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齒學碩士 學位論文

**Cpne7, a dental epithelium-derived factor,
regulates odontoblast differentiation via
Runx2-Nfic-Osx-Dspp pathway**

치아상피 유래인자 Cpne7의 Runx2-Nfic-Osx-
Dspp를 통한 상아모세포 분화 조절

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치의과학과 세포 및 발생생물학 전공

최 호 재

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이 논문을 치학석사학위논문으로 제출함

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**Cpne7, a dental epithelium-derived factor,
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June, 2015

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ABSTRACT

**Cpne7, a dental epithelium-derived factor,
regulates odontoblast differentiation via
Runx2-Nfic-Osx-Dspp pathway**

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Tooth development involves complex epithelial-mesenchymal interactions. In previous studies, disruption of these interactions is observed in nuclear factor I-C (*Nfic*)-deficient mouse incisors with an absence of the lingual root analog and formation of abnormal dentin along the epithelial structure in the labial crown analog. Interestingly, despite the aberrant and incomplete phenotype of dentin, the presence of dental epithelium seems to

partially rescue dentinogenesis when *Nfic* is deficient. The preceding analysis of factors released from dental epithelium yielded identification of Cpne7, a sole preameloblast-derived factor that could induce the promoter activity of the odontogenic marker dentin sialophosphoprotein (DSPP). However, its relationship with the *Nfic*-mediated odontogenic pathway is unknown.

Histologic staining and immunohistochemical analysis with Runt-related transcription factor 2 (RUNX2) and Osterix (OSX) were performed on sections of wild type (WT) and *Nfic*^{-/-} incisors. To confirm the odontogenic ability of epithelial factors, MDPC-23 odontoblastic cells were cultured in preameloblast-conditioned medium (PA-CM) obtained from mouse apical bud cells. Effects of dental epithelium of mouse incisors and molars on odontoblast differentiation were compared by culturing WT and *Nfic*^{-/-} mouse incisor and molar primary pulp cells. To investigate further the role of Cpne7 in the odontogenic signaling pathway, MDPC-23 cells were grown in PA-CM with CPNE7 Ab and human dental pulp cells (hDPCs) were treated with recombinant Cpne7 (rCPNE7). mRNA and protein expression of odontoblast-related genes were analyzed with real-time PCR and Western blot, respectively.

Histological analysis of *Nfic*^{-/-} mouse incisors showed strong expression of RUNX2 localized in abnormal hard tissue adjacent to the dental epithelium, whereas expression of OSX was not detected. Expression of *Runx2*, *Nfic*, and *Osx* was enhanced in MDPC-23 cells grown with PA-CM. Increased levels of *Runx2* and downregulation of *Osx* and *Dspp* were detected in *Nfic*^{-/-} mouse incisor pulp cells, whereas no significant change was observed in mouse molar pulp cells. Inactivation of Cpne7 in PA-CM inhibited the

stimulatory effect of PA-CM on *Runx2* and *Osx* in MDPC-23 cells. rCPNE7 treatment of hDPCs produced elevated levels of *Osx*, *Dmp1*, and *Dspp*.

These findings suggest that the dental epithelial factor Cpne7 controls odontoblast differentiation of dental mesenchymal cells by inducing the *Runx2-Nfic-Osx-Dspp* signaling pathway.

Keywords: epithelium-derived factor · CPNE7 · *Runx2* · *Nfic* · Odontoblast differentiation

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I. Introduction

Successful organogenesis requires interactions between epithelial and mesenchymal tissues. Likewise, epithelial-mesenchymal induction is necessary for tooth development (Bei, 2009; Thesleff et al., 1989). A tooth germ has an enamel organ of ectodermal origin consisting of two epithelial layers, the outer enamel epithelium (OEE) and inner enamel epithelium (IEE), which enclose stellate reticulum and stratum intermedium. The enamel organ caps neural crest-derived dental papilla, where mesenchymal stem cells lying adjacent to IEE differentiate to become odontoblasts (Nanci and Ten Cate, 2013; Thesleff and Sharpe, 1997). It has been established that differentiation of odontoblasts is induced by dental epithelium in both crown and root (Arakaki et al., 2012; Ten Cate, 1996), yet the precise molecular mechanisms of this developmental process remains unknown.

Nuclear factor I (NFI)-C, a member of NFI family that is highly conserved in vertebrates involving the development of various organs, is a transcription factor that plays an important role in tooth organogenesis (Chaudhry et al., 1997; Gronostajski, 2000). Expression of *Nfic* is detected in various stages of tooth formation, with strong expression in mesenchymal cells during the bud stage (Steele-Perkins et al., 2003). Genetic studies in dental papilla cells show that *Nfic* activates transcription of dentin sialophosphoprotein (DSPP), an odontogenic marker (Oh et al., 2012). In bone marrow stromal cells, *Nfic* is induced by the bone morphogenetic protein 2 (BMP2)-Runt-related transcription factor 2 (*Runx2*) pathway and controls Osterix (*Osx*) to promote differentiation of osteoblasts (Lee et al., 2014a). Moreover, *Osx* regulated by *Nfic* is

critical for root dentin formation (Zhang et al., 2015). Numerous studies with gene knockout animal models have been conducted to investigate the function of a gene through observation of their phenotype. Generation of *Nfic*^{-/-} mouse resulted in defective molar formation with an absence of roots (Chaudhry et al., 1997; Huang et al., 2010; Park et al., 2007; Steele-Perkins et al., 2003). Similar to the abnormal radicular structure found in *Nfic*^{-/-} mouse molars, aberrant morphology was observed in incisors of mice with a disrupted *Nfic* gene. The lingual area of *Nfic*^{-/-} incisors was open and poorly organized with missing dentin structure (Lee et al., 2009). Interestingly, incomplete hard tissue with cellular structure was present only adjacent to epithelial tissue consisting of a differentiated ameloblast layer and enamel.

Previous investigations demonstrate epithelial-mesenchymal induction pathways in tooth development incorporate several factors, including Bmp, fibroblast growth factor (Fgf), Sonic Hedgehog (Shh), and wingless (Wnt) (Steele-Perkins et al., 2003). A recent study identified Cpne7, a dental epithelial factor that induces differentiation of dental and non-dental mesenchymal stem cells into odontoblasts (Oh et al., 2015). In this study, odontogenic effects of dental epithelium on dental pulp cells were examined by using preameloblast-conditioned medium (PA-CM) as a source of epithelial factors. The results reveal that Cpne7 acts as a key dental epithelial factor that controls odontoblast differentiation by activating the *Runx2-Nfic-Osx-Dspp* signaling pathway.

II. MATERIALS AND METHODS

1. Animals, Tissue Preparation, and Immunohistochemistry

All experiments involving human cell lines were performed according to the Dental Research Institute guidelines and Institutional Animal Care and Use Committees of Seoul National University (SNU-111013-3). Mouse teeth were decalcified in 10% EDTA (pH 7.4), embedded in paraffin, and processed for immunohistochemistry. Sections were incubated overnight at 4°C with rabbit polyclonal *Runx2* and *Osx* (Santa Cruz, CA, USA) as the primary antibodies (dilutions of 1:100–1:200). Secondary anti-rabbit IgG antibodies were added to the sections for 30 min at room temperature and then reacted with the avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA, USA). Signals were converted using a diaminobenzidine kit (Vector Laboratories). Nuclei were stained with hematoxylin.

2. Primary Cell Culture and Cell Lines

The mandibles were removed from 14-day-old (P14) wild type (WT) and *Nfic*^{-/-} mice and primary pulp cell culture was conducted as described before (Couple et al., 2000; Lee et al., 2009). Briefly, after the incisors and molars were dissected out, they were cracked longitudinally using a 27-gauge needle on a 1-ml syringe. Pulp tissues were then minced to explants and placed in 60-mm culture dishes (Nunc, Rochester, NY, USA). Explants were weighed down with a sterile cover glass and cultured in Dulbecco's modified

Eagle's medium (DMEM; Gibco BRL, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco BRL) and antibiotics (Penicillin-G 100 U/ml, streptomycin 100 mg/ml, Fungizone 2.5 mg/ml, Gibco BRL). The cells were cultured at 37 °C in a humidified atmosphere containing 5% CO₂, and cells at passage 2 were used in the experiments.

Normal human impacted third molars were collected at the Seoul National University Dental Hospital (Seoul, Korea). The experimental protocol also was approved by the Institutional Review Board for Human Subjects of the Seoul National University (IRB No. S-D20140007). The experiments were performed with the understanding and written consent of each participating subject according to the Declaration of Helsinki. To isolate human dental pulp cells (hDPCs), the dental pulp tissue was separated from the pulp chamber and the root canal by cutting around the cement-enamel junction with sterilized dental fissure burs. The tissue was minced to approximately 1 mm³ and placed into 100-mm culture dishes. The explants were cultured in DMEM supplemented with 100 IU/ml penicillin, 100 mg/ml streptomycin (Gibco BRL, Carlsbad, CA, USA), and 10% FBS (Gibco BRL) at 37°C in a humidified atmosphere containing 5% CO₂.

MDPC-23 (a generous gift from Dr. J. E. Nör, School of Dental Medicine, University of Michigan, Ann Arbor, MI) cells were grown and maintained in DMEM supplemented with 10% FBS and antibiotics in a 5% CO₂ atmosphere at 37°C.

3. Preparation of CM, Recombinant Cpne7, and Inactivation of Cpne7

CM was harvested as described previously (Lee et al., 2011). Briefly, mouse apical bud cells were seeded at 7.5×10^5 cells/100 mM dish. When the cells reached 80% confluence, medium was replaced with differentiation medium. After 3 days of differentiation, the cells were washed twice with phosphate-buffered saline (PBS) and incubated in differentiation medium without FBS for 24 h. The serum-free conditioned medium was harvested and concentrated to 1 mg/ml by ammonium sulfate precipitation. Recombinant CPNE7 (rCPNE7) (NP 705900) was purchased from Origene (Rockville, MD, USA) and MDPC-23 cells were treated with rCPNE7 for 2 days (100 ng/ml). To inactivate CPNE7 in CM, 20 or 40 mg/ml anti-CPNE7 antibodies was mixed with CM for 2 h at 4 °C with rotation.

4. Real-time PCR Analyses

Total RNA was extracted from MDPC-23 cells as well as pulp tissue using TRIzol[®] reagent according to the manufacturer's instructions (Invitrogen). Total RNA (2 µg) was reverse transcribed for 1 h at 50°C with 0.5 mg Oligo dT and 1 µl (50 IU) Superscript III enzyme (Invitrogen) in a 20-µl reaction. One microliter of the RT product was PCR amplified using the primer pairs. For real-time PCR, the specific primers for *Runx2*, *Nfic*, *Osx*, *Dmp1*, and *Dspp* were synthesized as listed in Table 1. Real-time PCR was performed on an ABI PRISM 7500 sequence detection system (Applied Biosystems,

Carlsbad, CA, USA) using SYBR GREEN PCR Master Mix (Takara Bio Inc., Otsu, Shiga, Japan) according to the manufacturer's instructions. PCR conditions were 95°C for 1 min, 94°C for 15 s, and 60°C for 34 s for 40 cycles. All reactions were run in triplicate and were normalized to the housekeeping gene *GAPDH*. Relative differences in PCR results were calculated using the comparative cycle threshold (CT) method.

Table 1. Real-time PCR primer sequences

Gene name		Primer
<i>Runx2</i>	forward	5'-TTC TCC AAC CCA CGA ATG CAC-3'
	reverse	5'-CAG GTA CGT GTG GTA GTG AGT-3'
<i>Nfic</i>	forward	5'-GAC CTG TAC CTG GCC TAC TTT G-3'
	reverse	5'-CAC ACC TGA CGT GAC AAA GCT C-3'
<i>Osx</i>	forward	5'-CCC ACC CTT CCC TCA CTC AT-3'
	reverse	5'-CCT TGT ACC ACG AGC CAT AGG-3'
<i>Dspp</i>	forward	5'-ATT CCG GTT CCC CAG TTA GTA-3'
	reverse	5'-CTG TTG CTA GTG GTG CTG TT-3'
<i>Dmp1</i>	forward	5'-AGT GAAG TCA TCA GAA GAA AGT CA-3'
	reverse	5'-TAC TGG CCT CTG TCG TAG CC-3'
<i>mGapdh</i>	forward	5'-AGG TCG GTG TGA ACG GAT TTG-3'
	reverse	5'-TGT AGA CCA TGT AGT TGA GGT CA-3'
<i>hGapdh</i>	forward	5'-AGG TCG GTG TGA ACG GAT TTG-3'
	reverse	5'-TGT AGA CCA TGT AGT TGA GGT CA-3'

5. Western Blot Analyses

To prepare whole cell extracts, cells were washed three times with PBS, scraped into 1.5 ml tubes, and pelleted by centrifugation at 12,000 rpm for 2 min at 4°C. After removal of the supernatant, pellets were suspended in lysis buffer [50 mM Tris-Cl (pH 7.4), 150 mM NaCl, 1% NP-40, 2 mM EDTA (pH 7.4)] and incubated for 15 min on ice. Cell debris was removed by centrifugation. Proteins (30 µg) were separated by 10% SDS-PAGE and transferred to nitrocellulose membranes (Schleicher & Schuell BioScience, Dassel, Germany). Membranes were blocked for 1 h with 5% nonfat dry milk in PBS containing 0.1% Tween 20 (PBS-T), and incubated overnight at 4°C with the primary antibody diluted in PBS-T buffer (1:1000). Rabbit polyclonal anti-NFIC antibodies were produced as described previously (Lee et al., 2009). The anti-RUNX2 and -GAPDH antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). After washing, membranes were incubated for 1 h with anti-rabbit IgG secondary antibodies conjugated to horseradish peroxidase (Santa Cruz). Labeled protein bands were detected using an enhanced chemiluminescence system (Dogen, Cambridge, MA, USA).

6. Statistical Analysis

The data were analyzed for statistical significance using a non-parametric Mann-Whitney test.

III. Results

1. Effects of *Nfic* Disruption in Mouse Incisors

Hematoxylin and eosin staining of the WT mouse incisor cross-section showed distinct layers of well-differentiated cells forming hard tissue (Fig. 1A, C). A circular dentin surrounding pulp was lined inside by polarized, columnar odontoblasts. Enamel formed by ameloblasts was found in the labial area, which is a crown analog in mouse incisors. In *Nfic*^{-/-} incisors, incomplete and abnormal dentin was formed along with poorly differentiated odontoblasts (Fig. 1B, D). The lingual root analog was open with disrupted dentin formation and irregular hard tissue structure with embedded cellular components was found adjacent to the epithelial layer. Immunohistochemical staining was completed on sections of WT and *Nfic*^{-/-} incisors for the detection of RUNX2 and OSX, which are genes related to mineralization. Expression of RUNX2 was stronger in *Nfic*^{-/-} incisors than WT, especially within the abnormal hard tissue located next to the epithelium (Fig. 1E-H). Although WT incisors expressed OSX in differentiating pulp cells and predentin area, OSX was not detected in *Nfic*^{-/-} incisors (Fig. 1I-L).

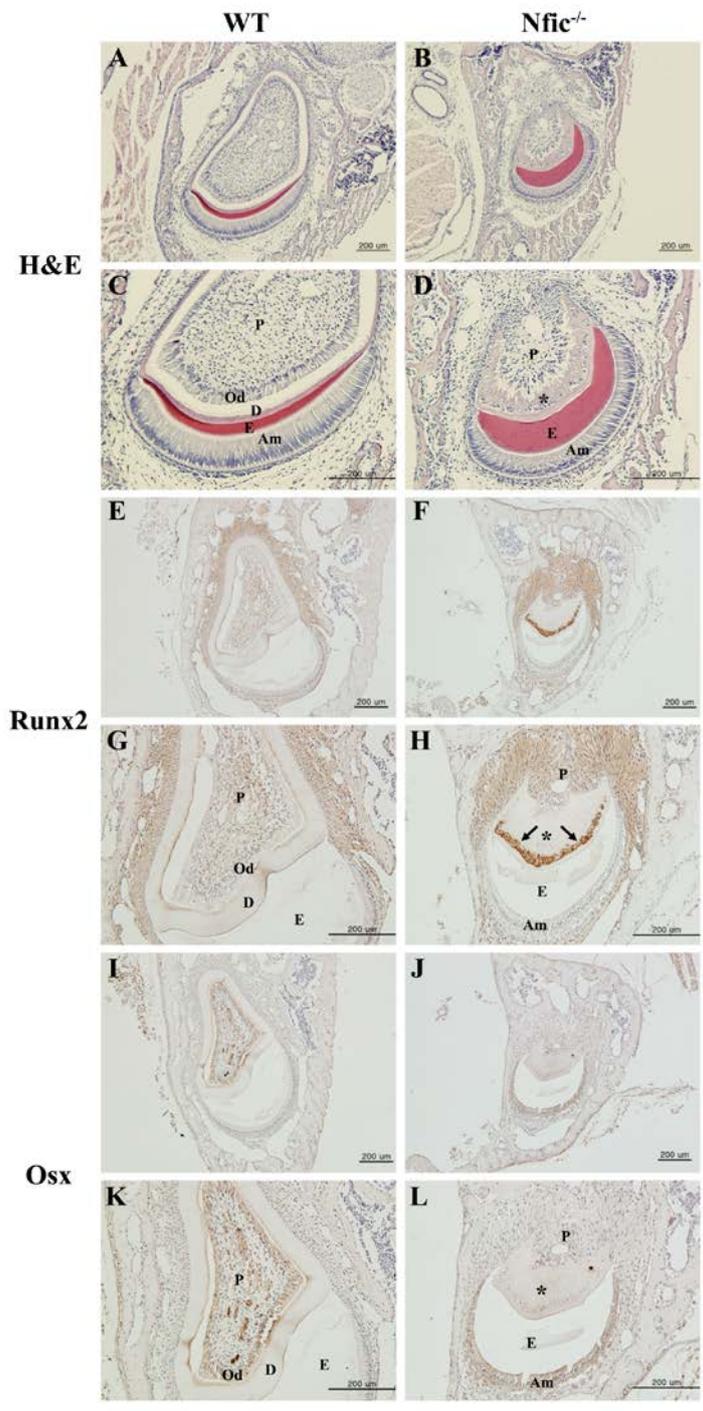


Figure 1. Light micrograph of cross-sections from wild type (WT) and *Nfic*^{-/-} mouse incisor at P13.

(A, C) In WT, a circular structure of dentin is lined inside by polarized, columnar odontoblasts and encloses pulp tissue. A layer of ameloblasts and enamel are located in the labial area. (B, D) The *Nfic*^{-/-} incisor shows disrupted dentin in the lingual area and formation of abnormal hard tissue with poorly differentiated odontoblasts. (E-H) Immunohistochemical staining shows strong expression of RUNX2 (*arrows*) in the abnormal hard tissue (*asterisk*) located next to enamel and ameloblasts in *Nfic*^{-/-} incisors. (I-L) OSX was detected in differentiating pulp cells and predentin of a WT incisor. OSX was not expressed in the *Nfic*^{-/-} incisor. *Asterisk*, abnormal hard tissue; Am, ameloblasts; E, enamel; D, dentin; Od, odontoblasts; P, pulp.

2. Expression of Runx2, Nfic and Osx in MDPC-23 Cells Induced by PA-CM

To determine effects of dental epithelial induction on odontoblast-related genes of the *Runx2-Nfic-Osx* axis, odontoblastic MDPC-23 cells were grown with PA-CM, which was obtained from mouse apical bud cell culture at day 4. Treatment of MDPC-23 cells with PA-CM significantly elevated mRNA expression of *Runx2*, *Nfic*, and *Osx* by 3.1, 6.6, and 3.3 folds, respectively, when compared to the control group (Fig. 2).

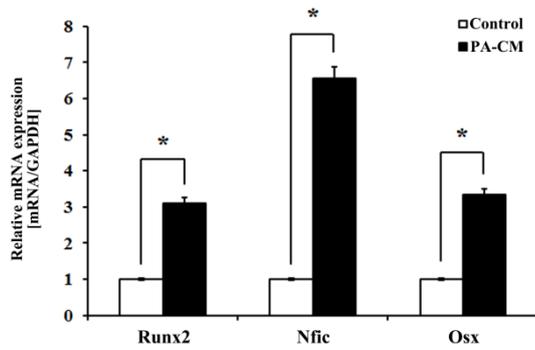


Figure 2. *Runx2*, *Nfic* and *Osx* expression in MDPC-23 cells induced by PA-CM.

After culturing MDPC-23 cells in control media or with PA-CM obtained from mouse apical bud cells, gene expression was quantified by real-time PCR. *Runx2*, *Nfic*, and *Osx* mRNA levels were 3.1, 6.6, and 3.3 folds higher, respectively, in the group treated with PA-CM compared to the control group. * denotes values significantly different from the control (P<0.05).

3. Expression of Odontogenic Markers in *Nfic*^{-/-} Mouse Incisors and Molars

Since previous studies highlight the importance of an epithelial component in odontogenesis (Arakaki et al., 2012; Ten Cate, 1996), the role of dental epithelium was investigated by comparing expression of odontogenic markers in primary pulp cells extracted from WT and *Nfic*^{-/-} mouse incisors and molars. mRNA and protein expression of Runx2 was significantly increased in *Nfic*^{-/-} incisor pulp cells (Fig. 3A, 3B). Disruption of *Nfic* down-regulated the levels of *Osx* and *Dspp*, which are downstream factors of *Nfic* (Fig. 3B). In contrast, there was no significant change in expression of odontogenic markers in *Nfic*^{-/-} molar pulp cells when compared to WT.

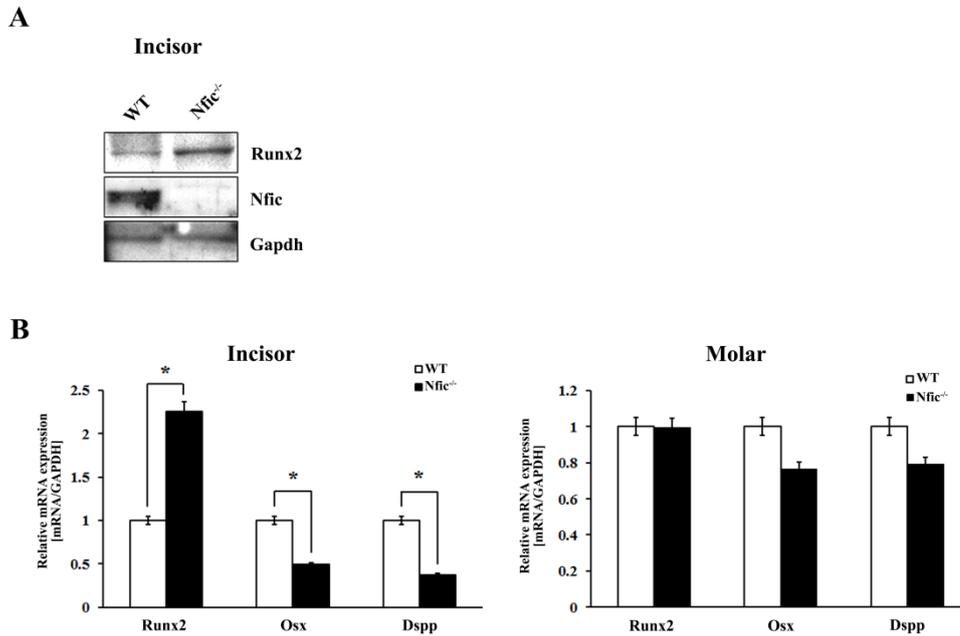


Figure 3. Comparison of odontogenic marker expression in primary pulp cells of WT and *Nfic*^{-/-} mouse incisors and molars.

(A) Western blot analysis demonstrated increased protein expression of RUNX2 in *Nfic*^{-/-} incisor primary pulp cells. (B) In *Nfic*^{-/-} incisor primary pulp cells, *Runx2* mRNA levels were increased and *Osx* and *Dspp* levels were diminished significantly. (C) No significant changes in gene expression were detected in *Nfic*^{-/-} molar primary pulp cells. * denotes values significantly different from the control (P<0.05).

4. Cpne7 Induces Expression of Odontogenic Genes in MDPC-23 cells and hDPCs

In a recent study, Cpne7 was identified as an epithelial factor that promotes odontoblast differentiation of mesenchymal cells (Oh et al., 2015). As dental epithelial factors of PA-CM could promote expression of *Runx2*, *Nfic*, and *Osx* (Fig. 2), the role of Cpne7 in the odontogenic pathway was further investigated. MDPC-23 cells were cultured in control differentiation media, PA-CM, or PA-CM treated by CPNE7-Ab, which inactivated Cpne7. Although PA-CM could up-regulate *Runx2* and *Osx* expression, inactivation of Cpne7 in PA-CM inhibited the stimulatory effect of PA-CM on *Runx2* and *Osx*, which were found close to control levels (Fig. 4A). In hDPCs, rCPNE7 treatment could significantly elevate mRNA levels of *Osx*, *Dmp1*, and *Dspp*, indicating that Cpne7 induces the *Runx2-Nfic-Osx-Dspp* pathway in dental pulp cell differentiation and mineralization (Fig. 4B).

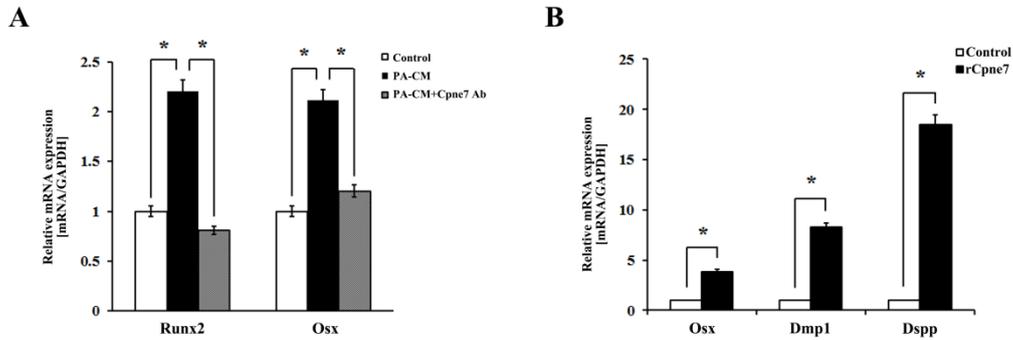


Figure 4. Effects of Cpne7 on odontogenic gene expression in MDPC-23 cells and hDPCs.

(A) Real-time PCR analysis was completed for *Runx2* and *Osx* mRNA expression in MDPC-23 cells cultured in control media, with PA-CM, or with PA-CM treated by CPNE7 Ab. Inactivation of Cpne7 in PA-CM inhibited the stimulatory effect of PA-CM on *Runx2* and *Osx* expression. (B) hDPCs treated by rCPNE7 exhibited significantly elevated mRNA levels of *Osx*, *Dmp1*, and *Dspp* compared to the control group. * denotes values significantly different from the control ($P < 0.05$).

IV. Discussion

The four members of the NFI family serve as site-specific transcription factors that are widely expressed in stem cells and regulate their proliferation and differentiation in a multitude of organs during development (Gronostajski, 2000; Harris et al., 2015). *Nfic* is reported to be highly involved in odontogenesis. *Nfic*^{-/-} mice have a dental phenotype distinct from WT mice, as gene deficiency results in molar formation without roots and reduction in incisor thickness (Steele-Perkins et al., 2003). The present study revealed *Nfic*^{-/-} mouse incisors with incomplete formation of the lingual area, which is comparable to *Nfic*^{-/-} molars lacking roots (Steele-Perkins et al., 2003). Hertwig's epithelial root sheath (HERS), an epithelial structure organized by integration of OEE and IEE, has been suggested to provide epithelial induction of odontoblast differentiation and root elongation in molars (Chen et al., 2014; Huang et al., 2010). Likewise, factors released from dental epithelium may have significance in the development of the lingual root analog. In the labial area, although hard tissue formation was incomplete with cellular components, the presence of epithelium seems to have played an important role in rescuing dentinogenesis by secreting dental epithelial factors.

Previous studies demonstrate several molecular signaling mechanisms involving *Nfic* in differentiation of mesenchymal cells. *Nfic* is recruited to the *Klf4* promoter to control both the *Dmp1-Dspp* pathway and *E-cadherin* during odontoblast differentiation (Lee et al., 2014c). In MDPC-23 cells, NFIC binds to the *Dspp* promoter and increases transcriptional activity (Oh et al., 2012). The BMP2-*Runx2-Nfic-Osx* axis is involved in

osteoblast differentiation from bone marrow stem cells (Lee et al., 2014a), and similarly, *Nfic* regulates *Osx* in tooth root dentinogenesis (Zhang et al., 2015). *Osx* promotes differentiation of pre-odontoblasts by binding to *Dmp1* and *Dspp* promoters and inducing their gene transcription (Liu and Lee, 2013). In C3H10T1/2 mesenchymal cells, 5.7-kb *Dspp* promoter activity is significantly increased by DMP1 (Gibson et al., 2013). In this study, culturing MDPC-23 cells with PA-CM resulted in enhanced expression of *Runx2*, *Nfic*, and *Osx*, which suggests that epithelial factors regulate an odontogenic pathway involving the three genes. In *Nfic*^{-/-} incisors, RUNX2 was strongly expressed in aberrant hard tissue adjacent to dental epithelium, indicating that dental epithelial factors allow compensation by elevating levels of RUNX2 in response to the absence of *Nfic* expression. Comparison of odontogenic gene expression in primary pulp cells of incisors and molars also substantiated positive effects of epithelial factors on *Nfic*-mediated dentinogenesis. *Nfic*^{-/-} incisor pulp cells showed changes in the expression of *Runx2*, *Osx*, and *Dspp*, whereas no significant difference was observed in *Nfic*^{-/-} molar pulp cells. As mouse incisors grow continuously, incisor pulp cells are exposed to constant epithelial induction from the stem cell-containing cervical loop, whereas there is limited epithelial influence in molars after crown formation (Harada et al., 1999; Harada et al., 2002; Li et al., 2015). Hence, such difference in gene expression patterns between mouse incisors and molars can be attributed to the length of period during which dental pulp cells are exposed to epithelial induction. Moreover, elevation of *Runx2* and downregulation of *Osx* and *Dspp* in *Nfic*^{-/-} incisor primary pulp cells support the hypothesis that epithelial factors induce a *Runx2-Nfic-Osx-Dspp* cascade in the differentiation of pulp cells.

Previously, various signaling mechanisms for dental epithelial-mesenchymal interaction have been proposed. BMP4 secreted from rat dental epithelium promotes differentiation of multipotent dental pulp stem cells into odontoblasts (Arakaki et al., 2012). Rescue experiments with FGF releasing beads demonstrate that Runx2 mediates epithelial FGF-induced expression of Fgf3 in dental mesenchyme (Aberg et al., 2004; D'Souza et al., 1999). Another study discovered phase-dependent interactions of FGF2 with BMP or WNT signaling pathways in dental pulp cells (Sagomyants and Mina, 2014). *Shh* from HERS regulates *Gli1*-induced mesenchymal *Nfic* for odontoblast differentiation (Huang et al., 2010). Nevertheless, experiments with the neurovascular bundle niche support that induction by the *Shh* signaling pathway is of glial origin rather than of epithelial origin (Zhao et al., 2014). Because secreted substances in CM acquired from culturing cells of interest can be identified further as biomarkers with specificity (Dowling and Clynes, 2011), proteomic analysis of PA-CM obtained from mouse apical bud cells led to identification of Cpne7, the only factor that could increase *Dspp* promoter activity in MDPC-23 cells (Lee et al., 2011). As mentioned previously, other factors including BMP, FGF, SHH, and WNT also participate in dental epithelial-mesenchymal interactions, yet the precise relationship between these factors and Cpne7 is undetermined and requires further studies. The present study demonstrated that inactivation of Cpne7 in PA-CM suppressed the up-regulatory effect of *Runx2* and *Osx* by PA-CM, indicating that Cpne7 is an essential dental epithelial factor that drives odontoblast differentiation.

In summary, this study substantiates that dental epithelial factors play a crucial role in regulation of odontoblast differentiation through an *Nfic*-mediated pathway. Moreover,

our results revealed that *Cpne7* activates the *Runx2-Nfic-Osx-Dspp* axis in the course of dental pulp cell differentiation. With these findings, future investigations on *Cpne7* may offer an insight into the molecular characterization of epithelial-mesenchymal interactions during odontogenesis.

V. References

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VI. ABSTRACT IN KOREAN

치아상피 유래인자 Cpne7의 Runx2-Nfic-Osx-Dspp를 통한 상아모세포 분화 조절

치아 발생은 복잡한 상피-간엽 상호작용에 의하여 일어난다. 이전 연구에서 Nfic^{-/-} 쥐의 전치 내 상피-간엽 상호작용의 중단으로 인하여 설측(뿌리) 부분은 아무것도 형성되지 않았고 순측(치관) 부분에 비정상적인 상아질이 치아 상피 구조를 따라 형성된 것을 관찰할 수 있었다. Nfic 유전자 결손 시 비록 상아질의 형태가 비정상적이지만 치아 상피의 존재가 부분적으로 상아질 형성을 회복시키는 것으로 보인다. 선행 연구의 치아 상피 유래 인자들의 분석을 통해 상아질 형성 표지자 Dspp를 유도할 수 있는 유일한 인자인 Cpne7을 발견하였으나, Cpne7에 의한 Nfic 매개 상아모세포 분화 기전에 관해서는 아직 알려지지 않았다. 본 연구에서는 Nfic^{-/-} 쥐 모델을 통하여 Cpne7을 포함하는 치아 상피 인자들의 역할 및 치아 간엽 세포의 분화와의 관계를 알아보고자 한다.

정상과 Nfic^{-/-} 쥐 전치의 구조와 유전자 발현 패턴을 알아보기 위하여 H&E 염색과 Runx2 및 Osx의 면역조직화학분석을 시행하였다. 상피 인자들의 치형성 능력을 평가하기 위하여 MDPC-23 세포를 ALC

법랑모세포주로부터 얻은 법랑모세포 조건 배지 (PA-CM)로 배양하였다. 정상과 $Nfic^{-/-}$ 쥐의 전치와 구치로부터 얻은 치수 세포를 분화 배지에서 배양하여 전치와 구치의 치아 상피의 영향을 비교하였다. 상아모세포 분화 신호전달 기전에서 상피 인자 $Cpne7$ 의 역할을 알아보기 위하여 PA-CM에서 $Cpne7$ 항체를 처리하여 $Cpne7$ 을 불활성화 시킨 배지를 사용하여 MDPC-23 세포를 배양하고, 사람치수 세포를 재조합 $Cpne7$ 단백질로 처리하여 보았다. 상아모세포 관련 유전자의 mRNA와 단백질 발현을 Real-time PCR과 Western blot으로 분석하였다.

$Nfic^{-/-}$ 쥐 전치의 조직학적 분석 시 치아 상피에 인접한 비정상 경조직에서 $Runx2$ 단백질의 강한 발현이 관찰되었으나, Osx 는 관찰되지 않았다. PA-CM과 함께 배양된 MDPC-23 세포에서 $Runx2$, $Nfic$, Osx 의 mRNA가 증가되었다. $Nfic^{-/-}$ 쥐 전치 치수 세포의 분화 시 $Runx2$ 의 발현은 증가하고, Osx 와 $Dspp$ 의 발현은 감소하였으나, 구치 치수 세포에서는 변화가 관찰되지 않았다. MDPC-23 세포에서 PA-CM에 의해 증가된 $Runx2$ 및 Osx 는 PA-CM 내 $Cpne7$ 의 불활성화로 인하여 그 효과가 억제되었고, 재조합 $Cpne7$ 단백질은 사람치수 세포에서 Osx , $Dmp1$, $Dspp$ 의 발현을 촉진하였다. 결과적으로 치아 상피 유래 인자 $Cpne7$ 은 $Runx2$ - $Nfic$ - Osx - $Dspp$ 신호전달 체계를 통하여 치아 간엽 세포의 상아모세포로의 분화를 조절하는 것을 알 수 있다.

주요어 : 치아상피 유래인자 · CPNE7 · Runx2 · Nfic · 상아모세포 분화

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