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Cytokine expression of stem cells originating from the apical complex and coronal pulp of immature teeth

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Cytokine expression of stem cells originating from the apical complex and coronal pulp of immature teeth

Directed by Professor Jae-Ho Lee

A Dissertation Thesis
Submitted to the Department of Dentistry
and the Graduate School of Yonsei University
in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Dental Science

Ki Hoon Joo

October 2017

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Abstract

Cytokine expression of stem cells originating from the apical complex and coronal pulp of immature teeth

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(Directed by Professor Jae-Ho Lee)

The aim of this study was to measure and compare the expression levels of cytokines from developing apical complex cells (DACCs) and dental pulp stem cells (DPSCs) of the immature tooth. DPSCs-conditioned medium (CM) and DACCs-CM were obtained from human young teeth, and 174 cytokines secreted from each CM were identified and compared. A cytokine membrane array and enzyme-linked immunosorbent assay were used to measure and compare the expression levels of the cytokines. Immunocytochemistry targeting insulin-like growth factor-1 and neurotrophin-3 was additionally performed. There were statistically significant differences in the expression

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levels of 25 cytokines between two groups: 22 and 3 were expressed more strongly in DPSCs-CM and DACCs-CM, respectively. Odontoblast-differentiation-related cytokines were more strongly expressed in DPSCs-CM, while cell-proliferation-related cytokines were more strongly expressed in DACCs-CM. Proinflammatory and anti-inflammatory cytokines were predominantly expressed in DPSCs-CM and DACCs-CM, respectively. DPSCs may exert a stronger paracrine effect than DACCs on regeneration of the dentin–pulp complex, in terms of odontoblast differentiation.

Keywords: apical complex, cytokine, DACC, dental pulp, dentin regeneration, DPSC, immature tooth

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I. INTRODUCTION

There is great interest in dental-related stem cells (DSCs) as important resources in tissue regeneration studies due to their high proliferation capacity and accessibility. DSCs can be obtained in various areas of extracted teeth such as the dental pulp, apical papilla, and dental follicle, and these are classified into dental pulp stem cells (DPSCs), stem cells from the apical papilla (SCAPs), and dental follicle precursor cells (DFPCs), respectively (Morsczeck, et al., 2008; Rodriguez-Lozano, et al., 2011). DPSCs were the first DSCs to

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be isolated and are known to have higher angiogenic, neurogenic, and regenerative potentials than conventional stem cells such as bone-marrow stem cells and adipose stem cells (Ishizaka, et al., 2012). SCAPs are a population of mesenchymal stem cells residing in the apical papilla that have demonstrated potential for root formation (Banchs and Trope, 2004; Sonoyama, et al., 2008). In addition to SCAPs, Hertwig's epithelial root sheath and follicular tissues exist as a single entity without definite boundaries and are called a developing apical complex that is known to be essential for the establishment of root/periodontal development. Since both SCAPs and DFPCs originate from the developing apical complex, they are also called developing apical complex cells (DACCs) (Xu, et al., 2009).

DSCs proliferate themselves or multiply into various cell types via a self-renewal capacity and multilineage differentiation potential. DPSCs have exhibited not only distinctive odontoblastic differentiation potential in previous *in vivo* studies but also angiogenic, osteogenic, and neurogenic activities in *in vitro* studies (Gronthos, et al., 2000; Iohara, et al., 2008). SCAPs are a novel population of multipotent stem cells with a higher proliferation potential than DPSCs (Bakopoulou, et al., 2011; Sonoyama, et al., 2008). DFPCs, another component of DACCs, have a demonstrated ability to differentiate into osteoblasts, cementoblasts, chondrocytes, and adipocytes in adequate *in vitro* environments, and are superior in expressing chondrogenesis-specific markers (Morsczeck, et al., 2005; Patil, et al., 2014).

Paracrine effect is a form of cell-to-cell communication in which a cell produces a signal

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to induce changes in nearby cells. Paracrine effects of DSCs such as cytokines and growth factors play an important role in promoting and controlling the differentiation and activation of peripheral cells. Cytokines commonly expressed in DSCs are involved in various mechanisms, such as dental development, neurogenesis, and inflammatory responses, and regeneration of the dentin–pulp complex (Nosrat, et al., 1998; Onishi, et al., 1999; Sloan and Smith, 1999; Tokuda, et al., 2002). Previous studies comparing the cell proliferation, mineralization capacity, and gene expression of DPSCs and DACCs showed that these two regions of cells play different roles (Kim, et al., 2016; Xu, et al., 2009). It can therefore be inferred that the cytokines secreted by each cell type are also different. However, there have been no comparative data published on the expression levels of cytokines from DPSCs and DACCs.

The aim of this study was to measure and compare the expression levels of cytokines from the developing apical complex and dental pulp of the immature tooth.

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II. Materials and Methods

1. Cell Culture and Preparation of DACCs- and DPSCs-Conditioned Medium

DACCs (*n*=7; three males and four females aged 4–18 years) and DPSCs (*n*=7; three males and four females aged 12–20 years) were obtained from human immature teeth, supernumerary teeth, or premolars or third molars having an immature root apex (Table 1), in accordance with guidelines approved by the Institutional Review Board of the Dental Hospital, Yonsei University (#2-2015-0021). DACCs and DPSCs were isolated by enzymatic disaggregation (Jeon, et al., 2014). In brief, the developing apical complex and pulp tissue were treated with collagenase type I (3 mg/ml; Invitrogen, Carlsbad, CA, USA) and dispase (4 mg/ml, Invitrogen) for 30 min at 37ºC and then were filtered through a 70-μm cell strainer. The DACCs and DPSCs were cultured in a cell culture medium comprising alpha-minimum essential medium (α-MEM, Invitrogen) containing 10% fetal bovine serum (FBS, Invitrogen), 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen), 2 mM L-glutamine (Invitrogen), and 10 mM L-ascorbic acid (Sigma-Aldrich, St Louis, MO, USA) at 37ºC in 5% CO_2 (Hilkens, et al., 2013; Jeon, et al., 2014; Xu, et al., 2009). The obtained DACCs and DPSCs were seeded, and after reaching 80% confluence they were washed and then cultured in a culture medium without FBS. After 48 hours, conditioned medium (CM) was collected and a protease inhibitor cocktail (Roche Diagnostic Systems, Branchburg, NJ, USA) was added.

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Table 1. Donor information of DACCs and DPSCs

Type of sample	Type of teeth	Age	Sex
DPSCs	Supernumerary tooth 1	6	Male
	Supernumerary tooth 2	4	Male
	Premolar 1	12	Female
	Premolar 2	10	Female
	Third molar 1	17	Female
DPSCs, DACCs	Premolar 3*	13	Female
	Third molar 2*	18	Male
DACCs	Premolar 4	12	Female
	Premolar 5	12	Male
	Premolar 6	13	Female
	Third molar 3	18	Female
	Third molar 4	18	Male

*Premolar 3 and third molar 2 are teeth that simultaneously extract DPSCs and DACCs.

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2. Cytokine Profiles of DACCs-CM and DPSCs-CM and Data Analysis

A human cytokine array (C2000, RayBiotech, Norcross, GA, USA) was used to identify and compare 174 cytokines secreted from each CM according to the manufacturer's instructions. Three independent samples of each CM were analyzed, and α-MEM containing 0.1% FBS and α-MEM without FBS were used for positive and negative controls, respectively. Signal intensities were quantified with Gel Quant Pro (DNR Bio-Imaging Systems, Jerusalem, Israel), and the intensities of the same cytokine from different samples were compared. For normalization, the average of the negative control spot intensities was subtracted from the measured intensity of each cytokine (duplicate spots) on the same membrane, including the positive control spot. Each experiment was repeated three times, and cytokines were selected that had statistically significant values for three averages. These cytokines were sorted according to their ratio and biological function based on previous studies, with a focus on studies of cytokine functions related to dental tissues or stem cells (Tables 2 and 3) (Cole, et al., 1998; Davideau, et al., 1995; Flaster, et al., 2007; Hutterer, et al., 2008; Imai, et al., 1997; Kaushansky, 2006; Konermann, et al., 2013; Krzysiek, et al., 2000; Kunkel, et al., 1991; Mizuno, et al., 2007; Nakashima, et al., 1994; Nosrat, et al., 1998; Ollmann, et al., 1997; Onishi, et al., 1999; Sawa, et al., 1998; Schwartzkopff, et al., 2002; Sloan and Smith, 1999; Tokuda, et al., 2002; Vicari, et al., 1997; Wang, et al., 1998; Yang, et al., 2012; Yasuda, et al., 2008; Yoshida, et al., 1996).

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Table 2. Up-regulated cytokines in developing apical complex cells (DACCs)-conditioned medium (CM) compared to dental pulp stem cells (DPSCs)-CM

Cytokine	DACCs	DPSCs	Ratio (DACCs/DPSCs)	Biological function
IGF-1	19.75	6.59	3.00	Cell proliferation (Onishi, et al., 1999)
IL-10	7.66	2.83	2.70	Immunosuppression (Tokuda, et al., 2002)
IGFBP-6	62.74	44.05	1.42	Cell proliferation (Konermann, et al., 2013)

Abbreviations: IGF, insulin-like growth factor; IL, interleukin; IGFBP, insulin-like growth factor binding protein.

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Table 3. Up-regulated cytokines in DPSCs-CM compared to DACCs-CM

Cytokine	DACCs	DPSCs	Ratio (DPSCs/ DACCs)	Biological function
NT-3	0.01	4.64	578.05	Odontoblast differentiation (Mizuno, et al., 2007), neurogenesis (Nosrat, et al., 1999)
BMP-4	0.02	9.45	489.94	Odontoblast differentiation (Nakashima, et al., 1994)
MIP-1δ	0.07	2.39	35.87	T-cell and monocyte chemotaxis (Wang, et al., 1998)
NAP-2	0.41	9.06	22.23	Neutrophil chemotaxis (Schwartzkopff, et al., 2002)
TGF-β3	1.02	16.69	16.38	Odontoblast differentiation (Sloan, et al., 1999)
TGF-β1	1.51	16.33	10.80	Odontoblast differentiation (Sloan, et al., 1999)
MIP-3α	0.62	6.17	9.91	T-cell and B-cell chemotaxis (Krzysiek, et al., 2000)
TNF-α	2.32	13.42	5.78	Proinflammatory, cell apoptosis (Kunkel, et al., 1991), induces odontogenic differentiation (Yang, et al., 2012)
TNF-β	2.94	11.28	3.83	Proinflammatory, cell apoptosis (Kunkel, et al., 1991), induces odontogenic differentiation (Yang, et al., 2012)
ICAM-1	15.57	26.70	1.72	Leukocyte immigration, cell adhesion (Sawa, et al., 1998)
NT-4	31.00	51.37	1.66	Odontoblast differentiation (Mizuno, et al., 2007), neurogenesis (Nosrat, et al., 2014)
I-TAC	37.98	62.28	1.64	T-cell chemotaxis (Cole, et al., 1998)

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TARC	20.06	32.79	1.63	T-cell chemotaxis (Imai, et al., 1997)
Axl	16.65	24.98	1.50	Cell growth, proliferation (Hutterer, et al., 2008)
THPO	45.39	65.21	1.44	Regulates the differentiation of megakaryocyte and platelet (Kaushansky, et al., 2006)
TECK	70.03	96.97	1.38	T-cell development, thymocyte and macrophage chemotaxis (Vicari, et al., 1997)
Acrp-30	15.80	21.63	1.37	Odontoblast differentiation (Yasuda, et al., 2008)
ICAM-3	29.14	38.72	1.33	Leukocyte immigration, cell adhesion (Sawa, et al., 1998)
EGFR	24.66	31.04	1.26	Cell proliferation, odontogenesis (Davideau, et al., 1995)
AgRP	30.70	38.38	1.25	Increase appetite, decrease metabolism (Ollmann, et al., 1997)
XCL-1	68.72	83.46	1.21	T-cell chemotaxis (Yoshida, et al., 1996)
MIF	80.23	90.25	1.12	Regulation of macrophage function (suppression of anti-inflammatory effects) (Flaster, et al., 2007)

Abbreviations: NT, neurotrophin; BMP, bone morphogenetic protein; MIP, macrophage inflammatory protein; NAP, neutrophil-activating peptide; TGF, transforming growth factor; TNF, tumor necrosis factor; ICAM, intercellular adhesion molecule; I-TAC, interferon-inducible T-cell alpha chemoattractant; TARC, thymus and activation-regulated chemokine; Axl, Tyrosine-protein kinase receptor UFO; THPO, thrombopoietin; TECK, thymus-expressed chemokine; EGFR, epidermal growth factor receptor; AgRP, agouti-related protein; MIF, macrophage migration inhibitory factor; XCL, X-C motif chemokine ligand1.

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3. Enzyme-Linked Immunosorbent Assay

The enzyme-linked immunosorbent assay (ELISA) was performed using the Mix and Match Custom ELISA Strip (Signosis, Santa Clara, CA, USA) according to the manufacturer's instructions. In brief, eight strips coated with different antibodies were incubated with DACCs-CM and DPSCs-CM for 1 hour at room temperature with gentle shaking. After the CM samples were decanted from the eight strips, the strips were washed with assay buffer three times and then incubated with diluted biotin-labeled antibody mixture for 1 hour at room temperature with gentle shaking. After washing the strips three times with assay buffer, they were incubated with diluted streptavidin–HRP conjugate for 45 minutes at room temperature with gentle shaking. After again washing the strips three times with assay buffer, they were incubated with substrate for 30 minutes at room temperature. After incubation, a stop solution was added and the absorbance at 450 nm measured within 30 minutes using a spectrophotometer (Benchmark Plus microplate spectrophotometer, Bio-Rad, Richmond, CA, USA). The quantity of each cytokine was normalized against the total protein quantity in the same lysate supernatant, as measured using a protein assay kit (Thermo Scientific Pierce BCA, Thermo Fisher Scientific, Rockford, IL, USA). The ELISA data were obtained from three independent experiments, each of which was performed five times.

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4. Immunocytochemistry

DACCs and DPSCs were cultured on coverslips (Marienfeld-Superior, Lauda-Königshofen, Germany) coated with poly-L-lysin (Sigma-Aldrich, St Louis, MO, USA). After washing with phosphate-buffered saline (PBS) (Invitrogen, Carlsbad, CA, USA), the samples were fixed with 4% paraformaldehyde (Biosesang, Seongnam, Korea). Fixed cells were permeabilized with PBST (PBS containing 0.25% Triton X-100; Bio Basic, Seoul, Korea), washed, and then incubated with 5% BSA (Sigma-Aldrich) in PBST to block nonspecific binding of the antibodies.

Cells were incubated in the primary antibody diluted in 5% BSA/PBST overnight at 4°C. The primary antibodies were a 1:1000 dilution of antihuman insulin-like growth factor-1 (IGF-1; rabbit polyclonal antibody, ab40657, Abcam, Cambridge, UK) and a 1:500 dilution of antihuman neurotrophin-3 (NT-3; rabbit polyclonal antibody, ab65804, Abcam). After washing, cells were incubated with the biotinylated secondary antibody (biotinylated antirabbit IgG, Vector Labs, Burlingame, CA, USA) in 5% BSA/PBST for 1 hour at room temperature. After washing, cells were incubated with streptavidin–HRP (Vector Labs) in 5% BSA/PBST for 30 minutes at room temperature. The color was developed using 3,3’-diaminobenzidine substrate (Abcam) and hematoxylin (Merck, Darmstadt, Germany). The coverslips were mounted with a drop of mounting medium (Vector Labs) and stored at room temperature. The negative control was stained in the same manner, but without including the primary antibody reaction procedure.

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5. Statistical Analysis

All experiments were performed in triplicate. The normality of the data was evaluated using the Shapiro-Wilk test. The *t* test and the Mann-Whitney *U* test were applied to normal and nonnormal distributions, respectively, using SPSS software (version 20.0, SPSS, Chicago, IL, USA). A probability value of $p < 0.05$ was considered to be indicative of statistical significance in all tests.

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III. Results

1. Cytokine membrane array analysis

Cytokine membrane array analysis was used to identify and compare the cytokines secreted from DACCs-CM and DPSCs-CM. In total, 174 human cytokines were analyzed in DACCs-CM and DPSCs-CM, which revealed that there were 25 strongly expressed cytokines (with a statistically significant difference in each CM): 22 and 3 were expressed more strongly in DPSCs-CM (Table 3) and DACCs-CM (Table 2), respectively.

NT-3 and bone morphogenetic protein 4 (BMP-4), which are both related to odontoblast differentiation, exhibited significantly higher expression ratios in DPSCs-CM (578.05) than in than DACCs-CM (489.94). Other cytokines related to odontoblast differentiation, such as transforming growth factor beta-1 (TGF-β1), TGF-β3, NT-4, and epidermal growth factor receptor, were only expressed in DPSCs-CM. Moreover, the inflammatory cytokines that were strongly expressed in DPSCs-CM have been confirmed to be mostly proinflammatory cytokines (Table 3).

The cytokines expressed more strongly in DACCs-CM than in DPSCs-CM were IGF-1, interleukin-10 (IL-10), and insulin-like growth-factor-binding protein 6 (IGFBP-6), with expression ratios of 3.00, 2.70, and 1.42, respectively. IGF-1 and IGFBP-6 are cytokines involved in cell proliferation, while IL-10 is a representative anti-inflammatory cytokine (Table 2).

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2. Enzyme-linked immunosorbent assay

The ELISA test was performed to confirm the protein levels of cytokine expression. TGF-β1 and tumor necrosis factor alpha (TNF-α), which are relatively strongly expressed in DPSCs-CM and known to be involved in odontoblast differentiation and proinflammatory, were selected as exemplars. TGF-β1 and TNF-α were expressed about 1.5- and 2-fold higher, respectively, in DPSCs-CM than in DACCs-CM ($p < 0.05$) (Figure 1). These results were consistent with those from the cytokine membrane arrays, in which TGF-β1 and TNF-α were expressed 10.80- and 5.78-fold higher in DPSCs than in DACCs, respectively.

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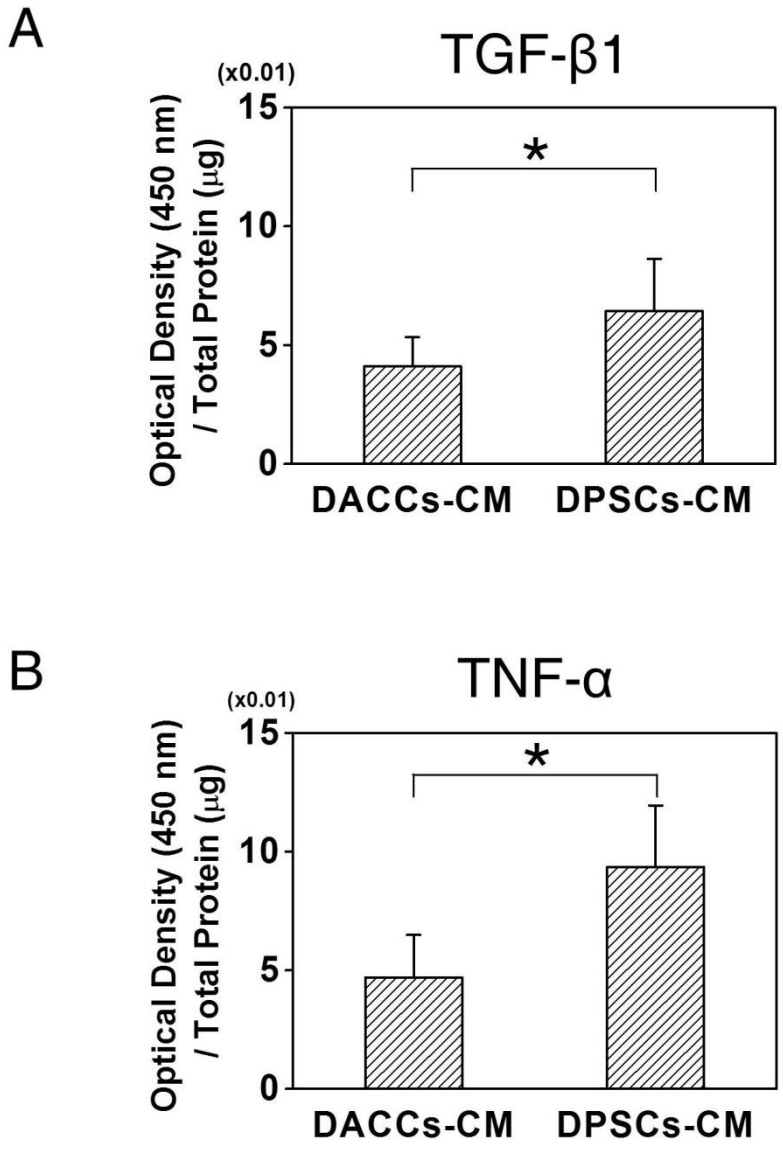

Fig. 1. Results obtained in the enzyme-linked immunosorbent assay with antibodies to transforming growth factor beta-1 (TGF-β1) and tumor necrosis factor alpha (TNF-α). The *Y*-axis indicates the optical density for the expression of each cytokine. The cytokine expression level was normalized to the amount of total protein. (A) TGF-β1 was

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expressed significantly dmore strongly in dental pulp stem cells (DPSCs)-conditioned medium (CM) than in developing apical complex cells (DACCs)-CM. (B) TNF-α was expressed significantly more strongly in DPSCs-CM than in DACCs-CM. The data are mean and standard-deviation values (*$p < 0.05$ in Mann-Whitney *U* test for TGF-β1 and *t*-test for TNF-α.).

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3. Immunocytochemistry

IGF-1 and NT-3 were stained in human DACCs and DPSCs to identify the staining characteristics of each protein. The staining patterns for IGF-1 were similar in the two cell types, but with the staining intensity being slightly higher in DACCs than in DPSCs. The cytoplasm was stained with antihuman NT-3 antibody in DPSCs but not in DACCs (Figure 2). These results are consistent with the results of the cytokine membrane arrays, in which IGF-1 was expressed 3-fold higher than in DACCs than in DPSCs, while NT-3 was expressed 578.05-fold higher in DPSCs than in DACCs.

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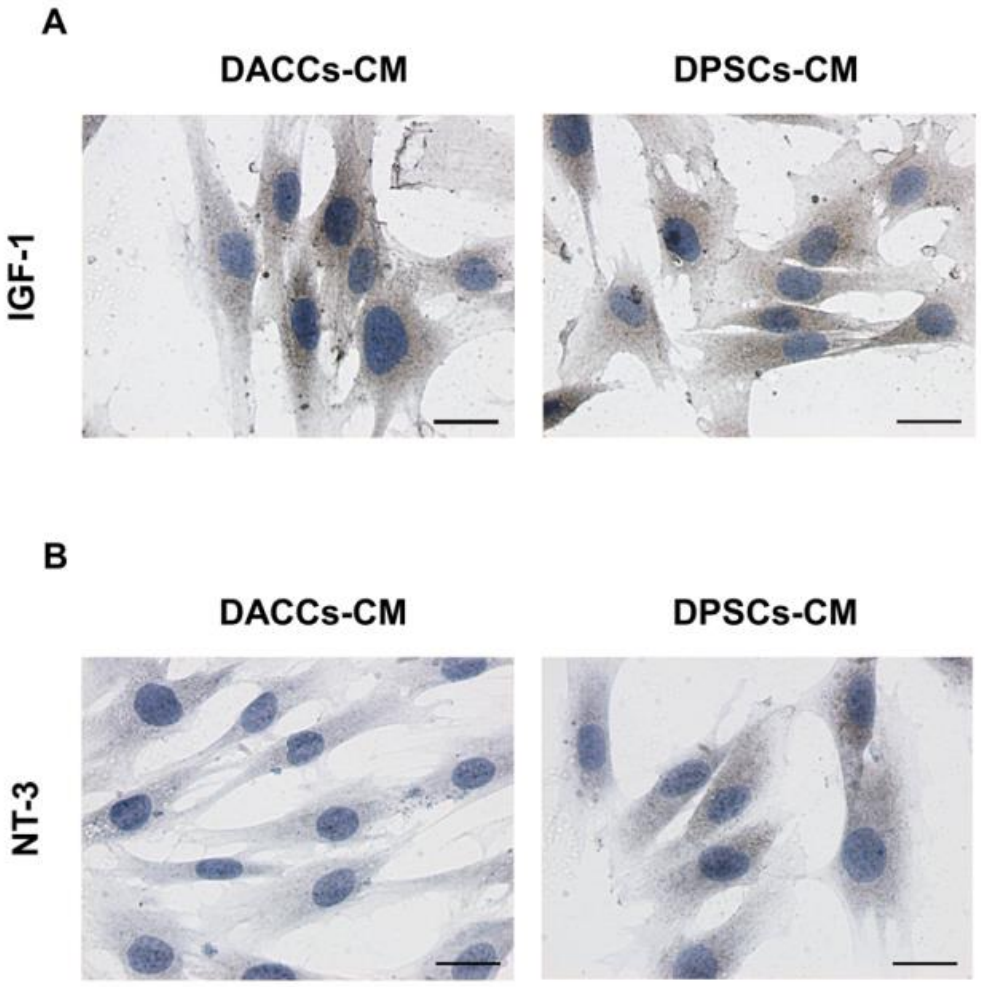

Fig. 2. Immunocytochemistry findings for insulin-like growth factor-1 (IGF-1) and neurotrophin-3 (NT-3). (A) DACCs and DPSCs immunostained with IGF-1. The antibody to IGF-1 was stained slightly more strongly in DACCs-CM than in DPSCs-CM. (B) DACCs and DPSCs immunostained with NT-3. The antibody of NT-3 was clearly stained much more strongly in DPSCs-CM than in DACCs-CM. (Scale bars = 50 μm.)

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IV. DISCUSSION

This study compared the expression levels of different cytokines using a cytokine membrane array, with additional ELISA and immunocytochemistry (ICC) tests performed to elucidate the paracrine effects of DPSCs and DACCs. High expression levels of odontoblast-differentiation-related and proinflammatory cytokines were observed in the DPSCs, whereas relatively high expression levels of proliferation-related and anti-inflammatory cytokines were observed in the DACCs.

Cytokines associated with odontoblast differentiation (NT-3, BMP-4, TGF-β1, and TGF-β3) were expressed more strongly in DPSCs than in DACCs. NT-3 is known to be a neurotrophic cytokine, and in human pulp cell culture it increased the mRNA level of dentin sialophosphoprotein, which is a phenotype marker for odontoblasts (Mizuno, et al., 2007). BMP-4 is also related to dentin formation, increasing the expression of alpha-I collagen mRNA that is essential for dentin formation in cell culture (Nakashima, et al., 1994). Likewise, reductions in mature odontoblast differentiation and dentin formation have been reported in a BMP-4-knockout mouse (Gluhak-Heinrich, et al., 2010). TGF-β1 and TGF-β3 are multifunctional cytokines belonging to the TGF superfamily that have been shown to signal the induction of odontoblast-like cell differentiation and up-regulation of their matrix secretion in the human dentin–pulp complex (Dobie, et al., 2002; Sloan and Smith, 1999). In particular, TGF-β1 is known to be an important promoter of both DPSC migration and dentin formation, and thus contributes to

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regeneration of the dentin–pulp complex (Howard, et al., 2010). The results of the present study show that cells from the dental pulp release more cytokines specific to mineralization than do cells from the apical complex. Previous studies have also shown differing calcifying potentials of the pulp and apical papilla tissues. Reparative dentin formation associated with odontoblast-like cell differentiation or osteodentin formation was found in pulpal sites around the implanted dentin or bone matrix. In contrast, apical papilla cells are never able to form a calcified matrix even when they are in contact with a specific dentinoinductive influence (Tziafas and Kolokuris, 1990). A study that compared the complex gene expression in the coronal pulp and apical pulp complex in human immature teeth found that genes related to dentin mineralization were expressed more strongly in the coronal pulp than the apical pulp (Kim, et al., 2016). Taken together with the results of this study, this suggests that cytokines expressed in DPSCs provide a more suitable environment for odontoblast differentiation for the same epigenetic signals.

The secretion level of cell-proliferation-related cytokines was higher for DACCs than for DPSCs. IGF-1, which is the cytokine expressed more strongly in DACCs than in DPSCs, increases cell proliferation, alkaline phosphatase activity, and DNA synthesis in serum-free culture (Onishi, et al., 1999). IGFBP-6 serves as a carrier protein of IGF-1 and helps to prolong the half-life of circulating IGFs in all tissues (Stewart, et al., 1993). Usually IGF-1 and IGF-binding proteins are found in the undifferentiated mesenchymal component of the human dental pulp, which is strongly expressed in the apical complex during root formation (Magnucki, et al., 2013). Elevated expression of IGF-1 and IGF-

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binding proteins in the developing apical complex could be confirmed in the results of cytokine array and ICC tests in the present study. The stronger expression of proliferation cytokines observed in this study corroborates the high proliferation activity of the DACCs found in previous *in vitro* studies (Huang, et al., 2008; Sonoyama, et al., 2008; Xu, et al., 2009). Thus, the developing apical complex of the young tooth acts as a growth center via the proliferation ability of cytokines expressed in the periphery.

Proinflammatory and anti-inflammatory cytokines were predominantly expressed in DPSCs and DACCs, respectively. In addition to TNF, which is a typical proinflammatory cytokine, various chemotactic cytokines were found to be predominant in DPSCs (Table 3). Interestingly, previous studies have shown that proinflammatory cytokines, including TNF, induce the odontogenic differentiation of DPSCs (Yang, et al., 2012). In the present study, both odontoblast-differentiation-related and proinflammatory cytokines were strongly expressed in DPSCs, suggesting a link between them, but further studies are needed to clarify the exact mechanism. IL-10, which is the only inflammatory cytokine prevalent in DACCs, is known to suppress pulpal inflammation via inhibition of lipopolysaccharide (LPS) activity (Tokuda, et al., 2002). The inhibition of LPS activity in DACCs is analogous to previous reports of anti-inflammatory responses of SCAPs and DFPCs via inhibition of activated T-cell reactions and the expression of osteoprotegerin (OPG), respectively (Ding, et al., 2010; Wise, et al., 2002). The apical complex contains both the continuous root formation of the dental papilla and the basal half of the dental follicle with compensatory bone formation due to tooth eruption (Marks and Cahill, 1987;

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Sonoyama, et al., 2008). In addition, exposure to bacterial LPS has been reported to decrease the proliferation activity of pulp progenitor cells (Nomiyama, et al., 2007). Together these findings imply that LPS inhibition and OPG expression of the apical complex reduce osteoclastic activity, thereby creating an environment that facilitates osteoblastic activity. It can be inferred that the anti-inflammatory cytokines strongly expressed in DACCs are designed to facilitate tissue development and osteogenesis in the apical complex.

This study is the first to attempt to measure and compare the expression levels of cytokines from DACCs and DPSCs of immature tooth. The findings suggest that DPSCs could provide a better environment than DACCs for regeneration of the dentin–pulp complex via the strong expression of odontoblast-differentiation-related cytokines. However, we cannot be certain that DPSCs are a better resource for dentin–pulp complex regeneration based on the analysis of cytokine expression performed in this study. The regeneration and differentiation abilities of DSCs are affected by various factors besides paracrine effects, which limits the validity of making inferences from the comparative analysis of cytokine expression alone. Moreover, multiple functions of the cytokines in dental tissue evaluated in this study remain unclear. A better understanding of the application of DPSCs and DACCs in regeneration of the dentin–pulp complex could be obtained by future investigations of the specific functions of cytokines from dental tissues.

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V. CONCLUSION

DPSCs may exert a stronger paracrine effect than DACCs on regeneration of the dentin–pulp complex, in terms of odontoblast differentiation.

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국문요약

미성숙 영구치의 치근단 복합체 및 치관부 치수 유래 줄기세포의 cytokine 발현

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이 연구의 목적은 미성숙 치아의 발달 치근단 복합체와 치관부 치수 줄기세포에서 유래한 cytokine의 발현 수준을 측정 및 비교하는 것이다. 인간의 미성숙 치아에서 획득한 치관부 치수 줄기세포 조정배지와 발달 치근단 복합체 조정배지에서 발현된 174개의 cytokine을 비교 분석하였다. Cytokine의 발현 수준을 측정하고 분석하기 위하여 cytokine membrane array와 효소면역측정법을 이용하였고, 인슐린 유사 성장인자 1과 뉴로트로핀 3를 표적으로 하는 면역세포화학법을 추가적으로 시행하였다. 25개의 cytokine에서 통계적으로 유의할만한 차이가 보였으며, 발달 치근단 복합체와 치관부 치수 줄기세포에서 상대적으로 높게 발현된 cytokine은 각각 22개, 3개로 나타났다. 치관부 치수 줄기세포에서는 상아질 모세포 분화 관련 cytokine이 강하게 발현되는

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경향이 있었고, 발달 치근단 복합체에서는 세포 증식 관련 cytokine이 강하게 발현되었다. 염증 유발 cytokine과 항염증 cytokine은 각각 치관부 치수 줄기세포와 발달 치근단 복합체에서 높게 발현되었다. 상아질 모세포 분화 측면에서 볼 때, 치관부 치수 줄기세포가 발달 치근단 복합체에 비해 상아질–치수 복합체 재생을 위한 더 강한 주변 분비작용을 수행하는 것으로 예상할 수 있다.

핵심되는 말: 치근단복합체, cytokine, DACC, 치수, 상아질 재생, DPSC, 미성숙 치아