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# Shared Cell Death and Immune Pathways Link Periodontitis and Rheumatoid Arthritis

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**Abstract:** Periodontitis and rheumatoid arthritis (RA) are chronic inflammatory diseases with striking clinical and pathological similarities. While periodontitis damages periodontal tissues and is linked to systemic conditions like cardiovascular disease and diabetes, RA is an autoimmune disorder causing joint inflammation, pain, and deformity. Both diseases involve dysregulated bone remodeling, inflammatory mediators, and cell death pathways, such as pyroptosis, necroptosis, and ferroptosis, yet their shared molecular mechanisms remain unclear. Using bioinformatics approaches, we analyzed GEO datasets from affected tissues and peripheral blood of patients with periodontitis and RA. Differential gene expression (DEGs), functional enrichment, and co-expression network analyses identified IKZF1, NLRP3, and TREM2 as upregulated cell death-related genes in both diseases. Single-cell RNA-Seq further revealed elevated IL7R and BTN3A2 expression in peripheral blood, implicating leukocyte migration and B-cell receptor signaling. Our findings highlight common DEGs tied to cell death and immune dysfunction in periodontitis and RA. Investigating these shared genes may uncover novel mechanistic links between the two diseases, offering potential therapeutic targets.

**Keywords:** periodontitis; rheumatoid arthritis; inflammation; cell death

## 1. Introduction

Periodontitis is a chronic inflammatory disease triggered by bacterial dysbiosis, characterized by gum inflammation, bleeding, and eventual tooth loss if untreated. Beyond its oral manifestations, growing epidemiological evidence ties periodontitis to systemic conditions, including cardiovascular disease, type 2 diabetes, rheumatoid arthritis (RA), and respiratory disorders [1–3]. This association stems from bacteremia and systemic dissemination of inflammatory mediators, which exacerbate conditions like atherosclerosis and impair glycemic control in diabetes [4,5]. Conversely, systemic diseases such as diabetes can worsen periodontitis by impairing immune responses, creating a vicious cycle [6,7]. The bidirectional nature of these interactions underscores the need to clarify whether these links reflect mere correlation or shared pathogenic mechanisms, a critical question for therapeutic and preventive strategies.

RA is a chronic autoimmune disorder marked by persistent joint inflammation, leading to pain, swelling, and progressive joint damage. Beyond its articular effects, RA manifests systemically, impacting cardiovascular, respiratory, and integumentary systems through widespread immune dysregulation. The disease arises from aberrant immune responses that target synovial tissues, driving inflammation and cartilage destruction. Intriguingly, emerging

evidence highlights a robust bidirectional link between RA and periodontitis, with shared inflammatory pathways underpinning both conditions [8,9].

In periodontitis, bacterial infection triggers localized inflammation, resulting in gingival erythema, bleeding, and tooth mobility. Similarly, RA involves immune-mediated joint inflammation, perpetuating a cycle of tissue damage. Crucially, both diseases exhibit dysregulated bone remodeling, where inflammatory cytokines skew the balance toward excessive bone resorption, exacerbating pathology in each condition [10,11]. Moreover, the overproduction of inflammatory mediators, a hallmark of both diseases, enables systemic spread. In periodontitis, these mediators enter circulation, amplifying systemic inflammation, while in RA, they fuel multi-organ involvement [12].

Notably, periodontal pathogens like *Porphyromonas gingivalis* may directly contribute to RA pathogenesis. Hematogenous dissemination of these bacteria can initiate distal inflammatory cascades [13], with *Porphyromonas gingivalis* specifically implicated in generating anti-citrullinated protein antibodies (ACPAs), a serological hallmark of RA [14]. This mechanistic overlap suggests that periodontitis may not merely coexist with RA but could actively participate in its onset or progression, revealing a profound biological interplay between the two diseases.

In RA, dysregulated programmed cell death (PCD) exacerbates joint inflammation. Pyroptosis, driven by NLRP3 inflammasome activation, promotes IL-1β/IL-18 release via succinate accumulation [15] and GSDMD cleavage triggered by C1q-PTX3 binding [16]. Necroptosis contributes to RA pathology through elevated 14-3-3η [17] and ASIC-1a-mediated RIPK1/RIPK3/MLKL signaling, reversible by necrostatin-1s [18]. Ferroptosis, linked to iron-dependent lipid peroxidation, is implicated in synovitis via RSL3-induced synovial cell death [19] and the ENO1-ACO1 regulatory axis [16]. These PCD pathways highlight shared mechanisms between RA and periodontitis, offering therapeutic potential for both diseases [20,21].

This study investigated shared cell death-related differentially expressed genes (DEGs) in local tissues and peripheral blood of periodontitis and RA patients. Using GEO datasets, we performed comprehensive bioinformatics analyses**,** including DEG profiling, functional enrichment, and weighted gene co-expression network analysis (WGCNA) of affected tissues. Additionally, we integrated single-cell RNA sequencing data from peripheral blood to identify common immune-mediated pathways. Our multi-omics approach reveals novel molecular crosstalk between these inflammatory diseases, providing potential mechanistic insights into their well-established clinical association.

## 2. Methods

### *2.1. Data Enrollment*

To investigate the molecular links between periodontitis and RA, we analyzed gene expression datasets from the GEO database. For periodontitis, we merged datasets GSE16134 and GSE10334 (total: 39 healthy vs. 75 diseased gingival samples) for WGCNA (Supplementary Materials). For RA, synovial tissue datasets GSE55235 (10 healthy vs. 10 RA) and GSE55457 (10 healthy vs. 13 RA) were analyzed. Single-cell RNA-seq data from peripheral blood included GSE174609 (4 periodontitis vs. 4 healthy controls) and GSE159117 (1 RA patient). Complete dataset characteristics are presented in Table 1. This integrated multi-dataset approach enabled systematic comparison of disease-associated gene expression patterns.

**Table 1.** The enrolled datasets in the current study.

| Datasets | Type | Platform (Homo Sapiens) | Sample size (Control/PD) | Sample Type |
|---|---|---|---|---|
| GSE16134 | Microarray | GPL570 | 12/12 | PD |
| GSE10334 | Microarray | GPL570 | 27/63 | PD |
| GSE55235 | Microarray | GPL96 | 10/10 | RA |
| GSE55457 | Microarray | GPL96 | 10/13 | RA |
| GSE174609 | scRNA sequencing | GPL20795 | 4/4 | PD |
| GSE159117 | scRNA sequencing | GPL23227 | 0/1 | RA |

**Note:** PD, periodontitis; RA, rheumatoid arthritis.

### *2.2. Identification of DEGs*

DEGs analysis was performed using the *limma* R package to compare disease versus control groups for both periodontitis and RA. We applied distinct statistical thresholds to account for biological differences between the diseases, identifying periodontitis DEGs using a $|\log_2 FC| > 0.5$ and $p < 0.05$ cutoff, while employing more stringent criteria ($|\log_2 FC| > 1$, $p < 0.05$) for RA due to its greater tissue heterogeneity. This analysis revealed disease-specific DEGs for each condition as well as shared DEGs between both diseases. The results were visualized through volcano plots to highlight significant expression changes, Venn diagrams to illustrate overlaps in up- and down-regulated genes, and clustered heatmaps generated with *ggplot2* and *pheatmap* packages to display expression patterns of

shared DEGs. This tiered analytical approach facilitated a comprehensive comparison of transcriptional profiles while addressing each disease's unique characteristics.

### *2.3. Functional and Pathway Enrichment Analysis of DEGs*

To investigate the biological significance of the identified DEGs in periodontitis and RA, we performed comprehensive functional enrichment analysis using the *clusterProfiler* R package. This approach enabled systematic evaluation of Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with the DEGs. We considered terms with a significance threshold of $p < 0.05$ as statistically enriched. Through this analysis, we sought to elucidate the molecular pathways and biological processes dysregulated in both diseases, with particular emphasis on identifying shared mechanisms that might explain their clinical association. The enrichment results provided valuable insights into potential functional connections between periodontal and joint inflammation at the molecular level.

### *2.4. Construction of Gene Co-Expression Networks and Module Identification*

We performed WGCNA to identify clusters of co-expressed genes in periodontitis and RA. For robust results, we merged datasets for each disease (periodontitis: GSE16134 + GSE10334, 114 samples; RA: GSE55235 + GSE55457, 43 samples). Data preprocessing included normalization, batch effect correction, and quality control (removing genes with missing values or insufficient variation, and outlier samples).

Network construction involved calculating gene correlations, transforming to an adjacency matrix using optimized soft power thresholds ($\beta = 8$ for periodontitis, $\beta = 5$ for RA) that satisfy scale-free topology ($R^2 = 0.9$). The adjacency matrix was converted to a topological overlap matrix (TOM) to measure network interconnectedness.

Module detection used average linkage hierarchical clustering of TOM-based dissimilarity, with parameters: minimum module size of 60 genes and module eigengene dissimilarity threshold of 0.3 for merging. Module-trait relationship analyses identified disease-associated modules, with hub genes pinpointed via gene significance and intramodular connectivity.

### *2.5. Single-Cell RNA-Seq Analysis of Key Gene Expression Patterns*

To characterize the cellular expression profiles of critical genes in periodontitis and RA, we analyzed single-cell RNA sequencing datasets GSE174609 (periodontitis) and GSE159117 (RA) using the *Seurat* pipeline. Initial quality control was performed with dplyr to filter cells, retaining those expressing 200 to 7500 genes with mitochondrial content below 20%. Additionally, we removed potential doublets using the Doublet Finder package to minimize artifacts from cell aggregates. Raw counts were normalized using SC Transform to account for technical variation and improve downstream clustering accuracy, followed by identification of the top 2000 highly variable genes. After dimensionality reduction via principal component analysis (PCA) and graph-based clustering, we visualized the cell clusters on UMAP plots. Cell type annotation was performed using *SingleR* with established marker genes and literature references. Finally, we mapped the expression distribution of key genes across identified cell populations in both disease and control groups to pinpoint cell-type-specific expression patterns [22,23].

### *2.6. Statistical Analysis*

All statistical analyses were conducted using R (version 4.2.1). Differential expression of key genes between healthy controls and disease groups was assessed using the non-parametric Wilcoxon rank-sum test, chosen for its robustness to non-normally distributed gene expression data. A two-sided significance threshold of $p < 0.05$ was applied to identify statistically significant differences in gene expression patterns.

## 3. Results

### *3.1. WGCNA Analysis of Periodontitis and RA*

The overall study design is presented in Figure 1. To elucidate the shared molecular networks underlying periodontitis and RA, we performed WGCNA on disease-specific transcriptomic datasets. Prior to conducting WGCNA, we integrated samples of periodontitis and RA and generated a sample dendrogram accompanied by a trait heatmap, as depicted in Supplementary Materials Figure S1. Supplementary Materials Figure S1 presents the hierarchical clustering results of the gene expression profiles for RA samples alongside corresponding phenotypic traits, revealing significant differences in gene expression profiles between the two groups. Notably, no distinct outlier samples were observed in the clustering results, indicating robust data quality. Subsequently, we proceeded with the

standard WGCNA workflow. Based on the scale-free topology criterion ($R^2 = 0.9$; Figure 2A,B), the optimal soft-thresholding power (β) was determined to be 8 for periodontitis and 5 for RA to construct the co-expression networks. Using a minimum module size of 60 genes, we identified 21 co-expression modules in periodontitis and 34 modules in RA, with their topological relationships depicted in weighted network heatmaps (Figure 2C,D). Module-trait correlation analysis revealed distinct disease-associated patterns: in periodontitis, the yellow module demonstrated strong positive correlation (r = 0.64), while RA showed significant associations with four modules—black (r = 0.59), turquoise (r = 0.88), brown (r = 0.66), and grey (r = 0.61). Notably, the turquoise module in RA exhibited the highest correlation, suggesting particularly robust co-regulation of these genes in disease pathogenesis.

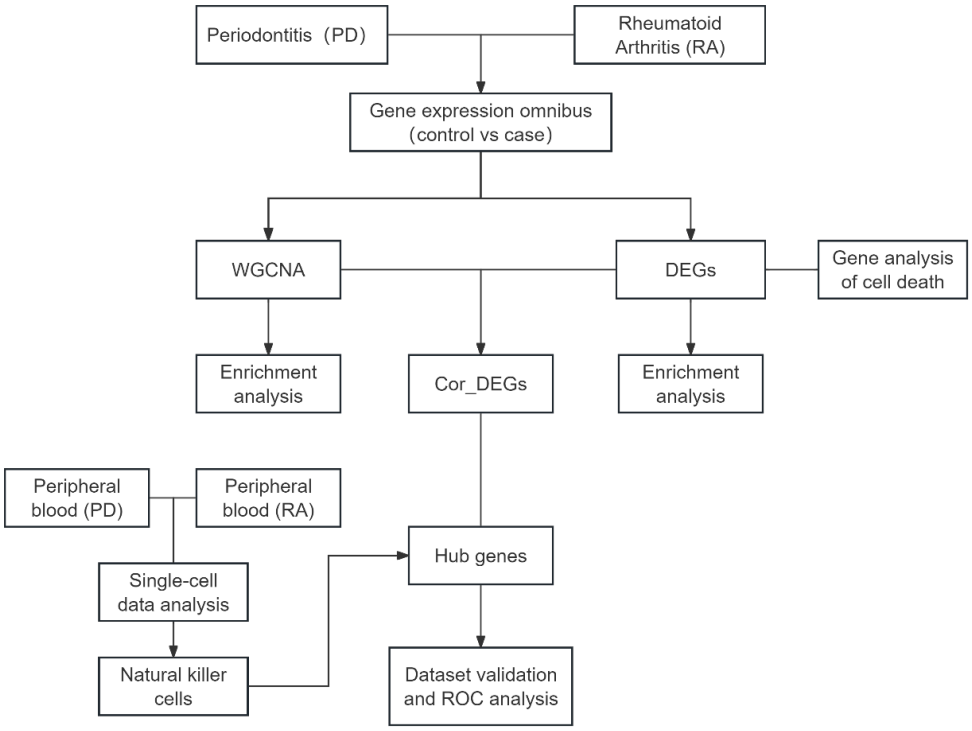


**Figure 1.** Bioinformatics workflow for identifying shared molecular signatures between periodontitis and rheumatoid arthritis: differential gene analysis using the limma R package, WGCNA using the WGCNA R package, and functional enrichment analysis using the clusterProfiler R package to uncover disease-associated genes and pathways. All analyses were performed using R (version 4.2.1).

### *3.2. Pathway Analysis Reveals Shared and Distinct Mechanisms in Periodontitis and RA*

KEGG pathway analysis of significant co-expression modules revealed distinct yet overlapping pathogenic mechanisms in periodontitis and RA. In periodontitis, the yellow module demonstrated strong enrichment for immune-related pathways, including natural killer cell-mediated cytotoxicity, leukocyte transendothelial migration, and osteoclast differentiation, along with B cell receptor signaling, suggesting coordinated regulation of both innate and adaptive immune responses in periodontal tissue destruction. The RA network showed more diverse pathway associations, with the turquoise module dominated by NF-κB and T cell receptor signaling, the grey60 module enriched for PI3K-Akt and Rap1 signaling, the brown module featuring Ras/MAPK cascades and TRP channel regulation, and the black module sharing periodontitis-associated pathways including osteoclast differentiation and B cell signaling.

A core set of 294 conserved genes intersected between disease modules, prominently overrepresented in leukocyte transendothelial migration, B cell receptor signaling, and osteoclast differentiation pathways. This shared molecular signature suggests these conserved pathways may represent fundamental mechanisms linking periodontal and joint inflammation. The particularly strong overlap in osteoclastic activity pathways highlights potential commonalities in inflammatory bone destruction between both diseases, providing mechanistic insights into their clinical association. The pathway convergence observed in B cell signaling and leukocyte migration further supports growing evidence of shared immunopathogenic mechanisms driving these chronic inflammatory conditions.

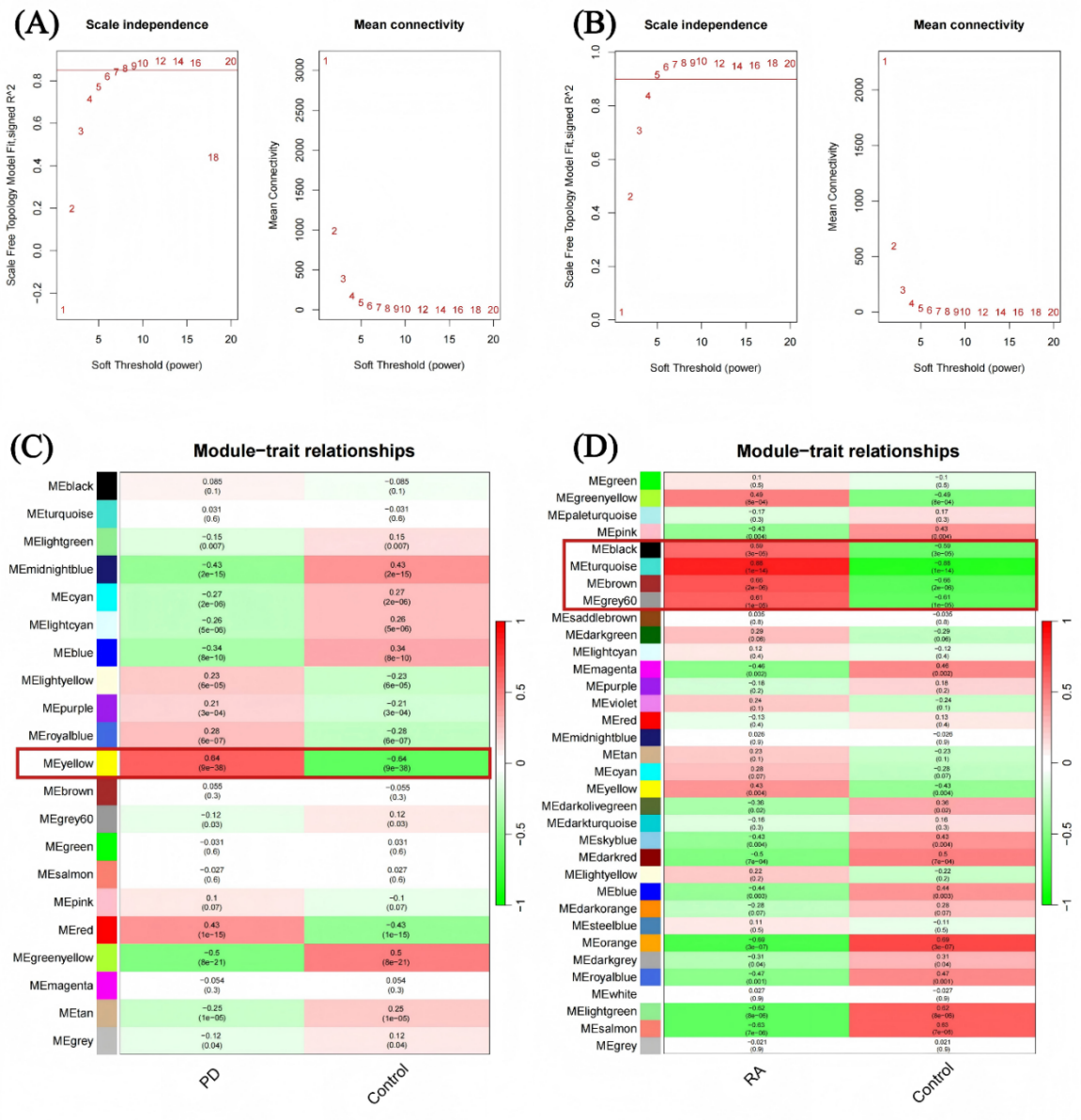


**Figure 2.** WGCNA reveals disease-associated modules in periodontitis and RA. (**A**,**B**) Determination of optimal soft-thresholding powers (β) based on scale-free topology fit for periodontitis (Figure 2A) and RA (Figure 2B) networks; (**C**,**D**) Module-trait correlation heatmap showing significant associations between co-expression modules and disease status. Red gradients represent positive correlations, while green gradients indicate negative correlations. The MEyellow module is a key positively correlated module associated with PD (Figure 2C); the MEblack, MEturquoise, MEbrown, and MEgrey modules are key positively correlated modules associated with RA (Figure 2D).

Functional enrichment analysis of key co-expression modules identified both disease-specific and shared pathogenic pathways. In periodontitis, the yellow module was significantly enriched for immune and bone remodeling pathways, including natural killer cell-mediated cytotoxicity, leukocyte transendothelial migration, B cell receptor signaling, and osteoclast differentiation (Figure 3A). RA modules showed more diverse pathway associations: the turquoise module was strongly linked to NF-κB and T cell receptor signaling, while the grey60 module involved PI3K-Akt and Rap1 signaling cascades. The brown module participated in Ras/MAPK signaling and inflammatory regulation of TRP channels, and the black module mirrored periodontitis pathways through its association with osteoclast differentiation, B cell signaling, and leukocyte migration (Figure 3B).

Notably, we identified 294 genes common to both diseases' key modules, with these shared genes predominantly involved in leukocyte transendothelial migration, B cell receptor signaling, and osteoclast differentiation pathways (Figure 3C,D). This substantial molecular overlap suggests these conserved pathways may represent core mechanisms driving inflammatory tissue destruction in both conditions. The convergence of osteoclast differentiation pathways is particularly compelling, providing a potential biological basis for the bone loss

characteristic of both diseases. Similarly, the shared involvement of B cell signaling and leukocyte migration pathways underscores common immunopathological features that may explain their frequent clinical co-occurrence.

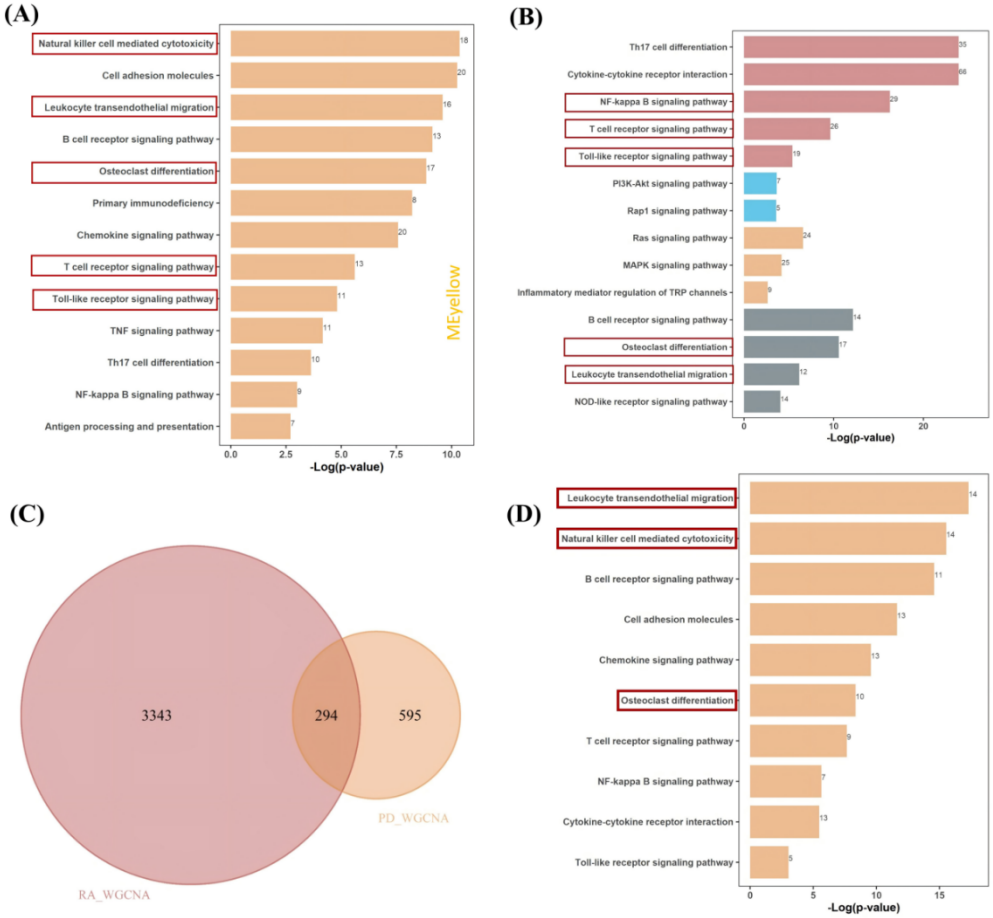


**Figure 3.** Functional pathway analysis of disease-associated gene modules reveals shared pathogenic mechanisms between periodontitis and RA. (**A**) Pathways involved by genes in the MEyellow module of periodontitis. The bar chart shows significantly enriched related pathways, with the number of genes labeled after each pathway. The color of the bars is chosen to be close to that of the corresponding module for easier reading; (**B**) Pathways involved by genes in the MEturquoise, MEbrown, MEgrey60, and MEblack modules of RA; other annotations have the same meanings as in Figure 3A; (**C**) Venn diagram of the intersection of shared genes in the key modules of PD and RA; (**D**) Pathways involved by the shared genes in the key modules of periodontitis and RA; annotations have the same meanings as in Figure 3A, and the color of the bars has no special significance.

### *3.3. Comparative Transcriptomic Profiling Reveals Shared Pathogenic Signatures*

Employing distinct significance thresholds to account for disease-specific biological variability, we identified 962 DEGs in periodontitis (GSE16134; 558 upregulated, 404 downregulated) and 1353 DEGs in RA (GSE55235; 756 upregulated, 597 downregulated) (Figure 4A,B). Strikingly, 143 genes showed conserved dysregulation patterns, comprising 12 co-downregulated and 131 co-upregulated transcripts (Figure 4C,D), suggesting fundamental molecular commonalities between these inflammatory disorders.

Functional characterization of these shared DEGs through integrated GO and KEGG analyses revealed their predominant involvement in immunoregulatory processes. The DEGs were significantly enriched for leukocyte migration responses to bacterial stimuli, intercellular adhesion, and chemotactic regulation. Pathway analysis further demonstrated their collective participation in leukocyte transendothelial migration, B cell receptor signaling, and osteoclast differentiation pathways (Figure 5A,B). Notably, the observed pathway convergence aligns with established clinical comorbidities, particularly the shared bone resorption phenotype mediated through RANKL-dependent osteoclast activation.

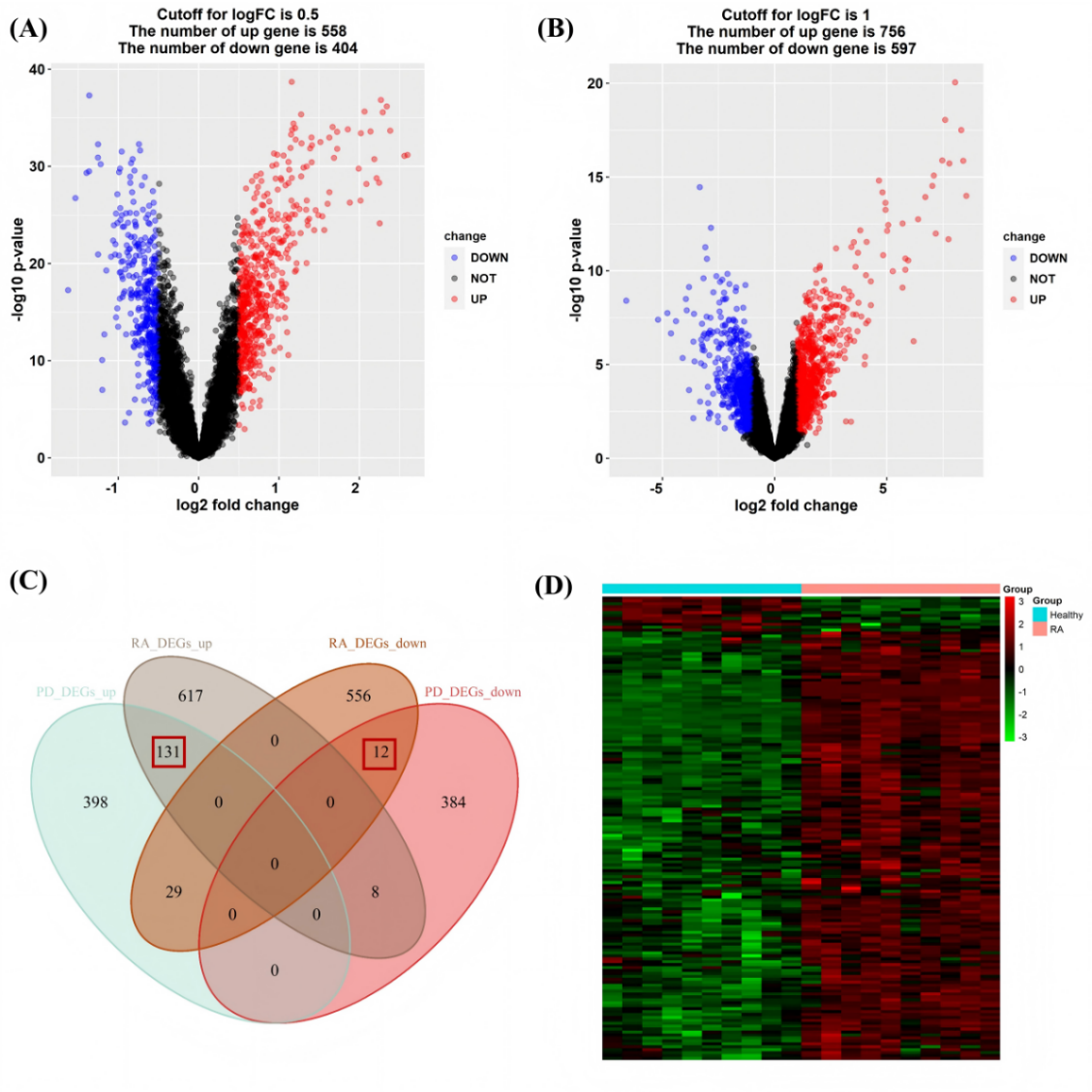


**Figure 4.** Comparative analysis of DEGs in periodontitis and RA. (**A**) Volcano plot of transcriptomic changes in periodontitis (GSE16134 dataset). Each point represents a gene, with red indicating 558 upregulated differentially expressed genes (DEGs) and blue indicating 404 downregulated DEGs (thresholds: $|\log_2 FC| > 0.5$, $p < 0.05$); (**B**) Volcano plot for RA (GSE55235 dataset). A total of 1353 DEGs were filtered out based on the criteria of $|\log_2 FC| > 1$ and $p < 0.05$ (red represents 756 upregulated genes, and blue represents 597 downregulated genes); (**C**) Venn diagram quantifying 143 DEGs shared between the two diseases, including 131 co-upregulated genes and 12 co-downregulated genes; (**D**) Hierarchical clustering heatmap of co-upregulated and co-downregulated genes in RA. Rows represent genes, and columns represent samples (control group vs. RA group). The color gradient (from green to red) represents the expression levels of genes in the samples (from low to high).

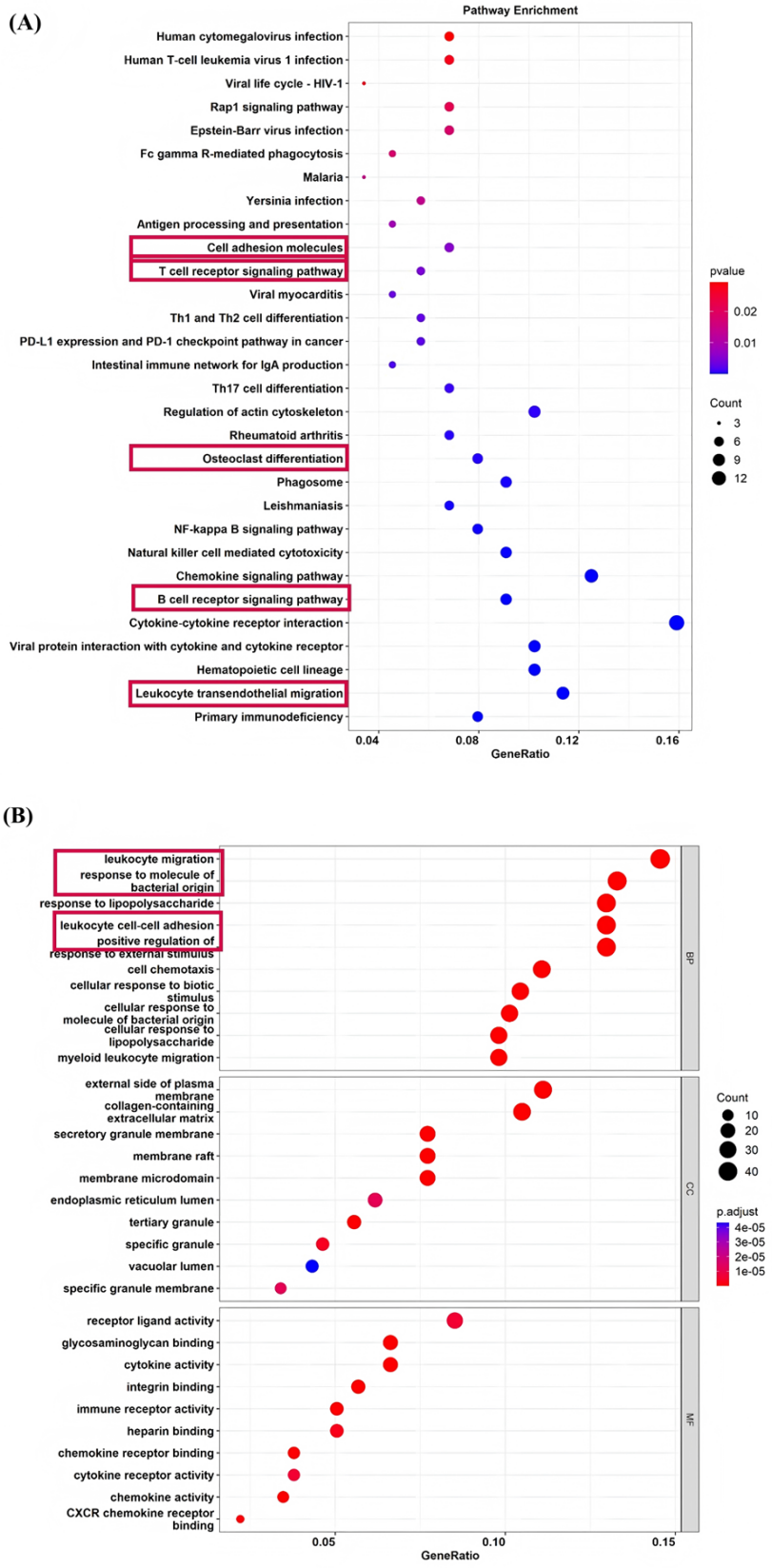


**Figure 5.** Functional enrichment analysis of shared differentially expressed genes. (**A**) KEGG pathway analysis reveals significant enrichment (top 30 pathways shown) for immune and bone metabolism-related processes, including leukocyte transendothelial migration; (**B**) Comprehensive Gene Ontology (GO) analysis demonstrating enrichment across biological processes (BP; e.g., leukocyte migration), cellular components (CC; e.g., immunological synapse), and molecular functions (MF; e.g., chemokine activity). The top 30 significantly enriched terms per category are displayed, with dot size representing gene count and color intensity indicating statistical significance.

### *3.4. Shared Cell Death-Related Genes in Periodontitis and RA Pathogenesis*

The chronic inflammatory microenvironment in RA, characterized by progressive synovitis and joint destruction, involves dysregulated cell death pathways that similarly contribute to periodontal tissue damage. Our analysis identified five key cell death-related genes with conserved roles in both diseases. Among these, IKZF1, NLRP3, and TREM2 showed consistent upregulation across both conditions, while ATF3 and SLC2A3 exhibited disease-specific regulation patterns, being upregulated in periodontitis but downregulated in RA.

Mechanistic characterization revealed that these genes participate in distinct cell death pathways. NLRP3 emerged as a central regulator mediating both necroptotic and pyroptotic pathways through its well-established role in inflammasome activation. IKZF1 and TREM2 were specifically associated with pyroptotic processes, suggesting their involvement in inflammatory programmed cell death. In contrast, ATF3 and SLC2A3 appeared to modulate ferroptosis, potentially through iron-dependent lipid peroxidation mechanisms.

This differential involvement of cell death modalities highlights both shared and disease-specific pathogenic mechanisms underlying inflammatory tissue destruction. The consistent upregulation of NLRP3 across both conditions is particularly noteworthy, as it positions inflammasome activation as a potential common therapeutic target for managing comorbid inflammatory bone loss. These findings provide new molecular insights into how different cell death pathways may contribute to the clinical association between periodontitis and RA, while also revealing potential targets for intervention in both diseases.

RA is a chronic inflammatory disease with synovitis as the main pathologic basis, which can lead to joint bone erosion and destruction, in which cell death plays an important role. In our previous studies [24], we have found the link between periodontitis and cell death, and identified the genes involved in cell death in periodontitis, based on which we further explored the link between periodontitis, RA and cell death. In this paper, we found that in periodontitis and RA, there are shared cell death-related differential genes that affect the development of these two diseases, namely ATF3, SLC2A3, IKZF1, NLRP3, and TREM2. among them, ATF3 and SLC2A3 are up-regulated in periodontitis, and down-regulated in RA. IKZF1, NLRP3, TREM2 were upregulated in both diseases, as shown in Figure 6. Meanwhile, it was found that NLRP3 was associated with necrotic apoptosis and pyroptosis, IKZF1 and TREM2 were associated with pyroptosis, and ATF3 and SLC2A3 were associated with iron death.

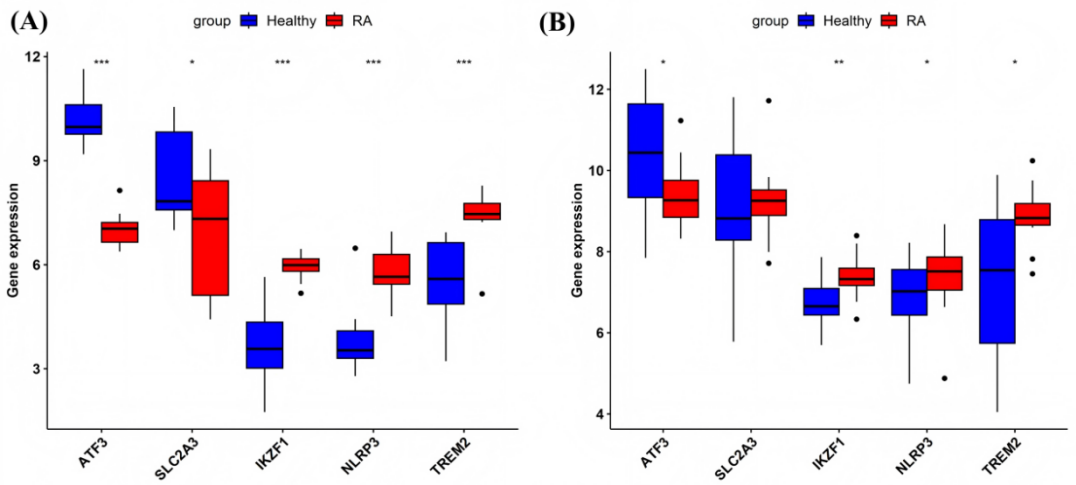


**Figure 6.** Differential expression of cell death-related genes in RA. (**A**) Expression levels of cell death genes in GSE55235; (**B**) Validation of cell death-related gene expression levels in the GSE55457 dataset. Statistical significance was determined using the Wilcoxon rank-sum test, with asterisks indicating the magnitude of differential expression (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

### *3.5. Single-Cell Transcriptomic Profiling Reveals Shared Immune Signatures in Peripheral Blood*

To validate tissue-derived DEGs and investigate their cellular origins, we analyzed single-cell RNA-Seq data from the peripheral blood of periodontitis (GSE174609) and RA (GSE159117) patients. Cell type annotation using SingleR identified six major populations in periodontitis (T cells, NK cells, monocytes, B cells, myeloid progenitors, and platelets), with NK cells showing significant expansion in disease states (Figure 7A–D).

Notably, NK cells from periodontitis patients exhibited 27 upregulated DEGs, including two genes previously identified in tissue analyses: *IL7R* and *BTN3A2* (Figure 7E). While both genes showed differential expression in peripheral NK cells, *BTN3A2* demonstrated particularly strong upregulation (Figure 7F). Interestingly, *IL7R*

displayed broader expression across T cell populations in both peripheral blood and periodontal tissues, suggesting distinct cellular roles for these markers.

Parallel analysis of RA peripheral blood revealed similar expression patterns, with *BTN3A2* again showing prominent upregulation in NK cells (Figure 8A,B). These findings align with established literature documenting NK cell expansion in both inflammatory conditions [22,23], and identify *BTN3A2* as a conserved marker of activated NK cells across diseases. The shared dysregulation of *IL7R* and *BTN3A2* in peripheral immune cells suggests these genes may participate in systemic inflammatory pathways linking oral and joint inflammation.

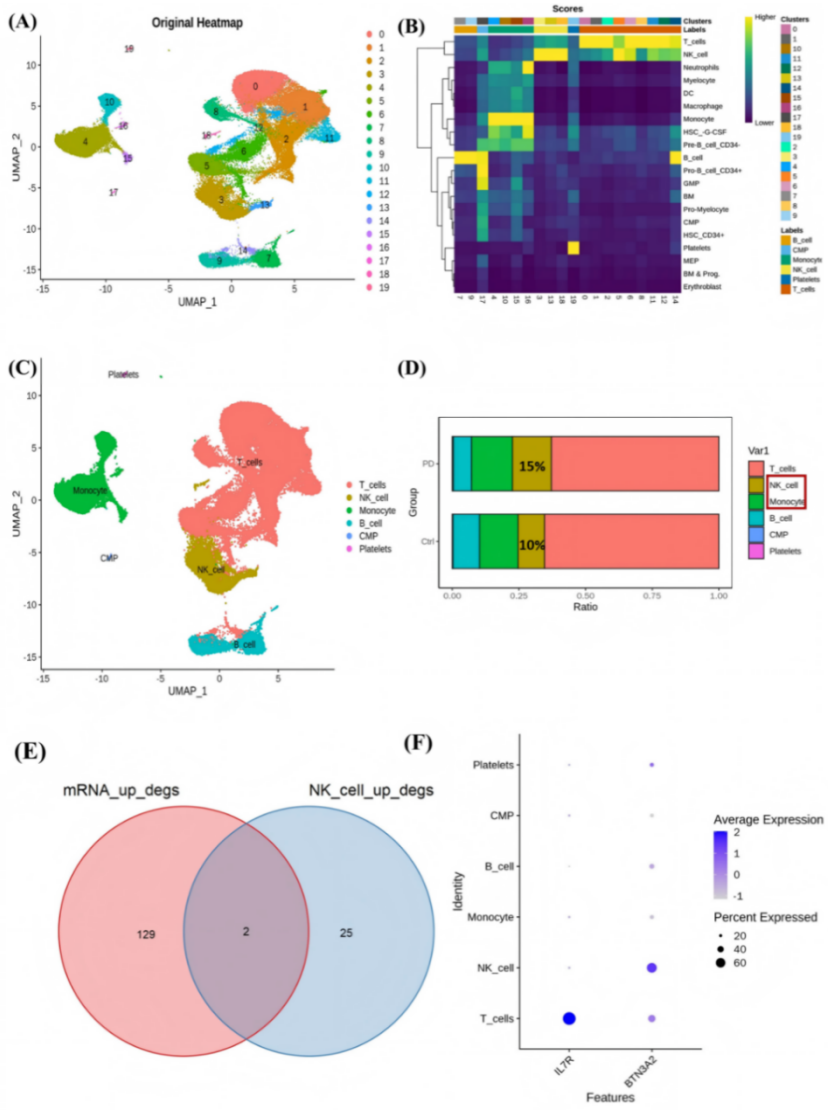


**Figure 7.** Single-cell transcriptomic landscape of peripheral blood in periodontitis. (**A**) UMAP projection of unsupervised clustering illustrating cellular heterogeneity in periodontitis peripheral blood mononuclear cells (PBMCs). Each point represents a single cell, with colors indicating distinct clusters (numbered 0–19); (**B**) Cell type annotation using SingleR, overlaid on the UMAP projection. Six major populations are identified: T cells ($CD3D^+$), NK cells ($NKG7^+$), monocytes ($CD14^+$), B cells ($MS4A1^+$), myeloid progenitors ($LYZ^+$), and platelets ($PPBP^+$), with distinct spatial separation of immune subsets; (**C**) UMAP visualization of annotated cell populations, confirming clear clustering of T cells, NK cells, monocytes, B cells, myeloid progenitors (CMP), and platelets; (**D**) Comparative cell quantification bar plot showing the proportion of each cell type in healthy controls vs. periodontitis patients. NK cells exhibit significant expansion in periodontitis (Wilcoxon rank-sum test, $p < 0.05$), with the percentage indicated; (**E**) Venn diagram showing that there are 2 upregulated genes shared by NK cells in periodontitis gingival tissue and peripheral blood; (**F**) Dot plot highlighting the distribution of IL7R and BTN3A2 in T cells and NK cells, respectively. The size of the dots represents the percentage of cells expressing the gene across all cell types, and the color of the dots indicates the expression level in the cells—The higher the expression level, the darker the color.

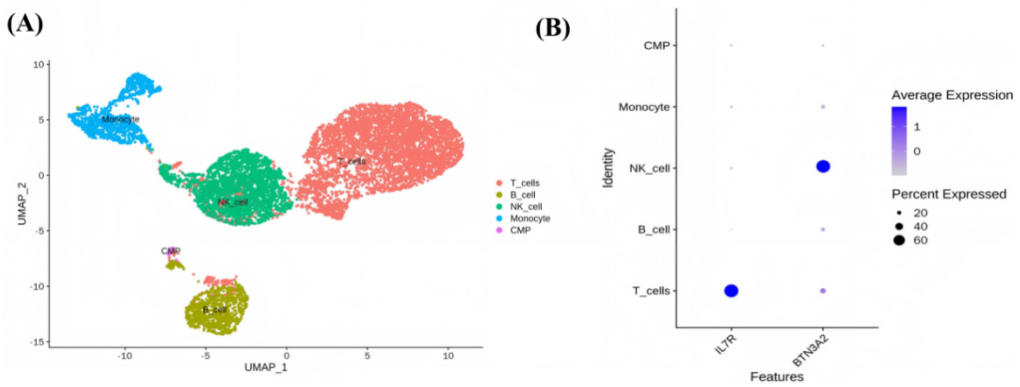


**Figure 8.** Single-cell transcriptomic profiling of peripheral blood in RA. (**A**) UMAP projection of annotated cell populations in RA peripheral blood, showing distinct clusters of T cells ($CD3D^+$), B cells ($MS4A1^+$), NK cells ($NKG7^+$), monocytes ($CD14^+$), and myeloid progenitors ($LYZ^+$); (**B**) Cell-type-specific expression patterns of differentially expressed genes, highlighting conserved upregulation of *BTN3A2* in NK cells and broader *IL7R* distribution across T cell subsets, mirroring periodontitis findings.

### *3.6. Independent Validation of Key Biomarkers in External Cohorts*

To confirm the robustness of our findings, we performed external validation using two independent human datasets from the GEO database (GSE10334 for periodontitis and GSE55457 for RA). Consistent with our primary results, both *IL7R* and *BTN3A2* showed significant upregulation in disease states compared to healthy controls (Figure 9A,B).

The diagnostic potential of these markers was further evaluated through receiver operating characteristic (ROC) curve analysis. Model performance was assessed on the validation sets, with the area under the curve (AUC) as the primary metric. The 95% confidence intervals (CI) for AUC estimates were calculated via 1000 bootstrap iterations. *IL7R* and *BTN3A2* demonstrated strong discriminatory power (AUC > 0.7 for both genes), with *BTN3A2* showing particularly high specificity for disease detection (Figure 9C,D). This validation across independent cohorts not only confirms the reliability of our initial observations but also positions these molecules as promising candidate biomarkers for both periodontitis and RA.

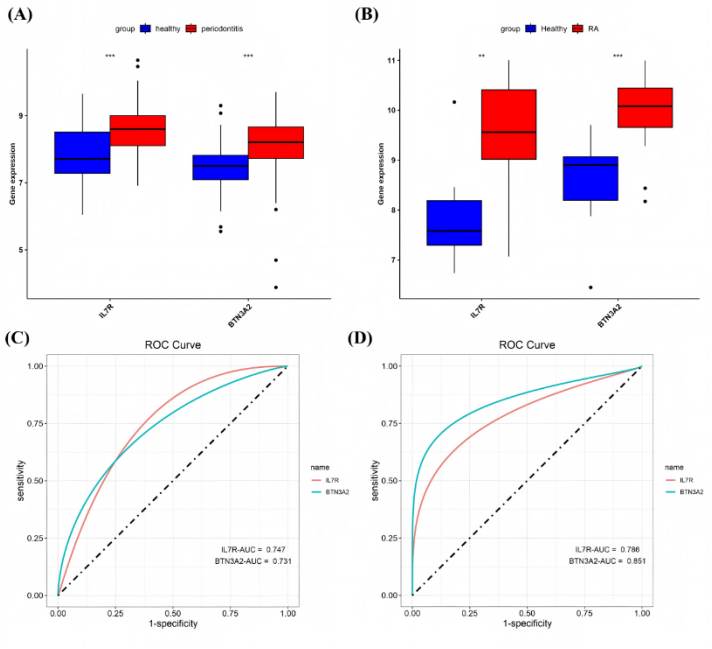


**Figure 9.** Validation of IL7R and BTN3A2 as cross-disease biomarkers. (**A**,**B**) Comparative expression analysis shows significant upregulation of *IL7R* and *BTN3A2* in both periodontitis (GSE10334) and RA (GSE55457) versus healthy controls (** $p < 0.01$ and *** $p < 0.001$, Wilcoxon rank-sum test). Box plots display median expression with IQR; (**C**,**D**) ROC curves demonstrate strong diagnostic performance of both markers (AUC > 0.85), with *BTN3A2* showing superior specificity (92.3%) for distinguishing disease states. The dashed line indicates a random prediction (AUC = 0.5).

## 4. Discussion

Our integrated bioinformatics analysis reveals novel molecular connections between periodontitis and RA, centered on dysregulated cell death pathways and immune activation. We identified consistent upregulation of key cell death regulators (*IKZF1*, *NLRP3*, and *TREM2*) in both diseases, implicating pyroptosis as a shared mechanism of inflammatory tissue destruction. Notably, we also discovered conserved overexpression of immune-related genes *IL7R* and *BTN3A2* across diseased tissues and peripheral blood, suggesting systemic immune dysregulation may link these clinically associated conditions.

The transcription factor *IKZF1* emerges as a particularly compelling molecular link, given its dual roles in immune regulation and cell death. As a member of the Ikaros family, *IKZF1* governs lymphocyte development and inflammatory responses through transcriptional regulation [25]. While its pathogenic role is well-established in hematologic malignancies [26,27] and autoimmune conditions like Systemic Lupus Erythematosus (SLE) [28], our study provides the first evidence of *IKZF1* upregulation in both periodontitis and RA. This finding aligns with recent reports identifying *IKZF1* as a hub gene in periodontal pathogenesis [29] and extends its potential involvement to joint inflammation. The convergent dysregulation of *IKZF1* in these diseases suggests it may coordinate shared inflammatory cascades through: Modulation of pyroptotic pathways via NLRP3 inflammasome interaction, regulation of leukocyte migration and activation, and disruption of immune homeostasis in mucosal and synovial environments.

The NLRP3 inflammasome emerges as a critical molecular node connecting periodontitis and RA pathogenesis. As a cytosolic pattern recognition receptor, NLRP3 orchestrates inflammatory responses to cellular stress by activating caspase-1 and promoting IL-1β/IL-18 maturation [30]. Our findings of NLRP3 upregulation in both diseases align with experimental evidence demonstrating its dual role in periodontal and joint destruction. In periodontitis, NLRP3 drives osteoclastogenesis and alveolar bone resorption, as demonstrated in ligature-induced models using NLRP3-deficient mice [31]. Similarly, in RA, synovial NLRP3 hyperactivation fuels disease progression through the NLRP3/IL-1β axis, promoting periarticular inflammation [32]. The concurrent upregulation of NLRP3 in both conditions suggests that shared inflammasome-mediated pyroptotic pathways may underlie the clinically observed comorbidity.

Complementing these findings, our analysis reveals TREM2 as another upregulated immune modulator in both diseases. This myeloid receptor operates at the intersection of inflammation and bone metabolism—In periodontitis, TREM2-mediated ROS signaling amplifies osteoclastogenesis [33], with clinical studies confirming elevated TREM2+ cells in diseased gingiva [34]. While TREM2's role in RA remains controversial, emerging evidence suggests its inhibition attenuates experimental arthritis [35], positioning it as a potential therapeutic target. The conserved dysregulation of TREM2 in our bioinformatics analysis implies it may coordinate similar osteo-immunological processes in both diseases, though mechanistic studies are needed to clarify its cell-type-specific functions.

The IL-7/IL-7R axis and BTN3A2 emerge as key immunomodulatory components linking periodontitis and RA pathogenesis. IL-7R, critically involved in lymphocyte development and homeostasis, demonstrates disease-aggravating effects in RA by promoting synovial angiogenesis through macrophage-endothelial crosstalk [36,37]. While its role in periodontitis remains unexplored experimentally, our identification of IL-7R upregulation in both diseases suggests it may similarly coordinate inflammatory bone loss through adaptive immune activation.

Our validation confirms IL7R and BTN3A2 have strong diagnostic potential (AUC > 0.7) for both conditions, but their clinical utility requires further study: their ability to distinguish primary from comorbid states needs assessment in stratified cohorts; dynamic expression during progression/treatment response, critical for monitoring, requires longitudinal validation; specificity against other inflammatory conditions (e.g., osteoarthritis) must be evaluated to rule out non-specific upregulation; and practicality of detection (peripheral blood vs. tissue) warrants investigation to translate these markers to clinical use.

A notable limitation of our study is the reliance on single-cell RNA sequencing data from only one RA patient (GSE159117) for peripheral blood analysis, which significantly constrains statistical power and generalizability to the broader RA population. While consistency with periodontitis findings (NK cell expansion and BTN3A2 upregulation) provides preliminary support, future studies with larger RA cohorts are needed to validate these observations.

Similarly, the immunoregulatory molecule BTN3A2 shows conserved dysregulation across conditions. Expressed on T and NK cells, BTN3A2 modulates immune synapse formation and cytotoxic responses [38], with demonstrated relevance in RA comorbidity [39] and cancer immunology [40]. Our findings extend its potential involvement to periodontitis, possibly mediating shared mechanisms of immune cell infiltration and activation. This study establishes novel molecular connections between periodontitis and RA through: (1) Shared upregulation

of pyroptosis regulators (*IKZF1*, *NLRP3*, *TREM2*) driving inflammatory tissue destruction; and (2) Conserved dysregulation of immune mediators (*IL7R*, *BTN3A2*) in both local and systemic compartments. These findings not only advance our understanding of disease comorbidity but also reveal several translational opportunities. Future studies should: Validate these targets in clinical cohorts, elucidate cell-type-specific functions using *in vitro* and animal models, and explore therapeutic modulation of these pathways. The convergence of cell death and immune activation pathways underscores the need for integrated treatment strategies targeting shared molecular mechanisms in these inflammatory disorders.

To validate the roles of IKZF1, TREM2, and NLRP3 in pyroptosis and ferroptosis, future studies could include: (1) In vitro assays using siRNA/CRISPR-Cas9 for gene silencing or specific inhibitors (e.g., MCC950 for NLRP3, Ferrostatin-1 for ferroptosis) in RA-related cells (e.g., synovial fibroblasts, THP-1), measuring pyroptosis markers (caspase-1 activity, IL-1β/IL-18) and ferroptosis indices (lipid peroxidation, iron accumulation); (2) In vivo experiments in RA models (e.g., CIA mice) via AAV-mediated gene knockout/overexpression, assessing joint pathology and protein levels of GSDMD (pyroptosis) or GPX4 (ferroptosis); (3) Clinical validation in larger RA cohorts, correlating gene expression with disease activity (e.g., DAS28) using qPCR, Western blot, or immunohistochemistry.

## 5. Conclusions

This study establishes molecular connections between periodontitis and RA through shared gene expression patterns, pathway activation, and cell death mechanisms. We identified consistent upregulation of pyroptosis-related genes (*IKZF1*, *NLRP3*, *TREM2*) in both diseases, implicating inflammasome activation as a key pathological link. Functional analyses revealed common involvement in leukocyte migration, B/T cell signaling, and osteoclast differentiation pathways that drive inflammatory tissue destruction. These findings provide a genetic and mechanistic basis for the clinical association between these conditions, highlighting potential therapeutic targets for comorbid disease management. While bioinformatics evidence strongly supports these connections, further experimental validation using preclinical models and clinical samples will be essential to translate these findings into targeted treatment strategies. Our results advance understanding of the shared pathophysiology between oral and joint inflammation while opening new avenues for dual-therapeutic development.

## Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/25091116441 12072/RMD-2508000043-Supplementary-materials-layout.pdf.

## Author Contributions

H.H.: Writing—original draft, Methodology, Software, Data curation; S.P.: Conceptualization, Methodology, Software, Data curation, Visualization; L.C.: Methodology. S.X.: Methodology; S.C.: Methodology; Q.Z.: Supervision; J.L.P.: Writing—review and editing, Conceptualization, Supervision. All authors have read and agreed to the published version of the manuscript.

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## Data Availability Statement

Publicly available datasets were analyzed in this study. This data can be found here: GSE16134, GSE10334, GSE55235, GSE55457, GSE174609, GSE159117.

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## Conflicts of Interest

The authors declare no conflict of interest.

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