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# Rodent incisor as a model to study mesenchymal stem cells in tissue homeostasis and repair

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The homeostasis of adult tissues, such as skin, hair, blood, and bone, requires continuous generation of differentiated progeny of stem cells. The rodent incisor undergoes constant renewal and can provide an extraordinary model for studying stem cells and their progeny in adult tissue homeostasis, cell differentiation and injury-induced regeneration. Meanwhile, cellular heterogeneity in the mouse incisor also provides an opportunity to study cell-cell communication between different cell types, including interactions between stem cells and their niche environment. More importantly, the molecular and cellular regulatory mechanisms revealed by the mouse incisor have broad implications for other organs. Here we review recent findings and advances using the mouse incisor as a model, including perspectives on the heterogeneity of cells in the mesenchyme, the niche environment, and signaling networks that regulate stem cell behavior. The progress from this field will not only expand the knowledge of stem cells and organogenesis, but also bridge a gap between animal models and tissue regeneration.

## KEYWORDS

rodent incisor, stem cell heterogeneity, stem cell niches, signaling networks, tissue homeostasis

## Introduction

Stem cells have an unlimited ability for self-renewal and the capability to differentiate into specialized cell types (1). Therefore, stem cells are the engine for supporting tissue homeostasis and regeneration because they are responsible for tissue renewal under normal conditions and in injury repairs (2, 3). Extensive progress has been made in recent decades regarding the mechanisms that regulate stem cell behavior (4, 5). Accumulating evidence suggests that stem cells are tightly regulated by both extrinsic signals from the surrounding niche cells, other tissue structures and intrinsic signals from the stem cells themselves (6, 7). Numerous organs have been utilized as models to investigate the function of stem cells in various scenarios (8).

At the cellular level, stem cells are highly heterogeneous and their interactions with the neighboring cells and structures will determine whether these stem cells will proliferate or differentiate into different cell types. Multiple signaling pathways have been shown to control the fate of stem cells during development, tissue homeostasis and repair. Specifically, FGF, BMP, Notch, WNT, and Hh signaling networks play crucial role in regulating the fate of stem

cells and providing important feedback to them (9–11). Transcription factors strategically teaming up with growth factor signaling mediators can activate or repress downstream signaling pathways to control the fate of stem cells in a cell type dependent manner (12). Furthermore, growing evidence has shown that epigenetic modifications play crucial roles in regulating the fate of stem cells in development, tissue homeostasis, injury repair and diseases (13, 14). Different tissues and organs have been used to investigate the regulatory mechanism of stem cell fate determination. Unlike most organs in adult animals, rodent incisors can continuously grow throughout the lifespan of the animal, making this organ an attractive model to study the role of stem cells in tissue homeostasis and repair.

Significant advances have been made using the incisor as a model to identify dental stem cells, understand their functions and characterize the molecular and cellular mechanisms that regulate their dynamic fate decisions (15–17). In the incisor, stem cells are compartmentalized into epithelial stem cells (ESCs), which reside in the cervical loop to support epithelial tissue renewal; and mesenchymal stem cells (MSCs), which encompass the territory between the cervical loops to fuel the homeostasis of the mesenchymal tissue in the incisor. Like in many tissues and organs, there is an undifferentiated population of cells that undergo mitosis, termed transit amplifying cells (TACs), positioned between the stem cells and terminally differentiated cells (18, 19). As the stem cells exit their quiescent state they immediately give rise to TACs, which then will differentiate into ameloblasts in the dental epithelium and odontoblasts and dental pulp cells in the dental mesenchyme of adult mouse incisor. The differentiation of these stem cells is highly organized along the proximal to distal axis, making incisor an ideal model to track stem cell fate determination and differentiation process, and to investigate the mechanisms through which stem cells support tissue homeostasis and repair. Lessons learned from stem cell studies in adult mouse incisor are highly informative for investigation of stem cells in other organs in maintaining tissue homeostasis and repair (20, 21). Furthermore, recent studies have clearly identified genes that are specifically expressed in stem cells, or TACs within the adult mouse incisor, making it possible to utilize these genes as markers to identify stem cells or TACs *in vivo* and perform cell lineage analysis in order to test the fate determination process of stem cells in maintaining tissue homeostasis or repair (19, 22, 23). Here we review the progress and current understanding of the rodent incisor as a model to investigate the regulatory mechanism and feedback on stem cells. We highlight some outstanding questions for future investigation. Specifically, we will address epithelial and mesenchymal stem cells in the adult mouse incisor, focusing more on the MSCs and their niche environment, signaling pathways and epigenetic regulators that control the fate of MSCs, and offer perspectives on how the adult mouse incisor will continue to serve as a highly informative model to advance our understanding of the regulatory mechanisms of stem cells in supporting tissue homeostasis, repair and regeneration.

## Epithelial and mesenchymal stem cells in the mouse incisor

Epithelial stem cells in the mouse incisor follow a classical stem cell paradigm, in which a few slow-cycling quiescent stem cells reside in the proximal region of the labial cervical loop (laCL), in particular the structures known as the outer enamel epithelium (OEE) and stellate reticulum (SR). Several signaling pathways have been shown to regulate the fate of these epithelial stem cells, including Hh, FGF, BMP and Activin, Hippo and others (24). This epithelial stem cell population gives rise to TACs in the inner enamel epithelium (IEE), which subsequently differentiate into ameloblasts to form enamel (25). Cell lineage and transplantation studies have definitively validated the stem cell status of these epithelial cells (16). The transition from epithelial stem cells to TACs, then to pre-ameloblasts and finally to fully differentiated ameloblasts provides a well-organized cell lineage differentiation process that can serve as a model to investigate the regulation of epithelial stem cells in maintaining tissue homeostasis and injury repair.

Recently, the identity of mouse incisor epithelial stem cells was characterized from different perspectives using a combination of single-cell RNA sequencing (scRNA-seq) and computational approaches. These studies revealed that dividing cells in the incisor IEE appear to undergo self-renewal during incisor epithelial tissue homeostasis and both ameloblasts and adjacent non-ameloblast cells are differentiated from these actively cycling epithelial progenitors, suggesting that these dividing cells orchestrate the homeostasis and repair of the incisor epithelium (26). Though well established, our understanding of the identity of epithelial stem cells in the incisor is still evolving, and future technologies may be utilized to study this question from different perspectives.

Similar to epithelial stem cells, MSCs residing in the proximal region are quiescent stem cells that can be activated into TACs, which differentiate into odontoblasts and dental pulp cells in the mouse incisor. MSCs are located close to the neurovascular bundle from which they receive signals to regulate their fate decision process (19). Using scRNA-seq analysis, studies have shown that MSCs are highly heterogeneous and may play multiple roles in maintaining incisor tissue homeostasis and repair (23). The anatomical locations of MSCs and TACs in the incisor, which are in close proximity to each other, are well defined, making the incisor an excellent model for studying the functions of these cell populations. Our study has also shown that MSCs in the proximal region of the adult incisor take about four weeks to populate the entire dental mesenchyme and reach the distal end of the incisor (19). This knowledge has provided the opportunity to measure the rate by which MSCs give rise to cells of the incisor mesenchyme, such as odontoblasts and dental pulp cells, which has facilitated the analysis of how genetic mutations, for example, may affect the migration of MSC progeny and

ultimately affect the fate of these cells in supporting tissue homeostasis and repair (27, 28). Similarly, the rate of odontoblast differentiation from the MSCs/TACs can also be measured. Because TACs serve as important intermediates between MSCs and pre-odontoblasts, we have a unique system to investigate MSC-TAC interaction and feedback in maintaining tissue homeostasis (29). Equally important, mouse incisor MSCs share some common characteristics with MSCs in the long bone. However, unlike in the long bone, the distribution of incisor MSCs, TACs, pre-odontoblasts, odontoblasts and dental pulp cells is well-organized along the proximal to distal axis of the incisor, providing an ideal environment to perform stem cell lineage tracing and differentiation analysis. Finally, because of the easy access to and well defined molecular markers in adult mouse incisors, we can measure the growth rate of the incisor and its ability to repair following injury. These analyses can be linked with the dynamic changes in MSCs or TACs in the proximal region of the incisor, making it possible to comprehensively evaluate the molecular and cellular processes involved in tissue homeostasis and repair (27, 28).

## Stem cell heterogeneity in the mouse incisor

Recent studies have shown that stem cells in the mouse incisor are very heterogeneous. This is especially the case in the dental mesenchyme. Different stem cell populations support the homeostasis and repair of the mouse incisor. The *in vivo* identities of these stem cells have been validated by lineage tracing experiments, which are the gold standard for identifying stem cells. For example, Ng2+ cells arising from pericytes can become odontoblasts after incisor damage (30). Gli1+ perivascular cells in the dental mesenchyme are Shh-responsive and can also contribute to incisor homeostasis and injury repair (19). To date, Gli1+ cells in adult mouse incisor represent the MSC population that can give rise to the entire dental mesenchyme while maintaining self-renewal in supporting incisor tissue homeostasis, highlighting the importance of the perivascular population in the incisor dental mesenchyme. Nerve associated Plp1+ glial cells can also give rise to odontoblasts and dental pulp cells during incisor growth and repair (31), suggesting the direct contribution of the nerve cells to the dental stem cells. Moreover, Thy1+ cells can contribute to incisor growth but not tissue homeostasis. Interestingly, a group of quiescent Celsr1+ cells in the dental mesenchyme can be activated and replenish Thy1+ cells upon injury (22). Recently, single-cell analysis of the mouse incisor has revealed that Foxd1+ cells near the labial cervical loop appear to possess self-renewal ability and thus can be considered another stem cell subpopulation in the incisor mesenchyme (23), suggesting that dental stem cell populations in the mouse incisor are quite heterogeneous.

Multiple epithelial stem cell populations in the proximal region of the OEE of the mouse incisor have been identified. For example,

Sox2+ cells can contribute to all the epithelial lineages in the mouse incisor (32). Bmi1+ stem cells are regulated by Bmi1-Ink4a/Arf axis (25). Shh-responsive Gli1+ cells in the incisor epithelium are also stem cells that contribute to incisor growth (33). Interestingly, the Acta2+ cell population can also contribute into the homeostasis of the incisor epithelium (23). In addition, Igfbp5+ and Lrig1+ cells in the mouse incisor reportedly give rise to the epithelial lineages in this organ (34).

## Incisor MSC niche

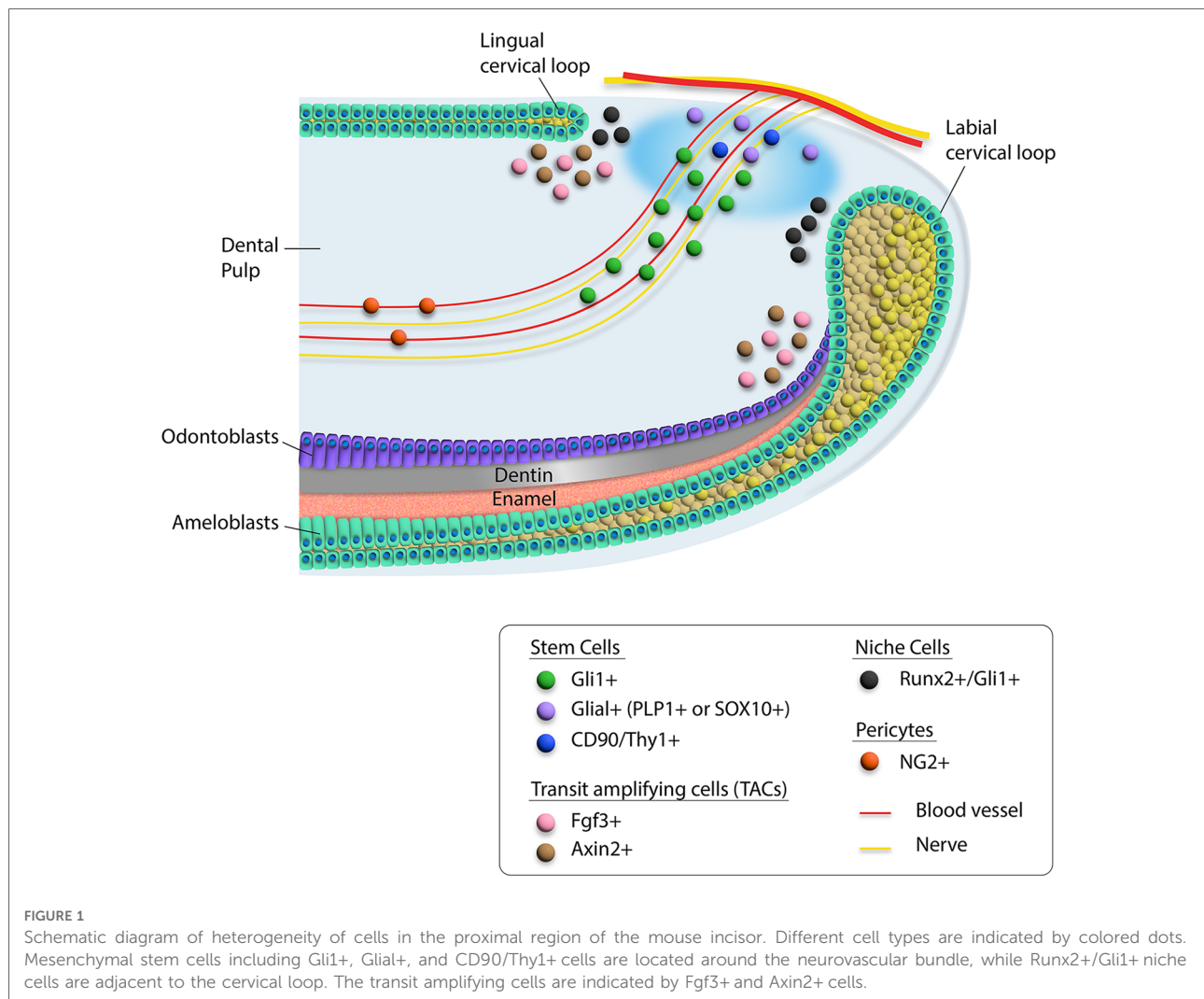
Stem cells are tightly controlled by their niche, which regulates how they participate in tissue homeostasis and repair. The neurovascular bundle serves as an important niche environment that integrates signals to mediate the balanced response of MSCs to the needs of homeostasis and repair. Recently, nerves have been found to regulate the MSC niches in other organs, such as skull and long bone. For example, Ngf is abundant in mesenchymal cells of cranial sutures, and directs sensory nerve transit and promotes nerve survival at suture sites (35). Moreover, sensory nerves secrete Fstl1 to regulate cell fate in the cranial suture and maintain MSCs in an undifferentiated state (36).

The interaction between MSCs and the neurovascular bundle niche component also creates the dynamic system necessary for sustaining tissues in the incisor. For example, Shh secreted by the inferior alveolar nerve activates Gli1+ cells in the mouse incisor to support its homeostasis. Interestingly, we found that a Runx2+ subpopulation of Gli1+ cells constitutes an important portion of the niche that regulates incisor homeostasis (27). These Runx2+ cells are not stem cells. Moreover, Axin2+ TACs function as a niche component, interacting with Gli1+ MSCs to regulate incisor homeostasis (29) (Figure 1).

Stem cell and niche cell populations were identified in the mouse incisor.

**Table 1** Stem cell and niche cell populations identified in the mouse incisor.

<i>Ng2</i>	Pericytes	<i>Ng2-CreER</i>	Growth and repair	Feng, J (30)
<i>Plp1</i>	Glial cells	<i>Plp1-CreER</i>	Growth and repair	Kaukua, N (31)
<i>Gli1</i>	Mixed population	<i>Gli1-CreER</i>	Homeostasis and repair	Zhao, H (19)
<i>Axin2</i>	TACs	<i>Axin2-CreER</i>	Homeostasis	Jing, J (29)
<i>Runx2</i>	Niche cells	<i>Runx2-rtTA</i>	Homeostasis	Chen, S (27)
<i>Thy1</i>	Mesenchymal cells	<i>Thy1-CreER</i>	Homeostasis	An, Z (22)
<i>Foxd1</i>	Subpopulation	<i>Foxd1-CreER</i>	Homeostasis	Krivanek, J (23)
<i>Pdgfrb</i>	Perivascular cells	<i>Pdgfrb-CreER</i>	Growth	Walker (37)
<i>Celsr1</i>	Mesenchymal cells	<i>Celsr1<sup>-/-</sup>; Celsr1<sup>fl/fl</sup></i>	Growth and repair	An, Z (22)
<i>Sox2</i>	OEE/SR	<i>Sox2-CreER</i>	Growth	Juuri, E (32)
<i>Bmi1</i>	OEE/SR	<i>Bmi1-CreER</i>	Homeostasis	Biehls, B (25)
<i>Igfbp5</i>	OEE/SR	<i>Igfbp5-CreER</i>	Homeostasis	Seidel, K (34)
<i>Lrig1</i>	OEE/SR	<i>Lrig1-CreER</i>	Homeostasis	Seidel, K (34)
<i>Acta2</i>	OEE/SR	<i>Acta2-CreER</i>	Homeostasis	Krivanek, J (23)



## Epigenetic regulation of stem cells in the incisor

Polycomb group (PcG) proteins are important epigenetic regulators involved in various biological processes. PcG proteins participate in two major multicomponent complexes, Polycomb Repressive Complexes 1 and 2 (PRC1 and PRC2) (38). Ring1a/b comprise the catalytic component of PRC1 complex, which mainly serves as a transcriptional repressor that deposits monoubiquitylation of histone H2A at lysine 119 (H2AK119ub1) (39). Ring1a/b are highly expressed in the proximal region of the dental mesenchyme in the incisor. Loss of *Ring1a/b* postnatally causes defects in the cervical loop and disturbs enamel and dentin formation, and it also causes a dramatic reduction of cell proliferation in the apical mesenchyme and cervical loop epithelium (40). Interestingly, downregulation of FGF signaling and its downstream targets is also observed in *Ring1a/b* mutant incisors. These results show that the PRC1

complex regulates the TACs and cell differentiation in developing mouse incisors (41).

The protein Bmi1, another member of the PRC1 family, is a well-recognized transcriptional suppressor and is capable of preventing premature senescence and maintaining the self-renewal of tissue-specific stem cells (42). Brian et al. found that Bmi1 is expressed by incisor epithelial stem cells and that deletion of Bmi1 results in diminished stem cells and defective enamel production. Mechanistically, they demonstrated that Bmi1-mediated repression of Hox genes preserves the undifferentiated state of incisor epithelial stem cells (25).

Ezh2 is one of the core enzymatic components of PRC2 and is important for the regulation of positional information of cranial neural crest cells (43). Interestingly, Ezh2 has been demonstrated to play an important function in determining the tooth root patterning of the mouse molar (44). Recently, Yu et al. found that loss of Ezh2 in Shh+ epithelial progenitor cells of the mouse incisor leads to impaired epithelial regeneration upon injury, suggesting



Ezh2 is indispensable for the lineage fidelity of epithelial stem cells in the mouse incisor (45).

BRG1/BRM-associated factor (BAF) is one of the most important chromatin remodelers belonging to mammalian SWI/SNF family, and plays an essential regulatory function in stem cell homeostasis (46). A recent study found that *Arid1a*, the largest subunit in the BAF complex, regulates mouse incisor tissue homeostasis through controlling proliferation of TACs and promoting cell cycle exit by inhibiting the Aurka-Cdk1 axis. After loss of *Arid1a*, the mitotic TAC population was expanded along and TAC differentiation was compromised (28).

MicroRNAs (miRNAs) are short strands of non-coding RNA that regulate protein function *via* post-transcriptional modifications. Selective silencing is achieved by complementary base-pairing between the miRNA and the mRNA's 3'-untranslated region (3'-UTR). Imperfect base-pairing allows miRNA to target a group of mRNA transcripts simultaneously, making miRNA a perfect tool for group-silencing related mRNA activities and maintaining stem cell homeostasis (47, 48).

Multiple studies have identified various miRNAs with roles in maintaining the tissue homeostasis of mouse incisors. The miR-200 family is one of the most studied miRNAs in the mouse incisor stem cell niche. It is highly expressed in the differentiating dental epithelial stem cells. The inhibition of the miR-200 family using the Plasmid-based miRNA Inhibitor System (PMIS) results in an expanded stem cell niche, reduced progenitor cell differentiation, and smaller incisor size (49). The miR-200 cluster maintains the stem cell homeostasis in the cervical loop through the proper compartmentalization of Sox2+ cells and epithelial differentiating cells, as well as by regulating the WNT and BMP signaling pathways (49, 50).

## Signaling pathways in regulating stem cells during incisor tissue homeostasis and repair

### FGF signaling

Numerous signaling pathways are involved in the tissue renewal and injury repair of the mouse incisor (Figure 2). Fibroblast growth factor (FGF) signaling induces the proliferation and differentiation of multiple cell types during embryonic development (51). Different ligands and receptors of FGF signaling participate in regulating stem cells in the incisor. For example, *Fgf3* and *Fgf10* are expressed in the incisor mesenchyme adjacent to the LaCL. Deletion of *Fgf3* and *Fgf10* leads to decreased proliferation of epithelial progenitor cells and a severely hypoplastic LaCL, suggesting functional redundancy of *Fgf3* and *Fgf10* in maintaining the epithelial stem cell pool in the incisor (52).

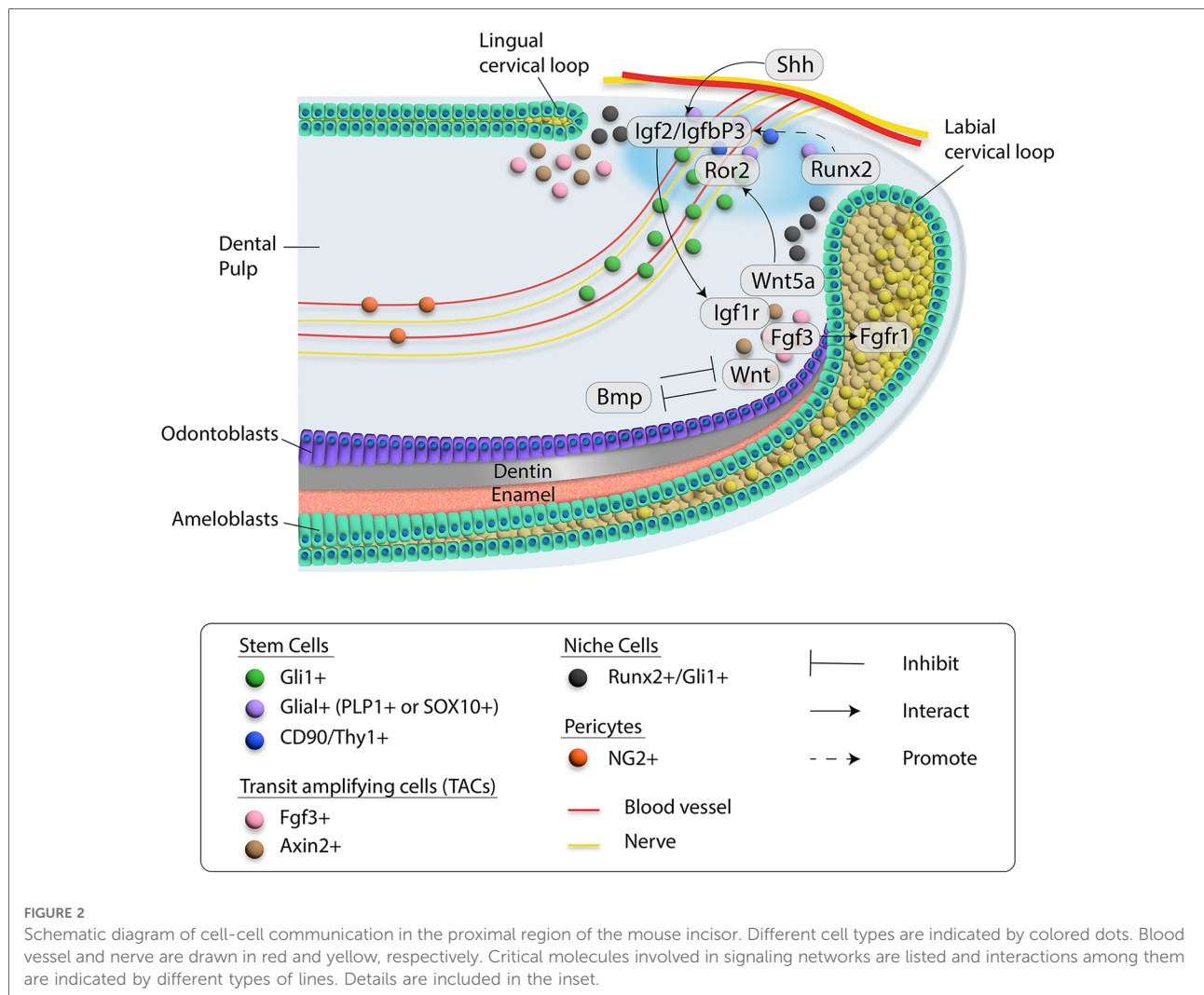
Furthermore, maintenance of epithelial stem cells in the developing incisor cervical loops is also regulated by the interaction between epithelial *Fgf9* and mesenchymal FGF signaling. Loss of *Fgf9* results in a lack of *Fgf3* and *Fgf10* expression in the dental mesenchyme (53). For this reason, it appears that *Fgf9* may keep progenitor cells from differentiating in the cervical loop by protecting them from being exposed to Shh signaling. The function of FGF signaling in regulating mesenchymal stem cells and pulp cells requires future studies.

### Hedgehog signaling

In the rodent incisor, Gli1+ cells are found in peri-NVB and epithelium of the cervical loop (33). Both of these two populations contain stem cells that can contribute to the dental mesenchyme and dental epithelium. HH signaling is among the most important signaling pathways that regulate embryonic development (54). *Ihh* and *Shh* are the two most studied HH ligands in the context of tooth development. Loss of *Ihh* in cranial neural crest cells can lead to reverse incisor occlusion, suggesting the vital role of *Ihh* in regulating incisor development (55). *Shh* is expressed in the dental epithelium and in the inferior alveolar nerve (IAN) innervating the tooth. Numerous studies have shown that *Shh* is involved in tooth morphogenesis (56, 57). Importantly, *Shh* is the major HH ligand that can activate the Gli1+ stem cell population in the mouse incisor. Previous studies have shown that Gli1+ cells are the HH-responsive epithelial stem cells that give rise to the epithelial lineages in the mouse incisor (33). They showed that HH signaling is not required for the survival of Gli1+ stem cells but is essential for their differentiation. Moreover, Li et al. showed that the fate of dental epithelial stem cells is controlled by a BMP-HH signaling network, which partially determines the postnatal growth potential of molars and incisors (58). As mentioned earlier in this review, *Shh* from the IAN can also activate the Gli1+ MSCs in the incisor, highlighting the importance of HH signaling in the regulation of stem cells in the mouse incisor (19, 59). Interestingly, loss of *Ptch2*, which is a receptor for HH signaling, can lead to increased Gli1+ MSCs and vascularization in the mouse incisor, adding more mechanistic insight into how HH signaling regulates stem cells in the incisor (60).

### BMP and TGF $\beta$ signaling

The transforming growth factor  $\beta$  (TGF $\beta$ ) signaling family plays diverse roles in embryonic development and adult tissue homeostasis by regulating various cellular behaviors (61). The TGF $\beta$  superfamily can be subdivided into four groups: the TGF $\beta$  subfamily (TGF $\beta$ 1, TGF $\beta$ 2, and TGF $\beta$ 3), the activin subfamily (inhibin/activin- $\beta$ A, inhibin/activin- $\beta$ B, and



inhibin/activin-βC), the BMP family (Bmp2, Bmp4, Bmp5, Bmp6, Bmp7, Bmp8, etc.), and divergent genes (Müllerian inhibiting substance, GDF-9, inhibin-α, GDNF, screw and lefty) (62–65). TGFβ signaling also has an important role in the development of the mouse incisor. For instance, TGFβ1, which is first observed in the dental epithelium and then extends to dental mesenchyme, is important for epithelial-mesenchymal interaction (66). Tgfβ2 is reported to regulate tooth size and stage without affecting cartilage, while Tgfβ3 regulates Meckel’s cartilage size without affecting tooth size and shape (67). In addition, mice lacking activin-βA are missing lower incisors (68). Meanwhile, the loss of TGF-β type I receptor (Alk5) in the dental mesenchyme leads to impaired proliferation of TA cells and the maintenance of dental epithelial stem cells (69). Guan et al. found that the mouse incisor displays “wavy” mineralized tissue caused by the premature differentiation of epithelial stem cells after the deletion of Tgfbr2 in the dental mesenchyme, highlighting the role of TGFβ signaling in mediating the cell-cell

interaction between the mesenchyme and epithelium of the incisor (70).

Bone morphogenetic proteins (BMPs) are one of the largest subgroups of the TGFβ family and play an essential role in many aspects of tissue homeostasis (71). BMP signaling is indispensable for tooth development. For example, loss of Bmpr1a signaling in the mouse molar can lead to compromised odontogenic differentiation (72–74). More interestingly, the inhibition of BMP signaling early in mandible development results in a transformation of tooth identity from incisor to molar (75). Shi et al. demonstrated that antagonistic interaction between BMP signaling and WNT and FGF signaling serves as a key regulator of MSC lineage commitment in the mouse incisor. Their study showed that maintenance of quiescent MSCs requires BMP signaling in the Gli1+ cell lineage, suggesting that BMP signaling has a dual role in incisor tissue homeostasis: it regulates odontoblast differentiation as well as provides feedback to the MSC population (76).

## WNT signaling

The WNT family comprises 19 WNT ligands that play essential roles during both embryonic development and tissue homeostasis by regulating stem cell behaviors including self-renewal, cell proliferation and differentiation. Members of the WNT family bind to transmembrane frizzled (FZD) receptors and various co-receptors to activate canonical ( $\beta$ -catenin dependent) and noncanonical ( $\beta$ -catenin-independent) signaling pathways (77–79). WNT signaling plays important functions in regulating various aspects of tooth development (80). Disrupting canonical WNT signaling in the incisor mesenchyme results in more apoptotic cells in the LaCL through inhibiting Fgf10 (81). Moreover, Wnt/ $\beta$ -catenin signaling in the dental mesenchyme can determine the number of mouse incisors (82), suggesting the importance of canonical WNT signaling at the early stage of incisor development. Noncanonical WNT signals are less well studied than canonical signals. Wnt5a is one of the typical noncanonical WNT ligands and can bind to its receptor Ror2, which belongs to the family of tyrosine-protein kinase transmembrane receptors (83, 84). Interestingly, deletion of Ror2 in the root progenitor cells of the mouse molar leads to a reduction in root length, suggesting noncanonical WNT signaling plays a vital role in tooth development (85). Wnt5a-Ror2 signaling has been reported to be involved with cell-cell interaction in the musculoskeletal system (84, 86). Our study has shown that loss of *Wnt5a* in Axin2+ TACs of the mouse incisor leads to diminished MSCs, suggesting Wnt5a secreted by TACs provides feedback to MSCs to regulate their maintenance. Indeed, deletion of *Ror2* in MSCs of the mouse incisor recapitulates this phenotype, implying the Wnt5a-Ror2 mediated cell-cell interaction between TACs and MSCs in the incisor is important for its homeostasis (29).

## Hippo signaling

The Hippo signaling pathway also regulates diverse developmental processes, although it was first appreciated for its critical role in organ size control in *Drosophila* (87). The Hippo pathway plays a crucial role in regulating an array of different types of stem cells during embryonic development, tissue homeostasis, repair and regeneration (88). Yap (Yes-associated protein) and Taz (transcriptional co-activator with a PDZ-binding domain) are important functional mediators for the Hippo signaling pathway. Their intracellular location and interaction with other signaling pathway molecules control cell proliferation, cell lineage determination, apoptosis, and tissue homeostasis. Yap/Taz are also sensitive to mechanical stress, which is an important part of the Hippo signaling function in regulating organogenesis and tissue homeostasis (89). In the craniofacial complex, Yap/Taz are

involved in regulating different stem cells, such as DPSC (dental pulp stem cells), PDLSC (periodontal ligament stem cells) and others (90). Hu and colleagues recently found that Yap/Taz are expressed in the TACs of the incisor epithelium and loss of Yap/Taz could lead to loss of TACs in the incisor, suggesting Yap/Taz are important for the maintenance of TACs (91). Specifically, Yap/Taz act through FAK/CDC42/PP1A1 to regulate mTOR signaling to promote TACs proliferation and prevent their differentiation. Through this mechanism, Yap/Taz signaling coordinates stem cell expansion and differentiation to maintain epithelial cell homeostasis in adult mouse incisor. Further studies will explore how Yap/Taz may be involved in mediating the mechanical stimulation in adult mouse incisor.

## Notch signaling

Canonical and non-canonical Notch signaling pathways are evolutionarily conserved and provide a mechanism that can regulate cell fate through ligand-receptor interaction (92). Previous studies of tooth development found that expression of canonical Notch receptors (*Notch1*, *Notch2*, *Notch3*, and *Notch4*) and their ligands (*Jag1*, *Jag2*, *Dll1*, *Dll3*, and *Dll4*) is spatiotemporally regulated during tooth formation, and is indispensable for regulating the reciprocal interactions between dental epithelium and CNC-derived mesenchymal cells. During early development of the mouse incisor (E11-E13), expression of *Notch1* is located in the dental epithelium, incisor furrow and condensed dental mesenchyme. Meanwhile, expression of *Notch1* is absent in the cells of epithelium adjacent to the incisor dental mesenchyme. On the other hand, expression of *Notch2* is found in the labial portion of the dental epithelium, the lingual side of the dental furrow, and the condensed dental mesenchyme (93). During the bell stage (E18.5), expression of *Notch1*, *Notch2*, and *Notch3* is asymmetric in the enamel (94). Ligands of Notch signaling also have unique expression patterns in the mouse incisor. *Jag2* expression is limited to the inner enamel epithelial cells from E17-E18.5 (95). At E18.5, expression of *Dll1* can be noticed in both the posterior outer dental mesenchyme and the lingual side of the inner dental epithelium. On the labial side, expression of *Dll1* can be detected in the both ameloblasts and odontoblasts, adjacent to cells expressing *Notch1*, *Notch2* and *Notch3* (94). These expression patterns of Notch ligands and receptors indicate that Notch signaling may have a very important role in regulating tooth development. More importantly, functional studies revealed that loss of genes involved in Notch signaling results in severe defects in the mouse incisor. *Lfng*, a Notch signaling regulator, is expressed in the dental epithelium and defines the lingual compartment of the developing mouse incisor, while the labial compartment is defined by epithelial *Notch2* (93, 96). Epithelial stem cells of the developing incisor

cannot maintain their viability without Notch signaling (97). Furthermore, inhibition of Notch signaling in adult mice results in impaired interaction between ameloblasts and the underlying stratum intermedium, which causes an enamel formation defect (98). Compared to its role in the dental epithelium, the function of Notch signaling in the dental mesenchyme is understudied. However, Walker et al. showed that loss of Notch signaling in *Collagen1 $\alpha$ 2-Cre;RBP-Jkappa<sup>fl/fl</sup>* mice results in premature differentiation of mesenchymal transit amplifying cells, highlighting the functional significance of Notch signaling in regulating dental mesenchyme (37).

## Conclusion and future perspective

Although stem cell studies using the rodent incisor as a model have made substantial progress, fundamental questions regarding how stem cells are regulated within the niche environment under homeostasis and injury repair remain to be elucidated. As the niche components of stem cells evolve, the studies highlighting the function of different niche cells regulating stem cell behavior receive more attention. For example, the sensory nerve serves as an important niche player that can regulate bone homeostasis and repair through secreting neural transmitters (99, 100). Moreover, various cell types comprising blood vessels play important functions in regulating stem cells (101). Recently, the lymphatic system, including the endothelial cells in lymphatic vessels, has been found important in regulating stem cells in the intestine, shedding light on new niche cells implicated in stem cell regulation (102, 103). Therefore, functional analysis of the niche environment from these perspectives will expand our understanding of stem cell regulation.

The stem cell regions within the rodent incisor are well established, enhancing the utility of the rodent incisor as a model to study the stem cell niche environment. Tissue clearing is a powerful technique that can make the target tissue transparent while retaining fluorescent signals, making the visualization of the niche environment of stem cells possible (104). Thus, combining tissue clearing with 3D reconstruction will enable us to perform more detailed analysis of stem cell regulation. Single-cell analysis has greatly advanced our understanding of the heterogeneity and hierarchy among different stem cell populations; therefore, spatiotemporal single-cell multiomic analysis of the rodent incisor will empower the mechanistic study of stem cell regulation in various respects (23, 105, 106). Together, these newly adopted strategies will provide us with a more comprehensive understanding of how stem cells are regulated in homeostasis and injury repair.

Transit amplifying cells are important intermediates between stem cells and their progeny (18). Although several studies using the mouse incisor have investigated the

contribution of TACs to homeostasis, their function as a niche component is yet to be determined (28, 29, 37, 91). Interestingly, Amnon et al. found that a group of cycling epithelial progenitors can give rise to ameloblasts and adjacent layers of non-ameloblast cells, highlighting the importance of actively proliferating cells contributing to homeostasis and injury repair (26). This finding calls into question the normal model of quiescent stem cells governing the tissue homeostasis in the mouse incisor. Therefore, the heterogeneity and function of stem cells in the mouse incisor will require more investigation.

## Author contributions

J.J. and Y.C. co-wrote the paper. M.Z., T.G. and F.P. provided critical comments for this manuscript. Y.Y. participated editing for the manuscript. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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