Research Article

Identifying Common Genes and Pathways Associated with Periodontitis and Aging by Bioinformatics Analysis

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Background. This work used bioinformatic analysis to identify the relationship between periodontitis (PD) and aging, which could lead to new treatments for periodontal disease in the elderly. Method. Four microarray datasets were obtained from the Gene Expression Omnibus (GEO) database and analyzed in R language to identify differentially expressed genes (DEGs). The common DEGs of PD and aging were evaluated as key genes in this investigation by a Venn diagram. These common DEGs were analyzed through additional experiments and analysis, such as pathway analysis and enrichment analysis, and a network of protein-protein interactions (PPIs) was constructed. Cytoscape was used to visualize hub genes and critical modules based on the PPI network. Interaction of TF-genes and miRNAs with hub genes is identified. Result. 84 common DEGs were found between PD and aging. Cytohubba was performed on the PPI network obtained from STRING tool, and the top 10 genes (MMP2, PDGFRB, CTGF, CD34, CXCL12, VIM, IL2RG, ACTA2, COL4A2, and TAGLN) were selected as hub genes. VIM may be a potential biomarker in the analysis of linked hub gene regulatory networks, and hsa-mir-21 and hsa-mir-125b are predicted to be associated in PD and aging. Conclusion. This study investigated the key genes and pathways interactions between PD and aging, which may help reveal the correlation between PD and aging. The current research results are obtained by prediction, and follow-up biological experiments are required for further verification.

1. Introduction

Periodontitis, an infectious and chronic inflammatory disease, is one of the world’s most common chronic diseases, affecting roughly 11% of adults globally [1, 2]. Without proper treatment, PD may cause damage to the periodontal soft and hard tissues, leading to the formation of periodontal pockets, loss of attachment, resorption of alveolar bone, and ultimately tooth loss [3].

Aging is a time-dependent process characterized by the progressive deterioration and loss of function of multiple organs of an organism and was recently introduced by the World Health Organization as a new disease in the International Classification of Diseases [4]. From a biological point of view, with age, many molecular pathways are deregulated due to the accumulation of various molecules and cellular damage, thereby affecting tissue homeostasis, causing structural damage, and affecting its biomechanical properties [5, 6].

In recent years, an increasing number of studies have found that PD is closely linked to the advancement of aging-related systemic disorders such as diabetes, coronary heart disease, and rheumatoid arthritis [7–9]. Although aging itself has not been classified as a contributing factor to periodontitis [10], recent studies have shown that similar to other systemic diseases, PD susceptibility changes with aging, and the degree of alveolar bone destruction is related to age factors [11]. The reason may be that one of the major changes occurring in the aging process is the dysregulation of the immune response, leading to the dysregulation of the proinflammatory mediators and the chronic systemic inflammatory state of [12]. However, the hyperactivation of the immune response in the host system can directly activate the osteoclast activity and the alveolar...
bone loss, which can further induce the progression of periodontal disease [13]. However, the underlying mechanisms between PD and aging are not fully understood. As a result, additional research on the function of aging in PD will contribute to a better understanding and treatment of periodontal disease in the elderly. It may provide new ideas for the study of aging-related systemic disorders as well as prospective novel tactics for the treatment of periodontal diseases in the elderly.

To address this question, we employed an integrative bioinformatics approach to identify common functional genes associated with PD and aging. Moreover, investigation of DEGs in four datasets from the GEO can discover common mechanisms of action and PPI nodes. It may provide a basic theoretical basis for the diagnosis and pathogenesis of periodontal diseases in the elderly. Figure 1 depicted the progressive workflow of our study.

### 2. Materials and Methods

#### 2.1. Data Sources

GEO (https://www.ncbi.nlm.nih.gov/geo) is a public database developed and maintained by the National Center for Biotechnology Information (NCBI), which stores gene expression data, microarrays, and other forms of high-throughput functional genomic data and makes it available to researchers for free [14]. This study retrieved the gene expression profiling datasets GSE10334, GSE16134, GSE23586, and GSE83382 from the GEO, based on the keywords "periodontitis and gingival tissue," "aging and gingival tissue," and "Homo sapiens." These datasets were screened based on inclusion criteria. The inclusion criteria were as follows: (1) interdental CAL detectable at ≥2 nonadjacent teeth or (2) buccal or oral with pocketing >3 mm detectable at ≥2 teeth. Details of the microarray dataset are presented in Table 1.

#### 2.2. DEG Identification

The primary goal of this work is to identify common DEGs from the GSE10334, GSE16134, GSE23586, and GSE83382 datasets. To identify the DEGs of GSE83382, we used the R language and the limma package (version 3.42.2) [15]. The GEO2R (https://www.ncbi.nlm.nih.gov/GEO2R/) web tool, which also employs the limma program to detect DEGs, was used to evaluate the DEGs from the GSE10334, GSE16134, and GSE23586 datasets. GEO2R (version 3.26.8) is an online GEO analysis application that allows users to analyze and compare data from two or more separate sample groups under similar experimental settings.
Figure 2: Continued.
DEGs were defined as genes with screening criteria $|\text{Log}_2\text{fold change (FC)}| > 0.5$ and $P$ value $< 0.05$. The intersection of common DEGs from the four datasets was defined as public DEGs in the following study and was found using the Venn diagram network tool (version 1.6.20) (http://bioinfogp.cnb.csic.es/tools/venny/), and finally, the primary experimental genes for the entire study were determined.

2.3. DEG Analysis at the Functional Level. Gene Ontology (GO) enrichment analysis is a technique for examining gene expression data. Enrichment is the process of classifying genes based on past knowledge, such as genome annotation information. GO is a method for identifying genes, gene products, and sequences as potential biological phenomena based on their roles in order to annotate and classify genes. It primarily consists of biological processes (BP), cellular components (CC), and molecular functions (MF) [17]. KEGG is a comprehensive database resource for biological interpretation of genomic sequences and other high-throughput data [18]. WebGestalt (http://www.webgestalt.org/), a long-standing and extensively used web program for functional enrichment analysis, has been regularly updated to fulfill the needs of biologists from various research fields [19]. WebGestalt (2019) was used in this study to perform GO and KEGG analysis on common DEGs. The usual measure was a $P$ value of 0.05.

2.4. PPI Network Analysis. The search tool to retrieve interacting genes/proteins (STRING) (https://string-db.org/) is an interactive network for studying gene and protein interactions, supplementing heuristic association and analysis methods, and investigating known and predicted PPI associations with PD and aging. In this investigation, we used a string database to build a PPI network of DEGs and interacted with a composite score of $>0.4$ as a crucial criterion [20]. In addition, we used Cytoscape (https://cytoscape.org/) to build the PPI network for visual representation and additional experimental testing. Cytoscape (version 3.9.1) is a free and open-source network visualization platform that combines biomolecular interaction networks, high-throughput expression data, and other molecular states into a cohesive conceptual framework [21].

2.5. Hub Gene Extraction and Module Analysis. The PPI network is made up of nodes, edges, and connections, with the most entangled nodes serving as hub genes. Cytohubba (http://apps.cytoscape.org/apps/cytohubba) is a new Cytoscape plugin for sorting and retrieving central or possible or potential target elements underpinning biological networks. The cellular landscape plugin Cytohubba can discover hub genes more precisely thanks to 11 algorithms [22]. Furthermore, the Molecular Complex Detection (MCODE) (http://apps.cytoscape.org/apps/mcode) plugin Cytoscape was used with a plugin with default parameters to discriminate the modules that best reflect DEG clusters [23].

2.6. Transcription Factors and miRNAs Interact with Hub Genes. A network analysis database was used to investigate the human transcription factors (TFs) of the discovered hub genes. TF-gene interactions with identified hub genes evaluated TF outcomes at the functional pathway and gene expression levels [24]. NetworkAnalyst (https://www.networkanalyst.ca/) (version 3.0) is a complete platform of web tools that allows researchers to execute a wide range of common and difficult meta-analyses on gene expression data via an easy-to-use web interface [25]. In addition, miRNAs targeting gene interactions were incorporated, and TF-miRNA coregulated interactions were collected from a network library [26]. Finally, the

Figure 2: Identification of gene expression profiles in the four datasets. (a–c) Volcano plot of PD microarray data. (d) Volcano plot of aging microarray data. (e) Venn diagram of the 84 common DEGs between PD and aging.
Figure 3: Continued.
Collagen-containing extracellular matrix

Extracellular matrix

Cell leading edge

Receptor complex

Cell surface

Integrin complex

Intrinsic component of plasma membrane

Endoplasmic reticulum

Protein complex involved in cell adhesion

Integral component of plasma membrane

Figure 3: Continued.
Extracellular matrix structural constituent
Protein-containing complex binding
Vascular endothelial growth factor binding
Integrin binding
Phosphatidylinositol 3-kinase binding
Actin filament binding
Transmembrane receptor protein tyrosine kinase activity
Growth factor binding
Extracellular matrix structural constituent conferring compression resistance
Transmembrane receptor protein kinase activity

Figure 3: Continued.
Cytoscape tool was used to show the network of interactions between TFs, miRNAs, and hub genes.

3. Results

3.1. DEG Identification. As described in the materials and methods section, we performed DEG analysis on the screened samples and used volcano plots to show the final screening results of DEGs (Figures 2(a)–2(d)). We identified 2000 DEGs in GSE10334, 2132 DEGs in GSE16134, 3467 DEGs in GSE23586, and 2268 DEGs in GSE83382. Whereas for extraction, all significant DEGs are based on $|\log_2 \text{fold change (FC)}| > 0.5$ and $P$ value $< 0.05$. A Venn diagram network tool was used to visualize common DEGs among the four datasets. These common genes were the main experimental gene for the entire study and were used to complete the next experiments. The Venn diagram revealed 84 shared differential genes (Figure 2(e)), including 24 upregulated genes and 51 downregulated genes.

3.2. DEG Enrichment Analysis by GO and KEGG Pathways. Based on the screened common DEGs, we ran GO enrichment and KEGG pathway analysis to assess the biological processes, cellular components, and molecular function of gene functions and their linked pathways. Figure 3 depicted the top ten substantially enriched phrases for each process with a linear connection in a histogram for each category. Extracellular structure organization, extracellular matrix organization, cell adhesion, biological adhesion, cell-substrate adhesion, blood vessel formation, blood vessel morphogenesis, and so on are...
all part of the BP category. These genes were abundant in collagen-containing extracellular matrix, extracellular matrix, cell leading edge, receptor complex, cell surface, and integrin complexes on the CC side. Extracellular matrix structural components, protein-containing complex binding, vascular endothelial growth factor binding, integrin binding, phosphatidylinositol 3-kinase binding, and actin filament binding were the most abundant genes in this category for MF. KEGG pathway analysis revealed that these genes were significantly involved in focal adhesion, PI3K-Akt signaling pathway, ECM-receptor interaction, regulation of actin cytoskeleton, leukocyte transendothelial migration, and cell adhesion molecules (CAMs).

3.3. PPI Network Identification Hub Genes and Module Analysis. To clarify the interactions between DEGs, we performed a PPI network analysis on all common DEGs in this study (Figure 4). The resulting PPI network contains 55 nodes and 133 edges. At the same time, we used Cytoscape to analyze the topological logic properties of the network. In the analysis results, we found highly connected genes, among which MMP2, PDGFRB, CTGF, CD34, CXCL12, VIM, IL2RG, ACTA2, COL4A2, and TAGLN were the top 10 highly connected genes (Supplementary Table 1). We then defined these 10 genes as hub genes. Subsequently, we reviewed the regulatory roles of these hub genes in PD and aging (Table 2).

The identified common DEGs were projected onto the Cytoscape MCODE plugin to assess key modules. The two most critical modules, which include the specified hub genes, are chosen from among all the modules. We used KEGG pathway analysis on the chosen modules and discovered that the genes in the two modules were considerably significantly enriched in the GnRH signaling route, leukocyte transendothelial migration, relaxin signaling pathway, cancer pathways, and actin cytoskeleton regulation (Figure 5).

3.4. TF-Gene Interaction Network. We examined the connections between hub genes to discover significant changes occurring at the transcriptional level and obtain insight into the most critical regulatory molecules among hub genes. NetworkAnalyst network analysis program found 109 nodes and 118 edges of 7 hub genes. The generated data were then put into Cytoscape software to visualize the interactions between TFs and hub genes (Figure 6). The expression values and characteristics of hub genes in individual cohorts are shown in Supplementary Table 2. The results showed that VIM was regulated by 75-TF genes, IL2RG was regulated by 17 TF-genes, and COL4A2 was regulated by 9-TF genes, which indicated that the above three genes may be key genes in the TF-target network of PD and aging.

3.5. TF-miRNA Coregulatory Network. A network analysis framework was used to build TF-miRNA coregulatory networks. The TF-miRNA coregulatory network splits TF-miRNA interactions and hub genes. This connection might be in charge of controlling hub gene expression. The TF-miRNA coregulation network was constructed with 204 nodes and
238 edges. Hub genes were interacted with by 109 miRNAs and 85 TF-genes (Figure 7). The expression values and characteristics of hub genes in individual cohorts are shown in Supplementary Table 3.

4. Discussion

With the global aging and the increase in the number of remaining teeth in the oral cavity, the demand for periodontal disease treatment and health care for the elderly population will continue to grow. A great number of studies have linked PD to age-related systemic disorders such as coronary heart disease and rheumatoid arthritis, indicating that chronic periodontal inflammation may increase with the aging of tissues and organs. Therefore, exploring the impact of aging on PD and its mechanism of action is critical [44, 45].

As an emerging interdisciplinary subject, bioinformatics has been widely used in the field of life sciences. Recently,
the use of bioinformatics tools to study the genetic relationship between aging and inflammatory diseases, studies have found some correlation in molecular pathogenesis and disease development [46–48]. In this study, the four datasets identified 84 common DEGs, including 24 common upregulated genes and 5 common downregulated genes. DEGs were subjected to GO functional enrichment analysis and KEGG pathway analysis at the same time. The protein interaction network and the regulatory network between the proteins expressed by the differential genes were visualized and studied, and 10 hub genes were screened out, including MMP2, PDGFRB, CTGF, CD34, CXCL12, VIM, IL2RG, ACTA2, COL4A2, and TAGLN.

By performing GO functional enrichment analysis on DEGs, GO items were selected according to the P value. For BP, it mainly focuses on extracellular structural organization, extracellular matrix organization, cell adhesion bioadhesion, cell-substrate adhesion, vascular development, and vascular morphogenesis. In vitro, it enhanced the secretion of proinflammatory cytokines by macrophages by upregulating the

| Genes | PD | Aging |
|-------|----|-------|
| MMP2  | Compared with healthy individuals (controls), metalloproteases (MMP-2, MMP-9) cascade in the initiation and progression of inflammatory bone resorption and periodontal soft tissue destruction in patients with periodontitis. [27]. | Skin aging with age or premature aging caused by light promotes cellular inflammation, ROS production, and the increase of hydrogen peroxide, and matrix metalloproteinase (MMP2) expression is upregulated. [28]. |
| PDGFRB | PDGF receptor- (PDGFR-) β on periodontal cells is a crucial element for various functions, such as wound healing in periodontal tissue, and their expression decreases when subjected to fluid shear stress [29]. | Oxidative stress-induced senescent vascular smooth muscle cells were obviously desensitized to stimulation by platelet-derived growth factor- (PDGF-) BB, which may have been caused by suppression of promoter activity, transcription, translation, and activation levels of PDGF receptor- (PDGFR-) β [30]. |
| CTGF  | CTGF promotes the fusion of preosteoclasts by downregulating Bcl6 and subsequently increasing the expression of dendritic cell-specific transmembrane protein in periodontitis [31]. | An upregulation of CTGF expression has been demonstrated in senescent cells [32]. |
| CD34  | The highest concentration of CD34+ cells was observed in the group of patients with advanced periodontal disease [33]. Treatment of periodontitis has neutral effects on peripheral endothelial function but significantly decreases circulating CD34(+) cell count [34]. | The absolute number of circulating CD34(+) cells progressively and significantly decreased with advancing age [35]. |
| CXCL12 | CXCL12 (SDF-1alpha) may be involved in the immune defense pathway activated during periodontal disease. Upon the development of diseased tissues, CXCL12 (SDF-1alpha) levels increase and may recruit host defensive cells into sites of inflammation [36]. | CXCR4 pathway stimulated by CXCL12 regulated AKT activation, CREB phosphorylation, and P53 level to affect the process of aging and Alzheimer’s disease [37]. |
| VIM   | VIM expression is also regulated by TGF and proinflammatory cytokines. VIM expression increased significantly as periodontitis developed severe [38]. | It was reported that VIM expression in was significantly increased in aged skin fibroblasts [39]. |
| ACTA2 | None | Seven aging molecular phenotype-relevant key genes (ACTA2, CALD1, LMOD1, MYH11, MYL9, MYLK, and TAGLN) were identified, which were specifically upregulated in tumors and in relation to dismal prognosis [40]. |
| COL4A2 | COL4A2 in the ECM promotes osteogenic differentiation of PDLSCs through negative regulation of the Wnt/β-catenin pathway, which can be used as a potential therapeutic strategy to repair bone defects [41]. | Aging suppresses the expression levels of Col4a1 and Col4a2 and affects basement membrane-related factors in the steady state [42]. |
| TAGLN | None | Seven aging molecular phenotype-relevant key genes (ACTA2, CALD1, LMOD1, MYH11, MYL9, MYLK, and TAGLN) were identified, which were specifically upregulated in tumors and in relation to dismal prognosis [40]. |
| IL2RG | None | The expression levels of IL7, IL2RG, and IL7R were significantly lower in the 90-year-old adults, as compared with the middle-aged offspring [43]. |
Figure 6: Construction of TF-hub gene interaction network from Cytoscape. The purple color octagons represent the hub genes, and the yellow nodes represent TF-genes.

Figure 7: The network presents the TF-miRNA coregulatory network. The nodes in red color are the hub genes, yellow nodes represent miRNAs, and blue nodes indicate TF-genes.
expression of endothelial cell adhesion molecules [49]. In the CC, vascular development and vascular calcification have long been recognized as a degenerative age-related pathology caused by passive deposition of extracellular matrix [50]. The most significant MF of GO is extracellular matrix structural constituent, protein-containing complex binding, vascular endothelial growth factor binding, integrin binding, phosphatidylinositol 3-kinase binding, and actin filament binding. Oral fibroblast aging has been discovered to affect ECM synthesis and, more crucially, the structure of ECM fibers, which may hinder wound healing in the elderly [51]. According to cellular composition, the top GO keywords are collagen-containing extracellular matrix, extracellular matrix, and cell leading edge. Cell sheets made from PDLCs from elderly donors have lower osteogenic potential than cell sheets made from PDLCs from young donors based on the synthesis of ECM proteins such as fibronectin, integrin 1, and collagen type I [52].

The investigation of KEGG pathways for common DEGs revealed that these DEGs were mostly engaged in the focal adhesion signaling route, the PI3K-Akt signaling pathway, and the ECM receptor interaction signaling system. Previous research has linked the focal adhesion signaling pathway and PI3K-Akt signaling to age-related diseases. It was observed that the PI3K-Akt-mTOR signaling pathway is active during hippocampus aging. Adhesion sites are made up of adhesion proteins such as integrins and extracellular proteins that attach cells to the ECM [53]. The PI3K-Akt signaling pathway is a pathway with extensive intracellular effects. PI3K mainly promotes cell survival and resists apoptosis by activating Akt. After activation of Akt signaling, it can inhibit a variety of proapoptotic factors [54]. Studies have shown that antiaging drugs play a balance between antiaging effects and oxidative stress by activating the PI3K-Akt signaling pathway [55].

In this study, two important modules of common DEGs were screened for KEGG signaling pathway analysis. The results showed that the modules are mainly related to GnRH signaling pathway, leukocyte transendothelial migration, relaxin signaling pathway, and other pathways and have high correlation with the occurrence of aging and the development of inflammation, which deserve further study [56].

To find the most important regulatory molecules among the 10 hub genes, we analyzed the correlation between the hub genes and constructed a regulatory network between the hub genes and the TFs and the miRNA. According to our analyses, most hub genes exhibited strong correlations, and VIM had direct or indirect connections with other genes in the TF target regulatory network. VIM stands for vimentin, which is a class III intermediate filament protein present in nonepithelial cells, notably mesenchymal cells, and plays a crucial role in intracellular structural stability. VIM, a biomarker of epithelial-to-mesenchymal transition, has also been used as a diagnostic, prognostic, and therapeutic marker for fibrotic diseases [57, 58]. VIM is associated with abilities responsible for signal transduction and kinase interactions, thereby exerting control over gene regulatory networks. According to its role in fibrosis, VIM expression is also regulated by TGF and pro-inflammatory cytokines [59].

The miRNAs regulate the expression of many genes in cells, so abnormal expression of miRNAs may have an impact on the development of diseases. By examining the interaction between hub genes, TFs and miRNAs, we discovered that several miRNAs are implicated in PD (such as hsa-mir-17, hsa-mir-21, hsa-mir-29a, hsa-mir-29b, hsa-mir-29c, hsa-mir-31, hsa-mir-125a-5p, and hsa-mir-125b) [60–63] and aging (such as hsa-mir-23a, hsa-mir-125b, hsa-mir-21, hsa-mir-137, and hsa-mir-144) [64–66].

Several limitations in this study should be acknowledged. First, this analysis is based on existing datasets to identify common DEGs. The datasets are relatively small in size, and the obtained results may be biased, which requires subsequent external experiments to verify in order to obtain reliable conclusions. Second, hub gene screening is based on the degree of node identification, and the results are purely hypothetical. Furthermore, functional studies are necessary to confirm the hub genes in PD and aging. Therefore, in future studies, we may provide a theoretical framework and basis based on existing research to explore the relationship between PD and aging through gain or loss of function on biological models.

5. Conclusion

In the present work, a new mechanism was proposed which explain that progression in pathogenesis of both diseases might due to the common hub genes that disturbs the pathways which ultimately leads to disease condition. Moreover, it was further found that the signaling systems such as adhesion signaling pathway, PI3K-Akt signaling pathway, and ECM receptor interaction may be mainly involved. Additionally, VIM may be a potential biomarker in the analysis of linked hub gene regulatory networks, and hsa-mir-21 and hsa-mir-125b are predicted to be associated in PD and aging. These findings may lay the theoretical foundation for future studies.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors’ Contributions

YN and WX conceived the research ideas and designed the workflow. QX, HL, and TW performed bioinformatics analysis and interpreted the data results. QX, JS, and YL wrote the draft of the paper. YN and WX review and edit manuscripts and manage and oversee the entire research project. All coauthors participated in the reading of this manuscript and approved submission of the final manuscript.

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Supplementary Materials

Supplementary Table 1: topological characteristics for top ten hub genes in PPI of DEGs. Supplementary Table 2: topological characteristics of hub genes in TF-gene interaction network. Supplementary Table 3: topological characteristics of hub genes in TF-miRNA coregulatory network. (Supplementary Materials)

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