Differentiation 87 (2014) 32–43

Contents lists available at ScienceDirect

Differentiation

journal homepage: www.elsevier.com/locate/diff

Initiation of teeth from the dental lamina in the ferret

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ARTICLE INFO

Available online 6 January 2014

Keywords:
Dental lamina
Tooth development
Tooth replacement
Ferret

ABSTRACT

Mammalian tooth development is characterized by formation of primary teeth that belong to different tooth classes and are later replaced by a single set of permanent teeth. The first primary teeth are initiated from the primary dental lamina, and the replacement teeth from the successional dental lamina at the lingual side of the primary teeth. An interdental lamina connects the primary tooth germs together. Most mammalian tooth development research is done on mouse, which does not have teeth in all tooth classes, does not replace its teeth, and does not develop an interdental lamina. We have used the ferret (*Mustela putorius furo*) as a model animal to elucidate the morphological changes and gene expression during the development of the interdental lamina and the initiation of primary teeth. In addition we have analyzed cell–cell signaling taking place in the interdental lamina as well as in the successional lamina during tooth replacement. By 3D reconstructions of serial histological sections we observed that the morphogenesis of the interdental lamina and the primary teeth are intimately linked. Expression of *Pitx2* and *Foxi3* in the interdental lamina indicates that it has odontogenic identity, and there is active signaling taking place in the interdental lamina. *Bmp4* is coexpressed with the stem cell factor *Sox2* at its lingual aspect suggesting that the interdental lamina may retain competence for tooth initiation. We show that when tooth replacement is initiated there is Wnt pathway activity in the budding successional lamina and adjacent mesenchyme but no active Fgf or Eda signaling. Genes associated with human tooth replacement phenotypes, including *Runx2* and *Il11rα*, are mostly expressed in the mesenchyme around the successional lamina in the ferret. Our results highlight the importance of the dental lamina in the mammalian tooth development during the initiation of both primary and replacement teeth.

1. Introduction

The main features of tooth development are shared between vertebrates from fish and reptiles to mammals. In all species, tooth development is initiated by formation of the primary dental lamina that in mammals is a horse-shoe shaped epithelial thickening along the embryonic jaws. All teeth develop within the dental lamina. After initiation, tooth germ epithelium proceeds through characteristic morphogenetic stages forming a bud, a cap and a bell shaped structure, and the dental mesenchyme condenses around the epithelium. Finally the development of the tooth crown is completed with the differentiation of tooth-specific cell types that secrete the hard tissues of a mature tooth. The genes and signaling pathways that regulate tooth development are largely similar among different species (Jussila and Thesleff, 2012; Richman and Handrigan, 2011; Fraser et al., 2009). There are, however, marked differences in the dentitions between species. Fish and reptiles replace their teeth throughout the life of the animal, and their teeth generally have more simple shapes. Mammals replace their teeth only once, and their teeth belong to different tooth classes, namely incisors, canine, premolars, and molars, each have their characteristic shapes and function.

Most research on tooth development is done on the laboratory mouse, but there are several characteristics of mammalian tooth development that the mouse is lacking. In most mammals, the primary dental lamina grows down into the underlying mesenchyme and connects the forming teeth to each other. Mice do not have this interdental lamina, as their primary dental lamina between the incisors and molars fragments during early tooth development. In mammals, teeth within each primary tooth class form in a temporal sequence. The three molars in the mouse develop in sequence from anterior to posterior from the epithelium associated with the previous tooth. Lineage tracing experiments have shown that the cells in the first molar epithelium expressing the stem cell factor *Sox2* give rise to the second and third molars (Juuri et al., 2013). However, not much is known about the initiation of primary teeth in the incisor and premolar classes, as mice have one pair of incisors and no canines or premolars. The process of the serial addition of molars resembles

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http://dx.doi.org/10.1016/j.diff.2013.11.004

the development of replacement teeth, which is initiated by budding of *Sox2*-positive successional lamina embedded on the lingual side of each primary tooth germ as a part of their enamel organ (Järvinen et al., 2009; Juuri et al., 2013). Mice do not replace their teeth, and therefore other model animals are needed to the study the development of the interdental lamina, initiation of primary teeth, and regulation of tooth replacement in mammals.

We have chosen to use the ferret (*Mustela putorius furo*) as a model animal of mammalian tooth development. Adult ferret has three incisors (I1-3), one canine (C), three premolars (P2-4) and two molars (M1-2) in the lower jaw (Fig. 1A). The timing of ferret tooth development has been described in detail previously (Berkovitz, 1973). The ferret genome has been sequenced (RefSeq Assembly ID GCF_000215625.1), and ferrets are used in biomedical fields such as respiratory infection research. There are some downsides in using ferrets as models in developmental biology. They have a seasonal estrus, and therefore embryos cannot be collected continuously throughout the year (Lindeberg, 2008). The gestation time varies between 39 and 42 days and this is why the embryos have to be staged carefully using morphological criteria (Fig. 1B). Previous work in our laboratory has addressed histological and molecular aspects of tooth replacement (Järvinen et al., 2009). That work showed for example that *Shh*, a marker of primary tooth placode initiation, is not expressed during replacement tooth initiation. In the present work we have extended the previous analysis in our laboratory on the gene expression patterns during tooth replacement in the ferret (Järvinen et al., 2009). In addition, as the dental lamina is a prominent histological structure in the ferret jaw, and the mammalian dental lamina development has not been studied extensively, we decided to examine its morphological and molecular aspects in more detail.

Even though mice do not replace their teeth, there are transgenic mouse models in which supernumerary tooth formation takes place. The most dramatic induction of supernumerary tooth formation has been observed in mice when Wnt signaling is activated in the epithelium, either by stabilizing the downstream effector β-catenin or knocking out the inhibitor *Apc* (Järvinen et al., 2006; Wang et al., 2009; Liu et al., 2008). The forced activation of Wnt signaling resulted in continuous development of successionally forming teeth (Järvinen et al., 2006). A milder phenotype is observed in the knock-out mouse of transcription factor *Osr2*, which develops a single ectopic tooth on the lingual side of the first molar (Zhang et al., 2009). In addition, a supernumerary tooth forms in front of the first molar in many mouse lines as a result of fine tuning of different signaling pathways (Jussila and Thesleff, 2012). There are also several known human mutations causing supernumerary or missing permanent teeth, suggesting that these genes regulate tooth replacement. Mutations in Wnt inhibitor *AXIN2* in humans cause missing permanent teeth including replacement teeth and molars (Lammi et al., 2004). As in mice, human *APC* mutations cause familial adenomatous polyposis syndrome, which involves supernumerary tooth formation (Fader et al., 1962).

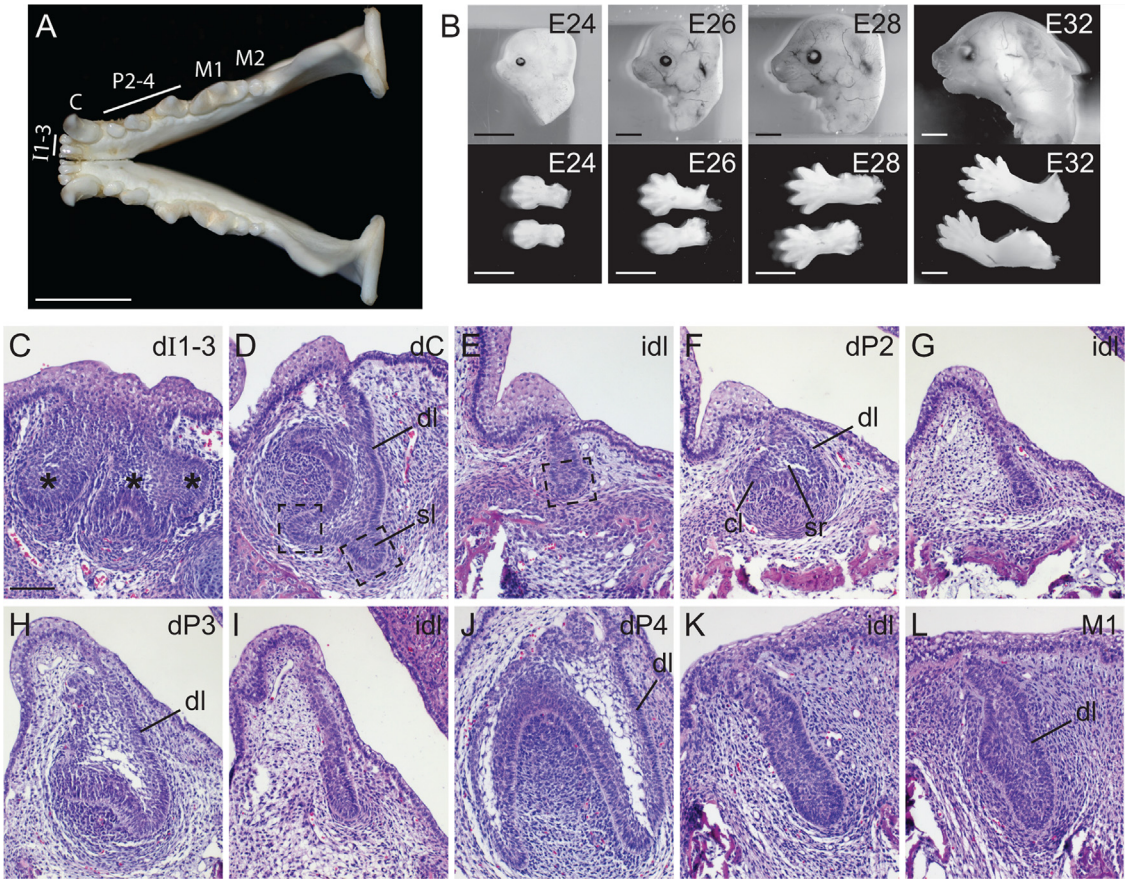

Fig. 1. The ferret dentition comprises teeth from all tooth classes. (A) The ferret permanent dentition in the lower jaw comprises three incisors (I1-3), one canine (C), three premolars (P2–P4) and two molars (M1-2). (B) Craniofacial and limb development were used as morphological criteria to stage the ferret embryos between E24 to E32. (C–L) Frontal histological sections of E32 ferret lower jaw. The ferret deciduous dentition at E32 is comprised of three incisors (dI1-3 in C, asterisks), canine (dC in D), three premolars (dP2-dP4 in F, H and J) and one molar (M1 in L). The interdental lamina (idl) runs between all teeth (E, G, I, and K) and is embedded as a dental lamina on the lingual side of each primary tooth (D, F, H, J, and L). Tooth replacement is initiated by formation of a successional lamina (sl in D). The core of the tooth germ epithelium is called stellate reticulum (sr), and the cervical loops (cl) grow down to participate in tooth shape formation (F). The cervical loop and the successional lamina of the developing primary canine and the interdental lamina resemble each other histologically (boxed areas in D and E). Lingual is to the right. Scale bar in A is 1 cm and 100 μm in others. Scale bar in C: C–L.