Identification of key genes and pathways for peri-implantitis through the analysis of gene expression data

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Abstract. The present study attempted to identify potential key genes and pathways of peri-implantitis, and to investigate the possible mechanisms associated with it. An array data of GSE57631 was downloaded, including six samples of peri-implantitis tissue and two samples of normal tissue from the Gene Expression Omnibus (GEO) database. The differentially expressed genes (DEGs) in the peri-implantitis samples compared with normal ones were analyzed with the limma package. Moreover, Gene Ontology annotation and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses for DEGs were performed by DAVID. A protein-protein interaction (PPI) network was established using Cytoscape software, and significant modules were analyzed using Molecular Complex Detection. A total of 819 DEGs (759 upregulated and 60 downregulated) were identified in the peri-implantitis samples compared with normal ones. Moreover, the PPI network was constructed with 413 nodes and 1,114 protein pairs. Heat shock protein *HSP90AA1* (90 kDa α, member 1), a hub node with higher node degrees in module 4, was significantly enriched in antigen processing, in the presentation pathway and nucleotide-binding oligomerization domain (NOD)-like receptor-signaling pathway. In addition, nuclear factor-κ-B1 (*NFKB1*) was enriched in the NOD-like receptor-signaling pathway in KEGG pathway enrichment analysis for upregulated genes. The proteasome is the most significant pathway in module 1 with the highest P-value. Therefore, the results of the present study suggested that *HSP90AA1* and *NFKB1* may be potential key genes, and the NOD-like receptor signaling pathway and proteasome may be potential pathways associated with peri-implantitis development.

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Introduction

Peri-implantitis is a destructive inflammatory disease that affects the tissues surrounding dental implants (1,2). It has been demonstrated that peri-implantitis is a crucial element in implant failure. In total, ~30% of patients that receive dental implants develop peri-implantitis (3). However, there are currently no effective therapeutic strategies against peri-implantitis. Moreover, the use of dental implants is constantly increasing, therefore an effective therapy to treat peri-implantitis is required. Thus, further investigations into the molecular pathophysiology of peri-implantitis are necessary in order to provide novel options for effective treatment (1).

Recently, a number of studies have investigated the pathological mechanisms underlying peri-implantitis progression. Becker *et al* (4) indicated that serglycin (*SRGN*) expression was significantly upregulated in peri-implantitis when compared with healthy individuals. It has been suggested that this gene may inhibit bone mineralization *in vitro* (5). Another study indicated that concentrations of the nuclear factor-κB (NF-B), soluble RANK ligand (sRANKL), osteoprotegerin (OPG) and sclerostin are significantly increased in patients with peri-implantitis (6). A number of typical bone matrix molecules, including collagen, type IX, α 1 (COL9A1), bone gamma-carboxyglutamate (Gla) protein (BGLAP) and secreted phosphoprotein 1 (SPP1) are decreased in the peri-implantitis tissues (1). Furthermore, it has been identified that fibroblasts are involved in the pathogenesis of peri-implantitis (7). The regulation of inflammatory mediators and matrix metalloproteinases (MMPs) in peri-implantitis fibroblasts function in the pathogenesis of the disease (8), and levels of the anti-inflammatory cytokine interleukin (IL)-10 are decreased in peri-implantitis (9). Furthermore, peroxisome proliferator-activated receptor γ (PPARγ) that can inhibit inflammation and promote osteoblast function is downregulated in the peri-implantitis tissues (10). However, other mechanisms associated with peri-implantitis have not been identified. Therefore, further research should focus on elucidating other potential mechanisms and investigate target genes for the treatment of peri-implantitis.

The microarray data of GSE57631 was used to confirm the similarities and differences of inflamed peri-implantitis tissues vs. normal peri-implantitis tissues at the mRNA level (1). In contrast to results from a previous study (1), the

array data of GSE57631 was downloaded and the differentially expressed genes (DEGs) associated with peri-implantitis were analyzed using a biological informatics approach. In addition, functional enrichment analyses were performed for DEGs. In addition, a protein-protein interaction (PPI) network was established and four significant modules were analyzed. The present study aimed to identify the key genes and pathways of peri-implantitis, and identify possible significant mechanisms associated with it.

Materials and methods

Affymetrix microarray data. The array data of GSE57631 was downloaded from the Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) database, which was deposited by Schminke *et al* (1). Six samples of peri-implantitis tissues and two samples of normal tissues were included in the present study. The raw data and annotation files were downloaded for subsequent analysis, based on the GPL15034 platform (Affymetrix Human Gene 1.0 ST Array [HuGene10stv1_Hs_ENTREZ, Brainarray v14]; Affymetrix, Inc., Santa Clara, CA, USA).

Data preprocessing. The raw expression data was preprocessed using the robust multiarray average (11) algorithm by applying an oligo (12) in the R statistical software program in Bioconductor (http://www.bioconductor.org/). Background correction, normalization and a calculating expression were included in the process of preprocessing. A total of 18,977 gene expression values were obtained.

DEG analysis. The limma package (13) in Bioconductor was used to analyze DEGs in peri-implantitis samples compared with controls. In the process of the analysis, the P-values of DEGs were calculated using a *t*-test in the limma package. $|\log2FC| \geq 1$ and $P<0.05$ were used as a cut-off criteria.

Gene Ontology (GO) and pathway enrichment analyses. GO is a tool that is used for gene annotation by collecting defined, structured, controlled vocabulary, which includes three main categories: Molecular function (MF), biological process (BP) and cellular component (CC) (14). Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database used for associating related gene sets with their pathways (15). Moreover, DAVID, an integrated data-mining environment, is used to analyze gene lists (16).

GO annotation and KEGG pathway enrichment analyses were conducted for upregulated and downregulated genes by DAVID. Moreover, EASE ≤0.05 and gene counts ≥ were set as the threshold value.

PPI network analysis. The Search Tool for the Retrieval of Interacting Genes (STRING) (17) database provides information regarding the predicted and experimental interactions of proteins. The prediction method of this database came from neighborhood, gene fusion, co-occurrence, co-expression experiments, databases and text mining. Moreover, the interactions of protein pairs in the database are presented with a combined score. In the present study, DEGs were mapped into PPIs and a combined score of >0.9 was used as the cut-off value. In addition, PPI networks were constructed using Cytoscape software version 3.2.1 (18).

Topological properties of the PPI network, including degree (19), subgraph (20) and betweenness (21) centralities were determined using the R software package igraph (22), in order to analyze key genes in the network. A degree was used for describing the importance of protein nodes in the network. Subgraph centrality based on combining network topology and information of protein complexes was used to measure the importance of nodes in the network. The higher the degree and subgraph values were, the more important the nodes were in the network. Moreover, the betweenness centrality is an index describing the global topological properties of the network and could be used to describe how the nodes affect the connectivity between two nodes. Betweenness was defined as the ratio of the number of every path passed per node and the number of the shortest paths. The higher the betweenness values are, the greater the impact of the node in the network is. In addition, R software package igraph version 1.0.1 (22) was used for these three methods.

Module analysis. Network module was one of the characteristics of the protein network and may contain specific biological significance. The Cytoscape software package Molecular Complex Detection (MCODE) (23) was used to analyze the most notable clustering module. Next, the KEGG pathway enriched by DEGs in different modules was analyzed using DAVID online tool. EASE ≤0.05 and count ≥2 were set as the cutoff values.

Results

Data processing and DEG analysis. As shown in Fig. 1, a total of 819 DEGs including 759 upregulated and 60 downregulated genes, were identified in the peri-implantitis samples compared with the control ones. As a result, the number of upregulated genes was found to be significantly higher than that of the downregulated ones.

GO and pathway enrichment analyses. GO and KEGG pathway analyses were performed for upregulated and downregulated DEGs, respectively. The GO terms of upregulated DEGs were mainly associated with the proteasomal protein catabolic process, endoplasmic reticulum, melanosome and structural constituent of the cytoskeleton (Table IA). The downregulated DEGs were mainly enriched in the epidermis and ectoderm development, and the extracellular region part and cadmium ion binding (Table IB).

Two pathways that were significantly enriched by upregulated DEGs were Alzheimer's disease and the proteasome (Table II). Moreover, an important gene *HSP90AA1* was significantly enriched in the antigen processing and presentation pathway and the nucleotide-binding oligomerization domain (NOD)-like receptor signaling pathway, and *NFKB1* was enriched in NOD-like receptor signaling pathway in KEGG pathway enrichment analysis for upregulated genes. However, downregulated DEGs did not significantly enriched any pathways.

PPI network analysis. Based on the STRING database, a total of 413 nodes and 1,114 protein pairs were obtained

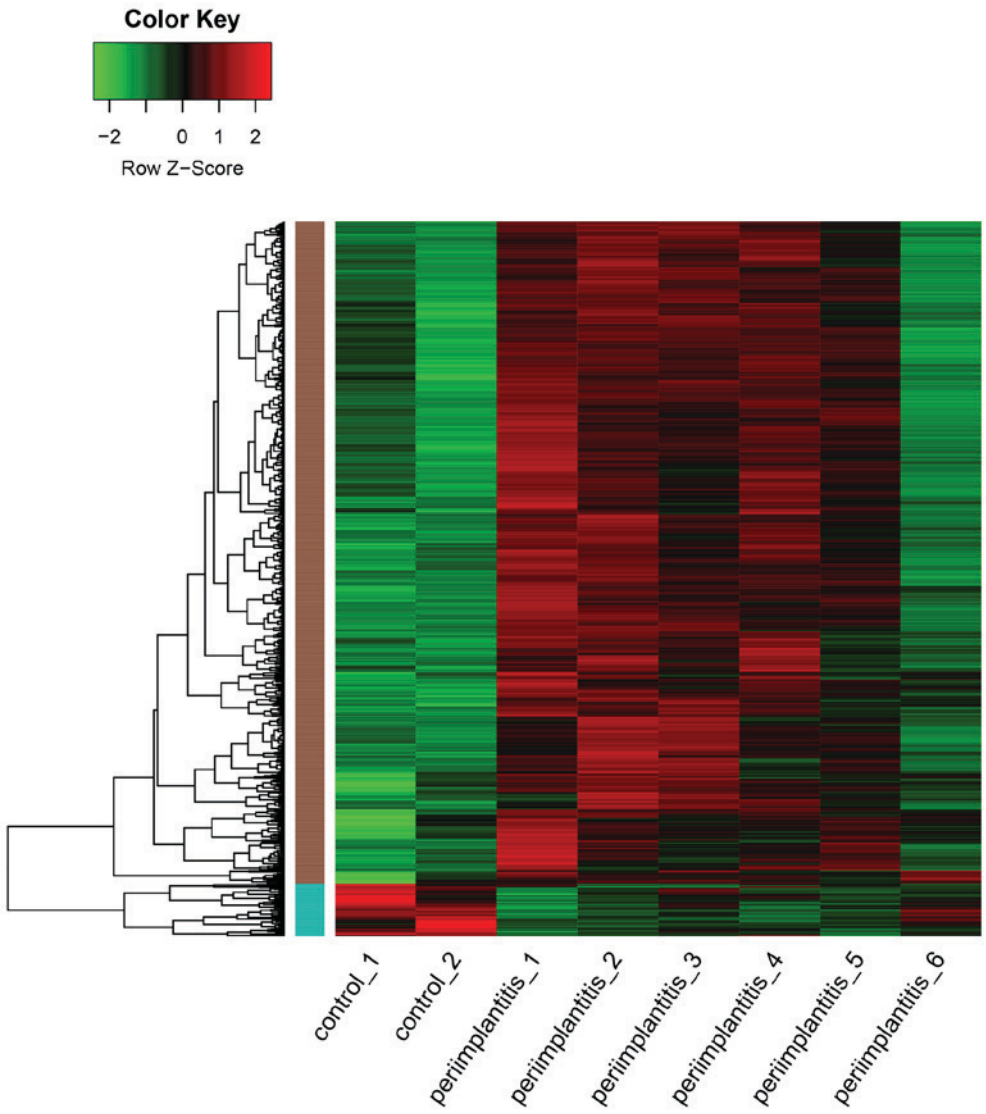

Figure 1. Heat map of differentially expressed genes. Green represents a lower expression level, red represents higher expression levels and black represents that there is no differential expression amongst the genes.

with a combined score >0.9 (Fig. 2). As shown in Fig. 2, the majority of the nodes in the network were upregulated DEGs in peri-implantitis samples. The top 20 most up- and dowregulated nodes are presented in Table III. A number of protein enzyme families, such as the proteasome subunit, α type 1 (PSMA1), proteasome subunit, beta type 1 (PSMB1) and proteasome subunit, α type 4 (PSMA4) were hub nodes based on the subgraph and degree centralities. Heat shock protein *HSP90AA1* (90 kDa α, member 1), Ras-related C3 botulinum toxin substrate 1, NFKB1, Jun proto-oncogene were hub nodes with higher betweenness values.

Module analysis. In total, four modules (modules 1, 2, 3 and 4) with score >6 were detected by MCODE (Fig. 3). As shown in Table IV, *PSMA1*, *PSMA4* and *PSMB1* were hub nodes with higher node degrees in module 1, and ribophorin 1 (*RPN1*), ribophorin 2 (*RPN2*) and dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit (*DDOST*) were hub nodes in module 2. The hub nodes with higher node degrees in module 3 were pre-mRNA processing factor 8 (*PRPF8*), small nuclear ribonucleoprotein D1 polypeptide (*SNRPD1;* 16 kDa) and small nuclear ribonucleoprotein polypeptides B and B1 (*SNRPB*), and the hub nodes in module 4 were *HSP90AA1*, ATP synthase, H^+ transporting, mitochondrial Fo complex, Subunit F2 (*ATP5J2*) and ATP synthase, H^+ transporting, mitochondrial F1 complex and beta polypeptide (*ATP5B*). Furthermore, the proteasome pathway was identified as the most significant pathway in module 1.

Discussion

In the present study, the gene expression patterns obtained from the GEO database revealed a total of 819 genes, including 759 upregulated and 60 downregulated genes, that were differently expressed in peri-implantitis samples compared with controls. The results of the present study demonstrated that *HSP90AA1*, which had the highest degrees in the PPI network, was significantly identified in module 4. In addition, *NFKB1* were also hub nodes with higher betweenness values in the PPI network. Moreover, the proteasome pathway was the most significant pathway in module 1, and may be key mechanisms associated with peri-implantitis progression.

Table I. GO for differentially expressed genes.

A, Upregulated

Terms	Description	Counts	P-value
GO-BP			
GO: 0010498	Proteasomal protein catabolic process	24	$5.32x10^{-11}$
GO: 0043161	Proteasomal ubiquitin-dependent protein catabolic process	24	$5.32x10^{-11}$
GO: 0032269	Negative regulation of cellular protein metabolic process	30	$9.32x10^{-10}$
GO-CC			
GO: 0005783	Endoplasmic reticulum	115	$1.37x10^{-21}$
GO: 0042470	Melanosome	30	$1.77x10^{-17}$
GO: 0048770	Pigment granule	30	$1.77x10^{-17}$
GO-MF			
GO: 0005200	Structural constituent of cytoskeleton	15	$2.22x10^{-6}$
GO: 0070003	Threonine-type peptidase activity	8	$1.13x10^{-5}$

B, Downregulated

Terms	Description	Counts	P-value
GO-BP			
GO:0008544	Epidermis development	20	$2.57x10^{-10}$
GO:0007398	Ectoderm development	20	$9.84x10^{-10}$
GO:0006954	Inflammatory response	19	$9.40x10^{-6}$
GO-CC			
GO:0044421	Extracellular region part	35	$1.05x10^{-5}$
GO:0005576	Extracellular region	54	$1.33x10^{-4}$
GO:0005615	Extracellular space	25	$2.79x10^{-4}$
GO-MF			
GO:0046870	Cadmium ion binding	4	$5.27x10^{-4}$
GO:0005507	Copper ion binding	6	$6.22x10^{-3}$

Terms represent the identification number of GO term; description represents the names of GO term; counts represent the number of genes enriched in GO terms. GO, Gene ontology; BP, biological process; CC: cellular component; MF, molecular function.

HSP90, a member of the heat shock family of proteins, is essential in determining cell cycle control and survival, hormone and a number of signaling pathways (24). In addition, released HSP90, which functions as a danger signal, can elicit secretion of inflammatory cytokines (25). A cell-impermeable HSP90 inhibitor can prevent inflammatory responses, indicating that extracellular HSP90 is involved in mediating and initiating sterile inflammatory responses (26). Moreover, previous data indicate that HSP90-targeted agents may be helpful for the treatment of inflammatory disease, and that an HSP90 inhibitor can affect multiple signaling processes associated with inflammation (27). Furthermore, inhibition of HSP90 is able to reduce innate immunity responses and diminishes proinflammatory mediator production in immune-stimulated macrophages (28,29). In addition, analysis of inflammatory mediators in crevicular fluid can be used to distinguish peri-implantitis from normal tissue (30).

Systemic markers of inflammation are increased in patients with peri-implantitis (30) and high levels of inflammatory cytokines, such as IL-1β, are associated with signs of early peri-implantitis development (31-33). Furthermore, it has been suggested that fibroblasts express HSP90 that also participates in the pathogenesis of peri-implantitis (7,34). Moreover, HSPs together with vascular and inflammatory biomarkers may be useful as biomarkers of peri-implantitis development (35,36). In the present study, *HSP90AA1*, a hub node with higher node degrees in module 4, was enriched in antigen processing and the presentation pathway, as well as the NOD-like receptor-signaling pathway. Therefore, the results of the present study are in line with results from former previous studies and indicate that *HSP90AA1* may be directly or indirectly important in peri-implantitis development.

In the present study, *NFKB1* was shown to also have hub nodes with higher betweenness values in the PPI network. The *NFKB1* gene is known to encode the NF-KB p105/p50 isoforms (37). The central pathological pattern of peri-implantitis is inflammatory osteoclastogenesis, which is mediated by proinflammatory mediators and performed

Table II. KEGG pathway enrichment analysis for upregulated differentially expressed genes.

Term	Description	Counts	P-value
hsa05010	Alzheimer's disease	25	$1.69x10^{-5}$
hsa03050	Proteasome	12	$5.36x10^{-5}$
hsa00190	Oxidative phosphorylation	18	$1.19x10^{-3}$
hsa05012	Parkinson's disease	17	$2.65x10^{-3}$
hsa05016	Huntington's disease	21	$3.41x10^{-3}$
hsa00510	*N*-Glycan biosynthesis	9	$4.31x10^{-3}$
hsa04142	Lysosome	15	$7.11x10^{-3}$
hsa00480	Glutathione metabolism	9	$7.27x10^{-3}$
hsa04612	Antigen processing and presentation	12	$7.74x10^{-3}$
hsa04621	NOD-like receptor signaling pathway	10	$8.63x10^{-3}$
hsa00970	Aminoacyl-tRNA biosynthesis	7	$2.89x10^{-2}$
hsa00020	Citrate cycle (TCA cycle)	6	$3.07x10^{-2}$
hsa01040	Biosynthesis of unsaturated fatty acids	5	$3.51x10^{-2}$
hsa05130	Pathogenic *Escherichia coli* infection	8	$4.45x10^{-2}$

Term represents the identification number of the KEGG pathway; Description represents the name of the KEGG pathway; Counts represent the number of genes enriched in the KEGG pathway. KEGG, Kyoto Encyclopedia of Genes and Genomes; NOD, nucleotide-binding oligomerization domain-like; TCA, tricarboxylic acid.

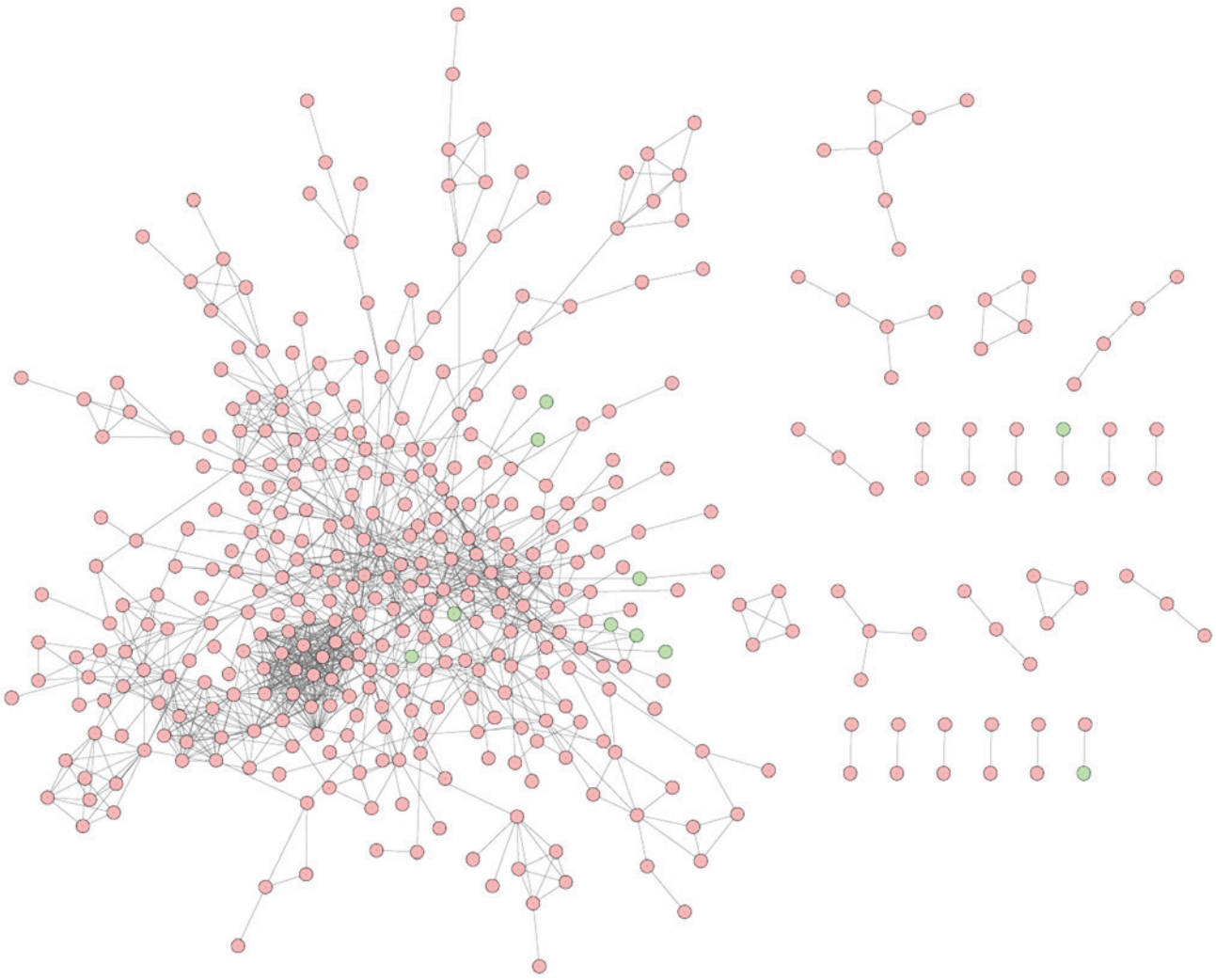

Figure 2. Protein-protein interaction network of differentially expressed genes in peri-implantitis samples compared with the control ones. Red nodes, upregulated genes; green nodes, downregulated genes.

Table III. Nodes with higher values in subgraph centrality, betweenness centrality and degree centrality.

Nodes	Subgragh	Nodes	Betweenness	Nodes	Degree
PSMA1	$2.35x10^7$	HSP90AA1	$2.78x10^4$	HSP90AA1	34
PSMB1	$2.32x10^7$	RAC1	$1.77x10^4$	JUN	27
PSMA4	$2.31x10^7$	NFKB1	$1.38x10^4$	RAC1	27
PSMB4	$2.30x10^7$	JUN	$1.21x10^4$	PSMA1	25
PSMA3	$2.30x10^7$	HIF1A	$1.07x10^4$	PSMA4	25
PSMB3	$2.30x10^7$	CDH1	$9.21x10^3$	PSMB1	25
PSMB6	$2.30x10^7$	SOS1	$8.93x10^3$	PSMC2	25
PSMC2	$2.16x10^7$	CANX	$8.55x10^3$	PSMB4	24
PSMD8	$2.12x10^7$	HDAC1	$8.35x10^3$	PSMA3	24
PSMD5	$2.08x10^7$	ATP5B	$8.24x10^3$	PSMB3	24
PSME4	$2.08x10^7$	GTF2B	$8.17x10^3$	PSMB6	24
PSMD10	$2.04x10^7$	HSPA5	$7.84x10^3$	PSMD10	24
PSME3	$1.96x10^7$	STAT3	$7.46x10^3$	PSMD8	23
UBE2D1	$1.54x10^7$	VCP	$7.32x10^3$	SEC61A1	23
CDC16	$1.36x10^7$	RPS5	$7.14x10^3$	RPN2	23
ANAPC5	$1.36x10^7$	NME1	$6.31x10^3$	NFKB1	23
BUB3	$1.35x10^7$	POLR2B	$5.90x10^3$	PSMD5	22
SEC61A1	$1.23x10^7$	P4HB	$5.90x10^3$	PSME4	22
SKP1	$1.16x10^7$	TXN	$5.67x10^3$	UBE2D1	22
HSPB1	$1.05x10^7$	RPN2	$5.67x10^3$	RPN1	22

PSM, proteasome subunit.

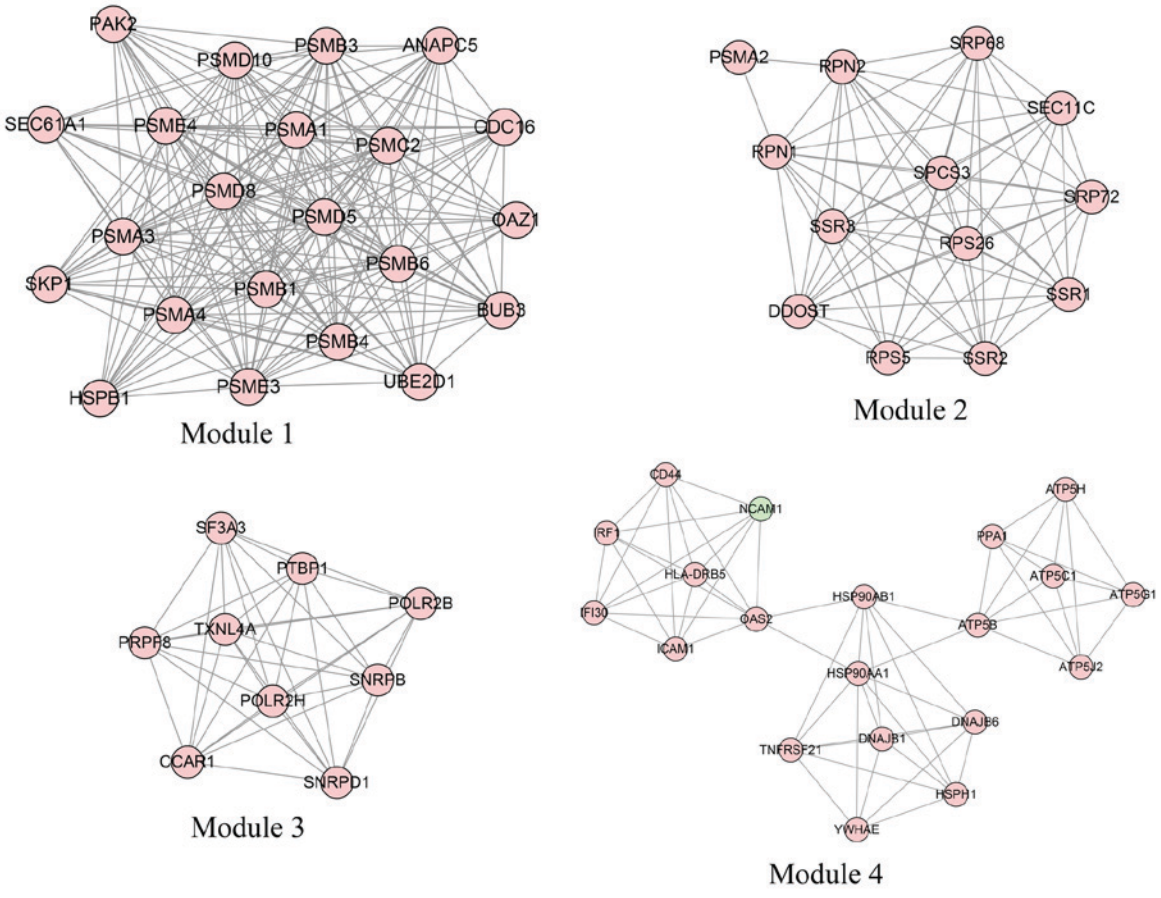

Figure 3. Four significant modules identified from the protein-protein interaction network using the molecular complex detection method with a score of >6.0. Module 1: MCODE score=19.238; Module 2: MCODE score=11.333; Module 3: MCODE score=9 and Module 4: MCODE score=6.316.

Table IV. KEGG pathway enriched by differentially expressed genes in different modules (P<0.05).

Term	Description	P-value	Genes
Module1			
hsa03050	Proteasome	$6.77x10^{-17}$	*PSMB4, PSMA1, PSMB6, PSMB1, PSMC2, PSMA4, PSMB3, PSMA3, PSME3, PSME4, PSMD8*
hsa04110	Cell cycle	$9.03x10^{-3}$	*ANAPC5, SKP1, CDC16, BUB3*
hsa04120	Ubiquitin mediated proteolysis	$1.16x10^{-2}$	*ANAPC5, SKP1, CDC16, UBE2D1*
Module2			
hsa00510	*N*-Glycan biosynthesis	$1.63x10^{-3}$	*RPN1, RPN2, DDOST*
hsa03060	Protein export	$1.10x10^{-2}$	*SRP68, SRP72*
Module3			
hsa03040	Spliceosome	$5.19x10^{-6}$	*PRPF8, SNRPD1, SNRPB, TXNL4A, SF3A3*
hsa03020	RNA polymerase	$3.26x10^{-2}$	*POLR2H, POLR2B*
Module4			
hsa00190	Oxidative phosphorylation	$1.68x10^{-5}$	*ATP5J2, ATP5B, ATP5C1, ATP5G1, ATP5H, PPA1*
hsa04612	Antigen processing and presentation	$1.34x10^{-3}$	*HSP90AB1, HSP90AA1, IFI30, HLA-DRB5*
hsa05012	Parkinson's disease	$4.63x10^{-3}$	*ATP5B, ATP5C1, ATP5G1, ATP5H*
hsa05010	Alzheimer's disease	$9.07x10^{-3}$	*ATP5B, ATP5C1, ATP5G1, ATP5H*
hsa05016	Huntington's disease	$1.19x10^{-2}$	*ATP5B, ATP5C1, ATP5G1, ATP5H*
hsa04514	Cell adhesion molecules	$5.00x10^{-2}$	*NCAM1, ICAM1, HLA-DRB5*

Term represents the identification number of the KEGG pathway; description represents the name of the KEGG pathway. KEGG, Kyoto Encyclopedia of Genes and Genomes.

by the regulators of osteoclastogenesis, including NF-B, sRANKL and OPG (38,39). A prior study indicated that the NF-B concentration increased 1.5-4-fold in patients with peri-implantitis compared to those with mucositis (6). Moreover, recent studies demonstrated that high levels of NF-B were associated with peri-implantitis (40,41). In addition, NF-B is upregulated in inflammatory bowel disease and is a transcription regulator of the immune response (42). Zou *et al* (43) reported that NFKB1 could regulate the transcription of genes in the immune response, and was also a key part in coordinating the immune system. Numerous studies have indicated that the NFKB1-94ins/del ATTG promoter polymorphism is associated with inflammatory disease (37,42,44,45). Furthermore, as mentioned in the aforementioned paragraph, inflammation is associated with the pathogenesis of peri-implantitis. Therefore, the results of the present study are in accordance with those from previous studies and suggest that *NFKB1* may be a key gene associated with peri-implantitis development. Notably in the current study, *HSP90AA1* and *NFKB1* were enriched in the NOD-like receptor-signaling pathway. Although the important roles of the NOD-like receptor-signaling pathway have not been fully discussed, it is speculated that *HSP90AA1* and *NFKB1* may be involved in peri-implantitis progression via the NOD-like receptor-signaling pathway.

In addition to the two aforementioned genes, the proteasome pathway containing the proteasome subunits (*PSM) A1*, *PSMA3*, *PSMA4*, *PSMB1*, *PSMB3*, *PSMB4*, *PSMB6*, *PSMC2*, *PSMD8*, *PSME3* and *PSME4,* were found to be enriched in the most significant pathway in module 1 and to have the highest P-value. Elliott *et al* (46) indicated that key proteins modulated by the proteasome were involved in controlling inflammatory processes. A prior study demonstrated that the activation of the ubiquitin-proteasome by macrophages is involved in an NF-B-dependent increase in inflammation (47). Moreover, previous studies have indicated that the proteasomal pathway as a targeting goal may be a potential way to treat autoimmune and inflammatory diseases (48-50). In addition, Wang and Maldonado (51) demonstrated that proteasome inhibitors could serve as novel drugs by regulating proinflammatory protein catabolism. Furthermore, as stated above, inflammation has been associated with the pathogenesis of peri-implantitis. Finally, the *PSMA1*, *PSMA3*, *PSMA4*, *PSMB1*, *PSMB3*, *PSMB4*, *PSMB6*, *PSMC2*, *PSMD8*, *PSME3* and *PSME4* proteins are essential subunits that contribute to the assembly of the proteasome complex (52-55). Therefore, the proteasome and its subunit genes may be important in peri-implantitis progression.

In conclusion, *HSP90AA1*, *NFKB1* and the NOD-like receptor signaling pathway, as well as the proteasome pathway and its subunits genes may be important in the development of peri-implantitis. However, there were certain limitations in the present study, such as no experimental verification and the relatively small sample size used. Therefore, further research investigating the potential mechanisms involved in peri-implantitis are required.

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