Identification of Key Immune-Related Genes, Molecular Pathways and Immune Infiltration as Diagnostic and Therapeutic Candidate Targets for RA: an integrated bioinformatics-based analysis

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Research Article

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Abstract

Relevance

Rheumatoid arthritis (RA) is a systemic autoimmune disease with an aggressive, chronic synovial inflammation as the main pathological change. However, the specific etiology, pathogenesis, and related biomarkers in diagnosis and treatment are still not fully elucidated. This study attempts to provide new perspectives and insights into RA at the genetic, molecular, and cellular levels through the tenet of personalized medicine.

Methods

Gene expression profiles of four individual knee synovial tissues were downloaded from a comprehensive gene expression database, R language was used to screen for significantly differentially expressed genes (DEGs), Gene Ontology Enrichment Analysis, Kyoto Gene Encyclopedia, and Gene Set Enrichment Analysis were performed to analyze the biological functions and signaling pathways of these DEGs, STRING online database was used to establish protein-protein interaction networks, Cytoscape software to obtain ten hub genes, Goplot to get six inflammatory immune-related hub genes, and CIBERSORT algorithm to impute immune infiltration.

Results

Molecular pathways that play important roles in RA were obtained: Toll-like receptors, AMPK, MAPK, TNF, FoxO, TGF-beta, PI3K and NF-κB pathways, Ten hub genes: *Ccr1, Ccr2, Ccr5, Ccr7, Cxcl5, Cxcl6, Cxcl13, Ccl13, Adcy2*, and *Pnoc*. among which *Adcy2* and *Pnoc* have not been reported in RA studies, suggesting that they may be worthy targets for further study. It was also found that among the synoviocytes in RA, the proportions of plasma cells, CD8 T cells, follicular helper T cells, monocytes, γ delta T cells, and M0 macrophages were higher, while the proportions of CD4 memory resting T cells, regulatory T cells (Tregs), activated NK cells, resting dendritic cells, M1 macrophages, eosinophils, activated mast cells, resting mast cells were lower in proportion, and each cell played an important role in RA.

Conclusions

This study may help understand the key genes, molecular pathways, the role of inflammatory immune infiltrating cells in RA's pathogenesis and provide new targets and ideas for the diagnosis and personalized treatment of RA.

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic synovial inflammation and vascular opacification with multiple, symmetrical, aggressive inflammation, immune cell infiltration, destruction of cartilage and bone tissue, deformation of affected joints, and eventual loss of function as the main pathological changes[1–3]. Genetic susceptibility and environmental changes, inflammation, immune dysfunction, altered gene expression, and abnormal molecular signaling pathways be involved in the

pathogenesis of RA. The synovial tissue plays the most critical role in the disease process. Many inflammatory and immune cells, such as neutrophils, macrophages, dendritic cells, T cells, and B cells, are gathered. These cells release chemokines, proteases, pro-inflammatory cytokines, and other substances that activate and regulate each other, forming a complex network. Simultaneously, these inflammatory and immune cells can lead to immune infiltration, activate the complement system, participate in antigen presentation reactions, induce abnormal activation of osteoclasts, abnormal vascular proliferation, and accelerate cartilage and bone erosion. These inflammatory immune storms lead to further development, evolution, and deterioration of the RA condition, ultimately leading to joint deformity and loss of function [4–7]. However, the specific etiology and pathogenesis are not fully clarified. Therefore, identifying key biomarkers and therapeutic targets from multiple perspectives, such as genes, signaling pathways, and immune cell infiltration, for accurate prediction, prevention, and personalized medicine (pppm) of RA is the current hot spot and development trend of RA research[8]. It also provides new ideas and methods for clinical diagnosis and personalized treatment.

Bioinformatics uses computer science to collect, process, and analyze big data obtained by gene chips and sequencing technologies. It allows us to perform large-scale screening of genetic changes in disease development at the microscopic level. It has become an effective method for studying the mechanisms of refractory diseases. Studies are increasingly using gene microarray technology to identify aberrantly expressed genes closely associated with disease pathogenesis. These genes regulate inflammatory factors, chemokines, various protein molecules, and cellular signaling pathways through transcription and translation, deriving different protein phenotypes that cause changes in normal biological functions and ultimately lead to the onset, development, and evolution of diseases[9–11]. These studies have mainly focused on tumor-related diseases[12–15]. And there are relatively few bioinformatics studies on non-tumor-related diseases. Meanwhile, due to samples' inconsistent gene expression profiles from different sequencing platforms, some bioinformatics analyses of rheumatoid arthritis are limited to one or two microarray data, or data from multiple platforms are used but not normalized. The results are more limited and less reliable, so the problem can be effectively solved by combining and normalizing multi-gene microarray data[16–19]. This study performed comprehensive bioinformatics analysis, screening, and identification of synovial tissue and infiltrating inflammatory immune cells that play a crucial role in the pathogenesis from synovitis to cartilage and bone destruction (Fig. 1). We expect to obtain more biologically significant diagnostic markers or object drug targets, providing new ideas and targets for RA's molecular mechanisms and a scientific basis and resources for effective diagnosis, prognosis assessment, and personalized treatment of RA PPPM.

Materials And Methods

Merging and batch normalization of data

We downloaded four RA synovial datasets (GSE1919, GSE55235, GSE77298, and GSE55457) from the GEO database. GSE1919[20]was based on GPL91 platforms, GSE55235[21] was based on GPL96 platforms,The GSE77298[22]data was based on GPL570 platforms,GSE55457[18]was based on GPL96 platforms. As the Normal group, we used gene expression profiling microarray of Synovial tissue from the healthy joint and expression profiling microarray of synovial tissue from rheumatoid arthritis joint as the RA group. The information regarding these datasets was shown in (Table 1).

We annotated these four raw data files separately with the Strawberry Perl software, transformed the line names into official gene name matrix files, deleted data without gene names, and merged the data with different names for the same gene by taking the average value. After that, we use the “sva” package [23, 24] of R language to batch normalize these four data files [25, 26]. So that these four files were combined into one normalized gene matrix file, these data sets are shown in (Table 2).

Identifying differentially expressed genes and assessing their enrichment in function and pathways

We used the R language limma package to analyze the above-normalized gene data files with log2 greater than one and p-values less than 0.05, yielding genes that were significantly differentially expressed in the RA group [27]. These data sets are shown in (Table 3).

We performed Gene Ontology’s (GO’s) enrichment analysis of differentially expressed genes obtained above, using the R language clustering package to determine that these genes are enriched among the following three classifications: biological processes, cellular composition, and molecular functions [28, 29], and then deduced the signaling pathways involved in RA by KEGG enrichment analysis[30, 31].

Analyzing of Gene-set enrichment

Gene-Set Enrichment Analysis (GSEA) analyzed gene expression matrix files, which retains more information than traditional GO and KEGG enrichment analysis by analyzing collections of genes rather than focusing on individual gene expression changes, avoiding omission genes[32]. In this study, the above four gene matrix files were analyzed by the GSEA-related packages “BiocManager,” “Dose,” “ClusterProfiler,” and “Stringi” in R language. Each analyzed gene set was executed 1000 times with enrichment score (NES) absolute value greater than one and p-value less than 0.05.[33–35].

Discovering the network of protein-protein interactions

The STRING database is an online resource applied to an organism-wide protein association network[36, 37]. It predicts protein-protein interactions and deciphers the functions between proteins by searching gene interactions. To find potential interactions between proteins in this study, we used the STRING (version 11.0) tool. The names of genes with significant expression differences obtained previously were entered, and the species was “Homo sapiens,” and the highest confidence level was chosen to be 0.9. In the network, each node corresponds to a protein, and each edge represents an interaction. The thickness of the edge represents the strength of the interaction. Select the “string_interactions.tsv” file in the exported options for subsequent analysis. Then we use the R language barplot to analyze and visualize the “string_interactions.tsv” protein interactions network file.

Identifying hub genes and screening for inflammation and immune-related hub genes

Cytoscape is an excellent tool for analyzing protein interactions, and the software has many functional extension plug-ins[38, 39]. We used Cytoscape (v3.8.0) software to analyze the above-exported protein interaction network files. The Cytohubba plug-in can use various algorithms to calculate the nodes and links in

the protein interaction network to obtain the hub genes. This study chose the MCC algorithm in the Cytohubba plug-in to obtain the top ten hub genes in the PPI network[40, 41].

We applied the GOplot package in R language, selected inflammation and immunity-related terms for gene prediction. We crossed the predicted genes with hub genes to get hub genes associated with inflammation and immunity[42–44].

Assessing the profile of immune cell infiltration

To infer the relative proportions of 22 infiltrating types of immune cells, we used the CIBERSORT algorithm, including neutrophils, eosinophils, T cells, macrophages, B cells, dendritic cells, and natural killer cells, etc. in RA synovial samples[45–47]. So we use the packages “e1071,” “parallel,” “preprocessCore,” and “CIBERSORT.R” to get the sample’s immune cell matrix. And use the barplot to show the proportion and composition of these immune cells. Then we use the R “corrplot” package to get the correlation heatmap of infiltrated immune cells. Next, We performed the heatmap mentioned above of immune cells to illustrate the various immune cells’ composition in different samples. In addition, we reduced the sample dimension to perform principal component analysis (PCA) on the differences between RA samples and normal samples. Finally, To show the differences in the abundance of infiltrating immune cells between them, we used the R language’ vioplot’ package.

Results

Identification of DEGs

After normalization of the four merged gene matrix files, the median line of the data before normalization was fluctuating up and down, showing that the median of each sample data was in a straight line after normalization, and the boxplot showed that the data met the criteria for further study as shown in Fig. 2. The DEGs were filtered in the R language using the limma package, as shown in Table 3. The blue points on the volcano map’s left side indicate genes with significantly down-regulated expression. The orange points on the right side indicate genes with up-regulated expression considerably. Heatmaps of the 20 DEGs with the highest and lowest gene expression rankings in RA were created using the Heatmap package in R language, as shown in Fig. 3 and Table 4.

Functional analysis of DEGs

On these DEGs, We performed GO enrichment analysis. These genes were enriched into three categories: molecular functions, cellular components, and biological processes, and the ten highest-ranked terms in each category were visualized using the GOBubble function of the R language GOplot package. They are shown in Fig. 4 (a) (b) (c) and Table 5, respectively.

The top 10 terms enriched by the molecular function are main in: immune receptor activity, chemokine activity, cytokine activity, chemokine receptor binding, cytokine binding, antigen binding, cytokine receptor activity, CXCR chemokine receptor binding, C-C chemokine receptor activity, chemokine receptor activity.

The top 10 terms enriched by the cellular components are main in: collagen-containing extracellular matrix, immunological synapse, endocytic vesicle, coated vesicle membrane, External side of the plasma membrane,

endocytic vesicle membrane, clathrin-coated endocytic vesicle membrane, specific granule, membrane microdomain, tertiary granule lumen, secretory granule membrane.

The top 10 terms enriched by the biological processes are main in: T cell activation and differentiation, regulation of leukocyte cell − cell adhesion, positive regulation of leukocyte activation, positive regulation of leukocyte cell − cell adhesion, lymphocyte differentiation, positive regulation of lymphocyte activation, lymphocyte chemotaxis, and proliferation, mononuclear cell proliferation, cell chemotaxis.

Pathway analysis of DEGs

On these DEGs, we performed KEGG enrichment analysis. Below are the primary signaling pathways: Cytokine-cytokine receptor interaction, Chemokine signaling pathway, Rheumatoid arthritis, Hematopoietic cell lineage, Primary immunodeficiency, Viral myocarditis, Graft-versus-host disease, Osteoclast differentiation, Cell adhesion molecules, Viral protein interaction with cytokine and cytokine receptor, Leishmaniasis, NF-kappa B signaling pathway, B cell receptor signaling pathway, Intestinal immune network for IgA production, Th17 cell differentiation, Staphylococcus aureus infection, Th1 and Th2 cell differentiation, Human T-cell leukemia virus 1 infection, Allograft rejection, Epstein-Barr virus infection, Type I diabetes mellitus, IL-17, and Phagosome signaling pathways. We used the GOBubble and GOCluster functions of the GOplot package to plot the pathway enrichment images, and they are shown in Fig. 4 (d) and Fig. 5.

Gene-set enrichment analysis in RA

Digging deeper into the RA group's signaling pathways by gene-set enrichment analysis(GSEA) (Fig. 6), we found the following additional signaling pathways: Intestinal immune network for IgA production, Natural killer cell-mediated cytotoxicity, Primary immunodeficiency, Rheumatoid arthritis, Th1, and Th2 cell differentiation, Th17 cell differentiation, B cell receptor, p53, TNF, Toll − like receptor, AMPK, T cell, ErbB, FoxO, Insulin, PI3K − Akt, TGF − beta, Chemokine, MAPK, NF − kappa B, Phospholipase D, cGMP − PKG, PPAR, and AGE − RAGE signaling pathways.

Construction protein-protein interaction networks

We logged into the STRING database website, entered these differentially expressed genes, and obtained the "string_interactions.tsv" file. For the "string_interactions.tsv" file, we used version 3.8.0 of Cytoscape software to construct the PPI network. This network demonstrates these DEGs' relationships and functions, and we set out to remove isolated nodes to derive the protein interaction network of RA, which contains 264 nodes and 999 links. We visualized the top 30 genes and displayed a bar chart of these 30 genes using R software. This situation implies that these 30 DEGs have more interconnections and interactions with other genes and have an important central role in biological processes. (Fig. 7b).

Identification of HUB genes and inflammation, immune-related HUB genes

The top ten hub genes were derived by selecting the MCC algorithm. these top ten hub genes include *Ccr1,Ccr2,Ccr5,Ccr7,Cxcl5,Cxcl6,Cxcl13,Ccl13, Adcy2* and *Pnoc*. shown in Fig. 7 (a).

We selected biofunctional terms related to inflammation and immunity for gene prediction and took intersections of these genes with hub genes. We derived the inflammation and immunity-related hub genes as

follows: *Ccr2, Ccr7, Cxcl5. Cxcl6, Ccl13*, and *Cxcl13*. as shown in Fig. 10

Analysis of immune infiltration in RA

We derived immune infiltration differences by the CIBERSORT algorithm from 22 immune cell subpopulations between RA and normal synovial tissue. Figure 8 (a) The descending PCA method allowed to find significantly biased clustering and individual differences in the proportion of immune cells in the RA group versus normal controls. Figure 8 (d) The result of the heat map correlation showed that neutrophils and resting NK cells correlated positively (value = 0.71). There was also a positive correlation between CD4 naive T cells and resting NK cells (value = 0.59), and CD8 T cells had a significant negative correlation with CD4 memory resting T cells (value = − 0.49). There was also a significant negative correlation between plasma cells and activated NK cells (value = -0.49). Figure 8 (b) By screening, the immune cell gene expression matrix of 71 samples were obtained, and the heatmap was plotted to visualize the difference in expression in the normal and RA groups of 22 immune cells. As shown in Fig. 8 (c) Finally, violin plots were drawn and analyzed for immune cells' content in the 27 normal and 44 RA groups. It was concluded that RA tissues contained higher proportions of Plasma cells, follicular helper T cells, Monocytes, CD8 T cells, M0 macrophages, and gamma delta T cells. In contrast, regulatory T cells (Tregs), activated NK cells, CD4 memory resting T cells, M1 Macrophages, resting Dendritic cells, Eosinophils, activated Mast cells, and resting Mast cells, were relatively low(Fig. 9).

Discussion

The pathogenesis of RA is centered on inflammation and immune response. One of RA's main clinical features is chronic synovitis, which progresses to cartilage and bone damage. Therefore, targeted analysis of these key genes, pathway molecules and immune infiltrations that are altered in disease will facilitate the discovery of valid and reliable biomarkers for early diagnosis, targeted personalized therapy and delayed disease progression, as well as facilitate the shift from a single-parameter model to a multi-parameter systemic model of traditional medical concepts[48, 49]. In this study, four knee synovial gene microarray expression data were downloaded from the GEO database. We mined these data to derive differentially expressed genes between RA synovial tissue and healthy controls. We found that 275 genes were significantly up-regulated, and 163 genes were significantly down-regulated among these differentially expressed genes. We expect that the hub genes, molecular pathways, and immune infiltration of inflammatory immune cells derived from the bioinformatics analysis of these differentially expressed genes will provide novel ideas and concepts for RA's diagnosis and treatment. The results of this study are summarized below.

Analysis of molecular pathways that play a diagnostic and therapeutic role in RA

Through KEGG and GSEA analysis, The molecular pathways in the knee synovial membrane of RA have been primarily involved in the biological processes of chemotaxis, adhesion, proliferation, differentiation, activation, and regulation of inflammatory immune cells leukocytes, T cells, and monocytes. Such results also confirm that RA is a biological process with an intricate regulatory network involving various inflammatory immune cytokine interactions. Furthermore, signaling pathways such as NF-κB, FoxO, Toll-like receptors, PI3K, TGF-beta, MAPK, PPAR, AGE-RAGE, and cGMP-PKG are also involved.

The NF-κB pathway is activated and up-regulated in the synovial tissue of patients with RA. Using specific antibodies that bind to IL-1 and IL-17, blocking the NF-κB signaling pathway reduces serum inflammatory cytokine levels and decreases the expression of IL-1, IL-2, IL-6, IL-17, TNF-a, interferon-γ, and matrix metalloproteinase-3. Increasing the expression of IL-10 inhibits bone destruction, reduces histological damage, decreases the severity of arthritis, and has a therapeutic effect on RA[50, 51].

During RA, the FoxO signaling pathway is down-regulated. One experiment found that in RA patients' peripheral blood, the expression of FoxO1 mRNA was significantly reduced. FoxO1 overexpression has induced apoptosis of RA synovial cells. Expression levels have been negatively correlated with the disease's activity, which is consistent with our results[52].

A suitable blockade of Toll-like receptor (TLR) activation has been reported to have an inhibitory effect on RA development during disease progression[53, 54]. The TLR4 inhibitor TAK-242, which inhibits IL-6, MMP-1, and VEGF expression, reduces serum IL-6 and VEGF and has an inhibitory effect on the NF-κB pathway. The inflammatory status of joint tissues has significantly improved, thus controlling RA progression[55].

It has been shown that transforming growth factor (TGF) in synovial tissue maintains the knee joint's normal physiological function, that increased expression of TGF in RA synovium may lead to abnormal synovial growth. That inhibition of the TGF signaling pathway reduces the survival and migration of synovial fibroblasts and attenuates pulmonary fibrosis in RA rats[56]. Betulinic acid (BA) inhibited the migration and invasion of RA synovial cells, suppressed vascular endothelial growth factor transcription and TGF. It also inhibited NF-3B inflammatory pathway-related protein expression, decreased IL-1 and IL-6 inflammatory mediator levels, and reduced RA symptoms in rats[57].

The PI3K/AKT signaling pathway is activated and up-regulated in RA. Shikonin(SKN) down-regulates PI3K and Akt's expression, inhibiting the phosphorylation and gene-level signaling molecules such as ERK1/2, JNK1/2, and p38, and exerts anti-RA angiogenic effects[58]. Cinnamaldehyde (CA) can block PI3K/AKT signaling pathway and inhibit the proliferation and infiltration of rheumatoid synovial cells, which has potential therapeutic effects on RA[59]. Berberine inhibits inflammatory proliferation of synovial cells, suppresses DC activation, regulates Th17/Treg balance, and prevents cartilage and bone destruction by regulating various signaling pathways involved in inflammatory responses, including PI3K/Akt. Such molecular targets may explore new RA treatment targets[60].

In RA synovial tissue, MAPK signaling pathway activation expression is increased. Its activation plays a regulatory role in differentiating monocytes and cell survival. In mice, members of the receptor families PAQR11, progesterone, and AdipoQ regulate in vitro monocyte and macrophage differentiation. In vivo Paqr11 knockdown deletion inhibits monocyte differentiation, encourages cell survival, and delays RA progression[61]. Silencing of lncRNA para nuclear assembly transcript 1 promotes miR-129 and miR-204, inhibits the MAPK signaling pathway. Lipoxin A4 (LXA4), a MAPK signaling pathway p38 inhibitor, decreased p38 expression in mouse synovial tissues, reduced proliferation of synovial tissue and inflammatory cell infiltration, reduced proliferation of RA severity, and produced a protective effect in arthritic mice[62].

In various diseases, peroxisome proliferator-activated receptors (PPAR) are involved in normal physiological and pathological processes. Expression of PPAR is significantly lower than normal in RA synovial tissues.

Down-regulation of PPAR promotes the expression of Cyclin D1, MMP1, and MMP9, and PPAR up-regulation may induce Wnt/β-catenin signaling activation, which plays a vital role in the synovial cells of RA. One study found that pioglitazone, a receptor agonist activated by peroxisome proliferators, promotes vasoprotective and anti-inflammatory effects in RA, significantly improves aortic elasticity, and decreases inflammation and activity of the disease[63, 64].

MiR-34a can activate the p53 signaling pathway and inhibit the abnormal growth of synovial tissue and inflammatory process in RA. Other studies have also found that mutations in the P53 signaling pathway, a traditional oncogene, may be associated with anti-rheumatic RA resistance[65].

AGE-RAGE signaling pathway and cGMP-PKG have not been reported in rheumatoid arthritis. Still, By activating NOx-1 and reducing SOD-1 expression, AGE/RAGE signaling has been shown to increase oxidative stress, thus promoting vascular calcification mediated by diabetes. And oxidative stress plays a key role in the synovial lesion process in RA, which needs to be further verified [66].

Natriuretic Peptides have been shown to cause inflammatory dissociation by activating the cGMP/PKG signaling pathway. By increasing intracellular cGMP levels, phosphodiesterases are activated while interfering with caspase-8 cleavage. It inhibits the activation pathway of inflammatory vesicles, thus acting as an anti-inflammatory and immunomodulatory agent. Therefore, we suggest that the signaling pathway of cGMP-PKG plays a significant role in RA and needs further research [67].

Analysis of Hub genes that play a diagnostic and therapeutic role in RA

We used STRING online database tool and R language to derive the top30 protein interaction networks, and Cytoscape software's Cytohubba plug-in was also used, and ten genes were filtered out such as *Ccr1, Ccr2, Ccr5, Ccr7, Cxcl5, Cxcl6, Cxcl13, Ccl13*, *Adcy2*, and *Pnoc*. We then used the R language GOplot package to derive six hub genes associated with inflammatory immunity: *Ccr2, Ccr7, Cxcl5. Cxcl6, Ccl13and Cxcl13*,

Our study revealed that these hub genes are mainly chemokine and receptor-related genes. Chemokines are produced by synovial macrophages and exert chemotactic effects on inflammatory immune cells such as neutrophils, lymphocytes, and monocytes and are closely associated with vasculitis development. CCR, CXCL, and CCL, as essential components and receptors of the chemokine family, are closely related to RA development.

The CC Chemokine Receptor (CCR) is an integral membrane protein. The targeting of chemokines or chemokine receptors is a promising therapeutic strategy for the treatment of chronic inflammation. Small CCR1 and CCR2 molecule antagonists are effective at blocking inflammatory immune cell migration. Reduces the inflammatory response and decreases pain caused by chronic inflammation [68].

The knockdown of CCR5 and the application of PD98059 (mitogen-activated protein kinase 1 inhibitor) in rats resulted in a significant decrease in interleukin-6, metalloproteinase-1, metalloproteinase-3, and tumor necrosis factor levels. In addition to substantial reductions in JNK1, ERK1, p38, Cyclin protein, and Bcl-2, CCR5 may inhibit synovial cell activity, promote synovial cell apoptosis, and suppress the inflammatory response of synovial cells in RA rats via the MAPK pathway. For RA, it may provide a new therapeutic target[69].

The CCR7 chemokine receptor plays a key role in health and disease by directing immune cells' migration, particularly DCs and T cells. Due to impaired CCR7 signaling, immune responses and misdirection of immune cells can lead to autoimmune diseases. [70, 71].

Inflammatory migration and erosive phenotypes have been observed in patients with RA. Identifying new pathways involved in various stages of the pathology of RA will provide valuable insights into different inflammatory immune cells' mechanical behavior and strategies to suppress their activity. CCR7 is a marker of RA synovial fluid macrophages, and in early RA, monocytes infiltrate synovial tissue. By blocking CCR7/ CCL21 in synovial fluid, monocyte migration is prevented. It increases the number of M1 macrophages and raises the levels of IL-6 and IL-23. Increased transcription of M1 macrophage cytokines leads to the differentiation of primary T cells into Th17 cells, and M1 macrophage-driven Th17 polarization promotes osteoclast formation in RA during the aggressive phase of the disease. They are extending joint inflammation to bone erosion. It also induces neovascularization, further aggravating disease progression. CCR7/CCL21 is an exciting emerging RA therapy target, and obstructing its function reduces or eliminates RA immune-inflammatory infiltration. They play a major role in delaying or blocking the RA pathological process[72, 73].

For how CCR7 signaling directs cell migration and infiltration, we need to develop new tools to assess and monitor CCR7 signaling. The latest study identifies human CCR7 by novel nanotechnology. It interacts with CCR7 through bimolecular fluorescence

complementary recognition without interfering with G protein coupling and downstream signaling. Targeted analysis of the migration and invasion process of CCR7 [74].

Chemokine C-X-C motif chemokine ligand 5 (CXCL5) has functions such as chemotaxis of neutrophils, pro-angiogenesis, and involvement in inflammatory responses. Its pathological angiogenesis is highly correlated with RA activity[75].

CXCL13 is a known B cell chemokine that promotes B lymphocyte migration by interacting with its receptor CXCR5. CXCL13 and CCL20 act synergistically to increase B cell migration, leading to autoimmune inflammation, closely associated with disease activity [76, 77].

By recruiting monocytes and lymphocytes, CC motif chemokines are thought to be involved in RA's pathogenesis. In both serum and synovial tissue, the expression of CCL13 is elevated in RA patients. Interestingly, tumor necrosis factor-alpha positively regulates CCL13 expression and inhibits synovial fibroblast apoptosis[78]. RA prevalence in women is higher than in men, and 17-estradiol mediates cellular activation signals in synovial fibroblasts via ERK-1/2, leading to uncontrolled apoptosis, increased matrix metalloproteinase-3 production, and excessive CCL13 production, leading to the progression of RA [79].

However, *Adcy2* and *Pnoc* genes have not been reported in RA studies. Adenylate cyclase 2 (*Adcy2*), a member of adenylate cyclase class B, plays a crucial role in promoting phosphorylation, glycogen synthesis, and catabolism. By studying the anti-migratory effect of adenylate cyclase-related protein 1 in pancreatic cancer cells, cyclic adenosine monophosphate was found to be a second messenger regulating the migration and invasion of pancreatic cancer cells. Its elevation prevented the migration and invasion of pancreatic ductal carcinoma cells [80]. We speculate that *Adcy2* may play a role in inflammatory immune cell migration and infiltration in RA and merits further investigation.

The prepronociceptin (*Pnoc*) gene encodes bioactive peptides involved in sensory, emotional, cognitive, and neurogenesis. The interaction between these active peptides and the corresponding receptors can modulate inflammation and immune diseases. Silencing the gene reduces inflammatory factor levels, oxidative stress, and glial fibrillary acidic protein expression. Conversely, overexpression of this gene reverses the changes in the above biochemical indicators[81]. Considering that it can regulate inflammation and immunity, we hypothesized that this gene might play an important role in RA's inflammatory response and potential therapeutic target.

Analysis of immune infiltrating cells that play a diagnostic and therapeutic role in RA

To further investigate the RA synovium role of inflammatory immune cell infiltration, we performed immune infiltration analysis on a combined dataset of four databases by the CIBERSORT algorithm. We found that CD8 T cells, Plasma cells, Monocytes, follicular helper T cells, gamma delta T cells, and M0 macrophages were significantly elevated in RA. In contrast, CD4 memory resting T cells, regulatory T cells (Tregs), M1 Macrophages,

activated NK cells, resting Dendritic cells, resting Mast cells, Eosinophils, and activated Mast cells were significantly reduced in the RA synovial tissue.

Plasma cells (PC) are also known as antibody-secreting cells. With antigen-presenting cells and Th cells, mature B cells become activated B cells after receiving antigen stimulation. They then differentiate into plasma cells, which synthesize different kinds of immunoglobulins and secrete them.β-ARR2 has been found to be a crucial protein mediating the desensitization and internalization of inflammatory and immune responses involving G protein-coupled receptors. Its deletion inhibits the endocytosis of Toll-like receptor 4 (TLR4) on B lymphocyte membranes while activating the NF-κB signaling pathway, increasing plasma cell differentiation and antibody production and exacerbating arthritis symptoms[82]. Myeloid suppressor cells derived from granulocytes secrete exosomes, transport different bioactive molecules, and play a regulatory role in immune cells. It also promotes the secretion of IL-10 from splenic B cells, reduces the proportion of plasma and follicular helper T cells and serum IgG levels, and mitigates rheumatoid arthritis symptoms in mice[83]. It is evident that plasma cells play an essential role in RA and deserve further study.

In RA, CD8 + CD28-T cells are significantly elevated and may be associated with T-lymphocyte homeostasis dysregulation. There is also an increase in their supracellular expression of programmed death receptor-1 (PD-1). When regulating T lymphocytes, PD-1 plays a key role. Although PD-1 induces immunosuppression of effector T cells in synovial inflammation, its expression is up-regulated in synovial inflammation, closely correlating with the severity of its disease [84]. Another study found that CD8 + T cells are involved in the pathogenesis of RA through the release of pro-inflammatory and cytolytic mediators and that targeting lactate dehydrogenase remodels CD8 + T cells and changes the metabolism of glucose and glutamine, thereby reducing the adipogenic, migratory and proliferative capacity of CD8 + T cells while losing the ability to induce a pro-inflammatory phenotype in B cells [85].

One of the potential mechanisms promoting RA is the imbalance in the ratio of M1 macrophages, which produce pro-inflammatory factors, to M2 macrophages, which produce anti-inflammatory factors.M2

macrophages may have a positive role in delaying the development of RA. In contrast, M0 macrophages may have a potential role in the immune imbalance associated with RA pathogenesis. Our results found a significant increase in the proportion of M0 macrophages and a substantial decrease in the proportion of M1 macrophages in RA's synovial tissue. The common knowledge contradicts this that M1 macrophages should be significantly up-regulated in the synovium of RA. It has been found that M0 macrophages can differentiate into M1 and M2 macrophages under certain circumstances, and these paradoxical phenomena and whether M0 macrophages are converted to M1 macrophages in the inflammatory setting in RA deserve further investigation [86].

Monocytes are attracted to pro-inflammatory mediators in response to inflammation or tissue injury, differentiate toward macrophages, activate macrophages, and the *Paqr11* gene regulates monocyte-to-macrophage differentiation. Knockdown of *Paqr11* in mice inhibited monocytes' differentiation and delayed RA progression [61]. Tumor necrosis factor-alpha and IL-6 were used in other experiments to induce osteoclasts with the ability to resorb bone matrix to stimulate peripheral blood monocytes. The expression of IL-1β, IL-12, TNF alpha, and MMP3 has been significantly increased in tumor necrosis, factor-alpha, and IL-6-induced osteoclasts. RA and other joint destruction-related inflammatory arthritis may involve these monocytes with the capacity to resorb bone matrix[87].

Dendritic cells (DCs) are the nexus of innate and acquired immunity, and increased expression of pro-inflammatory cytokines, chemokines, and adhesion molecules was found in DCs by culturing RA synovial tissue in vitro. Hydroxychloroquine (HCQ) is one of the most commonly used immunosuppressive agents in treating RA. HCQ inhibits DC maturation and migration to lymph nodes, decreases CXCR4 expression and interferon-α secretion, and DC activation is involved in RA's pathogenesis. HCQ protects against RA by blocking TLR9[88]. TARM1 is a member of the leukocyte immunoglobulin-like receptor family. Its expression is elevated in the joints of a mouse model of RA. It is an important stimulus for dendritic cell maturation, which may be a good target for the treatment of autoimmune diseases[89].

Mast cells are tissue-resident innate immune cells involved in the pathogenesis of many autoimmune diseases; they are mainly present in RA's synovial tissue. Their activation is associated with the amelioration of inflammation. However, a growing body of proof indicates that mast cells can act as modifiable immune cells with pro-and anti-inflammatory functions. Synovial mast cells are a potential research hotspot for this subtle, conflicting, and exciting hypothesis[90].

Substance P (SP) is a pro-inflammatory substance, and activated synovial mast cells rapidly degrade SP in RA while downregulating SP-mediated synovial cell activation. On the other hand, SP activates inflammatory mediators induced by synoviocytes, suggesting a dual regulation of SP-mediated synovial mast cell inflammation in RA[91]. It has been found that gene expression of synovial mast cells is negatively correlated with disease activity. When IL-33 activates synovial mast cells, they produce an immunomodulatory phenotype and inhibit monocyte activation[92]. However, a study found that mast cells are important targets of Toll-like receptor ligands and immune complexes. Mast cells greatly enhance the inflammatory response of synovial tissue in RA through these pathways' combined activation[93].

Eosinophils have a pro-inflammatory role in asthma. However, recent studies have shown that eosinophils have a pro-soluble effect in RA. After induction of eosinophil asthma, arthritis subsided, and joint tissue was

protected in mice. Single-cell RNA sequencing approaches identified a specific subpopulation of regulatory eosinophils in the joints, distinct from inflammatory eosinophils in the lungs. The reduction of eosinophils instead eliminated the beneficial effects of asthma on arthritis. Such a result is consistent with our study that concluded a significant down-regulation of eosinophils in RA[94].

The anti-inflammatory effect of eosinophils was also confirmed in another experiment. Eosinophil treatment reduced iNOS, TNF-α, IL-6, and IL-12 levels, while Arg1, transforming growth factor-β, IL-10, and IL-13, increased while inhibiting MAPK signaling polarizing M2 macrophages, exerting anti-inflammatory and reducing RA symptoms[95]. The double-edged role of these eosinophils needs to be studied in depth.

Conclusion And Expert Recommendations

In conclusion, we conducted a detailed bioinformatics study of RA gene expression profiles. Genes with significant differential expression were screened. Functional and signaling pathway enrichment analysis was performed on these genes. We found that Toll-like receptors, AMPK, MAPK, TNF, ErbB, P53, FoxO, TGF- beta, PI3K, and NF-κB pathways also play critical roles in RA in addition to signaling pathways closely related to inflammatory immune responses. Next, We imputed ten hub genes and six inflammatory immune-related hub genes from PPI, among which *Adcy2* and *Pnoc*. were not reported in RA studies, suggesting that they may be worthy targets for further research. Finally, We deduced the infiltration of different inflammatory immune cells in RA's synovial tissue by the CIBERSORT algorithm. In short, these results may contribute to the understanding of the biological processes associated with RA and the development of targeted therapeutic approaches.

We recommend this research article.RA is a chronic, complex disease with multi-gene, multi-pathway, and multi-cellular involvement, involving alterations in multi-level molecular networks of genomic, transcriptomic, proteomic, and various inflammatory immune cells. In clinical practice, patient samples can be collected for high-throughput sequencing. Through bioinformatics analysis, key genes and pathway molecules in the network can be obtained. The regulation and interactions between various genes and pathway molecules and cellular networks can be analyzed, integrated, and mined by computer science such as big data, machine learning, neural networks, artificial intelligence, biomaterials, and cell simulation. Targeted and rational prediction and analysis lead to effective personalized diagnosis and treatment. We also promote the transformation of traditional empirical and reactive medical services to predictive, targeted, and personalized medical services and improve RA patients' services by combining medicine with other disciplines such as mathematics, statistics, computers, and emerging materials under the innovative concept of the three elements of PPPM.

Abbreviations

RA: Rheumatoid arthritis,

KEGG: Kyoto Encyclopedia of Genes and Genomes pathway,

GO: Gene Ontology's enrichment analysis,

GSEA: Gene-set enrichment analysis,

PPPM: Predictive preventive personalized medicine,

DEGs: Differentially expressed genes,

PPI: protein-protein interaction networks,

SKN: Shikonin,

LXA4: Lipoxin A4,

PPAR: peroxisome proliferator-activated receptors,

CCR: The CC Chemokine Receptor,

CXCL: Chemokine C-X-C motif chemokine ligand,

Adcy2: Adenylate cyclase 2,

Pnoc: prepronociceptin,

TLR4: Toll-like receptor 4,

PC: Plasma cells,

PD-1: programmed death receptor-1,

DCs: Dendritic cells,

HCQ: Hydroxychloroquine,

SP: Substance P,

BP: Biological Process

CC: Cellular Component,

MF: Molecular Function,

Declarations

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Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest with the Gene Expression Omnibus (GEO) database used in this study.

Availability of data and material

All data is real and guarantee the validity of results.

Authors' contributions

Sheng Fang conceived the original idea and designed the outlines of the study. Xiao Fang, Xin Xu, Lin Zhong, and An-quan Wang Helped collect, organize, and check the data. Wei-lu Gao and Zong-Sheng Yin aided in revising the manuscript. All authors have read and approved the final manuscript.

Ethics approval

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Consent to participate

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Tables

TABLE 1 Basic information of the four datasets

GEO	Platform	Organism	Tissue	Normal	RA
GSE1919	GPL91	Homo sapiens	Synovial tissue	5	5
GSE55235	GPL96	Homo sapiens	Synovial tissue	10	10
GSE55457	GPL96	Homo sapiens	Synovial tissue	10	13
GSE77298	GPL570	Homo sapiens	Synovial tissue	7	16

TABLE 2 Sample numbers of each group

Group	GSE1919	GSE55235	GSE55457	GSE77298	Merged
Normal	5	10	10	7	32
RA	5	10	13	16	44

TABLE 3 Statistical results of DEGs in the four datasets

	ALL	Up-regulated	Down-regulated
DEGS	438	275	163

TABLE 4 The Top 20 Down-regulated and Up-regulated DEGs in RA

Gene symbol	logFC	P.Value	adj.P.Val
Down-regulated			
PLIN1	-3.75812	1.24E-09	4.45E-08
PCK1	-3.55264	5.28E-11	3.47E-09
ADH1B	-3.49749	6.66E-11	4.24E-09
ADIPOQ	-3.37143	6.87E-09	1.92E-07
APOD	-3.21412	2.60E-14	6.12E-12
FABP4	-3.11815	3.05E-11	2.13E-09
ANGPTL7	-3.08211	2.49E-10	1.21E-08
ZBTB16	-2.6968	1.32E-13	2.38E-11
ADH1C	-2.5479	1.11E-09	4.05E-08
RPS4Y1	-2.45545	0.000347	0.001991
MAOA	-2.4341	2.19E-14	5.64E-12
FOSB	-2.43366	4.28E-05	0.000338
MYOC	-2.38627	3.46E-10	1.54E-08
FKBP5	-2.38541	2.41E-08	5.55E-07
C7	-2.33321	4.26E-09	1.30E-07
CYP4B1	-2.30081	2.09E-06	2.53E-05
LEP	-2.12959	9.81E-06	9.59E-05
AKR1B10	-2.11575	1.59E-13	2.70E-11
GPD1	-2.05706	4.86E-07	7.24E-06
PDK4	-2.00167	7.59E-07	1.06E-05
Up-regulated			
IGJ	5.440321	5.19E-18	6.29E-15
CXCL13	5.200878	1.57E-21	1.33E-17
IGLC1	5.191091	5.53E-20	1.17E-16
IGHM	4.703149	1.01E-20	2.86E-17
IGLV1-44	4.641258	3.98E-17	3.07E-14
MMP1	4.518481	8.20E-12	7.17E-10
TNFRSF17	3.991615	1.27E-14	3.38E-12
IGKC	3.799941	9.21E-17	5.58E-14
ADAMDEC1	3.252949	1.39E-16	7.87E-14
NKG7	3.212648	2.35E-14	5.86E-12
CXCL9	3.038077	8.34E-14	1.57E-11
MMP13	2.937127	3.34E-10	1.51E-08
LRRC15	2.903438	5.53E-21	2.34E-17
LAMP3	2.898897	2.30E-12	2.53E-10
IGHD	2.85142	2.04E-12	2.31E-10
CXCL10	2.796226	5.91E-16	2.51E-13
CD52	2.746439	1.06E-14	3.07E-12
GUSBP11	2.713471	7.64E-18	7.20E-15
AQP9	2.687603	3.49E-11	2.41E-09
CCL18	2.668925	7.54E-12	6.87E-10

TABLE 5 GO analysis of RA

ID	Description	pvalue	p.adjust	geneID
BP				
GO:0007159	leukocyte cell-cell adhesion	2.51E-32	1.14E-28	RAC2/CCL5/CD27/BCL6/ITGA4/HLA-DMB/ZBTB16/RUNX3/ITGB2/LEF1/PTPRC/TNFSF11/
GO:0042110	T cell activation	4.25E-30	9.70E-27	RAC2/CCL5/CD27/BCL6/CD3D/HLA-DMB/ZBTB16/RUNX3/CD2/LEF1/PTPRC
GO:1903037	regulation of leukocyte cell-cell adhesion	7.89E-27	1.20E-23	CCL5/CD27/BCL6/ITGA4/HLA-DMB/ZBTB16/RUNX3/ITGB2/LEF1/PTPRC/TNFSF11/LCK
GO:0050863	regulation of T cell activation	1.20E-26	1.38E-23	RAC2/CCL5/CD27/BCL6/HLA-DMB/ZBTB16/RUNX3/CD2/LEF1/PTPRC/TNFSF11/LCK
GO:0030098	lymphocyte differentiation	1.74E-25	1.59E-22	CD27/BCL6/DOCK10/CD3D/ITGA4/ZBTB16/RUNX3/CD2/BLNK/LEF1/PTPRC
GO:1903039	positive regulation of leukocyte cell-cell adhesion	2.67E-25	2.03E-22	CCL5/CD27/BCL6/ITGA4/HLA-DMB/ZBTB16/RUNX3/ITGB2/LEF1/PTPRC/TNFSF11/LCK
GO:0022407	regulation of cell-cell adhesion	1.75E-24	1.14E-21	CXCL13/CCL5/CD27/BCL6/ITGA4/HLA-DMB/ZBTB16/RUNX3/ITGB2/LEF1/PTPRC/TNFSF11
GO:0022409	positive regulation of cell-cell adhesion	3.04E-24	1.73E-21	CXCL13/CCL5/CD27/BCL6/ITGA4/HLA-DMB/ZBTB16/RUNX3/ITGB2/LEF1/PTPRC/TNFSF11
GO:0050867	positive regulation of cell activation	1.19E-23	6.05E-21	IGHM/IGLC1/IGKC/CCL5/TRBC1/CD27/BCL6/HLA-DMB/ZBTB16/RUNX3/CD2/ITGB2
GO:0002696	positive regulation of leukocyte activation	1.60E-23	7.29E-21	IGHM/IGLC1/IGKC/CCL5/TRBC1/CD27/BCL6/HLA-DMB/ZBTB16/RUNX3/CD2/ITGB2
GO:0030217	T cell differentiation	4.87E-23	2.02E-20	CD27/BCL6/CD3D/ZBTB16/RUNX3/CD2/LEF1/PTPRC/LCK/IL7R/NCKAP1L/IL15
GO:0050870	positive regulation of T cell activation	8.99E-22	3.42E-19	CCL5/CD27/BCL6/HLA-DMB/ZBTB16/RUNX3/LEF1/PTPRC/TNFSF11/LCK/IL7R/NCKAP1L
MF				
GO:0140375	immune receptor activity	3.05E-13	1.94E-10	HLA-DOB/IL2RG/CCR5/IL7R/IL10RA/CSF2RB/CCR2/CTSH/LILRB2/IL1R1/CXCR3/GFRA2
GO:0042379	chemokine receptor binding	3.26E-11	7.90E-09	CXCL13/CCL5/CXCL10/STAT1/CXCL9/CCL18/CXCL11/CCR2/CXCL6/CCL25/CCL13/CCL19/CCL7/CXCL5/CXCL1
GO:0008009	chemokine activity	3.73E-11	7.90E-09	CXCL13/CCL5/CXCL10/CXCL9/CCL18/CXCL11/CXCL6/CCL25/CCL13/CCL19/CCL7/CXCL5/CXCL1
GO:0001664	G protein-coupled receptor binding	5.62E-10	8.36E-08	CXCL13/CCL5/CXCL10/STAT1/EDNRB/CXCL9/CCL18/SFRP1/CXCL11/CCR2/MYOC/CXCL6
GO:0005125	cytokine activity	6.58E-10	8.36E-08	CXCL13/CCL5/CXCL10/CXCL9/TNFSF11/IL15/CCL18/CXCL11/IL32/CXCL6/SPP1/ADIPOQ
GO:0030546	signaling receptor activator activity	6.48E-09	6.85E-07	CXCL13/CCL5/CXCL10/CXCL9/TNFSF11/IL15/CCL18/CXCL11/IL32/CXCL6/SPP1/ADIPOQ
GO:0003823	antigen binding	7.63E-09	6.92E-07	IGHM/IGLC1/IGLV1-44/IGKC/TRBC1/ITGA4/IGHD/IL7R/CD48/HLA-DPB1/TAP1/LILRB4
GO:0019955	cytokine binding	9.31E-09	7.39E-07	IL2RG/CCR5/ITGA4/GBP1/IL10RA/CCR2/IL1R1/CXCR3/TGFBR3/ZFP36/CCR1/CXCR4
GO:0048018	receptor ligand activity	1.76E-08	1.24E-06	CXCL13/CCL5/CXCL10/CXCL9/TNFSF11/IL15/CCL18/CXCL11/IL32/CXCL6/SPP1/ADIPOQ
GO:0019956	chemokine binding	3.11E-08	1.75E-06	CCR5/ITGA4/CCR2/CXCR3/ZFP36/CCR1/CXCR4/CCR7/CCR6
GO:0019957	C-C chemokine binding	3.18E-08	1.75E-06	CCR5/CCR2/CXCR3/ZFP36/CCR1/CXCR4/CCR7/CCR6
GO:0004896	cytokine receptor activity	3.31E-08	1.75E-06	IL2RG/CCR5/IL7R/IL10RA/CSF2RB/CCR2/IL1R1/CXCR3/GFRA2/CCR1/CXCR4/CCR7/GHR/CCR6
CC				
GO:0009897	external side of plasma membrane	3.67E-19	1.39E-16	IGHM/IGLC1/SDC1/IGKC/TRBC1/CXCL10/CD27/IL2RG/CCR5/CD3D/CXCL9/CD2
GO:0062023	collagen-containing extracellular matrix	2.85E-12	5.38E-10	LRRC15/ADAMDEC1/MXRA5/LAMA2/SFRP1/CTSH/ANGPTL7/COL3A1/MYOC/CTSC/COL5A2/ADIPOQ
GO:0070820	tertiary granule	3.96E-10	4.99E-08	ITGB2/GGH/NCKAP1L/CTSH/LILRB2/CD300A/CD53/CYBA/QPCT/CTSS/CYBB/VAMP8
GO:0030665	clathrin-coated vesicle membrane	6.11E-10	5.78E-08	CD3D/EGFR/IL7R/FZD2/HLA-DPB1/CEMIP/WNT5A/HLA-DPA1/NRGN/HBEGF/ROR2/VAMP8
GO:0001772	immunological synapse	6.83E-09	5.16E-07	LCK/CRTAM/GZMB/CORO1A/RHOH/LAT/CD53/GZMA/CD37/ZAP70
GO:0030139	endocytic vesicle	1.98E-08	1.25E-06	RAC2/EGFR/FZD2/HLA-DPB1/GSN/TAP1/CORO1A/SYK/CEMIP/CYBA/WNT5A/WASL
GO:0045121	membrane raft	5.28E-08	2.65E-06	EDNRB/EGFR/CD2/ITGB2/PTPRC/CD79A/LCK/CD48/ADCY2/CSK/LAT/THY1
GO:0098857	membrane microdomain	5.60E-08	2.65E-06	EDNRB/EGFR/CD2/ITGB2/PTPRC/CD79A/LCK/CD48/ADCY2/CSK/LAT/THY1

GO:0098589	membrane region	1.18E-07	4.96E-06	EDNRB/EGFR/CD2/ITGB2/PTPRC/CD79A/LCK/CD48/ADCY2/CSK/LAT/THY1
GO:0030662	coated vesicle membrane	4.62E-07	1.75E-05	CD3D/EGFR/IL7R/FZD2/HLA-DPB1/CEMIP/WNT5A/HLA-DPA1/NRGN/HBEGF/ROR2/VAMP8
GO:0030666	endocytic vesicle membrane	5.20E-07	1.79E-05	RAC2/FZD2/HLA-DPB1/TAP1/CORO1A/CYBA/WNT5A/WASL/HLA-DPA1/CYBB/HBEGF/ROR2
GO:0042613	MHC class II protein complex	6.31E-07	1.99E-05	HLA-DOB/HLA-DMB/HLA-DMA/HLA-DPB1/HLA-DPA1/HLA-DRB4

BP: Biological Process CC: Cellular Component, MF: Molecular Function

Figures

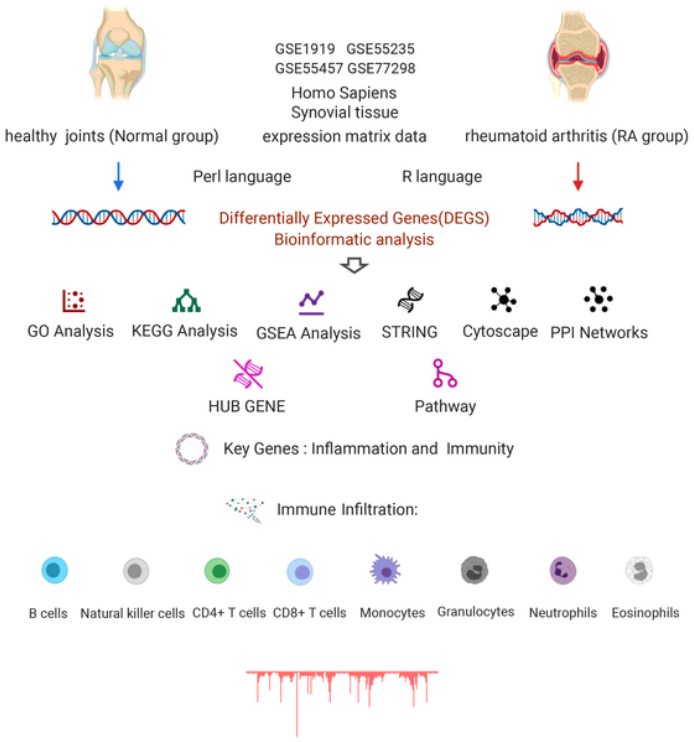

Figure 1

Schematic of the experimental strategy used to identify the hub gene between Rheumatoid arthritis(RA) group and Normal group, Raw data processing using Perl and R language, and identifying and screening differentially expressed genes applying R software limma packages. We assess DEGs' functional and pathway enrichment using GO Analysis, KEGG Pathway Enrichment Analysis, and GSEA, discovering the protein-protein interaction network using the STRING database and Cytoscape software. GOplot obtained hub genes associated with inflammation and immunity, and the CIBERSORT algorithm got infiltrative immunity in RA.

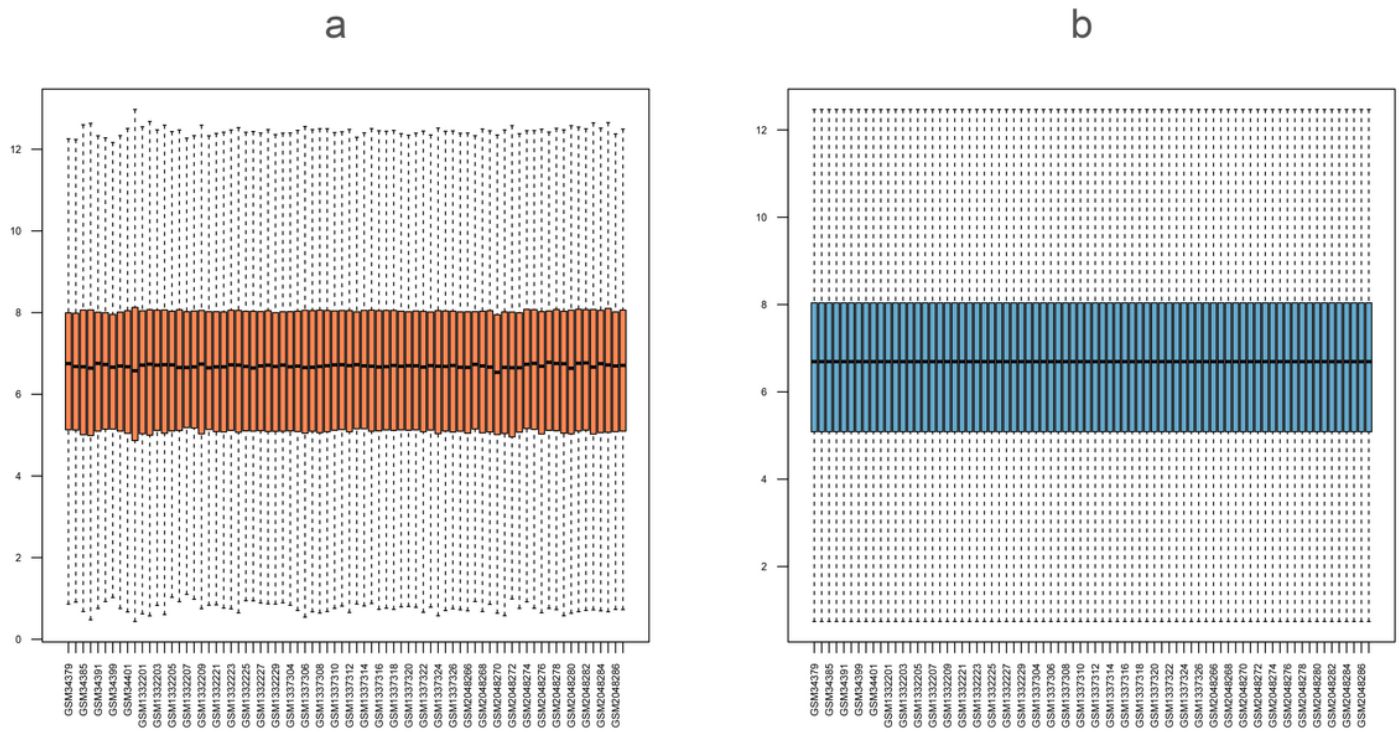

Figure 2

The boxplots for microarray data before(a) and after(b) normalization.

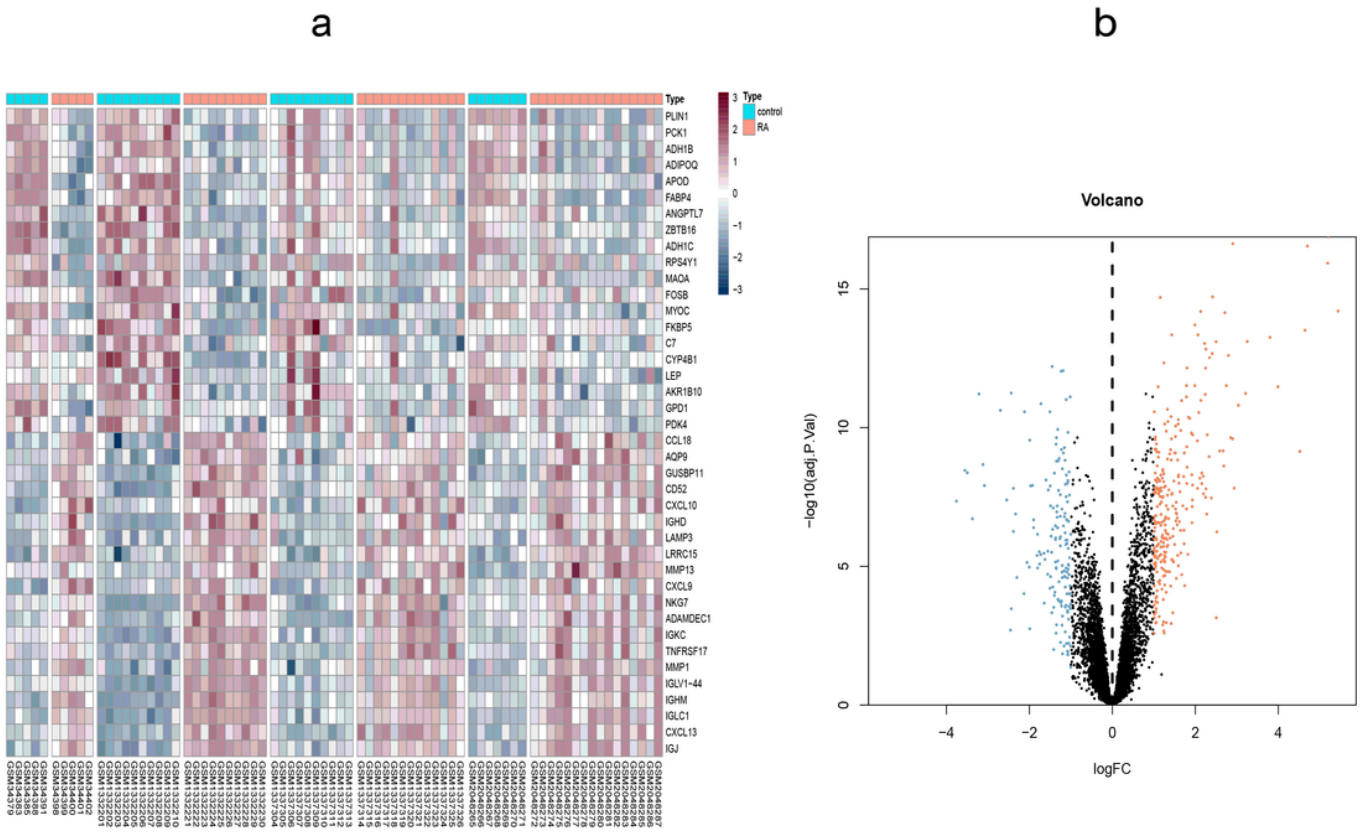

Figure 3

(a) Heatmap for the top 20 temporal genes. (b) Volcano plot for genes between the control and RA group.Red plots represent upregulated genes with |logFC| >1 and p < .05.group.blue plots represent downregulated genes with |logFC| <1 and p < .05.black plots represent the remaining genes with no significant difference.

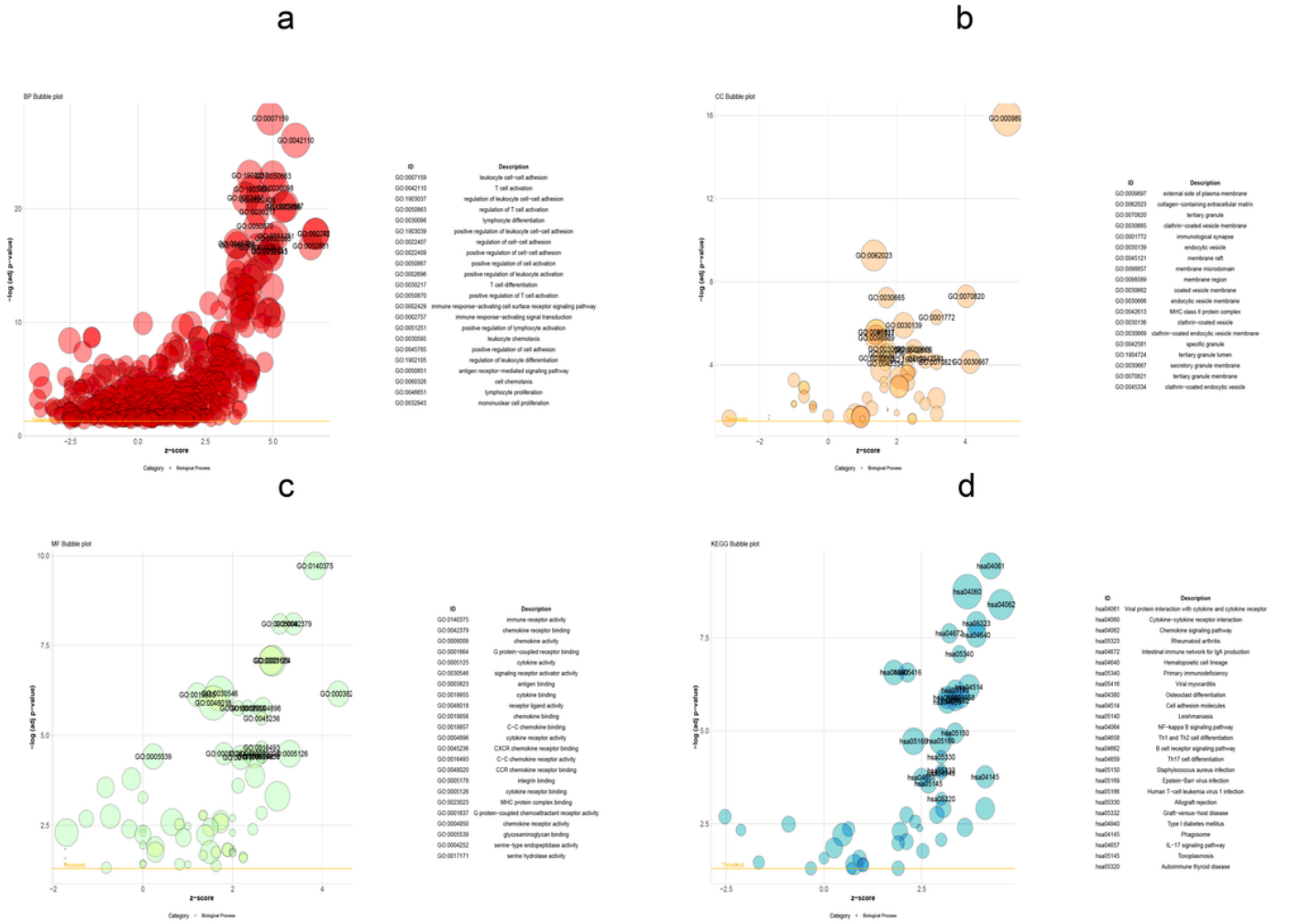

Figure 4

Gene Ontology (GO) enrichment analysis (a) biological process (BP), (b) cellular component (CC) and (c) molecular function (MF)) for the DEGs in RA. the circles are labeled with the term ID, a table connecting the IDs and terms is displayed on the right side. A threshold for the labeling is set based on the negative logarithm of the adjusted p-value < .05. (d) Kyoto Encyclopedia of Genes and Genomes(KEGG)terms enrichment analysis results for RA's DEGs.

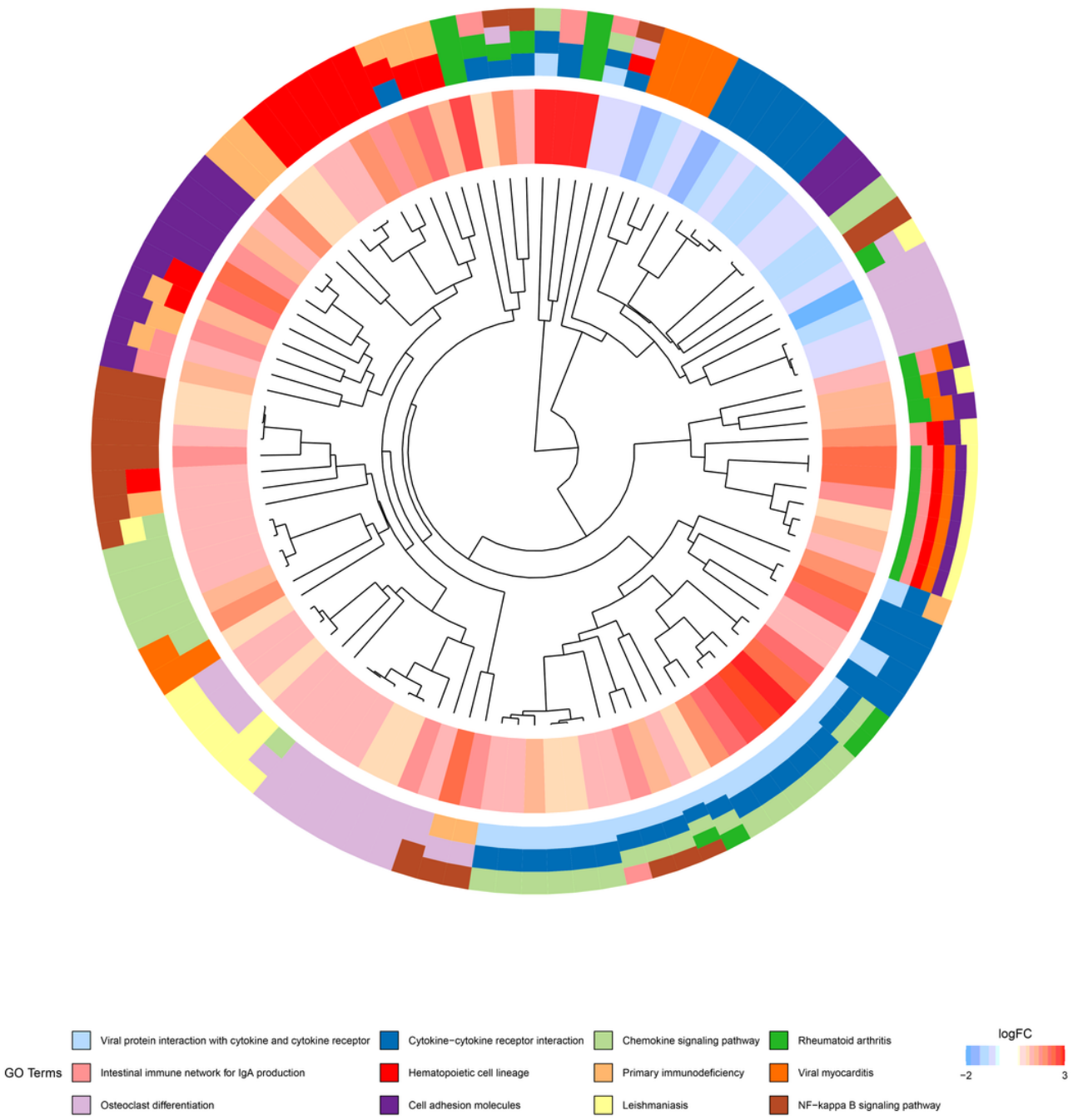

Figure 5

The KEGG enrichment analysis's GOcluster plot. The first ring next to the dendrogram represents the logFC of the genes, the clustering tree leaves. The next ring depicts the terms assigned to the genes.

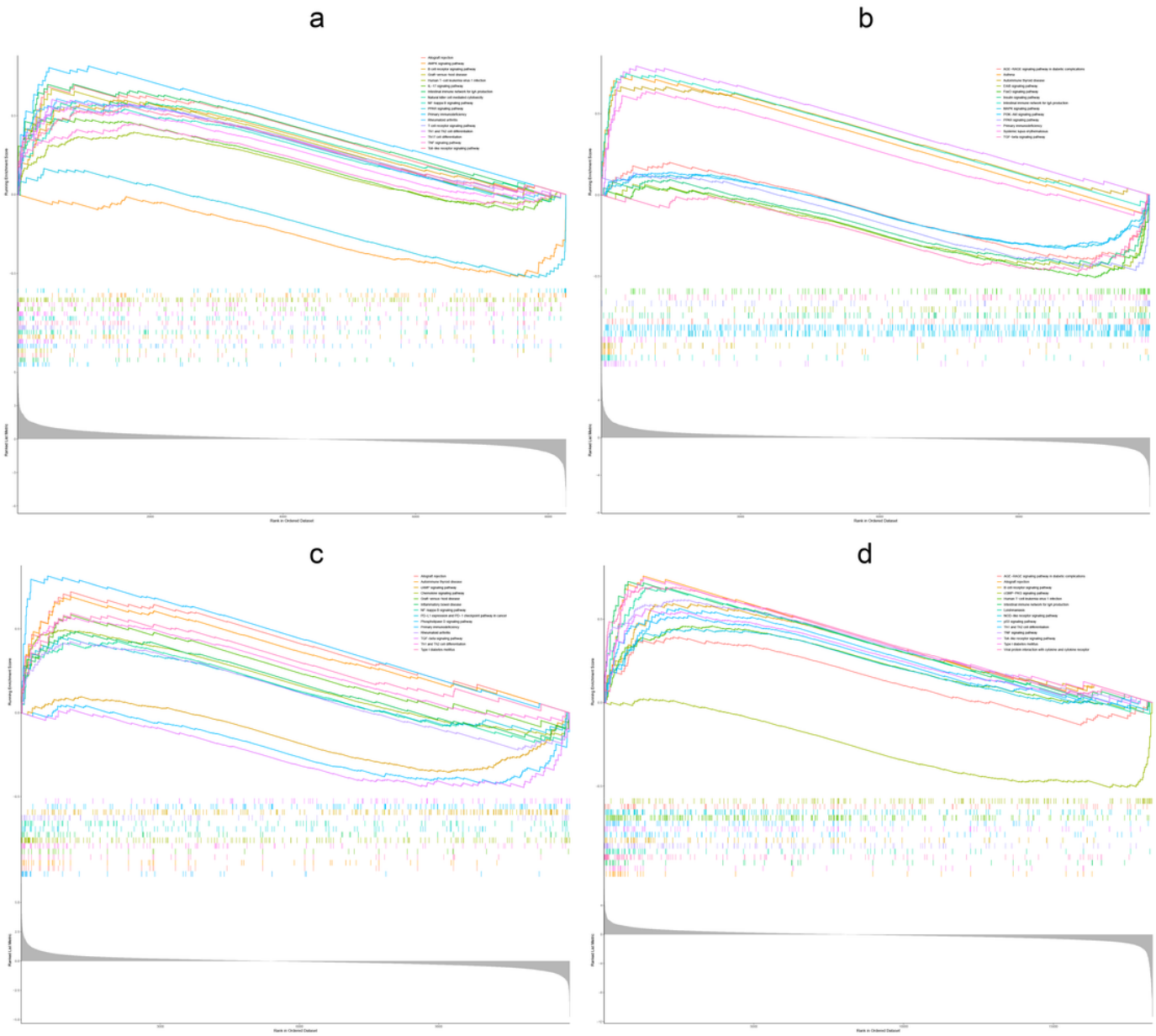

Figure 6

Gene-set enrichment analysis(GSEA)analysis in RA

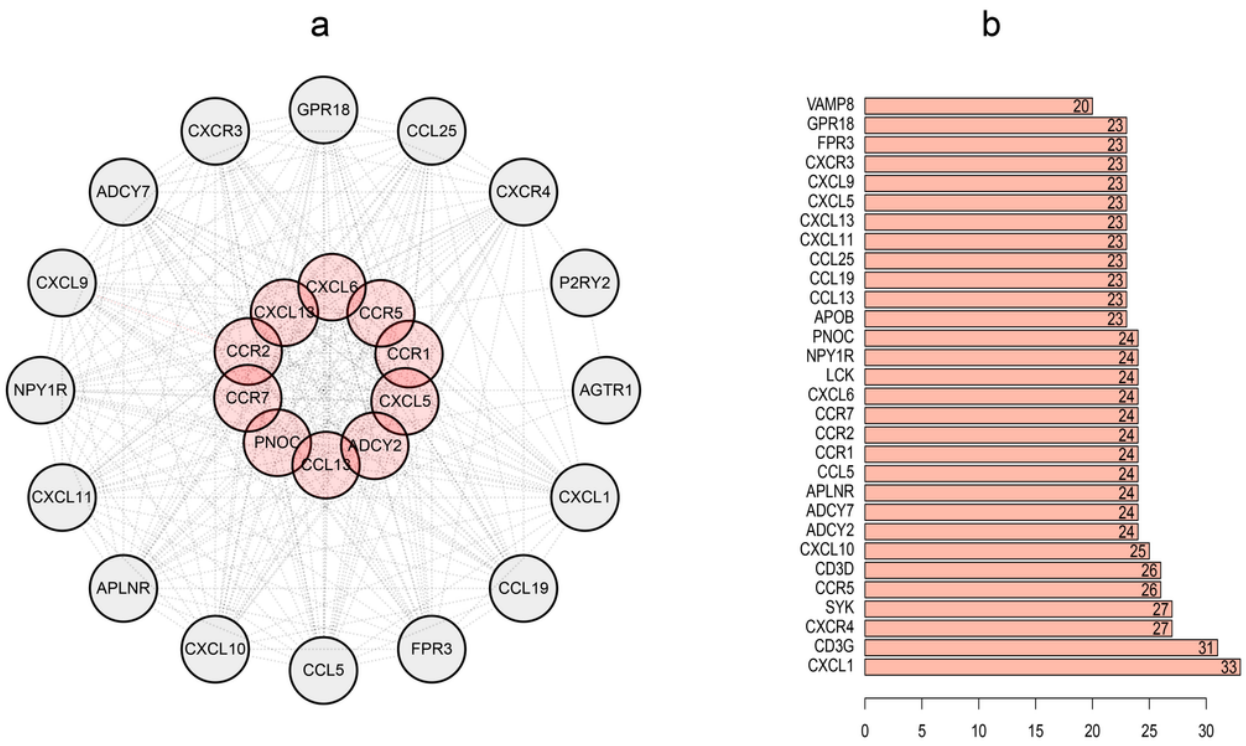

Figure 7

(a) Top 10 hub genes in RA using MCC algorithm. (b) Bar-plot of top 30 core genes with the most weight in RA.

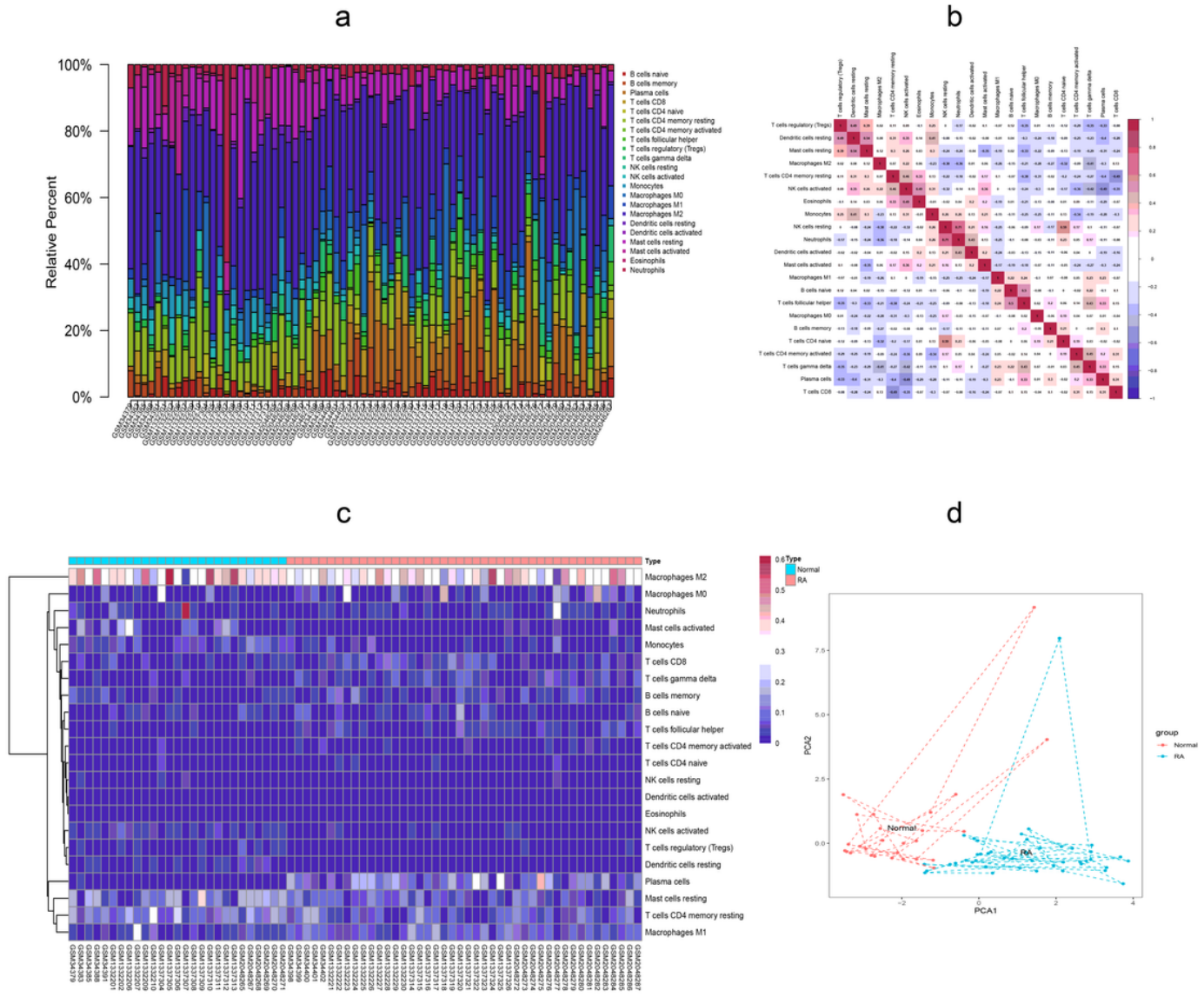

Figure 8

Results of CIBERSORT analysis. (a)The relative percentage of 22 subpopulations of immune cells in 71 samples from four datasets. (b)The correlation heat map graph is shown by the rate of correlation between individual immune cells. (c) Distribution of 22 immune cells in the gene matrix of 71 data samples. Immune infiltration expression of each immune cell in these samples: red indicates higher expression, and green shows lower expression. (d) Principal components analyses(PCA) were performed on all samples. The first two principal components, which explain most of the data variation,

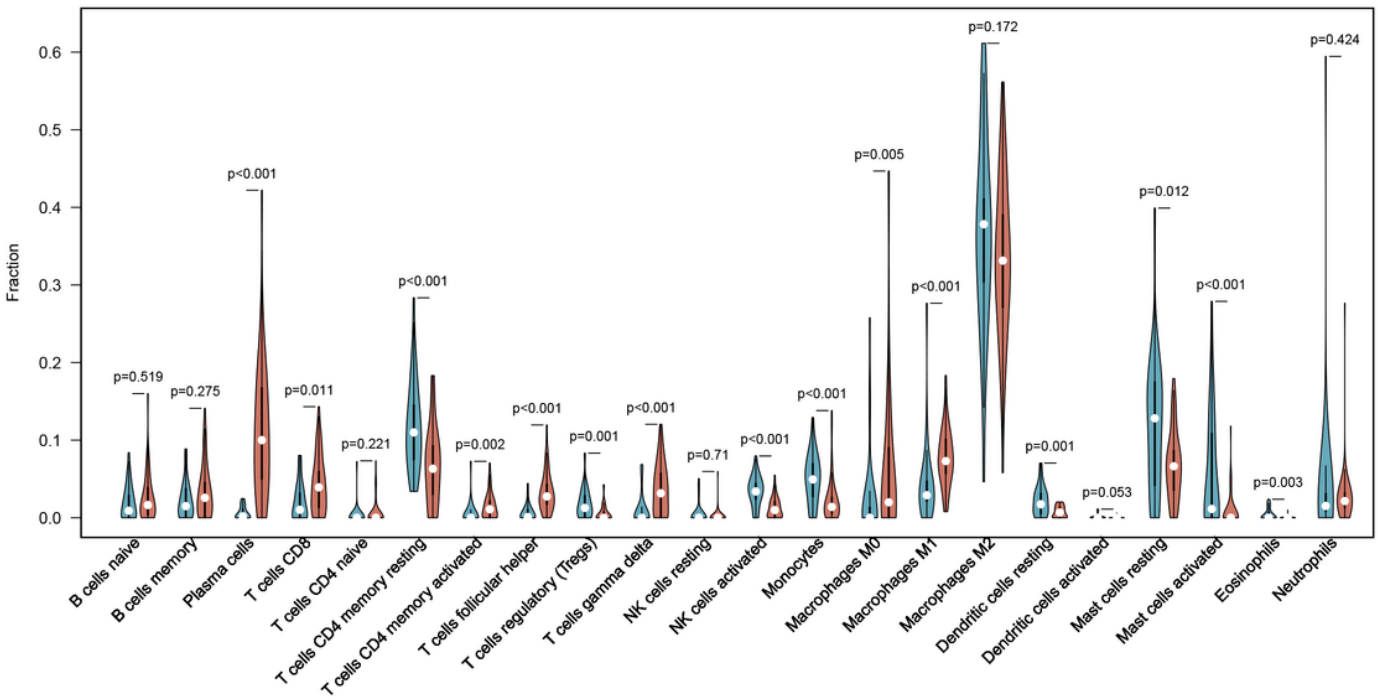

Figure 9

Immune infiltration between RA and normal groups.The violin plot groups of the two immune cell ratios. The blue spindle fraction on the left represents the normal group, and the red spindle fraction on the right represents the RA group.

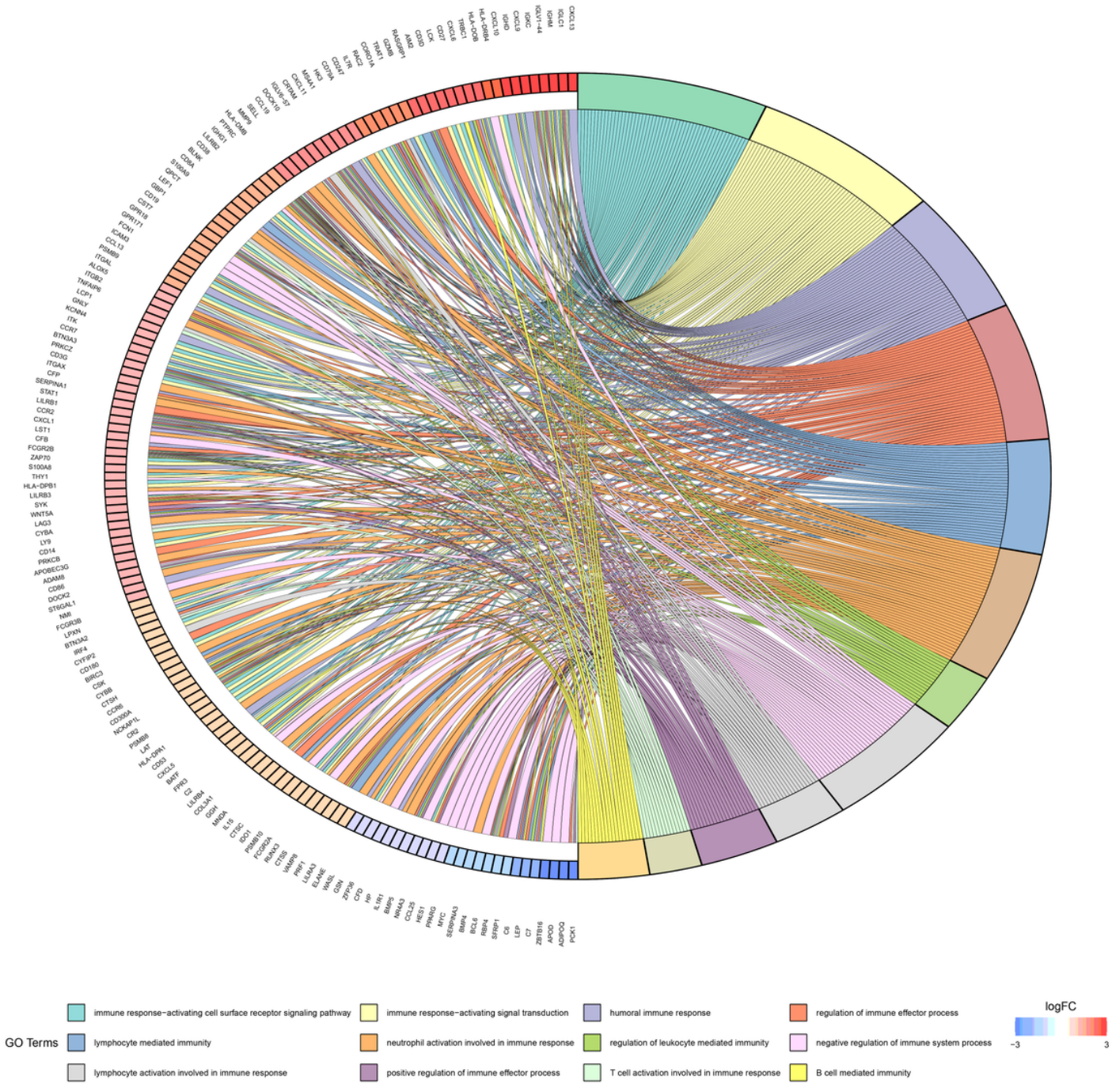

Figure 10

GOChord plot for Inflammatory immune-related HUB genes