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Original Article

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Exploration of the shared gene signatures and molecular mechanisms between periodontitis and inflammatory bowel disease: evidence from transcriptome data

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Abstract

Background: Periodontitis disease (PD) is associated with a systemic disorder of inflammatory bowel disease (IBD). The immune response is the common feature of the two conditions, but the more precise mechanisms remain unclear.

Methods: Differential expressed genes (DEGs) analysis and weighted gene co-expression network analysis (WGCNA) were performed on PD and Crohn's disease (CD) data sets to identify crosstalk genes linking the two diseases. The proportions of infiltrating immune cells were calculated by using Single-sample Gene Set Enrichment Analysis. In addition, a data set of isolated neutrophils from the circulation was performed via WGCNA to obtain PD-related key modules. Then, single-cell gene set enrichment scores were computed for the key module and grouped neutrophils according to score order in the IBD scRNA-seq data set. Single-cell gene enrichment analysis was used to further explore the biological process of the neutrophils.

Results: A total of 13 crosstalk genes (IL1B, CSF3, CXCL1, CXCL6, FPR1, FCGR3B, SELE, MMP7, PROK2, SRGN, FCN1, TDO2 and CYP24A1) were identified via DEGs analysis and WGCNA by combining PD and CD data sets. The enrichment analysis showed that these genes were involved in interleukin-10 signaling and inflammatory response. The immune infiltration analysis showed a significant difference in the proportion of neutrophils in PD and CD compared with healthy patients. Neutrophils were scored based on the expression of a periodontitis-related gene set in the scRNA-seq data set of IBD. The enrichment analysis demonstrated that inflammatory response, TNFα signaling via NF-κB and interferon-gamma response were upregulated in the high-score group, which expressed more pro-inflammatory cytokines and chemokines compared with the low-score group.

Conclusions: This study reveals a previously unrecognized mechanism linking periodontitis and IBD through crosstalk genes and neutrophils, which provides a theoretical framework for future research.

Keywords: periodontitis; inflammatory bowel disease; neutrophils; immune infiltration; inflammation; bioinformatics

Introduction

Periodontitis disease (PD) is a chronic inflammatory disease that progressively affects the integrity of the tissues supporting the teeth, with an estimated prevalence of 30%–50% in populations in developed countries [1]. Multiple interdisciplinary studies have shown that periodontitis is linked with various systemic diseases [2], such as type 2 diabetes mellitus [3]. Inflammatory bowel disease (IBD) is also one of the systemic disorders for which new significant evidence has been provided for an association with periodontitis. IBD is a group of immune-mediated, chronic inflammatory disorders of the gastrointestinal tract [4, 5], typically characterized by two primary entities: Crohn's disease (CD) and ulcerative colitis (UC) [6, 7]. Clinical studies have linked PD and IBD, suggesting that patients with IBD have higher prevalence, severity, and extent of periodontitis compared with those with no IBD [8]. Recent large-scale studies of Asian populations have also indicated a potential bidirectional relationship—that is, those being diagnosed with periodontitis have significantly increased risk of developing IBD, while those suffering from IBD are at a significantly increased risk of developing periodontitis [9, 10]. Both diseases have high prevalence around the world and affect the life quality of patients, although the precise mechanisms between PD and IBD have not been totally elucidated.

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Recent studies have shown that PD and IBD share common risk factors such as poor oral hygiene, smoking, diet, and psychosocial stress [11–13]. However, there is growing evidence that an essential link exists between PD and IBD beyond risk factors. Several pathophysiological mechanisms including microbiota, inflammatory cytokines, and immune cells were identified for the interplay between the two diseases [14]. Dysbiosis occurs in both periodontitis and IBD, suggesting microbiota is a connection mechanism of the two diseases [15, 16]. In the presence of IBD, physiologic oral microbiota is altered with a proliferation of pathobionts, reduction of commensals, and overall loss of diversity [17]. Moreover, amassed oral pathobionts (Klebsiella and Enterobacter species) in periodontitis are ingested and translocated to the gut, where they may elicit inflammatory responses in the colonic mucosa [18].

Furthermore, both PD and IBD involve an excessive inflammatory response in the intestinal or oral mucosa, respectively, to a microbial trigger in a susceptible host [19]. The inflammatory response of both diseases is characterized by massive tissue infiltration by neutrophils, cytokines activation including interleukin (IL)-1β, IL-6, IL17A, and tumor necrosis factor-alpha (TNF-α), and T helper 1 (Th1) and Th17 responses [20]. Higher expression of cytokines such as matrix metallopeptidase 8 (MMP8), IL-17A, IL-1β, and IL-21 in the periodontal tissues of IBD patients may increase the susceptibility to periodontal inflammation [21]. In addition, oral pathobiont-reactive Th17 cells generated in periodontitis can migrate to the inflamed gut and aggravate colitis [18]. Indeed, there also exists a similarity in the treatment for PD and IBD. Chi *et al.* [9] proposed that treatment of CD by steroids, aspirin, Plavix, and Licodin may protect against periodontitis. Exosomes derived from 3D-cultured mesenchymal stromal cells (MSCs) improve therapeutic effects in periodontitis and experimental colitis by restoring the Th17 cell/regulatory T cell (Treg) balance in inflamed periodontium [22]. These findings strongly suggested the interaction of these two pathological conditions, simultaneously exerting an influence on the development of PD and IBD. However, the relationship between PD and IBD at the genetic level has not been fully investigated. In view of this, we conducted a bioinformatics analysis of public databases to determine the shared pathogenesis of PD and IBD. Our results provide novel insights into the biological mechanisms underlying the development of these two diseases and may help in developing dual-purpose prevention methods.

Materials and methods

Data downloading and preprocessing

The gene expression data sets of periodontitis and IBD were obtained from the Gene Expression Omnibus by the R package "GEOquery." The accession numbers were GSE10334, GSE43525, and GSE208303 [23–25]. According to the annotation file downloaded from GEO, the probe matched to gene symbol, and the gene expression level for the same gene was normalized with the average gene expression. CD and UC single-cell RNA sequencing (scRNA-seq) data were downloaded from the single-cell portal (SCP259) [26]. We utilized the Seurat package to perform quality control, normalization, integration, batch correction, and t-distributed stochastic neighbor embedding (t-SNE) reduction [27], and the Celldex package for automated annotation of cell types [28].

Differentially expressed genes analysis

Differentially expressed genes (DEGs) in microarray data were analysed by using paired *t*-test with the limma R package and $|\log2FoldChange| > 1$, and adjust-*P*-value < 0.05 were set as the threshold [29]. RNA-seq data [23–25] were analysed using the edgeR R package [30]. The screening DEGs thresholds were $|\log2FoldChange| > 2$ and adjust-*P*-value < 0.05. Heat maps were generated by using the pheatmap R package. Ggplot2 plotted volcano maps. The hub gene network was distinguished through the protein–protein interaction (PPI) network and cluster analysis. The cluster analysis was performed using the "CytoHubba" algorithm with default parameters in Cytoscape software [31].

Immune cell infiltration analysis

The Single-sample Gene Set Enrichment Analysis (ssGSEA) method of the Gene Set Variation Analysis (GSVA) package was used to quantify the infiltration levels of immune cell types [32]. These specific gene sets of immune cells were obtained from the previous study [33]. The relative fraction of immunocytes between samples were compared using the Wilcox test. Venn diagrams were plotted using the R package VennDiagram.

Weighted gene co-expression network analysis

The weighted gene co-expression network analysis (WGCNA) algorithm aims to explore and reveal the correlations between gene modules and phenotypes [34]. In brief, the gene co-expression network was constructed based on the top 25% of genes with the highest variance and further transformed into a scale-free network by selecting an appropriate soft threshold β. Then the network was used to generate a topological overlap matrix, followed by hierarchical clustering to group genes with similar expression patterns. Finally, the eigengene was calculated to represent each module. The correlation between the gene module and specific traits was assessed using Pearson correlations, with *P*-values estimated using the corPvalueStudent function.

Gene functional annotation

Statistical analysis and visualization of functional profiles for genes from the WGCNA module were performed using the metascape webtool [35], which integrates various ontology sources such as the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway, Gene Ontology (GO) Biological Processes, and Reactome Gene Sets.

Identification of crosstalk genes

After identifying DEGs and the WGCNA module most relevant to PD and CD separately, they were imported into R software to obtain the intersection of PD DEGs, PD module, CD DEGs, and CD module. Those genes in PD and CD are supposed to be the key to the links between the two diseases, and we call them crosstalk genes. STRING databases were used to conduct the crosstalk genes PPI network [36, 37].

Single-cell gene set enrichment scoring and analysing

Single-cell gene set enrichment scores were computed for periodontitis-related WGCNA modules according to the methods of an earlier study [38]. We then grouped cells according to score order. The irGSEA package with the AUCell method was used to accomplish single-cell gene enrichment analysis.

Results

Identification and enrichment analysis of DEGs in GSE10334 and GSE208303

The workflow of data analysis is shown in Figure 1. To investigate the common mechanisms responsible for severe inflammation in PD and CD, we identified a total of 140 and 1,676 DEGs in PD and CD samples, respectively, compared with healthy control samples (Figure 2A and B). The top 50 upregulated genes and downregulated genes of PD and CD are visualized in Figure 2C and D. Next, we explored the related function and signal pathways of DEGs in GSE10334 and GSE208303. The enrichment analysis revealed that the DEGs in GSE10334 were enriched in response to bacterium, humoral immune response, leukocyte transendothelial migration, cell activation, neutrophil degranulation, etc. (Figure 2E). Similarly, inflammatory response, cell activation, regulation of cell activation, positive regulation of cytokine production, and regulation of immune effector process have been shown to be the important biological processes of the DEGs in GSE208303 (Figure 2F). The hub gene network shows that interleukin 1 beta (IL1B), C-X-C motif chemokine ligand 8 (CXCL8), and C-X-C motif chemokine receptor 4 (CXCR4) have the greatest significance among the DEGs in PD (Figure 2G), while IL1B, IL6, and IL10 have higher significance among DEGs in CD (Figure 2H). IL1B plays a central node in the hub gene network of both PD and CD. These results implied that both PD and CD were highly related to inflammatory and immune responses.

Identification and enrichment analysis of WGCNA in GSE10334 and GSE208303

To further explore the common feature of PD and CD, WGCNA of GSE10334 and GSE208303 was used to analyse the disease-related gene co-expression modules based on the correlation of gene expression profiles. After selecting appropriate soft-thresholding power and merging similar modules (Supplementary Figure 1), we found that the MEturquoise and MElightcyan modules were most relevant to PD and CD, respectively (Figure 3A and C). In addition, the scatter plot of gene significance vs module membership in the modules indicated that the genes were suitable for crosstalk gene mining (Figure 3B and D).

The genes in the MEturquoise module for PD were mainly enriched in cell activation and immune response-regulating signaling pathways (Figure 3E). Consistently with the enrichment analysis, we found that several immune-related signaling molecules such as the leukocyte common antigen (PTPRC, also known as CD45), the transmembrane immune signaling adaptor TYROBP, and the integrin subunit beta 2 (ITGB2) play central roles in the hub gene network of the MEturquoise module (Figure 3F). Functional enrichment analysis also indicated that genes of the MElightcyan module were enriched in inflammatory response, positive regulation of cytokine production, and cytokine signaling in the immune system (Figure 3G). Similarly, TNF, IL6, IL1B, and IL17A, which encode pro-inflammatory cytokines, were considered central genes with a higher degree in the MElightcyan module of CD (Figure 3H). Both DEG analysis and WGCNA demonstrated that PD and CD are both inflammatory diseases with strong ties to immune response.

Identification of crosstalk genes and immune infiltration

For more investigation of the relationship between PD and CD, the intersections of DEGs and WGCNA in PD and CD revealed 13 crosstalk genes, all of which were dysregulated in both PD and CD (Figure 4A). The STRING database was used to perform PPI network analysis of the crosstalk genes and the 13 crosstalk genes were acquired to visualize the PPI network (Figure 4B). In the PPI analysis, the 13 crosstalk genes may play pivotal physiological regulatory roles. These genes are significantly enriched in IL-10 signaling, inflammatory response, the complement system, and the regulation of defense response (Figure 4C).

In order to further investigate the potential function of neutrophils between PD and CD, immune cell infiltration was performed in GSE10334 and GSE208303. The analysis showed the differences in the proportion of various immune cells in gingival tissues from patients with periodontitis and intestinal mucosa from patients with CD. In our study, ssGSEA was used to identify the proportion of various immune cell types based on the gene expression profiles of GSE10334 and GSE208303. Significant differences in the proportions of neutrophils, monocytes, macrophages, and B cells were detected in GSE10334 (Figure 4D). In

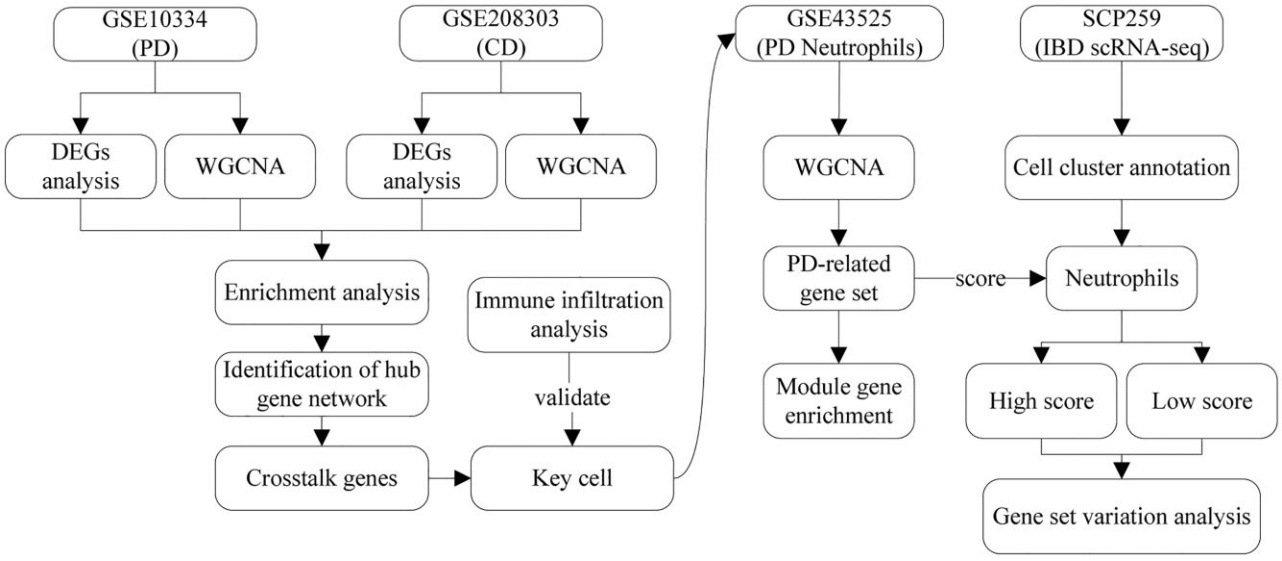

Figure 1. Flow chart of the data set analysis in this study. PD, periodontitis disease; CD, Crohn's disease; IBD, inflammatory bowel disease; scRNA-seq, single-cell RNA sequencing; DEGs, differential expressed genes; WGCNA, weighted gene co-expression network analysis.

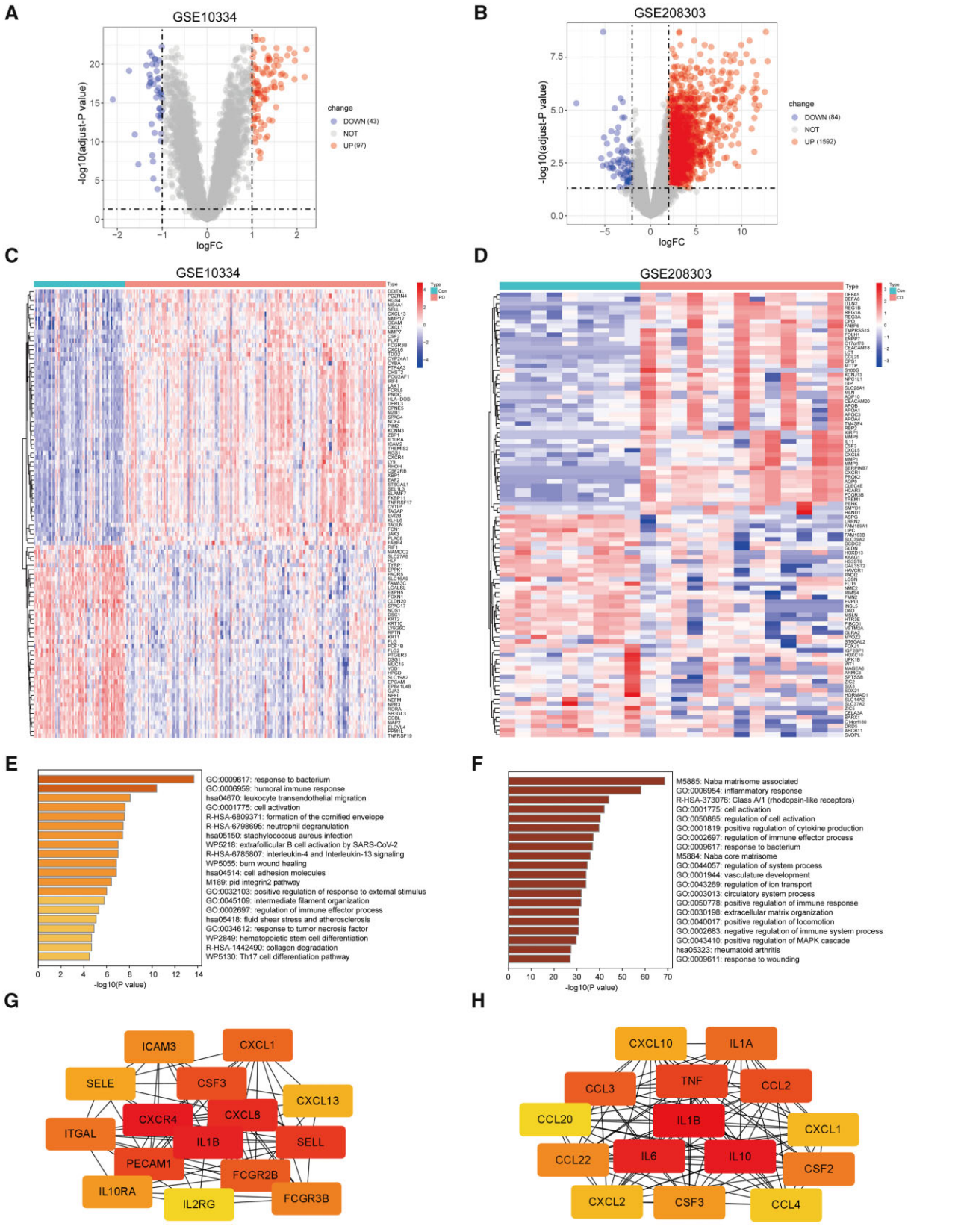

Figure 2. Identification and analysis of DEGs in GSE10334 and GSE208303. (A) and (B) Volcano plot of DEGs in GSE10334 and GSE208303 data set. (C) The heat map of the DEGs in GSE10334; PD represents the periodontitis affected gingival tissue samples and Con represents the periodontitis unaffected gingival tissue samples. (D) The heat map of the DEGs in GSE208303; CD represents inflamed intestinal mucosa samples from patients with active Crohn's disease and Con represents healthy intestinal mucosa samples from non-IBD patients. (E) and (F) The enrichment analysis of DEGs in GSE10334 (E) and GSE208303 (F). (G) and (H) The hub gene network of DEGs in GSE10334 (G) and GSE208303 (H). DEGs, differential expressed genes; IBD, inflammatory bowel disease.

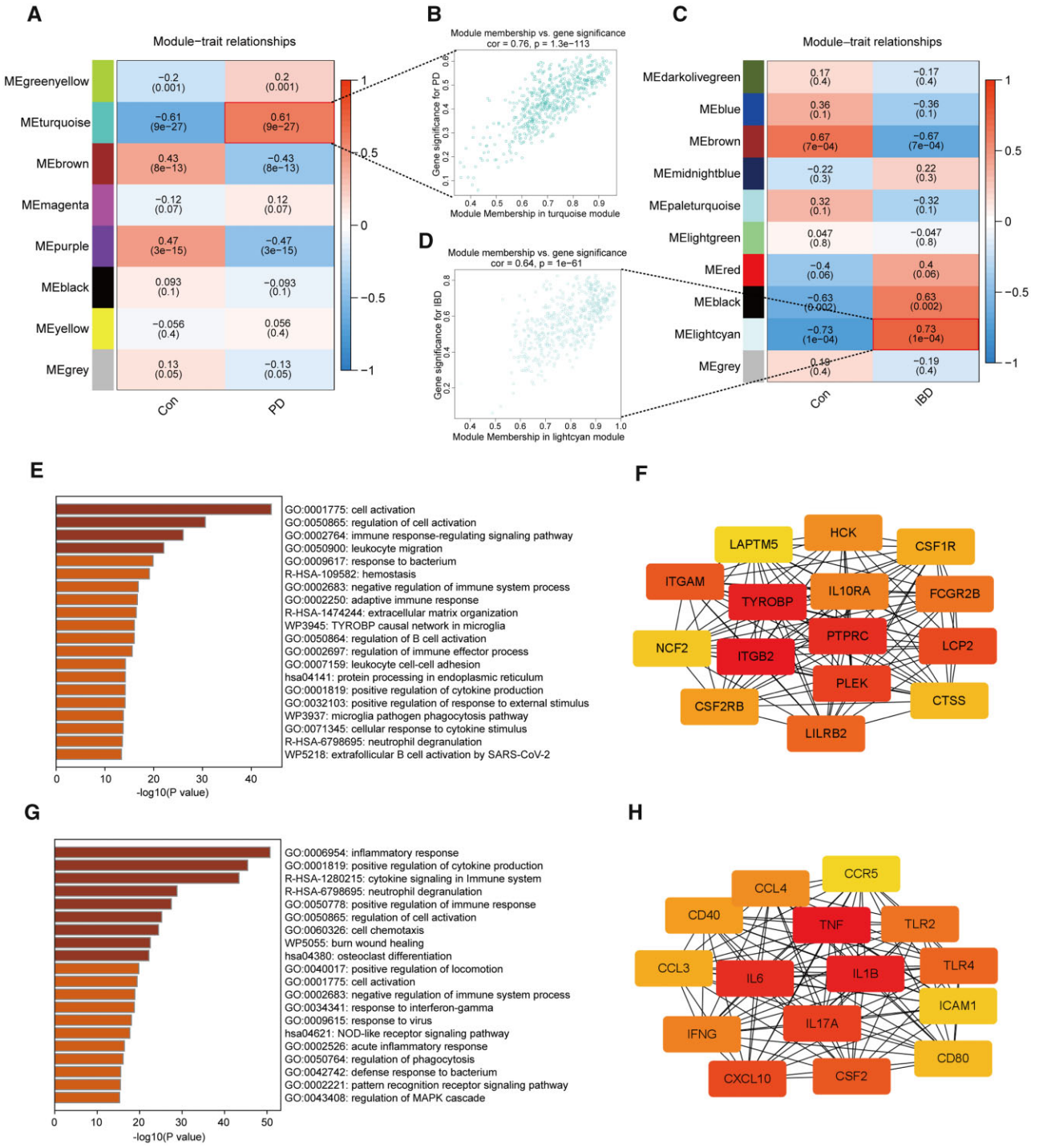

Figure 3. Identification and analysis of disease-related modules. (A) Heat map of module–trait correlations by WGCNA in GSE10334. (B) Scatter plot of gene significance for PD and module membership in the MEturquoise module. (C) Heat map of module–trait correlations by WGCNA in GSE208303. (D) Scatter plot of gene significance for CD and module membership in the MElightcyan module. (E) Enrichment analysis of MEturquoise module in PD. (F) Hub gene network of MEturquoise module in PD. (G) Enrichment analysis of MElightcyan module in CD. (H) Hub gene network of MElightcyan module in CD. WGCNA, weighted gene co-expression network analysis; PD, periodontitis disease; CD, Crohn's disease; Con, control.

addition, we found obvious differences between CD and normal intestinal mucosa tissue in 20 immune cell subpopulations (all *P*-values < 0.05) in GSE208303. Compared with normal tissue, CD tissue usually contained a higher proportion of neutrophils, monocytes, and macrophages (Figure 4E). Taken together, these results confirm the dysregulation of neutrophils not only in periodontitis but also in IBD. Due to the correlations among the immune cells and crosstalk genes above in PD and CD, we boldly assume that neutrophils may be the key cell linked to PD and CD together.

Identification of the periodontitis-related neutrophil gene set

To explore the potential connection of neutrophils between PD and IBD, we obtained a neutrophil gene set with a risk of periodontitis using WGCNA. The neutrophils with the gene set of

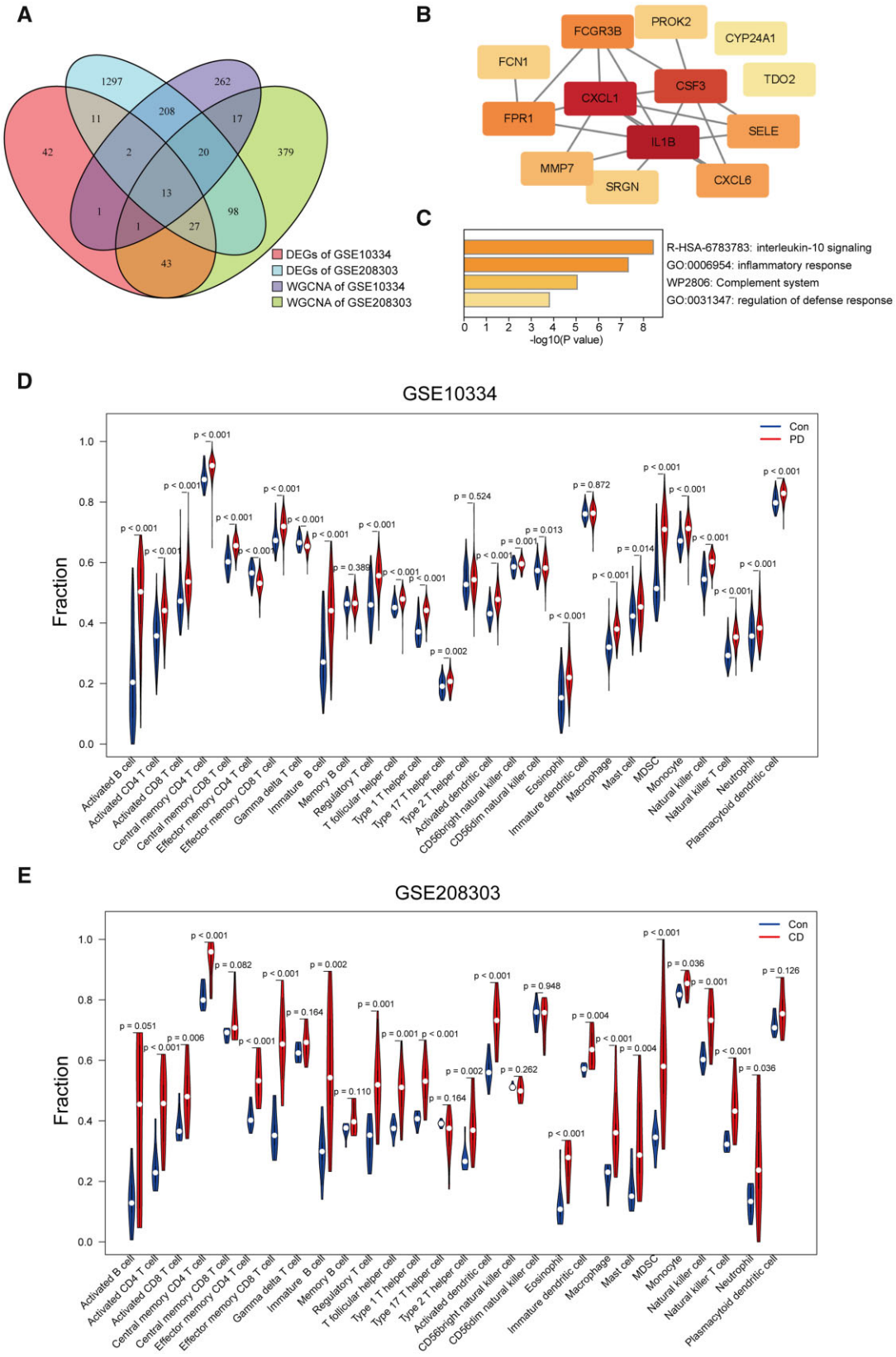

Figure 4. Identification of crosstalk genes and immune infiltration. (A) Venn diagram of crosstalk genes identified by WGCNA and DEGs in GSE10334 and GSE208303. (B) Protein–protein interaction network of crosstalk genes. (C) The enrichment analysis of crosstalk genes. (D) and (E) Violin plots of immune infiltration profiles in GSE10334 (D) and GSE208303 (E). The P-values of immune cell proportions between disease and control groups are marked on the top (Wilcoxon test). WGCNA, weighted gene co-expression network analysis; DEGs, differential expressed genes; PD, periodontitis disease; Con, control.

periodontitis may transmigrate to the gut, which could affect the severity of colitis. In the research, WGCNA and enrichment analysis of GSE43525, a PD data set of isolated neutrophils from the blood, were analysed. Selecting appropriate soft-thresholding power and merging similar modules are shown in Supplementary Figure 2. As for WGCNA in GSE43525, the two top modules displaying a strong correlation with periodontitis were MEwhite and MEmidnightblue (Figure 5A–C).

In the MEwhite module, GO and KEGG enrichment results showed that the genes were enriched in cellular homeostasis, regulation of calcium ion transmembrane transporter activity, protein maturation, and regulation of amine transport (Figure 5D). At the same time, the genes in the MEmidnightblue module were mainly enriched in neutrophil degranulation, positive regulation of protein phosphorylation, negative regulation of catalytic activity, negative regulation of cellular component organization, and negative regulation of phosphorylation (Figure 5E). In general, the genes in the MEmidnightblue module are associated with neutrophil degranulation, which was the most strongly correlated with periodontitis. Thus, the MEmidnightblue module was considered the periodontitis-related gene set of neutrophils and was selected for further exploration in IBD. The PPI network of this gene set is shown in Supplementary Figure 3.

Neutrophils with high expression of periodontitis-related genes in IBD exhibit enhanced inflammatory responses

To determine whether neutrophils with high expression of periodontitis-related genes in the gut can increase intestinal inflammation, we conducted a scRNA-seq analysis in data set SCP259 (including CD and UC data). The scRNA-seq immune cell profiles of healthy, inflamed, and uninflamed intestinal tissue were integrated using batch correction (Figure 6A) and the major cell types were labeled (Figure 6B).

We then classified neutrophils of IBD into two groups based on periodontitis-related gene set scoring. The enrichment analysis revealed that the biological processes, such as inflammatory response, TNFα signaling via NF-κB, interferon-gamma response, and p53 pathway, were activated in the high-score group (Figure 6C). Additionally, we showed the distinction in the inflammatory response between the high-score and the low-score groups (Figure 6D). Our analysis further demonstrated that genes encoding pro-inflammatory cytokines and chemokines such as IL18, IL1β, NLR family pyrin domain containing 3 (NLRP3), nuclear factor kappa B subunit 1 (NFKB1), oncostatin M (OSM), IL8, CXCL1, CXCL2, CXCL3, C–C motif chemokine ligand 2 (CCL2), and CCL4 were highly expressed in the neutrophils of the high-score group. In contrast, genes related to anti-inflammation such as transforming growth factor beta 1 (TGFB1), nuclear factor kappa B inhibitor alpha, and IL10 were mainly found in the neutrophils of the low-score group within IBD (Figure 6E). Collectively, these findings suggest that the neutrophils expressing risk genes for periodontitis may produce more pro-inflammatory cytokines in the gut, which may contribute to the severity of IBD.

Discussion

Periodontitis and IBD are both prevalent illnesses, posing a serious threat to public health. The shared pathogenesis of both diseases has been attracting intense research interest around the world. Several meta-analyses have integrated observational studies to show that patients with PD or IBD also have an increased risk of developing another disease [8, 39]. Patients with UC and CD have significantly increased odds for worse self-perceived oral health and severe periodontitis compared with controls, also losing more teeth [19]. Imai *et al.* [40] suggested that the short CD activity index increased in patients with CD with incipient periodontitis but declined or was unchanged in patients without periodontitis. Furthermore, differences in the oral and intestinal microflora have been reported, which may partly be due to specific characteristics of IBD and PD patients. IBD patients harbor higher numbers of bacteria related to opportunistic infections in the oral microflora [41]. Additionally, the gut microbiome of IBD patients is significantly more like the oral microbiome compared with that of IBD-free controls [40]. Indeed, PD and IBD share the inflammatory processes in which the key mediators involved in tissue damage are common, such as cytokines and immune cells [20]. These findings have increased our understanding of the oral–gut axis and may contribute to elucidating the molecular mechanism between IBD and PD. However, few studies have explored the common mechanism of IBD and PD at the genomic level.

Recently, several studies have focused on the interaction between different disease conditions from genomic perspectives by analysing transcriptome data from different tissues [42, 43]. This study is the first to integrate DEG, WGCNA, and single-cell GSEA to identify shared molecular and immune mechanisms associated with CD and PD. In this study, we first discussed the common mechanisms of CD and PD. The functional enrichment analysis of DEG and genes of hub modules showed that both PD and CD are correlated with immune cell infiltration, and their differential genes participate in biological processes such as the immune response-regulating signaling pathway, response to bacterium, and inflammatory response. *Porphyromonas* spp., a kind of complex oral pathogen for periodontitis, has been found in the presence of IBD intestinal specimens [44]. Simultaneously, oral administration of *Porphyromonas gingivalis* to mice is reported to disrupt the gut epithelial integrity and alter microbial composition [45]. Furthermore, a Th17 cell/regulatory T cells (Treg) imbalance has been reported in both periodontitis and IBD patients, characterized by overactivation of Th17 cells and a decrease in Treg [46, 47]. Kitamoto *et al.* [18] substantiated the ability of oral migratory Th17 cells to drive intestinal inflammation in mice, highlighting a shared inflammatory mechanism between the oral cavity and the intestine.

Then, 13 crosstalk genes, namely IL1B, CXCL1, colony stimulating factor 3 (CSF3), CXCL6, formyl peptide receptor 1 (FPR1), Fc gamma receptor IIIb (FCGR3B), selectin E (SELE), MMP7, prokineticin 2 (PROK2), serglycin (SRGN), ficolin 1 (FCN1), tryptophan 2,3-dioxygenase (TDO2), and cytochrome P450 family 24 subfamily A member 1(CYP24A1) were obtained through the intersection of genes of hub modules and DEG analysis. IL1B, CXCL1, and CSF3 are major genes that linked other genes together. IL1β is a classic pro-inflammatory cytokine primarily produced by monocytes or macrophages [48]. The elevations of IL-1β levels in IBD are associated with increased disease severity [49]. Further, serum IL-1β in patients with chronic periodontitis also reaches a high level, inducing a systemic effect [50]. Importantly, IL-1β in the inflammatory site accounts for increased local blood flow, leucocyte recruitment, and neutrophil infiltration [51]. Some scholars have suggested that the recruitment of neutrophils may require a sufficient release of mature IL-1β in periodontal or intestinal tissue to induce their chemoattraction [49]. Moreover, macrophages can attract neutrophils to the inflammatory sites and produce cytokines that control the lifespan and activity of the recruited cells [52]. In humans, CXCL1, with an elevated expression in both

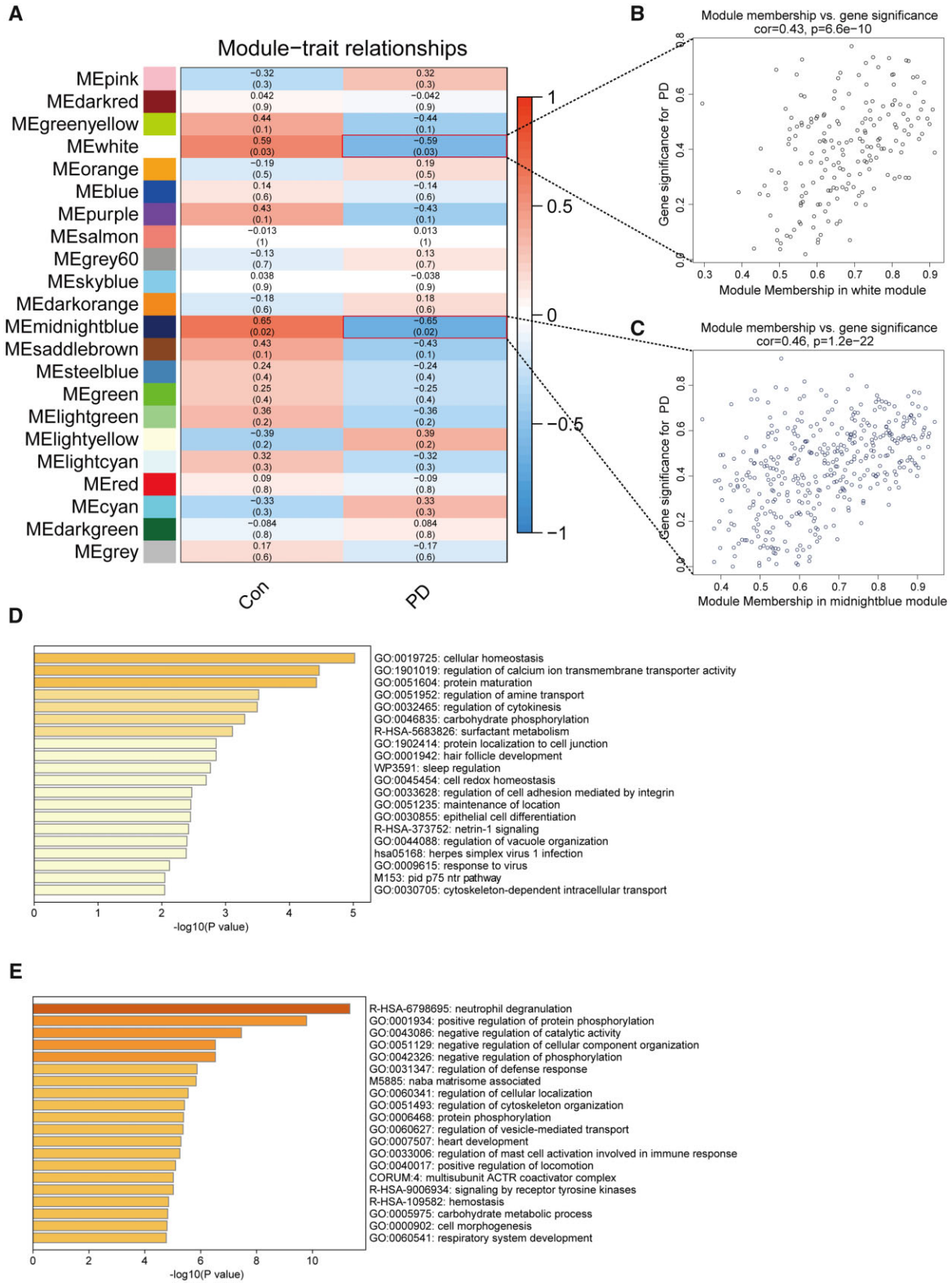

Figure 5. Identification of the periodontitis-related neutrophil gene set. (A) Heat map of module–trait correlations by WGCNA in GSE43525. (B) and (C) Scatter plot of gene significance for PD and module membership in the MEwhite module (B) and MEmidnightblue module (C). (D) and (E) The enrichment analysis of MEwhite module (D) and MEmidnightblue module (E). PD, periodontitis disease; Con, control.

inflamed gingiva and intestinal mucosa, can cause chemotaxis and infiltration of inflammatory response sites by neutrophils in PD and IBD [53]. CSF3 (also named granulocyte colony stimulating factor [G-CSF]) acts in hematopoiesis by controlling the production, differentiation, and function of the granulocytes. It is testified that CSF3 induces neutrophil release and

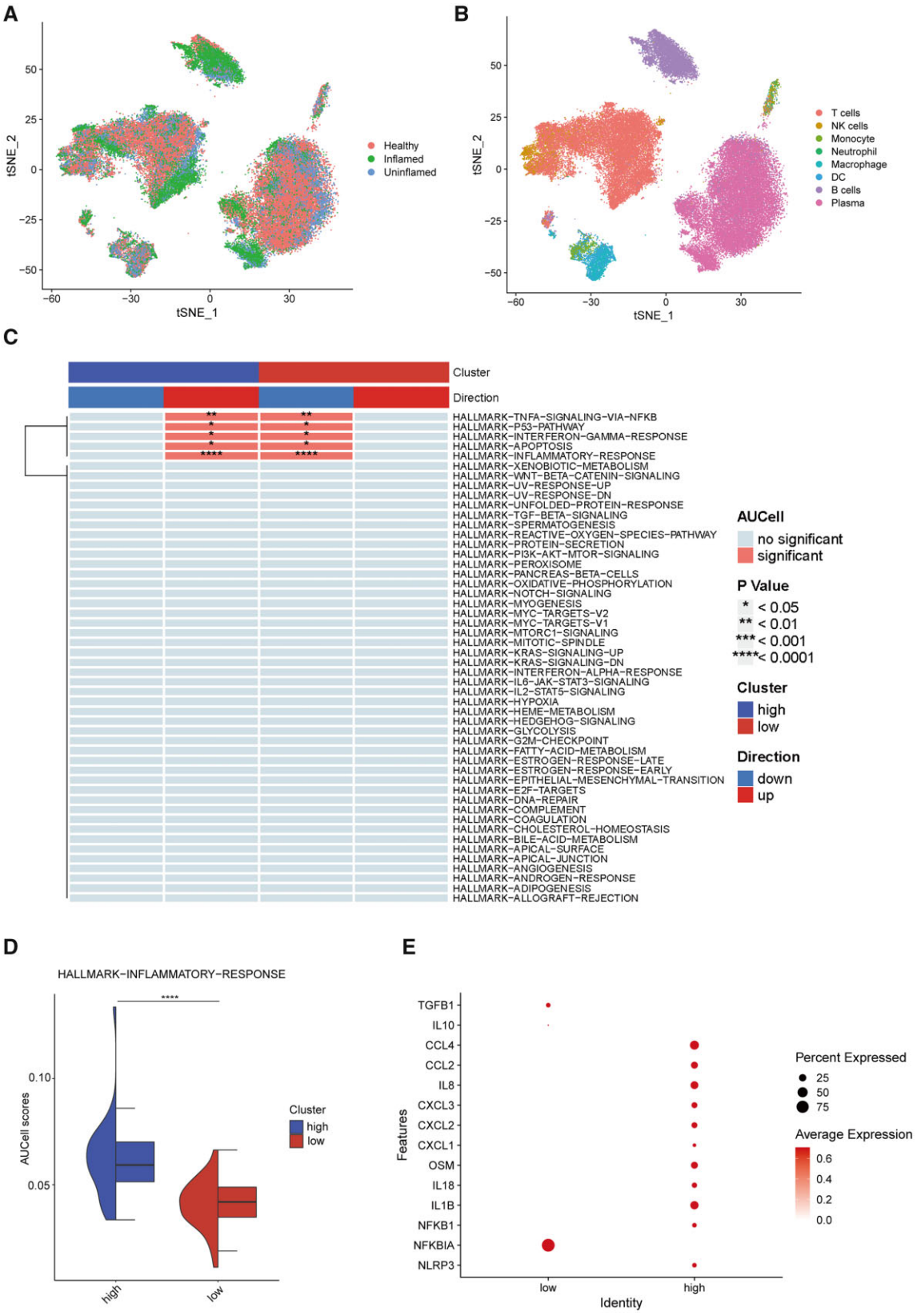

Figure 6. Neutrophils with high expression of periodontitis-related genes in IBD exhibit enhanced inflammatory responses. (A) t-Distributed Stochastic Neighbor Embedding (t-SNE) plot of immune cells colored according to the disease status of IBD. (B) Visualization of cell clustering by t-SNE plot identified by immune cell type (Wilcoxon test). (C) The enrichment analysis of the gene set with high-score and low-score neutrophils in SCP259 after PD-related gene set scoring. (D) Half-violin plots exhibiting the AUCell score of the gene set in inflammatory response compared by high-score group and low-score group. (E) Dot plot of cytokine and inflammatory-related genes expression in high-score group and low-score group. $P < 0.0001$; IBD, inflammatory bowel disease; NK, natural killer; DC, dentritic cell.

mobilization from the bone marrow [54]. Zhang *et al.* [55] found that CSF3 neutralization in periodontal inflammation could alleviate neutrophil infiltration and periodontal tissue destruction in experimental periodontitis. In addition, CXCL6 and FPR1 are also strong chemo-attractants involved in neutrophil recruitment and infiltration [56, 57]. A higher expression of them in the inflamed gingiva and intestinal mucosa have been ascertained. Interestingly, FPR1 can mediate its effects on chemoattracting macrophages [58], promoting phagocytosis, and increasing reactive oxygen species release [59]. Furthermore, various studies have identified SELE and MMP7 as participants in neutrophil production, differentiation, migration, and adhesion [60, 61]. In the crosstalk genes, FCN1, SRGN, and FCGR3B are also associated with immune responses, some of which are pattern-recognition receptors in innate immunity or involved in the formation of mast cell secretory granules [62, 63].

Next, we investigated the biological functions of crosstalk genes, revealing that these genes are enriched in the IL-10 signaling pathway, inflammatory response, complement system, and regulation of defense response. IL-10, a critical immunosuppressive cytokine, actively participates in the anti-inflammatory processes [64]. In human macrophages, IL-10 can suppress the production of IL-1β and, in the absence of this inhibitory signal, macrophages can produce IL-1β, which drives colitis [65]. IL-10 can also mitigate chemokine CXCL5 and CXCL1 expressions in IL-17-stimulated peripheral blood monocytic cells and peripheral blood monocytic cell-derived macrophages in periodontitis [66]. Notably, IL-10 is highly relevant to IBD and PD, as exhibited by causing severe intestinal inflammation and significantly more bone loss in IL-$10^{-/-}$ mice [66, 67]. In humans, polymorphisms in the IL10 locus confer susceptibility to IBD and periodontitis [68, 69]. Therefore, a disorder of IL-10 signaling might be the common mechanism of both diseases.

Neutrophils, a component of the innate immune system, are capable of producing several inflammatory cytokines and chemokines associated with intestinal and oral inflammation, such as IL-1β, IL-18, CXCL1, and CCL4 [56, 70]. Gustafsson *et al.* [71] have confirmed that neutrophils are hyper-responsive in PD. Recent studies have shown pathotype-associated neutrophils are increased in non-responders to several therapies of IBD [38]. Additionally, neutrophil infiltration regulated by IL-22 is associated with resistance to ustekinumab therapy in UC [72]. The majority of crosstalk genes identified in this study are associated with neutrophils, and we have also found that neutrophil infiltration was significantly upregulated in PD and IBD tissue compared with normal tissue. It has been reported that oral pathobiont-reactive Th17 cells that arise during periodontitis migrate to the gut and exacerbate colitis [18]. Although it is unclear whether neutrophils from periodontal tissue can transmigrate to the intestinal mucosa, prior studies have revealed that periodontal inflammation can prime circulating neutrophils in both mouse and human PD models [73]. Similarly to previous studies, our data have shown that neutrophils with elevated expression of periodontitis-related genes have promoted inflammatory responses in IBD. Further analysis revealed that pro-inflammatory cytokines and chemokines were mostly expressed in those neutrophils. Hence, neutrophils might play a critical role as a potential nexus in the crosstalk between periodontitis and IBD.

Some limitations to our study should be noted. First, our results are based on bioinformatics analysis and the samples may be biased, so further experiments are needed to confirm our conclusions. Second, the cohort in this study may be heterogeneous due to the inclusion of patients with both IBD and PD. Despite these limitations, our research offers new insights into the molecular and immune mechanisms underlying the oral–gut axis.

Supplementary Data

Supplementary data are available at *Gastroenterology Report* online.

Authors' Contributions

C.Z., Z.L., and Z.S. conceived and designed the study. C.Z., Z.Z., and Y.H. performed bioinformatics, analysed the data, and drafted the manuscript. S.H. generated the figures and interpreted the data. Z.L., F.H., and Z.S. supervised the study and reviewed the manuscript. All authors read and approved the final version of the manuscript.

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None.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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