Identification of Key Genes and Pathways in SARS-CoV-2 Infection using Bioinformatics Analysis

Zhe Wang
Shihezi University
Chenhao Jiang
Shihezi University
Xuxuan Zhang
Shihezi University
Yingna Zhang
Shihezi University
Yan Ren
Shihezi University
Xiangting Gao (gxt1523628@foxmail.com)
Shihezi University

Research

Keywords: SARS-CoV-2, Bioinformatics, Differentially expressed genes, hub genes, molecular mechanisms

Posted Date: September 14th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-72821/v1

Abstract

Background: Coronavirus disease 2019 (COVID-19) is a disease that causes fatal disorders including severe pneumonia. Our study aimed to utilize bioinformatics method to analyze the expression profiling by high throughput sequencing in human bronchial organoids/primary human airway epithelial infected with SARS-CoV-2 to identify the potentially crucial genes and pathways associated with COVID-19.

Methods: We analyzed microarray datasets GSE153970 and GSE150819 derived from the GEO database. Firstly, the Differentially expressed genes （DEGs） in human bronchial organoids/primary human airway epithelial infected with SARS-CoV-2. Next, the DEGs were used for GO and KEGG pathway enrichment analysis. Then, the PPI network was constructed and Cytoscape was used to find the key genes.

Results: Gene expression profiles of GSE153970 and GSE150819, in all 12 samples were analyzed. A total of 145 DEGs and 5 hub genes were identified in SARS-CoV-2. Meanwhile, we found that the 145 genes are associated with immune responses and the top 5 hub genes including CXCL8, CXCL1, CXCL2, CCL20, and CSF2 were mainly related to leukocyte migration, endoplasmic reticulum lumen, receptor ligand activity. In addition, the results also showed that the hub genes were associated with Cytokine−cytokine receptor interaction, IL−17 signaling pathway, and Rheumatoid arthritis in SARS-CoV-2 infection.

Conclusion: The five crucial genes consisting of *CXCL8*, *CXCL1*, *CXCL2*, *CCL20*, and *CSF2* were considered as hub genes of SARS-CoV-2, which may be used as diagnostic biomarkers or molecular targets for the treatment of SARS-CoV-2. It is evidenced that bioinformatics analyses in SARS-CoV-2 can be useful for understanding the underlying molecular mechanism and exploring effective therapeutic targets.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as a novel member of coronaviruses, was first reported in December 2019 in Wuhan, China, and rapidly spreads over the world[1, 2]. SARS-CoV-2, which belongs to the categories of severe acute respiratory syndrome-related coronaviruses, was first identified as an etiological pathogen of Coronavirus Disease 2019(COVID-19). SARS-CoV-2 has similar homologous sequences and structures with SARS-CoV and MERS-CoV[3]. As reported, the symptoms of SARS-CoV-2 infected patients are varying. Respiratory distress is the most typical symptom of patients with COVID-19, and some patients may lead to respiratory failure even death while some patients may be asymptomatic carrier[4]. In addition, some patients with COVID-19 also show flu symptoms, such as fever, dry cough, headache, fatigue, nausea, vomiting and diarrhea. So far, there is no specific antiviral treatment is available for COVID-19. Therefore, it is necessary to find potential therapeutics or effective vaccine for COVID-19. In this context, we try to explore molecular mechanisms of COVID-19 by bioinformatics analysis.

Previous studies has revealed that SARS-CoV-2 enters into host cell by binding with the SARS-CoV receptor angiotensin converting enzyme 2 (ACE2) and viral spike (S) protein priming is essential for viral entry into cells[5]. According to the results of single-cell RNA sequencing, the activated immune cells and inflammatory macrophages expressing, such as
CCL2, CCL3, CCL20, CXCL1, CXCL3, CXCL10, IL8, IL1B and TNF, might contribute to excessive inflammation by promoting monocyte recruitment in SARS-CoV-2 infection patients[6, 7]. In addition, SARS-CoV-2 infection could stimulate the immune response of host, leading to abnormal increase of cytokines and the decrease of lymphocytes in COVID-19 patients[8]. Therefore, It is convinced that the immune response is closely associated with the development of lung injury and severe pneumonia in COVID-19.

Our study aimed to utilize bioinformatics method to analyze the gene expression profiling based on high throughput sequencing in human bronchial organoids/primary human airway epithelial infected with SARS-CoV-2 to identify the potentially crucial genes and pathways associated with COVID-19. The Gene Ontology (GO)and Kyoto Encyclopedia of Genes and Genomes (KEGG) function enrichment analysis were used to analyze the differentially expressed gene (DEGs). PPI network was constructed and Cytoscape was used to find the key genes. Our results suggested that several molecular mechanisms may be involved in the development of COVID-19.

Materials And Methods

Microarray Database Sources

The mRNA expression file of GSE153970 and GSE150819 were downloaded from the National Centre of Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). The expression profiling arrays of GSE150819 were generated using the GPL18573 platform (Illumina NextSeq 500) and GPL24676 platform (Illumina NextSeq 6000). There 6 samples were choice in GSE150819 dataset, including 3 SARS-CoV-2-infected human bronchial organoids samples and 3 uninfected human bronchial organoids samples, were analyzed. Meanwhile, the expression profiling arrays of GSE153970 were generated using the GPL24676 platform (Illumina NextSeq 6000). A total of 6 samples in GSE153970 dataset, including 3 experiment group samples and 3 control group samples, were analyzed.

Identification of DEGs in SARS-CoV-2 infected model.

DEGs between human bronchial organoids/primary human airway epithelial infected with SARS-CoV-2 and control group were identified by using the limma o package in R [9]. The screening criteria of |log2 Fold Change| > 1 and adjusted P < 0.05 was considered as statistically significant. The analysis results were presented by heatmap and volcano map drawn in the pheatmap R package. In order to explore significant DEGs in both datasets, Venn map was made with the Venn online tool (http://bioin forma tics.psb.ugent .be/webto ols/Venn/) and after overlapping of DEGs for following research.

Functional Enrichment Analysis of DEGs

The package clusterProfiler was applied to carry out GO and KEGG pathway analysis for DEGs[10]. The P value < 0.05 adjusted by the Benjamini and Hochberg method was deemed to be statistically significant.

PPI Network Analyses and Identification of hub genes

In order to explore the interactions of DEGs, the protein-protein interaction (PPI) network analyse was constructed using String database (http://www.string-db.org)[11],and a comprehensive score over 0.4 was regarded as statistically significant. Cytoscape (version 3.7.2; https://cytoscape.org/)[12] was used to visualize biological network and integrate the data .The Cytoscape plugin CytoHubba was used to conduct module analysis and the top five genes were selected as hub genes in the study[13].

Results

Identification of DEGs

In the dataset of GSE150819, 954 genes were identified to be differentially expressed, including 559 up-regulated genes and 395 down-regulated genes (Fig. 1a, b). In the dataset of GSE153970, 940 genes were differentially expressed. Of the 940 DEGs, 497 genes were upregulated and 443 genes were downregulated (Fig. 1c, d). Screening criteria are |log2 FC| > 1, and adjusted $P < 0.05$. There are 145 DEGs by overlapping in two datasets (Fig. 1e).

Functional enrichment analysis of DEGs

To assess the biological roles of these DEGs a, KEGG and GO enrichment analyses were performed. The GO analysis consists of biological processes (BP), cellular component (CC), and molecular function (MF) categories. The results of GO analysis indicated that DEGs were mainly enriched in BPs, including granulocyte migration, neutrophil migration, myeloid leukocyte migration and granulocyte chemotaxis and so on. CC analysis revealed that the DEGs were significantly enriched in endoplasmic reticulum lumen, cornified envelope and anchored component of membrane. As for the MF, the DEGs were enriched in receptor ligand activity, cytokine activity, cytokine receptor binding and rchemokine activity and so on (Fig. 2a). Regarding the results of KEGG pathway analysis as shown in Fig. 2b, the DEGs were enriched in 13 pathways and significantly associated with the Cytokine–cytokine receptor interaction, IL−17 signaling pathway, Rheumatoid arthritis, Viral protein interaction with cytokine and cytokine receptor, TNF signaling pathway, and Chemokine signaling pathway.

Construction of protein-protein interaction network and selection of potential hub Genes.

In order to better understand the biological properties of DEGs, we utilized the string database to construct a PPI network containing 131 nodes and 248 edges (Fig. 3a). Then, Cytoscape was used to verify a vital module with the network and the most significant modules were selected (Fig. 3b). The cytoHubba plugin was used to analyze the hub genes, and the following genes with the top 5 grades were deemed to be hub genes: CXCL8, CXCL1, CXCL2, CCL20, and CSF2.

KEGG analysis of hub Genes

The five genes were re-analyzed by KEGG pathway enrichment analysis and the results showed that five hub genes were mainly associated with Cytokine–cytokine receptor interaction, IL–17 signaling pathway, and Rheumatoid arthritis (Fig. 4).

As shown in Fig. 4a, CXCL8, CXCL1, CXCL2, CXCL3 belong to the CXCL Subfamily, CXCL8 and CXCL1 can combine with CXCR1 and CXCR2 while CXCL2 and CXCL3 mainly bind to CXCR2 to exerts functions in Cytokine–cytokine receptor interaction signaling pathway. CCL20 belongs to the CC subfamily, and exerts its function by combining with CCR6. Moreover, CSF2 belonging to IL-4 like, works by binding to CSF2RA and CSF2RB. In IL–17 signaling pathway, the hub genes of CXCL8, CXCL1, CXCL2, CCL20, and CSF2 mainly play roles in autoimmune pathology, neutrophil recruitment, and immunity to extracellular pathogens (Fig. 4b). As shown in Fig. 4c, CXCL1 and CCL20 act on blood vessel to induce leukocyte migration and inflammatory cell infiltration in Rheumatoid arthritis.

Discussion

SARS-CoV-2 emerged in December 2019 in Wuhan, China, and rapidly spreads around the world[14]. Symptoms of SARS-CoV-2 patients are similar to acute respiratory syndrome, but little is known about specific molecular mechanism. Currently, there is no particular anti-nCoV treatment or effective vaccine, and COVID-19 management is mainly supportive[15]. However, the molecular mechanisms of the disease still remain unclear.

Identification of the key genes and signal pathways related to SARS-CoV-2 infection is important for understanding the molecular mechanisms in COVID-19. In our study, we first performed bioinformatics analysis based on two microarray datasets (GSE153970 and GSE150819). A total of 145 genes were identified as DEGs in SARS-CoV-2 infected model. Furthermore, among these DEGs, 5 hub genes were selected in the PPI network by cytoHubba, including CXCL8, CXCL1, CXCL2, CCL20, and CSF2. In addition, we found that the hub genes upregulated were related to Cytokine-cytokine receptor interaction, IL-17 signaling pathway, and Rheumatoid arthritis in SARS-CoV-2 infection.

As far as we know, CXCL8, CXCL1, CXCL2, CCL20, and CSF2 are cytokines, a category of small proteins that are secreted by cells associated with intercellular signaling, which consist of interferons, interleukins, chemokines, colony-stimulating factors, tumor necrosis factor. Chemokines are one of the largest family of cytokines, with over 40 members that combine with one or more of G-protein-coupled receptors[16]. In 1918 virus infected macaques, the cytokines of IL-6, IL-8, CCL2, and CCL5 were up-regulated in the

lungs[17, 18]. The H5N1 virus infection could cause severe inflammatory and innate immune responses, such as durable expression of CCL2, CXCL10, and CXCL9[19, 20]. Moreover, persistent expression of CXCL10, CCL2, IFNAR1, IFNGR1, and CD58 were also found in the lungs of SARS-CoV infected patients[21]. According to our knowledge, SARS-CoV-2 infection resulted in the storm of inflammatory response and proinflammatory genes, particularly chemokines, was significantly elevated in COVID-19 patients[22].

In the study, we found the top 5 genes containing CXCL8, CXCL1, CXCL2, CCL20, and CSF2 were up-regulated in SARS-CoV-2 infection. CXCL1, CXCL2, and CXCL8 belonging to the subfamily of CXC chemokines, which are involved in inflammatory response and occurrence of several tumors by binding with the G protein-coupled receptors. CXCL1/CXCL2 are critical for neutrophil recruitment in immune response[23]. CXCL8 is a pro-inflammatory cytokine by binding with the CXCR-1 and CXCR-2 involved in multiple intracellular signaling pathways[24]. It has been reported that serum CXCL-8 levels were significantly higher and correlate positively with depth of tumor invasion and CRP concentrations in oesophageal cancer patients, suggesting CXCL8 might be a tumor marker of oesophageal cancer[25]. CXCL1 and CXCL8 expression was significantly up-regulated, and through binding to CXCR2 participate in gastric carcinoma proliferation, migration and apoptosis[26]. CC chemokine receptor 6 (CCR6) as the only one known receptor of CC chemokine ligand 20 (CCL20), was involved in the humoral immunity and T-B cell immunobiology[27]. Colony stimulating factor-2 (CSF2), produced by various cells, which is considered to mediate inflammation, autoimmunity and host defense responses[28]. Consistent with previous findings, our study showed that the five hub genes induced immune response play important roles in the pathogenesis of SARS-CoV-2.

Cytokine–cytokine receptor interaction has been proved to play roles in multiple viral infections, such as megalocytivirus, HBV, HCV, and EBV[29-31]. The IL-17 family consists of six members, IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F, which is produced by activated T cells and involves in inflammatory response. IL-17 family of cytokines by combining with IL-17 receptor family activate extracellular signal-regulated protein kinase, c-jun N-terminal kinase, and p38 mitogen-activated protein kinases pathway, leading to the up-regulated expression of IL-1, IL-6, and NF-κB[32, 33]. IL-17A is contribute to clear extracellular pathogens, however, IL-17 signaling pathway may cause lung injury and respiratory failure in SARS-CoV-2 Infections[34]. Rheumatoid arthritis is a systemic autoimmune disease affecting multiple organs and tissues, which primary characteristics are systemic inflammation, synovitis[35]. Previous study has showed that CXCL8 is related to inflammation responses in Rheumatoid arthritis[34, 36]. It is worth noting that modifying anti-rheumatic drugs (bDMARDs) and target synthetic DMARDs (tsDMARDs), known to be small molecule inhibitors, are not only potential drugs to treat Rheumatoid arthritis, but also block the viral entry, inhibit the hyperimmune activation and reduce cytokine storm[37], suggesting that small molecule inhibitors might be potential drugs for SARS-CoV-2 treatment. Based on the above findings, it is convinced that the hub genes were associated with Cytokine–cytokine receptor interaction, IL–17 signaling pathway, and Rheumatoid arthritis in SARS-CoV-2 infection.

There are some limitations in our study. First, the microarray datasets was downloaded from the GEO database, and not made by the authors. Furthermore, more experimental and clinical studies should be performed to verify the function of hub genes in development of disease and clinical treatment of CIVID-19. However, our findings also provided some valuable clues in the CIVID-19.

Conclusion

In summary, we identified DEGs by bioinformatic analysis to find useful genes associated with SARS-CoV-2, which might serve as candidate diagnostic molecular and therapeutic targets of COVID-19. Our research predicted five hub genes and the results should be confirmed by further studies in COVID-19. Our results provided new insights of the molecular mechanisms with SARS-CoV-2.

Abbreviations

COVID-19: Coronavirus disease 2019

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

GEO: Gene Expression Omnibus

DEGs: Differentially expressed genes

PPI: Protein–protein interaction

GO: Gene Ontology

KEGG: Kyoto Encyclopedia of Genes and Genomes

STRING: Search Tool for the Retrieval of Interacting Genes

CXCL1: C-X-C motif chemokine ligand 1

CXCL2: C-X-C motif chemokine ligand 2

CXCL8: C-X-C motif chemokine ligand 8

CCL20: CC chemokine ligand 20

CSF2: Colony stimulating factor-2

IL-17: Interleukin 17

Declarations

Acknowledgements

Authors would like to thank help and support from hospital for the study.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests for this present study.

Funding

This work was supported by grands from the National Natural Science Foundation of China (Grant No 81760386) .The International Cooperation Promotion Project of Shihezi University(Grant No KX01860106).

Authors' contributions

Conceptualization:Xiangting Gao

Formal analysis：Yingna Zhang, Xuxuan Zhang

Data analysis and interpretation: Chenhao Jiang

Funding acquisition：Yan Ren

Writing -original draft：Zhe Wang

Writing -review and editing：Xiangting Gao

All authors read and approved the final manuscript.

References

1. 1. Riou, J. and C.L. Althaus, Pattern of early human-to-human transmission of Wuhan 2019 novel coronavirus (2019-nCoV), December 2019 to January 2020. Euro Surveill, 2020. 25(4).

2. 2. Zolfaghari, E.R., H. Nosrati and R.A. Taheri, Combination of Biodata Mining and Computational Modelling in Identification and Characterization of ORF1ab Polyprotein of SARS-CoV-2 Isolated from Oronasopharynx of an Iranian Patient. Biol Proced Online, 2020. 22: p. 8.
3. 3. Rabaan, A.A., et al., SARS-CoV-2, SARS-CoV, and MERS-COV: A comparative overview. Infez Med, 2020. 28(2): p. 174-184.
4. 4. Madabhavi, I., M. Sarkar and N. Kadakol, COVID-19: a review. Monaldi Arch Chest Dis, 2020. 90(2).
5. 5. Hoffmann, M., et al., SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell, 2020. 181(2): p. 271-280.e8.
6. 6. Wen, W., et al., Immune cell profiling of COVID-19 patients in the recovery stage by single-cell sequencing. Cell Discov, 2020. 6: p. 31.
7. 7. Chua, R.L., et al., COVID-19 severity correlates with airway epithelium-immune cell interactions identified by single-cell analysis. Nat Biotechnol, 2020. 38(8): p. 970-979.
8. 8. Li, K., et al., SARS-CoV-2 infection-induced immune responses: Friends or foes? Scand J Immunol, 2020. 92(2): p. e12895.
9. 9. Leek, J.T., et al., The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics, 2012. 28(6): p. 882-3.
10. 10. Yu, G., et al., clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS, 2012. 16(5): p. 284-7.
11. 11. Szklarczyk, D., et al., STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res, 2019. 47(D1): p. D607-D613.
12. 12. Smoot, M.E., et al., Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics, 2011. 27(3): p. 431-2.
13. 13. Chin, C.H., et al., cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol, 2014. 8 Suppl 4: p. S11.
14. 14. Huang, C., et al., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet, 2020. 395(10223): p. 497-506.
15. 15. Baghizadeh, F.M., What dentists need to know about COVID-19. Oral Oncol, 2020. 105: p. 104741.
16. 16. Comerford, I. and S.R. McColl, Mini-review series: focus on chemokines. Immunol Cell Biol, 2011. 89(2): p. 183-4.
17. 17. Cilloniz, C., et al., Lethal influenza virus infection in macaques is associated with early dysregulation of inflammatory related genes. PLoS Pathog, 2009. 5(10): p. e1000604.
18. 18. Kobasa, D., et al., Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. Nature, 2007. 445(7125): p. 319-23.
19. 19. Baskin, C.R., et al., Early and sustained innate immune response defines pathology and death in nonhuman primates infected by highly pathogenic influenza virus. Proc Natl Acad Sci U S A, 2009. 106(9): p. 3455-60.

20. 20. Tisoncik, J.R., et al., Into the eye of the cytokine storm. Microbiol Mol Biol Rev, 2012. 76(1): p. 16-32.
21. 21. Cameron, M.J., et al., Interferon-mediated immunopathological events are associated with atypical innate and adaptive immune responses in patients with severe acute respiratory syndrome. J Virol, 2007. 81(16): p. 8692-706.
22. 22. Zhou, Z., et al., Heightened Innate Immune Responses in the Respiratory Tract of COVID-19 Patients. Cell Host Microbe, 2020. 27(6): p. 883-890.e2.
23. 23. De Filippo, K., et al., Mast cell and macrophage chemokines CXCL1/CXCL2 control the early stage of neutrophil recruitment during tissue inflammation. Blood, 2013. 121(24): p. 4930-7.
24. 24. Holmes, W.E., et al., Structure and functional expression of a human interleukin-8 receptor. Science, 1991. 253(5025): p. 1278-80.
25. 25. Lukaszewicz-Zajac, M., et al., Comparison between clinical significance of serum CXCL-8 and classical tumor markers in oesophageal cancer (OC) patients. Clin Exp Med, 2019. 19(2): p. 191-199.
26. 26. Chen, X., et al., Complementary action of CXCL1 and CXCL8 in pathogenesis of gastric carcinoma. Int J Clin Exp Pathol, 2018. 11(2): p. 1036-1045.
27. 27. Lee, A. and H. Korner, The CCR6-CCL20 axis in humoral immunity and T-B cell immunobiology. Immunobiology, 2019. 224(3): p. 449-454.
28. 28. Li, Y., et al., Csf2 Attenuated Sepsis-Induced Acute Kidney Injury by Promoting Alternative Macrophage Transition. Front Immunol, 2020. 11: p. 1415.
29. 29. Xie, W., et al., Nine hub genes related to the prognosis of HBV-positive hepatocellular carcinoma identified by protein interaction analysis. Ann Transl Med, 2020. 8(7): p. 478.
30. 30. Wu, Q., et al., Transcriptome analysis reveals seven key immune pathways of Japanese flounder (Paralichthys olivaceus) involved in megalocytivirus infection. Fish Shellfish Immunol, 2020. 103: p. 150-158.
31. 31. Li, S.Y., et al., Transcriptional insights into the CD8(+) T cell response in mono-HIV and HCV infection. J Transl Med, 2020. 18(1): p. 96.
32. 32. Amatya, N., A.V. Garg and S.L. Gaffen, IL-17 Signaling: The Yin and the Yang. Trends Immunol, 2017. 38(5): p. 310-322.
33. 33. Awane, M., et al., NF-kappa B-inducing kinase is a common mediator of IL-17-, TNF-alpha-, and IL-1 beta-induced chemokine promoter activation in intestinal epithelial cells. J Immunol, 1999. 162(9): p. 5337-44.
34. 34. Orlov, M., et al., A Case for Targeting Th17 Cells and IL-17A in SARS-CoV-2 Infections. J Immunol, 2020. 205(4): p. 892-898.
35. 35. Scott, D.L., F. Wolfe and T.W. Huizinga, Rheumatoid arthritis. Lancet, 2010. 376(9746): p. 1094-108.
36. 36. Bartlett, D.B., et al., Habitual physical activity is associated with the maintenance of neutrophil migratory dynamics in healthy older adults. Brain Behav Immun, 2016. 56: p. 12-20.

37. Massalska, M., W. Maslinski and M. Ciechomska, Small Molecule Inhibitors in the Treatment of Rheumatoid Arthritis and Beyond: Latest Updates and Potential Strategy for Fighting COVID-19. Cells, 2020. 9(8).

Figures

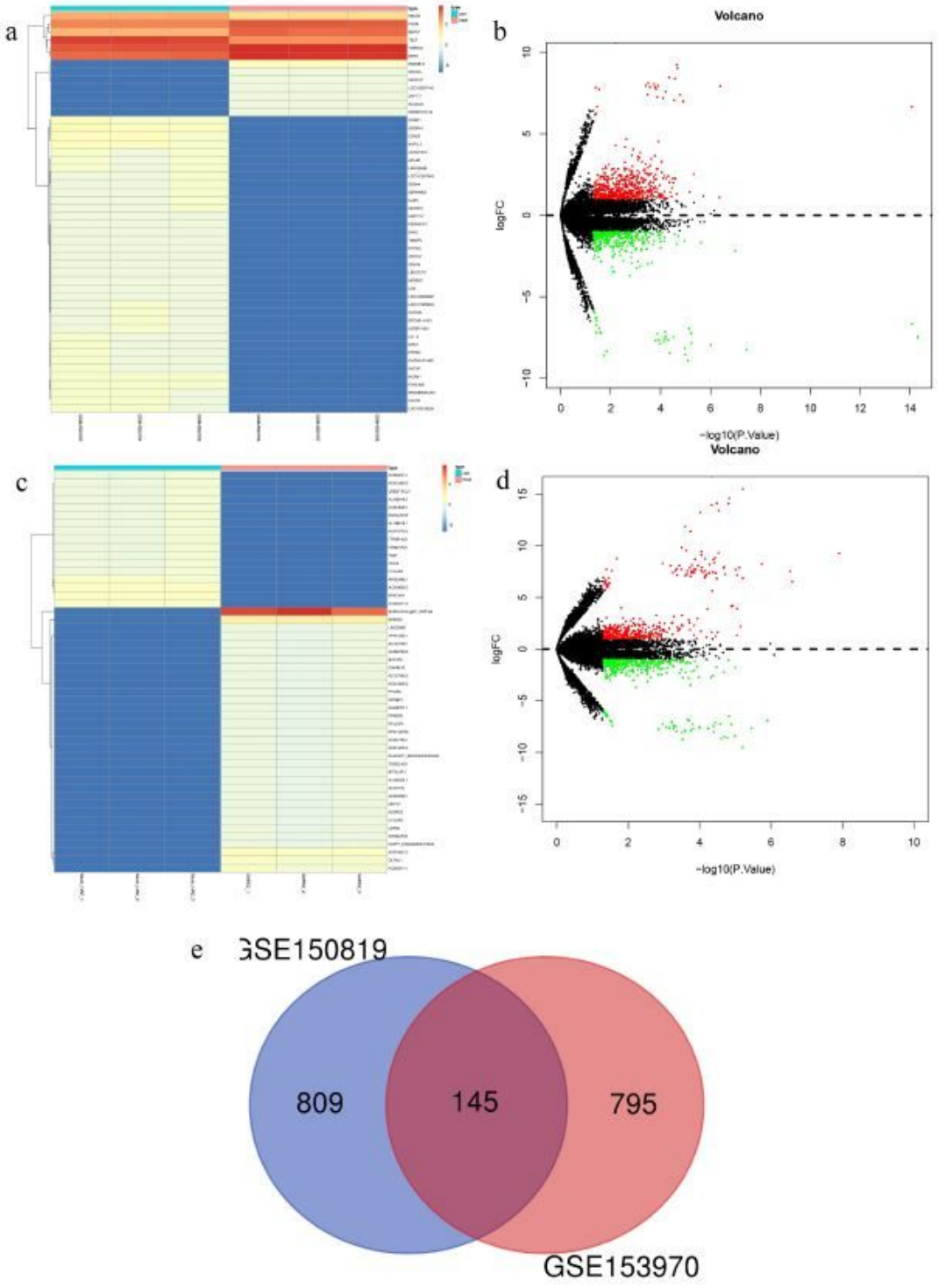

Figure 1

Identification of DEGs from 2 GEO datasets. Heatmap results of DEGs in dataset of GSE150819 (a) and GSE153970; (c) The red and blue represent up-regulation and down-regulation, respectively. Volcano plots for expression of DEGs in dataset GSE150819 (b) and GSE153970; (d) Red points represent up-regulated DEGs, green points represent down-regulated DEGs, and black points represent non-differentially expressed genes; (e) The Venn diagram of the overlapping DEGs among the two datasets.

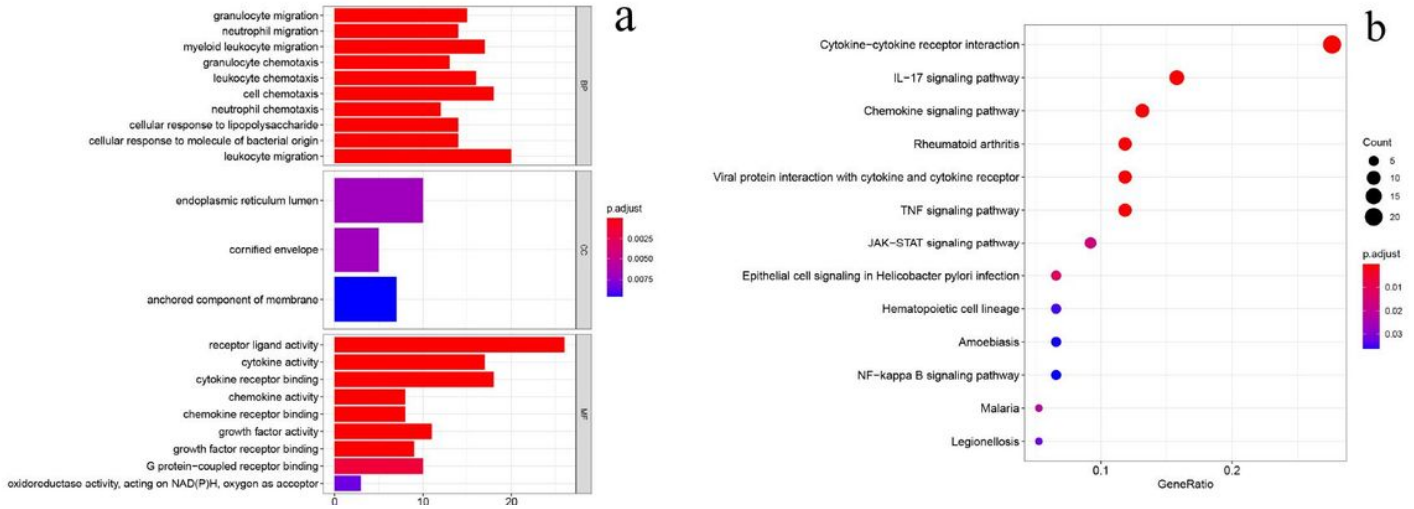

Figure 2

GO classification and KEGG pathway analysis of DEGs. (a) Integration of GO terms for DEGs in the biological process, cellular component, and molecular function terms; (b) KEGG pathway analysis of DEGs. The size of the circle represents the number of genes enriched in the entry, and the colour indicates the significance of the p value. The horizontal axis represents the ratio of genes and the vertical axis represents different terms in functional enrichment analysis.

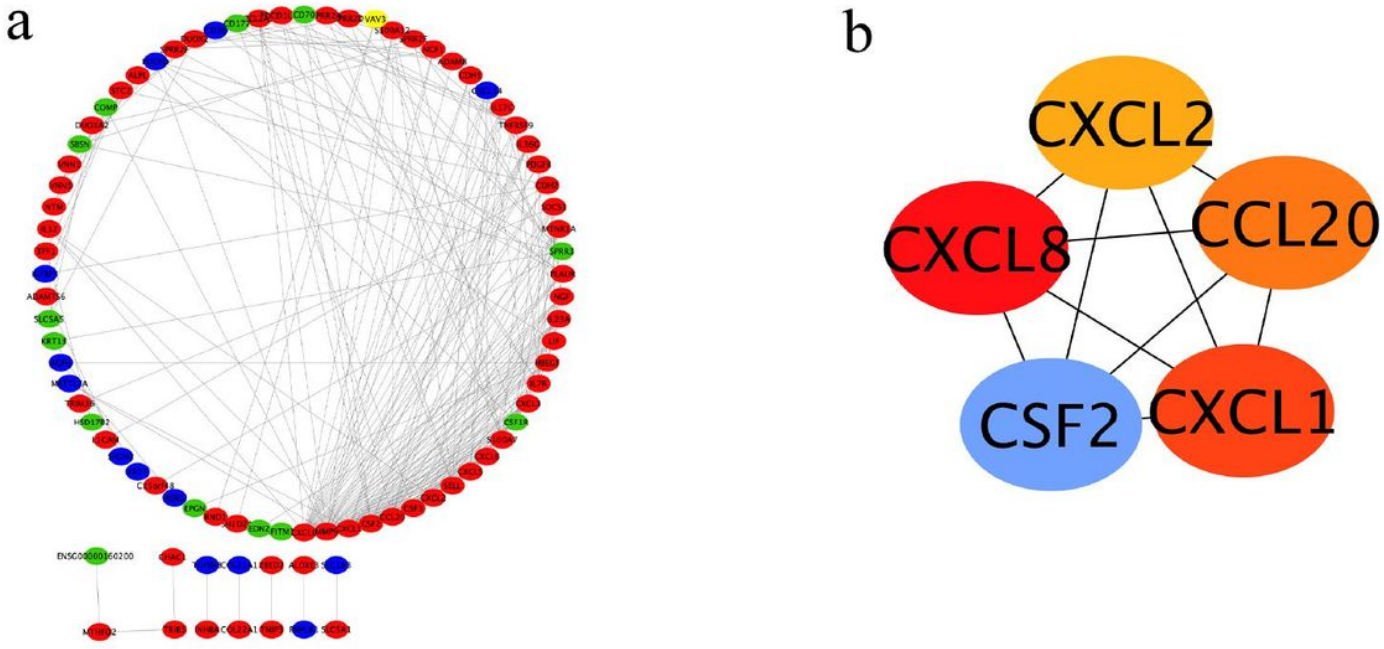

Figure 3

Protein-protein interaction (PPI) networks. (a) The PPI network was constructed by String on the interactions of DEGs. Red nodes represent the up-regulated DEGs; blue nodes represent the down-regulated DEGs; Green represents the opposite genes expressed in the 2 data sets; (b) The most significant modules were visualized by the cytoHubba plug-in in Cytoscape.

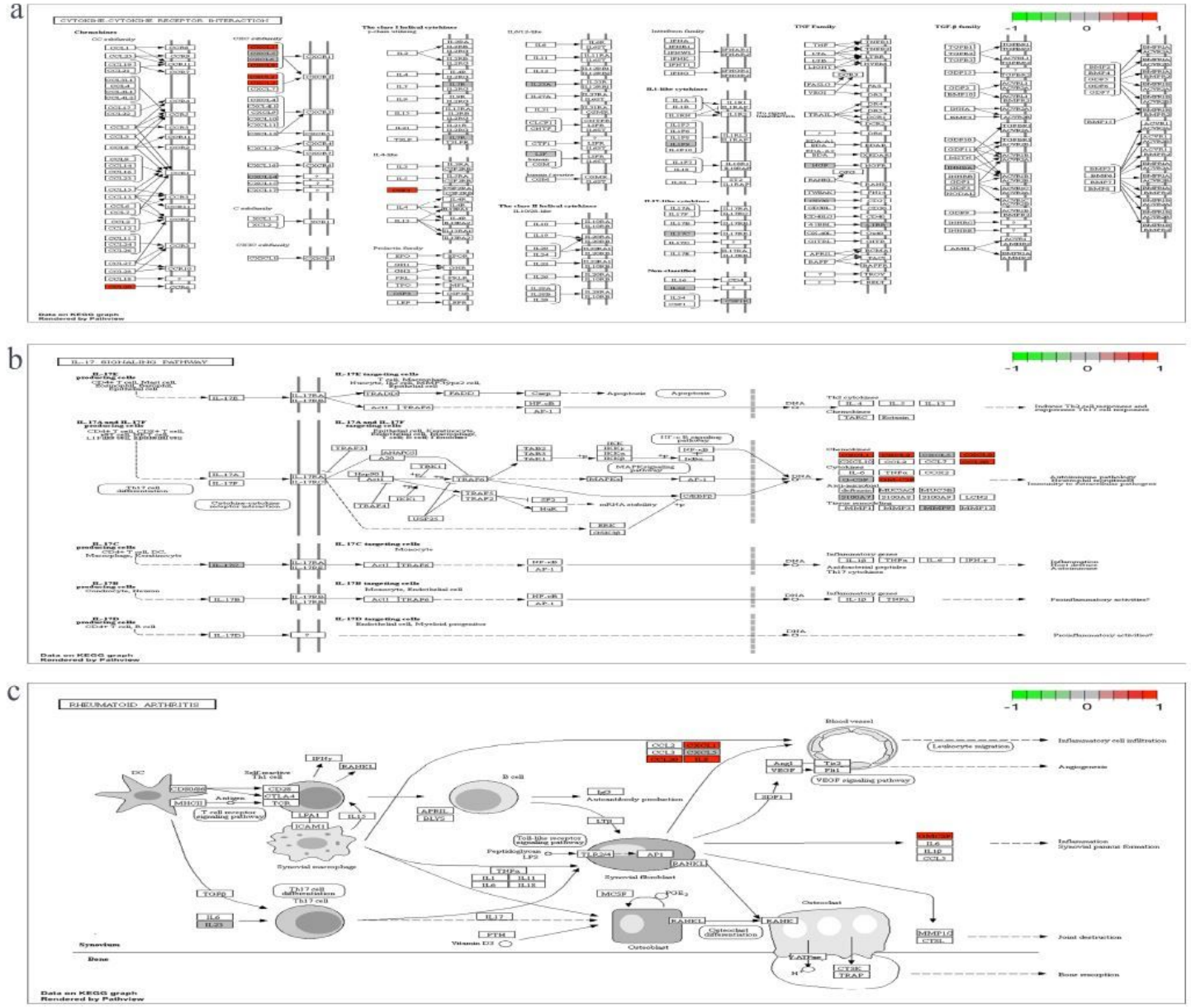

Figure 4

The relationship between five key genes and three major signaling pathways. Red represents up-regulated key genes, green represents down-regulated key genes, and gray represents the differential genes other than the five key genes. (a) Cytokine–cytokine receptor interaction; (b) IL−17 signaling pathway; (c) Rheumatoid arthritis.