

Comparative Gene-Expression Analysis of Periodontal Ligament and Dental Pulp in the Human Permanent Teeth

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Abstract

There is no genetic activity information with the functions of dental pulp and periodontal ligament in human. The purpose of this study was to identify the gene-expression profiles of, and the molecular biological differences between periodontal ligament and dental pulp obtained from human permanent teeth. cDNA microarray analysis identified 347 genes with a fourfold or greater difference in expression level between the two tissue types 83 and 264, of which were more plentiful in periodontal ligament and dental pulp, respectively. Periodontal ligament exhibited strong expression of genes related to collagen synthesis (FAP), collagen degradation (*MMP3*, *MMP9*, and *MMP13*), and bone development and remodeling (*SSP1*, *BMP3*, *ACP5*, *CTSK*, and *PTH1LH*). Pulp exhibited strong expression of genes associated with calcium ions (*CALB1*, *SCIN*, and *CDH12*) and the mineralization and formation of enamel and dentin (*SPARC/SPOCK3*, *PHEX*, *AMBN*, and *DSPP*). Among these genes, *SPP1*, *SPARC/SPOCK3*, *AMBN*, and *DSPP* were well known in dental research. However, the other genes are the newly found and it may help to find a good source of regenerative therapy if further study is performed.

Key words : Gene-Expression, Periodontal Ligament, Dental Pulp, Human Permanent Teeth, Korean

I . Introduction

Because of their significance in dental health, dental pulp and PDL have received considerable attention in the field of dental bioengineering¹⁻³⁾. One of the actively used methods of analysis in this regard is cDNA microarray, since it enables analysis of the expression of thousands of genes and proteins simultaneously and the comparison of the gene-expression profiles of two differ-

ent samples. In case of PDL, Song *et al.*⁴⁾ performed a comparative gene-expression analysis between the PDL of permanent and deciduous teeth. Xie *et al.*⁵⁾ and Lee *et al.*⁶⁾ compared the microRNA profiles of human periodontal diseased and healthy gingival tissues. Han and Amar⁷⁾ examined the fibroblasts of PDL and gingiva, and de Araujo *et al.*⁸⁾ used cDNA microarray to study PDL cells under mechanical stress. In addition, Paakkonen *et al.*⁹⁾ and McLachlan *et al.*¹⁰⁾ analyzed the gene-express-

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sion patterns of healthy and carious pulp, and Paakkonen *et al.*¹¹ further investigated the differences between odontoblasts and dental pulp. Other studies have compared pairs of related dental tissues, such as the ameloblastoma and tooth germ¹², and the PDL and tooth follicle¹³. According to these researches, the candidate genes in specific function or pathologic change have been revealed.

The main functions of the PDL are tooth support, regeneration of periodontal tissues, maintenance of homeostasis, and provision of the healing process required following periodontal disease or mechanical trauma¹⁴. In terms of mechanical trauma, ankylosed teeth cannot be moved by orthodontic force if the PDL is absent¹⁵. The mechanical stress loaded onto a tooth is transferred to the PDL, the cells of which respond to that mechanical stress, regulating the absorption and formation of the bone matrix by signaling the surrounding cells¹⁶. The main functions of dental pulp are the formation of tertiary dentin, nerve innervation, and nutrition supply¹⁷. Dental pulp cells also have the ability to differentiate into odontoblasts in order to repair dental damage caused by trauma, caries, or dental erosion¹⁸.

However, it is unknown about that the differences are made by which gene expression in spite of the functional differences of PDL and pulp. Each tissue has the specific gene expression pattern, so analysis of gene expression pattern of tissue enables to understanding the function of the tissue clearly. But no direct comparison of gene-expression profiles has yet been made between the healthy normal PDL and dental pulp tissues of human permanent teeth. And, most studies were conducted by gene expression analysis using cultured cells of PDL and dental pulp. Therefore, the purpose of this study is to identify and compare the gene-expression profiles and molecular biological differences between PDL and dental pulp tissues from human normally functioning teeth.

II. Materials and Methods

1. Sample collection

The experimental protocol was approved by the Institutional Review Board of the Yonsei University Dental Hospital (approval #2-2011-0050). Written informed consent to participate was obtained from all participants and from their next of kin, caregivers, or guardians on behalf of minors/children. The PDL sam-

ples ($n=9$; aged 11-19 years) and the pulp samples ($n=9$; aged 11-19 years) examined in this study were obtained from healthy, mature premolars extracted for orthodontic reasons from healthy persons. The extracted teeth were immediately frozen and stored in liquid nitrogen. Tissues were obtained carefully from the middle third of the PDL using sterile cures. The teeth were subsequently crushed with a bolt cutter and the pulp was carefully obtained using sterile tweezers. The PDL and pulp were then immediately submerged in RNA-stabilizing reagent (RNAlater, Qiagen, CA, USA).

2. RNA isolation

Total RNA was extracted from the PDL and pulp samples using the RNeasy Fibrous Minikit (Qiagen) according to the manufacturer's instructions. Prior to RNA extraction, the tissues were homogenized using a Bullet Blender Bead (Next Advanced, NY, USA). The extracted RNA was eluted in 25 μ l of sterile water. RNA concentrations were determined from absorbance values obtained at a wavelength of 260 nm using a spectrophotometer (NanoDrop ND-1000, Thermo Scientific, IL, USA). The RNA samples analyzed in this study had 260/280 nm ratios of ≥ 1.8 .

3. cDNA microarray analysis

The microarray procedure was conducted in triplicate for each sample, and using three samples from each group. Global gene expression analyses were conducted using Affymetrix GeneChip[®] Human Gene 1.0 ST oligonucleotide arrays (Affymetrix Inc., CA, USA). As recommended by the manufacturer's protocol, 300 ng were used. Briefly, 300 ng of total RNA from each sample was converted to double-strand cDNA, as described previously⁴. Fragmented end-labeled cDNA was hybridized to the GeneChip Human Gene 1.0 ST arrays for 16 hours at 45°C and 60 rpm, as described in the GeneChip Whole Transcript Sense Target Labeling Assay Manual (Affymetrix). After hybridization, the chips were stained and washed in a GeneChip Fluidics Station 450 (Affymetrix), and then scanned using Affymetrix Command Console software (version 1.1, Affymetrix). The raw file generated through this procedure provides expression intensity data, which were used for the next step.

4. Gene ontology analysis

Expression data were generated by Affymetrix Expression Console software version 1.1 (Affymetrix). The robust multichip average algorithm implemented in Affymetrix Expression Console software was used to normalize the data. One-way ANOVA was applied to the RMA expression values to determine whether genes were differentially expressed between the three groups. A multiple testing correction was applied to the *p* values of the *F* statistics to adjust for the false discovery rate. Genes with adjusted *F*-statistic *p* values of <0.05 were extracted. Strongly expressed genes in PDL or pulp that exhibited differences of over 6- or 11-fold relative to the signal value of the control and each test group were selected for the further study. In order to classify the co-expression gene group, for which the expression pattern was similar, hierarchical clustering and *k*-means clustering were performed using MultiExperiment Viewer software, version 4.4 (www.tm4.org; Dana-Farber Cancer Institute, MA, USA). The web-based tool Database for Annotation, Visualization, and Integrated Discovery (DAVID) was used to biologically interpret the differentially expressed genes (<http://david.abcc.ncifcrf.gov/home.jsp>). These genes were then classified based on information on gene function from the gene ontology, Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway database (<http://david.abcc.ncifcrf.gov/home.jsp>).

This microarray data set was approved by the Gene Expression Omnibus (<http://www.ncbi.nlm.gov/geo/>); its GEO accession number is GSE50639.

5. Quantitative polymerase chain reaction

The single-stranded cDNA required for the polymerase chain reaction (PCR) analysis was synthesized using 500 ng of extracted total RNA as a template for reverse transcription (Superscript III Reverse Transcriptase and random primer, Invitrogen, UK), as described previously⁴⁾.

The following TaqMan gene-expression assay primers were used (Applied Biosystems, CA, USA): ameloblastin (*AMBN*), calbindin 1 (*CALB1*), collagen, type XII, alpha (*COL12A*), dentin sialophosphoprotein (*DSPP*), matrix metalloproteinase (*MMP*)9, MMP20, secreted protein acidic and rich in cysteine (*SPARC*)/osteonectin 3 (*SPOCK3*), secreted phosphoprotein 1 (*SPP1*), and 18S rRNA. The results are plotted versus time, which was

quantified as the cycle number. A precise quantification of the initial target was obtained by examining the amplification plots during the early log phase of product accumulation above background [the threshold cycle (Ct) number]. Ct values were subsequently used to determine Δ Ct values (Δ Ct=Ct of the gene minus Ct of the 18S rRNA control), and differences in Ct values were used to quantify the relative amount of PCR product, expressed as the relative change by applying the equation $2^{-\Delta Ct}$.

6. Immunohistochemical staining

In preparation for immunohistochemical (IHC) staining, permanent teeth were fixed in 10% buffered formalin (Sigma, MO, USA) for 1 day, decalcified with 10% EDTA (pH 7.4; Fisher Scientific, TX, USA) for 8 weeks, embedded in paraffin, and sectioned at a thickness of 3 μ m. Specimens were subjected to IHC staining with anti-human CALB1 (rabbit polyclonal, diluted 1:400; Ab25085, Abcam, Cambridge, UK), MMP9 (rabbit polyclonal, diluted 1:800; Ab38898, Abcam), SPP1 (osteopontin, rabbit polyclonal, diluted 1:800; Ab8448, Abcam), and COL12A1 (rabbit polyclonal, diluted 1:800; Sc68862, Santa Cruz Biotechnology, Inc., CA, USA). Endogenous peroxidase activity was quenched by the addition of 3% hydrogen peroxide. Sections were incubated in 5% bovine serum albumin to block nonspecific binding. The primary antibodies were diluted to give optimal staining and the sections were incubated overnight. After incubation, EnVision+ System-HRP labeled Polymer Anti-rabbit (K4003, Dako North America Inc., CA, USA; ready to use) was applied for 20 min. Color development was performed using labeled streptavidin biotin kits (Dako) according to the manufacturer's instructions. The sections were counterstained with Gill's hematoxylin (Sigma). Control sections were treated in the same manner but without treatment with primary antibodies.

III. Results

1. Gene-expression profiles of PDL and dental pulp

The total data distribution and frequency were confirmed using density and box plots and M-A plots of the standardized log intensity ratio to the average intensity. The results revealed that there was a fourfold or greater

difference in expression of 347 out of 28,869 genes (1.20%) between the PDL and dental pulp tissues from permanent teeth: 83 and 264 genes were more strongly expressed in PDL and pulp tissue, respectively. Table 1 and 2 list the genes that were expressed most strongly in these two tissue types.

2. Gene ontology analysis

Gene Ontology Consortium (GO) grouping was used with the aid of DAVID to translate the microarray data into meaningful biologic functional terms and to characterize the groups of functionally related genes. The genes

Table 1. Up-regulated genes in the PDL tissue of permanent teeth

Name	Gene Symbol	Fold change	Gene Accession
matrix metalloproteinase 13	MMP13	43.56	NM_002427
matrix metalloproteinase 9	MMP9	28.53	NM_004994
keratin 5	KRT5	19.35	NM_000424
collagen, type XII, alpha 1	COL12A1	18.20	NM_004370
immunoglobulin superfamily, member 10	IGSF10	16.16	NM_178822
fibroblast activation protein, alpha	FAP	15.32	NM_004460
tenascin N	TNN	14.92	NM_022093
secreted phosphoprotein 1	SPP1	13.84	NM_001040058
collagen, type XI, alpha 1	COL11A1	12.94	NM_001854
RAB27B, member RAS oncogene family	RAB27B	12.28	NM_004163
plexin C1	PLXNC1	11.87	NM_005761
ATPase, H ⁺ transporting, lysosomal 38kDa, V0 subunit d2	ATP6V0D2	11.35	NM_152565
bone morphogenetic protein 3	BMP3	10.88	NM_001201
asporin	ASPN	10.80	NM_017680
leucine rich repeat containing 15	LRRC15	10.12	NM_001135057
platelet-derived growth factor receptor-like	PDGFRL	8.50	NM_006207
sushi-repeat-containing protein, X-linked 2	SRPX2	8.42	NM_014467
secreted frizzled-related protein 2	SFRP2	8.12	NM_003013
acid phosphatase 5, tartrate resistant	ACP5	7.85	NM_001111035
XG blood group	XG	7.66	NM_001141919
SH3 and cysteine rich domain	STAC	7.63	NM_003149
CD109 molecule	CD109	7.38	NM_133493
transmembrane 4L six family member 19	TM4SF19	7.36	NM_138461
odontogenic, ameloblast associated	ODAM	7.29	NM_017855
angiopoietin-like 2	ANGPTL2	7.25	NM_012098
collagen, type VI, alpha3	COL6A3	7.11	NM_004369
dipeptidyl-peptidase 4	DPP4	7.05	NM_001935
retinol binding protein 4, plasma	RBP4	6.92	NM_006744
prune homolog 2	PRUNE2	6.88	NM_015225
RAR-related orphan receptor B	RORB	6.85	NM_006914
integrin, alpha 11	ITGA11	6.81	NM_001004439
matrix metalloproteinase 3	MMP3	6.75	NM_002422
keratin 14	KRT14	6.61	NM_000526
cathepsin K	CTSK	6.60	NM_000396
fibronectin type III domain containing 1	FNDC1	6.41	NM_032532
prolyl 4-hydroxylase, alpha polypeptide III	P4HA3	6.39	NM_182904
sarcoglycan, gamma	SGCG	6.11	NM_000231
corin, serine peptidase	CORIN	6.07	NM_006587
parathyroid hormone-like hormone	PTHLH	6.05	NM_198965

PDL(periodontal ligament)

Table 2. Up-regulated genes in the dental pulp tissue of permanent teeth

Name	Gene Symbol	Fold change	Gene Accession
calbindin 1, 28kDa	CALB1	99.53	NM_004929
phosphate regulating endopeptidase homolog, X-linked	PHEX	68.41	NM_000444
sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 3	SPOCK3	67.93	NM_001040159
matrix metalloproteinase 20	MMP20	63.35	NM_004771
cadherin 12, type 2 (N-cadherin 2)	CDH12	62.88	NM_004061
transferrin	TF	55.52	NM_001063
ameloblastin	AMBN	48.59	NM_016519
sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphoring) 3E	SEMA3E	45.67	NM_012431
luciferin-rich repeat-containing G protein-coupled receptor 5	LGR5	42.99	NM_003667
dentin sialophosphoprotein	DSPP	41.24	NM_014208
abhydrolase domain containing12B	ABHD12B	32.82	NM_181533
lipid phosphate phosphatase-repeatd protein type 5	LPPR5	26.38	NM_001037317
scinderin	SCIN	26.17	NM_001112706
transmembrane protein 156	TMEM156	25.26	NM_024943
serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	SERPINA3	24.63	NM_001085
carboxypeptidase A6	CPA6	22.05	NM_020361
gamma-aminobutyric acid (GABA) A receptor, beta 1	GABRB1	18.98	NM_000812
bone morphogenetic protein receptor, type IB	BMPR1B	18.19	NM_001203
ceruloplasmin (ferroxidase)	CP	16.92	NM_000096
ATP-binding cassette, sub-family A (ABC1), member 6	ABCA6	16.76	NM_080284
RAN binding protein 3-like	RANBP3L	15.99	NM_001161429
potassium channel, subfamily K, member 2	KCNK2	15.81	NM_001017425
G protein-coupled receptor 37 (endothelin receptor type B-like)	GPR37	14.46	NM_005302
protocadherin 20	PCDH20	14.37	NM_022843
ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 1	ST8SIA1	14.28	NM_003034
coxsackie virus and adenovirus receptor	CXADR	13.64	NM_001338
solute carrier family 4, sodium bicarbonate cotransporter, member 4	SLC4A4	13.15	NM_001098484
G protein-coupled receptor 63	GPR63	12.88	NM_001143957
reelin	RELN	12.87	NM_005045
cytoplasmic FMR1 interacting protein 2	CYFIP2	12.51	NM_001037332
gastrin-releasing peptide receptor	GRPR	12.42	NM_005314
EPH receptor A5	EPHA5	12.2	NM_004439
sodium channel, voltage-gated, type VII, alpha	SCN7A	12.02	NM_002976
cerebellin 2 precursor	CBLN2	11.91	NM_182511
WD repeat domain 72	WDR72	11.72	NM_182758

were classified based on information regarding gene function in gene ontology from the KEGG Pathway database. GO classes with an F -statistic p value of < 0.05 following analysis on the basis of their biologic processes and molecular functions are shown in Figure 1 and 2, respectively. In broad outlines, the GO classes of the dental pulp tissue are relatively more counted those of the PDL tissue. Especially, there were notable differences of biologic processes in regulation of cell adhesion, neurological system process, signal transduction, and ion transport, and of molecular functions in nucleotide binding, ATP binding, and ion binding.

3. Quantitative PCR

Quantitative PCR (qPCR) was performed to verify the different gene-expression levels obtained through cDNA microarray analysis. The following six genes were selected for this verification procedure: *COL12*, *MMP9*, *SPP1*, *CALB1*, *SPOCK3*, and *DSPP*. The remaining six were compared between PDL and pulp in the form of gene-expression ratios (Table 3). The pulp: PDL ratios for *CALB1*, *SPOCK3*, and *DSPP* were high, at 4,600, 6,800, and 15,000, respectively, while those for *COL12*, *MMP9*, and *SPP1* were low, at 44, 8, and 9, respectively.

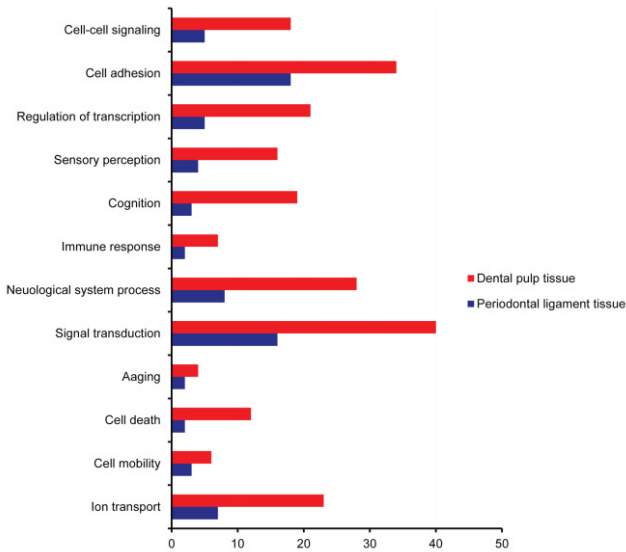


Fig. 1. Main categories of genes expressed in the PDL and dental pulp of human permanent teeth relative to their biologic processes (F -statistic: $p < 0.05$).

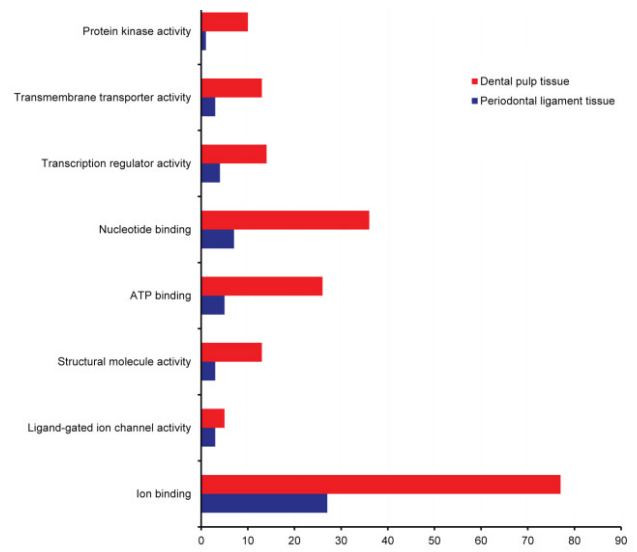


Fig. 2. Main categories of genes expressed in the PDL and dental pulp of human permanent teeth relative to their molecular processes (F -statistic: $p < 0.05$).

Table 3. The relative difference in gene mRNA expression in periodontal ligament tissue and dental pulp

Gene	Relative Gene Expression (Mean \pm SD)	
	Periodontal Ligament Tissues	Dental Pulp
<i>COL12</i>	44 \pm 6	1
<i>MMP9</i>	18 \pm 8	1
<i>SPP1</i>	9 \pm 2	1
<i>CALB1</i>	1	4,600 \pm 270
<i>SPOCK3</i>	1	6,800 \pm 270
<i>DSPP</i>	1	15,000 \pm 2,500

4. IHC staining

The following four proteins were the targets of the IHC study: *COL12A1*, *MMP9*, *SPP1*, and *CALB1* (Fig. 3). *COL12A1* was stained strongly in PDL tissues but was not stained in pulp tissues, while *MMP9* and *SPP1* were stained more strongly in all PDL tissues than in all pulp tissues, and staining was found only in the odontoblast layer of the pulp tissues. *CALB1* was located mainly in the odontoblast layer of the pulp tissue.

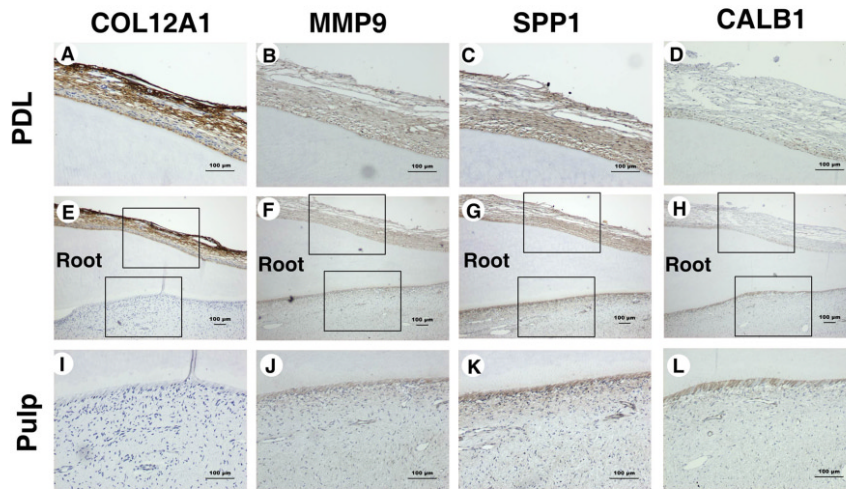


Fig. 3. Immunohistochemical staining of PDL and pulp samples (A-L). IHC staining for *COL12A1* in (A,E) PDL and (E,I) pulp; for *MMP9* in (B,F) PDL and (F,J) pulp; for *SPP1* in (C,G) PDL and (G,K) pulp; and for *CALB1* in (D,H) PDL and (H,L) pulp. The micrographs in A-D and I-L are higher-magnification views of the areas outlined by the upper and lower squares, respectively, in E-H. Scale bars: all 100 μ m.

IV. Discussion

Among the 28,869 genes that were analyzed using cDNA microarrays, 347 (1.20%) were expressed differentially by a factor of fourfold or more between PDL and pulp. According to the cDNA microarray and GO analyses, the genes that were up-regulated in the two tissue types were intimately related to their respective functions. PDL exhibited strong expression of genes related to collagen synthesis (*FAP*), collagen degradation (*MMP3*, *MMP9*, and *MMP13*), and bone development and remodeling (*SSP1*, *BMP3*, *ACP5*, *CTSK*, and *PTH1H*). In contrast, pulp exhibited strong expression of genes associated with calcium ions (*CALB1*, *SCIN*, and *CDH12*) and the mineralization and formation of enamel and dentin (*SPARC/SPOCK3*, *PHEX*, *AMBN*, and *DSPP*).

FAP alpha plays a major role in the production and turnover of extracellular matrix components, a process that is critical for wound healing and tissue remodeling. Research into *FAP* in dentistry is rare because of its lack of expression in normal tissues and induced expression in areas of tissue remodeling and tumor stroma¹⁹. *MMP3*, *MMP9*, and *MMP13* are members of the *MMP* family, which comprise proteolytic enzymes that mediate the degradation of extracellular matrix macromolecules and contribute not only to extracellular matrix homeostasis, but also to pathologic or therapeutic situations^{20,21}.

SSP1, widely known as osteopontin, has been implicated as an important factor in bone remodeling²². *BMP3* has been extensively investigated with regard to bone formation, and reportedly down-regulates bone mineralization and density, and acts as an antagonist of osteogenic *BMP*^{23,24}. *ACP5*, also known as tartrate-resistant acid phosphatase, is a glycosylated monomeric metalloenzyme that is expressed in mammals²⁵. *CTSK* is expressed predominantly in osteoclasts; high levels of *CTSK* are detected in patients with bone-destructive disease. *PTH1H* is a member of the parathyroid hormone family that represses bone sialoprotein, osteocalcin, and mineralization in PDL cells and, potentially, in cementoblasts²⁶.

In the dental pulp tissues, *CALB1* is an intracellular, soluble, vitamin-D-dependent calcium-binding protein and a member of the troponin C superfamily²⁷. *SCIN* is an actin-severing protein and that is found in abundance in secretory tissues. Very little research into the gene

that encodes this protein (*SCIN*) has been conducted in the field of dentistry, and work is needed to determine the function of *SCIN* in dental pulp function. *CDH12* mediates calcium-dependent cell-cell adhesion. This particular cadherin appears during a critical period of neuronal development, and perhaps specifically during synaptogenesis²⁸. It is thought that *CDH12* up-regulation is related to the nerve innervation of dental pulp.

As expected, genes related to the mineralization and formation of enamel or dentin were strongly expressed in pulp. *SPARC/SPOCK3* is a phosphorylated glycoprotein that is associated with development, tissue remodeling, and repair²⁹. Phosphate-regulating endopeptidase homolog, X-linked (encoded by *PHEX*) is expressed by human odontoblasts aligned at the margin of the dental pulp but not in the dental pulp cells, and its expression is up-regulated during odontoblast development³⁰.

AMBN is found in tooth enamel, and is produced by ameloblasts during the early secretory to late maturation stages of amelogenesis. However, it has recently been shown that reparative dentin formation is associated with the sequential expression of dentin-specific factors in wounded porcine pulp³¹. *DSPP* is a human gene that encodes two principal proteins of the dentin extracellular matrix of the tooth.

The qPCR findings were indeed consistent with those of the cDNA microarray analysis and the IHC findings supported the cDNA microarray data and revealed the locations of the expressed genes. These data also support previous research that has shown that *MMP9* may play an important role in the pathogenesis of pulpal inflammation³², and that the *SSP1* synthesized by odontoblasts is associated with the initial sites of calcification within mantle dentin³³.

Most microarray studies were about comparing the gene expression of bone marrow stem cells and stem cells derived from dental tissues. Another microarray researches were about confirming the gene expression patterns of cells under certain conditions. Although there were studies about comparative gene expression analysis of the PDL tissues in deciduous and permanent teeth and of the PDL and dental follicle tissues in humans, there is no study about comparing the relative gene expression of PDL and dental pulp tissues in humans. So gene expression analysis might help to explain the functional difference between PDL and dental pulp.

In conclusion, comparison of the PDL and the dental pulp tissues revealed genetic differences of actively for-

mationing gene. The gene-expression profiles presented here identify candidate genes that may distinguish specific functions of PDL and dental pulp. The critical point of this research is that RNA obtained from fresh functioning dental pulp and PDL tissues of permanent teeth, showed different specific genetic activities. And in tissue regenerative therapy, it might help to find the key regenerative factors in PDL and pulp regeneration if further studies are performed about genes with different expression pattern in two types of tissues.

V. Conclusion

This study was conducted to identify the gene-expression profiles and their molecular biological differences of PDL and dental pulp tissues from the human permanent teeth using cDNA microarray analysis, qPCR, and immunohistochemical stain. Genes associated with collagen degradation such as MMP3, MMP9, and MMP13 and with collagen synthesis such as FAP, and with bone development and remodeling such as SPP1, BMP3, ACP5, CTSK, and PTHLH were more strongly expressed in PDL tissues of permanent teeth. They are clinically related to PDL's functions of external force absorption and tooth supporting. In dental pulp tissues of permanent teeth, genes associated with calcium ion such as CALB1, CDH12, and SCIN and with mineralization and formation of enamel or dentin such as SPOCK3, PHEX, AMBN, and DSPP were more strongly expressed. They are clinically related to dental pulp's functions of secondary and tertiary dentin formation. The qPCR analysis and immunohistochemical staining analysis was also coincided with the cDNA microarray assay data.

References

1. Demarco FF, Conde MC, Cavalcanti BN, *et al.* : Dental pulp tissue engineering. *Braz Dent J*, 22:3-13, 2011.
2. Caton J, Bostanci N, Remboutsika E, *et al.* : Future dentistry: cell therapy meets tooth and periodontal repair and regeneration. *J Cell Mol Med*, 15:1054-1065, 2011.
3. Danesh-Meyer MJ : Tissue engineering in periodontics and implantology using rhBMP-2. *Ann R Australas Coll Dent Surg*, 15:144-149, 2000.
4. Song JS, Hwang DH, Kim SO, *et al.* : Comparative gene expression analysis of the human periodontal ligament in deciduous and permanent teeth. *PLoS One*, 8:e61231, 2013.
5. Xie YF, Shu R, Jiang SY, *et al.* : Comparison of microRNA profiles of human periodontal diseased and healthy gingival tissues. *Int J Oral Sci*, 3:125-134, 2011.
6. Lee YH, Na HS, Jeong SY, *et al.* : Comparison of inflammatory microRNA expression in healthy and periodontitis tissues. *Biocell*, 35:43-49, 2011.
7. Han X, Amar S : Identification of genes differentially expressed in cultured human periodontal ligament fibroblasts vs. human gingival fibroblasts by DNA microarray analysis. *J Dent Res*, 81:399-405, 2002.
8. de Araujo RM, Oba Y, Moriyama K : Identification of genes related to mechanical stress in human periodontal ligament cells using microarray analysis. *J Periodontal Res*, 42:15-22, 2007.
9. Paakkonen V, Ohlmeier S, Bergmann U, *et al.* : Analysis of gene and protein expression in healthy and carious tooth pulp with cDNA microarray and two-dimensional gel electrophoresis. *Eur J Oral Sci*, 113:369-379, 2005.
10. McLachlan JL, Smith AJ, Bujalska IJ, Cooper PR : Gene expression profiling of pulpal tissue reveals the molecular complexity of dental caries. *Biochim Biophys Acta*, 1741:271-281, 2005.
11. Paakkonen V, Vuoristo JT, Salo T, Tjaderhane L : Comparative gene expression profile analysis between native human odontoblasts and pulp tissue. *Int Endod J*, 41:117-127, 2008.
12. Heikinheimo K, Jee KJ, Niini T, *et al.* : Gene expression profiling of ameloblastoma and human tooth germ by means of a cDNA microarray. *J Dent Res*, 81:525-530, 2002.
13. Lee HS, Lee J, Kim SO, *et al.* : Comparative gene-expression analysis of the dental follicle and periodontal ligament in humans. *PLoS One*, 8:e84201, 2013.
14. Gottlow J, Nyman S, Karring T, Lindhe J : New attachment formation as the result of controlled tissue regeneration. *J Clin Periodontol*, 11:494-503, 1984.
15. Mitchell DL, West JD : Attempted orthodontic movement in the presence of suspected ankylosis. *Am J Orthod*, 68:404-411, 1975.
16. Lekic P, McCulloch CA : Periodontal ligament cell population: the central role of fibroblasts in creating a unique tissue. *Anat Rec*, 245:327-341, 1996.

17. Abrahao IJ, Martins MD, Katayama E, *et al.* : Collagen analysis in human tooth germ papillae. *Braz Dent J*, 17:208-212, 2006.
18. Gronthos S, Mankani M, Brahimi J, *et al.* : Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci U S A*, 97:13625-13630, 2000.
19. Shi M, Yu DH, Chen Y, *et al.* : Expression of fibroblast activation protein in human pancreatic adenocarcinoma and its clinicopathological significance. *World J Gastroenterol*, 18:840-846, 2012.
20. Boushell LW, Kaku M, Mochida Y, Yamauchi M : Distribution and relative activity of matrix metalloproteinase-2 in human coronal dentin. *Int J Oral Sci*, 3:192-199, 2011.
21. Hakki SS, Hakki EE, Nohutcu RM : Regulation of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases by basic fibroblast growth factor and dexamethasone in periodontal ligament cells. *J Periodontal Res*, 44:794-802, 2009.
22. Choi ST, Kim JH, Kang EJ, *et al.* : Osteopontin might be involved in bone remodelling rather than in inflammation in ankylosing spondylitis. *Rheumatology (Oxford)*, 47:1775-1779, 2008.
23. Daluiski A, Engstrand T, Bahamonde ME, *et al.* : Bone morphogenetic protein-3 is a negative regulator of bone density. *Nat Genet*, 27:84-88, 2001.
24. Choi S, Cho TJ, Kwon SK, *et al.* : 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*, 5:7-13, 2013.
25. Baumbach GA, Saunders PT, Ketcham CM, *et al.* : Uteroferrin contains complex and high mannose-type oligosaccharides when synthesized in vitro. *Mol Cell Biochem*, 105:107-117, 1991.
26. Ouyang H, McCauley LK, Berry JE, *et al.* : Parathyroid hormone-related protein regulates extracellular matrix gene expression in cementoblasts and inhibits cementoblast-mediated mineralization in vitro. *J Bone Miner Res*, 15:2140-2153, 2000.
27. Wasserman RH, Taylor AN : Vitamin d3-induced calcium-binding protein in chick intestinal mucosa. *Science*, 152:791-793, 1966.
28. Selig S, Bruno S, Scharf JM, *et al.* : Expressed cadherin pseudogenes are localized to the critical region of the spinal muscular atrophy gene. *Proc Natl Acad Sci U S A*, 92:3702-3706, 1995.
29. Yan Q, Sage EH : SPARC, a matricellular glycoprotein with important biological functions. *J Histochem Cytochem*, 47:1495-1506, 1999.
30. Buchaille R, Couble ML, Magloire H, Bleicher F : A subtractive PCR-based cDNA library from human odontoblast cells: identification of novel genes expressed in tooth forming cells. *Matrix Biol*, 19:421-430, 2000.
31. Nakamura Y, Hammarstrom L, Matsumoto K, Lyngstadaas SP : The induction of reparative dentine by enamel proteins. *Int Endod J*, 35:407-417, 2002.
32. Gusman H, Santana RB, Zehnder M : Matrix metalloproteinase levels and gelatinolytic activity in clinically healthy and inflamed human dental pulps. *Eur J Oral Sci*, 110:353-357, 2002.
33. Trowbridge HO : Pulp biology: progress during the past 25 years. *Aust Endod J*, 29:5-12, 2003.

국문초록

사람 영구치에서 치주인대 및 치수 조직의 유전자 발현에 대한 비교 연구

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이전 연구에서 사람의 치수와 치주인대의 기능에 대한 구체적인 3만 2천여개의 인체 유전자의 RNA 활성 정보는 없었다. 본 연구의 목적은 사람 영구치에서 얻은 치주인대와 치수 조직 내의 RNA 유전자 발현을 보고하고 각각의 분자생물학적인 차이를 알아보는 것이다. cDNA 미세배열분석에서 두 조직 사이의 유전자 발현 수준에서 4배 이상 차이 나는 유전자는 347개로 밝혀졌으며, 치주인대와 치수에서 각각 83개, 264개의 유전자 발현이 4배 이상 차이 난다는 것을 보여주었다. 치주인대는 교원질 합성 (*FAP*), 교원질 분해 (*MMP3*, *MMP9*와 *MMP13*), 골 형성 및 개조 (*SPP1*, *BMP3*, *ACP5*, *CTSK*와 *PTH1H*)와 관련된 유전자가 강하게 발현되었다. 반면 치수조직은 칼슘 이온 (*CALB1*, *SCIN*와 *CDH12*)과 법랑질 또는 상아질의 광화 및 형성 (*SPARC/SPOCK3*, *PHEX*, *AMBN*과 *DSPP*)와 관련한 유전자의 발현이 높게 나타났다. 이들 유전자 중 *SPP1*, *SPARC/SPOCK3*, *AMBN*, *DSPP* 등의 유전자는 치아의 기능과 관련해서 잘 알려져 있지만, 다른 유전자들은 microarray 분석을 통해서 새롭게 발견된 유전자이다. 이 유전자들은 추가적인 연구가 수행된다면 재생 치료의 좋은 요인을 찾는 데 도움이 될 것으로 생각된다.

주요어: 유전자 발현, 치주인대, 치수, 사람 영구치