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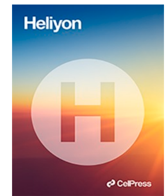

Research article

Transcriptome analysis on pulmonary inflammation between periodontitis and COPD

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ABSTRACT

Objective: The aim of this study is to investigate the correlation between periodontal disease and chronic obstructive pulmonary disease (COPD) from the perspective of gene regulation, as well as the inflammatory pathways involved.
Methods: Forty C57BL/6 mice were randomly divided into four groups: control group, chronic periodontitis (CP) group, COPD group, and CP&COPD group. Lung tissue samples were selected for messenger ribonucleic acid (mRNA) sequencing analysis, and differential genes were screened out. Gene enrichment analysis was carried out, and then crosstalk gene enrichment analysis was conducted to explore the pathogenesis related to periodontal disease and COPD.
Results: Results of enrichment analysis showed that the differentially expressed genes (DEGs) in the CP group were concentrated in response to bacterial origin molecules. The DEGs in the COPD group gene were enriched in positive regulation of B cell activation. The DEGs in the CP&COPD group were concentrated in neutrophil extravasation and neutrophil migration. The mice in the three experimental groups had 19 crosstalk genes, five of which were key genes.
Conclusions: Lcn2, S100a8, S100a9, Irg1, Clec4d are potential crossover genes of periodontal disease and COPD. Lcn2, S100a8, S100a9 are correlated with neutrophils in both diseases. Irg1 and Clec4d may bind to receptors on the surface of lymphocytes to produce cytokines and activate inflammatory pathways, this requires further research.

1. Introduction

Periodontal disease is one of the most common inflammatory diseases and the main cause of human tooth loss, characterized by periodontal pockets and alveolar bone resorption [1]. It can lead not only periodontal tissue destruction, but also to trigger systemic inflammatory response, thus promoting the occurrence and development of various systemic diseases [2]. Studies, both

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epidemiological and clinical, have established a correlation between periodontal disease and chronic obstructive pulmonary disease (COPD) [3,4]. Our research group has conducted multiple studies demonstrating a significant association between periodontal destruction and the severity of COPD [5]. Furthermore, we have found that high rates of tooth loss, poor oral hygiene, and infrequent brushing significantly increase the risk of acute COPD attacks [6]. COPD is the third leading cause of death worldwide, characterized by airway obstruction and dyspnea [7]. However, there are limited clinical studies exploring the correlation between periodontal disease and COPD, and the underlying mechanisms remain poorly understood. It is noteworthy that both diseases share common risk factors and pathogenesis [8]. Therefore, understanding the mechanisms underlying this correlation could help establish a causal relationship, providing a theoretical basis for the identification of COPD risk factors and the potential application of periodontal basic therapy in COPD prevention and treatment.

The immune response against periodontal pathogens is a key pathological mechanism in periodontal disease. Neutrophils play a pivotal role in the development of periodontal diseases, and periodontal disease is even called neutrophil-mediated disease [9]. Neutrophils produce enzymes, inflammatory factors and chemokines that mediate tissue destruction. These mediators not only damage the periodontal tissue but also enter the bloodstream, potentially contributing to the development of systemic diseases [10]. At advanced stage of periodontal disease, T lymphocytes and B lymphocytes become more dominant in the lesions. B lymphocytes interact with antigens through their immunoglobulin receptors and digest them, activating T lymphocytes. B cell activation is mediated by tumor necrosis factor family proteins and their corresponding receptors. Moreover, B cells exhibit a dual role, not only serving as effectors in antigen presentation but also functioning as mediators of immune responses. Through the secretion of cytokines, these cells play a pivotal role in reducing oxygen metabolites, nitric oxide, and antibodies, thereby shaping the immune landscape. As antigen-presenting cells, B cells have the remarkable ability to further amplify their activation state [11].

COPD is also a chronic inflammatory disease characterized by persistent airway limitation. It is further aggravated by the stimulation of toxic substances or gases, leading to a heightened inflammatory response [12]. The immune cells and cytokines are involved in the development of COPD. In patients at stable stage, there are numerous immune cells, including macrophages, neutrophils, T lymphocytes, B lymphocytes and dendritic cells, in airway and lung inflammation areas. A wide range of cytokines are also highly expressed, including tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin 1 beta (IL-1β), interleukin 6(IL-6), interleukin 17(IL-17), interleukin 18(IL-18), interleukin 32(IL-32) and thymic stromal lymphopoietin (TSLP) and growth factors, such as transforming growth factor-β(TGF-β) [13,14].

Periodontal disease and COPD are two chronic inflammatory diseases that share several common features and risk factors. The possible mechanism by which periodontal disease may contribute to the development or exacerbation of COPD involves direct and indirect pathways. The direct pathway involves the entry of periodontal bacteria into the lungs, where they can colonize and trigger a lung inflammatory response. Periodontal pathogens, such as *Fusobacterium nucleatum* (Fn) may affect the expression of matrix metalloproteinases 9(MMP9) through extracellular regulation kinase 1/2 and nuclear factor-k-gene binding (NF-κB) activation, thus inducing lung inflammation [15]. The indirect pathway involves the systemic circulation of inflammatory mediators released from periodontal tissue into the bloodstream. These mediators can reach the lungs and contribute to lung inflammation and injury. For example, periodontal diseases may induce the injury of lung tissue through macrophage TNF-α [16].

Previous studies conducted by our research team have also delved into the potential mechanisms connecting periodontitis and COPD. It has been found that 25-hydroxyvitamin D3(25-OHD3) can mitigate the concentration of RANKL, TNF-α and interleukin 1(IL-1) in serum, while increasing the concentration of interleukin 10(IL-10). This vitamin significantly reduces inflammation in periodontal tissue and also alleviates alveolar bone absorption, leading to improved lung function [17]. Moreover, genetic polymorphisms of Toll-like receptors 4 (TLR4)- rs1927907 and rs11536889-have been associated with the secretion levels of inflammatory factors IL-1β, IL-6 and TNF-α in the inflammatory pathway of chronic periodontitis and COPD. These gene variants may intensify the inflammation response [18]. Additionally, a bioinformatics analysis, utilizing available databases, has identified EPB41L4A-AS1, INSR and R3HDM1 as potential genes bridging the connection between COPD and periodontitis [19]. To gain a more comprehensive understanding of the correlation, tour team aims to explore the genes related to both diseases and the inflammatory pathways regulated by these genes through gene sequencing.

2. Materials and methods

2.1. Animal models building

Forty C57BL/6 male mice aged 6–8 weeks (purchased from Beijing Huafukang Biotechnology Co., LTD.) were fed for one week in the animal room of the Medical Research Center of Beijing Chaoyang Hospital under constant temperature of 26 °C and a 12-Hour light/dark cycle. During the experiment, the mice were provided with adequate food and water, as well as a quiet environment. The protocols in this study were approved by the Animal Experiments and Experimental Animal Welfare Committee of Capital Medical University (2020-067).

Forty mice were randomly assigned to four distinct groups, Group N(blank control), Group CP (chronic periodontitis), Group COPD (chronic obstructive pulmonary disease), and Group CC (periodontitis combined with COPD). The mice in Group N were maintained on a standard diet without any additional interventions. For Group CP mice, 7-0 sutures were placed around the maxillary second molars, and they continued to receive their regular diet. The COPD group mice were fed normally but were exposed to smoking for 2 h a day,5 days a week. The smoking sessions utilized Red Flag Canal cigarettes, each containing 11 mg tar. Over the course of the 2-h smoking period, a total of 2 packs of cigarettes (20 sticks/pack, 84mm/stick) were consumed using a specialized mouse smoking device (CIGARETTE SMOKE GENERATOR SG-300+NOSE-ONLY INHALATION SYSTEM, Shibata, Japan). The CC group mice underwent both

the ligation of their maxillary second molars and exposure to smoke, maintaining their regular diet throughout.

2.2. Sample collection

Fig. 1 outlines the experimental procedure. Six months later, mice were anesthetized, and their tracheas were intubated. Lung function was then assessed using Anires2005 system (Anires2005, Belambo Technology, Beijing, China). Following this, the right lung lobe was preserved in formalin for histopathological examination, while the left lobe was stored at −80 °C to facilitate RNA extraction and sequencing (Meiji Biology, Shanghai, China). Subsequently, maxillary alveolar bones and palatal gums were excised. The alveolar bones were soaked in hydrogen peroxide for 48 h, the gums were removed through boiling. The gums were then stained with 1% methyl blue (Zhongshan Jinqiao biological technology, Beijing, China), rinsed and air-dried. The absorption of alveolar bone on both sides of upper jaw was observed under a stereomicroscope (Leica M125C, Leica Microsystems, Germany) and photographs were taken under the same field of view. The height reduction was marked and measured using image J software for statistical analysis. The height was defined as the distance between the cemento-enamel junction and the lowest point of alveolar bone. Finally, paraffin sections were prepared and stained with hematoxylin and eosin (H&E) (hematoxylin and eosin, Zhongshan Jinqiao biological technology, Beijing, China) after fixing the gum and lung tissues. A Semi-quantitative analysis of inflammatory cells was performed on 4-μm sections. Inflammation scores were assigned to periodontal and lung tissues using the following scoring system: 0 = 0%–5% inflammatory cell (IC) infiltration,1 = 5%–25% ICs, 2 = 25%–50% ICs, and 3 = >50% ICs as recently reported [20]. additionally, the mean linear intercept of the alveoli in the lung and Bronchial wall thickness were measured and analyzed using Image-J software (Image J, National Institutes of Health and LOCI, America).

2.3. RNA extraction and sequencing

Total RNA was extracted from lung tissue samples using Trizol reagent(Trizol, Zhongshan Jinqiao biological technology, Beijing, China). The concentration and purity of the RNA were determined using the Nanodrop2000 spectrophotometer, with particular attention paid to the ratio of optical density at 260/280 nm (OD260/280). RNA integrity was further evaluated by agarose gel electrophoresis. RNA integrity number (RIN) was determined using the Agilent2100 Bioanalyzer with a minimum requirement of 1 μg of total RNA at a concentration of ≥50 ng/μL. The mRNA was then fragmented into small pieces of approximately 300 base pair. These fragments were converted into complementary DNA (cDNA) using reverse transcription for Illumina Hiseq sequencing.

2.4. Differently expressed gene analysis

Based on the quantitative expression data, we conducted an inter-group analysis of differentially expressed gene. Using the Deseq2 software (Max Planck Institute for Biinformatics, Germany), we analyzed the differential expression of genes based on read count data.

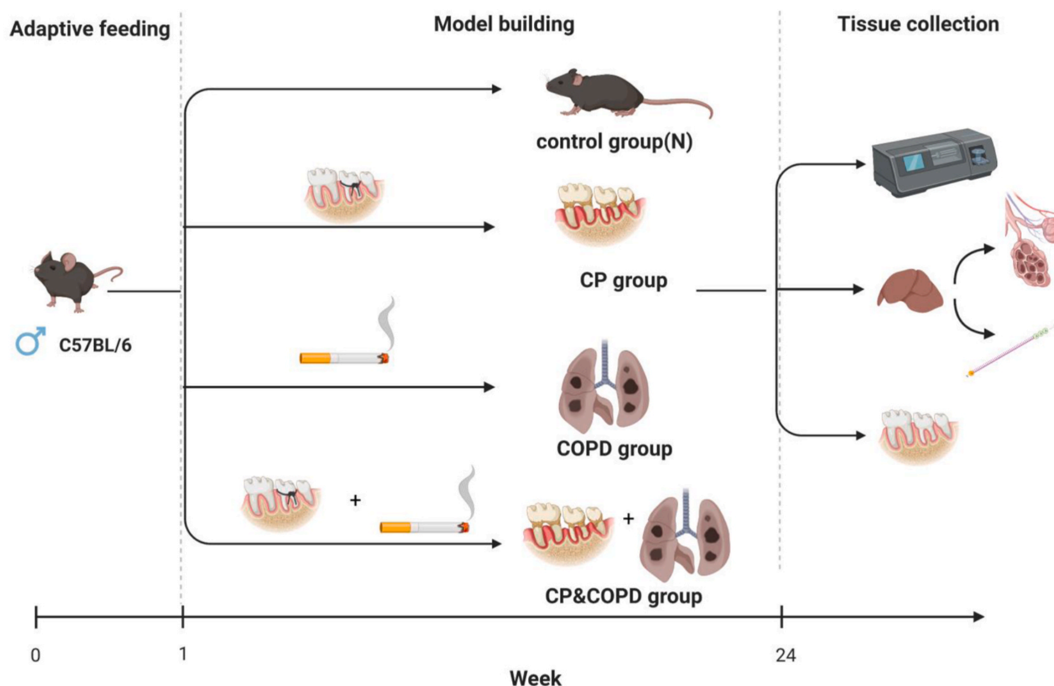


Fig. 1. Schematic diagram showing the design of the experiment.

This analysis allowed us to identify genes that were differentially expressed between the two groups. The screening criteria were set at a fold change of |log2FC| ≥1 and a statistically significant adjusted P-value of less than 0.05.

2.5. Bioinformatics analysis

Correlation and principal component analysis (PCA) were conducted using the corrplot and prcomp functions in R 3.6(R 3.6, Ross

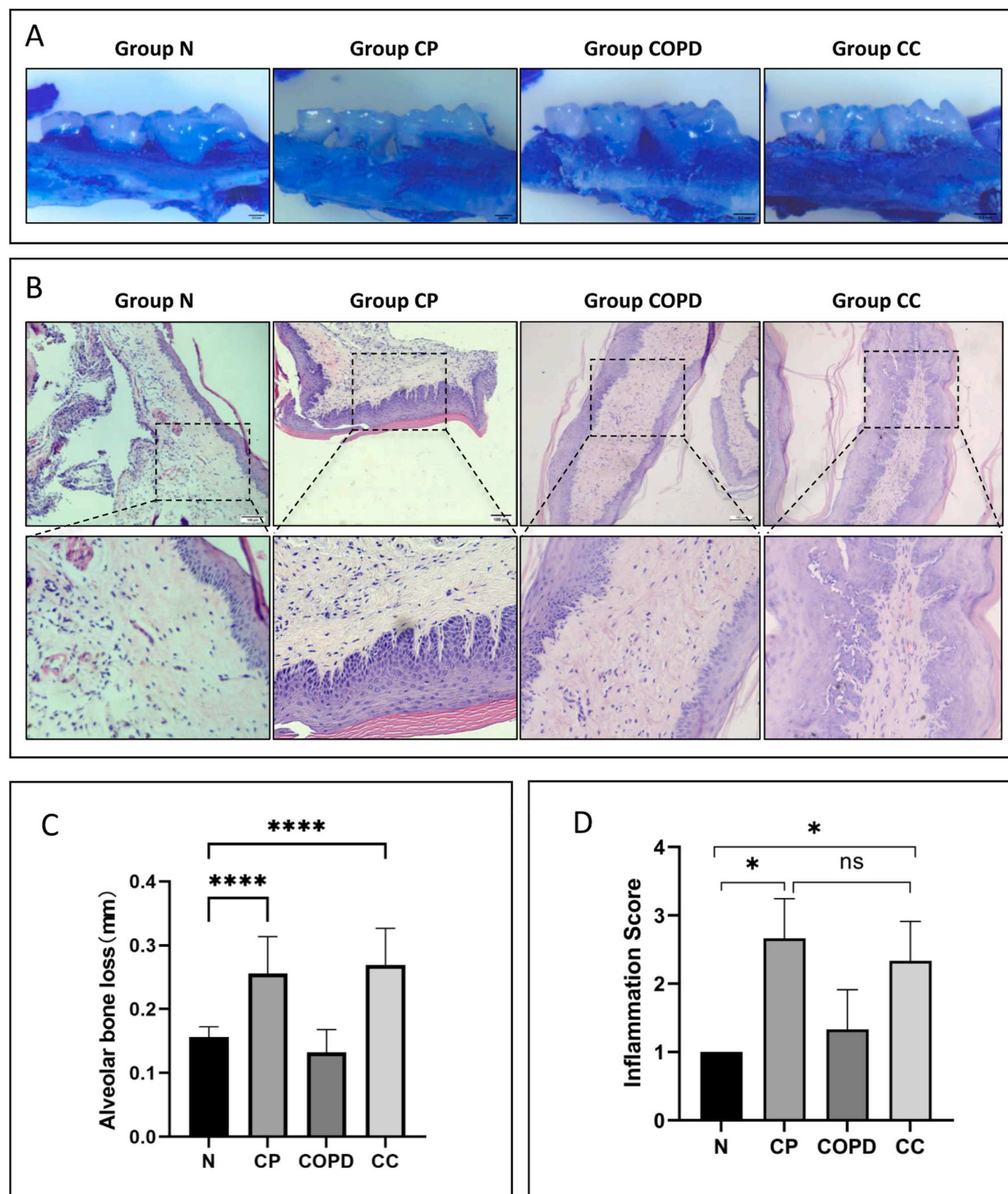


Fig. 2. Periodontal condition of mice in four groups. (A) Representative images from four group mice as indicated by alveolar bone loss (60 times, and the scale bar was 0.2 mm). (B) Representative images of HE staining of gingival tissues, the upper of photographs was taken under a microscope at 100× magnification (scale bar was 100 μm), with the same area magnified, and inflammatory cells (neutrophils, lymphocytes, macrophages) counted under the microscope. (C) Quantification analysis of alveolar bone resorption. (D) Inflammation score of the gingival tissues in HE pathology picture in the same area.

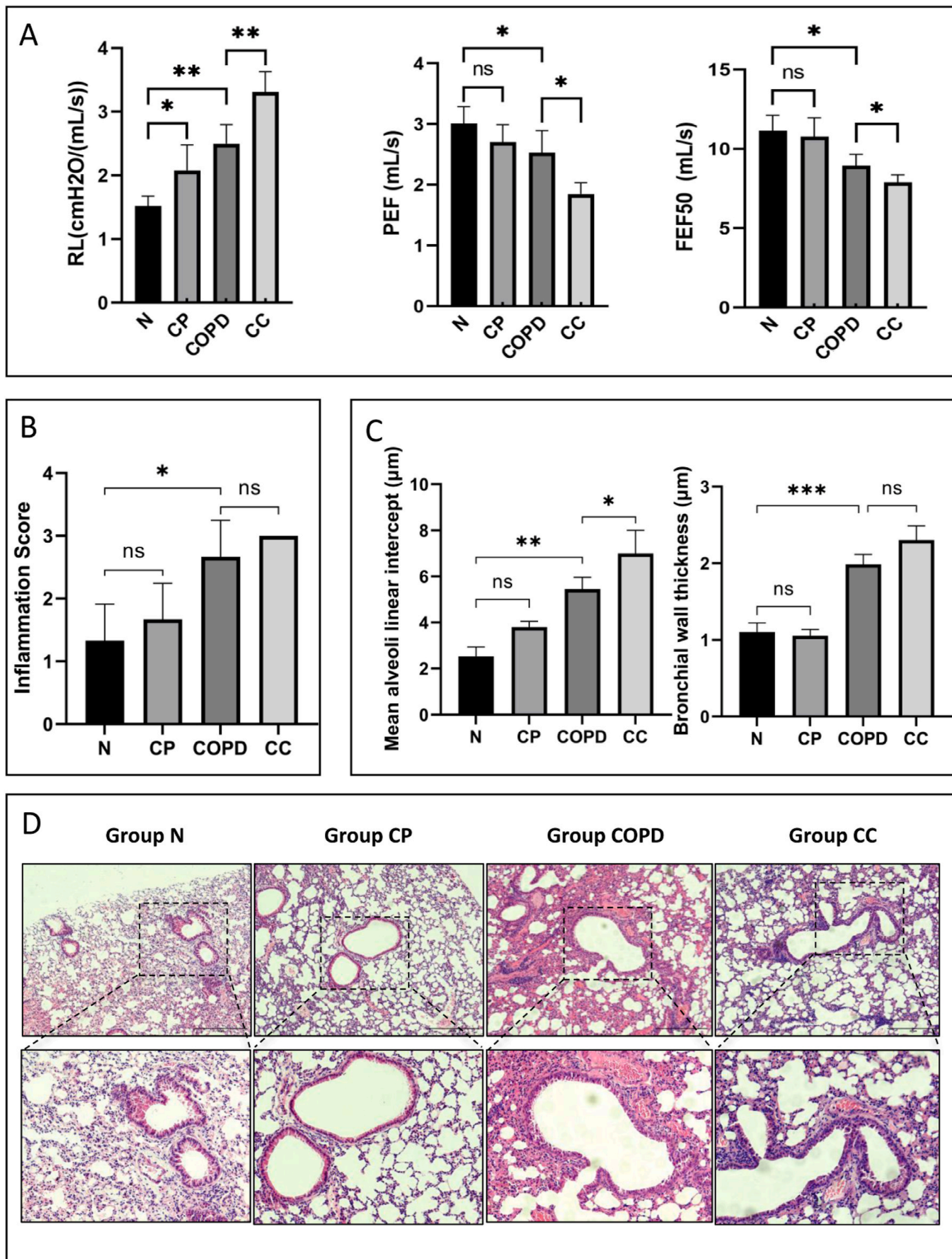


Fig. 3. Pulmonary condition of mice in four groups. (A) Quantifcation analysis of pulmonary function index, including RL,PEF,FEF50.(B) Inflammation score of the lung tissues in HE pathology picture in the same area.(C) Mean alveoli linear intercept and Bronchial wall thickness of the lung tissues in HE pathology picture with the magnified same area.(D) The left column of photographs was taken under a microscope at 100× magnification(scale bar was 100 μm), with the same area magnified, and inflammatory cells (neutrophils, lymphocytes, macrophages) counted under the microscope.

Ihaka and Robert Gentleman, University of Auckland, New Zealand), respectively. The volcano plot, Venn diagram, and heatmap were generated using ggplot2, Venn Diagram, and Complex Heatmap package in R3.6, respectively. Venn diagram of different genes was used to visualize the overlapping regions of different genes, by finding the intersection of different genes in each group. This representation highlights the relationship between genes in different group. Additionally, the quantity distribution of differential genes in samples of each group can be observed in Venn diagram.

Then, we conducted GO and KEGG enrichment analysis on the distinct gene sets of the three experimental groups. We initially explored unique signaling pathway of periodontal disease combined with COPD combined in the overlapping region. In the GO database, genes are classified according to the biological processes they participate in, the cells they constitute, and the molecular functions they perform. We utilized the Goatools to conduct the GO functional significance enrichment analysis for the differential genes, employing the Fisher exact test. To control the false positive rate, four multiple test correction methods (Bonferroni, Holm, Sidak and false discovery rate) were utilized to adjust the P-value. In general, a corrected P-value$\leq$0.05 was considered significant for GO function enrichment. For KEGG database analysis, genes were classified according to the participating pathway or function. KOBAS was used for enrichment analysis. The Fisher exact test was employed to calculate significance. The BH(FDR) method was used for multiple tests to control false positive rate. We defined the KEGG pathways were significantly enriched when the Corrected P-Value $\leq$0.05. Finally, we used CytoHubba software (CytoHubba, NCBI, India) to identify the top 5 key genes among 19 crosstalk genes from the perspectives of MCC, MNC, Degree and EPC.

2.6. Statistical analysis

Firstly, we used SPSS 17.0 program (SPSS, Chicago, IL, USA) to test the normality of the data, and the histogram combined with non-parametric test was used to verify the normal distribution of the data. Next, GraphPad Prism v8.0.2 (Prism, GraphPad company, San Diego, Calif., USA) was used for statistical analysis. All quantitative data are presented as M $\pm$ S(means $\pm$ standard). Statistical analyses were performed using one-way analysis of variance. T-test was used to identify statistically significant differences. Values of $P < 0.05$ were considered statistically significant.

3. Results

The data obtained from the experiment included alveolar bone resorption height, airway resistance, the peek expiratory flow (PEF) and forced expiratory flow 50% (FEF50), mean alveolar intercept and bronchial wall thickness were count data, gingival inflammation index and lung inflammation score were semi-quantitative data. The normality test was conducted on these data. Although they were not close to normal distribution, but did not appear very skewed.

4. Animal model

4.1. Periodontal status

The mice in the CP group and the CP&COPD group exhibited significantly lower alveolar bone height compared to the control group (Fig. 2A and C). The histopathological examination of the gingival tissue in the CP group and the CP&COPD group mice revealed notable hyperplasia of the gingival epithelial nail process, a thicker epithelial layer, and more pronounced inflammatory cell infiltration, which was concentrated in the epithelium and submucosa (Fig. 2B). As shown in Fig. 2D, inflammation scores were significantly higher in the gingival tissues of the CP and CC group mice than in the control group.

4.2. Lung function and pathology

As illustrated in Fig. 3A, the airway resistance in the CP group, COPD group, and CP&COPD group was significantly higher compared to the control group. Notably, the airway resistance was even higher in the CP&COPD group compared to the COPD group. Additionally, the peek expiratory flow (PEF) and forced expiratory flow 50% (FEF50) were significantly reduced in the COPD group compared to the control group. Moreover, these parameters were even further reduced in the CP&COPD group compared to the COPD group.

The histopathological examination of lung tissue revealed the alveolar structure in control group mice was intact, with only a small number of inflammatory cells present in the alveolar interval. In contrast, the alveolar structure of CP group mice exhibited partial broken, with slightly enlarged alveolar septum and increased inflammatory cell infiltration within the alveolar interval. In the lung tissue of COPD group mice, there was a notable enlargement of alveolar cavities and significant thickening of the bronchial wall with a large number of inflammatory cells were observed infiltrating the tissue, often accompanied by persistent bleeding. Alveolar space expanded largerly in CP&COPD group mice, along with notable bronchial wall thickening and mass of inflammatory cells infiltration (Fig. 3D). In Fig. 3B and C, the lung inflammation score, mean alveolar intercept and bronchial wall thickness in the COPD and CC groups were significantly elevated compared to the control group. Notably, the mean alveolar linear intercept was significantly wider, the inflammatory score and bronchial wall thickness tended to be higher in mice of the CC group than in the COPD group.

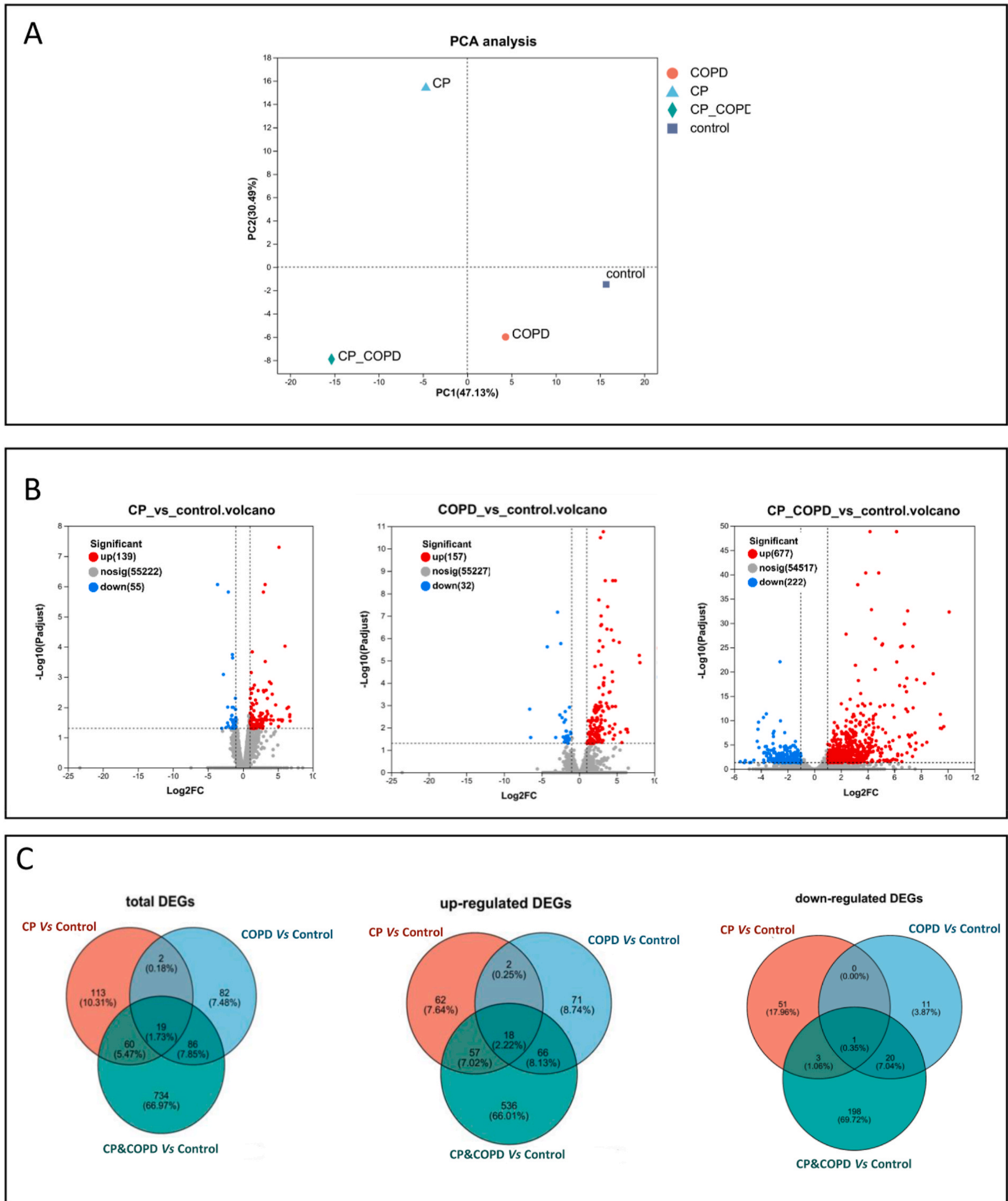


Fig. 4. Comprehensive analysis of differential expressed genes (DEGs). (A) The principal component analysis of four groups. (B) Comparison of DEGs in control, CP, COPD, and CP&COPD groups using volcano map. Red dots represent upregulated DEGs, blue dots represent downregulated DEGs. (C) The overlap and unique DEGs in each group using Venn diagram. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

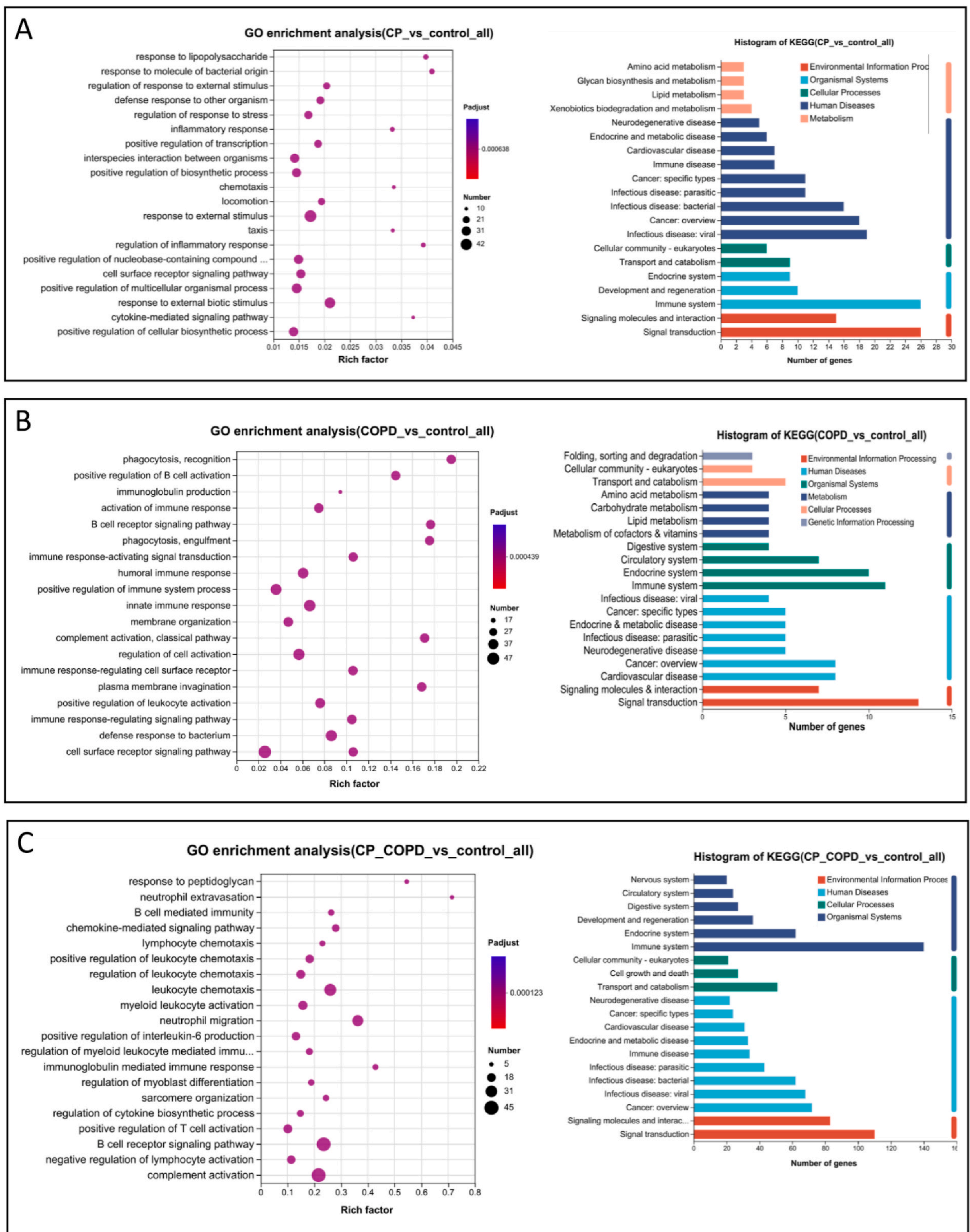


Fig. 5. GO and KEGG enrichment analysis of differentially expressed genes in CP group, COPD group, and CP&COPD group. (A) CP group vs Control. (B) COPD group vs Control. (C) CP&COPD group vs Control.

4.3. RNA-Seq transcriptome data of lung tissue

A total of 14996, 14018, 14530, 14462 genes were extracted from the lung tissue of mice in control group, CP group, COPD group and CP&COPD group mice, respectively. The percentage of Q30 bases exceeded 93.65%. The PCA results showed that PC1, which accounted for 47.13% of the variance, clearly distinguished the experimental groups from the control group, PC2, which accounted for 30.49% of the variance, separated the three experimental groups (Fig. 4A).

4.4. Differential expressed gene (DEG) analysis

Compared with control group, there were 194, 189, and 899 significantly differentially expressed genes (DEGs) reported in CP group, COPD group and CP&COPD group, which comprised 139, 157, and 677 upregulated genes and 55, 32, and 222 downregulated genes. The detailed information of significant DEGs is shown in Fig. 4B.

As showed in Fig. 4C, Venn diagrams revealed the overlapping and uniquely expressed genes in the three experimental groups. There were 19 DEGs shared by all groups; 79 DEGs overlapping between the CP&COPD and CP groups, and 105 DEGs overlapping between the CP&COPD and COPD group. Notably, the overlap ratio of DEGs between CP&COPD and COPD groups was more significant compared to that between the CP&COPD and CP groups. These results suggest that periodontitis and COPD share crosstalk genes. Combined with the pathological findings, we concluded that CP is likely to exacerbate COPD as a risk factor.

4.5. Potential signaling involving in the CP, COPD, CP&COPD model

We performed an enrichment analysis on differentially expressed genes in the CP group, COPD group, and CP&COPD group, aim to decipher the signaling pathways implicated in the regulation of the three diseases. Fig. 5A illustrates that the CP group exhibited significant enrichment in various pathways, including those related to responses to molecules of bacterial origin, lipopolysaccharide, regulation of inflammatory response, cytokine-mediated signaling pathway, inflammatory response, chemotaxis, response to external biotic stimulus, regulation of response to external stimulus, regulation of response to stress, cell surface receptor signaling pathway.

The COPD group exhibited significant enrichment in phagocytosis, B cell receptor signaling pathway, complement activation, classical pathway, plasma membrane invagination, positive regulation of B cell activation, immune response-activating cell surface receptor signaling pathway, immune response-activating signal transduction, immune response-regulating cell surface receptor signaling pathway, immune response-regulating signaling pathway, immunoglobulin production, defense response to bacterium, activation of immune response, positive regulation of leukocyte activation, innate immune response, humoral immune response, regulation of cell activation, positive regulation of immune system process, cell surface receptor signaling pathway (Fig. 5B).

The CP&COPD group was discovered to be significantly enriched in neutrophil extravasation, response to peptidoglycan, immunoglobulin mediated immune response, neutrophil migration, chemokine-mediated signaling pathway, B cell mediated immunity, leukocyte chemotaxis, B cell receptor signaling pathway, lymphocyte chemotaxis, complement activation, positive regulation of leukocyte chemotaxis, regulation of myeloid leukocyte mediated immunity, myeloid leukocyte activation, regulation of leukocyte chemotaxis, regulation of cytokine biosynthetic process, positive regulation of interleukin-6 production, positive regulation of T cell activation (Fig. 5C).

We observed that CP group and COPD group exhibited overlap or similarities in their signaling pathway, including response to bacterium, inflammatory response, immune response, cell surface receptor signaling pathway, a variety of immune cell and cytokine

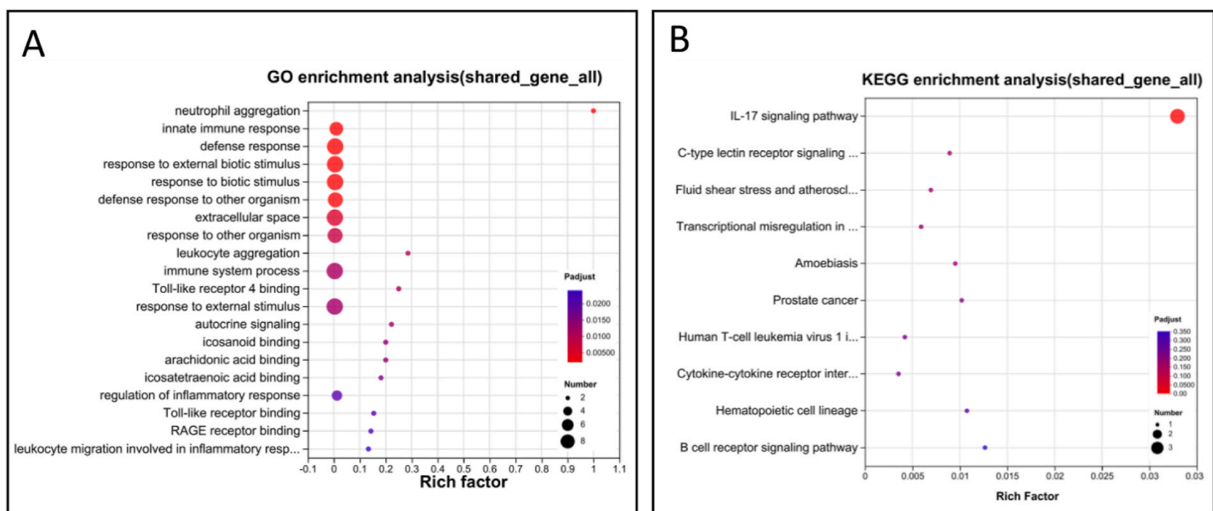
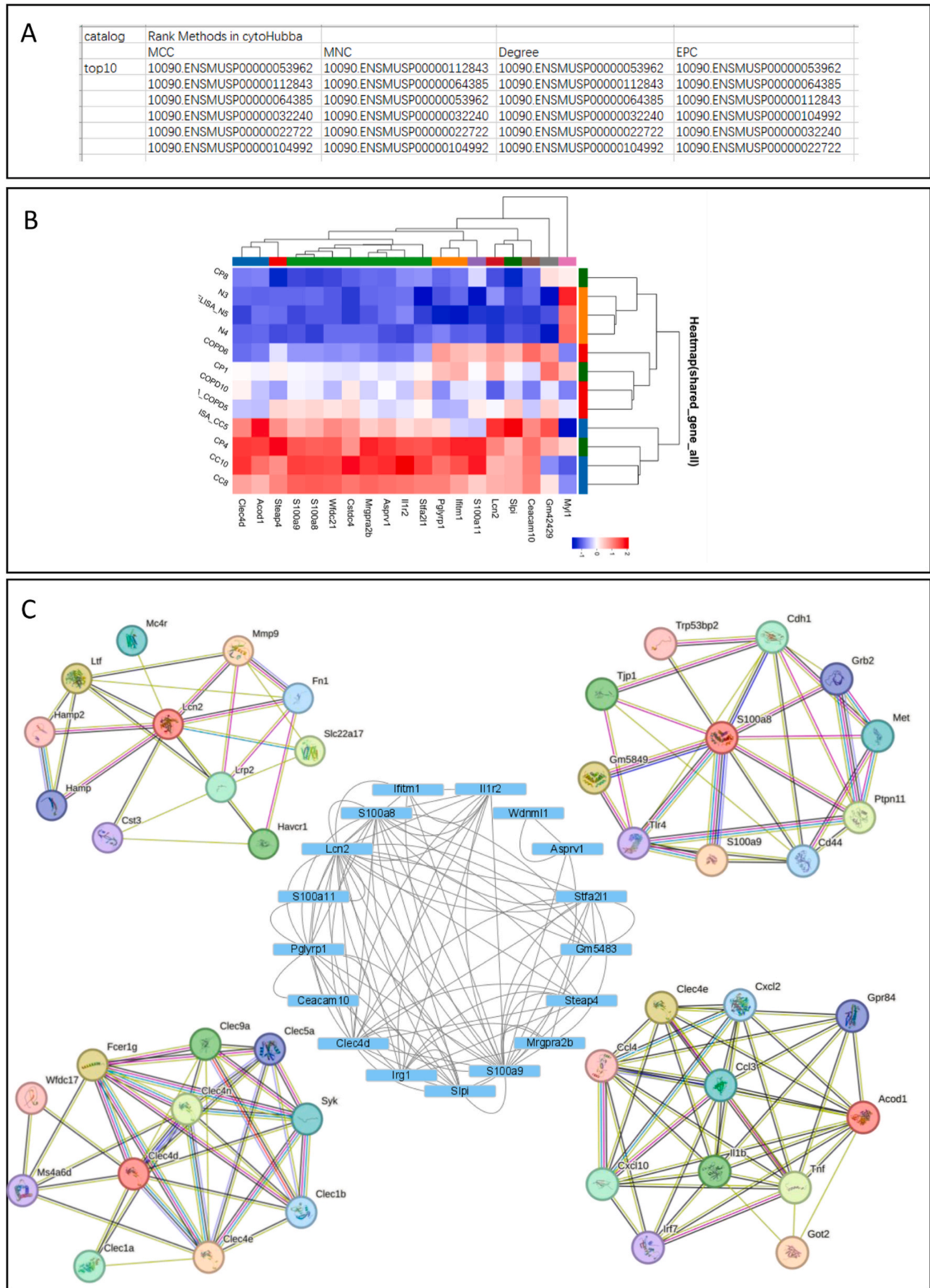


Fig. 6. GO and KEGG enrichment analysis of shared DEGs. (A) GO enrichment analysis. (B) KEGG analysis.



catalog	Rank Methods in cytoHubba			
	MCC	MNC	Degree	EPC
top10	10090.ENSMUSP00000053962	10090.ENSMUSP00000112843	10090.ENSMUSP00000053962	10090.ENSMUSP00000053962
	10090.ENSMUSP00000112843	10090.ENSMUSP00000064385	10090.ENSMUSP00000112843	10090.ENSMUSP00000064385
	10090.ENSMUSP00000064385	10090.ENSMUSP00000053962	10090.ENSMUSP00000064385	10090.ENSMUSP00000112843
	10090.ENSMUSP00000032240	10090.ENSMUSP00000032240	10090.ENSMUSP00000032240	10090.ENSMUSP00000104992
	10090.ENSMUSP00000022722	10090.ENSMUSP00000022722	10090.ENSMUSP00000022722	10090.ENSMUSP00000032240
	10090.ENSMUSP00000104992	10090.ENSMUSP00000104992	10090.ENSMUSP00000104992	10090.ENSMUSP00000022722

Fig. 7. Further analysis of crosstalk genes. (A) The top 5 key genes in 19 crosstalk genes from the perspectives of MCC, MNC, Degree and EPC. (B) the FPKM expression levels of 19 genes in every mice lung sample. (C) Protein interaction network diagram of crosstalk genes and respectively protein interaction network diagram of five key gene.

mediated signaling pathway. Combined with the result of gene enrichment in CP&COPD group, it suggests that the potential association mechanism between periodontal disease and COPD may focus on immune response, immune cell aggregation, B-cell-mediated immunity, B-cell receptor and related cytokine mediated signaling pathways.

4.6. Core genes in inflammation signaling

To delve deeper into the intricate relationship between periodontitis and COPD, we conducted a meticulous analysis of the 19 crosstalk genes identified through Venn diagram. First, we performed GO and KEGG enrichment analyses, which revealed that the shared DEGs were predominantly linked to inflammatory process. Notably, these genes were exhibited enrichment in hematopoietic cell lineage, immune cell activation, neutrophil aggregation, B cell receptor signaling pathway, toll-like receptor 4 binding (Fig. 6A and B).

Next, we employed the cytoHubba software to pinpoint the top five crucial genes among the 19 genes, based on various scores including MCC, MNC, Degree and EPC. The selected genes were Lcn2, S100A8, S100A9, Acod1 (Irg1), Clec4d. Finally, we conducted a heatmap analysis of these 19 genes and established protein networks to identify associated genes and the inflammatory pathways potentially involved. The results indicate that a robust interaction between adhesion chemokines with high expression level. S100a8 and S100a9 are regulate by Tlr4. Additionally, MYD88, TIRAP and TRAF6, TLR6. Lcn2, Steap4, Acod1 and Clec4d are highly expressed and regulated by CD14, TIr2, Syk and Clec4e (Fig. 7).

5. Discussion

At present, most studies on the correlation between periodontitis and COPD has been limited to epidemiological and clinical studies, with few exploring the underlying pathogenesis and causality of the two diseases. The current view is that periodontitis aggravates COPD by three ways [21]. First, periodontal pathogens can be directly inhaled into the lungs, leading to lung infection. Secondly, enzymes related to periodontal disease change the microenvironment of oral mucosa, making it easier for respiratory pathogens to colonize and enter the lungs through respiration. Finally, cytokines produced in periodontal tissue modify the microenvironment of respiratory epithelium, thereby facilitating the infection of respiratory pathogens. In this study, we delved deeper into the relationship between the two diseases, exploring their occurrence and development processes as well as the inflammatory pathways involved.

In terms of alveolar bone loss, when compared to the CP group, the alveolar bone resorption in the CP&COPD group did not increase significantly, indicating that COPD did not significantly promote periodontitis in a short period. However, as the results of lung function showed, compared to the COPD group, the CP&COPD group mice had significantly higher RL and significantly lower PEF and FEF50. Combined with the pathological findings, the mean alveolar linear intercept was significantly wider, and bronchial wall

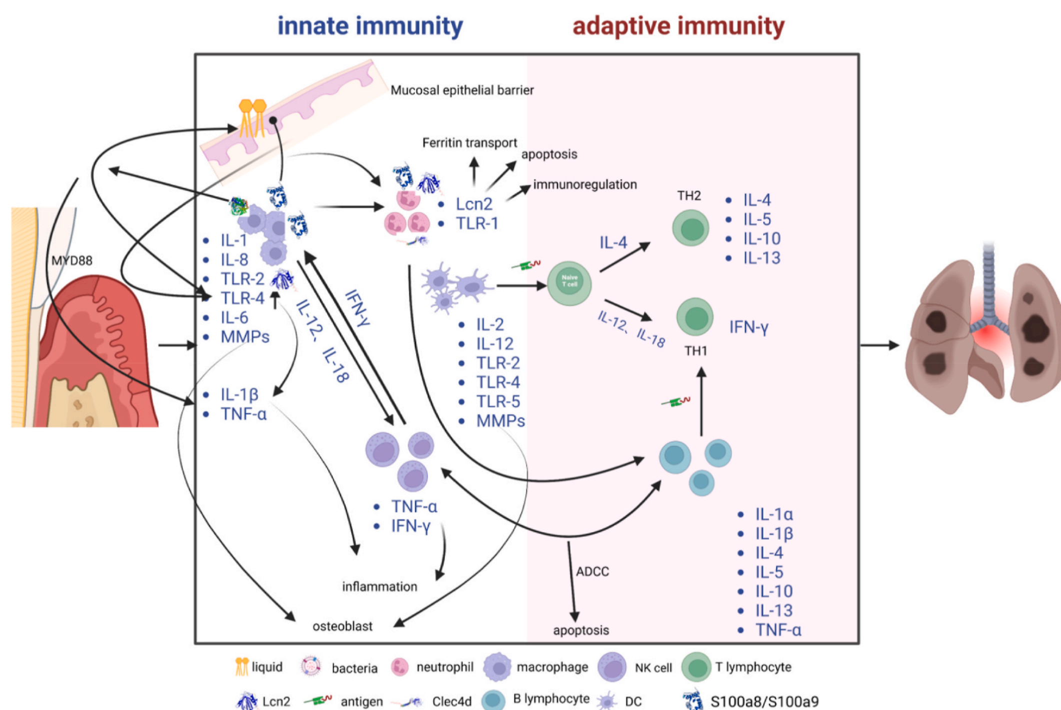


Fig. 8. Possible signaling pathways, genes and mediators involved and inflammatory factors associated with periodontitis and COPD.

thickness tended to be higher in the CC group compared to the COPD group. We speculated that periodontitis had a significant promoting effect on COPD. In terms of the lung inflammatory index, there was no significant increase observed in the CC group mice when compared to the mice in the COPD group. However, when considering the inflammatory score, an upward trend becomes evident. Given that periodontitis and COPD are both chronic progressive diseases, we postulate that the promotional effect of periodontitis on COPD will intensify as the disease progresses. This aligns with previous animal experiments results conducted by our team, which found that periodontitis can trigger pulmonary inflammation over the long term [22].

In venn diagram, we discovered that there were 19 genes that intersect between CP and COPD. After further screening and analysis, we narrowed down to 5 genes that were most closely associated. The results of enrichment analysis revealed that these genes were related to inflammation pathways. Although we did not conduct further verification experiments. several studies have indirectly supported the role of these genes in the pathophysiological association between CP and COPD. The lipid carrier protein Lcn2 has many physiological functions, including inducing apoptosis and glandular degeneration. Lcn2 is prevalent in tissues throughout the oral cavity, respiratory tract and digestive tract, with low expression under normal condition but high expression during inflammation condition [23]. Both periodontitis and COPD are inflammatory diseases, involving a large number of immune cells. Neutrophils are considered to be the most important cells in the pathogenesis of both diseases, with their activation and aggregation playing a pivotal role in the development of the diseases [24,25]. Lcn2 is mainly secreted by neutrophils, making it a proxy for neutrophils activity to a certain extent. Our study found that Lcn2 was co-expressed in both CP and COPD group, with its expression in CP&COPD group being higher compared to the COPD group, indicating that Lcn2 played a role in the process of periodontitis promoting COPD. A clinical study found that the level of Lcn2 in gingival crevicular fluid of individuals with periodontitis is significantly higher than that of individuals in normal group and the gingivitis group. This suggests Lcn2 can serve as a non-invasive screening tool for periodontitis [26]. Based on the results of enrichment analysis, we speculated that Lcn2 responds to pathogenic bacteria through inherent immune response. Additionally, it responsed to hydrogen peroxide, lipopolysaccharide, interleukin-1 and tumor necrosis factor, which are produced in the course of disease development through adaptive immune response., Lcn2 regulates the development of disease through exogenous apoptotic lacking ligands signaling pathways and iron death pathways (Fig. 8). Animal experiment and vitro cell experiment have verified this inference. Lcn2 is significantly increased in mice with lung injury. inhibition of Lcn2 effectively alleviates LPS-induced lung pathological injury, and silencing Lcn2 inhibit LPS-induced inflammatory response, oxidative stress and apoptosis in vivo and vitro [27]. As indicated by the protein network, Lcn2 is closely related to MMP9,which has a positive regulatory effect. The role of MMP9 in periodontitis and COPD has been previously verified [15]. MMPs play a major role in inflammation-related tissue damage, with MMP-9 being capable of cleaving a 62-amino acid peptide from interleukin 8(IL-8), thereby increasing its chemotactic activity towards neutrophils by 10 times [28]. Two studies have found elevated MMP9 level in tissue lesions of both periodontitis and lung disease [29,30].

The initial phase of chronic inflammation is the activation of the innate immune response. S100A8 can act as a pattern recognition receptor (PRR) to initiate this response by binding to bacterial lipopolysaccharide (LPS). Our findings indicate that S100A8 expression is significantly heightened during inflammation (periodontitis or COPD), and further elevates when both conditions coexist. Neutrophils serve as the primary line of defense in the innate immune response, and S100A8 plays a crucial role in inducing their chemotaxis and adhesion. In vitro experiment, they observed that a large number of neutrophils gathered 24 h later after injecting mS100A8 [31].In adaptive immune response, S100A8 contributes to disease development by participating in leukocyte migration and neutrophil chemotaxis. It also regulates Mitogen-activated protein kinase (MAPK) activity when combined with Toll-like 4 and S100A9 [32]. Maekawa et al. conducted an analysis of gum tissue sequencing in periodontitis mice and observed upregulated expression of S100A8 and S100A9. Notably, S100A8 was expressed only in the attached epithelium without any stimulation, and its expression was further heightened in mice exhibiting rapid bone loss in periodontal tissues [33]. The genes S100A8 and S100A9 have been identified as mediators of inflammation and injury of the lung, playing a critical roles in the pathogenesis of COPD [34]. The protein network reveals a close association between S100A8, S100A9 and TLR-4, exerting a positive regulatory effect. When lung tissue is injured, the level of S100A8/A9 proteins increases. These proteins are also involve in inflammation triggered by high tidal volume mechanical ventilation combined with lipopolysaccharide. However, without lipopolysaccharide, extracellular S100A8/A9 remain highly expressed and amplify ventilator-induced lung injury by TLR4 [35]. Autophagy plays an important role in the progression of various diseases. Furthermore, S100A8 is involved in apoptosis, influencing both intrinsic apoptotic signaling pathway and activation of cysteine-type endopeptidase activity, which are integral to the apoptotic process [36].

LPS has a significant stimulating effect in immune system, and the LPS-mediated signaling pathway can cause a series of pathological changes. It is worth mentioning that this signaling pathway is closely related to the pathological changes of many chronic inflammatory diseases, and it can make the body in a state of low-grade, persistent inflammatory [37]. The most critical step in the signaling pathway is LPS binds to myeloid differentiation protein 2 (MD2) on the surface of immune cells through toll-like receptor 4. This complex formation is recognized by immune system [38]. TLR 4 is a key mediator that plays an important role in periodontitis and COPD,as supported by a large number of studies [39,40]. Irg1 acts as a negative regulator of the TLRs-mediated inflammatory innate response by stimulating the tumor necrosis factor alpha-induced protein TNFAIP3 expression via reactive oxygen species (ROS) in LPS-tolerized macrophages. After LPS-TLR4-MD2 complex is recognized by immune system, it can activate MYD88-dependent signaling and the expression of transcription regulators such as NF-κB and activator protein 1(Fig. 7). Under normal circumstance, Irg1 is lower expression, but it significantly increases during inflammation. Our results also confirmed that Irg1 level was higher in CP group, COPD group and CP&COPD group mice compared to normal group. The expression of ACOD1 is upregulated by macrophages, monocytes, and DCs in the innate immunity response. Pathogen-associated molecular patterns (PAMPs), such as LPS, are structural components or products of microorganisms that can strongly upregulate ACOD1 expression by binding to different PRRs in macrophages [41].Ultimately, a large number of cytokines and chemokines are produced, including IL-1β, IL-6, IL-8,CCL2, TNF-α, CXCL10,

CCL5, IFN-β [42]. As the protein-protein interaction networks (PPI) showed, Irg1 was closely related to TNF, CXCL10 and IL-1β, and they exhibit a positive regulatory relationship. Through vitro cell experiment and knock gene mice experiment, Németh and Degrandi have confirmed that various inflammatory stimuli (e.g. PAMPs, TNF, and IL-1β) can induce ACOD1 expression by activating receptors (e.g. TLRs, TNFR, and IL1R) and transcription factors (such as NF-κB, IRFs, and STATs) in a context-dependent manner [43,44].

C-type lectin domain family 4 member D (Clec4d) acts as an endocytic receptor which is involved in antigen uptake at the site of infection, facilitating its processing and further presentation to T-cells. One of the three methods for T cells activate is antigen presentation, in which T cells regulate innate immune cells to initiate immune response or aggravate the inflammatory response by differentiating into different forms(Fig. 7) [45]. The hallmark features of periodontal disease include periodontal pocket formation and alveolar bone loss. Th1-T lymphocytes, B cells, macrophages and neutrophils promote bone loss by up-regulating the production of pro-inflammatory mediators TNF-α, IL-1 and prostaglandin E2(PGE2), as well as activating the RANK-L expression pathway [46]. In patients with stable COPD T lymphocytes, mainly $CD8^+$ T cells, are the dominate immune cells in the bronchial mucosa [47], and the number of $CD4^+$ T cells and $CD8^+$ T cells increases along with the severity of COPD progression [48]. Wilson et al. found that Clecsf8 (Clec4d)(−/−) mice displayed increased mortality rates after infection with M. tuberculosis. This Clecsf8 deficiency was associated with exacerbated pulmonary inflammation, manifesting as enhanced neutrophil aggregation [49]. Steichen et al. made a further discovery that the Clec4d(−/−) mice were highly susceptible and their lung tissue pathology presented severe inflammation. This observation was accompanied by a large number of neutrophils accumulation in lungs. It is worth noting that the neutrophils derived from Clec4d(−/−) mice did not exhibit any defect in in their ability to eliminate bacteria. These results suggest that Clec4d plays an important role in resolution of inflammation, potentially by promoting neutrophil turnover in lungs [50]. As the PPI showed that Clec4d is associated with spleen tyrosine kinase(Syk), Tlr2, Clec4e and CD14. Subsequently, Weng et al. identified a vital intracellular signaling, Trem2/DAP12/Syk, for amplifing reactive oxygen species (ROS) signals during osteoclastogenesis in periodontitis [51]. Through vivo and in vitro inhibition experiments, Yuan et al. confirmed that airway inflammation observed in COPD was significantly reduced after inhibiting of TLR4/Syk/PKC/NF-κB signaling pathway [52]. These studies indirectly confirmed that Clec4d plays an important role in inflammatory progression in both diseases.

6. Conclusions

Lcn2, S100a8, S100a9 are correlated with neutrophils in both diseases, suggesting that neutrophils may play an important role in the correlation between the two diseases. While from the results of enrichment analysis we speculated that Irg1 and Clec4d may bind to receptors on the surface of lymphocytes to produce cytokines and activate inflammatory pathways. In the follow-up experiments, we will further explore the related inflammatory factors and inflammatory pathways, which may provide further evidence for the study of the related mechanism of the two diseases.

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Data availability statement

Data will be made available on request.

Ethics statement

The animal experiments protocol was approved by the Animal Experiments and Experimental Animal Welfare Committee of Capital Medical University (2020-067), and all animal procedures were performed in accordance with the institutional and national guidelines.

CRediT authorship contribution statement

Kaili Wang: Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Xiaoli Gao:** Methodology, Formal analysis, Data curation. **Hongjia Yang:** Methodology, Data curation. **Huan Tian:** Methodology, Formal analysis, Data curation, Conceptualization. **Zheng Zhang:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Zuomin Wang:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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