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Review

Molecular patterning of the mammalian dentition

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ABSTRACT

Four conserved signaling pathways, including the bone morphogenetic proteins (Bmp), fibroblast growth factors (Fgf), sonic hedgehog (Shh), and wingless-related (Wnt) pathways, are each repeatedly used throughout tooth development. Inactivation of any of these resulted in early tooth developmental arrest in mice. The mutations identified thus far in human patients with tooth agenesis also affect these pathways. Recent studies show that these signaling pathways interact through positive and negative feedback loops to regulate not only morphogenesis of individual teeth but also tooth number, shape, and spatial pattern. Increased activity of each of the Fgf, Shh, and canonical Wnt signaling pathways revitalizes development of the physiologically arrested mouse diastemal tooth germs whereas constitutive activation of canonical Wnt signaling in the dental epithelium is able to induce supernumerary tooth formation even in the absence of Msx1 and Pax9, two transcription factors required for normal tooth development beyond the early bud stage. Bmp4 and Msx1 act in a positive feedback loop to drive sequential tooth formation whereas the Osr2 transcription factor restricts Msx1-mediated expansion of the mesenchymal odontogenic field along both the buccolingual and anteroposterior axes to pattern mouse molar teeth in a single row. Moreover, the ectodermal-specific ectodysplasin (EDA) signaling pathway controls tooth number and tooth shape through regulation of *Fgf20* expression in the dental epithelium, whereas Shh suppresses Wnt signaling through a negative feedback loop to regulate spatial patterning of teeth. In this article, we attempt to integrate these exciting findings in the understanding of the molecular networks regulating tooth development and patterning.

Contents

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1. Introduction

Cell–cell interactions through signal transduction pathways are crucial for the development of all multicellular organisms. Despite the enormous number of distinct cell types and a wide variety of tissue structures and patterns in the animal kingdom, a few conserved cell–cell signaling pathways, including the fibroblast growth factor (Fgf), hedgehog, transforming growth factor-beta (Tgfβ) and wingless-related (Wnt) signaling, are used repeatedly to regulate most of the developmental programs within individual animals and throughout vertebrate evolution [1]. Whereas decades of genetic and biochemical studies have identified many of the molecular components of each of these signaling pathways and revealed extensive cross-talk among them, the detailed mechanisms regarding how they are modulated and how new components are integrated into existing signaling networks to control morphogenesis and patterning during mammalian organogenesis remain to be elucidated.

Teeth, like many organs, form through sequential and reciprocal inductive interactions between the adjacent epithelium and mesenchyme [2–5]. Tooth development is largely independent from the rest of the body, and isolated, even dissociated and recombined, mammalian tooth germs can continue to develop to mineralized teeth upon transplantation to ectopic sites in adult animals [6,7], such as the renal capsule. Thus, tooth development has long been used as a model for studying inductive interactions regulating organogenesis. In addition to allowing detailed analysis of the mechanisms regulating initiation, morphogenesis, and maturation of the individual organ, teeth exhibit species-specific number, shape, and patterns, and therefore provide a general paradigm for the studies of molecular mechanisms of developmental patterning and of evolution [8,9]. Through combinations of experimental embryological manipulations and transgenic and gene knockout studies in mice, research in the past 20 years have investigated the roles of each of the major signaling pathways in tooth organogenesis [2–5,10–13]. Whereas many mutant mice exhibit tooth developmental arrest phenotypes and revealed requirements of particular genes and pathways for specific steps of tooth organogenesis [10], several transgenic or gene-knockout mutant mouse strains exhibit alterations in the number, shape, and/or pattern of teeth, of which recent studies have provided fascinating new insights into the integration of signaling networks regulating tooth organogenesis and dentition patterning [13]. Many review articles published previously provide excellent references on the progress in the studies of the molecular mechanisms of tooth development [2–5,9–13]. In this review, we highlight the integration of the actions of networks of activators and inhibitors of the Bmp (bone morphogenetic proteins, members of the Tgfβ superfamily), Fgf, Shh, and Wnt signaling pathways in the regulation of tooth morphogenesis and spatial patterning of the dentition.

2. The Bmp, Fgf, Shh, and Wnt signaling pathways are each repeatedly required for tooth initiation and morphogenesis

Whereas most of our understanding of the molecular mechanisms of tooth development has been derived from studies using mouse models, the basic steps of tooth organogenesis are similar in all vertebrates [8,9]. In mice, tooth development begins as a thickening of the oral epithelium, termed dental lamina (Fig. 1A), at 11 days of gestation (E11). The dental lamina proliferates and buds into the underlying neural crest-derived mesenchyme and induces the mesenchyme to condense around the epithelial bud from E12 to E13 (Fig. 1B). The dental mesenchyme in turn induces formation of an epithelial signaling center in the distal region of the epithelial bud, termed primary enamel knot, which drives tooth morphogenesis through the "cap" and "bell" stages (Fig. 1C and D). As development proceeds, the epithelial cells in contact with the dental papilla mesenchyme differentiate into ameloblasts and the adjacent mesenchymal cells differentiate into odontoblasts (Fig. 1E) [14]. The ameloblasts and odontoblasts deposit enamel and dentin matrices, respectively back-to-back and subsequent mineralization of these matrices forms the hard tissues of the tooth [6]. Thus, formation of each individual tooth, from its initiation through morphogenesis to cytodifferentiation, involves an extensive series of reciprocal interactions between the dental epithelium and the neural crest derived mesenchyme.

2.1. Regulation of tooth initiation and tooth bud formation

At the beginning of tooth development, multiple members of the Bmp, Fgf, and Wnt families, including *Bmp2*, *Bmp4*, Bmp7, *Fgf8*, *Fgf9*, *Wnt4*, *Wnt6*, *Wnt10a*, and *Wnt10b*, and *Shh* are expressed in the presumptive dental epithelium [15–22]. Blocking each of these four signaling pathways at the beginning of tooth development genetically or in explant culture causes tooth developmental arrest at the dental lamina or early bud stage [4,5,7,10–12]. Bmp and Fgf signaling is necessary for activation of expression of the Msx1 and Pax9 transcription factors, respectively, in the presumptive tooth mesenchyme [17,19,22,23]. Mice lacking either *Msx1* or *Pax9* function exhibit tooth developmental arrest at the bud stage [24,25]. Expression of *Bmp4* shifts from the presumptive dental epithelium to the developing tooth mesenchyme at the early bud stage during normal tooth development and is significantly reduced in the developing tooth mesenchyme in either $Msx1^{-/-}$ or $Pax9^{-/-}$ mutant mice [17,25,26]. In addition, Fgf8 induces *Fgf3* expression in the dental mesenchyme in an Msx1-dependent manner [27]. Although teeth develop nearly normally in $Fgf3^{-/-}$ mutant mice [28,29], mice homozygous for null mutations in both *Fgf3* and *Fgf10*, which are both expressed in the developing tooth mesenchyme, exhibit tooth developmental arrest at the bud stage [29]. Fgf8 also induces expression of *Inhibin-βA* (*Inhba*) (also known as *Activin-βA*), another member of the Tgfβ superfamily, in the developing tooth mesenchyme [30]. Mice lacking *Inhba* function exhibit early developmental arrest of incisors and mandibular molar tooth germs [30,31]. In addition, tissue-specific inactivation of the Bmp receptor gene *Bmpr1a* in either the neural crest lineage or the oral epithelium caused tooth developmental arrest at the bud stage [32–34]. Mice with a deletion of the epithelial isoform of the type-2 Fgf receptor also exhibit tooth developmental arrest at the bud stage [35]. Thus, both Bmp and Fgf signaling are critical for the reciprocal interactions between the epithelium and mesenchyme during early tooth development. On the other hand, although expression of the Wnt ligands is mostly restricted to the dental epithelium, with exception of expression of *Wnt5a* in the dental mesenchyme [21], tissue-specific inactivation of β-catenin, the obligatory intracellular mediator of the canonical Wnt signaling pathway, in either the dental epithelium or the dental mesenchyme also caused tooth developmental arrest at the bud stage [36,37].

Recently, O'Connell et al. [38] analyzed properties of the gene regulatory networks mediating the reciprocal epithelial–mesenchymal interactions during early mouse molar development through systematic analyses of previously reported gene expression data together with more than one hundred new microarray-based gene expression profiling datasets from isolated early tooth epithelial and mesenchymal tissues. They identified the Wnt and Bmp pathways as the two major mediators of epithelial–mesenchymal signaling in early tooth development. The Wnt and Bmp pathways collectively control the production of signaling molecules in all major pathways, including Bmp4, Shh, Fgfs, and Wnts in the epithelium and Fgfs, Bmp4, and Inhba in the mesenchyme of the early tooth germs [38]. Whereas a simple