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# Exploring the molecular mechanisms of ferroptosis-related genes in periodontitis: a multi-dataset analysis

Jili Chen<sup>1</sup>, Lijia Ou<sup>2</sup>, Weizhen Liu<sup>1</sup> and Feng Gao<sup>1\*</sup>

## Abstract

**Purpose** This study aims to elucidate the biological functions of ferroptosis-related genes in periodontitis, along with their correlation to tumor microenvironment (TME) features such as immune infiltration. It aims to provide potential diagnostic markers of ferroptosis for clinical management of periodontitis.

**Methods** Utilizing the periodontitis-related microarray dataset GSE16134 from the Gene Expression Omnibus (GEO) and a set of 528 ferroptosis-related genes identified in prior studies, this research unveils differentially expressed ferroptosis-related genes in periodontitis. Subsequently, a protein–protein interaction network was constructed. Subtyping of periodontitis was explored, followed by validation through immune cell infiltration and gene set enrichment analyses. Two algorithms, randomForest and SVM(Support Vector Machine), were employed to reveal potential ferroptosis diagnostic markers for periodontitis. The diagnostic efficacy, immune correlation, and potential transcriptional regulatory networks of these markers were further assessed. Finally, potential targeted drugs for differentially expressed ferroptosis markers in periodontitis were predicted.

**Results** A total of 36 ferroptosis-related genes (30 upregulated, 6 downregulated) were identified from 829 differentially expressed genes between 9 periodontitis samples and the control group. Subsequent machine learning algorithm screening highlighted 4 key genes: SLC1A5(Solute Carrier Family 1 Member 5), SLC2A14(Solute Carrier Family 1 Member 14), LURAP1L(Leucine Rich Adaptor Protein 1 Like), and HERPUD1(Homocysteine Inducible ER Protein With Ubiquitin Like Domain 1). Exploration of these 4 key genes, supported by time-correlated ROC analysis, demonstrated reliability, while immune infiltration results indicated a strong correlation between key genes and immune factors. Furthermore, Gene Set Enrichment Analysis (GSEA) was conducted for the four key genes, revealing enrichment in GO/KEGG pathways that have a significant impact on periodontitis. Finally, the study predicted potential transcriptional regulatory networks and targeted drugs associated with these key genes in periodontitis.

**Conclusions** The ferroptosis-related genes identified in this study, including SLC1A5, SLC2A14, LURAP1L, and HERPUD1, may serve as novel diagnostic and therapeutic targets for periodontitis. They are likely involved in the occurrence and development of periodontitis through mechanisms such as immune infiltration, cellular metabolism, and inflammatory chemotaxis, potentially linking the ferroptosis pathway to the progression of periodontitis. Targeted drugs such as flurofamide, L-733060, memantine, tetrabenazine, and WAY-213613 hold promise for potential therapeutic interventions in periodontitis associated with these ferroptosis-related genes.

**Keywords** Bioinformatic analysis, Multiomic datasets, Periodontitis, Ferroptosis, Immunoinfiltration

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## Introduction

Periodontal diseases are regarded as the most common diseases of mankind. The prevalence rate of periodontal disease assumes a clear growth tendency [1]. Globally, the severe form of the disease has a prevalence of 11%, affecting 743 million individuals [2, 3]. Periodontitis is a chronic multifactorial inflammatory disease associated with the accumulation of dental plaque (which will be referred to as dental biofilm/biofilm), and characterised by progressive destruction of the teeth-supporting apparatus, including the periodontal ligament and alveolar bone [4]. In advanced cases, periodontitis leads to a substantially negative effect on oral health related quality of life (OHRQoL), while successful management may improve patients' OHRQoL. The previous study has reported about the intricate molecular mechanism underlying this periodontitis [5]. However, the role of specific genes, cells, or cellular mechanisms involved in the pathogenesis of periodontitis are still unclear, and there are no pre-diagnostic markers or therapeutic targets available for such inflammatory lesions [6].

The investigation of molecular mechanism and diagnostic marker at the cellular or molecular level are important for periodontitis [7]. For example, R Ientile et al. observed that transglutaminase gene expression may be modified in response to chronic injury and play a key role in gingival remodelling/healing and adaptive processes [8]; Gaetano Isola et al. indicated that gingival crevicular fluid miRNAs is closely associated with the risk of periodontitis [9]; As is widely known that ferroptosis as an iron-dependent form of non-apoptotic cell death was described in 2012, and it was defined as the oxidative response of the intracellular microenvironment regulated by glutathione peroxidase 4 (GPX4), a form of regulatory cell death triggered by stimulation that can be inhibited by iron chelators and lipophilic antioxidants in 2018 [10]. Recently, there has been mounting interest in the function and role of ferroptosis in various diseases [11, 12]. Interestingly, several studies have confirmed that ferroptosis is indeed closely related to the inflammatory response including the bacterial inflammatory reaction in the oral cavity such as periodontitis [13, 14]. Wang et al. explored the relationship between antioxidant system and periodontitis, and considered ROS plays a key role in periodontitis due to excessive lipid peroxidation and tissue damage [15–17]; In Meuric's study, periodontitis is associated between serum iron biomarkers in genetic haemochromatosis patients, they also demonstrate that increased transferrin saturation is associated with oral dysbiosis characterized by elevated

proportions of periopathogens, which may participate in periodontitis severity [18]; Furthermore, Bains et al. found that periodontitis patients have the lower concentrations of reduced glutathione (GSH) in serum and gingival crevicular fluid, and periodontal therapy restores the redox balance [19, 20]. Based on the compelling evidence regarding the correlation between periodontitis and ferroptosis three elements (ROS, GSH and Iron) presented in the aforementioned studies, we reasonably hypothesize a necessary link between ferroptosis and the pathogenesis of periodontitis. Furthermore, we aim to explore the role of ferroptosis-related genes in clinical patients with periodontitis by utilizing bioinformatics techniques across multiple datasets.

In this study, we identified differentially expressed genes by analyzing a periodontitis-related microarray dataset from the Gene Expression Omnibus (GEO) database, alongside 528 ferroptosis-associated genes identified in prior research. This analysis was followed by the construction of a protein–protein interaction network. Subsequently, we investigated the subtypes of periodontitis, corroborating our findings through assessments of immune cell infiltration and gene set enrichment. To discern potential diagnostic markers of ferroptosis in periodontitis, we employed two machine learning algorithms. The diagnostic efficacy, potential therapeutic targets, and immune associations of these markers were then rigorously evaluated. Lastly, we constructed a microRNA (miRNA)-gene interaction network to further elucidate the molecular mechanisms at play. This finding could pave the way for an advanced comprehension of ferroptosis's role in the pathogenesis of periodontitis, thereby laying a theoretical foundation for subsequent investigations into the molecular mechanisms underlying the disease.

## Materials

### Data download

The GEO database (<https://www.ncbi.nlm.nih.gov/geo/info/datasets.html>), known as GENE EXPRESSION OMNIBUS, is a gene expression database created and maintained by the National Center for Biotechnology Information (NCBI) in the United States. The search in the NCBI GEO public database was conducted using keywords "periodontitis," "single-cell," "gingiva," and "transcriptome," with the species set to "Homo sapiens," resulting in the inclusion of GSE171213 and GSE16134. GSE171213 is a single-cell dataset comprising nine samples, with five samples from diseased subjects and four from control subjects. GSE16134 is a transcriptome

dataset comprising 310 samples, with the annotation file labeled as GPL570, including 241 samples from patients with the disease and 69 samples from healthy individuals. The ferroptosis gene set utilized in this analysis consists of 564 genes associated with ferroptosis.

#### GO and KEGG function notes

The functional annotation of crucial gene sets was conducted using the Metascape database ([www.metascape.org](http://www.metascape.org)), aiming to comprehensively explore the functional relevance of these genes. Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed for specific genes. Statistical significance was considered when Min overlap was  $\geq 3$  and  $p \leq 0.01$ .

#### Screening of key genes

Firstly, differential gene analysis was employed to identify significant variances within the GEO dataset ( $\text{adj.}P\text{-Val} < 0.05$  and  $|\log\text{FC}| > 0.585$ ). Subsequently, two distinct algorithms, RandomForest and SVM, were applied to refine the candidate gene set further. Among them, RandomForest is an integrated learning algorithm based on a decision tree, which selects multiple samples from the sample set as a training set by the method of sampling back and generates a decision tree from the sample set obtained by sampling.

At each generated node, features are randomly and non-repetitively selected. Utilizing these features, the sample set is partitioned, identifying the optimal feature for partitioning and determining the predicted outcome. This study assesses the importance of features using the random forest algorithm, constructing a total of 1000 classification trees with 50 iterations. The evaluation of feature importance is based on %IncMSE (% Increase in Mean Squared Error). SVM-RFE is a machine learning method based on Support Vector Machines (SVM). It identifies optimal variables by eliminating feature vectors generated by SVM. The Support Vector Machine model is established using the "e1071" package, facilitating the further recognition of these biomarkers' diagnostic value for the disease. Ultimately, the top 15 features are retained for subsequent analysis.

#### Development and verification of artificial neural network model

A neural network model was developed utilizing the R package "neuralnet" based on identifying key genes using machine learning. First, transform the expression levels of key genes into a "gene score" based on their respective expression levels. For a specific sample, the expression level of a particular gene is compared to the median

expression level across all samples. Subsequently, the hidden layer of the Artificial Neural Network (ANN) is configured as 5, and the gene weights are computed through the "gene score." Finally, an ANN diagnostic model is established, and its diagnostic performance is evaluated through the Area Under the Curve (AUC).

#### Single-cell analysis

First, the expression profile was imported using the Seurat package, and low-expressed genes were filtered out ( $\text{nFeature\_RNA} > 300$  &  $\text{percent.mt} < 30$ ). Subsequently, the data underwent standardization, normalization, and PCA analysis. The optimal number of principal components (20) was determined by observing the ElbowPlot. The t-SNE analysis was then employed to reveal the spatial relationships between clusters. The clusters were annotated using the annotation file, HumanPrimaryCellAtlasData, provided by the cellDex package. Each cluster was individually annotated to cells with significant relevance to disease occurrence.

#### Analysis of immune cell infiltration

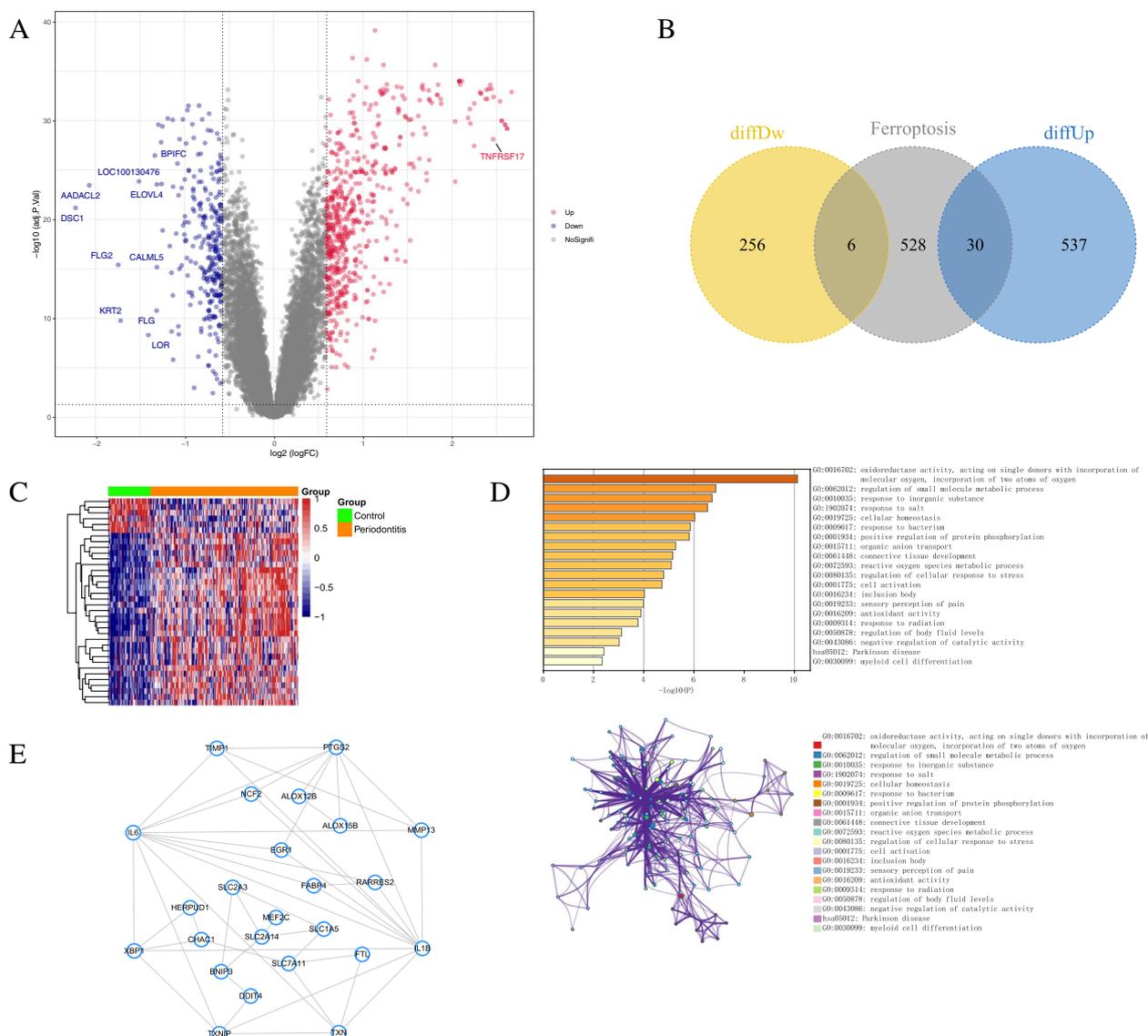
The CIBERSORT algorithm was employed to analyze RNA-seq data of periodontitis patients from different subgroups. This analysis aimed to infer the relative proportions of 22 immune infiltrating cell types. Subsequently, Pearson correlation analyses were conducted to assess the correlation between risk scores and the content of immune cells. A significance level of  $P < 0.05$  was considered statistically different.

#### GSEA enrichment analysis

Performing Gene Set Enrichment Analysis (GSEA; <http://www.broadinstitute.org/gsea>) on the expression spectrum of periodontitis patients to identify differentially expressed genes between the high-expression and low-expression groups. Utilizing maximum and minimum gene set sizes of 500 and 15 genes, respectively, we filtered the gene sets. Following 100 permutations, we obtained enriched gene sets based on a significance threshold of  $P < 0.05$  and a false discovery rate (FDR) of 0.25.

#### Drug targeting prediction analysis

The Connectivity Map (CMap) is a gene expression profile database developed by the Broad Institute based on interventions in gene expression. It is primarily utilized to elucidate functional connections between small-molecule compounds, genes, and disease states. The database encompasses gene expression data before and after the treatment with 1309 small-molecule drugs across



**Fig. 1** Screening of the differentially expressed ferroptosis related genes in periodontitis. **A** The volcano map of GEO dataset GSE16134. Upregulated genes are marked in red; downregulated genes are marked in blue; Not significant different genes are marked grey. **B** Venn diagram shows the overlap of genes between differentially expressed genes in GSE16134 and ferroptosis related genes. **C** Clustered heatmap of differentially expressed ferroptosis related genes. **D** KEGG enrichment analysis of differentially expressed ferroptosis related genes in GSE16134. **E** The PPI network constructed based on differentially expressed ferroptosis related genes in current study; the line represented interaction; the node represented different differentially expressed ferroptosis related genes

five human tumor cell lines. The treatment conditions are diverse, involving various drugs, concentrations, and treatment durations, among other factors. In this study, differential gene expression in periodontitis is employed to predict targeted therapeutic drugs for periodontitis.

**Statistical analysis**

All statistical analyses were conducted using R language (version 4.1). All statistical tests were two-sided,

with  $p < 0.05$  considered statistically significant. We used Pearson correlation coefficients to assess the relationships between different variables. Specifically, we calculated the correlation coefficients and their associated  $p$ -values. For comparisons between two groups, we employed the Wilcoxon test to examine whether there were significant differences in sample means between different groups.

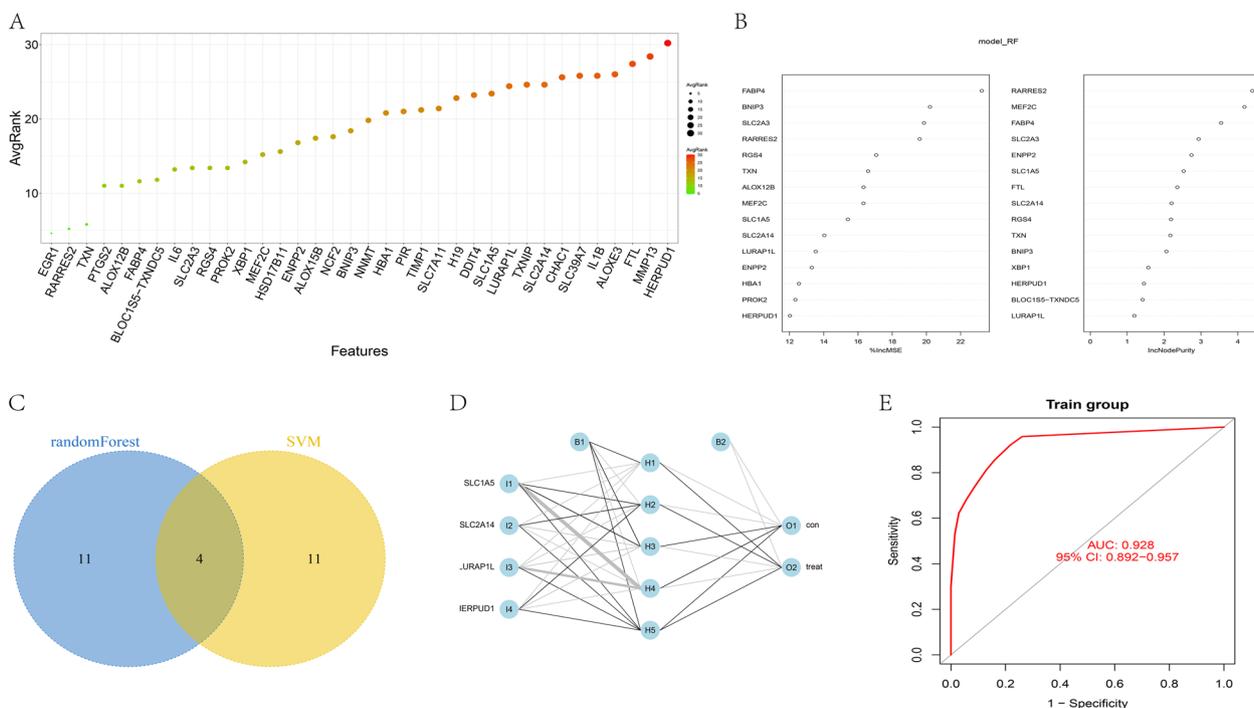
## Results

### Identifying differentially expressed candidate genes associated with ferroptosis in periodontitis

We conducted a differential gene analysis on the GEO dataset GSE16134 using the limma package, comparing control samples with periodontitis samples. The criteria for selecting differentially expressed genes were set as  $\text{adj.P.Val} < 0.05$  and  $|\log\text{FC}| > 0.585$ . Ultimately, we identified 567 upregulated genes and 262 downregulated genes (Fig. 1A). This includes 36 ferroptosis-related genes (30 upregulated genes; 6 downregulated genes), as illustrated in Fig. 1B-C. Ultimately, we consider these 36 differentially expressed genes related to ferroptosis as a candidate gene set for further analysis. Through pathway analysis using the Metascape database, it was revealed that these candidate genes are primarily enriched in pathways such as regulation of small molecule metabolic process, response to inorganic substances, and response to salt (Fig. 1D). Simultaneously, we conducted protein-protein interaction network analysis on these genes using Cytoscape software (Fig. 1E).

### Identifying key genes associated with ferroptosis in periodontitis

In order to further screen key genes associated with disease perturbations, we conducted random Forest and SVM analyses on the aforementioned 36 candidate genes (Fig. 2A-B). Among them, four genes consistently ranked among the Top 15 feature genes in both rounds of feature selection (Fig. 2C). These genes are SLC1A5, SLC2A14, LURAP1L, and HERPUD1. Utilizing the "neuralnet" R package, we constructed an artificial neural network model based on the GSE16134 dataset. The expression of key genes, identified through machine learning, was transformed into a "gene score" marked as 0/1. We computed the weights of all genes to best discriminate between normal and diseased samples. Subsequently, a diagnostic artificial neural network model was established based on these gene weights (Fig. 2D). The performance of this model, as indicated by the AUC in the dataset, was 0.928 (Fig. 2E), suggesting its robust predictive capability.



**Fig. 2** Screening of key genes. **A** Variable importance in the random forest models based on the above 36 candidate genes for the prediction of the role of disease perturbations. **B** The Top 15 genes revealed by SVM analyses according to Mean Decrease Accuracy (left) and Mean Decrease Gini (right). **C** The VENN diagram revealed the overlap of 4 differentially expressed ferroptosis related genes in current study. **D** Network structure diagram for 4 differentially expressed ferroptosis related genes. **E** The result of receiver operating characteristic (ROC) curve analysis for above 4 genes: X-axis represented specificity, Y-axis represented sensitivity

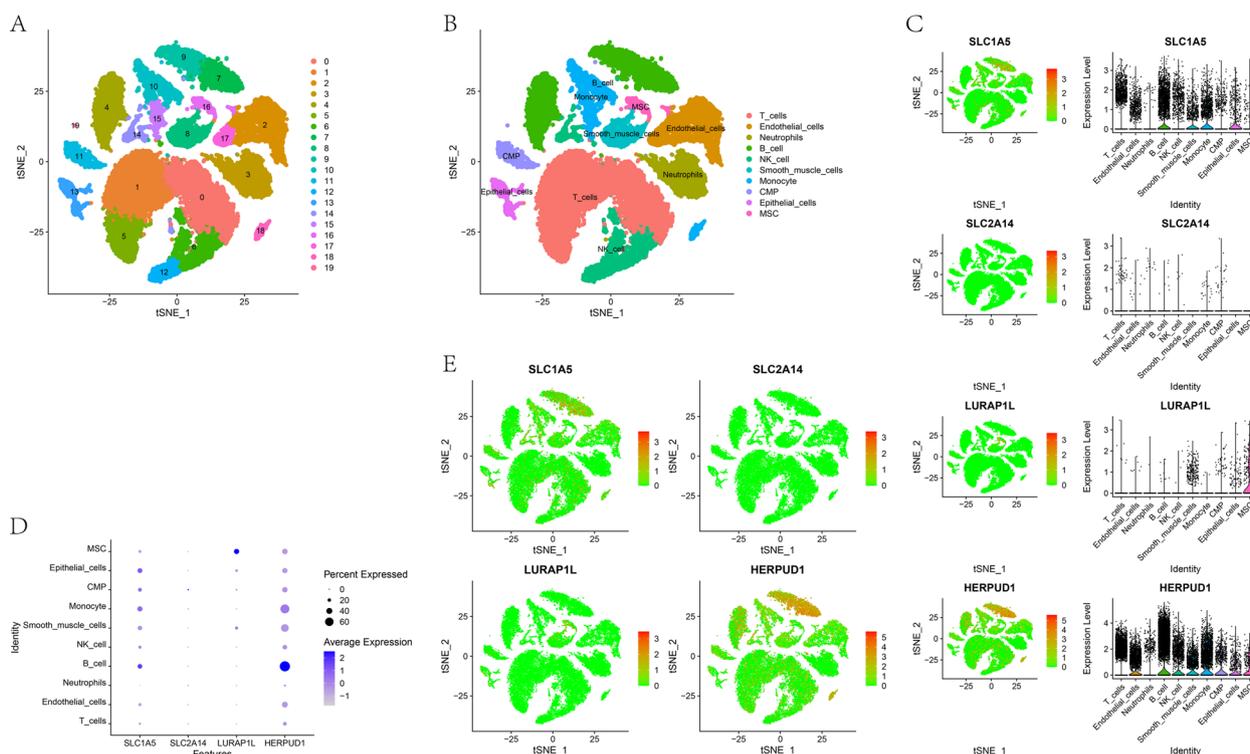
**scRNA analysis in periodontitis**

In this analysis, we utilized expression profiles from nine periodontitis-associated tissue samples. Employing the FindClusters algorithm, we identified 20 subtypes, as illustrated in Fig. 3A. We annotate each subtype through the R package "SingleR," and 20 clusters are annotated into 10 B cell categories: T cells, Endothelial cells, Neutrophils, B cells, NK cells, Smooth muscle cells, Monocyte, CMP, Epithelial cells and MSC (Fig. 3B). The expression of four key genes in cellular subtypes is illustrated in the figure (Fig. 3C-E).

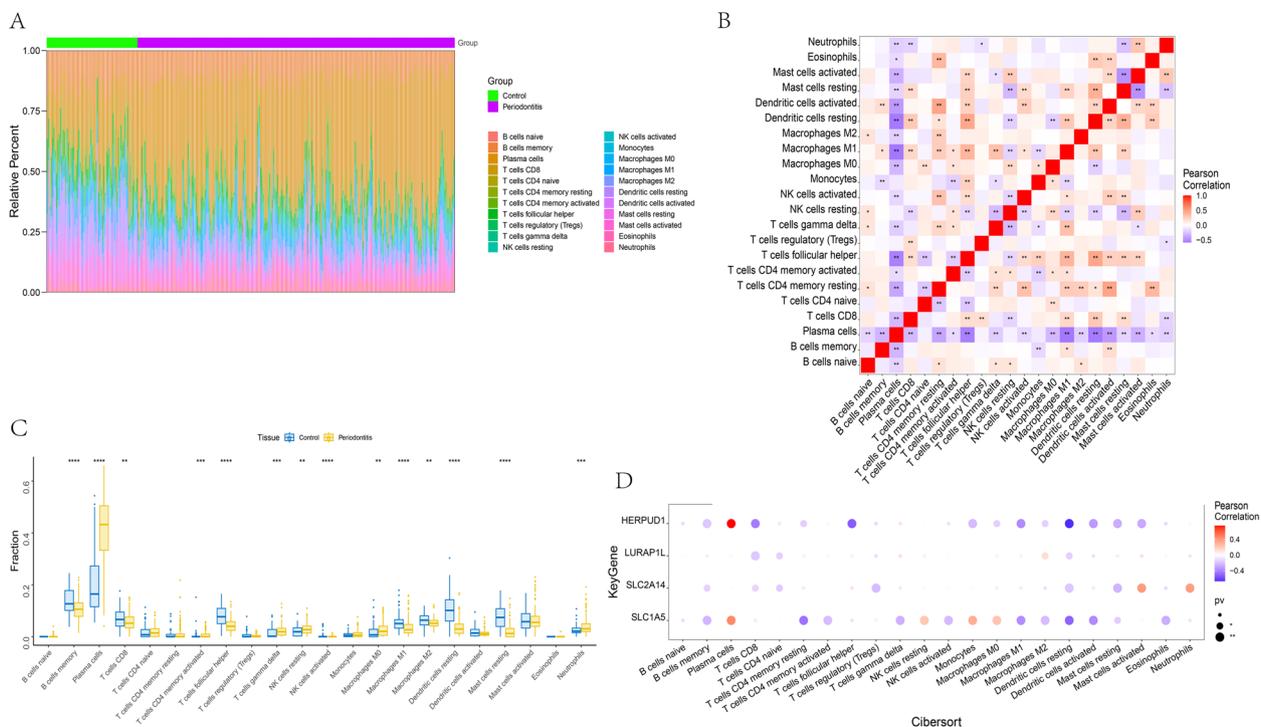
**Correlation analysis between four key genes and immune microenvironment characteristic factors**

The microenvironment is primarily composed of immune cells, extracellular matrix, various growth factors, inflammatory factors, and specific physicochemical features. It significantly influences the diagnosis and clinical treatment sensitivity of diseases. By analyzing the relationship between key genes and immune infiltration in the periodontitis dataset, we further explore the potential molecular mechanisms through which these key genes influence the progression of periodontitis.

The research findings indicate that the proportion of immune cell content in each patient and the correlations between immune cells are illustrated in the figures (Fig. 4A-B). Furthermore, compared to patients with periodontitis, the control group samples showed significantly elevated levels of B cells memory and T cells CD8, while the levels of Plasma cells and T cells gamma delta were significantly decreased (Fig. 4C). We further investigated the relationship between key genes and immune cells, revealing a strong correlation between these key genes and multiple levels of immune cell infiltration. The expression levels of the genes HERPUD1 and SLC1A5 were significantly positively correlated with the infiltration level of Plasma cells. In contrast, there was a significant negative correlation between the infiltration levels of B cell memory and Macrophages M1. The expression levels of genes LURAP1L and SLC2A14 show a significant negative correlation with the infiltration levels of T cells CD8 and T cells CD4 naive (Fig. 4D). We further obtained the correlation between these key genes and various immune factors from the TISIDB database, including immune modulators, chemokines, etc. The results revealed a strong correlation between



**Fig. 3** scRNA analysis in periodontitis. **A** The cluster analysis performed the t-distributed stochastic neighbor embedding (t-SNE) was employed for cluster visualization. Each distinct color in the plot corresponds to a cell cluster identified after clustering. Each data point in the scatter plot represents an individual cell, and the numerical labels in the figure indicate the cluster identifiers for each group. The graphical representation indicates a total of 20 distinct cell clusters identified from the clustering process. **B** The t-SNE analysis reveals the categorization of 20 clusters in distinct immune cell types. **C-E** The expression of SLC1A5, SLC2A14, LURAP1L, and HERPUD1 across various subtypes of immune cells



**Fig. 4** Correlation analysis between four key genes and immune cells. **A** Expression profiling of immune-infiltrating cells in periodontitis patients was conducted using heat map analysis; **B** Pearson correlation analysis was employed to assess the expression correlation among immune-infiltrating cells in periodontitis patients; **C** Comparison of the expression profiles of immune-infiltrating cells between inflammatory tissues of periodontitis patients and the control group; **D** Comparative analysis of the correlation between the expression of four key genes and immune-infiltrating cells

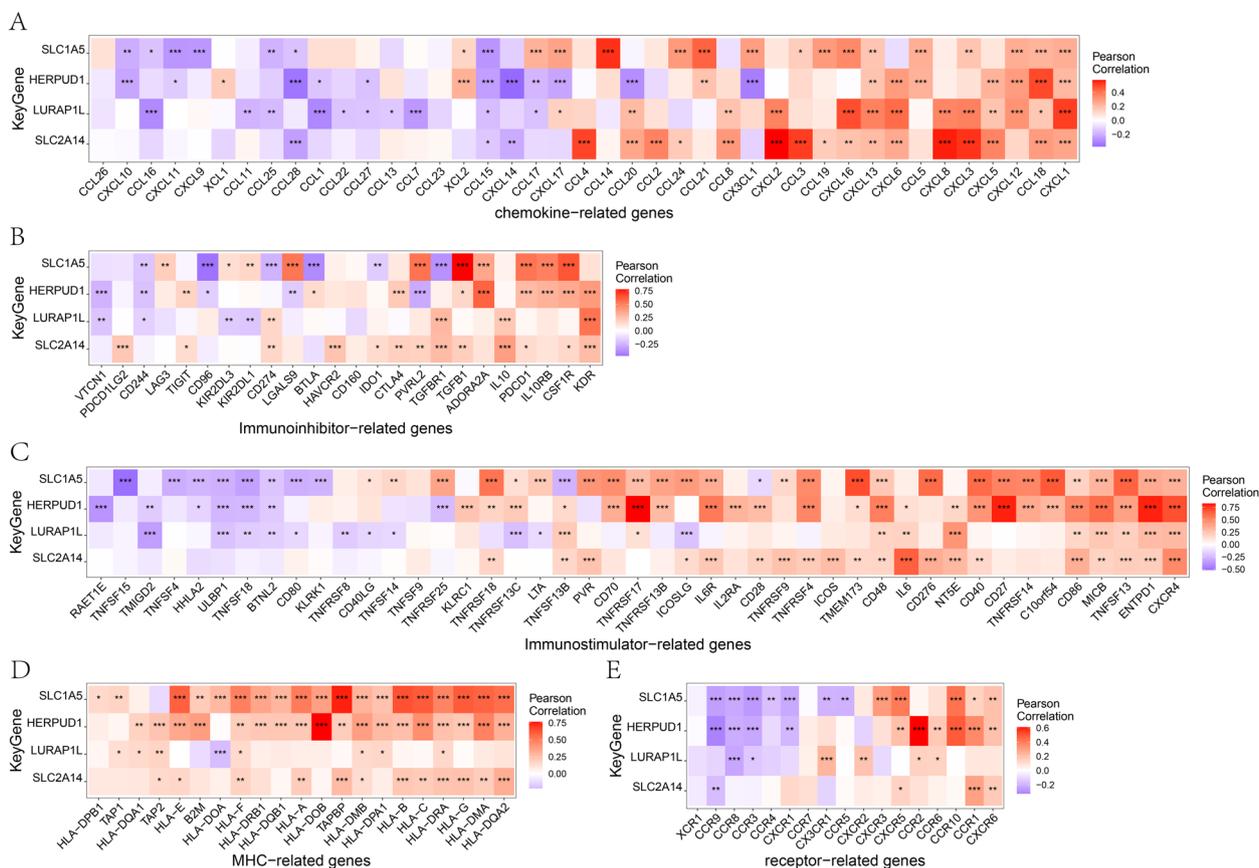
the key genes and immune factors such as chemokine-related genes, immuoinhibitor related genes, immuostimulator related genes, MHC-related genes and receptor related genes (Fig. 5A-E). These analyses confirmed the close association of these key genes with the level of immune cell infiltration, indicating their significant roles in the immune microenvironment.

**Pearson’s correlation analysis between four key genes and pathogenic genes associated with periodontitis**

We obtained pathogenic genes related to periodontitis from the GeneCards database (<https://www.genecards.org/>). We conducted an analysis of the expression levels of four key genes and the top 20 genes based on their Relevance score. The results revealed a significant correlation between the expression levels of the key genes and several genes associated with the disease (Fig. 6). Specifically, SLC2A14 and IL6 showed a significant positive correlation (Pearson  $r=0.646$ ), while SLC1A5 and TNFSF11 exhibited a significant negative correlation (Pearson  $r=-0.243$ ).

**Performing Gene Set Enrichment Analysis (GSEA) to enrich functional pathways associated with key genes**

In our subsequent research, we will investigate the specific signaling pathways enriched with four key genes, aiming to explore the potential molecular mechanisms through which these key genes impact the progression of periodontitis. We selected some highly significant pathways and showed them separately. The pathways enriched by SLC1A5 gene GO include the detection of chemical stimuli involved in sensor perception of taste and ARF PROTEIN SIGNAL TRANSDUCTION, and the pathways enriched by KEGG include GLIOMA and LYSOSOME. Pathways enriched by SLC2A14 gene GO include ERK1 AND ERK2 CASCADE, ENDOTHELIAL CELL CHEMOTAXIS, and those enriched by KEGG include ASCORBATE AND ALDARATE METABOLISM, APOPTOSIS, etc. The pathways enriched by LURAP1L gene GO include cardiac epithelial to mesochemical transition, atrio exotic valve development, etc. The pathways enriched by KEGG include glycosphindylinositol GPI anchor biosynthesis, neuroactive ligand-receptor



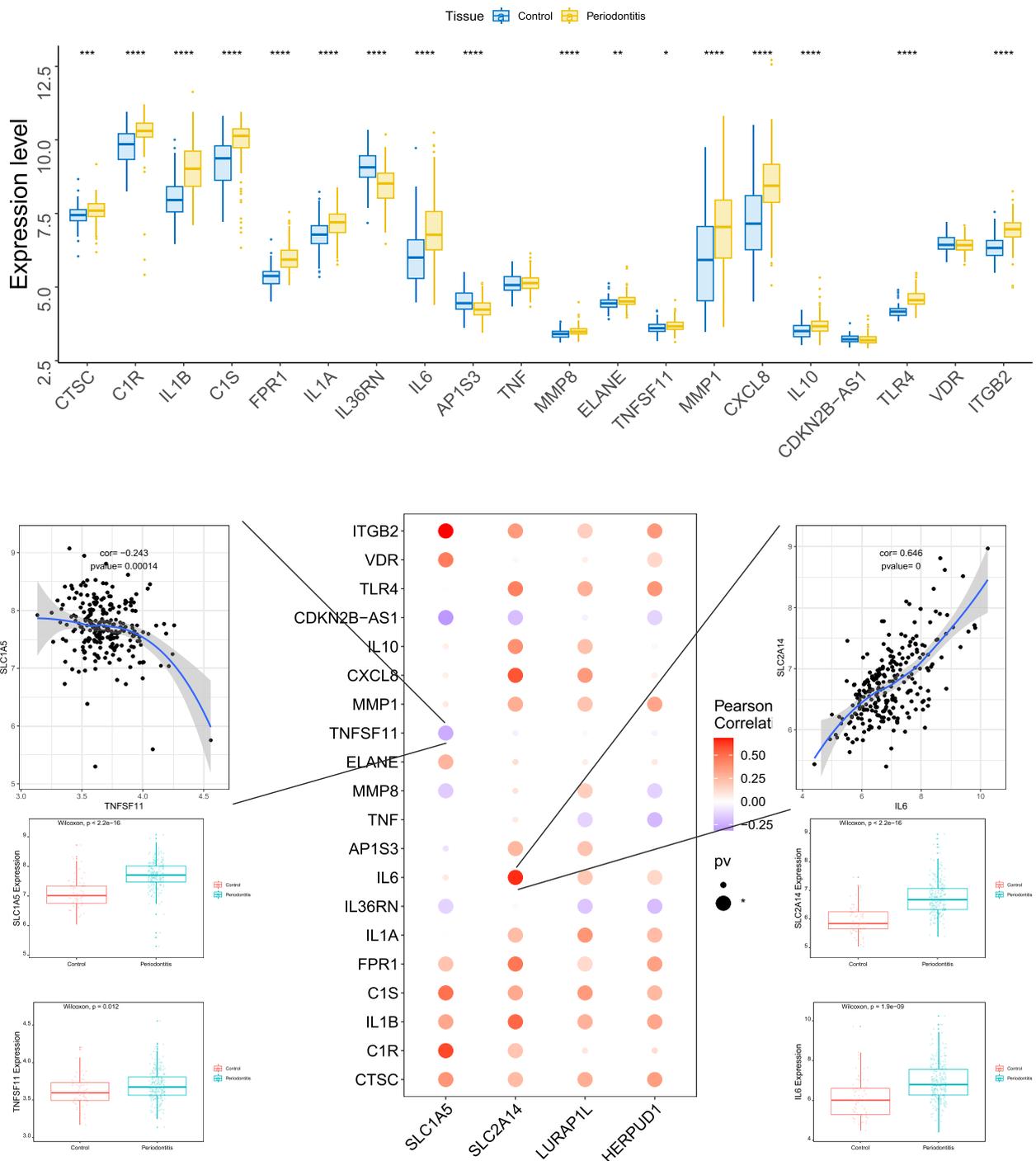
**Fig. 5** Heatmap between four key genes and immune microenvironment characteristic factors. Heatmap shows the relationship between key genes and chemokine-related genes(A)/immunoinhibitor-related genes(B)/immunostimulator-related genes(C)/MHC-related genes(D)/receptor-related genes(E). Red indicates a positive correlation, purple indicates a negative correlation. The number of asterisk designates the correlation coefficient between key genes and TME characteristic factors

interaction, and so on. Pathways enriched by HERPUD1 gene GO include branching involved in Mammary Gland duct morphogenesis and B CELL DIFFERENTIATION, while those enriched by KEGG include ALPHA LINOLENIC ACID METABOLISM and B CELL RECEPTOR SIGNALING PATHWAY (Fig. 7A-H). It is suggested that these signal pathways have a certain influence on the progress of the disease.

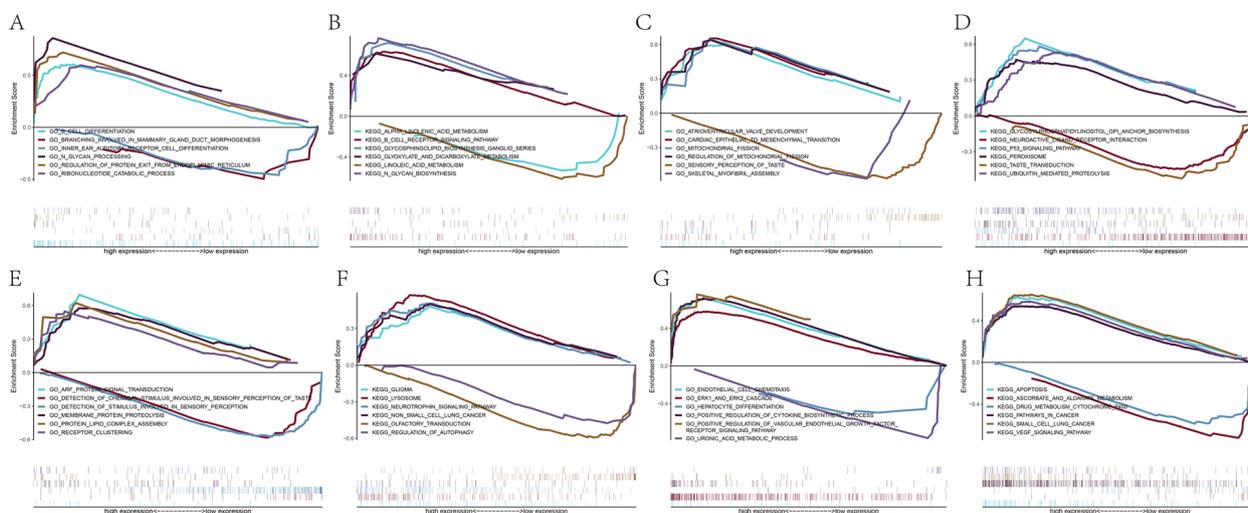
**The prediction of key genes’ transcriptional regulatory network and targeted drugs**

We employed this set of four key genes for the gene set analysis in the current study, aiming to further investigate the transcriptional regulatory networks associated with these critical genes. Through the Cistrome DB online database, we predicted the associated transcription factors. Specifically, SLC1A5 was predicted to have 81 transcription factors, SLC2A14 was associated with 69 transcription factors, LURAP1L showed predictions for 69 transcription factors, and HERPUD1 was linked

to 94 transcription factors. Finally, a transcriptional regulatory network related to periodontitis was visualized using Cytoscape (Fig. 8A). We predicted potential miRNAs and lncRNAs for four key genes using the miRWalk and ENCORI databases, respectively. Firstly, we extracted mRNA-miRNA pairs related to these four mRNAs from the miRWalk database, resulting in a total of 1,041 miRNAs. Subsequently, we retained only 54 mRNA-miRNA pairs detectable in either the TargetScan or miRDB databases, encompassing three mRNAs and nine miRNAs. Utilizing these nine miRNAs, we predicted interacting lncRNAs, identifying a total of 3,527 interaction pairs involving nine miRNAs and 1,497 lncRNAs. Finally, we constructed the ceRNA network using Cytoscape (v3.7), as illustrated in Fig. 8B. In the end, we categorized the differential mRNA into two groups: upregulated and downregulated. Using the Connectivity Map database for drug target prediction on differentially expressed genes, the results indicated a significant negative correlation between the expression



**Fig. 6** Pearson's correlation analysis between four key genes and pathogenic genes associated with periodontitis. The GeneCards database predicts the top 20 pathogenic genes in periodontal tissue. A box plot illustrates the expression patterns of these 20 genes in periodontitis and normal tissues, where yellow represents periodontal tissue, and blue represents normal tissue. Pearson's correlation analysis shows the relationship between key genes and pathogenic genes. Red indicates a positive correlation, purple indicates a negative correlation. \* $P < 0.05$ ; \*\* $P < 0.051$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$



**Fig. 7** GSEA enrichment analysis. **A-H** The GO and KEGG enrichment plot for the top pathway in the 4 key genes including SLC1A5, SLC2A14, LURAP1L, and HERPUD1

profiles of flurofamide, L-733060, memantine, tetrabenazine, WAY-213613, and the disease perturbation profiles. These drugs demonstrated the ability to alleviate or even reverse the pathological state (Figs. 8C-G).

**Discuss**

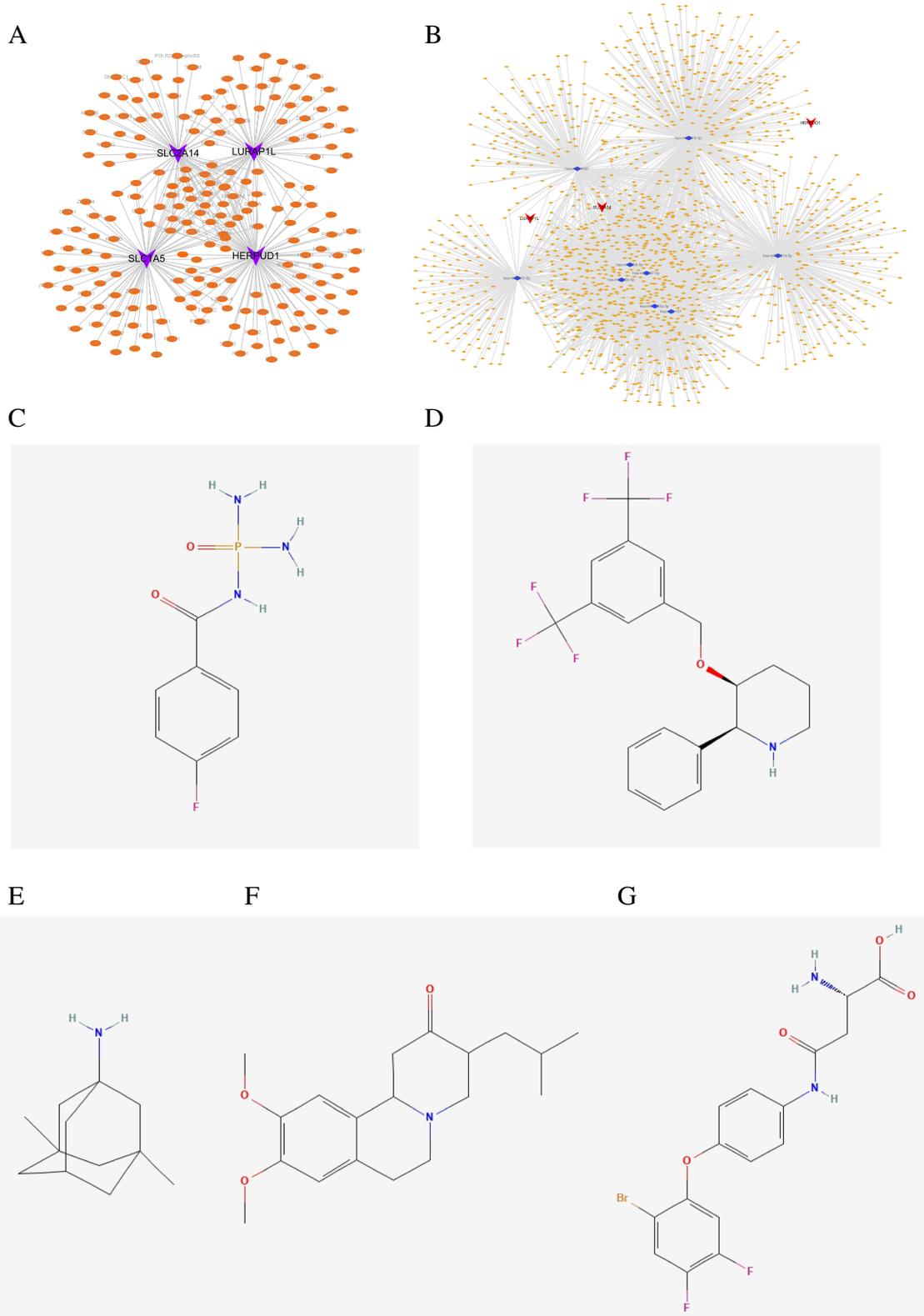
Although an increasing number of studies have gradually unveiled the role of ferroptosis, a novel and critical form of cell death, in the pathogenesis of periodontitis, the specific mechanisms through which it influences the onset and progression of this condition remain elusive [21–23]. Moreover, there is a scarcity of reports identifying key genes associated with ferroptosis that could serve as valuable clinical biomarkers for periodontitis. Therefore, in this study, we initially identified 36 candidate genes based on the differential expression of ferroptosis-related genes in normal versus periodontitis-affected samples. Subsequently, through protein–protein interaction networks and multi-dataset machine learning algorithms, we further narrowed down these candidates to four key ferroptosis-related genes differentially expressed in periodontitis, namely SLC1A5, SLC2A14, LURAP1L, and HERPUD1. We analyzed and validated the roles of these key genes in different subtypes of periodontitis through single-cell sequencing, focusing on their involvement

in immune infiltration and GSEA enrichment functions. This investigation further revealed the association of these ferroptosis-related key genes with molecular mechanisms involving immune infiltration and signal transduction in the disease progression of periodontitis. Ultimately, by predicting the ceRNA mechanisms or drug targets that may regulate these key genes through transcriptional regulatory networks, our study provides valuable and promising research directions for utilizing these key genes as diagnostic and therapeutic markers in the clinical management of periodontitis.

Programmed cell death encompasses apoptosis, necrosis, pyroptosis, and ferroptosis [24, 25]. The roles of apoptosis, necrosis, and pyroptosis-related genes in the pathogenesis of periodontitis have been extensively studied and reported in the literature [26–29]. In contrast, while some reports have shed light on the significance of ferroptosis in the pathogenicity of periodontitis, the specific genes and molecular mechanisms involved remain insufficiently understood, necessitating further experimental and bioinformatics research [30–32]. Therefore, this study primarily focuses on employing comprehensive bioinformatics analysis to identify ferroptosis-related genes in cells within human periodontal tissues affected by periodontitis. We conducted a bioinformatics

(See figure on next page.)

**Fig. 8** The prediction of key genes’ transcriptional regulatory network and targeted drugs. **A** The Cistrome DB online database shows the predicted transcription factors for key genes. **B** The miRWalk and ENCORI databases show the potential ceRNA net for four key genes. Dots represent gene or RNA names, and lines represent transcriptional regulatory relationships. **C-G** The Connectivity Map(CMap) revealed five potential targeted drugs flurofamide, L-733060, memantine, tetrabenazine, WAY-213613 for periodontitis. The diagram illustrates the chemical formulas of these five targeted drugs



**Fig. 8** (See legend on previous page.)

analysis of the GSE16134 dataset from the GEO database, employing Random Forest and SVM machine learning algorithms to identify four differentially expressed ferroptosis-related key genes: SLC1A5, SLC2A14, LURAP1L, and HERPUD1. Subsequently, we explored the relationship of these four key genes with immune infiltration. Our findings indicate that the expression of HERPUD1 and SLC1A5 positively correlates with plasma cell infiltration and negatively with M1 macrophage infiltration. Conversely, the expression levels of LURAP1L and SLC2A14 show significant negative correlations with the infiltration of CD8+ T cells and naive CD4+ T cells. Further analysis of the associations of these key genes with immune-stimulatory, immune-suppressive, and chemotactic factors revealed a positive correlation between these four crucial ferroptosis-related genes and the level of immune infiltration. These comprehensive results indicate the significant role of these key genes in immune processes within the context of periodontitis.

Existing research indicates that SLC1A5 is an amino acid transporter, primarily responsible for the transport of alanine, serine, and cysteine. It plays a crucial role in maintaining normal cellular growth and the immune functions related to T lymphocyte activity [33, 34]; SLC2A14, a member of the glucose transporter family, is involved in glucose transport and metabolic regulation [35, 36]; LURAP1L influences the tumorigenicity of fibroblasts through NF- $\kappa$ B signaling [37]; HERPUD1, a protein associated with endoplasmic reticulum (ER) stress, is involved in the ER stress response and linked to the ubiquitin degradation system [38, 39]. It may also regulate cellular lipid responses by affecting lipid synthesis and metabolic pathways [40]. A common characteristic of these four key genes is their involvement in the ferroptosis process by influencing metabolic pathways related to amino acids, glucose, and lipid synthesis. Of particular interest is recent study have found that Bomidin, a novel recombinant antimicrobial peptide, acts as a ferroptosis inhibitor by activating the Keap1/Nrf2 signaling pathway. It has been demonstrated to regulate Keap1 protein stability by binding and ubiquitin–proteasome pathway, thereby alleviating inflammatory responses for the treatment of periodontitis [40], and this suggests that targeting ferroptosis-related genes for the treatment of periodontitis is feasible. Consequently, we reasonably speculate that these four key genes can serve as genetic adjuvant therapy to conventional treatments, impacting periodontitis clinical management by modulating immune functionality and metabolic reprogramming during the validation process, thereby improving clinical treatment through the ferroptosis pathway [41–43].

The ANN diagnostic model comprising four key genes—SLC1A5, SLC2A14, LURAP1L, and HERPUD1—demonstrated a high predictive efficacy for periodontitis with an AUC of 0.928 in the dataset. Periodontitis, a chronic inflammatory disease associated with bacterial biofilms, is characterized by the loss of periodontal support and has been traditionally regarded as a "silent disease," adversely affecting the oral health of over 700 million people globally. The specific pathogenesis of this multifactorial disease remains elusive. Our study links the pathogenesis of periodontitis with the ferroptosis pathway, building a bridge through the bioinformatics-predicted key genes SLC1A5, SLC2A14, LURAP1L, and HERPUD1. Existing research indicates that periodontal tissue in periodontitis exhibits dysregulation of iron-containing compounds such as ferritin, transferrin, and heme [44, 45]. Excess free iron catalyzes the Fenton reaction, leading to radical generation and systemic ROS influx. The periodontal pocket, characterized by an anoxic environment due to the predominance of subgingival anaerobic bacteria, is influenced by HIF, a primary regulator of many genes related to iron homeostasis and inflammation, including TFR1, erythropoietin, and ferritin [14]. This facilitates fatty acid deposition in the microenvironment [46]. The "microbial infection-ROS-inflammation" feed-forward mechanism appears to accelerate the body's susceptibility to infection, inflammation, and oxidative stress [47]. Studies have shown a close association between oxidative stress, lipid peroxidation, and periodontitis. Changes in GPX, the glutathione/oxidized glutathione ratio, myeloperoxidase (MPO) activity, lipid peroxides, and other markers in periodontitis patients indicate that an increase in GPX may be an antioxidative compensation in response to oxidative stress [48]. The striking similarities in marker alterations between periodontitis and iron-induced cell death suggest a potential correlation. Based on this, our study hypothesizes that ferroptosis-related genes, including SLC1A5, SLC2A14, LURAP1L, and HERPUD1, could be novel biomarkers for periodontitis. However, this study has limitations, including a small sample size and lack of clinical validation analysis. Therefore, further validation analyses on a larger sample scale are necessary.

## Conclusion

The ferroptosis-related genes SLC1A5, SLC2A14, LURAP1L, and HERPUD1 may serve as novel biomarkers for the diagnosis of periodontitis. These genes are likely to influence the progression of periodontitis by affecting immune infiltration and metabolic reprogramming through the ferroptosis pathway.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12903-024-04342-2>.

Supplementary Material 1.

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### Authors' contributions

Chen performed the data analysis. Chen Liu and Gao wrote the main manuscript text and Chen and Ou prepared Figures 1, 2, 3, 4, 5, 6, 7 and 8. Gao performed the validation. All authors reviewed the manuscript.

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### Availability of data and materials

The authors acquired periodontitis-related data for single-cell analysis by downloading the GSE171213 dataset from the NCBI GEO public database. The dataset comprises a total of nine samples, including five from diseased subjects and four from control subjects. The Series Matrix File data for GSE16134 was downloaded from the NCBI GEO public database, with the annotation file labeled as GPL570. The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

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### References

- Yan Y, Zhan Y, Wang X, Hou J. Clinical evaluation of ultrasonic subgingival debridement versus ultrasonic subgingival scaling combined with manual root planing in the treatment of periodontitis: study protocol for a randomized controlled trial. *Trials*. 2020;21(1):113 (Published 2020 Jan 28).
- Kwon T, Lamster IB, Levin L. Current Concepts in the Management of Periodontitis. *Int Dent J*. 2021;71(6):462–76.
- Richards D. Review finds that severe periodontitis affects 11% of the world population. *Evid Based Dent*. 2014;15(3):70–1.
- Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Periodontol*. 2018;89(Suppl 1):S173–82.
- Slots J. Periodontitis: facts, fallacies and the future. *Periodontol* 2000. 2017;75(1):7–23.
- Qasim SSB, Al-Otaibi D, Al-Jasser R, Gul SS, Zafar MS. An Evidence-Based Update on the Molecular Mechanisms Underlying Periodontal Diseases. *Int J Mol Sci*. 2020;21(11):3829 (Published 2020 May 28).
- Sun Q, Zhu E. Molecular mechanism and diagnostic marker investigation of endoplasmic reticulum stress on periodontitis. *BMC Oral Health*. 2023;23(1):135 (Published 2023 Mar 9).
- Currò M, Matarese G, Isola G, et al. Differential expression of transglutaminase genes in patients with chronic periodontitis. *Oral Dis*. 2014;20(6):616–23.
- Isola G, Santonocito S, Distefano A, et al. Impact of periodontitis on gingival crevicular fluid miRNAs profiles associated with cardiovascular disease risk. *J Periodontol Res*. 2023;58(1):165–74.
- Galluzzi L, Vitale I, Aaronson SA, et al. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ*. 2018;25(3):486–541.
- Tang D, Chen X, Kang R, Kroemer G. Ferroptosis: molecular mechanisms and health implications. *Cell Res*. 2021;31(2):107–25.
- Jiang X, Stockwell BR, Conrad M. Ferroptosis: mechanisms, biology and role in disease. *Nat Rev Mol Cell Biol*. 2021;22(4):266–82.
- Kell DB. Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases. *BMC Med Genomics*. 2009;2:2 (Published 2009 Jan 8).
- Wessling-Resnick M. Iron homeostasis and the inflammatory response. *Annu Rev Nutr*. 2010;30:105–22.
- Wang Y, Andrukhov O, Rausch-Fan X. Oxidative Stress and Antioxidant System in Periodontitis. *Front Physiol*. 2017;8:910 (Published 2017 Nov 13).
- Kanzaki H, Wada S, Narimiya T, et al. Pathways that Regulate ROS Scavenging Enzymes, and Their Role in Defense Against Tissue Destruction in Periodontitis. *Front Physiol*. 2017;8:351 (Published 2017 May 30).
- Liu C, Mo L, Niu Y, Li X, Zhou X, Xu X. The Role of Reactive Oxygen Species and Autophagy in Periodontitis and Their Potential Linkage. *Front Physiol*. 2017;8:439 (Published 2017 Jun 23).
- Boyer E, Le Gall-David S, Martin B, et al. Increased transferrin saturation is associated with subgingival microbiota dysbiosis and severe periodontitis in genetic haemochromatosis. *Sci Rep*. 2018;8(1):15532 (Published 2018 Oct 19).
- Bains VK, Bains R. The antioxidant master glutathione and periodontal health. *Dent Res J (Isfahan)*. 2015;12(5):389–405.
- Grant MM, Brock GR, Matthews JB, Chapple IL. Crevicular fluid glutathione levels in periodontitis and the effect of non-surgical therapy. *J Clin Periodontol*. 2010;37(1):17–23.
- Xu Z, Tan R, Li X, Pan L, Ji P, Tang H. Development of a classification model and an immune-related network based on ferroptosis in periodontitis. *J Periodontol Res*. 2023;58(2):403–13.
- Wang Y, Lin H, Huang W, et al. Curcumin Attenuates Periodontal Injury via Inhibiting Ferroptosis of Ligature-Induced Periodontitis in Mice. *Int J Mol Sci*. 2023;24(12):9835 (Published 2023 Jun 7).
- Wang H, Qiao X, Zhang C, Hou J, Qi S. Long non-coding RNA LINC00616 promotes ferroptosis of periodontal ligament stem cells via the micro-RNA-370 / transferrin receptor axis. *Bioengineered*. 2022;13(5):13070–81.
- Bock FJ, Tait SWG. Mitochondria as multifaceted regulators of cell death. *Nat Rev Mol Cell Biol*. 2020;21(2):85–100.
- Dadsena S, Zollo C, García-Sáez AJ. Mechanisms of mitochondrial cell death. *Biochem Soc Trans*. 2021;49(2):663–74.
- Song B, Zhou T, Yang WL, Liu J, Shao LQ. Programmed cell death in periodontitis: recent advances and future perspectives. *Oral Dis*. 2017;23(5):609–19.
- Xu X, Zhang T, Xia X, et al. Pyroptosis in periodontitis: From the intricate interaction with apoptosis, NETosis, and necroptosis to the therapeutic prospects. *Front Cell Infect Microbiol*. 2022;12:953277 (Published 2022 Aug 16).
- Akhondian S, Fatemi K, Ebrahim Zadeh N, et al. Necroptosis has a crucial role in the development of chronic periodontitis. *J Oral Biol Craniofac Res*. 2023;13(4):465–70.
- Liu J, Zhang D, Cao Y, et al. Screening of crosstalk and pyroptosis-related genes linking periodontitis and osteoporosis based on bioinformatics and machine learning. *Front Immunol*. 2022;13:955441 (Published 2022 Aug 5).
- Qiao S, Li B, Cai Q, et al. Involvement of ferroptosis in Porphyromonas gingivalis lipopolysaccharide-stimulated periodontitis in vitro and in vivo. *Oral Dis*. 2023;29(8):3571–82.
- Zhao Y, Li J, Guo W, Li H, Lei L. Periodontitis-level butyrate-induced ferroptosis in periodontal ligament fibroblasts by activation of ferritinophagy. *Cell Death Discov*. 2020;6(1):119 (Published 2020 Nov 10).

32. Yang M, Shen Z, Zhang X, et al. Ferroptosis of macrophages facilitates bone loss in apical periodontitis via NRF2/FSP1/ROS pathway. *Free Radic Biol Med.* 2023;208:334–47.
33. Yoo HC, Park SJ, Nam M, et al. A Variant of SLC1A5 Is a Mitochondrial Glutamine Transporter for Metabolic Reprogramming in Cancer Cells. *Cell Metab.* 2020;31(2):267–283.e12.
34. Nachef M, Ali AK, Almutairi SM, Lee SH. Targeting SLC1A5 and SLC3A2/SLC7A5 as a Potential Strategy to Strengthen Anti-Tumor Immunity in the Tumor Microenvironment. *Front Immunol.* 2021;12:624324 (Published 2021 Apr 19).
35. Amir Shaghaghi M, Murphy B, Eck P. The SLC2A14 gene: genomic locus, tissue expression, splice variants, and subcellular localization of the protein. *Biochem Cell Biol.* 2016;94(4):331–5.
36. Amir Shaghaghi M, Zhouyao H, Tu H, et al. The *SLC2A14* gene, encoding the novel glucose/dehydroascorbate transporter GLUT14, is associated with inflammatory bowel disease. *Am J Clin Nutr.* 2017;106(6):1508–13.
37. Ren X, Li L, Wu J, Lin K, He Y, Bian L. PDGF-BB regulates the transformation of fibroblasts into cancer-associated fibroblasts via the lncRNA LURAP1L-AS1/LURAP1L/IKK/κB/NF-κB signaling pathway. *Oncol Lett.* 2021;22(1):537.
38. Nie X, Liu D, Zheng M, et al. HERPUD1 promotes ovarian cancer cell survival by sustaining autophagy and inhibit apoptosis via PI3K/AKT/mTOR and p38 MAPK signaling pathways. *BMC Cancer.* 2022;22(1):1338 (Published 2022 Dec 21).
39. Li W, Wang Y, Zhu L, et al. The P300/XBP1s/Herpud1 axis promotes macrophage M2 polarization and the development of choroidal neovascularization. *J Cell Mol Med.* 2021;25(14):6709–20.
40. Wu W, Li G, Dong S, et al. Bomidin attenuates inflammation of periodontal ligament stem cells and periodontitis in mice via inhibiting ferroptosis. *Int Immunopharmacol.* 2024;127:111423.
41. Han L, Zhou J, Li L, et al. SLC1A5 enhances malignant phenotypes through modulating ferroptosis status and immune microenvironment in glioma. *Cell Death Dis.* 2022;13(12):1071 (Published 2022 Dec 24).
42. Huang F, Zheng Y, Li X, Luo H, Luo L. Ferroptosis-related gene AKR1C1 predicts the prognosis of non-small cell lung cancer. *Cancer Cell Int.* 2021;21(1):567 (Published 2021 Oct 26).
43. Peng Y, Li N, Tang F, et al. Corosolic acid sensitizes ferroptosis by upregulating HERPUD1 in liver cancer cells. *Cell Death Discov.* 2022;8(1):376 (Published 2022 Aug 29).
44. Costa SA, Ribeiro CCC, Moreira ARO, Carvalho Souza SF. High serum iron markers are associated with periodontitis in post-menopausal women: A population-based study (NHANES III). *J Clin Periodontol.* 2022;49(3):221–9.
45. Liu LY, McGregor N, Wong BK, Butt H, Darby IB. The association between clinical periodontal parameters and free haem concentration within the gingival crevicular fluid: a pilot study. *J Periodontol Res.* 2016;51(1):86–94.
46. Ezzeddini R, Taghikhani M, Salek Farrokhi A, et al. Downregulation of fatty acid oxidation by involvement of HIF-1α and PPARγ in human gastric adenocarcinoma and related clinical significance. *J Physiol Biochem.* 2021;77(2):249–60.
47. Borges I Jr, Moreira EA, Filho DW, de Oliveira TB, da Silva MB, Fröde TS. Pro-inflammatory and oxidative stress markers in patients with periodontal disease. *Mediators Inflamm.* 2007;2007:45794.
48. Lahdentausta LSJ, Paju S, Mäntylä P, et al. Saliva and serum biomarkers in periodontitis and coronary artery disease. *J Clin Periodontol.* 2018;45(9):1045–55.

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