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Review

Salivary gland development: A template for regeneration

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ABSTRACT

The mammalian salivary gland develops as a highly branched structure designed to produce and secrete saliva. This review will focus on research on mouse submandibular gland development and the translation of this basic research toward therapy for patients suffering from salivary hypofunction. Here we review the most recent literature that has enabled a better understanding of the mechanisms of salivary gland development. Additionally, we discuss approaches proposed to restore salivary function using gene and cell-based therapy. Increasing our understanding of the developmental mechanisms involved during development is critical to design effective therapies for regeneration and repair of damaged glands.

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Contents

1. Introduction

The salivary system of mice and humans contains three major pairs of glands; the parotid, submandibular (SMG) and sublingual glands, which together secrete 90% of the saliva in the oral cavity. Additionally there are numerous (600–1000) minor salivary glands in the submucosa throughout the oral cavity. The reader is referred

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to recent extensive reviews on salivary glands [1–3]. The major function of salivary glands is to produce saliva, which aids in lubrication, digestion of food, taste, immunity and oral homeostasis. The acinar cells produce either serous or mucous secretion, which contains water, salts and proteins, while the ductal cells modify the secretion, primarily by reabsorbing the salt. The stellate myoepithelial cells, which surround the acini and intercalated ducts, are innervated and are proposed to facilitate secretion by contraction, although this has not been directly demonstrated. There are three types of ducts based on their morphology and histological appearance; intercalated, striated and granular. Saliva flows from the acinar units through the ductal system into the oral cavity. Readers are referred to reviews on the physiology of salivary secretion [4–6].

2. Mechanisms of development

2.1. Developmental origin

There is some controversy within the literature about the developmental origin of the epithelium of the major salivary glands, i.e. are they ectodermal or endodermal in origin? While it is apparent that all 3 pairs of major glands are primarily derived from the oral epithelium, the issue is which part of the oral epithelium they arise from and where this is in comparison to the junction of the oral ectoderm with the foregut endoderm. During development this border is marked by the oropharangeal membrane that separates the stomodeum from the cavity of the primordial pharynx [7], but the exact position of this line as compared to sites of gland initiation is unclear. The use of genetic lineage tracing using lineage-specific Cre drivers has helped clarify the lineage of some cell types within the glands. The mesenchyme and nerves in the gland are neural crest in origin as shown by lineage tracing with Wnt1-cre [8]. However, there are conflicting reports of the embryonic origin of the epithelium. In text books, it has been suggested that the parotid is ectodermal, whereas the SMG and sublingual are endodermal [9]. An endoderm origin was proposed to be supported by data showing that adult salivary gland progenitors can differentiate into pancreatic β-cells and hepatocytes when transplanted into hepatectomized liver [10]. However, while it is clear that salivary gland progenitors can differentiate into these cells types in the appropriate extracellular microenvironment, i.e. when transplanted into the liver, it is not proof that in vivo the salivary epithelium is derived from the endoderm. Recent genetic lineage tracing experiments using the Sox17-2A-iCre/R26R mouse, which marks endodermal cells, showed that the epithelia of all three major salivary glands are not of endoderm origin, suggesting an ectodermal lineage [11]. In addition, animal models and human mutations that cause ectodermal dysplasias, developmental syndromes that specifically affect ectodermal organs, suggest that the major salivary glands arise from common multipotent precursors residing in the embryonic ectoderm. Hypohidrotic ectodermal dysplasia (HED) patients have abnormal salivary glands and similar phenotypes are observed in mouse models *Tabby* (Eda^{Ta}) and *downless* ($Edar^{dl}$) [12,13]. Lineage tracing studies need to be performed with a specific ectodermal Cre to positively confirm the origin of the salivary gland epithelium.

2.2. Salivary gland initiation

Reciprocal interactions among the epithelium, and neural crest-derived mesenchyme, nerves, and blood vessels regulate the early events of SMG development (Fig. 1). It is not known what signals cause the migrating neural crest cells to form a mesenchymal condensation at the appropriate location beside the oral epithelium. The mesenchyme provides instructive signals, resulting in the thickening of the oral epithelium to form a placode at embryonic day 11 of development. Knockout mice for *Fgf10*, *Fgfr2b*, *Pitx1* and *p63* lack salivary glands, emphasizing that these genes are critical for salivary gland initiation and patterning. In organs such as the liver and pancreas the endothelial cells provide critical cues for organogenesis [14], however the role of endothelial cells in salivary gland initiation has not been investigated. By E12, the salivary placode invaginates into the mesenchyme, which begins to condense. The epithelial bud grows into the mesenchyme forming a primary bud on a stalk. The neural crest-derived neuronal precursors coalesce to form the parasympathetic submandibular ganglion (PSG), wrapping around the epithelial stalk that will become the major secretory duct. The signals that initiate this interaction have not been defined.

2.3. Branching morphogenesis

The major glands form by the developmental process of branching morphogenesis, which involves coordinated cell proliferation, clefting, differentiation, migration, apoptosis and reciprocal interactions between the epithelial, mesenchymal, neuronal and endothelial cells [15]. At E13 as the endbud enlarges, clefts in the epithelium delineate the first 3–5 buds, which correspond to major lobules of the gland, and in parallel, axons from the PSG extend along the epithelium to envelop the endbuds. By E14 the gland is highly branched and functional differentiation begins at E15 and continues to birth [1,16]. In the next sections we review specific mechanisms involved in branching morphogenesis.

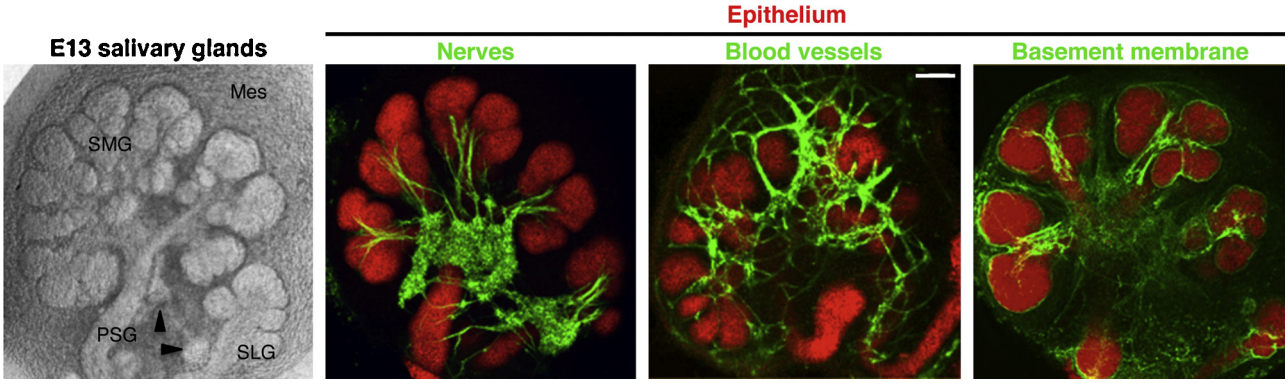

Fig. 1. Reciprocal interactions among the epithelium (E-cadherin staining red), nerves (Tubb3 staining green), blood vessels (Pecam staining green) and basement membrane (Perlecan staining green) regulate branching morphogenesis during submandibular (SMG) and sublingual gland (SLG) development. The brightfield image shows and E13 SMG and SLG cultured overnight, the mesenchyme (Mes) and parasympathetic ganglia (PSG) are also visible. The fluorescent images are projections of multiple confocal sections, Scale bar 100 μM.