Use of the Term Apical Bud to Refer to the Apical End of the Continuously Growing Tooth

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Abstract

Rodent incisors are known to be continuously growing teeth that are maintained by both cell-proliferation at the apical end of the tooth and the attrition of incisal edges. Our recent molecular biological studies have clearly demonstrated the existence of self-renewing adult stem cells in the apical region and its formation by the epithelial-mesenchymal interaction through fibroblast growth factor (FGF) signaling (Harada et al., J. Cell Biol. 147: 105-120, 1999; Development 129: 1533-1541, 2002). However, the proper term has not been found in referring to the epithelial stem cell compartment in the peculiar apical end of the rodent incisors. The morphology of serial cross sections of the apical region from the mouse mandibular incisors showed successively the morphological features equivalent to bud, cap and bell stages-tooth germs of molar teeth. These structures were composed of the cells of inner and outer enamel epithelium, and stellate reticulum. Furthermore, the three-dimensional features of the apical epithelial components observed by scanning electron microscopy clearly represented that the bud stage-tooth germ, appearing as a human head-like structure, existed in the apical end. The findings indicate that the tooth bud corresponding to the bud stage in developing tooth germ is eternally maintained at the apical end of dental epithelium. Thus, we would like to propose the new term "apical bud" for referring to the epithelial stem cell compartment in rodent incisors.

Introduction

Rodent incisors are continuously growing teeth, and all stages of odontogenesis including amelogenesis and dentinogenesis can be analyzed if we observe the tooth from the apical end to the incisal edge (Smith and Warshawsky, 1975a, 1976; Ohshima and Yoshida, 1992). This phenomenon is maintained by both cell-proliferation at the apical end of the tooth and the attrition of incisal edges. Smith and Warshawsky (1975b) proposed the term "odontogenic organ" to refer to an epithelial proliferative region present at the proximal end of the rodent incisor using a three-dimensional reconstruction of the epithelial tissue at the apical end of the lower rat incisor. However, there has been disagreement among researchers on the use of this term (Kallenbach et al., 1975). Nowadays, either "cervical loop" (Farges et al., 1991; Harada et al., 1999, 2002; Yoshiba et al., 2000) or "apical loop" (Smith, 1980; Nishikawa and Kitamura, 1986; Yoshiba et al., 1998; Toyosawa et al., 1996) has been used for referring to the epithelial tissue situated at the proliferative end of the rodent incisor. However, the "cervical loop" is the term referring to the junctional zone where the inner enamel epithelium meets the external enamel epithelium at the rim of the enamel organ (Ten Cate, 1998). Thus, the apical region of rodent incisors is totally different from "cervical loop" in

mouse molars or human teeth from the viewpoint of the morphology and biological significance.

Recent molecular biological studies have clearly demonstrated the existence of self-renewing adult stem cells in the apical region and its formation by the epithelial-mesenchymal interaction through fibroblast growth factor (FGF) signaling (Harada et al., 1999, 2002). The stem cells divide slowly to give rise to one daughter cell that remains in the apical region and another cell enters the zone of rapidly dividing inner enamel epithelial cells (transit-amplifying cell population) to differentiate into ameloblasts to deposit the enamel matrix (Fig. 1). Therefore, it is necessary to use the suitable term referring to the epithelial stem cell compartment in rodent incisors. The present study aims to clarify the three-dimensional architecture of the apical epithelial compartment including stem cells and propose the new term fitting this region.

Materials and Methods

The incisors were dissected carefully from the lower mandibles of 2 or 3-day-old mice. Dissected teeth were fixed in 2% paraformaldehyde + 2.5% glutaraldehyde + 1% acrolein in 0.01M phosphate buffer saline (PBS) (pH 7.2) overnight at 4°C. They were then post-fixed in 1% OsO_4 with 1.5% potassium ferrocyanide for 2 hours, dehydrated through a graded series of ethanol and embedded in Epon 812. Serial semithin sections were cut at a thickness of about 1 μm and stained with toluidine blue at both parallel and right angles to the long axis of the apical end of the epithelial compartments. Ultathin sections were also cut at a thickness of about 70 nm, double stained with uranyl acetate and lead citrate, and examined in a Hitachi H-7100 transmission electron microscope.

For the observation of three-dimensional features of the apical end of the epithelial compartments, the dissected incisor teeth were incubated in 2% collagenase Dulbecco's minimum essential medium (D-MEM) at 4°C for 8 hours and the epithelium were separated with the mesenchyme carefully using the forceps. The separated tissues were fixed in 2% paraformaldehyde + 2.5% glutaraldehyde + 1% acrolein in 0.01M PBS (pH 7.2) overnight at 4°C. They were then post-fixed in 1% OsO_4 with 1.5% potassium ferrocyanide for 2 hours, dehydrated through a graded series of ethanol and critical-point-dried with liquid CO_2 (Hitachi, HCP-1). The specimens were sputter-coated with gold in a vacuum evaporator (Eiko, IB-3) and observed under scanning electron microscope (Hitachi S-570) using an accelerating voltage of 5-15 kV.

Results

Semithin and ultrathin sections of the incisor tooth germ including the apical end

Sagittal semithin sections of the incisor tooth germ including the apical end showed that the labial epithelial compartment appeared as an oval-shaped structure. The morphology of serial transverse sections of the apical epithelial compartment showed the morphological features equivalent to bud (about 90 μm from the apical end), cap (120 - 180μm) and bell stages (210 - 300 μm) -tooth germs of molar teeth. These structures were composed of the cells of inner and outer enamel epithelium, and stellate reticulum, and these distinct epithelial compartments of the enamel organ were also confirmed by transmission electron microscopy. Cutting more incisally (about 500 μm from the apical end), the mesial and lateral Hertwig's epithelial root sheaths (HERS) elongated toward lingual side and met each other finally to encircle the dental pulp totally (about 600 μm).

Scanning electron microscopic views of the apical end of the epithelial compartment

The scanning electron microscopy clearly demonstrated that three-dimensional views of the apical epithelial compartment appeared as a human head-like structure equipped with his arm corresponding to the cervical loop (Fig. 2).

Discussion

The rodent incisors erupt throughout life, and the wear at the incisal edge is compensated for renewal in the apical end of tooth. This phenomenon is indicative of the proliferation of progenitor cells and their differentiation in the apical region, matrix deposition, and subsequent mineralization. Before half of century, Tsurushima (1955) studied on the developmental process of mandibular incisor of rats and represented three-dimensional developmental features of rodent incisor tooth germs obtained from serial paraffin sections. However, he failed to obtain the detailed three-dimensional views of the apical epithelial compartment including the stem cells, because of the limited resolution of paraffin

sections. Afterward, Smith & Warshawsky (1975b) reconstructed the outstanding three-dimensional views of the epithelial tissue at the apical end of the rat lower incisor, and they referred to this epithelial compartment as the "odontogenic organ". Although they clearly demonstrated the three-dimensional shape and the organization of the cells at the apical end of the tooth, those views were somehow erroneous due to the accidental error at the reconstruction of serial semithin sections. The serial semithin sections in the present study clearly demonstrated morphological features of the apical epithelial compartments from the mouse mandibular incisors, and showed that their morphological features were equivalent to bud, cap and bell stages-tooth germs of molar teeth. These structures were composed of the cells of inner and outer enamel epithelium, and stellate reticulum. Furthermore, the three-dimensional features of the apical epithelial components obtained by scanning electron microscopy clearly represented that the bud stage-tooth germ, appearing as a human head-like structure, existed in the apical end.

Concerning the molecular mechanism of continuously growing rodent incisors, our recent studies elucidated that expression of *Fgf-3* and *Fgf-10* were restricted to the mesenchyme underlying the apical epithelial cells and that FGF-3 and FGF-10 plays an important role in the formation and maintenance of stem cells in the development of mouse incisors (Harada et al., 1999, 2002). In the case of molar development, on the other hand, *Fgf-3* and *Fgf-10* were intensely expressed in the dental papilla mesenchyme during cap stage when the tooth grows rapidly and epithelium undergoes folding morphogenesis, and their expressions ceased gradually according to the progress of the tooth development (Kettunen et al., 2000). Taken together, the tooth bud corresponding to the bud stage in developing tooth germ is eternally maintained at the apical end of incisor dental epithelium. Thus, we would like to propose the new term "apical bud" for referring to the epithelial stem cell compartment in rodent incisors.

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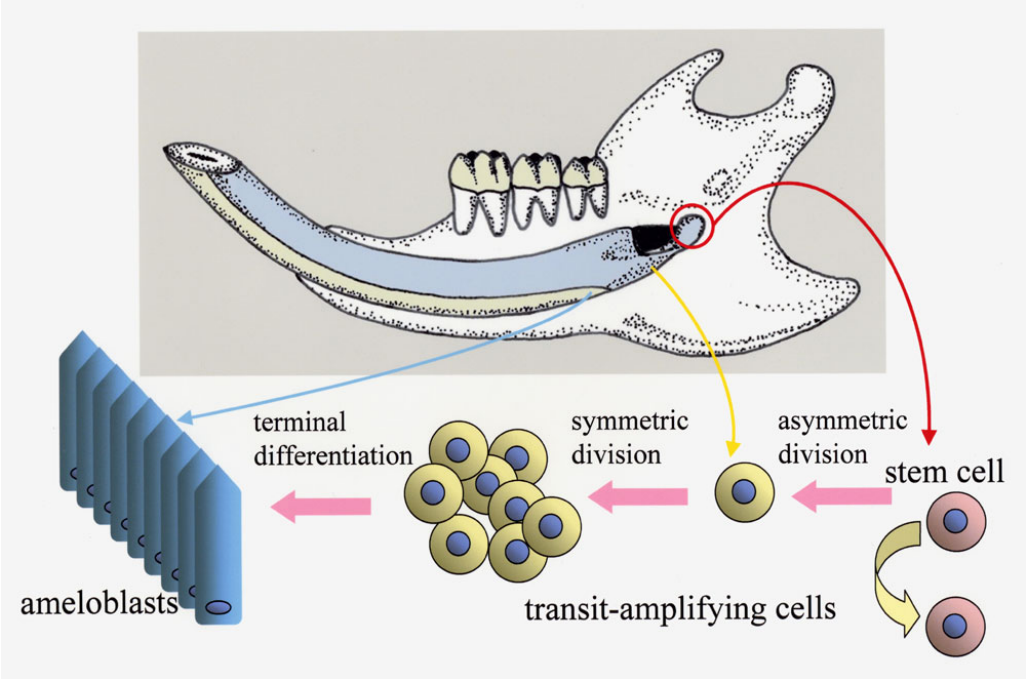

Fig. 1 Schematic drawing of a model for the generation of the ameloblast cell lineage from the stem cell. The stem cell divides slowly and gives rise to one daughter cell remaining in the stem cell pool in the apical epithelial compartment, whereas the other daughter cell enters the zone of rapidly dividing transit-amplifying cells. These cells move toward the incisal direction and differentiate into ameloblasts forming the enamel matrix.

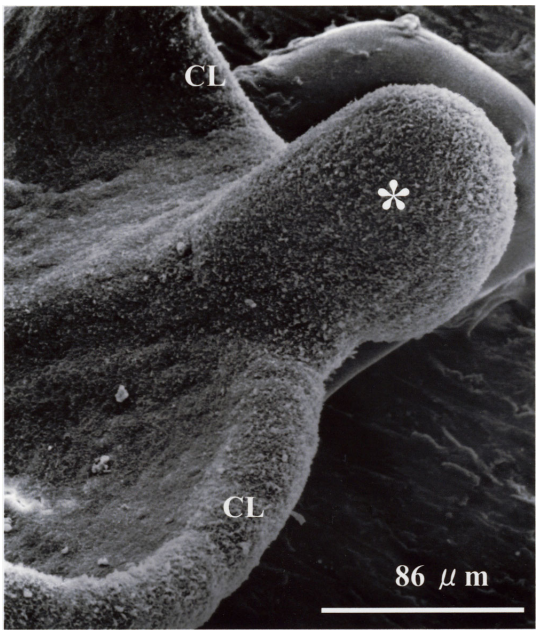

Fig. 2 The SEM image of a three-dimensional view of the apical epithelial compartment. The apical epithelial region (*) appears as a human head-like structure and the cervical loop (CL) corresponds to his arms. Bar=86 μm