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Abnormal root formation in *Nfic*-deficient mice

조선대학교 대학원

치의학과

박민주

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Nfic-결손 생쥐의 비정상적인 치근 형성

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이 논문을 치의학 박사학위 신청 논문으로
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박 민 주

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Contents

List of figures

List of table

ABSTRACT

Abnormal root formation in *Nfic*-deficient mice

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Nuclear factor I (NFI) genes play an important role in development of the brain, lung and roots of teeth. We had reported that *Nfic*-deficient mice form normal crowns, but abnormal roots of molar teeth. However, the mechanism by which the disruption of *Nfic* gene causes abnormal root formation remains unknown.

To understand this mechanism, the root formation in *Nfic*-deficient mice was examined and compared to that of wild-type mice by morphological, immunohistochemical and in situ hybridization analyses.

Nfic-deficient mice formed normal Hertwig's epithelial root sheath (HERS), but severely disrupted odontoblast differentiation, leading to the formation of aberrant odontoblasts in the early stage of root formation. They became dissociated and polygonal in shape, lost their orientation as well as polarity, and did not express dentin sialophosphoprotein. The abnormal roots contained trapped aberrant odontoblasts, thereby resembling osteodentin in overall morphology. No osteoclasts were associated with abnormal roots. Further, the abnormal roots exhibited a decreased number of cementoblasts and cementum formation on the root surface.

The loss of *Nfic* did not interfere with the formation of HERS, but disrupted odontoblast differentiation which resulted in the formation of short and abnormal roots, and decreased cementum. This finding also suggests that root dentin is required for normal cementum formation. *Nfic*, therefore, may be a key regulator of root odontoblast differentiation and root formation.

KEY WORDS

NFI-C; odontoblast; abnormal dentin formation; osteodentin; root formation

INTRODUCTION

During tooth development, neural crest-derived ectomesenchymal (EM) cells proliferate and form dental papilla with the ability to differentiate into odontoblasts through epithelial-mesenchymal interactions[1-3]. The inner enamel epithelial cells/preameloblasts induce the differentiation of dental papilla cells into odontoblasts during crown formation, while HERS cells induce their differentiation into odontoblasts during root formation[4]. Odontoblasts are responsible for the formation of crown and root dentin that is the major structural component of the teeth. However, the mechanism responsible for root dentin and thus root formation is not clearly understood.

The nuclear factor I family of transcription proteins consists of 4 members, NFI-A, NFI-B, NFI-C and NFI-X. They are expressed from four highly conserved genes (*Nfia, Nfib, Nfic and Nfix*) in mammals. All of the NFI proteins bind to the same DNA consensus sequence with similar apparent affinities[5]. NFI proteins appear to have unique cell-type specific transcriptional modulation properties, supporting unique functions for each gene in development. For example, disruption of the *Nfia* gene primarily causes brain development defects[6,7], while loss of *Nfib* interferes with prenatal brain and lung development, indicating crucial roles in development of the brain and lung[8,9]. An additional example is related to the unique role of the *Nfic* gene in root formation that is expressed primarily in odontoblasts, but not in preodontoblasts and EM cells[9]. When we disrupted the *Nfic* gene in mice by removal of its second exon that encodes the NFI-C/CTF DNA-binding and dimerization domain, *Nfic*-deficient mice exhibited normal crown formation, but short and abnormal root formation[10]. However, other tissues/organs in the body, including osteoblasts and ameloblasts that are responsible for bone and enamel formation, respectively, appear to be normal. In the present report, we describe the mechanism by which disturbance of *Nfic* gene causes short and abnormal root formation. We found that *Nfic*-deficient mice formed morphologically normal HERS, but failed to

differentiate normal odontoblasts in the early stage of root formation, resulting in short and abnormal root formation. This animal model provides invaluable information on understanding the molecular processes responsible for root and osteodentin formation.

MATERIALS AND METHODS

Mice

The homozygous *Nfic*$^{-/-}$ knockout (*Nfic*-deficient) mice were created by removal of its second exon, which encodes the NFI-C DNA-binding and dimerization domain as previously described[10]. Knockout mice were backcrossed into the *C57BL/6* background (The Jackson Laboratory, Bar Harbor, ME) and were bred and maintained at the Laboratory Animal Facility of the Chosun University. All the animals were housed in sterile microisolators and given water and autoclaved mouse chow.

Light Microscopy

Postnatal wild type (WT) and *Nfic*-deficient mice (P7, 10, 14, 21 and 28 days) were anesthetized by i.p. injection of ketamine/xylazine and perfused with 4% paraformaldehyde in phosphate-buffered saline (PBS). The mandibles and maxillae (P28 only) were removed, further fixed for 2 hours in the same fixative, demineralized in 0.1 M EDTA containing 1% paraformaldehyde, dehydrated and processed for embedding in paraffin[10] or Epon mixture[11]. For light microscopy, 5-μm thick paraffin or 1-μm thick Epon mesio-distal or bucco-lingual sections of the mesial roots were cut. Paraffin sections were stained with hematoxylin and eosin, while Epon sections were stained with 1% toluidine blue in 0.1 M veronal acetate buffer. Sections were photographed using an Axiophot light microscope (Carl Zeiss, Thornwood, NY).

Immunohistochemistry

HERS were localized by immunohistochemical identification of keratin according to the procedures of Park et al.[12] Briefly, 5-μm thick paraffin sections were incubated for 2 hours at room temperature with rabbit anti-human keratin polyclonal antibody reacting with 56- and 64-kDa keratins (DAKO Corporation, Carpintera, CA). After

washing with PBS, keratin molecules were then localized using an avidin-biotin-peroxidase complex kit (Vector Laboratory, Burlingame, CA.)

In situ Hybridization

To investigate the differentiation of odontoblasts and cementoblasts during root formation, the expression of dentin sialophosphoprotein (DSPP) and bone sialoprotein (BSP) mRNA was assessed by in situ hybridization using digoxigenin-labeled mouse DSPP and BSP cRNA sense and anti-sense cRNA probes as we described previously[10]. Briefly, sections were depaffinized, hydrated, treated for 20 minutes with Proteinase K, washed with PBS and fixed with 4% paraformaldehyde. After washing with PBS, they were incubated with 0.2 N HCl for 10 minutes to inhibit endogenous alkaline phosphatase and acetylated by incubation in 0.1M triethanolamine containing 0.25% acetic anhydride. Hybridization was performed overnight at 55°C in hybridization solution (50% deionized formamide, 10% dextran sulfate, 1× Dehart solution, 4× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 10 mM dithiothreitol, 1 mg of yeast tRNA/ml. 1 mg of salmon sperm DNA/ml) containing the DIG-UTP-labeled Dspp or Bsp cRNA sense or antisense probes. The probes were synthesized from the plasmids containing 434 bp Bsp and 543 bp Dspp sequences using the digoxigenin-labeled RNA Labeling kit (Boehringer Manhein, Manheim, Germany). After washing for 30 minutes with 2× SSC-50% formamide and 10 minutes each in 2× SSC and 0.2× SSC at 50 min, sections were incubated first with blocking solution to remove nonspecific probe binding and then with blocking solution containing 1:500 sheep anti-DIG-alkaline phosphate Fab fragments. DSPP and BSP mRNAs were detected by incubation of the sections with substrates until color developed. Sections were counterstained with methyl green.

Measurement of Root Length

To measure root length, a total of sixteen P21 and P28 WT and *Nfic*-deficient mice

(eight each: four P21 and four P28 mice) were used. At these ages, although they were in different stages of tooth eruption, the formation of the mesial roots of the first mandibular molars was nearly or completely finished in WT mice, thereby demonstrating a comparable root length. One mesial root that was cut through the midline of the root was selected from each mouse, photographed, printed, and measured on micrographs. Data were analyzed for statistical significance using student *t-test*.

RESULTS

In order to understand the mechanism for aberrant root formation in *Nfic*-deficient mice, our study focused on HERS formation and odontoblast differentiation in the early stage of root formation.

HERS Formation (P7)

It is well known that HERS induces the differentiation of EM cells into odontoblasts responsible for root dentin formation. To determine if the failure of HERS formation in the early stage of root formation caused the formation of short and abnormal roots in *Nfic*-deficient mice, the formation and morphology of HERS were examined. Upon completion of crown formation, both WT and *Nfic*-deficient mice exhibited normal HERS formation (Fig. 1a, b). It was composed of a bilayer of inner and outer epithelial cells, and was clearly identified after immunohistochemical localization of keratin filaments (Fig. 1b, c). EM cells were located along the pulp side of HERS (Fig. 1a).

Early (P10-14) Stage of Root Formation

Next, to investigate if *Nfic* gene disruption caused aberrant odontoblast differentiation in *Nfic*-deficient mice, we examined odontoblast differentiation in the early stage of root formation. At P10, both WT and *Nfic*-deficient mice revealed short roots. The pulp side of a newly formed root of a WT mouse was covered with elongated and well-organized odontoblasts and shorter preodontoblasts (Fig. 2a), while that of an *Nfic*-deficient mouse was covered with polygonal, disorganized and abnormal odontoblasts (Fig. 2b). Further, the abnormal root contained trapped cells (Fig. 2b). However, both WT and *Nfic*-deficient mice demonstrated the presence of normal HERS at the apical end of the developing roots (Fig. 2a, b).

At P14, the root of a WT mouse was long and well developed (Fig. 3a),

whereas that of an *Nfic*-deficient moue was short and abnormal (Fig. 3c). In WT mice, the proximal portion of roots was covered with elongated odontoblasts with a highly polarized distribution of cellular organelles (Fig. 3a). They were attached each other by terminal webs (Fig. 3b). The nuclei were located in the pulpal end of the cell bodies, while the Golgi complexes were situated between the nuclei and the dentin surface (Fig. 3b). The apical region of the developing root was covered with short columnar preodontoblasts that were continuous with polygonal EM cells located along the HERS (Fig. 3b). In contrast, the short and abnormal root of an *Nfic*-deficient mouse was covered with polygonal aberrant odontoblasts without any cellular polarity (Fig. 3c) that were continuous with polygonal EM cells in association with HERS (Fig. 3c). Normal HERS remained at the apical end of roots of both WT and *Nfic*-deficient mice, although the latter appeared to have longer HERS compared to that of WT (Figs. a, c). The most significant finding was the appearance of aberrant odontoblasts in the crown where the root began to form (Fig. 3d). In this region, highly polarized odontoblasts exhibited a reversed orientation. Unlike normal odontoblasts, their nuclei were located adjacent the dentin surface, while the Golgi complexes were situated away from the dentin surface (Fig. 3d). Uniquely, abnormal roots contained trapped cells and their surface was covered with polygonal cells (Fig. 3d). These changes that occurred during the early stage of root formation in *Nfic*-deficient mice led to the formation of short and abnormal roots that resemble osteodentin.

Odontoblasts in both the crown and root of a WT molar strongly expressed DSPP mRNA, while both preodontoblasts and EM cells did not (Fig. 4a). On the other hand, the odontoblasts in the crown of an *Nfic*-deficient molar highly expressed DSPP mRNA, but aberrant odontoblast on the abnormal root surface failed to do so (Fig. 4b), suggesting that the abnormal roots of *Nfic*-deficient mice may not contain DSPP.

In WT mice, the developing roots demonstrated the presence of numerous

cementoblasts expressing BSP mRNA (Fig. 5a) and small-sized ERs that were positively stained for keratin along the root surface (Fig. 5c). On the contrary, the abnormal roots of *Nfic*-deficient mice revealed a few cementoblasts (Fig. 5b) and large-sized aggregated ERs adjacent their root surface (Fig. 5d).

Fully Erupted Molars (P28)

The first maxillary molars of both WT and *Nfic*-deficient mice were fully erupted and had normal crowns. HERS were absent in both animals (Figs. 6, 7a). In WT mice, the molar had long and fully developed roots that were housed in the alveolar bone, and the periodontal ligament between the root and alveolar bone (Fig. 6). *Nfic*-deficient mice had fully erupted crowns, and short and abnormal roots that contain trapped cells. Noticeably, the abnormal roots had the apical foramens with a large diameter (Fig. 7a) and were covered with numerous polygonal cells of an unknown origin (Fig. 7c). The roots were not associated with odontoclasts (Fig. 7c), suggesting that short roots of *Nfic*-deficient mice were not resulted from root resorption by odontoclasts. The pulp contained numerous blood vessels (Figs. 7a, b). No noticeable PDL was present between the abnormal roots and the alveolar bone (Fig. 7c).

Measurement of Root Length

Nfic-deficient mice had significantly shorter mesial roots of the mandibular molars ($p<0.05$) when compared to those of WT mice. The average root length for WT and *Nfic*-deficient mice was 0.83 and 0.19 mm, respectively (Table 1).

Table 1.

Root length in WT and *Nfic*-deficient mice

	WT mice (n=8)	***Nifc*-deficient mice (n=8)**
Root Length (mm, mean ± SD)	0.83 ± 0.06*	0.19 ± 0.08

* Statistically Significant (student *t- test*, $p < 0.05$).

DISCUSSION

Nfic-deficient mice developed normal crowns, but abnormal roots on the molar teeth as a result of aberrant odontoblast differentiation during root formation. The mechanism for this differential odontoblast differentiation seen in the molar teeth is unknown. It is well known that the crown is formed during the embryonic stage, while the root is formed postnatally. Thus, we speculate that the function of *Nfic* in postnatal stages of tooth development is responsible for root odontoblast differentiation and dentin formation during molar development, and it does not have a role in crown formation. However, we do not exclude the possible involvement of other transcription factors or signaling pathways that may define the differences in gene activities between the crown and root.

Tissue recombination studies have demonstrated that teeth develop through the epithelial-mesenchymal interaction between the dental epithelium and cranial neural EM[13]. Upon completion of crown formation, the inner and outer enamel epithelial cells proliferate apically and form HERS[14,15]. It is generally agreed that HERS has a key role in determining the shape of the root and in the induction of EM differentiation into odontoblasts responsible for root dentin formation. The formation of morphologically normal HERS in *Nfic*-deficient mice soon after crown formation strongly suggests that the aberrant odontoblast differentiation observed in *Nfic*-deficient mice may not be the result of defects in HERS. However, we do not rule out possible functional defects of HERS at a molecular level due to the loss of *Nfic*. This may include a failure in the production of growth factors or signaling molecules necessary for normal odontoblast differentiation by HERS. However, our working model is that the loss of *Nfic* gene is directly responsible for aberrant cell-autonomous odontoblast differentiation in *Nfic*-deficient mice.

As EM cells differentiate into preodontoblasts and eventually odontoblasts, they become elongated and highly polarized. They have the nuclei at the base of cells, the Golgi complex at the supranuclear region and RER at the periphery of

cell bodies. As preodontoblasts differentiate into odontoblasts, they are joined and attached at their apical end of cell bodies by well-developed terminal webs of cytoskeletal actins, and associated tight as well as adherens junctions[16-18]. This junctional complex is responsible for alignment of odontoblasts as a single layer of cells functioning as a unit, maintaining a uniformly even dentin surface, and preventing their entrapment in the predentin[19,20]. Therefore, these junctional complexes appear to play a crucial role in the formation and maintenance of smooth-surfaced predentin and dentin. More importantly, unlike osteocytes and chondrocytes, odontoblasts are not trapped in mineralized tissue (osteodentin) and are always located on the pulpal surface of the dentin. The present study revealed that *Nfic*-deficient mice have aberrant odontoblasts that were polygonal in shape. In addition, they lost their cellular polarity, orientation and arrangement as a sheet of cells, and thus are very much disorganized. Further, they were trapped in dentin-like mineralized tissue that was formed by these aberrant odontoblasts. One of the most striking morphological changes observed in these cells was the absence of intercellular junction complexes known as the terminal web. On the basis of these morphological observations, we speculate that disruption of the *Nfic* gene may cause dissociation of odontoblasts by interfering with the formation of intercellular junctions that contribute to aberrant odontoblast differentiation and abnormal dentin. Indeed, the first sign of loss of intercellular junctions between aberrant odontoblasts in *Nfic*-deficient mice was observed in the junction between the crown and the newly forming root. Here, some of highly polarized odontoblasts revealed the loss of their cellular orientation, although the HERS are formed and present at the apical end of the crown. Unlike the WT odontoblasts, they have their nuclei close to and Golgi complex away from the predentin. As the formation of roots began, EM cells lost their orientation, polarity, and attachment/connection with neighboring cells. Consequently, they were dissociated and some of them were trapped in an abnormal mineralized tissue that was formed by these aberrant cells.

As EM cells differentiate into preodontoblasts, they synthesize and secrete type I and III collagen, osteopontin and dentin matrix protein-1[21]. Further, when preodontoblasts differentiate into odontoblasts, they actively synthesize and deposit DSPP, the odontoblast/dentin marker protein[21]. With the deposition of dentin matrix the odontoblast cell bodies move away from the predentin/dentin, leaving the odontoblastic processes embedded within dentinal tubules in the predentin/dentin. Odontoblasts are responsible for formation and maintenance of the predentin and dentin. The inability of aberrant odontoblasts to express DSPP indicates that EM cells failed to differentiate into odontoblasts after disruption of *Nfic* gene. On the basis of these findings, we postulate that NFI-C transcriptional factor may be the key regulator for root odontoblast differentiation and root formation.

Nfic-deficient mice appear to offer an invaluable animal model to determine the mechanism for osteodentin formation during dentin repair. When the odontoblastic layer has been destroyed by deep caries, cells in the underlying pulp migrate to this site and differentiate into odontoblast-like cells[22] and form osteodentin that shares morphological similarities to the abnormal roots found in *Nfic*-deficient mice. These cells had no odontoblastic processes, and failed to form intercellular junctions and associated terminal webs. The lack of intercellular junctions resulted in dissociation of the round osteoblast-like cells, loss of their cellular polarity and orientation, and their organization as a sheet of cells. As a result, these cells were trapped in the mineralized tissues, resembling osteocytes. As a result, these cells deposited an irregular and disorganized dentin-like mineralized tissue in which cells become trapped and the matrix lacks dentinal tubules. We postulate that osteodentin formation during dentin repair may be the result of the involvement of pulp cells which have silent *Nfic* gene during dentin repair.

We previously proposed that during normal root formation, chemoattractant substances that may be derived from the newly formed predentin/dentin of roots may cause directed cell migration of dental follicle cells towards the

predentin/dentin surface to form cementum[11]. As these cells migrate, they differentiate into precementoblasts, and actively invade and push away into the epithelial sheet. The dissociated cells of the HERS remain as epithelial rests in the PDL. Upon arrival of precementoblasts on the dentin surface, they differentiate into cementoblasts and form acellular cementum on the root dentin surface[11]. In the present study, we found the presence of large groups of cells of the HERS and a small number of cementoblasts on the short and abnormal roots in *Nfic*$^{-/-}$ mice. These findings support the notion that normal dentin formation is required for the formation of normal acellular cementum, epithelial rests and PDL.

Short-root anomaly generally in incisors and premolars has been reported[23-25]. The prevalence in Finnish healthy young adults and European children (no older than 14 years) was 1.3%[26] and 2.4-2.7%[27,28], respectively. Interestingly, individuals without recognized syndromes revealed short root anomaly[29,30], and some appear to be inherited in an autosomal dominant transmission[31]. Currently, the etiology of short root anomaly remains unknown. It is of great interest to examine if short root anomaly particularly in those without any syndromes is caused by the defect or functional loss of *Nfic* gene as seen in *Nfic*-deficient mice.

In conclusion, the *Nfic* gene may be the key regulator for root odontoblast differentiation and root formation. Further, the *Nfic*-deficient mice are an invaluable and unique experimental animal model that allows us to determine the apparent central role of NFI-C in odontoblast differentiation particularly during postnatal root dentin and osteodentin formation.

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FIGURE LEGENDS

Figure 1. Light micrographs showing the early stage of first mandibular molar root formation and HERS in P7 WT (b) and *Nfic*-deficient mice (a, c). (a) Note the presence of a newly formed, short and normal root (R), and HERS at the apical end of the root from an *Nfic*-deficient mouse. HERS in both WT (b) and *Nfic*-deficient (c) mice demonstrate normal morphology and keratin expression by IHC. Ab, ameloblasts; AB, alveolar bone; D, dentin; E, enamel; EM, ectomesenchymal cells; Od, odontoblasts. (Original magnification ×180).

Figure 2. Light micrographs showing developing first mandibular molar roots of P10 WT (a) and *Nfic*-deficient (b) mice. At this stage, both WT (a) and mutant (b) mice have relatively short roots (R). (a) The proximal portion of the root is covered with elongated and well-organized odontoblasts (Od), while the distal portion is covered with shorter preodontoblasts (Pod). The preodontoblasts are continuous with ectomesenchymal (EM) cells in association with HERS at the apical end of the root. (b) The abnormal root of an *Nfic*-deficient mouse is covered with polygonal, disorganized and aberrant odontoblasts (Od). Further, the root contains trapped cells (arrows). However, both WT (a) and *Nfic*-deficient (b) mice show normal HERS that are associated with ectomesenchymal (EM) cells. (Original magnification ×230). Ab, ameloblasts; D, dentin; E, enamel

Figure 3. Low (a, c) and high (b, d) magnification light micrographs showing the developing first mandibular molar roots from P14 WT (a, b) and *Nfic*-deficient (c and d) mice. (a) The pulp side of the root from a WT mouse is covered with elongated odontoblasts (Od) and preodontoblasts (Pod). Ectomesenchymal (EM) cells are closely located along HERS at the apical end of the root. (Original magnification ×130). (b) Odontoblasts are highly polarized and well oriented. They are connected each other via the terminal web (TW) at the apical end, and contain

the nuclei (N) at the distal end and the Golgi complexes (G) between them. (Original magnification ×450). (c) The root (R) of an *Nfic*-deficient mouse is short and abnormal. It is covered with disorganized, polygonal and aberrant odontoblasts (arrows). HERS remain at the apical end of the root. (Original magnification ×130). (d) The odontoblasts in the junction between the crown and root are elongated and highly polarized, but some show a reversed cell orientation (black arrows). Note the nuclei close to the dentin in these disoriented odontoblasts. The root contains trapped cells (thick arrows) and is covered with polygonal cells (rectangular area). (Original magnification ×467). Ab, ameloblasts; AB, alveolar bone; D, dentin; E, enamel; PDL, periodontal ligament; R, root.

Figure 4. In situ hybridization analysis of DSPP mRNA expression in odontoblasts in the developing first mandibular molar roots from P14 WT (a) and *Nfic*-deficient (b) mice. (a) The odontoblasts (Od) in both the crown and root (R) of a WT mouse show strong expression of DSPP mRNA, while preodontoblasts (Pod) and ectomesenchymal (EM) cells do not. (Original magnification ×230). (b) The odontoblasts (Od) in the crown show strong expression of DSPP mRNA, but not in the aberrant odontoblasts on the abnormal root surface (R) of a mutant mouse. (Original magnification ×340). D, dentin; E, enamel; EM, ectomesencymal cells.

Figure 5. In situ hybridization analysis of BSP mRNA expression (a, b) in cementoblasts and osteoblasts, and immunohistochemical localization of keratin (c, d) in epithelial rests and HERS in the developing first mandibular molar roots from P14 WT (a, c) and *Nfic*-deficient (b, d) mice. (a) Note the presence of numerous cementoblasts (Cb) along the root surface (R) and osteoblasts (Ob) on the alveolar bone (AB) surface that express BSP mRNA. (Original magnification ×270). (b) Note a few cementoblasts on the root (R) surface, but many osteoblasts (Ob) on the alveolar bone (AB) surface that express BSP mRNA. (Original magnification ×380). (c) Note the presence of small groups of epithelial rests (ER) along the root surface

and short HERS at the apical end of the developing root (R) from a WT mouse. (Original magnification ×260). (d) Note a large group of aggregated epithelial rests (ER) on the root surface and longer HERS at the end of the developing root (R) from a mutant mouse. (Original magnification ×300). Ab, ameloblasts; D, dentin; E, enamel.

Figure 6. Light micrograph of the first maxillary molar from a P28 WT mouse. Note the long and fully developed first maxillary roots that are anchored to the alveolar bone (AB) through the periodontal ligament (PDL). (Original magnification ×45). AB, alveolar bone.

Figure 7. Light micrographs of the first maxillary molar from a P28 *Nfic*-deficient mouse. (a) The tooth has a normal crown, but extremely short roots with widely opened apical foramens (arrows) and many blood vessels (*) in the pulp. (Original magnification ×45). (b) High magnification of the area marked with * in figure 7a. Note the presence of numerous blood vessels (arrows). (c) High magnification of the rectangular area in Figure 7a. Note a short root that contains trapped cells (arrows) and dense connective tissue (*) between a short root and the alveolar bone (AB), but no noticeable PDL is present. (Original magnification ×180). D, dentin; E, enamel; JE, junctional epithelium

FIGURE

Abstract in Korean

Nfic-결손 생쥐의 비정상적인 치근 형성

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Nuclear factor I 유전자들은 NFI-A, NFI-B, NFI-C, 및 NFI-X로 구성되어 있으며, 뇌와 폐 그리고 치근의 발생에 중요한 역할을 하는 것으로 알려져 있다. 그 중에서 *Nfic*가 결손 되면 구치의 치관은 정상적으로 형성되나 치근은 비정상적으로 형성된다고 하였다. 그러나 *Nfic* 유전자의 결손이 비정상적으로 치근을 형성하는 기전에 관하여는 아직까지 잘 알려져 있지 않다. 이 연구에서는 이 기전을 이해하는 과정의 일환으로 *Nfic*-결손 생쥐의 치근 형성 과정의 특징을 정상적인 생쥐와 비교하여 형태학적, 면역조직화학적 및 인사이투 하이브리드 방법으로 분석하였다.

실험 결과, *Nfic*-결손 생쥐는 Hertwig 상피근초(HERS)는 정상적으로 형성되어 있었으나, 상아모세포 분화의 이상으로 초기 치근형성과정에서 비정상적인 상아모세포가 분화되었다. 비정상적인 상아모세포들은 세포들이 서로 분리되어 있었으며 다각형 형태를 보이고 방향과 극성이 상실되었을 뿐만 아니라 DSPP도 발현하지 않았다. 비정상적인 치근에는 변이된 상아모세포들이 함입되어 치근의 상아질이 골양상아질과 유사한 소견을 보였다. 비정상적인 치근에서 파골세포는 관찰 할 수 없었으나, 특이하게도 비정상적인 치근에서는 치근 표면에 백악모세포의 수와 백악질의 양이 감소되었다.

결론적으로 *Nfic* 유전자가 결손되어도 HERS의 형성은 정상적으로 이루어지지만 상아모세포의 분화 과정에 이상이 초래되어 짧고 비정상적인 치근이 형성되고 백악질의 양도 감소하였다. 이 결과는 정상적인 백악질이 형성되기 위해서는 치근 상아질이 필

요하며, *Nfic* 유전자가 치근의 상아모세포 분화와 치근형성에 중요한 역할을 함을 시사한다.

저작물 이용 허락서

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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(V) 반대( )

2007년 2월 일

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