Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Potential Targets and Mechanisms of Saikosaponin D in Psoriasis: A Bioinformatic and Experimental Study on Oxidative Stress

Junrui Ding[1], Aiping Sun[2], Hua Hu[1], Jialin Li[1], Shuao Lu[2], Junlan Song[2], Juao He[1], Xiangfeng Song[2], Shaoju Qian[2], Zhongwei Tian[1,3]

[1]Department of Dermatology, The First Affiliated Hospital of Xinxiang Medical University, Xinxiang, Henan, 453000, People's Republic of China; [2]School of Basic Medicine School Sciences, Xinxiang Medical University, Xinxiang, Henan, 453000, People's Republic of China; [3]General Practice School, Xinxiang Medical University, Xinxiang, Henan, 453000, People's Republic of China

Correspondence: Zhongwei Tian; Shaoju Qian, Email zhonwt@xxmu.edu.cn; 211032@xxmu.edu.cn

Objective: This study aims to explore the potential therapeutic effect of Saikosaponin D (SSD) on psoriasis and elucidate its underlying mechanisms, focusing on oxidative stress modulation and immune regulation.

Methods: Network pharmacology, machine learning (LASSO, SVM-RFE, Random Forest), and molecular dynamics identified Saikosaponin D's core targets (STAT3, CCNB1) in psoriasis. Differential gene analysis (GEO datasets GSE6710, GSE50790, GSE14905), WGCNA, and PPI networks screened Saikosaponin D-psoriasis-oxidative stress intersecting genes. In vivo validation employed an imiquimod-induced psoriasis mice model with Saikosaponin D (2 mg/kg/day). Histology, qPCR, Western blot, and immune infiltration (CIBERSORT) assessed SAIKOSAPONIN D's effects on inflammation, JAK2/STAT3 signaling, and oxidative stress markers (GPX4, SLC7A11).

Results: Saikosaponin D markedly alleviated psoriasis-like symptoms, diminishing epidermal thickness and keratinocyte proliferation, accompanied by reduced Ki67 expression. Bioinformatics investigation revealed 25 intersecting genes, with STAT3 and CCNB1 identified as principal targets. Molecular docking revealed that Saikosaponin D consistently binds to STAT3 and CCNB1, with binding energies of −8.3 and −9.0 kcal/mol, respectively. Saikosaponin D suppressed JAK2/STAT3 phosphorylation, leading to the downregulation of IL-1, IL-6, and TNF-α expression. Saikosaponin D enhanced GPX4 expression and reduced SLC7A11 levels, restoring oxidative equilibrium. Moreover, Saikosaponin D regulated immune infiltration by reducing M1 macrophages and augmenting Tregs, hence enhancing the psoriatic immune microenvironment.

Conclusion: Saikosaponin D suppresses psoriasis by dual-targeting STAT3/CCNB1, disrupting JAK2/STAT3 signaling and oxidative stress. This study provides new insights into the mechanism of Saikosaponin D in psoriasis, offering a promising multi-pathway therapeutic candidate.

Keywords: traditional Chinese medicine, Saikosaponin D, oxidative stress, psoriasis, network pharmacology, machine learning

Introduction

Psoriasis is a chronic inflammatory skin disease with significant genetic susceptibility. It affects approximately 2–3% of the global population, characterized mainly by skin inflammation such as erythema and scales. Current treatment regimens for psoriasis include topical medications, phototherapy, systemic therapy, and biological agents. However, traditional therapies are often accompanied by adverse reactions like skin irritation and hepatotoxicity or nephrotoxicity. By contrast, natural components of Chinese patent medicines have shown multiple advantages and potential in recent studies. Triterpenoids such as celastrol,[1] quinones like shikonin and thymoquinone,[2,3] flavonoids including baicalin and quercetin,[4,5] phenols such as curcumin and salidroside,[6,7] and other natural Chinese medicine components have been confirmed by numerous studies to exert significant antioxidant and anti-inflammatory effects through various mechanisms. They also offer advantages of high safety, low cost, and wide availability.

Received: 3 September 2025
Accepted: 13 November 2025
Published: 26 November 2025

Bupleurum (known as "Chai Hu" in Traditional Chinese Medicine) has a long history of medicinal use in Asia for its anti-inflammatory properties.[8,9] Its main bioactive compounds, saikosaponins, are known to modulate immune responses and reduce oxidative stress.

Its main bioactive compounds, triterpene saponins (saikosaponins), have been extensively studied for their roles in modulating immune responses and reducing oxidative stress.[10] Xiao-Chai-Hu Decoction(XCHD) has been shown to treat psoriasis by regulating keratinocyte differentiation and inhibiting inflammation.[11] Saikosaponin D (SDD), a representative active component of the Chinese herb *Bupleurum*, has demonstrated extensive biological activities in modern pharmacological research.[12] In the field of oncology, Saikosaponin D can reduce the expression of tumor target genes and enhance cell apoptosis by inhibiting nuclear factor κB (NF-κB) signaling pathway, STAT3 phosphorylation, and activating p38 pathway.[13] Furthermore, Saikosaponin D can block the activation of NF-κB signaling pathway, inhibit pro-inflammatory cytokines (TNF-α and IL-6) to exert anti-inflammatory effects,[14] and eliminate ROS and counteract MAPK-mediated oxidative damage, indicating a certain antioxidant capacity.[15] Recent studies have proven that SAIKOSAPONIN D also regulates immunity and exhibits significant anti-fibrotic functions.[16,17] Therefore, Saikosaponin D can effectively treat diseases such as cardiopulmonary injury, liver fibrosis, glomerulonephritis, diabetes, depression, and cancer.[18] However, although existing studies have confirmed that quercetin, baicalein, wogonin, and kaempferol in Xiaochaihu Decoction are the main active components for treating psoriasis,[11] the efficacy and molecular mechanism of Saikosaponin D's in psoriasis remain unelucidated.

Given Saikosaponin D's known potent anti-inflammatory and antioxidant activities, particularly its documented effects on pathways like NF-κB and STAT3, we hypothesized that Saikosaponin D may ameliorate psoriasis by directly targeting the interplay between oxidative stress and these key inflammatory signaling pathways.

Oxidative stress (OS) refers to an imbalance between intracellular reactive oxygen species (ROS) production and antioxidant defense systems. At the cellular level, oxidative stress may induce ferroptosis, and exacerbate tissue damage by activating inflammatory signaling pathways and disrupting cellular metabolic networks.[19] In the pathological process of psoriasis, elevated lipid peroxidation levels have been observed in keratinocytes of psoriatic patients.[20,21] Thus, whether Chinese medicines and their active components act on psoriasis through oxidative stress will be a topic worth exploring.

In the present study, we first established a mice model of psoriasis-like dermatitis, finding that Saikosaponin D could effectively inhibit the psoriasis-like dermatitis phenotype and the expression of inflammatory factors. Based on network pharmacology, machine learning, and molecular dynamics, we further explored potential targets and pathways of Saikosaponin D in treating psoriasis (Figure 1). This study not only helps reveal the possible molecular mechanism of Saikosaponin D in treating psoriasis but also provides new evidence and directions for precise treatment of psoriasis with Chinese medicine components and the development of innovative drugs.

Materials and Methods

Data Sources

Three psoriasis-related datasets, including GSE6710, GSE50790, and GSE14905, were downloaded from the Gene Expression Omnibus (GEO) database. These datasets contained gene expression data from psoriatic lesional tissues and normal control samples. Meanwhile, potential targets of Saikosaponin D (SSD) were retrieved from databases such as PHARMAPPER, HERB, CTD, GENE CARDS, and Swiss Target Prediction. The three datasets (GSE6710, GSE50790, GSE14905) were merged. To remove batch effects from different studies, the "ComBat" function from the "sva" R package was applied. Principal Component Analysis (PCA) was performed before and after correction to verify the removal of batch effects.

Differential Gene Analysis

Differential expression analysis of the downloaded psoriasis datasets was performed using the LIMMA (Linear Models for Microarray Data) package. Genes with significantly differential expression between psoriasis patients and normal controls were screened ($|logFC| > 1.5$, adjusted $p < 0.05$). Volcano plots and heatmaps were generated to visualize the distribution of these differential genes.

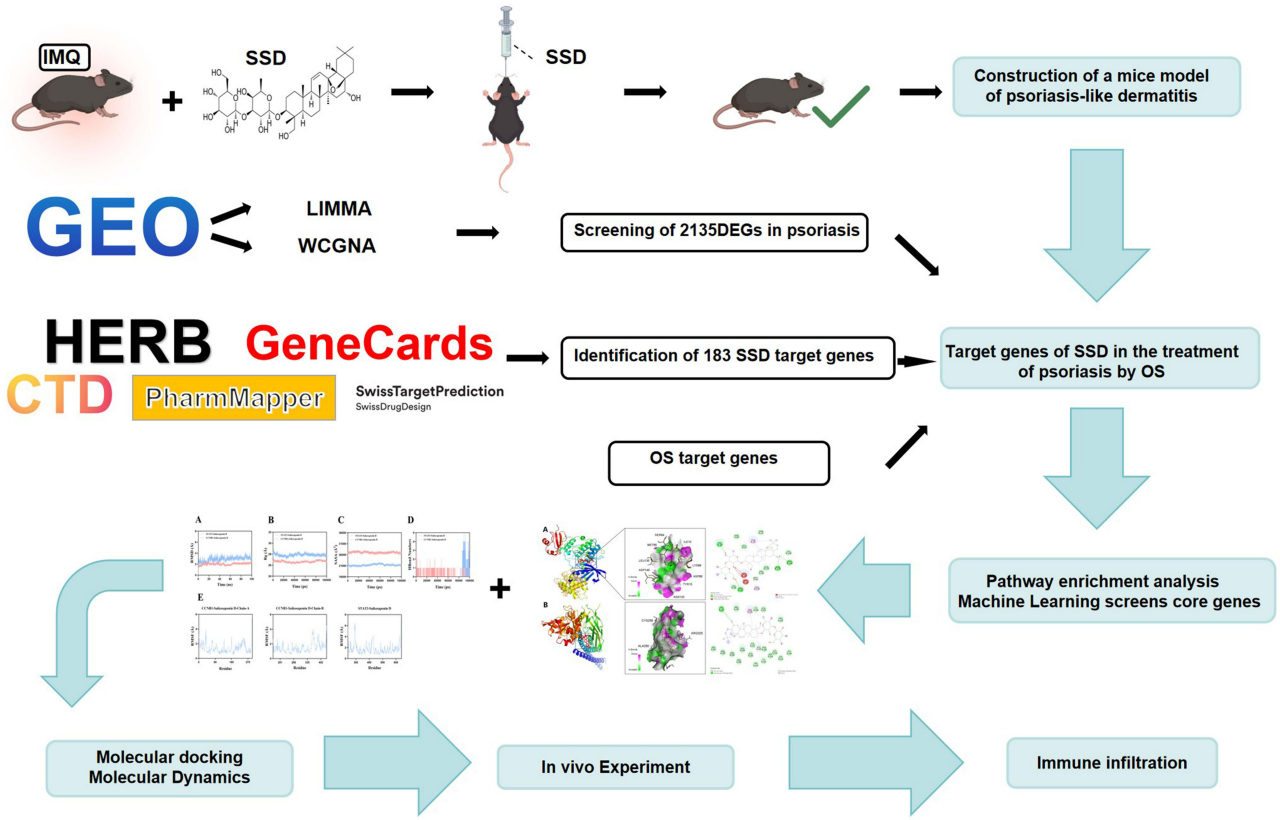

Figure 1 Study Flowchart.

Construction of Weighted Gene Co-Expression Network

Weighted Gene Co-Expression Network Analysis (WGCNA) was conducted to identify modules of highly correlated genes. This analysis summarized interconnections between modules and their associations with external sample traits, facilitating the identification of candidate biomarkers or therapeutic targets. A soft-thresholding power (β) of 6 was chosen to ensure a scale-free network topology ($R^2 > 0.85$). The adjacency matrix was converted into a topological overlap matrix (TOM), which measures gene network connectivity as the sum of adjacencies between a gene and all other genes in the network. Corresponding dissimilarity (1-TOM) was calculated. To cluster genes with similar expression profiles into modules, average linkage hierarchical clustering based on TOM similarity was applied. The minimum module size was set to 30. Notably, the grey module was classified as a gene set unassigned to any module.

Screening of Intersection Targets

Intersection analysis was performed among psoriasis differential genes, potential targets of Saikosaponin D, and Oxidative Stress-related target genes. This yielded potential common targets through which Saikosaponin D treats psoriasis via oxidative stress, laying a foundation for subsequent studies.

Construction and Analysis of Protein-Protein Interaction (PPI) Network

Intersection targets were imported into the STRING database (http://string-db.org). The PPI network was constructed and visualized using Cytoscape. Hub genes in the network were screened via the CytoHubba plugin based on the Degree algorithm. Additionally, a target gene network of Saikosaponin D -psoriasis-pathway was built.

https://doi.org/10.2147/JIR.S563272

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analyses

SANGERBOX was used to perform GO functional enrichment analysis on intersection targets, including Biological Process (BP), Cellular Component (CC), and Molecular Function (MF), as well as KEGG pathway enrichment analysis. These analyses explored potential biological processes, molecular functions, and signaling pathways involved in Saikosaponin D-mediated psoriasis treatment, with a significance threshold of $p < 0.05$.

Screening of Target Genes by Machine Learning

The combined and batch-corrected GEO dataset (containing 64 normal and 58 psoriatic samples) was randomly divided into a training set (70%) and a test set (30%). Three machine learning algorithms—LASSO regression, SVM-RFE, and **Random Forest**—were applied to the training set to screen for key target genes. 10-fold cross-validation was used during model training to optimize parameters and prevent overfitting. The diagnostic efficacy of the identified genes was then validated using the independent test set.

Establishment and Grouping of Animal Models

Saikosaponin D (SSD, purity 99.78%) was purchased from MedChemExpress (Cat No. HY-N0250). Female C57BL/6 mice (6–8 weeks old, 18–22 g body weight) were purchased from Henan Sibike Biotechnology Co., Ltd. (Henan, China). Animals were experimentally naïve and maintained under SPF conditions (22 ± 2 °C, 50–60% humidity, 12-h light/dark cycle, with ad libitum access to food and water). All mice underwent a 7-day acclimatization before experiments. The experimental unit was a single mouse.

Mice were randomly allocated into groups using a computer-generated random number list (block size = 6) by an investigator not involved in data collection. Cage position was rotated daily to minimize environmental bias.

A total of 18 mice were used (n=6 per group). Group 1 (WT): Only Vaseline (62.5 mg/day) was administered throughout the study as a baseline control. Group 2 (IMQ): Mice received topical application of 62.5 mg imiquimod (IMQ) on their shaved backs for 7 days to induce psoriasis-like skin lesions. Group 3 (Saikosaponin D): Mice were treated with IMQ daily and gavaged with Saikosaponin D (2.0 mg/kg/day) 30 minutes before IMQ application on days 2, 4, and 6. This intermittent dosing schedule (days 2, 4, 6) was chosen based on previous literature regarding the compound's stability and efficacy, to balance efficacy with minimizing stress from daily gavage.

The WT group served as the negative control for baseline phenotype, the IMQ group as the disease model control, and the Saikosaponin D group as the treatment group. On day 7, mice were sacrificed, and dorsal skin and ear tissues were collected for subsequent experiments.

Inclusion criteria were healthy female C57BL/6 mice (6–8 weeks, SPF). Exclusion criteria (predefined) were accidental injury, infection, or protocol deviations. No animals or data points were excluded during the study.

Blinding: Investigators performing PASI scoring, histological quantification, and molecular analyses (qPCR, WB) were blinded to group allocation until analysis was completed.

To evaluate the severity of skin inflammation, an objective scoring system based on the Psoriasis Area and Severity Index (PASI) was used. Erythema and scaling severity were scored as follows: 0, none; 1, mild; 2, moderate; 3, marked; 4, severe. The primary outcome was PASI score on day 7; secondary outcomes included epidermal thickness, Ki67 index, and expression of ferroptosis- and JAK-STAT-related proteins.

Ethics: All animals were kept in a pathogen-free environment and fed ad lib. The procedures for care and use of animals were approved by the Ethics Committee of the First Hospital of Xinxiang Medical University and all applicable institutional and governmental regulations concerning the ethical use of animals were followed. This study utilized public data published by others in the GEO database and no human experiments were conducted. The animal study was reviewed and approved by The First Hospital of XinXiang Medical University (EC-025-520). Euthanasia was performed by CO_2 inhalation followed by cervical dislocation. No analgesics were used to avoid confounding skin inflammation outcomes.

Histological and Immunohistochemical Staining

Mouse ear tissues were preserved in 4% paraformaldehyde. The tissues were then dehydrated using graded alcohols and fixed in paraffin blocks. Ultimately, 4-μm slices were subjected to staining with hematoxylin and eosin (H&E) to evaluate epidermal thickness and inflammation. Histopathological alterations in murine auricles were assessed using a light microscope.

H&E staining was conducted on murine skin samples to examine pathological morphological alterations. Ki67 immunohistochemistry staining was used to assess keratinocyte proliferation. The principal antibody used for immunohistochemistry was anti-Ki67 (1:400, GB111499-100, Cyagen, China). Specimens were then treated with a secondary antibody at 37°C for 20 minutes, washed with phosphate-buffered saline, and stained with DAB detection reagent (ZLI-9018, Zsbio, China) for 6 minutes. Samples were counterstained with hematoxylin, dried, and analyzed microscopically.

Detection of Gene Expression

Total RNA was extracted from skin tissues or cell samples using TRIzol reagent (G3013-100, Cwbio, China). cDNA was synthesized from 1 μg of total RNA using the Quantscript Reverse Transcription Kit (R333-01, BGI, China). Real-time PCR was performed on a 7500 Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) using SYBR Green PCR Master Mix (G3326-01, Cwbio, China). All values were normalized to GAPDH mRNA levels. Data were analyzed using the ΔΔCt method. Primer sequences used for RT-qPCR analysis are listed in Table 1. Each sample in this part of the experiment was biologically repeated three times.

Western Blotting (WB)

Protein samples were extracted from isolated mice tissues using RIPA lysis buffer (P0013, Beyotime Biotechnology). A total of 25 μg of protein was separated on 12.5% Yamei one-step gels and transferred to PVDF membranes (3010040001; Roche Applied Science, Mannheim, Germany) at 250 V for 60 minutes. Membranes were blocked with TBST containing 5% BSA at room temperature for 1 hour, then incubated overnight at 4°C with primary antibodies against GPX4 (ET1706-45, HUABIO, China; 1:10,000), SLC7A11 (HA601071, HUABIO, China; 1:5000), Phospho-STAT3 (60479-1-IG, Proteintech, China; 1:8000), STAT3 (10253-2-AP, Proteintech, China; 1:3000), JAK2 (R24775, ZENBIO, China; 1:2000), Phospho-JAK2 (R381556, ZENBIO, China; 1:2000), and GAPDH (Sanying, Wuhan; 1:3000). After incubation with secondary antibodies at room temperature for 1 hour, bands were visualized using ECL solution (Affinity, China) on a luminescent imaging workstation (Model 6600; Tanon, Shanghai, China). Additionally, ImageJ software (Version: 1.54 g, USA) was used to measure epidermal thickness and calculate the gray values of Western blot bands. Each sample in this part of the experiment was biologically repeated three times.

Immune Infiltration

The CIBERSORT algorithm was used to calculate 22 immune cell signatures, which were applied to evaluate immune infiltration patterns in psoriasis.

Molecular Docking and Molecular Dynamics

The 2D structure of the small molecule ligand was obtained from PubChem, converted into a 3D structure using Chem Office, and saved as a mol2 file; high-resolution protein crystal structures were selected from RCSB PDB, processed with PyMOL, and saved as PDB files. The protein and small molecule structures were processed via Autodock to determine

Table 1 Gene Primer

Genes	Forward	Revers
mTNF-α	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
mIL-1β	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
mIL-6	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG
mIL-10	TCAAGGCGCATGTGAACTCC	GATGTCAAACTCACTCATGGCT

https://doi.org/10.2147/JIR.S563272

the coordinates of the docking box, and molecular docking was performed using AutoDock Vina 1.1.2. The optimal conformation was identified based on the scoring results, and the interactions between the compound and key residues were visualized using PyMOL and Discovery Studio 2019. For molecular dynamics, force field parameters were obtained using Gromacs' pdb2gmx tool and the AutoFF webpage, with the CHARMM36 force field used for the receptor protein and the CGenff force field for the ligand. The system was constructed with the obtained force field parameters, subjected to treatments such as solvation and ion addition, and energy optimization. Simulations were conducted under specific conditions, during which relevant tools were used to calculate RMSD, RMSF, HBonds, Rg, and SASA.

Statistical Methods

Data distribution was first assessed for normality using the Shapiro–Wilk test. For normally distributed data comparing two groups, a two-tailed Student's *t*-test was used. For non-normally distributed data, the tests described below were applied. The Mann–Whitney *U*-test was used to compare the differential distribution of relevant variables between two subtypes or subgroups. Otherwise, the Kruskal–Wallis test was applied for analysis of variance. Spearman correlation analysis was adopted to verify the correlation between variables. The accuracy of the incidence model was determined by the Receiver Operating Characteristic (ROC) curve and the area under the curve (AUC). This study was performed using R version 4.2.2. The "pheatmap" package was used to draw heatmaps. The "ggplot2", "ggpubr", "ggExtra", "plyr" and "reshape2" packages were applicable for plotting multiple graphs, such as boxplots and scatter plots. Venn diagrams were developed using the "Venn" package. ROC curves were plotted by the "pROC" package. All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant. Where multiple comparisons were performed, p values were adjusted using the Benjamini–Hochberg method to control the false discovery rate.

Result

Saikosaponin D (SSD) Alleviates Skin Inflammation in Psoriasis-Like Mice

A model of psoriasis-like dermatitis was created with female C57BL/6 mice. The results indicated that prolonged administration of imiquimod cream led to significant erythema, infiltration, and scaling in the dorsal skin of mice in the psoriasis group, in contrast to the control group (Figure 2A). The erythema and scales infiltration (PASI score) of mice in the Saikosaponin D group were lower than those in the IMQ group (Figure 2B, $P \geq 0.05$). The psoriasis-like dermatitis symptoms were markedly reduced in the Saikosaponin D-treated group. The ear skin of mice underwent H&E staining. Marked epidermal thickening, acanthosis, downward extension of rete ridges, extensive inflammatory cell infiltration in the dermis, and a progressive notable rise in scratching frequency were seen. Moreover, the pathological alterations were diminished in the Saikosaponin D-treated group; epidermal thickness was reduced compared to the IMQ group (Figure 2C, $P<0.05$), and inflammatory cell infiltration was reduced. Concurrently, Ki67 immunohistochemistry staining was conducted to assess keratinocyte proliferation. The results indicated that the quantity of Ki67-positive cells in skin tissues was much greater in the model group compared to the normal control group. Furthermore, as comparison to the model group, the quantity of Ki67-positive cells in the Saikosaponin D -treated group was reduced compared to the model group (Figure 2D, $P \geq 0.05$).

Identification of Potential Target Genes and Pathway Enrichment Analysis for Saikosaponin D in Treating Psoriasis

After merging three psoriasis datasets and correcting for batch effects, bioinformatics analysis was performed to explore Saikosaponin D's target genes in psoriasis. Three psoriasis datasets (GSE6710, GSE50790, and GSE14905) were integrated, followed by LIMMA differential expression analysis (Supplementary File 1). This yielded 1314 upregulated genes and 1136 downregulated genes (Figure 3A). Furthermore, genes were divided into 15 modules via WGCNA analysis (Supplementary Files 2–14). Among these, the MEorange, MEgrey, and MEdarksteelblue modules showed significant correlation with the psoriatic phenotype (Figure 3B). By intersecting module genes with differentially expressed genes, 2135 psoriasis-related potential differential genes were obtained (Figure 3C and Supplementary File 15). Following the identification of psoriasis-related differential genes, 181 potential targets of Saikosaponin D were retrieved from databases

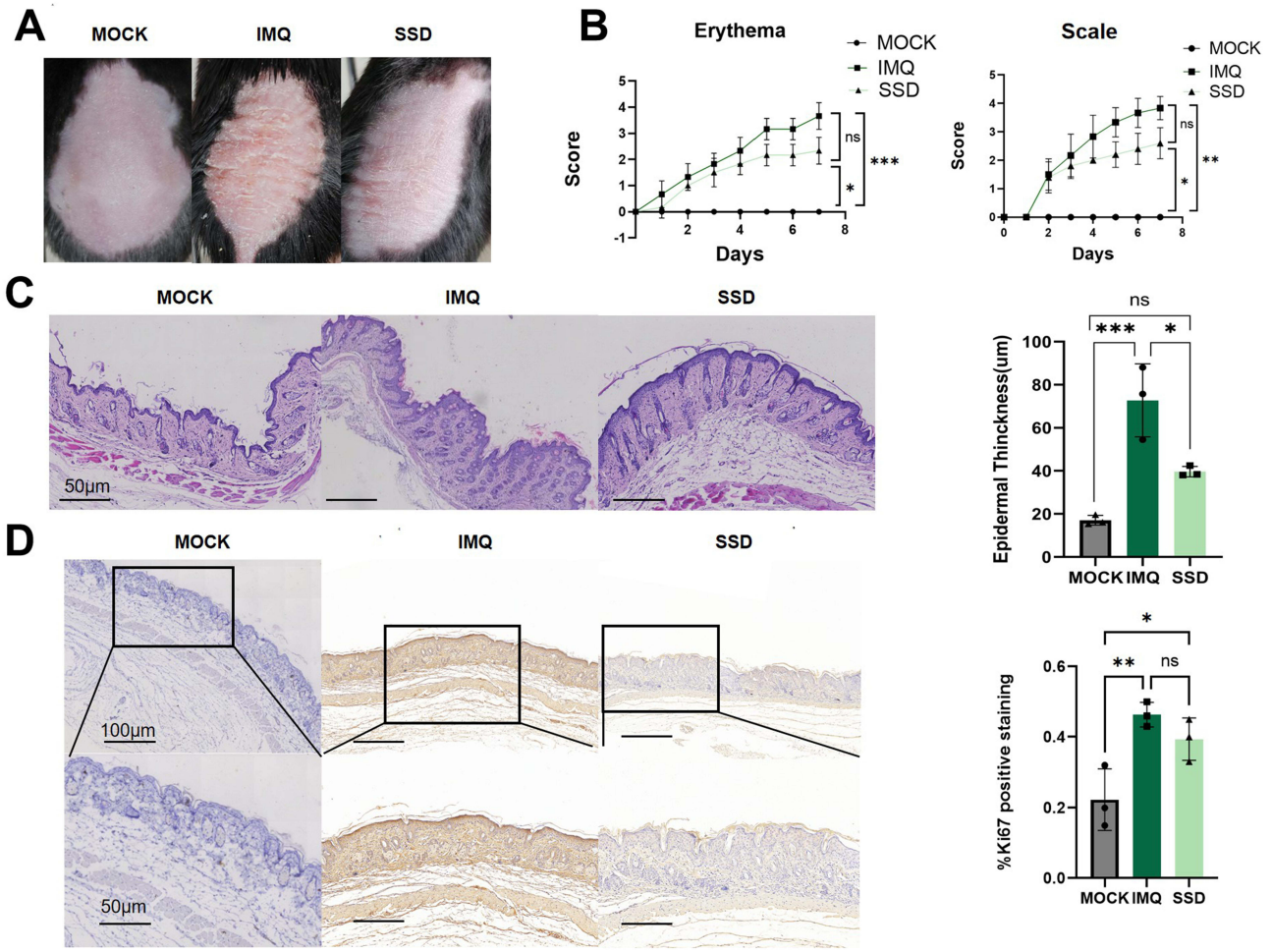

Figure 2 SAIKOSAPONIN D alleviates skin inflammation in psoriasis-like mice. (**A**) Differences in dorsal skin of mice in the MOCK group, IMQ group, and SAIKOSAPONIN D group on day 7 of the model. Differences in epidermal thickness based on H&E staining of mice auricle tissues. (**B**) Differences in scales and erythema of mice skin tissues from day 0 to day 7 of the model. (**C**) H&E staining images of mice auricle tissues on day 7 of the model and differences in epidermal thickness based on H&E staining of mice auricle tissues. (**D**) The Ki67 immunohistochemical staining image of the skin tissue of mice on the 7th day of the model and the difference of Ki67 expression in the skin tissue of mice based on immunohistochemical staining. P-value: *indicates $p < 0.05$, **indicates $p < 0.01$, and ***indicates $p < 0.001$ (n=6 per group).

including PHARMAPPER, HERB, CTD, GENE CARDS, and Swiss Target Prediction. To determine whether Saikosaponin D affects psoriasis pathogenesis through Oxidative Stress, 2912 Oxidative Stress-related genes were collected from various databases and literatures (Supplementary File 16). These were then subjected to intersection analysis with psoriasis differential genes and Saikosaponin D potential targets (Figure 3D), ultimately yielding 25 potential common targets. These 25 intersecting targets were imported into the STRING database to construct a Protein-Protein Interaction (PPI) network, which was visualized using Cytoscape software (Figure 3E and Supplementary File 17). These genes may play a core regulatory role in Saikosaponin D-mediated treatment of psoriasis. Additionally, verification of the expression differences of these 25 intersecting genes was performed (Figure 3F). Among them, genes such as JUN, FOS, and IL6 were significantly highly expressed in the normal group, whereas STAT3, CCNB1, and IL1B were overexpressed in the psoriasis group. Notably, STAT3 showed a marked high expression.

To further clarify the pathways potentially involved in these target genes, KEGG and GO pathway enrichment analyses were conducted on the 25 genes. In the KEGG analysis (Figure 4A), the Apoptosis and Peroxisome pathways exhibited the most significant enrichment. This suggests that the target genes may function by regulating programmed cell death and oxidative metabolism processes. Moreover, the JAK-STAT signaling pathway was significantly enriched, indicating that these genes are deeply involved in the inflammatory response regulatory network through the JAK-STAT3 pathway. The IL-17 signaling pathway and Th17 cell differentiation pathway showed the strongest enrichment signals, a finding highly consistent

https://doi.org/10.2147/JIR.S563272

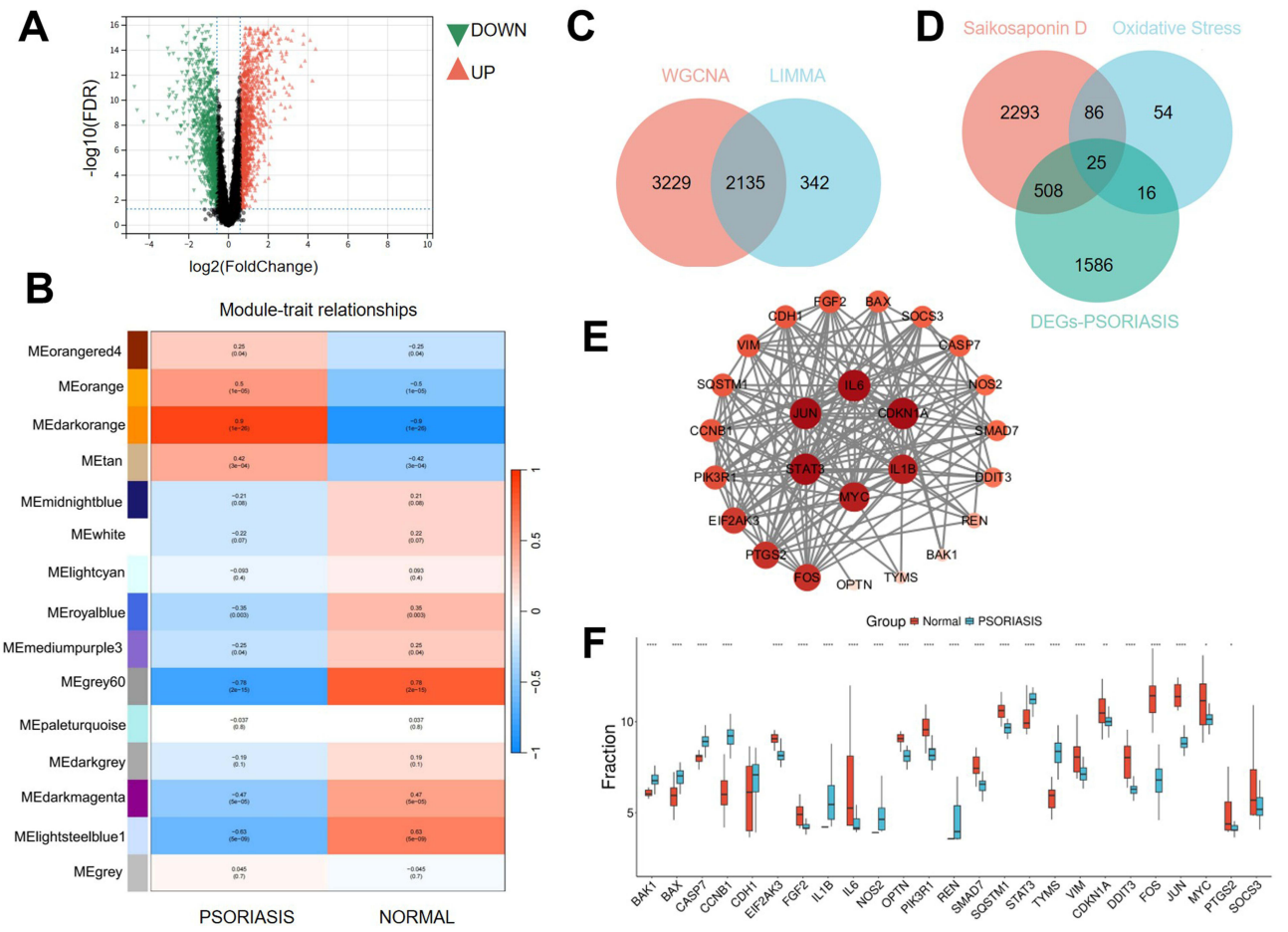

Figure 3 Identification of potential target genes analysis for saikosaponin D in treating psoriasis. (**A**) Volcano plot of differentially expressed genes (DEGs), with green dots representing downregulated DEGs and red dots representing upregulated DEGs. (**B**) Heatmap of module-trait relationships, where each row corresponds to a module (ME, module eigengene) and groups are divided into psoriasis and normal groups. (**C**) Venn diagram showing the overlap of DEGs identified by WGCNA and LIMMA methods. (**D**) Venn diagram displaying 25 core key target genes among DEGs, saikosaponin D targets, and oxidative stress (OS) target genes. (**E**) Key gene network. (**F**) Boxplots comparing gene expression differences between the normal group (blue) and psoriasis group (red).

with the typical Th17-type immunopathological characteristics of psoriasis. Besides, the co-enrichment of Toll-like receptor and C-type lectin receptor signaling pathways suggests that the target genes may participate in the cross-regulation of innate and adaptive immunity. GO analysis results revealed that "cytokine-mediated signaling pathway" and "immune system process" had the highest significance (Figure 4B). Meanwhile, terms such as "reactive oxygen species metabolic process" and "response to Oxidative Stress" were also significantly enriched. These genes are significantly involved in molecular functions such as "signal receptor binding" and "antioxidant activity". Collectively, the GO and KEGG analysis results indicate that these 25 core target genes are strongly associated with immune processes and Oxidative Stress. This suggests that Saikosaponin D may inhibit psoriasis through the JAK-STAT pathway, with Oxidative Stress potentially serving as a key pathway for Saikosaponin D's action in psoriasis.

Machine Learning Identifies Core Targets: CCNB1 and STAT3 as Key Targets of Saikosaponin D in Psoriasis Treatment

To validate the above hypothesis, gene sets (lassoGene, rfGenes, featureGenes) identified by three algorithms—LASSO, random forest, and SVM-RFE—were subjected to intersection analysis (Supplementary Files 18–20). This aimed to identify genes deemed important by all three methods. Initially, correlation analysis was used to explore preliminary associations between genes (Figure 5A).

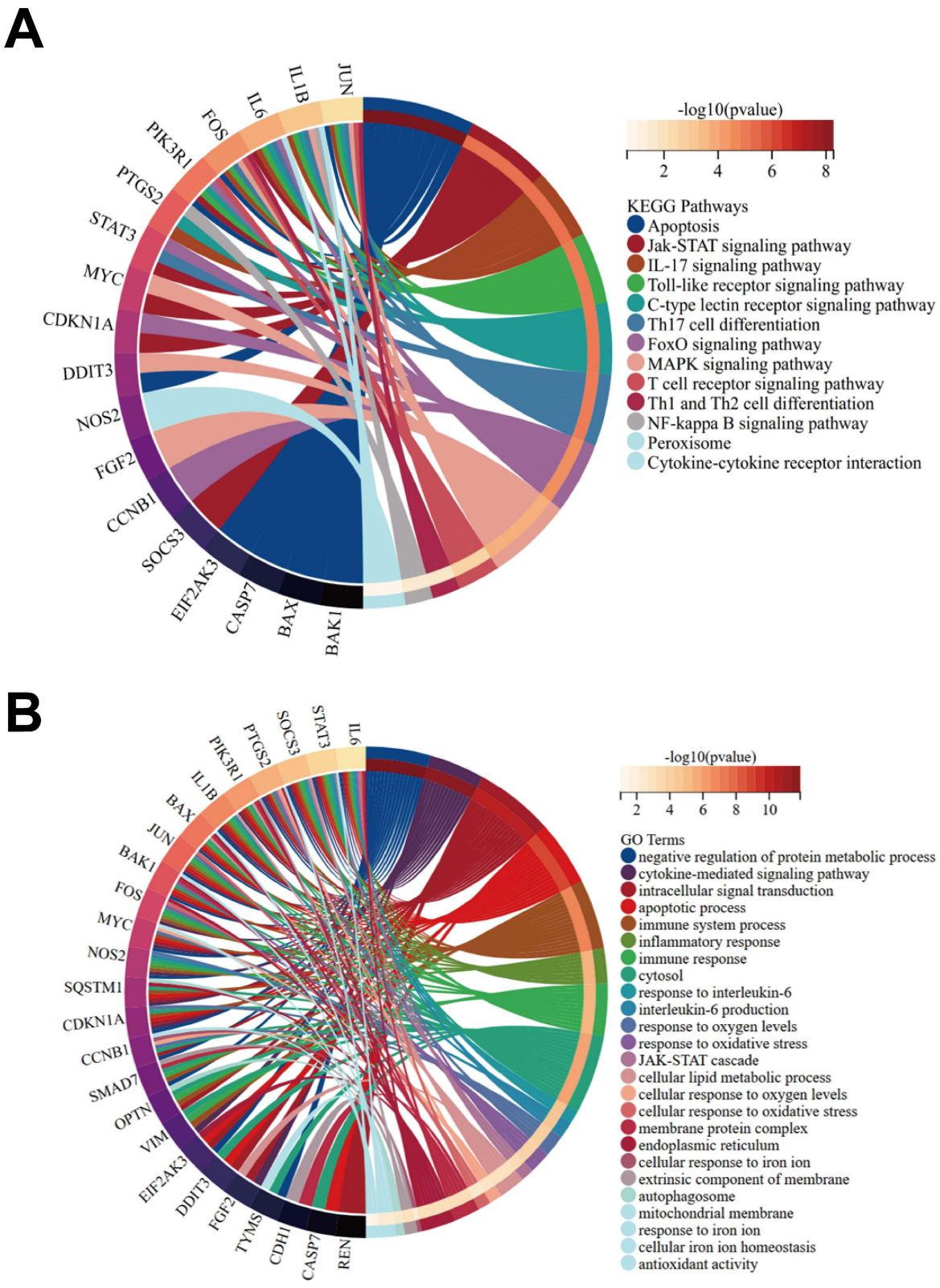

Figure 4 KEGG and GO pathway enrichment analysis for saikosaponin D in treating psoriasis. (**A**) Signal pathway enrichment analysis, showing pathways grouped by function with -log10 (*P*-value). (**B**) GO functional enrichment analysis, categorized into BP (Biological Process), CC (Cellular Component), and MF (Molecular Function), with results presented as -log10 (*P*-value).

https://doi.org/10.2147/JIR.S563272

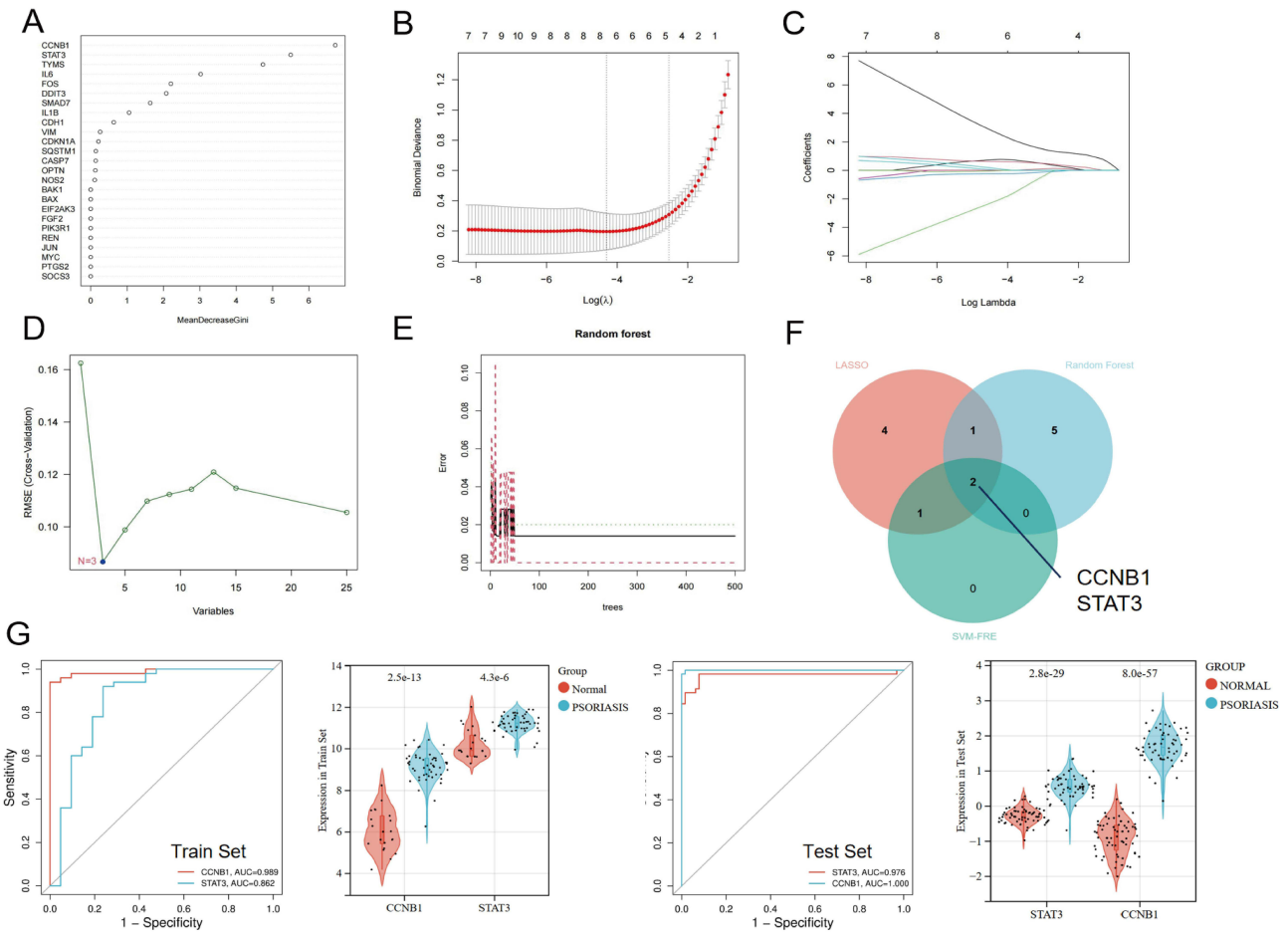

Figure 5 Screening of target genes by machine learning and verification of diagnostic efficacy using ROC. (**A**–**E**) Core genes screened by LASSO, SVM-RFE, and random forest. (**F**) Venn diagram of genes screened by LASSO, Random Forest, and SVM-RFE. (**G**) ROC curves of the training set and test set, and boxplots of gene expression differences between the normal group and psoriasis group, evaluated by the Mann–Whitney *U*-test (significance levels marked in the boxplots).

Subsequently, the LASSO regression algorithm was applied. Coefficient path plots (Figure 5B) and cross-validation graphs (Figure 5C) narrowed the range of key genes. This yielded CASP7, CCNB1, EIF2AK3, IL1B, STAT3, VIM, JUN, and MYC. Concurrently, the SVM-RFE algorithm identified three core genes: CCNB1, STAT3, and CASP7 (Figure 5D). The random forest model further pinpointed core genes including CCNB1, STAT3, TYMS, IL6, FOS, DDIT3, SMAD7, and IL1B (Figure 5E). Venn diagram integration (Figure 5F) of results from LASSO, random forest, and SVM-RFE confirmed CCNB1 and STAT3 as core candidate genes. Figure 4G displays ROC curves for the training and test sets, illustrating the diagnostic performance of CCNB1 and STAT3. For CCNB1, AUC values in the training and test sets were 0.989 and 1.000, respectively. For STAT3, the corresponding AUC values were 0.862 and 0.976. Furthermore, gene expression boxplots (Figure 5G) visually demonstrated that both CCNB1 and STAT3 were highly expressed in psoriatic lesion tissues with statistical significance. This consistency with earlier target identification analyses validated the reliability of the screening results. Taken together, through multi-step analysis and validation, key genes such as CCNB1 and STAT3 were identified. These findings provide directions for elucidating psoriasis pathogenesis and exploring diagnostic targets. Moreover, considering their enrichment in oxidative stress-related terms and the central role of the JAK-STAT pathway, STAT3 is hypothesized to act as a critical hub through which SAIKOSAPONIN D regulates oxidative stress and the JAK-STAT pathway.

Molecular Docking and Molecular Dynamics

To clarify the interaction mechanism between Saikosaponin D and CCNB1/STAT3, molecular docking and molecular dynamics simulations were performed to analyze their binding properties. Molecular docking results revealed specific

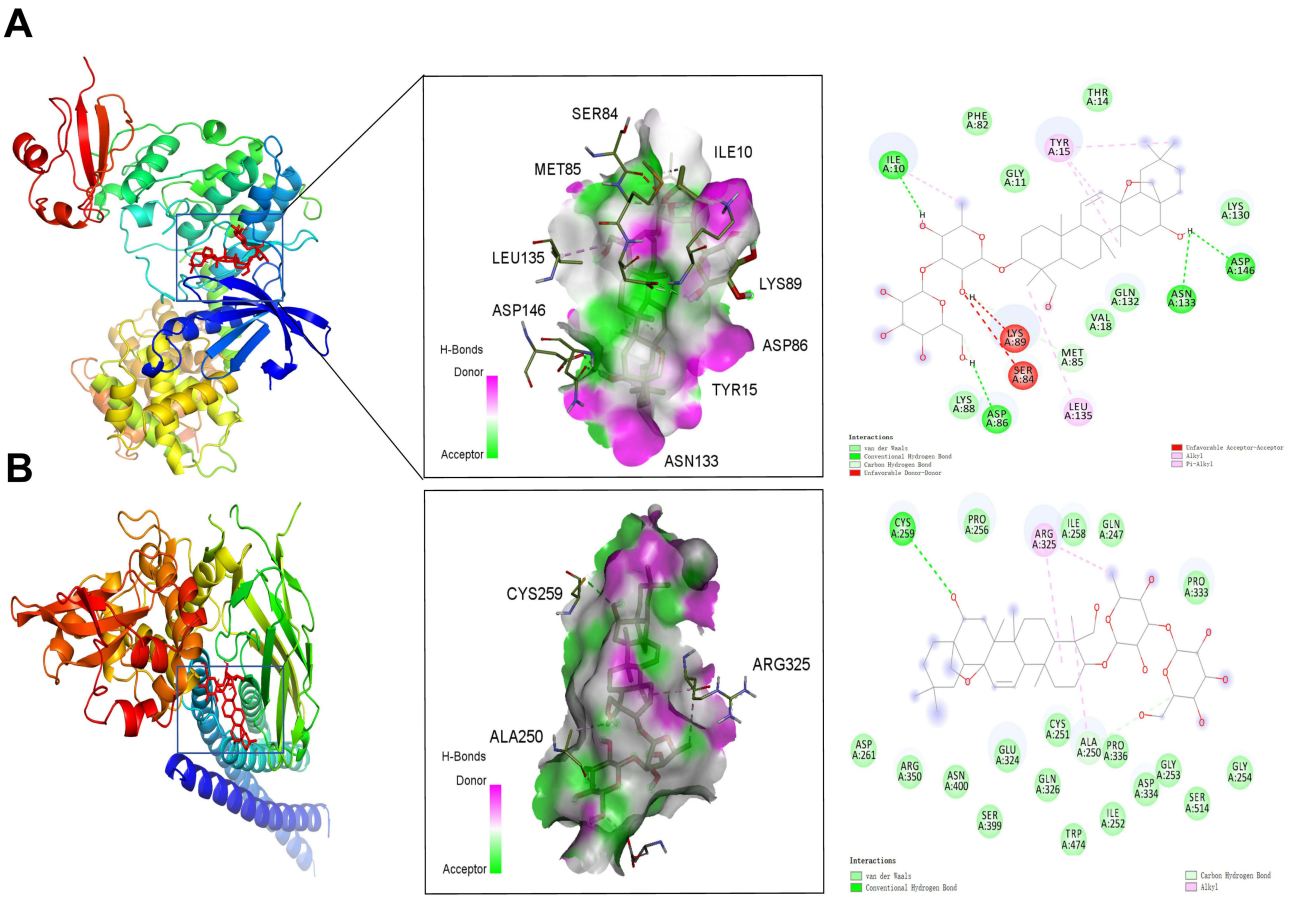

Figure 6 Molecular docking results. (**A**) Docking results of CCNB1 with SAIKOSAPONIN D. (**B**) Docking results of STAT3 with SAIKOSAPONIN D.

binding of Saikosaponin D to both STAT3 and CCNB1 (Figure 6). Notably, CCNB1 bound to Saikosaponin D via hydrogen bonds with ASN133 and ASP146 residues, hydrophobic interactions with LEU135 and TYR15 residues, and van der Waals forces across multiple residues (Figure 6A). Meanwhile, STAT3 interacted with Saikosaponin D through a hydrogen bond with CYS259, hydrophobic interaction with ARG325, and van der Waals forces involving multiple residues (Figure 6B). Their binding energies were −8.3 kcal/mol (STAT3- Saikosaponin D) and −9.0 kcal/mol (CCNB1-Saikosaponin D), respectively, indicating high binding stability and affinity.

Furthermore, molecular dynamics simulations further validated these findings (Figure 7 and Supplementary File 21). RMSD analysis showed that the STAT3-Saikosaponin D complex reached equilibrium after 20 ns (fluctuating around 3.1Å), while the CCNB1-Saikosaponin D complex stabilized after 10 ns (fluctuating around 2.3Å) (Figure 7A), demonstrating good conformational stability for both. In addition, slight fluctuations in the radius of gyration (Rg) and solvent-accessible surface area (SASA) (Figure 7B and C) suggested dynamic conformational adjustments but overall stability of the complexes. Hydrogen bond count analysis revealed sustained hydrogen bonding in both STAT3-Saikosaponin D (0–5 bonds) and CCNB1-Saikosaponin D (0–3 bonds) (Figure 7D). Most RMSF values were below 4Å (Figure 7E), indicating low flexibility and high stability of the complexes. Overall, Saikosaponin D forms stable bindings with STAT3 and CCNB1, providing a structural basis for its regulation of target protein functions.

In vivo Validation: Saikosaponin D Inhibits Inflammatory Factor Expression and JAK2/STAT3 Pathway Phosphorylation in IMQ-Induced Psoriasis-Like Mice Skin

In Figure 2, Saikosaponin D was shown to suppress the psoriasis-like dermatitis phenotype. In the present study, qPCR was performed to detect the mRNA expression levels of inflammatory factors IL1, IL6, IL10, and TNF-α in skin tissues.

https://doi.org/10.2147/JIR.S563272

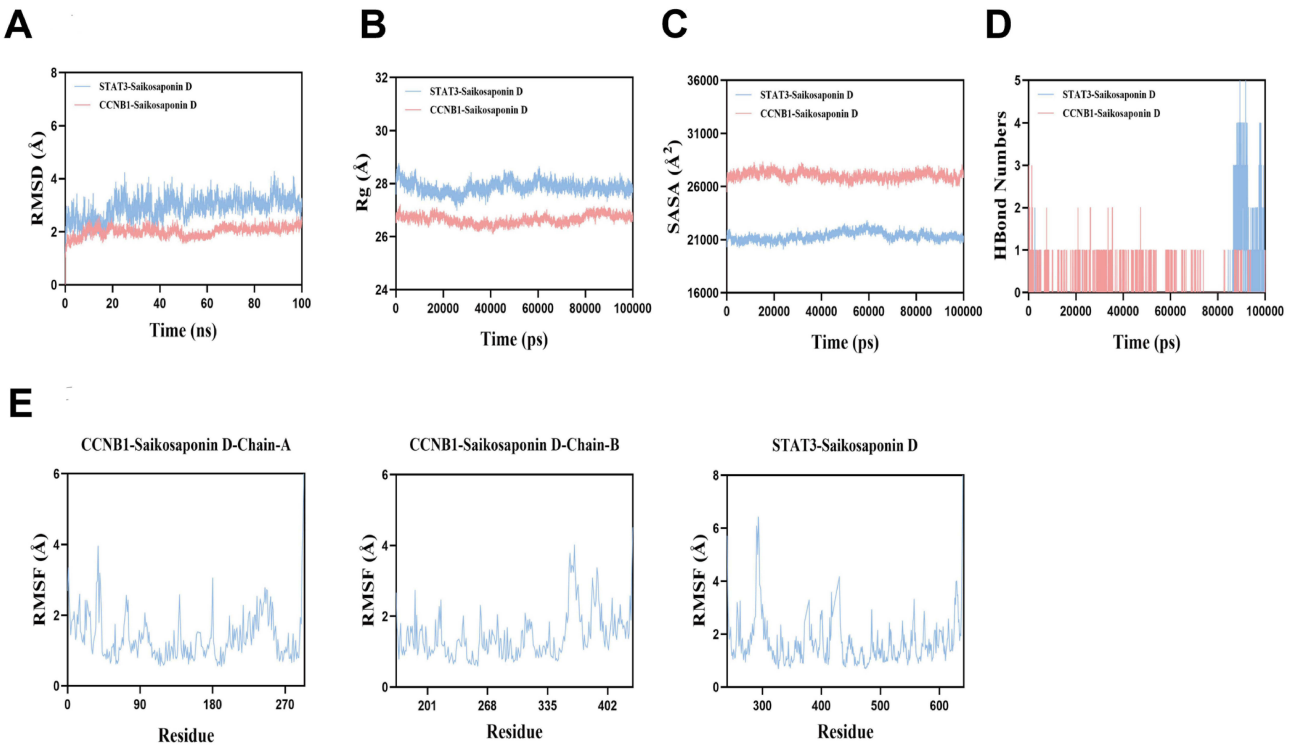

Figure 7 Molecular dynamics simulation analysis of interactions between saikosaponin D and target proteins. (**A**) Root mean square deviation (RMSD) of STAT3-saikosaponin D and CCNB1-saikosaponin D complexes over time (ns). (**B**) Radius of gyration (Rg) of the two complexes over time (ps). (**C**) Solvent-accessible surface area (SASA) of the two complexes over time (ps). (**D**) Number of hydrogen bonds (HBond) in the two complexes over time (ps). (**E**) Root mean square fluctuation (RMSF) of different chains (Chain-A of CCNB1-saikosaponin D, Chain-B of CCNB1-saikosaponin D, and STAT3-saikosaponin (**D**) as a function of residue number.

As shown in Figure 8A, compared with the normal control group, the mRNA levels of IL1, IL6, IL10, and TNF-α in the IMQ group were significantly upregulated compared with the normal control group. After Saikosaponin D treatment, the expressions of the mRNA levels of IL1 ($p < 0.05$), IL6 ($p < 0.01$), IL10 ($p < 0.05$), and TNF-α ($p < 0.001$) were markedly downregulated compared with the psoriatic group. These results indicate that Saikosaponin D can effectively inhibit inflammatory factor expression and alleviate inflammatory responses.

Core genes of Saikosaponin D in treating psoriasis were screened based on oxidative stress-related signature genes. Therefore, the expression levels of oxidative stress-related proteins SLC7A11 and GPX4 were further validated to explore whether Saikosaponin D affects oxidative stress in psoriatic skin tissues. As shown in Figure 8B, GPX4 was relatively highly expressed in the normal group, while SLC7A11 was overexpressed in the IMQ group, suggesting that oxidative stress is activated in psoriasis-like mice. After Saikosaponin D treatment, GPX4 expression was upregulated ($P<0.0001$), whereas SLC7A11 was significantly downregulated (Figure 8B, $P<0.001$). This suggests that Saikosaponin D can modulate these key markers related to oxidative stress.

Although machine learning identified both CCNB1 and STAT3 as key core target genes, and CCNB1 showed favorable binding energy with Saikosaponin D, the target pathway of Saikosaponin D in psoriasis remained unclear. In pathway enrichment analysis, core genes were mainly enriched in the JAK/STAT signaling pathway. Thus, emphasis was placed on differences in protein expression of the JAK-STAT pathway in the Saikosaponin D group (Figure 8C). Western blot (WB) results revealed significant changes in the phosphorylation levels of JAK2 and STAT3 in the Saikosaponin D-treated group. Specifically, P-STAT3/STAT3 ($P<0.0001$) and P-JAK2/JAK2 ($P<0.0001$) ratios were significantly increased in psoriatic mice skin tissues, indicating that phosphorylation of STAT3 and JAK2 was markedly activated, and the activity of the JAK2/STAT3 signaling pathway was significantly upregulated. In Saikosaponin D-treated psoriatic mice, decreased P-STAT3/STAT3 ($P<0.0001$) and P-JAK2/JAK2 ($P<0.0001$) ratios were observed. Saikosaponin D may thus inhibit psoriasis effectively by participating in the JAK2/STAT3 signaling pathway, regulating JAK2 and STAT3 phosphorylation, and thereby modulating oxidative stress (Supplementary File 22).

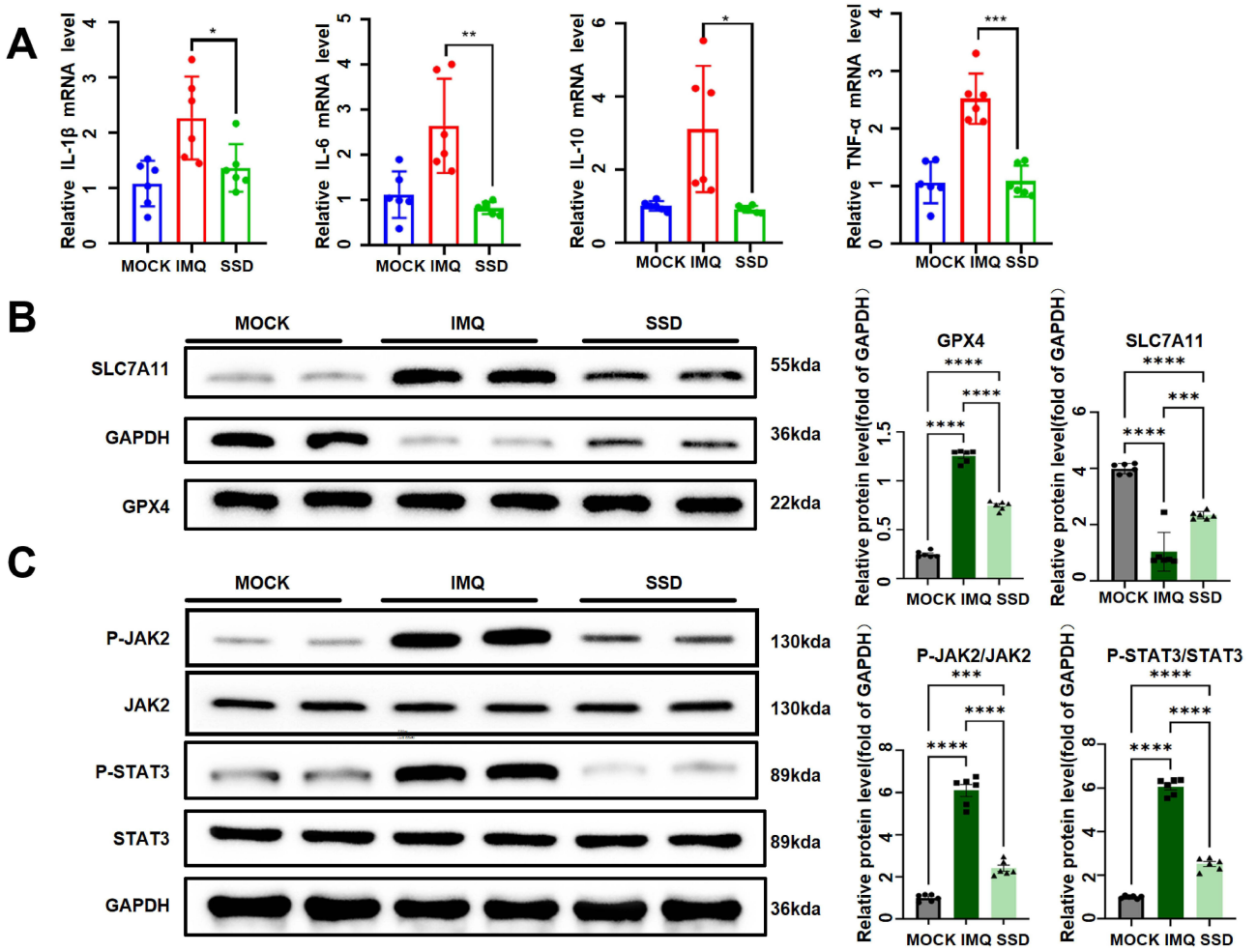

Figure 8 In vivo validation: SAIKOSAPONIN D inhibits inflammatory factor expression and JAK2/STAT3 pathway phosphorylation in IMQ-induced psoriasis-like mice skin. (**A**) Results of qPCR detecting mRNA expression levels of IL-1, IL-6, IL-10, and TNF-α. (**B** and **C**) WB validation of expression levels of oxidative stress core genes SLC7A11 and GPX4, and phosphorylation levels of the JAK2/STAT3 pathway. P-value: *indicates $p < 0.05$, **indicates $p < 0.01$, ***indicates $p < 0.001$, and ****indicates $p < 0.0001$. (n=6 per group).

Immune Infiltration Analysis

In KEGG and GO pathway enrichment analyses (Figure 4), these target genes were observed to correlate with immune-mediated inflammation. To further explore their precise relationship with immune cells, immune infiltration and immune cell correlation analyses were performed (Figure 9). Specifically, Figure 9A illustrates the distribution of various immune cells in normal and psoriatic groups, revealing distinct differences between the two groups.

Additionally, boxplots in Figure 9B further demonstrated that plasma cells, Treg cells, activated NK cells, and resting mast cells were highly expressed in normal skin tissues. In contrast, the infiltration proportions of Tfh cells, activated CD4+ T cells, activated dendritic cells, M0 and M1 macrophages, and resting NK cells were significantly higher in the psoriatic group than in the normal group. This indicates an imbalance in immune cell infiltration in psoriatic skin, with increased pro-inflammatory cells and decreased anti-inflammatory cells exacerbating the inflammatory response.

Furthermore, the correlation heatmap in Figure 9C presents infiltration correlations among different immune cells. Strong correlations were observed between some immune cells; for example, Th1 cells and Th17 cells showed a positive correlation, suggesting potential synergistic effects in inflammatory responses. In previous studies, Saikosaponin D was found to inhibit psoriasis progression via the JAK2/STAT3 pathway. Therefore, the correlation between STAT3 and immune cells was further analyzed.

https://doi.org/10.2147/JIR.S563272

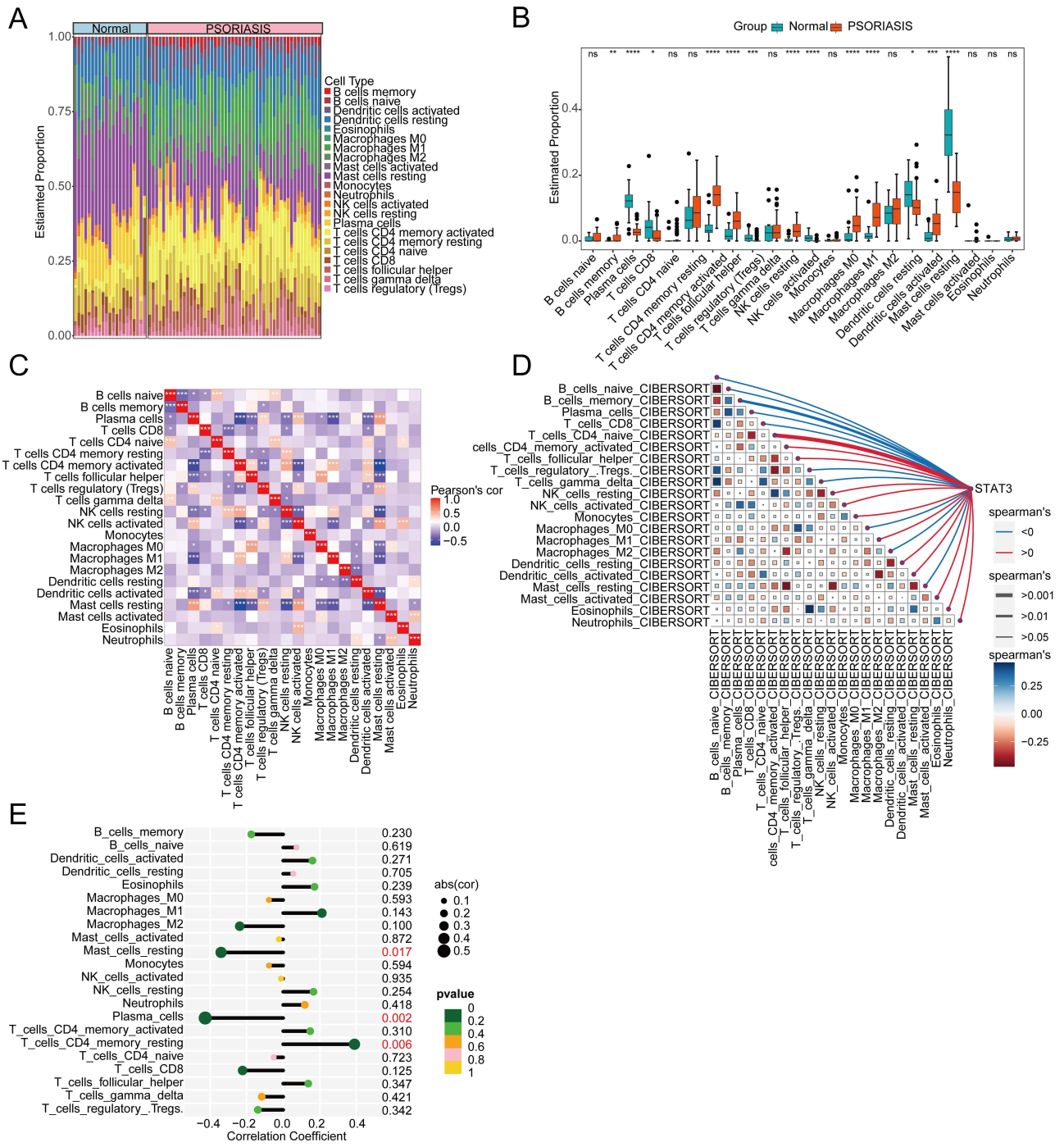

Figure 9 Immune infiltration analysis and correlation analysis between target genes and immunecell. (**A**) Heatmap of immune cell infiltration analysis. (**B**) Heatmap of immune cell correlations. (**C**) Boxplots of expression differences in 22 immune cell types between the normal group and psoriasis group. (**D**) Correlation plot between STAT3 and 22 immune cell types. (**E**) Lollipop plot of correlations between STAT3 and immune cells (abs, correlation coefficient; LS, skin lesions of Psoriasis).

Results in Figure 9D and E show the correlation analysis between different immune cell types and STAT3. Notably, STAT3 exhibited significant positive correlations with activated NK cells, activated mast cells, resting dendritic cells, naive B cells, and naive CD4+ T cells. This suggests that these cells may play important roles in environments with high STAT3 expression. These correlation results provide a basis for further investigating the role of STAT3 in the immune microenvironment, as changes in STAT3 expression levels may be closely associated with the infiltration and activation of specific immune cells.

Discussion

In recent years, the incidence of psoriasis has been on the rise. As a chronic immune-related skin inflammation, its exact pathogenesis remains incompletely understood, with a prolonged and recurrent course that severely impairs patients' quality of life. Traditional Chinese medicine (TCM) has demonstrated considerable application potential in exploring the mechanisms of psoriasis and in therapeutic practice. In recent studies, numerous TCM formulae (eg, Xiaochaihu Decoction,[11] Qingying Decoction,[22] Dihuang Decoction[23]), TCM herbs, and their monomer components (eg, quercetin,[24] curcumin)[25] have been confirmed to effectively inhibit the occurrence and progression of psoriasis. Saikosaponin D (SSD), an active component of the TCM herb Bupleuri Radix, exhibits anti-inflammatory and immune-regulatory effects.[12,16] However, to date, no study has clearly elucidated its specific role in psoriasis progression. Addressing this research gap where the mechanism of Saikosaponin D in psoriasis remains unclear, the present study aims to explore the potential mechanism of Saikosaponin D in treating psoriasis through network pharmacology, bioinformatics, machine learning, molecular docking, and molecular dynamics analyses. It is expected to provide references for developing new therapeutic strategies.

Multiple psoriasis datasets were retrieved from the GEO database in the present study. Differentially expressed genes were obtained via Limma differential analysis. Additionally, the WGCNA algorithm was used to construct co-expression networks and modules for healthy individuals and psoriasis patients, identifying gene sets with significant differences between different samples. By intersecting the differential genes from these two methods, 2135 psoriasis-related differential genes were obtained.

With advancing research on the pathogenesis of psoriasis, oxidative stress is considered closely associated with its occurrence and development. Therefore, it is necessary to explore the potential mechanism by which Saikosaponin D treats psoriasis by regulating oxidative stress. Initially, Saikosaponin D target genes were collected from multiple databases, along with 2912 oxidative stress-related genes. The intersection of these three gene sets yielded 25 target genes through which Saikosaponin D may treat psoriasis via oxidative stress, including JUN, FOS, IL6, STAT3, CCNB1, and IL1B.

In addition, GO enrichment analysis showed that its biological functions mainly involve negative regulation of protein metabolic process, cytokine-mediated signaling pathway, apoptotic process, intracellular signal transduction, immune process, etc. From the perspective of GO enrichment results, immune-related processes and Oxidative Stress-related items are significantly enriched. Oxidative stress in psoriasis leads to the activation of many signaling pathways, including NF-κB and MAPK, thus resulting in the activation of Th1 and Th17 cells, the secretion of pro-inflammatory cytokines, and then the excessive proliferation of keratinocytes, the infiltration of immune cells into the skin, and the alteration of vascular permeability through lipid peroxidation.[26] At present, studies have shown that natural antioxidants such as quercetin, curcumin, gingerol, resveratrol and other antioxidants are expected to become complementary therapies for psoriasis by inhibiting oxidative stress.[27] In addition to oxidative stress, immune processes have also attracted attention in GO analysis. During the occurrence and progression of psoriasis, immune processes require the collaboration of T lymphocytes, macrophages, etc., as well as the contributions of different cytokines and other cells, including neutrophils, macrophages, keratinocytes and B cells, to regulate the cooperation of immune cells and the release of inflammatory factors, promoting the process of psoriatic skin inflammatory damage and abnormal proliferation of keratinocytes.[28] At the cellular component level, it mainly includes cytosol, endoplasmic reticulum, mitochondrial membrane, etc. These structures are involved in oxidative stress signal transduction and material synthesis and metabolism, which may provide the intracellular environment basis for Saikosaponin D action targets. At the molecular function level, it involves the regulation of interleukin-6 production, signal transduction involved in JAK-STAT cascade, and the role of related gene targets such as antioxidant activity. Among them, the JAK-STAT pathway receives cytokine signals and interweaves with oxidative stress pathways, jointly affecting the pathogenesis of psoriasis.[29] It can be seen that Saikosaponin D may act on JAK-STAT pathway and oxidative stress-related targets by regulating the biological functions, cellular components and molecular functions involved in these GO enrichments, thus interfering with the pathological process of psoriasis. KEGG pathway enrichment analysis showed that Saikosaponin D may interfere with psoriasis by regulating oxidative stress, mainly involving Apoptosis, Jak-STAT signaling pathway, IL-17 signaling pathway, Toll-like receptor signaling pathway, Th17 cell differentiation pathway, etc. The cell apoptosis pathway can regulate programmed cell death. In the pathogenesis of psoriasis, the abnormal proliferation of keratinocytes is closely related to the imbalance of apoptosis, and the abnormality of this pathway may lead to the continuous development of skin lesions.[30] In psoriasis, in addition to the important role of the JAK-STAT signaling pathway, the Th17 cell

differentiation pathway is directly involved in the regulation of Th17 cell differentiation. Th17 cells are enriched and infiltrated in the circulation and skin lesions of psoriasis patients. Th17 cells are activated by upregulated factors in the psoriatic environment and express and secrete a variety of pro-inflammatory factors to participate in inflammation.[31] TLR-mediated activation of keratinocytes, pDC and/or cDC initiates early innate immune events, which is related to T cell activation and the development of autoimmunity in psoriasis.[32] These signaling pathways are intertwined and may play a synergistic role in the molecular mechanism of oxidative stress response regulated by Saikosaponin D in psoriasis. Among them, the association between the JAK-STAT signaling pathway and oxidative stress deserves special attention - oxidative stress can affect the signal transmission of the JAK-STAT pathway, change immune cell functions and the release of inflammatory factors. Saikosaponin D may interfere with the pathological process of psoriasis by regulating this pathway and oxidative stress-related targets. The results of GO and KEGG analyses suggest that as an active component of Bupleurum, Saikosaponin D has interactive effects with key nodes of the JAK-STAT pathway and oxidative stress regulatory factors. On the one hand, it may block abnormal immune signal transduction by inhibiting the phosphorylation of the JAK-STAT pathway, thereby alleviating skin inflammation; on the other hand, it may intervene in the pathogenesis of psoriasis in two dimensions by regulating oxidative stress-related targets.

To further explore the core targets, this study employed three machine learning methods and finally identified CCNB1 and STAT3 as key targets. Looking back at existing research, the role of CCNB1 in psoriasis has been verified by many studies: it may be involved in the process of IL-27 promoting the proliferation of psoriasis keratinocytes,[33] It may also participate in the pathogenesis of psoriasis together with CCNB2 and has a significant correlation with psoriasis-related immune targets.[34] Meanwhile, CCNB1 may be a common characteristic gene of psoriasis, atopic dermatitis, and inflammatory acne,[35] and has been identified as an exosome-related characteristic gene and is significantly overexpressed in the psoriasis cell model.[36,37] Notably, although there is no direct evidence that CCNB1 is involved in the occurrence and progression of psoriasis through oxidative stress, CCNB1 has been confirmed to be a ferroptosis-related gene in cutaneous squamous cell carcinoma,[38] and has been found to be one of the targets of berberine in regulating ferroptosis to inhibit prostate cancer.[39] STAT3 is an important signal transduction and transcription activation factor in the JAK/STAT signaling pathway. Existing studies have proven that some Chinese medicine monomer components, such as kaempferol,[40] total paeony glucosides, centella asiatica and rutin, can improve psoriasis through JAK/STAT signal transduction,[41,42] and can play a core role through the STAT3/SLC7A11 axis,[43] and can regulate the expression of a variety of pro-inflammatory cytokines, such as IL-6, IL-1β and TNF-α. Our study also confirmed the above conclusions. Both CCNB1 and STAT3 are highly expressed in psoriasis lesion tissues, are shared characteristic genes of Saikosaponin D, oxidative stress and psoriasis, and are relatively highly expressed in psoriasis lesion tissues. After ROC curve verification, they have good diagnostic significance. This suggests that CCNB1 and STAT3 are very likely to be key targets for regulating oxidative stress and affecting the progression of psoriasis, laying a solid target foundation for subsequent in-depth exploration of the molecular mechanism of Saikosaponin D in intervening psoriasis.

Following the identification of core targets, the present study performed molecular docking and molecular dynamics simulations focusing on CCNB1 and STAT3. Results from these analyses demonstrated that Saikosaponin D forms stable binding with high affinity to both STAT3 and CCNB1. For CCNB1, Saikosaponin D formed hydrogen bonds with ASN133 and ASP146 residues, and hydrophobic interactions with LEU135 and TYR15 residues of the receptor. Similarly, for STAT3, Saikosaponin D interacted with the receptor via a hydrogen bond with the CYS259 residue, hydrophobic interaction with the ARG325 residue, and van der Waals forces across multiple residues. Stable binding between small molecules and target proteins is a critical prerequisite for exerting pharmacological effects. Subsequent molecular dynamics simulations further confirmed that the complexes reached equilibrium in the later stage of simulation. Indicators such as hydrogen bond count and root mean square fluctuation (RMSF) also verified favorable interactions and stability between the ligand and target proteins. These findings fully indicate that Saikosaponin D can effectively bind to hub targets, providing direct molecular evidence for its anti-psoriatic effects.

At the in vitro experimental level, in the Saikosaponin D-treated group, psoriasis-like dermatitis symptoms in mice were alleviated, skin pathological changes were reduced, excessive keratinocyte proliferation was inhibited, and inflammatory factor expression was downregulated. Compared with the normal control group, the mRNA expression of inflammatory factors such as IL1 and IL6 in skin tissues of the model group was significantly upregulated; after

Saikosaponin D treatment, their expression was significantly downregulated. This strongly suggests that Saikosaponin D has the potential to alleviate psoriatic inflammation, consistent with existing research conclusions that TCM components like curcumin can treat psoriasis by downregulating inflammatory factors. Meanwhile, the present study noted that Saikosaponin D may exert its effects by regulating the JAK2/STAT3 signaling pathway. In psoriasis-like mice, the expressions of p-STAT3/STAT3 and p-JAK2/JAK2 were significantly increased, indicating activated phosphorylation of STAT3 and JAK2 and upregulated pathway activity. After Saikosaponin D treatment, both expressions decreased significantly. This suggests that Saikosaponin D may inhibit psoriasis by participating in the JAK2/STAT3 signaling pathway and regulating JAK2/STAT3 phosphorylation to modulate oxidative stress, supplementing key evidence for elucidating Saikosaponin D's mechanism of action. It also aligns with existing findings that TCM components such as kaempferol treat psoriasis by regulating the JAK/STAT pathway. Furthermore, Saikosaponin D's regulatory effects on oxidative stress signature proteins GPX4 and SLC7A11—with GPX4 upregulated and SLC7A11 downregulated after Saikosaponin D treatment—suggested it may inhibit oxidative stress in mice. This opened a new perspective for understanding Saikosaponin D's mechanism in treating psoriasis and identified oxidative stress-related directions for subsequent research.

Since both GO and KEGG enrichment analyses suggested that Saikosaponin D may regulate psoriasis by affecting immune processes, the present study aimed to further clarify differences in the immune microenvironment between normal and psoriatic skin tissues. Initially, an immune cell infiltration analysis heatmap was used to visually present the global landscape of immune cell infiltration in the normal and psoriatic groups. In the heatmap, different color gradients clearly showed the infiltration abundance distribution of 22 immune cell types across samples from the two groups. Changes in color intensity of specific immune cells in the psoriatic group initially indicated significant differences in their infiltration patterns compared with the normal group. Results revealed distinct differences in immune cell distribution between the two groups: plasma cells and Treg cells were highly expressed in normal skin tissues, while the infiltration proportions of Tfh cells and activated CD4+ T cells were significantly higher in the psoriatic group. Furthermore, boxplots of expression differences among 22 immune cell types showed that the infiltration proportions of immune cells such as Macrophages M1 and activated dendritic cells were significantly higher in the psoriatic group than in the normal group. These findings are consistent with previous studies,[44–47] supporting the critical role of such cells in initiating the immunopathological process of psoriasis. However, some immune cells, such as eosinophils, showed no significant differences between the two groups, which contradicts previous research.[44] This discrepancy may be attributed to inconsistent samples included in the datasets of the present study compared with prior studies.

To explore synergistic or antagonistic relationships among immune cells, an immune cell correlation heatmap was constructed. Results showed a significant positive correlation between T cell subsets (eg, naive CD4+ T cells and resting memory CD4+ T cells), suggesting potential synergistic effects in initiating and maintaining immune responses. In contrast, Macrophages M2 were negatively correlated with neutrophils, which is not entirely consistent with recent studies. In contrast, Macrophages M2 were negatively correlated with neutrophils. This finding, while seemingly contrasting with some studies indicating co-infiltration, may reflect the specific inflammatory snapshot of established psoriatic lesions. It is plausible that in this highly pro-inflammatory microenvironment, the persistent infiltration of neutrophils actively suppresses the differentiation or function of anti-inflammatory M2 macrophages, or vice-versa. This suggests a polarized, non-resolving inflammatory state that warrants further investigation.

One study found that both M2 macrophages and neutrophils are significantly infiltrated and interact; M2 macrophages can inhibit neutrophil-mediated inflammatory responses by secreting anti-inflammatory factors.[48] Another study confirmed that neutrophils in psoriasis activate macrophages via antimicrobial peptides, thereby further affecting the M1/M2 macrophage balance.[49] Finally, given the central role of the STAT3 signaling pathway in Saikosaponin D-mediated immune regulation of psoriasis identified in bioinformatics analyses, the present study focused on its association with immune cells. Through analyses of correlation plots and correlation lollipop plots between STAT3 and 22 types of immune cells, it was found that STAT3 exhibited a significant positive correlation with highly infiltrating M1 macrophages and activated dendritic cells in the psoriasis group. This suggests that STAT3 may drive local immune-inflammatory cascades in psoriasis by targeting the infiltration and activation of these immune cells. Meanwhile, STAT3 showed weak correlation with some immunosuppressive cells, such as regulatory T cells (Tregs). Through multi-dimensional immune cell analyses, the present

study not only validated the classical conclusion of pro-inflammatory cell enrichment in the psoriatic immune microenvironment but also revealed interaction mechanisms between key signaling pathways and immune cell networks via STAT3 correlation analysis. This provides a more targeted theoretical basis for immune-targeted therapy in psoriasis.

In summary, by integrating immune cell correlation analysis, molecular docking, molecular dynamics simulations, and in vitro experimental validation, the present study has deeply analyzed the roles of core genes CCNB1 and STAT3 in the psoriatic immune microenvironment. It has also clearly revealed the potential mechanism of action of Saikosaponin D. These findings not only provide new insights into understanding the immunopathological mechanisms of psoriasis but also offer potential targets and drug candidates for developing new psoriasis treatments. Furthermore, they echo existing research conclusions on TCM components in treating psoriasis at multiple levels, further consolidating the rationale for TCM-based psoriasis therapy. Looking forward, future studies could further explore the clinical application prospects of Saikosaponin D in psoriasis treatment, investigate its synergistic effects with other therapies, and aim to develop more effective treatment regimens for psoriasis patients.

However, the present study has limitations. First, bioinformatics analyses relied on public databases, making data biases and sample heterogeneity unavoidable. Additionally, differences between lesional and non-lesional areas of psoriasis were not addressed. Second, no cell experiments were conducted to verify Saikosaponin D's toxicity on HACAT cells; in vivo experiments were limited to mice models, requiring further validation of their applicability to humans. Subsequent studies could expand sample sizes and incorporate clinical samples. Fourth, the in vivo experiments were conducted with a small sample size (n=6 per group), which limits the statistical power and generalizability of the findings. Fifth, our machine learning models yielded high AUC values (eg, 1.000 in the test set). While promising, this strongly suggests a risk of overfitting, which is common in bioinformatics studies with limited sample sizes. These models should be considered exploratory and require robust validation in larger, independent external datasets. Additionally, the intermittent SSD dosing schedule (alternate days) was based on preliminary assessments, but its therapeutic optimization compared to daily dosing requires further study. Third, despite screening based on oxidative stress target genes and WB verification of expression differences in SLC7A11 and GPX4, the specific process by which Saikosaponin D regulates psoriasis via oxidative stress remains unelucidated. Future research intends to explore the specific molecular mechanisms by which Saikosaponin D regulates oxidative stress. This will strengthen the theoretical foundation for precise psoriasis treatment and is expected to fill gaps in mechanistic details within existing studies on TCM-mediated oxidative stress regulation in psoriasis.

Conclusion

Our research is the first to explore the role of the monomer component of traditional Chinese medicine, Saikosaponin D, at the level of skin inflammation. By integrating weighted gene co-expression network analysis (WGCNA), protein-protein interaction (PPI) network construction, machine learning algorithms, molecular docking and molecular dynamics simulation, we systematically investigated the potential mechanism by which Saikosaponin D regulates psoriasis. In addition, we also observed the changes in the activity of the JAK2/STAT3 signaling pathway through in vitro experiments, enriching the possibilities of treating psoriasis with single components of traditional Chinese medicine.

Highlights

1. Saikosaponin D (SSD) significantly alleviates psoriasis-like inflammation and keratinocyte hyperproliferation in an imiquimod-induced murine model.
2. Saikosaponin D exerts its anti-psoriatic effects through dual-targeting of STAT3 and CCNB1, identified via machine learning and validated by molecular docking/dynamics.
3. Mechanistically, Saikosaponin D suppresses JAK2/STAT3 signaling phosphorylation and rebalances oxidative stress by upregulating GPX4 and downregulating SLC7A11.
4. Saikosaponin D modulates the psoriatic immune microenvironment, reducing pro-inflammatory M1 macrophages while augmenting regulatory T cells (Tregs).

Abbreviations

SSD, Saikosaponin D; LIMMA, Linear Models for Microarray Data; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; LASSO, Least Absolute Shrinkage and Selection Operator; SVM-RFE, Support Vector Machine-Recursive Feature Elimination; GEO, Gene Expression Omnibus; JAK2, Janus Kinase 2; STAT3, Signal Transducer and Activator of Transcription 3; CCNB1, Cyclin B1; WGCNA, Weighted Gene Co-Expression Network Analysis; PPI, Protein-Protein Interaction; qPCR, Quantitative Polymerase Chain Reaction; WB, Western Blot; GPX4, Glutathione Peroxidase 4; SLC7A11, Solute Carrier Family 7 Member 11; IL-1β, Interleukin-1β; IL-6, Interleukin-6; IL-10, Interleukin-10; TNF-α, Tumor Necrosis Factor-α; M1, M2-macrophages, M1 and M2 Macrophages; Tfh cells, Follicular Helper T Cells; Th1 cells, T Helper 1 Cells; CD4+ T cells, CD4-Positive T Cells; TCM, Traditional Chinese Medicine; XCHD, Xiaochaihu Decoction; NF-κB, Nuclear Factor kappa-B; OS, Oxidative stress; C57BL/6, C57 Black 6; WT, Wild Type; IMQ, Imiquimod; DAB, 3,3′-Diaminobenzidine; JUN, Jun Proto-Oncogene; FOS, Fos Proto-Oncogene; Th17, T Helper 17 Cells.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author, Tian, upon reasonable request, all the supplementary files of this study can be seen in Supplementary Files 1–22, especially All original Western Blot gels images for the biological triplicates are provided in Supplementary File 22).

Ethics Statement

The GEO datasets used in this study are publicly available and de-identified; according to item 1 and 2 of Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects dated February 18, 2023, the use of such data does not require additional IRB/REC approval. All animals were kept in a pathogen-free environment and fed ad lib and following the ARRIVE guidelines. The procedures for care and use of animals were approved by the Ethics Committee of the First Affiliated Hospital of Xinxiang Medical University (EC-025-520) and all applicable institutional and governmental regulations concerning the ethical use of animals were followed. This study utilized public data published by others in the GEO database and no human experiments were conducted.

Acknowledgments

Thanks to Associate Professor Shaoju Qian for discovering and providing this meaningful topic, and to Professor Xiangfeng Song and Professor Zhongwei Tian for their writing suggestions.

Author Contributions

Junrui Ding: Writing-Original Draft, Validation, Software, Methodology, Formal analysis; Aiping Sun: Resources, Data Curation, Visualization; Resources; Writing-Original Draft; Hua Hu: Formal analysis, Writing-Original Draft; Shuao Lu: Data Curation, Visualization, Formal analysis; Writing - Review & Editing; Junlan Song: Investigation, Formal analysis, Writing-Original Draft; Jialin Li: Investigation, Validation, Software, Writing - Review & Editing; Juao He: Conceptualization, Data Curation, Writing - Review & Editing; Xiangfeng Song: Methodology, Writing - Review & Editing; Shaoju Qian: Project administration, Writing - Review & Editing, Supervision; Zhongwei Tian: Funding acquisition, Resources, Supervision, Writing - Review & Editing; All authors 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.

Funding

This work was supported by the Natural Science Foundation of Henan province (242300421306); the Xinxiang Medical University Graduate Research Innovation Support Program (YJSCX202440Y, YJSCX202414Y); the "EverPure" Skin Immunity Scientific Research Fund (HJ-DER-2024-16, HJ-DER-2024-19).

Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Park A, Heo TH. Celastrol regulates psoriatic inflammation and autophagy by targeting IL-17A. *Biomed Pharmacother*. 2024;172:116256. doi:10.1016/j.biopha.2024.116256
2. Zhang B, Yan G, Li F, et al. Qingxiong ointment and its active ingredient, Shikonin treat psoriasis through HIF-1 signaling pathway. *Curr Pharm Des*. 2024;30(24):1927–1938. doi:10.2174/0113816128287142240529120346
3. Modarresi Chahardehi A, Ojaghi HR, Motedayyen H, Arefnezhad R. Nano-based formulations of thymoquinone are new approaches for psoriasis treatment: a literature review. *Front Immunol*. 2024;15:1416842. doi:10.3389/fimmu.2024.1416842
4. Hung CH, Wang CN, Cheng HH, et al. Baicalin ameliorates imiquimod-induced psoriasis-like inflammation in mice. *Planta Med*. 2018;84 (15):1110–1117. doi:10.1055/a-0622-8242
5. Chen BL, Zhang WM, Dong XW, Liu JY, Bai YP. Quercetin induces keratinocytes apoptosis via triple inhibition of Notch, PI3K/AKT signaling and Glut1 in the treatment of psoriasis. *Biochim Biophys Acta Mol Basis Dis*. 2025;1871(6):167879. doi:10.1016/j.bbadis.2025.167879
6. Zhou T, Zhang S, Zhou Y, et al. Curcumin alleviates imiquimod-induced psoriasis in progranulin-knockout mice. *Eur J Pharmacol*. 2021;909:174431. doi:10.1016/j.ejphar.2021.174431
7. Su Z, Kang Y. Protective effect and regulatory mechanism of salidroside on skin inflammation induced by imiquimod in psoriasis mice. *J Pharmacol Sci*. 2024;154(3):192–202. doi:10.1016/j.jphs.2023.12.007
8. Cholet J, Decombat C, Vareille-Delarbre M, et al. In vitro anti-inflammatory and immunomodulatory activities of an extract from the roots of Bupleurum rotundifolium. *Medicines*. 2019;6(4):101. doi:10.3390/medicines6040101
9. Qi W, Tuerxun J, Li J, Wang C, Luo R, Liu C. Identification of Bupleurum (Apiaceae) seeds by allele-specific PCR based on ITS sequences. *3 Biotech*. 2020;10(6):240. doi:10.1007/s13205-020-02233-1
10. Zhu L, Liang ZT, Yi T, et al. Comparison of chemical profiles between the root and aerial parts from three Bupleurum species based on a UHPLC-QTOF-MS metabolomics approach. *BMC Complement Altern Med*. 2017;17(1):305. doi:10.1186/s12906-017-1816-y
11. He K, Wang Z, Liu M, et al. Exploring the effect of Xiao-Chai-Hu decoction on treating psoriasis based on network pharmacology and experiment validation. *Curr Pharm Des*. 2024;30(3):215–229. doi:10.2174/0113816128288527240108110844
12. Li P, Wu M, Xiong W, et al. Saikosaponin-d ameliorates dextran sulfate sodium-induced colitis by suppressing NF-κB activation and modulating the gut microbiota in mice. *Int Immunopharmacol*. 2020;81:106288. doi:10.1016/j.intimp.2020.106288
13. Zhou P, Shi W, He XY, Du QY, Wang F, Guo J. Saikosaponin D: review on the antitumour effects, toxicity and pharmacokinetics. *Pharm Biol*. 2021;59(1):1480–1489. doi:10.1080/13880209.2021.1992448
14. Lu CN, Yuan ZG, Zhang XL, et al. Saikosaponin a and its epimer saikosaponin d exhibit anti-inflammatory activity by suppressing activation of NF-κB signaling pathway. *Int Immunopharmacol*. 2012;14(1):121–126. doi:10.1016/j.intimp.2012.06.010
15. Lin X, Wu S, Wang Q, et al. Saikosaponin-D reduces H(2)O(2)-Induced PC12 cell apoptosis by removing ROS and blocking MAPK-dependent oxidative damage. *Cell Mol Neurobiol*. 2016;36(8):1365–1375. doi:10.1007/s10571-016-0336-5
16. Du P, Xu J, Jiang Y, et al. Saikosaponin-d attenuates Hashimoto's thyroiditis by regulating macrophage polarization. *J Immunol Res*. 2022;2022:7455494. doi:10.1155/2022/7455494
17. Sun J, Zheng J, Shi X, Qian H, Jin C, Xu L. Saikosaponin D inhibits proliferation and collagen production of human embryonic lung fibroblasts by regulating TGF-β1/Smads signaling pathway. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi*. 2019;35(3):256–261.
18. Gu S, Zheng Y, Chen C, et al. Research progress on the molecular mechanisms of Saikosaponin D in various diseases (Review). *Int J Mol Med*. 2025;55(3):37. doi:10.3892/ijmm.2024.5478
19. Galaris D, Barbouti A, Pantopoulos K. Iron homeostasis and oxidative stress: an intimate relationship. *Biochim Biophys Acta Mol Cell Res*. 2019;1866(12):118535. doi:10.1016/j.bbamcr.2019.118535
20. Shou Y, Yang L, Yang Y, Xu J. Inhibition of keratinocyte ferroptosis suppresses psoriatic inflammation. *Cell Death Dis*. 2021;12(11):1009. doi:10.1038/s41419-021-04284-5
21. Li S, Luo X, Zhang S, et al. Ferroptosis activation contributes to the formation of skin lesions in psoriasis vulgaris. *Antioxidants*. 2023;12(2):310. doi:10.3390/antiox12020310
22. Shen C, Liu X, Jia H, et al. Application of bioinformatics analysis and molecular docking to study the mechanism of Qingying decoction in treating psoriasis. *Hereditas*. 2025;162(1):52. doi:10.1186/s41065-025-00421-8
23. Song C, Yang C, Meng S, et al. Deciphering the mechanism of Fang-Ji-Di-Huang-Decoction in ameliorating psoriasis-like skin inflammation via the inhibition of IL-23/Th17 cell axis. *J Ethnopharmacol*. 2021;281:114571. doi:10.1016/j.jep.2021.114571
24. Liu Q, Liu J, Zheng Y, et al. Quercetin mediates the therapeutic effect of Centella asiatica on psoriasis by regulating STAT3 phosphorylation to inhibit the IL-23/IL-17A axis. *Nan Fang Yi Ke Da Xue Xue Bao*. 2025;45(1):90–99. doi:10.12122/j.issn.1673-4254.2025.01.12
25. Cai Z, Zeng Y, Liu Z, Zhu R, Wang W. Curcumin alleviates epidermal psoriasis-like dermatitis and IL-6/STAT3 pathway of mice. *Clin Cosmet Invest Dermatol*. 2023;16:2399–2408. doi:10.2147/ccid.S423922
26. Pleńkowska J, Gabig-Cimińska M, Mozolewski P. Oxidative stress as an important contributor to the pathogenesis of psoriasis. *Int J Mol Sci*. 2020;21(17):6206. doi:10.3390/ijms21176206
27. Ahmad Jamil H, Abdul Karim N. Unraveling mitochondrial reactive oxygen species involvement in psoriasis: the promise of antioxidant therapies. *Antioxidants*. 2024;13(10):1222. doi:10.3390/antiox13101222
28. Grän F, Kerstan A, Serfling E, Goebeler M, Muhammad K. Current developments in the immunology of psoriasis. *Yale J Biol Med*. 2020;93 (1):97–110.
29. Furtunescu AR, Georgescu SR, Tampa M, Matei C. Inhibition of the JAK-STAT pathway in the treatment of psoriasis: a review of the literature. *Int J Mol Sci*. 2024;25(9):4681. doi:10.3390/ijms25094681

30. Krawczyk A, Miśkiewicz J, Strzelec K, Wcisło-Dziadecka D, Strzalka-Mrozik B. Apoptosis in autoimmunological diseases, with particular consideration of molecular aspects of psoriasis. *Med Sci Monit*. 2020;26:e922035. doi:10.12659/msm.922035
31. Sieminska I, Pieniawska M, Grzywa TM. The immunology of psoriasis-current concepts in pathogenesis. *Clin Rev Allergy Immunol*. 2024;66 (2):164–191. doi:10.1007/s12016-024-08991-7
32. Sun L, Liu W, Zhang LJ. The role of toll-like receptors in skin host defense, psoriasis, and atopic dermatitis. *J Immunol Res*. 2019;2019:1824624. doi:10.1155/2019/1824624
33. Wu Z, Wang R, Liu Y, Yang B, Wang H. RNA sequencing reveals that the genes related to cell cycle and glycolysis play an essential role in IL-27-induced keratinocyte hyperproliferation. *J Inflamm Res*. 2024;17:8165–8180. doi:10.2147/jir.S481835
34. Li AH, Chen YQ, Chen YQ, Song Y, Li D. CCNB1 and CCNB2 involvement in the pathogenesis of psoriasis: a bioinformatics study. *J Int Med Res*. 2022;50(8):3000605221117138. doi:10.1177/03000605221117138
35. Wang W, Hwang S, Park D, Park YD. The features of shared genes among transcriptomes probed in atopic dermatitis, psoriasis, and inflammatory acne: S100A9 selection as the target gene. *Protein Pept Lett*. 2024;31(5):356–374. doi:10.2174/0109298665290166240426072642
36. Chen L, Zhu S, Zhao L, Ye W. Identification of exosome-related gene features in psoriasis and construction of a diagnostic model via integrated bioinformatics analysis. *Comput Methods Biomech Biomed Engin*. 2024;1–12. doi:10.1080/10255842.2024.2410224
37. Lin Z, Tan C, Qiu Q, et al. Ubiquitin-specific protease 22 is a deubiquitinase of CCNB1. *Cell Discov*. 2015;1:15028. doi:10.1038/celldisc.2015.28
38. Su W, Huang B, Zhang Q, et al. Exploring potential biomarkers, ferroptosis mechanisms, and therapeutic targets associated with cutaneous squamous cell carcinoma via integrated transcriptomic analysis. *J Healthc Eng*. 2022;2022:3524022. doi:10.1155/2022/3524022
39. Zou P, Li S, He Q, Zheng C. Berberine inhibits prostate cancer progression by inducing ferroptosis: evidence from network pharmacology. *Anticancer Drugs*. 2025;36(4):271–279. doi:10.1097/cad.0000000000001691
40. Li Y, Cui H, Li S, et al. Kaempferol modulates IFN-γ induced JAK-STAT signaling pathway and ameliorates imiquimod-induced psoriasis-like skin lesions. *Int Immunopharmacol*. 2023;114:109585. doi:10.1016/j.intimp.2022.109585
41. Lin P, Shi HY, Lu YY, Lin J. Centella asiatica alleviates psoriasis through JAK/STAT3-mediated inflammation: an in vitro and in vivo study. *J Ethnopharmacol*. 2023;317:116746. doi:10.1016/j.jep.2023.116746
42. Wu P, Liu Y, Zhai H, Wu X, Liu A. Rutin alleviates psoriasis-related inflammation in keratinocytes by regulating the JAK2/STAT3 signaling. *Skin Res Technol*. 2024;30(8):e70011. doi:10.1111/srt.70011
43. Qian S, Yang Z, Zhang X, et al. Novel therapeutic approach for psoriasis: upregulating FcRn to inhibit ferroptosis and alleviate lesional skin. *Free Radic Biol Med*. 2024;224:797–808. doi:10.1016/j.freeradbiomed.2024.09.010
44. Su W, Wei Y, Huang B, Ji J. Identification of Hub genes and immune infiltration in psoriasis by bioinformatics method. *Front Genetics*. 2021;12:606065. doi:10.3389/fgene.2021.606065
45. Armstrong AW, Read C. Pathophysiology, clinical presentation, and treatment of psoriasis: a review. *JAMA*. 2020;323(19):1945–1960. doi:10.1001/jama.2020.4006
46. Zou A, Kong Q, Sang H. Identification of key apoptosis-related genes and immune infiltration in the pathogenesis of psoriasis. *Hereditas*. 2022;159 (1):26. doi:10.1186/s41065-022-00233-0
47. Gong X, Wang W. Profiles of innate immune cell infiltration and related core genes in psoriasis. *Biomed Res Int*. 2021;2021(1):6656622. doi:10.1155/2021/6656622
48. Alshihmani AHH, Mahmoudi M, Kheder RK, Fadaee A, Esmaeili SA. The M2 macrophages importance role in psoriasis. *Immun Inflamm Dis*. 2025;13(6):e70211. doi:10.1002/iid3.70211
49. Zhang J, Feng Y, Shi D. NETosis of psoriasis: a critical step in amplifying the inflammatory response. *Front Immunol*. 2024;15:1374934. doi:10.3389/fimmu.2024.1374934