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Original Article

Aberrant gene expression profiles and related pathways in chronic periodontitis

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Abstract: Periodontitis is a major cause of tooth loss, which affects more than 80% of people. Thus far, little is known about the changes in mRNA expression in periodontal ligaments during chronic periodontitis. The objective of this study was to identify differentially expressed mRNAs related to chronic periodontitis, which may offer insight into chronic periodontitis treatment. The gene expression profiles of periodontal ligaments from five chronic periodontitis patients and five healthy controls were determined using oligonucleotide microarray. Bioinformatic analyses, including Gene Oncology (GO) and KEGG pathway, were performed to identify genes and pathways associated with chronic periodontitis. The signal transduction network was established to determine the core genes regulating chronic periodontitis processes. A set of genes was differentially expressed in periodontal ligaments from chronic periodontitis patients. A total of 317 GO terms and 145 common pathways were identified. All various gene interactions in the Signal-Net were analyzed. IGF1R, FGFR2, and PDGFRA were the most significantly altered genes. We identified a novel gene expression pattern for chronic periodontitis. Thus, further study could provide new targets for chronic periodontitis treatment.

Keywords: Microarray analysis, periodontitis, signal pathways

Introduction

Periodontitis is a major cause of tooth loss, which affects more than 80% of people globally [1]. The inflammatory micro-environment and chronic infection play important roles in damaging periodontal supportive tissues, including periodontal ligaments, cementum, and alveolar bone in periodontal disease. The development of periodontal disease is determined by complex interactions between various microorganisms and their hosts [2, 3]. Dental plaque is necessary, but not sufficient, for periodontitis. Studies have demonstrated that the presence of periodontal pathogens and their products in dental plaque biofilm is the initial factor in periodontal disease [4, 5], though the microorganisms do not directly cause destruction of periodontal tissue. However, non-specific plaque accumulation, which changes the micro-environment and is conducive to plaque growth, leads to gum tissue inflammation and gingivitis [6]. Such a condition results in more severe tissue changes and immune-mediated inflammatory reactions. Therefore, the host immune inflammatory response initiated by the bacteria and their toxic products is more important in the pathogenesis of periodontitis.

Due to the presence of inflammation, current clinical treatments cannot achieve full periodontal tissue regeneration in periodontitis. The discovery of periodontal ligament stem cells (PDLSCs) provides a new direction for periodontitis treatment [7]. PDLSCs isolated from patients with chronic periodontitis have impaired properties in multiple differentiation and immunomodulation [8]. However, the underlying mechanisms remain unclear, as the complex signaling pathways are involved in the determination of stem cell fate. Recently, β-catenin was shown to be highly expressed in PDLSCs from inflammatory periodontal tissue [9]. Moreover, PDLSCs in inflamed tissue have

Table 1. Clinical Features

Sample	Number	Age (years), mean ± SD	Gender (M/F)	Odontoseisis	PD	AL
Healthy control	5	24.2±7.5	1/4	None	2~4	0
Chronic periodontitis	5	40.8±14.2	2/3	II~III	3~9	5~9

Footnote: PD, probing depth; AL, attachment loss.

impaired osteogenic differentiation through the Wnt/β-catenin signaling pathway. In addition, glycogen synthesis kinase 3β (GSK3β), an important negative regulatory factor in the classic Wnt pathway, is involved in a variety of diseases, including the occurrence and development of inflammation [10]. Transcription factor NF-κB also plays a critical role in inflammation and the immune response. The regulation of IKK-NF-κB plays a dual role by triggering bone repair and regeneration and suppressing the inflammatory response [11]. In addition, IGFBP5 participates in the regulation of cell senescence and mesenchymal stem cell osteogenic differentiation [12]. Genome-wide reports on aberrant gene expression profiles and their related signaling pathways are still lacking for periodontal ligament tissues from periodontitis patients.

In this study, microarray analysis was performed to compare gene expression variations between periodontal ligaments from chronic periodontitis patients and healthy controls. Furthermore, to find the core genes and related pathways, Gene Ontology (GO) analysis, pathway analysis, Path-net, and signal-net methods were applied to determine the diverse gene target functions and regulatory networks.

Materials and methods

Patients

A total of 10 subjects, including 5 chronic periodontitis patients and 5 healthy controls, from the Department of Maxillofacial Surgery, Tianjin Medical University Stomatological Hospital between December 2013 and October 2014 were enrolled in the study. Tooth tissues were sampled after obtaining informed patient consent and following the guidelines set by the Tianjin Medical University Stomatological Hospital. The teeth were stored in a sterile centrifuge tube and precooled to extract periodontal ligaments.

The inclusion criteria for chronic periodontitis were age between 20 and 55 years the typical clinical manifestation of chronic periodontitis: probing depth (PD) ≥ 5 mm, alveolar bone adhesion loss (AL) ≥ 5 mm, X-ray showing alveolar bone absorption in at least two-thirds of the root, odontoseisis ≥ II, and tooth extraction and gingival repair needed. The inclusion criteria for healthy controls were age between 20 and 55 years and no typical clinical manifestation of gingivitis or periodontal disease: PD ≤ 3 mm, AL = 0, bleeding index (BI) = 0~1, gingival index (GI) = 0.2). A mandibular third molar with malocclusion or bicuspid requiring orthodontics without a carious lesion were sample to obtain periodontal membrane tissue. Patients with related systemic disease, smokers, and patients in gestation period or breast-feeding period were excluded.

RNA extraction and microarray

Total RNA was extracted from each frozen sample using Trizol Reagent (Life Technologies). Purification with an RNeasy mini kit (Qiagen, USA). To detect the integrity, concentration and purity of the RNA, cDNA was produced by using One-Cycle Target Labeling and Control Reagents (Affymetrix, Santa Clara, CA). The biotinylated and amplified cRNA was generated from the total RNA samples with a GeneChip IVT Labeling Kit (Affymetrix, Santa Clara, CA) and then was hybridized at 45°C to Affymetrix GeneChip Human Gene 1.0 ST arrays (Affymetrix, Santa Clara, CA) for 16 h The fluorescence signal was excited at 570 nm, and data were collected on a confocal scanner at 3 lm resolution. Raw data (CEL files) were normalized at the transcript level using the robust multi-average method (RMA workflow). The median summarization of transcript expressions was calculated. Gene-level data was then filtered to include only those probe sets that are in the 'core' metaprobe list, which represent RefSeq genes.

Significant differential gene analysis

Identified the differentially expressed genes were identified based on RVM t test. After the significant analysis, we selected the differen-

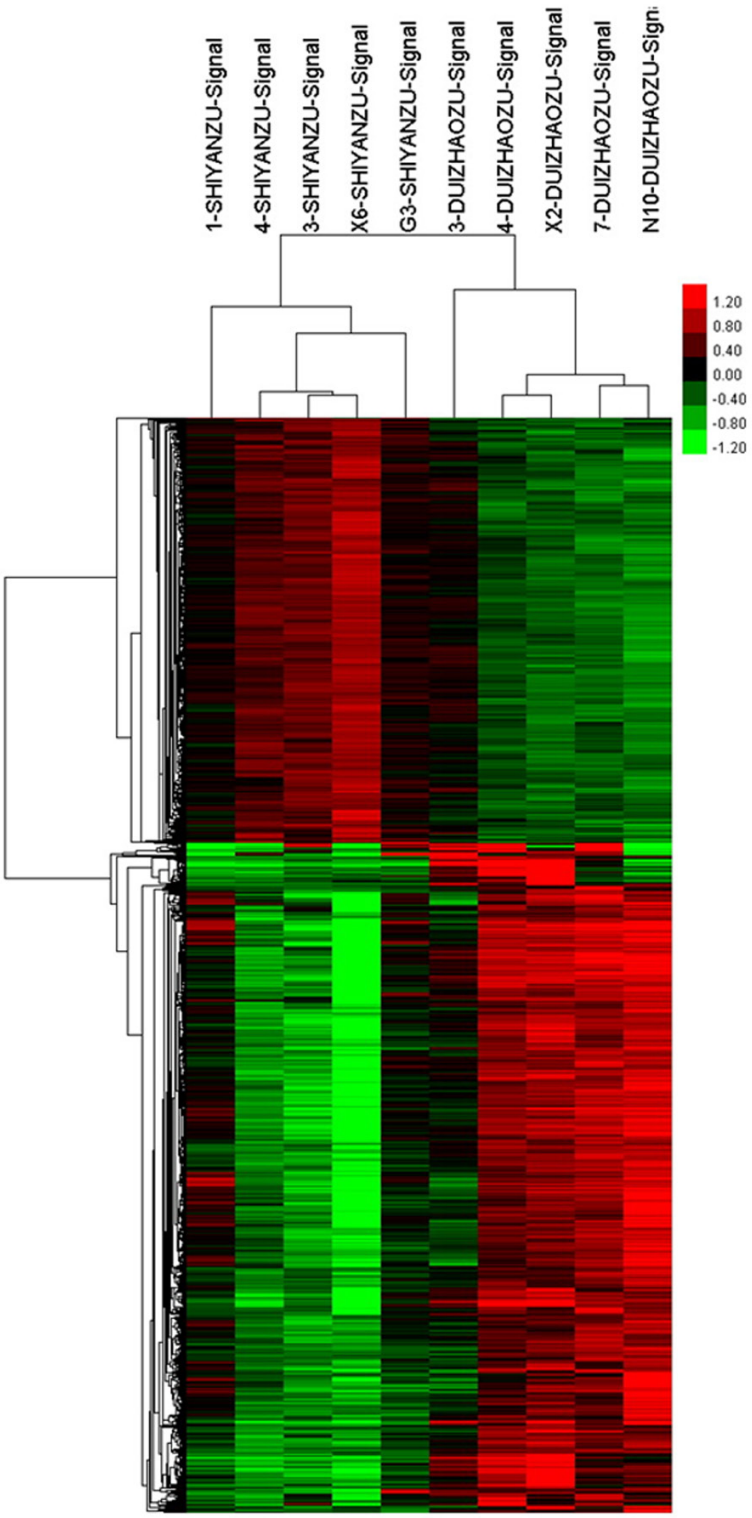

Figure 1. Gene expression profiling of chronic periodontitis and healthy control samples and classification of 10 periodontal ligament samples using the differentially expressed 6602-probe sets. Expression data are depicted as a data matrix in which each row represents a gene and each column represents a sample. Expression levels are depicted according to the color scale shown at the top. Red and green indicate expression above and below the median, respectively. The magnitude of deviation from the median is represented by the color saturation.

tially expressed genes according to the *p*-value threshold and fold change ≥ 1.5. *P* value < 0.05 was considered as significant difference [13-15]. The results of differentially expressed genes were subjected to unsupervised hierarchical clustering (Cluster 3.0) and TreeView analysis (Stanford University, Stanford, CA, USA).

Gene ontology (GO) enrichment analysis

GO Enrichment Analysis was applied to analyze the main function of the differential expression genes according to the Gene Ontology that can organize genes into hierarchical categories and uncover the gene regulatory network on the basis of biological process and molecular function [16, 17].

Specifically, two-side Fisher's exact test and x^2 test were used to classify the GO category, and the false discovery rate (FDR) [18] was calculated to correct the *P*-value, the smaller the FDR, the small the error in judging the *p*-value. The FDR was defined as $FDR = 1 - \frac{N_k}{T}$, where N_k refers to the number of Fisher's test *P*-values less than x^2 test *P*-values. We computed *P*-values for the GOs of all the differential genes. Enrichment provides a measure of the significance of the function: as the enrichment increases, the corresponding function is more specific, which helps us to find those GOs with more concrete function description in the experiment. Within the significant category, the enrichment Re was given by:

$$Re = (n_f/n)/(N_f/N)$$

Where "n_f" is the number of flagged genes within the par-

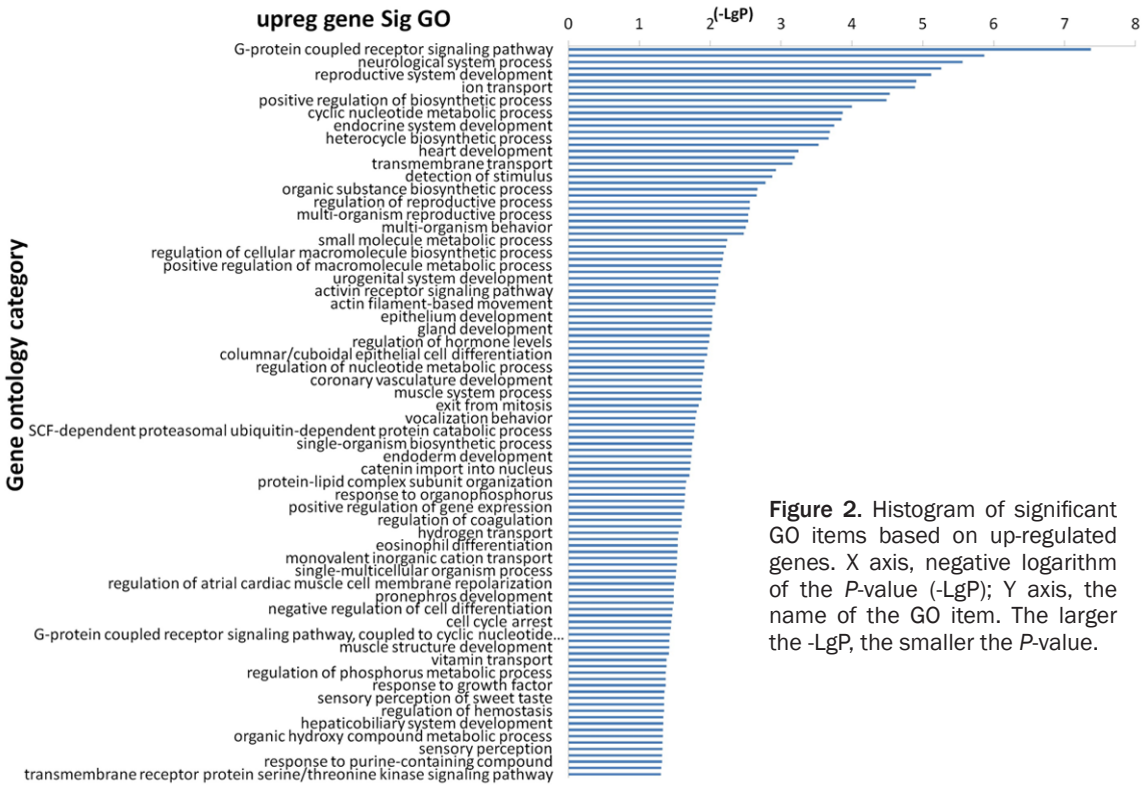

Figure 2. Histogram of significant GO items based on up-regulated genes. X axis, negative logarithm of the *P*-value (-LgP); Y axis, the name of the GO item. The larger the -LgP, the smaller the *P*-value.

ticular category, "*n*" is the total number of genes within the same category, "N_f" is the number of flagged genes in the entire microarray, and "*N*" is the total number of genes in the microarray [19].

Pathway enrichment analysis

Pathway Enrichment Analysis was performed to discover the significant pathway of the differential genes according to KEGG, Biocarta and Reatome. Still, we turn to the Fisher's exact test and x^2 test to select the significant pathway, and the threshold of significance was defined by *P*-value and FDR. The enrichment Re was calculated like the equation above [20-22].

Pathway-network

The Pathway-network was the interaction network of the significant pathways of the differential expression genes. It was built according to the KEGG database to find the interaction among the significant pathways directly and systemically. It could summarize the pathway interaction of differential expression genes under diseases and found out the reason why certain pathway was activated [21].

Global signal transduction network

Using java that allows users to build and analyze molecular networks, network maps were constructed. For instance, if there is confirmative evidence that two genes interact with each other, an interaction edge is assigned between the two genes. The considered evidence is the source of the interaction database from KEGG. Networks are stored and presented as graphs, where nodes are mainly genes (protein, compound, etc.) and edges represent relation types between the nodes, e.g. activation or phosphorylation. The graph nature of Networks raised our interest to investigate them with powerful tools implemented in R.

To investigate the global network, we computationally identify the most important nodes. To this end we turn to the connectivity (also known as degree) defined as the sum of connection strengths with the other network genes: $K_i = \sum_{U \neq 1} a_U$. In gene networks, the connectivity measures how correlated a gene is with all other network genes. For a gene in the network, the number of source genes of a gene is called the indegree of the gene and the number of target genes of a gene is its outdegree. The char-

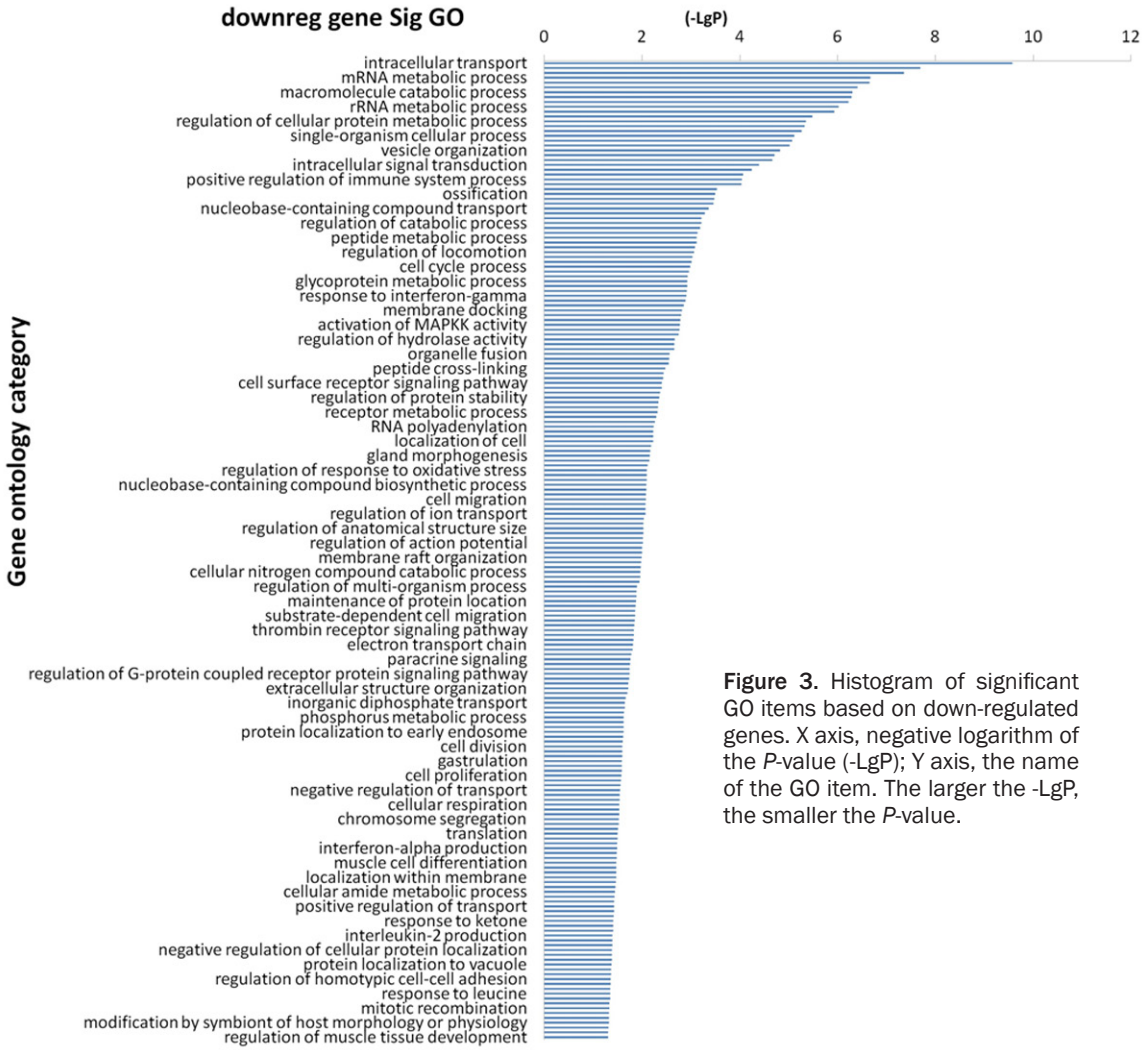

Figure 3. Histogram of significant GO items based on down-regulated genes. X axis, negative logarithm of the *P*-value (-LgP); Y axis, the name of the GO item. The larger the -LgP, the smaller the *P*-value.

acter of genes is described by betweenness centrality measures reflecting the importance of a node in a graph relative to other nodes. For a graph G: (V, E) with n vertices, the relative betweenness centrality $C_B'(v)$ is defined by: $C_B'(v) = \frac{2}{n^2 - 3n + 2} \sum_{\substack{s \neq v \neq t \in V \\ s \neq t}} \frac{\sigma_{\$}(v)}{\sigma_{\$}}$ where $\sigma_{\$}$ is the number of shortest paths from s to t, and $\sigma_{\$}(v)$ is the number of shortest paths from s to t that pass through a vertex v [23-27].

Statistical analysis

Every experiment was performed independently five times with similar results. Results are presented as mean ± SD. Significant differences were assessed by the Student's test. $P < 0.05$ was considered significant.

Results

Clinical characteristics

The features of the 10 subjects are provided in **Table 1**. Periodontal tissues were collected for mRNA microarray analysis. Smoking and gestation were ruled out for all enrolled patients. Chronic periodontitis patients had significantly higher PD and AL levels ($P < 0.05$).

Differential gene expression profiles in chronic periodontitis

We performed gene microarray studies to analyze the mRNA expression profiles in periodontal tissues from chronic periodontitis patients and healthy controls. A total of 6602 genes were significantly differentially expressed, am-

Table 2. Upregulated Significant Pathways

Pathway_name	(-LgP)
Small cell lung cancer	3.34169749
Neuroactive ligand-receptor interaction	14.2733485
Calcium signaling pathway	9.34707384
Maturity onset diabetes of the young	5.34465616
MAPK signaling pathway	4.43876687
Signaling pathways regulating pluripotency of stem cells	4.12878104
Glutamatergic synapse	3.94507386
Cholinergic synapse	3.67959827
Hedgehog signaling pathway	3.42312296
cAMP signaling pathway	3.3131454
Photo transduction	3.26084659
Amphetamine addiction	3.2286683
Basal cell carcinoma	3.16979854
Synaptic vesicle cycle	3.08328793
Amyotrophic lateral sclerosis (ALS)	2.94802629
Oxytocin signaling pathway	2.93596967
Circadian entrainment	2.86670466
Long-term potentiation	2.80642624
Dopaminergic synapse	2.76241755
Ras signaling pathway	2.66627311
Melanogenesis	2.60899403
Serotonergic synapse	2.42713016
Morphine addiction	2.27070803
Arginine biosynthesis	2.24640875
Nicotine addiction	2.19989822
Insulin secretion	2.17286998
GABAergic synapse	2.03462457
Cocaine addiction	2.01226325
Proximal tubule bicarbonate reclamation	1.92800803
Retrograde endocannabinoid signaling	1.84324759
Taste transduction	1.83602563
Adrenergic signaling in cardiomyocytes	1.74494221
Regulation of actin cytoskeleton	1.73861396
Melanoma	1.73497403
Protein digestion and absorption	1.6450725
Dilated cardiomyopathy	1.6450725
Inflammatory mediator regulation of TRP channels	1.63253194
Rap1 signaling pathway	1.58191087
Alanine, aspartate and glutamate metabolism	1.54426237
VEGF signaling pathway	1.44148837
Pancreatic secretion	1.42980144
Histidine metabolism	1.35430136
Salivary secretion	1.33180638
Sphingolipid metabolism	1.32189818
Glucagon signaling pathway	1.3012315

ong which 2632 were up-regulated in chronic periodontitis samples and 3970 were down-regulated. Hierarchical clustering revealed systematic variations in mRNA expression between the two groups (**Figure 1**). The results illustrate that these differential probes could separate the two groups accurately.

GO enrichment analysis of differentially expressed genes

GO is extremely useful for mining the functional and biological significance of genes from datasets. GO enrichment analysis of genes differentially expressed between the two groups found 115 up-regulated GO items and 202 down-regulated GO items (**Figures 2** and **3**). Items in the G-protein coupled receptor signaling pathway, reproductive system development, and intracellular transport had the highest enrichment. All of these items indicated that, except traditional knowledge about periodontal ligaments in chronic periodontitis processes, other mechanisms (e.g., Notch signaling pathway and biological adhesion) may also play important roles in regulating the pathological process.

Pathway enrichment analysis of differentially expressed genes

Related pathways were analyzed according to the functions and interactions of the differentially expressed genes. Tens of significant pathways were obtained using $P<0.05$ (**Tables 2** and **3**). The high-enrichment pathways targeted by over-expressed mRNAs included the calcium signaling pathway, MAPK signaling pathway, and signaling pathways regulating stem cell pluripotency. In contrast, significant pathways corresponding to down-regulated mRNAs appeared to be responsible for ubiquitin-mediated proteolysis, focal adhesion, and NOD-like receptor signaling. However, these pathways seem to not be relevant to chronic peri-

Table 3. Downregulated Significant Pathways

Pathway_name	(-LgP)
Ribosome	15.6535598
Phagosome	11.3135125
Endocytosis	11.2324496
Tuberculosis	9.33793291
Toxoplasmosis	8.93723601
Influenza A	8.76455621
Protein processing in endoplasmic reticulum	8.75598928
Epstein-Barr virus infection	8.58146875
Leishmaniasis	8.29206835
Ubiquitin mediated proteolysis	7.73324565
Focal adhesion	7.12732927
Proteoglycans in cancer	7.10415302
Proteasome	7.00415133
Adherens junction	6.89388534
Spliceosome	6.58582853
Herpes simplex infection	6.12806491
Chagas disease (American trypanosomiasis)	5.98869747
Salmonella infection	5.57259913
Pancreatic cancer	5.55606853
Osteoclast differentiation	5.26464148
Colorectal cancer	5.25539604
NOD-like receptor signaling pathway	5.14514118
RNA degradation	5.1397596
TGF-beta signaling pathway	5.12931979
Viral myocarditis	4.95429924
HTLV-I infection	4.81458259
Rheumatoid arthritis	4.687371
Antigen processing and presentation	4.68145037
Viral carcinogenesis	4.6659051
FoxO signaling pathway	4.57044744
Regulation of actin cytoskeleton	4.56712657
Pathways in cancer	4.54730193
Alzheimer's disease	4.45772528
Chronic myeloid leukemia	4.38817101
Hepatitis B	4.34495328
Parkinson's disease	4.3166699
Measles	4.23374256
Toll-like receptor signaling pathway	4.12039864
Type I diabetes mellitus	4.08745533
TNF signaling pathway	4.03095908
Legionellosis	3.97416788
Chemokine signaling pathway	3.90650674
Prostate cancer	3.88784556
Epithelial cell signaling in Helicobacter pylori infection	3.806178
Amoebiasis	3.79135842
B cell receptor signaling pathway	3.67747828
Sphingolipid signaling pathway	3.6688123
Renal cell carcinoma	3.66122652
Axon guidance	3.63138631

odontitis; thus, the pathway network was adopted to analyze the relationships between different pathways to find the most important (**Figure 4**). The MAPK signaling pathway had the largest degree in the pathnet, which means that, even though it is not the most significant variation pathway, it participates in most pathway regulation.

Global signal transduction networks in chronic periodontitis

According to the literature and experimental records in the databases, the genes appearing previously in 145 (45+100) pathways were collected to draw the gene interaction network (**Figure 5**). The total number of genes in the network was 114, and the particular relationships between them are listed in Supplemental Table 1. In the network, cycle nodes represent genes, and edges between two nodes represent interactions between genes. *IGF1R*, *FGFR2*, and *PDGFRA* were the three main genes with the highest interactions.

Discussion

Periodontists have investigated numerous ways to repair the tissue damage that occurs during periodontitis, including surgical procedures and grafting materials. However, their application in periodontal regeneration is questionable because of safety, effectiveness, and stability [28-30]. In addition, the emergence of tissue-engineering approaches provides prospective alternatives [7]. PDLSCs offer the potential for cell-based therapies to treat chronic periodontitis [28, 31, 32]. Previous *in vitro* and *in vivo* studies compared the proliferation and differentiation potentials of PDLSCs extracted from healthy controls and periodonti-

Leukocyte transendothelial migration	3.5426951
Malaria	3.52344151
Pathogenic Escherichia coli infection	3.50913003
Graft-versus-host disease	3.40793682
Oxidative phosphorylation	3.38989615
Lysosome	3.38468545
Hematopoietic cell lineage	3.38219917
Shigellosis	3.38120225
T cell receptor signaling pathway	3.30111417
Thyroid hormone signaling pathway	3.14131646
Apoptosis	3.13958468
Allograft rejection	3.10195769
RNA transport	3.02727009
Inflammatory bowel disease (IBD)	2.98522345
NF-kappa B signaling pathway	2.96029408
Fc epsilon RI signaling pathway	2.77414225
Neurotrophin signaling pathway	2.7679891
Endometrial cancer	2.65285601
Huntington's disease	2.5850446
Protein export	2.54774356
Non-alcoholic fatty liver disease (NAFLD)	2.53340141
Cell cycle	2.45782478
Platelet activation	2.45663632
Hepatitis C	2.31665668
Bacterial invasion of epithelial cells	2.29914484
Staphylococcus aureus infection	2.29396514
Pertussis	2.26682786
Fc gamma R-mediated phagocytosis	2.26045473
N-Glycan biosynthesis	2.24675159
MAPK signaling pathway	1.99902359
Transcriptional misregulation in cancer	1.94254448
Small cell lung cancer	1.91505193
Choline metabolism in cancer	1.84568867
Long-term potentiation	1.77739208
Signaling pathways regulating pluripotency of stem cells	1.76253229
PI3K-Akt signaling pathway	1.76049741
Vibrio cholerae infection	1.7240323
Estrogen signaling pathway	1.67560552
Wnt signaling pathway	1.67190743
Glioma	1.65864153
Vasopressin-regulated water reabsorption	1.6222486
ErbB signaling pathway	1.59066703
mRNA surveillance pathway	1.56265132
Hippo signaling pathway	1.52498182
Progesterone-mediated oocyte maturation	1.52472241
Dopaminergic synapse	1.42371395
Natural killer cell mediated cytotoxicity	1.40987178
Prolactin signaling pathway	1.3992308
Cell adhesion molecules (CAMs)	1.38455923
ECM-receptor interaction	1.36140553
Autoimmune thyroid disease	1.30951986

tis patients, finding significant differences [8]. However, the exact gene expression differences and related mechanisms in PDLSCs in chronic periodontitis have not been fully elucidated. Because PDLSCs are mainly stored in periodontal ligaments, the present study explored the differential gene expression between chronic periodontitis patients and healthy controls.

More than 6000 genes were differentially dysregulated in periodontal ligaments from chronic periodontitis patients in this study. The up-regulated gene with the largest fold change in chronic periodontitis, *SPANXE*, was only found in male germ cells and some tumor cells but was absent from other reports [33]. The second most significantly up-regulated gene, *TMEM229B*, was differentially expressed in pancreatic islets treated with streptozotocin [34]. The product of the third most up-regulated gene, *OR1D5*, is the ligand of an olfactory receptor, the role of which in periodontitis is poorly understood [35]. Most of the genes that were strongly down-regulated had close relationships with blood, including *HBB* and *HBA1*, which are different hemoglobin variants [36]. Most of the genes were not found to be associated with chronic periodontitis; thus, other methods should be used to excavate meaningful information from the expression profile. Limited by the sample size, we tried to investigate the microarray results in another way that can more accurately uncover the meaning of the gene expression profile.

GO analysis is widely known as the premier tool for molecule organization and functional annotation [37] and was applied to

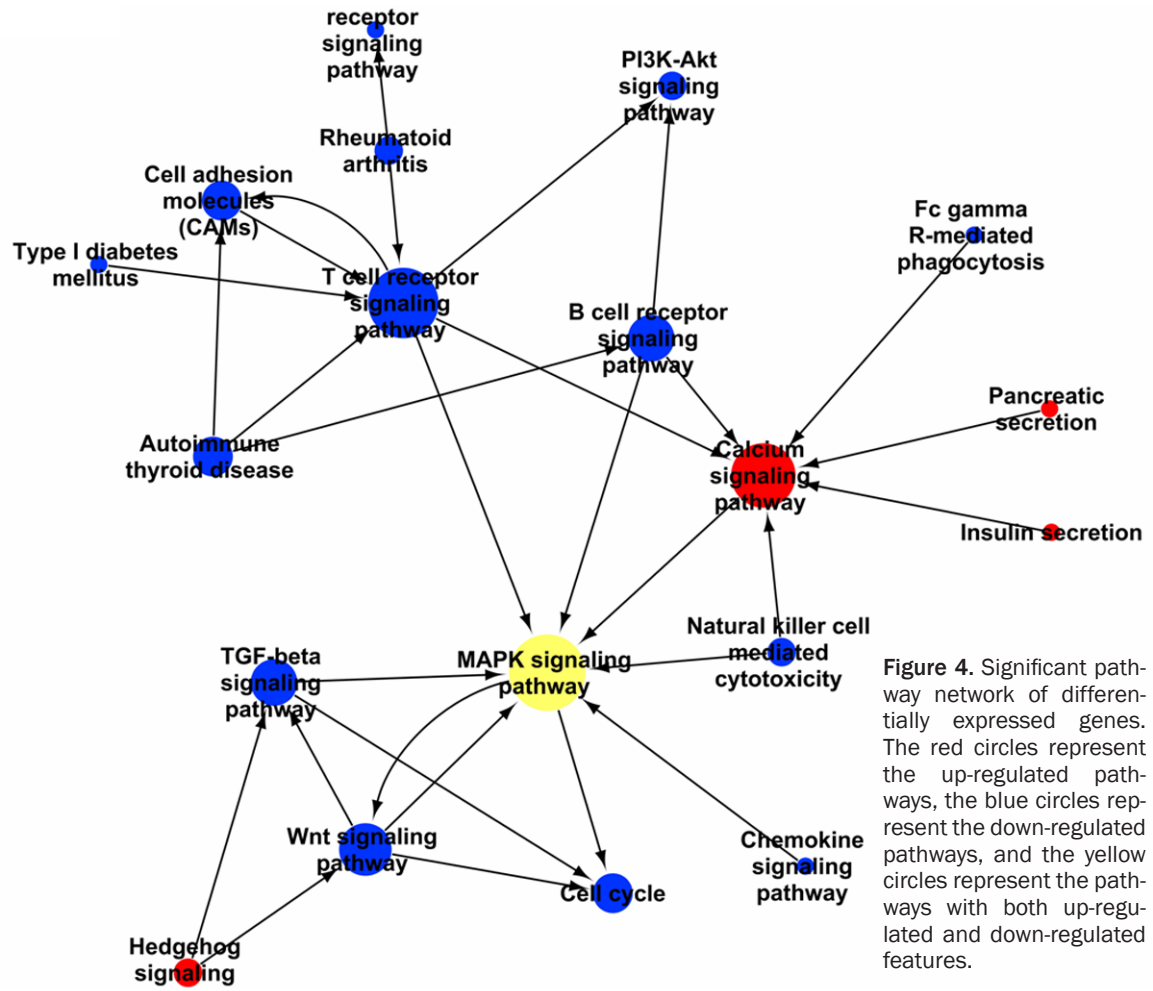

Figure 4. Significant pathway network of differentially expressed genes. The red circles represent the up-regulated pathways, the blue circles represent the down-regulated pathways, and the yellow circles represent the pathways with both up-regulated and down-regulated features.

analyze GO items for differentially expressed genes. GO terms about the reproductive process play an important role in periodontal ligaments from chronic periodontitis patients, indicating that PDLSCs may play critical roles in the disease. A variety of studies have reported stem cell differentiation into osteoblasts and osteoclasts, which depends on cell development and maturation and plays a vital role in tooth movement [38]. Sawada *et al [39]* reported that adipose tissue-derived multilineage progenitor cells transplanted into a defect in periodontal tissue can stimulate periodontal ligament cell differentiation to mineralized tissue-forming cells, such as osteoblasts and cementoblasts, which further the function of proven stem cells and their reproduction in chronic periodontitis. Therefore, even though the roles of other factors are still unclear, we think that they play roles in chronic periodontitis.

Pathway analysis presents more detailed distinct biological processes and significant pathways in which differentially expressed genes participate. The appearance of MAPK, Wnt, and TGF-β signaling pathways confirm their concordance with GO terms and central roles in chronic periodontitis. Travan *et al* demonstrated that MAPK is involved in human periodontal inflammation and severity by analyzing healthy and diseased periodontal tissues [40]. Furthermore, p38 MAPK was recently shown to participate in the inflammatory response of PDLSCs in the chronic inflammatory microenvironment [41]. Numerous studies have confirmed that the Wnt signaling pathway modulates the osteogenic differentiation of PDLSCs and affects chronic periodontitis [10, 42]. TGF-β3 was investigated in the mediation of chondrogenesis in PDLSCs, which may be applicable for cartilage and bone regeneration [43]. Though the characteristics of PDLSCs are still unclear, rare related signal-

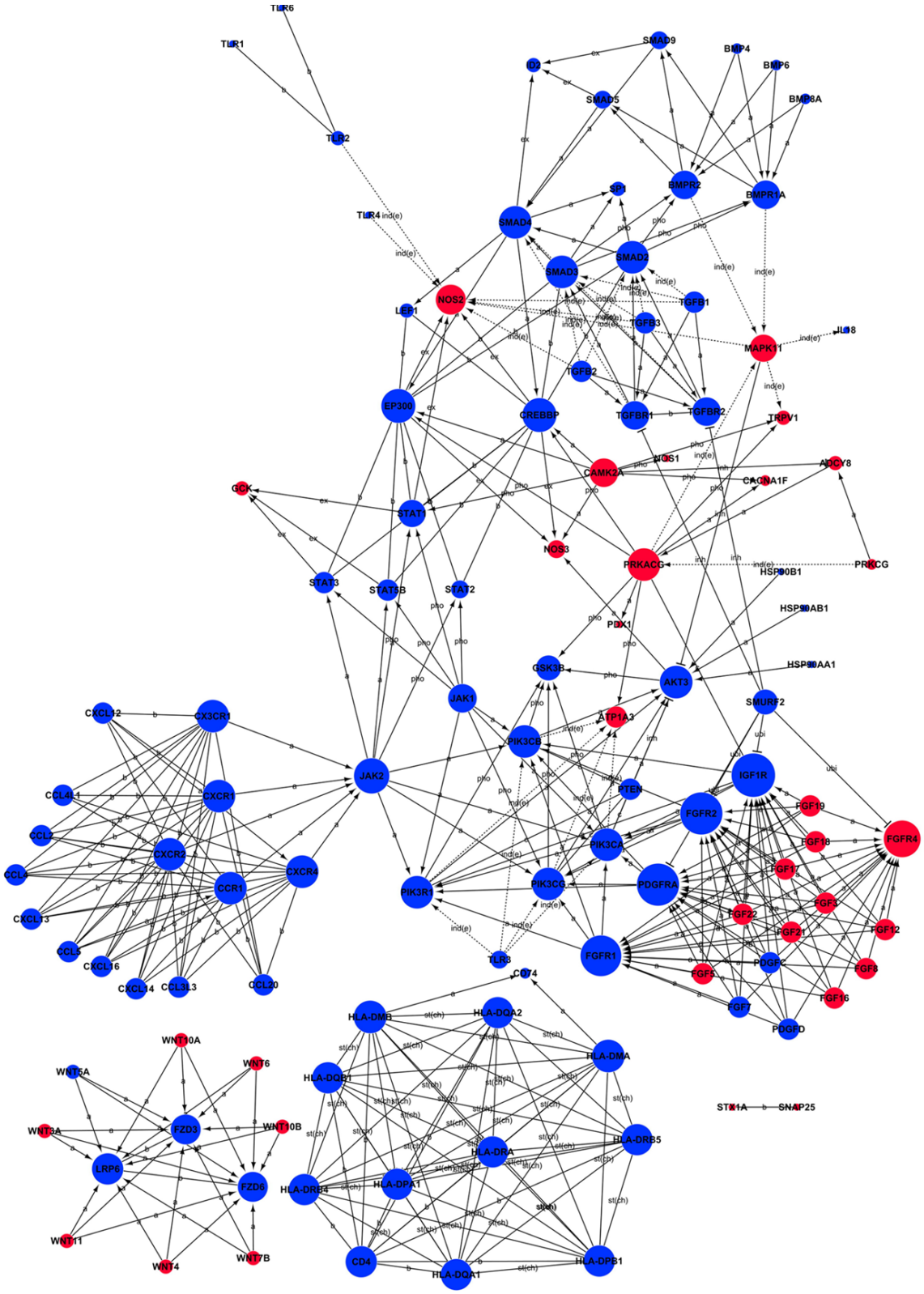

Figure 5. Global signal transduction networks of chronic periodontitis-related genes. Circles represent genes, red circles represent up-regulated genes, and blue circles represent down-regulated genes. The arrow represents the activation of (a); the straight line represents combine; dotted lines represent indirect effects (ind). a = activation; ex = gene expression; b = binding; c = compound; inh = inhibition; u = ubiquination, s = state change.

ing pathway information has been reported. Based on microarray and statistical analysis, we have reason to believe that the other seemingly irrelevant pathways play roles in chronic periodontitis.

Studying the genes involved in significant GOs and pathways, 114 common genes were found to be related to chronic periodontitis. IGF1R was differentially expressed in the periodontal lesions and positively correlated with tight-junction protein [44]. Fibroblast growth factor-inducible 14 protein has been shown to be an additional player in the pathogenesis of periodontitis [45]. Pericytes maintaining PDGF have been reported to be one of the characteristics of PDLSCs, showing another aspect of the central role of PDLSCs in periodontal ligaments [46, 47]. Though their functions are not well-investigated, many genes may regulate PDLSC function in chronic periodontitis. Based on these data, further investigation of gene expression and functions in a larger sample may provide more information about the role of PDLSCs in chronic periodontitis and help us better understand the pathophysiological mechanism of chronic periodontitis.

In conclusion, our results revealed that differences in gene expression exist between periodontal ligaments from chronic periodontitis patients and healthy controls. These genes are involved in different GOs and signaling pathways, the disruption of which may affect chronic periodontitis processes. Several genes, such as *IGF1R*, *FGFR2*, and *PDGFRA*, are potential targets for chronic periodontitis treatment.

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Disclosure of conflict of interest

None.

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References

[1] Sousa V, Mardas N, Farias B, Petrie A, Needleman I, Spratt D and Donos N. A systematic review of implant outcomes in treated periodontitis patients. Clin Oral Implants Res 2016; 27: 787-844.

[2] Deppe H, Mucke T, Wagenpfeil S, Kesting M, Karl J, Noe S and Sculean A. Are selected IL-1 polymorphisms and selected subgingival microorganisms significantly associated to periodontitis in type 2 diabetes patients? a clinical study. BMC Oral Health 2015; 15: 143.

[3] Feng X, Zhu L, Xu L, Meng H, Zhang L, Ren X, Lu R, Tian Y, Shi D and Wang X. Distribution of 8 periodontal microorganisms in family members of Chinese patients with aggressive periodontitis. Arch Oral Biol 2015; 60: 400-407.

[4] Morozumi T, Nakagawa T, Nomura Y, Sugaya T, Kawanami M, Suzuki F, Takahashi K, Abe Y, Sato S, Makino-Oi A, Saito A, Takano S, Minabe M, Nakayama Y, Ogata Y, Kobayashi H, Izumi Y, Sugano N, Ito K, Sekino S, Numabe Y, Fukaya C, Yoshinari N, Fukuda M, Noguchi T, Kono T, Umeda M, Fujise O, Nishimura F, Yoshimura A, Hara Y, Nakamura T, Noguchi K, Kakuta E, Hanada N, Takashiba S and Yoshie H. Salivary pathogen and serum antibody to assess the progression of chronic periodontitis: a 24-mo prospective multicenter cohort study. J Periodontal Res 2016; [Epub ahead of print].

[5] Salminen A, Kopra KA, Hyvarinen K, Paju S, Mantyla P, Buhlin K, Nieminen MS, Sinisalo J and Pussinen PJ. Quantitative PCR analysis of salivary pathogen burden in periodontitis. Front Cell Infect Microbiol 2015; 5: 69.

[6] Saffar JL. Development of periodontitis in the hamster. Quantitative macroscopic study of the accumulation of bacterial plaque and of alveolysis. J Biol Buccale 1982; 10: 183-190.

[7] Zheng W, Wang S, Wang J and Jin F. Periodontitis promotes the proliferation and suppresses the differentiation potential of human periodontal ligament stem cells. Int J Mol Med 2015; 36: 915-922.

[8] Liu D, Xu J, Liu O, Fan Z, Liu Y, Wang F, Ding G, Wei F, Zhang C and Wang S. Mesenchymal

stem cells derived from inflamed periodontal ligaments exhibit impaired immunomodulation. J Clin Periodontol 2012; 39: 1174-1182.
[9] Liang L, Zhou W, Yang N, Yu J and Liu H. ET-1 Promotes Differentiation of Periodontal Ligament Stem Cells into Osteoblasts through ETR, MAPK, and Wnt/beta-Catenin Signaling Pathways under Inflammatory Microenvironment. Mediators Inflamm 2016; 2016: 8467849.
[10] Liu W, Konermann A, Guo T, Jager A, Zhang L and Jin Y. Canonical Wnt signaling differently modulates osteogenic differentiation of mesenchymal stem cells derived from bone marrow and from periodontal ligament under inflammatory conditions. Biochim Biophys Acta 2014; 1840: 1125-1134.
[11] Chang J, Liu F, Lee M, Wu B, Ting K, Zara JN, Soo C, Al Hezaimi K, Zou W, Chen X, Mooney DJ and Wang CY. NF-kappaB inhibits osteogenic differentiation of mesenchymal stem cells by promoting beta-catenin degradation. Proc Natl Acad Sci U S A 2013; 110: 9469-9474.
[12] Liu D, Wang Y, Jia Z, Wang L, Wang J, Yang D, Song J, Wang S and Fan Z. Demethylation of IGFBP5 by Histone Demethylase KDM6B Promotes Mesenchymal Stem Cell-Mediated Periodontal Tissue Regeneration by Enhancing Osteogenic Differentiation and Anti-Inflammation Potentials. Stem Cells 2015; 33: 2523-2536.
[13] Clarke R, Ressom HW, Wang A, Xuan J, Liu MC, Gehan EA and Wang Y. The properties of high-dimensional data spaces: implications for exploring gene and protein expression data. Nat Rev Cancer 2008; 8: 37-49.
[14] Wright GW and Simon RM. A random variance model for detection of differential gene expression in small microarray experiments. Bioinformatics 2003; 19: 2448-2455.
[15] Yang H, Crawford N, Lukes L, Finney R, Lancaster M and Hunter KW. Metastasis predictive signature profiles pre-exist in normal tissues. Clin Exp Metastasis 2005; 22: 593-603.
[16] Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM and Sherlock G. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 2000; 25: 25-29.
[17] Gene Ontology Consortium. The Gene Ontology (GO) project in 2006. Nucleic Acids Res 2006; 34: D322-326.
[18] Dupuy D, Bertin N, Hidalgo CA, Venkatesan K, Tu D, Lee D, Rosenberg J, Svrzikapa N, Blanc A, Carnec A, Carvunis AR, Pulak R, Shingles J, Reece-Hoyes J, Hunt-Newbury R, Viveiros R, Mohler WA, Tasan M, Roth FP, Le Peuch C, Hope IA, Johnsen R, Moerman DG, Barabasi AL, Baillie D and Vidal M. Genome-scale analysis of in vivo spatiotemporal promoter activity in Caenorhabditis elegans. Nat Biotechnol 2007; 25: 663-668.
[19] Schlitt T, Palin K, Rung J, Dietmann S, Lappe M, Ukkonen E and Brazma A. From gene networks to gene function. Genome Res 2003; 13: 2568-2576.
[20] Draghici S, Khatri P, Tarca AL, Amin K, Done A, Voichita C, Georgescu C and Romero R. A systems biology approach for pathway level analysis. Genome Res 2007; 17: 1537-1545.
[21] Kanehisa M, Goto S, Kawashima S, Okuno Y and Hattori M. The KEGG resource for deciphering the genome. Nucleic Acids Res 2004; 32: D277-280.
[22] Yi M, Horton JD, Cohen JC, Hobbs HH and Stephens RM. WholePathwayScope: a comprehensive pathway-based analysis tool for high-throughput data. BMC Bioinformatics 2006; 7: 30.
[23] Jansen R, Greenbaum D and Gerstein M. Relating whole-genome expression data with protein-protein interactions. Genome Res 2002; 12: 37-46.
[24] Li C and Li H. Network-constrained regularization and variable selection for analysis of genomic data. Bioinformatics 2008; 24: 1175-1182.
[25] Spirin V and Mirny LA. Protein complexes and functional modules in molecular networks. Proc Natl Acad Sci U S A 2003; 100: 12123-12128.
[26] Wei Z and Li H. A Markov random field model for network-based analysis of genomic data. Bioinformatics 2007; 23: 1537-1544.
[27] Zhang JD and Wiemann S. KEGGgraph: a graph approach to KEGG PATHWAY in R and bioconductor. Bioinformatics 2009; 25: 1470-1471.
[28] Lin NH, Gronthos S and Bartold PM. Stem cells and periodontal regeneration. Aust Dent J 2008; 53: 108-121.
[29] Bartold PM, Xiao Y, Lyngstaadas SP, Paine ML and Snead ML. Principles and applications of cell delivery systems for periodontal regeneration. Periodontol 2000 2006; 41: 123-135.
[30] Benatti BB, Silverio KG, Casati MZ, Sallum EA and Nociti FH Jr. Physiological features of periodontal regeneration and approaches for periodontal tissue engineering utilizing periodontal ligament cells. J Biosci Bioeng 2007; 103: 1-6.
[31] Bartold PM, Shi S and Gronthos S. Stem cells and periodontal regeneration. Periodontol 2000 2006; 40: 164-172.

[32] Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, Liu H, Gronthos S, Wang CY, Wang S and Shi S. Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS One 2006; 1: e79.

[33] Zendman AJ, Zschocke J, van Kraats AA, de Wit NJ, Kurpisz M, Weidle UH, Ruiter DJ, Weiss EH and van Muijen GN. The human SPANX multigene family: genomic organization, alignment and expression in male germ cells and tumor cell lines. Gene 2003; 309: 125-133.

[34] Tonne JM, Sakuma T, Deeds MC, Munoz-Gomez M, Barry MA, Kudva YC and Ikeda Y. Global gene expression profiling of pancreatic islets in mice during streptozotocin-induced beta-cell damage and pancreatic Glp-1 gene therapy. Dis Model Mech 2013; 6: 1236-1245.

[35] Hassan SS, Choudhury PP, Pal A, Brahmachary RL and Goswami A. Designing exons for human olfactory receptor gene subfamilies using a mathematical paradigm. J Biosci 2010; 35: 389-393.

[36] Renoux C, Feray C, Joly P, Lacan P and Francina A. Description of Three New alpha Variants and Four New beta Variants: Hb Montluel [alpha110(G17)Ala --> Val; HBA1: c.332C > T], Hb Cap d'Agde [alpha131(H14)Ser --> Cys; HBA2: c.395C > G] and Hb Corsica [alpha-100(G7)Leu --> Pro; HBA1: 302T > C]; Hb Nimes [beta104(G6)Arg --> Gly; HBB: c.313A > G], Hb Saint Marcellin [beta112(G14)Cys --> Gly; HBB: c.337T > G], Hb Saint Chamond [beta80(EF4)Asn --> 0; HBB: c.241_243delAAC] and Hb Dompierre [beta29(B11)Gly --> Arg; HBB: c.88G > C]. Hemoglobin 2015; 39: 147-151.

[37] Lovering RC, Camon EB, Blake JA and Diehl AD. Access to immunology through the Gene Ontology. Immunology 2008; 125: 154-160.

[38] Jiang N, Guo W, Chen M, Zheng Y, Zhou J, Kim SG, Embree MC, Songhee Song K, Marao HF and Mao JJ. Periodontal Ligament and Alveolar Bone in Health and Adaptation: Tooth Movement. Front Oral Biol 2016; 18: 1-8.

[39] Sawada K, Takedachi M, Yamamoto S, Morimoto C, Ozasa M, Iwayama T, Lee CM, Okura H, Matsuyama A, Kitamura M and Murakami S. Trophic factors from adipose tissue-derived multi-lineage progenitor cells promote cytodifferentiation of periodontal ligament cells. Biochem Biophys Res Commun 2015; 464: 299-305.

[40] Travan S, Li F, D'Silva NJ, Slate EH and Kirkwood KL. Differential expression of mitogen activating protein kinases in periodontitis. J Clin Periodontol 2013; 40: 757-764.

[41] Nie J, Zhang B, Gu B and Liu N. Effects of p38 mitogen-activated protein kinase on osteogenic differentiation of human periodontal ligament stem cells in inflammatory microenvironment. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 2015; 37: 1-7.

[42] Napimoga MH, Nametala C, da Silva FL, Miranda TS, Bossonaro JP, Demasi AP and Duarte PM. Involvement of the Wnt-beta-catenin signalling antagonists, sclerostin and dickkopf-related protein 1, in chronic periodontitis. J Clin Periodontol 2014; 41: 550-557.

[43] Choi S, Cho TJ, Kwon SK, Lee G and Cho J. Chondrogenesis of periodontal ligament stem cells by transforming growth factor-beta3 and bone morphogenetic protein-6 in a normal healthy impacted third molar. Int J Oral Sci 2013; 5: 7-13.

[44] Choi YS, Kim YC, Ji S and Choi Y. Increased bacterial invasion and differential expression of tight-junction proteins, growth factors, and growth factor receptors in periodontal lesions. J Periodontol 2014; 85: e313-322.

[45] Kataria NG, Bartold PM, Dharmapatni AA, Atkins GJ, Holding CA and Haynes DR. Expression of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) and its receptor, fibroblast growth factor-inducible 14 protein (Fn14), in healthy tissues and in tissues affected by periodontitis. J Periodontal Res 2010; 45: 564-573.

[46] Hall AP, Ashton S, Horner J, Wilson Z, Reens J, Richmond GH, Barry ST and Wedge SR. PDGFR Inhibition Results in Pericyte Depletion and Hemorrhage into the Corpus Luteum of the Rat Ovary. Toxicol Pathol 2016; 44: 98-111.

[47] Iwasaki K, Komaki M, Yokoyama N, Tanaka Y, Taki A, Kimura Y, Takeda M, Oda S, Izumi Y and Morita I. Periodontal ligament stem cells possess the characteristics of pericytes. J Periodontol 2013; 84: 1425-1433.

Supplemental Table 1. The relationships between genes in the network

Gene1	Reaction	Gene2
FGF12	activation	FGFR1
FGF12	activation	IGF1R
FGF12	activation	PDGFRA
FGF16	activation	FGFR1
FGF16	activation	IGF1R
FGF16	activation	PDGFRA
FGF17	activation	FGFR1
FGF17	activation	IGF1R
FGF17	activation	PDGFRA
FGF18	activation	FGFR1
FGF18	activation	IGF1R
FGF18	activation	PDGFRA
FGF19	activation	FGFR1
FGF19	activation	IGF1R
FGF19	activation	PDGFRA
FGF21	activation	FGFR1
FGF21	activation	IGF1R
FGF21	activation	PDGFRA
FGF22	activation	FGFR1
FGF22	activation	IGF1R
FGF22	activation	PDGFRA
FGF3	activation	FGFR1
FGF3	activation	IGF1R
FGF3	activation	PDGFRA
FGF5	activation	FGFR1
FGF5	activation	IGF1R
FGF5	activation	PDGFRA
FGF7	activation	FGFR1
FGF7	activation	IGF1R
FGF7	activation	PDGFRA
FGF8	activation	FGFR1
FGF8	activation	IGF1R
FGF8	activation	PDGFRA
PDGFC	activation	FGFR1
PDGFC	activation	IGF1R
PDGFC	activation	PDGFRA
PDGFD	activation	FGFR1
PDGFD	activation	IGF1R
PDGFD	activation	PDGFRA
PIK3CA	activation	AKT3
PIK3CB	activation	AKT3
PIK3CG	activation	AKT3
PIK3R1	activation	AKT3
TGFB1	indirect effect	SMAD2
TGFB1	indirect effect	SMAD3
TGFB2	indirect effect	SMAD2
TGFB2	indirect effect	SMAD3
TGFB3	indirect effect	SMAD2
TGFB3	indirect effect	SMAD3
JAK1	phosphorylation	STAT1
TGFB1	indirect effect	NOS2
TGFB2	indirect effect	NOS2
TGFB3	indirect effect	NOS2
TLR2	indirect effect	NOS2
TLR4	indirect effect	NOS2
BMP4	activation	BMPR2
BMP6	activation	BMPR2
BMP8A	activation	BMPR2
SMAD2	activation	SMAD4
SMAD2	activation	SP1
SMAD3	activation	SMAD4
SMAD3	activation	SP1
SMAD4	activation	CREBBP
SMAD4	activation	EP300
SMAD4	activation	SP1
SMAD4	expression	ID2
SMAD5	activation	SMAD4
SMAD5	expression	ID2
SMAD9	activation	SMAD4
SMAD9	expression	ID2
SMURF2	inhibition	TGFBR1
SMURF2	inhibition	TGFBR2
TGFB1	activation	TGFBR2
TGFB2	activation	TGFBR2
TGFB3	activation	TGFBR2
BMP4	activation	BMPR1A
BMPR1A	activation	SMAD5
BMPR1A	activation	SMAD9
BMPR1A	indirect effect	MAPK11
BMPR2	activation	SMAD5
BMPR2	activation	SMAD9
BMPR2	indirect effect	MAPK11
IGF1R	activation	PIK3CA
IGF1R	activation	PIK3CB
IGF1R	activation	PIK3CG
IGF1R	activation	PIK3R1