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Excessive Wnt/β-catenin signaling disturbs tooth-root formation

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Background and Objective: Wingless-type MMTV integration site family (Wnt)/β-catenin signaling plays an essential role in cellular differentiation and matrix formation during skeletal development. However, little is known about its role in tooth-root formation. In a previous study, we found excessive formation of dentin and cementum in mice with constitutive β-catenin stabilization in the dental mesenchyme. In the present study we analyzed the molar roots of these mice to investigate the role of Wnt/β-catenin signaling in root formation in more detail.

Material and Methods: We generated *OC-Cre:Catnb*$^{+/lox(ex3)}$ mice by intercrossing *Catnb*$^{+/lox(ex3)}$ and *OC-Cre* mice, and we analyzed their mandibular molars using radiography, histomorphometry and immunohistochemistry.

Results: OC-Cre:Catnb$^{+/lox(ex3)}$ mice showed impaired root formation. At the beginning of root formation in mutant molars, dental papilla cells did not show normal differentiation into odontoblasts; rather, they were prematurely differentiated and had a disorganized arrangement. Interestingly, SMAD family member 4 was upregulated in premature odontoblasts. In 4-wk-old mutant mice, molar roots were about half the length of those in their wild-type littermates. In contrast to excessively formed dentin in crown, root dentin was thin and hypomineralized in mutant mice. Biglycan and dentin sialophosphoprotein were downregulated in root dentin of mutant mice, whereas dentin matrix protein 1 and Dickkopf-related protein 1 were upregulated. Additionally, ectonucleotide pyrophosphatase/phosphodiesterase 1 was significantly downregulated in the cementoblasts of mutant molars. Finally, in the cementum of mutant mice, bone sialoprotein was downregulated but Dickkopf-related protein 2 was upregulated.

Conclusion: These results suggest that temporospatial regulation of Wnt/β-catenin signaling plays an important role in cell differentiation and matrix formation during root and cementum formation.

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During tooth development, root formation follows crown development. At the late bell stage of tooth germ, the inner and outer enamel epithelium in the cervical loop extend apically to form Hertwig's epithelial root sheath (HERS). Mesenchymal cells in dental papilla, located inside HERS, start to differentiate into odontoblasts to produce root dentin (1). After the initiation of root-dentin formation, cementoblasts are differentiated and produce cementum, a thin mineralized tissue on the root surface, which assists in anchoring teeth to the surrounding alveolar bone (2).

While it is known that numerous growth factors and transcription factors such as sonic hedgehog, bone morphogenetic protein (BMP) and muscle segment homeodomain-homeobox 1, are extensively expressed in the dental epithelium and mesenchyme during root development (3,4), the molecular

mechanisms regulating this process are not yet fully understood. Recently, the roles of several signaling molecules in root development have been described. For instance, nuclear factor I/C, a CCAAT box-binding transcription factor, has been found to regulate odontoblast differentiation during root development (5). Additionally, SMAD family member 4 (SMAD4) and PTCH1 regulate root formation (4,6), and ablation of *Smad4* and *Ptch1* genes in the dental mesenchyme leads to disturbance of root formation. Altogether, these findings indicate that transforming growth factor beta (TGF-β)/BMP and sonic hedgehog signaling are involved in root formation through regulating odontoblast differentiation. Moreover, it has been reported that epithelial SMAD4 signaling is essential for HERS elongation and that it also regulates the fate of the dental mesenchyme through the SMAD4/sonic hedgehog/nuclear factor I/C signaling cascade (7). Nevertheless, the molecular mechanism underlying odontoblast differentiation during root development is not yet completely known.

Wingless-type MMTV integration site family (Wnt)/β-catenin signaling plays an essential role in the morphogenesis and cellular differentiation of many tissues (8). During tooth development, Wnt/β-catenin signaling plays multiple roles in various stages of tooth morphogenesis (9). However, little is known about the involvement of Wnt/β-catenin signaling in cellular differentiation and matrix formation during tooth formation. Recently, we showed that constitutive stabilization of β-catenin in the dental mesenchyme leads to the formation of excessive dentin and cementum (10). Although we did not explore the underlying mechanisms, the results of our study suggest that local modulation of Wnt/β-catenin signaling may play critical roles in the differentiation of odontoblasts and cementoblasts during tooth development. In the present study, we analyzed the molar roots and cementum of mice in which β-catenin was stabilized in the dental mesenchyme, in order to investigate the role of Wnt/β-catenin signaling in root and cementum formation in more detail.

Material and methods

Mouse strains and genotyping

All experimental procedures were approved by the Animal Welfare Committee of the Chonbuk National University. For stabilization of β-catenin in the dental mesenchyme, *Catnb*$^{+/lox(ex3)}$ mice were crossed with *OC-Cre* mice, as previously described (10).

Soft X-ray analysis and tooth isolation

Dissected mandibles from 4-wk-old *OC-Cre:Catnb*$^{+/lox(ex3)}$ (MT) and wild-type (WT) mice were examined on a soft X-ray system (SOFTEX CSM-2; Softex Co. Ltd., Kanagawa, Japan). The mandibles were incubated in 50 mM Tris–HCl (pH 8.0) containing 0.5% sodium dodecyl sulfate and 0.2 mg/mL of proteinase K at 55°C for 1 h. After briefly washing with water, the first molars were extracted and photographed under a stereomicroscope.

Histology and immunohistochemistry

For histological examination, mandibles from 8-d- and 4-wk-old mice were dissected and fixed in 4% paraformaldehyde at 4°C overnight. After rinsing with 0.01 M phosphate-buffered saline, the specimens were decalcified in 0.01 M phosphate-buffered saline containing

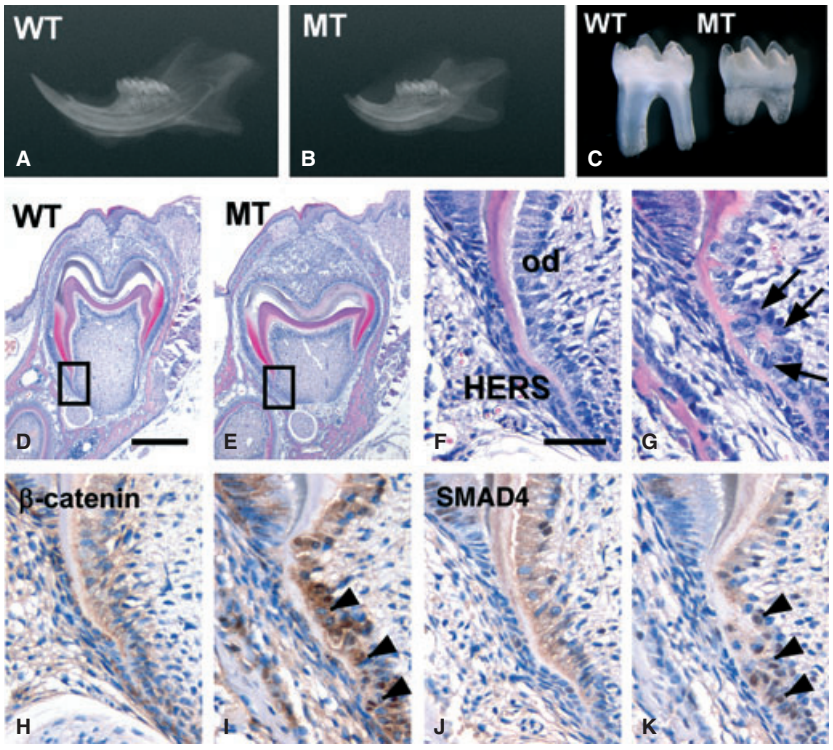

Fig. 1. Disturbances in root formation of *OC-Cre:Catnb*$^{+/lox(ex3)}$ (MT) mice. (A, B) In microradiography, small mandible and root abnormalities are observed in MT mice. (C) Short roots are observed in the isolated mandibular first molar of MT mice. (D, E) Hematoxylin and eosin-stained frontal sections of the developing mandibular first molar at postnatal day (P) 8. The boxed areas in panels D and E are shown at higher magnification in panels F and G, respectively. (F, G) Dental papilla cells are well differentiated and regularly arranged inside the Hertwig's epithelial root sheath (HERS) in wild-type (WT) mice, whereas the dental papilla cells (indicated by the arrows) are poorly differentiated and disorganized in MT mice. (H–K) β-catenin and SMAD family member 4 (SMAD4) are weakly expressed in the differentiating root odontoblasts of WT mice, but are strongly upregulated in the nucleus of the differentiating odontoblasts of MT mice (indicated by the arrowheads). HERS, Hertwig's epithelial root sheath; Od, odontoblasts. Scale bars: 500 μm in panel D and 50 μm in panel F.

10% ethylenediaminetetraacetic acid for 2–4 wk, dehydrated, embedded in paraffin and sectioned at a thickness of 5 μm. The slides were stained with hematoxylin and eosin.

For immunohistochemistry studies, frontal sections were treated with 3% hydrogen peroxide and then incubated with rabbit polyclonal antibodies against β-catenin (1 : 200 dilution; Thermo Scientific, Fermont, CA, USA), SMAD4 (1 : 300 dilution; Abcam, Cambridge, MA, USA), biglycan (1 : 800 dilution; kindly provided by Dr. Larry Fisher, NIDCR, Bethesda, MD, USA), dentin sialophosphoprotein (1 : 400 dilution; Dr. Larry Fisher), dentin matrix protein 1 (1 : 500 dilution; Takara Bio Inc., Shiga, Japan) and bone sialoprotein (Bsp) (1 : 1200 dilution; Abcam), and with goat polyclonal antibodies against ectonucleotide pyrophosphatase/phosphodiesterase 1 (E-NPP 1) (1 : 200 dilution; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), fibroblast growth factor 23 (1 : 200 dilution; Santa Cruz Biotechnology), Dickkopf-related protein (Dkk)-1 (1 : 100 dilution; R&D Systems Inc., Minneapolis, MN, USA) and Dkk-2 (1 : 100 dilution; R&D Systems). To mark the odontoblasts, mouse monoclonal anti-nestin Ig G (1 : 200 dilution; Chemicon, Temecula, CA, USA) was also used.

Histostain Plus Rabbit/Mouse Primary kits (Zymed Laboratories, San Francisco, CA, USA) and goat ImmunoCruz staining system (Santa Cruz Biotechnology) were used following each manufacturer's instructions.

Histomorphometry and statistical analysis

Histomorphometry was performed on the frontal sections obtained from hematoxylin and eosin staining and BSP immunohistochemistry using ANALYSIS software (Soft Imaging System GmbH, Muenster, Germany). Root dentin and cementum thickness were measured at a site 300 and 100 μm apical to the cemento–enamel junction of the mandibular first molars, respectively. The results are presented as mean ± standard error of the mean. All statistical analyses were performed using GRAPHPAD PRISM software (GraphPad Software, Inc., La Jolla, CA, USA). Statistical differences were determined using the Student's *t*-test and values of $p < 0.05$ were considered significant.

Results

Constitutive stabilization of β-catenin in the dental mesenchyme disturbs tooth-root formation

Radiographic examination revealed that the mandibles of MT mice were smaller than those of WT mice at the age of 4 wk (Fig. 1A, B). Incisors and molars developed and erupted normally in WT mice. In contrast, in MT mice incisors exhibited retarded eruption and molars had short roots. No significant difference was observed in the gross appearance and crown dimensions of isolated mandibular first molars of WT and MT mice. However, root formation was severely disturbed in MT mice and was about half the length of that of their WT littermates (Fig. 1C).

At the initial stage of root formation, odontoblasts were gradually differentiated from the dental papilla cells and were arranged along the inner enamel epithelium in WT mice (Fig. 1D, F). In MT mice, odontoblasts exhibited prematurely differentiated features, such as loose cellular polarities and a disorganized arrangement (Fig. 1E, G). In WT mice, β-catenin was expressed in the differentiating odontoblasts, but was more strongly expressed in the

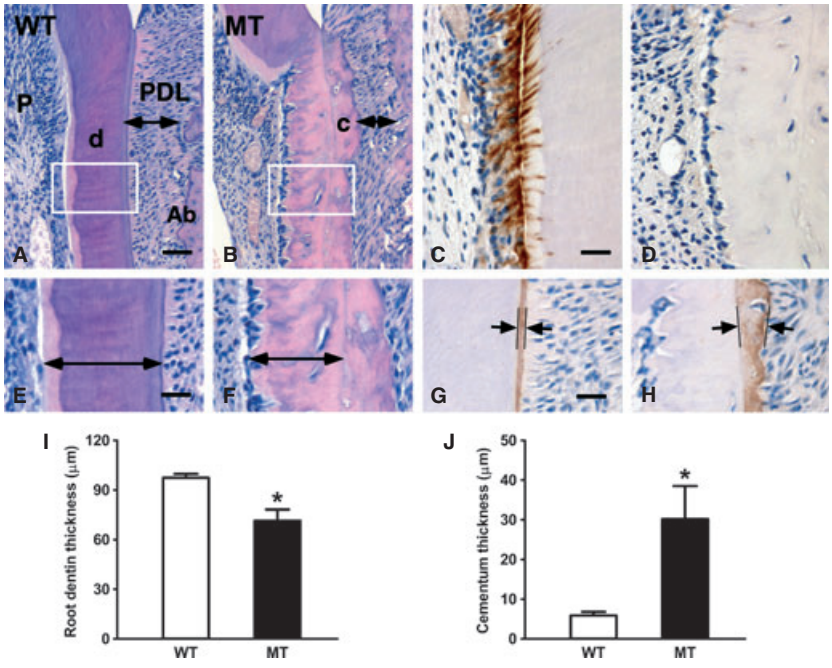

Fig. 2. Abnormal dentin and cementum formation in the roots of *OC-Cre:Catnb*$^{+/lox(ex3)}$ (MT) mice at postnatal day (P) 28. (A, B) In the frontal sections of the mandibular first molars, root dentin of MT mice is thin and hypomineralized, whereas that of wild-type (WT) mice is formed and mineralized normally. Panels E and F are higher magnifications of the boxed areas in panels A and B, respectively. (C, D) In immunohistochemistry, nestin is localized in the well-differentiated odontoblasts and their processes, but the prematurely differentiated odontoblasts of MT mice lose their cytoplasmic processes and nestin immunoreactivity. (G, H) Cementum is clearly marked by bone sialoprotein (BSP) immunohistochemistry. In WT mice, acellular cementum is regularly formed along the root surface, whereas a large amount of cementum is deposited in MT mice. (I, J) The decrease of root-dentin width and the increase of cementum deposition following the stabilization of β-catenin in the dental mesenchyme is statistically significant (*$p < 0.05$). Ab, alveolar bone; d, dentin; P, pulp; PDL, periodontal ligament. Scale bars: 50 μm in panel A; and 25 μm in panels C, E and G.

premature odontoblasts of MT mice (Fig. 1H, I). SMAD4, the common mediator of TGF-β/BMP signaling, was also expressed in the cytoplasm of differentiating odontoblasts of WT mice (Fig. 1J). Interestingly, SMAD4 was strongly expressed in the nuclei of premature odontoblasts in MT mice (Fig. 1K).

Thin root dentin and cementum hyperplasia in *OC-Cre:Catnb*$^{+/lox(ex3)}$ mice

In hematoxylin and eosin-stained sections of the mandibular first molar of 4-wk-old mice, mineralized root dentin with thin predentin produced by well-differentiated odontoblasts was observed (Fig. 2A). Thin acellular cementum was deposited along the root surface in WT mice (Fig. 2A). In MT mice, hypomineralized dentin matrix with premature odontoblasts was observed in some cells included within the matrix. In the root surface of MT molars, thick cementum was deposited along with whole root surface and some cementoblasts were also present in their matrix (Fig. 2B). Periodontal spaces between the root surface and the alvelolar bone were narrower in MT mice than in WT mice (Fig. 2A, B).

In WT mice, root odontoblasts were well differentiated with cytoplasmic processes and were specifically identified by nestin immunohistochemistry. However, the root odontoblasts of MT mice did not exhibit nestin immunoreactivity owing to the absence of cytoplasmic processes and a loss of cell polarity (Fig. 2C, D).

In the region 300 μm apical to the cemento–enamel junction, the mean dentin thickness of WT and MT mice was 97.40 ± 2.31 μm (n = 9) and 71.42 ± 6.78 μm (n = 9), respectively (Fig. 2E, F, I). BSP immunohistochemistry revealed deposition of a significantly larger amount of cementum in MT mice compared with WT mice ($p < 0.05$, Fig. 2G, H). In the region 100 μm apical from the cemento–enamel junction, the cementum thickness of WT and MT mice was 5.89 ± 0.93 μm (n = 9) and 30.19 ± 8. 32 μm (n = 9), respectively (Fig. 2J).

Thin and hypomineralized root dentin is caused by the dysregulation of noncollagenous proteins

To identify the changes of matrix protein expression in the root dentin following the persistent stabilization of β-catenin in the dental mesenchyme, we performed immunohistochemistry using antibodies to dentin matrix protein (Fig. 3). Biglycan and dentin sialophosphoprotein were localized in the predentin and dentin, respectively, in WT mice but were significantly downregulated in MT mice (Fig. 3A–D). In contrast, dentin matrix protein 1 was weakly expressed in the root dentin of WT mice, but was significantly upregulated in MT mice (Fig. 3E, F). Interestingly, Dkk-1, a Wnt inhibitor, was not expressed in the root dentin of WT mice, but was observed in MT mice.

Excessive deposition of cellular cementum is associated with downregulation of NPP1 in cementoblasts

NPP1, a cell-membrane protein that regulates the extracellular pyrophosphate level, was specifically localized in the cementoblasts located along the

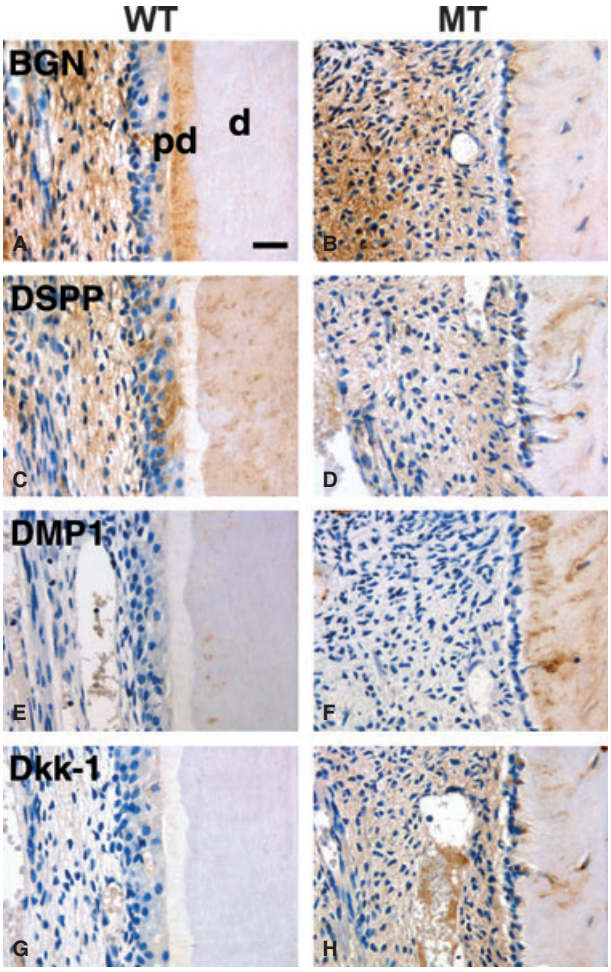

Fig. 3. Molecular changes in the root dentin of the *OC-Cre:Catnb*$^{+/lox(ex3)}$ (MT) mice. (A–D) In the roots of wild-type (WT) mice, biglycan and dentin sialophosphoprotein are localized in the predentin and mineralized dentin, respectively. However, both biglycan and dentin sialophosphoprotein are significantly decreased in the MT mice. (E–H) Dentin matrix protein 1 and Dickkopf-related protein 1 are weakly or not expressed in the root dentin of WT mice. However, they are upregulated in the root dentin of MT mice. BGN, biglycan; d, dentin; Dkk-1, Dickkopf-related protein 1; DMP1, dentin matrix protein 1; DSPP, dentin sialophosphoprotein; pd, predentin. Scale bar: 25 μm.

root surface of molars in WT mice (Fig. 4A). Interestingly, NPP1 expression was significantly downregulated in the cementoblasts of molars in MT mice (Fig. 4B). BSP was restricted to the cementum along the root surface of molars in WT mice (Fig. 4C), but was slightly decreased in the excessive deposited cementum of MT mice (Fig. 4D). An inhibitor of Wnt signaling, Dkk-2, was not expressed in the cementum of WT mice but was localized in the cementum around cementocytes in MT mice (Fig. 4E, F).

Discussion

Excessive dentin and cementum formed in the teeth of mice in which β-catenin was constitutively stabilized in the dental mesenchyme (10), suggesting that local modulation of the Wnt/β-catenin signaling pathway may be implicated in the regeneration of dentin and cementum. Here, we demonstrated that constitutive stabilization of β-catenin in the dental mesenchyme leads to the formation of abnormally short roots with thick cementum, associated with disrupted differentiation of odontoblasts and phosphate homeostasis.

Root formation is initiated with the extension of HERS following crown development (1). Cells in the dental papilla are gradually differentiated into pre-odontoblasts and then into odontoblasts, which form a regular arrangement inside the HERS and produce root dentin. In the molars of MT mice, HERS extended, as normal, from the crown, but dental papilla cells next to HERS were disorganized without cellular polarity and formed extremely short roots. These findings indicate that stabilization of β-catenin in the dental mesenchyme disrupts the differentiation of odontoblasts for root elongation. In our previous work, we observed that the crown size and the shape of mutant molars were similar to those of WT mice, but the prematurely differentiated odontoblasts observed in these mutants continuously produced a large amount of dentin in the pulp chamber (10). These phenotypic discrepancies indicate that prematurely differentiated odontoblasts, induced by persistent stabilization of β-catenin, may give rise to different disturbances in the crown and roots during development. To date, several signaling molecules, such as nuclear factor I/C, Ptch1 and SMAD4, have been associated with root formation (4–6). Interestingly, root abnormalities were observed in mice containing mutants of these genes, but there were no disturbances in the crown. Together, it could be suggested that different regulatory mechanisms underlying dentin formation may exist during crown and root development, and that Wnt/β-catenin signaling, along with these genes, may play critical roles in root elongation during tooth development.

In the present study, we also demonstrated that Smad4, the common mediator of TGF-β/BMP signaling, was significantly upregulated in the prematurely differentiated odontoblasts of MT molars during the initiation of root formation. This demonstrates that persistent stabilization of β-catenin in the differentiating odontoblasts leads to activation of SMAD4-mediated signaling. It has been reported that TGF-β/BMP signaling interacts with the Wnt/β-catenin signaling pathway through the regulation of Wnt inhibitors (e.g. Dkk-1 and sclerostin) during bone formation (11,12). Furthermore, Li *et al*. (13) recently reported that tissue-specific ablation of SMAD4 in the dental mesenchyme leads to defects in odontoblast differentiation and dentin formation. Loss of SMAD4 in odontoblasts results in the upregulation of Wnt/β-catenin signaling via the downregulation of Dkk-1 and Secreted frizzled-related protein. Conversely, our data showed that upregulation of SMAD4 was induced by the constitutive stabilization of β-catenin in

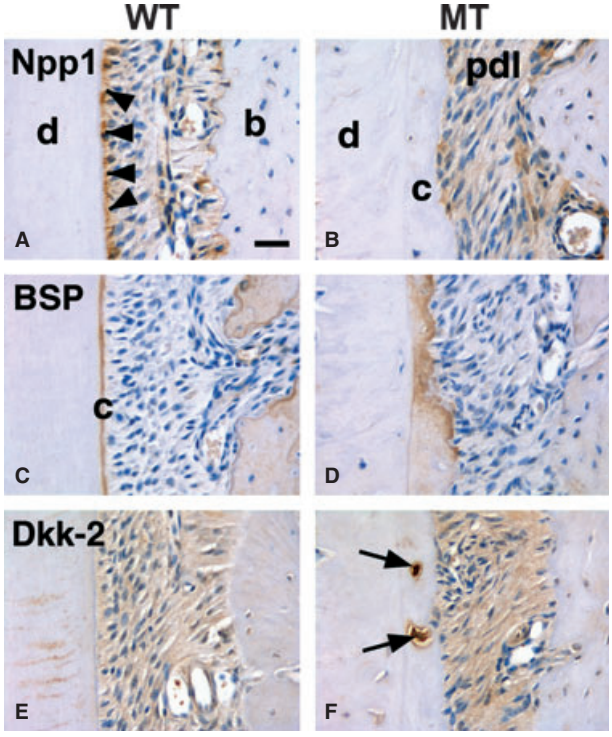

Fig. 4. Molecular changes in the cementoblasts and cementum of the *OC-Cre:Catnb*$^{+/lox(ex3)}$ (MT) mice. (A–D) In wild-type (WT) mice, ectonucleotide pyrophosphatase/phosphodiesterase 1 (NPP1) is specifically expressed in cementoblasts (indicated by arrowheads) but is significantly downregulated in MT mice. (C, D) Bone sialoprotein is also specifically expressed in the cementum of WT mice but is significantly downregulated in the cementum of MT mice. (E, F) Dickkopf-related protein 2 (an inhibitor of Wnt signaling) is not found in the cementum of WT mice, but is observed in the cementocytes (indicated by arrows) included in the cementum matrix of MT mice. b; bone; c, cementum; d, dentin; Dkk-2, Dickkopf-related protein 2; pdl, periodontal ligament. Scale bar: 25 μm.

odontoblasts. Thus, SMAD4 may be required for the upregulation of Wnt inhibitors to compensate for the persistent activation of Wnt/β-catenin signaling in odontoblasts. Our study also showed that Dkk1 was upregulated in the root dentin of mutants and this might have caused alterations in the levels of biglycan, dentin sialophosphoprotein and dentin matrix protein 1 in the root dentin. Thus, our findings, and those of others, suggest that dysregulation of SMAD4 may disturb the terminal differentiation of odontoblasts and dentin matrix formation, and that the molecular circuit between TGF-β/BMP and Wnt/β-catenin signaling, mediated by Wnt inhibitors, may participate in the differentiation of odontoblasts during root formation.

Cementum is known to be a phosphate-sensitive tissue (14). It has been reported that several molecules related to phosphate/pyrophosphate homeostasis are associated with cementum formation. Acellular cementum is not formed in alkaline phosphatase null mice (15), whereas a large amount of cementum is deposited in Npp1 and ANK mutants, as shown in *OC-Cre:Catnb*$^{+/lox(ex3)}$ mutants (10,14,16). Despite the observation that certain phenotypic similarities exist in the cementum of NPP1, Ank and *OC-Cre:Catnb*$^{+/lox(ex3)}$ mutants, the relationship between Wnt/β-catenin signaling and NPP1 and Ank proteins in cementum formation is not fully known. In this study, we demonstrated that NPP1 was significantly downregulated in the cementoblasts of *OC-Cre:Catnb*$^{+/lox(ex3)}$ mutant molars, indicating that stabilized β-catenin negatively regulates NPP1 expression in cementoblasts and leads to excessive cementum deposition. This observation is consistent with those in previous studies showing increased cementum depositions in NPP1 mutants (14). However, recently it was also reported that NPP1 was upregulated in the cementoblasts of Ank mutants (16). From these findings, it could be speculated that dysregulation of NPP1 in cementoblasts might cause large amount of cementum deposition. At any rate, however, further studies using *Npp1* mutant mice will be necessary to elucidate the roles of NPP1 in the formation of cementum. In view of these previous findings and those of the present study, inhibition of Wnt/β-catenin signaling may be required for expression of *Npp1* in cementoblasts in order to maintain the integrity of the cementum and the periodontium during development.

In conclusion, temporospatial regulation of Wnt/β-catenin signaling may play critical roles in root formation and cementum deposition. Inhibition of Wnt/β-catenin signaling may be required for root and cementum formation during tooth development.

Acknowledgements

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References

1. Thomas HF. Root formation. *Int J Dev Biol* 1995;**39**:231–237.
2. Saygin NE, Giannobile WV, Somerman MJ. Molecular and cell biology of cementum. *Periodontol 2000* 2000;**24**:73–98.
3. Yamashiro T, Tummers M, Thesleff I. Expression of bone morphogenetic proteins and Msx genes during root formation. *J Dent Res* 2003;**82**:172–176.
4. Nakatomi M, Morita I, Eto K, Ota MS. Sonic hedgehog signaling is important in tooth root development. *J Dent Res* 2006;**85**:427–431.
5. Steele-Perkins G, Butz KG, Lyons GE *et al*. Essential role for NFI-C/CTF transcription-replication factor in tooth root development. *Mol Cell Biol* 2003;**23**:1075–1084.
6. Gao Y, Yang G, Weng T *et al*. Disruption of Smad4 in odontoblasts causes multiple keratinocystic odontogenic tumors and tooth malformation in mice. *Mol Cell Biol* 2009;**29**:5941–5951.
7. Huang X, Xu X, Bringas P, Hung YP, Chai Y. Smad4-Shh-Nfic signaling cascade-mediated epithelial-mesenchymal interaction is crucial in regulating tooth root development. *J Bone Miner Res* 2010;**25**:1167–1178.
8. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol* 2004;**20**:781–810.
9. Liu F, Millar SE. Wnt/β-catenin signaling in oral tissue development and disease. *J Dent Res* 2010;**89**:318–330.
10. Kim TH, Lee JY, Baek JA *et al*. Constitutive stabilization of β-catenin in the dental mesenchyme leads to excessive dentin and cementum formation. *Biochem Biophys Res Commun* 2011;**412**:549–555.
11. Kamiya N, Ye L, Kobayashi T *et al*. BMP signaling negatively regulates bone mass through sclerostin by inhibiting the canonical Wnt pathway. *Development* 2008;**135**:3801–3811.
12. Kamiya N, Kobayashi T, Mochida Y *et al*. Wnt inhibitors Dkk1 and Sost are downstream targets of BMP signaling through the type IA receptor (BMPRIA) in osteoblasts. *J Bone Miner Res* 2010;**25**:200–210.
13. Li J, Huang X, Xu X *et al*. SMAD4-mediated WNT signaling controls the fate of cranial neural crest cells during tooth morphogenesis. *Development* 2011;**138**:1977–1989.
14. Nociti FH, Berry JE, Foster BL *et al*. Cementum: a phosphate-sensitive tissue. *J Dent Res* 2002;**81**:817–821.
15. Beertsen W, VandenBos T, Everts V. Root development in mice lacking functional tissue non-specific alkaline phosphatase gene: inhibition of acellular cementum formation. *J Dent Res* 1999;**78**:1221–1229.
16. Foster BL, Nagatomo KJ, Bamashmous SO *et al*. The progressive ankylosis protein regulates cementum apposition and extracellular matrix composition. *Cells Tissues Organs* 2011;**194**:382–405.