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Bcl11b transcription factor plays a role in the maintenance of the ameloblast-progenitors in mouse adult maxillary incisors

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ABSTRACT

Rodent incisors maintain the ability to grow continuously and their labial dentin is covered with enamel. Bcl11b zinc-finger transcription factor is expressed in ameloblast progenitors in mouse incisors and its absence in *Bcl11b*$^{KO/KO}$ mice results in a defect in embryonic tooth development. However, the role of *Bcl11b* in incisor maintenance in adult tissue was not studied because of death at birth in *Bcl11b*$^{KO/KO}$ mice. Here, we examined compound heterozygous *Bcl11b*$^{S826G/KO}$ mice, one allele of which has an amino acid substitution of serine at position 826 for glycine, that exhibited hypoplastic maxillary incisors with lower concentrations of minerals at the enamel and the dentin, accompanying the maxillary bone hypoplasia. Histological examinations revealed hypoplasia of the labial cervical loop in incisors, shortening of the ameloblast progenitor region, and impairment in differentiation and proliferation of ameloblast-lineage cells. Interestingly, however, juvenile mice at 5 days after birth did not show marked change in these phenotypes. These results suggest that attenuated Bcl11b activity impairs ameloblast progenitors and incisor maintenance. The number of BrdU label-retaining cells, putative stem cells, was lower in *Bcl11b*$^{S826G/KO}$ incisors, which suggests the incisor hypoplasia may be in part a result of the decreased number of stem cells. Interestingly, the level of *Shh* and *FGF3* expressions, which are assumed to play key roles in the development and maintenance of ameloblasts and odontoblasts, was not decreased, though the expressed areas were more restricted in ameloblast progenitor and mesenchyme regions of *Bcl11b*$^{S826G/KO}$ incisors, respectively. Those data suggest that the incisor maintenance by Bcl11b is not directly related to the FGF epithelial–mesenchymal signaling loop including Shh but is intrinsic to ameloblast progenitors and possibly stem cells.

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1. Introduction

The rodent incisor, the labial dentin of which is covered with enamel, grows continuously throughout the lifetime of the animals (Smith and Warshawsky, 1975). This continuous growth is maintained by proliferation and differentiation of dental epithelial and mesenchymal cells supplied by dental adult stem cells. The epithelial stem cells are thought to reside in the labial cervical loop (laCL) of incisors, which contains the stellate reticulum (SR) region surrounded by basal epithelium. The epithelial stem cells differentiate into inner enamel epithelium (IEE) and outer enamel epithelium (OEE), and the IEE cells provide preameloblasts. The IEE cells and early preameloblasts constitute rapidly dividing transit amplifying (TA) cells that differentiate and migrate toward the incisal edge to form enamel-forming ameloblasts (Harada et al., 1999). This special epithelial structure at the laCL is also referred to as the "apical bud" (Harada and Ohshima, 2004; Ohshima et al., 2005). On the other hand, the lingual cervical loop (liCL) is much smaller than laCL and constitutes Hertwig's epithelial root sheath (HERS)-like structure harboring few epithelial stem cells.

Various signaling molecules expressed in epithelial and mesenchymal cells regulate incisor development, which include Fibroblast growth factors (FGFs), bone morphogenetic proteins, and molecules on Notch and Wnt signaling pathways (Tummers and Thesleff, 2003, 2009; Harada and Ohshima, 2004). In particular, FGFs are crucial growth factors for not only tooth development during embryogenesis but also the proliferation and differentiation of epithelial cells in adult incisors (Harada et al., 1999, 2002). The expression of *FGF3* is restricted in the mesenchyme surrounding the laCL in adult incisors, whereas *FGF10* is expressed at a higher level in the mesenchyme beneath laCL than liCL (Harada et al., 1999; Wang et al., 2007). They act through the receptor, FGFR2, on the dental epithelium. Tissue-specific ablation of *FGFR2* in adult mice led to impaired incisor growth, failure of enamel formation and decreased proliferation of TA cells (Lin et al., 2009; Parsa et al., 2010). Shh, a member of the vertebrate Hedgehog family, is another important signaling required for the mouse incisor formation and maintenance, and the expression of *Shh* in TA cells is regulated by FGFR2 (Dassule et al., 2000; Seidel et al., 2010). The inhibition of Shh signaling prevents the proliferation and differentiation of ameloblast lineage cells from the stem cells (Seidel et al., 2010).

Bcl11b/Ctip2 encodes a transcriptional repressor that plays critical roles in the development of many different tissues, including thymus, skin, central nervous systems, and teeth (Wakabayashi et al., 2003; Golonzhka et al., 2007; Arlotta et al., 2008; Golonzhka et al., 2009). Studies of embryonic tooth development in $Bcl11b^{KO/KO}$ mice exhibit a developmental tooth defect due to the inappropriate differentiation of ameloblasts (Golonzhka et al., 2009). Further study showed that Bcl11b directly or indirectly regulates the expression of FGFs in mandibular incisors (Kyrylkova et al., 2012). Bcl11b expression is observed in not only embryonic tooth tissues but also in adult teeth. Therefore, Bcl11b may play a role in maintenance of growing incisors in adult mice. As for the T-cell development, Bcl11b regulates differentiation of immature thymocyte subsets in the thymus and also mature T cells in peripheries (Kominami, 2012). The absence of Bcl11b in $Bcl11b^{KO/KO}$ mice leads to death at birth, thus hampering the investigation of roles for this gene in incisor homeostasis in adult mice. To overcome this difficulty, we generated mice carrying a hypomorphic allele of $Bcl11b^{S826G}$ (S at the position 826 substituted for G) by mutagen treatment in the RIKEN large scale ENU Mutagenesis Program (Gondo, 2008). We analyzed incisors and laCL in adult $Bcl11b^{S826G/KO}$ mice. Here we show hypoplasia of adult maxillary incisors and laCL, together with decreased numbers of TA and label retaining cells (LRCs), suggesting that Bcl11b is required for generation of ameloblast progenitors and plays a role in incisor maintenance. However, $Bcl11b^{S826G/KO}$ incisors did not show changes in *FGF3* and *Shh* expression though the expression areas were more restricted. Hence, Bcl11b function in the incisor maintenance may be intrinsic to ameloblast progenitors and possibly stem cells, not directly related to the FGF epithelial-mesenchymal signaling loop.

2. Results

2.1. *Phenotypes of* $Bcl11b^{S826G/KO}$ *mutant mice*

We isolated a mutant mouse line carrying a hypomorphic allele of Bcl11b, $Bcl11b^{S826G}$, by screening mice from RIKEN ENU mutagenesis mouse library. $Bcl11b^{WT/S826G}$ mice were crossed with $Bcl11b^{WT/KO}$, and their progeny $Bcl11b^{S826G/KO}$ compound heterozygous mice were born at a frequency of 15/61, similar to the expected frequency of 25%. The $Bcl11b^{S826G/KO}$ mice were smaller at birth, and their weight was approximately 70–80% of their control littermates (Fig. 1A). Phenotypes of the mutant mice at postnatal day 21 (P21) were mostly analyzed because mouse incisors and molars are formed until 3 weeks after birth. $Bcl11b^{S826G/KO}$ mice at P21 showed a midface hypoplasia and relatively overgrown mandibular incisors that reached the nose (Fig. 1B). Those mice normally died around this age due to the difficulty in taking food because of elongated incisors in the mandible. However, cutting the elongated incisors and supplying small-sized food materials helped them take food and survive. This incisor elongation is probably due to lack of the attrition counterbalancing the mouse incisor growth. Consistently, 3D micro-CT scans of the skulls of $Bcl11b^{S826G/KO}$ mice revealed that a length of the anterior cranial base or maxillary bone was significantly shorter than that of control $Bcl11b^{WT/WT}$ or $Bcl11b^{WT/KO}$ mice (Fig. 1C), indicating the inability of lower incisors to engage with the upper incisors, resulting in severe anterior cross-bite phenotype.

The maxillary incisors were slender and exhibited chalky white in most of $Bcl11b^{S826G/KO}$ mice at P21, suggesting that the maxillary incisors of $Bcl11b^{S826G/KO}$ mice are hypoplastic. To further characterize this hypoplasia, we assayed the elemental mapping of calcium (Ca), phosphorus (P) and ferrum (Fe) in incisors by EPMA analysis (Fig. 1D). Concentrations of Ca and P in both enamel and dentin of $Bcl11b^{S826G/KO}$ mice were lower than those of control mice, and Fe deposition in