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The role of neutrophils, fibroblasts, and osteoclasts in periodontitis

A transcriptomic and single-cell analysis

Zhixiang Xu, MD[a], Zhenghao He, PhD[b], Chenglong Yin, MD[a], Qi Liu, PhD[c,a,*]

Abstract

This study aimed to elucidate the molecular mechanisms and regulatory pathways driving the onset and progression of periodontitis (PD). Four transcriptomic datasets from the Gene Expression Omnibus database were integrated to identify differentially expressed genes in gingival tissues of healthy individuals and patients with Pd. Common differentially expressed genes were determined using the RobustRankAggreg algorithm and subjected to pathway enrichment analysis. In parallel, transcriptome sequencing was performed on clinical gingival samples (three healthy controls and 3 patients with PD) to validate the findings. Single-cell RNA sequencing data from periodontal tissues, including healthy, diseased, and post-treatment groups, were further analyzed. Finally, in vitro experiments using human fibroblasts stimulated with *Interleukin-1 beta* were conducted to confirm key regulatory interactions. Core upregulated genes were enriched in pathways such as *Staphylococcus aureus* infection, osteoclast differentiation, and Nuclear factor kappa-light-chain-enhancer of activated B cells signaling. Validation with clinical samples supported these findings and highlighted key gene families including *LILRB* and *FCGR*. In monocytes, tumor necrosis factor receptor superfamily member 11A (RANK) expression was elevated in PD, with the highest levels in osteoclasts. Fibroblasts were identified as the predominant source of tumor necrosis factor ligand superfamily member 11 (RANKL) (*TNFSF11*), and *Interleukin-1 beta* secretion by neutrophils was shown to induce *TNFSF11* expression in fibroblasts. Among fibroblast subpopulations, clusters 1, 6, and 8 displayed abnormal differentiation phenotypes characterized by elevated *TNFSF11* and *IL1R1* expression. Our results define a neutrophil-fibroblast-osteoclast axis that contributes to periodontal tissue destruction and bone resorption. This study provides mechanistic insights into PD and identifies potential molecular targets for precision therapy.

Abbreviations: DEGs = differentially expressed genes, GEO = gene expression omnibus, HC = healthy controls, KEGG = Kyoto Encyclopedia of Genes and Genomes, *IL1B* = *Interleukin-1 beta*, NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells, PD = periodontitis, PDT = periodontitis post-treatment, RANK = receptor activator of nuclear factor κB, RANKL = receptor activator of nuclear factor κB ligand, S. aureus = Staphylococcus aureus, scRNA-seq = single-cell RNA sequencing, TNFRSF11A = Tumor necrosis factor receptor superfamily member 11A (RANK), TNFSF11 = tumor necrosis factor ligand superfamily member 11 (RANKL).

Keywords: biomarkers, fibroblast, neutrophils, osteoclast differentiation, periodontitis, *TNFSF11*

1. Introduction

Periodontitis (PD) is a chronic inflammatory disease initiated by bacterial infection and characterized by gingival bleeding, tooth mobility, and progressive loss of periodontal support, ultimately leading to tooth loss in advanced stages.[1–4] Its pathogenesis involves complex interactions among microbial communities, dysregulated host immune responses, and aberrant intercellular signaling.[5–7] While bacterial infection is recognized as the initiating factor, abnormal activation of immune cells such as neutrophils and monocytes, together with dysregulated cytokine networks, is a central drivers of disease progression.[8–10] Despite advances in clinical management, early diagnosis and targeted therapeutic strategies remain challenging. Therefore,

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

The use of all human gingival specimens was approved by the Ethics Committee of the First School of Clinical Medicine, Southern Medical University (approval No. NFEC-2022-261). All participants provided written informed consent.

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a deeper understanding of the pathology underlying PD is urgently needed, as its pathology is strictly related to other oral inflammatory conditions, as well as pericoronitis and infection sequelae.[11–15]

High-throughput transcriptomic technologies, combined with bioinformatic analyses, have facilitated the identification of numerous differentially expressed genes (DEGs) and signaling pathways associated with PD.[16–18] Pathways such as *Staphylococcus aureus* (*S. aureus*) infection, osteoclast differentiation, and Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling have been repeatedly implicated.[19–21] However, most studies rely on bulk tissue transcriptomics, which masks the specific roles of distinct cell subtypes and their interactions within the periodontal microenvironment. In particular, how immune and stromal cells communicate to drive disease progression remains poorly understood.

To address this gap, we systematically integrated multiple transcriptomic datasets from the Gene Expression Omnibus (GEO) database and validated the findings using clinical gingival samples. We further analyzed single-cell RNA sequencing (scRNA-seq) data to characterize cellular heterogeneity and intercellular communication across healthy, diseased, and post-treatment periodontal tissues. Special emphasis was placed on neutrophils, fibroblasts, and monocytes/osteoclasts. By mapping gene expression and regulatory relationships in these cells, we aimed to delineate the molecular mechanisms of PD and provide a theoretical foundation for precision therapy. Mechanism diagram drawn by Figdraw (Fig. 1).

2. Materials and methods

2.1. Data sources

Four transcriptome datasets related to PD were obtained from the GEO database: GSE10334, GSE16134, GSE23586, and GSE173078. These datasets include gingival tissue samples from healthy controls (HC) and patients with PD.[22–24] In addition, a scRNA-seq dataset (GSE171213) comprising samples from HC, PD, and post-treatment (PDT) groups was analyzed.[25]

For experimental validation, gingival tissues from 3 healthy individuals and 3 patients with PD were collected at the Southern Hospital of Southern Medical University. All procedures involving human gingival specimens were approved by the Ethics Committee of The First School of Clinical Medicine, Southern Medical University (approval No. NFEC-2022-261). Written informed consent was obtained from all participants.

2.2. Transcriptome data preprocessing and differential gene screening

Bulk RNA-seq data from GEO and clinical samples were standardized using the limma and edgeR packages (https://bioconductor.org/packages/edgeR/).[26] DEGs were defined by thresholds of $|\log2FC| \geq 0.8$ and $P < .05$. Results were visualized with volcano plots, and robust integration across datasets was performed using the RobustRankAggreg algorithm to identify consistently altered genes.[27]

2.3. Pathway enrichment analysis

Functional enrichment analysis was performed using the clusterProfiler package. Gene Ontology Biological Processes (GO-BP) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were analyzed, with significance set at $P < .05$.[28]

2.4. Processing of single-cell sequencing data

The initial scRNA-seq data were imported and subjected to quality control using the CreateSeuratObject function. Batch effect correction was conducted utilizing the Harmony package.[29] Uniform Manifold Approximation and Projection was applied for dimensionality reduction, followed by clustering and cell type annotation based on canonical marker genes. DEGs were identified using the FindMarkers function with thresholds of $|\log FC| \geq 0.5$ and minimum expression proportion ≥ 0.1.

2.5. Analysis of cell communication and pseudotime

Intercellular communication was inferred using the iTALK package (https://github.com/Coolgenome/iTALK) to characterize ligand–receptor interactions across cell subtypes. The

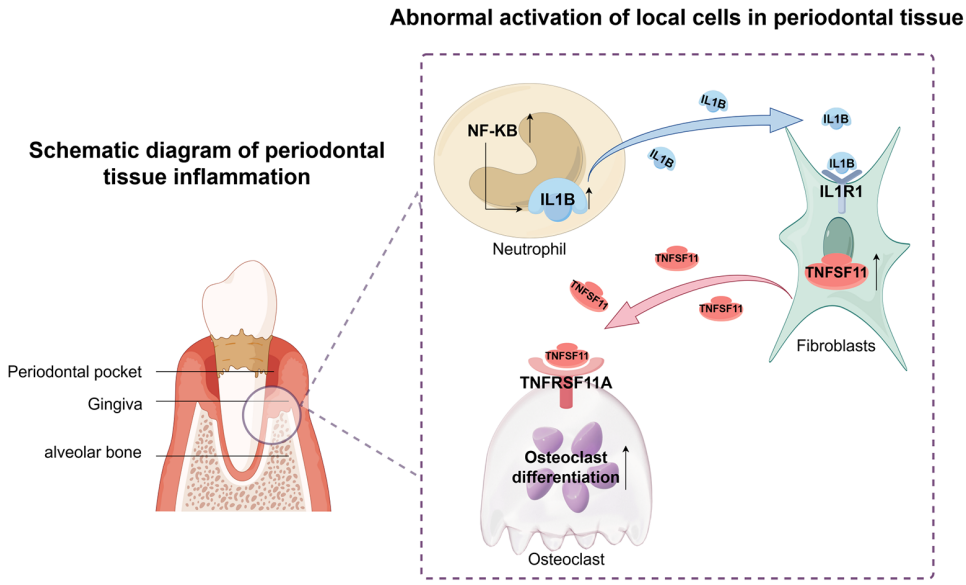

Figure 1. Schematic diagram of periodontal tissue inflammation and abnormal activation of local cells in periodontitis pathogenesis. Neutrophils secrete *IL1B*, which promotes the expression of *TNFSF11* in fibroblasts. Fibroblasts, as the main source of *TNFSF11*, secrete *TNFSF11* that acts on *TNFRSF11A* on osteoclasts, thereby facilitating osteoclast differentiation and participating in the pathological process of periodontal tissue inflammation.

correlation between *IL1R1* and tumor necrosis factor ligand superfamily member 11 (RANKL) (*TNFSF11*) expression in fibroblasts was assessed using Pearson's analysis. Pseudotime trajectory analysis of fibroblasts was performed with Monocle to investigate differentiation states and lineage relationships.[30]

2.6. In vitro cell experiments

Primary human gingival fibroblasts were cultured and divided into 2 groups: an *Interleukin-1 beta* (*IL1B*)-stimulated group (20 ng/mL, $n = 5$) and a control group ($n = 5$). After 48 hours, protein levels of *TNFSF11* receptor activator of nuclear factor κB ligand (RANKL) were measured by Western blotting to assess *IL1B*-induced regulatory effects.

2.7. Statistical analysis

All statistical analyses were conducted in R (version 4.2.0). Data are presented as mean ± standard deviation. Group differences were assessed by Student *t* test, and $P < .05$ was considered statistically significant.

3. Results

3.1. Identification of differential genes in PD patients and pathway enrichment analysis

Four transcriptomic datasets from the GEO database were analyzed to compare gingival tissues from HC and PD patients, with thresholds of $|\log2FC| \geq 0.8$ and $P < .05$. Volcano plots illustrated the distribution of DEGs, with upregulated genes shown in red and downregulated genes in blue. Specifically, GSE10334 contained 272 upregulated and 148 downregulated genes; GSE16134, 346 upregulated and 166 downregulated genes; GSE23586, 1550 upregulated and 1191 downregulated genes; and GSE173078, 297 upregulated and 228 downregulated genes (Figs. 2A-D). To identify robust signals, DEGs from all 4 datasets were integrated using the RobustRankAggregalgorithm. Enrichment analysis revealed significant associations with pathways including "*S. aureus* infection," "Osteoclast differentiation," and the "NF-κB signaling pathway" (Fig. 2E).

For validation, transcriptome sequencing was performed on clinical samples from 3 HC and 3 PD subjects. The results of the pathway enrichment analysis revealed that pathways significantly enriched with highly expressed genes in PD tissue include "Osteoclast differentiation" and "Neutrophil extracellular trap formation" (Fig. 3A). This finding is consistent with the results from the multi-dataset analysis in the previously referenced GEO database, underscoring the central role of these pathways in the progression of PD. Key genes involved included *NCF1*, *MAPK12*, members of the *LILRB* family (*LILRB1*, *LILRB2*, *LILRB4*), the *FCGR* family (*FCGR2A*, *FCGR3A*, *FCGR3B*), *CYBA*, *NCF2*, and NCF4 (Fig. 3B). Heatmaps demonstrated clear expression differences between PD and HC samples (Fig. 3C). These results highlight osteoclast differentiation and neutrophil activity as central processes in PD progression.

3.2. Single-cell sequencing snalysis and cell subtype annotation

scRNA-seq data from HC, PD, and PDT groups were analyzed to characterize cellular heterogeneity. After normalization and batch correction, Uniform Manifold Approximation and Projection revealed 24 clusters (Fig. 4A), which were annotated into 13 cell types using canonical markers: B cells, endothelial cells, epithelial cells, fibroblasts, mast cells, monocytes, neutrophils, plasmacytoid dendritic cells, pericytes, plasma cells, proliferating cells, T cells, and others (Figs. 4B–C). Cell composition analysis showed that neutrophils and endothelial cells were significantly increased in PD tissues compared with HC, but their proportions declined to near-normal levels after treatment (Fig. 4D). Notably, in the post-treatment group, the proportions of these cell types decreased to levels approximating those observed in the normal control group.

To further delineate molecular distinctions among cell subtypes, DEGs between HC and PD groups were identified using the FindMarkers function ($|\log FC| \geq 0.5$, $\min.pct \geq 0.1$) (Fig. 5A). GO and KEGG enrichment analysis was then performed (Fig. 5C). In fibroblasts, DEGs were significantly enriched in biological processes such as "extracellular matrix organization" and "extracellular structure organization," as well as the KEGG pathway "Focal adhesion." These findings suggest that fibroblasts contribute to structural degradation of periodontal tissues through aberrant remodeling of the extracellular matrix, potentially mediated by dysregulated collagen gene expression, thereby exacerbating gingival tissue instability. In neutrophils, DEGs were enriched in the GO pathway "cytokine-mediated signaling pathway" and KEGG pathways including "NF-kappa B signaling pathway" and the "NOD-like receptor signaling pathway." This indicates that neutrophils amplify local immune responses by activating inflammatory signaling cascades, consistent with their established role in releasing pro-inflammatory cytokines and recruiting additional immune cells. Endothelial cell DEGs were enriched in the KEGG pathway "Leukocyte transendothelial migration" and the GO pathway "positive regulation of cell adhesion," highlighting their role in facilitating immune cell infiltration into inflamed periodontal tissues. Collectively, these results demonstrate that pathological immune–stromal interactions underpin the persistence of local inflammation in PD.

We next compared PD and PDT groups to assess treatment effects (Fig. 5B). In neutrophils, differential expression of genes associated with the "NOD-like receptor signaling pathway" was reduced after treatment(Fig. 5D), suggesting that therapy suppresses innate immune receptor activation, decreases pro-inflammatory cytokine secretion, and attenuates local inflammatory responses. In fibroblasts, DEGs were enriched in the GO pathway "wound healing," implying that treatment promotes fibroblast-mediated repair, accelerates regeneration and remodeling of the gingival connective tissue, and supports restoration of periodontal architecture. Together, these findings demonstrate that PD is characterized by increased neutrophil- and fibroblast-driven inflammatory and degradative activity, while treatment partially reverses these changes by suppressing pro-inflammatory signaling and enhancing tissue repair programs.

3.3. Analysis of TNFRSF11A expression in monocytes

Building on the enrichment analysis that highlighted a strong association between PD and the "Osteoclast differentiation" pathway, and considering that osteoclasts originate from the monocyte lineage, we further examined the molecular characteristics of monocytes within this pathway. After batch correction and dimensionality reduction, monocyte populations were clustered into 15 subgroups (Fig. 6A). ased on canonical marker genes such as *CD1C*, *CD5*, *LAMP3*, and *ACP5*, these clusters were consolidated into 6 major subtypes: *CD1C* + dendritic cells (*CD1C* + DCs), *CD5* + dendritic cells (*CD5* + DCs), *LAMP3* + dendritic cells (*LAMP3* + DCs), macrophages, monocytes, and osteoclasts (Fig. 6B). The dimensionality reduction map illustrated the spatial distribution of these 6 subtypes within the monocyte compartment (Fig. 6C).

Analysis of Tumor necrosis factor receptor superfamily member 11A (RANK) (TNFRSF11A) expression revealed that overall expression was significantly elevated in the PD group compared with both HC and PDT groups (Fig. 6D). Subtype-specific assessment further demonstrated that osteoclasts exhibited the highest *TNFRSF11A* expression among all monocyte-derived

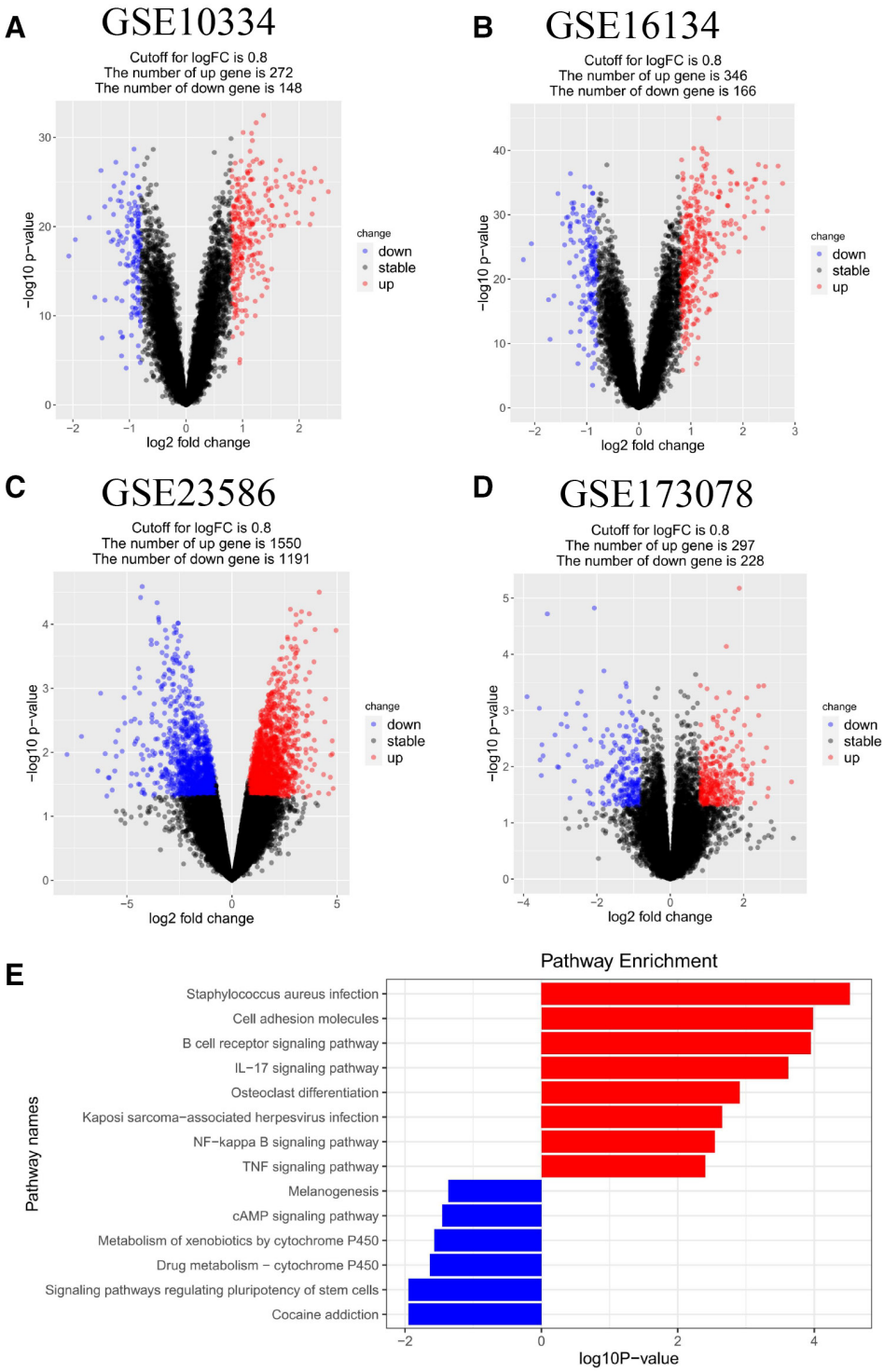

Figure 2. Outcomes of differential gene identification and pathway enrichment analysis. (A–D). Depict volcano plots illustrating differentially expressed genes across the GSE10334, GSE16134, GSE23586, and GSE173078 datasets, respectively. Genes exhibiting upregulation are marked in red, downregulation in blue, and those with stable expression in gray. The selection criteria applied were |log2FC| ≥ 0.8 and $P < .05$. (E) presents the results of pathway enrichment analysis for genes commonly differentially expressed. The vertical axis denotes the names of the enriched pathways, highlighting the principal signaling pathways enriched and their corresponding significance levels.

populations in PD (Fig. 6E). These findings underscore the pivotal role of the "Osteoclast differentiation" pathway in PD and suggest that osteoclasts actively drive this process through elevated *TNFRSF11A* expression. This provides direct molecular evidence for the contribution of osteoclasts to periodontal bone resorption.

3.4. The regulation of IL1B on fibroblasts TNFSF11 expression and its impact on osteoclast differentiation

Following the identification of elevated *TNFRSF11A* expression in osteoclasts, we next investigated the expression characteristics of its ligand, *TNFSF11*. Comprehensive analysis across all cell types demonstrated that *TNFSF11* expression was

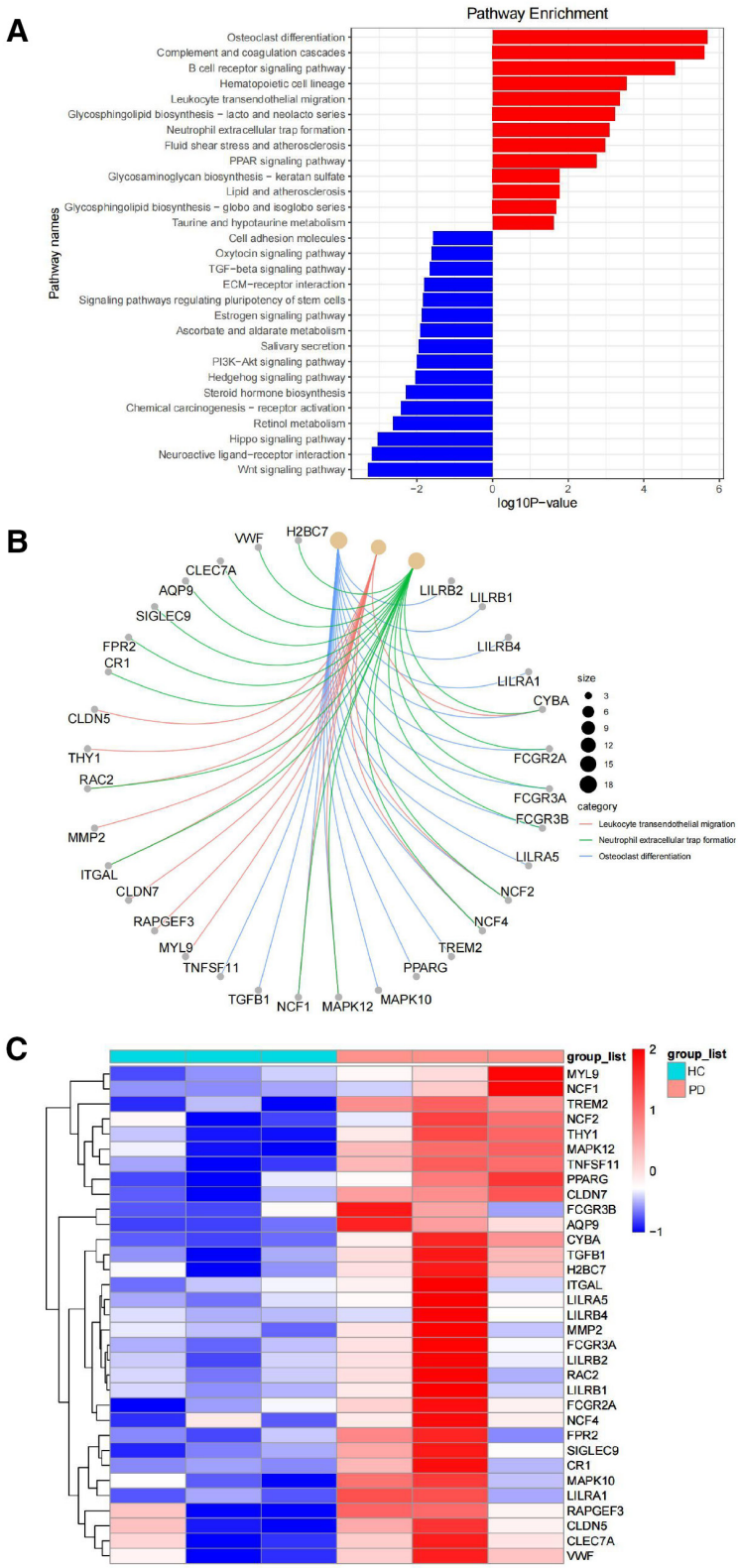

Figure 3. Pathway enrichment and key gene expression analysis of clinical sample transcriptome sequencing. (A) Pathway enrichment analysis was performed on transcriptome data comparing the periodontitis patient group (PD group) with the healthy control group (HC group). (B) The association network of key genes with enriched pathways is illustrated, where nodes represent genes, highlighting the strong connections between genes such as *NCF1*, *MAPK12*, the *LILRB* family, the *FCGR* family, and their associated pathways. (C). An expression heatmap of key genes in the periodontitis group (PD) and the healthy control group (HC) is presented. The color variations indicate differences in gene expression levels, providing a visual representation of the differential expression of key genes between the 2 groups.

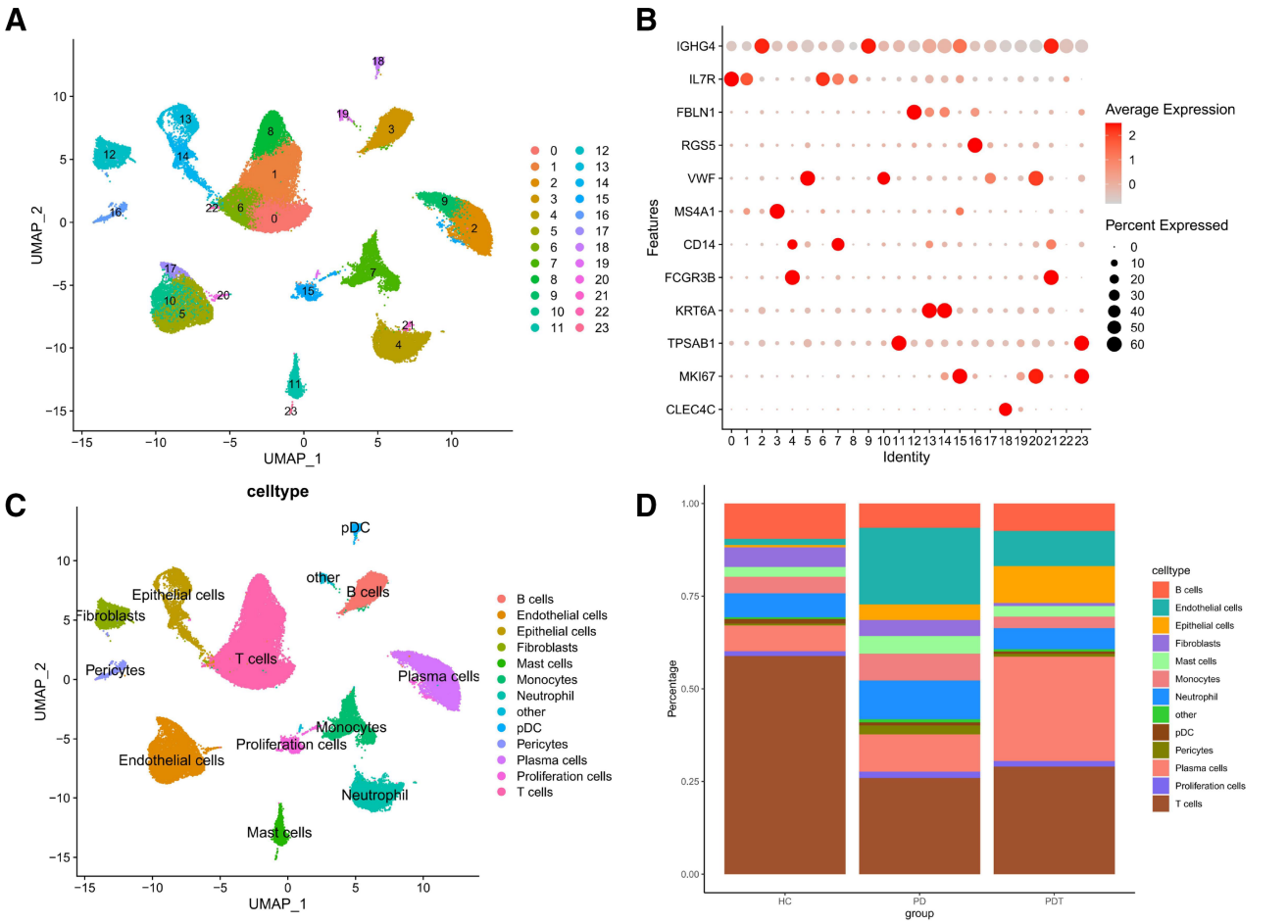

Figure 4. Analysis of dimensionality reduction clustering, cell subtype annotation, and group distribution in single-cell sequencing data. (A) graph depicting the results of dimensionality reduction clustering analysis, illustrating the distribution of 24 distinct cell clusters. (B) A graph detailing the expression characteristics of cell marker genes, where the horizontal axis denotes various clusters and the vertical axis represents cell markers. (C) A UMAP diagram illustrating cell subtype annotations, with colors indicating 13 primary cell subtypes, thereby demonstrating the clustering distribution of each subtype. (D) A statistical graph presenting the proportion of each cell subtype across the 3 sample groups. UMAP = uniform manifold approximation and projection.

significantly upregulated in the PD group compared with both HC and PDT groups (Fig. 7A). Subtype-level analysis within PD tissues revealed that fibroblasts exhibited the highest *TNFSF11* expression, both in terms of average expression levels and proportion of expressing cells (Fig. 7B). These results indicate that fibroblasts are the primary source of *TNFSF11* in PD and may facilitate osteoclast differentiation through interactions with *TNFRSF11A* on osteoclasts, thereby contributing to disease progression.

To explore the regulatory mechanism underlying *TNFSF11* upregulation in fibroblasts, we performed intercellular communication analysis. Compared with HC, neutrophil-derived *IL1B* in PD tissues was predicted to interact with *IL1R1* on fibroblasts (Figs. 7C–D). Co-expression analysis confirmed a positive correlation between *IL1R1* and *TNFSF11* expression in fibroblasts ($R = 0.32$, $P = .029$) (Fig. 7E). Considering the increased proportion of neutrophils in PD, we hypothesized that neutrophil-derived *IL1B* may induce fibroblasts to upregulate *TNFSF11*.

To validate this hypothesis, in vitro stimulation experiments were performed. Fibroblasts were treated with recombinant *IL1B* (experimental group) or left untreated (control group), and RANKL (encoded by *TNFSF11*) protein levels were assessed. Western blot analysis demonstrated that *IL1B* stimulation significantly increased RANKL expression compared with controls (Fig. 7F), directly confirming that *IL1B* promotes *TNFSF11* expression in fibroblasts.

Collectively, these findings support a mechanistic model in which neutrophil-derived *IL1B* activates *IL1R1* on fibroblasts, leading to upregulation of *TNFSF11*. The secreted *TNFSF11* subsequently binds *TNFRSF11A* on osteoclasts, activating the osteoclast differentiation pathway, thereby accelerating osteoclastogenesis and contributing to periodontal tissue destruction.

3.5. Subpopulation analysis of fibroblasts in HC and PD groups and identification of aberrant differentiation phenotypes

To explore the heterogeneity and functional attributes of fibroblasts in PD, fibroblasts were isolated from HC and PD groups and reanalyzed. Dimensionality reduction and clustering identified 11 distinct fibroblast subpopulations (Fig. 8A). Gene expression profiling revealed that clusters 1, 6, and 8 displayed markedly elevated expression of *TNFSF11* and *IL1R1* (Figs. 8B–C), suggesting that these subpopulations may play specialized roles in disease progression.

To further assess differentiation dynamics, pseudotime trajectory analysis was performed, which categorized fibroblasts into 7 distinct differentiation states (Fig. 8D). Group-specific mapping showed that fibroblasts in state 1 were predominantly derived from the PD group (Fig. 8E). Correspondence analysis demonstrated that state 1 aligned closely with clusters 1, 6,

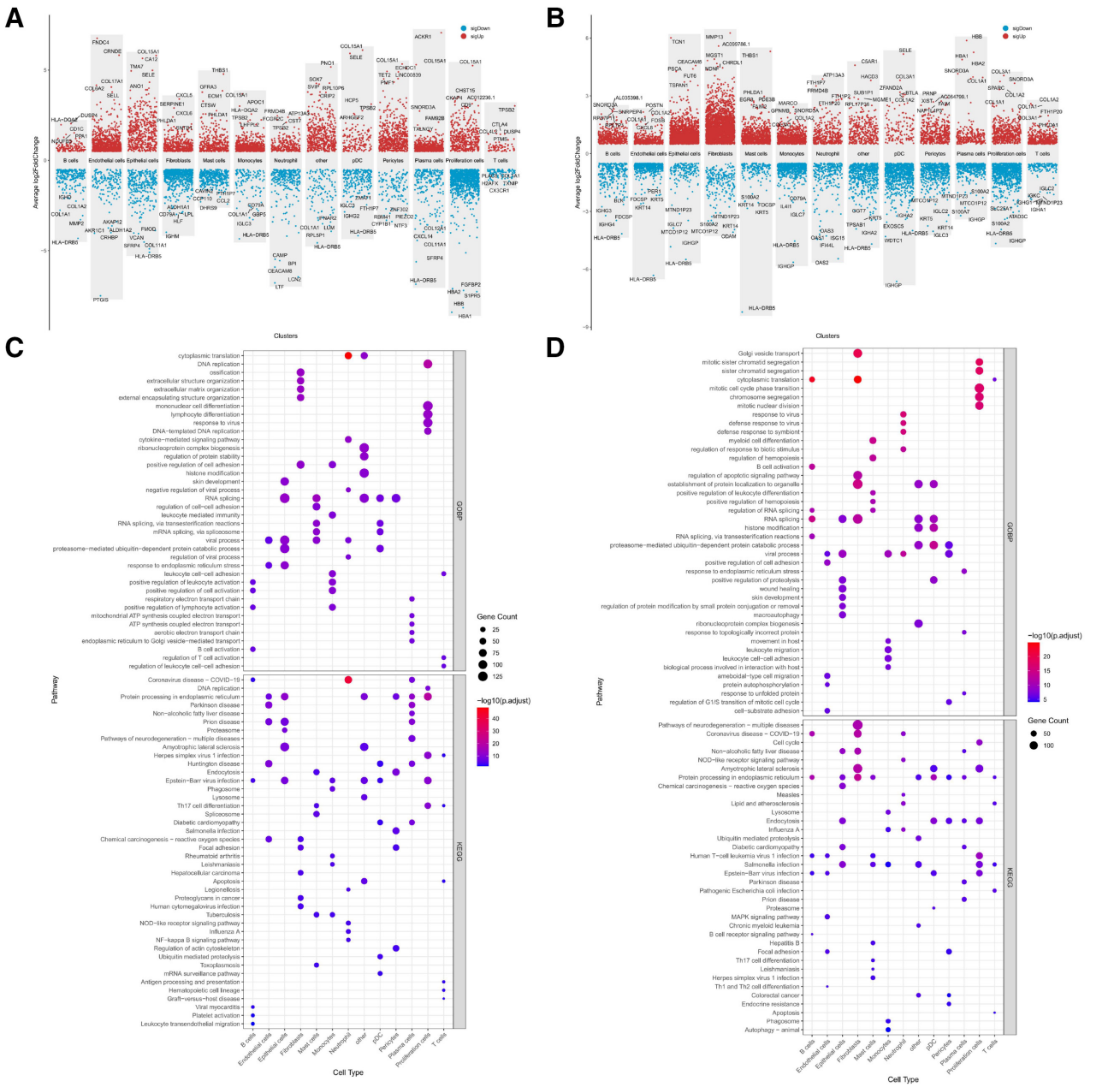

Figure 5. Analysis of differentially expressed genes and enriched pathways in various cell subtypes. (A–B). Differentially expressed genes between the PD group and the HC group (A), as well as between the PD group and the PDT group (B), were identified using single-cell sequencing data. (C–D). Pathways were derived from GOBP and KEGG enrichment analyses of the differentially expressed genes in each cell subtype identified in panels A and B. GOBP = Gene Ontology Biological Processes, KEGG = Kyoto Encyclopedia of Genes and Genomes.

and 8, the same subgroups characterized by high *TNFSF11* and *IL1R1* expression (Fig. 8F).

These findings indicate that in PD, fibroblasts undergo aberrant differentiation, giving rise to a distinct subgroup (clusters 1, 6, and 8) defined by co-upregulation of *IL1R1* and *TNFSF11*. This dysfunctional fibroblast phenotype may represent a critical driver of pathological fibroblast activity and disease progression in PD.

3.6. Elevated IL1B expression and NF-κB pathway enrichment in neutrophils of the PD

To investigate *IL1B* expression in neutrophils and its potential regulatory mechanisms, a targeted analysis was performed on neutrophils from both HC and PD groups. Compared with HC, neutrophils in PD exhibited significantly higher mean *IL1B* expression levels, as well as an increased proportion of *IL1B*-positive cells (Fig. 8G).

Pathway enrichment analysis of DEGs between HC and PD neutrophils revealed that genes upregulated in PD were significantly enriched in "NF-κB signaling pathway," "Osteoclast differentiation" and "Cytokine-cytokine receptor interaction" (Fig. 8H). Prior studies have established that the NF-κB pathway can positively regulate *IL1B* transcription, suggesting that the elevated *IL1B* expression observed in PD neutrophils is closely linked to NF-κB activation. These findings indicate that NF-κB–mediated upregulation of *IL1B* in neutrophils represents a key molecular mechanism by which neutrophils amplify inflammation and promote periodontal tissue destruction in PD.

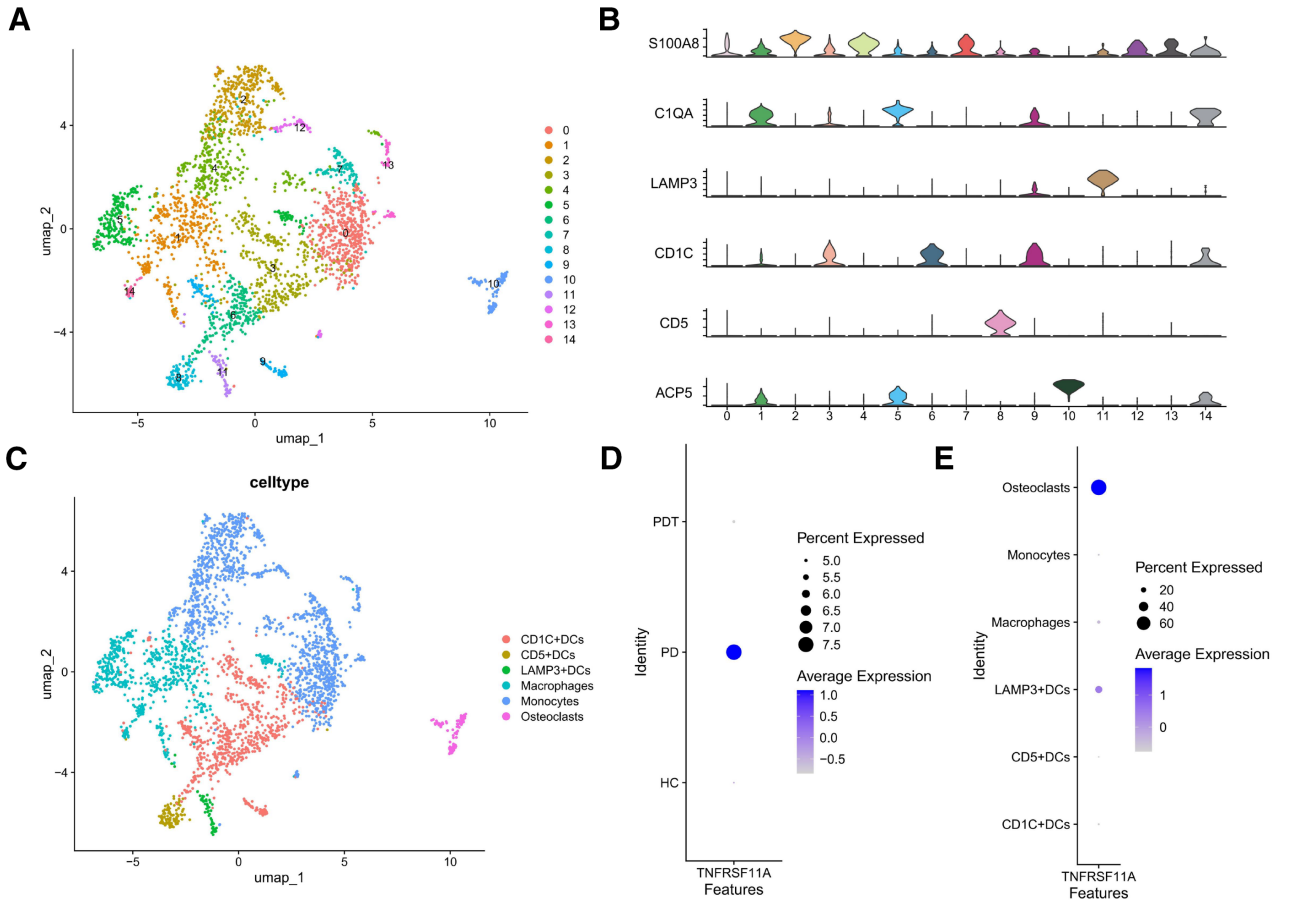

Figure 6. Analysis of mononuclear cell subsets and expression characteristics of *TNFRSF11A*. (A) Dimensionality reduction clustering plot of mononuclear cells, illustrating the distribution of 15 distinct cell clusters. (B) Annotation of each mononuclear cell cluster based on the expression of classical marker genes, including *CD1C*, *CD5*, and *LAMP3*. (C) A dimensionality reduction plot depicting the spatial distribution of 6 mononuclear cell subtypes, including *CD1C* + DCs, *CD5* + DCs, *LAMP3* + DCs, macrophages, monocytes, and osteoclasts. (D) Comparative analysis of *TNFRSF11A* expression in total mononuclear cells across different groups (HC, PD, PDT). (E) Comparative analysis of *TNFRSF11A* expression in the 6 mononuclear cell subtypes within the PD group, highlighting the highest gene expression in osteoclasts. HC = healthy controls, PD = periodontitis, PDT = Periodontitis post-treatment.

4. Discussion

This study systematically analyzed the molecular characteristics and cellular functional heterogeneity of gingival tissues affected by PD by integrating multi-omics data with experimental validation. The results highlight the pivotal role of the neutrophil-fibroblast-osteoclast axis in driving disease progression. Collectively, our findings provide experimental evidence that deepens the understanding of the pathological mechanisms of PD and informs the development of targeted therapeutic strategies.

By analyzing 4 GEO datasets, we identified that DEGs in PD patients were predominantly enriched in pathways such as "*S. aureus* infection," "Osteoclast differentiation" and the "NF-κB signaling pathway." Previous studies have confirmed a strong association between *S. aureus* infection and PD pathogenesis, as this pathogen disrupts the local immune environment by inducing inflammatory mediators and immune activation.[31]The enrichment of the "Osteoclast differentiation" pathway further reflects the enhanced bone resorption characteristic of PD, likely exacerbated by interactions among osteoclasts, fibroblasts, and neutrophils. These observations underscore the central role of osteoclast activity in periodontal tissue destruction and identify potential therapeutic targets within this pathway.

Single-cell transcriptomic analysis revealed a significant increase in endothelial cells and neutrophils in PD samples, both of which returned to near-normal levels after treatment. This suggests that these cell populations may serve as biomarkers for disease activity and therapeutic response. Among them, neutrophils exhibited particularly aberrant activation. Consistent with previous reports, our data confirm that NF-κB signaling plays a central role in PD by regulating inflammatory responses, immune cell activation, and osteoclast differentiation.[32] Specifically, we observed that neutrophils released large amounts of *IL1B* through NF-κB activation. *IL1B* subsequently bound *IL1R1* on fibroblasts, inducing robust upregulation of *TNFSF11* (RANKL). This paracrine interaction between neutrophils and fibroblasts establishes a mechanistic cascade of inflammatory cytokine release → *TNFSF11* induction → osteoclast activation and bone resorption.

Fibroblasts, as the predominant matrix cells of periodontal supporting tissues, play a dual role in both tissue homeostasis and disease.[33–35] In PD samples, fibroblast DEGs were significantly enriched in pathways such as extracellular matrix organization and focal adhesion, implicating them in structural degradation via aberrant ECM remodeling. Importantly, fibroblasts were identified as the primary source of *TNFSF11*, with specific subpopulations (clusters 1, 6, and 8) characterized by elevated *TNFSF11* and atypical differentiation phenotypes. These findings reveal the functional heterogeneity of fibroblasts and highlight that certain subsets not only lose their tissue-supporting function but also directly facilitate osteoclastogenesis by secreting RANKL. This establishes fibroblasts as a critical link between inflammation and bone resorption and suggests that targeting pathogenic fibroblast subsets may represent a novel therapeutic approach.

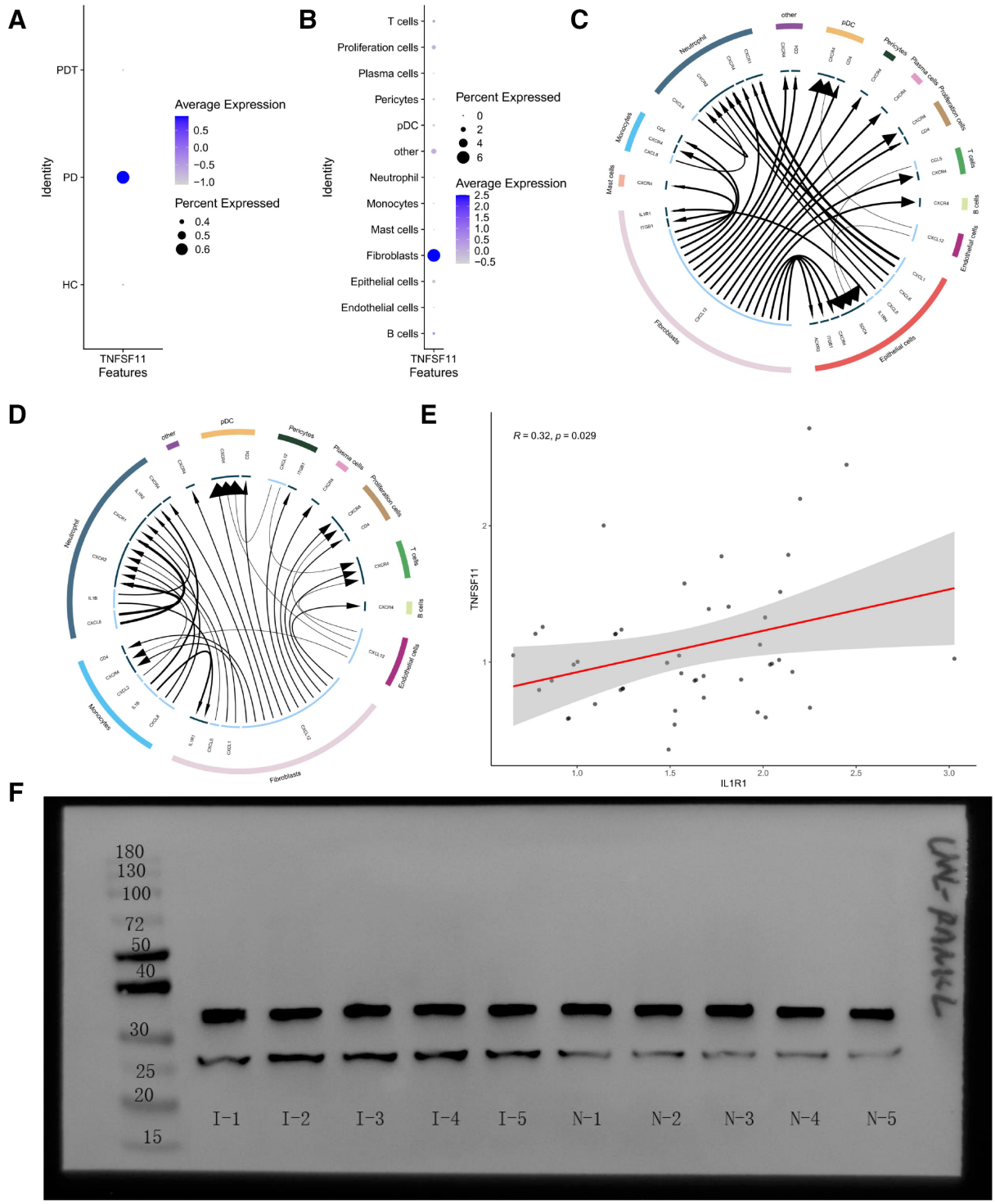

Figure 7. Mechanistic analysis of *IL1B* regulation on fibroblast *TNFSF11* expression. (A) The expression levels of *TNFSF11* were compared across the HC, PD, and PDT groups, revealing a notably elevated expression in the PD group. (B). The distribution of *TNFSF11* expression across different cell subtypes within the PD group was analyzed, indicating that fibroblasts exhibit the highest levels of gene expression. (C–D). Results from the cell communication analysis illustrate the interaction between *IL1B* secreted by neutrophils in the PD group and *IL1R1* located on the surface of fibroblasts. (E) A co-expression correlation analysis between *IL1R1* and *TNFSF11* in fibroblasts yielded an *R* value of 0.32 and a *P*-value of 0.029, indicating a positive correlation between these 2 factors. (F). The expression of RANKL, the protein encoded by *TNFSF11*, was compared in fibroblasts treated with *IL1B* in vitro against control groups, demonstrating that *IL1B* significantly induces elevated RANKL expression. HC = healthy controls, PD = periodontitis, PDT = Periodontitis post-treatment.

Analysis of monocyte subsets further demonstrated that *TNFRSF11A* (RANK) expression was highest in osteoclasts, and its overall expression was significantly elevated in PD compared with HC and PDT groups. This strongly supports the importance of the *TNFSF11–TNFRSF11A* axis in osteoclast differentiation. Taken together, our findings support a regulatory model of PD: neutrophils secrete *IL1B* → fibroblasts upregulate *TNFSF11* → osteoclasts undergo activation and differentiation

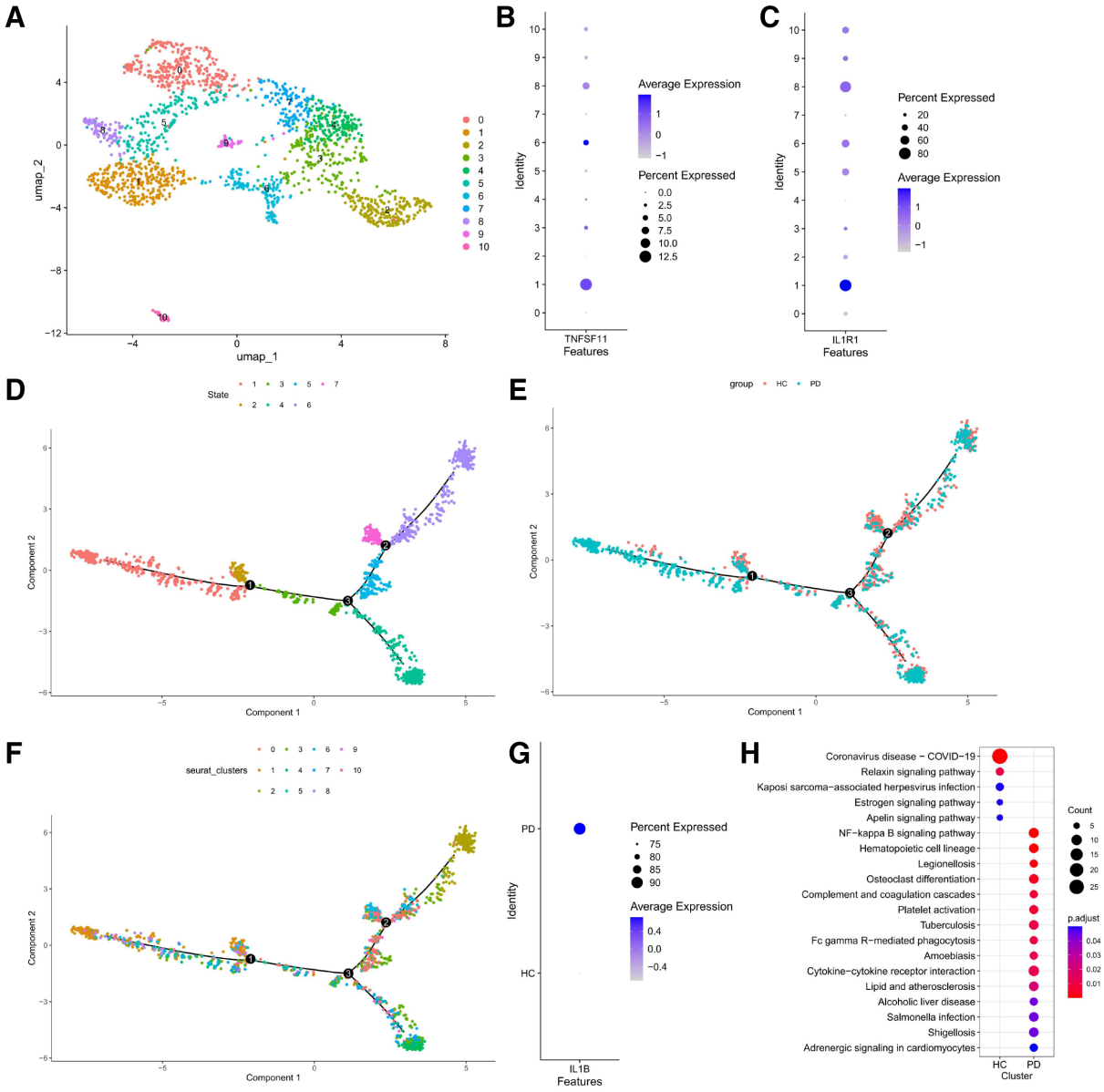

Figure 8. Analysis of fibroblast subpopulations and the regulatory mechanism of *IL1B* in neutrophils. (A). The results of dimensionality reduction clustering for fibroblasts derived from the HC group and the PD group illustrate the distribution of 11 distinct fibroblast subpopulations. (B–C). Expression profiles of *TNFSF11* (B) and *IL1R1* (C) across each fibroblast subpopulation, indicating elevated expression levels in clusters 1, 6, and 8. (D–E). Pseudotime analysis results for fibroblasts; panel D depicts the distribution of 7 differentiation states, while panel E reveals that state 1 predominantly originates from the PD group. (F). The relationship between fibroblast subpopulations and differentiation states demonstrates a significant overlap of state 1 with clusters 1, 6, and 8. (G). A comparative analysis of *IL1B* expression in neutrophils between the HC and PD groups, indicating a marked increase in expression within the PD group. (H). Pathway enrichment analysis of differentially expressed genes in neutrophils between the HC and PD groups. HC = healthy controls, PD = periodontitis, PDT = periodontitis post-treatment.

via *TNFRSF11A*. This model integrates multiple lines of evidence and provides a mechanistic framework for understanding bone resorption in PD.

The novelty of this study lies in coherently linking immune and stromal dimensions to establish a directional bridge between *IL1B*–NF-κB–driven inflammatory amplification and *TNFSF11* (RANKL)–mediated osteoclastogenesis. At the cellular level, we delineate disease-specific fibroblast and monocyte subpopulations and connect them to bone resorption through an interpretable, actionable pathway. Centering on these nodal molecules also yields translation-ready therapeutic entry points: inhibiting *TNFSF11* or *IL1B*, modulating osteoclast differentiation, and attenuating NF-κB signaling: thereby enabling risk stratification and individualized treatment. Compared with prior correlation-focused observations, our work advances mechanistic depth, source-to-effector clarity, and clinical translatability.

We acknowledge several limitations. The sample size and source could be expanded to enhance generalizability, and key inferences derived from transcriptional readouts warrant further functional and longitudinal confirmation to consolidate causality. Moreover, in vitro evidence may not fully recapitulate in vivo tissue ecology; future studies integrating spatiotemporal profiling and interventional testing will be valuable to validate and refine the proposed axis and implicated cell subsets.

In conclusion, this study identifies critical genes and signaling pathways central to PD progression, with particular emphasis on *TNFSF11*, *IL1B*, and NF-κB within immune and stromal cells. Targeted therapeutic strategies: such as inhibiting

TNFSF11 or *IL1B*, modulating osteoclast differentiation, or attenuating NF-κB–mediated inflammatory responses, may provide novel avenues for precision treatment. Furthermore, the identification of fibroblast and monocyte subpopulations with disease-specific functions establishes a foundation for future investigations into their pathogenic roles and therapeutic potential in PD.

Author contributions

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