Transcriptome analysis identifies activation of IL-17A and STAT3 circuit activation for the pathogenesis of psoriasis (#82783)

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I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.
Transcriptome analysis identifies activation of IL-17A and STAT3 circuit activation for the pathogenesis of psoriasis

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Background: Psoriasis is an autoimmune skin disease characterized by immunocyte activation, excessive proliferation, and abnormal differentiation of keratinocytes. Signal transducers and activators of transcription 3 (STAT3) link activated keratinocytes and immunocytes in the development of psoriasis. T helper (Th) 17 cells and their associated cytokines have been recognized to contribute to the pathogenesis of psoriasis significantly.

Methods: In this study, we intradermally induced recombinant interleukin (IL) -17A into STAT3 transgenic mice and performed transcriptome analyses in the skin of wild type (WT), STAT3, and IL-17A induced STAT3 mice. The functional enrichment analysis of differentially expressed genes (DEGs) was performed by bioinformatics to predict biological pathways. Meanwhile, the morphological and pathological features of skin lesions were observed, and the DEGs were verified by qPCR.

Results: IL-17A induced STAT3 mice skin lesions displayed the pathological features of hyperkeratosis and parakeratosis. The DEGs between IL-17A induced STAT3 mice and WT mice were highly consistent with those observed in psoriasis patients and included S100A8, S100A9, Sprr2, and LCE. Gene ontology (GO) analysis of the core DEGs concentrated in immune response, chemotaxis, and cornified envelope, et al. The major KEGG enrichment pathways were IL-17 and Toll-like receptor signaling pathways.

Conclusion: IL-17A and STAT3 circuit activation plays a crucial role in the pathogenesis of psoriasis.
Transcriptome analysis identifies activation of IL-17A and STAT3 circuit activation for the pathogenesis of psoriasis

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ABSTRACT

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Keywords: psoriasis, IL-17A, STAT3, bioinformatics

INTRODUCTION

Psoriasis is a genetically determined chronic inflammatory skin disease mediated by the innate and adaptive immune system. It is characterized by erythema, scales, punctate bleeding, and inflammatory infiltration. The pathogenesis is complicated and yet to be deciphered. The pathogenesis of psoriasis was demonstrated to be mediated through the activation of immune-
mediated T helper (Th) 1/Th17/Th22 cells together with excessive hyperplasia and abnormal
differentiation of keratinocytes resulting in the release of inflammatory cytokines.{Egeb erg, 2020 #119}

Signal transducers and activators of transcription (STAT) 3 has emerged as an essential factor for
numerous biological processes, including cell proliferation, survival, and migration. Sano et al.
demonstrated that STAT3 links activated keratinocytes and immunocytes in the development of
psoriasis. They constructed a K5. STAT3C transgenic mouse expresses skin lesions resembling
human psoriasis.(Sano et al. 2004) This animal model was used to administer 12-O-tetra-
decanoylphorbol-13-acetate (TPA) to the dorsal skin topically. The psoriasiform skin lesions
took shape that closely resembles psoriatic patients, which strongly indicated that STAT3
activation was required for psoriasis.(Vinita et al. 2012) STAT3 is not only a critical factor for T
lymphocyte differentiation but plays an essential role in keratinocyte hyperplasia.(Calautti et al.
2018) Besides, they demonstrated that psoriatic lesions were recapitulated in K5.STAT3C mice
by intradermal injection of activated T cells and developed more aberrant parakeratosis.
K5.STAT3C mice and other psoriasiform transgenic mouse models, such as K5.Tie2, K14.
vascular endothelial growth factor (VEGF), K5. transforming growth factor (TGF)-β1 transgenic
mouse and imiquimod induced mouse models were compared by whole-genome transcriptional
profiling to human psoriatic skin. The profiling study supported the value of the K5.STAT3C
mouse model as a research model for psoriasis with identifiable convergence to human
psoriasis.(Swindell et al. 2011)

Increasing evidence suggests that the interleukin (IL)-23/IL-17 axis plays a central pathogenic
role in the development of psoriasis.(Sakkas & Bogdanos 2017) IL-17 is the major cytokine
produced by Th17 cells. IL-17 family members include IL-17A, IL-17B, IL-17C, IL-17D, IL-
17E, and IL-17F. IL-17A and IL-17F are isoforms that form homodimers or heterodimers with
each other. The differentiation of naive CD4+ T cells to Th17 cells results from regulating STAT3
genes. (Tripathi et al. 2017) Th17 cells release various cytokines, especially IL-17A, which
activates intracellular signaling pathways in keratinocytes such as the STAT3 pathway
responsible for epidermal hyperplasia.(Shi et al. 2011)

Here, we established a psoriasis mouse model by intradermal injection of recombinant IL-17A
into the dorsal skin of STAT3 transgenic mice. Transcriptome analysis was performed to
determine the role of IL-17A and STAT3 dual activation in psoriasis pathogenesis.

MATERIALS AND METHODS

Animals and experimental design

We established the transgenic overexpression of STAT3 in mice following the protocol
established by Sano.(Sano et al. 2004) STAT3 plasmid was synthesized from Invitrogen
(Invitrogen, Life Technologies Corp., Carlsbad, CA, U.S.A.). STAT3 transgenic mice were made
by the Institute of Laboratory Animal Sciences, CAMS&PUMC. STAT3 cDNA was transferred
into the male prokaryote and used to fertilize the ovum by microinjection. The fertilized cells
were transplanted into pseudo-pregnant female mice. The STAT3 gene was identified using
polymerase chain reaction (PCR). STAT3 primer sequences were as follows: STAT3-L: 5’-
GAGAGTCAAGACTGGGCATATGC-3', STAT3-R: 5'--
CCAGCTCACTCACAATGCTTCTC-3', 550bp (NM_0114686.4). The PCR reaction conditions were: 94°C 5min, (95°C 30s, 55°C 30s) × 30 cycles, and 72°C for 5 min. STAT3 protein expression in the dorsal skin of transgenic mice were verified by western blot (Supplementary Figure 1). Positive STAT3 transgenic mice at 16-18 weeks of age were used in all experiments. Non-positive animals were euthanized with CO₂. All mice were raised at 18-22°C and 40-60 % humidity under alternating 12 light/dark conditions. All animal experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH). All experimental procedures were approved by the appropriate animal welfare committee of Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University (No. 2017120101).

After 7 days of adaptive feeding, mice were anesthetized by intraperitoneal injection of 80 mg/kg pentobarbital sodium and dorsal hair shaved, then feed in a single cage. The next day intradermal were induced with recombinant murine IL-17A 100μg/kg (Peprotech, San Diego, CA, USA). At the same time, wild type (WT) (C57BL/6J) mice and STAT3 transgenic mice were intradermally induced with equal volumes of PBS (n=10 per group). After 24 hours, all mice were euthanized with CO₂ inhalation, and skin samples were harvested and stored at -80°C for processing.

RNA-seq and data analysis
Total RNA was extracted using a Qiagen RNeasy column by the manufacturer's protocol. The concentrations of RNA were determined by a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). RNA integrity was evaluated using the RNA Nano 6000 test kit running on Agilent BioAnalyzer 2100 System (Agilent, CA, USA). RNA samples of good quality were used for downstream processing and analysis. All samples were cDNA synthesized, and size was measured using the Agilent 2100. The concentration of the library was quantified by qPCR. According to the manufacturer's instructions, the index-coded samples were clustered on a cBot system using the TruSeq PE Cluster kitv3-cBot-HS(Illumina, Santiago, CA, USA). The Illumina Hiseq2500 (Illumina, Santiago, CA, USA) platform was applied for sequencing and generating paired-end reads.

Differential expressed genes (DEGs) analysis
DEGs analysis was conducted by DESeq R package (1.10.1). DEGs were defined by Genes with an adjusted P-value <0.01 and the absolute value of log2 (Fold change) ≥1.2. DEGs were used to generate Venn diagrams using the online software Venny 2.1. (http://bioinfogp.cnb.csic.es/tools/venny/)

Bioinformatics Analysis
To identify the potential core genes, the DEGs (|log2FC|≥1.2) were inputed into the STRING database (https://string-db.org/) to construct the network and displayed by cytoscape software 3.9.0. Core genes were obtained by CytoNCA analysis (degree greater than two times the median). Gene Ontology (GO) biological process (BP) and Kyoto Encyclopedia of Genes and Genomes pathway (KEGG) analysis were performed using DAVID functional
annotation web resource (https://david-d.ncifcrf.gov). Pathways were performed with bioinformatics Toolbox (http://www.bioinformatics.com.cn/).

**Histological staining and immunofluorescent assays**

The dorsal skin of mice was fixed in 10% buffered formalin and embedded in paraffin for Hematoxylin and eosin (HE) and immunofluorescence staining. Histopathological changes were imaged using the Aperio CS2 scanner (Leica, San Diego, CA, USA). Epidermal thickness was calculated using the Image-Pro Plus software (version 6.0) (Media Cybernetics, Rockville, MD, USA). Paraffin sections were incubated with proliferating cell nuclear antigen (PCNA) (1:800, #13110; CST, Danvers, MA) and involucrin (1:100, ab53112, Abcam, Cambridge, UK) and then subsequently incubated with their corresponding secondary antibodies. Sections were mounted with DAPI Fluoromount-G™ and observed using the Zeiss LSM 710 confocal microscope (Zeiss, Jena, Germany). Images were acquired using excitation wavelengths of 488 nm and 405 nm, and then merged.

**Quantitative Real-Time Polymerase Chain Reaction (qPCR)**

Total RNA was isolated using the RNeasy Mini Kit. cDNA was synthesized with QuantiTect Reverse Transcription Kit. qPCR was performed in triplicate with QuantiTect SYBR Green RT-PCR Kit (Qiagen, Hilden, Germany) in 7500 PCR System (Applied Biosystems, Thermo Fisher, USA). The reaction conditions were as follows: 95°C 30s, 95°C 5s, 60°C 40s for 40 cycles, followed by melting curve. β-actin as an internal gene using the 2^−ΔΔCt method. The primer sequences for qPCR are shown in Supplementary Table 1.

**Statistical analysis**

Epidermal thickness and DEGs were presented as “mean ± SEM”. Comparisons between data were performed using t-tests (and nonparametric tests) by GraphPad Prism 9.0.0 Software. Differences were considered statistically significant at * P < 0.05, ** P< 0.01, and *** P < 0.001.

**Results**

Evaluating skin phenotypes in WT, STAT3 and IL-17A induced STAT3 mice

There was no significant difference in the morphology of the dorsal skin between STAT3 and WT mice. However, IL-17A induced STAT3 mice dorsal skin showed thickening and flaky scaling (Figure 1A). By histopathology, the skin of IL-17A induced STAT3 mice showed epidermal thickening and dermal inflammatory infiltration. The number of PCNA expressions in the epidermis's basal layer increased and the involucrin expression in the stratum corneum up-regulated (Figure 1B). STAT3 and IL-17A induced STAT3 mice significantly differed significantly in epidermal thickening compared to WT mice (Figure 1C).

Alterations in DEGs in WT, STAT3 and IL-17A induced STAT3 mice

RNA expression profiling was performed from samples from the dorsal skin of WT, STAT3 and IL-17A induced STAT3 mice. Cluster analysis of the differentially regulated transcripts were shown in the heatmap image (Figure 2A). The Illumina HiSeq sequence reads generated in this study have been deposited at BioProject accession number PRJNA431348 in the NCBI-SRA database. The venn diagram depicted overlaps of significant DEGs among the three comparisons indicated (Figure 2B).
The major up-regulated DEGs in pairwise comparison and Validation

The top ten up-regulated DEGs for each pairwise comparison are presented in Table 1. The DEGs between WT and IL-17A induced STAT3 mice included S100a protein family such as S100a8 (calgranulin A, MRP-8) and S100a9 (calgranulin B, MRP-14), small proline-rich protein 2 (Sprr2) family such as Sprr2e, Sprr2g and Sprr2d, late cornified envelope-3 (LCE) genes such as LCE3d, LCE3e and LCE3f, et al. Some of the DEGs in STAT3 mice compared with WT mice have been previously reported to be involved in psoriasis, such as IL-1β and chemokine (C-C motif) ligand (CCL) 4.

DEGs (S100a protein, Sprr2 and LCE genes) from pairwise comparisons of skin samples from WT, STAT3 and IL-17A induced STAT3 mice were validated using qPCR. We selected eight genes for validation, which included S100a8, S100a9, Sprr2e, Sprr2g, Sprr2d, LCE3d, LCE3e, LCE3f. The validation results were concordant with RNAseq data for the DEGs. The expression of S100a8, S100a9, Sprr2e, Sprr2d, LCE3d, and LCE3f in IL-17A induced STAT3 were significantly up-regulated compared to WT mice (Figure 3).

Functional Enrichment Analysis

The DEGs from IL-17A induced STAT3 compared to WT mice were used to develop an interactive network of genes using the STRING database and further annotated and modified using Cytoscape. Forty-nine core genes were obtained by CytoNCA analysis (degree greater than two times the median) (Figure 4A and B). Then core DEGs functional analysis and Gene Ontology (GO) analysis were performed by DAVID database. GO terms for biological processes (BP), cellular component (CC), and molecular function (MF) were used. Among the BP, DEGs were mainly concentrated in ‘immune system process’ and ‘neutrophil chemotaxis’. CC were most enriched ‘extracellular region’, ‘cornified envelope’ and ‘keratin filament’. MF like ‘cytokine/chemokine activity’ was significantly enriched (Figure 4C).

Enriched pathways after cluster analysis of the DEGs were shown in Figure 4D. The most significant pathways were ‘IL-17 signaling pathway’, ‘Coronavirus disease-COVID-19’, ‘Viral protein interaction with cytokine and cytokine receptor’, ‘Lipid and atherosclerosis’, ‘Toll-like receptor signaling pathway’, ‘C-type lectin receptor signaling pathway’, ‘NOD-like receptor signaling pathway’, ‘Cytokine-cytokine receptor interaction’, ‘NF-kappa B signaling pathway’, ‘Chemokine signaling pathway’ and ‘TNF signaling pathway’. Among those, ‘IL-17 signaling pathway and Toll-like receptor signaling pathway’ as the main enriched pathways demonstrate the primary pathogenesis mechanism of psoriasis, involving significant genes in the pathway (Figure 4E and F).

Confirmation of core DEGs by qPCR

The core DEGs derived from CytoNCA and functional enrichment analysis were validated using qPCR. We selected eight genes for validation, which included TNF-α, IL-1β, chemokine (C-X-C motif) ligand (CXCL) 1, CXCL2, CCL3, CCL4, CD14, and TLR7. The expressions of TNF-α, IL-1β, CXCL1, CXCL2, CCL3, CCL4, and CD14 in IL-17A induced STAT3 were significantly up-regulated compared to WT mice. The expressions of TLR7 also have an increasing trend (Figure 5).
DISCUSSION

Psoriasis is a chronic inflammatory skin disease characterized by immune-mediated keratinocytes excessive proliferation and differentiation. In this study, we demonstrated the essential role of IL-17A and STAT3 in the pathogenesis of psoriasis. IL-17A induced STAT3 mice dorsal skin displayed pathological manifestations of epidermal thickening and silvery lamellar scales.

The expressions of PCNA and involucrin were increased, indicating the pathological features of hyperkeratosis and parakeratosis. The DEGs identified between IL-17A induced STAT3 mice versus WT mice included S100a8, S100a9, Sprr2 protein, and Lce genes and have been identified in previous studies to be associated with psoriatic patients. The core genes including TNF-α, IL-1β, CXCL1, CXCL2, CCL3, CCL4, CD14, and TLR7 were increased in IL-17A induced STAT3 mice. Bioinformatics analysis of the core DEGs enriched for the IL-17 signaling pathway, immune inflammation, cell chemotaxis, and keratinization reflected the pathological features of psoriasis. Hence our study demonstrated that activation of the IL-17A and STAT3 pathways are crucial for the pathogenesis of psoriasis.

Psoriatic hallmark features include epidermal acanthosis, hyperkeratosis, and parakeratosis. IL-17A is involved in keratinocyte differentiation. We found a dozen epidermal differentiation complex (EDC) genes that were differentially expressed after IL-17A injection into STAT3 mice. The EDC contains several genes that include loricrin, involucrin, S100a calcium-binding proteins, Sprr2 proteins and Lce genes. They are located on human chromosome 1q21 with character traits that are involved in epidermal differentiation. These gene clusters are located on mouse chromosome 3 and have the same differentiation functions as observed in humans. S100a8 and S100a9 are known for their antimicrobial function and are expressed in differentiated keratinocytes. They colocalize with differentiation markers such as involucrin.

In addition, S100a8 and S100a9 are potent pro-inflammatory mediators and are triggered by IL-17A. Sprr2 encodes for a family of cornified cell envelope precursor proteins and is strongly induced during psoriatic hyperproliferation. The Sprr2 family of genes in mice consists of 11 isoforms (Sprr2a–2k) and are differentially expressed in epithelial tissues. Sprr2 was found to be non-coordinately up-regulated by IL-6/gp130/STAT3 signaling and functions in cell migration and wound healing.

The Lce gene cluster has been reported to be a susceptibility locus for psoriasis through genome-wide analysis. Lce genes encode stratum corneum proteins of the cornified envelope that function in epidermal terminal differentiation and are modulated by Th17 cytokines. We found Lce3d-f transcript to be overexpressed in both STAT3 and IL-17 induced STAT3 mice. The strong association of Lce3a and Lce3d locus with the severity of psoriasis has been reported. Lce3e is significantly up-regulated to compensate for LCE3a/b/c/d-deletions, while the role of Lce3f has not been reported. We observed terminal differentiation in
granular keratinocytes and incomplete squamous corneocyte formation in IL-17 induced STAT3 mice, resulting in characteristic psoriasis lesions.

The core DEGs found by CytoNCA analysis were inflammatory cytokines (TNF-α and IL-1β) and chemokines (CXCL1, CXCL2, CCL3, and CCL4), which were mainly secreted by IL-17A-induced keratinocytes. They were verified by qPCR to be highly expressed in IL-17A-induced STAT3 mice. TNF-α is a proinflammatory cytokine produced by multiple cells, including T cells and keratinocytes. It amplifies inflammation through the induction of expression of other proinflammatory cytokines and neutrophil chemokines, and the effects of other cytokines, including IL-17. (Chiricozzi et al. 2011) TNF-α inhibitors or monoclonal antibodies have been proven to be effective treating of psoriasis. (Yiu et al. 2022) IL-1β is a well-known inflammation inducer and effector. In psoriasis, IL-1β is mediated by an apoptosis-associated speck-like protein containing a caspase recruitment domain, such as NLRP3 and AIM2. (Zwicker et al. 2017) CXCL1 is a neutrophil-activating protein-3 derived from keratinocytes. CXCL2 is also called macrophage inflammatory protein-2a (MIP-2α). CXCL1 and CXCL2 recruitment to sites of inflammation by binding to CXC chemokine receptor 2 (CXCR2). (Sellau & Groneberg 2020) CCL3 (MIP-1α) and CCL4 (MIP-1β) produced by stimulated leukocytes, fibroblasts, and tumor cells, and induce chemotaxis of T cells, monocytes, NK cells, and dendritic cells, have diverse effects on various types of immune and nonimmune cells under its interaction with its specific receptor, CCR5. (Yazdani et al. 2020) CD14 is a co-receptor for several other toll-like receptors (TLRs), including TLR2, TLR4, TLR7, and TLR9. The overexpression of CD14 in pathological epidermis results in potentiated inflammatory signaling, leading to diminished epidermal barrier function. (Dolivo et al. 2022)

The BP of GO analysis was enriched for the immune system process and chemotaxis. CC was enriched for the extracellular region, cornified envelope, and keratin filament. MF was enriched for cytokine and chemokine activity. All of the above present the pathological features of psoriasis. (Mehta et al. 2017) KEGG analyses of the clustered DEGs enriched for the IL-17 signaling pathway and Toll-like receptor signaling pathway. The enrichment for pathways and terms was similar to RNA sequencing on skin biopsy samples from psoriasis patients and healthy controls. (Dou et al. 2017) (Niehues et al. 2017) TLRs are generally upregulated and promote the transcription and release of proinflammatory cytokines. (Chen et al. 2016) In addition, DEGs were found to be clustered for Lipid and atherosclerosis, which was identified that could provide a mechanistic link between psoriasis and an increased risk of cardiovascular diseases. (Hu & Lan 2017; Li et al. 2014)

CONCLUSIONS

STAT3 is a critical factor in Th17 cell differentiation and keratinocyte proliferation. Administration of exogenous IL-17A significantly hyperactivates STAT3 resulting in proliferation and differentiation of keratinocytes. Keratinocytes, in turn, produce some cytokines and chemokines to trigger immune-inflammatory responses and promote Th17 cells to secrete endogenous IL-17A. IL-17A and STAT3 circuit activation ultimately result in psoriatic skin lesions. We established an animal model by injecting IL-17A into STAT3 transgenic mice and
validated the IL-17A and STAT3 circuit activation as a critical pathway in the pathogenesis of psoriasis (Figure 6).

**Data Availability**

The following information was supplied regarding data availability. The raw measurements are available in the Supplemental Files.

**Competing Interests**

The authors declare that they have no competing interests.

**Funding**

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**REFERENCES**


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Sellau J, and Groneberg M. 2020. Androgens predispose males to monocytomediated immunopathology by inducing the expression of leukocyte recruitment factor CXCL1. 11:3459. https://doi.org/10.1038/s41467-020-17260-y


Table 1 (on next page)

The top ten up-regulated DEGs in each pairwise comparison
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Figure 1

Skin phenotypes in WT, STAT3, and IL-17A induced STAT3 mice.

STAT3 mice and IL-17A induced STAT3 mice versus wild type (C57BL/6) mice, respectively (n=10). (A) Images of psoriasiform skin. (B) Skin biopsies were stained with hematoxylin and eosin (H&E). Mitotic basal cells are shown in the insets. Scale bar=100μm. Representative PCNA and involucrin immunostained images of the dorsal skin. Scale bar=20μm. (C) Epidermal thickness was measured using the image analysis system. Results are shown as mean+SEM. *P<0.05, **P<0.01, and ***P<0.001.
Figure 2

Differential gene expression in WT, STAT3, and IL-17A induced STAT3 mice.

(A) Hierarchical clustering of DEGs from the skin of WT, STAT3, and IL-17A induced STAT3 mice. Genes ordered based on k-meanclustering (FDR<0.05, log2FC>1.5-fold difference) (n=3). (B) Venn diagram depicts the overlap of DEGs between each pairwise comparison.
Figure 3

qPCR validation of the top ten up-regulated genes in WT, STAT3, and IL-17A induced STAT3 mice (n=3).

Eight mRNAs were selected for qPCR validation of the sequencing analysis data. The expressions of S100A8, S100A9, Sprr2e, Sprr2d, LCE3e, and LCE3f in IL-17A induced STAT3 mice were significantly up-regulated compared to WT mice. The expressions of S100A9, Sprr2e, Sprr2d, LCE3d, LCE3e, and LCE3f in STAT3 mice were up-regulated compared to WT mice. The ratio of each mRNA relative to β-actin was calculated using the $2^{-\Delta\Delta Ct}$ method. Differences were considered statistically significant at *$P<0.05$, **$P<0.01$, and ***$P<0.001$. 
Figure 4

Bioinformatics analysis of DEGs

(A) Protein-protein interaction networks analysis. The network construct from the DEGs (|log2FC|≥1.2) in IL-17A induced STAT3 mice compared to WT mice. The nodes represent proteins. Edges stand for protein-protein associations. The disconnected nodes in the network were hidden. (B) Forty-nine core genes were obtained by CytoNCA analysis (degree greater than two times the median) using Cytoscape (www.cytoscape.org). (C-D) GO analysis and KEGG pathway enrichment analysis of DEGs using the online DAVID database (https://david-d.ncifcrf.gov). (C) GO enrichment analysis for biological process, cellular component, and molecular function. (D) KEGG pathway enrichment analysis. (E) IL-17 signaling pathway. (F) Toll-like receptor signaling pathway. Red boxes are represented upregulated DEGs detected by RNA-seq.
Figure 5

qPCR validation of the core genes in WT, STAT3, and IL-17A induced STAT3 mice (n=3).

Eight mRNAs were selected for qPCR validation of the enrichment pathway. The expressions of TNF-α, IL-1β, CXCL1, CCL3, CCL4, and CD14 in IL-17A induced STAT3 mice were significantly up-regulated compared to WT mice. The expressions of TNF-α, IL-1β, CCL4, and CD14 in STAT3 mice were up-regulated compared to WT mice. The ratio of each mRNA relative to β-actin was calculated using the $2^{-\Delta\Delta Ct}$ method. Differences were considered statistically significant at $^*P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$. 
Figure 6

Schematic diagram illustrating the mechanism. STAT3 is a critical nuclear transcription factor in Th17 cell differentiation and keratinocyte proliferation.

Administration of exogenous IL-17A significantly hyperactivates STAT3 resulting in hyperkeratosis and parakeratosis. Keratinocytes, in turn, produce some cytokines and chemokines to promote Th17 cells to secrete endogenous IL-17A. IL-17A and STAT3 circuit activation ultimately result in psoriatic skin lesions.