Original Article

General gene expression patterns and stemness of the gingiva and dental pulp

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KEYWORDS
Dental pulp tissue; Gene expression patterns; Gingiva; Inducible pluripotent stem cells; Mesenchymal stem cells

Abstract Background/purpose: Due to the unique properties of healing processes and cellular differentiation, the gingiva and dental pulp have attracted attention as a potential source of mesenchymal stem cells (MSCs). The purpose of this study was to obtain molecular-level information on these tissues in terms of their function and differentiation processes and investigate stemness.

Materials and methods: Healthy gingival tissues were collected from patients (n = 9; aged 7–12 years) who underwent simple surgical procedures, and normal dental pulp tissues were obtained from patients (n = 25; aged 11–25 years) undergoing tooth extraction for orthodontic reasons. Complementary DNA microarray, qRT-qPCR, and immunohistochemical staining were performed to assess general and MSC gene expression patterns.

Results: In the gingival tissue, genes related to keratinization, the formation of epithelial cells and ectoderm, and immune and/or inflammatory responses were highly expressed. Meanwhile, in the dental pulp tissue, genes related to ion transport, neuronal development and axon guidance, bone and enamel mineralization, extracellular matrix organization, and angiogenesis were highly expressed. When focusing on the expression of MSC genes, induced pluripotent stem (iPS) cell genes, such as Sox2, c-Myc, and KLF4, were expressed at higher levels in the gingival tissue, whereas dental stem cell genes, such as NT5E and VCAM1, were expressed in dental pulp tissue.

Conclusion: We found different general and MSC gene expression patterns between the gingival and dental pulp tissue. These results have implications for future regenerative medicine, considering the application of gingival tissue as a potential source of iPS cells.

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Introduction

Mesenchymal stem cells (MSCs) are cells that maintain multipotency, can differentiate into various cell types, and have high capacity for immune regulation and structural regeneration. Therefore, these cells have attracted attention due to their possible application to regenerative medicine; specifically, MSCs can be differentiated before transplantation and replace the defected tissue. These cells can be isolated from various tissues, such as bone marrow, cord blood, and adipose. Recently, dental tissue cells, such as gingival-derived mesenchymal stem cells (GMSCs) and dental pulp stem cells (DPSCs) have emerged as an alternative source of MSCs. The gingiva comprises the gingiva comprises cells, such as gingival-derived mesenchymal stem cells (GMSCs) and dental pulp stem cells (DPSCs) have emerged as an alternative source of MSCs. The gingiva comprises ample mucosal tissue lining the masticatory area of the oral mucosa. Therefore, GMSCs can be obtained in large numbers, are convenient to isolate, and reportedly have effective wound healing ability without a scar formation. The dental pulp is an unmineralized connective tissue located in the central pulp cavity of the teeth. It was reported to play an important role in producing structures such as the extracellular matrix, dental pulp, dentin, and periodontal ligament, and accordingly, dental damage is healed through the mineralization and differentiation of DPSCs.

Compared with MSCs from other sources, GMSCs and DPSCs are reported to have fast self-renewal and differentiation capabilities. However, general and specific MSC gene expression patterns have not been investigated thoroughly with respect to gingival and dental pulp tissue. Therefore, to obtain molecular-level information on the characteristics of these tissues in terms of function and differentiation process, we investigated the general gene expression patterns and stemness of the gingival and dental pulp tissue.

Materials and methods

Samples and RNA isolation

Healthy gingival tissues were obtained during extraction of the supernumerary tooth and odontoma and flap surgeries from nine patients aged 7–12 years. Normal dental pulp tissues were obtained from 25 patients aged 11–25 years who received orthodontic extraction of premolars. The experimental protocol was approved by the Institutional Review Board of the Yonsei University Dental Hospital (#2-2012-0001 and #2-2015-0005).

These samples were immediately frozen and stored in liquid nitrogen after extraction and submerged in buffer RLT, a component of the RNeasy Fibrous Tissue Mini kit (Qiagen, Valencia, CA, USA). The gingival and dental pulp tissues were homogenized using a Bullet Blender (Next Advance, Averill Park, NY, USA), and the total RNA was extracted using the RNeasy Fibrous Tissue Mini kit (Qiagen). The extracted RNA was eluted in 25 μl of sterile water. RNA concentrations were determined from absorbance values at a wavelength of 260 nm using a spectrophotometer (NanoDrop ND-2000; Thermo Scientific, Rockford, IL, USA).

Analysis of cDNA microarray data

To compare gene expression between gingival and dental pulp tissues, we analyzed public microarray data (GSE588480, gingival data; GSE75644, dental pulp data) published on the Gene Expression Omnibus (GEO). The unit for gene expression in cDNA microarray analysis was 'Robust Multi-array Average (RMA)'. The web-based tool DAVID (the Database for Annotation, Visualization, and Integrated Discovery) was used to analyze the biological characteristics of differentially expressed genes. Then, these genes were classified based on gene functions in Gene Ontology and KEGG Pathway databases.

Quantitative reverse transcriptase-polymerase chain reaction

The single-stranded cDNA required for PCR analysis was produced using 500 ng of extracted total RNA as a template for reverse transcription (Superscript III Reverse Transcriptase and random primer; Invitrogen, Paisley, UK). The RT reaction was performed at 65 °C for 5 min, followed by 25 °C for 5 min, 50 °C for 1 h, and 70 °C for 15 min to inactivate the activity of the reverse transcriptase. The synthesized cDNA was diluted 10:1 in distilled water and used as a template for quantitative RT-PCR, which was performed using the ABI7300 RT-PCR system (Applied Biosystems, Warrington, UK). Samples of 25 μl containing 1 × Universal TaqMan Master Mix (4,369,016; Applied Biosystems), PCR primers at a concentration of 0.9 μM, and the diluted cDNA were prepared in triplicate. The amplification conditions were 50 °C for 2 min and 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The following TaqMan gene expression assay primers (Applied Biosystems) were used: CALB1, c-Myc, DMP1, DSG1, DSP, KLF4, KRT76, NT5E, Sox2, SPK7, VCAM1, and 18S rRNA. Primer information is listed in Table 1. ABI 7300 SDS 1.3.1 software (Applied Biosystems) recorded the fluorescence intensity of the reporter and quencher dyes; the results are plotted versus time and quantified as the cycle number. 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 ΔCt values (ΔCt = 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^ΔCt.
diluted 1:400, rabbit monoclonal against c-Myc (Ab32072; Abcam) diluted 1:25, and rabbit polyclonal against NT5E (Ab175396; Abcam) diluted 1:100. Protease K (Dako, Carpinteria, CA, USA) was used to retrieve the antigen for NT5E staining, whereas no such treatment was performed for other staining. 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 provide optimal staining, and the sections were incubated overnight. After incubation, EnVision + System-HRP Labelled Polymer Anti-rabbit (K4003; Dako) was applied for 20 min. Color development was performed using labeled streptavidin biotin kits (Dako), and samples were counterstained with Gill’s hematoxylin (Sigma-Aldrich). Controls were stained in the same way without antibodies.

### Statistical analysis

We explored the general gene expression patterns using the cDNA data. The normality of the RMA expression values was confirmed through the Shapiro–Wilk test (p-values > 0.05). To determine whether genes were differentially expressed between the gingival and dental pulp tissue, we conducted t-tests for gene expression values. We considered inflated alpha error due to multiple testing by applying a false discovery rate method (Benjamini and Hochberg, 1995). We selected differentially expressed genes in the gingiva and dental pulp that showed > 4-fold or 20-fold differences when comparing the signal value of gene expression. We investigated the stemness of the gingival and dental pulp tissue using the quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) and IHC staining.

| Table 1 | Specific primers used for quantitative RT-PCR analysis. |
|---------|----------------------------------------------------------|
| Gene symbol | Gene description | Functions | Assay ID | Product size (bp) |
| CALB1 | Calbindin 1 | Dentin formation and mineralization | Hs00191821_m1 | 90 |
| c-Myc | Myc | Regulation of transcription, DNA-dependent | Hs00153408_m1 | 107 |
| DMP1 | Dentin matrix protein 1 | Regulation of osteogenic differentiation | Hs00109391_g1 | 106 |
| DSG1 | Desmoglein 1 | Cell-cell junction assembly | Hs00355084_m1 | 87 |
| DSPP | Dentin sialophosphoprotein | Extracellular matrix organization | Hs00171962_m1 | 67 |
| KLF4 | Kruppel-like factor 4 | Mesodermal cell fate determination, regulation of transcription | Hs00358836_m1 | 110 |
| KRT76 | Keratin 76 | Cytoskeleton organization | Hs00210581_m1 | 80 |
| NT5E | 5’-Nucleotidase | Regulation of transcription, DNA-dependent | Hs00159686_m1 | 107 |
| Sox2 | Sex-determining region Y-box 2 | Negative regulation of transcription from RNA polymerase II promoter, osteoblast differentiation | Hs01053049_s1 | 91 |
| SPINK7 | Serine peptidase inhibitor, Kazal type 7 | Epithelial inflammatory process | Hs00261445_m1 | 93 |
| V CAM1 | Vascular cell adhesion molecule 1 | Acute/chronic inflammatory response | Hs01003372_m1 | 62 |
| 18S | 18S rRNA | | Hs03003631_g1 | 69 |

![Figure 1](image_url)  
**Figure 1**  The main classification of genes expressed in the dental pulp and gingiva according to biological features.
Table 2  Representative differentially expressed genes with higher expression levels in gingiva than in dental pulp.

| Functional category          | Gene symbol | Biological process                        | Accession number | Absolute food change |
|------------------------------|-------------|-------------------------------------------|------------------|----------------------|
| Cascade of healing           | S100A8      | Wound healing                             | NM_002964        | 213.59               |
|                              | SPRR3       | Wound healing                             | NM_005416        | 120.20               |
|                              | SERPINB2    | Wound healing                             | NM_001143818     | 65.28                |
|                              | BNC1        | Wound healing                             | NM_001717        | 24.94                |
|                              | GRHL3       | Wound healing                             | NM_001195010     | 21.10                |
| Structural process           | DSG1        | Cell–cell junction assembly               | NM_001942        | 334.26               |
|                              | SPRR2A      | Keratinocyte differentiation              | NM_005988        | 242.38               |
|                              | DSC3        | Cell–cell adhesion                        | NM_001941        | 232.72               |
|                              | DSG3        | Cell adhesion                             | NM_001944        | 221.51               |
|                              | KRT76       | Keratinocyte differentiation              | NM_015,848       | 177.93               |
|                              | KRT10       | Keratinocyte differentiation              | NM_000421        | 163.88               |
|                              | KRTDAP      | Keratinocyte differentiation              | NM_001,244,847   | 148.29               |
|                              | TGM3        | Keratinocyte differentiation              | NM_003245        | 133.95               |
|                              | SPRR2E      | Keratinocyte differentiation              | NM_001,024,209   | 112.32               |
|                              | CNFN        | Keratinization                            | NM_032,488       | 105.27               |
| Developmental process        | DSC1        | Cell adhesion                             | NM_004948        | 79.81                |
|                              | SCEL        | Epidermis development                     | NM_003843        | 179.77               |
|                              | KRT6B       | Ectoderm development                      | NM_005555        | 130.24               |
|                              | KRT5        | Epidermis development                     | NM_000424        | 90.52                |
|                              | KRT14       | Epidermis development                     | NM_000526        | 70.56                |
|                              | KRT15       | Epidermis development                     | NM_002275        | 69.68                |
|                              | TACSTD2     | Regulation of epithelial cell proliferation| NM_002353        | 64.8                 |
| Immune and inflammatory      | SPINK5      | Epidermal cell differentiation            | NM_001,127,698   | 41.37                |
| process                      | SPINK7      | Immune and inflammatory response          | NM_032566        | 419.58               |
|                              | GBP6        | Immune and inflammatory response          | NM_198,460       | 130.9                |
|                              | S100A9      | Chronic inflammatory response             | NM_002965        | 73.29                |
|                              | IL1RN       | Immune response                           | NM_173,842       | 67.95                |
|                              | S100A7      | Innate immune response                    | NM_002963        | 60.53                |
|                              | ADH7        | Response to bacterium                     | NM_000673        | 49.05                |
|                              | IL36A       | Immune and inflammatory response          | NM_014,440       | 48.01                |
|                              | IL18        | Immune and inflammatory response          | NM_001,243,211   | 36.07                |
| Protein modification and      | TMPRSS11A   | Proteolysis                               | NM_001114,387    | 258.58               |
| maintenance                  | TMPRSS11D   | Proteolysis                               | NM_004262        | 237.03               |
|                              | SERPINB3    | Proteolysis                               | NM_006,919       | 197.05               |
| Metabolism and catabolism    | SERPINB5    | Proteolysis                               | NM_002639        | 186.93               |
|                              | A2ML1       | Regulation of endopeptidase activity      | NM_001,282,424   | 117.61               |
|                              | KLK10       | Proteolysis                               | NM_001,077,500   | 44.78                |
|                              | KLK13       | Proteolysis                               | NM_015,596       | 41.72                |
| Transport activity           | AKR1B10     | Metabolic process                         | NM_020,299       | 132.04               |
|                              | LIPK        | Lipid catabolic process                   | NM_001,080,518   | 109.82               |
|                              | MUC15       | Cellular protein metabolic process        | NM_001,350,91    | 93.3                 |
|                              | MUC21       | Metabolic process                         | NM_010,019,09    | 60.87                |
|                              | CERS3       | Metabolic process                         | NM_001,290,341   | 57                   |
|                              | CLCA2       | Transport                                 | NM_006,536       | 147.78               |
|                              | RHCG        | Transport                                 | NM_016,321       | 93.26                |
|                              | CLCA4       | Transport                                 | NM_012,128       | 77.48                |
|                              | AQP3        | Water transport                           | NM_004,925       | 60.77                |
|                              | SLC5A1      | Transport                                 | NM_000,343       | 31.07                |
| Signal transduction and      | GJB2        | Cell communication                        | NM_004,004       | 49.86                |
| regulation                   | CEACAM6     | Signal transduction                       | NM_002,483       | 48.62                |
|                              | GJB6        | Cell communication                        | NM_001,110,219   | 48.01                |
|                              | GPR87       | Signal transduction                       | NM_023,915       | 33.68                |
| Cell cycle and apoptosis     | EHF         | Positive regulation of transcription      | NM_001,206,615   | 105.06               |
|                              | CRCT1       | Apoptosis                                 | NM_019,060       | 90.52                |
|                              | GRHL1       | Transcription, DNA-templated              | NM_198,182       | 43.33                |
|                              | DYNAP       | Regulation of apoptotic process           | NM_173,629       | 42.92                |
|                              | MACC1       | Regulation of transcription               | NM_182,762       | 28.55                |

(continued on next page)
Meanwhile, the stratum granulosum and spinosum. Meanwhile, the genes upregulated more than 20-fold higher expression in the gingival tissue than in the dental pulp tissue, whereas levels of 644 (1.9%) were higher in the dental pulp tissue than in the gingival tissue. When we focused on the genes with a more prominent difference in expression, we identified 180 (0.5%) showing more than 20-fold higher expression in the gingival tissue than in the dental pulp tissue and 65 (0.2%) with higher levels in the dental pulp tissue than in the gingival tissue. The genes upregulated more than 20-fold in the gingival tissue ($n = 180$) were functionally related to structural process, developmental process, immune and inflammatory process, and protein modification and maintenance (Fig. 1; Table 2). Meanwhile, the genes upregulated more than 20-fold higher in the dental pulp tissue ($n = 65$) were functionally related to transport activity, developmental process, biominal tissue development, structural process, and physiological process (Fig. 1; Table 3).

The results from qRT-PCR analysis of the selected genes (CALB1, c-Myc, DMP1, DSG1, DSPP, KLF4, KRT76, NT5E, SOX2, SPINK7, VCAM1, and 18S rRNA) were consistent with those of cDNA microarray analysis. Through qRT-PCR analysis of the dental pulp tissue, we did not identify SPINK7, DSG1 and KRT76, which were found to be expressed higher in the gingival tissue than in the dental pulp tissue in the cDNA microarray analysis (Table 2). Similarly, qRT-PCR in the gingival tissue did not detect CALB1, DMP1, and DSPP.

We also found that Sox2, c-Myc, and KLF4, which are induced pluripotent stem (iPS)-associated genes, were expressed at higher levels in the gingival tissue in both cDNA microarray and qRT-PCR analyses (Fig. 2B). Meanwhile, NT5E and VCAM1, which are dental pulp stem cell genes, were expressed at higher levels in the dental pulp tissue in both cDNA microarray and qRT-PCR analyses (Fig. 2B). Based on IHC staining, levels of DSG1 and c-Myc were more prominent in the gingival tissue, especially on the stratum granulosum and spinosum. Meanwhile, the expression of DSPP and NT5E was more prominent in dental pulp tissue, which is also consistent with other results from cDNA microarray and qRT-PCR analyses (Fig. 3).

**Results**

We performed cDNA microarray analysis to compare the general gene expression patterns of the gingival and dental pulp tissues. Results indicated that among a total of 33,297 examined genes, 596 (1.8%) exhibited more than 4-fold higher expression in the gingival tissue than in the dental pulp tissue, whereas levels of 644 (1.9%) were higher in the dental pulp tissue than in the gingival tissue. When we focused on the genes with a more prominent difference in expression, we identified 180 (0.5%) showing more than 20-fold higher expression in the gingival tissue than in the dental pulp tissue and 65 (0.2%) with higher levels in the dental pulp tissue than in the gingival tissue. The genes upregulated more than 20-fold in the gingival tissue ($n = 180$) were functionally related to structural process, developmental process, immune and inflammatory process, and protein modification and maintenance (Fig. 1; Table 2). Meanwhile, the genes upregulated more than 20-fold higher in the dental pulp tissue ($n = 65$) were functionally related to transport activity, developmental process, biominal tissue development, structural process, and physiological process (Fig. 1; Table 3).

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**Discussion**

Dental pulp is defined as vascularized and innervated connective tissue of dental papilla origin enclosed by dentin. Meanwhile, gingiva, which is composed of epithelium and connective tissue, originates from the neural crest ectomesenchyme of dental papilla connected to the periodontal ligament and is originates from the perifollicular mesenchyme and periodontal ligament stem/progenitor cells.20 The developmental origin and anatomical and functional differences are widely known, and in this study, we focused more on characteristic gene expression patterns in gingival and dental pulp tissues.

In the gingival tissue, genes related to structural processes, developmental processes, immune and inflammatory processes, and protein modification and maintenance were expressed prominently. Meanwhile, in the dental pulp tissue, genes related to transport activity, developmental processes, biominal tissue development, structural processes, and physiological processes were expressed prominently. When we focused on the expression of stemness markers, iPS-related genes, such as Sox2, c-Myc, and KLF4, were expressed at higher levels in the gingival tissue, whereas dental-derived stem cell genes, such as NT5E and VCAM1, were expressed at higher levels in the dental pulp tissue.

The gingiva is an actively regenerating mucosal tissue that reacts to external stimuli and defends against pathogens through immune functions and inflammation. Gingival tissue has high wound healing properties due to the regulation of inflammatory cytokines and interleukin21 and maintains immune homeostasis.22 Therefore, it is plausible that genes functionally related to keratinization (e.g. SPRR2A, KRT76, KRT10, KRTDAP, TGM3, SPRR2E, and CNFN ), the formation of epithelial cells and the ectoderm (e.g. SCIL, KRT6B, KRT5, KRT14, KRT15, TACSTD2, and SPINK5), immune and/or inflammatory responses (e.g. SPINK7, GBP6, S100A9, IL1RN, S100A7, ADH7, IL36A, and IL18), and proteolysis (e.g. TMMP5S11A, TMPPS11D, SERPINB3, SERPINB5, A2ML1, KLK10, and KLK13) were expressed at higher levels in the gingival tissue.

Especially, SPINK7, which is involved in epithelial inflammatory processes and the negative regulation of peptidase,23,24 and DSG1, DSC3, and DSG3, which are key components of cell–cell junction assembly,25–27 were
Table 3: Representative differentially expressed genes with higher expression levels in dental pulp than in gingiva.

| Functional category               | Gene symbol | Biological process                          | Accession number | Absolute food change |
|-----------------------------------|-------------|---------------------------------------------|------------------|----------------------|
| **Biomineral tissue development** | PHEX        | Bone mineralization                         | NM_000444        | 110.47               |
|                                   | CALB1       | Dentin formation and mineralization         | NM_004929        | 101.9                |
|                                   | GPC3        | Bone mineralization                         | NM_001164617     | 97.99                |
|                                   | AMBN        | Enamel mineralization                       | NM_016519        | 63.12                |
|                                   | WDR72       | Enamel mineralization                       | NM_182758        | 45.21                |
|                                   | NES         | Formation of dentin matrix                 | NM_006617        | 38.82                |
|                                   | PDGFD       | Odontoblastic differentiation              | NM_025208        | 34.07                |
|                                   | BMP6        | Regulation of bone mineralization          | NM_001718        | 20.47                |
| **Structural process**            | DMP1        | Extracellular matrix organization           | NM_001079911     | 89.12                |
|                                   | DSPP        | Extracellular matrix organization           | NM_014208        | 84.04                |
|                                   | MMP20       | Extracellular matrix disassembly           | NM_00771        | 71.09                |
|                                   | PCDH20      | Cell adhesion                              | NM_022843        | 46.21                |
|                                   | ADAM22      | Cell adhesion                              | NM_004194        | 22.49                |
|                                   | ITGA10      | Extracellular matrix organization           | NM_003637        | 22                    |
|                                   | COCH        | Extracellular matrix organization           | NM_001135058     | 21.38                |
|                                   | PVRL3       | Cell adhesion                              | NM_001243286     | 20.53                |
|                                   | FERMT2      | Cell—matrix adhesion                       | NM_001134999     | 20.18                |
| **Developmental process**         | CNTN4       | Neuron projection development               | NM_001206955     | 33.47                |
|                                   | MAP1B       | Positive regulation of axon extension      | NM_005909        | 31.97                |
|                                   | RELN        | Neuron migration                           | NM_005045        | 31.08                |
|                                   | FAT3        | Multicellular organismal development        | NM_001008781     | 29.17                |
|                                   | SLIT2       | Axon guidance                              | NM_001289135     | 21.72                |
| **Immune and inflammatory process** | C7          | Innate immune response                     | NM_000587        | 46.16                |
| **Protein modification and maintenance** | MERTK     | Phagocytosis                                | NM_006343        | 24.66                |
|                                   | SCUBE3      | Protein homooligomerization                | NM_152753        | 37.95                |
|                                   | PTPRD       | Protein dephosphorylation                  | NM_001040712     | 25.77                |
| **Metabolism and catabolism**     | ST8S1A1     | Glycosphingolipid metabolic process         | NM_003034        | 44.91                |
|                                   | LPPR5       | Metabolic process                          | NM_001010861     | 22.74                |
|                                   | LPL         | Phospholipid metabolic process             | NM_000237        | 22.34                |
|                                   | ST8S1A1     | Glycosphingolipid biosynthetic process      | NM_003034        | 21.29                |
| **Transport activity**            | TF          | Ion transport                              | NM_001063        | 82.29                |
|                                   | SCN7A       | Sodium ion transport                       | NM_002976        | 68.39                |
|                                   | KCNK2       | Ion transport                              | NM_001017424     | 53.43                |
|                                   | RANBP3L     | Intracellular transport                     | NM_001161429     | 38.92                |
|                                   | CP          | Transport                                  | NM_000096        | 31.47                |
|                                   | ABCA6       | Transport                                  | NM_080284        | 29.08                |
|                                   | ATP1A2      | Ion transport                              | NM_000702        | 24.82                |
| **Signal transduction and regulation** | SPOCK3   | Signal transduction                        | NM_001040159     | 96.76                |
|                                   | WIF1        | Wnt signaling pathway                      | NM_007191        | 62.31                |
|                                   | LGR5        | Signal transduction                        | NM_001277226     | 59.22                |
|                                   | AKAP12      | Signal transduction                        | NM_005100        | 36.08                |
|                                   | Gfra1       | Cell surface receptor signaling pathway     | NM_001145453     | 25                    |
|                                   | CRABP1      | Signal transduction                        | NM_004378        | 20.53                |
|                                   | RGS5        | Signal transduction                        | NM_001195303     | 20.48                |
| **Cell cycle and apoptosis**      | CLU         | Regulation of neuron death                 | NR_038,335       | 29.1                  |
|                                   | CDK14       | Regulation of cell cycle                   | NM_001287135     | 21.54                |
| **Physiologic process**           | SEMA3E      | Sprouting angiogenesis                      | NM_001178129     | 108.78               |
|                                   | CYP1B1      | Angiogenesis                               | NM_000104        | 60.07                |
|                                   | NRXN1       | Angiogenesis                               | NM_00135659      | 28.4                  |
|                                   | HEY2        | Blood vessel development                   | NM_012259        | 27.07                |
|                                   | TFPI        | Blood coagulation                          | NM_001032281     | 23.93                |
|                                   | FGFR1       | Angiogenesis                               | NM_001174063     | 21.54                |
|                                   | ENPEP       | Angiogenesis                               | NM_001977        | 20.63                |
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Figure 2 Complementary DNA microarray and quantitative RT-PCR of stem cell markers (A) The relative gene expression of induced pluripotent stem cell and dental-derived stem cell markers using cDNA microarray. (B) The relative fold-differences in the expression levels of five selected stem cell marker genes between the gingiva and dental pulp using quantitative RT-PCR. The data are presented as the mean ± standard deviation (A, B) and are expressed as the relative change by applying the equation 2^−ΔΔCt. (b). **p < 0.05.

Significantly upregulated. This confirmed that the gingiva is specialized as an intrusion barrier and to the outside. S100A8 has been reported as an innate immune response-related gene in the gingiva,⁵¹ and it has been shown to be deeply involved in wound healing. According to Iglesias-Bartolome,⁵² the regulation of inflammation, structure formation, and epithelial cell differentiation comprises the comprehensive process of wound healing.

The dental pulp is known to play an important role in nutrition supply, nerve innervation, angiogenesis, and tertiary dentin formation.⁵³ Therefore, our results suggest that genes functionally related to ion transport (e.g. TF, KCNK2, and ATP1A2), neuronal development and axon guidance (e.g. CNNT4, MAP1B, RELN, and SLIT2), bone and enamel mineralization (e.g. PHEX, CALB1, DMP1, DSPP, AMBN, WDR72, NES, and BMP6), extracellular matrix organization (e.g. MMP20, ITGA10, and COCH), and angiogenesis (e.g. SEMA3E, CYP1B1, NRXN1, HEY2, FGFR1, and ENPEP) were expressed at higher levels in the dental pulp tissue is biologically plausible. Especially, PHEX, which is involved in bone mineralization, is a causative gene in X-linked dominant rickets when mutated.⁵⁰ SEMA3E, a key regulator for angiogenesis and axon guidance of axon,⁵¹,⁵² and CALB1, which is functionally related to dentin formation and mineralization,⁵³ showed high absolute fold-changes. Other than these genes, SPOCK3, TF, and MMP20 were identified as genes with absolute fold-changes > 70.

Although stemness of the gingiva and dental pulp were reported to be associated with multi-lineage differentiation and immunomodulatory capacity,⁵⁴ the gingiva showed higher expression of iPS-related genes, which is consistent with the results of a previous study.⁵⁵ Because iPS cells have higher growth capacity than traditional MSCs,⁵⁶ they can be a rich source of stem cells with the strong capacity to differentiate into osteoblasts, adipocytes, chondroblasts, and other tissues.⁵⁶ Further studies are warranted to investigate and utilize iPS cells of gingiva and dental tissue. Especially, Sox2, which we identified in the present study, was reported to affect the healing properties of the gingiva⁵⁷ and needs to be considered as a research priority.

Meanwhile, in the dental pulp tissue, dental stemness markers (NT5E and VCAM1) were overexpressed, consistent with the results of previous studies. Specifically, NT5E was reported to be more ubiquitously expressed in dental tissues, whereas VCAM1 was reported to be more specifically expressed in the dental pulp.⁵⁸,⁵⁹ Although DPSCs were determined to be superior to MSCs derived from bone marrow and adipose tissue in terms of the production of mineralized matrix and dentin,⁶⁰ their relative clinical significance in other contexts, as compared with MSCs from other sources, has not been evaluated comprehensively.

Although we found distinctive general and MSC gene expression patterns between the gingiva and dental pulp, ambiguity remains regarding the function of each identified gene and the interpretation of the results. Because most genes have a wide range of functions rather than just one, it is, to some extent, arbitrary to select the most relevant function of specific genes and to classify each gene into one functional category, which raises the possibility of selection bias and the concern of limited generalizability. However, this study directly investigated and compared the gene expression and stemness patterns between the gingival and dental pulp tissue and showed the stemness of and differences between the two tissues in terms of healing and regeneration capacity, which can serve as a basis for further research.

In conclusion, different general and MSC gene expression patterns between gingival and dental pulp tissues were found. iPS genes were expressed at higher levels in the gingival tissue, whereas dental-derived stemness markers were expressed at higher levels in the dental pulp tissue. These distinctive molecular-level characteristics of the gingival and dental pulp tissues could be considered to select more proper tissue sources of stem cells in future regenerative medicine.
Declaration of competing interest

The authors have no conflict of interest to declare.

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