[UCI]I804:24011-200000235859

2008년 2월
석사학위논문

Disturbance of the Nuclear Factor I-C Gene Induces Apoptosis in Odontoblast During Tooth Root Development

조선대학교 대학원

치의공학과

한 평 호

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Nuclear factor I-C 유전자 결핍이 치근발생 동안 세포사멸 유도

*2008*년 *2*월 일

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이 논문을 치의학 석사학위신청 논문으로 제출함.

*2007*년 *10*월 일

조선대학교 대학원

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A thesis submitted of the faculty of the Chosun University in partial fulfillment of the requirements for the degree of Master of Science in the Department of Dental Engineering

Gwangju, Korea
2008. 2.
Approved by
Professor Heung-Joong Kim
Major Advisor :

한평호의 석사학위 논문을 인준함

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*2007*년 *11*월 일

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ABSTRACT

Disturbance of the Nuclear Factor I-C Gene Induces Apoptosis in Odontoblast During Tooth Root Development

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Nfic (-/-) mice demonstrated aberrant odontoblast differentiation, abnormal dentin formation, and thus molar lacking roots. However, other tissues/organs in the body including ameloblasts appeared to be unaffected and normal. In the present study, first we observed the expression of p-Smad2/3 and TGFβ-RI in *Nfic* (-/-) mice. Second, we investigated if disruption of *Nfic* gene causes apoptosis of odontoblasts. Third, we examined to uncover the molecular mechanism of apoptosis in *Nfic* (-/-) odontoblasts. Initial studies demonstrated that disturbance of the *Nfic* gene increased both TGFβ-RI and p-Smad2/3 expression in aberrant odontoblasts and pulp cells in the sub-odontoblastic layer. Analysis of apoptotic cells in the sub-odontoblastic layer of the pulp in

Nfic (-/-) mice exhibited an increased apoptotic activity. Further, *Nfic* (-/-) primary pulp cells increased not only the expression of Fas and FasL but also the activation of caspase-8 and -3, while the cleaved form of Bid was hardly detected. These findings indicate that NFI-C plays important roles in regulation of differentiation and apoptosis in odontoblasts during tooth root formation.

I. INTRODUCTION

Tooth development is a complex and well-coordinated developmental process that is achieved through a series of reciprocal interactions between dental epithelium and neural crest-derived ectomesenchyme (EM). The dental epithelium gives rise to the outer and inner enamel epithelium from which ameloblasts differentiate. On the other hand, EM cells differentiate into odontoblasts. The critical roles of some transcription factors and growth factors in crown formation have been relatively well documented (1, 2). After completion of crown formation, the inner and outer enamel epithelial cells proliferate and form Hertwig's epithelial root sheath (HERS) that plays a key role in root formation. It is believed on the basis of the information derived from crown development that HERS induces the differentiation of EM cells in the radicular pulp area into odontoblasts responsible for root dentin formation. However, the molecular mechanisms responsible for root development are not well understood (3-5).

The nuclear factor I (NFI) family of transcriptional factor/replication factors was first discovered as protein required for the replication of adenovirus DNA *in vitro* (6). The NFI gene family encodes the site-specific transcription factors essential for the development of a number of organ systems (7). There are four NFI gene family members in vertebrates (*Nfia, Nfib, Nfic,* and *Nfix*) and a single NFI gene in *Drosophila melanogaster* and *Caenorhabditis elegans* (NFI-I) (7, 8). The consequences after disruption of four Nfi genes in mice have been reported. *Nfia* (-/-) mice exhibit defects in brain development (9), whereas *Nfib* (-/-) mice show defects in lung maturation and brain development (10, 11). *Nfix* (-/-) mice reveal defects in brain and skeleton

development (12). Uniquely, *Nfic* (-/-) mice demonstrate aberrant odontoblast differentiation during root formation, short root formation and severe defects in incisors (13). However, the exact roles of NFI-C in root formation remain unknown.

Transforming growth factor-β (TGF-β), a prominent member of the TGF-β superfamily of ligands including TGF-βs, activins and BMPs, regulates a broad spectrum of biological responses in a variety of cell types (14, 15). The exposure of cells to TGF-β1 can trigger a variety of cellular responses including the cell growth arrest, differentiation and apoptosis (16, 17). Upon binding of TGF-β1 to the TGF-β receptor II (TGFβ-RII), TGFβ-RIs are heterodimerized and phosphorylated. The activated TGFβ-RI phosphorylates Smad2 and Smad3, and forms a complex with a common partner, Smad4, which translocates into the nucleus for transcriptional regulation18). During mouse tooth development, TGF-β1 has been implicated as a key mediator in odontoblasts differentiation and dentinmineralization (19). Interestingly, conditional overexpression of TGF-β1 in mouse odontoblasts under the control of the dentin sialophosphoprotein promoter with TGF-β1 cDNA revealed the same phenotypic changes as seen in *Nfic* (-/-) mice. These include the presence of aberrant odontoblasts and their entrapment in abnormal dentin (20). Further, treatment of immortalized preodontoblastic MDPC-23 cells derived from a mouse molar dental papilla (21, 22) with TGF-β1 induced the expression of Smad2, Smad3 and Smad4, and apoptosis (23).

In the present study, we investigated the expression of p-Smad2/3 and TGFβ-RI in *Nfic* (-/-) mice. Further, we investigated if disruption of *Nfic* gene causes apoptosis of odontoblasts. Finally, we examined the molecular mechanism for apoptosis in *Nfic* (-/-) odontoblasts.

II. EXPERIMENTAL PROCEDURES

1. Antibodies

An antiserum against NFI-C was produced by immunization of rabbit with the synthetic peptides (NH2)-RPTRPLQTVPLWD-(COOH) (amino acid residues 427～439 of NFI-C). Mouse monoclonal anti-Fas antibodies were purchased from R&D System and Cell Signaling Technology. All other antibodies were purchased from Santa Cruz Biotechnology.

2. Tissue preparation and Immunohistochemistry

Tissue preparation and immunohistochemistry were performed as described previously (24). Briefly, mice were cardiac-perfused with 4% paraformaldehyde-phosphate-buffered saline (PBS), and heads were removed, decalcified in a 10% EDTA (ethylenediaminetetra-acetic acid, pH 7.4) solution at 4°C, and processed for embedding in paraffin. The deparaffinized sections were immersed in 0.6% H_2O_2/methanol for 20 min to quench the endogenous peroxidase activity. They were then pre-incubated with 1% BSA in PBS for 30 min, and incubated for overnight at 4°C with the rabbit polyclonal TGFβ-RI (1:100, Santa Cruz Biotechnology) or p-Smad2/3 (1:200, Santa Cruz Biotechnology) antibodies. The sections were incubated for 1 h at room temperature with the secondary antibody, and reacted with the avidine-biotine-peroxidase complex (Vector Lab) in PBS for 30 min. After color development with 0.05% 3,3′-diaminobenzidine tetrahydrochloride, (DAB, Vector

Lab), they were counter stained with hematoxylin.

3. Primary pulp cell culture

The mandibles were removed from 17-days old wild type and *Nfic* (-/-) mice. After the incisors were dissected out, they were cracked longitudinally using a 27 G needle on a 1ml syringe. The pulp tissues were removed gently with forceps, cut into several pieces, and placed on 60 mm culture dishes (Nunc). The explants were weight down with a sterile cover glass and cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL) supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin (Gibco BRL) and 10% fetal bovine serum (FBS, Gibco BRL). The cells were cultured at 37°C in a humidified atmosphere containing 5% CO2 and cells at the passage 2 were used in the experiments. To characterize primary pulp cells, the expression of collagen type I, ALP and DSPP was assessed using RT-PCR. The genes were down-regulated in *Nfic* (-/-) primary pulp cells compared to normal (data not shown).

4. TUNEL POD staining

The apoptotic cells were detected in paraffin sections using a terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) kit (In Situ Cell Death Detection Kit, POD) according to the manufacture's instruction (Roche Molecular Biochemicals). The endogenous peroxidase in tissue sections was inactivated by incubating them for 10 min in 3% H_2O_2 before enzymatic labeling. The TUNEL POD staining was achieved by incubation with DAB

after enzymatic labeling and sections were counter-stained with methyl green.

5. Reverse transcription-polymerase chain reaction (RT-PCR) analysis

The total RNA was extracted from the primary pulp cells with the TRIzol® reagent according to the manufacture's instruction (Invitrogen). The total RNA (2㎍) was subjected to reverse transcription with 0.5㎍ of Oligo d(T) and 1㎕ (50IU) of Superscript III enzyme (Invitrogen) in a 20㎕ reaction mixture at 50℃ for 1 h. The resulting mixture was amplified by PCR. One microliter of the reverse transcription products were subjected to PCR using the following cycling conditions: 94℃, 0.5 min 55℃, 0.5 min 72℃, 1 min for 32 cycles, except FasL (38 cycles). The primer sequences used are as follows: 5′-GAC CTG TAC CTG GCC TAC TTT G-3′ and 5′-TTT CCA CCA AAA ATG CAG GCT GG-3′ for NFI-C; 5′-GAC TGC AAA ATG AAT GGG GGT-3′ and 5′-AGT GTC TGG GGT TGA TTT TC-3′ for Fas; 5′-TCA GTT TTT CCC TGT CCA TCT -3′ and 5′-TGG GGT TGG CTA TTT GCT TT-3′ for FasL; 5′-TTC GGG ATG GAG TAA ACT GG-3′ and 5′-TGG ATC CAA GGC TCT AGG TG-3′, Bcl-xL (forward and reverse). As the quantitative control, GAPDH PCR (forward 5′-ACC ACA GTC CAT GCC ATC AC-3′ and reverse 5′-TCC ACC ACC CTG TTG CTG T-3′) was also performed for 20 or 25 cycles using the same cycle profile as used for NFI-C. The PCR products were electrophoresed on a 1.2% agarose gel, stained with ethidium bromide, and visualized under ultraviolet (UV) light.

6. Western blot analysis

To prepare whole cell extracts, the cells were washed 3 times with PBS, scraped into 1.5 ml tubes and pelleted by centrifugation at 1,000 × g for 5 min at 4°C. After removal of the supernatant, the pellet was resuspended in a lysis buffer (100 mM Tris, pH 7.4, 350 mM NaCl, 10% glycerol 1% Nonidet P-40, 1 mM EDTA, 1 mM dithiothreitol, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg /ml pepstatin) and incubated for 15 min on ice. The cell debris was removed by centrifugation at 16,000 × g for 15 min at 4°C. The proteins (30 μg) were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane (Schleicher & Schuell). The membranes were blocked for 1 h with 5% nonfat dry milk in PBS containing 0.1% Tween-20 (PBS-T), washed with the PBS-T and incubated overnight with primary antibody diluted in PBS-T buffer (1:1000) at 4°C. After washing, the membranes were then incubated with anti-mouse, rabbit or goat-IgG conjugated horseradish peroxidase (Santa Cruz Biotechnology) for 1 h. Labeled protein bands were detected using an enhanced chemiluminescence system (Amersham Biosciences).

Ⅲ. *RESULTS*

1. Expression of TGFβ-RI and p-Smad2/3 was increased in Nfic (-/-) mice.

As a first step to understand the mechanism by which disturbance of the *Nfic* gene causes short root formation, the expression of TGFβ-RI and p-Smad2/3 was investigated using immunohistochemistry. The immunoreactivity for the TGFβ-RI was barely observed in odontoblasts and cells in the subodontoblastic layer of incisors from normal mice (Fig. 1A, C), while that of those cells from *Nfic* (-/-) mice was evidently increased (Fig. 1B, D). Similarly, immunoreactivity for p-Smad2/3 was barely detected in those cells of the incisors from normal mice (Fig. 1E, G), but strongly in incisors from *Nfic* (-/-) mice (Fig. 1F, H). The similar results were also found in the molars from normal and *Nfic* (-/-) mice (data not shown).

2. Apoptosis of pulp cells in Nfic (-/-) mice.

In order to examine if short root formation in *Nfic* (-/-) mice would be resulted from the apoptosis of pulp cells and odontoblasts during root development, TUNEL-POD staining in incisor histological sections from P17 normal and *Nfic* (-/-) mice was performed. Normal mice showed fewer apoptotic cells (Fig. 2A, C), whereas *Nfic* (-/-) mice demonstrated many apoptotic pulp cells, preodontoblastic cells in the sub-odontoblastic layer, and aberrant odontoblasts trapped in abnormal dentin. (Fig. 2B, D).

3. Expression of Fas and FasL were increased in primary pulp cells of Nfic (-/-) mice.

To determine if disturbance of the *Nfic* gene induces odontoblastic cell apoptosis by expressing the death receptor, the expression of Fas and FasL in primary pulp cells were assessed by RT-PCR and western blot. The level of Fas and FasL expression was higher in *Nfic* (-/-) primary pulp cells (Fig. 3A, B).

4. Apoptosis induced by caspases activation in primary pulp cells of Nfic (-/-) mice.

The expression of caspases 3 and 8, the central players of apoptosis, in *Nfic* (-/-) primary pulp cells were examined by western blot analysis. The expression of caspase-8 known as the general initiator caspase was higher in *Nfic* (-/-) primary pulp cells compared to that of control cells. The level of caspase-8 cleavage was also higher in *Nfic* (-/-) primary pulp cells (Fig. 4A). Active caspase-8 can cleave and activate procaspase-3, which leads to apoptosis. The level of caspase-3 expression was higher in *Nfic* (-/-) primary pulp cells, and cleaved caspase-3 was detected in *Nfic* (-/-) primary pulp cells (Fig. 4B).

We next examined the expression of a mitochondria-related apoptosis effector Bid that involves cleavage of the BH3-only protein Bid by caspase-8 (25). The level of Bid expression was unchanged in normal and *Nfic* (-/-) primary pulp cells, and no Bid cleavage was detected (Fig. 4C). The anti-apoptotic Bcl-2

family, including Bcl-2 and Bcl-XL, was also examined by RT-PCR and western blot analysis. The level of Bcl-XL remained unchanged, but the level of Bcl-2 was higher in *Nfic* (-/-) primary pulp cells than normal cells (data not shown). xIAP and cIAP1/2 are direct caspase inhibitors. They are known to bind to and inhibit the active caspase-3, -7, and procaspase-9 (25). Western blot analysis showed that the expression level of cIAP1/2 was lower in *Nfic* (-/-) primary pulp cells than controls (Fig. 4C).

Ⅳ. *DISCUSSION*

We previously reported that *Nfic* (-/-) mice develop short roots with aberrant odontoblasts that exhibited unique morphological features (24). Unlike normal odontoblasts, they had a round shape, and lost their cellular polarization and organization as a sheet of odontoblasts layer. Further, aberrant odontoblasts become dissociated and trapped in an osteodentin-like mineralized tissue. Interestingly, when TGF-β1 is overexpressed predominantly in odontoblasts using the transgenic construct consisting of a dentin sialophosphoprotein regulatory sequence and a TGF-β1 cDNA, the transgenic mice revealed the same phenotypic changes of odontoblasts as seen in *Nfic* (-/-) mice (20). These transgenic animal studies strongly suggest a possible functional relationship between NFI-C and Smads in odontoblasts. In the present study, *Nfic* (-/-) mice demonstrated a higher expression level of TGFβ-RI and p-Smad2/3 than normal mice. These findings led us to speculate that the increased TGF-β signaling in *Nfic* (-/-) mice may be resulted from inactivation of the *Nfic* gene, and responsible for aberrant odontoblasts and short root formation. This speculation is supported by a recent study that compared the DNA binding domains of Smads and NFI transcriptional factors (26). According to a sensitive PSI-Blast database research of the DNA binding domains of these transcriptional factors, they share significant similarities in the DNA binding domains, and may belong to a new superfamily of genes (27). The possible functional relationship between these two transcriptional factors in odontoblasts differentiation and functions during root formation is under investigation.

Apoptosis is an essential physiological process that plays a critical role in

development and tissue homeostasis (28). During tooth development, apoptosis occurs at all the stages: early tooth morphogenesis (29), amelogenesis (30), dentinogenesis (31), and tooth eruption (32). Especially, the specific temporospatial appearance of apoptotic cells during tooth development suggests its important role in odontogenesis (33). In the present study, the appearance of apoptotic cells was evident in the sub-odontoblastic region of developing roots from *Nfic* (-/-) mice, and more prominent in the area where preodontoblasts are located. However, little is known about the causes and signaling pathways responsible for odontoblasts apoptosis. In general, cell death signals are communicated through two main biochemical pathways in mammalian cells: the mitochondrial and the death receptor pathways (28). One of significant findings in this study was the up-regulated expression of Fas and FasL in both *Nfic* (-/-) primary pulp cells compared to that of their normal counterparts. Previous studies showed that TGF-β1 induces apoptosis through different signaling pathways depending on cell types. For example, TGF-β1 causes the apoptosis of MDPC-23 cells in a SMAD-dependent pathway (34), while it not only up-regulates FasL expression in endometrial cells (35), but also induces apoptosis of lung epithelial cells (36) and gilioma cells (37) in a Fas-dependent pathway. Further, it also causes apoptosis of SNU-620 cells in Smad3- and Fas-dependant pathways (38). Our findings suggest that inactivation of the *Nfic* gene appears to up-regulate the expression of TGF-β1 that induces the expression of Smad 2/3, Fas and FasL in odontogenic cells. It is, therefore, conceivable to speculate that odontogenic cells in *Nfic* (-/-) mice may undergo apoptosis in Smad3- and Fas-dependant pathways, and that would be the major contributor to short root formation.

It is well known that activated death receptors recruit adaptor molecules like

Fas-associating death domain (FADD) which recruits procaspase-8 to the receptor complex (DISC). And, the N-terminus of procasepase-8 then binds to and activates other downstream caspases such as caspase-3 and -7 (28). The caspase execution can proceed in two different pathways (25), i.e., direct cleavage or indirect effect communicated by release of mitochondrial factor (25, 28). c-FLIP resembles procaspase-8, but dose not have the active proteinase site. It can block Fas-mediated apoptosis (38). Also, IAPs (inhibitor of apoptosis, cIAP1/2 and xIAP) are direct caspase inhibitors. They all bind to and inhibit active caspase-3 and -7 that are key effectors of apoptosis (25). Recently, activated caspase-3 was detected in primary enamel knot of the filed vole (39), but little is known about the activation of other caspases such as caspase-8 and -9. In the present study, we found that the expression of caspase-3 and -8 was increased, while that of c-FLIP and c-IAP1/2 was decreased in *Nfic* (-/-) primary pulp cells. It is known that the mitochondrial apoptotic pathway is initiated through caspase-8-mediated Bid cleavage (40), but we could not detect any cleaved form of Bid. Also, even though the expression of Bcl-XL, an anti-apoptotic member of Bcl-2 family, remained unchanged, the expression of Bcl-2 was increased in *Nfic* (-/-) primary pulp cells (data not shown). These findings suggest that procaspase-3 that is cleaved directly by activated caspase-8, but not by mitochondrial apoptotic pathway, may also be involved in apoptosis of *Nfic* (-/-) primary pulp cells.

In conclusion, NFI-C appears to play an important role in differentiation into odontoblasts, and odontoblasts survival during root formation. Therefore, inactivation of the *Nfic* gene may result in short root formation.

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Ⅵ. *FIGURE LEGENDS*

Fig. 1. TGFβ-RI and p-Smad2/3 were expressed strongly in the incisors of the Nfic (-/-) mice. Expression of TGFβ-RI and p-Smad2/3 in P17 incisors from the normal and *Nfic* (-/-) mice were analyzed by immunohistochemistry. (***A-D***) TGFβ-RI positive immunoreactivity was clearly observed in the sub-odontoblast layer of the *Nfic* (-/-) mice (B, D) compared with the normal (A, C). (***E-H***) the number of p-Smad2/3 immunoreactive cells were increased in the sub-odontoblast and pulp of *Nfic* (-/-) mice (F, H) than in the normal cells (E, G). Panels C, D, G and H are higher magnifications of panels A, B, E and F, respectively. Sagittal sections. Am, ameloblasts; Od, odontoblasts; P, pulp. A, B scale bar = 50 ㎛. C, D scale bar = 20 ㎛. E, F scale bar = 100 ㎛. G, H scale bar = 20 ㎛.

Fig. 2. Disruption of the Nfic gene increased the apoptotic activity in primary pulp cells. TUNEL-POD staining of the P15 incisors from the normal and *Nfic* (-/-) mice. (***A***, ***C***) TUNEL-positive cells were detected fewer in the odontoblasts of the normal mice (Od). (***B***, ***D***) However, apoptotic cells were detected in a sub-odontoblastic location, and were more numerous in the differentiating abnormal preodontoblasts (arrows) of the root-forming area than in the crown region of the pulp of *Nfic* (-/-) mice. Panels C and D show the higher magnifications of panels A and B, respectively. Coronal sections. Am, ameloblasts; Od, odontoblasts; P, pulp. A scale bar = 100 ㎛. B scale bar = 50 ㎛. C, D scale bar = 20 ㎛.

Fig. 3. Disruption of the Nfic gene increased the expression of the Fas

receptor and Fas Ligand (FasL) in primary pulp cells. (***A***, ***B***) Evaluation of Fas, FasL mRNA (A) and protein expression (B) were analyzed by RT-PCR and western blotting, as described MATERIALS AND METHODS.

Fig. 4. Disruption of the Nfic gene induced apoptosis through the activation of caspases in primary pulp cells. (***A***) Activation of caspase-8 and (***B***) caspase-3 during apoptosis in odontoblasts. (***C***) Evaluation of the Bid and cIAP1/2 protein. The whole cell lysates were obtained from the primary pulp cells and separated by SDS-PAGE. Western blot analysis was carried out using the anti-caspase-8, anti-caspase-3, anti-Bid and anti-cIAP1/2 antibodies.

Ⅶ. *FIGURES*

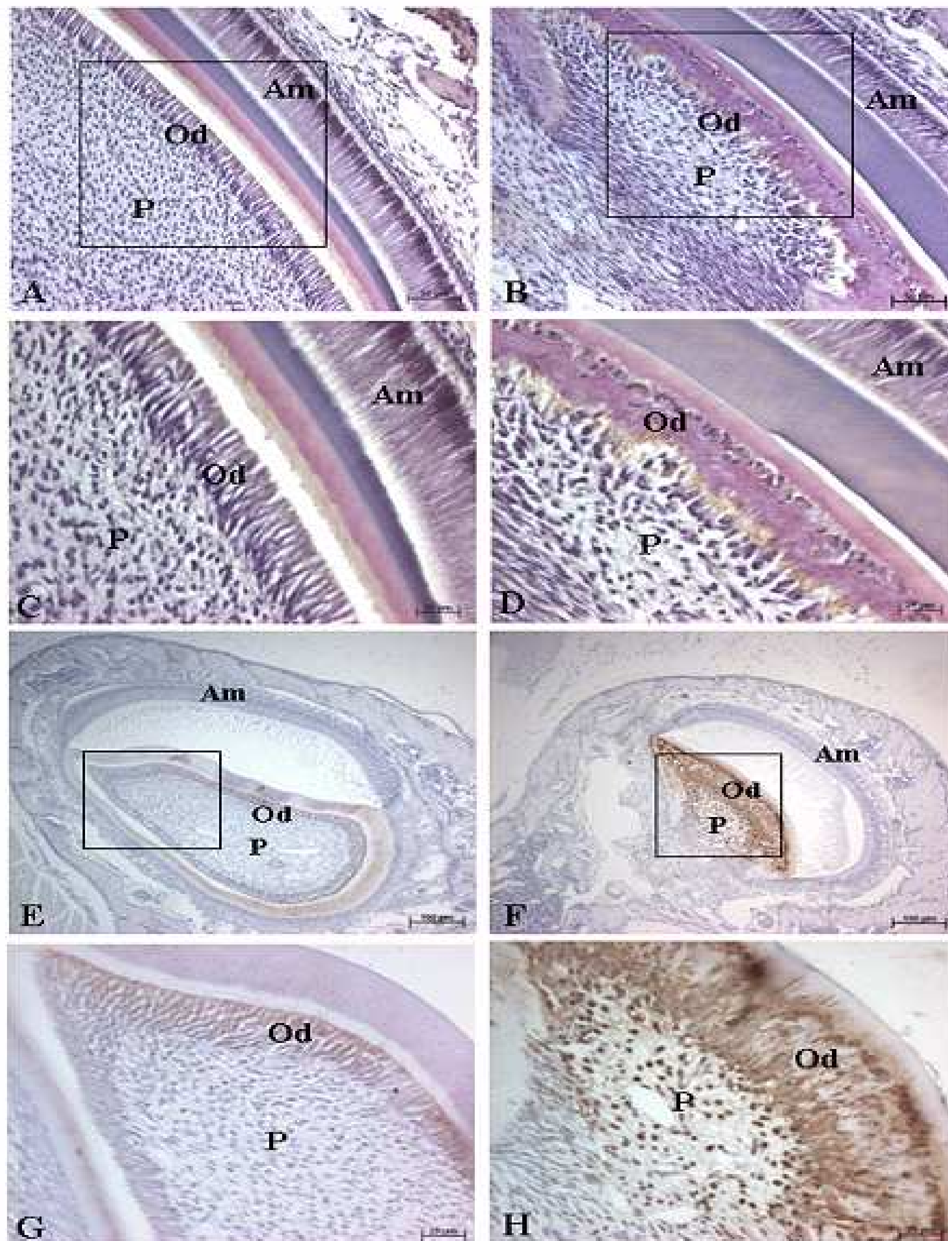

Fig. 1. TGFβ-RI and p-Smad2/3 were expressed strongly in the incisors of the *Nfic* (-/-) mice.

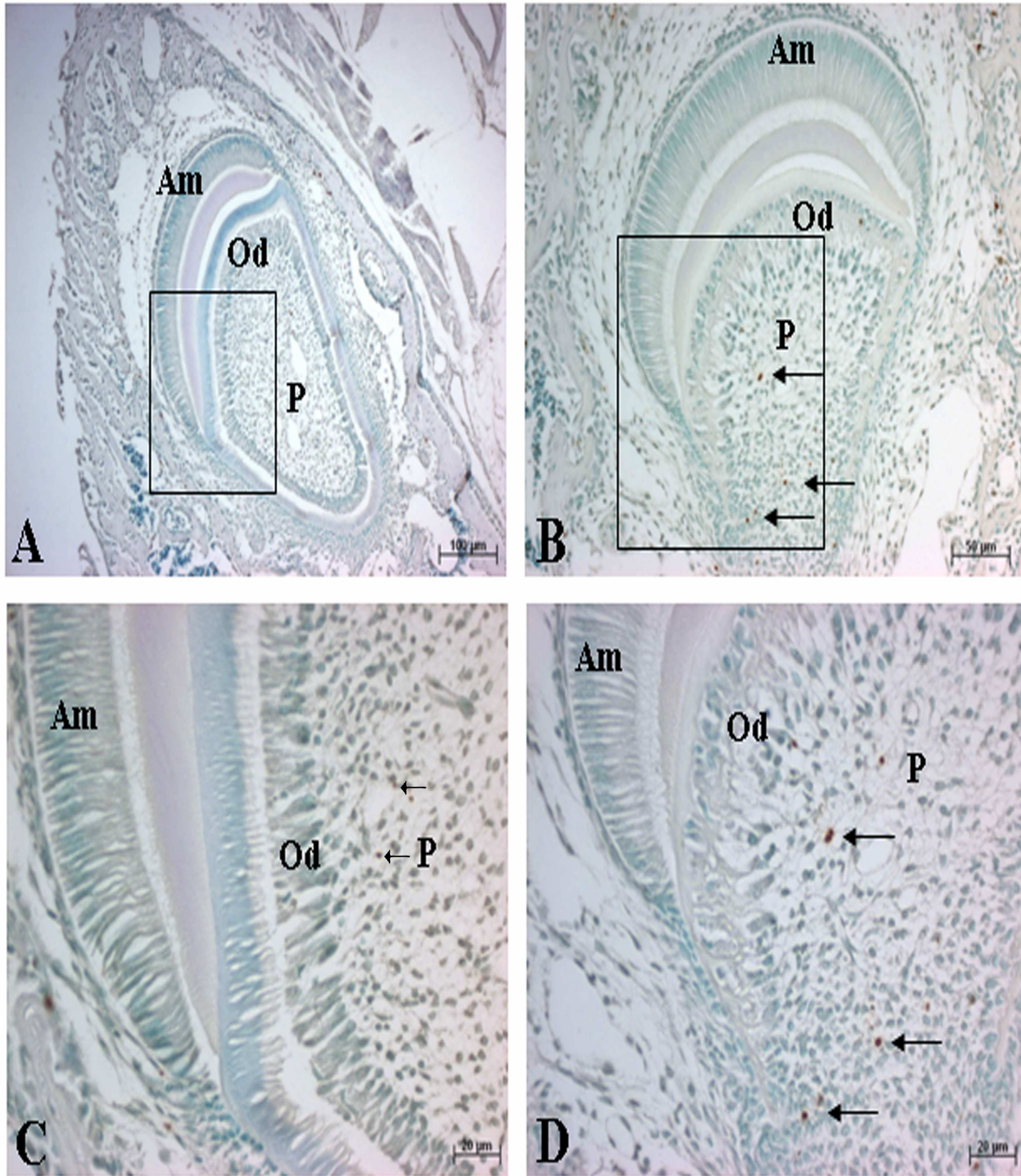

Fig. 2. Disruption of the Nfic gene increased the apoptotic activity in primary pulp cells.

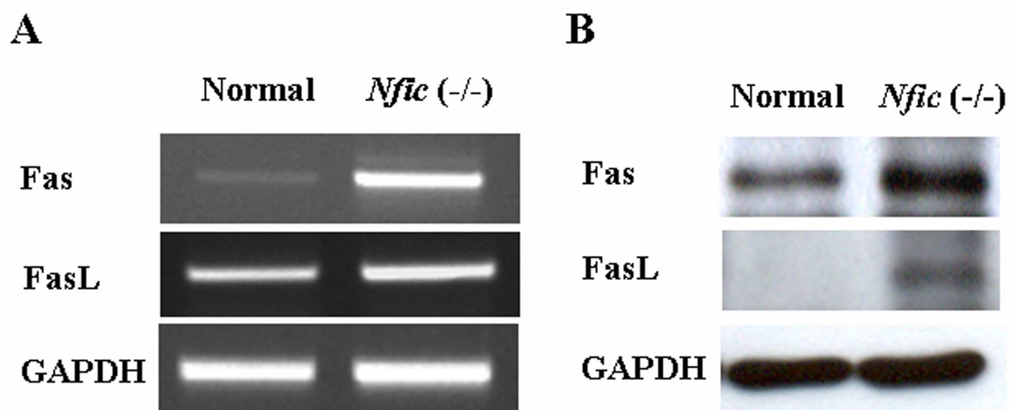

Fig. 3. Disruption of the Nfic gene increased the expression of the Fas receptor and Fas Ligand (FasL) in primary pulp cells.

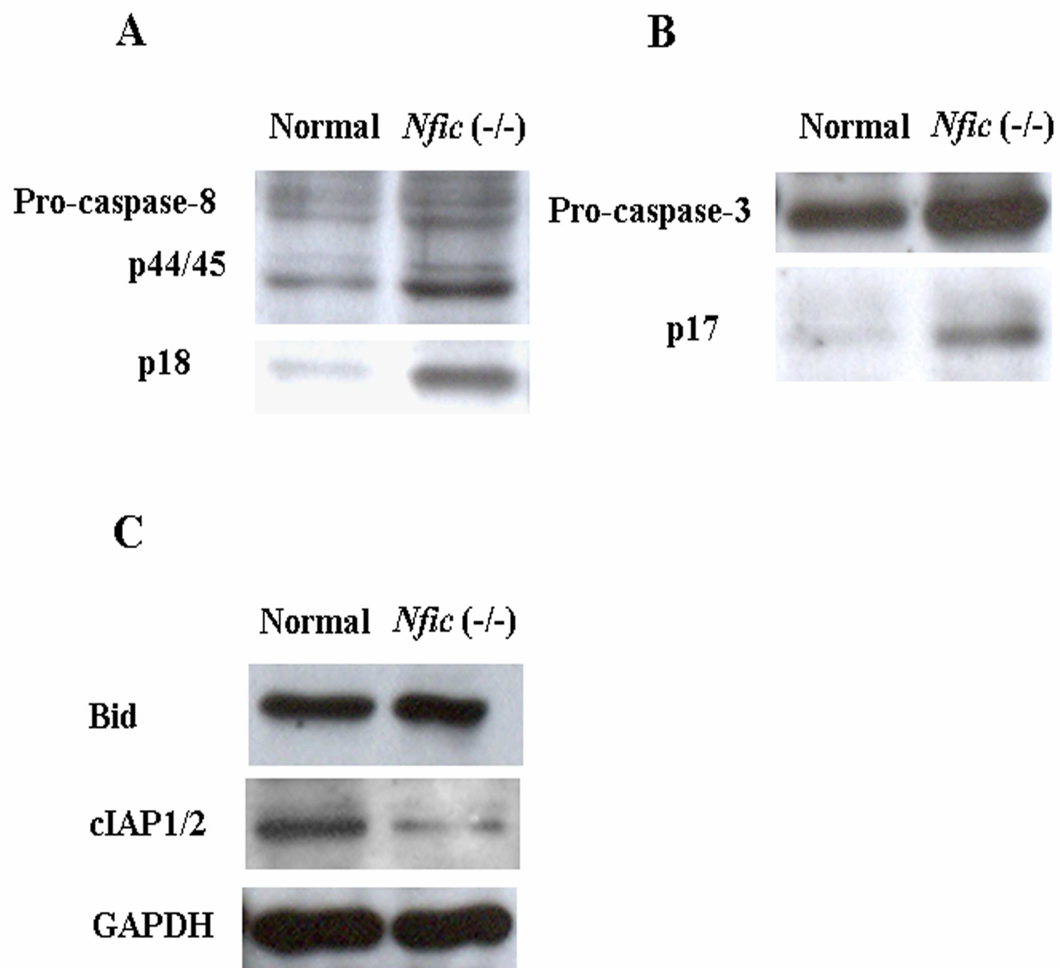

Fig. 4. Disruption of the Nfic gene induced apoptosis through the activation of caspases in primary pulp cells.

ABSTRACT in KOREAN

Nuclear factor I-C 유전자 결핍이 치근발생 동안 세포사멸 유도

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(지도교수 : 김흥중)

NFI-C는 정상적으로 상아모세포에 존재하는데, NFI-C가 없으면 치아의 치근 상아질 형성과정과 상아모세포의 분화과정에 이상을 초래하여 비장상적인 상아질이 형성되는 것으로 알려져 있다. 그러나 법랑모세포를 포함한 다른 조직에서는 영향을 나타내지 않았다.

따라서 본 연구는 NFI-C 결손 생쥐에서 비정상적인 상아질 형성이 NFI-C 결손이 TGF-β1을 과발현 시킨 결과로 나타나는지 알아보기 위하여 TGF-β1, TGFβ-R1 그리고 Smad2/3의 발현을 연구하였다. 또한 NFI-C 결손 생쥐의 비정상적인 상아모세포가 TGF-β1과 Smad 경로를 통하여 세포사멸을 유도하는지 조사하였다.

정상 생쥐에서는 치수의 상아모세포와 상아모세포밑 세포층에서 약하게 Smad2/3의 발현이 관찰되었다. 그러나 NFI-C 결손 생쥐에서는 전치부에서 상아모세포밑 세포층과 치수에서 Smad2/3의 발현이 강하게 검출 되었다. 정상 생쥐의 치아에서는 TGFβ-R1의 발현을 뚜렷이 관찰할 수 없었으나, NFI-C 결손 생쥐의

비정상적인 상아질 아래의 세포들에서 TGFβ-R1의 발현을 관찰할 수 있었다. 세포 사멸에 관여하는 세포 사멸 수용체와 ligand인 Fas, FasL의 발현은 NFI-C 결손 생쥐에서 증가하였다. 또한 이들 세포 사멸 경로에 관여하는 caspase-8과 -3의 발현과 활성이 NFI-C 결손 생쥐의 일차배양 세포에서 증가하였다. 미토콘드리아 경로를 통한 세포사멸에 관여하는 Bid는 변화를 보이지 않았으며, caspase-3 억제인자인 cIAP1/2의 발현은 NFI-C 결손 생쥐에서 감소하였다.

따라서 이들 결과들을 종합하여 볼 때 NFI-C가 생쥐의 상아모세포 분화와 세포사멸을 조절하는 중요한 인자라는 것을 시사한다.

저작물 이용 허락서

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논문제목	한글 : Nuclear factor I-C 유전자 결핍이 치근발생 동안 세포사멸 유도 영어 : Disturbance of the nuclear factor I-C gene induces apoptosis during tooth root development				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

\- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집・형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포・전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송・출력을 허락함.

동의여부 : 동의(○) 반대( )

2007 년 월 일

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