Comparative gene expression analysis of the coronal pulp and apical pulp complex in human immature teeth

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Comparative gene expression analysis of the coronal pulp and apical pulp complex in human immature teeth

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감사의 글

레지던트 1년차 2학기 때 박사과정을 시작하여 어느새 수련을 마치고 1년이 지난 지금 드디어 학위를 마치게 되었습니다. 박사학위를 받기까지 정말 많은 분들께서 도와 주셔서 오늘의 학위 이수가 가능했습니다.

먼저 저의 지도교수님인 송제선 교수님께 감사의 말씀을 전하고 싶습니다. 처음 교수님의 지도학생으로 들어와 다양한 연구와 논문에 참여하면서 많은 경험을 쌓을 수 있었고 과정 중에 앎에 대한 강한 욕구와 연구에 대한 열정을 불러일으켜 주심에 조기에 학위을 마칠 수 있었습니다. 레지던트 수련시절부터 따뜻한 격려와 지도를 해주셨던 손흥규 교수님, 환자의 마음을 어루만지시는 참된 치과의사를 보여주셨던 최병재 교수님, 수련의의 마음을 세세하게 들여다봐주시며 함께 해주셨던 최형준 교수님, 환자를 보면서 가장 많이 의지했던 이제호 교수님, 논문에서 처음 접했던 microarray에 대한 개념에 대한 가르침을 주신 김성오 교수님, 좋은 싹이 보인다고 항상 격려해주셨던 이효설 교수님, 힘든 순간마다 큰 도움을 주셨던 김승혜 교수님, 동아리 선후배로 만나 함께 나눈 시간만큼이나 소중한 격려와 조언을 해주신 강정민 선생님 모두 감사드립니다. 또한 박사 논문이 나올 수 있도록 관심 있게 지켜 봐주신 마연주 교수님께도 감사를 드립니다. 아울러 소아치과학교실의 연구원으로 계신 전미정 박사님, 많은 도움을 주시고 조언해 주심에 정말 감사드립니다.

마지막으로 저희 가족에게 감사의 마음을 전합니다. 아버지, 어머니, 항상 제 편에서 생각해 주시며 언제나 든든한 후원자로서 물심양면 지원해주셔서 감사합니다. 준비된 자는 기회를 얻을 수 있으며 항상 긍정적으로 생각하라는 삶의 가르침을 주시어 인내하며 오늘의 자리에 올 수 있었습니다. 남편 태웅씨, 당신 덕분에 살면서 힘든 고비를 쓰러지지 않고 넘을 수 있었습니다. 사랑하고 사랑합니다. 딸 서원아, 네가 있기에 오늘의 학위가 더욱 더 감격스럽고 영광스럽단다. 배려와 사랑으로 저를 지켜봐주시는 시부모님, 진웅도련님, 경하아가씨, 동생 한성이 감사합니다.

많은 분들의 도움과 사랑으로 학위를 받게 되어 감사합니다.

2016년 12월

김수현 드림

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Abstract

Comparative gene expression analysis of the coronal pulp and apical pulp complex in human immature teeth

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This study determined the gene expression profiles of the human coronal pulp (CP) and the apical pulp complex (APC) with the aim of explaining differences in their functions.

Total RNA was isolated from the CP and the APC, and gene expression was analyzed using complementary DNA microarray technology. Gene ontology analysis was used to classify the biological function. Quantitative reverse-transcription polymerase chain reaction and immunohistochemical staining were performed to verify microarray data. In the microarray analysis, expression increases of at least 2-fold were present in 125 genes in the APC and 139 genes in the CP out of a total of 33,297 genes. Gene ontology class

processes found more genes related to immune responses, cell growth and maintenance, and cell adhesion in the APC, whereas transport and neurogenesis genes predominated in the CP. Quantitative reverse-transcription polymerase chain reaction and immuno-histochemical staining confirmed the microarray results, with *DMP1*, *CALB1*, and *GABRB1* strongly expressed in the CP, whereas *SMOC2*, *SHH*, *BARX1*, *CX3CR1*, *SPP1*, *COL XII*, and *LAMC2* were strongly expressed in the APC. The expression levels of genes related to dentin mineralization, neurogenesis, and neurotransmission were higher in the CP in human immature teeth, whereas those of immune-related and tooth development-related genes were higher in the APC.

Keywords: apical pulp complex, complementary DNA microarray, coronal pulp, human immature teeth, immunohistochemical staining

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I. Introduction

The dental pulp, which is originated from dental papilla, is an unmineralized oral tissue composed of soft connective tissue, vascular, lymphatic and nervous elements that occupy the central pulp cavity of dental apparatus. In immature teeth, the dental pulp is composed of coronal pulp (CP) and apical pulp (AP). The AP is located at the apex of developing human teeth and smooth-surfaced soft tissue was easily detached from the apex exposing dental pulp tissue in the canal space. The AP originally comes from dental

papilla of dental organ, which is early tooth bud state, and there is an apical cell-rich zone lying between the CP and the apical papilla. The apical papilla appears to contain less blood vessels and extracellular matrix relative to the CP and the apical cell-rich zone (Sonoyama et al., 2008).

After crown formation, root development begins via the interaction between Hertwig's root sheath (HERS) and the dental papilla, Which differentiates into odontoblasts and forms dentin and pulp. HERS is associated with the number of roots and their morphology (Huang and Chai, 2012). The stem cells from the apical papilla appear to be the source of odontoblasts that are responsible for the formation of root dentin. Conserving these stem cells when treating immature teeth may allow for the continuous formation of the root to completion (Huang et al., 2008); Otherwise, cells in covering the follicular tissue can differentiate into cementoblasts that are induced by the stimulation of root dentin (Srinivasan et al., 2015). The AP, HERS, and covering follicular tissues are all essential for root development. Despite the heterogeneity of this region, it exists as a single entity in which the interaction and functions of these components are essential for the establishment of a structurally intact root-periodontal complex (Xu et al., 2009); we call this structure the apical pulp complex (APC) (Figure 1A-E).

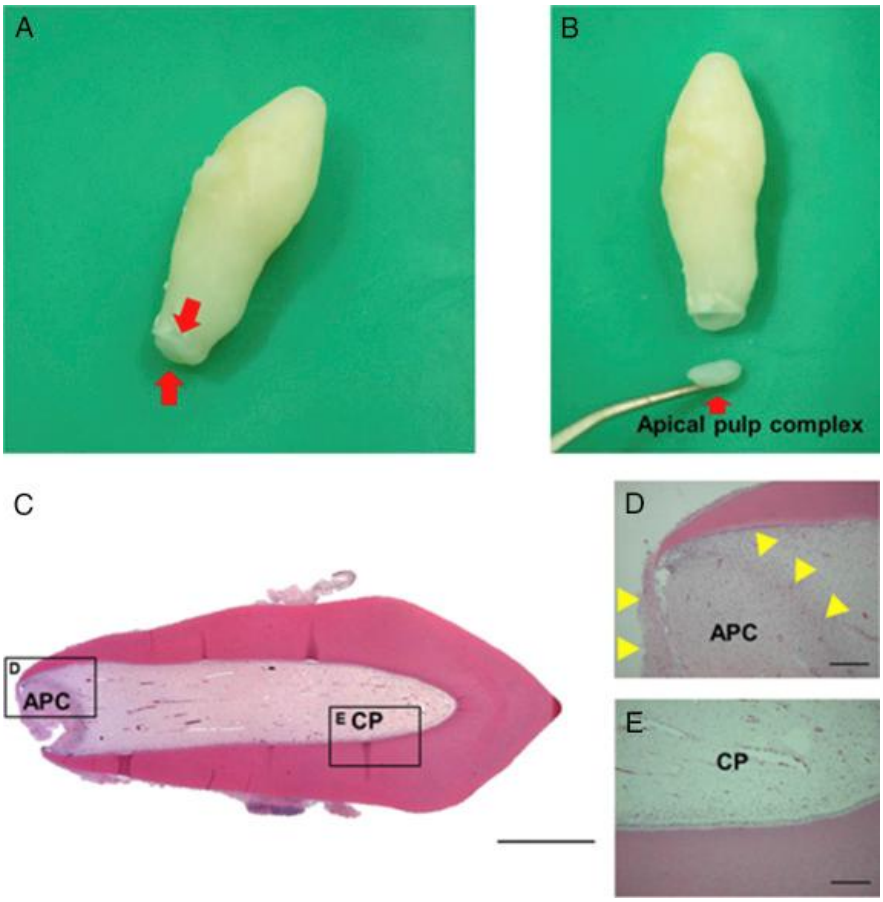

Figure 1. Anatomy of the apical pulp complex and coronal pulp. (A) An extracted human supernumerary tooth with an immature root with the APC (arrows), (B) the APC removed from the apex (on the explorer), (C) hematoxylin-eosin staining of a supernumerary tooth with an immature root apex, (D) a magnified view of the area indicated by the rectangle at the APC (arrowheads), and (E) a magnified view of the area indicated by the rectangle at the CP. (Scale bars: (C) 2mm and (D and E) 100μm).

The CP contains more differentiated cells than the AP with mature odontoblast, and their cellular processes extending into dentinal tubules are the first to encounter the caries bacterial antigens (Hahn and Liewehr, 2007). Many genes characterizing mature odontoblasts have been identified, with transforming growth factor beta, which is important in dentinogenesis, mineralization, and proinflmmation, recruiting immune cells such as dendrite cells (Byers, 1991; Rodd and Boissonade, 2001). Nestin, which produces the hard tissue matrix of dentin and repairs carious and injury teeth, was expressed more strongly in mature odontoblasts of the crown cusp region (About et al., 2000). Dentin sialophosphoprotein, which is expressed by matured odontoblasts, is important in dentinogenesis and is a specific marker for odontoblastic differentiation (Zhang et al., 2001).

Several recent studies of pulp biology have used complementary DNA (cDNA) microarray technology to compare the gene expression profiles in different subjects including comparing pulp tissue between carious and sound teeth (McLachlan et al., 2005), pulp tissue, and odontoblast to determine the characteristics of odontoblasts among pulp tissues (Pääkkönen et al., 2008) and evaluating age-related changes in human dental pulp tissue (Tranasi et al., 2009). This technology is a useful method for screening new genes, which contrasts with only fragmented information being used in the past. Although the CP and APC are complex and have different cellular composition, such investigations can provide useful insights into APC functions in root development and biological process of the pulp tissue maturation.

Previous studies have used animal models to investigate root development and the differentiation of pulp tissue. In contrast, this study compared the gene expression profiles of the human CP and APC in immature teeth, with the aim of elucidating whether any of the differences found can explained by differences in their functions.

II. Materials and Methods

1. Preparation of Pulp Samples and RNA Isolation

The experimental protocol was approved by the Institutional Review Board of the Yonsei University Dental Hospital, and informed consent was obtained from all children enrolled in our study and their parents (approval no. 2-2013-0007). The CP and APC tissues were obtained from healthy immature premolar or supernumerary teeth or third molars having an immature root apex (APC, n=18 from 13 males and 5 females, aged 4-20 years; CP, n=11 from 8 males and 3 females, aged 4–20 years). The extracted teeth were washed in saline and then immediately frozen and stored in liquid nitrogen.

After thawing, apical pulp complex tissues were isolated and crushed with a bolt cutter, and the pulp tissues were carefully obtained using sterile tweezers. The tissues were homogenized using a Bullet Blender Bead (Next Advance, NY, USA). Total RNA was purified using the RNeasy Fibrous Mini kit (Qiagen, CA, USA) in accordance with the manufacturer's instructions. RNA quality was assessed using the Agilent 2100 bioanalyzer using the RNA 6000 Nano Chip (Agilent Technologies, Amstelveen, The Netherlands), and its quantity was determined using a NanoDrop ND-2000 device (Thermo Scientific, IL, USA). The RNA samples used in this study had 260/280 nm ratios of at least 1.8.

2. cDNA Microarray and Data Analysis

Global gene-expression analysis was performed using Affymetrix GeneChip Human Gene 1.0 ST oligonucleotide arrays (Affymetrix, CA, USA) following the instructions and recommendations provided by the manufacturer. The Affymetrix procedure followed the manufacturer's protocol (http://www.affymetrix.com). Briefly, 300 ng of total RNA from each sample was converted into double-strand cDNA. Using random hexamers with a T7 promoter, amplified RNA (cRNA) was generated from the double-stranded cDNA template though an in-vitro transcription reaction and purified with the Affymetrix sample cleanup module. cDNA was regenerated through a random-primed reverse transcriptase using a dNTP mix containing dUTP. The cDNA was then fragmented by uracil-DNA glycosylase and apurinic/apyrimidinic endonuclease 1 restriction endonucleases, and end-labeled by a terminal transferase reaction incorporating a biotinylated dideoxynucleotide. 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 scanned using a GeneChip Array scanner 3000 G7 (Affymetrix), and the image data were extracted using Affymetrix Command Console software (version 1.1, Affymetrix). The raw file generated by this procedure yielded expression intensity data that were used in the next processing step. And a Web-based tool, the Database for Annotation, Visualization, and Integrated

Discovery (DAVID), was used to assess the biological interpretation of differentially expressed genes. These genes were then classified based on the gene function in the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway database (http://david.abcc.ncifcrf.gov/home.jsp). All experiments were performed at least in triplicate. The normality of the data was evaluated using the Shapiro-Wilk test ($p<0.05$). The one-way ANOVA ($p<0.05$) was used for c-DNA microarray analysis

3. Quantitative RT-PCR

The quantitative RT-PCR was performed using modified method from the previous studies (Kim et al., 2014; Lee et al., 2013; Song et al., 2013). Diluted cDNA was used as a template for quantitative RT-PCR (qPCR), which was performed using the ABI 7300 Real-Time PCR system (Applied Biosystems, Warrington, UK). Total RNA used in the microarray analysis was also used to synthesize cDNA with Superscript III reverse transcriptase and random primer (Invitrogen, Warrington, UK). Total RNA (250 ng) was used as a template for the RT reaction, which was performed at 65ºC for 5 minutes, and then the sample was incubated at 25ºC for 5 minutes, 50ºC for 1 hour, and 70ºC for 15 minutes to inactivate the activity of the reverse transcriptase, and the synthesized cDNA was diluted 1:5 in distilled water. Reaction volumes of 25 μl containing 1× Universal TaqMan Master Mix (4369016, Applied Biosystems), PCR primers at a concentration of 0.9 μM, and the diluted cDNA were prepared in triplicate. The amplification conditions were 50ºC for 2 minutes and 95ºC for 10 minutes, followed by 40 cycles of 95ºC for 15 seconds and 60ºC for 1 minute. The specific TaqMan gene-expression assay primers (Applied Biosystems) are listed in Table 1. The values for each gene were normalized to the expression levels of the gene encoding 18S rRNA, and the relative expression levels of the studied genes were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). All these qPCR procedures were done obtaining triplicated data. The t-test ($p<0.05$) was performed for qPCR using SPSS software (19.0 SPSS, IL, USA).

Table 1. TaqMan gene-expression assay primers.

Gene symbol	Primer Assay ID	Amplicon length (bp)
AMTN	Hs00418384_m1	62
CALB1	Hs00191821_m1	90
DMP1	Hs01009391_g1	106
LAMC2	Hs01043711_m1	79
LGR5	Hs00173664_m1	112
MMP13	Hs00233992_m1	91
ODAM	Hs00215292_m1	81
PHEX	Hs01011692_m1	118
SPOCK3	Hs01553242_m1	74
SPP1	Hs00959010_m1	84
18S rRNA	Hs03003631_g1	69

4. Immunohistochemical Staining

Selected genes, *BARX1*, *CALB1*, *COL XII*, *CX3CR1*, *DMP1*, *GABRB1*, *LAMC2*, *SPP1*, *SHH*, and *SMOC2* were stained in human immature teeth. The information of primary antibodies was given in Table 2. For immunohistochemical staining, immature teeth were fixed in 10% buffered formalin for 1 day, decalcified with 10% EDTA (pH 7.4; Fisher Scientific, TX, USA) for 8 weeks, embedded in paraffin, and then sectioned at a thickness of 3 μm. The sections were deparaffinized in xylene, rehydrated, and rinsed with distilled water. Protease K (Dako, CA, USA) was used to retrieve the antigen for the *SMOC2*, *SHH*, and *CX3CR1* staining, while no such treatment was performed for the other staining protocols. The sections were first immersed in 3% hydrogen peroxide for 10 minutes to inactivate endogenous peroxidase activity and were then incubated with the primary antibody overnight. *COL XII* staining was performed using antibodies obtained from Santa Cruz Biotechnology (CA, USA), while the other staining protocols were performed using antibodies from Abcam (Cambridge, UK). After incubation, EnVision+System-HRP Labeled Polymer Anti-rabbit antibody (ready to use; K4003, Dako) was applied for 20 minutes, or Vectastain Elite ABC Kit (goat IgG, diluted 1:200; PK-6105, Vector Laboratories, CA, USA) was applied for 30 minutes. Color development was achieved using 3,3'-diaminobenzidine substrate (Dako) and counterstainung with Gill's hematoxylin solution (Merck, Darmstadt, Germany). Negative control sections were treated in the same manner but without applying primary antibodies.

Table 2. Primary antibodies for immunohistochemistry.

Antibodies	Catalog number	Host species	Dilution factor
BARX1	Ab26156	Rabbit	1:2000
CALB1	Ab25085	Rabbit	1:400
COLXII	Sc-68862	Rabbit	1:800
CX3CR1	Ab8020	Rabbit	1:500
DMP1	Ab82351	Rabbit	1:100
GABRB1	Ab51123	Rabbit	1:50
LAMC2	Ab85578	Goat	1:800
SPP1	Ab8448	Rabbit	1:800
SHH	Ab53281	Rabbit	1:25
SMOC2	Ab78069	Rabbit	1:100

III. Results

1. Gene-expression Profiles of the Coronal Pulp and Apical Pulp Complex in Human Immature Teeth

The results demonstrate that there was a twofold or greater difference in expression of 258 out of 33,297 genes between the apical pulp complex and coronal pulp from immature teeth; 124 and 134 genes were more strongly expressed in the apical pulp complex and coronal pulp, respectively. Table 3 and 4 list the genes that were expressed more strongly in these two tissue types, by greater than two folds.

Table 3. Most up-regulated genes in the coronal pulp of immature teeth as compared to apical pulp complex (absolute fold change >2.0).

Gene Description	Gene Symbol	Fold change	Gene Accession
dentin matrix acidic phosphoprotein 1	*DMP1*	9.23	NM_004407
leucine-rich repeat-containing G protein-coupled receptor 5	*LGR5*	5.83	NM_003667
adherens junctions associated protein 1	*AJAP1*	5.03	NM_018836
KIAA1199	*KIAA1199*	4.79	NM_018689
hyaluronoglucosaminidase 4	*HYAL4*	4.16	NM_012269
solute carrier family 38, member 11	*SLC38A11*	3.73	NM_173512
microRNA 95	*MIR95*	3.69	NR_029511
WD repeat domain 72	*WDR72*	3.60	NM_182758
sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3D	*SEMA3D*	3.49	NM_152754
v-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian)	*ERBB4*	3.34	NM_005235
WNT1 inducible signaling pathway protein 1	*WISP1*	3.26	NM_003882
anoctamin 1, calcium activated chloride channel	*ANO1*	3.22	NM_018043
EPH receptor A5	*EPHA5*	3.21	NM_004439
protocadherin 7	*PCDH7*	3.17	NM_032456
fin bud initiation factor homolog (zebrafish)	*FIBIN*	3.15	NM_203371
transmembrane protein 229A	*TMEM229A*	3.05	NM_001136002

solute carrier family 12 (sodium/potassium/ chloride transporters), member 2	*SLC12A2*	3.00	NM_001046
cadherin 4, type 1, R-cadherin (retinal)	*CDH4*	2.99	NM_001794
protein tyrosine phosphatase, receptor-type, Z polypeptide 1	*PTPRZ1*	2.99	NM_002851
calbindin 1, 28kDa	*CALB1*	2.98	NM_004929
transmembrane protein 156	*TMEM156*	2.95	NM_024943
chromosome 8 open reading frame 4	*C8orf4*	2.95	NM_020130
phospholipase C, delta 4	*PLCD4*	2.93	NM_032726
phosphate regulating endopeptidase homolog, X-linked	*PHEX*	2.93	NM_000444
DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	*DDX3Y*	2.91	NM_001122665
lipid phosphate phosphatase-related protein type 5	*LPPR5*	2.90	NM_001037317
CD36 molecule (thrombospondin receptor)	*CD36*	2.83	NM_001001548
sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 3	*SPOCK3*	2.82	NM_001040159
ADAM metallopeptidase with thrombospondin type 1 motif, 12	*ADAMTS12*	2.79	NM_030955
G protein-coupled receptor 155	*GPR155*	2.74	NM_001033045
myozenin 1	*MYOZ1*	2.72	NM_021245
GDNF family receptor alpha 1	*GFRA1*	2.70	NM_005264
eukaryotic translation initiation factor 1A, Y-linked	*EIF1AY*	2.70	NM_004681

actin binding LIM protein family, member 2	*ABLIM2*	2.70	NM_001130083
connector enhancer of kinase suppressor of Ras 2	*CNKSR2*	2.69	NM_014927
glutamate decarboxylase-like 1	*GADL1*	2.69	NM_207359
taxilin beta	*TXLNB*	2.69	NM_153235
UDP glycosyltransferase 3 family, polypeptide A2	*UGT3A2*	2.68	NM_174914
ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 1	*ST8SIA1*	2.66	NM_003034
cytoplasmic FMR1 interacting protein 2	*CYFIP2*	2.66	NM_001037332
integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)	*ITGA2*	2.63	NM_002203
family with sequence similarity 134, member B	*FAM134B*	2.62	NM_001034850
ceruloplasmin (ferroxidase)	*CP*	2.61	NM_000096
amine oxidase, copper containing 3 (vascular adhesion protein 1)	*AOC3*	2.60	NM_003734
ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	*UTY*	2.60	NM_007125
matrix metallopeptidase 20	*MMP20*	2.57	NM_004771
microtubule-associated protein tau	*MAPT*	2.56	NM_016835
membrane associated guanylate kinase, WW and PDZ domain containing 2	*MAGI2*	2.54	NM_012301
collagen, type XI, alpha 2	*COL11A2*	2.52	NM_001163771
cerebellin 2 precursor	*CBLN2*	2.52	NM_182511
protein tyrosine phosphatase, receptor type, K	*PTPRK*	2.51	NM_001135648

peptidyl arginine deiminase, type II	*PADI2*	2.50	NM_007365
gamma-aminobutyric acid (GABA) A receptor, beta 1	*GABRB1*	2.49	NM_000812
collagen, type XI, alpha 2	*COL11A2*	2.49	NM_080680
solute carrier family 4, sodium bicarbonate transporter, member 10	*SLC4A10*	2.47	NM_001178015
KIAA1324	*KIAA1324*	2.46	NM_020775
sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3E	*SEMA3E*	2.45	NM_012431
desmoplakin	*DSP*	2.45	NM_004415
syntabulin (syntaxin-interacting)	*SYBU*	2.42	NM_001099750
ermin, ERM-like protein	*ERMN*	2.41	NM_001009959
family with sequence similarity 107, member B	*FAM107B*	2.41	BC072452
early growth response 3	*EGR3*	2.37	NM_004430
solute carrier family 13 (sodium-dependent citrate transporter), member 5	*SLC13A5*	2.37	NM_177550
lysyl oxidase	*LOX*	2.37	NM_002317
met proto-oncogene (hepatocyte growth factor receptor)	*MET*	2.36	NM_001127500
deleted in azoospermia 2	*DAZ2*	2.36	NM_020363
deleted in azoospermia 1	*DAZ1*	2.36	NM_004081
amine oxidase, copper containing 2 (retina-specific)	*AOC2*	2.35	NM_009590

docking protein 6	*DOK6*	2.35	NM_152721
CD52 molecule	*CD52*	2.32	NM_001803
glycine receptor, beta	*GLRB*	2.31	NM_000824
cordon-bleu homolog (mouse)	*COBL*	2.30	NM_015198
ubiquitin specific peptidase 9, Y-linked	*USP9Y*	2.29	NM_004654
reelin	*RELN*	2.29	NM_005045
WD repeat domain 62	*WDR62*	2.29	NM_001083961
glycerophosphodiester phosphodiesterase domain containing 5	*GDPD5*	2.28	NM_030792
transforming growth factor, alpha	*TGFA*	2.27	NM_003236
potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4	*KCNN4*	2.27	NM_002250
glutamate receptor, ionotropic, AMPA 1	*GRIA1*	2.25	NM_000827
transmembrane protein 144	*TMEM144*	2.24	NM_018342
ATPase, Ca++ transporting, plasma membrane 1	*ATP2B1*	2.23	NM_001001323
G protein-coupled receptor 77	*GPR77*	2.21	NM_018485
limb bud and heart development homolog (mouse)	*LBH*	2.21	NM_030915
neuropeptide Y receptor Y1	*NPY1R*	2.20	NM_000909
death associated protein-like 1	*DAPL1*	2.19	NM_001017920
klotho	*KL*	2.18	NM_004795
scleraxis homolog A (mouse)	*SCXA*	2.17	NM_001008271
bone morphogenetic protein 8a	*BMP8A*	2.16	NM_181809
potassium channel, subfamily K, member 2	*KCNK2*	2.16	NM_001017425

KIAA1161	*KIAA1161*	2.16	NM_020702
synaptotagmin XVII	*SYT17*	2.15	NM_016524
zinc finger protein, Y-linked	*ZFY*	2.15	NM_003411
heat shock 22kDa protein 8	*HSPB8*	2.14	NM_014365
lysine (K)-specific demethylase 5D	*KDM5D*	2.14	NM_001146705
proprotein convertase subtilisin/kexin type 5	*PCSK5*	2.12	NM_001190482
zinc finger protein 42 homolog (mouse)	*ZFP42*	2.12	NM_174900
muscle, skeletal, receptor tyrosine kinase	*MUSK*	2.12	NM_005592
chromosome Y open reading frame 15A	*CYorf15A*	2.12	NM_001005852
neuropeptide Y receptor Y5	*NPY5R*	2.11	NM_006174
wingless-type MMTV integration site family, member 10A	*WNT10A*	2.11	NM_025216
interferon, kappa	*IFNK*	2.11	NM_020124
endoplasmic reticulum aminopeptidase 2	*ERAP2*	2.08	NM_022350
prokineticin 2	*PROK2*	2.07	NM_001126128
UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 6 (GalNAc-T6)	*GALNT6*	2.06	NM_007210
GRAM domain containing 1B	*GRAMD1B*	2.06	NM_020716
rhophilin, Rho GTPase binding protein 2	*RHPN2*	2.06	NM_033103
transmembrane protein 154	*TMEM154*	2.06	NM_152680
actin filament associated protein 1	*AFAP1*	2.04	NM_198595
regulator of calcineurin 1	*RCAN1*	2.03	NM_004414
doublecortin-like kinase 1	*DCLK1*	2.03	NM_004734

sorbin and SH3 domain containing 2	*SORBS2*	2.03	NM_021069
glutathione S-transferase mu 1	*GSTM1*	2.03	NM_000561
neuroligin 4, Y-linked	*NLGN4Y*	2.03	NR_028319
potassium voltage-gated channel, subfamily H (eag-related), member 5	*KCNH5*	2.03	NM_139318
isthmin 1 homolog (zebrafish)	*ISM1*	2.03	NM_080826
ADAM metallopeptidase with thrombospondin type 1 motif, 2	*ADAMTS2*	2.02	NM_014244
secretogranin II	*SCG2*	2.01	NM_003469
inositol 1,4,5-triphosphate receptor interacting protein-like 2	*ITPRIPL2*	2.01	NM_001034841
alpha-2-macroglobulin	*A2M*	2.01	NM_000014
guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1	*GNAI1*	2.01	NM_002069
tetraspanin 13	*TSPAN13*	2.01	NM_014399
tripartite motif-containing 36	*TRIM36*	2.01	NM_018700
ribosomal protein S4, Y-linked 1	*RPS4Y1*	2.01	NM_001008

Table 4. Most up-regulated genes in the apical pulp complex of immature teeth as compared to coronal pulp (absolute fold change >2.0).

Gene Description	Gene Symbol	Fold change	Gene Accession
matrix metallopeptidase 13 (collagenase 3)	*MMP13*	20.07	NM_002427
secreted frizzled-related protein 4	*SFRP4*	9.08	NM_003014
secreted phosphoprotein 1	*SPP1*	7.33	NM_001040058
secreted frizzled-related protein 1	*SFRP1*	7.09	NM_003012
odontogenic, ameloblast associated	*ODAM*	7.05	NM_017855
immunoglobulin heavy constant delta	*IGHD*	6.81	BC021276
amelogenin, X-linked	*AMELX*	6.74	NM_182680
immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides	*IGJ*	6.20	NM_144646
Amelotin	*AMTN*	6.08	NM_212557
collagen, type XII, alpha 1	*COL12A1*	5.96	NM_004370
immunoglobulin heavy constant mu	*IGHM*	5.88	BC020240
immunoglobulin kappa constant	*IGKC*	5.67	AF113887
hemoglobin, alpha 2	*HBA2*	5.61	NM_000517
hemoglobin, alpha 1	*HBA1*	5.61	NM_000558
Aspirin	*ASPN*	5.59	NM_017680
immunoglobulin lambda joining 3	*IGLJ3*	4.95	AB001736
integrin-binding sialoprotein	*IBSP*	4.76	NM_004967
hemoglobin, beta	*HBB*	4.33	NM_000518

RAB27B, member RAS oncogene family	*RAB27B*	4.15	NM_004163
immunoglobulin heavy constant alpha 1	*IGHA1*	4.14	AK128476
ST6 beta-galactosamide alpha-2,6-sialyltranferase 2	*ST6GAL2*	4.10	NM_032528
sushi-repeat-containing protein, X-linked	*SRPX*	4.10	NM_006307
Ovostatin	*OVOS*	4.04	BX647938
matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	*MMP9*	3.77	NM_004994
fatty acid binding protein 4, adipocyte	*FABP4*	3.77	NM_001442
Lysozyme	*LYZ*	3.64	NM_000239
dipeptidase 1 (renal)	*DPEP1*	3.60	NM_004413
cadherin 1, type 1, E-cadherin (epithelial)	*CDH1*	3.59	NM_004360
keratin 5	*KRT5*	3.51	NM_000424
chemokine (C-X-C motif) ligand 9	*CXCL9*	3.40	NM_002416
Ig kappa chain V-I region HK102-like	*LOC652493*	3.38	ENST00000493819
Fraser syndrome 1	*FRAS1*	3.31	NM_025074
immunoglobulin kappa locus	*IGK@*	3.30	BC032451
immunoglobulin heavy constant alpha 1	*IGHA1*	3.16	BC073771
prostaglandin E receptor 2 (subtype EP2), 53kDa	*PTGER2*	3.15	NM_000956
prostaglandin F receptor (FP)	*PTGFR*	3.12	NM_001039585
elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 3	*ELOVL3*	3.07	NM_152310

immunoglobulin superfamily, member 10	*IGSF10*	3.06	NM_178822
regulator of G-protein signaling 1	*RGS1*	3.05	NM_002922
cytochrome P450, family 26, subfamily A, polypeptide 1	*CYP26A1*	3.05	NM_000783
immunoglobulin kappa constant	*IGKC*	3.01	BC029444
Enamelin	*ENAM*	2.97	NM_031889
SPARC related modular calcium binding 2	*SMOC2*	2.94	NM_022138
protein tyrosine phosphatase, non-receptor type 22 (lymphoid)	*PTPN22*	2.91	NM_015967
insulin-like growth factor 1 (somatomedin C)	*IGF1*	2.90	NM_001111283
ectonucleoside triphosphate diphosphohydrolase 3	*ENTPD3*	2.89	NM_001248
eyes absent homolog 1 (Drosophila)	*EYA1*	2.84	NM_000503
laminin, gamma 2	*LAMC2*	2.80	NM_005562
carboxylesterase 1	*CES1*	2.79	NM_001025195
chromosome 20 open reading frame 103	*C20orf103*	2.79	NM_012261
glutamate receptor, ionotropic, kainate 1	*GRIK1*	2.77	NM_175611
immunoglobulin kappa constant	*IGKC*	2.77	BC073772
protease, serine, 12 (neurotrypsin, motopsin)	*PRSS12*	2.76	NM_003619
pannexin 3	*PANX3*	2.66	NM_052959
serpin peptidase inhibitor, clade B (ovalbumin), member 5	*SERPINB5*	2.65	NM_002639
thrombospondin 4	*THBS4*	2.62	NM_003248

granzyme K (granzyme 3; tryptase II)	*GZMK*	2.58	NM_002104
Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide	*FCER1A*	2.58	NM_002001
FRAS1 related extracellular matrix protein 2	*FREM2*	2.56	NM_207361
GRB2-binding adaptor protein, transmembrane	*GAPT*	2.50	NM_152687
basonuclin 2	*BNC2*	2.50	NM_017637
NEL-like 2 (chicken)	*NELL2*	2.48	NM_006159
retinol binding protein 4, plasma	*RBP4*	2.47	NM_006744
Acid phosphatase 5, tartrate resistant	*ACP5*	2.46	NM_001111035
actin, gamma 2, smooth muscle, enteric	*ACTG2*	2.45	NM_001615
ectonucleotide pyrophosphatase/phosphodiesterase 1	*ENPP1*	2.44	NM_006208
coagulation factor II (thrombin) receptor-like 2	*F2RL2*	2.43	NM_004101
melanocortin 2 receptor accessory protein 2	*MRAP2*	2.42	NM_138409
plexin C1	*PLXNC1*	2.42	NM_005761
cholinergic receptor, nicotinic, alpha 5	*CHRNA5*	2.40	NM_000745
keratin 14	*KRT14*	2.40	NM_000526
sonic hedgehog	*SHH*	2.35	NM_000193
signal peptide, CUB domain, EGF-like 2	*SCUBE2*	2.31	NM_020974
ectonucleotide pyrophosphatase/phosphodiesterase 6	*ENPP6*	2.30	NM_153343
EPH receptor B1	*EPHB1*	2.28	NM_004441
carboxypeptidase M	*CPM*	2.27	NM_001874

secreted frizzled-related protein 2	*SFRP2*	2.25	NM_003013
Lix1 homolog (chicken)	*LIX1*	2.25	NM_153234
Dermatopontin	*DPT*	2.23	NM_001937
ADAM metallopeptidase with thrombospondin type 1 motif, 15	*ADAMTS15*	2.23	NM_139055
prominin 1	*PROM1*	2.22	NM_006017
CD1c molecule	*CD1C*	2.22	NM_001765
mohawk homeobox	*MKX*	2.20	NM_173576
retinoblastoma binding protein 8	*RBBP8*	2.19	NM_002894
Kv channel interacting protein 4	*KCNIP4*	2.19	NM_147182
thymidylate synthetase	*TYMS*	2.18	NM_001071
retinoid X receptor, gamma	*RXRG*	2.18	NM_006917
endothelin 3	*EDN3*	2.18	NM_207032
tripartite motif-containing 29	*TRIM29*	2.17	NM_012101
synaptotagmin XIV	*SYT14*	2.15	NR_027458
Osteoglycin	*OGN*	2.14	NM_033014
immunoglobulin kappa constant	*IGKC*	2.14	BC073763
signal peptide, CUB domain, EGF-like 1	*SCUBE1*	2.14	NM_173050
BARX homeobox 1	*BARX1*	2.14	NM_021570
placenta-specific 8	*PLAC8*	2.13	NM_016619
synaptotagmin II	*SYT2*	2.11	NM_177402
annexin A3	*ANXA3*	2.11	NM_005139
leucine-rich repeat LGI family, member 2	*LGI2*	2.11	NM_018176

complement component 3	*C3*	2.11	NM_000064
Chondrolectin	*CHODL*	2.10	NM_024944
SH3-domain GRB2-like 2	*SH3GL2*	2.10	NT_003026
popeye domain containing 3	*POPDC3*	2.10	NM_022361
mucolipin 2	*MCOLN2*	2.10	NM_153259
protease, serine, 35	*PRSS35*	2.09	NM_001170423
integrin, alpha 11	*ITGA11*	2.09	NM_001004439
gremlin 2	*GREM2*	2.08	NM_022469
lymphoid-restricted membrane protein	*LRMP*	2.07	NM_006152
chemokine (C-X3-C motif) receptor 1	*CX3CR1*	2.05	NM_001337
G protein-coupled receptor 183	*GPR183*	2.05	NM_004951
collagen, type VI, alpha 3	*COL6A3*	2.05	NM_004369
matrix Gla protein	*MGP*	2.04	NM_001190839
collagen, type XIV, alpha 1	*COL14A1*	2.03	NM_021110
KIAA1024	*KIAA1024*	2.03	NM_015206
chromosome 4 open reading frame 7	*C4orf7*	2.01	NM_152997
chemokine (C-C motif) ligand 5	*CCL5*	2.01	NM_002985
PDZ domain containing ring finger 4	*PDZRN4*	2.01	NM_013377
actin, alpha 2, smooth muscle, aorta	*ACTA2*	2.01	NM_001141945
Synaptoporin	*SYNPR*	2.00	NM_144642

2. Gene Ontology Analysis

Gene Ontology (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 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 biological processes are shown in Figure 2. Those GO-class processes found more frequently in the APC including immune, cell growth and maintenance, and cell adhesion. Genes associated with the immune reaction such as *IGHD*, *IGHM*, *IGKC*, *IGHA1*, *LYZ*, *CXCL9*, *LOC652493*, *IGK@*, *CES1*, *GZMK*, *FCER1A*, *C3*, *CX3CR1*, *GPR183*, and *CCL5* were found. In contrast, the GO-class processes found more frequently in the coronal pulp including transport and neurogenesis. Cell communication and signal transduction classification in the Go-class of CP contains neurotransmission genes such as *ANO1*, *ST8SIA1*, *GLRB*, *GRIA1*, *NPY1R*, *KCNN4*, *KCNK2 and KCNK5*. Especially, there were notable biologic differences about immune in APC and neurogenesis in CP.

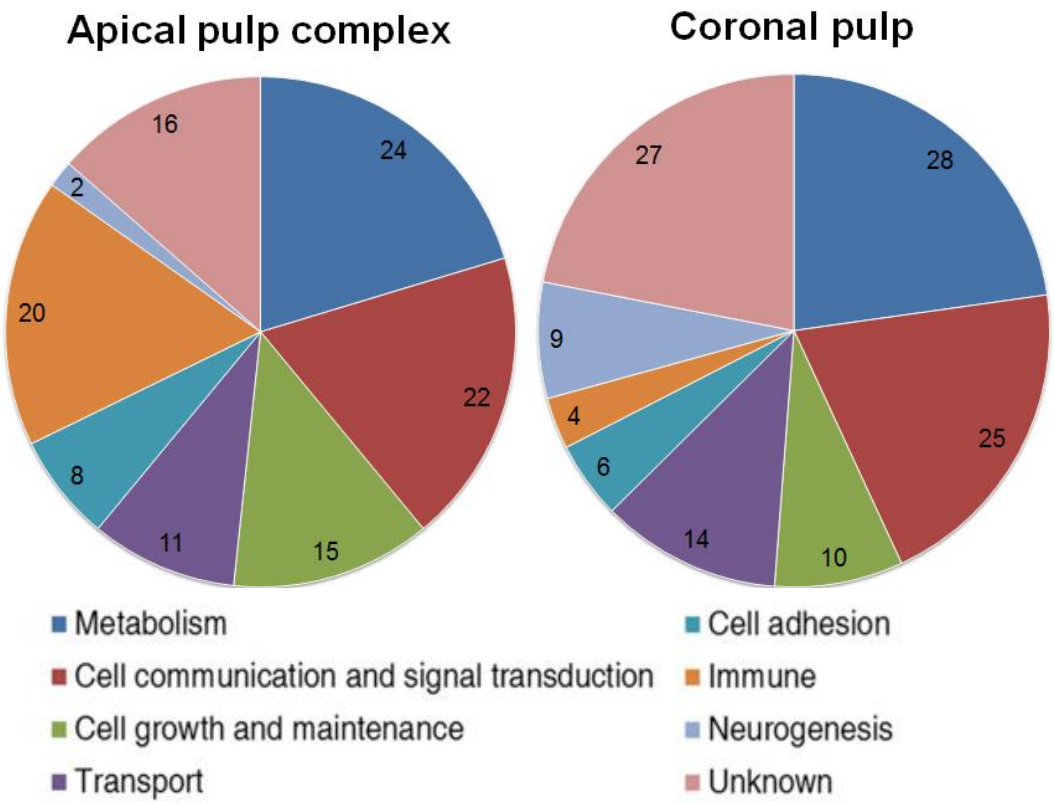

Figure 2. Main categories of genes and their expressed frequencies specifically in the apical pulp complex and the coronal pulp on the basis of their biological processes.

3. Quantitative RT-PCR

Quantitative RT-PCR analysis was performed to verify the differential expression levels determined via cDNA microarray analysis. The ten genes (i.e., *MMP13, SPP1, ODAM, AMTN, LAMC2, DMP1, LGR, CALB1, PHEX*, and *SPOCK3*) that were selected for this verification procedure exhibited an increase of at least fivefold in the gene expression level compared to the other tissue type (Figure 3). The results are consistent with those of the cDNA microarray analysis.

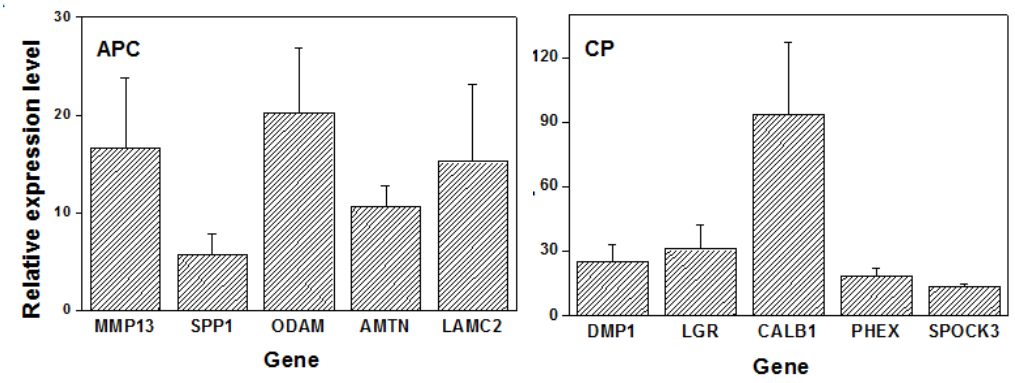

Figure 3. Relative gene-expressions in apical pulp complex and coronal pulp. (**APC**) Relative expression levels of the genes encoding matrix metallopeptidase 13 (*MMP13*); secreted phosphoprotein 1 (*SPP1*); Odontogenic, ameloblast-associated (*ODAM*), amelotin (*AMTN*); and laminin, gamma 2 (*LAMC2*) in APC tissues. The expression level of each gene was calculated relative to base level of 1 in coronal pulp tissues. (**CP**) Relative expression levels of the genes encoding Dentin matrix acidic phosphoprotein 1 (*DMP1*); leucine-rich repeat-containing G protein-coupled receptor 5 (*LGR5*); calbindin 1 (*CALB1*); Phosphate-regulating endopeptidase homolog, X-linked (*PHEX*), and Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 3 (*SPOCK3*) in CP tissues. The expression level of each gene was calculated relative to base level of 1 in APC tissues. (APC, n=18; CP, n=11.) The data are mean and standard deviation values.

4. Immunohistochemical Staining

DMP1, calbindin, and *GABA1* were strongly expressed in the predentin, odontoblast area, and the CP, but they were barely expressed in the APC. In particular, *DMP1* was strongly expressed in the coronal odontoblast layer and predentin (Figure 4). *SMOC2*, *BARX1*, *SHH*, and *CX3CR1* were strongly expressed in the apical odontoblast layer, HERS, and APC, but they were barely expressed in the CP (Figure 4BA-BT). *SPP1*, *COL12A1*, and *LAMC2* were highly expressed in APC, but little expressed in the CP. Especially, *SPP1* was expressed between apical pulp and covering follicular tissue. *COL12A1* and *LAMC2* were expressed around the border of the APC (Figure 4CA-CO). These findings were consistent with the microarray results.

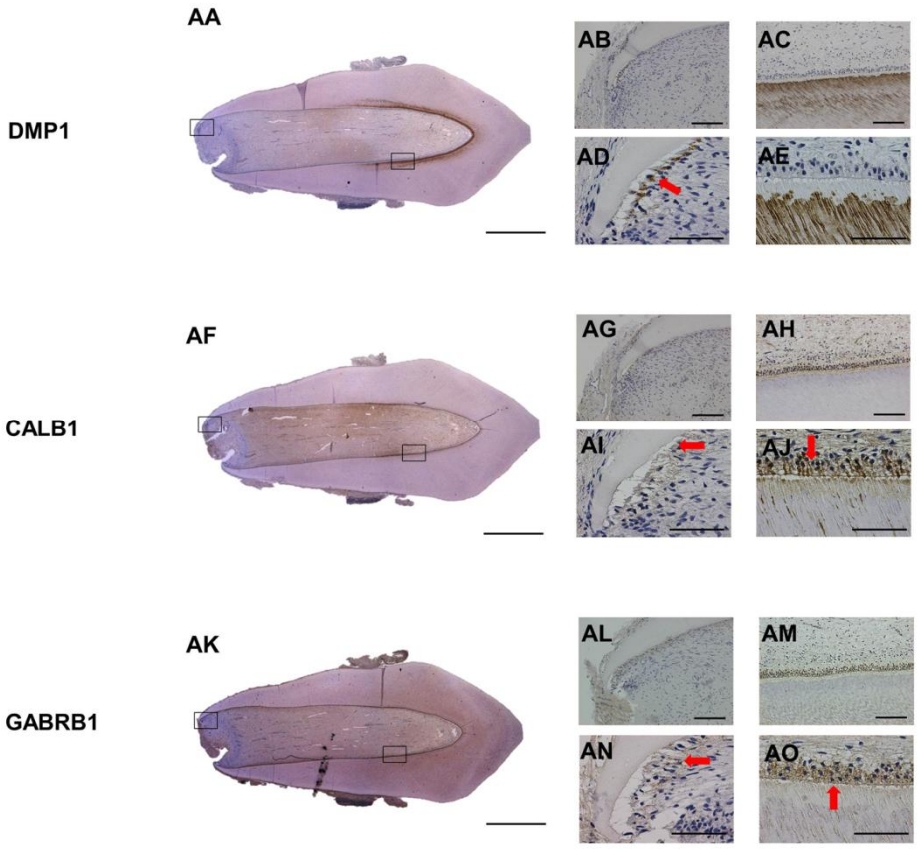

Figure 4. Immunohistochemical (IHC) staining of a tooth with an immature root. (AA–AE), *DMP1* is strongly expressed in the CP and coronal dentin but is barely expressed in the APC. (AF–AJ), *CALB1* is strongly expressed in the CP and coronal odontoblast but is barely expressed in the APC. (AK–AO), *GABRB1* is strongly expressed in the CP, coronal dentin, and odontoblast layer, but is barely expressed in the APC. The red arrows indicate examples of positively immunostained tissues. Scale bars: 2 mm (AA, AF, and AK); 100 μm (AB, AC, AG, AH, AL, and AM); 40 μm (AD, AE, AI, AJ, AN, and AO).

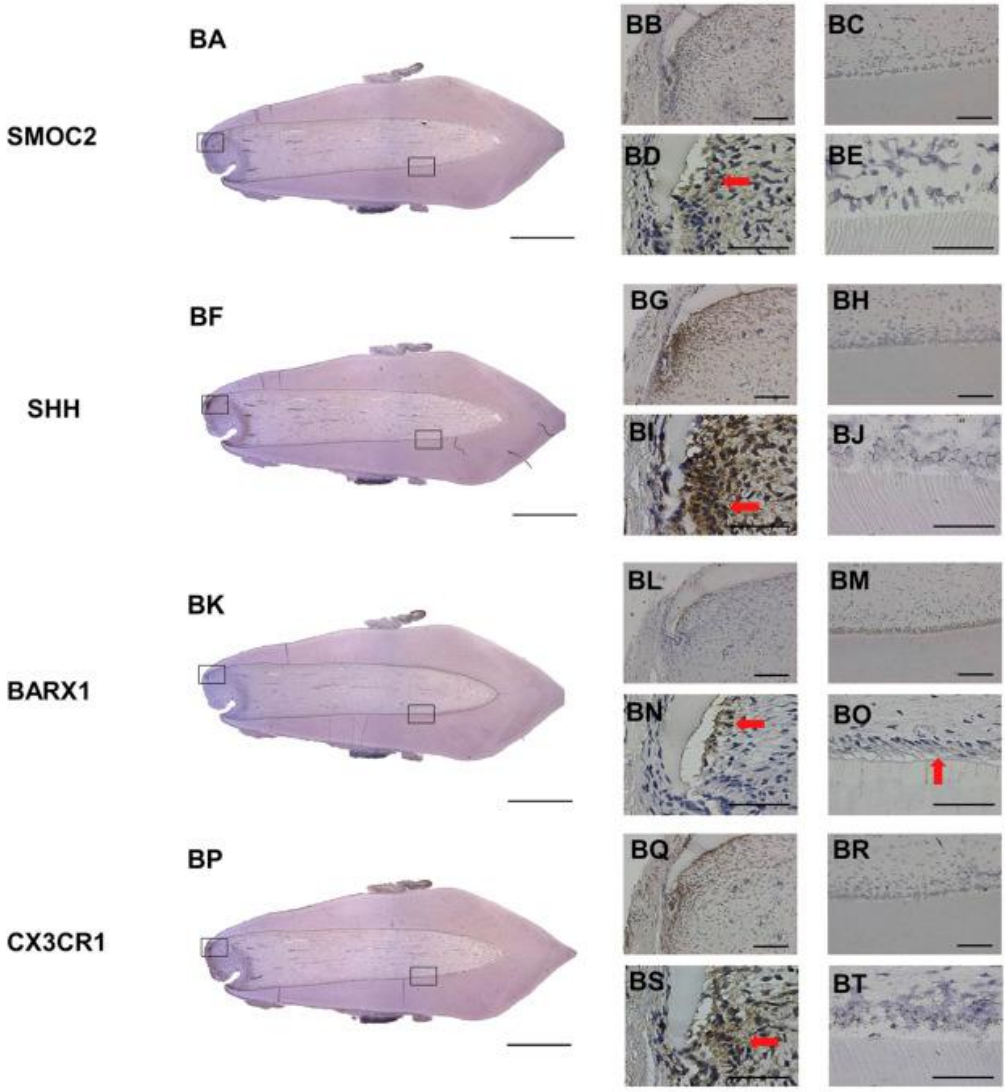

Figure 4 continued. IHC staining of a tooth with an immature root. (BA–BE), SPARC-related modular calcium binding 2 (*SMOC2*) is strongly expressed in the APC and apical odontoblasts but is barely expressed in the CP. (BF–BJ), Sonic hedgehog (*SHH*) is strongly expressed in the APC, apical odontoblasts, and Hertwig's root sheath (*HERS*), but is barely expressed in the CP. (BK–BO), BARX homeobox 1 (*BARX1*) is strongly

expressed in the APC and apical odontoblasts but is barely expressed in the CP. (BP–BT), Chemokine (C-X3-C motif) receptor 1 (*CX3CR1*) is strongly expressed in the APC and apical odontoblasts but is barely expressed in the CP. The red arrows indicate examples of positively immunostained tissues. Scale bars: 2 mm (BA, BF, BK, and BP); 100 μm (BB, BC, BG, BH, BL, BM, BQ, BR); 40 μm (BD, BE, BI, BJ, BN, BO, BS, and BT).

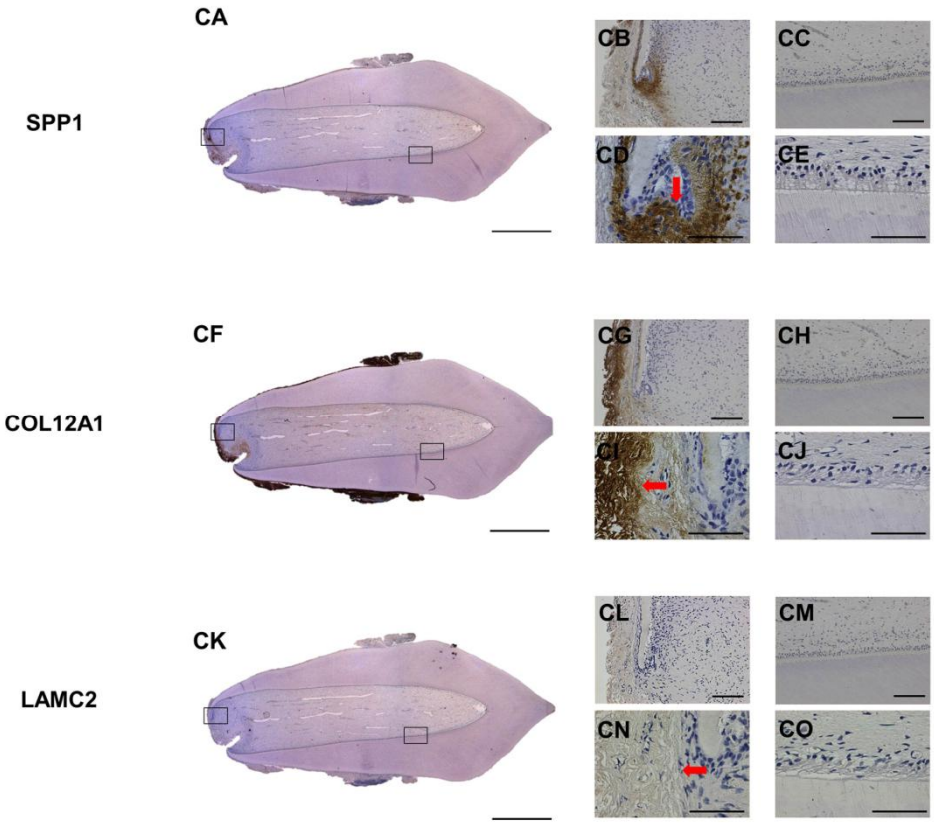

Figure 4 continued. IHC staining of a tooth with an immature root. (CA–CE), *SPP1* is strongly expressed in the apical papilla and region covered by the dental follicular tissues, but is barely expressed in the CP. (CF–CJ), *COL12A1* is strongly expressed in the covering follicular tissues that extend to the periodontal ligament but is barely expressed in the CP. (CK–CO), *LAMC2* is strongly expressed in the APC, especially when it covers the follicular tissue around HERS, but is barely expressed in the CP. The red arrows indicate examples of positively immunostained tissues. Scale bars: 2 mm (CA, CF, and CK); 100 μm (CB, CC, CG, CH, CL, and CM); 40 μm (CD, CE, CI, CJ, CN, and CO).

IV. Discussion

This study revealed different gene expression patterns of the CP and APC in human immature teeth. CP have higher expression of genes related to neurotransmission, neurogenesis and dentin mineralization than APC, whereas APC showed increased expression of immune, and tooth development-related genes.

Genes related to dentin mineralization were expressed more strongly in CP than in the APC, especially *DMP1*, which is well known to be related to mineralized tissues (Feng et al., 2003). This protein was more strongly expressed in the predentin and CP (middle half) than in the APC in this study, which implies that odontoblast in CP tends to be mature than in those in the APC. A stronger expression of *CALB1* in CP tissues and in the cytoplasm of odontoblasts in the coronal portion suggests that dentin mineralization is more important in the CP than in the APC (Figure 4AF-AJ). *CALB1* is related to dentin mineralization and is an intracellular, soluble, vitamin-D-dependent calcium binding protein (Wasserman and Taylor, 1966). Although the function of *CALB1* in dentin formation and mineralization remains to be determined, some authors have suggested that the calcium play a role in odontoblast activity via the direct communication of both the microvasculature and odontoblasts, suggesting a metabolic role for these cells through shunting of calcium to odontoblasts (Farahani et al., 2011). Moreover, *CALB1* may have a physiological role as a neuroprotector in conditions related to Ca2+ overload, which is expected for Ca2+ sensors (Berggård et al., 2002).

Genes related neurotransmission were more strongly expressed in the CP. Although the functions of almost all of them have not been fully explained in dental pulp tissues, this finding can explains the increased sensitivity of the CP or odontoblasts to stimuli compared to the APC. Some genes and their functions have been reported for dental pulp tissue. Some authors have suggested that the odontoblast expression of mechanosensitive ion channels TWIK-related K1 channel (*KCNK*) is an additional indicator of sensory function. TWIK-related K1 channels might also be involved in the K+ homeostasis of odontoblasts after dentin injury. This process modifies the dentinal fluid flow, changes the local microcirculation of the pulp tissue, an increase in the pulp pressure, and, consequently, tissue ischemia (Magloire et al., 2003). Anoctamin-1, also known as transmembrane member 16A, is a voltage-sensitive calcium-activated chloride channel that is expressed more strongly in differentiating odontoblasts than in preodontoblasts (Rock, 2008). Among the neurogenesis-related genes that are strongly expressed in the CP but barely found in the APC, The GABA A receptor (*GABRB1*) is a multisubunit chloride channel that mediates the fastest inhibitory synaptic transmission in the central nervous system (Jones and Harrison, 1993). Semaphorin 3D was related to immature neuron and growth cone marker, and Semaphorin 3E (*SEMA3E*) was found to be related to vascular and neural development, especially axon guidance (Tamagnone and Comoglio, 2000). Cerebellin 2 precursor is related to synaptogenesis induction, the presynaptic differentiation of cortical neurons, and the interaction with neurexins (Joo et al., 2011). Syntabulin is related to intercellular junction and anterograde axonal transport (Cai et al.,

2007), Ermin is related to myelinogenesis and neuron maturation (Brockschnieder et al., 2006). Cordon-Bleu regulates neuron morphogenesis (Ahuja et al., 2007). Reelin controls cell to cell communication and neuronal migration during brain development (Rice and Curran, 2001). WD repeat domain 62 is related to cerebral cortical organization (Mirzaa and Paciorkowski, 2014), prokineticin 2 is related to olfactory bulb neuronal precursor cell's chemoattractor (Dodé and Rondard, 2013), and neuroligin 4 (Y-linked) is related cell-adhesion molecules in synapse (Arese et al., 2011).

Most of the genes that are expressed more abundantly in the APC have barely been discussed with respect to human teeth and dental pulp tissues although most of them have been found to be associated with genes related to tooth development in animal studies. Our microarray analysis found that the expression levels of secreted frizzled-related protein 4 (*SFRP4*) and secreted frizzled-related protein 1 were higher in the APC. Some authors have suggested that *SFRP4* modulates Wnt gene signals in the pregnant uterus (Fujita et al., 2002). Wnt signaling is essential to tooth crown development, from initiation to the early bell stage, but Wnt expression is barely detectable during root development (Huang and Chai, 2012; Sarkar and Sharpe, 1999). *SFPR4* might be associated with tooth root development via a Wnt signaling inhibitor. SPARC-related modular calcium binding 2 (*SMOC2*) stimulates the mitogenic and angiogenic effects of vestibular endothelial growth factor (*VEGF*) and platelet-derived growth factor (*PDGF*) (Rocnik et al., 2006). *SMOC2* expression was found in the oral ectoderm, the outer dental epithelium, and the mesenchymal papilla facing the epithelial loops of molars (Bloch-

Zupan et al., 2011). In a mouse model, *SMOC2* appeared to have a developmental function like in the human root, which requires a greater metabolic circulation for nutrition supply. Sonic hedgehog (*SHH*) was strongly expressed in AP and especially in HERS, which contains apical odontoblast. *SHH* signaling mediates epithelial and mesenchymal interaction during root development in mouse (Huang et al., 2010), strongly suggesting that *SHH* signaling is involved in the regulation of tooth root elongation in the mouse (Nakatomi et al., 2006). In the present study, the expression of *SHH* in the APC indicated actively generating root formation in the human tooth. BARX homeobox 1 (*BARX1*) was expressed more strongly in the APC than in the CP. *BARX1* is a homeobox gene that acts as a transcription factor associated with regulators of regionally specific tooth morphogenesis in the murine molar. *BARX1* was found to be involved in mesenchyme of the developing molar after crown formation (Mitsiadis et al., 1998). The expression of *BARX1* in the APC indicates that it has a certain function in human root development like animal models.

Genes associated with the immune responses were expressed more strongly in the APC. This study indicated that the immune-related genes included chemokine-related genes (*CXCL9*, *CX3CR1*, and *CCL5*), B-cell antigen-binding-related genes (*IGHD*, *IGHM*, *IGKC*, *LOC652493*, *IGK@*, and *GPR183*), detoxification by carboxylesterase in the liver and monocyte (*CES1*) (Markey, 2010), granzyme family for proapoptic protease (*GZMK*) (Cooper et al., 2011), initiator of allergic reaction (*FCER1A*) (Weidinger et al., 2008), and complement for danger signal (*C3*) (Gasque, 2004). Recent studies showed that *CX3CR1*

is related to the development of periapical lesions. This chemokine and its receptor may be involved in the progression of tissue destruction (including bone resorption) during periapical inflammation (Wang et al., 2014). A recent study found that the gene expression in the dental follicle was related to bone development and remodeling, apoptosis, and chemotaxis (Lee et al., 2013). This might also suggest that the immune responses of the APC are related to the development and remodeling of tissue.

Other genes that were expressed more abundantly in the APC have been found in association with the dental follicular tissues that extend to the periodontal ligament, such as secreted phosphoprotein 1; collagen, type XII, alpha 1 (*COL12A1*), and laminin, gamma 2 (*LAMC2*). Secreted phosphoprotein 1, widely known as osteopontin, is a major phosphoprotein and suggests that the synthesis of *OPN* could be used as a characteristic marker of bone cells (Yokota et al., 1992). This might explain why the APC has multipotent functions such as modulating crypt bone remodeling. *COL12A1* encodes the collagen XII, alpha 1 protein (*Col XII*). Intense expression of *Col XII* is observed at the mature stage of periodontal ligament development (Karimbux et al., 1992). The expression of *COL12A1* in the APC was strong when the APC covering the follicular tissue extended to periodontal ligament. *LAMC2* encodes the laminin family of proteins, which are components of the basement membrane. Laminin is known to be involved in a wide variety of biological processes, including cell adhesion, differentiation, migration, signaling, neurite outgrowth, and metastasis (Kallunki et al., 1992). Root development starts during the late stages of crown morphogenesis, all subunits of laminin-5 were

detected in the basement membrane closely associated with the inner and outer epithelia (Yoshiba et al., 1998). *LAMC2* was strongly expressed in the APC, especially when it was covering the follicular tissue around HERS. This could indicate that the APC facing the outer epithelium of HERS is actively involved in epithelial-mesenchymal interactions.

In this study, we did not exclude the covering follicular tissue from the apical papilla. However, this covering follicular tissue originated from the dental follicle, which expressed genes associated with cell adhesion, differentiation, migration, signaling, and morphogenesis (Morsczeck et al., 2009). Because the original aim was characterize tooth development and differentiation, not only the apical papilla but also the covering follicular tissue needed to be considered. The AP and the covering follicular tissue are inseparably related to each other and can function as a unit like periodontium, which consist of cementum, periodontal ligament, and alveolar bone (Xu et al., 2009).

There are still much aspects of the biological control mechanisms underlying cellular activity and root development that remain to be studied. However, comparing the expression profiles of the CP and the APC may yield useful information about the mechanisms involved in the differentiation, growth, and evolution of human dental pulp in both normal and pathologic condition. Such information could be applied in dental tissue engineering applications, clinical problem such as immature teeth with pulp necrosis, and regenerative endodontic procedures.

V. Conclusion

This study found that the expression levels of genes related to dentin mineralization, neurogenesis, and neurotransmission are higher in the CP in human immature teeth, whereas the expression levels of immune-related, and tooth development-related genes are higher in the APC.

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국문요약

사람의 미성숙 치아에서 치관부 치수와 근단부 치수복합체의 유전자 발현 비교연구

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김수현

지도교수: 송제선

치수는 치유두에서 기원한, 비광화된 구강 내 조직으로 결합 조직과 혈관, 림프, 그리고 신경 조직으로 구성되서 치아내부의 중심인 치수강을 차지하고 있는 조직이다. 미성숙한 치아에서 치수는 치관부 치수(CP)와 근단부 치수복합체(APC)로 구성되어 있다. 이 중 치관부 치수는 근단부 치수복합체보다 성숙한 상아모세포를 포함한 분화된 조직으로 이는 상아질발생, 광화에 관련하며, 근단부 치수복합체는 치근의 발생에 필수적인 역할을 담당하고 있다. 기존의 동물 연구에서는 치수의 분화와 치근의 발생에 대한 연구를 진행하였으나 사람을 대상으로 하는 치관부 치수와 근단부 치수복합체에 대한 연구는 없었다. 이에 본 연구는 사람의 미성숙 치아의 치관부 치수와 근단부 치수복합체의 유전자 발현의 차이를 비교함으로써 두 조직간의 기능의 차이를 설명하고자 한다.

각각의 분자생물학적 차이를 알아보기 위하여 cDNA분석과 역전사효소 중합효소 연쇄반응분석과 면역화학염색법을 시행하여 다음과 같은 결론을 얻었다.

1. 치관부 치수와 근단부 치수복합체의 cDNA 미세배열 분석 결과, 스크리닝한 33297개의 유전자 중 치관부 치수에서는 139개의 유전자가, 근단부 치수복합체에서는 125개의 유전자가 2배 이상의 차이로 발현되었다.
2. 치관부 치수에서는 상아질 광화에 관련한 *DMP1*, *CALB1*, 신경발생에 관련한 *ANO1*, *ST8SIA1*, *GLRB*, *GRIA1*, *NPY1R*, *NPY1R*, *KCNN4*, *KCNK2*, *KCNK5* 유전자가 높게 발현되었다. 이는 치관부 치수 주위의 상아모세포가 좀 더 분화하였으며 외부 자극에 근단부 복합체보다 민감하게 반응할 수 있음을 의미한다.
3. 근단부 치수복합체에서는 면역반응에 관련한 *IGHD*, *IGHM*, *IGKC*, *IGHA1*, *LYZ*, *CXCL9*, *LOC652493*, *IGK@*, *CES1*, *GZMK*, *FCER1A*, *C3*, *CX3CR1*, *GPR183*, *CCL5*와 치아발생 관련한 *SFPR4*, *SFPR1*, *SMOC2*, *SHH*, *BARX1*, 그리고 치주인대로 뻗어있는 치낭조직과 관련한 *COL12A1*, *LAMC2* 유전자가 높게 발현되었다. 이는 조직의 발생과 재형성에 있어 근단부 치수복합체의 면역반응이 관련되고 치아발생과정에서 근단부 치수복합체가 중요한 역할을 하고 있음을 확인할 수 있었다.

본 연구 통해 사람의 미성숙 치수의 치관부 치수와 근단부 치수복합체의 차이점를 이해할 수 있었고, 향후 조직공학에 적용할 뿐 아니라 미성숙치아의 치수괴사에 대한 치료 및 재생적 근관 치료술식 등 임상에서 마주치는 문제에 응용할 수 있을 것으로 기대한다.

핵심되는 말: 근단부 치수복합체, cDNA 미세배열, 치관부 치수, 사람 미성숙 치아, 면역형광염색법