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ABSTRACT

Mice carrying a knock-in mutation (Phe377del) in the *Ank* gene replicate many skeletal characteristics of human craniometaphyseal dysplasia, including hyperostotic mandibles. *Ank*^{KI/KI} mice have normal morphology of erupted molars and incisors but excessive cementum deposition with increased numbers of Ibsp- and Dmp1-positive cells on root surfaces. The cervical loops of adult *Ank*^{KI/KI} lower incisors are at the level of the third molars, while they are close to the mandibular foramen in *Ank*^{+/+} mice. Furthermore, *Ank*^{KI/KI} incisors show decreased eruption rates, decreased proliferation of odontoblast precursors, and increased cell apoptosis in the stellate reticulum. However, their capability for continuous elongation is not compromised. Quantification of TRAP-positive cells in the apical ends of *Ank*^{KI/KI} incisors revealed decreased osteoclast numbers and osteoclast surfaces. Bisphosphonate injections in *Ank*^{+/+} mice replicate the *Ank*^{KI/KI} incisor phenotype. These results and a comparison with the dental phenotype of *Ank* loss-of-function mouse models suggest that increased cementum thickness may be caused by decreased extracellular PPi levels and that the incisor phenotype is likely due to hyperostosis of mandibles, which distinguishes *Ank*^{KI/KI} mice from the other *Ank* mouse models.

KEY WORDS: craniometaphyseal dysplasia; dental cementum; incisor; Ankh protein, mouse; hyperostosis; bone remodeling.

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Dental Abnormalities in a Mouse Model for Craniometaphyseal Dysplasia

INTRODUCTION

Craniometaphyseal dysplasia (CMD) is a genetic bone disorder characterized by hyperostosis of craniofacial bones and flared metaphyses of long bones. A few studies have reported dental anomalies in CMD patients, including delayed tooth eruption, enamel hypoplasia, and excess mineralization of the crowns of primary maxillary second molars (Hayashibara *et al.*, 2000; Zajac *et al.*, 2010; Zhang *et al.*, 2007). It has been suggested that retention of primary teeth or slower eruption of permanent teeth is likely caused by hyperostosis of the jaw (Hayashibara *et al.*, 2000). However, it has been unclear whether dental abnormalities in CMD patients are caused by direct effects of the CMD gene mutation or are secondary to abnormal bone remodeling.

Autosomal-dominant mutations in CMD patients have been identified in the ankylosis gene *ANKH* (Nurnberg *et al.*, 2001; Reichenberger *et al.*, 2001). ANK is a transmembrane protein known to transport intracellular pyrophosphate (PPi) into the extracellular matrix (Ho *et al.*, 2000). PPi inhibits mineralization, while phosphate (Pi) promotes hydroxyapatite deposition. A balance of Pi and PPi is needed to regulate mineralization of bone and teeth.

We have generated a knock-in (KI) mouse model by introducing one of the most common CMD mutations, a Phe377 deletion into the *Ank* gene (Chen *et al.*, 2009). *Ank*^{KI/KI} mice develop many skeletal characteristics of human CMD, including hyperostotic mandibles and club-shaped femurs. Two *Ank* loss-of-function mouse models (*Ank*^{ank/ank} and *Ank*^{null/null} mice) develop joint stiffness, cranial hyperostosis, and a narrowed foramen magnum (Ho *et al.*, 2000; Gurley *et al.*, 2006b), but miss certain CMD features like mandibular hyperostosis and flaring metaphyses of long bones. This phenotypic difference suggests that CMD mutations may cause more functional changes in ANK than purely loss of PPi transport and may indicate a novel role for ANK as a signaling protein.

Here we describe the dental abnormalities in *Ank*^{KI/KI} mice and compare differences in tooth phenotype between *Ank*^{KI/KI} mice and *Ank*^{null/null} mice. We investigate whether the differences are due to reduced extracellular PPi (ePPi) levels or to CMD-specific effects of the *Ank* mutation on tooth development.

MATERIALS & METHODS

Mice

Ank^{KI/KI} mice have previously been described (Chen *et al.*, 2009). This CMD mouse model carries a deletion of TTC1130–1132 (phenylalanine 377) in exon 9 of *Ank*. *Ank*^{KI/KI} mice were crossed with transgenic fluorescence reporter mice expressing Ibsp-Topaz/Dmp1-mCherry (Maye *et al.*, 2009), a

kind gift of Dr. Peter Maye. *Ank^{null/null}* mice (Gurley *et al.*, 2006a) were generously provided by Dr. David Kingsley and maintained in a mixed background of FVB and C57Bl/6. Procedures were approved by the Animal Care Committee of the University of Connecticut Health Center.

Radiography, Micro-computed Tomography (micro-CT), and Scanning Electron Microscopy (SEM)

Radiographs of the mandibles were obtained with the use of a MX20 Radiography System (Faxitron X-Ray LLC, Lincolnshire, IL, USA). Micro-CT analysis was performed in mandibles of 1-, 2-, and 12-week-old mice at the Micro-CT facility at UCHC (mCT20; ScanCo Medical, Bassersdorf, Switzerland). For SEM imaging, mandibles of 10-week-old mice were sectioned at the sagittal plane, cleaned in 5% sodium hypochlorite, dehydrated in aqueous ethanol solutions, and observed under a TM1000 Tabletop Microscope (Hitachi High-Technologies Europe GmbH, Krefeld, Germany).

Eruption Rate of Incisors

A groove was made at the gingival margin of the lower incisors of 13-week-old *Ank^{+/+}*, *Ank^{+K1}*, *Ank^{K1/K1}*, and *Ank^{null/null}* male mice, by means of a rotary tool (Lavelle, 1969). The distance between the groove and the gingival margin was measured 9 days later.

Dynamic Histomorphometry

Calcein (10 mg/kg body weight) and demeclocycline (50 mg/kg) were intraperitoneally injected into 13-week-old *Ank^{+/+}*, *Ank^{+K1}*, *Ank^{K1/K1}*, and *Ank^{null/null}* male mice at seven-day intervals. Mice were sacrificed 2 days after the second injection. Newly mineralized dentin was examined in undecalcified frozen embedded sections. The distance between the apical end of the first label (calcein) and the second label (demeclocycline) in incisors was measured by AxioVision LE (Carl Zeiss MicroImaging, LLC, Thornwood, NY, USA).

Proliferation and Apoptosis Assay

Bromodeoxyuridine (BrdU; Sigma-Aldrich, St. Louis, MO, USA) was injected into seven-day-old *Ank^{+/+}*, *Ank^{+K1}*, *Ank^{K1/K1}*, and *Ank^{null/null}* mice 1.5 hrs before their death. BrdU (Invitrogen Corporation, Carlsbad, CA, USA) and TUNEL staining (Terminal Transferase dUTP Nick End Labeling; Promega, Madison, WI, USA) was performed in sagittal sections of the lower incisors, and the number of labeled cells within dental epithelial and mesenchyme surrounding the cervical loop was quantified with Adobe Photoshop.

Incisor Organ Culture

Incisor tooth germs were dissected from the lower jaws of five-day-old mice. The incisors were then placed on Millicell culture plate inserts with a pore size of 0.4 μ m (Millipore Corporation, Billerica, MA, USA). Incisors were cultured in DMEM with

10% FBS, 100 IU/mL penicillin, 100 mg/mL streptomycin, and 100 μ g/mL ascorbic acid for 11 days. Images were taken on days 0, 3, 5, 7, and 11. Elongation of the dental epithelium was measured with ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Osteoclast Quantification

Sagittal paraffin sections of two-week-old *Ank^{+/+}*, *Ank^{+K1}*, and *Ank^{K1/K1}* mice were stained for tartrate-resistant acid phosphatase (TRAP) and analyzed with Osteomeasure software (OsteoMetrics, Decatur, GA, USA).

Bisphosphonate Treatment

Alendronate (Sigma-Aldrich, St. Louis, MO, USA) was injected intraperitoneally into *Ank^{+/+}* mice (1 μ m/g of body weight). Injections were administered once a week for 5 wks starting in mice at the age of 1 wk. Mice were then sacrificed, and radiographs of the mandibles were taken.

RESULTS

Ank^{K1/K1} Mice Present Excessive Cementum Deposition in Molars and Incisors

Histological evaluation by H&E staining shows excessive deposition of cementum in molars of *Ank^{K1/K1}* mice as early as 2 wks of age with progressive thickening (Fig. 1A; Appendix Fig. 1). Excessive cementum is cellular (Appendix Fig. 2) in a region where a thin acellular cementum is normally found. Micro-CT images of *Ank^{K1/K1}* molar roots indicate excessive mineralization, which overlaps the enamel at the cement-enamel junction (Fig. 1B). Incisors of *Ank^{K1/K1}* mice show thicker cementum as well (Appendix Fig. 3). Heterozygous *Ank^{+K1}* molars present an intermediate phenotype of cementum thickness (Fig. 1A). Therefore, the cementum phenotype in *Ank^{K1/K1}* mice is regulated by a time- and dose-dependent effect. Cementum deposition in *Ank^{K1/K1}* mice resembles that of *Ank^{null/null}* mice (Fig. 1A), suggesting that decreased ePPi levels may lead to this phenotype.

It has been reported that BSP and DMP1 are markers for cementoblasts (D'Errico *et al.*, 1997) and for cementocytes (Cao *et al.*, 2012), respectively. Crossing of *Ank^{K1/K1}* mice with reporter mice expressing Ibsp-Topaz/Dmp1-mCherry revealed increased numbers of Ibsp- and Dmp1-positive cells in the cementum of *Ank^{K1/K1}* molars (Fig. 1C). This finding suggests that the *Ank* mutation may induce elevated DMP1 expression in cementoblasts, which is shown by increased numbers of BSP- and DMP1-double-positive cells on root surfaces and by increased numbers of cells with strong DMP1 label embedded in the cementum (Appendix Fig. 2).

Abnormal Incisor Development in *Ank^{K1/K1}* Mice

During normal mandibular incisor development, the apical end (cervical loop) moves backward from the anterior part of the mandible toward the mandibular foramen (Addison and Appleton, 1915). At post-natal day 5, the labial cervical loop of

Figure 1. Thicker cementum deposition in *Ank^{KI/KI}* mice. **(A)** Representative sections of 1st and 2nd molars of 10-week-old *Ank^{+/+}*, *Ank^{+/KI}*, *Ank^{KI/KI}*, and *Ank^{null/null}* mice stained with H&E. **(B)** Micro-CT images of 12-week-old *Ank^{+/+}* and *Ank^{KI/KI}* molars. Red arrow indicates excessive cementum deposition of molar roots of *Ank^{KI/KI}* mice. **(C)** Increased Ibsp- and Dmp1-positive cells on root surfaces of *Ank^{KI/KI}* molars (4-week-old *Ank^{+/+}* and *Ank^{KI/KI}* /Ibsp-Topaz/Dmp1-mCherry mice). Dentin (D); acellular cementum (AC); periodontal ligament (PDL); alveolar bone (B); cellular cementum (CC); cementoblasts (Cb); osteoblasts (Ob); osteocytes (Ost). Scale bar = 100 μ m.

the lower incisor is located at the distal surface of the second molar in both *Ank^{+/+}* and *Ank^{KI/KI}* mice shown by radiographs and histology (Appendix Fig. 4). However, in *Ank^{+/+}* mice, the apical end of the lower incisor begins to move posteriorly as early as post-natal day 7, while the cervical loop in *Ank^{KI/KI}* mice remains close to the starting point (Figs. 2A, 2C). From 2 to 4 wks of age, the incisor in *Ank^{+/+}* mice elongates and the cervical loop almost reaches its final position anterior to the mandibular foramen, while the cervical loop fails to substantially change its position in *Ank^{KI/KI}* mice (Fig. 2A). At 10 wks, the cervical loop in *Ank^{KI/KI}* mice is still at the level of the third molar and does not move from this position as the mice get older (Figs. 2A-2C). The mandibular sizes of *Ank^{KI/KI}* and *Ank^{+/+}* mice are comparable (Appendix Fig. 5). The development of upper incisors in *Ank^{KI/KI}* mice is normal (data not shown).

The cervical loop of the lower incisor in adult heterozygous *Ank^{+/KI}* mice is positioned in an intermediate position between the cervical loop locations of *Ank^{+/+}* and *Ank^{KI/KI}* mice (data not shown). Adult *Ank^{null/null}* mice present normal cervical loop positions, suggesting that lack of PPI transport is not the primary cause of this incisor phenotype (Fig. 2C).

We next evaluated whether smaller incisors of the *Ank^{KI/KI}* mouse differ in the rate of continuous eruption. We compared incisor eruption rate and dentin mineralization by double-labeling with calcein and demeclocycline of *Ank^{+/+}*, *Ank^{+/KI}*, *Ank^{KI/KI}*, and *Ank^{null/null}* mice. The eruption rate and the distance between the first and second labels were significantly decreased in *Ank^{KI/KI}* mice in comparison with *Ank^{+/+}*, *Ank^{+/KI}*, and *Ank^{null/null}* mice (Fig. 2D), suggesting a slower incisor growth rate in *Ank^{KI/KI}* mice.

Decreased Proliferation of Odontoblast Precursors and Increased Cell Apoptosis in the Stellate Reticulum of the Incisor but Normal Elongation of the Dental Epithelium in *Ank^{KI/KI}* Mice

Tooth development involves proliferation of progenitor cells, cell differentiation, and matrix deposition. We hypothesized that CMD mutations in *Ank* may inhibit cell proliferation and therefore result in smaller incisors with cervical loops positioned anterior to the normal position. We examined cell proliferation by BrdU staining in post-natal day 7 *Ank^{+/+}*, *Ank^{+/KI}*, *Ank^{KI/KI}*, and *Ank^{null/null}* mice. We observed cells positive for BrdU in the

Figure 2. Short lower incisors, abnormal position of the cervical loop, and decreased eruption rate in *Ank*^{KI/KI} mice. **(A)** Radiographs of mandibles from 1-, 2-, 4-, and 20-week-old *Ank*^{+/+} and *Ank*^{KI/KI} mice. **(B)** Comparison of 10-week-old *Ank*^{+/+}, *Ank*^{KI/KI}, and *Ank*^{null/null} incisors by x-ray imaging and histology (H&E). Scale bar = 200 μm. **(C)** Cervical loop sections of 1-, 2-, and 20-week-old *Ank*^{+/+} and *Ank*^{KI/KI} mice (H&E). Scale bar = 200 μm. **(D)** Histomorphometry of incisors from 13-week-old *Ank*^{+/+} and *Ank*^{KI/KI} mice. Arrows represent the beginning of the first (calcein; green) and second labels (demeclocycline; yellow), respectively. Histograms show eruption rates and distance between the first and second labels in *Ank*^{+/+} (n = 5), *Ank*^{+/KI} (n = 5), *Ank*^{KI/KI} (n = 5), and *Ank*^{null/null} (n = 3) mice. *p < 0.005. Mandibular foramen (MF), distal root of the second molar (2M), root of the third molar (3M), position of the cervical loop (CL). Scale bar = 500 μm.

epithelium (stellate reticulum, inner enamel epithelium, and pre-ameloblasts) and in the mesenchyme (pulp cells, pre-odontoblasts, and polarizing odontoblasts) (Fig. 3A). *Ank*^{KI/KI} mice showed significantly decreased proliferation of odontoblast-lineage cells compared with *Ank*^{+/+}, *Ank*^{+/KI}, and *Ank*^{null/null} mice. However, the number of proliferative cells of the epithelial lineage was not different (Fig. 3A). EdU (5-ethynyl-2'-deoxyuridine) quantification in 2-week-old *Ank*^{+/+} and *Ank*^{KI/KI} mice showed reduced proliferation of odontoblast precursors and dental epithelial cells (Appendix Fig. 6). We detected apoptotic cells in the stellate reticulum in both *Ank*^{+/+} and *Ank*^{KI/KI} mice, but not in other regions of the incisor (Fig. 3B). Quantification of TUNEL-positive cells revealed that *Ank*^{KI/KI} mice have significantly increased cell apoptosis in the stellate reticulum compared with *Ank*^{+/+} mice (Fig. 3B).

We applied an organ culture system to determine whether the developmental defect is intrinsic to the cervical loop or secondary to defective bone resorption. Without the confinement of bone, the dental epithelium of *Ank*^{+/+} and *Ank*^{KI/KI} cervical loops extended at a similar rate during 11 days of culture (Figs. 3C, 3D). Analysis of these data suggests that the ability for continuous elongation is not compromised in dental epithelium of *Ank*^{KI/KI} incisors.

Reduced Mandibular Bone Remodeling in *Ank*^{KI/KI} Mice

The remodeling of jawbones is harmonized with tooth development (Marks and Schroeder, 1996). We hypothesized that hyperostotic mandibles in *Ank*^{KI/KI} mice can inhibit the apical elongation of mandibular incisors. We analyzed 1-, 2-, and

Figure 3. Decreased proliferation of odontoblast precursors and increased apoptosis in the stellate reticulum, but normal elongation of dental epithelium of *Ank^{Kl/Kl}* lower incisors. **(A)** Sections of one-week-old *Ank^{+/+}* and *Ank^{Kl/Kl}* cervical loops of incisors stained for BrdU. Graphs represent percentages of proliferative cells in the apical end of *Ank^{+/+}* ($n = 3$), *Ank^{Kl/Kl}* ($n = 4$), *Ank^{Kl/Kl}* ($n = 4$), and *Ank^{null/null}* ($n = 3$) mice. Odontoblast-lineage cells include pre-odontoblasts (PrO) and polarizing odontoblasts (PoO). Epithelial cells are stellate reticulum (SR), inner enamel epithelium (IE), and pre-ameloblasts (PA). * $p < 0.05$. Scale bar = 100 μ m. **(B)** Sections of one-week-old *Ank^{+/+}* and *Ank^{Kl/Kl}* cervical loops stained for TUNEL and percentage of apoptotic cells in the stellate reticulum (SR) of *Ank^{+/+}* ($n = 5$) and *Ank^{Kl/Kl}* ($n = 5$) incisors. # $p < 0.01$. Scale bar = 50 μ m. **(C)** *Ank^{+/+}* and *Ank^{Kl/Kl}* incisor organ cultures. Dotted line indicates the location of cervical loop (CL). Scale bar = 200 μ m. **(D)** Elongation rates of dental epithelium between *Ank^{+/+}* ($n = 5$) and *Ank^{Kl/Kl}* ($n = 5$) incisor tooth germs are comparable.

12-week-old *Ank^{+/+}* and *Ank^{Kl/Kl}* mandibles by micro-CT and observed increased bone volume in *Ank^{Kl/Kl}* mice as early as 1 wk of age, especially in the body of the mandible (Fig. 4A). Bone around the incisor progressively thickens, and bone marrow spaces in the mandible are markedly reduced as *Ank^{Kl/Kl}* mice age (Fig. 4A). We next examined bone at the apical end of the incisor using scanning SEM. *Ank^{+/+}* mice had a smooth bone surface and well-defined delimitation between the mandibular canal and the apical end of the incisor (Fig. 4B). In contrast, SEM images of *Ank^{Kl/Kl}* mice showed a rough and disorganized bone surface, suggesting abnormal bone remodeling of this region (Fig. 4B).

Previous studies demonstrated that osteoclasts from *Ank^{Kl/Kl}* mice are smaller and resorb less bone *in vitro* (Chen *et al.*, 2009). To study whether bone resorption was reduced and negatively

affects the extension of cervical loops in *Ank^{Kl/Kl}* mice, we analyzed the osteoclast numbers and surfaces in the apical region of lower incisors. Analysis of histological data from two-week-old mice showed TRAP-positive cells in the alveolar bone surrounding the cervical loop of *Ank^{+/+}* and *Ank^{Kl/Kl}* incisors (Fig. 4C). However, osteoclast numbers and surfaces were decreased and the bone volume increased in the apical ends of the *Ank^{Kl/Kl}* incisors (Fig. 4D).

To further assess the association between bone resorption and cervical loop positioning of the lower incisor, we inhibited the activity of osteoclasts in *Ank^{+/+}* mice by bisphosphonate (alendronate) injections. Treatment of *Ank^{+/+}* mice resulted in short lower incisors with incomplete cervical loop migration toward the mandibular foramen, similarly to *Ank^{Kl/Kl}* mice (Fig. 4E). Analysis of these data suggests that abnormal positioning

Figure 4. Abnormal bone remodeling in mandibles of *Ank*^{KI/KI} mice. **(A)** Micro-CT images show excessive bone volume and decreased bone marrow spaces in *Ank*^{KI/KI} mandibles at 1, 2, and 12 wks. **(B)** SEM images of bone at the apical end of the incisor (10-week-old *Ank*^{+/+} and *Ank*^{KI/KI} mice). **(C)** TRAP staining of two-week-old *Ank*^{+/+} and *Ank*^{KI/KI} mandibles. Arrows indicate TRAP-positive cells. Scale bar = 100 μ m. **(D)** Bone volume is increased and osteoclast number decreased in *Ank*^{KI/KI} mice. [BV/TV (%): bone volume/total volume. Oc.S/BS (%): osteoclast surface/bone surface. N.Oc.T.Ar (n/mm²): number of osteoclasts/tissue area. N.Oc: number of osteoclasts.] *Ank*^{+/+} (n = 5), *Ank*^{KI/KI} (n = 6). **p* < 0.01. **(E)** Alendronate injections in *Ank*^{+/+} mice replicate the failure of cervical loop migration similar to the *Ank*^{KI/KI} phenotype. Six-week-old control (no injection) and alendronate-treated *Ank*^{+/+} mice. Mandibular foramen (MF), mandibular canal (MC), root of the third molar (3M), distal root of the second molar (2M), position of the cervical loop (CL).

of cervical loops of *Ank*^{KI/KI} mouse incisors is primarily due to decreased bone resorption in this region.

DISCUSSION

We observed that a CMD-causing mutation in *Ank* results in excessive cementum deposition of molars and shorter lower incisors with abnormal positioning of the cervical loops. The cementum phenotype is shared by *Ank* loss-of-function mouse models (*Ank*^{ank/ank} and *Ank*^{null/null} mice), but the lower incisor phenotype is unique to *Ank*^{KI/KI} mice.

Increased cementum deposition has been described in mouse models with mutations in genes that lead to increased ratios of Pi/PPi in *Ank*^{ank/ank} and *Ank*^{null/null} mice and *Enpp1* (PC-1) null

mice (PC-1 plasma cell membrane glycoprotein 1, an enzyme that generates extracellular and intracellular PPi by ATP hydrolysis). They present with a cementum phenotype similar to that of the *Ank*^{KI/KI} mice, with a more than 10-fold cellular cementum increase compared with wild-type mice (Nociti *et al.*, 2002; Popowics *et al.*, 2005; Foster *et al.*, 2011, 2012). In contrast, TNAP knock-out mice (TNAP hydrolyzes PPi to generate Pi) have a thinner acellular cementum (Beertsen *et al.*, 1999; Foster *et al.*, 2012). *In vitro* experiments suggested that cementoblasts, the cells that produce cementum, are sensitive to Pi (Foster *et al.*, 2006; Rutherford *et al.*, 2006). The similarity between *Ank*^{KI/KI} mice and mouse models with decreased transport or generation of ePPi (*Ank*^{ank/ank}, *Ank*^{null/null}, and *PC-1*^{null/null} mice) suggests that the increased cementum formation is likely caused by a decrease

of ePPI. Excessive cementum deposition has not been described in CMD patients, but excessive mineralization of the crowns of some primary teeth has been reported (Zhang *et al.*, 2007). We are currently investigating tooth mineralization in a group of CMD patients.

Delayed tooth eruption has been reported in CMD patients (Hayashibara *et al.*, 2000; Kornak *et al.*, 2010). Rodent incisors are continuously growing, while molars have limited eruption, similar to human teeth. Both molars and incisors in *Ank^{KI/KI}* mice can erupt comparably with those in *Ank^{+/+}* mice, but with a slower eruption rate of incisors. Sustained growth of incisors is maintained by proliferation of progenitor cells at the cervical loop. Our BrdU assay showed decreased proliferation of odontoblast precursors in one-week-old *Ank^{KI/KI}* mice, but no difference in the proliferation of epithelial cells (cells that differentiate into ameloblasts) compared with *Ank^{+/+}* and *Ank^{null/null}* mice. In addition, we observed increased cell apoptosis in the stellate reticulum of the incisors. The stellate reticulum is considered to be the stem cell niche of incisors (Harada *et al.*, 1999). Interestingly, incisor organ cultures demonstrated that the capacity for continuous elongation is not compromised in *Ank^{KI/KI}* dental epithelium. Analysis of our data, taken together, suggests that the ratio of some cell populations in the cervical loop of the *Ank^{KI/KI}* incisors is altered due to abnormal proliferation and apoptosis rates and other extrinsic factors, such as insufficient bone resorption at the apical end of the incisor, which may contribute to the lack of incisor elongation.

Ank^{KI/KI} bone-marrow-derived monocytes (BMM) cultured with M-CSF and RANKL show less osteoclast formation and reduced resorption on bone chips as well as on calcium-phosphate-coated plates (Chen *et al.*, 2009). Therefore, insufficient bone resorption in *Ank^{KI/KI}* mice may play a major role in the abnormal dental phenotype, specifically in preventing migration of the cervical loop and slowing tooth eruption. Bisphosphonate-treated *Ank^{+/+}* mice also had shorter lower incisors, suggesting a strong correlation between reduced bone resorption and abnormal position of the cervical loop. The mechanism for reduced bone resorption in *Ank^{KI/KI}* mice still needs to be investigated.

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