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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.

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ABSTRACT

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

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.{Egeberg, 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℃ 5min, (95℃ 30s, 55℃ 30s) × 30 cycles, and 72℃ 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 CO_2. All mice were raised at 18-22℃ 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 CO_2 inhalation, and skin samples were harvested and stored at -80℃ 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^{TM} 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 syntheized 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℃ 30s, 95℃ 5s, 60℃ 40s for 40 cycles, followed by melting curve. β-actin as an internal gene using the $2^{-\Delta\Delta Ct}$ 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.(Bhattacharya et al. 2018; D'Erme et al. 2015) 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.(Pfaff et al. 2017) 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.(Mischke et al. 1996) They are located on human chromosome 1q21 with character traits that are involved in epidermal differentiation.(Motomu et al. 1997) 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.(Schmidt et al. 2001) In addition, S100a8 and S100a9 are potent pro-inflammatory mediators and are triggered by IL-17A.(Podgorska et al. 2018)·(Jin et al. 2014) Sprr2 encodes for a family of cornified cell envelope precursor proteins and is strongly induced during psoriatic hyperproliferation.(Hohl et al. 1995) 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.(Lambert et al. 2017; Nozaki et al. 2004) The Lce gene cluster has been reported to be a susceptibility locus for psoriasis through genome-wide analysis.(Zhang et al. 2009) Lce genes encode stratum corneum proteins of the cornified envelope that function in epidermal terminal differentiation and are modulated by Th17 cytokines.(Bergboer et al. 2011) 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.(Julia et al. 2012) Lce3e is significantly up-regulated to compensate for LCE3a/b/c/d-deletions (Karrys et al. 2018), 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.

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REFERENCES

Bergboer JG, Tjabringa GS, Kamsteeg M, van Vlijmen-Willems IM, Rodijk-Olthuis D, Jansen PA, Thuret JY, Narita M, Ishida-Yamamoto A, Zeeuwen PL et al. . 2011. Psoriasis risk genes of the late cornified envelope-3 group are distinctly expressed compared with genes of other LCE groups. *The American journal of pathology* 178:1470-1477.https://doi.org/10.1016/j.ajpath.2010.12.017

Bhattacharya S, Kim JC, Ogawa Y, Nakato G, Nagle V, Brooks SR, Udey MC, and Morasso MI. 2018. DLX3-Dependent STAT3 Signaling in Keratinocytes Regulates Skin Immune Homeostasis. *Journal of Investigative Dermatology* 138:1052-1061.https://doi.org/10.1016/j.jid.2017.11.033

Calautti E, Avalle L, and Poli V. 2018. Psoriasis: A STAT3-Centric View. *Int J Mol Sci* 19:1-14.https://doi.org/10.3390/ijms19010171

Chen JQ, Szodoray P, and Zeher M. 2016. Toll-Like Receptor Pathways in Autoimmune Diseases. *Clin Rev Allergy Immunol* 50:1-17.https://doi.org/10.1007/s12016-015-8473-z

Chiricozzi A, Guttman-Yassky E, Suarez-Farinas M, Nograles KE, Tian S, Cardinale I, Chimenti S, and Krueger JG. 2011. Integrative responses to IL-17 and TNF-alpha in human keratinocytes account for key inflammatory pathogenic circuits in psoriasis. *Journal of Investigative Dermatology* 131:677-687.https://doi.org/10.1038/jid.2010.340

D'Erme AM, Wilsmann-Theis D, Wagenpfeil J, Hölzel M, Ferring-Schmitt S, Sternberg S, Wittmann M, Peters B, Bosio A, Bieber T et al. . 2015. IL-36γ (IL-1F9) Is a Biomarker for Psoriasis Skin Lesions. *Journal of Investigative Dermatology* 135:1025-1032.https://doi.org/10.1038/jid.2014.532

Dolivo D, Rodrigues A, Sun L, Galiano R, Mustoe T, and Hong SJ. 2022. Reduced hydration regulates pro-inflammatory cytokines via CD14 in barrier function-impaired skin. *Biochim Biophys Acta Mol Basis Dis* 1868:166482.https://doi.org/10.1016/j.bbadis.2022.166482

Dou J, Zhang L, Xie X, Ye L, Yang C, Wen L, Shen C, Zhu C, Zhao S, Zhu Z et al. . 2017. Integrative analyses reveal biological pathways and key genes in psoriasis. *British Journal of Dermatology* 177:1349-1357.https://doi.org/10.1111/bjd.15682

Egeberg A, Gisondi P, Carrascosa JM, Warren RB, and Mrowietz U. 2020. The role of the interleukin-23/Th17 pathway in cardiometabolic comorbidity associated with psoriasis. *J Eur Acad Dermatol Venereol* 34:1695-1706.https://doi.org/10.1111/jdv.16273

Hohl D, de Viragh PA, Arniguet-Barras F, Gibbs S, Backendorf C, and Huber M. 1995. The Small Proline-Rich Proteins Constitute a Multigene Family of Differentially Regulated Cornified Cell Envelope Precursor Proteins. *Journal of Investigative Dermatology* 104:902-909.https://doi.org/10.1111/1523-1747.ep12606176

Hu SC, and Lan CE. 2017. Psoriasis and Cardiovascular Comorbidities: Focusing on Severe Vascular Events, Cardiovascular Risk Factors and Implications for Treatment.

International Journal of Molecular Sciences 18:2211.https://doi.org/10.3390/ijms18102211

Jin S, Park CO, Shin JU, Noh JY, Lee YS, Lee NR, Kim HR, Noh S, Lee Y, Lee J-H et al. . 2014. DAMP molecules S100A9 and S100A8 activated by IL-17A and house-dust mites are increased in atopic dermatitis. *Experimental Dermatology* 23:938-941.https://doi.org/10.1111/exd.12563

Julia A, Tortosa R, Hernanz JM, Canete JD, Fonseca E, Ferrandiz C, Unamuno P, Puig L, Fernandez-Sueiro JL, Sanmarti R et al. . 2012. Risk variants for psoriasis vulgaris in a large case-control collection and association with clinical subphenotypes. *Hum Mol Genet* 21:4549-4557.https://doi.org/10.1093/hmg/dds295

Karrys A, Rady I, Chamcheu RN, Sabir MS, Mallick S, Chamcheu JC, Jurutka PW, Haussler MR, and Whitfield GK. 2018. Bioactive Dietary VDR Ligands Regulate Genes Encoding Biomarkers of Skin Repair That Are Associated with Risk for Psoriasis. *Nutrients* 10:1-20.https://doi.org/10.3390/nu10020174

Lambert S, Swindell WR, Tsoi LC, Stoll SW, and Elder JT. 2017. Dual role of Act1 in keratinocyte differentiation and host defense: TRAF3IP2 silencing alters keratinocyte differentiation while inhibiting IL-17 responses. *Journal of Investigative Dermatology* 137:1501-1511.https://doi.org/10.1016/j.jid.2016.12.032

Li RC, Krishnamoorthy P, DerOhannessian S, Doveikis J, Wilcox M, Thomas P, Rader DJ, Reilly MP, Van Voorhees A, Gelfand JM et al. . 2014. Psoriasis is associated with decreased plasma adiponectin levels independently of cardiometabolic risk factors. *Clinical and Experimental Dermatology* 39:19-24.https://doi.org/10.1111/ced.12250

Mehta NN, Teague HL, Swindell WR, Baumer Y, Ward NL, Xing X, Baugous B, Johnston A, Joshi AA, Silverman J et al. . 2017. IFN-gamma and TNF-alpha synergism may provide a link between psoriasis and inflammatory atherogenesis. *Scientific Reports* 7:13831.https://doi.org/10.1038/s41598-017-14365-1

Mischke D, Korge BP, Marenholz I, Volz A, and Ziegler A. 1996. Genes Encoding Structural Proteins of Epidermal Cornification and S100 Calcium-Binding Proteins Form a Gene Complex ("Epidermal Differentiation Complex") on Human Chromosome 1q21. *Journal of Investigative Dermatology* 106:989-992.https://doi.org/10.1111/1523-1747.ep12338501

Motomu M, Masayuki M, Hajime S, and Hideoki O. 1997. Epidermal structural proteins in skin disorders. *Journal of Dermatological Science* 15:143-165.

Niehues H, Tsoi LC, van der Krieken DA, Jansen PAM, Oortveld MAW, Rodijk-Olthuis D, van Vlijmen I, Hendriks W, Helder RW, Bouwstra JA et al. . 2017. Psoriasis-Associated Late Cornified Envelope (LCE) Proteins Have Antibacterial Activity. *Journal of Investigative Dermatology* 137:2380-2388.https://doi.org/10.1016/j.jid.2017.06.003

Nozaki I, Lunz JG, Specht S, Stolz DB, Taguchi K, Subbotin VM, Murase N, and Demetris AJ. 2004. Small proline-rich proteins 2 are noncoordinately upregulated by IL-6/STAT3 signaling after bile duct ligation. *Laboratory Investigation* 85:109-123.https://doi.org/10.1038/labinvest.3700213

Pfaff CM, Marquardt Y, Fietkau K, Baron JM, and Luscher B. 2017. The psoriasis-associated IL-17A induces and cooperates with IL-36 cytokines to control keratinocyte differentiation and function. *Scientific Reports* 7:15631.https://doi.org/10.1038/s41598-017-15892-7

Podgorska M, Oldak M, Marthaler A, Fingerle A, Walch-Ruckheim B, Lohse S, Muller CSL, Vogt T, Ustav M, Wnorowski A et al. . 2018. Chronic Inflammatory Microenvironment in Epidermodysplasia Verruciformis Skin Lesions: Role of the Synergism Between HPV8 E2 and C/EBPbeta to Induce Pro-Inflammatory S100A8/A9 Proteins. *Frontiers in microbiology* 9:1-12.https://doi.org/10.3389/fmicb.2018.00392

Sakkas LI, and Bogdanos DP. 2017. Are psoriasis and psoriatic arthritis the same disease? The IL-23/IL-17 axis data. *Autoimmunity Reviews* 16:10-15.https://doi.org/10.1016/j.autrev.2016.09.015

Sano S, Chan KS, Carbajal S, Clifford J, Peavey M, Kiguchi K, Itami S, Nickoloff BJ, and DiGiovanni J. 2004. Stat3 links activated keratinocytes and immunocytes required for development of psoriasis in a novel transgenic mouse model. *Nature Medicine* 11:43-49.https://doi.org/10.1038/nm1162

Schmidt M, Gillitzer R, Toksoy A, Bröcker E-B, Rapp UR, Paus R, Roth J, Ludwig S, and Goebeler M. 2001. Selective Expression of Calcium-Binding Proteins S100A8 and S100A9 at Distinct Sites of Hair Follicles. *Journal of Investigative Dermatology* 117:748-750.https://doi.org/10.1046/j.0022-202x.2001.01485.x

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

Shi X, Jin L, Dang E, Chang T, Feng Z, Liu Y, and Wang G. 2011. IL-17A Upregulates Keratin 17 Expression in Keratinocytes through STAT1- and STAT3-Dependent Mechanisms. *Journal of Investigative Dermatology* 131:2401-2408.https://doi.org/10.1038/jid.2011.222

Swindell WR, Johnston A, Carbajal S, Han G, Wohn C, Lu J, Xing X, Nair RP, Voorhees JJ, Elder JT et al. . 2011. Genome-wide expression profiling of five mouse models identifies similarities and differences with human psoriasis. *PLoS One* 6:e18266.https://doi.org/10.1371/journal.pone.0018266

Tripathi SK, Chen Z, Larjo A, Kanduri K, Nousiainen K, Aijo T, Ricano-Ponce I, Hrdlickova B, Tuomela S, Laajala E et al. . 2017. Genome-wide Analysis of STAT3-Mediated Transcription during Early Human Th17 Cell Differentiation. *Cell Rep* 19:1888-1901.https://doi.org/10.1016/j.celrep.2017.05.013

Vinita B, Zanobia S, Jennifer N, Gill., Malari A, Coburn., Patrick A, John D, J M, Mathis., Runhua S, John L, Clifford., and Heather E, Kleiner,Hancock. 2012. Effects of the tropical ginger compound,1'-acetoxychavicol acetate, against tumor promotion in K5.Stat3C transgenic mice. *Journal of Experimental & Clinical Cancer Research* 31:1-14.

Yazdani Z, Mousavi Z, Ghasemimehr N, Kalantary Khandany B, Nikbakht R, Jafari E, Fatemi A, and Hassanshahi G. 2020. Differential regulatory effects of chemotherapeutic protocol on CCL3_CCL4_CCL5/CCR5 axes in acute myeloid leukemia patients with monocytic lineage. *Life Sci* 240:117071.https://doi.org/10.1016/j.lfs.2019.117071

Yiu ZZN, Becher G, Kirby B, Laws P, Reynolds NJ, Smith CH, Warren RB, and Griffiths CEM. 2022. Drug Survival Associated With Effectiveness and Safety of Treatment With Guselkumab, Ixekizumab, Secukinumab, Ustekinumab, and Adalimumab in Patients With Psoriasis. *JAMA Dermatol*.https://doi.org/10.1001/jamadermatol.2022.2909

Zhang XJ, Huang W, Yang S, Sun LD, Zhang FY, Zhu QX, Zhang FR, Zhang C, Du WH, Pu XM et al. . 2009. Psoriasis genome-wide association study identifies susceptibility variants within LCE gene cluster at 1q21. *Nature genetics* 41:205-210.https://doi.org/10.1038/ng.310

Zwicker S, Hattinger E, Bureik D, Batycka-Baran A, Schmidt A, Gerber PA, Rothenfusser S, Gilliet M, Ruzicka T, and Wolf R. 2017. Th17 micro-milieu regulates NLRP1-dependent caspase-5 activity in skin autoinflammation. *PLoS One* 12:e0175153.https://doi.org/10.1371/journal.pone.0175153

Table 1(on next page)

The top ten up-regulated DEGs in each pairwise comparison

Table 1:

The top ten up-regulated DEGs in each pairwise comparison

	WT vs STAT3		WT vs STAT3+IL-17A		STAT3 vs STAT3+IL-17A	
	Gene symbol	**logFC**	**Gene symbol**	**logFC**	**Gene symbol**	**logFC**
1	Mup1	2.71	Sprr2e	7.56	Gm5414	4.38
2	Clec4e	2.68	Sprr2g	6.32	Sh2d5	4.18
3	Mup7	2.53	Chil3	6.05	Lcn2	3.13
4	Il1b	2.46	S100A8	6.04	Krt84	3.11
5	Irg1	2.27	Gm5414	5.36	Ca4	2.99
6	Ccl4	2.25	Lce3d	5.34	Sprr2b	2.96
7	Mup14	2.16	S100A9	5.28	Saa3	2.90
8	Trem3	2.15	Lce3f	5.12	Sprr1b	2.86
9	Mup19	2.15	Lce3e	5.00	Sprr2h	2.82
10	Trim30b	2.15	Sprr2d	4.94	Timp1	2.74

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.

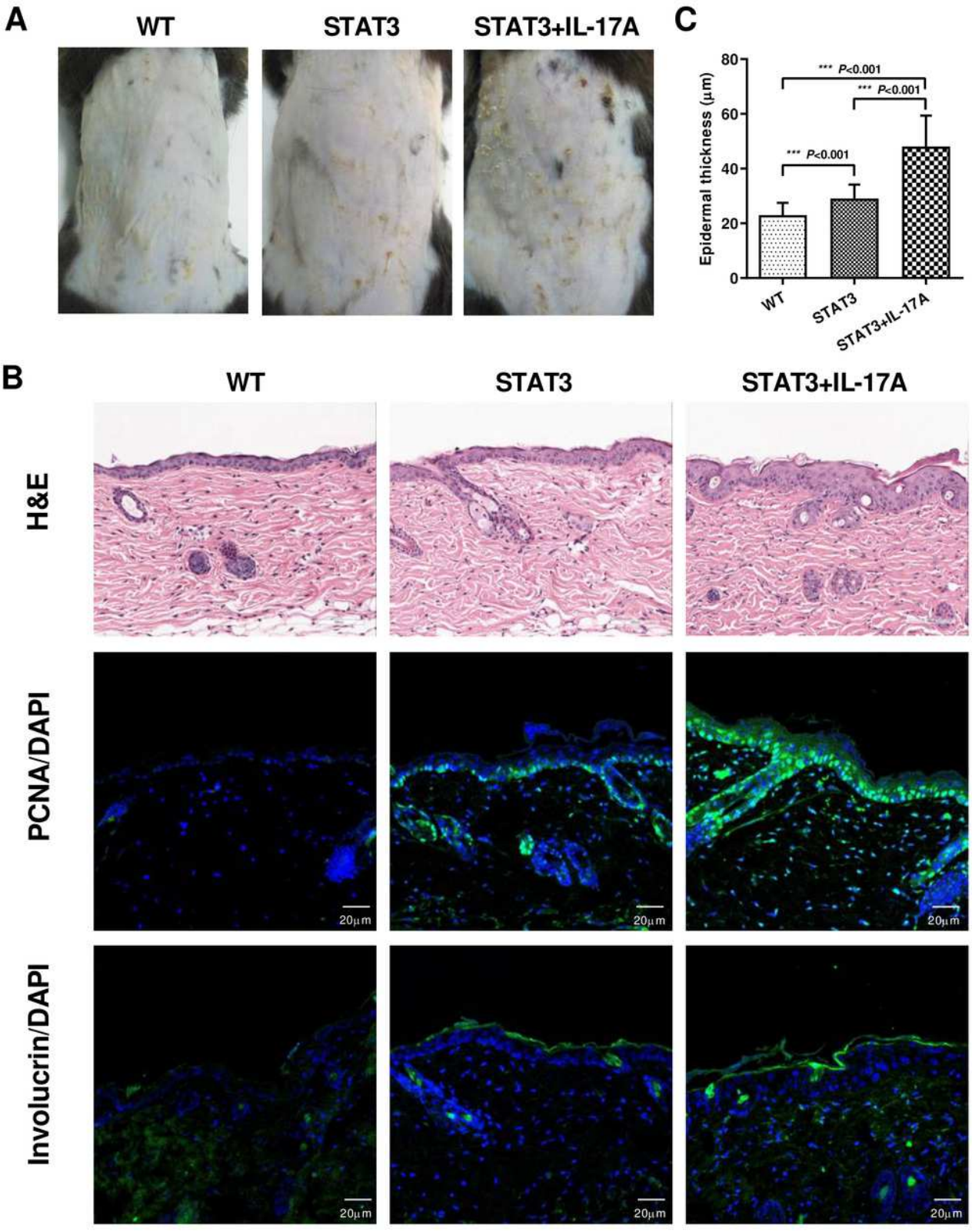
A
WT
STAT3
STAT3+IL-17A
C
Epidermal thickness (μm)
80
60
40
20
0
*** P<0.001
*** P<0.001
*** P<0.001
WT
STAT3
STAT3+IL-17A
B
WT
STAT3
STAT3+IL-17A
H&E
PCNA/DAPI
Involucrin/DAPI
20μm

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 onk-meanclustering (FDR<0.05, log2FC>1.5-fold difference) (n=3). (B) Venn diagram depicts the overlap of DEGs between each pairwise comparison.

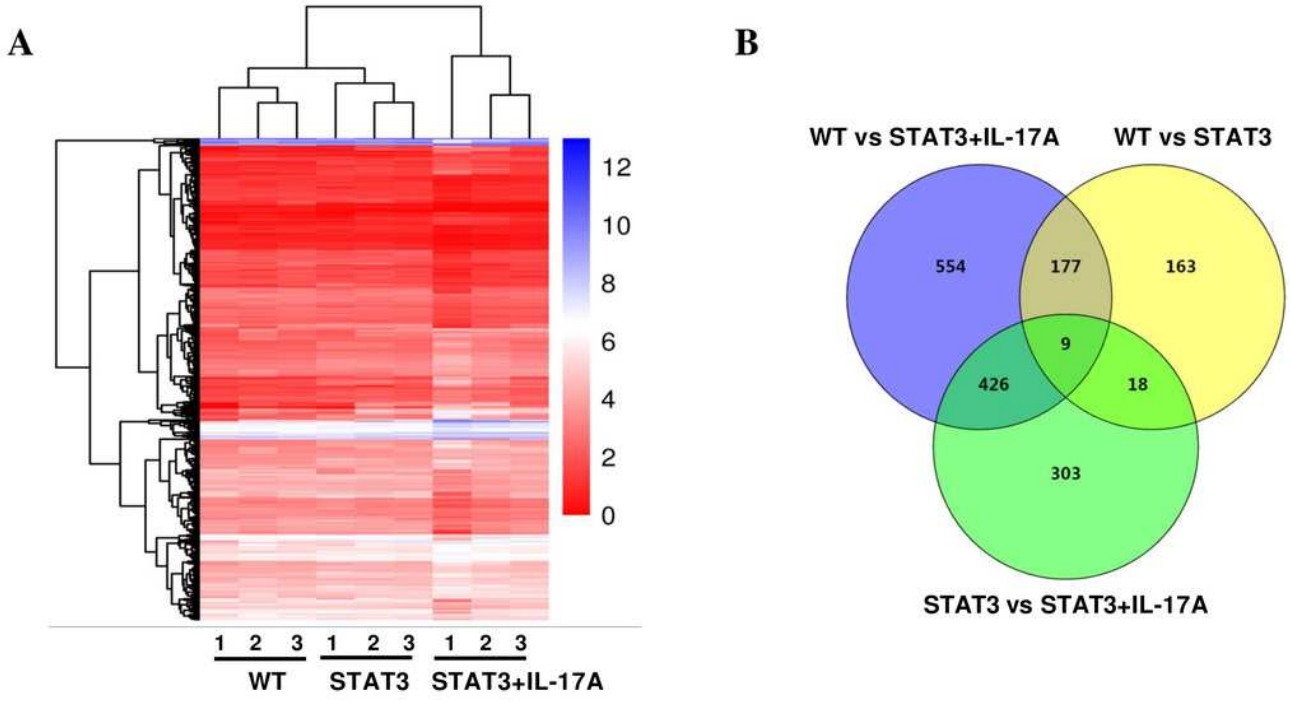

Figure 3

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 $^{***}P<0.001$.

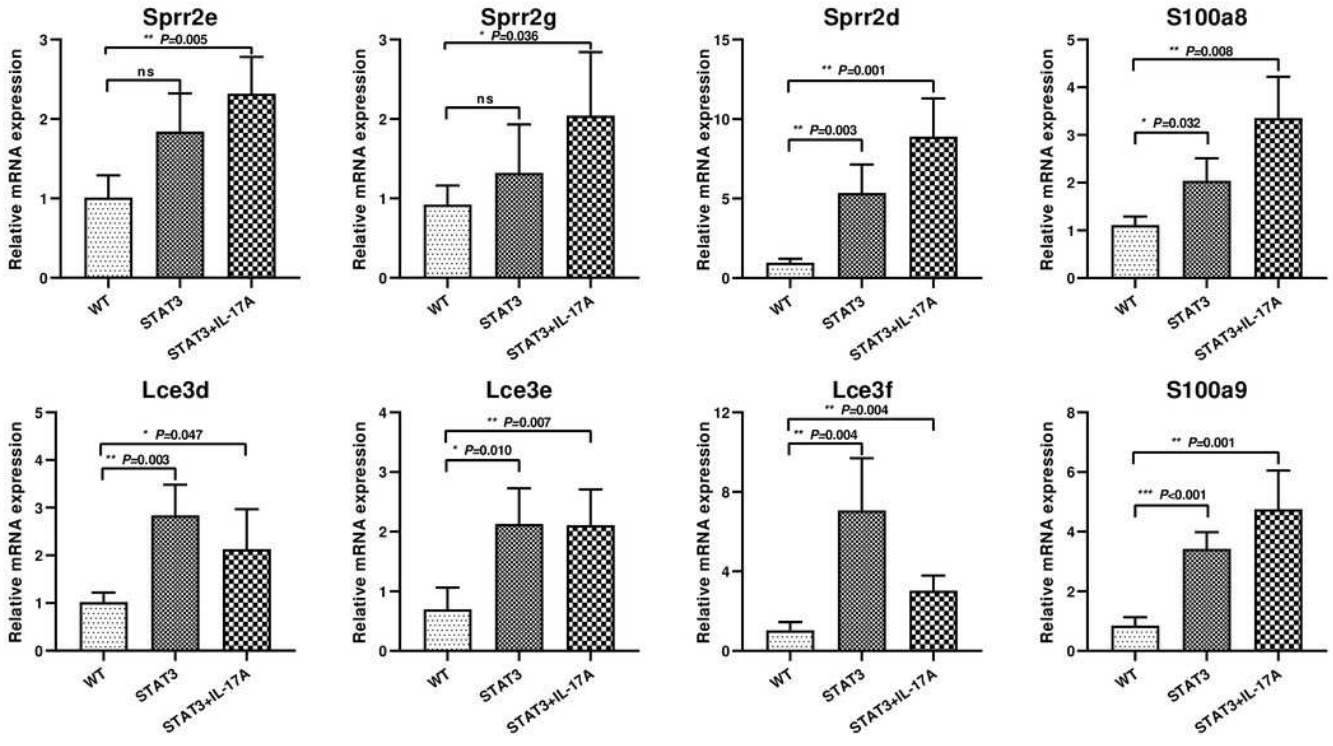

Figure 4

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) usingCytoscape (www.cytoscape.org). **(C-D)** GOanalysisand KEGG pathway enrichmentanalysisof DEGs using the online DAVID database (https://david-d.ncifcrf.gov). **(C)** GOenrichmentanalysisfor biological process, cellular component, and molecular function. **(D)** KEGGpathway enrichment analysis.**(E)** IL-17 signaling pathway. **(F)** Toll-like receptor signaling pathway. Red boxes arerepresented upregulated DEGs detected by RNA-seq.

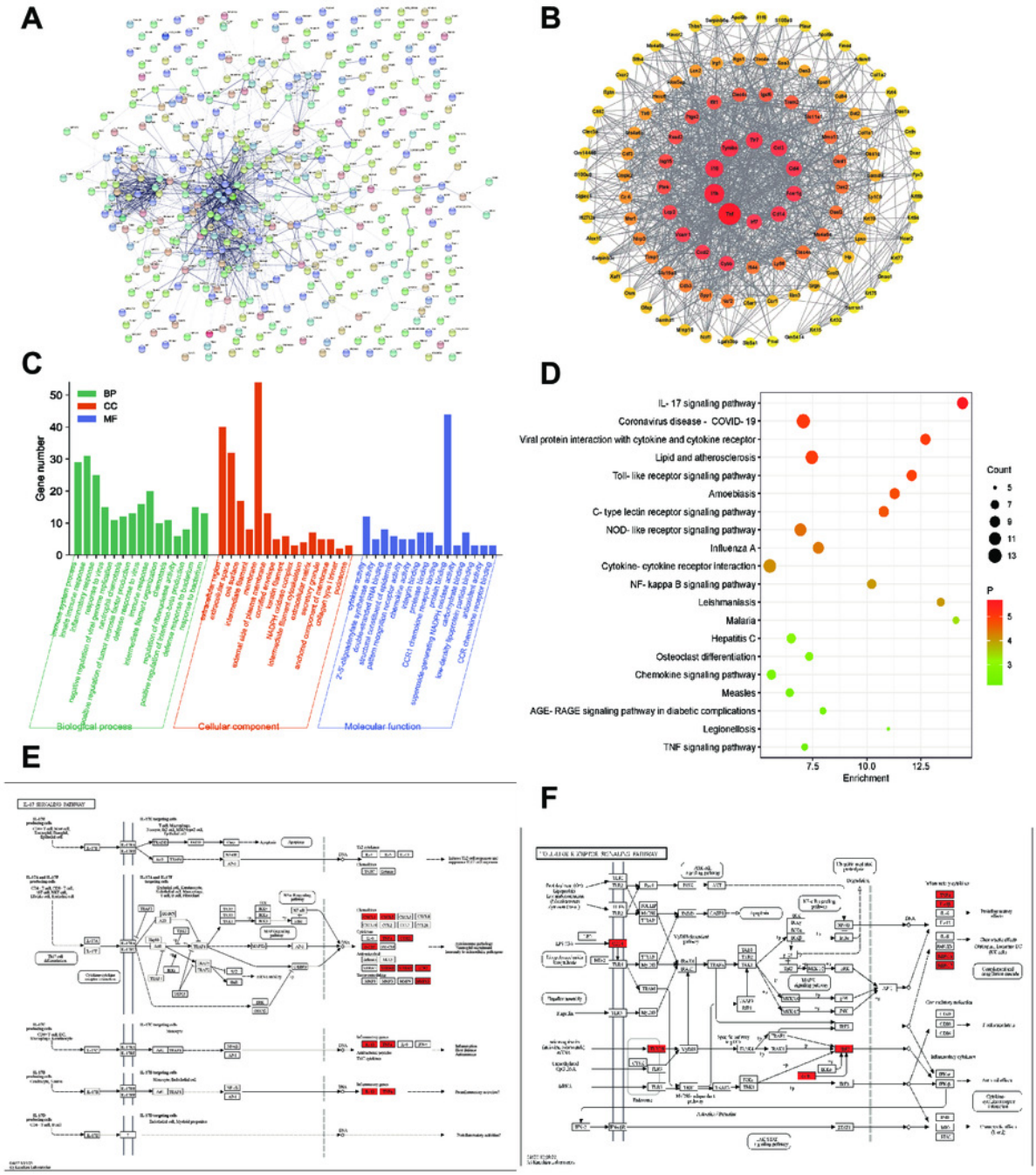
A
B
C
BP
CC
MF
Gene number
50
40
30
20
10
0
Biological process
Cellular component
Molecular function
D
IL- 17 signaling pathway
Coronavirus disease - COVID- 19
Viral protein interaction with cytokine and cytokine receptor
Lipid and atherosclerosis
Toll- like receptor signaling pathway
Amoebiasis
C- type lectin receptor signaling pathway
NOD- like receptor signaling pathway
Influenza A
Cytokine- cytokine receptor interaction
NF- kappa B signaling pathway
Leishmaniasis
Malaria
Hepatitis C
Osteoclast differentiation
Chemokine signaling pathway
Measles
AGE- RAGE signaling pathway in diabetic complications
Legionellosis
TNF signaling pathway
7.5
10.0
12.5
Enrichment
Count
5
7
9
11
13
P
5
4
3
E
F

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.

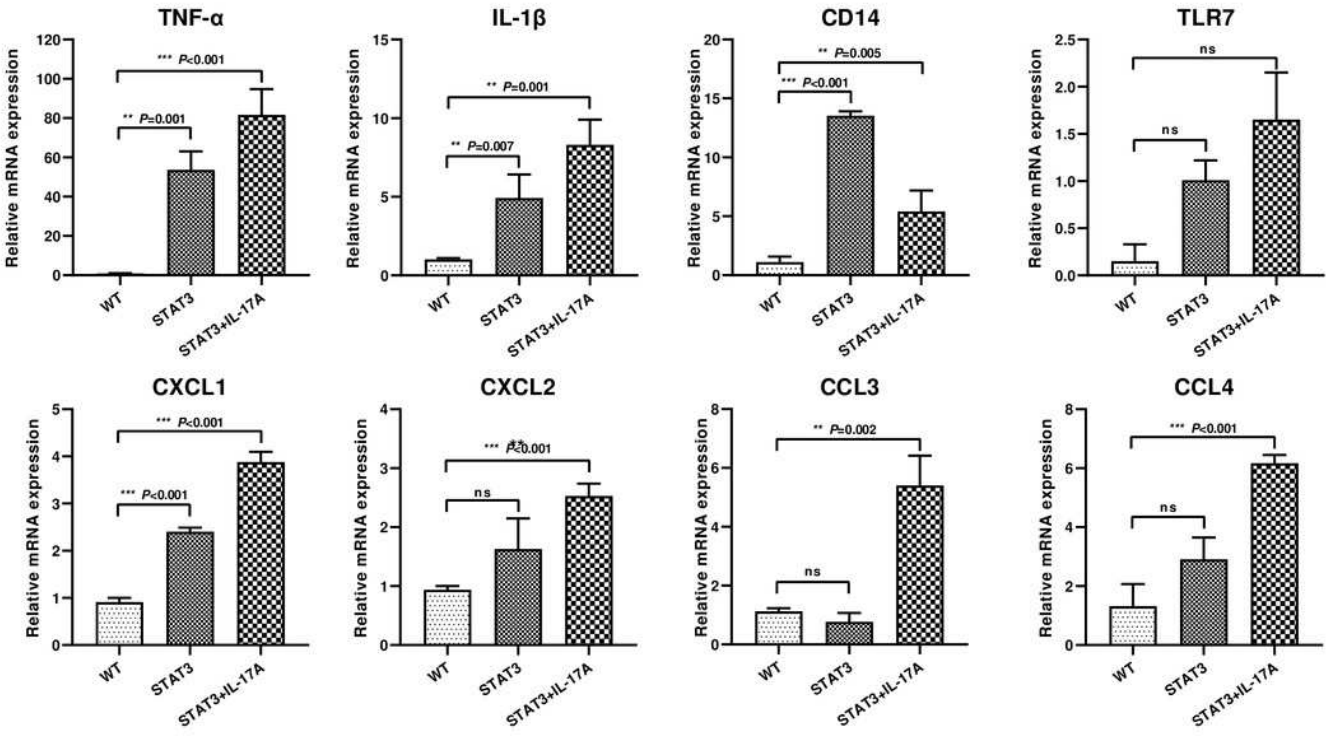

Figure 6

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.