Comparative Gene Expression Analysis of the Coronal Pulp and Apical Pulp Complex in Human Immature Teeth

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Abstract

Introduction: This study determined the gene expression profiles of the human coronal pulp (CP) and apical pulp complex (APC) with the aim of explaining differences in their functions. **Methods:** Total RNA was isolated from the CP and 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. **Results:** In the microarray analyses, 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 immunohistochemical 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. **Conclusions:** The expression levels of genes related to dentin mineralization, neurogenesis, and neurotransmission are higher in the CP in human immature teeth, whereas those of immune-related and tooth development–related genes are higher in the APC. *(J Endod 2016;42:752–759)*

Key Words

Apical pulp complex, complementary DNA microarray, coronal pulp, human immature teeth, immunohistochemical staining

The dental pulp, which originates from the dental papilla, is an unmineralized oral tissue composed of soft connective tissue and vascular, lymphatic, and nerve components that occupy the central pulp cavity of the 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 permanent teeth, and smooth-surfaced soft tissue was easily detached from the apex to expose dental pulp tissue in the canal space. The AP originates from the dental papilla of the dental organ, which is the 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 fewer blood vessels and less extracellular matrix relative to the CP and the apical cell–rich zone (1).

After crown formation, root development begins via the interaction between the Hertwig 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 (2). 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 (3); otherwise, cells in covering the follicular tissue can differentiate into cementoblasts that are induced by the stimulation of root dentin (4). 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 (5); we call this structure the apical pulp complex (APC) (Fig. 1*A–E*).

The CP contains more differentiated cells than the AP with mature odontoblasts, and their cellular processes extending into dentinal tubules are the first to encounter caries bacterial antigens (6). Many genes characterizing mature odontoblasts have been identified, with transforming growth factor beta, which is important in dentinogenesis, mineralization, and proinflammation, recruiting immune cells such as dendritic cells (7, 8). Nestin, which produces the hard tissue matrix of dentin and repairs carious and injured teeth, was expressed more strongly in mature odontoblasts of the crown cusp region (9). Dentin sialophosphoprotein, which is expressed by matured odontoblasts, is important in dentinogenesis and is a specific marker for odontoblastic differentiation (10).

Several recent studies of pulp biology have used complementary DNA (cDNA) microarray technology to compare the gene expression profiles in different subjects

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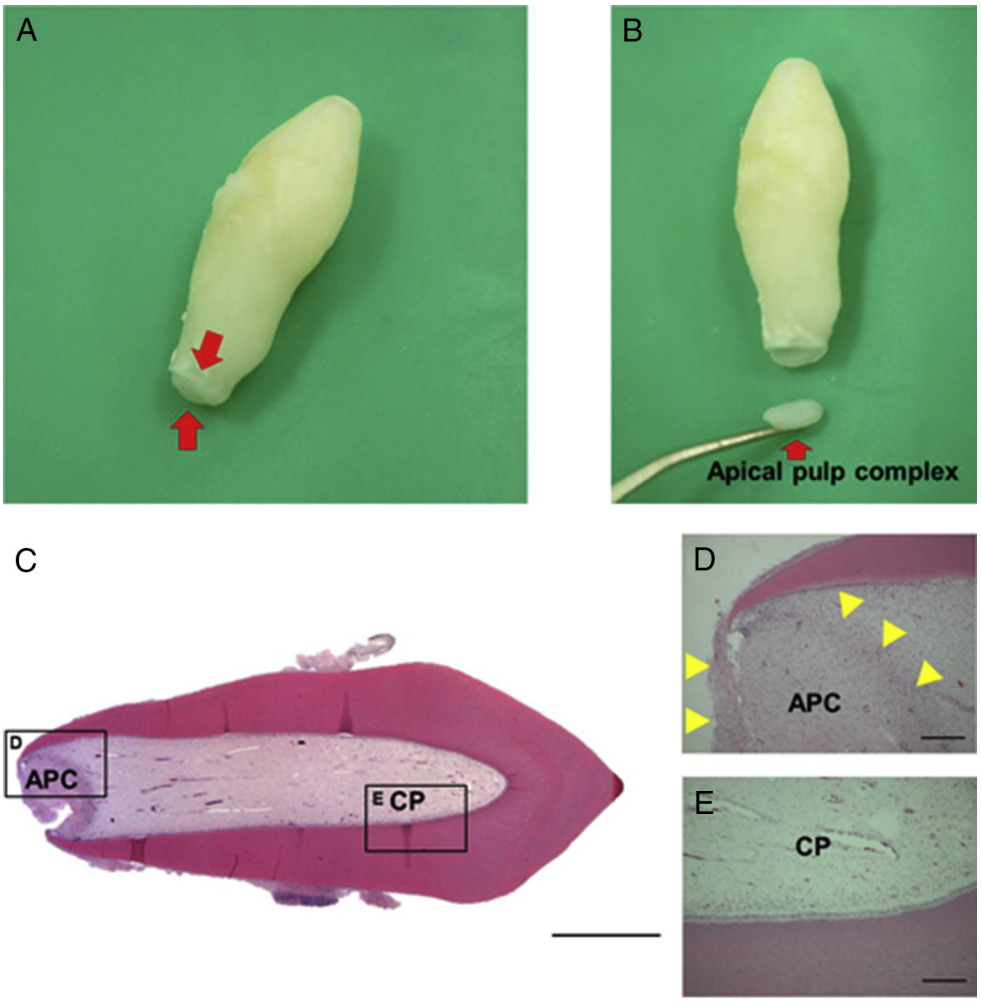

Figure 1. Anatomy of the APC and CP. (*A*) An extracted human supernumerary tooth with an immature root with the APC (*arrows*), (*B*) the APC removed from the apex (on the explorer), (*C*) hematoxylin-eosin staining of a supernumerary tooth with an immature root apex, (*D*) a magnified view of the area 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: (*A*) 2 mm and (*D* and *E*) 100 μm.

including comparing pulp tissue between carious and sound teeth (11), pulp tissue, and odontoblasts to determine the characteristics of odontoblasts among pulp tissues (12) and evaluating age-related changes in human dental pulp tissue (13). 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 compositions, such investigations can provide useful insights into APC functions in root development and biological processes of 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, with the aim of elucidating whether any of the differences found can explained by differences in their functions.

Materials and Methods

Preparation of Pulp Samples

The experimental protocol was approved by the Institutional Review Board of Yonsei University Dental Hospital, Seoul, Republic of Korea, and informed consent was obtained from all children enrolled in our study and their parents (approval #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.

RNA Isolation

After thawing, the APC tissue samples were isolated and crushed with a bolt cutter, and the pulp tissues were carefully obtained using sterile tweezers. The APC tissue samples were pooled from 6 or 7 teeth samples, and the pulp tissues were pooled from 3 or 4 teeth samples. The tissues were homogenized using a Bullet Blender Bead (Next Advance, Averill Park, NY), and total RNA was purified using the RNeasy Fibrous Mini kit (Qiagen, Valencia, CA) in accordance with the manufacturer's instructions. RNA quality was assessed using the Agilent 2100 bioanalyzer with the RNA 6000 Nano Chip (Agilent Technologies, Amstelveen, The Netherlands), and its quantity was determined using a NanoDrop ND-2000 device (Thermo Scientific, Wilmington, DE). The RNA samples used in this study had 260/280 nm optical ratios of at least 1.8.

cDNA Microarray and Data Analyses

Global gene expression analyses was performed using Affymetrix GeneChip Human Gene 1.0 ST oligonucleotide arrays (Affymetrix, Santa