Rheumatoid arthritis (RA) is the most common chronic inflammatory joint disease, characterized by the simultaneous inflammation of the synovium of multiple joints, joint damage and systemic immune response. Early diagnosis and treatment are the key to effectively mitigate disease activity and improve the condition of RA patients [1]. Rituximab is an anti-CD20 monoclonal antibody that suppresses inflammation effectively in autoimmune diseases. It was initially approved for the treatment of B-cell lymphomas and leukemia and later for RA. It exerts remarkable curative effects on patients exhibiting signs of the active phase of serious diseases and RA patients who are resistant to other antirheumatic drugs [2, 3], and can improve conditions of RA patients with pulmonary interstitial diseases [4]. Nevertheless, not all RA patients demonstrate a good disease response to RTX., for RTX therapy is ineffective for treatment of a considerable number of RA patients, and even produce serious adverse reactions such as renal amyloidosis [5], or result in serious infection events [6]. Therefore, the early identification of RA patients for whom RTX therapy is not effective allows treatment to be started before the development of irreversible joint damage and the occurrence of adverse reactions, and it is of substantial significance.

In this study, a comprehensive and systemic analysis on the molecular mechanism of related genes was performed using a bioinformatics analysis method and the gene expression data associated with therapeutic effects of RTX on RA patients to screen out core genes related to the therapeutic effects of RTX, facilitating the therapeutic application of RTX targeting RA patients.

1 Materials and methods

1.1 Acquisition and processing of microarray data

The gene expression profile GSE24742 associated with RTX treatment of RA patients was obtained from the gene expression database (http: //www.ncbi.nlm.nih.gov/geo/), including synovial membrane samples of 8 RA patients who responded well to RTX treatment and synovial membrane samples of 3 RA patients who responded poorly to RTX treatment. The chip platform file was GPL570.

The microarray data was analyzed using the online analysis tool GEO2R [7] to compare the differentially expressed genes (DEGs) between the group of RA patients with excellent RTX effects and the group of RA patients poor RTX effects. $P < 0.05$ and fold change $|\log2\ FC| \geq 2$ were used as the screening conditions for differential genes.

1.2 Enrichment analysis of GO function of differential genes and enrichment analysis of KEGG pathway

The screened DEGs were uploaded to DAVID database （https://david.ncifcrf.gov/） for gene ontology (GO) function enrichment analysis and kyoto encyclopedia of genes and genomes (KEGG) gene pathway enrichment analysis [8], and the enrichment analysis results are significant ($P<0.05$).

1.3 PPI network construction and network centrality analysis

The protein-protein interaction (PPI) network analysis on the screened DEGs was performed using the STRING database（https://string-db.org/）[9], the minimum efficient binding score was set to 0.7, the analysis result was imported into the Cytoscape（http://www.cytoscape.org/）, the degree of each node in the network was calculated using the cytoHubba plug-in[10], the calculated results were sorted from high to low, and the top 10 node degree genes was selected as the network core genes.

2 Results

2.1 Screening results of differentiated genes from microarray data

The chip data GSE24742 was analyzed using GEO2R to compare the DEGs between the group with excellent **therapeutic effects of RTX** and the group with poor therapeutic effects of RTX, and 727 DEGs were obtained, including 522 up-regulated genes and 205 down-regulated genes ($|\log2FC| \geq 2$, $P < 0.05$), as shown in Fig. 1.

2.2 Enrichment analysis of GO and KEGG pathways

The analysis on the functional enrichment and pathway enrichment of 727 DEGs was performed using DAVID database. GO enrichment analysis primarily includes three modules: biological process, cell composition and molecular function. As shown in Fig. 2A, the results of GO enrichment analysis indicated that DEGs were

primarily enriched in positive regulation of gene expression, positive regulation of transcription and intracellular signal transduction in the biological process module (Fig. 2B), DEGs were mainly enriched in extracellular region and endosome in the cellular component module (Fig. 2C), and DEGs were primarily enriched pertaining to NFAT protein binding, transport, and activities, etc. in the molecular function module (Fig. 2D).

KEGG pathway analysis results revealed that DEGs were primarily enriched in PI3K-Akt signal pathway and Rap1 signal pathway, etc. The results were visualized as shown in Fig 3.

2.3 PPI Network construction and network centrality analysis

The PPI network was constructed using 727 DEGs screened using STRING database, as shown in Fig. 4A. The DEGs interaction results were imported into Cytoscape, and the degree value of each node was calculated using cytoHubba plug-in. the top 10 node degree genes were defined as network core genes, namely, EGFR, HIST1H2BB, MMP9, CXCR3, MEIS1, CXCL5, IL17A, WASL, KMT2C, and PGF (Fig. 4B).

3 Discussion

In this study, the chip data GSE24742 associated with RTX treatment of RA patients was selected and processed using bioinformatics technology. 727 DEGs were screened out in the two groups with excellent and poor RTX effects, including 522 up-regulated genes and 205 down-regulated genes. GO function enrichment analysis and KEGG pathway enrichment analysis were performed on DEGs, and PPI interaction network was constructed to identify 10 core genes, i.e., EGFR, HIST1H2BB, MMP9, CXCR3, MEIS1, CXCL5, IL17A, WASL, KMT2C, and PGF.

The therapeutic effects of RTX on RA patients involves multiple genes and cellular pathways. The identification of the network core genes associated with therapeutic effects of RTX and the understanding of its molecular mechanism in the treatment of RA patients can help determine RA patients suitable for RTX treatment and facilitate the prediction of therapeutic effects of RTX at the genetic level.

GO enrichment analysis suggested that DEGs were primarily involved in the positive regulation of gene expression and transcription, and intracellular signal transduction. The enrichment analysis of KEGG pathway indicated that DEGs were mainly enriched in PI3K-Akt signal pathway and Rap1 signal pathway, etc. The aforementioned results demonstrated that the differential genes associated with RTX effects were primarily involved in proliferation and apoptosis of RA inflammation-related cells.

PPI network was constructed using the screened DEGs to identify genes at the core of the network. Among them, the expression difference of MMP9 was the most significant. MMP is a matrix metalloproteinase produced by macrophages and synovial fibroblasts, which can degrade extracellular matrix components and cause joint damage [11]. Previous studies suggested that MMP was highly expressed in the damaged cartilage of RA patients, the accumulated MMP level was associated with joint inflammation, reduction of MMP level improved joint swelling and local inflammation and avoided aggravating joint injury [12]. In this study, compared with that of the group with poor RTX effects, the expression level of MMP of the group with poor RTX effects was significantly higher.

As the epidermal factor growth receptor, EGFR, the core gene in PPI network, participates in DNA synthesis and cell proliferation, its expression level in serum of RA patients is higher than that of normal people, and it is closely associated with the development of RA diseases [13-15]. As a G protein-coupled receptor, the chemokine receptor CXCR3 is notably expressed in peripheral blood and synovium of RA patients. CXCR3 can mediate the regulation of immune cells and participate in the B cell migration to synovium and the local proliferation of B cells in synovium [16], and then trigger immune and inflammatory reactions through antigen presentation, autoantibody production, etc. and participate in the pathogenesis of RA [17]. IL-17 is a cytokine produced by CD4+T cells, and its expression increases in autoimmune diseases. As an inflammatory factor, IL-17 can cause bone and cartilage damage in RA patients [18], and plays a key role in the pathogenesis of RA. IL-17 can also activate synovial fibroblasts of RA patients to produce a large number of CXC

chemokines, and induce neutrophil invagination and particle formation leading to inflammatory response [19]. Particularly, CXCL5 is closely associated with the inflammatory response induced by IL-17 [20].

Citrullinated CXCL5 can recruit monocytes to cause inflammation and swelling of joint tissues and participate in the occurrence and development of RA [21]. As a biological preparation for the treatment of RA, RTX can inhibit T cells stimulated by B cells and further hinder the activation of pro-inflammatory cytokines, thus mitigating the diseases of RA patients [22]. Nevertheless, multiple RA patients have different levels of core gene expression and various degrees of inflammatory reactions, and the therapeutic effects of RTX therapy very greatly. Further studies on network core genes may provide a genetic basis for determining whether RA patients are suitable for RTX therapy.

In summary, an analysis on chip data associated with the therapeutic effects of RTX on RA patients using a bioinformatics method is performed to deepen our understanding of potential molecular events related to the therapeutic effects of RTX, and the core genes screened using network construction may be of great significance for determining the therapeutic effects of RTX in advance. Nevertheless, the specific functions of these core genes remain to be further explored.