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REVIEW

Integration of tooth morphogenesis and innervation by local tissue interactions, signaling networks, and semaphorin 3A

Keijo Luukko[a] and Päivi Kettunen[b]

[a]Section of Orthodontics, Department of Clinical Dentistry, University of Bergen, Bergen, Norway; [b]Craniofacial Developmental Biology Group, Department of Biomedicine, University of Bergen, Bergen, Norway

ABSTRACT

The tooth, like many other organs, develops from both epithelial and mesenchymal tissues, and has proven to be a valuable tool with which to investigate organ formation and peripheral innervation. Tooth formation is regulated by local epithelial-mesenchymal tissue interactions, and is closely integrated with stereotypic dental nerve navigation and patterning. Recent analyses of the function and regulation of semaphorin 3A (SEMA3A) have shed light on the regulatory mechanisms that coordinate organogenesis and innervation at the tissue and molecular levels. In the tooth, SEM3A acts as a developmentally regulated secretory chemo-repellent, that controls tooth innervation during embryonic and postnatal development. The tooth germ governs its own innervation by a combination of local tissue interactions and SEMA3A expression. SEMA3A signaling, in turn, is controlled by a number of conserved signaling effectors, including TGF-β superfamily members, FGF, and WNT; all function in embryo and organ development, and are essential for tooth histo-morphogenesis. Thus, SEMA3A driven axon guidance is integrated into key odontogenic signaling networks, establishing this protein as a critical molecular tether between 2 distinct developmental processes (morphogenesis and sensory innervation), both of which are required to obtain a functional tooth.

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Introduction

The developing tooth germ has turned out to be a useful model system with which to decipher the regulatory mechanisms that underpin organ development and stem cell biology.[1-4] Moreover, the tooth is a well-defined target organ for peripheral innervation.[5-9] Previous investigations have demonstrated how 2 apparently distinct processes, tooth organ formation and innervation, take place in a highly coordinated fashion. This integration is achieved through local, organ specific tissue interactions, that are mediated by distinct molecular signaling pathways.

The adult tooth nerve supply and its functions

The teeth are unique, greatly specialized organs, which in mammals develop in the 1st branchial arch and are exclusively found in the oral cavity. In addition to 1st branchial arch, some mammalian teeth develop from the frontonasal process. The principal function of teeth in man is mastication, while also making a substantial contribution to articulation and appearance. Teeth receive protective sensory trigeminal innervation from the trigeminal ganglion,[10] with an abundant number of sensory nerve endings located in the soft tissue pulp of the crown. The crown itself is the visible part of the tooth within the oral cavity, and is responsible for its masticatory functions. The highest concentration of plentiful number of intricately arranged, sensory nerves, in the crown pulp is distributed in the pulp-dentin border- area of the crown pulp, toward the occlusal surface containing the subodontoblastic region, odontoblast layer, and dentin tubules. These sensory nerves mediate the sensation of pain.[10-12] The periodontal space between the root of the tooth and the adjacent alveolar bone, is the second major target area for sensory nerves, with these mediating pain, as well as pressure and touch.[10-12] Nerves emanating from the sympathetic superior cervical ganglion are found in association with dental blood vessels in both the dental pulp, and periodontal space, and are involved in vasoregulatory functions.[10-13]

Peripheral nerves engage in additional, non-neuronal functions, in the developing and adult tooth. Dental

CONTACT Keijo Luukko Keijo.Luukko@iko.uib.no Section of Orthodontics, Department of Clinical Dentistry, University of Bergen, Årstadveien 19, 5009 Bergen, Norway; Päivi Kettunen Paivi.Kettunen@uib.no Craniofacial Developmental Biology Group, Department of Biomedicine, University of Bergen, Jonas Lies vei 91, 5009 Bergen, Norway.

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nerves are involved in inflammatory responses of the adult tooth, with more extensive pulp necrosis evident in injured denervated teeth than innervated.[12-14] Trigeminal nerves have also been shown to be indispensable for the continuous generation of teeth in fish.[15] Innervation of the periodontium is required to inhibit the pathological fusion of teeth roots to the surrounding alveolar bone (dentoalveolar ankylosis), and root resorption.[16] Recently, the neurovascular bundle was identified as a mesenchymal stem cell niche in the mouse incisor.[17] Similarly, the glial cells that envelope neurons, are a source of mesenchymal stem cells that can contribute to the repair of injured pulp.[18]

Tooth histomorphogenesis and innervation are tightly spatio-temporally integrated

During embryogenesis, the development of tissues, organs, and the nervous system, takes place contemporaneously. Given the specific localization of sensory nerve endings in key areas of the mature tooth, and their important functions, it is plausible to assume that the development of tooth-supporting innervation, together with the tooth germ itself, does not occur haphazardly, but is instead strictly choreographed. Indeed, earlier developmental biology studies on mammalian teeth established that the development of the tooth's shape and dental cells, takes place in a strictly regulated manner.[19] This process is characterized by complex epithelial and mesenchymal tissue histomorphogenesis, including a condensation of the mesenchyme, coordinated cell proliferation, folding of the dental epithelium, and a gradual determination and differentiation of tooth-specific cells that includes the enamel and dentin producing ameloand odontoblasts in the crown.[2,3,20]

Tooth innervation is tightly linked to advancing tooth development, and occurs stereotypically in a developmentally regulated manner across different species.[10] Recent investigations used the mouse mandibular first molar tooth germ as a model system to show that dental sensory axons must grow in precisely defined mesenchymal pathways in order to properly reach the mesenchymal tooth-target areas (Fig. 1).[5] Histologically, the pioneer dental axons have been shown to emerge from the deep mandibular inferior alveolar nerve, where they grow toward the first molar tooth germ at embryonic (E) day 12.5.[21-24] Approximately half a day later, the first dental axons reach the target area of the bud-stage tooth germ. During the tooth-specific morphogenetic cap and bell stages, nerves innervate the tooth germ, to surround

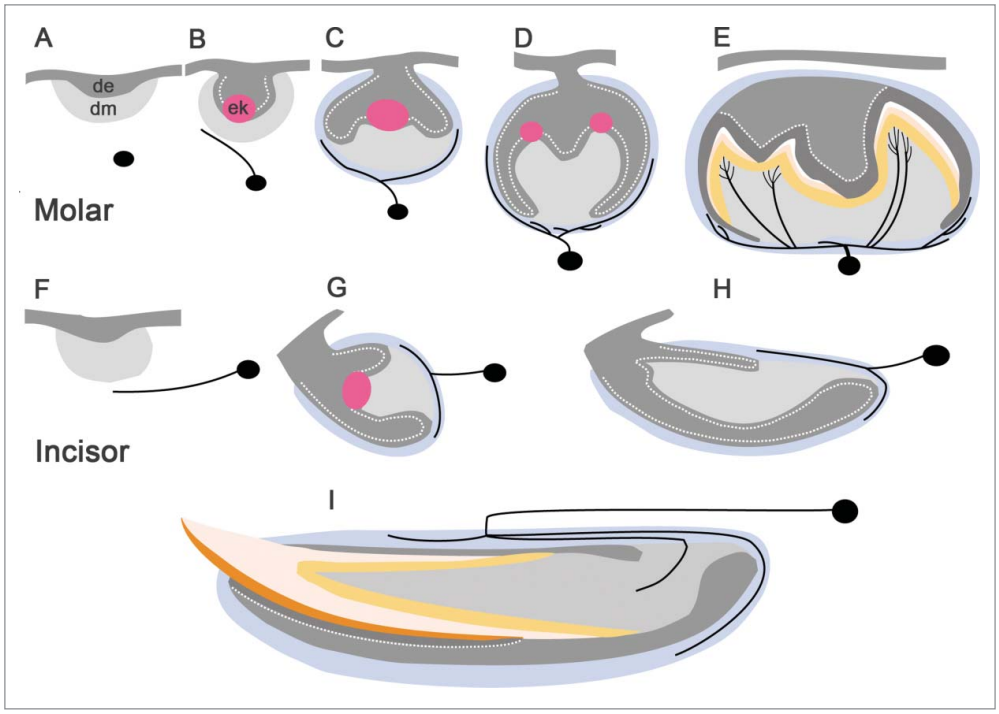

Figure 1. A comparison of nerve localization during embryonic and early postnatal crown morphogenesis of the mouse mandibular incisor, and 2-rooted first molar. Tooth innervation takes place in a stereotypic manner in both tooth types, and is linked to the advancing tooth histomorphogenesis and dental cell differentiation. See main text for a description of the figure. (A, F) initiation stage; (B), bud stage; (C, G) cap stage; (D, E, H, I) bell stage. Abbreviations: cm, condensed dental mesenchyme; de, dental epithelium; dp, dental pulp; p, dental papilla; ek, enamel knot; pm, presumptive dental mesenchyme. Nerve fibers are indicated in black. This schematic is based on data from previous reports.[32,23,13,25,26]

the mesenchymal dental follicle target area, which ultimately forms the periodontium (during root formation and tooth eruption) that attaches roots to alveolar bone.[10,13,23,25,26] Of note, although the nerves already surround the developing tooth organ during embryonic stages, axons will only grow into the mesenchymal dental pulp post-natally. Typically this occurs after a delay of about 10 days (from the first encounter of axons with the tooth target), on postnatal day 3–4. This timing is immediately after the onset of enamel formation of the tooth crown.[13] It is also intriguing to note that the ingrowth of axons into the pulp does not take place randomly, but instead axons navigate the mandibular molar tooth pulp solely through defined mesial and distal areas, around which the roots of the 2-rooted molar tooth are formed by odontoblasts.[7,13] The roots commence to form after the floor of the pulp has been laid down, and at this stage ingrowth of the sympathetic nerve fibers occurs into the dental pulp for the first time.[13] The periodontal space receives its final nerve supply after tooth eruption and termination of root growth during later postnatal development. Mouse tooth innervation is developmentally regulated in the embryo and newborn pup, and is not complete until late postnatal stages. Importantly, the timing of axon navigation, the encounter of axons with the tooth germ, as well as patterning of the axons within the mesenchymal tissue compartment of the tooth germ, take place concomitantly with tooth morphogenesis and cell differentiation. These events are therefore closely integrated with the key developmental steps of odontogenesis.

The tooth germ controls it own innervation, using local signals

Earlier neurobiological investigations have provided fundamental data on the mechanisms of tooth innervation. It has been demonstrated that experimentally separated tooth germs, and adult denervated teeth, undergo re-innervation.[27-29] In line with this, explants of dental mesenchyme can influence axonal growth in a developmental manner.[30] The expression of neuro-regulatory molecules appears to be independent of nerves in culture, with innervation of the adult tooth regenerating after trauma.[12,31] Moreover, rudimentary tooth germs, which degenerate in the mouse diastema, never become innervated.[32] To sum up, these data provide evidence that the developing tooth regulates the establishment of its own nerve supply, in a similar fashion to its control of morphogenesis,[3] and that the genetic control of these events is mediated by locally expressed effectors.[5]

The regulation of tooth innervation appears to involve signaling derived from both secretory and membrane-bound neuro-regulatory families; both are critical for the development of the central and peripheral nervous system.[5,6] Many neuro-regulatory molecules exhibit spatio-temporal changes in their cellular expression in the tooth germ, with these changes correlating with the growth of axons. Besides the neurotrophin and glial cell line-derived neurotrophic factor (GDNF) protein families (which serve critical roles in tooth innervation), other neuro-regulatory families are also expressed in the developing tooth germ, and are implicated in the development of the tooth's nerve supply.[5,6] These include the cell membrane-bound ephrin ligands, their Eph receptors, netrins, laminins, and cell-adhesion molecules (CAMs). Members of these families are likely to control various aspects of tooth innervation such as axon navigation, target field recognition and innervation, as well as nerve survival, and maturation.[5,6] Although the exact functions of the majority of neuro-regulatory molecules in tooth innervation are yet to be discovered, there is evidence that the prototypic member of the neurotrophin family, NGF (nerve growth factor),[33] serves a key role in the development of the nerve supply. For example, dynamic expression patterns of NGF mRNA during the embryonic and postnatal development of the incisor and molar teeth, correlate with pivotal phases of tooth innervation. These data suggest that NGF may control dental axon guidance, and target field innervation,[31,34,25] in addition to its functions in neuronal survival.[35,36,35,37]

Locally expressed SEMA3A regulates tooth innervation

In addition to being a significant regulator of nervous system development, axonal guidance, and fasciculation, SEMA3A signaling is involved in the development and physiology of many non-neuronal tissues.[38,39,40,41,42,43] mRNAs for SEMA3A, which repels both sensory and sympathetic nerves,[41] displays developmentally regulated and distinct cellular expression patterns in both epithelial and mesenchymal tissue during mouse molar and incisor development.[23,26,44,45] *Sema3A* is specifically expressed in sites that are devoid of navigating axons, suggesting functions in tooth innervation. During the onset of tooth formation and innervation, *Sema3A* is expressed in the dental and jaw mesenchyme areas, adjacent to the pioneer nerve branch (the trigeminal 'molar nerve') growing toward the early tooth germ. Later, during the bud, cap and bell stages, *Sema3A* is seen around the tooth germ, flanking the mesenchymal dental follicle target area, where the number of nerves gradually increases in both embryonic and postnatal molar and incisor tooth germs.[7,23,26,45] In

the dental pulp, although *Sema3A* is located in the middle part of the base of the 2-rooted mandibular molar tooth, transcripts are specifically absent from the future sites of the mesial and distal roots where nerve fibers grow into the dental pulp. Similarly, in the single-rooted incisor tooth germ, *Sema3* transcripts are lacking in the pulp/root areas through which nerves navigate the pulp.[7,23,26,45]

Analyses of *Sema3A*-deficient transgenic mice confirmed that SEMA3A is a critical regulator of tooth innervation, that is serially exploited at critical embryonic and postnatal stages of tooth innervation.[23,25,26] In both *Sema3A*-deficient incisor and molar tooth germs, pioneer axons reach the tooth germ prematurely and show apparent disturbances in nerve patterning and fasciculation. Although dental mesenchymal target areas become innervated, nerve fibers frequently overshoot their targets, to enter abnormal ectopic locations within mesenchymal exclusion areas, which would suggest a failure of the surrounding repulsion mechanisms (Fig. 2).[23,25,26] The dental pulp of *Sema3A*$^{-/-}$ molars are also prematurely innervated, with axons exhibiting abnormal patterning and fasciculation, in particular within the enlarged and defective subodontoblastic nerve plexus.[25] In contrast, the innervation of the dental pulp in *Sema3A*$^{-/-}$ incisors appears normal, reflecting the finding that *Sema3A* is largely nonexistent in the postnatal incisor pulp.[26,45] However, an abnormal, elevated number of axons and their arborization, is observed in the incisor periodontium, particularly at the labial side.[26]

The finding that some degree of correction occurred in the dental nerve patterning of *Sema3A*$^{-/-}$ molars[25] suggests that other neuro-regulatory molecules may compensate for the lack of SEMA3A signaling. For example, the expression of NGF and GDNF (that exert positive influences on axon growth and tooth target innervation), and their receptors, are unaltered in the trigeminal ganglion during various stages of tooth innervation in *Sema3A*-mutant teeth.[23,25,26] Similarly, mRNA expression of LANR, NCAM, and NET3, all implicated in tooth innervation, appears to be independent of SEMA3A signaling. This supports a model in which SEMA3A's control of dental axon growth, navigation, patterning, and fasciculation, is independent on many tooth-expressed neuro-regulatory molecules, and that tooth innervation involves redundant and independent signaling from neuro-regulatory proteins of different families.[5,6,8,9]

Collectively, these results establish that SEMA3A mediates dental nerve-tooth target interactions, and is an essential signal needed for the timing and patterning of embryonic and postnatal tooth innervation, as well as dental nerve fasciculation and sprouting.[23,25,26] Moreover, these results provide significant evidence for a model in which the tooth germ itself controls its own innervation by local concerted, and apparently redundant signals. Several expression domains of *Sema3A*, such as the epithelial cervical loops, which contribute to root formation, and preodontoblasts, which later differentiate into

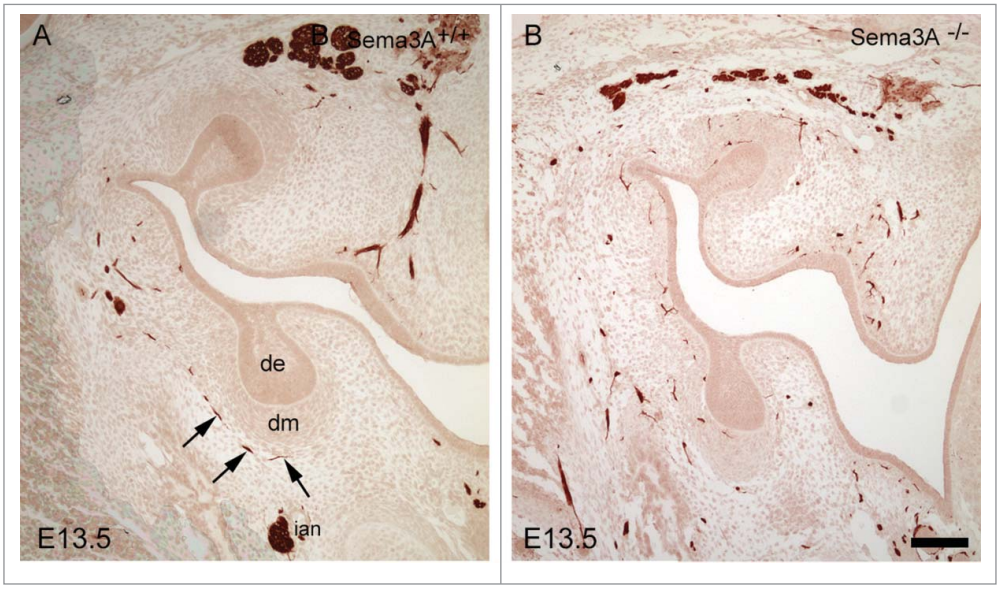

Figure 2. Immunohistochemical localization of nerve fibers in *Sema3A*$^{+/+}$ (A) and *Sema3A*$^{-/-}$ (B) bud stage molar tooth germs at E13.5. Nerves in the *Sema3A*$^{-/-}$ molars and mandible mesenchyme exhibit apparent defasciculation as well as abnormal patterning and tooth target innervation (e.g. ectopic expression in the condensed dental mesenchyme and next to the dental epithelium). In contrast, in the *Sema3A*$^{+/+}$ tooth, germ dental nerves show organized, appropriate target innervation (arrows). Abbreviations: cm, condensed dental mesenchyme; de, dental epithelium. Scale bar: 100 μm.

dentin producing odontoblasts, imply non-neuronal functions not yet revealed by genetic analyses.[23,25,26] Recently, however, SEMA3A was shown to be able to induce mesenchymal-stem-like properties in human periodontal ligament cells in culture conditions.[46] Thus, further studies regarding putative non-neuronal functions of SEMA3A signaling in tooth-formation are now warranted.

The regulation of dental Sema3A expression and tooth nerve supply by local tissue interactions

Classic developmental biology studies have demonstrated that tooth formation is regulated by sequential and reciprocal interactions between epithelial and mesenchymal cells. This chain of inductive interaction is defined as secondary induction and regulates all aspects of tooth development.[19,7] Recent molecular and genetic studies, particularly with mice, have elaborated on the molecular signatures that define relevant interactions, together with signaling pathways, and networks that control odontogenesis.[3]

Spatio-temporal changes in the *Sema3A* expression domain in the incisor and molar tooth germs, especially in the early dental mesenchymal compartments, correlated with inductive signaling between the dental epithelium and mesenchyme.[23,45] Analyses of *Sema3A* regulation using organotypic cultures and tissue separation, and recombination experiments, demonstrated that rather than being controlled by, or being dependent on, peripheral nerves, mesenchymal *Sema3A* expression was instead regulated by the dental epithelium during early tooth development.[23] Thus, these results demonstrated, for the very first time, that local tissue interactions regulate mesenchymal Sema3A expression, and thereby the development of the sensory nerve supply in the tooth germ. Consequently, local inductive signaling within the developing tooth germ provides an explanation as to how the tooth is able to control and orchestrate its own histomorphogenesis and innervation.[23] Interestingly, the early dental epithelium has been shown to possess the odontogenic information needed to determine the requisite number of teeth, their size, and shape.[21,47,48] Data regarding the regulation of *Sema3A* suggests that the tooth germ proper possesses the instructions needed to guide innervation and the establishment of a tooth-specific sensory nerve supply, until finalized late after birth.[23,25,26]

SEMA3A as a link integrating organogenesis and innervation

Tooth formation is dependent on, and controlled by, the activity of a limited number of conserved secreted signaling proteins (and their downstream pathways), including the TGF-β superfamily, FGFs, Hedgehog, and WNT,[49,3] all of which are commonly exploited in developmental processes during embryogenesis.[50] Certain family members have been shown to mediate reciprocal tissue interactions, and to control the expression of various signals and transcription factors essential for tooth formation. These effectors are collectively integrated into a complex network whose fine-tuning is suggested to underlie the creation of teeth with various morphologies and evolutionary transitions.[20,3]

Studies of *Sema3A* regulation during early tooth development revealed its integration into key odontogenic pathways. WNT and TGF-β signals were found to regulate *Sema3A* expression in the dental mesenchyme during early stages of pioneer dental axon navigation.[5] Many WNT signaling components are present in developing tooth tissues, with WNT signaling acting at multiple stages, including initiation, morphogenesis, and episodes of hard-tissue formation.[51-54] Analyses of *Sema3A* regulation also revealed that WNT4 expressed on early dental epithelium induces not only *Sema3A*, but also the *Msx1* transcription factor. MSX1 is essential for tooth formation in mouse and man, acting within the presumptive dental mesenchyme prior to the arrival of the first dental nerve fibers.[23] Moreover, TGF-beta1 stimulates *Sema3A* expression in the dental mesenchyme at the time of the initial nerve encounter with the tooth target field. TGF-beta1 expression arises at the onset of tooth morphogenesis,[55] and during dentinogenesis,[57] and it regulate *Ngf* and *Nt3* mRNA expression in maxillary process cells in culture.[56] GF signaling regulates embryonic development and organogenesis.[58] In the developing tooth, epithelial FGFR2b mediates the signaling of the mesenchymal FGFs requisite for tooth formation.[24,59] FGFR2b is essential for tooth morphogenesis from an early stage, as shown by an arrested development at the bud stage when this growth factor receptor is inactivated.[24] In FGFR2b$^{-/-}$ molars, *Sema3A* shows defective expression domains, and is down-regulated from the bud and cap stage in Fgfr2b$^{-/-}$ dental mesenchymes.[24] Notably, dental axons show defective patterning as demonstrated by the finding that the trigeminal molar nerve failed to establish its lingual branch at the bud stage.[59] Furthermore, it was found that the enamel knot signaling center expressed *Fgf4*,[60-62] which was able to indirectly regulate *Sema3A* by controlling *Tgfß1*.[24] These results indicate that FGF signaling is essential for tooth morphogenesis, and, by regulating *Sema3A*, also controls tooth innervation. Collectively, these data show that SEMA3A is regulated by, and integrated into, the TGF-β, FGF, and WNT signaling pathways, as well as more extensive odontogenic signaling networks that control tooth formation (Fig. 3).[24,63-65]

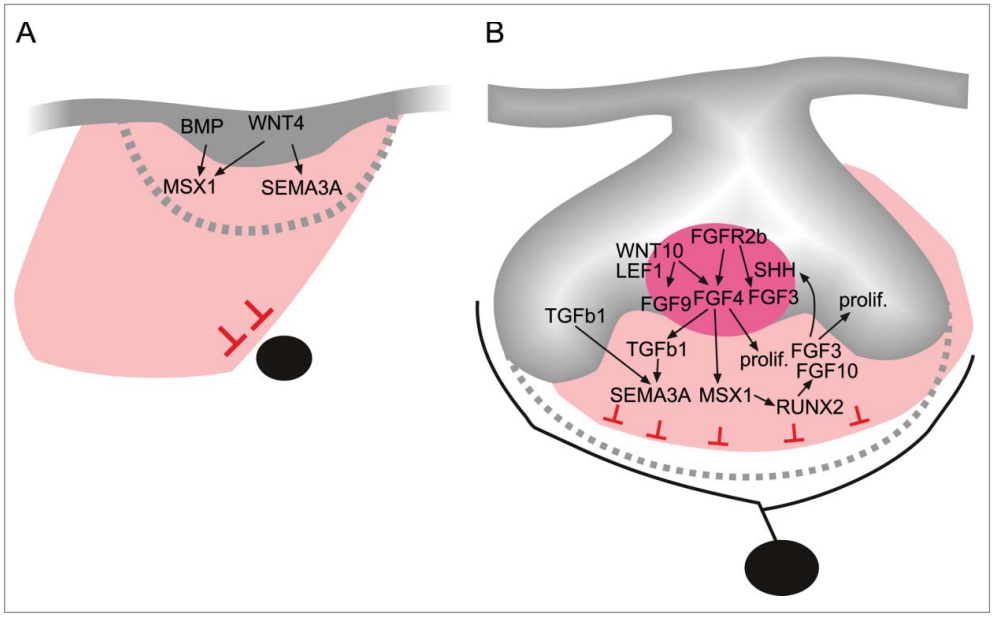

Figure 3. A model showing select signaling pathways and networks involved in the coordination of tooth morphogenesis and innervation during initiation (A), and the early morphogenetic cap stage (B). Tooth formation is crucially dependent on epithelial-mesenchymal interactions, which also regulate mesenchymal Sema3A, and the subsequent timing and patterning of tooth target innervation. Members of the conserved FGF (FGF4), Wnt (WNT4), and TGF-β superfamily (TGFß1) regulate SEMA3A expression. These signaling pathways are part of a larger odontogenic signaling network involving genes that are absolutely necessary for tooth formation in man and mouse, such as the MSX1 transcription factor.[23,24,66-68,69,70,71]

Summary

The developing tooth, like many organs, arises from both epithelial and mesenchymal tissues, and is a valuable model organ with which to investigate the regulation of organ histomorphogenesis at the tissue, genetic, and molecular levels.[64,65] Studies of the functions and regulation of SEMA3A have unraveled novel developmental regulatory mechanisms regarding tooth innervation. The developing tooth germ controls its own innervation by local epithelial-mesenchymal tissue interactions, with the regulation of SEMA3A achieved via different conserved signaling effector families. These studies have established SEMA3A as a key regulator of innervation for the tooth. In particular, SEMA3A, as part of an integrated molecular network, is employed at consecutive stages of embryonic and postnatal innervation to control tooth development. Importantly, SEMA3A is proposed to serve as a molecular link between the development of the tooth and its specific nerve supply. Further detailed studies regarding the functions of SEMA3A signaling in tooth innervation, and how these signals are integrated within larger regulatory networks, are now warranted.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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