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LEF1 is a critical epithelial survival factor during tooth morphogenesis

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Abstract

LEF1 is a cell-type-specific transcription factor and mediates Wnt signaling pathway by association with its co-activator β-catenin. Wnt signaling is known to be critical for the specification of cranial neural crest (CNC) cells and may regulate the fate diversity of the CNC during craniofacial morphogenesis. Loss of *Lef1* results in arrested tooth development at the late bud stage and LEF1 is required for a relay of a Wnt signaling to a cascade of FGF signaling activities to mediate the epithelial–mesenchymal interaction during tooth morphogenesis. It remains unclear, however, what is the cellular mechanism of LEF1 signaling in regulating tooth morphogenesis. To test the hypothesis that LEF1 signaling regulates the fate of the dental epithelial and the CNC-derived mesenchymal cells during tooth morphogenesis, we investigated and compared the cellular migration, proliferation, and apoptotic activity within the tooth germ between the wild-type and *Lef1* null mutant mice. Using the *Wnt1-Cre/R26R* transgenic system for indelibly marking the progenies of CNC cells, we show that there is no CNC migration defect in the *Lef1* null mutant mice, indicating that the arrest in tooth development is not the result of shortage of the CNC contribution into the first branchial arch in the *Lef1* mutant. Furthermore, there is no alteration in cell proliferation or condensation of the CNC-derived dental mesenchyme in the *Lef1* null mutant, suggesting that LEF1 may not affect the cell cycle progression of the multipotential CNC cells during tooth morphogenesis. Importantly, apoptotic activity is significantly increased within the dental epithelium in the *Lef1* null mutant mice. As the result of this increased cell death, the bud stage tooth germ fails to advance to the cap stage in the absence of *Lef1*. Inhibition of apoptotic activity by FGF4 rescues the tooth development in the *Lef1* null mutant. Our studies suggest that LEF1 is a critical survival factor for the dental epithelial cells during tooth morphogenesis.

Keywords: Apoptosis of dental epithelium; Cranial neural crest (CNC); LEF1 signaling; Tooth morphogenesis

Introduction

Tooth morphogenesis results from an ordered series of gene interactions, each in turn designating individual cell type proliferation, apoptosis, and differentiation. These genetic interactions form the molecular basis of heterologous tissue interactions between the ectodermally derived enamel organ epithelium and the cranial neural crest (CNC)-derived ectomesenchyme during tooth morphogenesis. Multiple growth and transcription factors belonging to several signaling families have been identified as critical regulators at the initiation and subsequently throughout all stages of tooth development (Chai and Slavkin, 2003; Thesleff and Sharpe, 1997). Significantly, most of the signaling networks that are used reiteratively throughout tooth development are in common with the regulatory systems that are critical for governing the development of other organs, such as feather, hair, mammary gland, salivary gland, and pancreas morphogenesis. The growing

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scientific evidence suggests a highly conserved biological mechanism in regulating organogenesis. More importantly, specific growth and transcription factor signaling cascades have been identified as critical regulators in determining the physiological site and location for tooth initiation and in defining patterns of formation of various types of teeth (for review, see Cobourne and Sharpe, 2003; Jernvall and Thesleff, 2000).

Lymphoid enhancing factor 1 (LEF1) is a cell-type-specific transcription factor expressed in lymphocytes of the adult mouse, and in the neural crest, mesencephalon, tooth bud, whisker follicles, and other sites during embryogenesis (Oosterwegel et al., 1993; Travis et al., 1991; van Genderen et al., 1994; Waterman et al., 1991; Zhou et al., 1995). Targeted inactivation of the *Lef1* gene in the mouse germ line results in a pleiotropic phenotype in which the development of teeth, whiskers, hair follicles, and mammary glands is severely impaired (van Genderen et al., 1994). Tooth development is initiated in *Lef1* null mutant embryos; however, it is arrested at the late bud stage before the formation of a mesenchymal dental papilla. Recent study suggests that LEF1 is required in the dental epithelium during tooth development. The biological function of LEF1 is to induce the CNC-derived dental mesenchyme to become competent to form dental papilla, an important component indicating the successful advancement of the tooth germ development into the cap stage (Chai et al., 1998; Kratochwil et al., 1996, 2002). At the molecular level, LEF1 is responsible for a direct regulation of *Fgf4* expression to relay a Wnt signaling to a cascade of FGF signaling activities that mediate the sequential and reciprocal interactions between the dental epithelium and the CNC-derived dental mesenchyme during tooth development (Kratochwil et al., 2002). However, despite this molecular information demonstrating the biological importance of LEF1 signaling in regulating tooth morphogenesis, the cellular mechanism of LEF1 signaling in regulating cell fate determination during tooth morphogenesis remains unclear.

During embryonic development, LEF/TCF family of DNA-binding factors form transcriptional regulatory complex with β-catenin to mediate Wnt signaling to control cell proliferation, intercellular adhesion, cell survival, and cell fate determinations (for review, see Fuchs et al., 2001; van Noort and Clevers, 2002; Willert and Nusse, 1998). In addition, LEF1-mediated Wnt signaling is also critical for pattern formation and axis specification (Laurikkala et al., 2002; Moon et al., 1997a,b; Siegfried and Perrimon, 1994). Dysregulation of components of the Wnt signaling pathway blocks hair follicle, tooth, and mammary gland development during embryogenesis and may also have oncogenic effects in tissues such as colon and breast (Andl et al., 2002; Morin et al., 1997; Nusse and Varmus, 1982). Collectively, these studies suggest that LEF1-mediated Wnt signaling may play a critical role to regulate the progression of cell cycle of progenitor cells to control the cell fate during embryonic and postnatal development.

To investigate the role of LEF1 signaling in regulating the progression of cell cycle of the dental epithelium and the CNC-derived dental mesenchyme cells during tooth morphogenesis, we compared the cellular proliferation and apoptotic activity in the tooth germ between the wild-type and *Lef1* null mutant mice. Our study shows that loss of *Lef1* results in a significant increase in the apoptotic activity within the dental epithelium, suggesting that LEF1 is a critical survival factor for the dental epithelial cells during the advancement of the tooth germ from the bud to the cap stage. In addition, our gene expression analyses have discovered a specific *Lef1* expression in the cervical loop of the enamel organ epithelial cells. These findings suggest that LEF1 may also have an important role in regulating the progression of cell cycle and fate determination of the cervical loop progenitor cells during later stages of tooth morphogenesis.

Materials and methods

Two-component genetic system for marking the progeny of CNC cells

Both *Wnt1-Cre* transgenic line and *R26R* conditional reporter allele have been described previously (Danielian et al., 1998; Soriano, 1999). Mating *Wnt1-Cre* and *R26R* mice generated transgenic mice with progenies of neural crest cells labeled with β-gal because once *Wnt1-Cre* expression commences in premigrating neural crest cells, the β-galactosidase expression is indelible. Detection of β-galactosidase (*LacZ*) activity in both whole embryos and tissue sections was done as previously described (Chai et al., 2000). All animals used in this study were maintained in a C57BL/6J background. We first crossed either *Wnt1-cre* or *R26R* transgenic mice with *Lef1* heterozygous mutant to generate mice carrying $Wnt1cre^{Tg/+}/Lef1^{+/-}$ or $R26R^{Tg/+}/Lef1^{+/-}$, respectively. Upon crossing $Wnt1cre^{Tg/+}/Lef1^{+/-}$ with $R26R^{Tg/+}/Lef1^{+/-}$ mice, we generated *Lef1* null mutant carrying *Wnt1cre/R26R* transgene, which allowed us to follow the progeny of CNC cells indefinitely. Embryonic age was determined with noon of the day of plug observation as E0.5. External staging was used to define embryonic development according to the number of somite pairs (Theiler, 1989). Genotyping of the *Lef1* mutant embryos carrying *Wnt1cre/R26R* transgene was done as previously described (Chai et al., 2000; Soriano, 1999; van Genderen et al., 1994).

Detection of β-galactosidase (lacZ) activities

Whole embryos (E9.5 and E10.5) were stained for β-galactosidase activity according to standard procedures. Embryos were fixed for 20 min at RT in 0.2% glutaraldehyde in PBS. Fixed embryos were washed three times in rinse solution (0.005% Nonidet P-40 and 0.01% sodium