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Original Article

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

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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.[1] 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.[2,3] These cells can be isolated from various tissues, such as bone marrow, cord blood, and adipose.[4] 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.[5,6] The gingiva comprises ample mucosal tissue lining the masticatory area of the oral mucosa.[7] Therefore, GMSCs can be obtained in large numbers, are convenient to isolate, and reportedly have effective wound healing ability without a scar formation.[8,9] 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,[10] and accordingly, dental damage is healed through the mineralization and differentiation of DPSCs.[11,12]

Compared with MSCs from other sources, GMSCs and DPSCs are reported to have fast self-renewal and differentiation capabilities.[13–16] 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® Bead (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 (GSE58480, gingival data; GSE75644, dental pulp data) published on the Gene Expression Omnibus (GEO).[17] 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.[18,19]

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*, *DSPP*, *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 $2^{-\Delta Ct}$.

Immunohistochemical staining

For immunohistochemical (IHC) staining, the gingival tissue and premolars were embedded in paraffin and sectioned at a thickness of 3 μm. Prior to this, the premolars were fixed in 10% buffered formalin (Sigma–Aldrich, St. Louis, MO, USA) for more than a 1day and decalcified with 10% EDTA (pH 7.4; Thermo Fisher Scientific, Waltham, MA, USA) for 12 weeks. Specimens were subjected to IHC staining with rabbit polyclonal against *DSG1* (Ab64883; Abcam, Cambridge, UK) diluted 1:100, rabbit polyclonal against *DSPP* (sc-33586; Santa Cruz Biotechnology, Santa Cruz, CA, USA)

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	Hs01009391_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
VCAM1	Vascular cell adhesion molecule 1	Acute/chronic inflammatory response	Hs01003372_m1	62
18S	18S rRNA		Hs03003631_g1	69

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

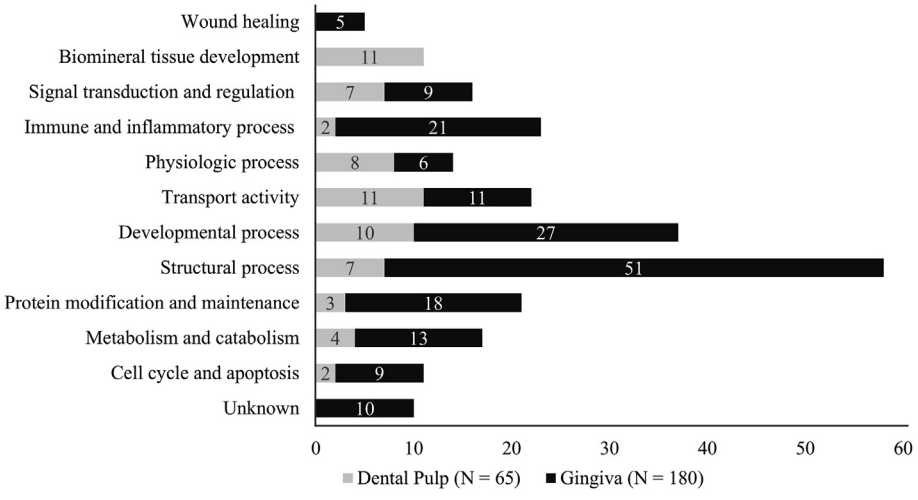

Figure 1 The main classification of genes expressed in the dental pulp and gingiva according to biological features.

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
	DSC1	Cell adhesion	NM_004948	79.81
Developmental process	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	68.4
	SPINK5	Epidermal cell differentiation	NM_001,127,698	41.37
Immune and inflammatory process	SPINK7	Immune and inflammatory response	NM_032566	413.58
	GBP6	Immune and inflammatory response	NM_198460	130.9
	S100A9	Chronic inflammatory response	NM_002965	73.29
	IL1RN	Immune response	NM_173842	67.95
	S100A7	Innate immune response	NM_002963	60.53
	ADH7	Response to bacterium	NM_000673	49.05
	IL36A	Immune and inflammatory response	NM_014440	48.01
	IL18	Immune and inflammatory response	NM_001243211	36.07
Protein modification and maintenance	TMPRSS11A	Proteolysis	NM_001114387	258.58
	TMPRSS11D	Proteolysis	NM_004262	237.03
	SERPINB3	Proteolysis	NM_006919	197.05
	SERPINB5	Proteolysis	NM_002639	186.93
	A2ML1	Regulation of endopeptidase activity	NM_001282424	117.61
	KLK10	Proteolysis	NM_001077500	44.78
	KLK13	Proteolysis	NM_015596	41.72
Metabolism and catabolism	AKR1B10	Metabolic process	NM_020299	132.04
	LIPK	Lipid catabolic process	NM_001080518	109.82
	MUC15	Cellular protein metabolic process	NM_001135091	93.3
	MUC21	Metabolic process	NM_001010909	60.87
	CERS3	Metabolic process	NM_001290341	57
Transport activity	CLCA2	Transport	NM_006536	147.78
	RHCG	Transport	NM_016321	93.26
	CLCA4	Transport	NM_012128	77.48
	AQP3	Water transport	NM_004925	60.77
	SLC5A1	Transport	NM_000343	31.07
Signal transduction and regulation	GJB2	Cell communication	NM_004004	49.86
	CEACAM6	Signal transduction	NM_002483	48.62
	GJB6	Cell communication	NM_001110219	48.01
	GPR87	Signal transduction	NM_023915	33.68
Cell cycle and apoptosis	EHF	Positive regulation of transcription	NM_001206615	105.06
	CRCT1	Apoptosis	NM_019060	90.52
	GRHL1	Transcription, DNA-templated	NM_198182	43.33
	DYNAP	Regulation of apoptotic process	NM_173629	42.92
	MACC1	Regulation of transcription	NM_182762	28.55

(*continued on next page*)

Table 2 (*continued*)

Functional category	Gene symbol	Biological process	Accession number	Absolute food change
Physiologic process	KRT1	Regulation of angiogenesis	NM_006121	129.13
	CD177	Blood coagulation	AJ310433	125.34
	ANXA8	Blood coagulation	NM_001040084	35.74

data. All analyses were performed using 25.0 SPSS software (SPSS Inc., Chicago, IL, USA). Two-sided p-values < 0.05 were considered significant.

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, biomineral 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).

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, biomineral 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 interleukin[21] 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. *SCEL*, *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. TMPRSS11A, TMPRSS11D, *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_004771	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
	MERTK	Phagocytosis	NM_006343	24.66
Protein modification and maintenance	SCUBE3	Protein homooligomerization	NM_152753	37.95
	PTPRD	Protein dephosphorylation	NM 001040712	25.77
Metabolism and catabolism	ST8SIA1	Glycosphingolipid metabolic process	NM_003034	44.91
	LPPR5	Metabolic process	NM_001010861	22.74
	LPL	Phospholipid metabolic process	NM_000237	22.34
	ST8SIA1	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_001135659	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

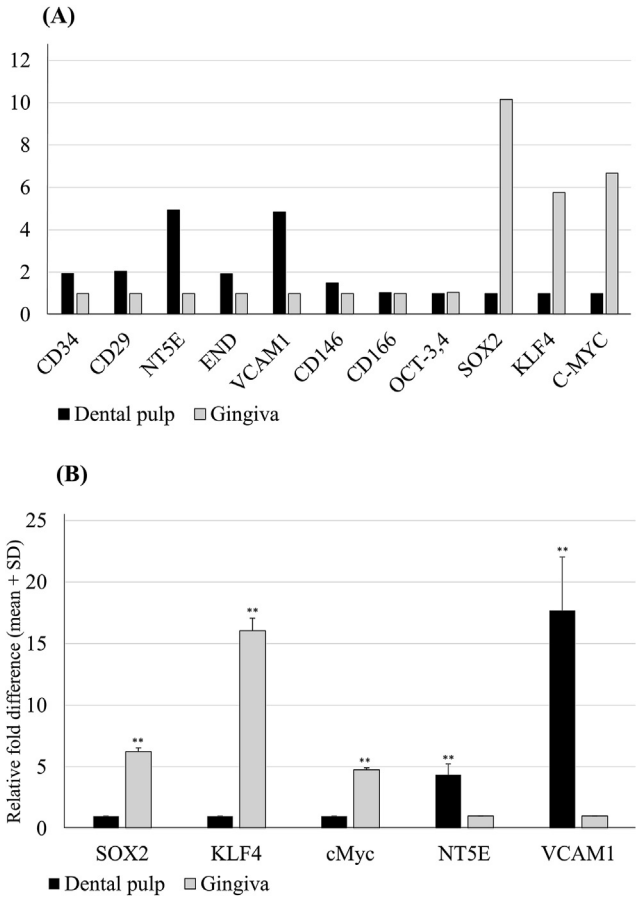

Figure 2 Complementary DNA microarray and quantitative RT-PCR of stem cell markers (A) The relative gene expression of induced pluripotent stem cell and dental-derived stem cell markers using cDNA microarray. (B) The relative fold-differences in the expression levels of five selected stem cell marker 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 $2^{-\Delta\Delta Ct}$.(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,[28] and it has been shown to be deeply involved in wound healing. According to Iglesias-Bartolome,[24] 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.[29] Therefore, our results suggesting that genes functionally related to ion transport (e.g. *TF*, *KCNK2*, and *ATP1A2*), neuronal development and axon guidance (e.g. *CNTN4*, *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.[30] *SEMA3E*, a key regulator for angiogenesis and axon guidance of axon,[31,32] and *CALB1*, which is functionally related to dentin formation and mineralization,[33] 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,[6] the gingiva showed higher expression of iPS-related genes, which is consistent with the results of a previous study.[34] Because iPS cells have higher growth capacity than traditional MSCs,[35] they can be a rich source of stem cells with the strong capacity to differentiate into osteoblasts, adipocytes, chondroblasts, and other tissues.[36] 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 gingiva[24] 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.[37–39] 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,[40] 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.

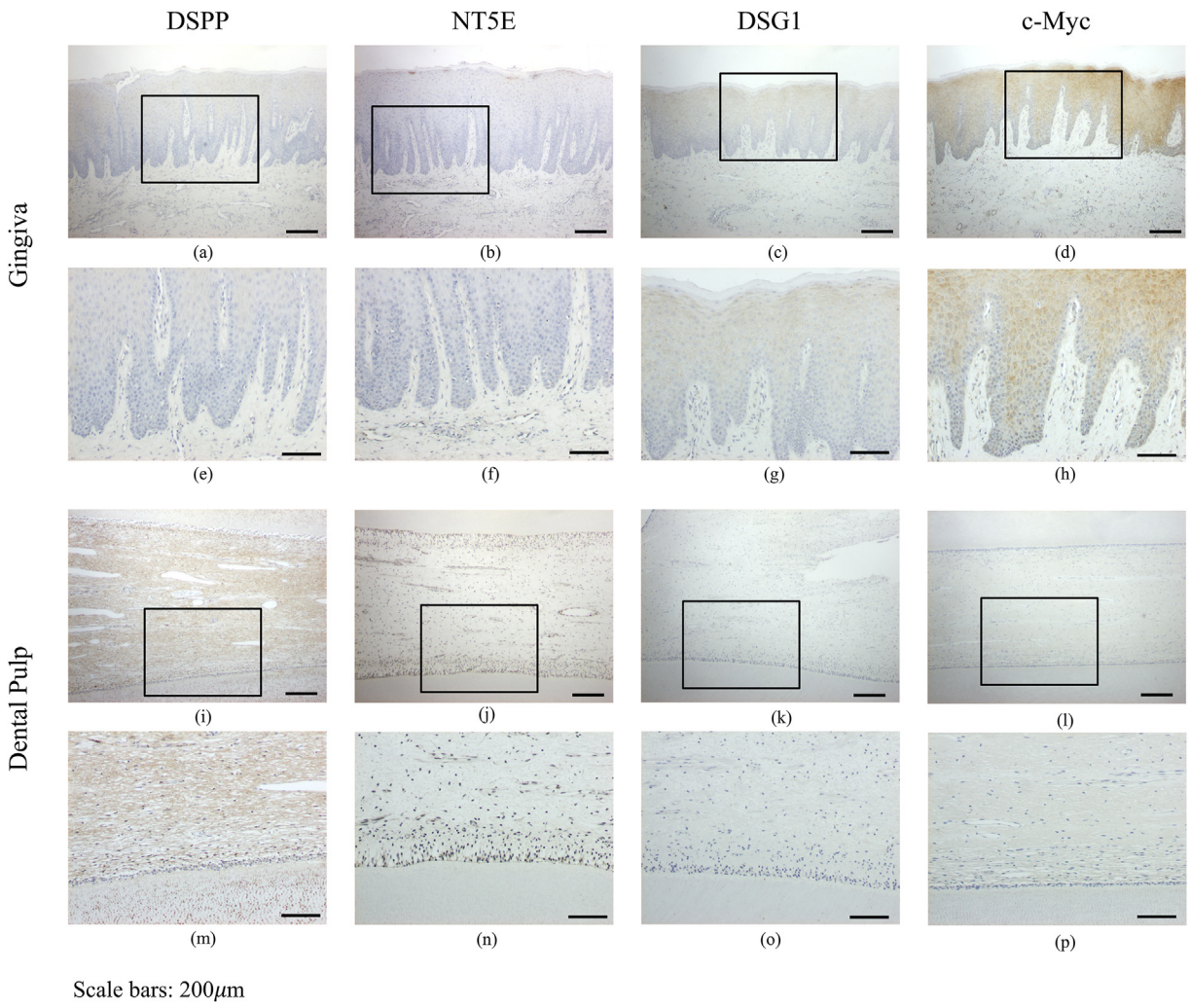

Figure 3 Immunohistochemical (IHC) staining of dental pulp and gingiva. IHC staining for DSPP in the gingiva (A, E) and dental pulp (DP) (I, M). IHC staining for NT5E in the gingiva (B, F) and DP (J, N). Staining for DSPP and NT5E was markedly high in the DP. IHC staining for DSG1 in the gingiva (C, G) and DP (K, O). IHC staining for c-Myc in the gingiva (D, H) and DP (L, P). DSG1 and c-Myc staining was observed in the gingiva, especially on the stratum granulosum and spinosum (scale bars: 200 μm).

Declaration of competing interest

The authors have no conflict of interest to declare.

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