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Key genes and immune infiltration in chronic spontaneous urticaria: a study of bioinformatics and systems biology

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Background: Chronic spontaneous urticaria (CSU) is defined by the spontaneous occurrence of wheals and/or angioedema for >6 weeks. The pathogenesis involves skin mast cells, but the complex causes of their activation remain to be characterized in detail.

Objectives: To explore disease-driving genes and biological pathways in CSU.

Methods: Two microarray data sets, e.g., GSE57178 and GSE72540, with mRNA information of skin from CSU patients, were downloaded from the Gene Expression Omnibus (GEO) database. An integrated bioinformatics pipeline including identification of differentially expressed genes (DEGs), functional enrichment analysis, protein-protein interaction (PPI) network analysis, co-expression and drug prediction analysis, and immune and stromal cells deconvolution analyses were applied to identify hub genes and key drivers of CSU pathogenesis.

Results: In total, we identified 92 up-regulated and 7 down-regulated genes in CSU lesions. These were significantly enriched in CSU-related pathways such as TNF, NF-κB, and JAK-STAT signaling. Based on PPI network modeling, four genes, i.e., IL-6, TLR-4, ICAM-1, and PTGS-2, were computationally identified as key pathogenic players in CSU. Immune infiltration analyses indicated that dendritic cells, Th2 cells, mast cells, megakaryocyte-erythroid progenitor, preadipocytes, and M1 macrophages were increased in lesional CSU skin.

Conclusion: Our results offer new insights on the pathogenesis of CSU and suggest that TNF, NF-κB, JAK-STAT, IL-6, TLR-4, ICAM-1, and PTGS-2 may be candidate targets for novel CSU treatments.

Introduction

CSU is usually characterized by typically short-lived, itchy, and fleeting wheals, angioedema, or both, occurring spontaneously for longer than 6 weeks (1). CSU affects about 1-4% of the world population, across all ages, mostly young and middle-aged women (2). CSU carries a significant emotional and economic burden for the patient (3),often lasting a long time and affecting many areas of daily life. To date, only antihistamines and omalizumab are licensed for the treatment of CSU, but there are still many patients who do not use these therapies and need more effective treatment.

The development of novel and better treatments for CSU is very much needed. Possible pathophysiological mechanisms now known for CSU include autoantibodies and other mast cell activation signals that lead to skin MC degranulation, release of pro-inflammatory mediators, and recruitment of inflammatory cells to the skin. Beyond this, the pathogenesis of urticaria is poorly understood (4–6). Insights into the complexities and drivers of the pathogenesis of CSU can point to promising targets of newer and more efficacious targeted biological treatments for severe, refractory CSU.

Microarray analysis can detect thousands of gene expression changes in a short time (7). Bioinformatic analyses of microarray gene expression datasets can identify DEGs and relevant functional pathways (8–10). As of now, two CSU studies have assessed skin gene expression (11–13), but joint analyses of these microarray datasets, GSE57178 and GSE72540 are limited. One recent study of these datasets recently reported that the IL-6/miR-149-5p/ZBTB20-AS1 axis may be involved in mast cell activation in CSU (14). The lesional immune cell infiltrate and core genes, however have not been investigated by bioinformatics analyses.

In this study, we performed deep bioinformatic analyses of the merged CSU microarray datasets GSE57178 and GSE72540 available from the GEO, which contain cutaneous gene expression data from 16 CSU patients and 13 healthy individuals. The aim of this study was to identify key genes and immune cells related to the pathological mechanism of CSU.

Methods

Microarray studies and datasets from GEO

Two microarray datasets (GSE57178 (11) and GSE72540 (12)) were downloaded from GEO (http://www.ncbi.nlm.nih.gov/geo).

Abbreviations: BP, Biological processes; COX-2, Cyclooxygenase 2; CRP, C-reactive protein; CSU, Chronic spontaneous urticarial; DCs, Dendritic cells; DEGs, Differentially expressed genes; DGIdb, Drug-Gene Interaction database; FC, Fold-changes; GEO, Gene Expression Omnibus; GSEA, Gene set enrichment analysis; ICAM-1, Intercellular adhesion molecule 1; IL-6, Interleukin 6; LS, Lesional skin; MEP, Megakaryocyte-erythroid progenitor; NL, Non-lesional skin; NS, Normal skin; PGD2, Prostaglandin D2; PPI, Protein-protein interaction; PTGS-2, Prostaglandin-endoperoxide synthase 2; sIL-6R, Soluble IL-6 receptor; TF, Tissue factor; TLR-4, Toll-like receptor 4; TLRs, Toll-like receptors; TNF, Tumor necrosis factor; TNFR1, TNF receptor 1; UAS7, urticaria activity score 7.

The details are shown in Table 1. GSE57178 contains the mRNA information of lesional skin (N=6) and non-lesional skin (N=7) of CSU patients, and of skin from healthy individuals (N=5). GSE72540 also contains information on lesional CSU skin (N=10), non-lesional CSU skin (N=13), and the skin of healthy control subjects (N=8). All included patients showed active CSU, i.e. urticaria activity score 7 (UAS7) ≥ 11 for at least 3 months, and were refractory to antihistamine treatment.

Data preprocessing and screening for differentially expressed genes

Background correction and data normalization were completed through the "Affy" package and the RMA algorithm.The limma package was applied to identify the DEGs between CSU skin and the skin of healthy controls. |LogFC| > 1 and P-value < 0.05 were defined as the cut-off.

Enrichment analyses

GSEA (Gene Set Enrichment Analysis) is a calculation method to determine whether a set of genes defined in advance are differentially expressed in different samples (15). In order to explore whether most of the genes in the predefined gene set are highly expressed or poorly expressed in the sample, the H. All. V6.2. Symbols.gmt [Hallmarks] gene set database in GESA software is used for analysis. Significantly enriched biological process (BP) items of the DEGs were obtained by DAVID (version 6.8) and Metascape. Biological pathways analysis was completed by the functional enrichment analysis tool (Funrich) (16). The pathway enrichment analyses of DEGs were evaluated by two databases, including "KEGG pathway" and "Reactome".

PPI network construction and modules analysis

The PPI network of DEGs was predicted by STRING (version 11.0; http://string-db.org) and visualized by Cytoscape (version 3.7.2). Key modules were identified by using The Molecular

TABLE 1 Details of the GEO datasets. Lesional skin (LS), non-lesional skin (NL), normal skin (NS).

Dataset	Platform	No. of Samples (LS, NL, NS)	References
GSE57178	GPL6244 Platforms (Affymetrix Human Gene 1.0 ST Array)	6,7,5	Patel OP et al. (11)
GSE72540	GPL16699 Platforms (Agilent-039494 SurePrint G3 Human GE v2 8x60K Microarray 039381)	10,13,8	Giménez-Arnau A et al. (12)

Complex Detection (MCODE; version 1.5.1) of Cytoscape (MCODE scores > 5, degree cut-off = 2, node score cut-off = 0.2, max depth = 100 and k-score = 2).

Identification of hub genes

The cytoHubba plug-in in the Cytoscape software was used to analyze the topological attributes of nodes in the network, and the parameters are set to no weight. By sorting the score of each node, we identified the important nodes involved in protein interaction in the network. We selected six commonly used parameters to calculate the top ten genes and their respective rankings and obtained the common hub genes through the Venn diagram. GeneMANIA (http://www.genemania.org/) was used to explore the co-expression genes and functions of hub genes. The Drug-Gene Interaction database (DGIdb 3.0; http://www.dgidb.org/) was used to discover the existing targeted drugs for hub genes. All the network map was formed by Cytoscape.

Immune and stromal cells deconvolution analyses

xCell is an evaluation tool of immune cell infiltration based on gene expression data, which can identify potential immune and stromal cell subsets and calculate their relative abundance in tissues (17). We evaluated the immune cell infiltration of two microarray data by xCell. Finally, their common differential immune and stromal cells were obtained by Venn diagrams.

Correlation analysis of hub genes and infiltrating immune cells

Spearman correlation analysis was used to explore the relationship between hub genes and infiltrating immune cells. The results were visualized by using the "ggplot2" software package.
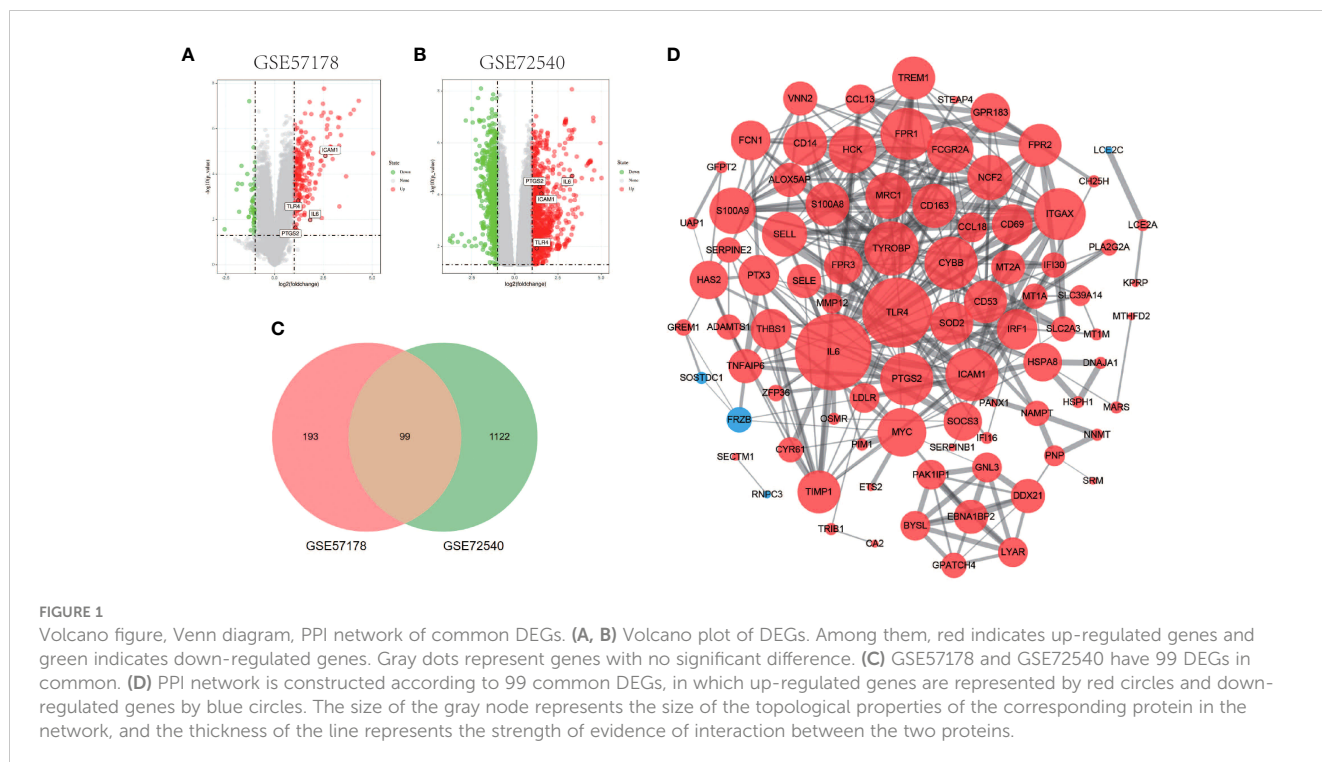
Results

In CSU, gene expression signatures of lesional skin are different and distinct from healthy skin

Comparing lesional CSU skin and healthy control samples, 292 and 1221 DEGs were identified in GSE57178 and GSE72540, respectively, of which 99 were in both (Figure 1), 92 upregulated and 7 downregulated DEGs (Figure 1). The heat map shows that these DEGs can basically distinguish CSU lesion samples from healthy control samples (Figure 2).

In CSU lesions, gene sets of TNF/NF-κB, IL-6/JAK/STAT3 and IFN-γ pathways are enriched

Gene set enrichment analyses showed that four common gene sets were significantly enriched in CSU and healthy controls samples (Figure 3), including TNF-mediated NF-κB pathway, IL-6/JAK/STAT3 signaling pathway, and responses to interferon-

FIGURE 1
Volcano figure, Venn diagram, PPI network of common DEGs. **(A, B)** Volcano plot of DEGs. Among them, red indicates up-regulated genes and green indicates down-regulated genes. Gray dots represent genes with no significant difference. **(C)** GSE57178 and GSE72540 have 99 DEGs in common. **(D)** PPI network is constructed according to 99 common DEGs, in which up-regulated genes are represented by red circles and down-regulated genes by blue circles. The size of the gray node represents the size of the topological properties of the corresponding protein in the network, and the thickness of the line represents the strength of evidence of interaction between the two proteins.
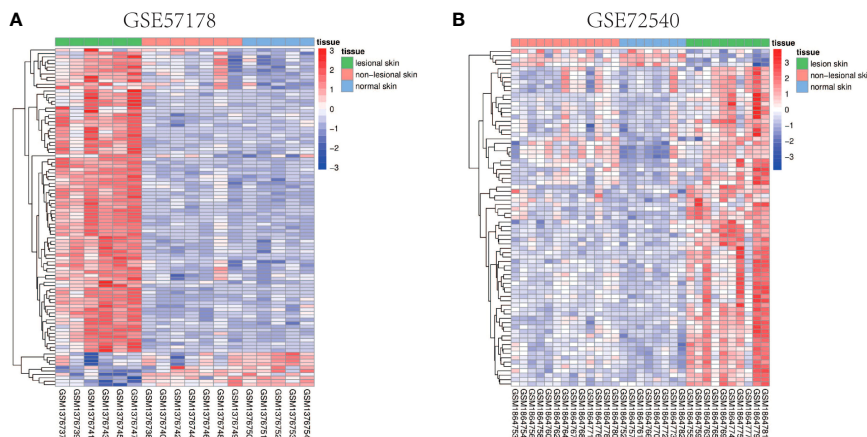
FIGURE 2

Heat map of common DEGs. **(A, B)** Heat maps of common DEGs between GSE57178 and GSE72540. Up-regulated genes are shown in red, down-regulated genes are shown in blue, and genes with little change are shown in white.

gamma. Biological processes enrichment analysis indicated that these were mainly linked to inflammation and leukocyte migration (Figure 4 and Table S1). Enrichment analyses of biological pathways demonstrated that DEGs in lesional vs non lesional skin were mainly enriched in IL-6-mediated signaling events and the biological process by which formyl peptide receptors bind to formyl peptides and many other ligands (Figure 5). Moreover, our KEGG analysis showed that 4 pathways were significantly different in lesional vs healthy skin of the control group, including Phagosome, Malaria, TNF signaling pathway, and Staphylococcus aureus infection. Reactome enrichment analyses revealed that the most significantly enriched pathways in CSU lesions vs non lesional skin were Immune System, Innate

Immune System, Neutrophil degranulation, and Interleukin-4 and Interleukin-13 signaling (Figures 6A, B, Tables S2 and S3).

Development of CSU lesions is linked to TNF, NF-κB, and JAK-STAT signaling pathways

The PPI network of DEGs generated by Cytoscape consists of 87 nodes and 347 interaction pairs (Figure 1D). Two important gene modules were generated by MCODE plug-ins (Figures 7A, C). KEGG pathway enrichment analysis showed that three pathways are closely related to CSU, including TNF signaling pathway, NF-
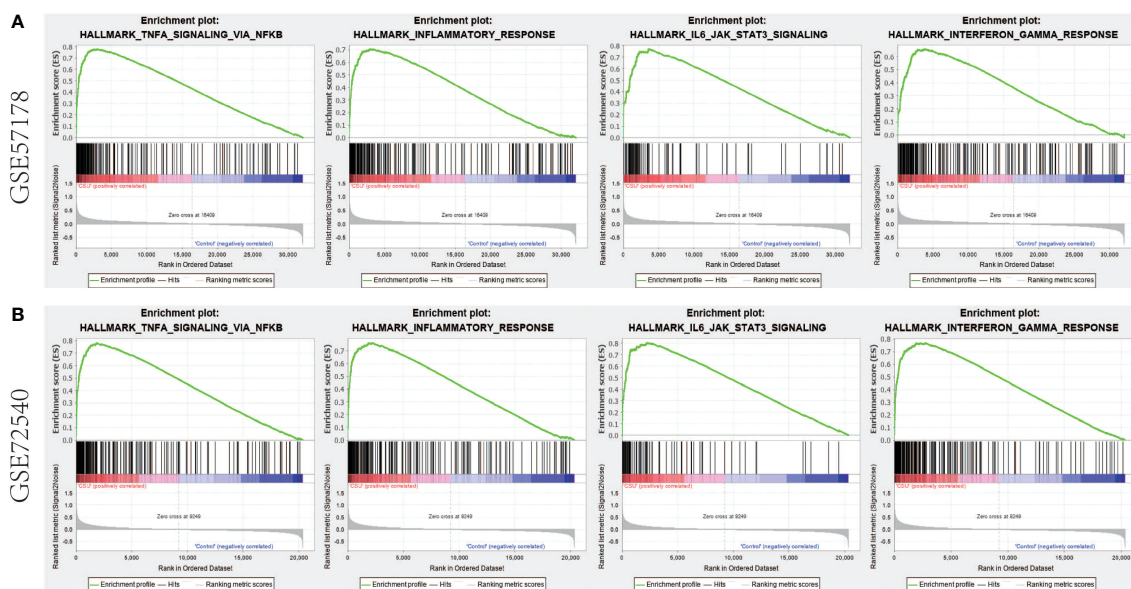

FIGURE 3

GSEA enrichment analysis. **(A, B)** The common gene set pathway obtained by GESA analysis of GSE57178 and GSE72540 respectively.
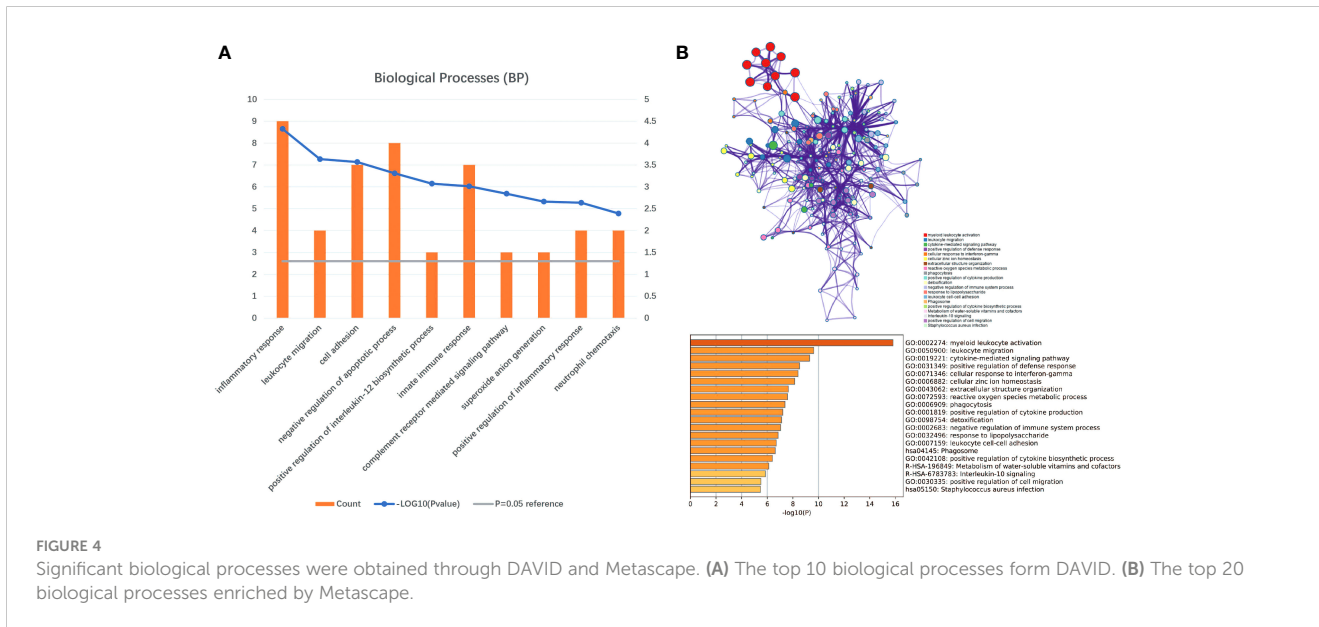
FIGURE 4

Significant biological processes were obtained through DAVID and Metascape. **(A)** The top 10 biological processes form DAVID. **(B)** The top 20 biological processes enriched by Metascape.

κB signaling pathway and JAK-STAT signaling pathway (Figure 7B, D).

Four hub genes IL-6, TLR-4, ICAM-1, and PTGS-2 are significantly linked to CSU lesion formation

Using the 6 algorithms (MNC, Radiality, Stress, Degree, Closeness, and EPC) in the cytoHubba plugin, we obtained 4 lesional skin-expressed hub genes: IL-6, TLR-4, ICAM-1, and PTGS-2 (Figure 8A and Table 2). Then, a network of these 4 common hub genes and their co-expression genes were analyzed by GeneMANIA online platform. The analysis of co-expression network showed that hub genes and co-expression genes were mainly involved in the process of leukocyte migration and inflammatory response (Figure 8B). Furthermore, we obtained 12 drugs (FDA-listed + Immunotherapies) that paired with these hub genes from the DGIdb (Figure 8C).
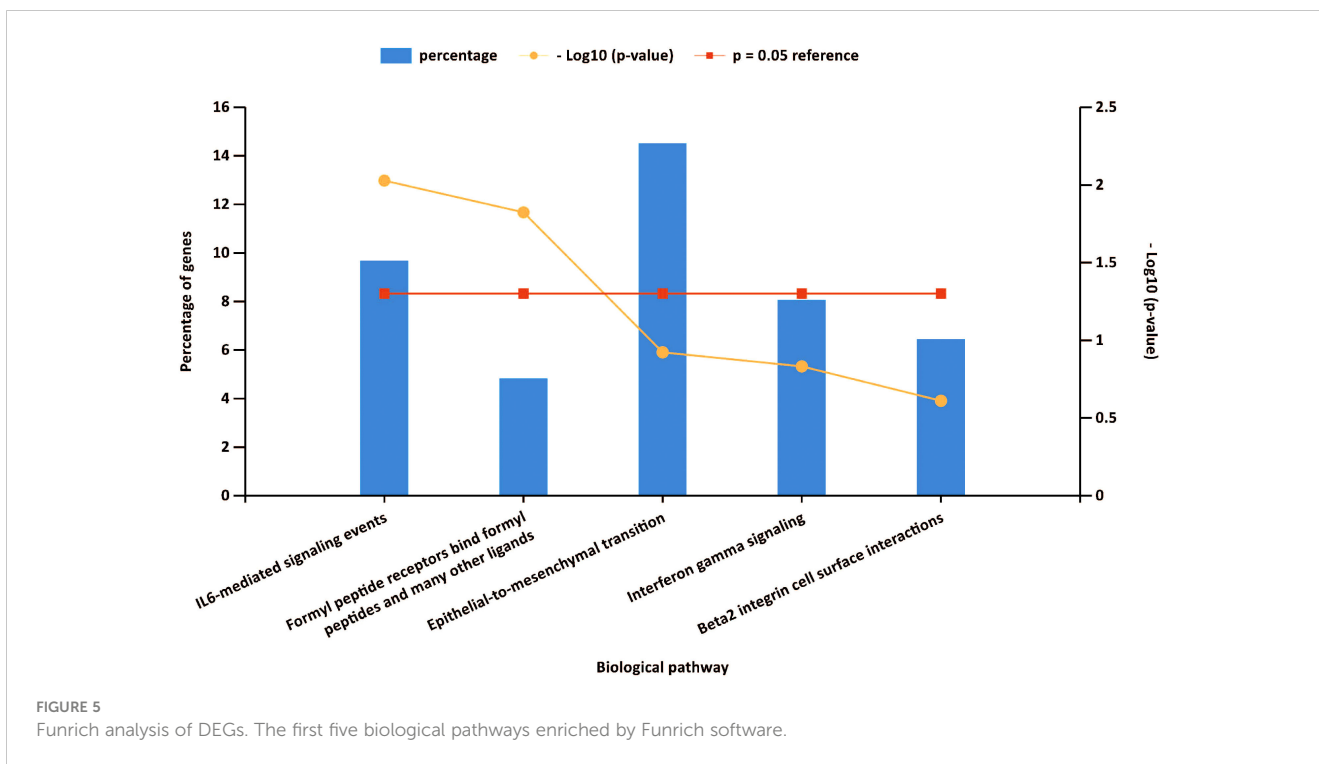

FIGURE 5

Funrich analysis of DEGs. The first five biological pathways enriched by Funrich software.
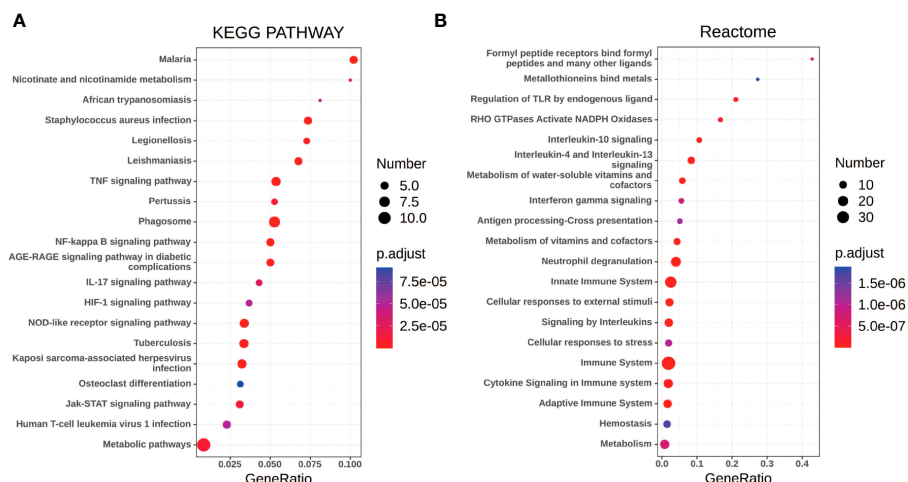
FIGURE 6

The pathway analysis of DEGs by KOBAS 3.0. **(A, B)** The pathway analysis of all the DEGs via KEGG and Reactome database. The size of the dot represents the number of enriched genes, the color represents the size of the P-value, the abscissa represents the proportion of the enriched genes to the total number of gene sets, and the ordinate represents the name of the pathway.

The spectrum of infiltrating cells in CSU lesions is different from healthy skin

To investigate the immune cell types that may be infiltrated in CSU lesions, cell enrichment analyses done with xCell showed marked differences between CSU and healthy controls samples in both datasets, GSE57178 and GSE72540. Eight cell types showed much higher expression in lesional CSU skin: immune cells, sensory nerves, dendritic cells (DC), preadipocytes, megakaryocyte-erythroid progenitor (MEP), mast cells, M1 macrophages, microvascular (mv) endothelial cells, and Th2 cells (Figure 9A).

Specifically, both in GSE57178 and GSE72540, compared with normal healthy skin, CSU lesions showed higher infiltrations of DC, preadipocytes, Th2 cells, MEP, mast cells, and macrophages M1(Figure 9B, C), respectively.

Lesional CSU hub genes correlate with immune and stromal cell infiltration

Correlation analysis between hub genes and infiltrating immune cells in CSU lesions showed that 1) IL-6 (Figure 10A)
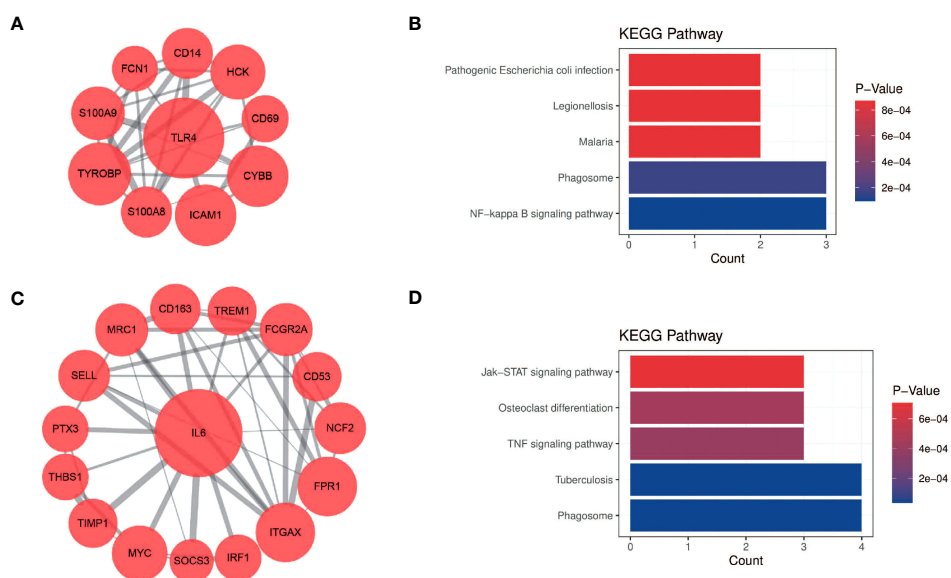

FIGURE 7

KEGG pathway analysis of modular genes. **(A, B)** Module 1 and the KEGG pathway enrichment analysis. **(C, D)** Module 2 and the KEGG pathway enrichment analysis.
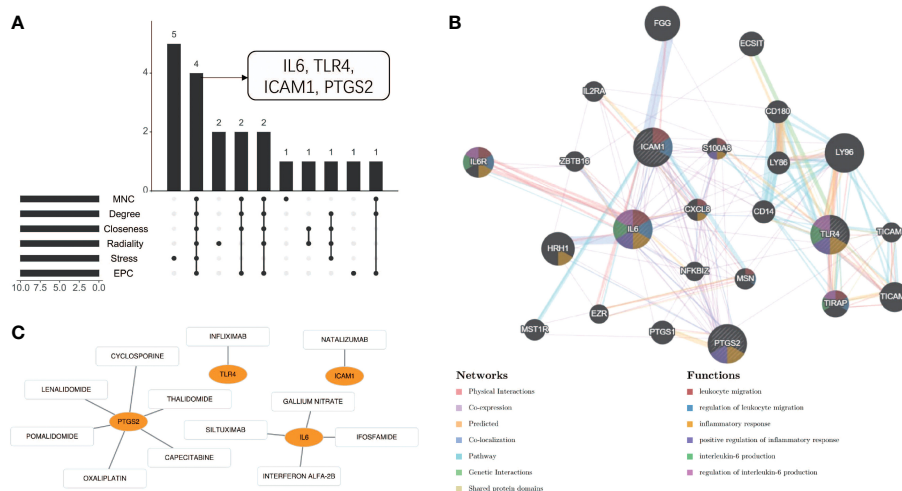
FIGURE 8

Co-expression analysis and drug-gene interaction analysis of hub genes. **(A)** Four common hub genes obtained by six algorithms. **(B)** The network diagram of Hub genes and their co-expressed genes. **(C)** Drug-gene interaction diagram.

was positively correlated with activated Th2 cells, preadipocytes, and mv Endothelial cells; 2) PTGS-2 (Figure 10B) was positively correlated with Th2 cells, neurons, and macrophages M1; 3) ICAM-1 (Figure 10C) was positively correlated with Th2 cells, mast cells, macrophages M1, mv Endothelial cells, MEP, and preadipocytes; 4) TLR-4 (Figure 10D) was positively correlated with mv Endothelial cells, MEP, macrophages M1, and preadipocytes.
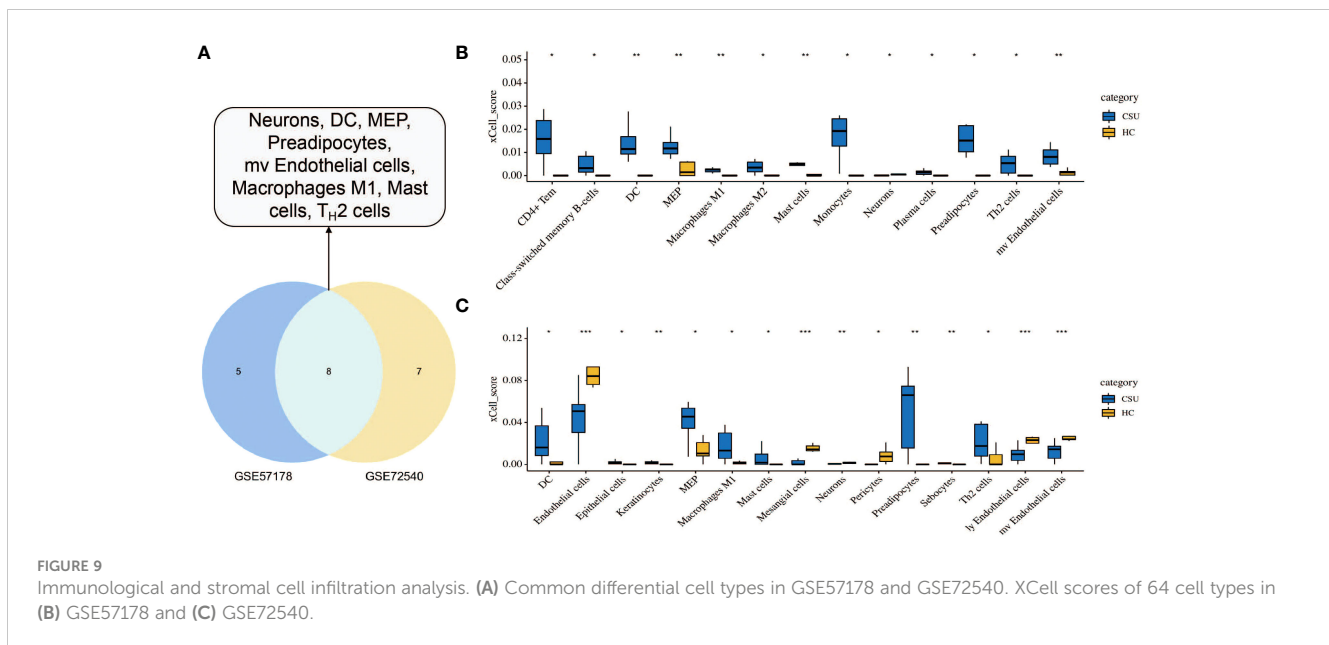
Discussion

This study investigated potential disease driving gene signatures and functional networks in patients with CSU through microarray analysis. In total, 99 DEGs including 92 up-regulated genes and 7 down-regulated genes between CSU and healthy controls were identified. By enrichment analysis we identified core modules and three signaling pathways i.e. TNF, NF-κB, and JAK-STAT, that are closely related to the development of CSUs. In addition, we

identified four hub genes, including IL-6, TLR-4, ICAM-1, and PTGS2.In addition, xCell is often used to assess local immune cell infiltration in skin biopsies of CSU patients. CSU lesions contained higher proportions of DCs, Th2 cells, MCs, megakaryocyte-erythroid progenitors, preadipocytes, and M1 macrophages compared to healthy skin. Finally, the above four hub genes IL-6, TLR-4, ICAM-1, and PTGS2 were found to be correlate with immune and stromal cell infiltration in CSU lesions.

TNF is a key inflammatory mediator in CSU inflammation

CSU is not only associated with autoimmune processes but also systemic inflammatory responses (18). The release of various inflammatory mediators (histamine, protease, leukotriene and TNF, etc.) is closely related to the wheals of CSU. TNF, which also can be secreted by T cells, natural killer (NK) cells, monocytes,

TABLE 2 Top 10 hub genes among the six algorithms.

MNC	Degree	Closeness	Radiality	Stress	EPC
HCK	HCK	FPR1	IRF1	HSPA8	HCK
FPR1	FPR1	PTGS2	PTGS2	PTGS2	FCGR2A
PTGS2	PTGS2	ITGAX	TIMP1	ICAM1	FPR1
ITGAX	ITGAX	ICAM1	IL6	IL6	PTGS2
IL6	IL6	IL6	ICAM1	EBNA1BP2	ITGAX
ICAM1	ICAM1	MYC	MYC	LDLR	IL6
MRC1	MYC	TLR4	TLR4	GNL3	ICAM1
TLR4	TLR4	SOD2	SOD2	MYC	TLR4
CYBB	CYBB	CYBB	CYBB	TLR4	CYBB
TYROBP	TYROBP	TYROBP	TYROBP	HAS2	TYROBP

FIGURE 9
Immunological and stromal cell infiltration analysis. **(A)** Common differential cell types in GSE57178 and GSE72540. XCell scores of 64 cell types in **(B)** GSE57178 and **(C)** GSE72540.

macrophages, basophils, eosinophils, keratinocytes, and fibroblasts, et al. (19). Dysregulation of the TNF pathway is a key feature and causative factor in many autoimmune and inflammatory diseases (20, 21). The formation of wheals in CSU is closely related to the activation of TNF signaling pathway, marked by 1) increased circulating concentrations of TNF-R, sTNF-R1, and sTNF-R2, 2) serum TNF level was positively correlated with disease activity in the samples of patients with CSU, 3) TNF is upregulated in the lesional and non-lesional skin of patients with CSU (22, 23). In addition, the use of TNF inhibitors (Etanercept, Adalimumab, Infliximab) for the treatment of CSU have also been validated in clinical care (24). Consistent with these findings, in the present study, we also observed that TNF is a hub gene of CSU. In sum, all these findings provide a theoretical basis for the potential of TNF targets in the treatment of CSU. Building on these foundations, larger and more rigorous trials are needed to make a stronger case that TNF for CSU is safe and effective.

NF-κB activation may link to chronic inflammation in CSU

CSU is also associated with chronic inflammation. And most chronic diseases, caused by lifestyle factors, appear to be linked to inflammation (25). As we all know, NF-κB is a pivotal pathway that mediates the pathogenesis of inflammatory skin diseases (26). When cells are subjected to various intracellular and extracellular stimuli, NF-κB dimers are released, which are further activated and translocated to the nucleus, leading to the production of inflammatory cytokines such as IL-6 and TNF (27, 28). In turn, these inflammatory cytokines promote the activation of NF-κB signalling, leading to a complex inflammatory cascade *in vivo* (27). In the present study, we have demonstrated that both IL-6 and TNF are hub genes of CSU. Besides, our enrichment analysis results showed that the NF-κB signaling pathway was significantly related
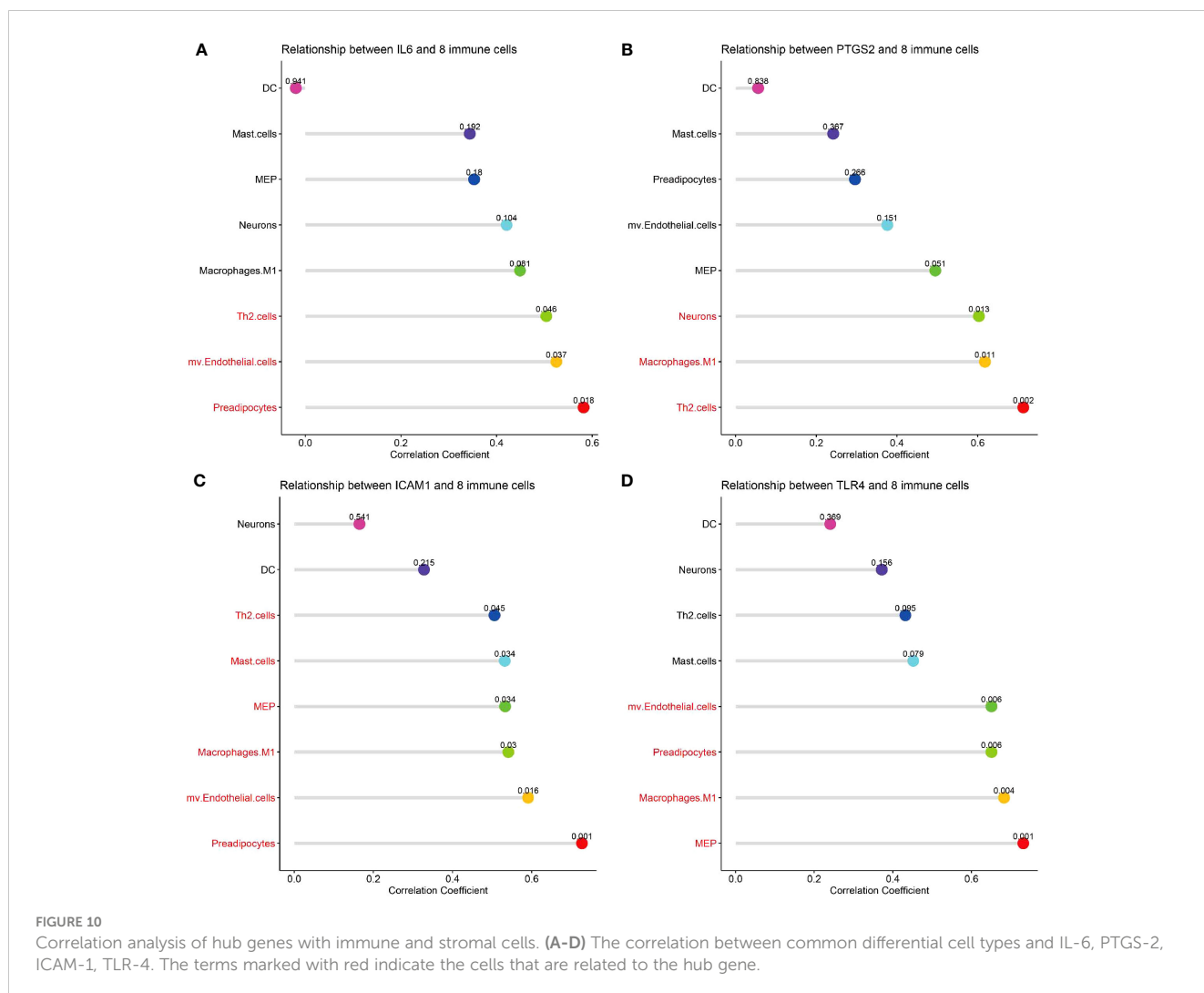
to the occurrence and development of CSU. Based on these findings, we speculate that IL-6/TNF-mediated over-activation of the NF-κB signaling pathway is a new therapeutic target for CSU.

JAK/STAT3 signaling pathway involves in inflammatory reactions and allergic responses of CSU

It has been shown in recent years that the JAK/STAT3 signalling pathway is associated with the pathogenesis of CSU. In the lesions of CSU patients, the high expression of OSMR gene can promote the expression of JAK/STAT3 signalling pathway related genes (29). They further observed that OSMR gene silencing can significantly inhibit the activity of JAK/STAT3 signal pathway, promote cell proliferation and migration, and further inhibit epithelial cell apoptosis in a chronic autoimmune urticaria mouse model. Other studies have also found that IL-9 and IL-10 can promote CSU through activation of the JAK/STAT3 signalling pathway (30). Our GSEA enrichment analysis based on both datasets showed that the IL-6/JAK/STAT3 pathway was also highly expressed in the CSU patient group. Additionally,the IL-6/ JAK/STAT3 signalling pathway has been shown to be associated with mast cell degranulation and allergic reactions (31). All aforementioned findings indicate that blocking the IL-6/JAK/ STAT3 signalling pathway may be a novel approach for the treatment of CSU.

IL-6, the interleukin related with CSU

IL-6 is an important regulator of inflammation and immune responses and is associated with a variety of autoimmune and chronic inflammatory diseases (32–34). Although the imbalance of inflammatory cells was detected in skin biopsies of CSU patients,

FIGURE 10
Correlation analysis of hub genes with immune and stromal cells. **(A-D)** The correlation between common differential cell types and IL-6, PTGS-2, ICAM-1, TLR-4. The terms marked with red indicate the cells that are related to the hub gene.

the source of IL-6 in CSU has not been identified (6, 35). Previously, several studies have reported higher IL-6 levels in patients with CSU compared to those observed in the healthy group (36–39). IL-6 levels could be linked to CSU disease activity (40). In addition, IL-6 levels in CSU patients were reduced by omalizumab therapy.

Besides, IL-6/soluble IL-6 receptor (sIL-6R) complex can stimulate the synthesis of acute phase proteins (such as CRP) and promote the production of autoantibodies. On the other hand, it promotes the differentiation of Th2 and Th17 cells while inhibiting the differentiation of Th1 and Treg cells, ultimately leading to an imbalance of Th1/Th2 cells (32, 41). These findings suggest that IL-6 might contribute to CSU pathology, even though the utility of IL-6 as biomarker in CSU is still questionable (42).

TLR-4 is associated with CSU

Toll-like receptors (TLRs) are a group of pattern recognition receptors that recognise microbe-associated PAMPs and damage or tissue repair hazard-associated sub-DAMPs (43).. TLR-4 was one of the most important TLRs which play a key role in pathogen recognition and activation of various inflammatory signaling

pathways (44). TLR-4 signaling also has been suggested to participate in allergic diseases as well as autoimmune responses (45, 46). TLR-4 expression was significantly upregulated in allergic rhinitis patients (47). Lately, although contradictory observations have also been published, TLR-4 polymorphisms have been reported to associate with atopy and asthma (48, 49). Interestingly, a decreased TLR-4 expression on CD14^{+} cells from chronic idiopathic urticaria patients was observed in ex vivo conditions (50). In the present study, for the first time, we identified TLR-4 as a hub gene in CSU. Taken together, these findings indicate that TLR-4 may be involved in the development of CSU. As for the specific mechanisms, clinical sample verification and laboratory data are still needed.

ICAM-1 might contribute to tissue edema formation in CSU

In CSU, although MCs initiate and remain in early-phase wealing response, cellular adhesion molecules (CAMs) are thought to sustain the late phase (51). CAMs are well documented in the selective recruitment and activation of

inflammatory cells, which ultimately lead to tissue damage (52). Among the CAMs, ICAM-1 plays an important role in accelerating tissue oedema formation (53): 1) Overexpression of ICAM-1 in endothelial cells alters cell morphology and increases endothelial cell permeability or vascular leakage (54); 2) Moreover, antibody cross-linking specific for ICAM-1 also increased albumin passage from intact cremaster micro vessels (55). It has been demonstrated that ICAM-1 is the hallmark of allergic inflammation (56). Although it has been reported that ICAM-1 was not always significantly elevated in CU sera. ICAM-1, as a biomarker of endothelial dysfunction, is increased in sera and detected in spontaneous wheals of patients with CIU (35). Moreover, one study, which included a large number of CSU patients, showed a significant increase in soluble ICAM-1 level in sera (57). A recent study also demonstrated that specific knockout of ICAM-1 can inhibits the expression of IgE, ECP, CD62L, and CD11a, thereby reducing the adhesion between vascular endothelial cells in a mouse model of immune contact urticaria (58). In this study, we observed that ICAM-1 was a hub gene in CSU for the first time. Thus, the above previous findings combined with our data, all indicate that ICAM-1 plays an important role in tissue edema formation of CSU, where relevant clinical and laboratory evidence remains to be further explored.

PTGS-2(COX-2) contributes to non-histamine-mediated symptoms in CSU

In clinic, the effect of using H1 antihistamine alone in the treatment of CSU is not satisfactory (59). A reasonable explanation is that some inflammatory mediators can show similar functions to histamine, such as prostaglandins and coagulans, has been overlooked (60). Arachidonic acid forms prostaglandins and thromboxanes through the actions of the enzymes cyclooxygenases (COXs). Inducible COX-2 is associated with pseudoallergy and other inflammatory reactions (61). Besides, COX-2 is predominantly induced in activated leukocytes and inflammatory sites (62). Meanwhile, Nonsteroidal anti-inflammatory drug (NSAID)-sensitivity is very common in patients with chronic urticaria (63). The physiological and pathological processes of respiratory and skin reactions may involve the increase of cysteinyl leukotrienes (64). COX-2 selective inhibitor is a major alternative treatment to control pain and inflammation in these patients (65). In the present study, we further confirmed PTGS-2 as a hub gene in CSU through PPI network characterization. Thus COX-2 may be an important target for the treatment of CSUs.

Overview of infiltrating cells in lesional skin of patients with CSU

The study found that CSU lesions contained a higher proportion of DCs, Th2 cells, mast cells, MEP, preadipocytes, and M1 macrophages compared with healthy skin. It was also discovered that increased expression of Th-2 cytokines (IL-4/IL-

5) in CSU than in normal healthy controls (66). Mast cells are key effector cells in the pathogenesis of CSU, and when activated, they release inflammatory mediators such as histamine and PGD2, which in turn lead to an increase in vascular permeability, causing flare-ups, itching, angioedema (66). In CSU, IgE-mediated type I hypersensitivity is closely related to mast cell activation (67). A predominance of activated M2 macrophages was also observed in CSU patients with refractory for antihistamines (68). Thus, our experimental data are consistent with previous findings on the overview of immune cell infiltration in the CSU, which in turn demonstrates the accuracy of our analysis.

It's worth noting that, so far there have been no reports on MEP or preadipocytes in CSU, and these potential connections were observed for the first time in the present study. Thus, the specific mechanism of MEP or preadipocyte involvement in the development of CSU still needs further verification and exploration. Moreover, in this study, we found that hub genes IL-6, TLR-4, ICAM-1, and PTGS-2 were positively correlated with immune and stromal cell infiltration in CSU lesions. These findings indicate that these hub genes also contribute to the infiltration of these immune cells to the lesions of patients with CSU. However, the specific mechanism of these DEGs on the immune invasion of CSU lesions remains to be further studied.

Limitations of this study

However, this study still has certain limitations. First, the sample size we analyzed is relatively small. Cause the amount of samples uploaded to public databases is currently valid, and we have analyzed the only two databases available until now. Second, all our data comes from publicly available databases. Third, the potential pathway involved in the CSU was not examined and verified. Future studies should be performed to identify the detailed pathway that participated in the progression of CSU. Nevertheless, our results provide a new insight into the pathological mechanism of CSU. These findings do require further substantiation such as by *in vivo* or *in vitro* experiments.

Conclusions

In summary, in the present study, a total of 4 hub genes (IL-6, TLR-4, ICAM-1, and PTGS-2) have been identified, which has the potential to be used as markers or as therapeutic targets for CSU. TNF signaling pathway, NF-κB signaling pathway, and JAK-STAT signaling pathway are thought to be closely related to the pathological mechanism of CSU. We observed higher proportions of DCs, Th2 cells, mast cells, MEP, preadipocytes, and M1 macrophages in CSU lesions. Finally, the positive correlation of hub genes IL-6, TLR-4, ICAM-1, and PTGS-2 and these immune cells infiltration in CSU lesions were found. Our findings will help to encourage further studies on the biological functions of these hub genes, immune infiltration profiles, as well as the relationship between them in CSU.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

WS: Software, Visualization, Writing – original draft, Writing – review & editing. YT: Conceptualization, Data curation, Formal Analysis, Writing – review & editing. YW: Data curation, Writing – review & editing. FH: Project administration, Supervision, Writing – review & editing. JJ: Data curation, Project administration, Supervision, Writing – review & editing.

Funding

Acknowledgments

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1279139/full#supplementary-material

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