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ORIGINAL RESEARCH

Tanshinone I Ameliorates Psoriasis-Like Dermatitis by Suppressing Inflammation and Regulating Keratinocyte Differentiation

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Background: Psoriasis is an immune-related inflammatory systemic condition characterized by dysregulated keratinocyte proliferation and chronic inflammation. Tanshinone I (Tan-I) has recently been discovered to have immunomodulatory properties, but its role and mechanisms in treating psoriasis remain unclear.

Objective: To evaluate the efficacy of Tan-I in the treatment of psoriasis and to determine the mechanisms involved.

Methods: An imiquimod (IMQ)-induced psoriasis-like mouse model was treated topically with Tan-I (7.5 mg/kg/d) or a vehicle. Disease severity was evaluated using the Psoriasis Area and Severity Index (PASI), and histological changes were assessed via H&E staining and Ki67 immunofluorescence. TNF-α-stimulated HaCaT keratinocytes were used for in vitro analyses, including apoptosis, cell cycle progression, and inflammatory gene expression via RT-qPCR. RNA sequencing (RNA-seq) was performed to investigate Tan-I's mechanisms in vivo and in vitro, while keratin expression was analyzed by immunofluorescence and Western blot.

Results: Tan-I treatment significantly alleviated psoriasis-like lesions in the IMQ mouse model, improving skin pathology and reducing Ki67-positive cells. RNA-seq revealed that Tan-I modulated immune pathways, keratinocyte differentiation, and barrier function. In TNF-α-stimulated HaCaT cells, Tan-I induced G1-phase cell cycle arrest, reduced apoptosis, and suppressed inflammatory gene expression. RNA-seq further showed that Tan-I normalized cell cycle signaling and apoptosis pathways disrupted by TNF-α. Additionally, Tan-I restored keratin expression patterns, increasing K1 and decreasing K6 and K17 levels in both mouse skin and HaCaT cells.

Conclusion: Tan-I is a promising therapeutic candidate for psoriasis, effectively mitigating inflammation, normalizing keratinocyte differentiation, and inhibiting abnormal keratinocyte apoptosis.

Keywords: psoriasis, tanshinone I, immune modulation, keratinocyte, apoptosis

Introduction

Psoriasis is a systemic disease resulting from the interplay of genetic, environmental and immunologic factors, with incidence rates rising annually.[1] It is marked by recurring red, thick, scaly skin plaques, causing discomfort like itching and pain.[2] In addition, patients with psoriasis often suffer from comorbidities, such as psoriatic arthritis, cardiovascular disease, psychiatric disorders, and metabolic syndrome.[3] Therefore, effective treatments for psoriasis are needed to alleviate symptoms and reduce complications.

The pathogenesis of psoriasis is intricate, characterized by sustained inflammation and uncontrolled keratinocyte proliferation, with interaction involving genetic susceptibility and environmental factors.[4,5] Treatments for psoriasis includes topical medications, systemic medications, phototherapy, and biologics.[6] However, high recurrence rates persist, and these

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therapies are often constrained by adverse effects, treatment resistance, financial costs, and infection risk, resulting in ongoing management challenges for psoriasis.[7]

Tanshinone I (Tan-I) is a major component of the Chinese medicine Danshen.[8] Accumulating evidence demonstrates the diverse therapeutic properties of Tan-I across multiple organ systems, particularly in addressing disorders affecting blood vessels of the heart and brain, as well as various neoplastic conditions.[9,10] Recent research has increasingly focused on its anti-inflammatory and immunomodulatory effects. For example, Tan-I acts on multiple inflammation-related pathways, including NF-κB and JAK/STAT3 signaling.[11–13] The compound exerts immunomodulatory activity by targeting both macrophage and lymphocyte populations, resulting in attenuated release of multiple cytokines such as TNF-α, IL-1β, IL-12, and interferon-γ.[14,15] However, the precise influence of Tan-I on psoriasis, along with its mechanistic pathways, remains to be elucidated.

The study revealed that Tan-I treatment alleviated psoriasis-like inflammatory symptoms in both in vivo and in vitro models.These findings suggest that topical application of Tan-I may offer a therapeutic effect on psoriasis.

Materials and Methods

Targets Screening

The spatial structure data for Tan-I was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov). The targets of Tan-I were predicted on the SwissTarget website, guided by its structure (http://swisstargetprediction.ch/). Furthermore, psoriasis-associated genes were queried on the GeneCards database website (https://www.genecards.org/).

Bioinformatics and Functional Enrichment Analysis

Intersecting targets across multiple datasets were visualized using Venny 2.1.0. Functional annotation and pathway analysis were performed through the DAVID platform (https://david.ncifcrf.gov/). The enrichment analysis encompassed three GO domains (biological processes, cellular components, and molecular functions) and KEGG pathways, providing comprehensive insights into the biological roles and molecular mechanisms of the identified genes.

Animal Models of Psoriasis and Intervention

BALB/c female mice (aged 6–8 weeks) were maintained under specific pathogen-free conditions. The psoriasis-like mouse model was established using 5% IMQ cream (Sichuan Med-Shine Pharmaceutical Co., Ltd). The mice were randomly assigned to three groups, each comprising seven mice: untreated controls (Sham), IMQ with vehicle (Control), and IMQ with Tan-I treatment (Selleck, Shanghai; 7.5 mg/kg/d, dissolved in 100 μL of a 3:1 acetone to olive oil solution). Topical applications were administered daily for 6 days, with the experiment ending on day 7. All procedures were performed in compliance with guidelines established by the Animal Care and Use Committee of Central South University (Approval No.20230734).

Dermatological Severity Assessment in Psoriasis-Like Mouse Model

The intensity of dermatological manifestations in the psoriasis-like mouse model was assessed using a modified Psoriasis Area and Severity Index (PASI) scoring system,[16] assessing erythema, scaling, and thickness ranging from 0 (absent) to 4 (very pronounced).The sum of these scores, ranging from 0 to 12, constituted the final PASI score.

Cell Culture

HaCaT cells (Cell Resource Center, IBMS,CAMS/PUMC) were cultured in DMEM with 10% FBS at 37°C in a 5% CO_2 humidified incubator. When cell confluence exceeded 90%, they were passaged at a 1:3 ratio.

Cell Viability Assay

HaCaT cells at over 60% confluence in a 96-well plate were treated with varying concentrations of Tan-I. The wells were supplemented with CCK8 solution (Dojindo, Kumamoto, Japan) and kept in darkness at 37°C for a 40-minute period. Cell viability was determined through absorbance readings at 450 nm.

Cell Apoptosis Assay

HaCaT cells at over 60% confluence in 12-well plates were treated with Tan-I for 6–8 hours, followed by TNF-α (100 ng/mL, Sino Biological, Beijing, China) co-stimulation for 24 hours. Cells were collected and subjected to 0.25% EDTA-free trypsin, and stained with Annexin V-FITC/PI Apoptosis Detection Kit (Yeasen Biotechnology, Shanghai, China). After a 10–15 minute dark incubation, apoptosis was quantified using a BD Canto II flow cytometer, with data analyzed via FlowJo software version 10.

Cell Cycle Assay

To assess the cell cycle, we utilized the BD Cycletest™ Plus kit. HaCaT cells at over 60% confluence in 12-well plates were treated with Tan-I for 6–8 hours, followed by TNF-α (100 ng/mL, Sino Biological, Beijing, China) co-stimulation for 24 hours. Collected cells were fixed with ice-cold 70% ethanol at −20°C for 30 minutes, then centrifuged, stained with PI and RNase, and incubated in the dark for 30 minutes. Flow cytometry (BD Canto II) was used to analyze cell cycle distribution, with data processed using ModFit software version 3.1.

RT-qPCR

Total RNA was extracted using AG RNAex Pro RNA Reagent (Catalog AG21101). The RNA was then reverse transcribed to cDNA with AG Evo M-MLV Reagent Premix (Catalog AG11705). RT-qPCR was performed with RPLP0 as the internal control for normalization. Experimental data were analyzed using the 2^-ΔΔCt method to determine relative gene expression, normalizing differences between samples with the internal reference gene RPLP0. The primer sequences are listed in Supplemental Table 1.

Histological Analysis and Immunofluorescence

Skin lesions were fixed in 4% paraformaldehyde for 24 hours, dehydrated, paraffin-embedded, and cut into 4–5 μm sections. Sections were stained with hematoxylin and eosin (H&E). For immunofluorescence, antigen retrieval and blocking were performed, followed by incubation with rabbit anti-mouse Ki-67 (1:200, Cell Signaling, Catalog 12202), rabbit anti-Cytokeratin 1 (K1) polyclonal antibody (1:400, Proteintech, Catalog 16848-1-AP), rabbit anti-Cytokeratin 6A (K6) polyclonal antibody (1:400, Proteintech, Catalog 10590-1-AP), or rabbit anti-Cytokeratin 17 (K17) polyclonal antibody (1:400, Proteintech, Catalog 17516-1-AP) overnight at 4°C. Afterwards, a goat anti-rabbit secondary antibody (1:2000, Abcam, Catalog 205722) was applied for 1 hour at room temperature. Nuclear staining was completed with DAPI, and images were captured using a PerkinElmer Vectra imaging system, then analyzed with inForm 2.3.1 software.

Western Blot

HaCaT cells were lysed using RIPA lysis buffer supplemented with protease/phosphatase inhibitor cocktail to extract total protein. The lysate was then mixed with loading buffer and denatured at 100°C for 10 minutes. The samples were separated using a 10% SDS-PAGE gel and subsequently transferred to a PVDF membrane. After blocking, the membranes were incubated overnight at 4°C with primary antibodies against K1, K6, K17, or GAPDH, followed by the corresponding secondary antibodies. The protein bands were visualized using an enhanced chemiluminescence detection system and quantified by Image J software (NIH, Bethesda, MD, USA).

RNA-Sequencing (RNA-Seq)

Total RNA was extracted from mouse skin samples (vehicle-treated and Tan-I-treated groups) and HaCaT cells (treated with or without Tan-I, followed by TNF-α stimulation) using AG RNAex Pro RNA reagent. RNA sequencing was performed by Beijing Novigen Technology. After cDNA synthesis and library construction, sequencing was conducted on the Illumina HiSeq X Ten platform. Raw data were quality-filtered to obtain clean reads, which were aligned to the reference genome with HISAT2 and quantified using Cufflinks to produce FPKM values. Differential expression was defined by q-values <0.05 with fold changes >2 or <0.5, and DEG enrichment analysis was conducted via hypergeometric distribution testing.

Statistical Analysis

The statistical examination of experimental data was accomplished through GraphPad Prism v9.0 (GraphPad Inc., CA, USA). Results are manifested as mean values accompanied by their standard deviations (±SD). To ensure reliability, each experiment was independently replicated at least three times. For binary group comparisons, we employed the two-tailed unpaired Student's *t*-test when data met parametric assumptions. In cases where data exhibited non-normal distribution or heterogeneous variances, we opted for the two-tailed Mann–Whitney *U*-test. For multi-group statistical evaluation, we employed one-way ANOVA coupled with post hoc testing. Results achieving $P < 0.05$ were considered statistically meaningful, using asterisk notation (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$).

Results

Topical Application of Tan-I Alleviates IMQ-Induced Psoriasis

Tan-I is a lipophilic diterpene quinone that represents one of the major bioactive constituents in Danshen (*Salvia miltiorrhiza*). The molecular structure of Tan-I is illustrated in Figure 1A. We performed in vivo experiments on IMQ-treated mice, applying Tan-I or a vehicle topically once daily for six days to examine the effects of Tan-I (Figure 1B). Tan-I ameliorated the psoriasis-like lesions, as evidenced by reduced superficial redness, scaling, and crust formation, compared to the vehicle group (Figure 1C). The PASI scores, which assess erythema, scaling, and dorsal skin thickness, were decreased in Tan-I group compared to the vehicle group (Figure 1D–G). H&E staining of the skin lesions (Figure 1H) showed that Tan-I-treated mice displayed significantly reduced epidermal acanthosis (Figure 1I) and reduced dermal inflammatory infiltrates (Figure 1J) in histopathology, compared with vehicle-treated mice. Additionally, an increase in epidermal Ki67-positive cells was observed in IMQ-treated mice compared to the sham group, but this proliferation was inhibited by Tan-I treatment (Figure 1K and L). These results indicate that Tan-I relieved skin inflammation and mitigated keratinocyte proliferation in IMQ-induced psoriasis-like dermatitis.

Tan-I Modulates Inflammatory Pathways and Keratinocyte Function in Psoriasis

A network pharmacology approach was employed to investigate the possible mechanism of Tan-I in treating psoriasis. Initially, we screened 100 potential targets associated with the chemical structure of Tan-I utilizing the SwissTarget platform. Concurrently, we extracted 5428 unique psoriasis-related targets from the GeneCards database. Venn diagram analysis revealed 44 overlapping genes between the targets of Tan-I and those implicated in psoriasis (Supplemental Figure 1A), which represents a significant finding of this study. Subsequently, we performed enrichment analysis on these 44 overlapping genes, elucidating the mechanism of action of Tan-I within the context of network pharmacology. To further explore the roles of these key targets, we conducted GO enrichment analysis using the DAVID database. The results demonstrated that these targets were primarily involved in biological processes such as phosphorylation, protein kinase B signaling, and MAP kinase activity (Supplemental Figures 1B-D).

To further elucidate the mechanism of Tan-I in treating psoriasis, we performed RNA-seq on lesional skin. There were 91 significantly up-regulated genes and 107 significantly down-regulated genes in the Tan-I group relative to those in vehicle-treated mice (Figure 2A, P-value ≤ 0.05 and fold-change ≥ 2). Heat map analysis revealed that the representative differentially expressed genes (DEGs) involved inflammatory mediators related to psoriasis, keratinocyte proliferation, and skin barrier function (Figure 2B). GO analysis indicated that these downregulated genes were primarily associated with inflammatory and chemokine processes, including chemokine production, receptor-ligand, and receptor regulator activities, cytokine activity, as well as the stabilization of keratinocytes, including keratinocyte differentiation and the cornified envelope (Figure 2C). KEGG analysis suggested downregulated genes involved in cytokine-cytokine receptor interaction, JAK-STAT, IL-17A, and TNF signaling pathways (Figure 2D). GSEA further indicated the inhibition of cytokine-cytokine receptor interaction, IL-17A, and TNF signaling pathways (Figure 2E–G). These findings suggest that Tan-I exerts its therapeutic effects on psoriasis by modulating inflammatory responses, regulating keratinocyte proliferation and differentiation, and enhancing skin barrier function.

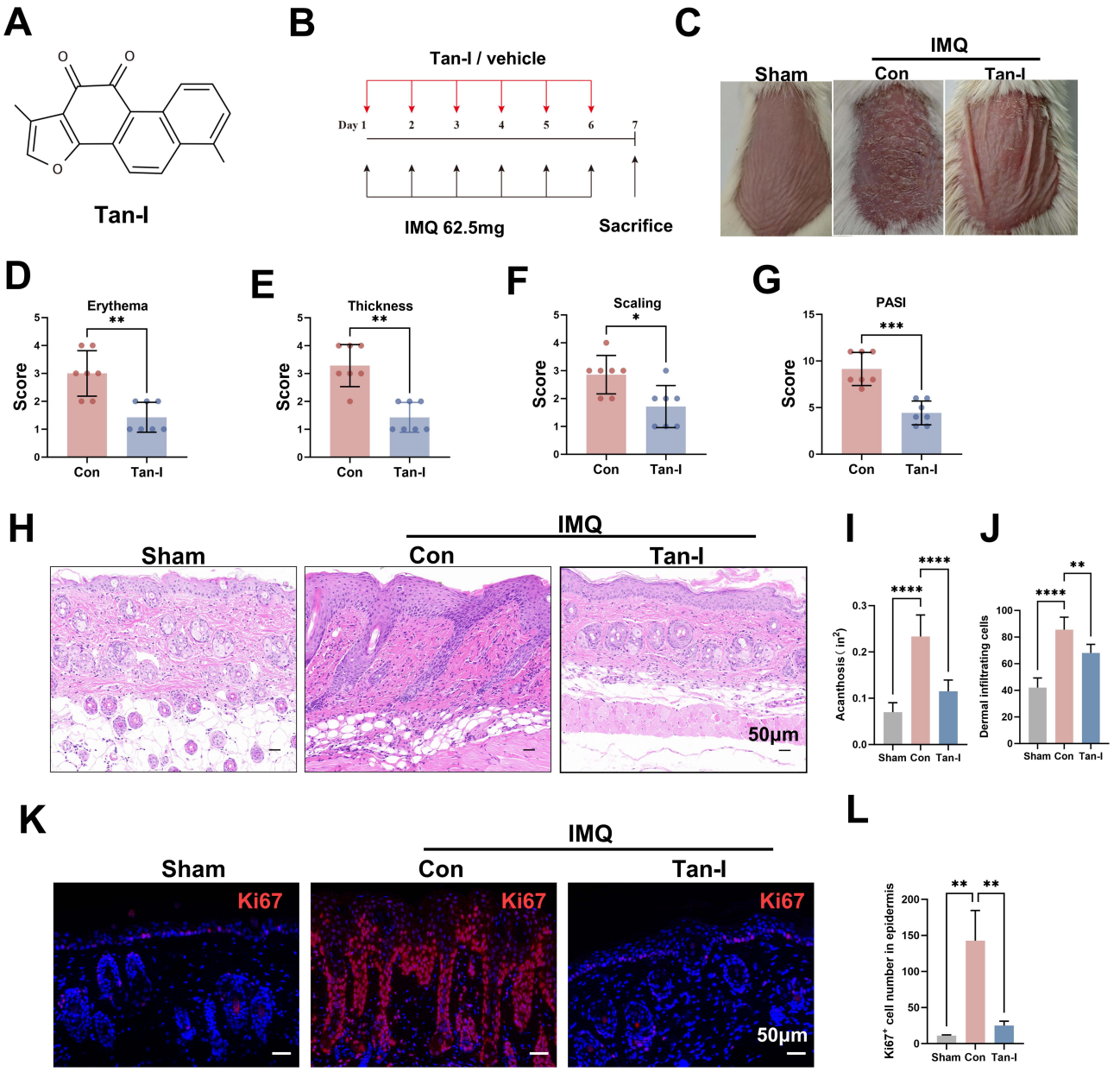

Figure 1 Tan-I alleviates psoriasis-like dermatitis. (**A**) Molecular structure of Tan-I. (**B**) Scheme of the in vivo experiments on IMQ-induced mice model and animal treatments. (**C**) Appearance of skin lesions in IMQ-induced mice after 6 days treatment (one representative mouse shown). (**D-G**) Statistical charts of psoriasis-like dermatitis severity (PASI) scores of sham group, control group and Tan-I-treated group, including erythema, scales, skin thickness and total PASI score. Red dots indicate individual records in the control group and blue dots indicate individual records in the Tan-I treated group. (**H**) H&E-stained presentation of murine back skin. (**I, J**) Statistical charts of acanthosis (**I**) and dermal infiltrating cells in (**H**). (**K**) Image of the Immunofluorescence analysis of epidermal Ki-67 positive cells in sham group, control group, and Tan-I treated group. (**L**) Statistical charts of Ki67-positive cells. Data are presented as mean ± SD. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

Tan-I Modulates Abnormal Apoptosis, Cell Cycle and Inflammatory Mediators in TNF-α-Stimulated HaCaT Cells

Using the HaCaT cell model in vitro, the study explored the direct effects of Tan-I on keratinocytes. Tan-I exhibited no cytotoxicity to HaCaT cells at concentrations ranging from 2.5 to 10 μM, while inhibitory or cytotoxic effects were observed at 20 μM (Supplemental Figure 2A–C). Based on these findings, 2.5 and 5 μM were selected as the optimal concentrations for further experiments. To elucidate the impact of Tan-I, we employed an in vitro psoriasis mode using TNF-α (100 ng/mL) for 24 hours. Consistent with previous reports, following stimulation of TNF-α, HaCaT keratinocytes exhibited increased apoptosis, replicating the abnormal cellular behavior observed in psoriasis (Figure 3A). Tan-I demonstrated inhibitory effects

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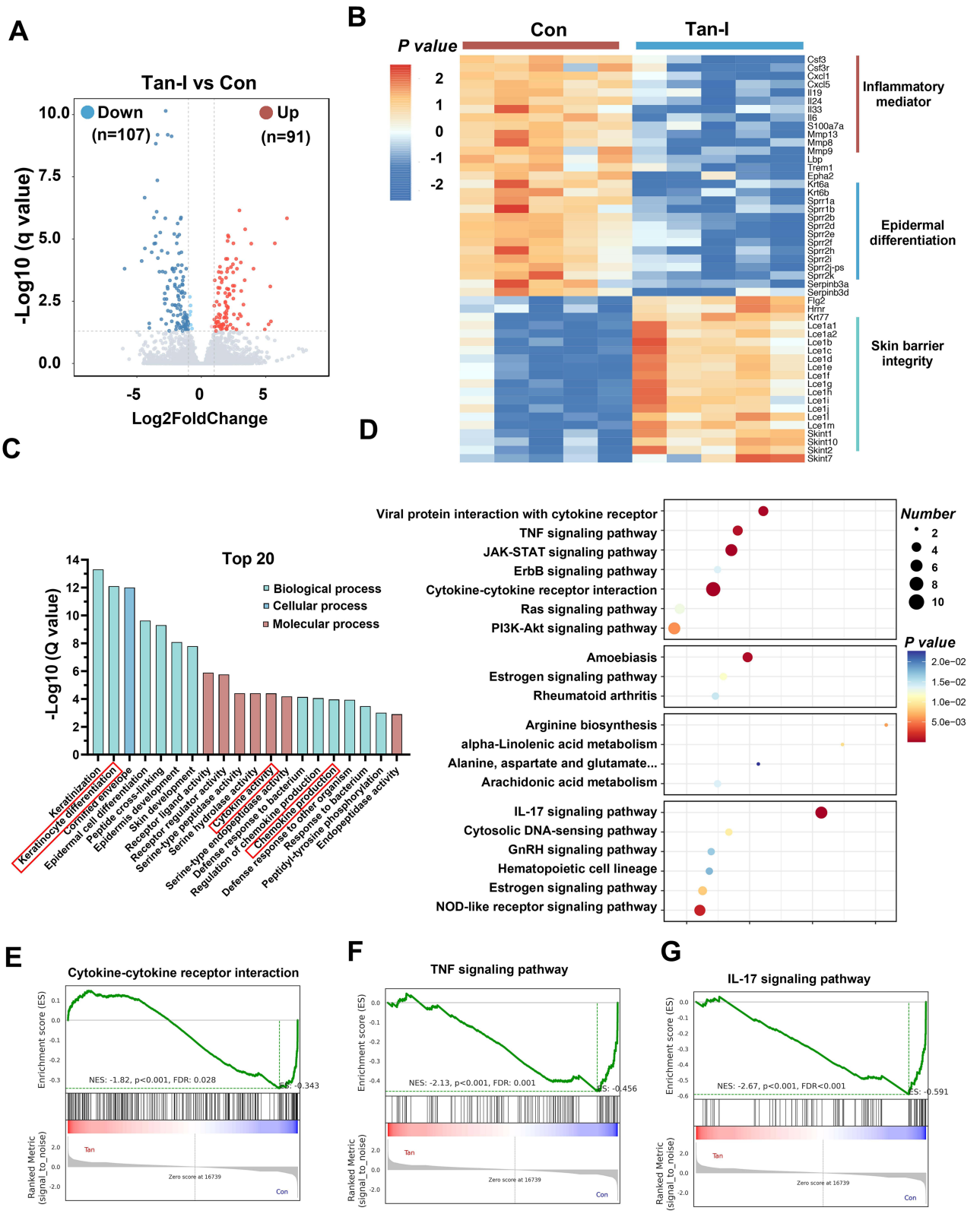

Figure 2 RNA-Seq analysis elucidates the mechanism of Tan-I in treating psoriasis. (**A**) Volcano plot of down-regulated (blue) and up-regulated (red) differentially expressed genes (DEGs) between Tan-I treated and untreated IMQ-induced psoriatic lesions. (**B**) Representative DEGs revealed in heat map. (**C**) Gene Ontology (GO) analysis (The red box represents the signaling pathways related to psoriasis influenced by Tan-I). (**D**) Representative KEGG pathways enriched in downregulated genes in mouse skin. (**E-G**) Downregulated pathways detected by Gene Set Enrichment Analysis (GSEA).

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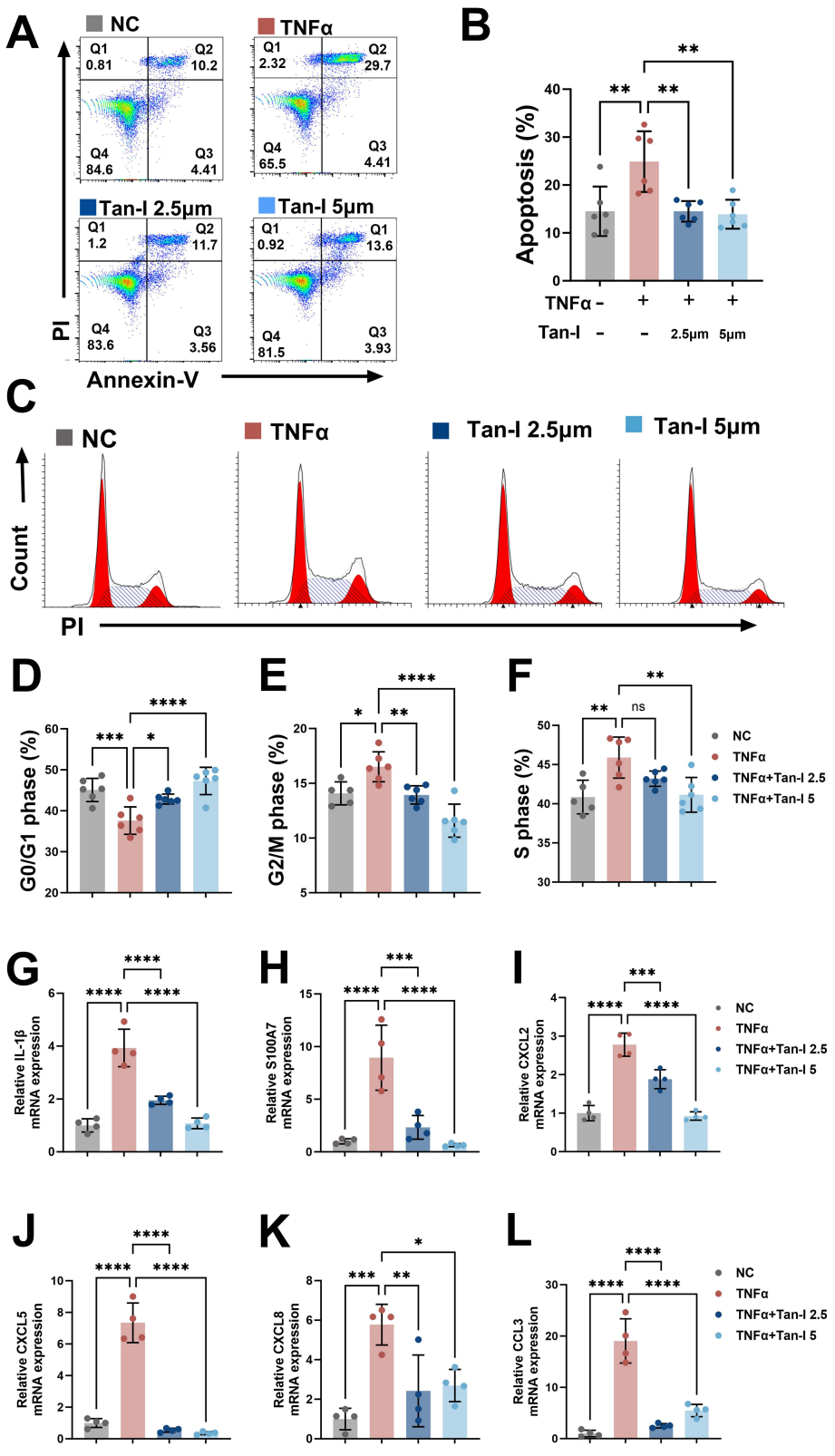

Figure 3 Effects of Tan-I on TNF-α-induced apoptosis, cell cycle distribution, and cytokine expression in HaCaT cells. (**A**) Flow cytometry analysis of apoptosis using Annexin-V/PI staining. Cells were treated with TNF-α alone or in combination with Tan-I at 2.5 μM and 5 μM. NC represents non-treated control. (**B**) Quantification of apoptotic cells (%) under different treatment conditions. (**C**) Representative histograms of PI staining for cell cycle analysis. (**D-F**) Quantification of cell populations in G0/G1 (**D**), G2/M (**E**), and S (**F**) phases of the cell cycle. (**G-L**) The mRNA expression levels of *IL-1β, S100A7, CXCL2, CXCL5, CXCL8*, and *CCL3*. Data are presented as mean ± SD. Statistical significance is indicated as **P* <0.05, ***P* <0.01, ****P* <0.001, *****P* <0.0001, ns = not significant.

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on TNF-α-induced-apoptosis in HaCaT cells (Figure 3A–B). Additionally, Tan-I interfered with cell cycle advancement by promoting G1 phase accumulation while decreasing G2/M phase entry (Figure 3C–F). Further, Tan-I reduced the mRNA expression of several chemokines and antimicrobial peptides, such as *CXCL2, CXCL5, CXCL8, CCL3, S100A7*, and *IL-1β*, which is induced by TNF-α (Figure 3G–L). These findings confirm that Tan-I effectively disrupts abnormal apoptosis while modulating the inflammatory mediators implicated in the pathogenesis of psoriasis.

Tan-I Regulated Genes in TNF-α-Induced HaCaT Cell

To investigate the downstream target modulated by Tan-I in HaCaT cells, we examined the mRNA profiles of TNF-α-stimulated HaCaT cells treated with or without Tan-I (5 μM) in vitro for 24 hours. Principal Component Analysis (PCA) of RNA-Seq analysis revealed distinct mRNA profiles among the NC (non-treated), CON (TNF-α-treated), and Tan-I (TNF-α and Tan-I-treated) groups (Figure 4A). RNA-seq analysis revealed that TNF-α stimulation significantly altered 1834 genes in HaCaT cells compared to the NC group. Subsequent Tan-I intervention further identified 494 DEGs compared to the CON group. Among these, 181 common genes were found between the two datasets, mainly related to cell division, proliferation, and apoptosis (Figure 4B and C). RNA-Seq analysis identified 275 up-regulated and 237 down-regulated genes in Tan-I-treated cells compared with the CON group (q value<0.05 and fold-change≥2) (Figure 4D and E). These DEGs are profoundly related to cell cycle regulators, including CCNB and CDC, in TNF-α-stimulated HaCaT cells (Figure 4E). The DEGs were significantly enriched in GO terms related to cell division and microtubule dynamics (Figure 4F), while KEGG enrichment analysis revealed suppressed activity in cell cycle and p53 signaling pathways (Figure 4G).These findings indicate that Tan-I exerts its therapeutic effects in psoriasis by modulating cell cycle signals.

Tan-I Regulates Keratinocyte Differentiation

To investigate the potential of Tan-I in modulating keratinocyte differentiation, we first employed immunofluorescence to assess keratin expression in mouse skin lesions. Compared to the sham group, the epidermis of mice in the IMQ group exhibited decreased K1 expression, whereas the Tan-I group showed a notable increase in K1 levels (Figure 5A and B). Conversely, K6 and K17 expressions were elevated in the IMQ group but significantly reduced in the Tan-I group (Figure 5C–F). Subsequently, we utilized Western blot analysis to evaluate keratin expression in HaCaT cells. TNF-α-stimulated HaCaT cells led to a significant reduction in K1 expression and increased levels of K6 and K17 compared to the NC group. Notably, Tan-I treatment effectively reversed these TNF-α-induced keratin expression alterations (Figure 5G–J).

Discussion

As a widespread cutaneous disorder, psoriasis manifests as persistent inflammatory lesions characterized by abnormal keratinocyte proliferation and the infiltration of various immune cells.[4,17] Psoriasis treatment focuses on disease management, slowing progression, and reducing symptoms and recurrence.[6,18] Recent advances in biological agents and small-molecule targeted therapies have yielded encouraging clinical outcomes.[19] However, prolonged use can lead to unwanted effects, treatment resistance and high costs.[20]

Recently, natural plants and their active components have emerged as a new option for treating psoriasis. Diverse plant-based monomers have been proved to have the effect of anti-psoriasis.[21] For example, acetyl-11-keto-beta-boswellic acid (ABKA) therapeutically impacts psoriasis by suppressing Th17 cell differentiation.[22] The anti-psoriasis effects of sappanone A are attributed to its inhibition of the expression of MMP8 and activation of the IL-17 pathway.[23] Nonetheless, the majority of traditional Chinese medicine ingredients and compounds are limited by insufficient clinical validation and unclear molecular mechanisms, hindering their broader application in psoriasis therapy.

Tan-I is a natural bioactive compound derived from *Salvia miltiorrhiza* (Danshen) and multiple studies have shown that Tan I has a superior alleviating and inhibiting effect on inflammation events.[24] Evidence suggests that Tan-I mitigates non-alcoholic steatohepatitis progression in mice by disrupting NLRP3 inflammasome activation.[25] It also exhibits substantial Inflammation-reducing activity against bone loss due to estrogen deficiency by inhibiting MAPK and NF-κB signaling pathways.[26] Treatment with Tan-I resulted in significant downregulation of inflammatory cytokine signatures (TNF-α, IL-1β, IL-6) in LPS-challenged microglial cells.[27] Despite this, it remains unclear how it works in psoriasis.

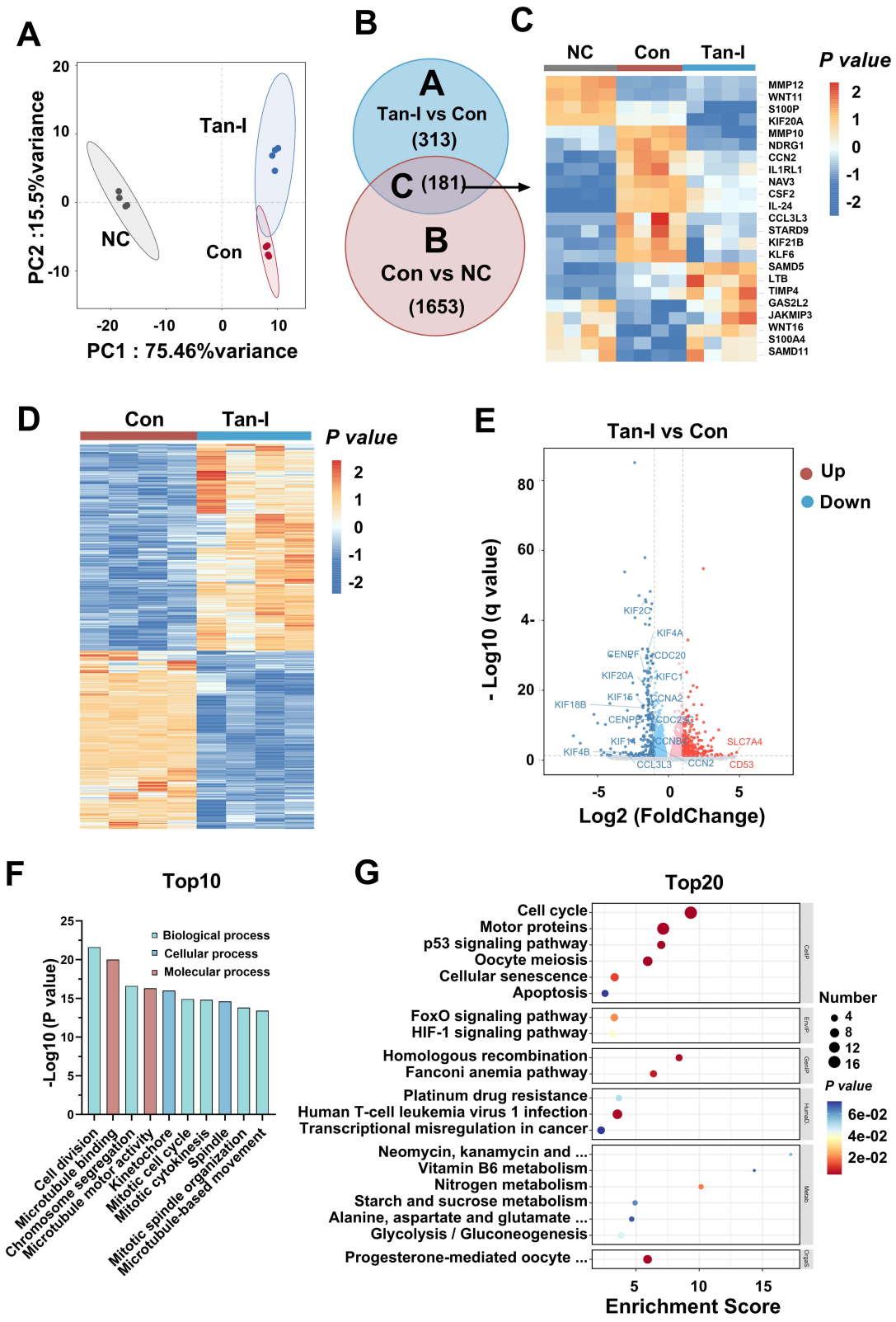

Figure 4 RNA-seq analysis of Tan-I effects on TNF-α-stimulated HaCaT cells. (**A**) Principal Component Analysis (PCA) plot showing the distribution of samples from NC (non-treated), CON (TNF-α-treated), and Tan-I (TNF-α and Tan-I-treated) groups. (**B**) Venn diagram illustrating the number of differentially expressed genes between Tan-I vs Con (313 genes) and Con vs NC (1653 genes), with 181 genes overlapping. (**C**) Heatmap of selected DEGs across NC, Con, and Tan-I groups. (**D**) Heatmap showing global gene expression changes between Con and Tan-I groups. (**E**) Volcano plot of down-regulated (blue) and up-regulated (red) DEGs in Tan-I vs Con. (**F**) Top10 enriched GO terms categorized into biological process, cellular component, and molecular function. (**F**) The DEGs show significant enrichment in GO terms related to cell division and microtubule dynamics. (**G**) KEGG pathway analysis reveals downregulation in pathways such as cell cycle and p53 signaling.

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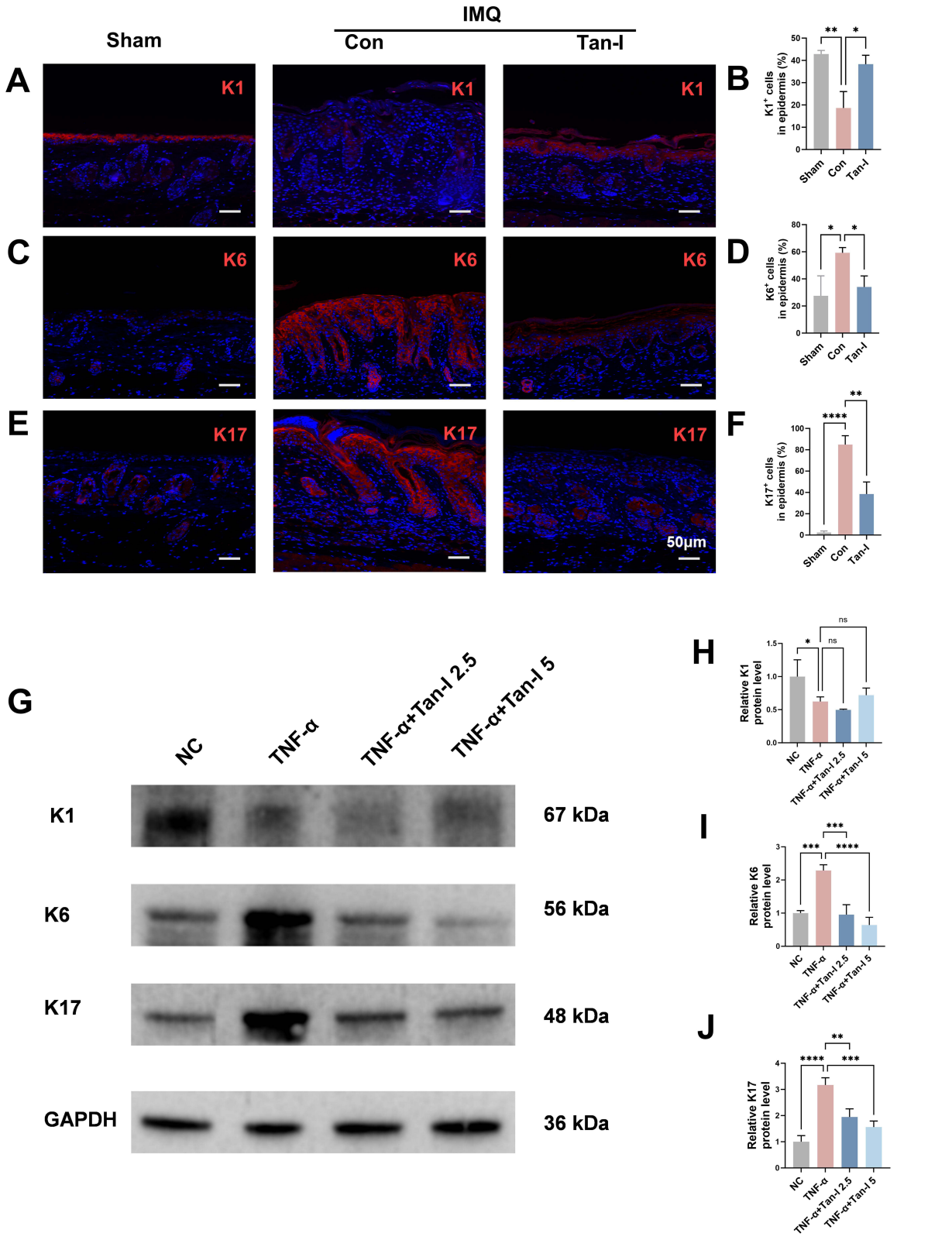

Figure 5 Effects of Tan I on keratinocyte differentiation. (**A-F**) Representative immunofluorescence staining of K1 (**A**), K6 (**C**), and K17 (**E**) from the skin lesions of mice in the sham group, control group, and Tan-I treated group and quantification of $K1^+$ (**B**), $K6^+$ (**D**) and $K17^+$ (**F**) cells percent in epidermis cells. (**G**) Immunoblotting of K1, K6 and K17 in HaCaT cells. Cells were treated with TNF-α alone or in combination with Tan-I at 2.5 μM and 5 μM. NC represents non-treated control. (**H-J**) Quantification of K1, K6, and K17 protein expression under different treatment conditions. Data are presented as mean ± SD. Statistical significance is indicated as **P* <0.05, ***P* <0.01, ****P* <0.001, *****P* <0.0001, ns = not significant.

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The present study represents the first attempt to systematically characterize the therapeutic potential and molecular mechanisms of Tan-I in psoriasis. Topical Tan-I alleviated key features of psoriatic symptoms in vivo, including epidermal hyperplasia and skin inflammation. Notably, Tan-I inhibits the expansion and cell-cycle progression of keratinocytes, which has not been reported before. These results support Tan-I's potential application in psoriatic skin inflammation and abnormal proliferation, suggesting that its topical application may be a simple and viable treatment option.

Tan-I, a diterpene quinone derived from the traditional Chinese medicinal herb Danshen, has demonstrated notable anti-inflammatory, anti-tumor, and broad-spectrum antibacterial properties.[24,28] In our study, Tan-I treatment led to substantial improvements in IMQ-treated mice, notably reducing inflammation. RNA seq revealed that Tan-I downregulated several immune and inflammation-related genes, including *TREM2, IL-17C, CXCL2, CXCL5, CXCL8, CCL3, S100A7*, and *IL-1β*, thereby further elucidating its potent anti-inflammatory effects. Recent investigations have revealed that Tan-I inhibits LPS-induced PGE2 formation in RAW macrophages and suppresses TNF-α-induced adhesion molecules.[14] In this study, we similarly observed that Tan-I significantly inhibited the TNF-α signaling pathway, a key driver in psoriasis. Moreover, Tan-I also decreased the activity of the IL-17 signaling pathway, which is another important pathway in psoriasis pathogenesis. Abnormal expression of inflammatory factors and the activation of IL-17 pathways markedly influenced disease progression. The major sources of IL-17 in psoriatic patients are T_H17 cells, γδ T cells and ILC3 (group 3 innate lymphoid cells) in lesional skin.[29,30] IL-17 interacts with IL17RA in keratinocytes, stimulating the production of inflammatory chemokines, cytokines, and antimicrobial peptides, including *CXCL2, CXCL5, CXCL8*, and *IL-1β*.[31] Local inflammation increases as a result of these pro-inflammatory mediators.[32] In our study, RNA-seq analysis confirmed that Tan-I exhibits anti-psoriasis effects by modulating IL-17 and various inflammatory pathways, including cytokine-cytokine receptor interaction, JAK-STAT, and TNF signaling, highlighting its therapeutic potential for psoriasis.

The anti-psoriatic mechanism of Tan-I involves the dysfunction of keratinocytes. Tan-I significantly downregulated several key genes associated with psoriasis, including inflammatory mediator such as *CXCL1, CXCL5*, and *CXCL8*, which are critical for maintaining inflammatory storm of the skin.[33] DEG enrichment analysis reveals that epithelial cells and the epidermal barrier play important roles in epithelial proliferation and differentiation.Notably, SPRR2B, KRT6 and S100A7, which are overexpressed in psoriatic lesions, are recognized as antigens by immune cells, particularly pattern recognition receptor-expressing antigen-presenting cells.[33–36] The activated immune cells then secrete pro-inflammatory cytokines, such as IL-17 and TNF-α, which in turn reinforce keratinocyte activation, creating a vicious cycle between immune cells, inflammatory cytokines, and keratinocytes.[37] The potential of Tan-I to disrupt this pathological feedback loop indicates its promise as a therapeutic agent in managing psoriasis.

Our in vitro studies using HaCaT keratinocytes have provided robust evidence for the keratinocyte-targeting properties of Tan-I. In previous studies, Tan-I inhibited the expression of cyclin A and cyclin B proteins, thereby retarding cell proliferation during S and G2/M phases.[38] In addition, research indicates that Tan-I causes G0/G1 phase arrest by decreasing cyclin D1 expression and increasing p21 expression.[39] Tan-I is also capable of causing apoptosis in HepG2 and Huh7 cells by inducing reactive oxygen species in the endoplasmic reticulum (ERS). In this study, Tan-I was shown to induce G1 phase cell cycle arrest and inhibit TNF-α-induced apoptosis in HaCaT cells. These are crucial mechanisms for mitigating aberrant keratinocyte proliferation in psoriasis. In addition, the expression of inflammation-related genes, including *IL-1β, S100A7, CXCL2, CXCL5, CXCL8*, and *CCL3*, was significantly upregulated in TNF-α-induced HaCaT cells. Tan-I treatment significantly downregulated mRNA levels of these proinflammatory genes.These results demonstrate that Tan-I significantly mitigates the inflammatory response in TNF-α-induced HaCaT cells. Furthermore, RNA-seq analysis revealed that Tan-I downregulated genes enriched in cell cycle and apoptosis signaling pathways, underscoring its potential in modulating keratinocyte function within a psoriatic context.[40,41]

Although the simplified in vitro environment led to minimal alterations in TNF or IL-17 signaling, Tan-I significantly modulated the expression of inflammatory genes, including IL-24 and CSF2, which may contribute to the overactivation of immune cells and keratinocytes.[37,42] This dual impact on both keratinocyte proliferation and inflammation underscores Tan-I's therapeutic potential in addressing the complex cellular mechanisms underlying psoriasis.

Building upon our previous findings, we further demonstrated the effects of Tan-I on modulating keratinocyte differentiation. Normal keratinocyte differentiation is crucial for maintaining skin barrier function, while abnormal proliferation and differentiation of keratinocytes are considered core pathological features of psoriasis. Our experimental results in the IMQ-

induced psoriasis mouse model showed that Tan-I treatment significantly increased K1 expression while reducing the levels of K6 and K17. Furthermore, the effects of Tan-I on TNF-α-stimulated HaCaT cells reinforce these findings. Under normal conditions, K1 is a marker of terminal keratinocyte differentiation,[43] indicating that keratinocytes are effectively completing their maturation process. In contrast, K6 and K17 are typically upregulated in psoriasis skin lesions,[35,44] signaling abnormal or immature keratinocyte states. These observations suggest that Tan-I may play a role in restoring skin barrier function by promoting normal keratinocyte differentiation. Specifically, Tan-I might enhance the terminal differentiation process of keratinocytes, thereby reducing inflammatory responses and keratinocyte hyperproliferation.

Despite the promising findings, several limitations of this study should be acknowledged. First, our research was primarily conducted using an IMQ-induced psoriasis-like mouse model and in vitro HaCaT cell experiments, which may not fully capture the complex immunological interactions in human psoriasis. The sample size in both animal and cell experiments was relatively small, which could potentially limit the generalizability of our results. Additionally, while we demonstrated Tan-I's potential therapeutic effects, further investigation is needed to elucidate its long-term efficacy, potential side effects, and precise molecular mechanisms in human psoriasis. The current study focused on a single concentration of Tan-I (7.5 mg/kg), and additional dose-response studies would be valuable to optimize therapeutic strategies. Moreover, our mechanistic insights were primarily derived from RNA sequencing and in vitro experiments, necessitating validation through more comprehensive immunological and clinical studies. Future research should include larger cohorts, extended treatment periods, and comparative studies with existing psoriasis treatments to fully establish Tan-I's therapeutic potential.

Conclusion

Tan-I presents a novel and effective strategy for managing psoriasis by concurrently targeting inflammatory processes and keratinocyte differentiation. With its broad spectrum of effects and natural origin, Tan-I represents a promising candidate for psoriatic therapies. Future studies are supposed to focus on elucidating the molecular interactions between Tan-I and its target pathways, particularly within the inflammation-related pathways, such as TNF and IL-17 signaling cascade, and evaluating its efficacy in clinical settings.

Ethics Statement

This study and included experimental procedures were approved by the Animal Care and Use Committee of Central South University (Approval No.20230734). All animal housing and experiments were performed following relevant named institutional and national guidelines and regulations.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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