Research Article

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ARG1 and CXCL2 are potential biomarkers target for psoriasis patients

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Abstract

Background: Psoriasis is a common chronic skin inflammatory disease. Understanding the pathogenesis of psoriasis and identifying novel therapeutic targets are under investigation.
Methods: Gene expression profiles were obtained from GSE13355, GSE30999 and GSE54456 datasets to identify differentially expressed genes (DEGs) between psoriasis and normal controls. Enrichment analysis was used to identify the biological functions and pathways of common genes from three groups of DEGs. Protein-protein interaction (PPI) network was then constructed to identify key genes according to degree of connectivity. Expression of genes was detected by the method of qRT-PCR and immunohistochemistry. The infiltration of immune cells of psoriasis were quantified and detected by flow cytometry.
Results: A total of 146 common genes were identified between psoriasis and normal controls. They were significantly enriched in IL-17, chemokine, and NOD-like receptor (NLR) signaling pathway. Ten key genes were selected with bigger degree of connectivity through PPI network, and ARG1 and CXCL2 had better predictive ability based on ROC curves. Increased expression of ARG1 and CXCL2 in psoriasis patients were verified by qRT-PCR and immunohistochemistry method. In addition, a lot of immune cells were upregulated in psoriasis compared to healthy controls through ssGSEA and flow cytometry.
Conclusion: ARG1 and CXCL2 may serve as biomarkers and potential therapy for psoriasis. This may be related to the immune response and NLR pathway.

Keywords

psoriasis, bioinformatics, NOD-like receptor, ARG1, CXCL2

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Introduction

Psoriasis is a chronic inflammatory skin disease with strong genetic predisposition and autoimmunity, causing severe damage to patients' quality of life.[1] The worldwide prevalence of psoriasis is estimated to be 2–4%.[2] Psoriatic patients often present with concomitant psoriatic arthritis, autoimmune diseases, cardiovascular diseases as well as metabolic diseases.[3] Despite the variety of methods for treating psoriasis, 52.3% of psoriatic patients reported that they were not satisfied with the treatment due to ineffective methods and adverse effects.[4] Previous studies have shown that life expectancy of patients with moderate to severe psoriasis is reduced by approximately 5 years.[5] Therefore, it is important to explore the molecular dysregulation mechanisms of psoriasis and thereby develop effective means of evaluation and treatment.

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The dermatological manifestations of psoriasis are diverse, and psoriasis vulgaris, also known as plaque psoriasis, is the most common type of psoriasis, which may be associated with the disturbance of keratinocytes.[6] While keratinocyte differentiation and proliferation may result from the interaction between immune cells and keratinocytes. Multiple genetic variations and interactions occurring between immune cells and keratinocytes in the microenvironment determine the development of psoriasis, the severity of the disease, as well as therapeutic responses.[7] With advances in molecular biology, various novel targets for the treatment of psoriasis are under investigation. Indeed, there is accumulating clinical and experimental evidence that both autoimmune and autoinflammatory mechanisms are central to psoriasis, including T cells, macrophages, and dendritic cells, among others.[8,9]

Moreover, the complexity of gene expression alterations during the development of psoriasis is an important pathological mechanism.[10] Currently, multiple biological therapies for the treatment of psoriasis are approved by the FDA to exert therapeutic effects through the regulation of immune and inflammatory responses.[11] Herein, we sought to explore the pathomolecular mechanisms of psoriatic patients, as well as the related gene targets. The aberrant activation of immune cells was identified, and the correlation between gene targets and immune cells was further recognized. Thus providing a new direction for immunotherapy of psoriasis patients.

Materials and methods

Data processing

Gene expression profiles were collected from GSE13355,[12] GSE30999[13] and GSE54456[14] datasets in the Gene Expression Omnibus (GEO) database. GSE13355 included gene expression profiles of punch biopsies from 58 psoriatic patients and 64 normal healthy controls profiled by array based on GPL570 platform. GSE30999 contains 85 paired lesional and non-lesional samples, which profiling by array based on GPL570 platform. For GSE54456, 92 psoriatic and 82 normal skin samples were profiling by high throughput sequencing based on GPL9052 platform. For array data processing, the Robust Multichip Average (RMA) method[15] was used. For high throughput sequencing, the number of reads per kilobase per million mapped reads (RPKM) was used to normalize the expression. Reads were aligned to the reference genome NCBI build 37 using TopHat and counting using Cufflinks.[16]

Differentially expressed genes (DEGs) between psoriasis and normal controls in GSE13355 and GSE30999 datasets were analyzed using *limma* package[17] in R. DEGs between psoriasis and normal controls in GSE54456 dataset were identified using *DEseq2* package[18] in R. All DEGs were obtained by setting a filtering threshold of |log2 fold change (FC)| >1 and $p < 0.05$.

Enrichment analysis

Enrichment analysis of Gene Ontology (GO) functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for DEGs using *clusterProfiler* package[19] in R. In the results, terms with $p < 0.05$ were selected as significantly enriched. Gene set variation analysis (GSVA) package[20] in R was used to calculate KEGG pathways that were activated or inhibited in the psoriasis compared to controls.

Construction of protein-protein interaction (PPI) network

The PPI network was constructed using DEGs through STRING database (https://string-db.org) and displayed by Cytoscape software. The key genes were selected according to the higher degree of connectivity in the network. The predictive values of key genes were determined using receiver operating characteristic (ROC) curves by calculating area under ROC curve (AUC) values.

Immune infiltration score

Single-sample gene set enrichment analysis (ssGSEA) was used to quantify the infiltration score of immune cells in psoriasis based on 28 immune cell signatures.[21] The differences between psoriasis and normal controls were determined using *limma* R package. Correlations between immune cells and key genes were calculated using Pearson's correlation.

Sample collection

Five paired lesional and non-lesional samples were collected from adult psoriasis patients. In addition, peripheral blood samples were also collected from five adult psoriasis patients and five healthy controls. All studied were approved by the Ethics Committee of General Hospital of Xinjiang Military Command (No. XJJQZYY100). All participants gave written informed consent.

Quantitative real-time PCR

Total RNA of lesional and non-lesional samples was extracted using Trizol reagent (Invitrogen, CA, USA). Then the cDNA was obtained from RNA reverse transcription reactions using cDNA synthesis kit (Takara, Dalian, China). Quantitative real-time PCR (qRT-PCR) was performed subsequently using SYBR Premix Ex Taq II kit (Takara, Dalian, China). The relative expression of target genes was normalized to GAPDH using $2^{-\Delta\Delta Ct}$ method. Primers used in this study were as follows: ARG1 (F: 5′-TTGGCTTGAGAGACGTGGAC-3′; R: 5′-GTGCCAGTAGCTGGTGTGAA-3′); CXCL2 (F: 5′-GCTTGTCTCAACCCCGCATC-3′; R: 5′-TGGATTTGCCATTTTTCAGCATCTT-3′); GAPDH (F: 5′-GCACCGTCAAGGCTGAGAAC-3′; R: 5′-GGATCTCGCTCCTGGAAGATG-3′).

Flow cytometry

Peripheral blood samples were used to determine the proportion of immune cells in psoriasis patients and healthy controls using flow cytometry. The peripheral blood samples were washed twice with PBS after incubation using red blood cell lysate. The samples were stained immediately with various antibodies for 30 min at 4°C. Antibodies for flow cytometry were as follows: anti-CD3 (PC5.5-A), anti-CD19 (FITC-A), anti-CD8 (ECD-A), anti-CD4 (PE-A), anti-CD45 (FITC-A), anti-CD56 (PE-A), anti-CD4 (FITC-A), anti-CXCR5 (PE-A), anti-CD183 (PC5.5-A), anti-CD196 (PE-A). All antibodies were purchased from BD Biosciences. Data were analyzed by Kaluza or FlowJo software.

Immunohistochemistry

For immunohistochemistry (IHC), five paired lesional and non-lesional samples were fixed in 4% formalin overnight and embedded in paraffin with standard techniques. 4 μm sections were deparaffinized with xylene and rehydrated in graded ethanol. Hydrogen peroxide was used to inactivate the intrinsic peroxidases. Antigen retrieval was performed using citrate EDTA buffer (10 mM citric acid, 2 mM EDTA, 0.05% Tween 20, pH 6.2) in a water bath. Subsequently, the sections were incubated with primary antibodies (ARG1 and CXCL2, Abcam, Cambridge, UK) overnight in a humidified chamber at 4°C. Biotin conjugated secondary antibodies were incubated for 30 min. Staining was visualized after sections were counterstained with hematoxylin.

Statistics

Statistical analysis was performed with SPSS19.0 statistical software. The data was shown as mean ± standard deviation (SD). Comparison analyses were performed using t test. $p < 0.05$ was considered statistically significant.

Results

DEGs in psoriasis

To identify the DEGs in psoriasis, we performed differential expression of genes between psoriasis and normal controls. We identified 163 DEGs between psoriasis and normal controls in GSE13355 dataset (Figure 1(a)), 1275 DEGs in GSE30999 dataset (Figure 1(b)), and 2092 DEGs in GSE54456 dataset (Figure 1(c)). Through intersection analysis, we obtained 146 common genes from these three groups of DEGs (Figure 1(d)). The common genes may have great relationship with psoriasis.

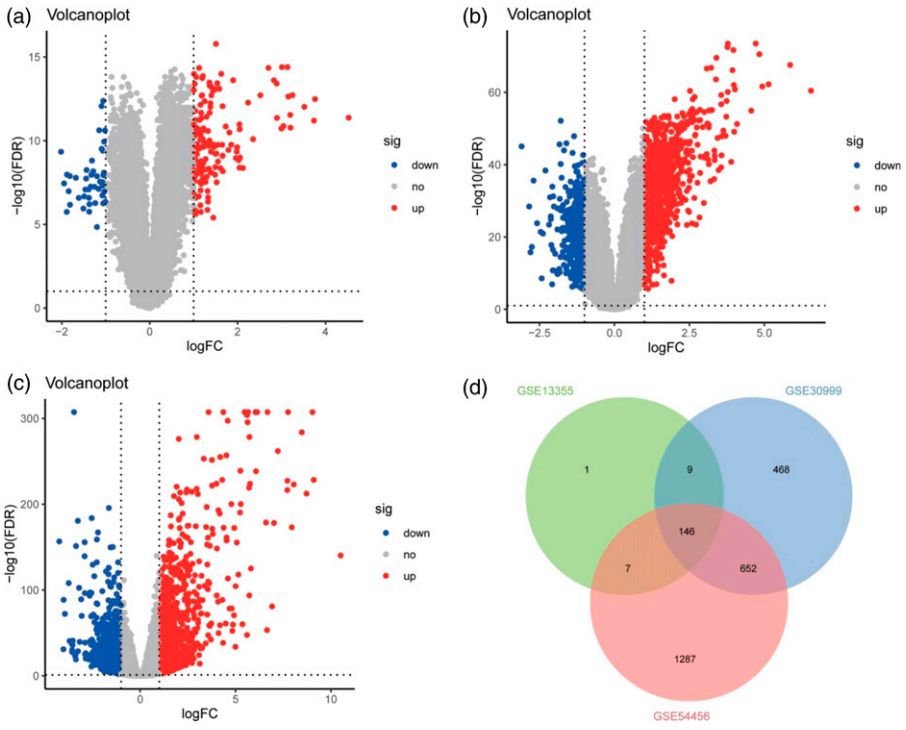

Figure 1. Differentially expressed genes between psoriasis and normal controls. (a) Volcano plot of DEGs between psoriasis and normal controls in GSE13355 dataset. (b) Volcano plot of DEGs between psoriasis and normal controls in GSE30999 dataset. (c) Volcano plot of DEGs between psoriasis and normal controls in GSE54456 dataset. Red is up regulated and blue is down regulated. FDR, false discovery rate; FC, fold change; sig, significant. (d) Intersection analysis of the three sets of DEGs. Intersected 146 common genes were identified.

Biological functions of common genes

To identify the biological functions in which common genes involved, we performed enrichment analysis. In the biological processes (BP) of GO results, keratinocyte differentiation, cellular response to type I interferon, and type I interferon signaling pathway were significantly enriched (Figure 2(a)). For the cellular components (CC), specific granule lumen, secretory granule lumen, and specific granule were enriched by common genes (Figure 2(a)). Then, chemokine activity, chemokine receptor binding, and CXCR chemokine receptor binding were involved in the molecular

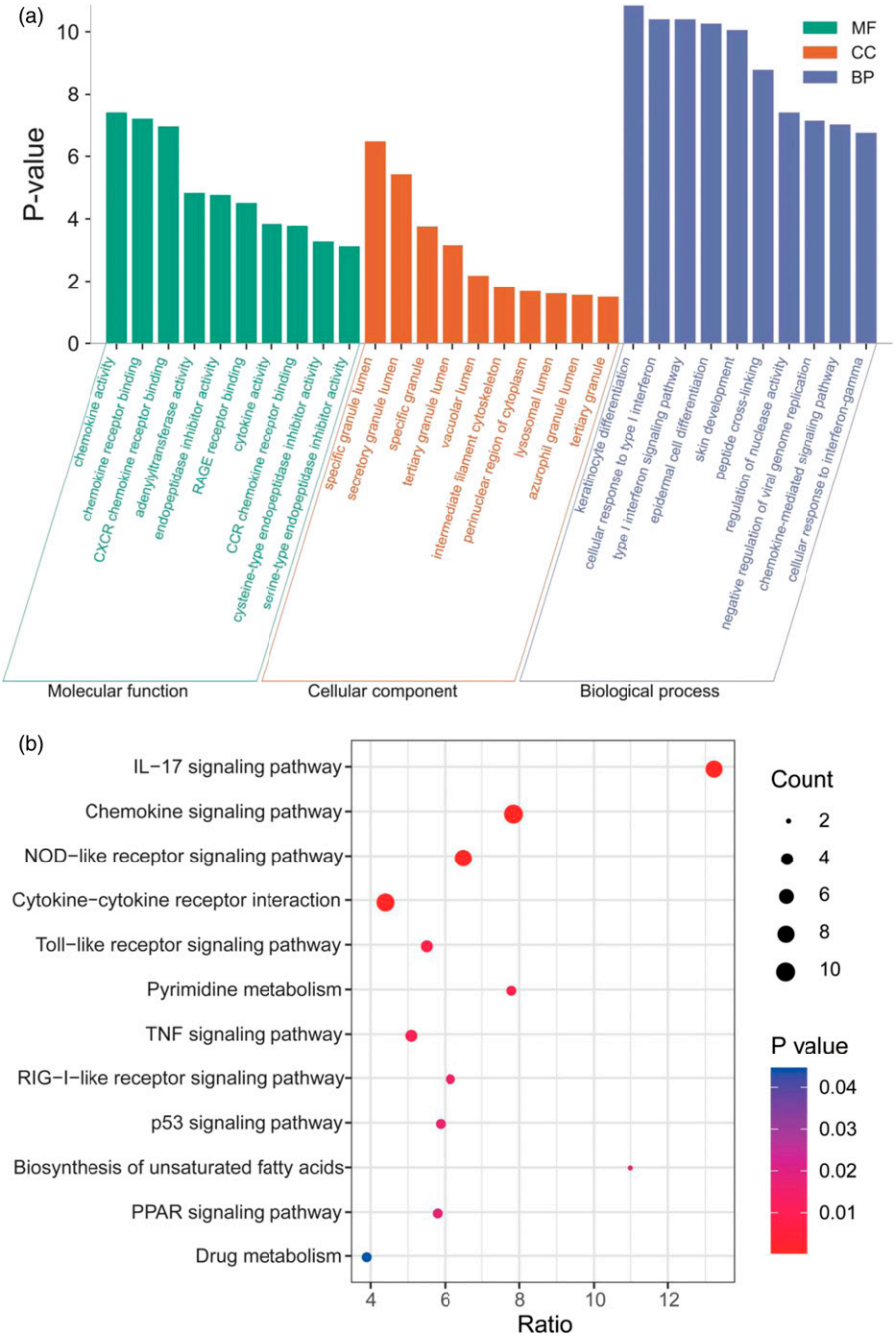

Figure 2. GO and KEGG pathways of common genes. (a) Important GO enriched in the common genes. Which included biological processes, cellular components, and molecular functions. BP, biological processes; CC, cellular components; MF, molecular functions. (b) Important Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched by the common genes. The larger the dot, the greater count of genes involved; the redder the color, the more significant the *p* value.

functions (MF) (Figure 2(a)). In addition, we also found IL-17 signaling pathway, chemokine signaling pathway, and NOD-like receptor signaling pathway were mainly enriched in KEGG pathways (Figure 2(b)).

PPI network of common genes

To identify genes with significant roles in psoriasis, we constructed a PPI network for common genes (Supplemental Figure S1(a)). Then, we identified the top 10 genes (CXCL10, STAT1, CXCL1, CXCL9, ARG1, LCN2, ISG15, CCL20, LTF, and CXCL2) with biggest degree of connectivity in the network as key genes (Figure 3(a)). Through ROC curves, we found that ARG1 and CXCL2 may be more important with bigger AUC values among key genes in GSE13355 (Figure 3(b)), GSE30999 (Figure 3(c)) and GSE54456 (Figure 3(d)) datasets, especially in GSE13355 dataset.

In addition, we calculated the activation differences of KEGG pathways between psoriasis patients and controls using GSVA method. We found that NOD-like receptor signaling pathway was activated in psoriasis, while circadian rhythm mammal was inhibited (Figure 3(e)). Correlation analysis results showed significant positive between NOD-like receptor signaling pathway and ARG1 or CXCL2 (Figure 3(f), Supplemental Figure S1(b) and S1(c)).

Immune cell infiltration in psoriasis

According to single-sample GSEA results, we found that psoriasis samples contained higher proportion of immune cell types than controls (Figure 4(a), Supplemental Figure S2(a), and S2(b)). Differential analysis results showed that most immune cells were significantly upregulated between psoriasis patients and controls (Figure 4(b), Supplemental Figure S2(c), and S2(d)). Importantly, ARG1 and CXCL2 were increased expression in psoriasis compared to controls in all three datasets (Figure 4(c)). Activated CD4 T cell, neutrophil, activated B cell, type 17 T helper (Th17) cell, type 2 T helper cell (Th2), activated CD8 T cell, T follicular helper cell (Tfh), macrophage, MDSC, activated dendritic cell, and CD56dim natural killer (NK) cell were correlated positively with ARG1 and CXCL2 (Figure 4(d), Supplemental Figure S3(a), and S3(b)).

Molecular experimental validation

To validate key results in clinical samples, we performed molecular experiments. In the results of qRT-PCR, we

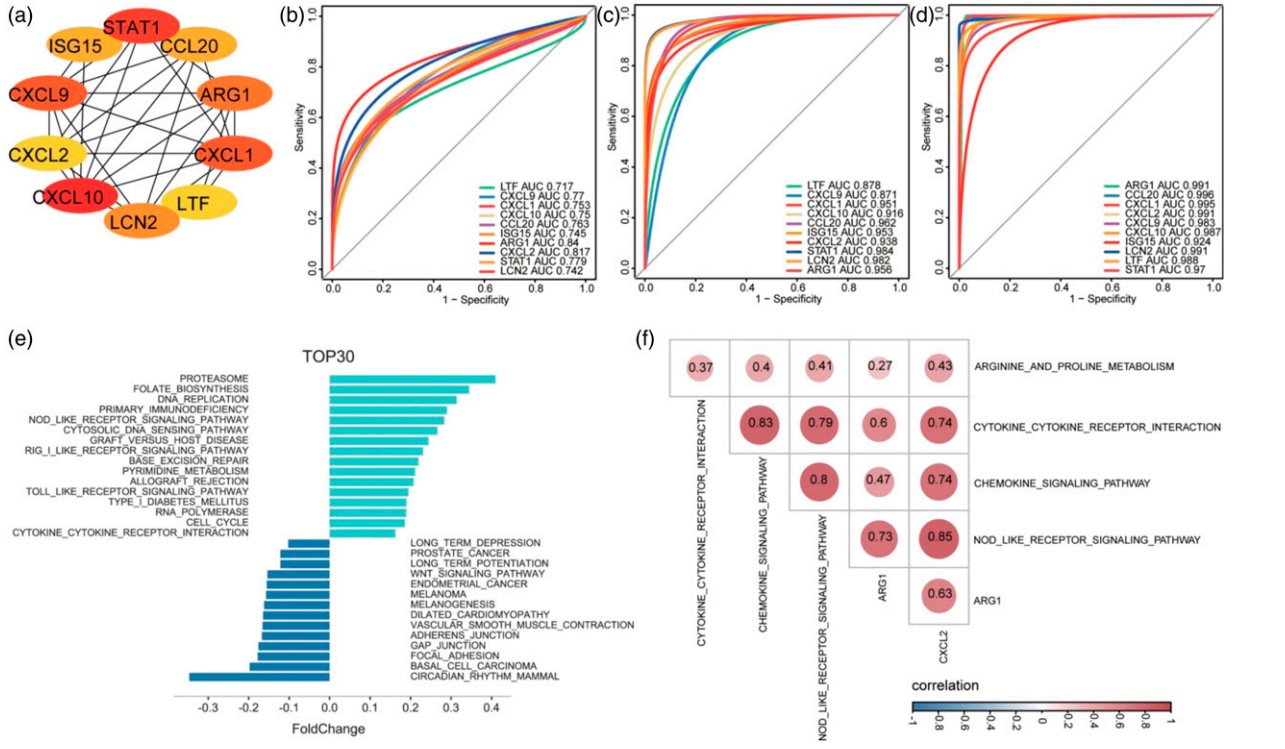

Figure 3. Identification of key genes through PPI network and ROC curves. (a) Genes with top ten degree of connectivity in PPI network. The redder the color, the greater the connectivity. The ROC curves of key genes in GSE13355 (b), GSE30999 (c) and GSE54456 (d) datasets. AUC, area under ROC curve. (e) The top 30 activated or inhibited KEGG pathways in psoriasis. The longer the column, the higher the fold change. (f) Correlation between activated or inhibited KEGG pathways and ARG1 and CXCL2 in GSE13355.

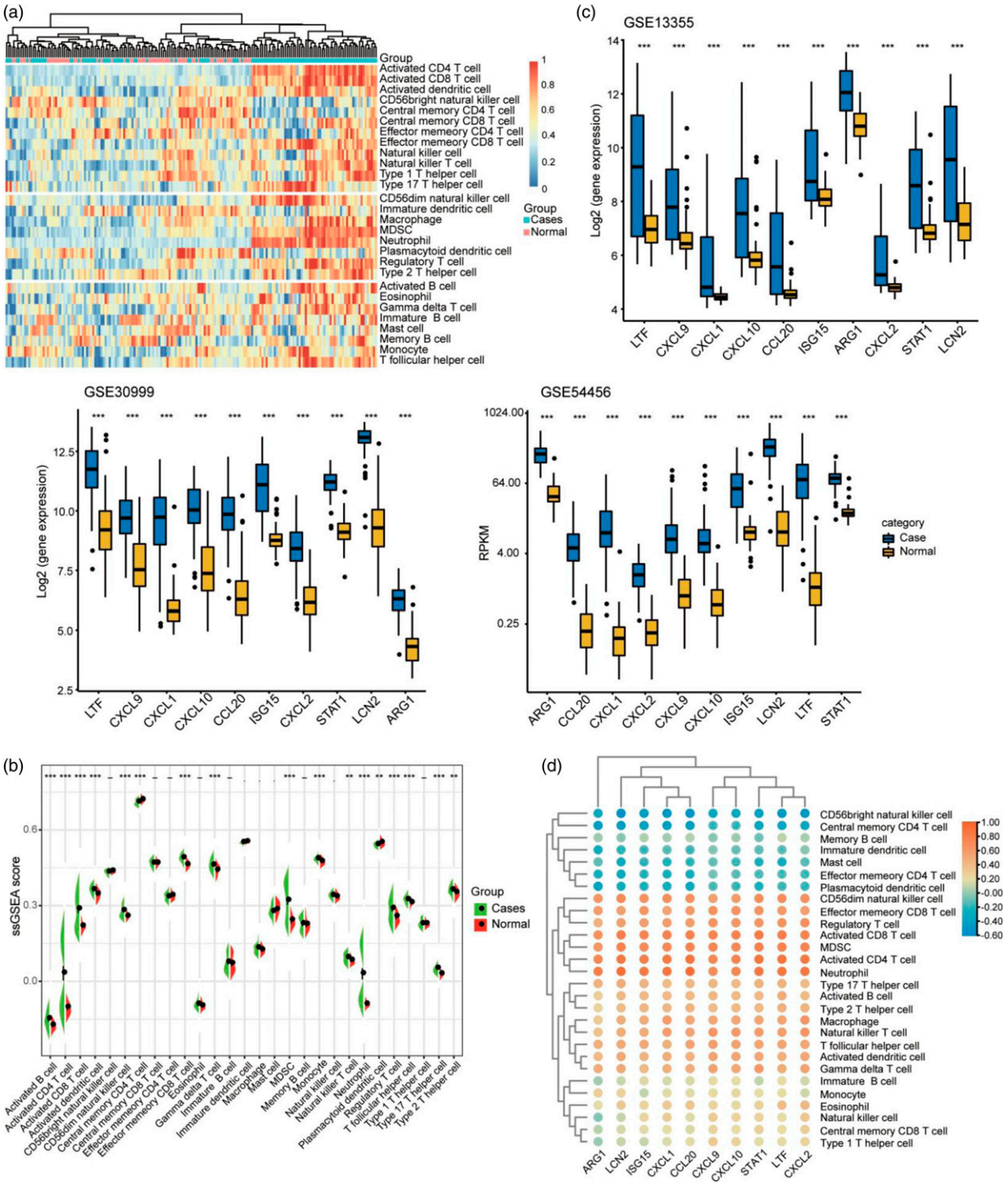

Figure 4. Immune cell infiltration in psoriasis. (a) Heatmap of different infiltration of immune cell in psoriasis and controls of GSE13355. Red is high expression and blue is low expression. (b) Differences in immune cell infiltration between psoriasis and controls of GSE13355. $^{**}p < 0.01$, $^{***}p < 0.001$,. $p < 0.1$, - $p > 0.1$. (c) The expression of key genes in psoriasis and controls of GSE13355, GSE30999 and GSE54456 datasets. $^{***}p < 0.001$. RPKM, reads per kilobase per million mapped reads. (d) Correlations between ARG1, CXCL2 expression and infiltration levels of different immune cell types in psoriasis of GSE13355. Red is positive correlation and blue is negative correlation.

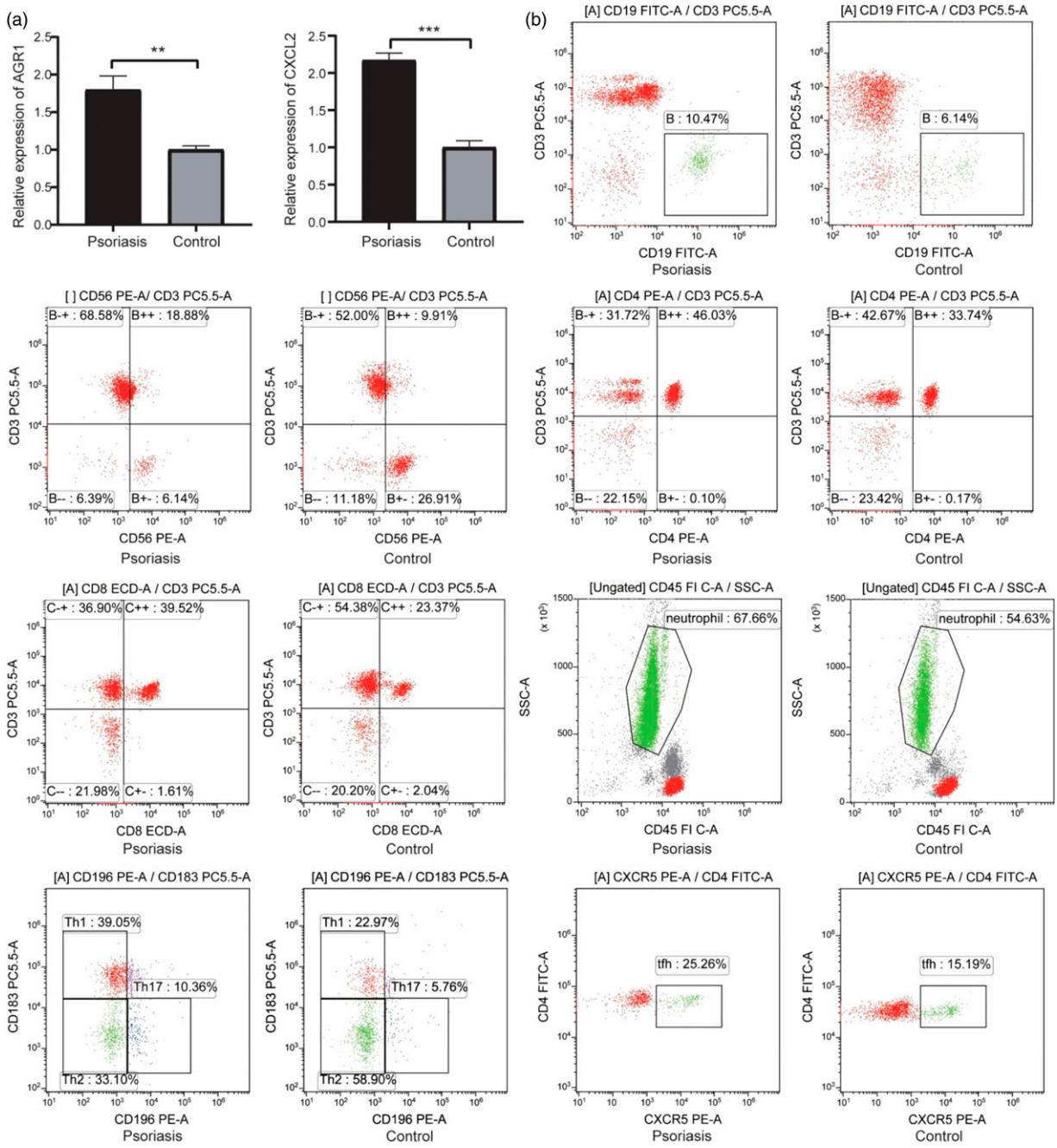

Figure 5. Key results were verified by molecular experiments. (a) The expression of ARG1 and CXCL2 between psoriasis and healthy controls which detected by qRT-PCR method. $^{**}p < 0.01$, $^{***}p < 0.001$. (b) The proportion of significantly changed immune cells in psoriasis compared to controls which detected by flow cytometry.

verified the high expression of ARG1 and CXCL2 in patients with psoriasis compared to controls (Figure 5(a)). We examined the protein expression of ARG1 and CXCL2 by IHC. They were increased expression in psoriasis patients than that in controls (Figure 6). Through flow cytometry detection, we found that the proportion of CD4 T cell, neutrophil, B cell, Th17, Th1, CD8 T cell, Tfh, and NK cell were all significantly higher in psoriatic patients than in controls (Figure 5(b)). However, Th2 was decreased in psoriasis.

Discussion

Psoriasis, as one of the most common skin diseases, has received much attention from clinicians and basic scientists.

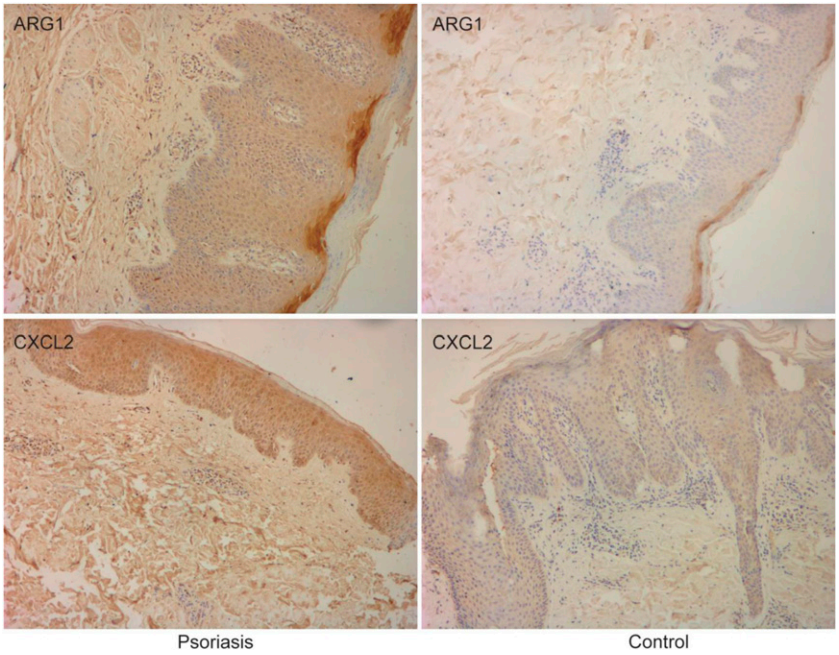

Figure 6. The protein expression of ARG1 and CXCL2 in psoriasis and healthy controls. The expression of ARG1 and CXCL2 detected by IHC.

There have been numerous studies elucidating many of the underlying pathogenic mechanisms and being translated into novel therapeutic strategies.[22] However, there remains a constant need for new, effective, and safe decisions in the management of psoriasis, leading to improved patient care. This study used bioinformatics to screen ARG1 and CXCL2 as diagnostic and therapeutic targets in psoriasis, and to identify the signaling pathways and immune cells associated with them. Key results were further validated utilizing molecular experiments in clinical samples.

The differentially expressed genes between psoriasis and normal controls may be associated with pathological alterations. The enrichment results of common genes may indicate the pathological mechanism of psoriasis. We found that type I interferon was significantly enriched in biological processes. Previous studies have shown the potential role of type I interferon in the pathogenesis of psoriasis.[23] Plasmacytoid dendritic cells (pDCs) in psoriatic skin activate and produce type I interferon, evoking autoimmune T cell responses in patients with psoriasis.[24] Among the enrichment results of KEGG pathways, the IL-17 signaling pathway has been confirmed by numerous studies for its therapeutic role in psoriasis.[25,26] IL-17 family cytokines are overexpressed in psoriatic skin, induce psoriasis associated chemokines and inflammatory pathways, and promote keratinization.[27,28] Nod like receptor (NLR) signaling pathways are significantly activated in psoriasis, thereby mediating immune responses and participating in the onset and progression of psoriasis.[29,30] Keratinocytes are the main cell type of the epidermis with widespread expression of NLRs involved in protection against harmful threats.[31] However, the function of NLRs in keratinocytes has not been clearly evaluated.[32] Therefore, we need continued exploration to better understand specific NLR functions in the skin and to understand their contribution to skin disease.

On the other hand, the results of our analysis suggest that arginase 1 (ARG1) and CXCL2 may have a predictive role in psoriasis. Myeloid derived suppressor cells (MDSCs) express ARG1 which is involved in regulating Th17 cell proliferation and is involved in the disease process of psoriasis.[33,34] Previous studies have suggested that ARG1 overexpression, possibly by limiting iNOS activity, is a molecular mechanism underlying the hyperproliferation of psoriatic keratinocytes.[35] ARG1 is recognized as an inflammatory associated genes with up-regulated expression in psoriasis, accompanied by the production of a large number of proinflammatory Th1 cytokines.[36,37] The neutrophil chemokine CXCL2 is up-regulated in the skin of patients with psoriasis and participates in innate immune system when activated by IL-17A.[1,3] Correlation results showed that ARG1 and CXCL2 were significantly positively correlated with NLR signaling, suggesting that they may further contribute to the pathology of psoriasis through NLR signaling.

Psoriasis is an autoimmune skin disease, so we also identified the abnormalities of immune cells in psoriatic patients and detected them using flow cytometry. Among them, the proportion of immune cells with higher correlation to ARG1 and CXCL2 were all significantly increased in psoriatic

patients. Many researchers have now shown that psoriatic lesions contain more T cells.[38] IL-17-producing CD4 and CD8 T cells have been found in psoriatic lesions.[39,40] The involvement of Th1 cells, Th17 cells and their cytokines in the pathogenesis of psoriasis has been well established.[41,42] In addition, an increased Th1/Th2 ratio in psoriasis patients stimulates keratinocyte proliferation and promotes angiogenesis.[43,44] Psoriatic neutrophils are recruited to the site of inflammation following inflammatory signals, produce and release large amounts of ROS, enhancing psoriatic symptoms.[45,46] NK cells are markedly increased in psoriatic lesions and may be involved in the pathogenesis of psoriasis.[47] Studies have shown that B cells are elevated in lesional skin of patients with psoriasis compared with nonlesional skin weight.[48] B cells promote CD4 + T cell activation and produce IL-17 to promote inflammation in psoriasis [20974990]. These results suggest a novel mechanism of immune cells in psoriasis, although whether ARG1 and CXCL2 regulate immune dysfunction in psoriasis requires further investigation.

Conclusion

New and effective therapies need to be continuously developed to eradicate psoriasis. Our study utilized a combination of bioinformatics and molecular experiments to analyze potential markers and therapeutic targets for psoriasis. These data suggest that ARG1 and CXCL2 are significantly altered in psoriatic patients and may play a key role in the pathogenesis of psoriasis. This may be associated with activation of the NLR pathway and increased immune responses. Further studies are needed to elucidate whether significantly increased levels of ARG1 and CXCL2 can predict the risk of psoriasis as well as their clinical therapeutic value.

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Author contributions

Research conception and design: Huilin Wang, and Wenjun Chen. Data analysis and interpretation: Caihua Lie and Yijie Zhang. Performed experiments: Huilin Wang, and Caihua Lie. Statistical analysis: Jiajia Li, and Jilong Meng. Drafting of the manuscript: Nan Zhang. Critical revision of the manuscript: Huilin Wang. All authors read and agreed to the published version of the manuscript.

Declaration of conflicting interests

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Data availability

All datasets generated for this study are included in the GSE13355, GSE30999 and GSE54456 datasets.

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Supplemental Material

Supplemental material for this article is available online.

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