that in the normal group, and both were significantly reduced after TXL and CsA treatment, which was consistent with the changing trend of Th17 cells. Perhaps we can think that TXL improves psoriasis like dermatitis by inhibiting Th17 cells and relative pro-inflamatory cytokines via JAK2/stat3 signaling pathway.

We conclude that TXL can inhibit aberrant proliferation of keratinocytes in vitro and in vivo. It also effectively relieves the clinic symptoms of mouse psoriasis-like skin inflammation, possibly through the regulation of Notch1 and JAK2/stat3 signaling pathways on Th1, Th2, and Th17 cells and there transcript factors, Fig. 10. This may provide new strategy for the treatment of psoriasis in the future, as well as TXL can also be potential drug for the treatment of psoriasis.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

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