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The contribution of specific cell subpopulations to submandibular salivary gland branching morphogenesis

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Branching morphogenesis is the developmental program responsible for generating a large surface to volume ratio in many secretory and absorptive organs. To accomplish branching morphogenesis, spatiotemporal regulation of specific cell subpopulations is required. Here,we review recent studies that define the contributions of distinct cell subpopulations to specific cellular processes during branching morphogenesis in the mammalian submandibular salivary gland, including the initiation of the gland, the coordination of cleft formation, and the contribution of stem/progenitor cells to morphogenesis. In conclusion, we provide an overview of technological advances that have opened opportunities to further probe the contributions of specific cell subpopulations and to define the integration of events required for branching morphogenesis.

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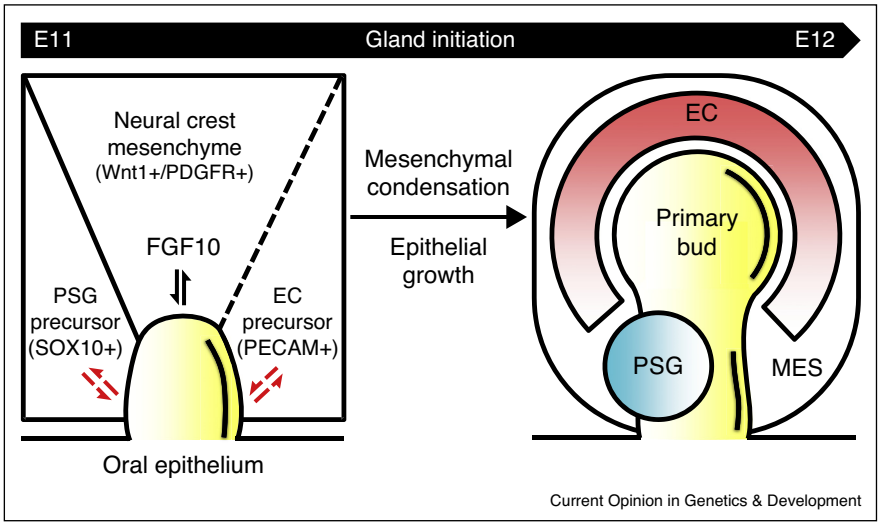
Introduction

The developmental process by which the arborized structure of branched organs develops has fascinated biologists for decades. As with other branched organs, the major salivary glands (submandibular, sublingual, and parotid) all undergo a complex process known as branching morphogenesis during embryonic development to yield adult organs having a large surface area to volume ratio. While there are some commonalities in the genes and pathways that drive branching morphogenesis, each organ is unique; thus, it is worthwhile to study each organ. In this review, we focus on development of the mouse submandibular salivary gland (SMG), which has historically been the most studied of the salivary glands since these paired embryonic organs can be cultured *ex vivo*, mimicking *in vivo* morphogenesis [1–4] and cellular differentiation [5,6,7••]. For a comprehensive overview, the reader is referred to several recent reviews on salivary gland development [8–13]. Here, we examine several specific questions regarding salivary gland branching morphogenesis towards which recent studies have provided significant insights. We discuss recent insights into the initiation of the gland, the coordination of cleft formation, and the contribution of stem/progenitor cells to branching morphogenesis. We conclude by highlighting the importance of defining and manipulating specific cell subsets within the epithelium and the mesenchyme, and with an overview of resources and emerging technologies that will facilitate the elucidation of molecular mechanisms driving branching morphogenesis.

How is the submandibular salivary gland initiated?

The submandibular salivary placode initiates from the oral epithelium at embryonic day 11.0 (E11.0) and invaginates into the surrounding mesenchyme by E12, which subsequently condenses around the epithelium (Figure 1). Recent Sox17 lineage tracing studies of oral endoderm indicate that all of the major salivary glands of the mouse are not endodermal but instead must be ectodermal in origin [14•] (reviewed in [12]), and Wnt1-Cre lineage tracing demonstrated that the mesenchyme is derived from neural crest [15]. Early tissue recombination studies in which epithelium and mesenchyme derived from various sources and developmental time-points were recombined indicated that the mesenchyme has an instructive role in epithelial patterning but that the epithelium retains some autonomy in the control of its own cytodifferentiation, as reviewed in [8]. The epithelium is instructive up until E12.5 and is required to induce fibroblast growth factor 10 (Fgf10) production by the mesenchyme [16], which is then required for subsequent salivary gland development [3,17]. Since the mesenchyme becomes instructive at E11.5 [16,18], as distinct cell subpopulations arise, some of these cells may have a function in gland initiation. Acetylcholine-positive neurons that are not neural crest-derived, but rather are derived from nerve-associated Sox10-positive Schwann cell precursors, condense to form the parasympathetic ganglion (PSG) [19•]. An endothelial plexus of unconfirmed origin is also present at gland initiation. Since endothelial cells are critical to the initiation of the liver [20•], pancreas [21], lung [22•,23], and testis [24,25] in a

Figure 1



Involvement of multiple cell subpopulations in salivary gland initiation. At E11 the epithelial placode initiates from the oral epithelium and protrudes into the surrounding mesenchyme. Salivary mesenchyme is composed of subpopulations derived from distinct origins, including Wnt1+/PDGFR+ cranial neural crest cells, Sox10+ Schwann cell-derived parasympathetic ganglion (PSG) precursors, and PECAM+ endothelial cell (EC) precursors of unknown origin. The epithelium has an instructive role to promote mesenchymal Fgf10 expression, which subsequently induces epithelial morphogenesis (black half arrows). Possible contributions of the PSG and EC precursors to initiation are not known (red half arrows). By E12, the epithelium forms the primary bud and stalk as the mesenchyme condenses around it. The PSG surrounds the epithelial stalk and a primitive capillary system is established in the mesenchyme.

non-perfusional manner, the endothelial cell plexus may also be required for salivary gland initiation. The mechanisms by which the epithelium and mesenchyme mediate instructive signaling and the requirements for mesenchymal cell subpopulations in salivary gland initiation merit further investigation.

How is cleft formation coordinated?

Following initiation of the primary epithelial bud at E12, small invaginations of the basement membrane known as clefts form in the surface of this solid epithelial cell mass to start the process of branching morphogenesis. The mechanisms through which clefts initiate remain unclear but mesenchymally-induced activation of autocrine EGF signaling in the epithelium appears to promote cleft initiation [26], and lysophosphatidic acid (LPA) can synergize with EGF [27] (Figure 2). Semaphorin 3A/3C acting through the neuropilin receptor 1 (Nrp1) [28] may also be involved. Deposition of fibronectin (FN) in clefts is required [29•] but may not be the initial symmetry-breaking signal. Ectopic cleft initiation can occur with inhibition of Rho-associated protein kinase (ROCK) or non-muscle myosin (NMM) II function [30•], suggesting that low contractility facilitates cleft initiation and that effective morphogenesis requires a contractility-dependent cleft-stabilization step. How the placement of cleft initiations is determined remains unknown. Since computational modeling predicts that an optimal level of epithelial cell contractility is required to generate a progressing cleft [31], stabilization of clefts may be the critical event for effective branching morphogenesis.

Cleft progression occurs with the replacement of cell–cell adhesions by cell–matrix adhesions as basement membrane is assembled adjacent to the outer polarized epithelial cell layer in this very narrow structure [32]. Polarized deposition of basement membrane by these cells is maintained by ROCK1 in a Microtuble affinity-regulating kinase 2 (MARK2)/Partitioning-defective 1b (Par1b)-dependent manner [33]. Cleft progression requires FN assembly, which is regulated by ROCK1 in a NMM II-dependent manner to activate integrin β1, and FAK, which is required to recruit focal adhesion proteins at the basal surface of the polarized outer epithelial cell population [30•,34]. This pathway may generate a feed-forward signal to propagate clefts [30•]. Myosin light chain phosphatase 1 (MYPT1) can balance regulatory myosin light chain (rMLC) or microtubule deacetylase (HDAC6), to control contractility and microtubule acetylation, respectively, which are required for integrin α5β1 function and FN assembly [35]. Additionally, LIM kinase (LIMK)-mediated control of the actin and microtubule cytoskeletons facilitates FN assembly [36]. Mechanical signals applied by the mesenchyme also likely influence the progress of branching since a low environmental compliance is required to facilitate branching [5,37]. The assembled epithelial basement membrane translocates from the tip of the endbud into progressing