Transcriptomic profiles and their correlations in saliva and gingival tissue biopsy samples from periodontitis and healthy patients

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감사의 글

4년간의 치주과 대학원 생활의 졸업을 앞두고, 많은 추억들이 떠올라 감회가 새롭습니다. 그동안 치주과의 일원으로 즐겁고 행복했으며 더불어 개인적으로 많이 성장할 수 있었습니다. 학위논문 출판을 앞두고, 무한한 지지와 격려를 보내주신 이중석 교수님께 진심으로 존경과 감사의 마음을 전합니다. 따뜻한 눈빛으로 인생의 멘토가 되어주셧던 조규성 교수님께도 감사드립니다. 저에게 치주과 의사로써 올바른 가르침을 주신 김종관 교수님, 채중규 교수님, 최성호 교수님, 김창성 교수님, 정의원 교수님, 차재국 교수님, 백정원 교수님, 송영우 교수님께 깊은 감사를 드립니다.

부족함이 많은 저와 함께 해준 의국 동기들, 선후배님들께도 감사 드립니다. 덕분에 수련생활을 마치고 연세대학교 치주과라는 마음의 고향이 생겨 더없이 행복합니다.

제가 여기까지 올 수 있도록 큰 믿음과 사랑을 보여주신 어머니, 아버지, 동생에게 감사합니다. 마지막으로 인생의 즐거운 모험을 함께해주는 영관에게 사랑한다고 전하고 싶습니다.

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Abstract

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Purpose: This study was conducted to analyze specific RNA expression profiles in gingival tissue and saliva samples in periodontitis patients and healthy individuals, and to determine their correlations in light of the potential use of microarray-based analyses of saliva samples as a periodontal monitoring tool.

Materials and methods: Gingival tissue biopsies and saliva samples from 22 patients (12 with severe periodontitis and 10 with a healthy periodontium) were analyzed using transcriptomic microarray analysis. Differential gene expression was assessed, and pathway and clustering analyses were conducted for the samples. The correlations

between the results for the gingival tissue and saliva samples were analyzed at both the gene and pathway levels.

Results: There were 621 differentially expressed genes (DEGs; 320 upregulated and 301 downregulated) in the gingival tissue samples of the periodontitis group, and 154 DEGs (44 upregulated and 110 downregulated) in the saliva samples. Nine of these genes overlapped between the sample types. The periodontitis patients formed a distinct cluster group based on gene expression profiles for both the tissue and saliva samples. Database for Annotation, Visualization and Integrated Discovery analysis revealed 159 enriched pathways from the tissue samples of the periodontitis patients, as well as 110 enriched pathways in the saliva samples. Thirty-four pathways overlapped between the sample types.

Conclusions: The present results indicate the possibility of using the salivary transcriptome to distinguish periodontitis patients from healthy individuals. Further work is required to enhance the extraction of available RNA from saliva samples.

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Key words: Microarray analysis; Periodontitis; Saliva; Transcriptome

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I. Introduction

The progression of periodontitis is characterized by a hyperinflammatory response to pathogens to which each patient has a different inherent susceptibility (Al-Yahfoufi, Mombelli et al. 1995, Trombelli, Tatakis et al. 2004). The progression rate of periodontitis varies, and can be clinically classified as rapid, moderate, or no progression according to the loss of attachment (Loe, Anerud et al. 1986). However, the clinical symptoms of periodontitis vary widely, and predicting the progression of the disease using clinical

parameters is subject to several limitations (Halazonetis, Haffajee et al. 1989). This dilemma has prompted various attempts to diagnose or predict periodontitis by detecting tissue responses based on proteomics or genomics (Gonçalves, Soares et al. 2010).

Transcriptome analysis is a powerful tool for detecting the overall pattern of tissue responses at the gene level, and it has been shown that gene expression profiles differ in the gingival tissue of periodontitis patients (Demmer, Behle et al. 2008, Jönsson, Ramberg et al. 2011, Kebschull, Guarnieri et al. 2013, Taiete, Casarin et al. 2017). Offenbacher *et al.* (Offenbacher, Barros et al. 2009) reported that the genes related to immune responses, neural processes, and epithelial defenses were selectively overexpressed during the induction of experimental gingivitis. Davanian *et al.* (Davanian, Stranneheim et al. 2012) also found that the genes involved in immune and inflammatory responses were overexpressed in periodontitis-affected tissue. Periodontitis can be diagnosed or detected based on these distinct expression patterns of periodontitis-affected tissue.

Even though a definitive diagnosis of periodontitis should be made by professional experts based on clinical parameters, monitoring periodontal conditions can have a critical impact on detecting disease onset and preventing disease progression from both the clinical and research perspectives. The differential gene expression pattern of *in situ* samples may provide clues for monitoring periodontitis, which is a chronic and unspecific disease. Most previous transcriptome studies have analyzed periodontal tissue

samples, but repetitive sampling using periodontal tissue biopsies is problematic due to their inherent invasiveness and patient discomfort.

Saliva has recently entered the spotlight as a sampling source for genomic or proteomic research due to the convenience, safety, and noninvasiveness of collecting saliva samples from patients (Kaczor-Urbanowicz, Martin Carreras-Presas et al. 2017). Mediators of tissue responses such as cytokines, enzymes, hormones, and antibodies are released into the oral fluid and can be used as a pool for biomarkers to detect tissue conditions (Zhang, Henson et al. 2009). Li *et al.* (Li, Zhou et al. 2004) reported that thousands of human messenger RNAs (mRNAs) are present in saliva, and that they can be isolated, amplified, and profiled from cell-free saliva. Several recent studies have also found that specific mRNAs in saliva may serve as biomarkers for detecting Sjögren syndrome (Hu, Wang et al. 2007), oral cancer (Li, John et al. 2004), and other systemic diseases such as diabetes (Javaid, Ahmed et al. 2016). Therefore, saliva sampling could also be a candidate methodology for monitoring periodontal conditions and could allow the onset of periodontitis to be detected using regular base-sequential sampling.

Previous studies of the salivary transcriptome have independently analyzed the transcriptome of saliva in patients but did not compare the associations of salivary transcriptional profiles with those of tissue samples taken from the same individuals. The aim of this study was to determine the expression profiles of RNA in gingival tissue and saliva samples and to evaluate their correlations in order to assess the feasibility of using saliva samples in microarray analyses for monitoring periodontal conditions.

II. Materials and methods

1. Study design

Thirty-five patients (23 with severe periodontitis and 12 with a healthy periodontium) who visited the Department of Periodontology of the Yonsei University Dental Hospital for periodontal treatment (periodontitis patients) or a crown-lengthening procedure (healthy periodontium patients) between September 2014 and September 2015 were originally enrolled in this study. The experimental protocols and informed consent forms were designed according to the Declaration of Helsinki (Tokyo version, revisited in 2004) and Good Clinical Practice guidelines, and approved by the Institutional Review Board for Clinical Research of the Dental Hospital of Yonsei University (approval No. 2-2014-0026). The manuscript was prepared following the guidelines of the Strengthening the Reporting of Observational Studies in Epidemiology statement.

Saliva sampling and a gingival tissue biopsy were performed immediately before and during the periodontal procedures, respectively. Thirteen participants withdrew before sample acquisition; therefore, the gingival tissue biopsy and saliva collection were completed in a final total of 22 patients (12 with severe periodontitis and 10 with a healthy periodontium). RNA extraction and microarray analysis were conducted using the obtained gingival tissue and saliva samples.

2. Inclusion and exclusion criteria

Patients underwent clinical and radiographic evaluations at their first visit. Those who showed generalized severe loss of periodontium with familial aggregation were enrolled into the periodontitis group. The detailed inclusion criteria for the periodontitis group were as follows: (1) generalized signs of inflammation, such as gingival swelling, redness, and bleeding on probing; (2) generalized severe probing depth (>7 mm) and clinical attachment loss (>5 mm); (3) generalized severe alveolar bone loss in radiography (>50% of the root length); (4) angular bony defects at multiple sites; and (5) familial aggregation. Individuals with a healthy periodontium (henceforth referred to simply as "healthy patients") were enrolled from among patients requiring crown-lengthening procedures who had no sign of gingival/periodontal inflammation, no bleeding on probing, and no evidence of bone loss.

The following exclusion criteria were also applied: (1) current pregnancy or planning pregnancy, (2) any history of systemic diseases that may affect the periodontal status (e.g., diabetes, malignancy, immune-related disease or respiratory diseases), (3) history of taking drugs that may affect the periodontal status (e.g., nifedipine, phenytoin, or cyclosporine), (4) history of smoking (excluding smoking cessation more than 10 years previously), or (5) periodontitis patients with endo-perio combined lesions.

3. Collection of samples

Saliva samples

Saliva samples were acquired immediately before the periodontal surgery in all patients.

Patients were asked to refrain from eating and drinking for at least 30 minutes before saliva collection. Unstimulated saliva samples (5 ml) were collected by a spitting method(Navazesh 1993) and immediately stored in RNA-preserving solution (ORAGENE-RNA RE-100, DNA Genotek, Ottawa, ON, Canada). The collection vial was stored for 24 hours at room temperature after sample acquisition, and then at –80°C until being analyzed.

Gingival tissue samples

The tissue biopsy procedure was performed during flap operation surgery in the periodontitis group. During the flap operation, samples were collected from gingival tissue with clinical attachment loss >5 mm, probing depth >7 mm, and the presence of bleeding on probing. The gingival tissue samples measured 3 mm^2 and included connective tissue, epithelium, and granulation tissue. Tissue was harvested in the healthy group during crown-lengthening procedures. Healthy gingival tissues (3 mm^2) were sampled from sites with a probing depth of <3mm and without bleeding on probing. All of the gingival biopsy samples were immediately stored in RNAlater solution (RNAlater Stabilization Solution AM7020, Life Technologies, Carlsbad, USA) at room temperature for 24 hours, and then stored at –80°C until being analyzed.

4. RNA isolation

Total RNA was isolated from the gingival tissues of each patient using TRIzol LS Reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. RNA was

isolated from the saliva using the Oragene RNA purification protocol with the Qiagen RNeasy Micro kit (Qiagen, Valencia, USA).

5. Quantity and quality of purified RNA

Quality and quantity control tests were applied to the isolated total RNA samples before performing the microarray analysis. The RNA concentration (in nanograms per microliter) and purity (absorbance ratios at 260 nm/280 nm and 260 nm/230 nm) were determined using a spectrophotometer (ND-1000, NanoDrop, Wilmington, USA). The quality of the RNA was assessed using the Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, USA) based on the RNA integrity number.

6. Microarray analysis

Total RNA was amplified and purified using a TargetAmp-Nano Labeling Kit for the Illumina Expression BeadChip device (EPICENTRE, Madison, WI, USA) to yield biotinylated complementary RNA (cRNA) according to the manufacturer's instructions. Briefly, 500 ng of total RNA was reverse transcribed to cDNA using a T7 oligo(dT) primer. Second-strand cDNA was synthesized, transcribed *in vitro*, and labeled with biotin-NTP. After purification, the cRNA was quantified using a spectrophotometer (ND-1000, NanoDrop).

Biotinylated cRNA was prepared from 0.55 μg of total RNA using the Illumina TotalPrep RNA Amplification Kit (Ambion, Austin, USA). Labeled cRNA samples (750

ng) were hybridized to individual Human HT-12 (version 4.0) Expression BeadChip devices for 17 hours at 58°C according to the manufacturer's instructions (Illumina, San Diego, CA, USA). The array signals were detected using Amersham fluorolink streptavidin-Cy3 (GE Healthcare Bio-Sciences, Little Chalfont, UK) following the bead-array manual. Arrays were scanned with an Illumina bead-array confocal scanner according to the manufacturer's instructions.

7. Raw data preparation and statistical analysis

The quality of hybridization and the overall chip performance were monitored by visual inspection of both internal quality control checks and the raw scanned data. Raw data were extracted using the software provided by the manufacturer (Illumina GenomeStudio, version 2011.1; Gene Expression Module, version 1.9.0). The measured intensities of the array probes were transformed by taking the logarithm and normalizing values into quartiles.

A comparative analysis was used to identify differences in the expression data in tissue and saliva samples between periodontitis and healthy patients. The statistical significance of relative differences in the expression data was determined using the independent t-test, with the null hypothesis that there would be no intergroup difference. The false discovery rate was controlled by adjusting the *P* value using the Benjamini-Hochberg algorithm. The cutoff criterion for probes was a change of at least twofold, with a *P* value of <0.05. Additionally, for each probe, the number of samples for which the *P* value ≥ 0.05 was

considered to be the fail count. If fail count exceeded 50% of the total number of samples, the probe was low quality and was therefore excluded.

Unsupervised hierarchical clustering analysis was performed using complete linkage and a Euclidean distance metric with the expression profile of differentially expressed genes (DEGs). Pathway enrichment analysis for significant probes was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov/home.jsp) (Huang, Sherman et al. 2008). The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases were used as functional annotation tools.

Results

1. Patients and samples

The 12 patients in the periodontitis group comprised 6 male and 6 female aged 30 to 50 years, with a mean age of 42.16 years. The 10 patients in the healthy group comprised 7 male and 3 female aged 17 to 71 years, with a mean age of 43.7 years (Figure 1).

All the tissue samples passed the RNA quality and quantity tests, whereas 8 of the saliva samples did not pass the tests (4 each from the periodontitis and healthy groups). The final microarray analysis was performed using 22 tissue samples (12 for periodontitis and 10 for healthy patients) and 14 saliva samples (8 for periodontitis and 6 for healthy patients).

2. Differentially expressed gene analysis of gingival tissue and saliva samples

In total, 621 genes were differentially expressed in the tissue samples: 320 were upregulated and 301 were downregulated in the periodontitis group compared to the healthy group. The top 20 upregulated and downregulated genes are listed in Table 1. The top 20 upregulated genes included immune-response-related genes (CD79A, IGLL3, and TNFRSF17), a protease gene (MMP7), protein kinase genes (CAMK1G and CRKRS), and a cell-signaling-related gene (RGS1).

Applying the same cutoff standard to the results for the saliva sample yielded 154 DEGs in the periodontitis group (44 upregulated and 110 downregulated). The top-20 up-regulated and downregulated genes are listed in Table 1; these genes included a cellular-transportation-related gene (VPS41) and an immune-system-related gene (NLRP8).

A comparison of DEGs in the gingival tissue and saliva samples revealed that there were 9 overlapping genes, five of which (SPRR2G, LOC643161, LOC647993, LOC647987, and CRCT1) were downregulated in both gingival tissue and saliva samples, while the other 4 genes (ALOX5, IL1B, SRGN, and RAC2) were upregulated in gingival tissue samples and downregulated in saliva samples.

3. Cluster analysis

The results of the cluster analysis according to the similarity of the gene expression pattern of each sample using the total probe sets are shown in Figure 2. The branch

heights in the horizontal dendrogram indicate similarity relationships between entities. Reading the dendrogram from top to bottom, it is divided into 2 cluster groups from the first branch at the top of the structure. Two different clusters were produced for each set of saliva and tissue samples.

The gene expression pattern in the tissue samples (Figure 2A) showed distinct clusters corresponding to specific clinical features. The saliva samples (Figure 2B) similarly generated 2 clustered groups of samples based on the gene expression patterns that corresponded to periodontitis and healthy patients. However, 2 exceptional samples were found in the clusters that did not match the clinical diagnoses: 1 tissue/saliva sample from a periodontitis patient and 1 saliva sample from a healthy patient.

4. Pathway enrichment analysis of differentially expressed genes

The DAVID analysis identified that 159 terms (146 GO terms and 13 KEGG terms) were enriched in periodontitis patients with P values of <0.05 in the results for the tissue samples, while 110 terms (104 GO terms and 6 KEGG terms) were enriched in the results for the saliva samples. There were 34 overlapping enriched pathways between the tissue and saliva samples (33 GO terms and 1 KEGG term) (Figure 3). These 34 overlapping pathways included immune responses, inflammatory responses, cytokine regulation, and response to wounding, and immune-response-related pathways were prominent (Figure 4).

III. Discussion

This study specifically compared gene expression in gingival tissue and saliva in both healthy and periodontitis patients with the aim of determining the feasibility of detecting pathological responses in tissue samples based on only saliva samples, and with the eventual goal of developing a noninvasive screening tool. Transcriptomic analysis showed that the RNA expression pattern of saliva could be discriminated between periodontitis and healthy patients, in a manner comparable to the results obtained for the tissue biopsy samples. The cluster analysis of gene expression revealed that periodontitis patients clustered together both in the tissue samples and in the saliva samples, which implies that salivary transcriptome data can be used to distinguish between periodontitis patients and healthy controls.

Several previous transcriptome analyses using tissue samples found that patients with periodontitis were clustered together(Davanian, Stranneheim et al. 2012, Kebschull, Guarnieri et al. 2013, Becker, Beck-Broichsitter et al. 2014), with gingival tissue from periodontitis patients showing a significantly different gene expression pattern that formed distinct clusters compared to that of healthy patients. Kebschull et al.(Kebschull, Guarnieri et al. 2013) also demonstrated similarly clustered results based on DEGs between diseased and healthy periodontal tissues regardless of phenotype of periodontitis. The present study included periodontitis patients who showed familial aggregation and clear clinical features of severe periodontal destruction and produced a specific expression pattern of genes that was in agreement with previous studies. However, this is

the first study to obtain clustering results from the salivary transcriptome in periodontitis patients in addition to analyzing tissue samples. Our clustering results showed that the transcriptome of saliva samples could be used to distinguish periodontitis patients from healthy patients. Based on hierarchical clustering and the DEGs between saliva samples from diseased and healthy patients, salivary RNA expression could provide clues for identifying systemic disease(Zhang, Farrell et al. 2010) and salivary disease(Hjelmervik, Petersen et al. 2005).

Among the genes that were significantly overexpressed in the tissue samples, the TNFRSF17 gene was noteworthy, because its product specifically binds to the tumor necrosis factor family and plays roles in the immune responses, especially signal transduction, B-cell development, and cell differentiation. These results also support reports of a relation between up-regulation of the TNFRSF17 gene and the periodontitis phenotype(Davanian, Stranneheim et al. 2012, Guo, Wang et al. 2015, Guzeldemir-Akcakanat, Sunnetci-Akkoyunlu et al. 2016). Another meaningful gene found in the present study was MMP7, which is strongly correlated with the tissue inflammation level. MMP7 plays important roles in bone resorption and remodeling, and the possibility of detecting periodontitis based on the upregulation of MMP7 has been reported previously. These significant genes in the present data from periodontal tissue support the findings of previous functional studies of periodontitis, as well as whole-genome and microarray studies.

At the functional pathway level, 34 enriched pathways overlapped in the gingival tissue and saliva samples obtained from periodontitis patients compared to healthy patients, and 12 of those pathways were related to the immune responses. Although different sets of genes were detected in the tissue and saliva samples, it is meaningful that the immune-response pathway was commonly detected at the functional level. Similar results have also been found in other studies of the salivary transcriptomes of periodontitis patients, with cellular immunity pathways being significantly up-regulated(Zhang, Lin et al. 2015). Other studies of periodontal tissue samples have found that important roles are played by immune-response pathways, such as the leukocyte extravasation signaling pathway (Guzeldemir-Akcakanat, Sunnetci-Akkoyunlu et al. 2016), the pathway involving the chemotaxis and trans-endothelial migration of leukocytes(Offenbacher, Barros et al. 2009), and immune-system processes including defense responses(Davanian, Stranneheim et al. 2012). Chronic inflammation due to persistent infection of the periodontium causes hyperactivation of immune responses (both innate and adaptive). Changes in the homeostasis of immune responses around the periodontium may affect various sources of salivary mRNA(Giannobile, Beikler et al. 2009). In detail, gingival crevicular fluid is directly affected by the state of tissue(Loos and Tjoa 2005), and major salivary glands may be affected by circulating inflammatory mediators(Teng, Sodek et al. 1992, Giannobile, Beikler et al. 2009).

The present results revealed only small numbers of overlapping DEGs between periodontitis and healthy patients (1.5% from the entire DEG lists of the tissue samples

and 5.8% from the saliva samples). This limitation might have been caused by the total amount of mRNA detected from the whole saliva being less than that detected from the tissue samples, due to the nature of saliva and limitations of the present analytical technology (Figure 5). The present results are in agreement with those of a previous study showing only small amounts of total RNA, with wide variations and contamination from bacterial sources, in microarray analyses of samples taken from patients with chronic periodontitis(Hidayat, Milne et al. 2018). Salivary mRNA is stabilized by interactions with salivary proteins and apoptotic bodies(Hasselmann, Rappl et al. 2001), and RNases and proteases from microbial/human sources can rapidly degrade mRNA. These features have prompted the developed of various collection methods and RNase inhibitors (Li, Zhou et al. 2004, Park, Yu et al. 2006, Hu, Wang et al. 2007), but there are no standardized methods for obtaining acceptable amounts of mRNAs from saliva and for their amplification(Park, Zhou et al. 2007).

Of particular note, 4 genes (ALOX5, IL1B, SRGN, RAC2) overlapped between the saliva and tissue samples, but with the opposite expression pattern. These findings clearly demonstrate limitations or hurdles to be overcome in the degree to which saliva samples can be currently used as a diagnostic tool based on specific biomarkers. The scant quantity of the transcriptome from the periodontal pocket in saliva samples and the unclear pathophysiological classification of periodontitis may cause noise within the data, inducing discordant results. In addition, the expression levels of several genes, such as IL1B and ALOX5, have been found to show a temporal pattern during the process of

inflammation(Paula-Silva, Petean et al. 2016, Belstrøm, Damgaard et al. 2017). Periodontitis has multiple pathological steps, with repeated cycles of rest and active phases, and further studies are needed on the pathophysiological mechanism and the specific gene expression patterns to clarify a specific biomarker. However, the present study identified specific clustered patterns of gene expression in severe periodontitis based on the analysis of either saliva samples or tissue samples. In addition, there were more overlapping results in the pathway enrichment analysis between gingival tissue and saliva samples than in the individual gene-level analysis, as overlap was found for 12.8% of all enriched pathways from the tissue samples and for 22.5% of enriched pathways from the saliva samples of periodontitis patients. Therefore, further studies focusing on the pathways and changes in their categorical genes would be helpful for identifying specific markers for use in diagnosis and disease monitoring.

The present study identified specific clustering patterns of gene expression in both saliva samples and tissue samples. Based on these results, saliva samples can be considered as candidate biospecimens for monitoring periodontitis at the level of immunity-related pathways. These findings represent an initial demonstration of the possibility of using saliva samples for transcriptome-based periodontal monitoring; however, further studies are needed to determine how to enhance the yield of RNA from saliva samples and how to perform pathway-specific analyses.

IV. Conclusion

The present results indicate the possibility of using the transcriptome from saliva sample to distinguish periodontitis patients from healthy individuals. However, further work is required to enhance the extraction of available RNA from saliva samples.

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Figure legends

Figure 1. Panoramic radiographic views of patients in (A) the periodontitis patient group and (B) the healthy patient group.

Figure 2. Hierarchical clustering results and heat map of the gene expression data from (A) gingival tissue samples and (B) saliva samples. The horizontal dendrogram displays similarities between samples. Samples with similar patterns of gene expression are clustered together and there were 2 different cluster groups in each result. Under the dendrogram, the clinical features of the samples are indicated by colored blocks. The samples obtained from the same patients were given the same number for gingival tissue and saliva sample listed under the heat map. The heat map is colored according to the relative expression of a probe, scaled by z-score normalized values.

Figure 3. (A) Venn diagrams showing the number of differentially expressed genes and enriched pathways of gingival tissue and saliva sample. The intersection of the 2 circles refers to genes and pathways commonly detected in the results from the gingival tissue and saliva samples. There were 9 overlapping genes and 34 overlapping pathways between the tissue and saliva samples. (B) Interactive maps of overlapping GO biological process terms between the tissue and saliva samples. The size of each node represents the frequency of GO terms in the underlying GO annotation database and the thickness of the lines indicates similarity between GO terms. The length of the lines is arbitrary. The data

suggest that immune response-related terms were prominent in overlapping pathways. The maps were visualized using REVIGO (http://revigo.irb.hr). GO: Gene Ontology.

Figure 4. Interactive maps of significantly identified GO biological process terms from the results of (A) tissue samples and (B) saliva samples. The color of the nodes represents the P values in each data set (more intense colors indicate lower P values). The size of each node represents the frequency of GO terms in the underlying GO annotation database, and the thickness of the lines indicates similarity between GO terms. The length of the lines is arbitrary. The maps were visualized using REVIGO (http://revigo.irb.hr). GO: Gene Ontology.

Figure 5. The number of differentially expressed genes detected with different FCs (FC ≥2 or ≥1.5 in absolute value) is indicated by a horizontal bar. The results for each type of sample are shown in different colors: gingival tissue (blue) and saliva samples (green). FC: fold change.

Table

Table 1. Top 20 differentially expressed genes from each sample from periodontitis patients compared to healthy patients

Gene symbol	Definition	Fold change	*P* value
Upregulated genes: gingival tissue			
CD79A	CD79a molecule, immunoglobulin-associated alpha	10.70	4.38E-06
IGLL3	Immunoglobulin lambda-like polypeptide 3	9.83	3.49E-05
JSRP1	Junctional sarcoplasmic reticulum protein 1	9.05	4.86E-05
LOC649923	Similar to Ig gamma-2 chain C region	9.01	9.24E-04
CAMK1G	Calcium/calmodulin-dependent protein kinase IG	8.99	4.01E-06
MMP7	Matrix metallopeptidase 7	8.76	3.50E-05
LOC652694	Similar to Ig kappa chain V-I region HK102	8.46	9.60E-05
FAM46C	Family with sequence similarity 46,member C	7.70	7.30E-06
MGC29506	Hypothetical protein MGC29506 (MGC29506)	7.59	8.92E-05
LOC652102	Ig heavy chain V-I region HG3 precursor	7.53	3.16E-04
LOC647506	Hypothetical LOC647506	7.44	4.67E-04
MMP7	Matrix metallopeptidase 7	7.28	2.82E-05
LOC651751	Similar to Ig kappa chain V-II region RPMI 6410 p	7.07	1.10E-03
LOC647450	Similar to Ig kappa chain V-I region HK101	6.98	1.21E-03
TNFRSF17	Tumor necrosis factor receptor superfamily, member 17	6.81	1.75E-06
LOC652493	Similar to Ig kappa chain V-I region HK102	6.45	1.69E-03
CRKRS	Cdc2-related kinase, arginine/serine-rich	6.29	1.45E-06
RGS1	Regulator of G-protein signaling 1 .	6.11	7.35E-06
RNF126P1	Ring finger protein 126 pseudogene 1.	6.11	1.29E-05
LOC642113	Similar to Ig kappa chain V-III region HAH precursor	5.93	2.92E-03
Downregulated genes: gingival tissue			
LOR	Loricrin	−22.22	6.52E-05

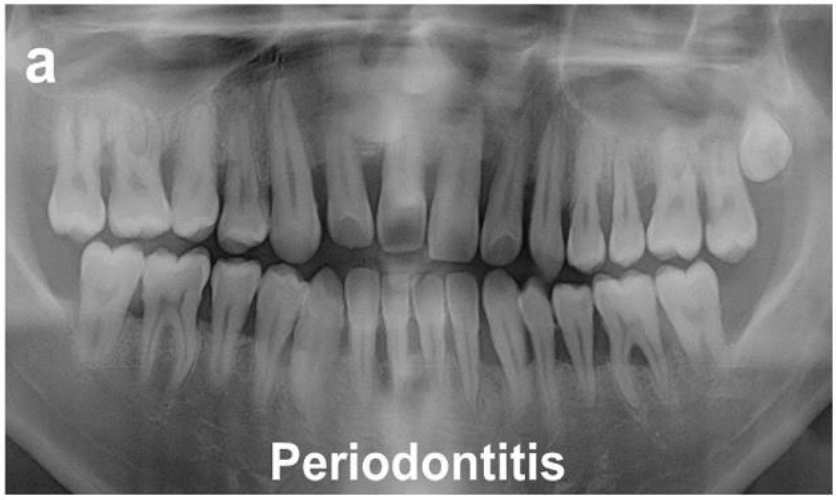
KRT1	Keratin 1	−20.30	3.89E-05
C6orf15	Chromosome 6 open reading frame 15	−13.06	4.69E-06
KRT2	Keratin 2	−12.62	5.18E-05
ASPRV1	Aspartic peptidase, retroviral-like 1	−11.42	7.54E-06
LCE2B	Late cornified envelope 2B	−10.52	2.20E-05
CDSN	Corneodesmosin	−9.91	7.31E-06
RPTN	Repetin (RPTN)	−9.89	1.26E-04
LCE2D	Late cornified envelope 2D	−9.45	1.16E-05
KRT76	Keratin 76	−9.25	1.07E-04
KPRP	Keratinocyte proline-rich protein	−8.83	3.07E-06
LCE2A	Late cornified envelope 2A	−8.65	6.05E-05
LCE2C	Late cornified envelope 2C	−8.54	1.15E-05
LCE3A	Late cornified envelope 3A	−8.19	6.30E-05
KRT3	Keratin 3 (KRT3)	−7.29	1.31E-04
FLG	Filaggrin (FLG)	−6.58	3.58E-04
LCE3D	Late cornified envelope 3D	−6.56	3.34E-05
LY6G6C	Lymphocyte antigen 6 complex, locus G6C	−6.47	6.12E-07
LCE3E	Late cornified envelope 3E	−6.44	4.89E-06
SLURP1	Secreted LY6 / PLAUR domain containing1	−5.70	6.30E-07
Up-regulated genes: saliva			
VPS41	Vacuolar protein sorting 41 homolog	3.18	6.00E-03
ZNF786	Zinc finger protein 786	2.81	2.61E-02
LOC389765	Similar to KIF27C	2.79	1.23E-02
ZNF738	Misc_RNA	2.65	1.40E-02
LOC100132585	Hypothetical protein LOC100132585	2.59	1.58E-02
LOC100129502	Hypothetical protein LOC100129502	2.57	1.09E-02
PNPT1	Polyribonucleotide nucleotidyltransferase 1	2.54	1.02E-02
C15orf63	Chromosome 15 open reading frame 63	2.49	2.83E-02
LOC100131718	Misc_RNA	2.48	3.52E-02
NLRP8	NLR family, pyrin domain containing 8	2.46	2.04E-02
FLJ36131	Hypothetical protein FLJ36131	2.41	2.22E-02

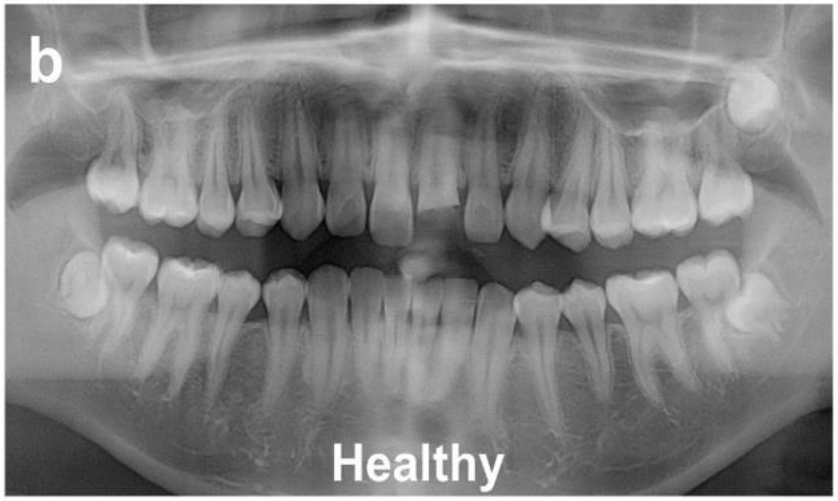
ZNF223	Zinc finger protein 223	2.40	1.75E-02
SLC35E1	Solute carrier family 35, member E1	2.39	3.21E-02
C5orf28	Chromosome 5 open reading frame 28	2.36	1.69E-02
LOC644250	Hypothetical protein LOC644250	2.33	1.75E-02
CCDC125	Coiled-coil domain containing 125	2.33	9.45E-03
DUXAP3	Double homeobox A pseudogene 3	2.33	4.95E-02
FAM73A	Family with sequence similarity 73, member A	2.32	2.74E-02
FLJ40722	Hypothetical protein FLJ40722	2.30	1.83E-02
N4BP2	Nedd4 binding protein 2	2.25	1.08E-02
Down-regulated genes: saliva			
SPRR3	Small proline-rich protein 3	−15.26	3.10E-03
IL1B	Interleukin 1, beta	−14.12	1.33E-02
FTHL7	Ferritin, heavy polypeptide-like 7	−12.53	8.73E-03
FTHL16	Misc_RNA	−10.08	7.71E-03
SPRR2D	Small proline-rich protein 2D	−9.91	4.98E-03
ACTB	Actin, beta	−9.89	3.44E-03
SPRR2A	Small proline-rich protein 2A	−9.67	5.49E-03
KRT13	Keratin 13	−9.55	4.85E-03
S100A9	S100 calcium binding protein A9	−8.39	1.01E-02
IL8	Interleukin 8	−8.19	2.95E-02
CRNN	Cornulin	−7.40	3.32E-03
ZFP36	Zinc finger protein 36, C3H type	−6.46	1.67E-02
TPT1	Tumor protein, translationally-controlled 1	−6.45	1.67E-02
SPRR2F	Small proline-rich protein 2F	−6.21	1.70E-02
KRT6A	Keratin 6A	−6.03	1.72E-02
IL1RN	Interleukin 1 receptor antagonist	−5.89	4.54E-03
S100A8	S100 calcium binding protein A8	−5.72	2.97E-02
B2M	Beta-2-microglobulin	−5.64	1.74E-02
ACTG1	Actin, gamma 1	−5.59	2.57E-02
PLEK	Pleckstrin	−5.37	3.08E-02

Upregulated genes in gingival tissue samples; downregulated genes in gingival tissue samples;

upregulated genes in saliva samples; downregulated genes in saliva samples. Genes involved in overlapped enriched pathways between the results from the gingival tissue and saliva samples are gray-shaded.

Figures





	Periodontitis patients	Healthy patients
N	12	10
Gender (F/M)	6/6	3/7
Age (mean±SD;)	42.16 ± 7.2	43.7 ± 16.95

Figure 1

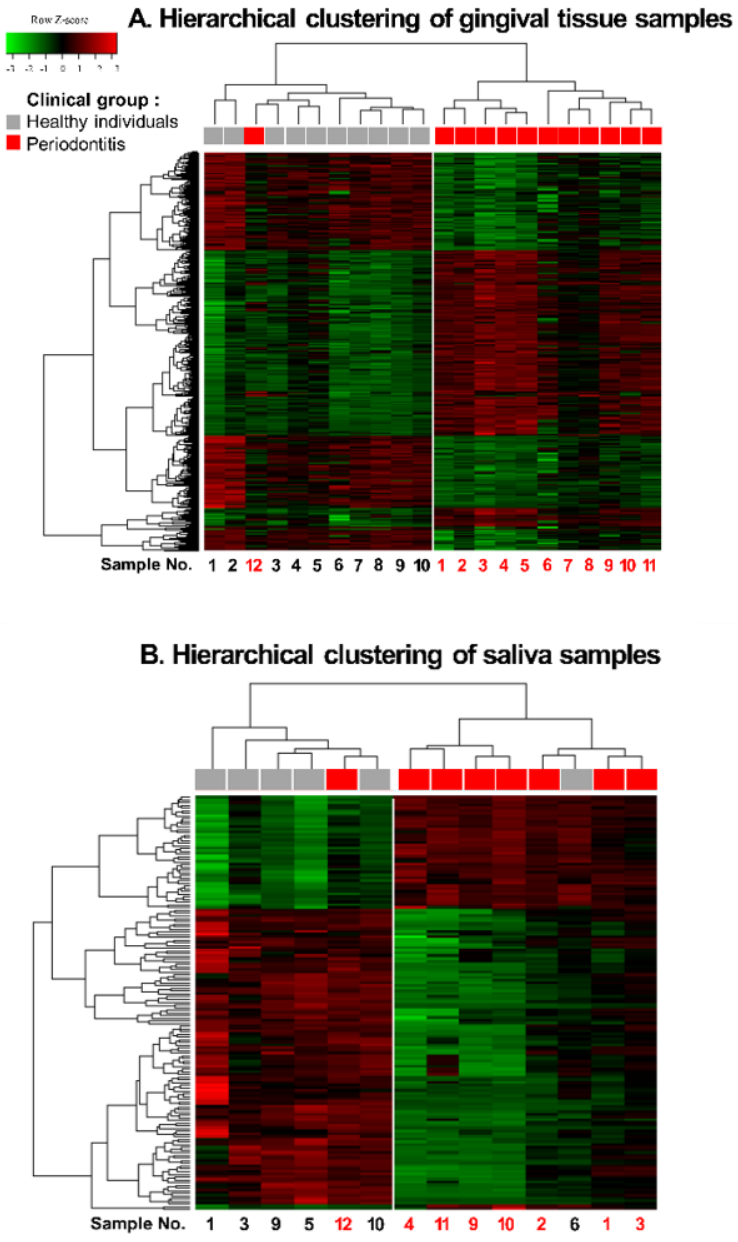


Figure 2

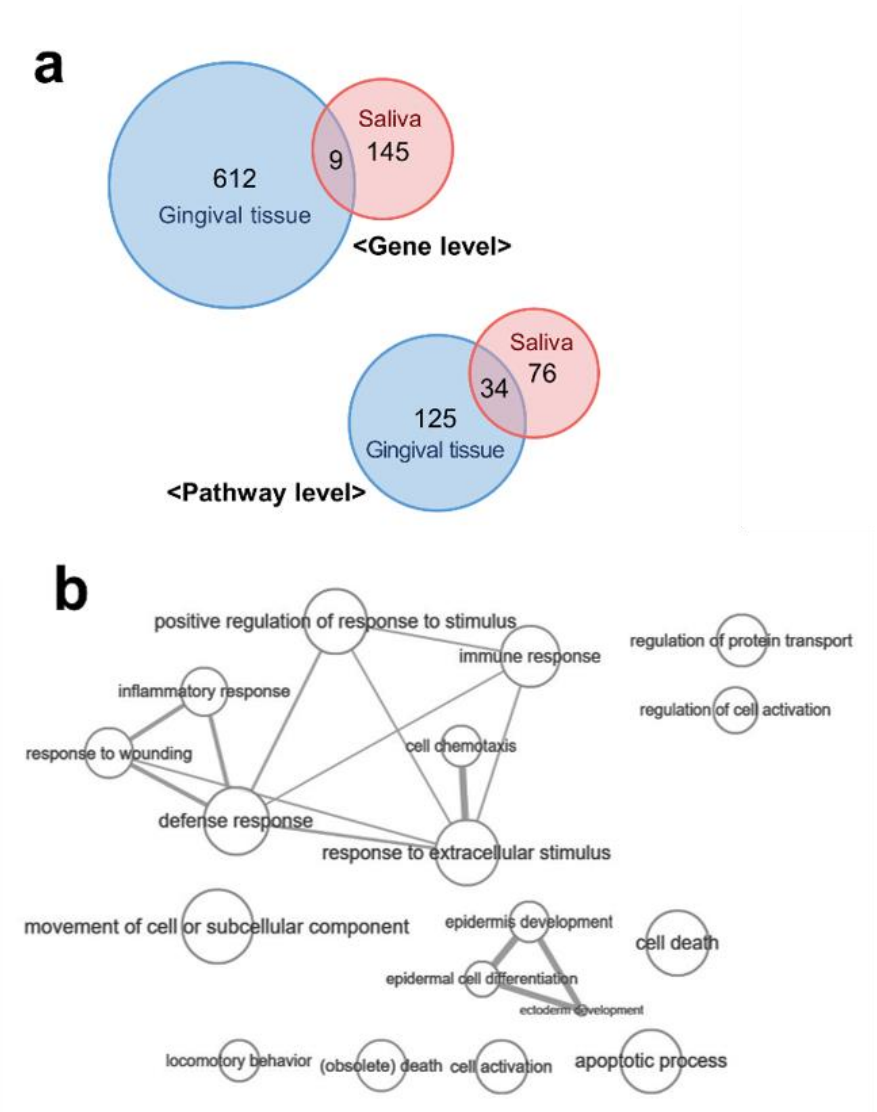


Figure 3

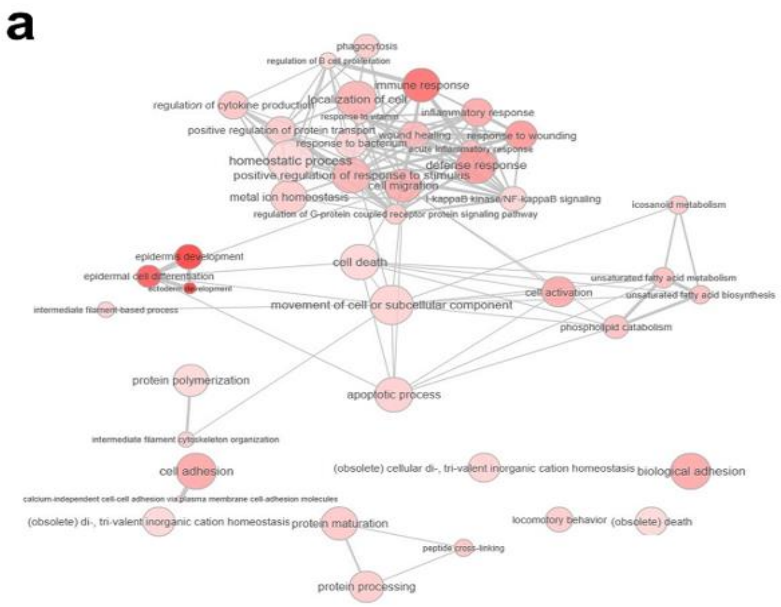


Figure 4

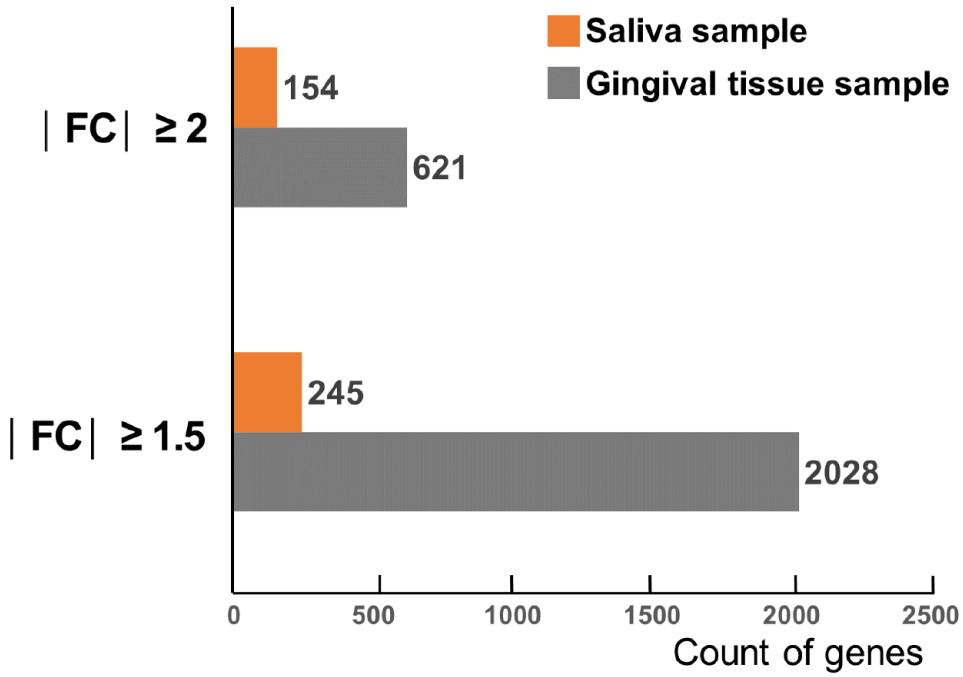


Figure 5

국문요약

치주염 및 건강한 환자의 타액과 치주 조직 생검에서의 전사체 발현양상과 두가지의 상관 관계

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전사체 분석은 유전자 단위에서 전반적인 조직의 반응을 검출하는데 유용한 분석 방법이다. 이를 이용한 기존의 연구들에서 치주염 환자의 치주 조직에서 특이적인 조직 반응을 나타내는 전사체 발현 양상이 보고되어왔다. 그러나, 기존의 연구들은 대부분 치주 조직 생검을 이용한 전사체 분석을 진행하였으며, 이러한 샘플은 반복적인 채취가 불가능하다는 단점이 있다. 이러한 단점을 극복하기 위하여, 타액을 유전학적 및 단백학적 분석의 시료로 사용하는 방법이 주목받고 있다. 치주염 환자의 타액 샘플에서 RNA 발현 프로파일의 타당성을 평가하기 위해서는, 동일 환자의 치은조직 샘플의 RNA 발현 프로파일과의 비교가 필요하다. 이 연구의 목적은 치은 조직 및 타액 샘플에서 RNA 발현 프로파일을 분석하고 이들의 상관 관계를 평가하는 것이다.

본 연구는 12명의 severe 치주염 환자와, 10명의 건강한 환자를 대상으로

진행하였다. 치주 조직 생검과 타액 채취가 모든 환자에게 진행되었으며, 이를 이용하여 전사체 마이크로 어레이 분석을 시행하였다. 전사체 발현 양상 및 생체 경로 분석, 클러스터 분석을 시행하였다. 유전자 단위와 생체 경로 단위에서 치주 조직생검 샘플과 타액 샘플 결과의 연관성을 분석하였다.

치주조직 샘플의 분석 결과 621개의 차별 발현유전자(과발현 유전자 320개, 저발현 유전자 301개)가 검출되었고, 타액샘플의 분석 결과 154개의 차별발현유전자(과발현 유전자 44개, 저발현 유전자 110개)가 검출되었다. 두가지 샘플의 분석 결과상 공통된 차별발현유전자는 9개였다. 클러스터 분석 결과 치주 조직 샘플과 타액 샘플 모두에서 유전자 발현 양상을 기초로 하여 치주염 환자들끼리 특정한 클러스터 그룹을 형성하였다. 생체 경로 분석 결과로는 치주 조직 샘플의 결과에서 159개의 생체 경로가 검출되었고, 타액 샘플의 결과에선 110개의 생체 경로가 검출되었다. 이중에서 두가지 샘플의 결과끼리 겹치는 것은 총 34개 생체 경로였다.

이번 연구 결과를 통해 타액의 전사체 분석 결과를 통해 건강한 환자로부터 치주염 환자를 구별할 수 있는 가능성을 확인하였다. 이를 위해선 타액 샘플로부터 RNA추출을 효과적으로 진행할 수 있는 방법에 대한 추가적인 연구가 필요하다.

핵심되는 말 : 전사체 분석; 치주염; 타액; 전사체