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Original Article

Comparative gene expression analysis of stemness between periodontal ligament and umbilical cord tissues in humans

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Abstract *Background/purpose:* Due to their regenerative potential, periodontal ligament (PDL) and umbilical cord (UBC) tissues are an attractive potential mesenchymal stem cells (MSCs) source. This study compared the expression patterns of genes related to stemness between fresh PDL and UBC tissues.
Materials and methods: PDL tissues were collected from 38 permanent premolars extracted for orthodontic purposes, and UBC tissues were obtained from three newborns. Each sample was immediately frozen to prevent RNA degradation. cDNA microarray analysis, quantitative real-time polymerase chain reaction (PCR), and immunohistochemical staining were performed. Gene expression patterns associated with dental stemness (DS) and induced pluripotent stemness (iPS) were compared between PDL and UBC tissues.
Results: In the cDNA microarray analyses, the expressions of most iPS genes were greater in the PDL than in the UBC. Meanwhile, the expressions of most DS genes were greater in the UBC than in the PDL. Quantitative real-time PCR analyses showed that the expression levels of matrix metallopeptidase 13 (MMP13), ADAM metallopeptidase domain 22 (ADAM22), vascular cell adhesion protein 1 (VCAM1), and kruppel-like factor 4 (KLF4) genes were greater in the PDL

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than in the UBC, while the expressions of melanoma cell adhesion molecule (MCAM) and activated leukocyte cell adhesion molecule (ALCAM) were greater in the UBC than in the PDL.
Conclusion: These results suggest that UBC and PDL tissues showed slightly different expression patterns of genes related to stemness, which warrants further investigation to use these tissues for future regeneration and implantation therapies.

Introduction

Mesenchymal stem cells (MSCs) are used for injury treatment due to their regenerative potential in muscle, fat, nerve, blood vessel, cartilage, and bone tissues.[1–6] Self-regeneration is a vital stem cell property, and the cellular and molecular mechanisms of self-regeneration have been extensively studied.[7–9] MSCs are a possible alternative to embryonic stem cells, especially in cell-based therapeutic applications, but little is known about their nature, *in vivo* function, and developmental features.

Periodontal ligament (PDL), a connective tissue that surrounds the tooth-root surfaces, attaches the tooth to the alveolar bone. The success of healing traumatized, periodontal damage and tooth replantation treatment are closely related to the survival of PDL cells which has nutritive, sensory, formative, and remodeling function.[10–12] The PDL contains MSCs and exhibits the regenerative potential of tooth root surfaces.[13–15]

MSCs can also be obtained from the umbilical cord (UBC),[16, 17] which is easily obtained from abandoned tissue after birth.[18] The UBC is suggested to be a good source of cells for regenerative therapy.[17, 19] However, although a recent study compared stemness between fresh UBC cells and dental pulp,[20] no study has compared the expression patterns of genes related to stemness between the UBC and the PDL tissues.

Therefore, the present study aimed to compare the expression patterns of dental stemness (DS) and induced pluripotent stemness (iPS) genes between the PDL and UBC tissues. In addition, because most microarray studies have used purified RNA from cultured cells,[21–23] which may be problematic given that media types can affect cultured cell development,[24] this study aimed to establish a method for obtaining qualified RNA from fresh tissues to analyze gene expression.

Materials and methods

Sample preparation

PDL samples (n = 38; 4 men and 10 women, ages 10—19) were obtained from healthy permanent premolars extracted for orthodontic reasons. UBCs (2 cm in length) were obtained from three newborns. The experimental protocol was approved by the Institutional Review Board of Yonsei University Dental Hospital (#220120001) and Severance Hospital (#420120408). Extracted teeth and UBCs were immediately frozen and stored in liquid nitrogen.

RNA isolation via rapid thawing method

Total RNA was extracted from PDL and UBC tissues using the RNeasy Fibrous Mini Kit® (Qiagen, Valencia, CA, USA). The traditional thawing method could not extract any RNA from the fresh UBCs. Therefore, we designed a rapid thawing method (Fig. 1). A Cryostat (CM3050S, Leica Biosystems, Newcastle Upon Tyne, UK) was set to −20 °C. The frozen UBC tissues were placed in the chamber for 30 min and then embedded in an optimal cutting temperature compound. Tin foil was placed underneath the blade of the cryotome to collect the sliced sections. Approximately 300 frozen slices were harvested and submerged in RLT buffer solution. The tissues were homogenized using Bullet Blender® Bead (Next Advanced Inc., Averill Park, NY, USA). RNA concentrations were determined using a spectrophotometer (Nanodrop ND-1000®, Thermo Scientific, Rockford, IL, USA).

cDNA microarray analysis

Global gene expression analyses were performed using Affymetrix Gene Chip Human Gene 1.0 ST oligonucleotide arrays (Affymetrix Inc., Santa Clara, CA, USA). To compare gene expression between PDL and UBCs tissues, we analyzed public GSE75642 and GSE75695 microarray data could be accessed on the gene expression omnibus (GEO). The average amount of RNA isolated from PDL and UBC tissues was 1 μg. RNA quality was assessed using an Agilent 2100 bioanalyzer with the RNA 6000 Nano Chip® (Agilent Technologies, Amstelveen, Netherlands), and quantification was conducted using a NanoDrop ND-1000 (Thermo Scientific). The RNA samples were used as input for the Affymetrix procedure, as recommended by the protocol (http://www.affymetrix.com).

Quantitative reverse-transcription real-time polymerase chain reaction

Quantitative reverse transcription real-time polymerase chain reaction (qPCR) was performed in triplicate as follows. Single-stranded cDNA was produced using 500 ng of extracted total RNA as a template for reverse transcription (RT; Superscript III Reverse Transcriptase and random

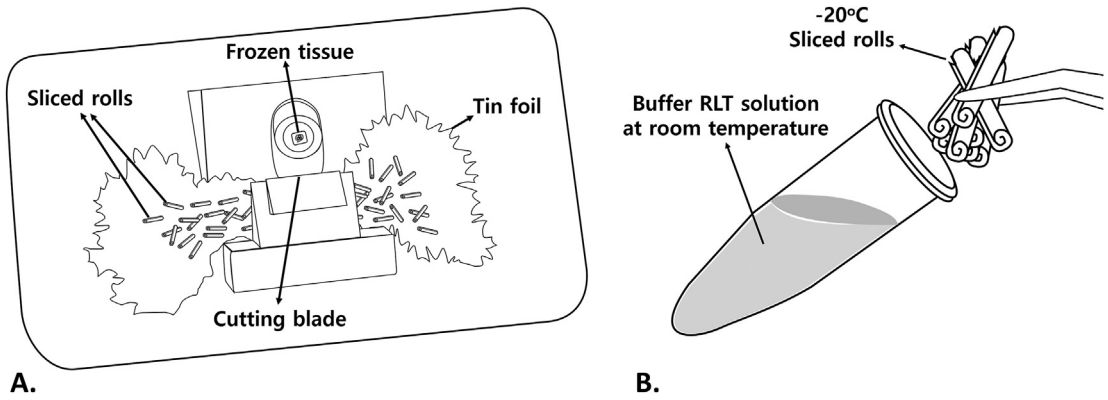

Figure 1 Schematic view of the cryotome-based rapid thawing method. A. About 300 frozen tissue samples were sliced into 14-μm-thick sections and collected on tinfoil at −20 °C using a cryotome. B. Three aliquots of 350 μL RLT buffer solution prepared in microtubes were transferred into the cryotome. Collected samples were submerged in RLT buffer solution at room temperature. This method enabled frozen tissue to be thawed rapidly, minimizing the degradation of RNA. The procedure was repeated several times until the microtubes were filled.

primers, Invitrogen, Paisley, UK). The RT reaction was performed at 65 °C for 5 min, followed by 5 min at 25 °C, 1 h at 50 °C, and 15 min at 70 °C to inactivate the reverse transcriptase. The synthesized cDNA was diluted 10:1 in distilled water and used as a template for qPCR, performed using an ABI7300 RT-PCR system (Applied Biosystems, Warrington, UK). 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. TaqMan gene expression assay primers (Applied Biosystems) were used (Table 1). Ct values were subsequently used to determine ΔCt values (ΔCt = Ct of the gene − Ct of the 18 S rRNA control), which were used to quantify the relative amount of PCR product, expressed as the relative change using equation $(2)^{-\Delta Ct}$.

Immunohistochemical staining

For immunohistochemical (IHC) staining, permanent teeth and UBC tissues were fixed in 10% buffered formalin (Sigma–Aldrich, St. Louis, MO, USA) for 1 d. Permanent teeth were decalcified with 10% EDTA (pH 7.4; Thermo Fisher Scientific, Waltham, MA, USA) for eight weeks. Tissues were embedded in paraffin and cut into 3-μm-thick sections. Specimens were subjected to IHC staining with the following seven antibodies: anti-human matrix metallopeptidase 13 (MMP13) (rabbit polyclonal, diluted 1:500; Ab39012, Abcam), tenascin-N (TNN) (rabbit polyclonal, diluted 1:400; Ab121887, Abcam), ADAM metallopeptidase domain 22 (ADAM22) (rabbit polyclonal, diluted 1:2000; Ab66479, Abcam), vascular cell adhesion protein 1 (VCAM1) (rabbit polyclonal, diluted 1:1000; Ab10677, Abcam), melanoma cell adhesion molecule (MCAM) (rabbit polyclonal, diluted 1:400; Ab75769, Abcam), activated leukocyte cell adhesion molecule (ALCAM) (rabbit polyclonal, diluted 1:1000; Ab78649, Abcam), and kruppel-like factor 4 (KLF4) (rabbit monoclonal, diluted 1:100; Ab, Abcam).

Endogenous peroxidase activity was quenched by the addition of 3% hydrogen peroxide. The sections were incubated in 5% bovine serum albumin to block nonspecific binding. The primary antibodies were diluted to obtain optimal staining, and the sections were incubated overnight. Next, EnVision + System-HRP Labelled Polymer Anti-rabbit (K4003, Dako North America Inc., Carpinteria, CA, USA; ready-to-use) was applied for 20 min. Color development was performed using labeled streptavidin-biotin kits (Dako North America Inc.), according to the manufacturer's instructions. The sections were counterstained with Gill's hematoxylin (Sigma–Aldrich). Control sections were treated in the same manner but without primary antibody treatment.

Data analysis

Expression data were generated using Affymetrix Expression Console software version 1.1® (Affymetrix Inc.). The robust multi-average (RMA) algorithm implemented in the

Table 1 Specific primers used for quantitative RT-PCR analysis.

Gene symbol	Gene description	Assay ID	Amplicon length
MMP13	matrix metallopeptidase 13 (collagenase 13)	Hs00233992_m1	91
VCAM1	vascular cell adhesion molecule 1	Hs01003372_ml	62
ALCAM	activated leukocyte cell adhesion molecule	Hs00977641_m1	103
MCAM	melanoma cell adhesion molecule	Hs00174838_m1	77
KLF4	Kruppel-like factor 4	Hs00358836_ml	110
18 S	18 S rRNA	Hs03003631_g1	69

Table 2 Representative differentially expressed genes with higher expression levels in the periodontal ligament than in the umbilical cord.

Gene symbol	Gene description	Accession number	Fold difference
MMP13	matrix metallopeptidase 13 (collagenase 3)	NM_002427	148.67
TNN	tenascin N	NM_022093	94.64
SFRP4	secreted frizzled-related protein 4	NM_003014	52.59
MOXD1	monooxygenase, DBH-like 1	NM_015529	39.96
IBSP	integrin-binding sialoprotein	NM_004967	36.99
IGSF10	immunoglobulin superfamily, member 10	NM_178822	35.03
WIF1	WNT inhibitory factor 1	NM_007191	31.49
EPHA7	EPH receptor A7	NM_004440	31.42
ALPL	alkaline phosphatase, liver/bone/kidney	NM_000478	29.73
SLC7A2	solute carrier family 7	NM_003046	29.32
EPHA3	EPH receptor A3	NM_005233	29.25
EYA1	eyes absent homolog 1 (Drosophila)	NM_000503	29.12
MMP9	matrix metallopeptidase 9	NM_004994	27.92
EYA4	eyes absent homolog 4 (Drosophila)	NM_004100	26.06
PDGFRL	platelet-derived growth factor receptor-like	NM_006207	25.52
DMP1	dentin matrix acidic phosphoprotein 1	NM_004407	23.36
SATB2	SATB homeobox 2	NM_015265	22.94
PRRX1	paired related homeobox 1	NM_006902	22.23
AK5	adenylate kinase 5	NM_174858	20.06
BMP3	bone morphogenetic protein 3	NM_001201	20.03

Affymetrix Expression Console software was used for normalization. One-way ANOVA was performed for the RMA expression values to determine whether the genes were differentially expressed among the three groups. Multiple testing corrections were applied to the p-values of the F-statistics to adjust the false discovery rate. Genes with adjusted F-statistics ($p < 0.05$) were extracted. Highly expressed genes with over a two-fold difference between the signal values of the control and each test group were selected for further study. To classify the co-expression gene groups comprising genes with a similar expression pattern, we performed hierarchical clustering and K-mean clustering using Multi Experiment Viewer version 4.4 (www.tm4.org, Dana-Farber Cancer Institute, Boston, MA, USA). The Database for annotation, visualization, and integrated discovery was used for the biological interpretation of differentially expressed genes. These genes were then classified based on gene function data in the Gene Ontology (GO) and KEGG Pathway databases (http://david.abcc.ncifcrf.gov/home.jsp).

Results

Gene expression profiles of umbilical cord and periodontal ligament tissues

In the cDNA microarray analysis, out of 28,869 genes, 152 (0.53%) were upregulated 10-fold or more in the PDL and UBC tissues; among them, 51 and 101 genes were upregulated in the PDL and UBC, respectively (Tables 2 and 3).

GO classes with an *F*-statistic p-value of <0.05 are shown in Figs. 2 and 3. Generally, in terms of biological processes, there were significant differences between the tissue types in transport, signal transduction, response to stimulus, drug, ion transport, inflammatory response, and cell—matrix adhesion. In terms of molecular functions, there were differences in zinc ion binding, receptor activity, protein metal ion binding, and DNA binding.

Stemness characterization using surface protein markers

DS and iPS markers were compared between the tissue types (Table 4). Among the DS markers, *MCAM* and *ALCAM* were upregulated in UBC tissues, and *VCAM1* was upregulated in PDL tissues. Meanwhile, all iPS markers were upregulated in PDL tissues compared to those in UBC tissues.

Quantitative reverse-transcription real-time polymerase chain reaction analysis

The following seven genes were selected for verification: *MMP13*, *KLF4*, *VCAM1*, *MCAM*, and *ALCAM*. The expression of *MMP13*, a PDL-specific marker, was increased in PDL tissues only, as expected. The expression of *KLF4*, an iPS-specific marker, and *VCAM1*, a DS-specific marker, was higher in PDL tissues. The expression of *MCAM* and *ALCAM*, both of which are DS-specific, was higher in UBC tissues (Table 5).

Immunohistochemical staining assessment

Seven antibodies exhibiting more than a two-fold difference in the microarray data were used to target the following proteins: MMP13, tenascin-N, ADAM22, VCAM1, MCAM, ALCAM, and KLF4. Only four proteins showed different staining patterns between the PDL and UBC:

Table 3 Representative differentially expressed genes with higher expression levels in the umbilical cord than in the periodontal ligament.

Gene symbol	Gene description	Accession number	Fold difference
HBG1	hemoglobin, gamma A	NM_000559	92.89
HSD17B6	hydroxysteroid (17-beta) dehydrogenase 6 homolog (mouse)	NM_003725	90.93
ACTG2	actin, gamma 2, smooth muscle, enteric	NM_001615	90.07
ACTC1	actin, alpha, cardiac muscle 1	NM_005159	85.63
MFAP5	microfibrillar associated protein 5	NM_003480	85.01
PRLR	prolactin receptor	NM_000949	61.93
DES	Desmin	NM_001927	58.23
PLN	phospholamban	NM_002667	54.75
SERPINB2	serpin peptidase inhibitor, clade B (ovalbumin), member 2	NM_001143818	54.14
DLK1	delta-like 1 homolog (Drosophila)	NM_003836	45.24
CNN1	calponin 1, basic, smooth muscle	NM_001299	43.27
UPK1B	uroplakin 1 B	NM_006952	42.20
MYOCD	Myocardin	NM_001146312	41.67
DKK1	dickkopf homolog 1 (*Xenopus laevis*)	NM_012242	39.63
SCRG1	stimulator of chondrogenesis 1	NM_007281	35.25
SULT1E1	sulfotransferase family 1 E, estrogen-preferring, member 1	NM_005420	34.41
C8orf84	chromosome 8 open reading frame 84	NM_153225	32.94
P2RX1	purinergic receptor P2X, ligand-gated ion channel, 1	NM_002558	31.93
PDLIM3	PDZ and LIM domain 3	NM_014476	30.26
IL1RL1	interleukin 1 receptor-like 1	NM_016232	28.88
SERPINB7	serpin peptidase inhibitor, clade B (ovalbumin), member 7	NM_003784	26.99
TGM2	transglutaminase 2	NM_004613	26.24
ART4	ADP-ribosyltransferase 4 (Dombrock blood group)	NM_021071	25.96
HTR2A	5-hydroxytryptamine (serotonin) receptor 2 A	NM_000621	25.85
HBB	hemoglobin, beta	NM_000518	25.71
SCEL	Sciellin	NM_144777	23.60
FLJ34690	hypothetical protein FLJ34690	NR_034,145	23.59
C5orf46	chromosome 5 open reading frame 46	NM_206966	23.00
STXBP5L	syntaxin binding protein 5-like	NM_014980	22.43
MYH11	myosin, heavy chain 11, smooth muscle	NM_022844	22.09
PDE1C	phosphodiesterase 1C, calmodulin-dependent 70 kDa	NM_001191058	21.70
HSPB8	heat shock 22 kDa protein 8	NM_014365	20.83

MMP13, TNN, ADAM22, and MCAM. A possible cause of unsuccessful IHC staining is detailed in the Discussion. Strong staining for MMP13, TNN, and ADAM22 was observed in PDL tissues but not in UBC tissues, whereas strong staining for MCAM was observed in the venous wall of the UBC tissues (Fig. 4).

Discussion

In the cDNA microarray analyses, the expressions of most iPS genes were greater in the PDL than in the UBC, and the expressions of most DS genes were greater in the UBC than in the PDL. In the Quantitative real-time PCR analyses, the expressions of *MMP13*, *ADAM22*, *VCAM1*, and *KLF4* genes were greater in the PDL than in the UBC, and the expressions of *MCAM* and *ALCAM* were greater in the UBC than in the PDL.

PDL is a very important part of dental function and survival, and both treatment of PDL and treatment of dental pulp are inevitable part in case of tooth trauma.[25] However, although expression patterns of genes related to stemness were compared between dental pulp and UBC, to the best of our knowledge, no studies compared the stemness gene expression patterns between PDL and UBC.

UBC has been expected to be a rich resource for MSCs, because MSCs can be easily obtained through non-invasive methods.[26] In addition, MSCs collected from the UBC are less likely to show immune reactions during transplantation due to having less mature tissues.[27] Therefore, the UBC has been widely facilitated in regenerative medicine and dental medicine.

The up-regulated genes observed in the PDL (*MMP13, matrix metallopeptidase 9 (MMP9), TNN, alkaline phosphatase, biomineralization associated (ALPL), dentin matrix acidic phosphoprotein 1 (DMP1), and bone morphogenetic protein 3 (BMP3))* are known to be associated with PDL functions, such as rapid metabolism and bone and collagen regeneration. Enriched biological process terms included "cell differentiation," "skeletal system development," "ossification," and "bone resorption," indicating that the main PDL functions are associated with bone and collagen development, which are important for clinical regenerative activity.

The up-regulated genes observed in the UBC, including *hemoglobin subunit gamma 1 (HBG1), actin gamma 2 (ACTG2), actin alpha cardiac muscle 1 (ACTC1), myocardin*

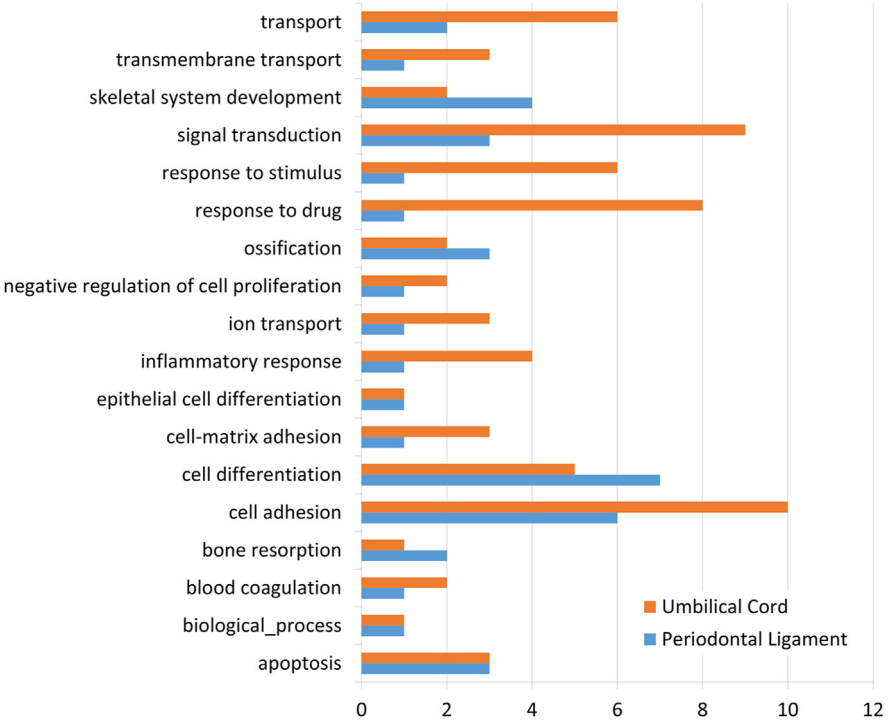

Figure 2 The main categories of genes expressed specifically in the periodontal ligament and umbilical cord tissues on the basis of their biological processes.

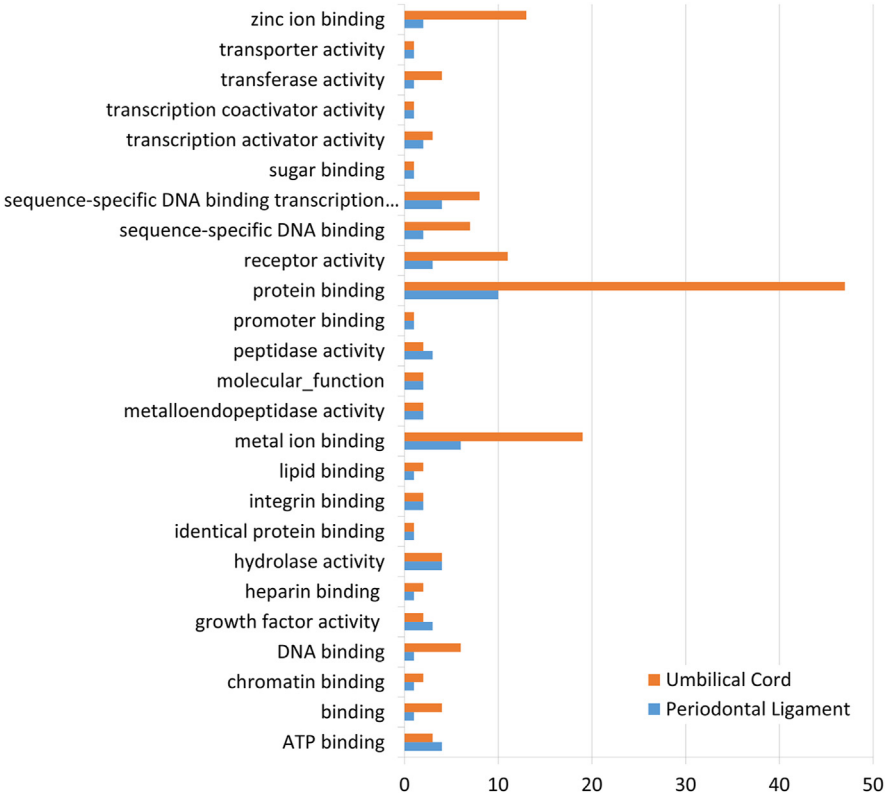

Figure 3 The main categories of genes expressed specifically in the periodontal ligament and umbilical cord tissues on the basis of their molecular functions.

Table 4 Relative comparison of stemness by microarray analysis between the periodontal ligament and umbilical cord.

Type of gene	RNA	Synonym	Fold difference	
			PDL	UBC
Dental Stemness (DS)	STRO-1	CD34	1.00	1.00
	ITGB1	CD29	1.00	**1.20**
	NT5E	CD73	1.00	**1.19**
	ENG	END	1.00	**1.04**
	VCAM1	CD106	**2.36**	1.00
	MCAM	CD146	1.00	**5.17**
	ALCAM	CD166	1.00	**5.49**
induced Pluripotent Stemness (iPS)	OCT-3,4	POU5F1	**1.31**	1.00
	SOX2	ANOP3	**1.39**	1.00
	KLF4	EZF	**2.28**	1.00
	C-MYC	MYC	**1.45**	1.00

PDL: periodontal ligament, UBC: umbilical cord, STRO-1: stromal cell surface marker-1, ITGB1: integrin subunit beta 1, NT5E: 5′-Nucleotidase Ecto, ENG: endoglin, VCAM1: vascular cell adhesion protein 1, MCAM: melanoma cell adhesion molecule, ALCAM: activated leukocyte cell adhesion molecule, OCT-3,4: octamer-binding transcription factor 3,4, SOX2: sex determining region Y, KLF4: kruppel-like factor 4, C-MYC: myelocytomatosis.

Table 5 Relative gene expression determined by quantitative real-time PCR between the periodontal ligament and umbilical cord.

Gene	Character	PDL (mean ± standard errors)	UBC (mean ± standard errors)
MMP13	iPS specific	7028 ± 1900	1
KLF4	iPS specific	5.01 ± 0.70	1
VCAM1	DS specific	5.32 ± 0.67	1
MCAM	DS specific	1	19.96 ± 2.16
ALCAM	DS specific	1	13.44 ± 1.33

DS: dental stemness, iPS: induced pluripotent stemness, PDL: periodontal ligament, UBC: umbilical cord, MMP13: matrix metallopeptidase 13, KLF4: kruppel-like factor 4, VCAM1: vascular cell adhesion protein 1, MCAM: melanoma cell adhesion molecule, ALCAM: activated leukocyte cell adhesion molecule.

(MYOCD), stimulator of chondrogenesis 1 (SCRG1), hemoglobin subunit beta (HBB), and myosin heavy chain 11 (MYH11), are associated with functions of the blood, cartilage, and muscle. Enriched biological process terms included "cell adhesion," "signal transduction," "response to drug," "transport," "response to stimulus," and "inflammatory response." In addition, enriched molecular function terms included "protein binding," "metal ion binding," and "zinc ion binding." These results suggest that the main UBC functions are associated with blood, cartilage, and muscle development.

We investigated seven markers of DS and four markers of iPS using microarray analysis. The expression levels of most DS markers (*stromal cell surface marker-1* (*STRO-1), integrin subunit beta 1 (ITGB1), 5′-Nucleotidase ecto (NT5E), endoglin (ENG), MCAM, and ALCAM*) in UBC tissues were equal to or higher than those in PDL tissues, except for that of *VCAM1*. In contrast, the expression levels of all iPS markers in PDL tissues were similar to or higher than those in UBC tissues. The expression of *VCAM1* among DS markers and *KLF4* among iPS markers was significantly increased in PDL tissues, whereas the expression of *MCAM* and *ALCAM* was significantly increased in UBC tissues. qPCR and IHC staining analyses confirmed these results.

In the qPCR analysis, *MMP13* exhibited enhanced expression in PDL tissues. In the microarray analysis results, among the iPS markers, the expression of *KLF4* differed the most between PDL and UBC tissues. *VCAM1* was the only DS marker that exhibited higher stemness in PDL tissues. Finally, in the microarray data, *MCAM* and *ALCAM* exhibited the biggest difference in expression between the two tissue types; the expression of these genes was more than five times higher in UBC tissues.

KLF4 is a zinc-finger transcription factor[28] involved in regulating proliferation, differentiation, apoptosis, and somatic cell reprogramming.[29] *KLF4* is highly expressed in non-dividing cells, and overexpression induces cell cycle arrest,[30] which is particularly important in preventing cell division when DNA damage occurs.[31] The high expression of *KLF4* observed in PDL tissues in this study suggests that cell division is more inhibited in the PDL than in the UBC. VCAM1 contains immunoglobulin domains.[32] In this study, the expression of *VCAM1* was higher in the PDL than in the UBC, which may indicate that the PDL exhibits higher levels of vascular activity. ALCAM were reported to exhibit upregulated expression in a cell line derived from a metastasizing melanoma.[33,34] In the present study, *ALCAM* was highly expressed in the UBC, indicating that this tissue type features more immune cell adhesion activity than the PDL. MCAM is highly expressed by cells in the vascular wall.[35,36] *MCAM* is a mesenchymal stem cell marker, and its expression may be linked to multipotency.[37,38] In this study, the expression of *MCAM* was higher in the UBC than that in the PDL, suggesting that the multipotency of the vascular wall is higher in the UBC.

In IHC staining, antibodies against MMP13, TNN, and ADAM22 were detected only in PDL tissue and were strongly stained, as expected. Although IHC was performed for all markers of DS and iPS, only MCAM exhibited a difference between PDL and UBC. MCAM is highly concentrated in the vascular endothelial cells of the UBC vein wall, suggesting

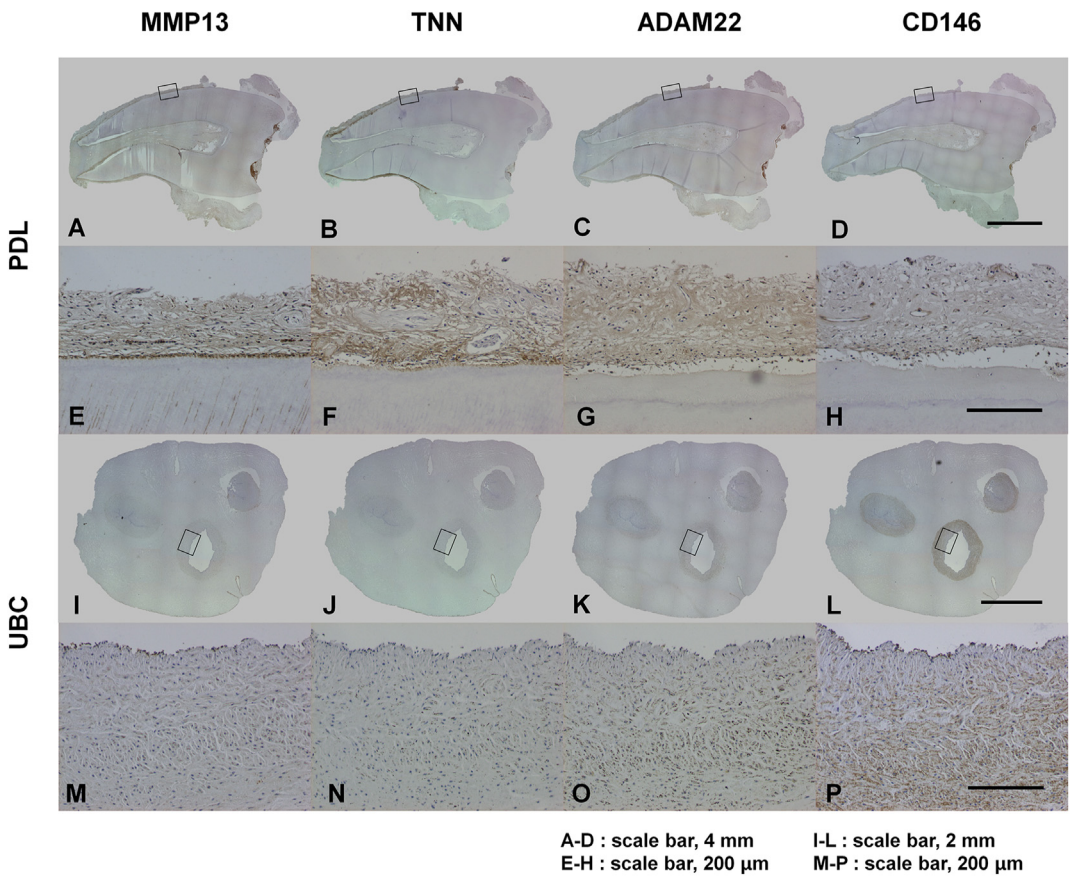

Figure 4 Immunohistochemical (IHC) staining of the umbilical cord (UBC) and permanent periodontal ligament (PDL) tissues (A—P). IHC staining for matrix metallopeptidase 13 (MMP13) in PDL (A, E) and UBC (I, M) tissues. IHC staining for tenascin-N (TNN) in PDL (B, F) and UBC (J, N) tissues. IHC staining for ADAM metallopeptidase domain 22 (ADAM22) in PDL (C, G) and UBC (K, O) tissues. IHC staining for melanoma cell adhesion molecule (MCAM, CD146) in PDL (D, H) and UBC (L, P) tissues. Scale bar: 100 μm in A—D and I—L; 20 μm in E—H and M—P.

stem cells originate form blood vessels.[39] Aside from the differences observed in MCAM, the absolute amounts of the markers might be too small for successful IHC staining. Through elaborated method, comparative studies with other UC stem cells, such as Wharton's jelly will be needed.

Although both PDL and UBC are known for their good regenerative ability, a cryotome-based rapid thawing method developed in the present study is clinically meaningful because rapid degradation of RNA at room temperature makes the use of PDL and UBC in the clinical practice. Because this method has not been widely used in previous studies, further studies are needed to confirm the effectiveness of this method in the clinical setting.

In conclusion, this study presented comparative gene expression data for PDL and UBC tissues. Because slightly different expression patterns of genes related to stemness were observed between PDL and UBC tissues, further studies are needed to use these tissues for future regeneration and implantation therapies.

Declaration of competing interest

The authors report no conflicts of interest related to this study.

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