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Structure, morphology and signalling development mechanisms of human salivary glands

Margherita Sisto*, Domenico Ribatti and Sabrina Lisi

Department of Basic Medical Sciences, Neurosciences and Sensory Organs (SMBNOS), Section of Human Anatomy and Histology, University of Bari "Aldo Moro", Bari, Italy

Abstract

The human salivary gland (SGs) develops as a highly branched structure designed to produce and secrete saliva indispensable to maintain the health of the oral cavity and for carry out physiological functions like mastication, taste perception and speech. Here we review the anatomy and cytoarchitecture of SGs and the most recent literature that has enabled a better understanding of the molecular signalling pathways of SGs development to translate this basic research towards therapy for patients suffering from salivary hypo function.

Keywords

Salivary gland development, acinar cells, ductal cells, myoepithelial cells, branching morphogenesis, EGF, FGF, EDA.

Introduction

The salivary glands (SGs) are multicellular exocrine glands that synthesize and secrete saliva into the mouth, maintaining several physiological functions ranging from the protection of teeth and surrounding soft tissues to the lubrication of the oral cavity, crucial for speech and perception of taste sensitivity (Carpenter, 2013; Feller et al., 2013). The SGs are divided into the major paired and minor SGs. Humans have three paired major SGs [parotid (PG), submandibular (SMG), and sublingual (SLG)] as well as hundreds of minor SGs. (Edgar et al., 2012). SGs can be affected by infection, inflammation, autoimmune disease, and tumorigenesis. Indeed, advances in routine imaging have played an important role in visualization of morphology and function and have led to improved sensitivity in the diagnosis of several diseases that involve the major and minor SGs. Here we aim to provide a perspective on what is currently known about the anatomical findings on SGs, as well as the recent progresses in the identification of the signalling pathways involved in SGs morphogenesis. Understanding the molecular mechanisms involved in gland biogenesis provides a template for regenerating, repairing or reengineering SGs which will hopefully one day restore SGs function in patients who suffer from xerostomia.

* Corresponding author. E-mail: margherita.sisto@uniba.it

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Anatomical structure of the major SGs

The largest of the three major SGs is the PG. It is located superficially, below the external acoustic meatus between the sternocleidomastoid muscle and the masseter extending from the mastoid tip to just below the angle of the mandible. The gland is enveloped by the superficial layer of the deep cervical fascia that splits to constitutes the parotid space delimited anteriorly by the masticator space. The PG is divided into a superficial and deep lobe by the facial nerve, which passes through the gland (Chaurasia's, 2006; Som & Brandwin-Gensler, 2011).

The secretions of the PG are transported to the oral cavity by the Stensen's duct. It arises from the anterior border of the gland traversing ventrally the superficial surface of the masseter muscle. The duct that perforates the buccinators muscle, moving medially, and it opens out into the oral cavity in the buccal mucosa near the second maxillary molar. It is important to note that a relevant number of individuals have an accessory duct that drains directly into the main parotid duct (Carpenter, 2013; Kessler & Bhatt, 2018).

The SMG is the second largest of the three major SGs. It is positioned deeply and inferiorly to the mandible, precisely in the posterior part of the submandibular triangle, which borders are anterior and posterior bellies of the digastric muscle and the lower border of the mandibular body; SMG overlies both bellies of the digastric muscle. A line drawn through the SMG at the level of the posterior margin of the mylohyoid muscle can be used to separate the submandibular (superficial) portion of the SMG from the sublingual (deep) portion of the SMG (Carlson, 2000). The excretory Wharton's duct, extends from the anterior aspect of the SMG deep to mylohyoid on the lateral surfaces of the hyoglossus muscle and genioglossus muscle, which are lateral to the hypoglossal nerve (Johns, 1977; Carlson, 2000). Laterally to the Wharton's duct lies the SLG, the smallest of the three major SGs. It is situated submucosally in the floor of the mouth and deeply to the body of the mandible, precisely in the sublingual space. The sublingual space, bounded between the mylohyoid muscle and the geniohyoid and genioglossus muscles, contains the lingual artery and nerve, the hypoglossal nerve, the glossopharyngeal nerve, Wharton's duct, and the SLG, which drains into the oral cavity through several small excretory ducts in the floor of the mouth and a major duct known as Bartholin's duct. (Johns, 1977; Carlson, 2000).

Anatomical structure of the minor SGs

The mucosa of the upper aerodigestive tract is lined by hundred small, minor SGs spread throughout the submucosa of the sinonasal cavity, oral cavity, pharynx, larynx, trachea, lungs, and middle ear cavity. However, the minor SGs are ubiquitous but most concentrated along the buccal mucosa, labial mucosa, lingual mucosa, soft/hard palate, and floor of mouth. They lack a distinct capsule, instead mixing with the connective tissue of the submucosa or muscle fibres of the tongue or cheeks (Nanci, 2013; Kessler, 2018). Minor SGs also are formed from a complex ductal network similar to those of the major glands although constitute from small ducts. Minor salivary glands contribute substantially to the amount of secreted saliva within the oral cav-

ity that usually occurs through several short ducts, instead of being collected by a single large duct as the major SGs (Ferraris & Muñoz, 2006; Nanci, 2013). Therefore, paradoxically, the minor SGs have an efficient system of salivary production that is considered the most important for the mucosal protective and lubricant functions for the oral cavity (Edgar, 1990).

Cytoarchitecture of SGs

Acini

Saliva is secreted by the SGs end-pieces, the acinar lobules, which are composed by acinar cells (Figure 1). There are three main types of acini: serous, mucinous and seromucous, (Berkovitz et al., 1992; Tandler & Phillips, 1998). Serous acini have a spherical morphology and produce a watery secretion containing proteins that are modified and stored in secretory, or zymogen, granules abundant at the apex of the cell. In contrast, mucinous acini store a glycoprotein mixture (mucous, like mucins), which becomes hydrated upon exocytosis to form mucus. Lastly, seromucous acini contain secretions of both types (Tandler & Phillips, 1998).

Serous and mucinous acini are characterized by a distinct cellular architecture; the serous cells are pyramidal or triangular in shape, are distinguished by basophilic basal cytoplasm, a centrally-located nucleus, and variously-staining secretory vesicles (zymogen granules) in apical cytoplasm. These cells, arranged in a spherical structure with a narrow apex that forms a central lumen, secrete pre-packaged secretory granules located in the apical cytoplasm that contain salivary molecular components (Berkovitz et al., 1992; Carpenter, 2013). The mucous saliva provides oral lubrication and form a relevant glycan barrier in mucosal protection (Munger, 1964; Carpenter, 2013). Serous acini secrete protein and glycoprotein and high levels of amylase, ions and water. (Ligtenberg et al., 2015).

Ductal system

The ductal system modifies the composition of the primary hypotonic saliva into an isotonic fluid through ionic changes between saliva and ductal cells (Figure 1). These events occur into three different types of ducts known as intercalated, striated and excretory ducts (Carlson, 2000). The intercalated ducts, connecting directly to the acini, are the first to receive the primary hypotonic saliva since that the lumen of the secretory acini is contiguous with the lumen of the intercalated ducts. These ducts are constituted by simple cuboidal epithelial cells, partially covered by contractile myoepithelial cells that contribute to the salivary flow. Intercalated ductal cells present microvilli pointing towards the lumen space and in the apical region, contain granules of lysozyme and lactoferrin that are secreted in the saliva (Berkovitz et al., 1992; Carpenter, 2013; Ellis & Auclair, 2008).

Striated ducts, considered as intralobular ducts, are specialized in promoting the essential salivary modification from isotonic to hypotonic saliva trough the secretion and reabsorption of electrolytes in a bidirectional way between the lumen and the extracellular space. In the striated duct cells are present a large amount of mitochon-

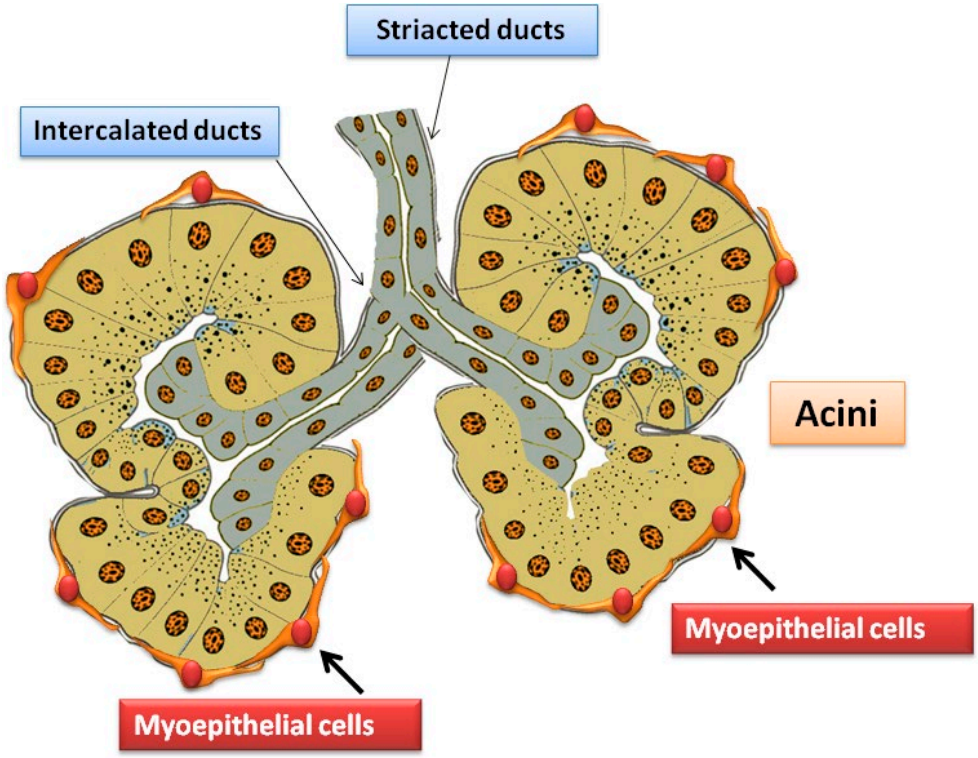

Figure 1. Schematic representation of the structural features of major salivary glands.

dria along their basolateral membrane that characterizes the striated aspect by their multiple folding (Berkovitz et al., 1992; Carpenter, 2013).

The final collecting ducts, interlobular excretory ducts, are formed by pseudostratified epithelium and insert between the glandular lobules. These ducts reabsorb the sodium and potassium secretions in a continuous way, and subsequently are responsible to drive the final saliva production versus oral cavity (Ligtenberg & Veerman, 2014).

Myoepithelial cells

Myoepithelial cells have dual epithelial and contractile properties and play an essential role in acinar salivary secretion (Figure 1). These cells present in their cytoplasm keratin filaments and contractile proteins such as actin, caldesmon, calponin and smooth muscle actin (Redman, 1994; Ianez et al., 2010; Chitturi et al., 2015). They have variable distribution between types of glands and also even within the same gland during the development (Redman, 1994; Hardy & Kramer, 1998; Ogawa, 2003; Chitturi et al., 2015). The myoepithelial cells are stellate or spiderlike, with a flattened nucleus scanty, perinuclear cytoplasm and numerous branching processes that

embrace the secretory and duct cells. These cells rhythmically contract to squeeze saliva from the acinar units upon stimulation by nerves, through the duct system, and into the oral cavity (Shah et al., 2016). Furthermore, it has been suggested that myoepithelial cells play a role in propagation neural stimuli transport of metabolites and in inflammatory state of SGs (Caselitz et al, 1986; Redman., 1994; Ogawa, 2003; Ianez et al., 2010; Chitturi et al., 2015; Shah et al., 2016; Sisto et al. 2018).

Salivary gland innervation and vascularization

The composition and volume of secreted saliva depends on neural stimulation, and the normal secretion is associated with the autonomic nerve supply, that is important to understand autonomic effects on not only salivation, but also biogenesis (Proctor & Carpenter 2007; Ferreira & Hoffman, 2013). Parasympathetic stimulation results in secretion of serous, or watery, salivary secretion and ions, whereas sympathetic stimulation increases the secretion of proteins. In the central nervous system, the salivatory nuclei are the pontine superior salivatory nucleus responsible for the innervation of SMG and SLG, and the pontine inferior salivatory nucleus that innervate PG. From the superior salivatory nucleus preganglionic parasympathetic fibers are distributed via the chorda tympani and lingual nerves to the submandibular and sublingual ganglia, which are within the glands. The SMG and SLG are innervated by post-ganglionic fibres that stimulate saliva secretion and innervate myoepithelial cells (Ishizuka et al. 2010). From the inferior salivatory nucleus, the preganglionic parasympathetic fibres originate in the glossopharyngeal nerve. They leave the glossopharyngeal nerve by its tympanic branch and then pass via the tympanic plexus and the lesser petrosal nerve to the otic ganglion. Here, the fibres synapse, and the postganglionic fibres pass by communicating branches to the auriculotemporal nerve, a branch of the mandibular nerve (Tosios et al., 2010), which conveys them to the parotid gland. For the sympathetic innervation, the cell bodies of the are located in the superior cervical ganglion in the neck and post-ganglionic fibres innervate the SGs through the blood vessels of the carotid plexus (Kahle & Frotscher, 2003).

Regards the vascularization, for the PG the blood is supplied by the posterior auricular and superficial temporal arteries, both branches of the external carotid artery, which arise within the parotid gland itself (Ten cate, 1998) Venous drainage is achieved via the retromandibular vein. It is formed by unification of the superficial temporal and maxillary veins. For SMG, blood supply is via the submental arteries which arise from the facial artery; a branch of the external carotid artery. Venous drainage is through the submental veins which drain into the facial vein and then the internal jugular vein (Fehrenbach MJ, Herring SW, 2012). For SLG, blood supply is via the sublingual and submental arteries which arise from the lingual and facial arteries respectively; both of the external carotid artery. Venous drainage is through the sublingual and submental veins which drain into the lingual and facial veins respectively; both then draining into the internal jugular vein. (Nanci, 2013) The lymphatic system of the parotid gland differs from that of SMG and SLG, because in that there is a high density of lymphnodes in and around it. PG contains two nodal layers, draining into both the superficial and deep cervical lymph systems (Garatea-Crelgo J, 1993).

Development of human SGs

Morphogenesis of SGs requires the cooperation of signalling pathways that coordinately direct cell proliferation, cell quiescence, apoptosis, and histological differentiation (Melnick and Jaskoll, 2000; Melnick et al., 2001 a, b, c, d; Davidson et al., 2002; Gardner et al., 2003). The development of the major SGs in humans begins the sixth to eighth embryonic week. The SMG of the mouse shows a classic organogenetic and branching morphogenesis process and is commonly used as a model to study human organogenesis (Borghese, 1950). The highly branched structure of SGs development is regulated by multiple stage-specific growth factors, cytokines, and transcription factors which are expressed at specific time points to trigger the organogenesis process (Kashimata and Gresik, 1997; Jaskoll and Melnick, 1999; Melnick et al., 2001 a, b, c, d; Jaskoll et al., 2002). SGs organogenesis involves epithelial, mesenchymal, neuronal, lymphatic, and endothelial cells, together with their corresponding stem and progenitor cells. These cell types and their extracellular matrix microenvironment interact spatiotemporally to induce a program of genetic and epigenetic tissue patterning and cellular differentiation, ultimately resulting in functional SGs. There is some controversy within the literature about the developmental origin of the epithelium of the major SGs; while it is accepted that major SGs are primarily derived from the oral epithelium, it is unclear 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 (Avery, 2002; Hisatomi et al., 2004). During oral cavity development, a transient formation begins, that initially defines the boundaries of the ectoderm and endoderm and furthermore it separates the oral cavity from the cavity of the primordial pharynx (Patel and Hoffman, 2014), but the exact position of this formation as compared to sites of SGs initiation remains to be clarified. Using the genetic Cre-loxP system, in which expression of Cre-recombinase in neural crest cells genetically enables the expression of a Cre-reporter allele, to permanently mark neural crest-derived cells, the fate of neural crest cells has been determined (Debbache et al., 2018), demonstrating that the mesenchyme and nerves in the SGs are neural crest in origin as shown by lineage tracing with Wnt1-cre (Jaskoll et al., 2002). However, many authors agree that the parotid is ectodermal, whereas the SMG and sublingual are endodermal (Avery, 2002). The endoderm origin was supported by data showing that adult SGs progenitors can differentiate into pancreatic β-cells and hepatocytes when transplanted into hepatectomized liver (Hisatomi et al., 2004), even if there is no evidence to prove 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 SGs are not of endoderm origin, suggesting an ectodermal lineage (Rothova et al., 2012). In addition, animal models and human mutations that cause ectodermal dysplasia, developmental syndromes that specifically affect ectodermal organs, suggest that the major SGs arise from common multipotent precursors residing in the embryonic ectoderm (Jaskoll et al., 2003; Thesleff and Mikkola, 2002).

The SMG placode is visible as a localized thickening of the oral epithelium adjacent to the tongue around at embryonic day (ED) 11,5 of development, known as the. prebud stage (Tucker, 2007). Migrating neural crest cells coalesce adjacent to the salivary placode. These neural crest-derived mesenchymal cells contain Schwann

cell precursors that migrate along nerves, differentiate into neurons, and coalesce within their target tissue to form parasympathetic ganglia (Knosp et al., 2015). By ED12, the salivary proof enlarges and invaginates into the underlying mesenchyme which begins to condense leading to the formation of a primary bud; a duct secures the link to the oral surface and this duct will become the major secretory duct. By ED13, known as the pseudoglandular stage, the final part of the bud grows in size and undergoes a process of cluster formations resulting in ramification of the SMG. At this point, the epithelium is characterized by a high level of proliferation unlike the mesenchyme which shows a relatively low grade of proliferation in all stages of gland development (Tucker, 2007). At ED13.5 the epithelium begins a process termed branching morphogenesis. These buds continue branching producing a multi-lobed gland by ED14.5. Lumen formation of the primary duct occurs by ED13.5, while lumenization of the secondary and tertiary ducts starts after ED14, and end bud lumenization occurs by ED15. The majority of the ducts develop lumen at the canalicular stage, from about ED15.5. After ED15.5, the polarized end buds begin secretory cytodifferentiation, while the cells locate around at the lumens are undergoing apoptosis. Around ED17.5, the branches and terminal buds are delved to form the ductal and acinar system and at this point, the terminal bud stage is completed and exhibits distinct lumina and presumptive ducts (Melnick and Jaskoll, 2000). SGs development carry on after birth with the final differentiation of the granular convoluted tubules until at puberty (Gresik et al., 2009). By 13-16 weeks in humans, the SMG appears well differentiated, and continue to develop up to 28 weeks, at which stage secretory products can be seen in acini. At birth the glands are functional to secrete saliva (Holmberg and Hoffman, 2014), (Figure 2).

Signalling mechanisms controlling SGs morphogenesis

The SGs development is a progressive process involving complex multiple reciprocal interactions between epithelial and its surrounding mesenchyme. The recent literature reports that a series of cross talk between mesenchyme and epithelium drive the migrating neural crest cells to control placode initiation in mice SGs. Multiple molecules, including components of the extracellular matrix, cell adhesion receptors, proteases, and growth factors, mediate these instructive interactions crucial to govern organ branching by providing structural integrity and regulating cell shape, cell motility and cell growth (Jaskoll & Melnick, 1999). Different experimental studies conducted on SMG, demonstrated that SMGs, as well as lung and the mammary glands, are formed during embryonic development by epithelial branching, which establishes the architecture of these organs (Patel et al. 2006). Branching involves repetitive formation of epithelial clefts and buds that invade surrounding embryonic ECM, which changes in composition and distribution over time. In these sequential events, the mesenchyme and mesenchyme-secreted factors control the glandular pattern formation and the branching of the glands (Patel et al. 2006). The extracellular matrix, through integrin engagement, collaborates with growth factors in cell signaling and, as clearly demonstrated, the EGF system acts as key regulator of development of mouse SMG and $\alpha 6$ integrin expression is coordinated by the level of EGF, which in turn control the interactions between epithelial cells and the extracellular

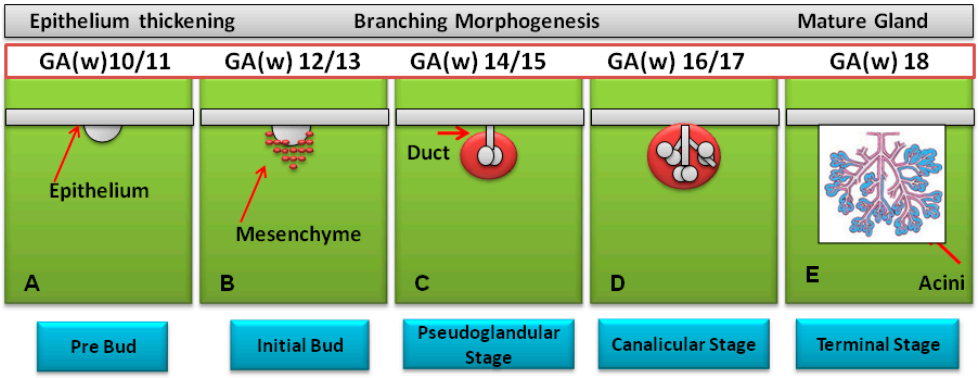

Figure 2. Embryonic branching morphogenesis of human salivary glands (GA: gestional age; W: week).

matrix (Kashimata & Gresik, 1997). The EGFR is strongly expressed in developing ducts and EGF can act as ligand (Gresik, 1997). In the EGFR mutant mice, the SGs have a substantial reduction of number of terminal buds indicating that the EGF-EGFR ligand-receptor system is fundamental for physiological SMG development (Jaskoll & Melnick, 1999). Advanced genetic studies have demonstrated that branching morphogenesis appears to be controlled by molecular conserved regulators, including FGF family. Indeed, the FGF/FGFR system has an essential key role for the development processes branching morphogenesis of the SGs (Hoffman et al., 2002), as demonstrated, for example, from the evidence that FGF/FGFR transgenic mice display altered MSG phenotype (De Moerlooze et al., 2000; Ohuchi et al., 2000; Ornitz and Itoh, 2001; Jaskoll et al., 2004).Therefore, FGFR cleavage seems to be increased by MMPs activity allowing localized spread of the epithelium at sites where proliferation occurs (Simian et al., 2001). These interesting results suggest that FGFR pathway involves a regulatory network that triggers bud formation and duct elongation during branching morphogenesis (Steinberg et al., 2005). In human patients, mutations in FGF/FGFR pathway are linked with aplasia of the SGs demonstrating that the normal development of the glands depends on balance of signalling triggered by this system (Shams et al., 2007). The critical role of BMPs (2, 4, 7) to control initial stages of embryonic SMG branching morphogenesis was also reported by innovative studies. In particular, BMP7 mutant mice exhibit an altered phenotype, the mesenchymal tissue of the SGs is disorganized with reduced branching and lumen formation (Jaskoll et al., 2002). The TNF/TNF-R1 signal transduction represents another widely studied pathway playing a critical role in balancing pro- and anti-apoptotic factors during SMG ducts and acini formation (Melnick et al., 2001c). Results obtained derived from the study of a genetic disease known as hypohidrotic ectodermal dysplasia caused by mutations in ectodysplasin (EDA) gene (Kere et al., 1996; Mikkola, 2008). EDA and its receptor EDAR are members of the TNF superfamily critically involved during teeth, hair and sweat glands development (Srivastawa et al., 1997; Monreal et al., 1998) and in the EDA knockout gene mice, a loss or reduction in lumen formation is evident (Kere et al., 1996; Mikkola, 2008). Indeed, EDA and EDAR mutant mice have hypoplastic and dysplastic glands

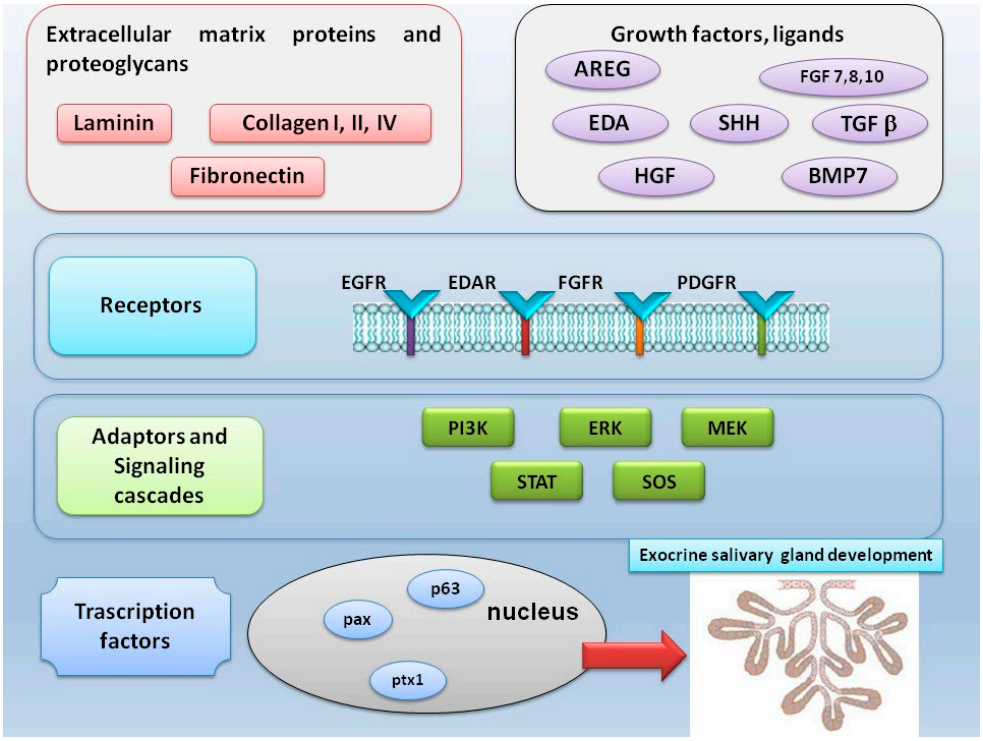

Figure 3. Representation of the best known molecular regulatory mechanisms of branching morphogenesis in human salivary glands.

like as lack lumens and acini (Melnick et al., 2009); in addition, when EDA recombinant is added to SMG organotypic cultures branching is increased, while soluble form of EDAR supplemented in embryonic SMG cultures abrogates EDA/EDAR signalling resulting in a significant decrease in branching morphogenesis (Mikkola, 2008; Melnick et al., 2009). Further studies focusing on the effects of signals through EGFR on *in vitro* differentiation recognize EGFR as a critical regulator during the final stages of the SMG development, when the EGF/TGFα/EGFR pathway was activated that controls the rate of branching and histodifferentiation and progression from the canalicular stage to the terminal bud stage. The increased expression of TGFα and EGFR suggests the importance of this signalling pathway during the development of the terminal bud stage (Melnick et al., 2000). It is clear that, while many details regarding cell physiology of adult acinar and ductal SGs cells have been identified, further studies are required to investigate new aspects of the SGs human developmental process providing new methods to interpret glandular health and disease A scheme of signalling pathways involved in SGs development is represented in Figure 3.

Conclusion

SGs development requires the interaction of multiple cell types including epithelial, mesenchymal, endothelial and neuronal cells and the coordination of many signalling pathways to direct the cell shape changes, cell movements, and cell-cell interactions. Although much progress has been made in the past several years, we remain in the early stages of the understanding of the specific molecular pathways that mediate the development of the SGs. This review is not exhaustive and there is still much to learn but our hope is that a better understanding of molecular development pathways will inform efforts to provides a template for regenerating, repairing or reengineering diseased or damaged adult human SGs.

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Disclosure

The authors declare that they have no conflict of interests.

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