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Comprehensive analysis of the expression pattern of peripheral blood mononuclear cells in rheumatoid arthritis before and after anesthesia

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Abstract

Rheumatoid arthritis (RA) is a chronic systemic disease was characterized by inflammatory synovitis. The common causes may be related to heredity, infection and sex hormones. However, the potential effect of rheumatoid arthritis anesthesia on the expression pattern of peripheral blood mononuclear cells has no idea. Therefore, in this study, we explored the expression pattern of peripheral blood mononuclear cells in rheumatoid arthritis patients before and after anesthesia with a variety of analytical strategies. First, download the relevant data in the GEO database, and analyze the differences between normal and disease samples to get the pertinent differences of microRNAs. Then, retrospective targeting of these differentially expressed microRNAs was carried out to obtain the related differentially expressed genes. Then, the protein interaction network of these differential genes is analyzed and the related functional modules are constructed. Finally, a hypergeometric test was carried out to calculate the potential regulatory effects of multiple factors on the module, and a series of ncRNA and TF was identified. 2571 differentially expressed genes were obtained and clustered into 6 functional expression disorder modules. These modules have a tendency to regulate biological processes such as regulation of protein ubiquitousness, regulation of neuron apoptosis process. At the same time, they are mainly involved in PI3K-Akt signaling pathway, FoxO signaling pathway and JAK-STAT signaling pathway. In addition, in view of pivot analysis, 76 ncRNAs and 4 TFs were found to drive dysfunctional expression modules in rheumatoid arthritis. Based on the results of this study, we can provide a new way for biologists and pharmacists to study rheumatoid arthritis, and provide valuable reference for follow-up treatment programs.

Keywords: rheumatoid arthritis, anesthesia, dysfunctional expression module, peripheral blood mononuclear cells, expression pattern

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease affecting millions of people worldwide. It is seen as a chronic multi-system disease (Chehade et al., 2019; Fatel et al., 2018). It is characterized by persistent synovitis, systemic inflammation and autoantibodies (Scott et al., 2010). Depression is the most frequent complication in patients with rheumatoid arthritis (RA). Recession has a negative effect on the health-related quality of life, physical function, mental function, mortality and the severity of pain and symptoms in patients with RA (Li et al., 2019). In addition, patients with rheumatoid arthritis may be at greater risk of falling

due to disease-induced changes, such as muscle weakness, joint injury, decreased mobility and postural instability (Lourenco et al., 2018). In addition, rheumatoid arthritis mainly affects the working population and may cause critical function and work limitations. With the development of diseases, individuals are increasingly unable to carry out daily activities, which have a significant impact on the individual and socio-economic (Gomes et al., 2018). However, there are many pathogenic mechanisms of rheumatoid osteoarthritis, and little knows about it. Therefore, in order to diagnose and treat rheumatoid osteoarthritis more effectively, most biologists have

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been implicated in the pathological study of rheumatoid osteoarthritis. Miyazaki Y et al. reported that Th22 cells isolated from peripheral blood had the ability to promote osteoclast differentiation by producing IL-22. Thus, playing a key role in bone destruction in RA patients (Miyazaki et al., 2018). Previous studies have shown that interleukin-38 (IL-38) may also be implicated in rheumatoid arthritis and may be a potential biomarker of RA (Xu et al., 2018). In addition, fibroblast-like synovial cells (FLS) produce inflammatory cytokines and participate in the migration and invasion of porous tissue, leading to joint damage in RA (Wu et al., 2018). FLS may also actively drive joint inflammation and degradation by producing inflammatory cytokines and matrix degradation molecules, making it a key factor in the pathogenesis of RA (Zhang et al., 2018). On the one hand, serum level is closely related to RA activity, suggesting that sCD25 may participate in the inflammatory process of RA, and may become a recent inflammatory marker of RA (Xu et al., 2018). In addition, the serum levels of lnc-Cox2 and HOTAIR can also be used as a different non-invasive biomarker for the diagnosis of RA (Shaker et al., 2019). On the other hand, ADAM-17 in RA serum is much higher than that in normal subjects. These data suggest that ADAM-17 is expressed in RA ST and has a role in RA inflammation by regulating the adhesion of monocytes to RA FLS. Therefore, ADAM-17 may be a key inflammatory mediator in inflammatory diseases such as RA (Ishii et al., 2018). Masuoka S and other scholars found that EBV infection was linked to RA in synovial tissue and chronic inflammatory attacks (Masuoka et al., 2018). Further studies have shown that the increased expression of IL7R and STAT1 in synovial tissues and primary immunodeficiency may be related to the occurrence of RA (Zhang et al., 2019).

It has been proved that the TNF signaling pathway is also involved in RA synovitis, and CCR5, CCR7, CXCR4, CCL5 and CCR4 chemokines may play a key role in RA FLS mediated cell migration, invasion and chemotactic release (Cai et al., 2019). Bone marrow sirtuin 6 (Sirt6) is a key determinant of phenotype transition and macrophage migration. PBMC and monocyte/macrophage in RA patients show lower expression of Sirt6 than those in osteoarthritis patients, and their Sirt6 activity is negatively correlated with the severity of disease (Woo et al., 2018). According to Guo C et al, it was found that activation of NLRP3 inflammatory bodies mainly occurred in infiltrating monocytes/macrophages in synovium, and it is likely to be involved in the pathogenesis of RA (Guo et al., 2018). Anderson JR et al. found that the metabolites of glycolysis and tricarboxylic acid cycle in RA were lower than those in OA. These results demonstrated that higher levels of inflammation were noted in RA (Anderson et al., 2018). In preceding studies, RAS guanine nucleotide releasing protein 2 (RASGRP 2) was expressed in FLS of RA synovium and participated in the pathogenesis of RA by promoting FLS adhesion, migration and IL-6 production (Nakamura et al., 2018). Some studies have shown that there is a link between human microbiome and the progress of rheumatoid arthritis. Ceccarelli F et al. confirmed the significant correlation between the percentage of Porphyromonas gingivitis to total tongue biofilm and RA disease activity (DAS28), indicating that oral microbial status may play a role in the pathogenesis of inflammation (Ceccarelli et al., 2018). After understanding the pathogenesis of rheumatoid arthritis, biologists and physicians from all over the world have taken an active part in the research of its treatment. For example, Bustamante MF et al. found that hexokinase (HK2) specifically expressed in the synovial lining of RA and regulated FLS invasion. In the treatment of RA, HK2 may be a safer selective metabolic target than global glycolysis (Bustamante et al., 2018). In addition, studies by Duan W et al have confirmed that sirml and F-siRML have unique efficacy and good safety in arthritis mice, which may be a promising method for the treatment of rheumatoid arthritis (Duan et al., 2018). Recent studies have shown that TNF-alpha plays an important role in synovial fibroblasts of osteoarthritis, suggesting that inhibition of TNF-alpha can reduce inflammation in mouse models through ERK/AKT signaling pathway mediated by TLR-3 (Yu et al., 2018). Upregulation of calcium binding protein Bcl-XL and MCL-1 promotes apoptosis of fibroblast-like synthesise and resists rheumatoid arthritis by activating PI3K/Akt and STAT3 pathways in rheumatoid arthritis (Jiao et al., 2018). Contemporary studies have shown that microRNA-143-3p can regulate cell proliferation and apoptosis by targeting the expression of IGF1R and IGFBP5 and regulating Ras/p38MAPK signal transduction pathway. Therefore, mir-143-3p may be a novel therapeutic target in RA (Yang et al., 2018).

Here, we study the pathogenesis of rheumatoid arthritis based on comprehensive strategies, including co-expression analysis and enrichment analysis of functional pathways. In addition, we also focused on predicting a series of core ncRNA and transcription factors to explore the expression pattern of peripheral blood mononuclear cells

before and after rheumatoid arthritis anesthesia.

Materials and methods

Differential expression analysis

We collected expression microarray data sets of rheumatoid arthritis disease samples from NCBI Gene Expression Omnibus (GEO) database (Barrett et al., 2013), numbered GSE58458. The different analysis of the collected disease samples was carried out. The basic data processing packages (including R.utils, R.oo, R.methodsS3 and hgu133plus 2cdf) of the R language expression profile chip were used to construct the disease and normal sample expression profiles, and the R language limma package was used to calculate (Ritchie et al., 2015). For chip data, we first use backgroundCorrect function for background correction and standardization. Then, based on the quantile normalization method of normalize Between Array function, the control probes and low-expression probes are filtered out to obtain high-quality standardized data. Using the lmFit and eBayes functions of limma package with default parameters. The differential genes related to rheumatoid arthritis were obtained.

Protein Interaction Network Identification Disorder Module

Observing the target interaction of genes in the module is useful to understand the core molecules of driving module function and dysfunction. Based on String database, we constructed a protein interaction network (PPIs) for each module (Athanasios et al., 2017). Unlike genes related to rheumatoid arthritis were introduced into the protein interaction network to identify the different roles played by different modules.

Functional and Pathway Enrichment Analysis and Identification of Dysfunctional Modules

Exploring the function and signal pathway of gene is often an effective means to study the molecular mechanism of disease, while the function and pathway of module gene participation can characterize the dysfunction mechanism of the module in the process of disease occurrence. Therefore, we used R language Cluster profile package (Yu et al., 2012) to enrich and analyze the functions of Go (p value Cutoff = 0.01, q value Cutoff = 0.01) and KEGG pathway (p value Cutoff = 0.05, q value Cutoff = 0.2) respectively. According to the function and pathway of module gene participation, it was identified as a probable dysfunctional expression module of rheumatoid arthritis.

Identification of transcription factors and regulation of ncRNA on modules

Data on the relationship between transcriptional and post-transcriptional target regulation were collected from TRRUST v2.0 and RAID v2.0 databases (Han et al., 2018; Yi et al., 2017). Pivot analysis was carried out under the background of TF Pivot data, and p value$<$0.01 was screened. A total of 76 ncRNA and 115 ncRNA-Module interaction pairs were obtained. These regulatory factors often mediate the occurrence of diseases. In order to explore the driving forces of functional modules of rheumatoid arthritis-related genes, pivot analysis is built on these interactive data. Pivot analysis refers to finding at least two interacting drivers with the module in target pairs and calculating the significance of the interaction between the driver and the module based on the hypergeometric test. Under the background of TF Pivot data, four transcription factors and four TF-Module interaction pairs were obtained by Pivot analysis (finding the point of regulatory module), p value$<$0.01. Finally, pivot is identified as the core pivot by statistical analysis. Based on the target data of ncRNA and TF as background set prediction, pivot regulators of regulatory dysfunction module are obtained.

Result

Analysis of differential gene expression in rheumatoid arthritis

In order to explore the fundamental pathogenic factors of rheumatoid arthritis, we selected the disease samples and normal samples of rheumatoid arthritis to construct gene expression profiles, which were studied in depth. 31 differentially expressed microRNAs were obtained and 2571 differentially expressed genes were retrospectively identified (Table S1). These differentially expressed genes may be potentially associated with rheumatoid arthritis. However, the analysis of differential gene expression in rheumatoid arthritis needs further study.

Construction of protein interaction network in rheumatoid arthritis

Through the differential gene expression of diseases, we obtained the related pathogenic genes of rheumatoid arthritis, but the regulatory mechanism and the interaction between genes remain unclear. To this end, we are still analyzing the protein interaction network of different genes in rheumatoid arthritis, thus mining six functional modules (Figure 1). In addition, we integrate rheumatoid arthritis-related genes and import

them into the network module. The genes of the same module are clustered together. These functional modules may participate in different functions and pathways, and may represent different regulatory mechanisms to mediate the occurrence and development of rheumatoid arthritis.

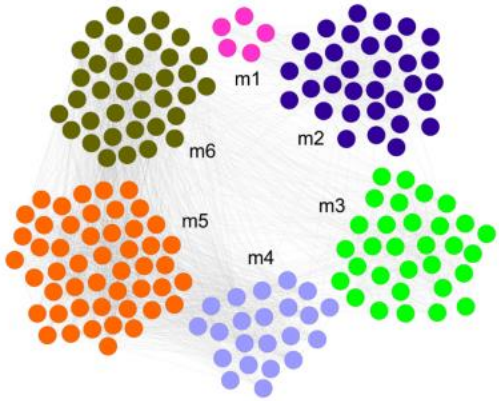


Figure 1 Protein Interaction Network Map to Characterize the Potential Pathogenesis of Rheumatoid Arthritis.

It involves a network of six functional modules clustered by 242 genes.

Identification of rheumatoid arthritis related dysfunction module

Studying the function and pathway of gene involvement is an essential means to identify its mediated pathogenesis. In order to study possible dysfunction caused by module gene disorder, we analyzed GO function and KEGG pathway enrichment in six modules. We collected a wealth of GO terms, and obtained 645 cell composition items, 725 molecular functional terms and 7400 biotic processes (Figure 2A). Based on functional analysis, we observed that functional modules tend to enrich multiple disease-related functions. For example, regulation of protein ubiquitination, dephosphorization and regulation of neuron apoptosis process is biological processes. On the other hand, 413 KEGG pathway enrichment results (Figure 2B) reflect that functional module genes are mainly involved in PI3K-Akt signaling pathway, FoxO signaling pathway and JAK-STAT signaling pathway, which affect the occurrence and progress of rheumatoid arthritis. In view of the close relationship between the function and pathway of module gene enrichment and the pathogenesis of rheumatoid arthritis, we identified these six modules as dysfunction modules. Modular genes can regulate a series of functions and pathways, and module disorders may be a significant factor in the pathogenesis. In retrospect of the overall effect of these modules, we constructed a functional network of all modules (Figure 3), which may represent the global dysfunction mechanism of rheumatoid arthritis. Gene imbalance within the module lead to dysfunction of the module, which affects the function and pathway of its participation, leading to the occurrence and progression of disease.

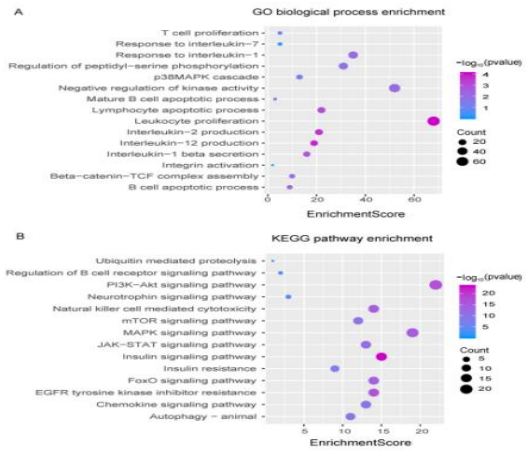


Figure 2. **Modular gene participates in function and pathway identification of rheumatoid arthritis dysfunction module.**

A. Modular genes are involved in GO function. The darker the color, the stronger the significance of enrichment. The larger the circle, the larger the proportion of module genes in GO functional entry genes. B. Modular genes are involved in KEGG pathway. The darker the color, the stronger the significance of enrichment. The larger the circle, the larger the proportion of module genes to KEGG pathway entry genes.

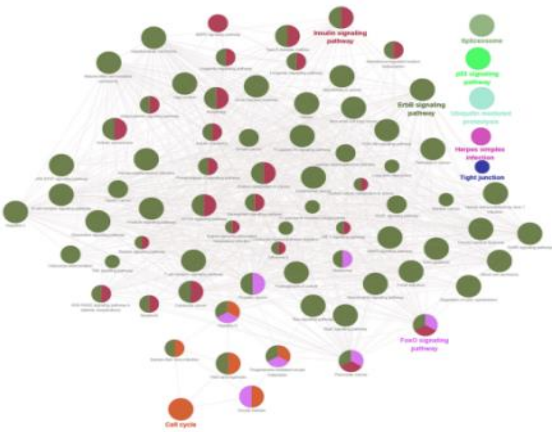


Figure 3. **functional network of rheumatoid arthritis related dysfunction module (excerpts).**

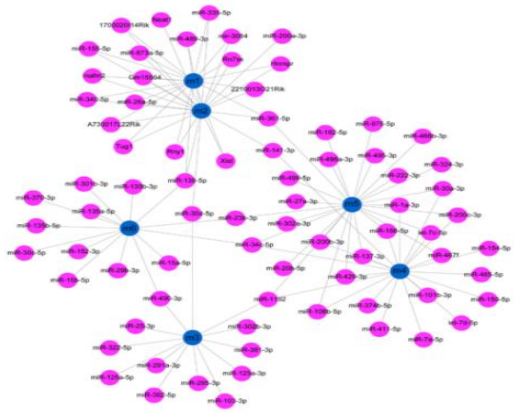
According to the relationship between modules, the corresponding function and pathway network is

constructed, and the proportion of modules participating in the corresponding function and pathway is identified.

NcRNA driving rheumatoid arthritis

Gene transcription and post-transcriptional regulation have been considered as the key factors regulating the occurrence and development of diseases, while ncRNA is considered as an essential regulator. Scientific prediction of ncRNA regulating dysfunction module genes is helpful for us to explore the transcriptional regulation mechanism of rheumatoid arthritis. Therefore, we use pivot analysis of ncRNA to explore ncRNA regulators that cause module dysfunction. The predicted consequences (Table S2, Figure 4) show that 76 ncRNAs have significant regulatory effects on modules, involving 115 ncRNA-Module regulatory pairs. These ncRNAs affect the condition of rheumatoid arthritis in varying degrees. In addition, the statistical analysis of the results showed that the three dysfunctional modules were significantly regulated by microRNA-1192, microRNA-139-5p, microRNA-34c-5p and microRNA-361-5p, which played an important part in module dysfunction. Other ncRNAs also show significant regulatory effects on dysfunction modules and play an important role in the regulation mechanism of rheumatoid arthritis.

Figure 4. **ncRNA regulatory network maps of rheumatoid arthritis.**



The blue circle represents the module and the pink circle represents the ncRNA corresponding to the module.

TF mediates the occurrence and development of rheumatoid arthritis

The occurrence and development of rheumatoid arthritis is strongly related to the dysfunction of transcription factors, which is also reflected in the regulation of dysfunction module by transcription factors. Therefore, we use pivot analysis to predict the module according to the regulation relationship of transcription factors to genes. The results showed that (Table S3, Figure 5), a total of four transcription factors play a transcriptional regulatory role in the dysfunction module of rheumatoid arthritis, involving four TF-Module regulatory roles. Statistical analysis of these transcription factors showed that Esr1, Esr2, Foxo3 and Trp53 all regulated one dysfunction module, which may be a possibility regulator for promoting rheumatoid arthritis. These data indicate that transcription factors may play an indispensable role in the disorder mechanism of rheumatoid arthritis.

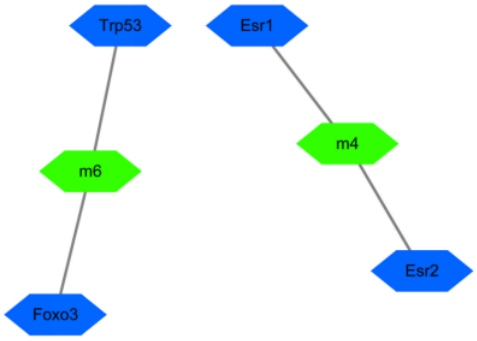


Figure 5. **Regulation network of transcription factors in rheumatoid arthritis.**

The green node represents the module, and the blue node represents the transcription factor corresponding to the module.

Discussion

Rheumatoid arthritis (RA) is just an autoimmune inflammatory disease characterized by peripheral and symmetrical polyarthritis (Gomes et al., 2018). RA is also a chronic inflammatory rheumatic disease, with changes in lipid characteristics and increased risk of cardiovascular events associated with inflammation (Audo et al., 2018). At present, the most common types of arthritis include osteoarthritis and rheumatoid arthritis (Williams, 2019). Clinically, musculoskeletal pain, swelling and stiffness is common symptoms, which are likely to increase the burden of medical care. Therefore, it is urgent to carry out in-depth research (Sparks, 2019; Ackerman et al., 2018). Fortunately, in recent years, the exploration of rheumatoid arthritis has concentrated on some genes or proteins, as well as related signaling pathways, and achieved some results. However, the global regulation of these genes, proteins and signaling pathways in rheumatoid arthritis remain unclear. In order to

fully explore the mechanism of potential pathogenic genes in rheumatoid arthritis, we first integrated these potential pathogenic genes and their interacting genes, and constructed a protein interaction network. Thus, we have excavated six functional modules. In view of the results of functional enrichment, these modules are substantially involved in PI3K-Akt signaling pathway, FoxO signaling pathway and JAK-STAT signaling pathway. Among them, PI3K-Akt signaling pathway was found to be significantly involved in the signaling pathway of three modules, which also show that PI3K-Akt signaling pathway plays an important role in disease signaling pathway of rheumatoid arthritis. In RA synovial tissue, it was found that nystatin may induce the expression of TNF-alpha by the PI3K-Akt signaling pathway in rheumatoid arthritis synovial fibroblasts (Su et al., 2019). In addition, FoxO signaling pathway was also found to be substantially involved in three modules of rheumatoid arthritis. Kok SH et al. have shown that simvastatin inhibits the expression of cysteine-rich protein 61 in rheumatoid arthritis synovial fibroblasts by regulating sirtuin-1/FoxO3a signaling pathway, suggesting that FoxO signaling pathway may be involved in the inflammatory response of rheumatoid arthritis (Kok et al., 2013). JAK-STAT signaling pathway was also found to be involved in two signaling pathways, so it is inferred that the JAK-STAT signaling pathway plays a potential regulatory role in the disease signaling pathway of rheumatoid arthritis. According to the research of Malemud CJ et al., the continuous activation of JAK/STAT signal transduction in RA synovial joint leads to the increase of matrix metalloproteinase gene expression level and the frequency of apoptosis chondrocytes, and the most prominent "apoptosis resistance" in inflammatory synovial tissue (Malemud, 2018).

On the other hand, we identified the transcription factors involved in these dysfunction modules for rheumatoid arthritis-related genes, and obtained four transcription factors involving four Pivot-Module interaction pairs. Statistical analysis of these transcription factors showed that Esr1, Esr2, Foxo3 and Trp53 all regulated a dysfunction module, which may be the key transcription factors involved in the probable pathogenesis of rheumatoid arthritis. Esr1 was found to be involved in RA through cancer-related and immune-related pathways, and may play a critical role in the development of disease (Yin et al., 2013). Rheumatoid arthritis is mainly related to cell apoptosis deficiency, and FoxO3a is a transcription factor implicated in cell cycle regulation and cell apoptosis. FoxO3a was mainly detected in inflammatory aggregates in synovium of patients with RA, and may also regulate the protracted survival of T lymphocytes (Turrel-Davin et al., 2010). In addition, Trp53 gene mutation and subsequent dysfunction may also lead to chronic inflammation and proliferation in RA patients (Abou-Shousha et al., 2005). Meanwhile, Trp53 over expression was found in apoptosis rheumatoid arthritis fibroblast-like synovial cells (Firestein et al., 1996). As noted above, these transcription factors can be considered as key transcription factors regulating the potential pathogenesis of rheumatoid arthritis. In addition, ncRNA has been thought to be an important regulator of disease occurrence and development. We conduct pivot analysis based on the targeting relationship between ncRNA and genes. The predicted results show that 76 ncRNAs have significant regulatory effects on modules, including 115 ncRNA-module interaction pairs. The results of statistical analysis showed that the three dysfunction modules were significantly regulated by microRNA-1192, microRNA-139-5p, microRNA-34c-5p and microRNA-361-5p. Therefore, rheumatoid arthritis is thought to be the core of the key regulators. Although there is no specific literature for reference, these factors play an important potential regulatory role and participate in the disease process in rheumatoid arthritis. This study not only explores the strategic factors affecting the process of rheumatoid arthritis, but also provides new insights into future research directions and research targets.

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