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Lymphocyte infiltration and key differentially expressed genes in the ulcerative colitis

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Abstract

Background: Ulcerative colitis (UC) was a type of inflammatory bowel diseases, which was difficult to cure and even would malignant turn into colon cancer. The specific etiology and molecular mechanism of UC were unclear to date. The purpose of this study was to search for new targets for the diagnosis and treatment of UC.

Methods: Firstly, we downloaded the gene expression data of UC from the gene expression omnibus database database (GSE107499), and used multiple bioinformatics methods to find differently expressed genes (DEGs) in UC. Subsequently, we evaluated the lymphocyte infiltration in UC inflamed colon tissue by using the cell type identification by estimating relative subset of known RNA transcripts method.

Results: We obtained 1175 DEGs and 8 hub genes (IL6, TNF, PTPRC, CXCL8, FN1, CD44, IL1B, and MMP9) in this study. Among them, 903 DEGs were up-regulated and 272 DEGs were down-regulated. Compared with non-inflamed colon tissues, the inflamed colon tissues had higher levels of memory B cells, activated memory $CD4^+$ T cells, follicular helper T cells, M1 macrophages, resting dendritic cells, activated dendritic cells, activated mast cells, and neutrophils, whereas the proportions of plasma cells, resting memory $CD4^+$ T cells, gamma delta T cells, activated NK cells, M2 macrophages and resting mast cells were relatively lower.

Conclusions: The DEGs, hub genes and different lymphatic infiltration conditions can provide new targets for diagnosis and treatment of UC. However, these were just predictions through some theoretical methods, and more basic experiments will be needed to prove in the future.

Abbreviations: CIBERSORT = cell type identification by estimating relative subset of known RNA transcripts, DEGs = differential expression genes, GEO = Gene Expression Omnibus Database, PPI = protein protein interact, UC = ulcerative colitis.

Keywords: cell type identification by estimating relative subset of known RNA transcripts, differently expressed genes, lymphocyte infiltration, ulcerative colitis

1. Introduction

Ulcerative colitis (UC) was a type of inflammatory bowel diseases (IBD).[1] Its main clinical manifestations were gastrointestinal disorders; such as abdominal pain, diarrhea, tenesmus, bloody diarrhea with mucus, and these were varied in duration and severity.[2] UC was sometimes difficult to cure, it was easy to relapse, and then the patients could form polyps, even malignant turn into colon cancer.[3] Epidemiology showed that the incidence of UC was increasing year by year globally.[4] It was widespread in 20 to 40 years old adults, and there was no gender difference.[5] The specific etiology and molecular mechanism of UC were unknown to date. Studies had shown that the disease was related to genetic, environmental, immune and infectious factors, etc.[1] There were approximately 8% to 14% of UC patients had a family history of IBD, and their first-degree relatives had a 4-fold risk of this disease.[2] The mucosal layer of the intestinal wall of UC patients obviously showed thickened and there were densely infiltrated neutrophils, macrophages, dendritic cells, T lymphocytes, and other immune cells.[6]

With the advancement and innovation of science and technology, microarray gene chip detection technology can simultaneously detect the expression levels of a large number of genes, thereby obtaining a large amount of gene expression profile data.[7] The Gene Expression Omnibus (GEO) database was a comprehensive library of gene expression from the National Center for Biotechnology Information (NCBI) of America; it was one of the largest gene chip databases in the world, and scholars can download the disease-related expression profile data for free, and then used the bioinformatics methods to analyze and reveal the molecular mechanism of disease.[8] Cell type identification by estimating relative subset of known RNA transcripts (CIBERSORT) was a deconvolution algorithm, which

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The datasets generated during and/or analyzed during the current study are publicly available.

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first published in the journal Nature methods in 2015.[9] It could calculate the cellular composite of complex tissues based on standardized gene expression data. This method energized the abundance of 22 specific immune cell types and had been well validated in the breast and liver cancer tissues.[9–12]

In this study, we downloaded the gene expression data of UC from the GEO database (GSE107499), and used bioinformatics methods to seek abnormally expressed genes in UC inflamed colon tissues. Subsequently, we evaluated the lymphocyte infiltration in UC inflamed colon tissues by using the CIBERSORT method. The purpose of this study was to explore new targets for the diagnosis and treatment of UC.

2. Materials and methods

2.1. Microarray data

The gene expression profiles of GSE107499, which contained 119 samples (including 75 inflamed colon tissues and 44 non-inflamed colon tissues from UC subjects), were downloaded from the GEO database.[8] The annotation platform was GPL15207 [primeview] Affymetrix Human Gene Expression Array. Due to the analysis of publicly available online data used in this study, there were no ethical issues and no ethics committee approval was required. There was no need to obtain the patient's informed consent.

2.2. Data processing

R software was utilized to analyze and process the data, including data normalization, background correction, and supplement missing values. The limma package was used to identify differentially expressed genes (DEGs) between the inflamed colon tissues and non-inflamed colon tissues from UC subjects. The criterions of DEGs were adjusted P value $< .05$ and $|\log2\text{fold change (FC)}| \geq 1$. The pheatmap package of R software was used to draw the heatmap of the DEGs.

2.3. GO analysis and KEGG pathway analysis

The whole DEGs was put in the online analysis database, Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/, Version 6.8), to make the Gene Ontology annotation (GO) and KEGG pathways enrichment analysis.[13,14] The criterion was P-value $< .05$ and gene counts > 2.

2.4. PPI network construction

The STRING version 10.5 (https://string-db.org/) database was used to predict the possible protein-protein interaction (PPI) network of DEGs, and the selected confidence score was higher than 0.4.[15] Cytoscape 3.6.1 (http://www.cytoscape.org/) software was used for visual analysis of the PPI network, and the CentScaPe 2.2 plug-in of Cytoscape 3.6.1 software was to analyze the degree centrality and betweenness centrality of DEGs, which were further used to screen the hub genes.[16]

2.5. Immune infiltration by CIBERSORT analysis

The CIBERSORT algorithm was used to evaluate the composition of 22 different lymphoid immune cells (naive B cells, memory B cells, plasma cells, $CD8^+$ T cells, naive $CD4^+$ T cells, resting memory $CD4^+$ T cells, activated memory $CD4^+$ T cells, follicular helper T cells, regulatory T cells (Tregs), gamma delta T cells, resting NK cells, activated NK cells, monocytes, M0 macrophages, M1 macrophages, M2 macrophages, resting dendritic cells, activated dendritic cells, resting mast cells, activated mast cells, eosinophils, and neutrophils) by through analyzing of the gene expression profile data obtained before.[7,9] In this study, the percentage of 22 different lymphoid immune cells in each sample was evaluated, and the significant samples ($P < .05$) were selected for the subsequent differential infiltration immune cells analysis between UC inflamed colon tissues and non-inflamed colon tissues.

3. Results

3.1. Differentially expressed genes

According to the criterions of DEGs, 1175 DEGs were detected between the UC inflamed colon tissues and non-inflamed colon tissues. Among them, 903 DEGs were up-regulated and 272 DEGs were down-regulated. And the top 50 up-regulated DEGs and 50 down-regulated DEGs were shown in the Figure 1. As shown in the Table 1, the top 10 up regulated genes were SAA1, DEFB4A, DUOX2, MMP3, DEFA6, REG1A, SLC6A14, REG3A, CHI3L1, and REG1B; the top 10 down regulated genes were AQP8, PITX2, CYP2B7P, HSD3B2, MEP1B, HSPB3, GBA3, CDKN2B-AS1, ABCG2, and OTOP2 (Table 1).

3.2. GO analysis and KEGG pathway

DEGs were mainly enriched in 404 GO terms (biological process, 311; cellular component, 28 and molecular function, 65). The top 10 significant biological process, cellular component and molecular function, which with the lowest P value, were shown in the Table 2, respectively.

DEGs were mainly enriched in 44 KEGG pathways, the top 10 significant KEGG pathways were shown in the Table 3, including cytokine-cytokine receptor interaction pathway ($P = 5.15\text{E}{-16}$), rheumatoid arthritis pathway ($P = 2.42\text{E}{-11}$), malaria pathway ($P = 2.70\text{E}{-11}$), chemokine signaling pathway ($P = 1.44\text{E}{-09}$), staphylococcus aureus infection pathway ($P = 1.25\text{E}{-08}$), cell adhesion molecules (CAMs) pathway ($P = 3.68\text{E}{-08}$), hematopoietic cell lineage pathway ($P = 8.99\text{E}{-08}$), pertussis pathway ($P = 6.66\text{E}{-07}$), leishmaniasis pathway ($P = 1.30\text{E}{-06}$), and NF-kappa B signaling pathway ($P = 7.30\text{E}{-06}$)

3.3. PPI network construction

As showed in the Figure 2, we constructed a PPI network which was included 1069 nodes and 10,691 edges. The top 10 genes, which the highest degree centrality and betweenness centrality were displayed in the Table 4, respectively. Finally, we took the 8 intersection genes as the hub genes, including IL6, TNF, PTPRC, CXCL8, FN1, CD44, IL1B, and MMP9, all of them were upregulated.

3.4. Immune infiltration analyses

After screened by the P value $< .05$, we used the CIBERSORT algorithm to calculate the percentage of the 22 immune cells in the 74 significant UC inflamed colon tissues and 27 significant non-inflamed colon tissues (Fig. 3A). As shown in the Figure 3B, compared with non-inflamed colon tissues, the inflamed colon

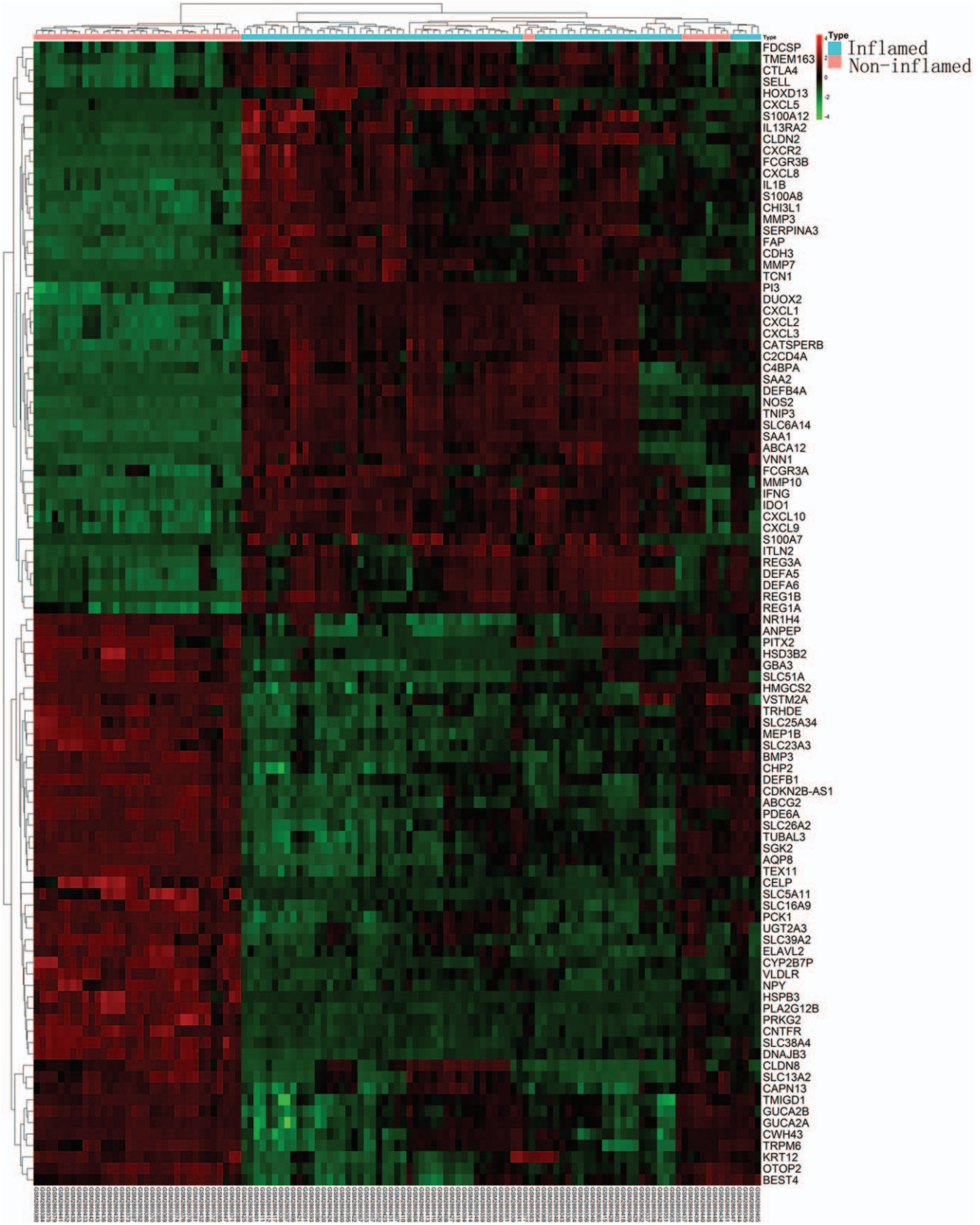

Figure 1. Heat map of differential expression genes (DEGs) between ulcerative colitis inflamed colon tissues and non-inflamed colon tissues (top 50 up-regulated and down-regulated DEGs). Colors from green to red mean increasing expression of DEGs between the 2 groups. DEGs = differentially expressed genes.

Table 1

The top 10 upregulated and downregulated differential expression genes.

GENE	LogFC	Adj.*P*-value
Upregulated genes		
SAA1	5.611816	3.94E–34
DEFB4A	5.574448	3.73E–26
DUOX2	5.141979	7.92E–40
MMP3	5.111792	3.65E–28
DEFA6	4.733108	1.94E–20
REG1A	4.712302	8.29E–22
SLC6A14	4.709493	5.13E–38
REG3A	4.623626	6.57E–19
CHI3L1	4.257901	4.34E–31
REG1B	4.23256	7.57E–14
Downregulated genes		
AQP8	–4.64048	9.91E–26
PITX2	–4.61373	6.06E–18
CYP2B7P	–3.34506	1.12E–22
HSD3B2	–3.17381	5.23E–20
MEP1B	–3.13332	1.58E–27
HSPB3	–3.10365	2.43E–29
GBA3	–3.05836	2.81E–21
CDKN2B-AS1	–2.93584	2.70E–29
ABCG2	–2.89902	3.03E–31
OTOP2	–2.86712	1.34E–27

tissues had higher levels of memory B cells, activated memory CD4$^+$ T cells, follicular helper T cells, M1 macrophages, resting dendritic cells, activated dendritic cells, activated mast cells, and neutrophils, whereas the proportions of plasma cells, resting memory CD4$^+$ T cells, gamma delta T cells, activated NK cells, M2 macrophages, and resting mast cells were relatively lower (Fig. 3B, $P < .05$).

4. Discussion

UC was an incurable and recurrent IBD disease, that eventually could progress to colon cancer.[3] Although a large number of studies had demonstrated that it was related to many factors, such as genes, environment, immunity, and infection, its exact etiology had not been clearly elucidated so far.[1] The histopathology showed that lots of immune cells would infiltrate in the mucosal layer of the intestinal wall, such as neutrophils, macrophages, dendritic cells, and T lymphocytes, etc.[6] In this study, we used bioinformatics method to find the possible targets for new diagnosis or treatment of UC, and we utilized the CIBERSORT algorithm to obtain the status of lymphocyte infiltration in UC inflamed colon tissues. Finally, compared with UC non-inflamed colon tissues, 1175 DEGs were found in the UC inflamed colon tissues, including 903 up-regulated DEGs and 272 down-regulated DEGs. For the status of lymphocyte infiltration, the inflamed colon tissues had higher levels of memory B cells, activated memory CD4$^+$ T cells, follicular helper T cells, M1 macrophages, resting dendritic cells, activated dendritic cells, activated mast cells and neutrophils, whereas the proportions of plasma cells, resting memory CD4$^+$ T cells, gamma delta T cells, activated NK cells, M2 macrophages and resting mast cells were relatively lower.

Table 3

KEGG pathways analysis for DEGs (top 10).

KEGG Term	Count	*P*-value
Cytokine-cytokine receptor interaction	57	5.15E–16
Rheumatoid arthritis	28	2.42E–11
Malaria	21	2.70E–11
Chemokine signaling pathway	39	1.44E–09
Staphylococcus aureus infection	19	1.25E–08
Cell adhesion molecules (CAMs)	31	3.68E–08
Hematopoietic cell lineage	23	8.99E–08
Pertussis	20	6.66E–07
Leishmaniasis	19	1.30E–06
NF-kappa B signaling pathway	20	7.30E–06

Subsequently, we performed an enrichment analysis on those DEGs. And we found that those DEGs mostly related to the immune-related processes, such as immune response (inflammatory response, immune response, response to lipopolysaccharide, and innate immune response) and immunochemotaxis (chemotaxis, chemokine-mediated signaling pathway, neutrophil chemotaxis, leukocyte migration, and cell adhesion). For the KEGG pathway analysis, the DEGs also significantly involved in the chemokine signaling pathway. This stated that the future treatment strategy of UC maybe focused on regulating the body's immune response and chemotaxis-related factors.

While we conducted a PPI network analysis, and we found that IL6, TNF, PTPRC, CXCL8, FN1, CD44, IL1B, and MMP9 were the hub genes among these DEGs. Previous studies have shown that IL6, TNF, CXCL8, and IL1B were UC-related proinflammatory cytokines, and they were thought to contribute to the development of UC diseases.[17] Parisinos et al indicated that when there were a single nucleotide polymorphism rs2228145 in

Table 2

GO analysis for differential expression genes (top 10).

Biological process	Count	*P*-value	Cellular component	Count	*P*-value	Molecular function	Count	*P*-value
Inflammatory response	92	8.34E–31	Extracellular space	205	3.15E–36	Chemokine activity	21	1.26E–12
Immune response	94	1.68E–28	Extracellular region	194	2.65E–21	Heparin binding	32	3.99E–09
Chemotaxis	44	3.84E–22	Plasma membrane	375	5.31E–19	Calcium ion binding	83	5.98E–09
Chemokine-mediated signaling pathway	32	1.57E–19	Integral component of Plasma membrane	162	1.94E–15	Carbohydrate binding	34	4.75E–08
Angiogenesis	47	1.90E–13	External side of plasma membrane	47	2.92E–14	Receptor activity	35	1.81E–07
Neutrophil chemotaxis	25	2.85E–13	Integral component of membrane	412	3.87E–11	RAGE receptor binding	8	6.66E–07
Leukocyte migration	33	1.05E–12	Proteinaceous extracellular matrix	48	4.10E–11	CXCR chemokine receptor binding	7	3.05E–06
Response to lipopolysaccharide	37	1.22E–11	Extracellular matrix	45	3.30E–08	Cytokine activity	28	4.90E–06
Innate immune response	65	2.56E–11	Cell surface	66	1.12E–07	Extracellular matrix structural constituent	15	3.03E–05
Cell adhesion	67	5.81E–11	Extracellular exosome	231	5.78E–07	Serine-type endopeptidase inhibitor activity	18	4.96E–05

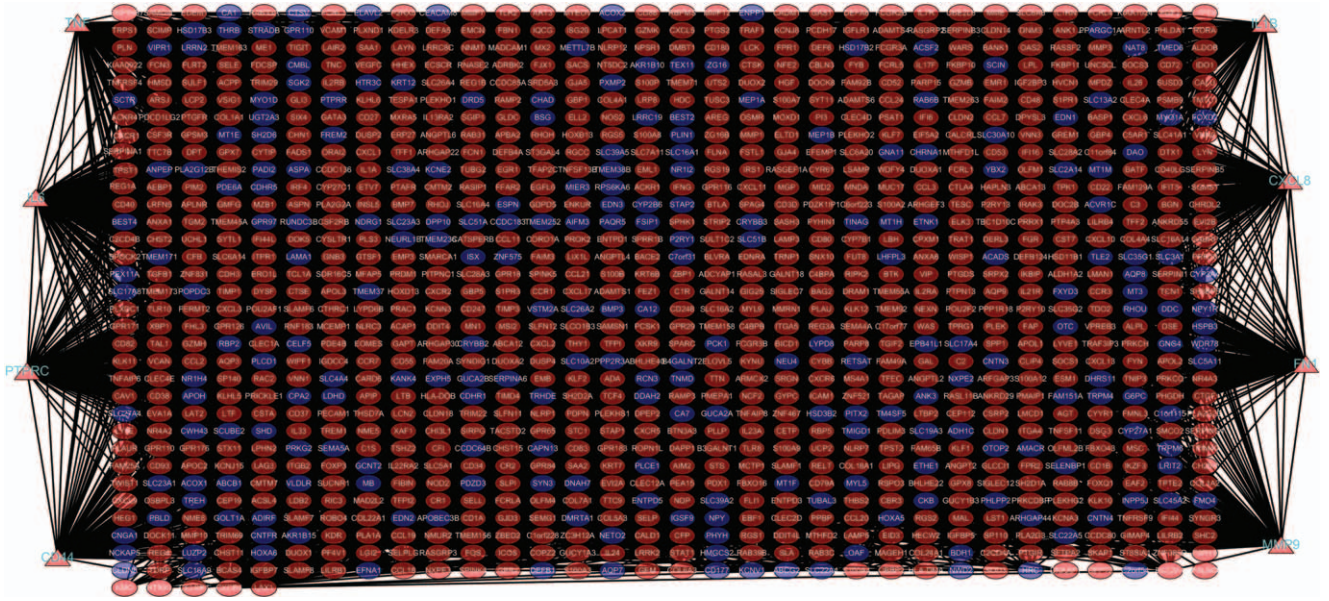

Figure 2. Protein protein interact network of differential expression genes. Hub genes are labeled by triangles. Red indicates upregulated genes, and blue indicates downregulated genes. DEGs = differentially expressed genes.

the receptor of interleukin 6 (IL6R), the expression of soluble IL6R would increase, meanwhile, the corresponding expression level of IL6R will decrease, and then the risk of UC inflammatory disorders was decreased; and they thought that blocking the IL6R signaling pathway would be a new therapeutic direction to treat UC.[18] Infliximab, adalimumab, and golimumab were 3 drugs currently used in clinical treatment of UC, and their mechanism of action was to specifically block tumor necrosis factor[19]; several international clinical studies had shown that the application of these anti-TNF biologics to the UC patients can make effective clinical remission and and mucosal healing.[20–22] C-X-C Motif Chemokine Ligand 8 (CXCL8), also known as IL-8, which was produced by a variety of immune cells and intestinal epithelium.[23] It can induce neutrophil chemotactic to UC inflamed colon tissues, and its expression level was linked with the severity and duration of UC.[24] Walana at al found that when they used the CXCL8 antagonist G31P to treat the dextran sulfate sodium induced UC mice, it could decreased the expression of proinflammatory cytokines (including IL-β, IL-6, IL-8, TNF-α, and IFN-γ, etc), and had an potential therapeutic protective effect on UC disease.[25] Interleukin-1β (IL-1β, also known as IL1B) was involved in the pathogenesis of IBD diseases, and Guzmán et al found that when IL1B was highly expressed in the UC serum, the patients would be resistant to the treatment by anti-TNF biologics (infliximab), and the patients would presented with a poor treatment effects.[26] Crohn disease (CD) was another type of IBD disease. Some scholars conducted bioinformatics analysis on the data of CD disease gene chip; they also concluded that CXCL8 and IL1B are highly expressed in CD disease. And these 2 genes are also considered to be important hub genes.[27] Fibronectin 1 (FN1) was a high-molecular-weight glycoprotein in the extracellular matrix. It played an important role in cell migration, adhesion, proliferation, hemostasis, and tissue repair.[28] Yan et al used whole exon sequencing method to detect the tumor tissues and paired adjacent nondysplastic tissues of UC–associated colorectal cancer patients (CRC); they found that a deleterious mutation in the FN1 may be related to the UC–associated CRC.[29] AbdElazeem et al found that the expression of CD44 and MMP-9 was significant correlation in the UC dysplasia and neoplastic colon mucosa tissues, and the elevated level of these molecules indicated a poor clinical outcome.[30] Previous studies had shown that MMP played a major role in intestinal tissue damage and inflammation in IBD disease.[31,32] At the same time, MMP was involved in lymphocyte chemotaxis and pro-inflammatory cytokine secretion of the UC inflammation intestinal tissue.[32,33] Therefore, scholars believed that it may be another method for treating IBD disease by inhibiting MMP.[34,35] However, Sandborn et al compared the efficacy between the anti-MMP-9 antibody (andecaliximab) treatment and placebo for UC patients, and the results showed that after 8 weeks of treatment with 150 mg andecaliximab, UC patients did not perform better clinical remission.[36] There were comparatively few articles related to PTPRC and UC.

CIBERSORT was a deconvolution algorithm, which was first reported in the journal Nature methods in 2015.[9] It could

Table 4

The top 10 genes in protein protein interact network by network topology parameters.

GENE	Degree centrality	GENE	Betweenness centrality
IL6	234	IL6	0.08001179
TNF	229	TNF	0.07787476
PTPRC	176	FN1	0.04651836
CXCL8	173	GPR110	0.03176785
FN1	162	CD44	0.03137941
TLR2	151	MMP9	0.02974559
CD44	148	PTPRC	0.02782289
IL1B	147	CXCL8	0.02425323
CXCR4	142	FOS	0.02246342
MMP9	140	IL1B	0.02185226

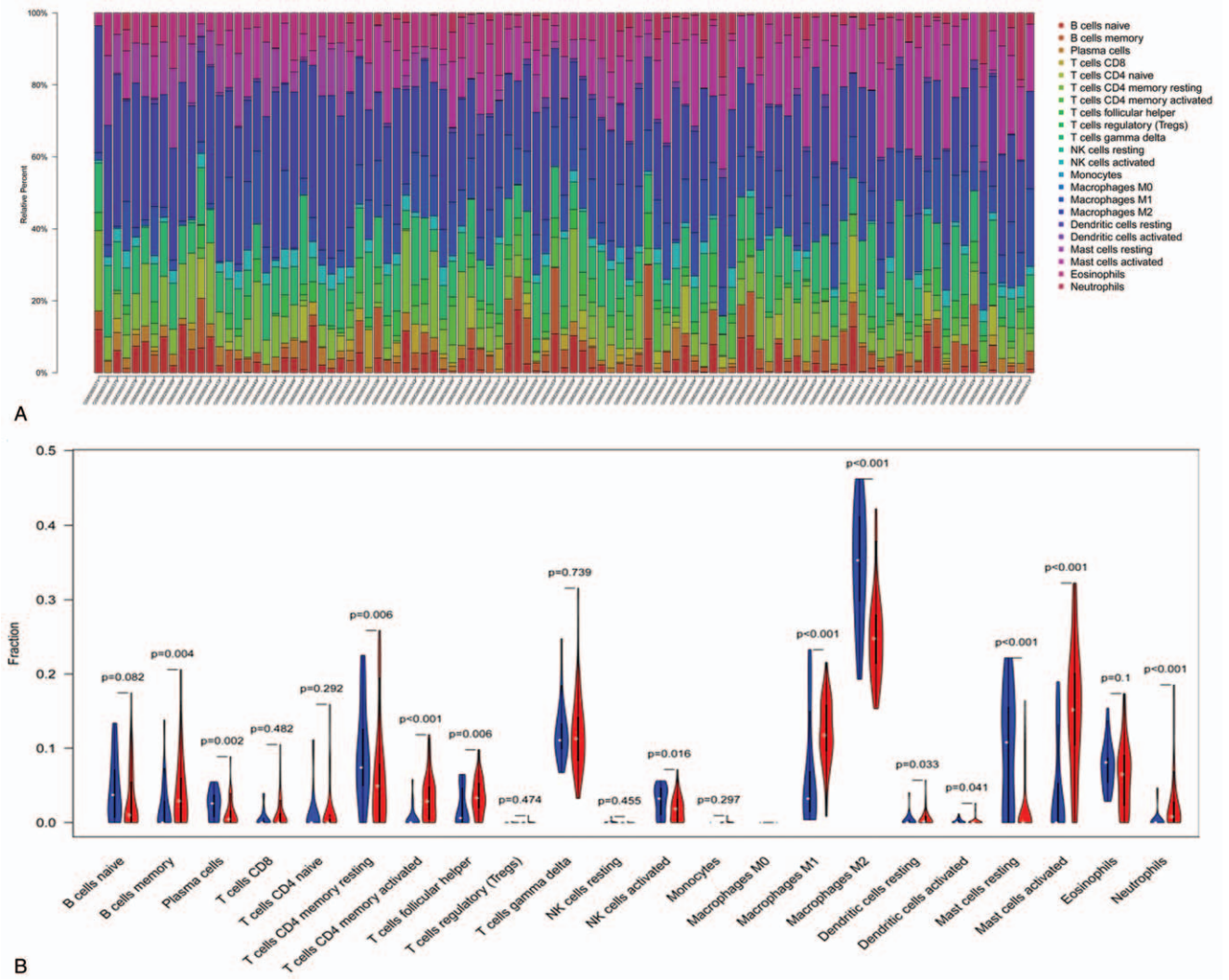

Figure 3. The landscape of immune infiltration between ulcerative colitis (UC) inflamed colon tissues and non-inflamed colon tissues. (A) The relative percentage of 22 subpopulations of immune cells in the 74 significant UC inflamed colon tissues and 27 significant non-inflamed colon tissues. (B) The difference of immune infiltration between UC inflamed colon tissues and non-inflamed colon tissues. The non-inflamed colon tissues were marked as blue color and inflamed colon tissues was marked as red color. *P* values $< .05$ were considered as statistical significance.

calculate the cell composite and energized the abundance of specific cell types of complex tissues based on standardized gene expression data; the composition of immune cells in breast and liver cancer tissues were successfully evaluated and well verified.[10–12] Subsequently, scholars had applied it to the study of lymphocyte infiltration in the tumor microenvironment of various tumor diseases.[37–39] Recently, many non-tumor diseases had also begun to use this algorithm to explore lymphatic infiltration, such as osteoarthritis and systemic lupus erythematosus, etc.[7,40] To our knowledge, our article was the first time application of the CIBERSORT algorithm to explore the lymphatic infiltration status of UC. Our results shown that compared with UC non-inflamed colon tissues, the UC inflamed colon tissues had higher levels of memory B cells, activated memory $CD4^+$ T cells, follicular helper T cells, M1 macrophages, resting dendritic cells, activated dendritic cells, activated mast cells and neutrophils. Surprisingly, the proportions of plasma cells, resting memory $CD4^+$ T cells, gamma delta T cells, activated NK cells, M2 macrophages, and resting mast cells were relatively lower in the inflamed colon tissues. This result may reveal the composition of lymphocytes in the microenvironment of lymphatic infiltration in colon tissues of UC. Coincidentally, when Chen et al studied the lymphatic infiltration of CD disease, their findings were broadly similar to our results. They also found that CD inflammatory intestinal tissue highly expressed activated memory $CD4^+$ T cells, M1 macrophages, resting dendritic cells, activated mast cells, and neutrophils, but low gamma delta T cells, activated NK cells, M2 macrophages, and resting mast cells expressing.[27] However, it also required to verify by experiments and histopathological tests in the future. And it can also get more accurate results by using the single-cell RNA sequencing to analyze the type of lymphoid infiltrating cells.

In summary, we obtained 1175 DEGs and 8 hub genes in this study. In addition, we first used the CIBERSORT algorithm to analyze the lymphatic infiltration in UC inflamed colon tissues, and we found that the types of lymphocytes infiltrated in the UC inflamed colon tissues and adjacent non- inflamed colon tissues were very different. However, these were just predictions through

some bioinformatics methods, and more basic experiments will be needed to prove in the future.

Author contributions

Junhui Zhang analyzed the data and wrote the first draft, Guixiu Shi revised the manuscript.

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