Current Science
CS 5 (3), 2206-2226 (2025)
Received 10 Feb 2025 | **Revised 11 March** 2025 | **Accepted 12 April**. 2025 | **Online Available 10 May** 2025
https://doi.org/ 10.52845/CS/2025-5-3-14

Current Science

Current Science

OPEN ACCESS

ISSN (O) 2795-8639

Orignal Article

Bioinformatics and Systems Biology Approaches Identify the Potential Common Pathogenesis of Periodontitis and Rheumatoid Arthritis

Qingqing Yin[1,2], Zhengxian Shen [1,2], Guoying Wang [1,2], Xinyu Wei [1,2], Yifan Wang[1,2], Dongqing Ye [1,2], Bin Wang [1,2]

[1]Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Hefei, Anhui, China

[2]Anhui Province Key Laboratory of Major Autoimmune Diseases, Hefei, Anhui, China

[*]Corresponding Author: Bin Wang, Dongqing Ye

Abstract:

Rheumatoid arthritis (RA) and periodontitis (PD) are chronic inflammatory disorders with significant comorbidity, yet their shared pathogenic mechanisms remain poorly understood. **Methods:** This study employed bioinformatics approaches to identify common molecular pathways and diagnostic biomarkers linking RA and PD. Datasets for RA and PD were obtained from the GEO database. Common differentially expressed genes (DEGs) were identified, followed by gene and pathway enrichment analysis, protein-protein interaction (PPI) network construction, hub gene identification, and immune infiltration analysis. Interaction networks involving transcription factor (TF)-gene, gene-miRNA, gene-disease, and protein-drug were established based on the identified hub genes.Diagnostic accuracy of hub genes was evaluated using receiver operating characteristic (ROC) curve analysis, and expression profiles were validated using external datasets.

Results:A total of 251 common DEGs were identified, with 15 hub genes (PTPRC, ITGB2, CXCL8, CCL5, CXCR4, MMP9, FCGR3B, CD2, SELL, CXCL1, LYN, FCER1G, CXCL2, IL7R, and CD69) showing high diagnostic performance (AUC > 0.7) across independent datasets. Immune infiltration analysis revealed significant correlations between hub genes and immune cells, particularly B cells and CD4+ T cells. TFs, miRNAs, and potential therapeutic drugs associated with hub genes were also predicted.

Conclusion: This study identified shared molecular mechanisms between RA and PD, highlighting potential diagnostic biomarkers and therapeutic targets. The findings provide a foundation for future research into comorbid disease management.

Keywords: periodontitis, rheumatoid arthritis, differentially expressed genes, bioinformatics, hub genes

Introduction

RA and PD are chronic inflammatory diseases that significantly impact global health, leading to progressive tissue destruction and substantial socioeconomic burdens[1]. RA is a systemic autoimmune disorder primarily characterized by synovial inflammation and joint destruction[2], driven by dysregulated immune responses, including aberrant activation of immune cells, excessive production of pro-inflammatory cytokines, and osteoclast-mediated bone resorption. In contrast, PD is a localized chronic inflammatory condition initiated by microbial plaque accumulation[2-4], resulting in the destruction of periodontal supporting tissues, including alveolar bone and periodontal ligaments, due to an imbalanced host immune response.

Emerging evidence suggests a bidirectional relationship between RA and PD, with epidemiological studies indicating a higher prevalence of PD among RA patients and vice

versa. Notably, the coexistence of these conditions may exacerbate disease severity, as evidenced by the correlation between increased periodontal inflammation and elevated RA disease activity scores (de Smit et al., 2012)[5]. This interplay suggests shared pathological mechanisms, potentially involving common inflammatory pathways, immune cell dysregulation, and molecular cross-talk[6-10]. For instance, both diseases are characterized by the infiltration of immune cells such as neutrophils, monocytes, and T lymphocytes, as well as the overexpression of pro-inflammatory mediators, including IL-17, TNF-α, and matrix-degrading enzymes like matrix metalloproteinases (MMPs). Despite these observations, the molecular and genetic underpinnings of the comorbid relationship between RA and PD remain poorly understood, particularly at the intersection of immune dysregulation and tissue destruction.

Investigating the comorbid mechanisms of RA and PD is of paramount importance for several reasons. First, elucidating shared pathological pathways can provide insights into the systemic nature of chronic inflammatory diseases, offering a framework for understanding other comorbid conditions . Second, both RA and PD impose significant burdens on patients' quality of life and healthcare systems, underscoring the need for improved diagnostic and therapeutic strategies . By identifying common molecular targets, it may be possible to develop novel therapeutic interventions with dual efficacy, thereby reducing treatment costs and improving patient outcomes . Finally, understanding the interplay between these diseases could inform early screening protocols and integrated management approaches for patients with comorbid RA and PD .

This study employs an integrative bioinformatics approach to uncover shared molecular mechanisms between RA and PD, identifying key hub genes and signaling pathways. By constructing interaction networks and validating diagnostic potential, our findings provide a foundation for future research and highlight potential therapeutic targets for comorbid RA and PD management.

Materials and Methods

Data Search and Extraction

Gene expression datasets for RA and PD were obtained from the GEO database (http://www.ncbi.nlm.nih.gov/geo/). A systematic search was conducted to identify microarray studies using the keywords "rheumatoid arthritis," "periodontitis," and "Homo sapiens." The GSE77298 dataset was selected to represent the transcriptional profile of synovial tissue in RA patients compared to healthy controls, while the GSE10334 dataset was used to illustrate the transcriptional profile of gingival tissue in PD patients relative to healthy individuals. To validate the mRNA expression of the identified hub genes, the GSE55235 and GSE16134 datasets were utilized. Detailed information regarding each GEO dataset, including platform annotations (GPL96, HG-U133A Affymetrix Human Genome U133A Array; GPL570, HG-U133_Plus_2 Affymetrix Human Genome U133 Plus 2.0 Array), is provided in Table 1.

Table 1 Datasets for HT and PD.

	PD		RA	
Datasets	GSE10334	GSE16134	GSE77298	GSE55235
Platform	GPL570	GPL570	GPL570	GPL96
Experimental	Array	Array	Array	Array
Case sample	183	241	16	10
Control sample	64	69	7	10
Total sample	247	310	23	20

Identification of DEGs and Common DEGs in RA and PD

The preliminary analysis of the datasets was conducted using R software (version 4.1. 1; https://cran.r-project.org). Prior to data analysis, data preprocessing was performed, which included normalization using the "normalizeBetweenArrays" function and log2 transformation. DEGs were identified using the "Limma" package . For the GSE77298 dataset, a cutoff threshold of *P*-value < 0.05 and |logFC| >

1.0 was applied to identify significant DEGs. For the GSE10334 dataset, a cutoff threshold of *P*-value < 0.05 and |logFC| > 0.6 was used. Common DEGs shared between RA and PD were identified by intersecting the DEGs from both datasets using an online Venn diagram tool (version 2.1 .0; https://bioinfogp.cnb.csic.es/tools/venny).

Volcano plots and heatmaps were generated to visualize the DEGs using GraphPad Prism 8.0 and the online platform Microbioinformatics (https://www.bioinformatics.com.cn/), respectively. These visualizations provide a comprehensive overview of the differentially expressed genes and their expression patterns across the datasets.

Functional Enrichment Analysis of Intersected DEGs

To elucidate the biological functions and pathways associated with the intersected DEGs, Gene Ontology (GO)[11] and Kyoto Encyclopedia of Genes and Genomes (KEGG) [12, 13]pathway enrichment analyses were performed using the DAVID online tool (https://david.ncifcrf.gov/). The GO enrichment analysis provided insights into the biological processes, molecular functions, and cellular components associated with the DEGs, while the KEGG pathway analysis identified key signaling pathways involved in the pathogenesis of the diseases .

The results of the GO and KEGG enrichment analyses were visualized using bubble plots generated via the Microbioinformatics online platform (https://www.bioinformatics.com.cn/). The enrichment results were sorted based on P-values and gene counts, with a P-value < 0.05 considered statistically significant.

Protein–Protein Interaction Network Analysis and Hub Gene Extraction

To construct and analyze the PPI [14]network, the STRING database (version 11.5; www.string-db.org) was utilized, with interactions restricted to a median confidence score of 0.7 to ensure high reliability . The resulting network was visualized and further analyzed using Cytoscape software [15](version 3.9.1) . To identify densely connected regions within the PPI network, the Molecular Complex Detection (MCODE) [16]plugin was applied, with default parameters set as follows: Degree Cutoff = 2, Node Score Cutoff = 0.2, K-Core = 2, and Max Depth = 100. This approach enabled the extraction of significant sub-networks from the shared DEGs.

Hub genes, defined as highly influential nodes within the PPI network, were identified using the CytoHubba[17] plugin, a robust tool embedded within Cytoscape for network analysis . CytoHubba employs multiple topological algorithms, including Maximum Clique Centrality (MCC), Degree, Maximum Neighborhood Component (MNC), and Edge Percolated Component (EPC), to rank and identify key genes. The intersection of hub genes identified by these algorithms was visualized using an online Venn diagram tool (version 2.1.0; https://bioinfogp.cnb.csic.es/tools/venny) .

To further explore the functional relevance of the identified hub genes, a gene co-expression network was constructed using the GeneMANIA[18] online tool (http://genemania.org). This analysis provided insights into genes co-expressed or functionally interacting with the hub genes in the context of RA and PD. Additionally, GeneMANIA facilitated the prediction of gene set functions and their potential roles in the pathogenesis of these diseases, offering a comprehensive view of the molecular interactions underlying RA and PD comorbidity.

Candidate Biomarker Expression Levels and Diagnostic Value

To evaluate the diagnostic potential of the candidate hub genes, ROC curves were generated, and the area under the curve (AUC) was calculated using the pROC package in R. This analysis was performed separately for the PD (GSE10334) and RA(GSE77298) datasets to assess the diagnostic accuracy of the identified genes. Additionally, the expression levels of these pivotal genes were validated using two independent external datasets (GSE10334 and GSE55235). Boxplots were constructed using the "ggplot2" package in R to visualize and compare the expression profiles of the candidate biomarkers in periodontitis and RA samples, further confirming their diagnostic efficiency.

Immune Cell Infiltration Analysis

To further validate the bioinformatics findings and explore the potential immunological relationship between RA and PD, immune cell infiltration analysis was conducted using single-sample gene set enrichment analysis (ssGSEA) implemented in

the GSVA package. The infiltration levels of 28 immune cell types were calculated based on gene sets associated with immune cell signatures. To visualize the infiltration patterns of immune cells across different samples, a clustering heatmap was generated using the heatmap software package in R, illustrating the relative abundance of immune cells in disease and control groups.

Additionally, group boxplots were constructed using the ggplot2 package in R to compare the expression levels of different immune cell types between disease and control samples. Statistical significance between groups was assessed using the Student's t-test or Wilcoxon rank-sum test, with a threshold of $P<0.05$ considered statistically significant.

To investigate the relationship between hub genes and immune cell infiltration, the expression matrix of hub genes was extracted and correlated with the immune cell abundance matrix. Correlation coefficients and P-values were calculated, with $P<0.05$ defined as statistically significant. A correlation heatmap was generated using the heatmap software package in R to visualize the associations between hub genes and immune cells, providing insights into the potential regulatory roles of these genes in immune cell infiltration.

TF and miRNA Interaction Analysis

TFs are proteins that specifically recognize and bind to target gene sequences, regulating the rate of gene transcription (Rustad et al., 2014). To construct the gene–TF interaction network, we utilized NetworkAnalyst (https://www.networkanalyst.ca) (Zhou et al., 2019). The topologically significant TFs, predicted to bind to hub genes, were identified using the JASPAR database, a publicly accessible repository of TF binding profiles across six taxonomic groups (Fornes et al., 2020).

Additionally, miRNA–gene interactions were analyzed to identify miRNAs potentially regulating hub genes at the post-transcriptional level. The miRTarBase database (v8.0), a well-established resource for experimentally validated miRNA–gene interactions (Huang et al., 2020), was employed to retrieve miRNAs targeting the hub genes. These interactions were integrated and visualized using Cytoscape, enabling the construction of comprehensive gene–TF and gene–miRNA interaction networks.

Gene-disease Association Analysis

DisGeNET[19] is a comprehensive platform that integrates information on genes and variants associated with human diseases and can be used to investigate the molecular basis of specific human diseases and their comorbidities. We also examined the gene-disease relationship using DisGeNET database via NetworkAnalyst to disclose associated diseases and their complications with hub genes.

Evaluation of Applicant Drugs

In this research, prediction protein–drug interaction (PDI) or drug molecules identification is one of the significant parts. Drug Signatures database (DSigDB) containing 22,527 gene sets was used to generate the small molecules which could downregulate the expression of hub genes[20]. The access to the DSigDB database is acquired through Enrichr (https://amp.pharm.mssm.edu/Enrichr/) platform[21]. Drug molecules were identified using the DSigDB via Enrichr based on the selected hub genes.

Result

Identification of DEGs and Shared DEGs between RA and PD

The flowchart summarizing the key steps of this study is presented in Figure 1.

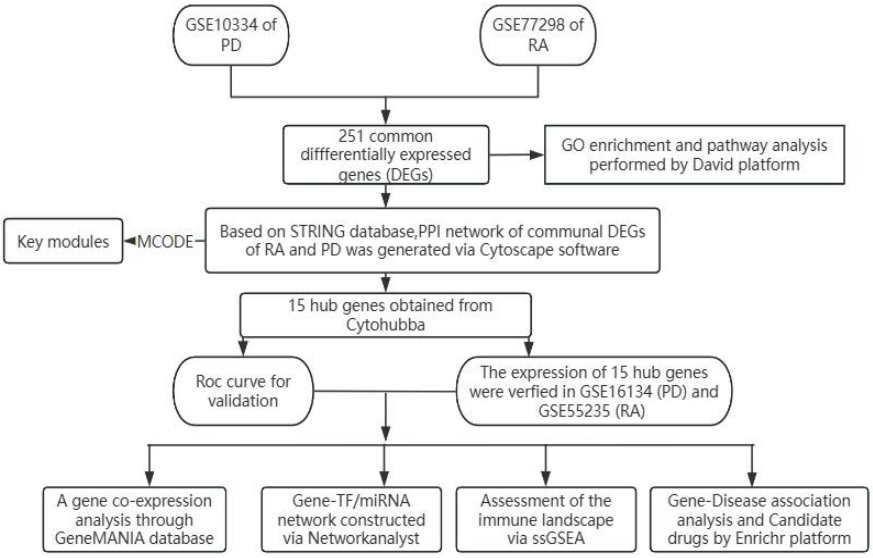

Figure 1 The research design flowchart

To investigate the shared pathogenic mechanisms between RA and PD, we identified DEGs from transcriptomic datasets and determined the common DEGs associated with both diseases. In the GSE77298 dataset, a total of 2,311 DEGs were identified, comprising 926 up-regulated and 1,385 down-regulated genes (Supplementary Table S1). In the GSE10334 dataset, 917 DEGs were detected, including 503 up-regulated and 414 down-regulated genes (Supplementary Table S2). Intersection analysis revealed 251 common DEGs shared between RA and PD (Figure 2A; Supplementary Table S3), of which 172 genes were up-regulated and 56 genes were down-regulated in both diseases (Figure 2B, C).

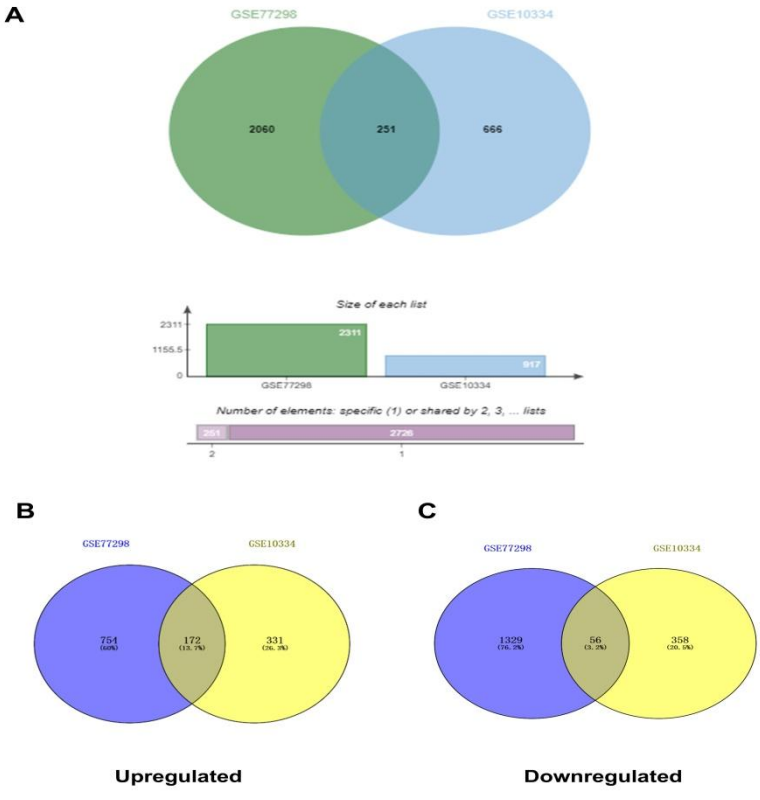

Figure 2 The study incorporates RA (GSE77298) and PD (GSE10334). (A)The Venn diagram

revealed 251 common DEGs for RA and PD.(B) Venn diagram of the 172 upregulated communal DEGs between RA and PD. (C) Venn diagram of the 56 downregulated communal DEGs between RA and PD.

The top 100 DEGs for each disease are displayed in heatmaps (Figure 3B, D), while the expression patterns of DEGs are illustrated in volcano plots (Figure 3A, C).

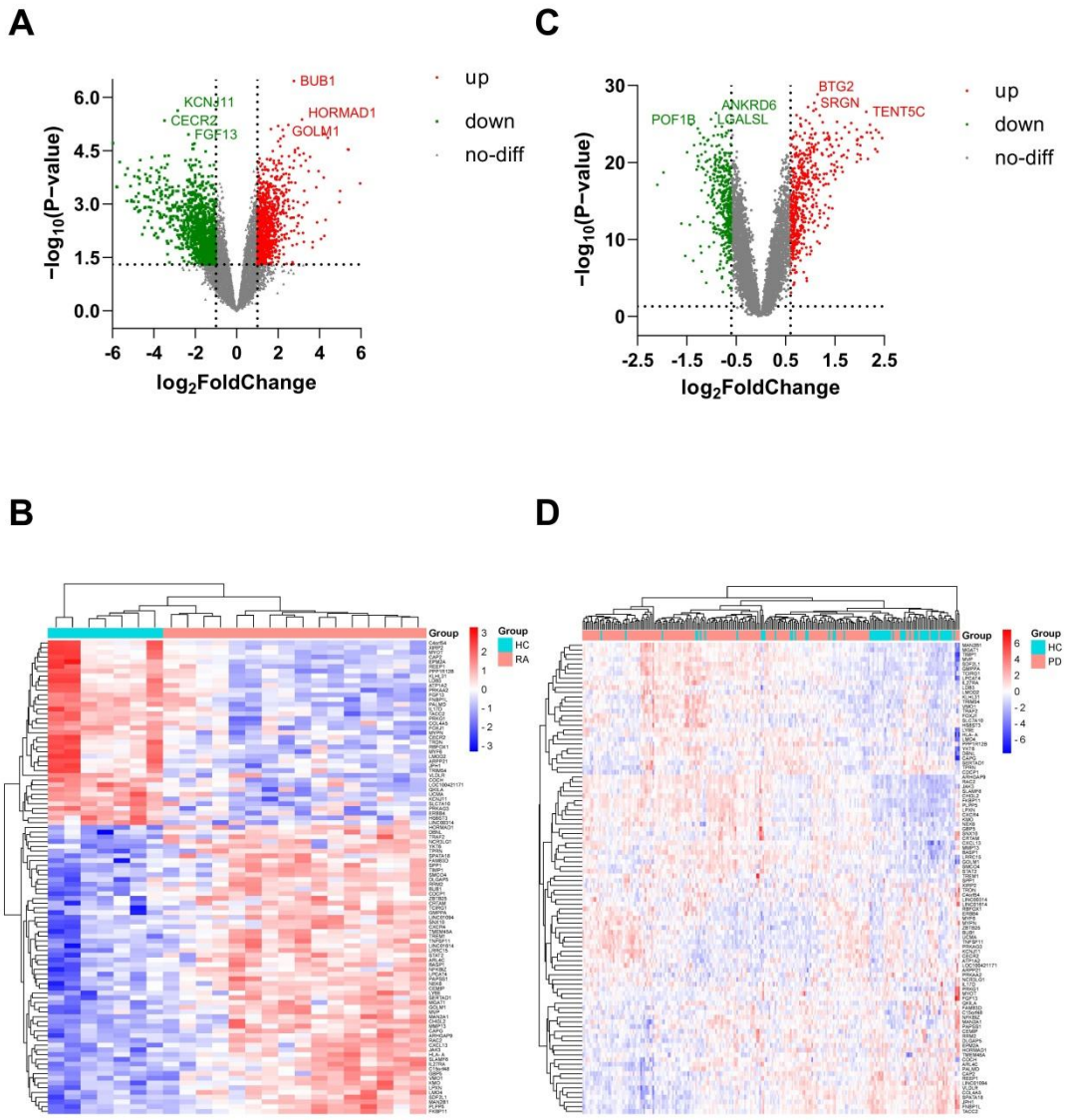

Figure 3 Identification of differentially expressed genes.(A) A volcano plot of DEGs in GSE77298. (B) A heatmap of the top 100 DEGs in GSE77298. (C) A volcano plot of DEGs in GSE10334. (D) A heatmap of the top 100 DEGs in GSE10334. HC: healthy control;

These findings indicate a high degree of consistency in the directional changes of gene expression, suggesting potential overlapping molecular mechanisms underlying the pathogenesis of periodontitis and rheumatoid arthritis.

Gene Ontology and Pathway Enrichment Analysis

GO analysis and KEGG analysis provide insights into the biological traits and enrichment pathways of the shared DEGs.GO analysis was performed to categorize the DEGs into three domains: biological process (BP), cellular component (CC), and molecular function (MF). The top 10 enriched terms for each category are presented in Table 2 and visualized in Figure 4A-C. In the BP category, DEGs were significantly enriched in immune response, B cell receptor signaling

pathway, and neutrophil chemotaxis. In the CC category, DEGs were primarily associated with the extracellular region and plasma membrane. In the MF category, DEGs were enriched in chemokine activity and CXCR chemokine receptor binding, all of which are closely related to immunotherapy-related functional pathways.

KEGG pathway analysis identified the top 10 enriched pathways, including the chemokine signaling pathway, viral protein interaction with cytokine and cytokine receptor, lipid and atherosclerosis, rheumatoid arthritis, NF-kappa B signaling pathway, IL-17 signaling pathway, leishmaniasis, cytokine-cytokine receptor interaction, Fc epsilon RI signaling pathway, and epithelial cell signaling in Helicobacter pylori infection. The detailed KEGG enrichment results are listed in Table 3 and further illustrated using bubble plots (Figure 4D).

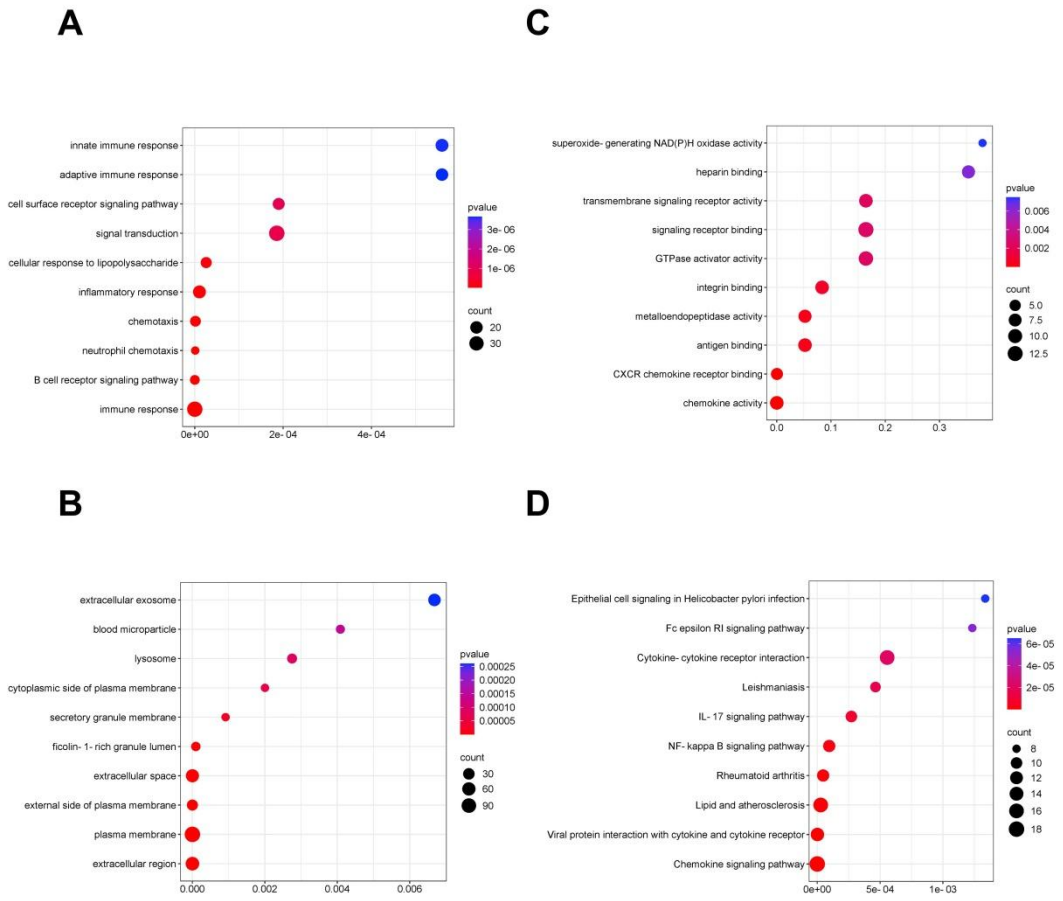

Figure 4 GO and KEGG enrichment analysis of the common genes. (A) Biological processes in bubble chart. (B) Cellular component in bubble chart. (C) Molecular function in bubble chart. (D) The top 10 signaling pathways of KEGG in bubble chart. The color of the dots reflects the size of the p-values, and the size of the dots reflects the number of annotated genes in the bubble charts.

PPI Network and Hub Gene Identification

To investigate potential interactions among the shared target genes, a PPI network was constructed using the STRING database (https://string-db.org/), with an interaction score threshold set at > 0.7. The resulting network comprised 95 nodes and 588 edges (Figure 5).

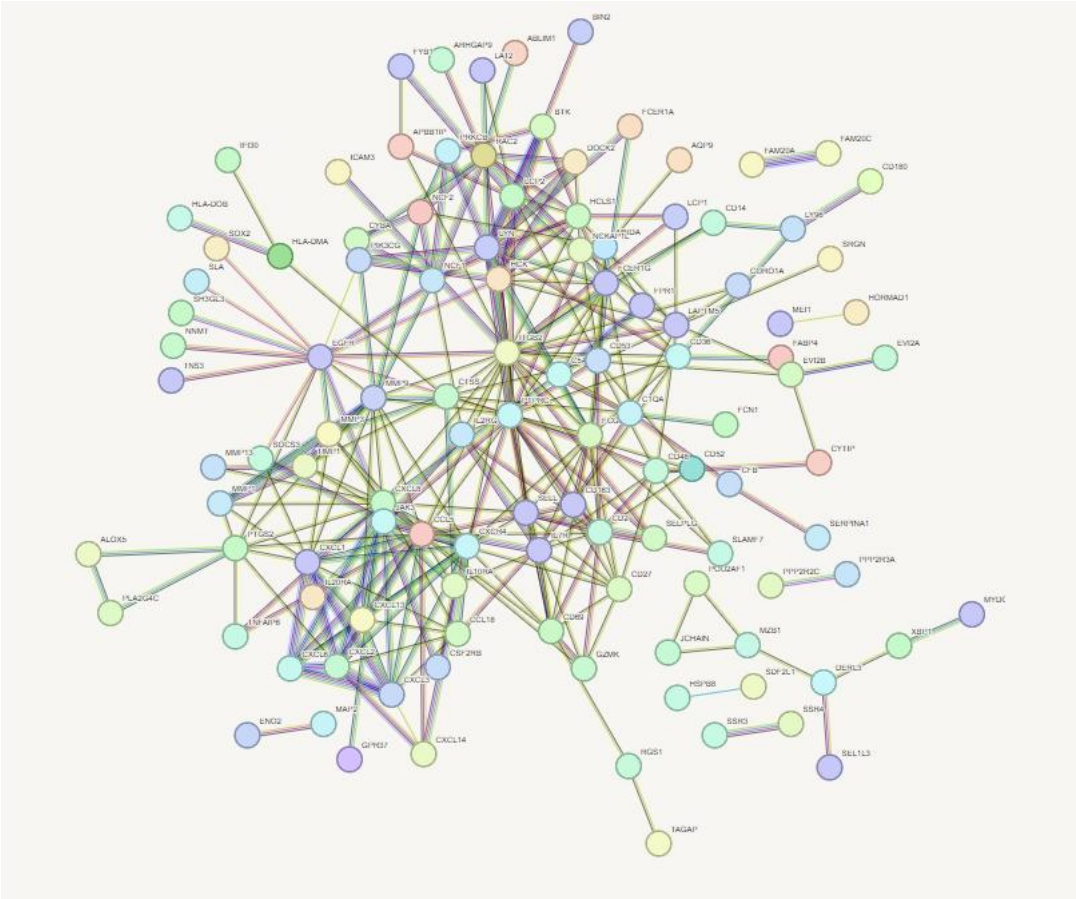

Figure 5 PPI network of common DEGs between RA and PD. PPI protein–protein interwork, DEGs differentially expressed genes, RA Rheumatoid osteoarthritis,PD periodontitis.

To identify hub genes potentially implicated in the comorbidity of RA and PD, we utilized the CytoHubba plugin within the Cytoscape platform. Given the inherent heterogeneity of biological networks, we employed four distinct topological analysis algorithms—MCC, MNC, Degree, and EPC—to comprehensively assess network centrality. The intersection of the top 20 hub genes identified by these algorithms yielded 15 consensus hub genes: PTPRC, ITGB2, CXCL8, CCL5, CXCR4, MMP9, FCGR3B, CD2, SELL, CXCL1, LYN, FCER1G, CXCL2, IL7R, and CD69 (Figure 6A). These genes represent robust candidates for further investigation into their potential roles in the shared pathophysiology of RA and PD.

Module analysis was performed using the MCODE plugin in Cytoscape to identify densely connected subnetworks. Four significant modules were detected: Module 1, containing 16 nodes and 128 edges with a cluster score of 8.533; Module 2, containing 6 nodes and 20 edges with a score of 4; Module 3, containing 8 nodes and 28 edges with a score of 4; and Module 4, containing 3 nodes and 6 edges with a score of 4 (Figure 6B).

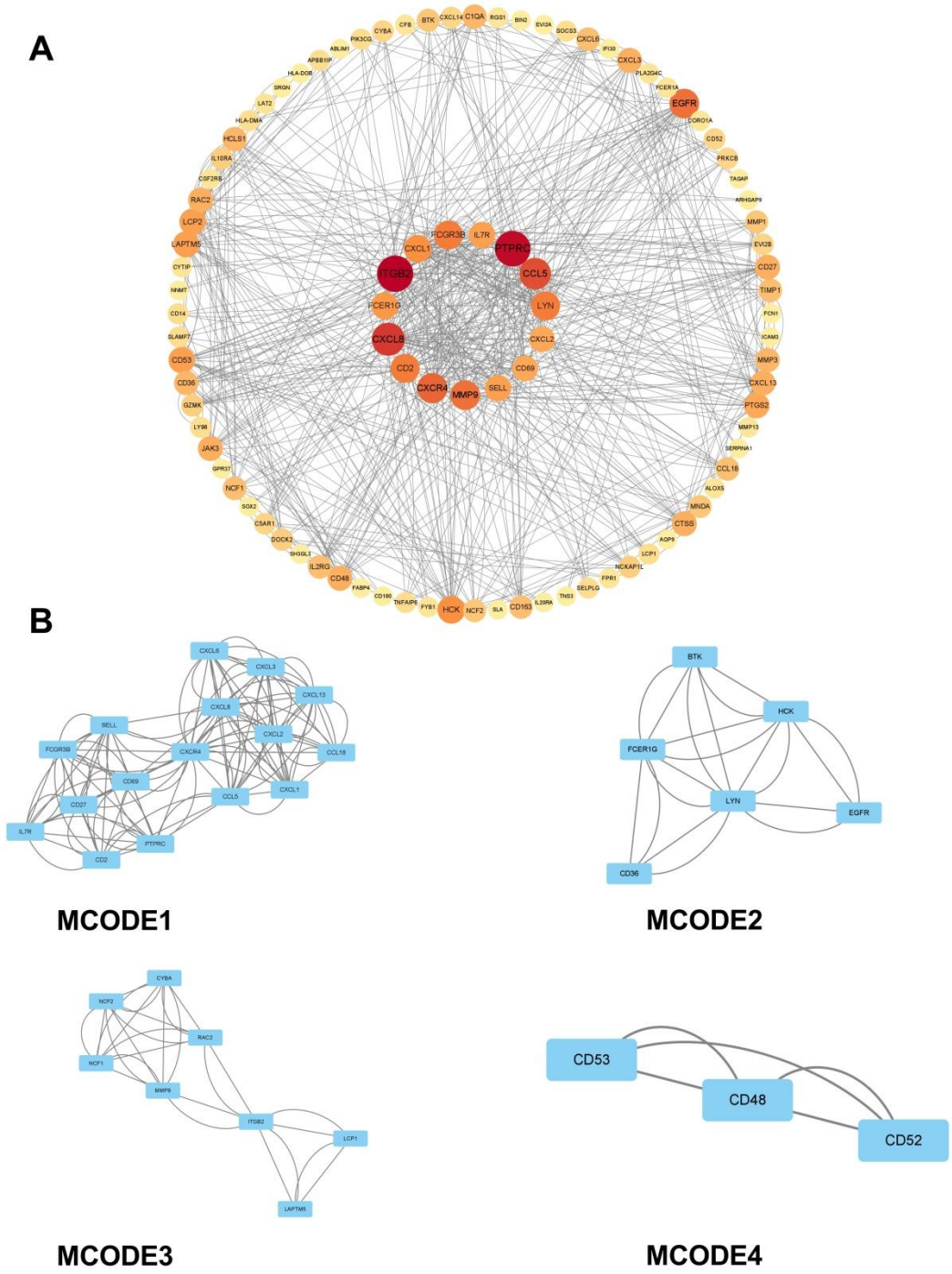

Figure 6 (A) The fifteen hub genes from MCC, MNC, Degree and EPC 4 algorithms.Significant modules and roles of PPI network (B) The top four key gene modules identified by MCODE plug-in of Cytoscape.

A GeneMANIA biological function analysis was adopted to investigate the genes with common properties and similar functions to the above 15 hub genes, as well as to demonstrate the interactive functional association network between genes. A total of 20 molecules were most associated with the15 hub genes. The results revealed that 61.72% co-expression between genes, physical interactions of 16.27%, co-localization of 8.56%, prediction of 7.61%, shared protein domains of 2.59% and pathway of 3.25%(Figure 7).

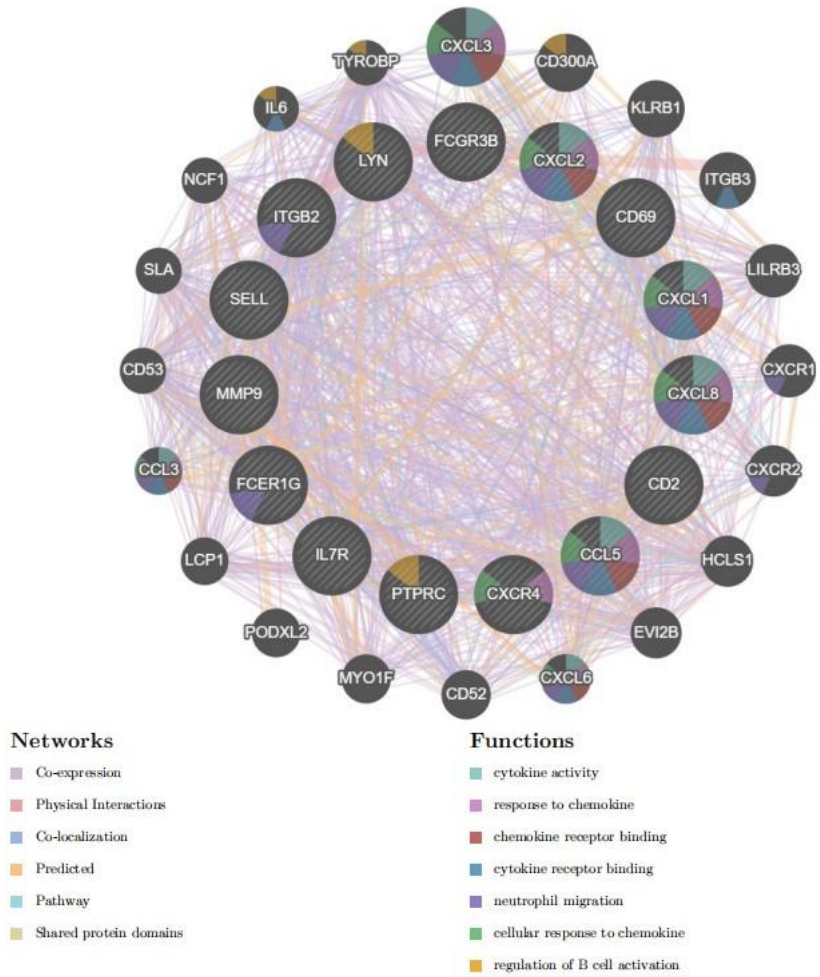

Figure 7 GeneMANIA analysis of the hub genes. The biological functions of the genes are shown. The hub genes are located in the inner circle and the genes correlated with the hub genes are located in the outer circle.

These genes functions were mainly associated with immune response-regulating cell surface receptor signaling pathway involved in cytokine activity, response to chemokine, chemokine receptor binding, cytokine receptor binding, neutrophil migration, cellular response to chemokine , and regulation of B cell activation.

Diagnostic and Validation Analysis of Hub Genes

To evaluate the diagnostic efficacy of the identified hub genes, ROC curve analysis was conducted on the RA and PD datasets. The AUC values for all 15 hub genes surpassed 0.7 in both datasets, indicating robust diagnostic performance (Supplementary File 1).

For further validation, the expression profiles of these hub genes were examined in independent external datasets: GSE55235 for RA and GSE16134 for PD. In the GSE16134 dataset, all hub genes demonstrated significant differential expression between case and control groups (p-value < 0.001), with consistent up-regulation observed in the case groups. Similarly, in the GSE55235 dataset, 13 out of 15 hub genes (excluding CD69 and CXCL1) exhibited significant differential expression (p-value < 0.01) between case and control groups (Supplementary File 2).Although CD69 and CXCL1 did not show significant differential expression in the GSE55235 dataset, their critical roles in immune responses and support from existing literature justify their inclusion for further investigation.

These results highlight the shared molecular underpinnings of RA and PD and provide a foundation for the development of targeted therapeutic interventions.

Immune Cell Infiltration and its Correlation with Candidate Biomarkers

Immune and inflammatory responses are crucial in the molecular pathways of RA and PD.

ssGSEA was further applied to analyze the differences in immune cell infiltration between these two diseases and healthy controls. Figure 8A and Figure 9A shows the distribution of 28 immune cells in the GSE77298 sample. Figure 8B and Figure9B shows the distribution of 28 immune cells in the GSE10334 sample.

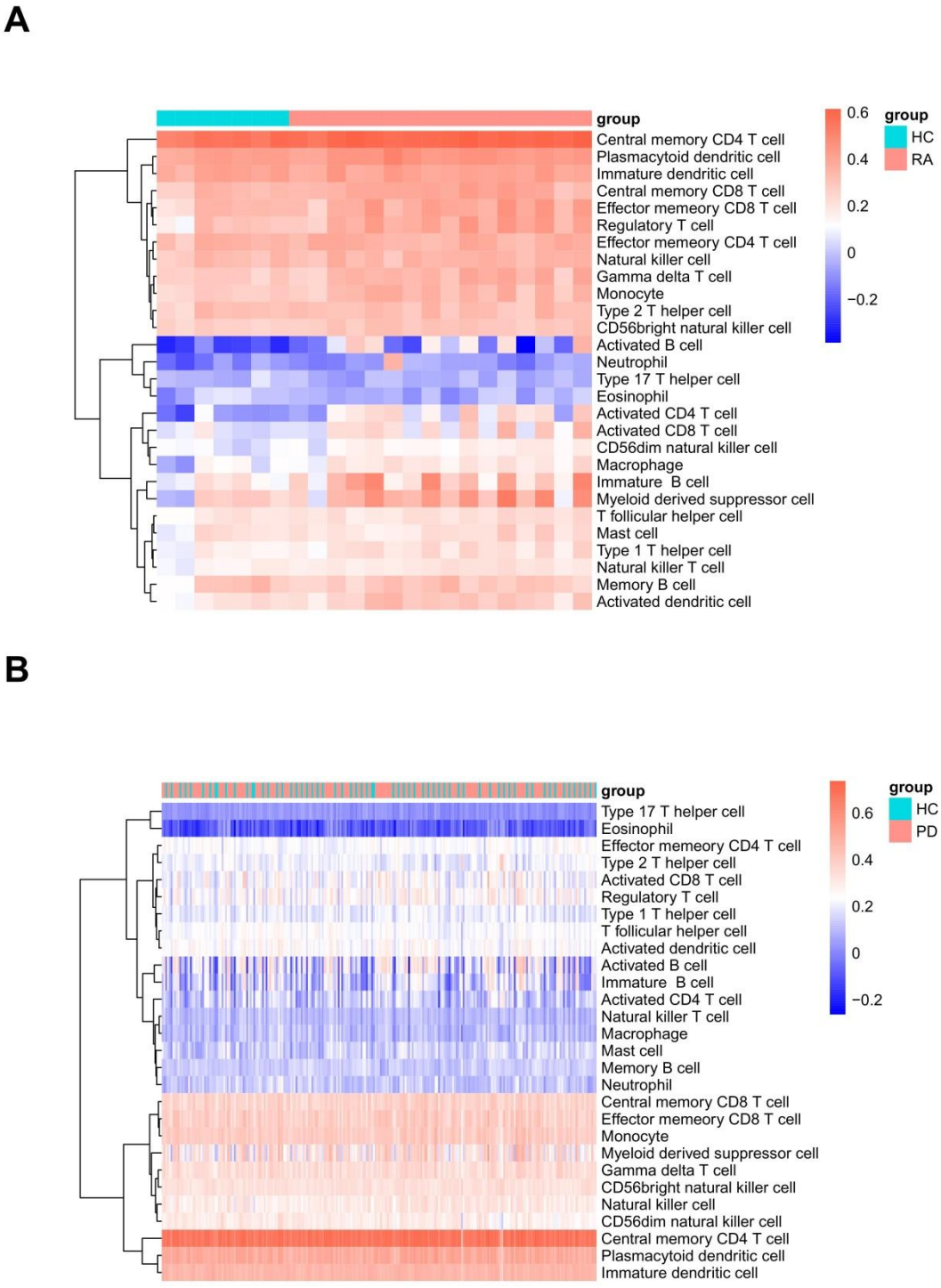

Figure 8 Open in a new tab Expression analysis of infiltrated immune cells by ssGSEA algorithm among two diseases. (A) Heat map of RA. (B) Heat map of PD.

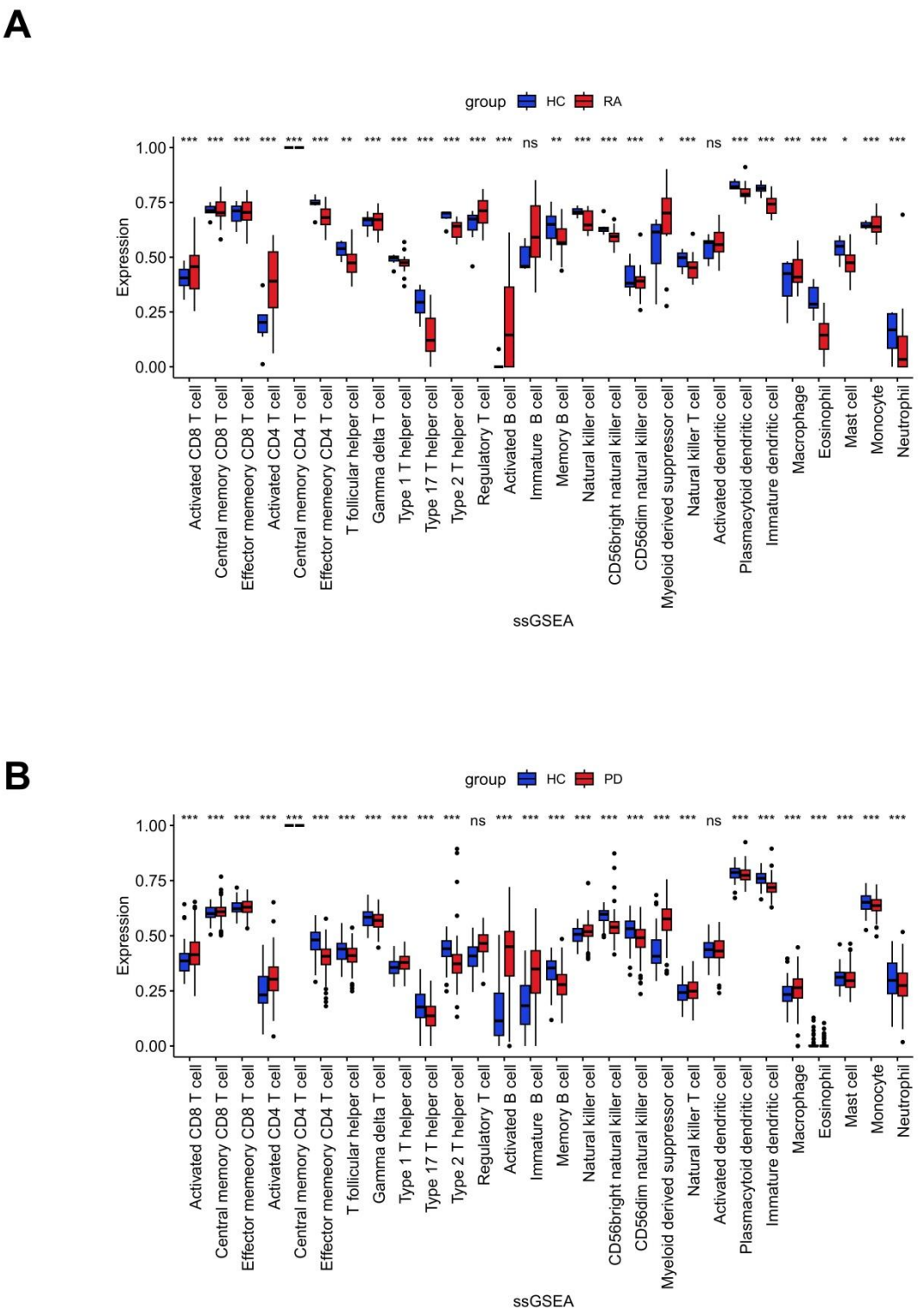

Figure 9 Group comparison graphs of infiltrated immune cells by ssGSEA algorithm among two diseases. (A) Infiltrated immune cells expression levels of RA. (B) Infiltrated immune cells expression levels of PD. (*p<0.05, **p<0.01, ***p<0.001, ns meant no significant difference vs. Healthy Control).

According to the immune infiltration results, we found that immature B cells, activated B cellss, regulatory T cells and activated CD4 T cell increased more significantly in both diseases. Furthermore, the correlation analysis results of immune cells with the hub genes suggested that multiple immune cells had a positive correlation with the hub genes in RA samples (Figure 10A) and PD samples (Figure 10B), and active B cells, immature B cells, regulatory T

cells,MDSCs,active CD4 T cells and active CD8 T cells were more highly correlated with the hub genes.

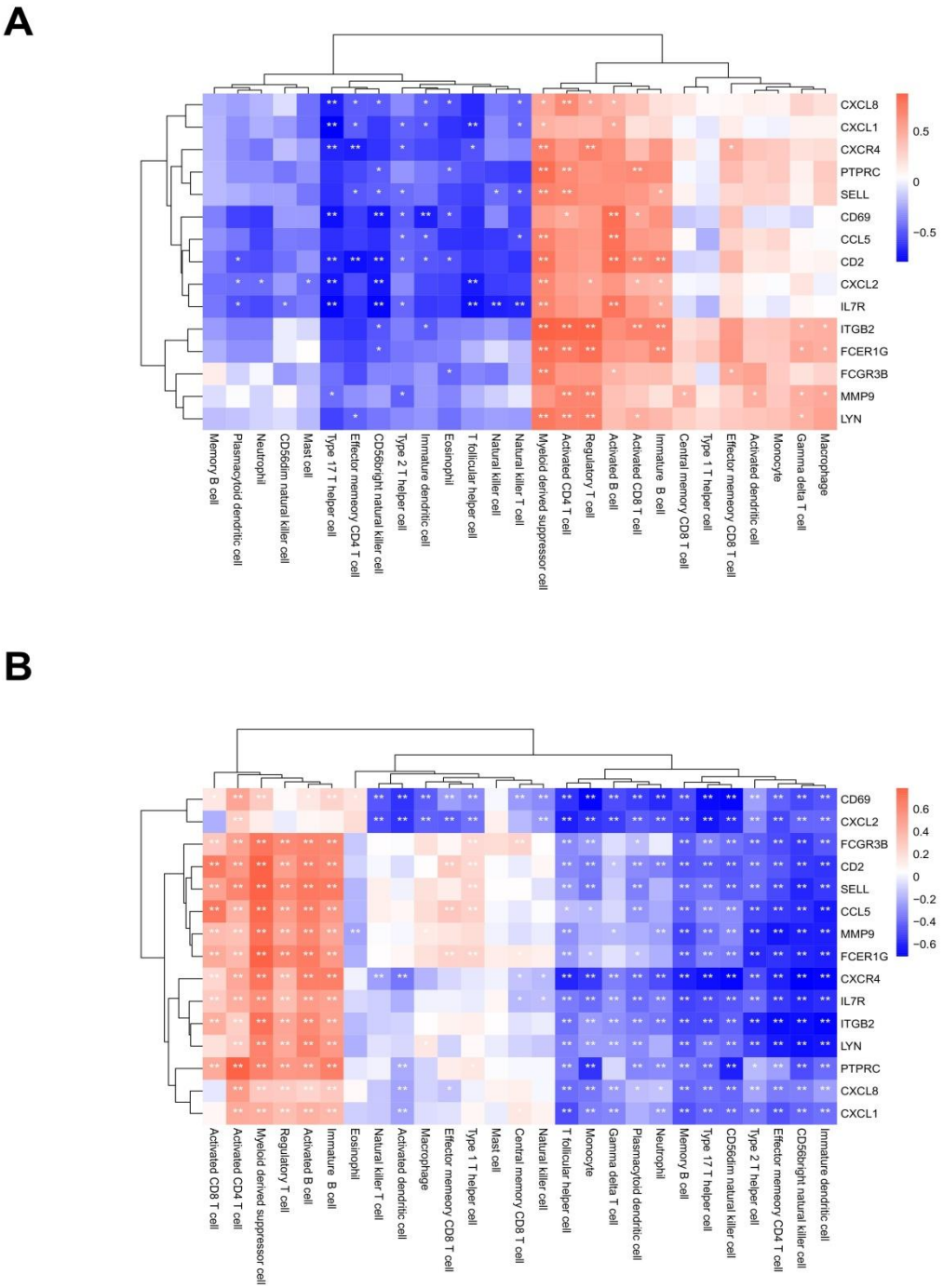

Figure 10 Correlation heat map analysis of infiltrated immune cells by ssGSEA algorithm among two diseases. (A) Correlation heat map of RA. (B) Correlation heat map of PD . (*p<0.05, **p<0.01, ***p<0.001, ns meant no significant difference vs. Healthy Control)

Recognition of Transcription Factors and Mirnas Associated with Hub Genes

To investigate the transcriptional regulation of hub genes, a network-based approach was employed to identify the regulatoryTFs and miRNAs. Figure 11 illustrates the interactions between TF regulators and hub genes, which include 66 nodes and 123 edges.

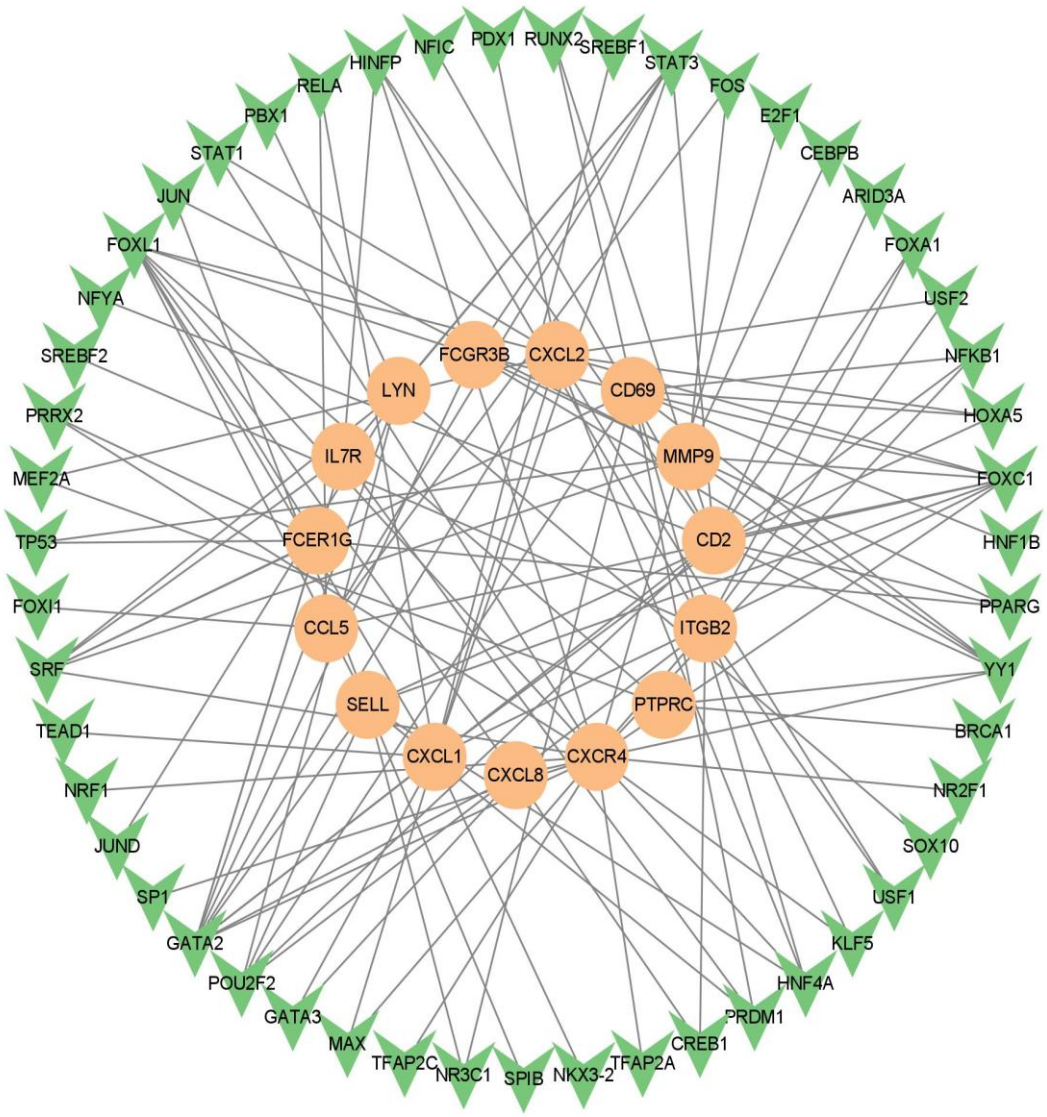

Figure 11 The interconnected regulatory interaction network of Hub genes–TFs. Hub genes–TFs,orange nodes indicate Hub genes and green nodes represent TFs (66 nodes,123 edges).

Additionally, Figure 12 depicts the interactions between miRNAs and hub genes, comprising 195 nodes and 431 edges. From the TF-gene and miRNA-gene interaction networks, 61 TFs, such as GATA2, FOXC1, YY1, FOXL1, SRF, STAT3, NFκB1, and POU2F2, as well as 29 miRNAs, including hsa-miR-98-5p, hsa-miR-146a-5p, hsa-let-7e-5p, hsa-miR-26b-5p, hsa-miR-204-5p, hsa-miR-1-3p, and hsa-let-7a-5p, were identified.

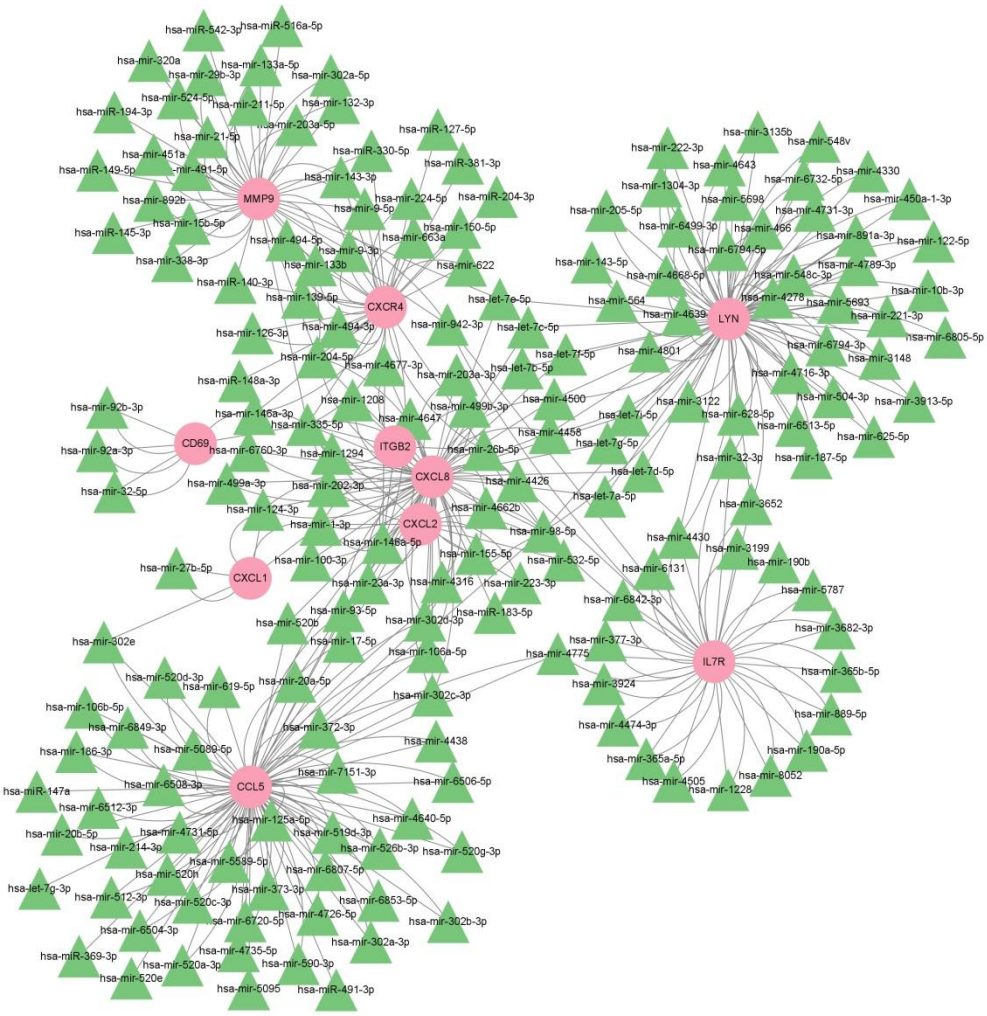

Figure 12 The interconnected regulatory interaction network of Hub genes–miRNAs . Hub genes–miRNAs,red nodes indicate Hub genes and green nodes represent miRNAs (195nodes,431 edges).

Gene-disease Association Analysis

The shared DEGs between RA and periodontitis were uploaded to DisGeNET for further analysis, revealing potential connections between these diseases and a range of other conditions, including pneumonia, systemic lupus erythematosus, autoimmune disorders, pneumonitis, T-cell leukemia, arthritis, rheumatoid arthritis, and neutrophilia, among others (Figure 13).

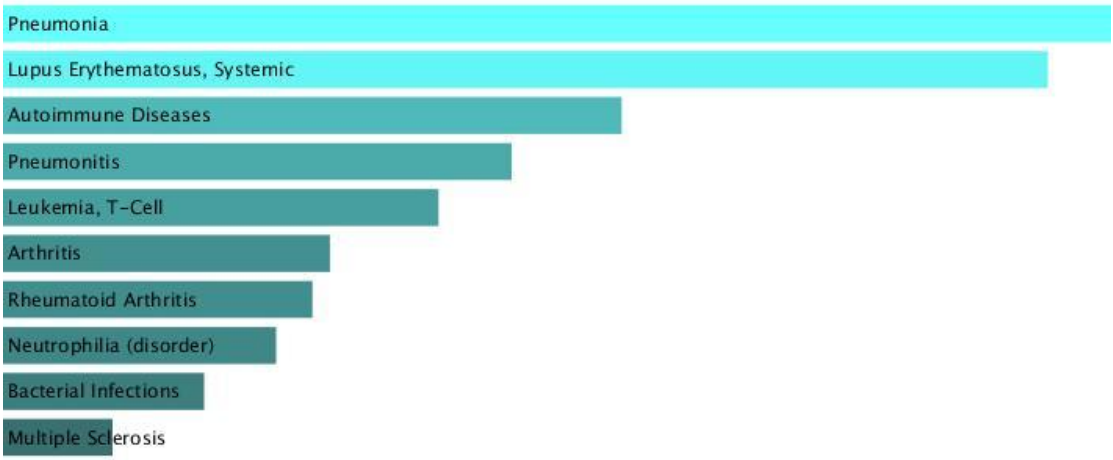

Figure 13 Gene-disease association analysis identifies the leading ten associated diseases.

These results underscore the genetic overlap and interplay between periodontitis, RA, and related diseases, providing important insights into their pathogenesis and potential therapeutic strategies for future research.

Evaluation of Applicant Drugs

Small molecule drugs regulating the expression of hub genes were collected from the DSigDB database on Enrichr platform. The results from the potential small molecules were generated based on their P values to represent the closeness between the small molecules and genes. Table 2 pointed out the top 6 potential small molecule drugs for hub genes.

Table 2 List of the suggested drugs for patients with both RA and PD.

Term	P-value	Adjusted P-value	Odds Ratio	Genes
triprolidine PC3 UP	1.4860762518099782E-9	6.197206865235402E-7	403.37373737373736	CXCL8;CXCL1;IL7R;CXCL2
Tamibarotene CTD 00002527	1.9183699570932357E-9	6.197206865235402E-7	40.30904848327716	LYN;FCER1G;PTPRC;SELL;CCL5;ITGB2;CXCR4;CD69
aspirin CTD 00005447	1.9730706737023413E-9	6.197206865235402E-7	40.15913200723327 5	LYN;CXCL8;FCER1G;SELL;ITGB2;CXCL1;IL7R;MMP9
Calcimycin CTD 00005287	7.747035847799265E-9	1.3742262528251656E-6	105.80319148936171	CXCL8;SELL;CCL5;ITGB2;CD69
promethazine PC3 UP	9.372155849730614E-9	1.3742262528251656E-6	241.87878787878788	CXCL8;CXCL1;IL7R;CXCL2
dexamethasone CTD 00005779	9.692818397669663E-9	1.3742262528251656E-6	41.36382850241546	LYN;CXCL8;CCL5;CXCR4;CD69;IL7R;MMP9

Discussion

Our findings demonstrate that immune response is significantly enriched in the biological processes of both RA and PD, suggesting that the overactivation of immune responses is a common feature of these two diseases. Immune cell infiltration analysis further revealed that B cells and CD4 T cells exhibit high activity in both diseases.

Interestingly, despite the well-established role of neutrophils in the literature, we did not observe significant neutrophil activity in the immune infiltration analysis of our study[1]. This discrepancy may stem from variations in the public datasets used for the analysis, including differences in sample characteristics or inherent biases in data reporting. Importantly, B cells and CD4 T cells remain critical contributors to immune dysregulation in both RA and PD.

B cells are known to play a crucial role in RA by producing autoantibodies, such as anti-citrullinated protein antibodies (ACPA)[22-24], which contribute to the pathological immune response. In PD, B cells exacerbate local inflammation through the secretion of pro-inflammatory cytokines such as IL-6. Our analysis further corroborates the essential role of B cells in both diseases by identifying a significant positive correlation between B cells and key immune response-related genes, including CXCL1 and CXCR4.

The involvement of CD4+ T cells, particularly Th17 cells, has received increasing attention in both RA and PD. Th17-driven inflammation characterizes the pathogenesis of both diseases. IL-17, although a weak inflammatory inducer in isolation, exerts powerful inflammatory effects by interacting synergistically with other cytokines and recruiting inflammatory cells, including neutrophils. Beyond its inflammatory role, IL-17's osteoclastogenic properties make it a key factor in bone destruction[25, 26]. In RA, IL-17 enhances the expression of RANKL (Receptor Activator of Nuclear Factor kappa-B Ligand) in fibroblasts and osteoblasts, triggering osteoclast formation and promoting joint degradation. In PD, IL-17 induces RANKL expression in gingival

fibroblasts and stimulates macrophages to release TNF, leading to periodontal tissue damage. Importantly, IL-17 levels are positively correlated with RANKL expression in periodontal ligament cells, further emphasizing its role in periodontal bone loss[27, 28].

Neutrophils, though not identified as significantly active in our immune infiltration analysis, have been extensively documented in the literature for their infiltration in both RA and PD. Neutrophils are particularly abundant in the synovial fluid of RA patients, accounting for 80-90%[29] of cells, and in the gingival crevicular fluid (GCF) in PD, where they make up 80-95% of the leukocyte population. Neutrophil activation, prolonged survival, and impaired clearance can lead to neutrophil necrosis, degranulation, and the formation of neutrophil extracellular traps (NETs)[30]. NETs have been implicated in exacerbating tissue damage and oxidative stress in both RA and PD, contributing to the degradation of cartilage in RA and the destruction of periodontal tissues in PD. Notably, MMPs, such as MMP-2 and MMP-9 in RA[31], and MMP-8 in PD[32-34], play a critical role in this process by degrading the extracellular matrix, leading to tissue degradation. MMP-9 levels in GCF are considered a reliable marker of disease progression in PD[35].

The CC part of the GO analysis further supports the key role of immune cells in local inflammation, with significant enrichment observed in the extracellular region and plasma membrane components. These findings underscore the importance of immune cell interactions with the extracellular matrix and plasma membrane receptors, which sustain the inflammatory response. Receptors such as CXCR4 and CD69, critical for immune cell migration and activation, are prominently involved in the inflammatory processes of both RA and PD.

Our KEGG pathway analysis revealed significant enrichment in both the NF-κB and IL-17 signaling pathways. These pathways are central to immune cell activation and cytokine production. IL-17 plays a key role in the inflammatory response in RA, PD, and other inflammatory diseases[36]. The IL-17 pathway promotes osteoclast activation and immune cell recruitment, which contribute to tissue destruction. While IL-17 inhibitors have demonstrated efficacy in RA treatment, their impact on periodontal tissues remains largely unexplored, likely due to limited indications, high costs, and potential side effects. Further research into the effect of biologics on gingival and periodontal tissues is needed to clarify the role of Th17 cells and the IL-17 pathway in the pathogenesis of both diseases.

NF-κB, a central pro-inflammatory pathway, is involved in the upregulation of inflammatory cytokines and chemokines, playing a critical role in cytokine storms in RA and PD[37, 38]. Persistent NF-κB activation in RA leads to synovial cell proliferation and excessive secretion of inflammatory mediators, resulting in joint inflammation and destruction. Similarly, in PD, NF-κB activation contributes to alveolar bone resorption and MMP expression, driving periodontal tissue damage.

In addition to the key immune players discussed, our research identifies other potential therapeutic targets for RA and PD. For instance, PTPRC (CD45) is associated with TNF-targeted therapies in RA[39], indicating its potential role in guiding personalized treatment approaches. The expression of CD2 in RA synovial tissues correlates with inflammation, and polymorphisms in CD2 may contribute to the development or persistence of joint autoimmunity[40]. Furthermore, FCGR3B polymorphisms have been linked to a higher risk of aggressive PD[41]. SELL, involved in immune cell adhesion and migration[42], is also critical in both RA and PD. CD69 is an early activation molecule[43, 44] that plays a significant immune role in both RA and PD.In RA, CD69 is closely associated with the activation of T cells, while in PD, it may promote inflammatory responses through the activation and migration of immune cells. Therefore, CD69 may serve as a bridging factor in the shared pathogenic mechanisms of RA and PD.Targeting chemokines and inflammatory factors holds promise in modulating immune overactivation and inflammation, potentially slowing disease progression in both conditions.

RA treatment currently involves NSAIDs, corticosteroids, DMARDs, and biologics. Our findings are consistent with these therapeutic strategies. DMARDs have also shown positive effects on clinical and immune parameters in periodontal tissues, although the effects of newer

DMARDs on inflammation and immune-mediated joint damage in the periodontal microenvironment remain debated[45]. Emerging therapies, such as GPCR-targeted drugs and RNA-based therapies[46], are actively being explored. Given the significant role of chemokine receptors in both RA and PD, targeting these receptors represents a potential therapeutic intervention. Chemokine receptors, a subset of GPCRs, have been extensively studied in RA, with therapeutic strategies focusing on both chemokine receptor ligands and the receptors themselves. Despite the widespread expression of chemokines in human cells, their functions may vary at different stages of RA, posing challenges in the development of GPCR-based therapies. RNA therapies, particularly RNA interference (RNAi), which involves silencing miRNA-mediated mRNA expression, show promising potential for RA treatment. Using databases such as MiRwalk and TRRUST, we constructed a regulatory network for hub genes, providing possible avenues for RNA-based therapies.

While the identified genes offer new insights into potential therapeutic targets for RA and PD, it is important to acknowledge that our findings are based on bioinformatics analyses. Experimental validation of these hub genes and candidate drug efficacy is essential. This remains a key limitation of our current study and an area for future research.

In conclusion, we have identified common DEGs and hub genes in RA and PD, providing valuable insights into their shared pathogenesis. Our findings lay the groundwork for further exploration of the molecular mechanisms, drug discovery, and the development of personalized therapies for RA and PD patients.

Conclusions

This study uncovered shared molecular pathways and hub genes between between rheumatoid arthritis and periodontitis.

The PTPRC, ITGB2, CXCL8, CCL5,CXCR4, MMP9, FCGR3B, CD2, SELL, CXCL1, LYN, FCER1G, CXCL2, IL7R, and CD69 genes were the most important cross-talk genes between rheumatoid arthritis and periodontitis. T-cell and B-cell-driven immune responses may play an important role in the association between rheumatoid arthritis and periodontitis.

Funding

No funding was received.

Availability of Data and Materials

The datasets analyzed during the present study are available in the GEO.

Authors' Contributions

QQY and ZXS designed the study and wrote the initial draft of the manuscript. QQY, ZXS, GYW and XYW contributed to the analysis and interpretation of data. XYW and YFW helped to design the study and acquire data, QQY, ZXS and GYW participated in interpreting the results and revised the writing of manuscript. BW and DQY supervised the project.All authors have reviewed and approved the final manuscript.

Competing Interests

The authors declare that they have no competing i nterests

Reference

1. De Molon R S, Rossa C, Jr., Thurlings R M, et al. Linkage of Periodontitis and Rheumatoid Arthritis: Current Evidence and Potential Biological Interactions [J]. Int J Mol Sci, 2019, 20(18).http://doi.org/10.3390/ijms20184541
2. Radu A F, Bungau S G. Management of Rheumatoid Arthritis: An Overview [J]. Cells, 2021, 10(11).http://doi.org/10.3390/cells10112857
3. Kinane D F, Stathopoulou P G, Papapanou P N. Periodontal diseases [J]. Nat Rev Dis Primers, 2017, 3: 17038.http://doi.org/10.1038/nrdp.2017.38
4. Palioto D B, Finoti L S, Kinane D F, et al. Epigenetic and inflammatory events in experimental periodontitis following systemic microbial challenge [J]. J Clin Periodontol, 2019,46(8):819-29. http://doi.org/10.1111/jcpe.13151
5. De Smit M, Westra J, Vissink A, et al. Periodontitis in established rheumatoid arthritis patients: a cross-sectional clinical, microbiological and serological study [J]. Arthritis Res Ther, 2012, 14(5): R222.http://doi.org/10.1186/ar4061
6. Scher J U, Bretz W A, Abramson S B. Periodontal disease and subgingival microbiota as contributors for rheumatoid arthritis pathogenesis: modifiable risk factors?

[J]. Curr Opin Rheumatol, 2014, 26(4): 424-9.http://doi.org/10.1097/bor.0000000000000076
7. Firestein G S, Mcinnes I B. Immunopathogenesis of Rheumatoid Arthritis [J]. Immunity, 2017, 46(2): 183-96.http://doi.org/10.1016/j.immuni.2017.02.006
8. Kaur S, White S, Bartold P M. Periodontal disease and rheumatoid arthritis: a systematic review [J]. J Dent Res, 2013, 92(5): 399-408. http://doi.org/10.1177/0022034513483142
9. Araújo V M, Melo I M, Lima V. Relationship between Periodontitis and Rheumatoid Arthritis: Review of the Literature [J]. Mediators Inflamm, 2015, 2015: 259074. http://doi.org/10.1155/2015/259074
10. Fuggle N R, Smith T O, Kaul A, et al. Hand to Mouth: A Systematic Review and Meta-Analysis of the Association between Rheumatoid Arthritis and Periodontitis [J]. Front Immunol, 2016, 7: 80.http://doi.org/10.3389/fimmu.2016.00080
11. Gene Ontology Consortium: going forward [J]. Nucleic Acids Res, 2015, 43(Database issue): D1049-56.http://doi.org/10.1093/nar/gku1179
12. Kanehisa M, Goto S. Kegg: kyoto encyclopedia of genes and genomes [J]. Nucleic Acids Res, 2000, 28(1): 27-30.http://doi.org/10.1093/nar/28.1.27
13. Kanehisa M. Toward understanding the origin and evolution of cellular organisms [J]. Protein Sci, 2019, 28(11): 1947-51.http://doi.org/10.1002/pro.3715
14. Szklarczyk D, Kirsch R, Koutrouli M, et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest [J]. Nucleic Acids Res, 2023, 51(D1): D638-d46.http://doi. org/10.1093/nar/gkac1000
15. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks [J]. Genome Res, 2003, 13(11): 2498-504.http://doi.org/10.1101/gr.1239303
16. Bader G D, Hogue C W. An automated method for finding molecular complexes in large protein interaction networks [J]. BMC Bioinformatics, 2003, 4: 2. http://doi.org/10.1186/1471-2105-4-2
17. Chin C H, Chen S H, Wu H H, et al. cytoHubba: identifying hub objects and sub-networks from complex interactome [J]. BMC Syst Biol, 2014, 8 Suppl 4(Suppl 4): S11. http://doi. org/10.1186/1752-0509-8-s4-s11
18. Franz M, Rodriguez H, Lopes C, et al. GeneMANIA update 2018 [J]. Nucleic Acids Res, 2018,46(W1):W60-w4. http://doi.org/10.1093/nar/gky311
19. Piñero J, Bravo À, Queralt-Rosinach N, et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants [J]. Nucleic Acids Res, 2017, 45(D1): D833-d9.http:// doi.org/10.1093/nar/gkw943
20. Yoo M, Shin J, Kim J, et al. DSigDB: drug signatures database for gene set analysis [J]. Bioinformatics, 2015, 31(18):3069-71. http://doi.org/10.1093/bioinformatics/btv313
21. Kuleshov M V, Jones M R, Rouillard A D, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update [J]. Nucleic Acids Res, 2016, 44(W1): W90-7. http://doi.org/10.1093/nar/gkw377
22. Äyräväinen L, Leirisalo-Repo M, Kuuliala A, et al. Periodontitis in early and chronic rheumatoid arthritis: a prospective follow-up study in Finnish population [J]. BMJ Open, 2017, 7(1): e011916.http://doi.org/10.1136/bmjopen-2016-011916
23. Mankia K, Cheng Z, Do T, et al. Prevalence of Periodontal Disease and Periodontopathic Bacteria in Anti-Cyclic Citrullinated Protein Antibody-Positive At-Risk Adults Without Arthritis [J]. JAMA Netw Open, 2019, 2(6): e195394.http://doi.org/10.1001/jamanetworkopen.2019.5394
24. Gonzalez S M, Payne J B, Yu F, et al. Alveolar bone loss is associated with circulating anti-citrullinated protein antibody (ACPA) in patients with rheumatoid arthritis [J]. J Periodontol, 2015, 86(2): 222-31.http://doi.org/10.1902/jop.2014.140425
25. Kurebayashi Y, Nagai S, Ikejiri A, et al. Recent advances in understanding the molecular mechanisms of the development and function of Th17 cells [J]. Genes Cells, 2013,18(4):247-65. http://doi.org/10.1111/gtc.12039
26. Sato K, Suematsu A, Okamoto K, et al. Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction [J]. J Exp Med, 2006, 203(12):

2673-82.http://doi.org/10.1084/jem.20061775
27. Lin D, Li L, Sun Y, et al. IL-17 regulates the expressions of RANKL and OPG in human periodontal ligament cells via TRAF6/TBK1-JNK/NF-κB pathways [J]. Immunology, 2014, 144(3): 472-85.http://doi.org/10.1111/imm.12395
28. Dutzan N, Gamonal J, Silva A, et al. Over-expression of forkhead box P3 and its association with receptor activator of nuclear factor-kappa B ligand, interleukin (IL) -17, IL-10 and transforming growth factor-beta during the progression of chronic periodontitis [J]. J Clin Periodontol, 2009, 36(5): 396-403. http://doi.org/10.1111/j.1600-051X.2009.01390.x
29. Delima A J, Van Dyke T E. Origin and function of the cellular components in gingival crevice fluid [J]. Periodontol 2000, 2003, 31: 55-76.http://doi.org/10.1034/j.1600-0757.2003.03105.x
30. Vitkov L, Klappacher M, Hannig M, et al. Neutrophil fate in gingival crevicular fluid [J]. Ultrastruct Pathol, 2010, 34(1): 25-30.http://doi.org/10.3109/01913120903419989
31. Itoh T, Matsuda H, Tanioka M, et al. The role of matrix metalloproteinase-2 and matrix metalloproteinase-9 in antibody-induced arthritis [J]. J Immunol, 2002, 169(5): 2643-7.http://doi.org/10.4049/jimmunol.169.5.2643
32. Marcaccini A M, Meschiari C A, Zuardi L R, et al. Gingival crevicular fluid levels of MMP-8, MMP-9, TIMP-2, and MPO decrease after periodontal therapy [J]. J Clin Periodontol, 2010, 37(2): 180-90.http://doi.org/ 10.1111/j.1600-051X.2009.01512.x
33. NISSINEN L, Kähäri V M. Matrix metalloproteinases in inflammation [J]. Biochim Biophys Acta, 2014, 1840(8): 2571-80.http://doi.org/10.1016/j.bbagen.2014.03.007
34. Checchi V, Maravic T, Bellini P, et al. The Role of Matrix Metalloproteinases in Periodontal Disease [J]. Int J Environ Res Public Health, 2020, 17(14).http://doi.org/10.3390/ijerph17144923
35. Batool A, Vaithilingam R D, Mohamad Hassan N H, et al. Evaluating the potential of matrix metalloproteinase as a diagnostic biomarker in rheumatoid arthritis and periodontitis: A systematic review and meta-analysis [J]. Medicine (Baltimore), 2023, 102(41): e35340.http://doi.org/10.1097/md. 0000000000035340
36. Komatsu N, Takayanagi H. Immune-bone interplay in the structural damage in rheumatoid arthritis [J]. Clin Exp Immunol, 2018, 194(1): 1-8.http://doi.org/10. 1111/cei. 13188
37. Sun X, Gao J, Meng X, et al. Polarized Macrophages in Periodontitis: Characteristics, Function, and Molecular Signaling [J]. Front Immunol, 2021, 12: 763334.http://doi.org/10.3389/fimmu.2021.763334
38. Guan X, He Y, Li Y, et al. Gremlin aggravates periodontitis via activation of the nuclear factor-kappa B signaling pathway [J]. J Periodontol, 2022, 93(10): 1589-602. http://doi.org/10.1002/jper.21-0474
39. Al Barashdi M A, Ali A, Mcmullin M F, et al. Protein tyrosine phosphatase receptor type C (PTPRC or CD45) [J]. J Clin Pathol, 2021, 74(9): 548-52.http://doi.org/10.1136/jclinpath-2020-206927
40. Fernandez Lahore G, Förster M, Johannesson M, et al. Polymorphic estrogen receptor binding site causes Cd2-dependent sex bias in the susceptibility to autoimmune diseases [J]. Nat Commun, 2021, 12(1): 5565. http://doi.org/10.1038/s41467-021-25828-5
41. Song G G, Lee Y H. Associations between FCGR2A rs1801274, FCGR3A rs396991, FCGR3B NA1/NA2 polymorphisms and periodontitis: a meta-analysis [J]. Mol Biol Rep, 2013,40(8):4985-93. http://doi.org/10.1007/s11033-013-2599-y
42. Malinowski D, Zawadzka M, Safranow K, et al. SELL and GUCY1A1 Gene Polymorphisms in Patients with Unstable Angina [J]. Biomedicines, 2022, 10(10). http://doi.org/10.3390/biomedicines10102494
43. Kimura M Y, Hayashizaki K, Tokoyoda K, et al. Crucial role for CD69 in allergic inflammatory responses: CD69-Myl9 system in the pathogenesis of airway inflammation [J]. Immunol Rev, 2017, 278(1): 87-100. http://doi.org/10.1111/imr.12559
44. Miki-Hosokawa T, Hasegawa A, Iwamura C, et al. CD69 controls the pathogenesis of allergic airway inflammation [J]. J Immunol, 2009,183(12): 8203-15. http:// doi.org/10.4049/jimmunol.0900646
45. Martu M A, Maftei G A, Luchian I, et al. The Effect of Acknowledged and Novel Anti-Rheumatic Therapies on Periodontal Tissues-A Narrative Review [J]. Pharmaceuticals

(Basel), 2021, 14(12).http://doi.org/10.3390/ph14121209
46. Yuan W, Shi X, Lee L T O. Rna therapeutics in targeting G protein-coupled receptors: Recent advances and challenges [J]. Mol Ther Nucleic Acids, 2024, 35(2): 102195.http://doi.org/10.1016/j.omtn.2024.102195