ORIGINAL RESEARCH

Analysis of Enrichment Pathway, Hub Gene, and Protein-Protein Interaction Network in Rheumatoid Arthritis and Construction of Molecular Subtypes in Peripheral Blood

Shi-Guo Yuan, PhD; Kai Zheng, MD; Meixiong Chen, BD; Zhiwei Zhang, MD

ABSTRACT

Objective • To analyze the enrichment pathway, hub gene, and Protein-protein interaction (PPI) network of rheumatoid arthritis (RA) and construct peripheral blood subtypes based on integrated bioinformatics analysis.

Methods • Suitable datasets were screened from the GEO database based on titles and abstracts, batch positive analysis was performed using R language, and KEGG enrichment analysis and GO enrichment analysis were performed. After screening the differential genes, the PPI network was constructed, and the hubba plug-in of Cytoscape software was used to obtain the top 10 hub genes(key regulatory genes). hub genes were used as the typing condition to identify the molecular subtypes of synovial tissue and peripheral blood of arthritis.

Results • GSE12021 and GSE93272 have been chosen for analysis. GSE12021 presents the transcriptome analysis of human joint synovial tissue, comprising 12 samples from patients with rheumatoid arthritis and 9 samples from normal healthy individuals. On the other hand, GSE93272 includes human peripheral blood samples, comprising 232 samples from patients with rheumatoid arthritis and 43 samples from normal healthy individuals. The main results of GSE12021 KEGG enrichment were Parathyroid hormone synthesis, Relaxin signaling pathway, TNF signaling pathway, Rheumatoid arthritis, T cell receptor signaling pathway, Th1 and Th2 cell differentiation, Th17 cell differentiation, Toll–like receptor signaling pathway and so on. The main results of GSE12021 GO enrichment were regulation of feeding behavior, regulation of neuron death, positive regulation of cell–cell adhesion, and positive regulation of leukocyte activation. The top 10 hub genes were CD8A, JUN, CTLA4, CD19, LCK, FOS, CCL5, IL7R, CCR7 and CD247. Synovial tissue and peripheral blood subtypes of rheumatoid arthritis showed that the two classification methods maintained consistency.

Conclusion • Identifying the Hub gene in peripheral blood helps screen molecular subtypes of rheumatoid arthritis. (*Altern Ther Health Med.* 2024;30(1):134-141).

Shi-Guo Yuan, PhD; Kai Zheng, MD; Meixiong Chen, BD; Zhiwei Zhang, MD; Department of Orthopaedic, Hainan Traditional Chinese Medicine Hospital, School of Chinese Medicine, Hainan Medical University, Haikou, Hainan Province, China.

Corresponding author: Shi-Guo Yuan, PhD
E-mail: ysg@smu.edu.cn
Corresponding author: Kai Zheng, MD
E-mail: Kaizheng0228@163.com

BACKGROUND

Rheumatoid arthritis (RA) is a chronic autoimmune disease, that will lead to limb deformation in the severe stage of development, but there are individual differences in disease and disease progression.[1] Sometimes accompanied by multiple system damage, the main pathological change is joint deformity and, eventually loss of function. At present, the etiology of rheumatoid arthritis is still under exploration, which may be related to immune factors, genetic factors, environmental factors, infectious factors, and many other factors.[2] The clinical characteristics of rheumatoid arthritis are mainly manifested in the infiltration and growth of synovial cells, leading to cartilage injury, synovial thickening, bone erosion, and local inflammatory cell infiltration of joints, resulting in chronic inflammation.[3]

However, because the pathogenesis of rheumatoid arthritis is not yet clear, some experts speculate that rheumatoid arthritis is driven by antigens and the emergence of the "trigger-chain" reaction. Under the interaction and influence of a variety of immune cells and immune molecules, a very complex and huge network is formed, resulting in the pathogenesis of rheumatoid arthritis, so rheumatoid arthritis has a very complex pathogenesis.[4]

The GEO database is a comprehensive database containing transcriptome information on many diseases.

Through mining-related transcriptome data, pathological pathways related to diseases can be obtained, key genes related to them can be mined, and the pathological mechanism can be revealed.[5] Identification of molecular subtypes of rheumatoid arthritis is of great significance for clinical diagnosis and treatment. Compared with synovial tissue samples, peripheral blood samples are easy to obtain. At the same time, RNA from injured tissue can enter peripheral blood at the early stage of the disease, so peripheral blood detection is relatively sensitive.[6] Therefore, based on bioinformatics methods, the enrichment pathway, the hub genes, PPI network analysis, and peripheral blood subtype identification of RA were conducted in this study.

MATERIALS AND METHODS

Screening GEO datasets

"Rheumatoid arthritis" was searched in the GEO database, and the species was selected as "Homo sapiens". GSE12021 and GSE93272 were selected after inspecting the full text of the dataset abstract and link and checking each sample data. GSE12021 was a transcriptome analysis of human joint synovial tissue, from which 12 samples from rheumatoid arthritis patients and 9 samples from normal healthy people were selected. GSE93272 were human peripheral blood samples, including 232 samples from rheumatoid arthritis patients and 43 samples from normal healthy people.

GSE dataset preprocessing method

The program "GEO2R" of the GEO database was used to rectify the data set samples in batches, and R language(V3.2.5) was used to check the data set, check and remove the missing values. View the GPL(GEO Platform) corresponding to each GSE dataset from the GEO database, and download the GPL platform annotation file. GSE data were annotated by GPL platform annotation file through the R language "limma" program package, that is, gene ID conversion.

Differential gene screening and visualization

R language "limma" program package, GSE12021 according to the logFC ≥2, $P < .05$ standard for data processing, GSE93272 according to the logFC ≥1.3, $P < .05$ standard for data processing analysis results, "ggplot2" program package was used to visualize the differential gene results. Including volcano map, heat map, and PCA map. In addition, the box diagram was drawn by R language to compare the expression of the hub genes.

KEGG enrichment analysis and GO enrichment analysis

Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway enrichment analysis were performed for significantly differentially expressed genes using the package "clusterProfiler". Among them, GO enrichment analysis includes three parts: molecular function, cellular component, and biological process.

PPI network construction and hub gene screening

The GSE12021 differential gene was imported into a STRING database to obtain the protein interaction (PPI) network diagram. The STV files(SageTV application package file) were exported from STRING database and analyzed by the Cutohubba plug-in of Cytoscape software(V3.10). The top 10 hub genes were obtained according to the degree of evaluation. R language was used to analyze the correlation of the hub genes after data standardization and batch correction.

Analysis of molecular subtypes in tissue and peripheral blood

According to the hub gene, PCA results were used to identify a default cluster tree through R language, data standardization, and batch correction, without limiting the number of subtypes. , and show the results; At this time, the appropriate number of clustering clusters can be selected according to the operation results (all possible grouping situations are included in the HD vector diagram). The selection principles are as follows: 1. The sample distribution is uniform, that is, there are not too few or too many samples in some groups; 2. The color difference in the clustering heat map is significant. Generally speaking, the cleanest map is found, that is, the whiter blocks appear and the blue is not mixed as much as possible, the better the clustering result. 3. According to the cumulative CDF curve results, the smoother the CDF curve, and the larger the area under the line, the better. At the same time, the Delta area is the K value at the inflection point, which is the best number of clustering clusters. 4. It is still not clear how many clusters are suitable, so you can also try to adjust the default clustering result up and down. If the default clustering result is identified as 4, you can try the result of 3 or 5 respectively.

Statistical Methods

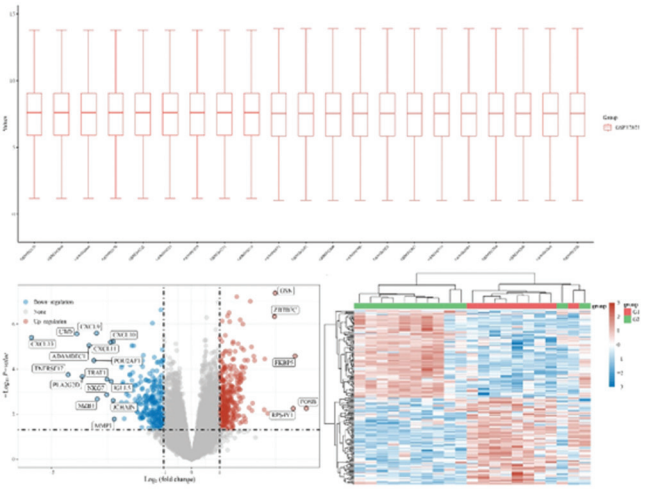
Use R language for data processing. Measurement data were expressed as ($\bar{x} \pm s$). t test was used for comparison of normal distribution data between groups, nonparametric test was used for non-normal distribution data, ANOVA was used for comparison between multiple groups, and Wilcoxon rank sum test was used for correlation analysis. $P < .05$ was considered statistically significant.

RESULTS

GSE12021 differential gene analysis and enrichment analysis

As shown in Figure 1, the sample batches of GSE12021 have good tidiness and are at the same level as the median line. The volcano map showed that there were 317 up-regulated genes and 259 down-regulated genes. Red nodes represent upregulated genes, including GSN, ZBTB7C, FKBP5, FOSB, RPS4Y1, etc. Blue nodes represent down-regulated genes, including CXCL13, UBD, CXCL9, CXCL10, ADAMDEC1, CXCL11, TNFRSF17, POU2AF1, TRAT1, PLA2G2D, NKG7, IGLL5, MZB1, JCHAIN, and MMP1. Gray nodes represent indifference genes. The results of the heat map showed that

Figure 1. Positive and differential gene analysis of GSE12021 sample batches



the differential genes of synovial tissues of patients with RA were different from those of healthy people, and there was a certain correlation between different samples.

The main results of KEGG enrichment of upregulated genes were Osteoclast differentiation, Parathyroid hormone synthesis, Qs and action, Regulation of lipolysis in adipocytes, Relaxin signaling pathway, Renal cell carcinoma, TNF signaling pathway, cAMP signaling pathway, and other signaling pathways. The main results of KEGG enrichment of down-regulated genes were Primary immunodeficiency, Rheumatoid arthritis, T cell receptor signaling pathway, Th1 and Th2 cell differentiation, Th17 cell differentiation, Toll–like receptor signaling pathway, Viral protein interaction with, cytokine and cytokine receptor. The main results of up-regulated gene GO enrichment were regulation of feeding behavior, regulation of neuron death, regulation of vasculature development, and response to alcohol, response to mechanical stimulus, response to peptide hormone, response to transforming growth factor beta, tissue Regeneration. The main results of GO enrichment of down-regulated genes were positive regulation of T cell activation, positive regulation of cell activation, positive regulation of cell–cell adhesion, positive regulation of leukocyte activation, positive regulation of lymphocyte activation, and regulation of T cell activation, regulation of cell–cell adhesion, and regulation of leukocyte cell–cell adhesion. As shown in figure 2.

PPI network establishment and hub gene screening

The PPI network revealed extensive interactions among GSE12021 differential genes. According to the hubba plug-in of Cytoscape software, the top 10 hub genes were CD8A, JUN, CTLA4, CD19, LCK, FOS, CCL5, IL7R, CCR7, and CD247, respectively. The degree values were 87, 73, 71, 64, 57, 56, 55, 54, 52 and 50, respectively. Gene correlation analysis showed the correlation between hub genes, with a positive value representing a positive correlation and a negative value representing a negative correlation. CD8A, CTLA4, CD19, LCK, CCL5, IL7R, CCR7, and CD247 were

Figure 2. KEGG and GO enrichment analysis of GSE12021 differential gene

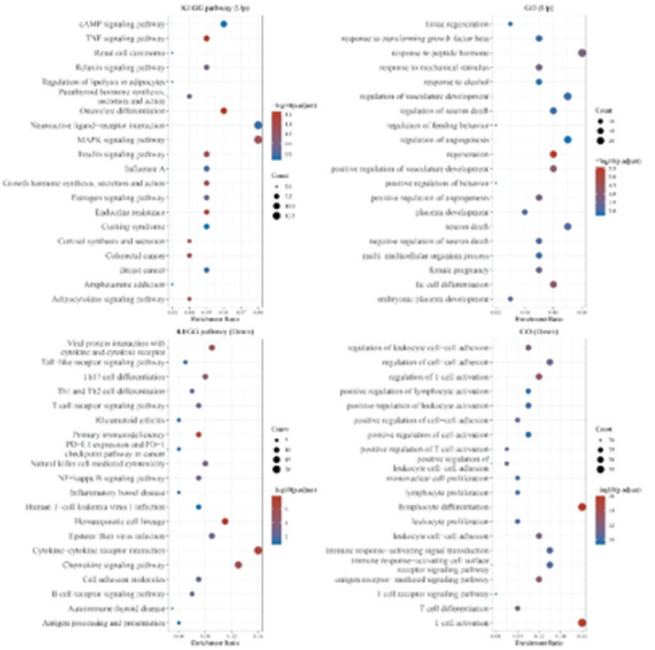
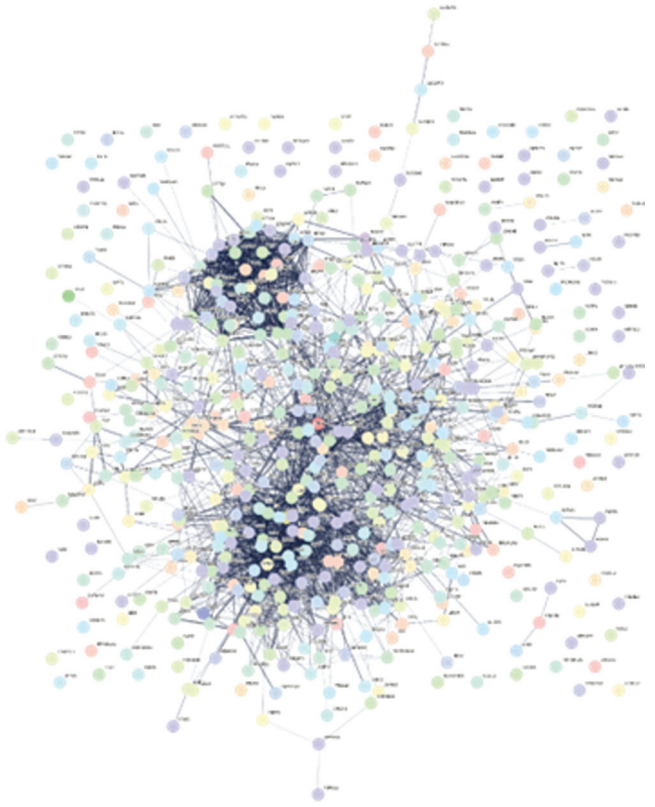


Figure 3. GSE12021 differential gene PPI network



positively correlated, JUN and FOS were positively correlated, and the genes between the above two groups were negatively correlated. The results of gene expression analysis showed that hub genes in synovial tissues of patients with RA were different from those of healthy subjects.

Figure 4. Hub gene analysis of GSE12021 differential gene PPI network

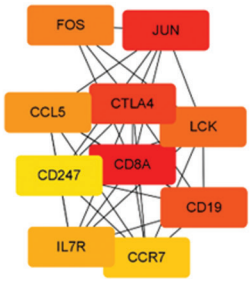


Figure 5. Correlation analysis of GSE12021 hub gene

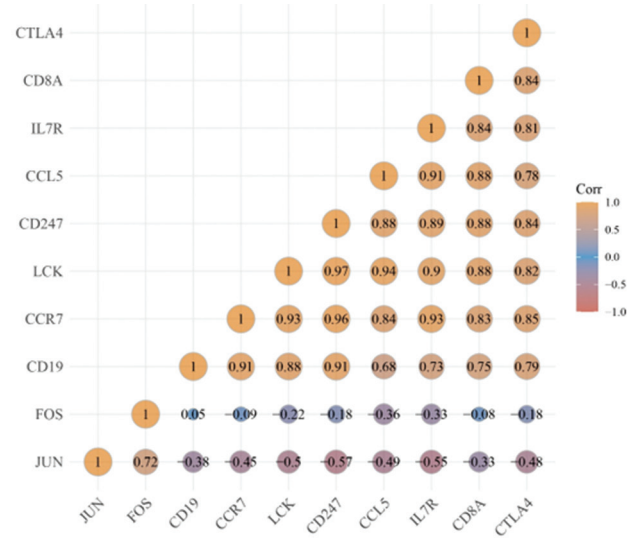


Figure 6. Analysis of relative expression of GSE12021 hub gene

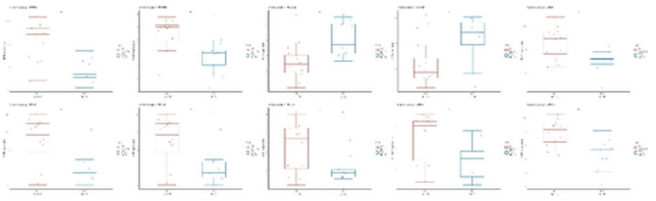


Figure 7. Molecular subtype analysis of synovial tissue in rheumatoid arthritis

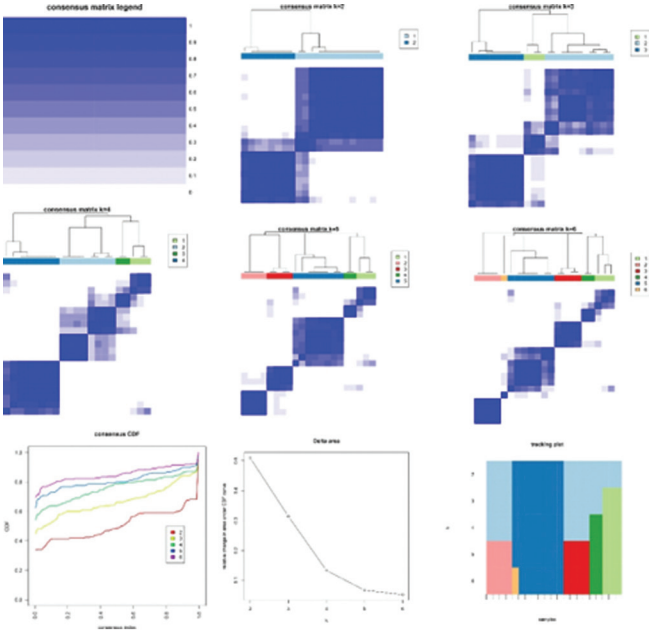
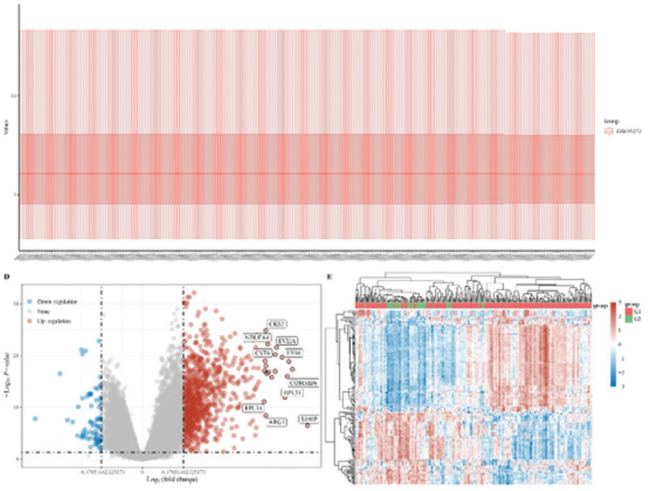


Figure 8. Positive and differential gene analysis of GSE93272 samples



Molecular subtype analysis of synovial tissue in rheumatoid arthritis based on GSE12021

The results of molecular subtype analysis showed that the gene types of the synovial tissue of RA could be divided into type 3 or type 4 according to the hub gene, with good discrimination.

GSE93272 differential gene analysis and enrichment analysis

The sample batch of GSE93272 has good tidiness, and the batch is at the same level as the median line. The volcano map showed that there were 823 up-regulated genes and 72 down-regulated genes. Red nodes represent up-regulated genes, including CKS2, NDUFA4, EV12A, CSTA, LY96, COMMD6, RPL31, RPL34, ARG1, S100P, etc. Blue nodes represent down-regulated genes, including RAI1, MT-ND2, GLG1, DCHS1, PTPRO, NCOR2, ZBTB4, VCP, ESYT1, ANKRD17, SPOCK2, PRAG1, QPRT. Gray nodes represent indifference genes. The results of the heat map showed that the differentially expressed genes in the peripheral blood of patients with RA and healthy people were distinguished, and there was a certain correlation between the different samples. KEGG enrichment of up-regulated genes mainly resulted in Thermogenesis, Prion disease, Protein export, RIG–I–like receptor signaling pathway, Ribosome, and other signaling pathways. The main results of KEGG enrichment of down-regulated genes were Thyroid hormone signaling pathway, TGF–beta signaling pathway, Proteoglycans in cancer, and Rap1 signaling Pathway and Regulation of actin cytoskeleton, Regulation of lipolysis in adipocytes and Serotonergic

synapse. The main results of up-regulated gene GO enrichment were protein localization to the endoplasmic reticulum, protein targeting to ER, and respiratory electron transport chain, response to virus, translational, translational initiation, translational termination, and viral transcription. The main results of down-regulated gene GO enrichment were cell cycle phase transition, positive regulation of transforming growth, and factor beta receptor signaling pathway, regulation of megakaryocyte differentiation, regulation of myeloid cell differentiation, regulation of response to wounding, regulation of wound ranging, the response to misfolded protein.

Molecular subtype analysis of rheumatoid arthritis peripheral blood based on GSE93272

The results of molecular subtype analysis showed that the gene types of the synovial tissue of RA could be divided into type 3 or type 4 according to the hub gene, with good discrimination. The correlation map showed that the peripheral blood molecular subtypes of rheumatoid arthritis based on the hub gene were consistent with the tissue subtypes, indicating that the peripheral blood molecular subtypes were helpful for the identification of the tissue subtypes of rheumatoid arthritis, which was helpful for clinical rapid and convenient subtype identification, to facilitate more rapid and accurate treatment.

DISCUSSION

Rheumatoid arthritis (RA) is a common chronic and systemic autoimmune disease characterized by chronic synovitis and cartilage destruction. Patients are often accompanied by persistent joint pain, swelling, stiffness, and in severe cases, cardiovascular, pulmonary, skeletal, and other complications.[7] The incidence of RA is reported to be high globally and in China, and it can occur at any age. The condition of RA is repeated and difficult to be cured. Although it does not pose a major threat to the lives of patients, it will seriously affect the daily life and social activities of patients and bring heavy mental and economic burden to patients and their families.[8] At present, the drugs commonly used in the treatment of RA mainly include non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids (GCs), disease-modifying anti-rheumatic drugs (DMARDs), biological products, and traditional Chinese herbs. Rheumatoid arthritis is a destructive joint disease and symmetry, joint synovial inflammation, chronic autoimmune disease characterized by joint deformity, morning stiffness, wrist of hand, foot, and ankle conditions such as temporal jaw arthritis is given priority to, not only can reduce the patient's motor function, also the cumulative breathing, kidney, heart, such as system, make the patient's life, work is affected by the serious.[9] If the patient's condition is not controlled in time and effectively, it will lead to Sjogren's syndrome, pericarditis, anemia, necrotizing vasculitis, and other complications, threatening their life safety.[10] At present, the pathogenesis of rheumatoid arthritis in the medical field is still in the stage of research, but some scholars believe that genetics and environment are the main factors causing rheumatoid arthritis. At the same time, there are more immune cells and immune molecules in the synovial tissue and synovial fluid of patients with this disease. This indicates that the occurrence of rheumatoid arthritis may be related to the activation or release of such substances, or accelerate the

Figure 9. KEGG and GO enrichment analysis of GSE93272 differential genes

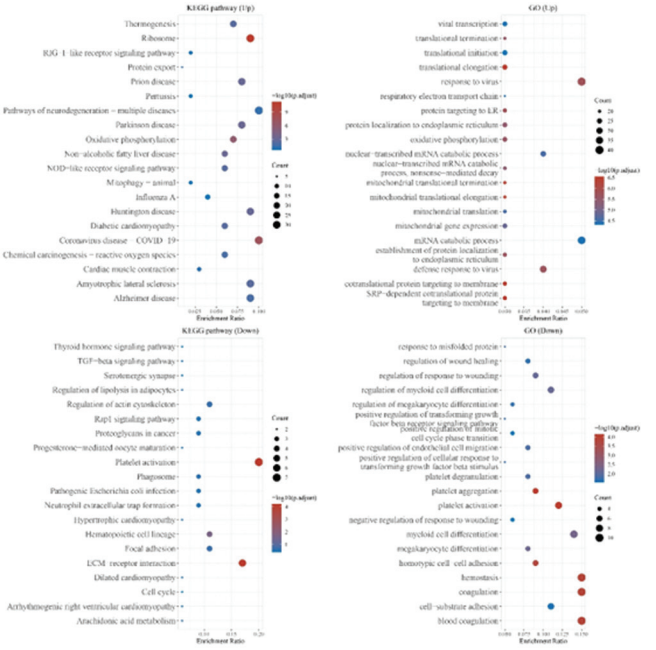
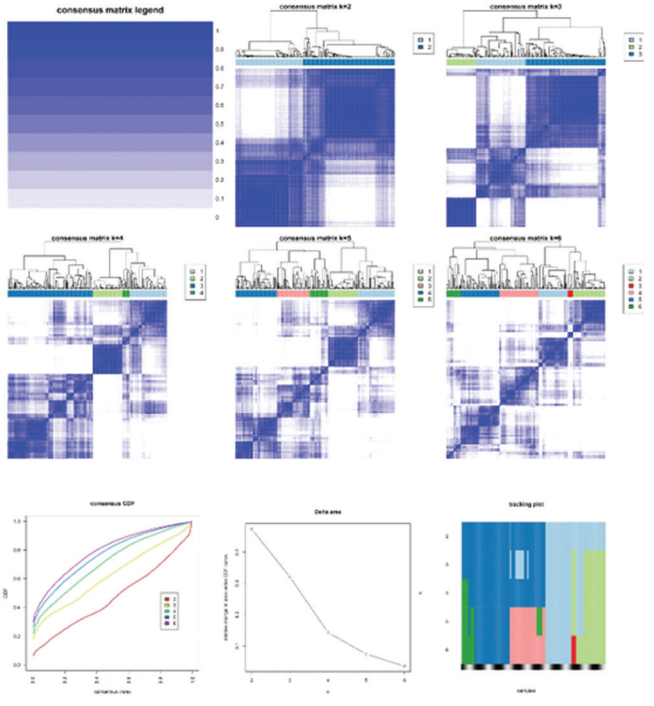


Figure 10. Molecular subtype analysis of GSE93272 in rheumatoid arthritis peripheral blood



progress of patients' disease under the action of the above substances.[11]

Bioinformatics is a discipline that studies the collection, processing, storage, dissemination, analysis, and interpretation of biological information. It is also a new discipline formed by the combination of life science and computer science with the rapid development of life science and computer science.[12] It makes comprehensive use of biology, computer science, and information technology to reveal the biological secrets of a large number of complex biological data. Bioinformatics is a science that uses computers as a tool to store, retrieve, and analyze biological information in life science research. It is one of the major frontiers of life science and natural science, and it will also be one of the core fields of natural science in the 21st century. Bioinformatics research focus is mainly reflected in genomics and proteomics, which is to analyze the biological information of structure and function expressed in nucleic acid and protein sequences. To be specific, bioinformatics, as a new discipline, takes genomic DNA sequence information analysis as the source simulates and predicts protein spatial structure after obtaining protein coding region information, and then carries out necessary drug design according to the function of specific proteins. Genome informatics, protein spatial structure simulation, and drug design are three important components of bioinformatics. From the perspective of the specific contents of bioinformatics research, bioinformatics should include three main parts: (1) Research on new algorithms and statistical methods; (2) Analysis and interpretation of various types of data; (3) development of new tools for effective use and management of data.

The main results of GSE12021 KEGG enrichment were Parathyroid hormone synthesis, Relaxin signaling pathway, TNF signaling pathway, Rheumatoid arthritis, T cell receptor signaling pathway, Th1 and Th2 cell differentiation, Th17 cell differentiation, Toll–like receptor signaling pathway and so on. The main results of GSE12021 GO enrichment were regulation of feeding behavior, regulation of neuron death, positive regulation of cell–cell adhesion, and positive regulation of leukocyte activation.[13] The pathogenesis of RA involves many aspects. Kunkel's pioneering work proposed that RA may be an autoimmune disease caused by autoreactive antibodies. Zvaifler's study demonstrated the main pathogenic potential of rheumatoid factors in RA.[14] Experiments by Stastny et al. demonstrated genetic similarity between RA patients and found that specific human leukocyte antigen-DR genes residing in the major histocompatibility complex (MHC) and involved in antigen presentation were associated with disease. Environmental factors also have an impact on the induction, degree, and progression rate of diseases.[15] Data indicate that smoking is an important environmental risk factor for the development of human leukocyte antigen-DR4-positive individuals.[16] In recent years, more and more evidence has shown that various immune cells are involved in the pathogenesis of RA. T lymphocytes are closely related to the pathogenesis of RA, and a large number of T cells can be detected in the inflammatory synovium. Cumulative evidence suggests that CD4 + T cells, especially those polarized toward T helper (Th1) /Th17 subsets, play a key role in the pathogenesis of RA.[17] Th17 cells are involved in various key processes in the development of arthritis by activating synovial fibroblasts to form pannus and by inducing osteoclasts to destroy the joint. Data have confirmed the enhancement of B-cell activation in the synovium of RA inflammation.[18] The presence of B lymphocytes in tissues is one of the key factors to determine the functional activity of follicular CD4 + T cells. Given the ability of B cells to specifically capture antigens through their receptors and present them to T cells, B cells may have a unique position to stimulate proinflammatory T cells in RA synovitis.[19] Studies have found that a humanized anti-CD20 monoclonal antibody (IgG1) depletes B cells through complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity.[20] In clinical trials, Edwards and Cambridge used a humanized anti-CD20 monoclonal antibody to treat B-cell depletion in RA patients and showed significant improvement. Studies of anti-CD20 therapy in RA have provided evidence that B cells may play an important role in the pathogenesis of RA.[i] Macrophages play a central role in RA, coordinating the cytokine environment that enhances inflammation and leads to cartilage and bone destruction. Studies have shown that macrophages in the synovial lining layer exhibit high levels of activation, and activated synovial tissue macrophages show increased expression and transcription of interleukin 1b(IL-1b), tumor necrosis factor-α (TNF-α) and C-C monocyte chemoattractant protein 1(MCP1). Macrophages are one of the most abundant cell types in the inflammatory site of RA. The key cytokines released by macrophages (Mφ) include IL-12 and IL-23. Potential functions of IL-12 in the pathogenesis of RA include Th1 cell proliferation and maturation, T cell and NK cell toxicity, and B cell activation. IL-23 promotes Th17 cell proliferation. In addition, macrophage migration-inhibitory factor (MIF) released by macrophages can promote the release of NO, activation of T cells, proliferation of fibroblasts, expression of COX and PLA2, and activity of intrinsic oxidoreductase. Tumor necrosis factor, IL-6 and IL-1 derived from macrophages can further enhance osteoclast function directly or indirectly. Increased numbers of MCs have been found in synovial tissues and body fluids from RA patients, as well as at sites of cartilage erosion. A large proportion of MCs have been reported to be activated in rheumatic specimens. Given the nature and function of mast cells in the synovium, it is hypothesized that mast cells may provide a critical cellular link between soluble factors (autoantibodies, complement, cytokines) and the synovium. Lee et al. used mast cell-deficient mice to investigate the functional role of mast cells in inflammatory arthritis. Our results suggest that the potential function of mast cells as a cellular link between soluble components and subsequent arthritic development leads to

i. Edwards and Cambridge used a humanized anti-CD20 monoclonal antibody to treat B-cell depletion in RA patients and showed significant improvement. Studies of anti-CD20 therapy in RA have provided evidence that B cells may play an important role in the pathogenesis of RA

their activation and rapid release of available granule components after serum transfer, and that degranulation is the most obvious histological marker of mast cell activation. In addition, because mast cells continue to exhibit a degranulation phenotype during the more chronic stages of arthritis, they may also play an ongoing role in the arthritic process. Mast cell granules are also rich in proteases that activate matrix metalloproteinases and mMCP-6. They also produce numerous other inflammatory molecules, including histamine, eicosanoids, fibroblast growth factor, and angiogenic factor (VEGF), which may further contribute to the arthritic process. In addition, histological analysis of human synovial sections demonstrated the presence of numerous mast cells and immune complexes, complement fragments, and SCFS in synovial fluid and tissues from RA. Together, these findings suggest that mast cells may be involved in the pathogenesis of erosive arthritis in the synovium.

The top 10 hub genes were CD8A, JUN, CTLA4, CD19, LCK, FOS, CCL5, IL7R, CCR7 and CD247. CD8 molecule is a leukocyte differentiation antigen, a glycoprotein on the surface of some T cells, which is used to assist the T cell receptor (TCR) to recognize antigens and participate in the transduction of T cell activation signal, also known as the co-receptor of TCR.[21] Cd8-expressing T cells (CD8+T cells) usually differentiate into cytotoxic T cells (CTL) upon activation, which can specifically kill target cells. Naturally, CD8 usually forms dimers, and dimers can have two kinds of structures. The first is a heterodimer (CD8α, β)[22] composed of one alpha and one beta chain, encoded by the leu-2a and leu-2b genes, respectively. The second is a homodimer (CD8α) composed of two alpha strands, both of which are encoded by the leu-2a gene. The α-chain size is about 34-37 kD and the β-chain size is 32kD. The two peptide chains are transmembrane proteins and their extracellular parts are linked by disulfide bonds. They belong to the immunoglobulin superfamily (IgSF) because their extracellular structure is similar to immunoglobulin. There is a variable region (V-like region) in the extracellular part of both α and β chains of the CD8 molecule, and between the V-like region and the membrane part is a hinge region (linker peptide) rich in proline, threonine, and serine residues. The V-like region of the α chain can bind to the α3 domain of the heavy chain of major histocompatibility complex class I molecules (MHCI molecules). The CTLA4 gene, a member of the immunoglobulin superfamily, encodes a protein that transmits inhibitory signals to T cells. The protein contains a V domain, a transmembrane domain, and a cytoplasmic tail. Alternate transcribed splice variants, encoding different isoforms, have been characterized. Membrane-bound isomers act as homodimers linked by disulfide bonds, whereas soluble isomers act as monomers. Mutations in this gene are associated with insulin-dependent diabetes mellitus, Graves' disease, Hashimoto's thyroiditis, celiac disease, systemic lupus erythematosus, thyroid-associated orbital disease, and other autoimmune diseases. CD19 is a CD molecule (leukocyte differentiation antigen) expressed by B cells and belongs to the Ig superfamily.[23] All B cell lines except plasma cells, malignant B cells, and FDC express this molecule. It is an important membrane antigen involved in the proliferation, differentiation, activation, and antibody production of B cells, and can also promote the signal transduction of BCR. IL-7 binds to the IL-7 receptor, which is a heterodimer composed of IL-7 receptor alpha and a common γ-chain receptor. Binding leads to a series of signals that are important for T-cell development in the thymus and survival in the periphery. The cytokine IL-7 and its receptor IL-7R are essential for T cells, and in mice are essential for B-cell development, differentiation and survival of naive T cells, and generation and maintenance of memory T cells.[24] They are also required for the development and maintenance of innate lymphoid cells (ILC) and therefore for the generation of lymphoid structures and barrier defense. These results indicate that rheumatoid arthritis is closely related to inflammatory cell infiltration and dysfunction.

Molecular diagnostic technology refers to a technology that uses DNA or RNA nucleic acid as diagnostic material and uses molecular biological methods such as nucleic acid amplification to detect the presence, defect, or abnormal expression of target genes, to diagnose diseases. Currently, molecular diagnostic technology is rapidly advancing with various innovative breakthroughs. In addition to real-time PCR technology, novel techniques and methods have been developed in the field of molecular diagnosis in recent years, and their application scope is constantly expanding. The development and application of molecular diagnostic technologies have played an extremely important role in the diagnosis and treatment of infectious diseases, genetic diseases, and tumors, especially the rise of new molecular diagnostic technologies represented by isothermal amplification technology, dPCR technology, and high-throughput sequencing technology and their clinical application. It greatly makes up for the shortcomings of traditional methods in detection range, sensitivity, specificity, and turnaround time. In the future development direction, molecular diagnostic technology will continue to optimize and transform in the direction of rapid and convenient, high sensitivity, high accuracy, high automation and integration, and high throughput, to promote early accurate diagnosis and treatment of clinical diseases. But it must be clear, when these emerging molecular diagnostic techniques are introduced when used in the detection of clinical specimens, clinical laboratory, be sure to communicate with the internal quality management, performance qualification, clinical and results explain, to ensure that the test system is suitable for fitting test specimens of intended use, promote the development of technology in the clinical application of orderly and fair use. Synovial tissue and peripheral blood subtypes of rheumatoid arthritis showed that the two typing methods maintained consistency, and identification of hub genes in peripheral blood helped screen molecular subtypes of rheumatoid arthritis.[25]

FUNDING

Project supported by Hainan Province Clinical Medical Center ([2021] No. 276)

ACKNOWLEDGMENTS

We thank every participant who contributed to the study. The authors' responsibilities were as follows—Yuan Shiguo and Zheng Kai: designed the research; Chen Meixiong and Zhang Zhiwei: had primary responsibility for the final content; and all authors: read, reviewed, and approved the final manuscript.

REFERENCE

1. Scherer HU, Häupl T, Burmester GR. The etiology of rheumatoid arthritis. *J Autoimmun*. 2020;110(110):102400. doi:10.1016/j.jaut.2019.102400
2. Cush JJ. Rheumatoid Arthritis: Early Diagnosis and Treatment. *Med Clin North Am*. 2021;105(2):355-365. doi:10.1016/j.mcna.2020.10.006
3. van Delft MAM, Huizinga TWJ. An overview of autoantibodies in rheumatoid arthritis. *J Autoimmun*. 2020;110:102392. doi:10.1016/j.jaut.2019.102392
4. Dedmon LE. The genetics of rheumatoid arthritis. *Rheumatology (Oxford)*. 2020;59(10):2661-2670. doi:10.1093/rheumatology/keaa232
5. Barrett T, Wilhite SE, Ledoux P, et al. NCBI GEO: archive for functional genomics data sets--update. *Nucleic Acids Res*. 2013;41(Database issue):D991-D995. doi:10.1093/nar/gks1193
6. Aletaha D. Precision medicine and management of rheumatoid arthritis. *J Autoimmun*. 2020;110:102405. doi:10.1016/j.jaut.2020.102405
7. Radu AF, Bungau SG. Management of Rheumatoid Arthritis: an Overview. *Cells*. 2021;10(11):2857. doi:10.3390/cells10112857
8. Cush JJ. Rheumatoid Arthritis. *Rheum Dis Clin North Am*. 2022;48(2):537-547. doi:10.1016/j.rdc.2022.02.010
9. Smith MH, Berman JR. What Is Rheumatoid Arthritis? *JAMA*. 2022;327(12):1194-1194. doi:10.1001/jama.2022.0786
10. Figus FA, Piga M, Azzolin I, McConnell R, Iagnocco A. Rheumatoid arthritis: extra-articular manifestations and comorbidities. *Autoimmun Rev*. 2021;20(4):102776. doi:10.1016/j.autrev.2021.102776
11. Bhamidipati K, Wei K. Precision medicine in rheumatoid arthritis. *Best Pract Res Clin Rheumatol*. 2022;36(1):101742. doi:10.1016/j.berh.2022.101742
12. Zhou S, Lu H, Xiong M. Identifying Immune Cell Infiltration and Effective Diagnostic Biomarkers in Rheumatoid Arthritis by Bioinformatics Analysis. *Front Immunol*. 2021;12:726747. doi:10.3389/fimmu.2021.726747
13. Buch MH, Eyre S, McGonagle D. Persistent inflammatory and non-inflammatory mechanisms in refractory rheumatoid arthritis. *Nat Rev Rheumatol*. 2021;17(1):17-33. doi:10.1038/s41584-020-00541-7
14. Klein K, Gay S. Epigenetics in rheumatoid arthritis. *Curr Opin Rheumatol*. 2015;27(1):76-82. doi:10.1097/BOR.0000000000000128
15. Zhou S, Huang G. Some important inhibitors and mechanisms of rheumatoid arthritis. *Chem Biol Drug Des*. 2022;99(6):930-943. doi:10.1111/cbdd.14015
16. Scott TE, Wise RA, Hochberg MC, Wigley FM. HLA-DR4 and pulmonary dysfunction in rheumatoid arthritis. *Am J Med*. 1987;82(4):765-771. doi:10.1016/0002-9343(87)90013-1
17. Jiang Q, Yang G, Liu Q, Wang S, Cui D. Function and Role of Regulatory T Cells in Rheumatoid Arthritis. *Front Immunol*. 2021;12:626193. doi:10.3389/fimmu.2021.626193
18. Yang P, Qian FY, Zhang MF, et al. Th17 cell pathogenicity and plasticity in rheumatoid arthritis. *J Leukoc Biol*. 2019;106(6):1233-1240. doi:10.1002/JLB.4RU0619-197R
19. Wu F, Gao J, Kang J, et al. B Cells in Rheumatoid Arthritis Pathogenic Mechanisms and Treatment Prospects. *Front Immunol*. 2021;12:750753. doi:10.3389/fimmu.2021.750753
20. Kaegi C, Wuest B, Crowley C, Boyman O. Systematic Review of Safety and Efficacy of Second- and Third-Generation CD20-Targeting Biologics in Treating Immune-Mediated Disorders. *Front Immunol*. 2022;12:788830. doi:10.3389/fimmu.2021.788830
21. Brummelman J, Pilipow K, Lugli E. The Single-Cell Phenotypic Identity of Human $CD8^+$ and $CD4^+$ T Cells. *Int Rev Cell Mol Biol*. 2018;341:63-124. doi:10.1016/bs.ircmb.2018.05.007
22. Ramos MI, Garcia S, Helder B, et al. cDC1 are required for the initiation of collagen-induced arthritis. *J Transl Autoimmun*. 2020;3:100066. PMID:33015599 doi:10.1016/j.jtauto.2020.100066
23. Tedder TF. CD19: a promising B cell target for rheumatoid arthritis. *Nat Rev Rheumatol*. 2009;5(10):572-577. doi:10.1038/nrrheum.2009.184
24. Churchman SM, Ponchel F. Interleukin-7 in rheumatoid arthritis. *Rheumatology (Oxford)*. 2008;47(6):753-759. doi:10.1093/rheumatology/ken053
25. Savvateeva E, Smoldovskaya O, Feyzkhanova G, Rubina A. Multiple biomarker approach for the diagnosis and therapy of rheumatoid arthritis. *Crit Rev Clin Lab Sci*. 2021;58(1):17-28. doi:10.1080/10408363.2020.1775545