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Signaling Pathways Critical for Tooth Root Formation

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Abstract

Tooth is made of an enamel-covered crown and a cementum-covered root. Studies on crown dentin formation have been a major focus in tooth development for several decades. Interestingly, the population prevalence for genetic short root anomaly (SRA) with no apparent defects in crown is close to 1.3%. Furthermore, people with SRA itself are predisposed to root resorption during orthodontic treatment. The discovery of the unique role of *Nfic* (nuclear factor I C; a transcriptional factor) in controlling root but not crown dentin formation points to a new concept: tooth crown and root have different control mechanisms. Further genetic mechanism studies have identified more key molecules (including Osterix, β-catenin, and sonic hedgehog) that play a critical role in root formation. Extensive studies have also revealed the critical role of Hertwig's epithelial root sheath in tooth root formation. In addition, Wnt10a has recently been found to be linked to multirooted tooth furcation formation. These exciting findings not only fill the critical gaps in our understanding about tooth root formation but will aid future research regarding the identifying factors controlling tooth root size and the generation of a whole "bio-tooth" for therapeutic purposes. This review starts with human SRA and mainly focuses on recent progress on the roles of NFIC-dependent and NFIC-independent signaling pathways in tooth root formation. Finally, this review includes a list of the various Cre transgenic mouse lines used to achieve tooth root formation–related gene deletion or overexpression, as well as strengths and limitations of each line.

Keywords: dentin, odontogenesis, cell signaling, tooth regeneration, NFIC, osterix

Introduction

Tooth is composed of 2 major components: the enamel-covered crown and the cementum-covered root. There has been substantial progress in understanding crown formation and its regeneration since the molecular biology era began (Lan et al. 2014), while we know relatively little about root formation. Because there is only 1 type of odontoblast, it would be very hard to reason that odontoblasts would behave differently in crown and root. Currently, the interaction of the epithelial and mesenchymal cells is considered responsible for the initiation of root dentin formation (Nanci 2007). Based on this theory, epithelial cells from the cervical loop proliferate to form 2 layers of Hertwig's epithelial root sheath (HERS), which then induces the adjacent dental mesenchymal cells to differentiate into odontoblasts for dentin formation (Nanci 2007; Fig. 1).

Importantly, there are numerous hereditary syndromes and chromosomal anomalies that mainly affect the root structure. Technically, tooth crown regeneration does not appear to be an issue, but it is a great challenge to regrow roots for treatment of dental trauma, tooth agenesis, and periodontal diseases. In the recent decade, root research has gained substantial attention from different researchers in genetics and developmental biology. As a result, there has been impressive progress in this area, which has been extensively covered by several researchers in excellent review articles (Kumakami-Sakano et al. 2014; Luder 2015; Li et al. 2017). In the present short review, we focus on short root anomaly (SRA; the clinic relevance of the tooth root study) and roles of NFIC-dependent and NFIC-independent signaling pathways in tooth root formation.

Short Root Anomaly

The term "short root anomaly" (also called "root dwarfism") was initially described by Lind (1972) based on radiographic measurements of the ratio of the crown versus the root in 112 children with abnormally short roots of the maxillary central incisors as compared with 100 children with normally developed roots. Currently, SRA is defined as a developmental anomaly characterized by full but short root formation with a normal crown. The diagnosis is commonly made when such a short root case appears in some family members but no other known cause is found. The etiology of SRA is largely unknown, although a familial clustering is linked to this syndrome (Lind 1972). The prevalence of SRA appears variable in different

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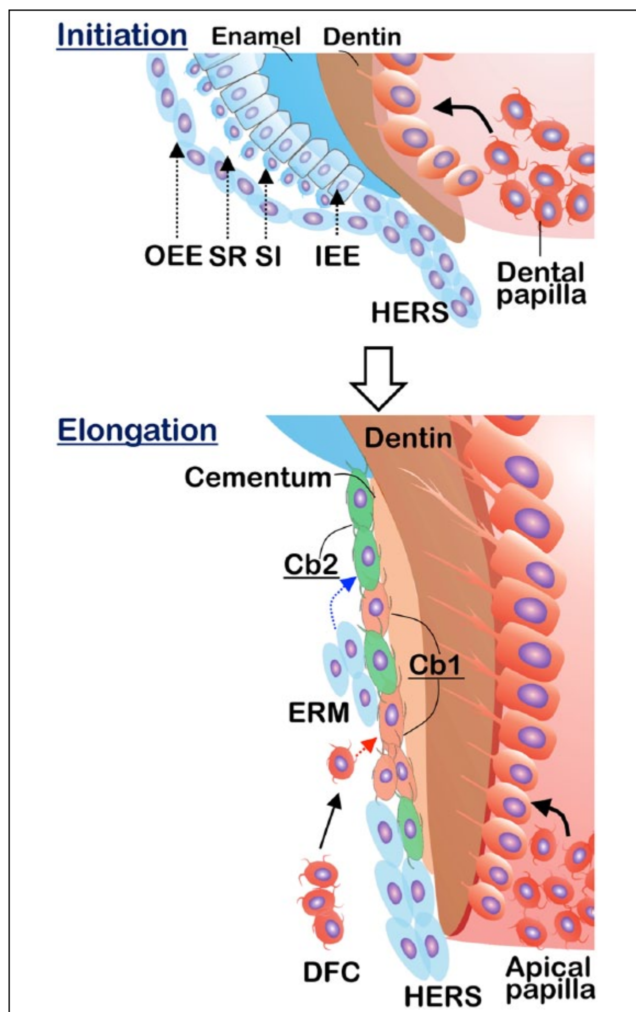

Figure 1. Diagram showing root morphogenesis in 2 stages: root initiation and root elongation. HERS is formed by fusion of outer enamel epithelium (OEE) and inner enamel epithelium (IEE), which marks the initiation of root formation. The odontoblasts derived from the dental papilla (apical papilla at root elongation stage) form dentin, whereas there are 2 stem cell niches giving rise to cementoblasts (Cb), including the dental follicle (dashed red arrow) and HERS (dashed blue arrow). Some HERS cells eventually become epithelial rests of Malassez (ERM). DFC, dental follicle cell; HERS, Hertwig's epithelial root sheath; SI, stratum intermedium; SR, stellate reticulum.

populations and ranges from 0.6% to 2.4% (Valladares Neto et al. 2013), but a Japanese research group reported a much higher population affected (Ando et al. 1976). Interestingly, all case reports of SRA are linked to permanent dentition with no such a case in primary dentition (Newman 1975).

SRA can be associated with systemic changes or syndromes (Baccetti 1998). For example, Tananuvat et al. (2014) recently identified a homozygous gene mutation in plasminogen (c.1193G>A missense mutation, leading to type I plasminogen deficiency) from a Thai girl. This affected girl displayed tapered incisor roots and thin root dentin with generalized short tooth roots and mandibular prognathism (i.e., the lower incisors and the upper incisors overlapped). The immunohistochemistry stain showed a higher level of plasminogen in the early root development versus its expression in the mouse crown odontoblast layer. Given these data, the authors proposed a specific role of plasminogen in root dentin formation. However, the plasminogen-deficient mouse model did not show teeth abnormalities but severe periodontitis, suggesting that root dentin anomaly seems not to be a causal phenotype of plasminogen deficiency and that plasminogen is not a true candidate gene for SRA (Kurtulus-Waschulewski et al. 2015).

Generally, SRA has little impact on children's health except for a high risk of root resorption that occurs in children undergoing orthodontic treatment, which requires more careful biomechanical adaptations, periodic radiographic monitoring, clinical monitoring of tooth mobility, and permanent retention, especially for the incisors. However, orthodontic treatment is contraindicated in only extreme cases (Valladares Neto et al. 2013).

Discovery of the Specific Role of NFIC in Root Formation Points to a Different Control Mechanism in Root Formation from Crown Formation

For many years, dentin was considered 1 component in the entire tooth until the discovery of *Nfic*, the master gene that specifically controls tooth root rather than crown (Steele-Perkins et al. 2003; Park et al. 2007; Lee et al. 2009), which suggests a unique regulation mechanism in root formation.

NFIC belongs to the nuclear factor I family of transcription proteins, which include 3 additional members: NFIA, NFIB, and NFIX. All of these nucleus proteins bind to the consensus DNA sequence with similar affinities (Gronostajski 2000). However, each member has unique targeted tissues. For example, NFIA mainly controls brain development (das Neves et al. 1999); NFIB regulates brain and lung development (Steele-Perkins et al. 2005); and NFIX defines neural stem cell lineage (Zhou et al. 2015) and regulates neural stem cell quiescence (Martynoga et al. 2013).

NFIC is expressed in odontoblasts of crown and root, but the deletion of *Nfic* leads to short tooth roots with no apparent changes in molar crowns (Steele-Perkins et al. 2003). This unique root phenotype suggests a different regulation mechanism, which is distinguished from the crown. Although HERS plays an essential role in tooth root formation (detailed later), there is no gross defect in HERS in the *Nfic* knockout (KO) mice (Park et al. 2007). To better understand the mechanism by which the abnormal molar root occurs, Lee et al. (2009) used the *Nfic* KO incisor as a model to investigate the detailed changes in the cellular structure and molecular mechanisms. This group found that removing *Nfic* results in a great change in cell cycles, including a decrease in cell proliferation and an increase in apoptosis, which interfere with the formation of intercellular junctions and cellular polarity of the odontoblast cells. In addition, there was an increase in p-Smad2/3 and BSP expressions in the *Nfic*-null incisor pulp cells. In contrast, a recent report suggested that a lack of root furcation between

the mesial and distal roots in the *Nfic*-null molar is due to an increase in cell proliferation and a reduction in cell differentiation (Kim, Bae, Yang, et al. 2015).

In fact, the role of NFIC is not restricted only in tooth root formation. An important study showed that NFIC is expressed in bone cells and that the disruption of *Nfic* greatly reduces osteoblast differentiation and bone formation and increases bone marrow adipocytes (Lee et al. 2014). However, overexpressing *Nfic* in bone marrow stem cells greatly enhances osteoblast differentiation but inhibits adipocyte differentiation, leading to more new bone formation. These results indicate that NFIC controls the cell fate between osteoblast and adipocyte differentiation. Furthermore, a lower NFIC expression in osteogenic cells is found in patients with osteoporosis.

Taken together, these findings indicate that NFIC is likely the real master gene that is required for tooth root elongation, although there has been no *NFIC* mutation reported in human SRA cases so far.

Osterix, One of the Key Downstream Molecules of NFIC, Plays a Critical Role in Root but Not Crown Formation

Osterix (Osx), a zinc finger–containing transcription factor, has long been known as the key factor in skeletal and cementum formation (Cao et al. 2012). A recent study demonstrated that Osx is the key downstream molecule of NFIC, based on the following evidence: 1) there is a sharp reduction in Osx expression in *Nfic*-null mice; 2) *Osx*-cKO (2.3-kb *Col1α1-Cre, Osx*$^{fl/fl}$) mice recapture the *Nfic*-null root phenotypes, displaying modest changes in the tooth crown but severe defects in the molar root and incisor root analog; and 3) the in vitro overexpression of *Nfic* leads to a dosage-dependent increase in Osx expression that is not linked to the change in cell numbers (Fig. 2; Zhang et al. 2015). Similarly, Kim, Bae, Lee, et al. (2015), using 2 *Osx*-cKO mouse models (3.6-kb *Col1α1-Cre, Osx*$^{fl/fl}$*; osteocalcin-Cre, Osx*$^{fl/fl}$), demonstrated a crucial role of Osx in root but not crown dentin formation. Furthermore, *Osx*-cKO pulps displayed a lack of polarized odontoblasts but significantly more proliferative cells when compared the age-matched control, indicating an inhibitory role of Osx in cell proliferation but an enhancing role in the odontoblast differentiation (Kim, Bae, Lee, et al. 2015; Zhang et al. 2015). There is a sharp reduction of 2 critical dentin matrix proteins: DMP1 (dentin matrix protein 1; Feng et al. 2003) and DSPP (dentin sialophosphoprotein; Sreenath et al. 2003) in the cKO root rather than in the crown, supporting the notion that Osx is responsible for root-polarized odontoblast formation, partly through DMP1 and DSPP (Zhang et al. 2015). The in vitro study further confirmed that *Osx* overexpression increased *DSPP* transcription with mouse odontoblast-like cells (Chen et al. 2009; Zhang et al. 2015). Of note, there is disagreement in interpreting the *Osx*-cKO root phenotype. Kim, Bae, Lee, et al. (2015) claimed that the cause of the root phenotype is due to a site-specific role of Osterix in the root. This view does not address the fact that there is no apparent crown phenotype, while Osx is indeed expressed in the crown and root regions (Feng et al. 2015). It is more likely that there are other factors that compensate for Osterix function in crown but not root, which agrees with studies on *Nfic*, in which *Nfic* is expressed in the crown and root odontoblasts (Feng et al. 2015). Conventional *Nfic*-KO mice displayed short tooth root with limited impact on the tooth crown, indicating that NFIC and Osterix (the key downstream molecule of NFIC) are essential for root formation but their function can be compensated for by other factors in crown.

In summary, as the key downstream molecule of NFIC, Osx functions to promote odontoblast differentiation in root elongation but not crown formation.

Vital Roles of Wnt/β-catenin in Root Formation

It is known that β-catenin, the key downstream component of the canonical Wnt signaling pathway, is vital for bone formation (Long 2011). Interestingly, the regulation of Osx on bone cell differentiation happens via an inhibition of Wnt/β-catenin signaling (Zhang et al. 2008), suggesting that a high level of Wnt/β-catenin signaling may not be ideal for bone formation. Because bone and dentin share many similarities, several independent studies found a great impact of changes in the β-catenin levels on postnatal tooth formation. By crossing the *osteocalcin-Cre* that is active in all odontoblasts starting from the embryonic stage and beyond, removing β-catenin in odontoblasts completely disrupted tooth root formation with little impact on the tooth crowns (Kim et al. 2013; Zhang et al. 2013). Similarly, Han et al. (2011) reported the short root with a thin dentin layer phenotype in the overexpressed *Dkk1* (a potent inhibitor of Wnt signaling) transgenic mice. Mechanism studies by Zhang et al. (2013) showed that these cKO mice displayed sharp reductions in cell proliferation by BrdU and PCNA immunostaining and a lack of expression of odontoblast markers such as *Col 1, DSPP*, osteocalcin, and *BSP* in roots with little change in crowns with the in situ hybridization assay. Thus, Zhang et al. concluded that Wnt/β-catenin signaling is functionally significant to root odontogenesis, whereas Kim et al. (2013) proposed a cell-autonomous requirement for Wnt/β-catenin signaling in the dental mesenchyme for root formation. Because neither of these groups compared its findings to the short root phenotype in the *Nfic* KO or tried to explain why the crown is largely unaffected in the β-catenin cKO model, it is unclear whether there is any connection between NFIC and Wnt/β-catenin signaling in root formation.

Constitutively stabilizing β-catenin studies by crossing the osteocalcin-Cre; Catnb+/lox(ex3) mice led to an acceleration in root dentin formation (including a lack of predentin and thicker dentin), although the root is short (less than half of the control root length) and disrupted (Kim et al. 2011; Bae et al. 2013). The gain of function data appears to be in agreement with the cKO data.

These studies demonstrate that Wnt/β-catenin signaling, which appears independent from NFIC, is vital for tooth root initiation, while the suitable level of Wnt/β-catenin signaling is required for proper tooth root formation.

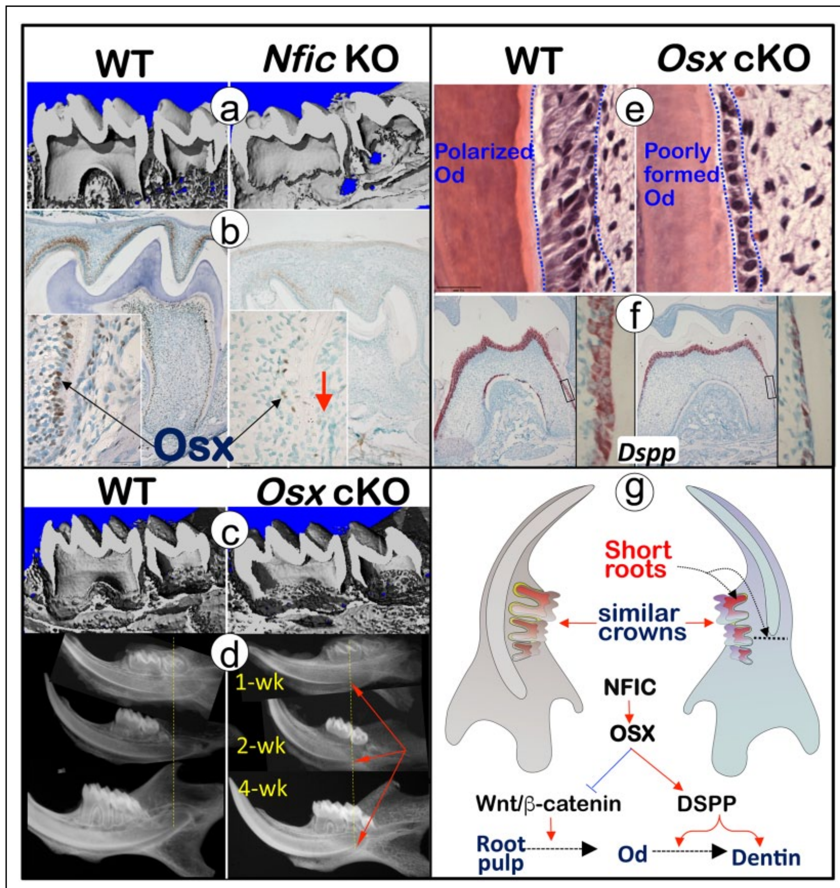

Figure 2. Osterix, 1 of the key downstream molecules of NFIC, plays a crucial role in root but not crown formation. (**a**) The 2-wk-old *Nfic*-KO displayed short molar roots as viewed by micro–computed tomography (right panel). (**b**) Immunohistochemistry stains showed few positive Osx signals in the *Nfic*-KO odontoblasts (right panel). Red arrow indicates few positive OSX signals in the KO odontoblasts. (**c**) The 2-wk-old *Osx*-cKO molar roots (right) were short and thin according to micro–computed tomography images. (**d**) The *Osx*-cKO showed short incisor root analogue according to x-ray images. Red arrows indicate the *Osx*-cKO short incisor root-analogue. (**e**) The hematoxylin and eosin stain documented an immature odontoblast cell layer with no sign of polarization morphology in the *Osx*-cKO root (right panel). (**f**) The in situ hybridization assays displayed a great decrease in *Dspp* in the *Osx*-cKO root (right panel). (**g**) Osx is the key downstream molecule of NFIC, which controls root but not crown formation via its inhibitory role in cell proliferation and stimulatory function in the cell differentiation/odontoblast process formation, partially through DSPP (right panel). DSPP, dentin sialophosphoprotein; KO, knockout; Od, odontoblast; OSX, Osterix; WT, wild type. Panels A to G adapted and reproduced with permission from Zhang et al., *J Bone Mineral Res*, 2015, copyright John Wiley & Sons.

Complex Role of TGF-β and BMP Signaling in Tooth Root Formation

Similar to Wnt/β-catenin signaling, TGF-β (transforming growth factor β) and BMP (bone morphogenetic protein) signaling is universally vital for almost all cell types during development. An in vitro study showed a complex relationship between TGF-β and NFIC in odontoblast cell reactions, in which TGF-β promotes the degradation of NFIC while NFIC downregulates the TGF-β/Smad signals (Lee et al. 2011). An in vivo study showed major defects in root formation with the impaired crown dentin formation when *Tgfbr2* (TGF-β receptor 2, the key receptor for TGF-β signaling) is conditionally deleted by crossing with *Sp7 (Osx)-Cre* in dental mesenchyme (Wang et al. 2013). Furthermore, there is no apparent change in the expression levels of NFIC in the *Tgfbr2-cKO* mice, excluding the direct impact of TGF-β signaling on NFIC regulation in tooth root formation. With the same *Osx-Cre* line, conditional deletion of the *BMP2* gene in a subset of dental pulp cells (preodontoblasts) and a subset of dental follicle cells leads to major defects in root and periodontium formation, including short roots, odontoblast dysmorphic differentiation, and the failure to form cellular cementum (Rakian et al. 2013). Interestingly, the *Nfic* mRNA expression is dramatically decreased in *BMP2-cKO* root odontoblasts, with the reduced expression of DMP1, DSPP, and Col1α1.

Overall, the TGF-β signaling is important not only for postnatal tooth root formation but also for continuous crown dentin formation without changing the NFIC signal pathway. In contrast, BMP signaling is more specific for the root formation in which NFIC is likely a key downstream molecule for root elongation.

Impact of HERS on Proper Root Formation via the *Nfic* Pathway

HERS is an epithelial bilayer structure formed after crown morphogenesis, which consists of the inner enamel epithelium and the outer enamel epithelium (Diekwisch 2001). The formation of HERS without a stratum intermedium or a stellate reticulum is considered to be the start of the root formation at approximately postnatal days 3 to 5 in mice (Kumakami-Sakano et al. 2014). It is currently believed that the interaction between the migrating HERS cells and the dental mesenchymal cells triggers root odontoblast formation via Smad4, the key mediator of TGF-β and BMP signaling.

The overexpression and KO studies in HERS showed that TGF-β and BMP signaling is vital for tooth root formation. A recent well-done study clearly showed that HERS cells regulate the dental mesenchyme for root formation through the *Smad4-Shh-Nfic* pathway (Huang et al. 2010). In this study, the authors conditionally inactivated *Smad4* in the epithelial cells using *K14-Cre*, which led not only to abnormal enamel defects as expected but also to a severe root phenotype: a complete lack of roots in molars. This rootless phenotype is even worse than the short root phenotype in the *Nfic*-null mice. Further

molecular studies have documented a sharp reduction in *Shh* in the epithelial cells and *Nfic* in the dental mesenchymal cells. Importantly, the ectopic application of Shh in the ex vivo culture partially restored the tooth roots and the *Nfic* expression in the dental mesenchyme (Huang et al. 2010). These data support the notion that HERS regulates root dentin formation partially through Smad4-Shh, which activates NFIC expression in the dental mesenchymal cells. However, future studies are required to address how Shh activates mesenchymal cells, as the direct targeting molecule of Shh is Gli1 (Briscoe and Therond 2013).

Interestingly, NFIC can downregulate the Shh signaling activity as a feedback loop through *Nfic-Hhip-Shh*, based on the following evidence (Liu et al. 2015): 1) Shh activity is elevated in *Nfic*-null mice; 2) constitutive activation of Hh signaling in dental mesenchymal progenitor cells leads to reduced proliferation and shorter roots with *Gli1-CreER*^{T2}*; R26SmoM2*$^{fl/fl}$ mice, which are similar to the phenotype of *Nfic*-null mice; and 3) treating *Nfic*-null mice with an Hh inhibitor partially restores cell proliferation and root development. In addition, ChIP and RNAscope analyses suggest that NFIC binds to the promoter region of the hedgehog interacting protein (Hhip), which functions as an Hh attenuator. It is noteworthy that the inhibition of Hh signaling by injecting Hh inhibitors can also lead to short roots, with reduced apical papilla cell proliferation and impaired odontoblast differentiation (Liu et al. 2015). On the whole, these findings indicate that Shh signaling precisely regulates the root formation as Nfic and Wnt/β-catenin do.

The cell lineage tracing approach with *K14-Cre; R26R* mice showed that many HERS cells are detected on the surface of the root from the beginning of the root formation with a small subset of HERS cells becoming epithelial rests of Malassez (Huang et al. 2009). This study suggests that the HERS cells may directly differentiate into cementoblasts and play important roles in cementum formation. An in vitro study revealed that the HERS cells initially synthesize and secrete some enamel-related proteins and then change their morphology and produce a mineralized extracellular matrix resembling acellular cementum. This indicates that HERS cells are capable of deriving cementoblasts (Zeichner-David et al. 2003). As a consequence, the epithelial rests of Malassez, remnants of HERS in the periodontal ligament, may play a role as a stem cell niche that can give rise to new cementoblasts (Bosshardt et al. 2015).

As for the dental papilla, the apical papilla has received more attention recently, since it harbors stem cells from the apical papilla (Huang et al. 2008). Evidence is accumulating to support the hypothesis that the apical papilla appear to be the source of primary odontoblasts responsible for the root dentin formation, whereas dental pulp stem cells are likely the source of replacement odontoblasts (Huang et al. 2008; Liu et al. 2015). Cells in the apical papilla proliferate 2- to 3-fold more than those in pulp in the organ culture, with the strong potential to give rise to osteogenic/odontogenic cells (Sonoyama et al. 2008).

To sum up, 3 stem cell niches contribute to tooth root formation: the apical papilla (forming odontoblasts in root), HERS, and dental follicle (contributing to cementoblasts for cementum formation; Fig. 1).

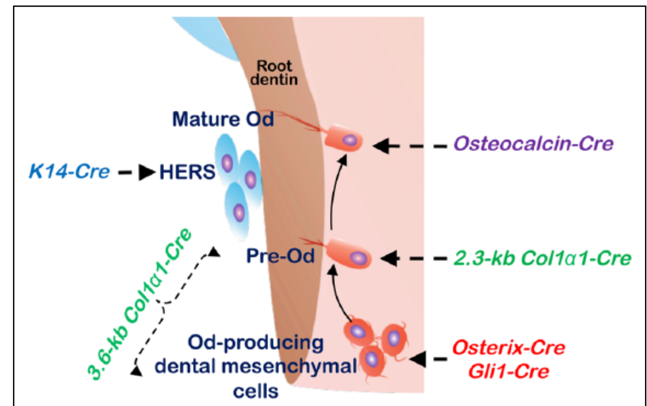

Figure 3. Diagram showing the Cre transgenic mouse lines applied in tooth root formation studies. HERS, Hertwig's epithelial root sheath; Od, odontoblast.

Genes Involved in Multirooted Tooth Furcation Formation

Perhaps, one of the most intriguing challenges in root formation is to understand the mechanism by which some teeth develop a single root while others form 2 or 3 single roots. Although the formation of tooth furcation (the place where the roots fork or separate) is largely unknown at present, Hu and Simmer's (Yang et al. 2015) laboratory recently discovered a notable taurodontism (a condition whereby the pulp chamber is vertically enlarged at the expense of the roots) with essentially no root furcation in mice lacking *Wnt10a* (a signaling molecule important for tooth development). Importantly, these authors found that in *WNT10A* mutation families, some heterozygotes exhibited molar root taurodontism. This finding may greatly stimulate more research interest in this area.

Cre Transgenic Mouse Lines Applied in Studies in Tooth Root Formation

Currently, there is no Cre transgenic mouse line that specifically targets targeting tooth root cells. However, multiple Cre transgenic lines have been used to investigate how root dentin is formed by targeting either HERS- or dental mesenchyme–derived cells (Fig. 3). At present, K14-Cre is the only transgenic line available for targeting genes expressed in HERS cells (Huang et al. 2010). The Cre lines targeting early dental mesenchyme–derived cells include *Gli1-CreER*^{T2} and *Osterix-CreER*^{T2} (mainly active in pulp cells). The cell lineage tracing (*Gli1-CreER*^{T2}*; R26*$^{tdTomato/+}$) and cell ablation (*Gli1-CreER*^{T2}*; R26*$^{DTA/+}$) assays revealed that the Gli1^{+} cells appear to be root progenitor cells and are crucial for root development, although the Gli1-derived cells are also responsible for crown dentin

Table. Main Cre Transgenic Lines Used for the Analysis of Tooth Root Formation–Related Genes.

Cre Transgenic Line	Examples of Gene Targeted	Root Phenotype	Reference	Original Description
Conventional knockout	*Nfic*$^{-/-}$	Short roots	Steele-Perkins et al. 2003	
Gli1-CreER^{T2}	*Gli1-CreER*^{T2}*; R26SmoM2*$^{fl/fl}$	Short roots	Liu et al. 2015	Ahn and Joyner 2004
Osterix-CreER^{T2}	*Osx-CreER*^{T2}*; PPR*$^{fl/fl}$	Short roots	Ono et al. 2016	Maes et al. 2010
	Bmp2-cKO$^{Sp7\text{-}Cre\text{-}EGFP}$	Short roots	Rakian et al. 2013	
	Osx-CreER^{T2}*; Tgfbr2*$^{fl/fl}$	Short roots	Wang et al. 2013	
3.6-kb *Col1α1-Cre*	3.6-kb *Col1α1-Cre, Osx*$^{fl/fl}$	Short roots	Kim, Bae, Lee, et al. 2015	Liu et al. 2004
2.3-kb *Col1α1-Cre*	2.3-kb *Col1α1-Cre, Osx*$^{fl/fl}$	Short roots	Zhang et al. 2015	Liu et al. 2004
Osteocalcin-Cre	*OC-Cre; Catnb*$^{+/lox(ex3)}$	Short roots	Bae et al. 2013; Zhang et al. 2013	Dacquin et al. 2002
	OC-Cre; Ctnnb1$^{fl/fl}$	No roots	Kim et al. 2013	
	OC-Cre; Wls$^{CO/CO}$	Short roots	Bae et al. 2015	
K14-Cre	*K14-Cre; Smad4*$^{fl/fl}$	No roots	Huang et al. 2010	Dassule et al. 2000
UBC-CreER^{T2}	*UBC-CreER*^{T2}*; Bmp1*$^{fl/fl}$*, Tll1*$^{fl/fl}$	Short roots	Wang et al. 2017	Ruzankina et al. 2007

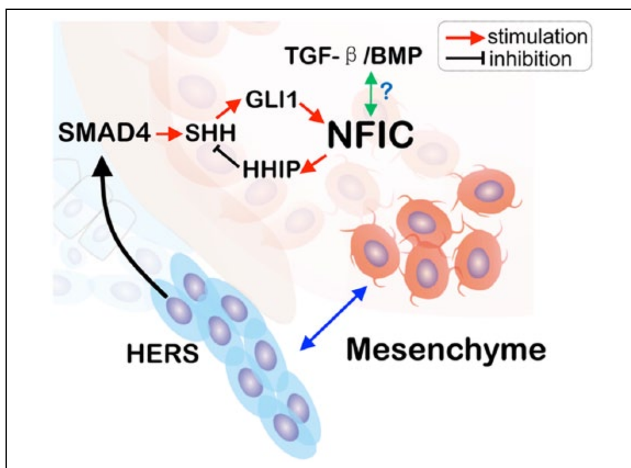

Figure 4. Diagram showing the current major molecular pathways regulating root formation via an interaction between Hertwig's epithelial root sheath (HERS) and dental mesenchymal cells. BMP, bone morphogenetic protein; TGF-β, transforming growth factor β.

formation (Liu et al. 2015). Similarly, the cell lineage tracing technique showed a large number of Osx^{+} cells in the tooth pulp. Many of their progeny cells become root odontoblasts, as well as odontoblasts in crown, cementoblasts, alveolar bone osteoblasts, and periodontal ligament fibroblasts (Rakian et al. 2013).

The most common Cre lines targeting root odontoblasts are 1) 2 noninducible Cre lines—2.3-kb *Col1α1-Cre* (more specific to preodontoblasts) and 3.6-kb *Col1α1-Cre* (some pulp cells, preodontoblasts, and odontoblasts; Elefteriou and Yang 2011)—and 2) the tamoxifen-inducible 3.2-kb *Col 1α1-CreER*^{T2}, which is active in preodontoblasts and odontoblasts (Maes et al. 2010). Another Cre line to target mature odontoblasts is the *osteocalcin-Cre*, whose activity was first observed in the developing tooth germ prior to postnatal day 0. By P4 and P6, *osteocalcin-Cre* was highly expressed in crown odontoblasts. At P10, the Cre was strongly expressed in the root odontoblasts (Bae et al. 2015). Additionally, some ubiquitous Cre lines, such as *UBC-CreER*^{T2}, which is under the control of the human ubiquitin C promoter, have been used in root formation as well (Wang et al. 2017).

Conclusion

In the last 2 decades, there has been substantial progress in tooth root studies with the application of new techniques (including cell lineage tracing) and the gain- or loss-of-function studies of different genes (Table). These exciting findings suggest that root formation is regulated in a different and unique way when compared with crown formation. Furthermore, the new evidence demonstrates that NFIC, as the key master gene for tooth root formation, directly or indirectly interacts with Osx-Wnt/β-catenin (Fig. 2) and TGF-β-BMP-SMAD4 via an interaction between the HERS and dental mesenchyme (Fig. 4). For a potential link of the tooth root study to clinical relevance, we included the subject of the short root anomaly in this review.

Future studies in this area will not only shed new light on understanding signaling pathways involved in tooth root formation but also provide a solid foundation for tooth regeneration as "the problem with tooth regeneration is the root problem" (Dr. Martha Somerman, director of National Institute of Dental and Craniofacial Research / National Institutes of Health).

Author Contributions

J. Wang, J.Q. Feng, contributed to conception, design, and data interpretation, drafted and critically revised the manuscript. Both authors gave final approval and agree to be accountable for all aspects of the work.

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