Chapter 2

Epithelial Development Based on a Branching Morphogenesis Program: The Special Condition of Thymic Epithelium

Juan José Muñoz and Agustín G. Zapata

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.81193

Abstract

Numerous epithelia undergo tubulogenesis and branching morphogenesis during their development (i.e., lung, salivary gland, pancreas) in order to establish sufficient available surface for their proper functioning. The thymus is a primary lymphoid organ constituted by pharyngeal-derived epithelium necessary to produce immunocompetent lymphocytes whose mechanisms of development are not fully known. In the current chapter, we review histological, cellular, and molecular mechanisms governing early thymic epithelium development emphasizing its resemblance with the process of branching morphogenesis and tubulogenesis occurring in other epithelial organs in which epithelial-mesenchyme interactions determine the tissue patterning through specific combinations of common molecular signaling pathways.

Keywords: branching morphogenesis, epithelium, tubulogenesis, thymus, thymic epithelium

1. Introduction

Many epithelia, particularly those derived from the gut, organize tubular structures (i.e., mammary gland, salivary glands, lungs, kidneys, pancreas) that repeatedly fold to reach an enlarged area necessary to perform their major functions (i.e., gas exchange, excretion, nutrient transport, etc.). Whereas a branching morphogenesis pattern of development is well established in the case of the respiratory system or in the exocrine glands, it appears to be less evident for other endoderm-derived organs, such as the endocrine glands or the lymphoid organs.

In the present chapter, we will examine prior studies supporting the claim that the development of other branching organs as lung, salivary gland, pancreas, or kidney, despite their morphological and functional differences, follows common patterning programs under the control of epithelium-underlying mesenchyme interactions governed by a few families of molecules (FGF/FGFR, Wnt, BMP/TGFβ, Shh), and that the thymus, an epithelial primary lymphoid organ derived from the ventral endoderm of the third pharyngeal pouch, despite following the same pattern, constitutes a special case. Remarkably, its functions are not related to those of other epithelia of similar origin but rather to the establishment of a 3D epithelial network necessary for the functional maturation of thymocytes. Before acquiring their specific features, distinct epithelial organs, therefore, follow a common complex pattern of development which includes different processes. After a first step of specification from the original embryonic layer, they undergo a process of **tubulogenesis** consisting of outgrowth and extension of the epithelial primordium forming a tubular structure. A complex program of **branching morphogenesis helps to** increase the functional area of the organ. Finally, **terminal epithelial differentiation** prepares the primordium to become a functional organ.

2. The early development of endodermal epithelial organs

2.1. Specification and primordium development

The process of development followed by these epithelial tissues is well exemplified by the early development of salivary glands. The primordium of submandibular glands raises from an evaginated thickening of ectoderm-derived oral epithelium into the neural crest-derived mesenchyme at the base of tongue [1]. The evaginated epithelium proliferates forming an epithelial "stalk" and a terminal bud. The stalk will evolve into excretory duct cells, and the buds will establish the named "pseudoglandular" area by repeated elongation and branching morphogenesis, which will finally differentiate into functional acini (**Figure 1**) [2].

In mice, mammary placodes are visible at E11-E12 and become buds at E13 when surrounded by several layers of mesenchyme. Signals from the mesenchyme of cardia and septum transversum determine the hepatic fate in the foregut endoderm, [3] inducing expression of the transcription factor Hhex, but not Pdx1, whereas the Hhex-Pdx1+ foregut endoderm will differentiate into the extrahepatic bile ducts and the ventral pancreas [4]. Apart from this ventral area, the embryonic pancreas in vertebrates forms from a dorsal protrusion of the primitive gut epithelium, which express Mnx1 [5]. These two pancreatic buds grow, branch, and fuse to form a multilayered epithelium (E9.0 to E11.5), which forms the definitive pancreas. This stratified epithelium consists of two domains: an outer layer of semipolarized "cap" cells, which express only basal markers, and an inner "body" of nonpolarized cells [6, 7] (**Figure 1**).

At E9.0-E9.5, Nkx2–1, a transcription factor specific of the lung, is determined on the ventral side of the anterior foregut by Wnt ligands expressed in the surrounding ventral mesoderm that activates the canonical Wnt pathway in the epithelium. One day later, Nkx2–1+ cells extend ventrally forming a primitive trachea and two lung buds, whereas Sox 2 expression restricted on the distal foregut endoderm will determine the esophagus. Next, the trachea and the esophagus become fully separated [8] (**Figure 1**). Thus, absence of Wnt signaling

http://dx.doi.org/10.5772/intechopen.81193

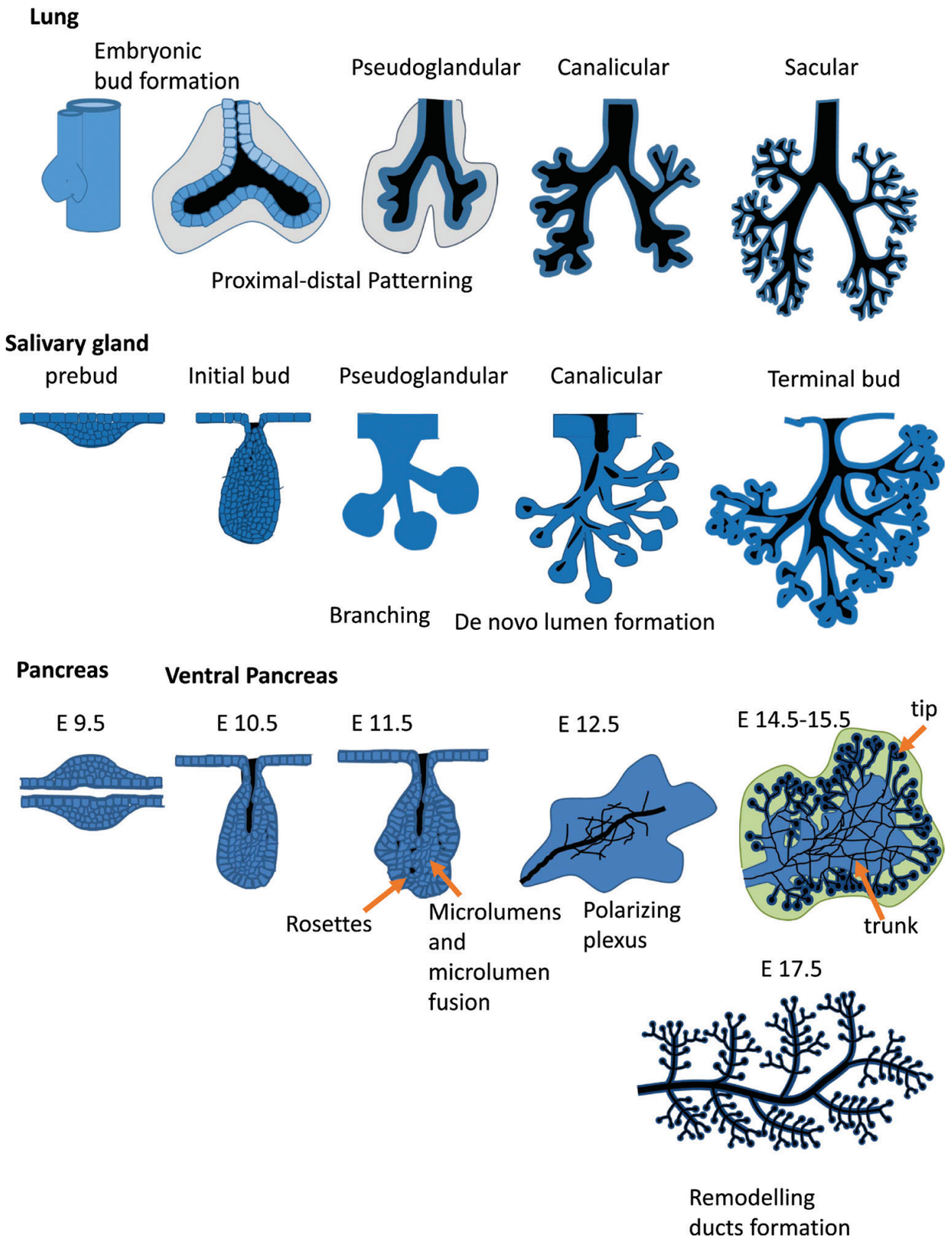

Figure 1. Different models of branching morphogenesis occurring during development of lung (branching of tubes), salivary gland (branching of an unpolarized primordium and later de novo lumen formation), and pancreas (polarization and remodeling of an unpolarized mass resulting in more synchronous branching, lumen formation, and differentiation). Modified from [5, 6, 140].

courses with lack of Nkx2–1 expression, absence of tracheal morphogenesis, and lung agenesis [9]. BMP expression in the ventral mesoderm is necessary to establish a proper location of the lung along the proximal distal axis of the foregut [8]. Also, FGF molecules expressed in the ventral mesoderm reinforce early lung specification of the foregut endoderm [8]. Murine embryos deficient in either FGF10 or FGFR2b exhibit a stopped salivary gland development at the initial bud stage [10], and conditioned deletion of FGF8 in the ectoderm results in arrest of salivary gland development [11]. The process could be more complex because specific overexpression of FGF7 in the salivary gland epithelium produces small glands that exhibit delayed differentiation [12], and elimination of FGF signaling antagonists, Sprouty 1 and 2, impairs salivary epithelium development [13]. Indeed, multiple branching organs undergo agenesis after deletion of either FGF10 or its receptor FGFR2b [10].

2.2. Tubulogenesis

Both mono- and pluristratified epithelia have the capacity to fold and form tubes [14]. Distinct mechanisms of cellular binding have been reported, including the orientation of cells via cell-to-cell and cell-ECM interactions, the establishment of apical-basal polarity, changes in the cellular shape and migration capacities, and formation and expansion of the luminal spaces, which eventually fuse establishing a unique cavity [14, 15].

Although some tubules show lumens surrounded by a single cell, they normally consist of multicellular lumens sealed by cell junctions. In addition, tubules may branch (see later) and/or differentiate into end buds or cap-like structures, as acini (pancreas, mammary, and salivary glands) or alveoli (lung) [16] (**Figure 1**). Further variability in the process of tubulogenesis is provided by the distinct mechanisms used for the formation of lumens. Budding, wrapping, entrapment, cavitation, and hollowing have been described in organs, which undergo tubulogenesis during their development. Budding or wrapping occur in polarized epithelia, as is the case of the lung, whereas the formation of tubules by entrapment, cavitation, or hollowing is performed by nonpolarized cells [16]. In the entrapment, migrating cells trap an extracellular space and form a lumen [17]. By contrast, the formation of lumens by cavitation, reported in mammary and salivary glands, implies programmed cell death to create a cell-free space [18], whereas in the hollowing, the luminal space is organized de novo via exocytosis of intracellular vesicles [19]. The salivary glands, the liver, or the pancreas undergo polarization from unpolarized primordia (**Figure 1**). In the pancreas, E10.5–11.5 individual cells within the inner body of pancreatic buds acquire apico-basal polarity and rearrange to form microlumina by fusion of apical membrane-containing vesicles with the cell membrane. During this process, the asynchronous apical constriction of individual polarized cells generates rosettes with a central lumen that later expand and eventually fuse to generate an immature, highly interconnected tubular plexus, consisting of stratified epithelial cells surrounded by an epithelial periphery [7]. Their reorganization will form the ductal system and primordial endocrine islets and the acinar exocrine cells, respectively (**Figure 1**) [5, 6].

In the salivary gland, lumen formation takes place and evolves along the forming branched structure, following branching progression. Initially, epithelial cell polarization results in multiple microlumens that fuse to form a contiguous lumen [20] (**Figure 1**). The signaling events controlling microlumen fusion to establish a common single lumen are just beginning to emerge [15].

As above indicated, the establishment of cell polarity, in which cues provided by neighboring cells and ECM play major roles, has particular relevance for epithelial tubulogenesis. These cues activate signaling pathways, particularly those mediated by Wnt ligands and their receptors [15], which modify the cytoskeleton, cell contractibility, and trafficking, as well as the transcription program. Wnt ligands and receptors arrange in the epithelial cells in a polarized manner. Wnt5a and 3a are released specifically throughout the basolateral cell surface, where their specific receptors, Fz2, LRP6, and Rer2 are expressed [21]. In the embryonic midgut, Wnt5a is produced by mesenchyme cells under the basement membrane and it activates Wnt5a receptor (Fz2, Rer2) on the basolateral domains of epithelial cells, resulting in Rac-dependent adhesion, establishment of apical/basal polarization, formation of cell junctions, and organization of intracellular molecule trafficking necessary to establish different apical and basal domains.

The developing submandibular gland expresses numerous Wnt ligands and receptors, as well as antagonists in both epithelium and mesenchyme and are accurately regulated spatially and temporally [13]. Wnt signaling promotes duct development by coordinating canonical and noncanonical pathways. Canonical activation through Wnt/β-catenin signaling inhibits end bud formation, whereas Wnt 5b activates the noncanonical Wnt pathway to determine duct formation with the concourse of the transcription factor, TFCP2L1. Inhibition of end bud formation is a consequence of the absence of Wnt distally, regulated by FGF signaling that represses Wnt5b expression and upregulates the Wnt inhibitor, SFRP1, (secreted related frizzled protein 1), which sequesters Wnt proteins [22].

Likewise, retinoid acid produced by foregut mesenchyme before lung specification [23] signals through retinoid acid receptor B in the mesoderm to regulate FGF expression [24]. Shh signaling in the mesoderm is also a regulator of the initial lung bud outgrowth [25].

Distinct members of the EGF family (EGF, TGFα) and their receptors (EGFR 1, 2, 3) as well as heparin-binding EGF (HBEGF) and neuregulin are differentially expressed in the salivary glandular epithelium and mesenchyme, and the activation of EGF receptors modulates ductal morphogenesis by governing progenitor cell differentiation and expansion [26].

Hedgehog proteins, mainly Shh and their receptors Patched1 and Smoothed, also participate in the organization of a salivary duct and a preacinar end bud (prostate, sebaceous glands, mammary glands, lung) [11], but its effects on these organs are indirect because Hh signals in the mesenchyme, whereas in the salivary gland, the action is directly exerted on duct epithelium [26]. On the other hand, overexpression of Gli-1, one effector of Hh pathway in keratin+ epithelial cells, results in large lumens, duct expansion, and loss of acini [27]. Again, Hh and FGF8 appear to cooperate in these processes. FGF is a Gli3-mediated target of Hh signaling pathway. Both FGF8 and Shh positively upregulate each other [28], and the former rescues defects in salivary gland development produced by cyclopamine, a blocker of Hh signaling [11]. Shh could collaborate with other molecules, such as ectodysplasin [29] or TGFβ [30] in the formation of the salivary gland duct, but results are contradictory.

2.3. Branching morphogenesis

Branching morphogenesis constitutes a developmental program that induces the building of an arborized network, in which new tubules arise from the pre-existing ones by repeated

rounds of sprouting [15]. Two morphological models can be distinguished: de novo branching from the surface of a primordial epithelium or the lateral side of a pre-existing branch (budding) and the splitting of a pre-existing branch tip into several tips (clefting) [31]. Moreover, branching morphogenesis can be stereotypic as occurs in the kidney branches [32] or stochastic, without a defined pattern, as reported in mammary gland or salivary gland [31]. At the cellular level, new branch formation can be driven by collective cell migration, patterned cell proliferation and differential growth, coordinated cell deformation or epithelial folding, and/or cell arrangement and matrix-driven branching [31]. Budding in blood vessels and Drosophila trachea follows an invasive form of collective cell migration, whereas in mammalian epithelial organs (i.e., mammary gland) budding appears to be powered by a noninvasive form of collective cell migration along with cell proliferation [33].

Cell proliferation is related to organ growth, and differential cell proliferation may be related to branched budding [31]. Blocking cell proliferation abolishes budding in cultured mouse lung [34] and mammary gland [33], whereas clefting in salivary gland still proceeds [35] mediated by cytoskeleton and ECM remodeling [36]. Clefting at the branch tip in lung and kidney requires proliferation to enlarge the tip, which deforms and splits [37]. In kidney, other factors contribute because less mesenchyme cells correlate with less branching [38], and studies on the 3D morphology of fetal organs demonstrate that the local geometry of the epithelial buds determine the pattern of branching [32].

In lung, branching involve cytoskeleton-mediated constriction of the apical surface of cells [39], with the concourse of Rho GTPases and the involvement of Wnt-dependent planar cell polarity pathway [15].

ECM elements also play an important role in branching morphogenesis. Thus, fibronectin accumulates at branch point constriction and its block inhibits cleft formation [40]. In addition, the loss of β1 integrin that interacts with fibronectin blocks the branching morphogenesis inducing a multilayered epithelium [41]. Degradation of collagen 1 and collagen 3 reduces cleft formation and, therefore, branching [42], and the blockade of laminin α1 or γ1 inhibits branching in culture [43], whereas laminin α5−/− embryos show reduced branching [44].

In many organs, branching occurs through repetitive clefting and elongation of epithelial end buds at distal ends, but whereas in some of them, such as the pancreas, lumen formation occurs concomitantly with branching [7]; in others (i.e., salivary glands), there is a substantive delay between the two processes [45] (**Figure 1**). In pancreas, lumen formation gives rise to a plexus and, at the same time, the epithelial bud is progressively transformed into a lobulated surface of multiple minor protruding tips interrupted by epithelial ridges. Progressive remodeling of the pancreatic plexus in an outside-in continuous manner, eventually leading to a single-layered epithelial network surrounding a single lumen [6, 46] (**Figure 1**).

In the same manner as previous stages of epithelium development, branching morphogenesis is controlled through epithelial-mesenchyme interactions mediated by a network of signaling pathways that includes largely Wnt, FGF, Shh, and TGFβ/BMP. Mammary glands undergo several processes of branching morphogenesis, associated with their physiological cycle, under control of Wnt signaling [15]. In virgin glands, Wnt2, Wnt5a, and Wnt7b

are strongly expressed but downregulated in pregnancy [47] and overexpression of Wnt 4 increases branching while lacking results in delayed ductal branching [48].

Both canonical and noncanonical Wnt signaling pathways are necessary for lung branching morphogenesis. Reduced canonical Wnt/β-catenin signaling in the pulmonary epithelium causes enlarged bronchioles and reduced epithelial branches and alveoli [49], and conditional deletion of β-catenin or overexpression of Wnt inhibitor Dickkopf 1 severely impairs branching morphogenesis [49, 50] by regulating FGFR2 and BMP4 effects on lung epithelium [50]. BMP4 seems to limit FGF10-mediated lung epithelial outgrowth [51]. Wnt5a appears to be a key for determining the effects of noncanonical Wnt pathway in lung branching morphogenesis. Wnt5a−/− mice exhibit increased formation of peripheral airways [52]. In this case, effects of Wnt5a are mediated through Shh: Wnt5a regulates Shh expression in the lung epithelium and, in turn, Shh regulates FGF10 signaling in the mesenchyme [52].

Several members of the FGF family and their receptors are expressed in the renal stroma whereas FGF stimulation induces the appearance of branched tubular structures [53], and the lack of FGFR2b generates significantly smaller kidneys with reduced branches [54].

FGFR1 and FGFR2 are expressed in pubertal and adult mammary glands, and the specific deletion of FGFR1 in keratin 14+ cells produces a transitional delay of the gland development with reduced ductal outgrowth and branch points [55], whereas the deletion of FGFR2 produces an incomplete branching [56]. In addition, FGF10 directs the early stages of epithelial migration and branching, whereas FGF2 is responsible for epithelial expansion and duct elongation [57].

FGF/FGFR signaling is also a key for generating new branches in the developing lung [58]. FGFR2b, which binds four ligands (FGF1, FGF3, FGF7, and FGF10) detected on mesenchymal cells [58] is largely expressed in the airway epithelium, and FGF signaling in lung is associated with Shh pathway [59]. Activation of FGFR2b on epithelial cells by FGF10 secreted by mesenchyme cells induces Shh expression that creates a negative feedback loop by regulating FGF10 levels [60].

Recently, results on the effects of TGFβ1 have been contradictory. TCFβ1 that accumulates in mesenchyme inhibits the branching by inducing components of ECM [61], but its in vivo elimination does not result in altered branching, perhaps due to the existence of other similar factors as TGFβ2 and TGFβ3 [62].

2.4. Cell differentiation

At the end of the development, specialized epithelial cell types appear and gradually mature. In the case of salivary glands, final differentiation of end buds into secretory acini is followed by further growth and functional differentiation [26] (**Figure 1**).

Complex signaling established between lung epithelium, mesenchyme, ECM and vasculature is essential for normal alveolar space organization. Between E16.5 and 3-5PN, lungs develop at the distal end of branches saccules that finally form alveoli (3-14PN in mice) establishing a proximal-distal polarity in the just formed branches (**Figure 1**). Thus, whereas Sox-2

expressing endoderm progenitors that differentiate into ciliated cells, secretory cells and basal cells concentrate in the proximal zone, pluripotent Sox9/Id2+ progenitor cells that will form types 1 and 2 alveolar cells do so in the distal zone [63].

Pancreatic progenitors simultaneously proliferate and differentiate into the endocrine, ductal and acinar cell lineages. In the E9.5, early primordium, multipotent, unipotent endocrine, and duct-endocrine bipotent precursor cells are present, while a wave of acinar precursor differentiation takes place at the peripheral portion around E11.5–12 as branching morphogenesis initiates and tip differentiation is induced [46] (**Figure 1**). Mesenchymal factors and ECM components increase acinar/tip formation, whereas the interconnection between epithelium and endothelial cells favors trunk development [46].

In both organs, lung and pancreas, notch signaling plays an essential role in the differentiation of distinct cell types. Its chemical inhibition in lung causes expansion of distal progenitor cells and decreased numbers of proximal precursors [64]. On the other hand, during development, increased notch signaling correlates with preferential production of secretory cells versus ciliated and neuroendocrine cells [65]. In addition, activation of Notch in keratin 5+ basal cells promotes secretory cell fate whereas its inhibition favors the differentiation toward ciliated cells [66]. In pancreas, Notch activity regulates tip-trunk patterning. Inducing trunk formation via Nkx6.1 activation and blocking tip fate through Ptf1a repression [6] and regulates the differentiation of Ngn3/Pdx1-positive endocrine progenitors versus Sox9/ Hnf1b-expressing ductal cells from trunk bipotent precursors. The specification, differentiation, and maintenance of acini from tips are regulated mainly by Ptf1a [6, 67].

After branching morphogenesis, there are changes in the epithelial cells and cap mesenchyme cells of the developing kidney [32]. Remarkably, Wnt ligands are asymmetrically distributed in the epithelial branches. Wnt 9d is extensively expressed in the ureteric epithelium but downregulated in the tips where Wnt 11 is expressed. Also, Six 2-expressing cells show zonation in the cap mesenchyme: a slow dividing Six 2^{hi} cell population occurs in the periphery of cap, whereas fast cycling Six 2^{lo} cells are intimately associated with the pretubular aggregate that will govern the nephron formation [32]. Moreover, at the beginning of branching morphogenesis, four Six 2+ cap cells exist for every one of the epithelial tip cells, but during branching, the ratio falls to 2:1 and continues to decrease until the end of nephron formation [32].

3. The development of the thyroid

The condition of endocrine tissues is special because they do not show a ductal system, and the secretion is closely associated with the vascular system. Thyroid fate is induced in the anterior endoderm by the concerted action of FGF2 and BMP4 [68], probably derived from cardiogenic mesoderm [69]. A thyroid initial bud is generated in the midline of the pharyngeal floor under control of Tbx1/FGF8 dependent signals [70]; later it detaches from endoderm, cells proliferate and the primordium bifurcates and grows laterally to generate a bilobulated organ with two lateral thyroid bodies formed by fusion with the paired ultimobranchial bodies (UBB), which provide C cell precursors to the embryonic thyroid [71].

Afterward, at a prefolicular growth stage, the thyroid grows by branching morphogenesis of epithelial cords radiating from the UBB remnant, reminding the pseudoglandular stage of salivary gland before duct generation [72]. Finally, cells polarize locally forming cystic lumens leading to cords of back-to-back connected follicles. This folliculogenesis occurs synchronously, not in a proximal/distal direction and is related to Sox9 expression, which is firstly expressed in some cells in the placode and finally accumulates in the distal portions as in other branching organs, remaining in mature follicular cells [72].

Therefore, thyroid development is equivalent to that of an exocrine gland (i.e., salivary gland) in which the ductal system is not fully differentiated but is regressed, and the endocrine portion detached. An initially forming continuous branching structure polarizes locally leading to isolated cystic lumens and to the generation of isolated follicles [72].

4. The early development of the thymus: phases and involved molecules

Similarly, the thymus follows a pattern of development whose stages resemble the specification, tubulogenesis, and branching morphogenesis previously described. Remarkably, they appear to be regulated by many molecular families reported to be involved in the early development of other branching epithelial organs. Although the thymus development has been profusely studied [73–75], few studies have highlighted its resemblance with a process of tubulogenesis and branching morphogenesis the way we do in this review. As known, thymus development occurs in two steps: an early organogenesis, independent of the transcription factor Foxn1, in which the pharyngeal endoderm is specified to thymus fate and a later organogenesis in which thymic epithelium differentiates and is organized under the control of Foxn1 and the lymphoid progenitor cells that seed the thymic epithelial primordium [76].

4.1. Early thymus development

The first step for the thymic rudiment formation is the segmentation of the posterior pharynx that culminates with the specification of endodermal cells into thymic epithelial cells (TECs) [75]. At these early stages, an inner sheet of endodermal tissue of the third pharyngeal pouch and an outer layer of ectodermal cells of the third branchial cleft contact and fuse [77]. Although pioneer morphological studies pointed out that the thymic epithelium derived from these two embrionary layers [78, 79], further experiments in birds and mice demonstrated that all TECs have an endodermal origin [80, 81]. Moreover, clonal analysis determined the existence of a bipotent common thymic epithelial progenitor cell capable of giving rise to both cortical (c) and medullary (m) TECs [82]. In fact, many of ectodermal cells die in the contact limits with the endoderm and they could just be inductors of thymus tissue or even not contribute to the thymus rudiment [80].

Thymic rudiment appears at E10–11 in mice constituting a simple epithelial structure surrounded by mesenchyme largely derived from the neural crests (NC). Earlier (E9.5), the endoderm evagination has formed a common primordium that expresses Glial cells missing

homolog 2 (Gcm2), the earliest marker of parathyroid, in the anterodorsal domain. In the ventral domain, Foxn1 expression will be detected at E11 [80, 83]. From E 11.5, the common primordium initiates the detachment from the lateral surface of the pharynx through apoptosis [80]. Presumably, NC-derived mesenchyme cells are actively involved in this process because Splotch mutants that lack NC cells show delayed or no pharyngeal detachment of parathyroid/thymus rudiment [84, 85]. Nevertheless, other molecules are also concerned because mutants deficient in either Shh, Pax 9, or Frs 2a also maintain the pharynx connection [86–88]. At E12, the rudiment is totally separated from the pharynx and begins to individualize into two different organs. Then, the lateral thymic lobes descend caudally and medially until the midline, above the heart and behind the sternum. NC-derived mesenchyme as well as BMP4, Ephrin B2, and Hoxa3 are involved in the migration of thymic lobes [84, 85, 89].

In the branchial arches, the mesenchyme derives from both mesoderm and neural crests [90], although presumably the role of NC-derived cells is more important [91]. NC-derived mesenchyme contributes to organize the outer connective tissue capsule and interlobular septae of developing thymus [92], but their relevance decreases in the adult thymus where mesenchyme could derive from mesoderm [91]. Accordingly, NC-derived mesenchyme is not required for the initial specification of endoderm but, as in other branching suffering epithelial organ, it is important for thymus development participating in the determination of the third pharyngeal pouches, the establishment of the limits between thymus and parathyroid domains, and the signaling necessary for the separation from common rudiment of the pharyngeal endoderm and later of the parathyroid and thymic lobes [85]. Finally, NC-derived cells are involved in the migration of thymic lobes into the thoracic cavity [93].

4.2. Molecules involved in early thymic development

It is difficult to establish the temporal sequence of functioning of distinct molecules, particularly because any defect in the formation and/or organization of pharyngeal pouches or arches will finally affect the thymus development, even though this development is not regulated directly by it. Furthermore, several molecules act at different stages of the thymus development even exerting opposite effects. Two main systems seem to govern the early thymus development: the one constituted by Hoxa3 and Pax1/9, together with other related molecules, Eya 1 and Six1/4 [73], and Tbx1. Tbx1 is related to human defects in chromosome 22q11.2, responsible of three phenotypes: Di George syndrome (DGS), velocardiofacial syndrome, (SCFS) and conotruncal anomaly face syndrome [94]. Both systems target morphogens of the families FGF, BMP/TGFβ, Shh, and Wnt, which in turn regulate transcription factor activity making it difficult to establish a conclusive picture. As in other branching organs, many of these molecules are regulated by retinoid acid that would diffuse from adjacent NC-derived mesenchyme specifying pharyngeal endoderm [95]. In support of this, treatment with retinoid acid antagonists or mutant deficient in retinoid acid signaling courses with thymus agenesis [95, 96].

4.2.1. The Tbx1 complex

Tbx1 is expressed in the third pharyngeal pouch endoderm and surrounding mesenchyme, and its lack produces thymic hypoplasia and defects in other derivatives of third and fourth

pharyngeal pouches [97]. Apart from retinoid acid, Pbx1, which acts in cooperation with several Hox proteins, regulates Tbx1 expression [98]. Also, BMPs appear to affect Tbx1 indirectly. Mice deficient in Chordin, a BMP antagonist, shows reduced Tbx1 expression in both pharyngeal endoderm and head mesenchyme [99]. In addition, FGF8 expression disappears in the pharyngeal endoderm of these mice, suggesting a relationship between Tbx1 and FGF8. Indeed, the FGF family is a target of Tbx1. The expression of Tbx1and FGF8 overlap within the secondary heart field [100] and Tbx1-deficient mice exhibit reduced FGF8 expression in the pharyngeal endoderm but not in tissue where Tbx1 is not expressed [101]. The lack of FGF8 courses with failure of mesoderm to migrate at the primitive steak and then absence of embryonic endoderm tissue [102]). Therefore, Tbx1 acts downstream of Chordin/BMPs but upstream of FGF8. Thus, specific deletion of FGF8 in Tbx1-expressing cells phenocopies the DG and VCF syndromes [103]. Presumably, FGF8 models pharyngeal arches and pouch-derived structures, additionally affecting survival of pouch endoderm and NC cell migration [104].

Consequently, Tbx1 homozygous mutants show thymus aplasia [94] but indeed, the lack of Tbx1 results in absence of pharyngeal pouches. As a result, the thymus absence seems to be rather a consequence of this defective pouch formation. More recent studies demonstrate that ectopic Tbx1 expression in the ventral third pharyngeal pouch, the domain in which thymic primordium will be formed, suppresses Foxn1 expression and inhibits TEC proliferation and differentiation but does not reverse thymus fate [105]. Moreover, Tbx1 is downregulated in the ventral domain of wt third pharyngeal pouch [98, 101] and ectopic activation of Shh signaling in the third pharyngeal pouch endoderm (see later) induces Tbx1 expression that results in Foxn1 blockade [105]. All these results suggest that actually Tbx1 negatively regulates TEC growth and differentiation and its disappearance from third pharyngeal pouch endoderm is a requisite for proper thymic organization.

4.2.2. The Eya/Hoxa/Pax complexes

There are nine Pax (Paired box) proteins in mammals, subdivided in four groups. Pax 1 and Pax 9, belonging to the same group, and Pax 3 are necessary for early thymus development [88, 106]. In addition, Pax function is closely related to that of Hoxa3, Eya1, and Tbx1, suggesting that they share common signaling pathways or follow parallel, complementary routes [107, 108].

Pax 3 specifies third pharyngeal pouch endoderm to TEC fate [109]. Pax3−/− mice (Splotch mutants), that have severe deficiency of NC cells, organize the thymus and the parathyroid normally but from E11.5 onward a change in the limits of parathyroid/thymus domains produces an enlarged thymus and a small parathyroid. In addition, the common rudiment does not detach from the pharynx [85].

Pax1 appears firstly in the foregut endoderm (E 8.5) and 2 days later in the endoderm of the third pharyngeal pouch remaining in the developing thymus. In the adult thymus, Pax1+ cells are restricted to a small group of cTECs [106]. Pax9 expression follows the same pattern but is also detected in NC-derived mesenchyme [110]. Pax1 mutants exhibit smaller thymic than those of wt mice and contain large cysts accumulating DP thymocytes [106], whereas Pax 9−/− embryos do not fold away from foregut and the thymus rudiment does not move

vetrocaudally remaining in the larynx. Although the primordium is colonized by lymphoid progenitors, it shows decreased proportions of proliferating cells and increased apoptosis finally resulting, as Pax1-deficient thymi, in small thymi [111].

The control exerted by Pax1, perhaps also by Pax 9, and Hoxa3 on early thymus development presumably follows a common pathway [107, 108]. Hoxa 3 is expressed in both endoderm of third pharyngeal pouch and NC-derived mesenchyme [107]. When this expression is downregulated in E10.5–11 Hoxa3−/− mice, the formation of parathyroid/thymus rudiment is blocked, increases the proportion of apoptotic endodermal cells and there is reduced proliferation of mesenchyme cells [112]. More importantly, the expression of both Pax1 and Pax9 decreases in the third pharyngeal pouch of E10,5 Hoxa3-deficient embryos [107], suggesting that Pax 1/9 act downstream of Hoxa3 but all three molecules have synergistic and dose-dependent effects on early thymus maturation [88, 113, 114]. Thus, Hoxa3+/− Pax1+/− double heterozygous mice have a similar phenotype as Pax1−/− mutants, but Hoxa3+/− Pax1−/− hypoplastic thymi exhibit a more severe phenotype than Pax1−/− [114].

Eya1 is involved in the regulation of genes controlling cell growth, activating the repressor Six (Sine oculis). The expression of Eya1, Six, and Pax genes colocalizes in the NC cells and the pharyngeal endoderm [115]. In the absence of Eya1, the third pharyngeal pouch does not detach from the pharyngeal endoderm, and consequently, the thymic primordium is not formed. Foxn1, Pax1, and Pax3 are not expressed in the thymic area, but Hoxa3, Pax1, and Pax3 appear in the E10.5 pouch endoderm [115]. On the other hand, endodermal Six expression is Eya1 dependent, and loss of Six1 in Eya1−/− embryos contributes to the induced thymic defects [116]. Accordingly, Six1 acts downstream of Eya1, whereas Hoxa3, Pax1, and Pax3 do it upstream or independently of Eya 1 [117].

4.2.3. FGF family

FGF is an extensive family of molecules that influences cell survival, proliferation, and differentiation of many epithelial organs, as repeatedly mentioned in this review. FGF8 is expressed in the pouch epithelium, whereas FGF10 is produced by underlying NC-derived mesenchyme both being involved in the maturation of endoderm [104]. After pouch formation, FGF7 and again FGF10, activate FGFR2iiib receptor on fetal TECs for inducing their proliferation. Accordingly, deficient mice either in the receptor or FGF10 show severe thymic hypoplasia and reduced TEC proliferation [118–120]. Likewise, removal of surrounding mesenchyme from E12 fetal thymus inhibits the growth but not the differentiation of epithelial cells [119, 121]. Other studies demonstrate that FGF7 produced by thymic blood vessels also promotes expansion but not differentiation of TECs [118].

4.2.4. Shh

Shh is a promoting factor for parathyroid development via Tbx1 [122], whereas negatively regulating the growth of thymus domain. Consequently, Shh functions as an antagonist of BMP4 signaling [87]. Shh is expressed early in the posterior endoderm of second pouch and then in the third arch endoderm, acting upstream of Tbx1 [123] and affecting the patterning of pharyngeal pouches [77].

4.2.5. BMP family

Particularly relevant is the role played by BMPs and Wnt molecules in the early thymic development, as direct controllers of Foxn1 expression, the key transcription factor mandatory for the late embryogenesis of thymus [124]. In addition, both signaling pathways constitute the major means for NC-derived mesenchyme to signal thymic epithelial rudiment [93, 124, 125]. Possibly, FGF8 produced by the primordial endoderm signals to the adjacent mesenchyme inducing BMP4 expression [126]. BMP4 and its antagonist Noggin govern the parathyroid/thymus individualization and the Foxn1-dependent TEC maturation. In general, BMP4 is essential for the early stages of thymus development prior to the onset of Foxn1 expression [93]. BMP4 is expressed in the ventral domain of the pouch and Noggin in the dorsal area colocalizing with Gcm2 in the parathyroid domain [83]. Furthermore, BMP4 seems to be also involved in the full parathyroid/thymus separation, as BMP4 deletion delays the process [93]. Inhibition of BMP signaling provokes decline of Foxn1 expression in the zebrafish thymic primordium [83, 125], and BMP4 signaling promotes Foxn1 expression in early chicken thymus [126], as well as in mouse FTOCs [127]. Loss of BMP4 from pharyngeal endoderm and underlying mesenchyme prior to the onset of Foxn1 expression does not affect patterning, separation from the pharynx, or initial organ formation, although it alters some important morphogenetic processes such as lumen closure, organ separation and migration, initial lymphoid seeding, and formation of mesenchyme thymic capsule [93]. The sequence established between BMP and Foxn1 is the following: FGF8-mediated mesenchymal BMP signaling initiates the expression of both Foxn1 and BMP4 in the endodermal cells [126]. Then, endodermal BMP4 expression targets a regulatory feedback loop [128] for maintaining BMP4 and Foxn1 expression in the future thymic epithelium rather to directly affect Foxn1 [129]. In these conditions, if BMP signaling is blocked, the expression of both molecules ceases and nonfunctional Foxn1-TECs would remain in the thymus. If this occurs during concrete periods of midgestation, thymopoiesis will irreversibly fail [129]. Therefore, the balance between BMP4 and its inhibitors (i.e., Noggin) becomes critical for a proper maturation of thymic epithelium.

4.2.6. Wnt family

Wnt family members are extensively expressed in developing and adult thymi in both TECs and fibroblasts [130], whereas their receptors are only detected on TECs [124]. Particularly, the noncanonical Wnt4 and Wnt5b, but also the canonical Wnt10b, coexpress with Foxn1 in third pharyngeal pouch and later in E13 and adult thymus [131] and are involved in its control [124]. Thus, overexpression of Dkk1, a Wnt4 inhibitor, in TECs induces thymic atrophy with reduced epithelial progenitors and TEC proliferation and appearance of TEC proliferation [132]. However, recent results indicate that a proper thymus development can only occur when β-catenin-dependent Wnt signaling is low or lacking [133]. Thus, β-catenin-deficient thymi exhibit Foxn1 expression, and stabilized β-catenin overexpression shows decreased rather than increased Foxn1 transcripts [133, 134]. Therefore, these results suggest that β-catenin is dispensable for Foxn1 expression in fetal TECs. Remarkably, during branching morphogenesis of lung and lacrimal glands, Wnt overexpression, stimulated Wnt signaling and conditional overexpression of β-catenin all result in decreased branching morphogenesis [135]. However, it is important to remark that sustained Wnt signaling promotes the production of secreted

Wnt antagonisms [136] that block thymocyte development in FTOCs [137]. On the other hand, other signaling pathways involved in TEC differentiation, such as those mediated by BMPs, modulate the effects produced by Wnt4 overexpression [133].

5. After acquisition of thymus fate, thymic primordium undergoes tubulogenesis and branching morphogenesis

E11.5 thymic primordium consists of a bi/pluristratified epithelium polarized with respect to a ramified central lumen resulting from the evagination of pharyngeal epithelium where K5+ Cld 3/4+ cells line the lumen [138, 139]. In the following days, the thymus grows and the K5+ Cld 3/4 + cell cords increase their total length and branching degree. At the same time, external clefts determine an incipient lobulation that became clearly evident by day E14.5. Beyond E12.5, the initial lumen is almost totally closed although a central lumen is still visible at E12,5 and to some extent at E13,5 [139]. Secondary forming lumens can be observed in the K5 + Cld 3/4+ branching cell cords up to E13.5 (**Figure 2**) [139]. Thus, in these initial steps of thymus development, its histological organization is quite similar to that of organs undergoing branching morphogenesis in which the lumen formation and elongation take place within a proliferating bud (**Figure 2**) [20, 140]. These results indicate that between E11.5 and E13.5, a primary lumen connects with secondary and growing order lumens through branched microlumens or polarized canals, giving rise to a continuous formed or forming luminal structure that grows hierarchically (**Figure 2**). Therefore, in the thymus, clefting/branching and lumen formation seems to be more synchronic and not as regionally separated as in the salivary gland, similar to the pancreas condition (**Figure 2**).

However, in the thymus, a definitive duct is not developed, nor is terminal end buds, acini or other differentiated distal secreting structures, but instead the thymus remains as a concentric structure in which the central Cld + K5+ cells will differentiate into the thymic medulla [138, 139]. This central medulla does not apparently present lumen and is surrounded by the thymic cortex differentiated from Cld 3/4- cells (**Figure 2**) [139]. On the other hand, the fact that the branching pattern of the K5 + Cld 3/4+ cell cords appears to be similar between different mice [139] and that rat adult thymic medulla has also been described as a 4–5 order ramified structure in which the ratio of branches sizes is mathematically constant [141], suggesting that thymus development follows a programmed branching pattern.

If, as above indicated, the branching morphogenesis of the developing thymus has some particular features, as the lack of a definitive duct or terminal end buds or acini, the early lymphoid seeding introduces in the thymus development other important differences with respect to other epithelial organs inducing the specific three-dimensional network formed by dendritic-shaped TECs. Thymocyte precursors enter the thymus at around E11.5 through the surrounding mesenchyme [142], and at E12.5 $CD45^{+}$, lymphoid progenitors appear associated with nonpolarized TECs that express little or no K5 [139]. This 3D arrangement of thymic epithelium is, to some extent, precluded in the absence of thymocytes [143] in which the presence of medullary luminal or cystic structures becomes more evident, presumably representing a default pathway of epithelial differentiation when thymocytes are missing [143].

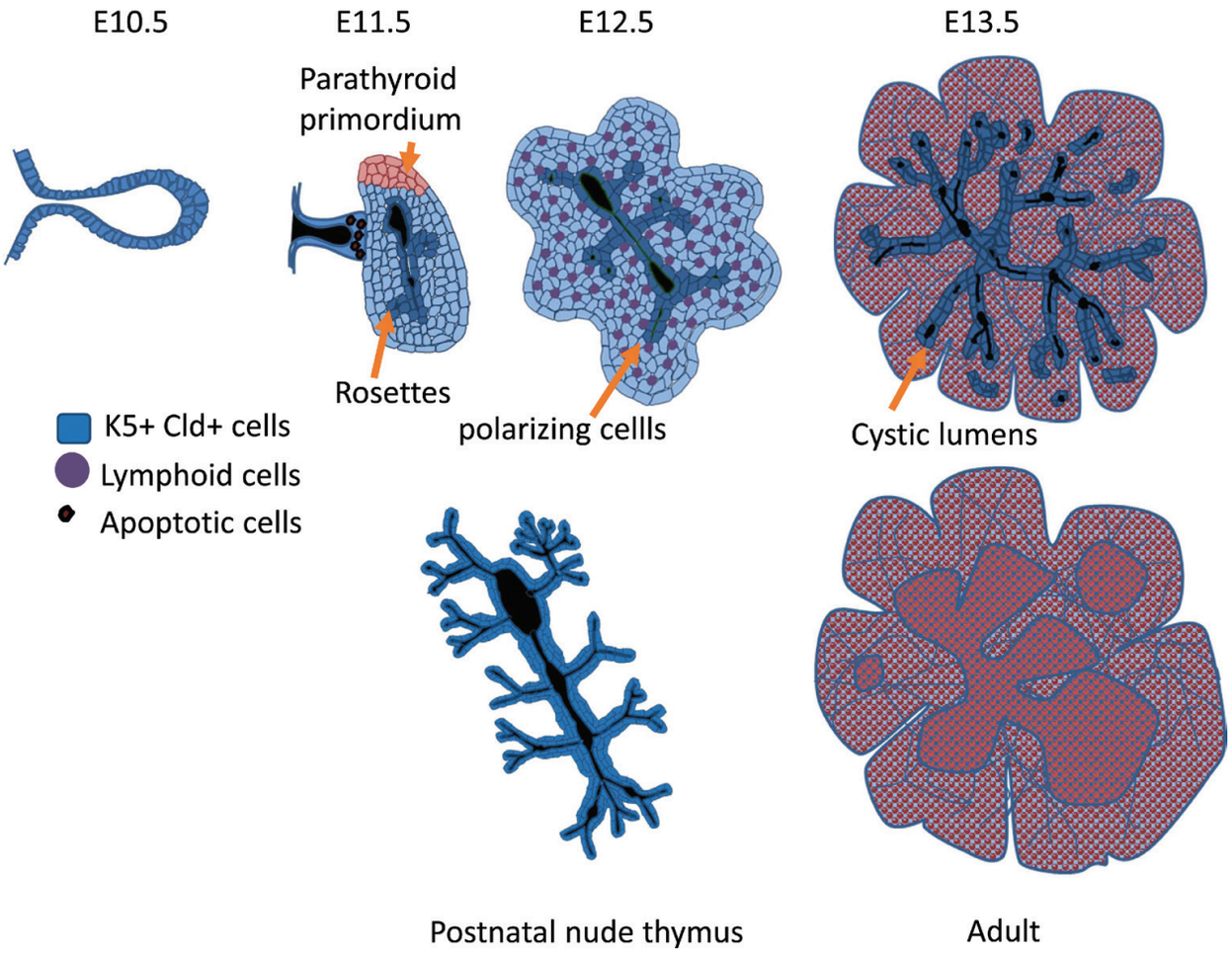

Figure 2. Thymus development follows a branching morphogenesis process similar to those of salivary gland and pancreas (see **Figure 1**).

On the other hand, in the absence of Foxn1, the existence of a tubular-branched structure in which both ductal and acinar components can be distinguished [144] and that cannot be colonized by lymphoid progenitors [76] is clearly evident. This is the situation of Nude (Foxn1−/−) thymi, in which the transcription factor Foxn1 central for thymic epithelial differentiation, lacks. The earliest stages of Foxn1−/− thymus development appear to occur in the same way as those of wt thymus, and the expression of claudin 3/4 and wt thymus takes place in similar ways [145], suggesting that wt thymus organogenesis might be considered as a modification of the tubulogenesis and epithelial branching morphogenesis, which occur in the nude thymus (**Figure 2**). Thus, FoxN1 expression would preclude lumen formation and generate concentric layers of distinct TEC subsets (Muñoz et al., 2018 submitted). Moreover, the conditioned removal of FoxN1 in K14+ epithelial cells results in the progressive polarization of medullary cells, Cld3/4 expression, and lumen formation [146]. Other defects affect mainly thymic branching morphogenesis without importantly altering thymic-specific differentiation. Transgenic expression of Noggin under the control of a FoxN1 promoter leads to a hypoplastic spheric thymus always containing big cystic structures [125]. These structural alterations seem to be the result of a branching defect in consonance with the known role of BMP signaling in regulating branching morphogenesis of different organs [26] and to affect Foxn1 expression [147].

Acknowledgements

This work was supported by grants BFU2013-41112-R from the Spanish Ministry of Economy and Competitiveness and Cell Therapy Network (RD12/0019/0007) from the Spanish Ministry of Health and Consume and Avancell-CM (S2017/BMD-3692) from Community of Madrid.

Conflict of interest

Authors declare no competing or financial interests.

Author details

Juan José Muñoz[1] and Agustín G. Zapata[1,2]*

*Address all correspondence to: zapata@ucm.es

1 Center for Cytometry and Fluorescence Microscopy, Complutense University of Madrid, Madrid, Spain

2 Department of Cell Biology, Complutense University of Madrid, Madrid, Spain

References

[1] Knosp WM, Knox SM, Hoffman MP. Salivary gland organogenesis. Wiley Interdisciplinary Reviews: Developmental Biology. 2012;**1**:69-82. DOI: 10.1002/wdev.4

[2] Harunaga J, Hsu JC, Yamada KM. Dynamics of salivary gland morphogenesis. Journal of Dental Research. 2011;**90**:1070-1077. DOI: 10.1177/0022034511405330

[3] Ober EA, Verkade H, Field HA, Stainier DY. Mesodermal Wnt2b signalling positively regulates liver specification. Nature. 2006;**442**:688-691. DOI: 10.1038/nature04888

[4] Bort R, Signore M, Tremblay K, Martinez Barbera JP, Zaret KS. Hex homeobox gene controls the transition of the endoderm to a pseudostratified, cell emergent epithelium for liver bud development. Developmental Biology. 2006;**290**:44-56. DOI: 10.1016/j.ydbio.2005.11.006

[5] Larsen HL, Grapin-Botton A. The molecular and morphogenetic basis of pancreas organogenesis. Seminars in Cell & Developmental Biology. 2017;**66**:51-68. DOI: 10.1016/j.semcdb.2017.01.005

[6] Bastidas-Ponce A, Scheibner K, Lickert H, Bakhti M. Cellular and molecular mechanisms coordinating pancreas development. Development. 2017;**144**:2873-2888. DOI: 10.1242/dev.140756

[7] Villasenor A, Chong DC, Henkemeyer M, Cleaver O. Epithelial dynamics of pancreatic branching morphogenesis. Development. 2010;**137**:4295-4305. DOI: 10.1242/dev.052993

[8] Swarr DT, Morrisey EE. Lung endoderm morphogenesis: Gasping for form and function. Annual Review of Cell and Developmental Biology. 2015;**31**:553-573. DOI: 10.1146/annurev-cellbio-100814-125249

[9] Harris-Johnson KS, Domyan ET, Vezina CM. Sun X: Beta-catenin promotes respiratory progenitor identity in mouse foregut. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**:16287-16292. DOI: 10.1073/pnas.0902274106

[10] De Moerlooze L, Spencer-Dene B, Revest JM, Hajihosseini M, Rosewell I, Dickson C. An important role for the IIIb isoform of fibroblast growth factor receptor 2 (FGFR2) in mesenchymal-epithelial signalling during mouse organogenesis. Development. 2000; **127**:483-492

[11] Jaskoll T, Witcher D, Toreno L, Bringas P, Moon AM, Melnick M. FGF8 dose-dependent regulation of embryonic submandibular salivary gland morphogenesis. Developmental Biology. 2004;**268**:457-469. DOI: 10.1016/j.ydbio.2004.01.004

[12] Guo L, Yu QC, Fuchs E. Targeting expression of keratinocyte growth factor to keratinocytes elicits striking changes in epithelial differentiation in transgenic mice. The EMBO Journal. 1993;**12**:973-986

[13] Knosp WM, Knox SM, Lombaert IM, Haddox CL, Patel VN, Hoffman MP. Submandibular parasympathetic gangliogenesis requires sprouty-dependent Wnt signals from epithelial progenitors. Developmental Cell. 2015;**32**:667-677. DOI: 10.1016/j.devcel.2015.01.023

[14] Pearl EJ, Li J, Green JB. Cellular systems for epithelial invagination. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 2017;**372**. DOI: 10.1098/rstb.2015.0526

[15] Bernascone I, Hachimi M, Martin-Belmonte F. Signaling networks in epithelial tube formation. Cold Spring Harbor Perspectives in Biology. 2017;**9**. DOI: 10.1101/cshperspect.a027946

[16] Marciano DK. A holey pursuit: Lumen formation in the developing kidney. Pediatric Nephrology. 2017;**32**:7-20. DOI: 10.1007/s00467-016-3326-4

[17] Santiago-Martinez E, Soplop NH, Patel R, Kramer SG. Repulsion by slit and roundabout prevents shotgun/E-cadherin-mediated cell adhesion during Drosophila heart tube lumen formation. The Journal of Cell Biology. 2008;**182**:241-248. DOI: 10.1083/jcb.200804120

[18] Mailleux AA, Overholtzer M, Brugge JS. Lumen formation during mammary epithelial morphogenesis: Insights from in vitro and in vivo models. Cell Cycle. 2008;**7**:57-62. DOI: 10.4161/cc.7.1.5150

[19] Datta A, Bryant DM, Mostov KE. Molecular regulation of lumen morphogenesis. Current Biology. 2011;**21**:R126-R136. DOI: 10.1016/j.cub.2010.12.003

[20] Walker JL, Menko AS, Khalil S, Rebustini I, Hoffman MP, Kreidberg JA, et al. Diverse roles of E-cadherin in the morphogenesis of the submandibular gland: Insights into the formation of acinar and ductal structures. Developmental Dynamics. 2008;**237**:3128-3141. DOI: 10.1002/dvdy.21717

[21] Yamamoto H, Awada C, Hanaki H, Sakane H, Tsujimoto I, Takahashi Y, et al. The apical and basolateral secretion of Wnt11 and Wnt3a in polarized epithelial cells is regulated by different mechanisms. Journal of Cell Science. 2013;**126**:2931-2943. DOI: 10.1242/jcs.126052

[22] Yamaguchi Y, Yonemura S, Takada S. Grainyhead-related transcription factor is required for duct maturation in the salivary gland and the kidney of the mouse. Development. 2006;**133**:4737-4748. DOI: 10.1242/dev.02658

[23] Malpel S, Mendelsohn C, Cardoso WV. Regulation of retinoic acid signaling during lung morphogenesis. Development. 2000;**127**:3057-3067

[24] Desai TJ, Malpel S, Flentke GR, Smith SM, Cardoso WV. Retinoic acid selectively regulates Fgf10 expression and maintains cell identity in the prospective lung field of the developing foregut. Developmental Biology. 2004;**273**:402-415. DOI: 10.1016/j.ydbio.2004.04.039

[25] Motoyama J, Liu J, Mo R, Ding Q, Post M, Hui CC. Essential function of Gli2 and Gli3 in the formation of lung, trachea and oesophagus. Nature Genetics. 1998;**20**:54-57. DOI: 10.1038/1711

[26] Mattingly A, Finley JK, Knox SM. Salivary gland development and disease. Wiley Interdisciplinary Reviews: Developmental Biology. 2015;**4**:573-590. DOI: 10.1002/wdev.194

[27] Fiaschi M, Kolterud A, Nilsson M, Toftgard R, Rozell B. Targeted expression of GLI1 in the salivary glands results in an altered differentiation program and hyperplasia. The American Journal of Pathology. 2011;**179**:2569-2579. DOI: 10.1016/j.ajpath.2011.07.033

[28] Aoto K, Nishimura T, Eto K, Motoyama J. Mouse GLI3 regulates Fgf8 expression and apoptosis in the developing neural tube, face, and limb bud. Developmental Biology. 2002;**251**:320-332

[29] Pummila M, Fliniaux I, Jaatinen R, James MJ, Laurikkala J, Schneider P, et al. Ectodysplasin has a dual role in ectodermal organogenesis: Inhibition of bmp activity and induction of Shh expression. Development. 2007;**134**:117-125. DOI: 10.1242/dev.02708

[30] Hall BE, Zheng C, Swaim WD, Cho A, Nagineni CN, Eckhaus MA, et al. Conditional overexpression of TGF-beta1 disrupts mouse salivary gland development and function. Laboratory Investigation. 2010;**90**:543-555. DOI: 10.1038/labinvest.2010.5

[31] Wang S, Sekiguchi R, Daley WP, Yamada KM. Patterned cell and matrix dynamics in branching morphogenesis. The Journal of Cell Biology. 2017;**216**:559-570. DOI: 10.1083/jcb.201610048

[32] Short KM, Smyth IM. The contribution of branching morphogenesis to kidney development and disease. Nature Reviews. Nephrology. 2016;**12**:754-767. DOI: 10.1038/nrneph.2016.157

[33] Ewald AJ, Brenot A, Duong M, Chan BS, Werb Z. Collective epithelial migration and cell rearrangements drive mammary branching morphogenesis. Developmental Cell. 2008;**14**:570-581. DOI: 10.1016/j.devcel.2008.03.003

[34] Goldin GV, Hindman HM, Wessells NK. The role of cell proliferation and cellular shape change in branching morphogenesis of the embryonic mouse lung: Analysis using aphidicolin and cytochalasins. The Journal of Experimental Zoology. 1984;**232**:287-296. DOI: 10.1002/jez.1402320216

[35] Nakanishi Y, Morita T, Nogawa H. Cell proliferation is not required for the initiation of early cleft formation in mouse embryonic submandibular epithelium in vitro. Development. 1987;**99**:429-437

[36] Daley WP, Yamada KM. ECM-modulated cellular dynamics as a driving force for tissue morphogenesis. Current Opinion in Genetics & Development. 2013;**23**:408-414. DOI: 10.1016/j.gde.2013.05.005

[37] Schnatwinkel C, Niswander L. Multiparametric image analysis of lung-branching morphogenesis. Developmental Dynamics. 2013;**242**:622-637. DOI: 10.1002/dvdy.23961

[38] Cebrian C, Asai N, D'Agati V, Costantini F. The number of fetal nephron progenitor cells limits ureteric branching and adult nephron endowment. Cell Reports. 2014;**7**:127-137. DOI: 10.1016/j.celrep.2014.02.033

[39] Kim HY, Varner VD, Nelson CM. Apical constriction initiates new bud formation during monopodial branching of the embryonic chicken lung. Development. 2013;**140**:3146-3155. DOI: 10.1242/dev.093682

[40] Sakai T, Larsen M, Yamada KM. Fibronectin requirement in branching morphogenesis. Nature. 2003;**423**:876-881. DOI: 10.1038/nature01712

[41] Chen J, Krasnow MA. Integrin Beta 1 suppresses multilayering of a simple epithelium. PLoS One. 2012;**7**:e52886. DOI: 10.1371/journal.pone.0052886

[42] Grobstein C, Cohen J. Collagenase: Effect on the morphogenesis of embryonic salivary epithelium in vitro. Science. 1965;**150**:626-628

[43] Kadoya Y, Nomizu M, Sorokin LM, Yamashina S, Yamada Y. Laminin alpha1 chain G domain peptide, RKRLQVQLSIRT, inhibits epithelial branching morphogenesis of cultured embryonic mouse submandibular gland. Developmental Dynamics. 1998;**212**:394-402. DOI: 10.1002/(sici)1097-0177(199807)212:3<394::Aid-aja7>3.0.Co;2-c

[44] Menko AS, Kreidberg JA, Ryan TT, Van Bockstaele E, Kukuruzinska MA. Loss of alpha-3beta1 integrin function results in an altered differentiation program in the mouse submandibular gland. Developmental Dynamics. 2001;**220**:337-349. DOI: 10.1002/dvdy.1114

[45] Andrew DJ, Ewald AJ. Morphogenesis of epithelial tubes: Insights into tube formation, elongation, and elaboration. Developmental Biology. 2010;**341**:34-55. DOI: 10.1016/j.ydbio.2009.09.024

[46] Larsen HL, Martin-Coll L, Nielsen AV, Wright CVE, Trusina A, Kim YH, et al. Stochastic priming and spatial cues orchestrate heterogeneous clonal contribution to mouse pancreas organogenesis. Nature Communications. 2017;**8**:605. DOI: 10.1038/s41467-017-00258-4

[47] Bradbury JM, Edwards PA, Niemeyer CC, Dale TC. Wnt-4 expression induces a pregnancy-like growth pattern in reconstituted mammary glands in virgin mice. Developmental Biology. 1995;**170**:553-563. DOI: 10.1006/dbio.1995.1236

[48] Brisken C, Heineman A, Chavarria T, Elenbaas B, Tan J, Dey SK, et al. Essential function of Wnt-4 in mammary gland development downstream of progesterone signaling. Genes & Development. 2000;**14**:650-654

[49] Mucenski ML, Wert SE, Nation JM, Loudy DE, Huelsken J, Birchmeier W, et al. Beta-catenin is required for specification of proximal/distal cell fate during lung morphogenesis. The Journal of Biological Chemistry. 2003;**278**:40231-40238. DOI: 10.1074/jbc.M305892200

[50] Shu W, Guttentag S, Wang Z, Andl T, Ballard P, Lu MM, et al. Wnt/beta-catenin signaling acts upstream of N-myc, BMP4, and FGF signaling to regulate proximal-distal patterning in the lung. Developmental Biology. 2005;**283**:226-239. DOI: 10.1016/j.ydbio.2005.04.014

[51] Weaver M, Dunn NR, Hogan BL. Bmp4 and Fgf10 play opposing roles during lung bud morphogenesis. Development. 2000;**127**:2695-2704

[52] Li C, Hu L, Xiao J, Chen H, Li JT, Bellusci S, et al. Wnt5a regulates Shh and Fgf10 signaling during lung development. Developmental Biology. 2005;**287**:86-97. DOI: 10.1016/j.ydbio.2005.08.035

[53] Qiao J, Bush KT, Steer DL, Stuart RO, Sakurai H, Wachsman W, et al. Multiple fibroblast growth factors support growth of the ureteric bud but have different effects on branching morphogenesis. Mechanisms of Development. 2001;**109**:123-135

[54] Zhao H, Kegg H, Grady S, Truong HT, Robinson ML, Baum M, et al. Role of fibroblast growth factor receptors 1 and 2 in the ureteric bud. Developmental Biology. 2004;**276**:403-415. DOI: 10.1016/j.ydbio.2004.09.002

[55] Pond AC, Bin X, Batts T, Roarty K, Hilsenbeck S, Rosen JM. Fibroblast growth factor receptor signaling is essential for normal mammary gland development and stem cell function. Stem Cells. 2013;**31**:178-189. DOI: 10.1002/stem.1266

[56] Parsa S, Ramasamy SK, De Langhe S, Gupte VV, Haigh JJ, Medina D, et al. Terminal end bud maintenance in mammary gland is dependent upon FGFR2b signaling. Developmental Biology. 2008;**317**:121-131. DOI: 10.1016/j.ydbio.2008.02.014

[57] Zhang X, Martinez D, Koledova Z, Qiao G, Streuli CH, Lu P. FGF ligands of the postnatal mammary stroma regulate distinct aspects of epithelial morphogenesis. Development. 2014;**141**:3352-3362. DOI: 10.1242/dev.106732

[58] Peters K, Werner S, Liao X, Wert S, Whitsett J, Williams L. Targeted expression of a dominant negative FGF receptor blocks branching morphogenesis and epithelial differentiation of the mouse lung. The EMBO Journal. 1994;**13**:3296-3301

[59] Hirashima T, Iwasa Y, Morishita Y. Distance between AER and ZPA is defined by feed-forward loop and is stabilized by their feedback loop in vertebrate limb bud. Bulletin of Mathematical Biology. 2008;**70**:438-459. DOI: 10.1007/s11538-007-9263-4

[60] Herriges M, Morrisey EE. Lung development: Orchestrating the generation and regeneration of a complex organ. Development. 2014;**141**:502-513. DOI: 10.1242/dev.098186

[61] Zhou L, Dey CR, Wert SE, Whitsett JA. Arrested lung morphogenesis in transgenic mice bearing an SP-C-TGF-beta 1 chimeric gene. Developmental Biology. 1996;**175**:227-238. DOI: 10.1006/dbio.1996.0110

[62] Letterio JJ, Geiser AG, Kulkarni AB, Roche NS, Sporn MB, Roberts AB. Maternal rescue of transforming growth factor-beta 1 null mice. Science. 1994;**264**:1936-1938

[63] Rawlins EL, Clark CP, Xue Y, Hogan BL. The Id2+ distal tip lung epithelium contains individual multipotent embryonic progenitor cells. Development. 2009;**136**:3741-3745. DOI: 10.1242/dev.037317

[64] Tsao PN, Chen F, Izvolsky KI, Walker J, Kukuruzinska MA, Lu J, et al. Gamma-secretase activation of notch signaling regulates the balance of proximal and distal fates in progenitor cells of the developing lung. The Journal of Biological Chemistry. 2008;**283**: 29532-29544. DOI: 10.1074/jbc.M801565200

[65] Tsao PN, Vasconcelos M, Izvolsky KI, Qian J, Lu J, Cardoso WV. Notch signaling controls the balance of ciliated and secretory cell fates in developing airways. Development. 2009;**136**:2297-2307. DOI: 10.1242/dev.034884

[66] Guseh JS, Bores SA, Stanger BZ, Zhou Q, Anderson WJ, Melton DA, et al. Notch signaling promotes airway mucous metaplasia and inhibits alveolar development. Development. 2009;**136**:1751-1759. DOI: 10.1242/dev.029249

[67] Krapp A, Knofler M, Ledermann B, Burki K, Berney C, Zoerkler N, et al. The bHLH protein PTF1-p48 is essential for the formation of the exocrine and the correct spatial organization of the endocrine pancreas. Genes & Development. 1998;**12**:3752-3763

[68] Kurmann AA, Serra M, Hawkins F, Rankin SA, Mori M, Astapova I, et al. Regeneration of thyroid function by transplantation of differentiated pluripotent stem cells. Cell Stem Cell. 2015;**17**:527-542. DOI: 10.1016/j.stem.2015.09.004

[69] Serls AE, Doherty S, Parvatiyar P, Wells JM, Deutsch GH. Different thresholds of fibroblast growth factors pattern the ventral foregut into liver and lung. Development. 2005;**132**:35-47. DOI: 10.1242/dev.01570

[70] Lania G, Zhang Z, Huynh T, Caprio C, Moon AM, Vitelli F, et al. Early thyroid development requires a Tbx1-Fgf8 pathway. Developmental Biology. 2009;**328**:109-117. DOI: 10.1016/j.ydbio.2009.01.014

[71] Johansson E, Andersson L, Ornros J, Carlsson T, Ingeson-Carlsson C, Liang S, et al. Revising the embryonic origin of thyroid C cells in mice and humans. Development. 2015; **142**:3519-3528. DOI: 10.1242/dev.126581

[72] Liang S, Johansson E, Barila G, Altschuler DL, Fagman H, Nilsson M. A branching morphogenesis program governs embryonic growth of the thyroid gland. Development. 2018;**145**. DOI: 10.1242/dev.146829

[73] Gordon J, Manley NR. Mechanisms of thymus organogenesis and morphogenesis. Development. 2011;**138**:3865-3878. DOI: 10.1242/dev.059998

[74] Manley NR, Richie ER, Blackburn CC, Condie BG, Sage J. Structure and function of the thymic microenvironment. Front Bioscience (Landmark Ed). 2011;**16**:2461-2477

[75] Takahama Y, Ohigashi I, Baik S, Anderson G. Generation of diversity in thymic epithelial cells. Nature Reviews. Immunology. 2017;**17**:295-305. DOI: 10.1038/nri.2017.12

[76] Vaidya HJ, Briones Leon A, Blackburn CC. FOXN1 in thymus organogenesis and development. European Journal of Immunology. 2016;**46**:1826-1837. DOI: 10.1002/eji.201545814

[77] Graham A. The development and evolution of the pharyngeal arches. Journal of Anatomy. 2001;**199**:133-141

[78] Cordier AC, Haumont SM. Development of thymus, parathyroids, and ultimo-branchial bodies in NMRI and nude mice. The American Journal of Anatomy. 1980;**157**:227-263. DOI: 10.1002/aja.1001570303

[79] Cordier AC, Heremans JF. Nude mouse embryo: Ectodermal nature of the primordial thymic defect. Scandinavian Journal of Immunology. 1975;**4**:193-196

[80] Gordon J, Wilson VA, Blair NF, Sheridan J, Farley A, Wilson L, et al. Functional evidence for a single endodermal origin for the thymic epithelium. Nature Immunology. 2004;**5**:546-553. DOI: 10.1038/ni1064

[81] Le Douarin NM, Jotereau FV. Tracing of cells of the avian thymus through embryonic life in interspecific chimeras. The Journal of Experimental Medicine. 1975;**142**:17-40

[82] Rossi SW, Jenkinson WE, Anderson G, Jenkinson EJ. Clonal analysis reveals a common progenitor for thymic cortical and medullary epithelium. Nature. 2006;**441**:988-991. DOI: 10.1038/nature04813

[83] Patel SR, Gordon J, Mahbub F, Blackburn CC, Manley NR. Bmp4 and noggin expression during early thymus and parathyroid organogenesis. Gene Expression Patterns. 2006;**6**:794-799. DOI: 10.1016/j.modgep.2006.01.011

[84] Chen L, Zhao P, Wells L, Amemiya CT, Condie BG, Manley NR. Mouse and zebrafish Hoxa3 orthologues have nonequivalent in vivo protein function. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**:10555-10560. DOI: 10.1073/pnas.1005129107

[85] Griffith AV, Cardenas K, Carter C, Gordon J, Iberg A, Engleka K, et al. Increased thymus- and decreased parathyroid-fated organ domains in splotch mutant embryos. Developmental Biology. 2009;**327**:216-227. DOI: 10.1016/j.ydbio.2008.12.019

[86] Kameda Y, Ito M, Nishimaki T, Gotoh N. FRS2alpha is required for the separation, migration, and survival of pharyngeal-endoderm derived organs including thyroid, ultimobranchial body, parathyroid, and thymus. Developmental Dynamics. 2009;**238**:503-513. DOI: 10.1002/dvdy.21867

[87] Moore-Scott BA, Manley NR. Differential expression of sonic hedgehog along the anterior-posterior axis regulates patterning of pharyngeal pouch endoderm and pharyngeal endoderm-derived organs. Developmental Biology. 2005;**278**:323-335. DOI: 10.1016/j.ydbio.2004.10.027

[88] Peters H, Neubuser A, Kratochwil K, Balling R. Pax9-deficient mice lack pharyngeal pouch derivatives and teeth and exhibit craniofacial and limb abnormalities. Genes & Development. 1998;**12**:2735-2747

[89] Foster KE, Gordon J, Cardenas K, Veiga-Fernandes H, Makinen T, Grigorieva E, et al. EphB-ephrin-B2 interactions are required for thymus migration during organogenesis. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**:13414-13419. DOI: 10.1073/pnas.1003747107

[90] Jiang X, Rowitch DH, Soriano P, McMahon AP, Sucov HM. Fate of the mammalian cardiac neural crest. Development. 2000;**127**:1607-1616

[91] Manley NR, Blackburn CC. A developmental look at thymus organogenesis: Where do the non-hematopoietic cells in the thymus come from? Current Opinion in Immunology. 2003;**15**:225-232. DOI: 10.1016/s0952-7915(03)00006-2

[92] Le Lievre CS, Le Douarin NM. Mesenchymal derivatives of the neural crest: Analysis of chimaeric quail and chick embryos. Journal of Embryology and Experimental Morphology. 1975;**34**:125-154

[93] Gordon J, Patel SR, Mishina Y, Manley NR. Evidence for an early role for BMP4 signaling in thymus and parathyroid morphogenesis. Developmental Biology. 2010;**339**:141-154. DOI: 10.1016/j.ydbio.2009.12.026

[94] Baldini A. DiGeorge syndrome: An update. Current Opinion in Cardiology. 2004;**19**: 201-204

[95] Wendling O, Dennefeld C, Chambon P, Mark M. Retinoid signaling is essential for patterning the endoderm of the third and fourth pharyngeal arches. Development. 2000; **127**:1553-1562

[96] Begemann G, Schilling TF, Rauch GJ, Geisler R, Ingham PW. The zebrafish neckless mutation reveals a requirement for raldh2 in mesodermal signals that pattern the hindbrain. Development. 2001;**128**:3081-3094

[97] Lindsay EA, Vitelli F, Su H, Morishima M, Huynh T, Pramparo T, et al. Tbx1 haploinsufficieny in the DiGeorge syndrome region causes aortic arch defects in mice. Nature. 2001; **410**:97-101. DOI: 10.1038/35065105

[98] Manley NR, Selleri L, Brendolan A, Gordon J, Cleary ML. Abnormalities of caudal pharyngeal pouch development in Pbx1 knockout mice mimic loss of Hox3 paralogs. Developmental Biology. 2004;**276**:301-312. DOI: 10.1016/j.ydbio.2004.08.030

[99] Bachiller D, Klingensmith J, Shneyder N, Tran U, Anderson R, Rossant J, et al. The role of chordin/bmp signals in mammalian pharyngeal development and DiGeorge syndrome. Development. 2003;**130**:3567-3578

[100] Xu H, Morishima M, Wylie JN, Schwartz RJ, Bruneau BG, Lindsay EA, et al. Tbx1 has a dual role in the morphogenesis of the cardiac outflow tract. Development. 2004; **131**:3217-3227. DOI: 10.1242/dev.01174

[101] Vitelli F, Taddei I, Morishima M, Meyers EN, Lindsay EA, Baldini A. A genetic link between Tbx1 and fibroblast growth factor signaling. Development. 2002;**129**:4605-4611

[102] Sun X, Meyers EN, Lewandoski M, Martin GR. Targeted disruption of Fgf8 causes failure of cell migration in the gastrulating mouse embryo. Genes & Development. 1999;**13**:1834-1846

[103] Brown CB, Wenning JM, Lu MM, Epstein DJ, Meyers EN, Epstein JA. Cre-mediated excision of Fgf8 in the Tbx1 expression domain reveals a critical role for Fgf8 in cardiovascular development in the mouse. Developmental Biology. 2004;**267**:190-202. DOI: 10.1016/j.ydbio.2003.10.024

[104] Frank DU, Fotheringham LK, Brewer JA, Muglia LJ, Tristani-Firouzi M, Capecchi MR, et al. An Fgf8 mouse mutant phenocopies human 22q11 deletion syndrome. Development. 2002;**129**:4591-4603

[105] Reeh KA, Cardenas KT, Bain VE, Liu Z, Laurent M, Manley NR, et al. Ectopic TBX1 suppresses thymic epithelial cell differentiation and proliferation during thymus organogenesis. Development. 2014;**141**:2950-2958. DOI: 10.1242/dev.111641

[106] Wallin J, Eibel H, Neubuser A, Wilting J, Koseki H, Balling R. Pax1 is expressed during development of the thymus epithelium and is required for normal T-cell maturation. Development. 1996;**122**:23-30

[107] Manley NR, Capecchi MR. The role of Hoxa-3 in mouse thymus and thyroid development. Development. 1995;**121**:1989-2003

[108] Su D, Ellis S, Napier A, Lee K, Manley NR. Hoxa3 and pax1 regulate epithelial cell death and proliferation during thymus and parathyroid organogenesis. Developmental Biology. 2001;**236**:316-329. DOI: 10.1006/dbio.2001.0342

[109] Blackburn CC, Manley NR. Developing a new paradigm for thymus organogenesis. Nature Reviews. Immunology. 2004;**4**:278-289. DOI: 10.1038/nri1331

[110] Hetzer-Egger C, Schorpp M, Haas-Assenbaum A, Balling R, Peters H, Boehm T. Thymopoiesis requires Pax9 function in thymic epithelial cells. European Journal of Immunology. 2002;**32**:1175-1181. DOI: 10.1002/1521-4141(200204)32:4<1175::Aid-immu1175>3.0.Co;2-u

[111] Peters H, Wilm B, Sakai N, Imai K, Maas R, Balling R. Pax1 and Pax9 synergistically regulate vertebral column development. Development. 1999;**126**:5399-5408

[112] Chisaka O, Kameda Y. Hoxa3 regulates the proliferation and differentiation of the third pharyngeal arch mesenchyme in mice. Cell and Tissue Research. 2005;**320**:77-89. DOI: 10.1007/s00441-004-1042-z

[113] Neubuser A, Koseki H, Balling R. Characterization and developmental expression of Pax9, a paired-box-containing gene related to Pax1. Developmental Biology. 1995;**170**:701-716. DOI: 10.1006/dbio.1995.1248

[114] Su DM, Manley NR. Hoxa3 and pax1 transcription factors regulate the ability of fetal thymic epithelial cells to promote thymocyte development. Journal of Immunology. 2000;**164**:5753-5760

[115] Zou D, Silvius D, Davenport J, Grifone R, Maire P, Xu PX. Patterning of the third pharyngeal pouch into thymus/parathyroid by six and Eya1. Developmental Biology. 2006;**293**:499-512. DOI: 10.1016/j.ydbio.2005.12.015

[116] Laclef C, Souil E, Demignon J, Maire P. Thymus, kidney and craniofacial abnormalities in six 1 deficient mice. Mechanisms of Development. 2003;**120**:669-679

[117] Xu PX, Zheng W, Laclef C, Maire P, Maas RL, Peters H, et al. Eya1 is required for the morphogenesis of mammalian thymus, parathyroid and thyroid. Development. 2002; **129**:3033-3044

[118] Erickson M, Morkowski S, Lehar S, Gillard G, Beers C, Dooley J, et al. Regulation of thymic epithelium by keratinocyte growth factor. Blood. 2002;**100**:3269-3278. DOI: 10.1182/blood-2002-04-1036

[119] Jenkinson WE, Jenkinson EJ, Anderson G. Differential requirement for mesenchyme in the proliferation and maturation of thymic epithelial progenitors. The Journal of Experimental Medicine. 2003;**198**:325-332. DOI: 10.1084/jem.20022135

[120] Revest JM, Suniara RK, Kerr K, Owen JJ, Dickson C. Development of the thymus requires signaling through the fibroblast growth factor receptor R2-IIIb. Journal of Immunology. 2001;**167**:1954-1961

[121] Jenkinson WE, Rossi SW, Parnell SM, Jenkinson EJ, Anderson G. PDGFRalpha-expressing mesenchyme regulates thymus growth and the availability of intrathymic niches. Blood. 2007;**109**:954-960. DOI: 10.1182/blood-2006-05-023143

[122] Bain VE, Gordon J, O'Neil JD, Ramos I, Richie ER, Manley NR. Tissue-specific roles for sonic hedgehog signaling in establishing thymus and parathyroid organ fate. Development. 2016;**143**:4027-4037. DOI: 10.1242/dev.141903

[123] Garg V, Yamagishi C, Hu T, Kathiriya IS, Yamagishi H, Srivastava D. Tbx1, a DiGeorge syndrome candidate gene, is regulated by sonic hedgehog during pharyngeal arch development. Developmental Biology. 2001;**235**:62-73. DOI: 10.1006/dbio.2001.0283

[124] Balciunaite G, Keller MP, Balciunaite E, Piali L, Zuklys S, Mathieu YD, et al. Wnt glycoproteins regulate the expression of FoxN1, the gene defective in nude mice. Nature Immunology. 2002;**3**:1102-1108. DOI: 10.1038/ni850

[125] Bleul CC, Boehm T. BMP signaling is required for normal thymus development. Journal of Immunology. 2005;**175**:5213-5221

[126] Neves H, Dupin E, Parreira L, Le Douarin NM. Modulation of Bmp4 signalling in the epithelial-mesenchymal interactions that take place in early thymus and parathyroid development in avian embryos. Developmental Biology. 2012;**361**:208-219. DOI: 10.1016/j.ydbio.2011.10.022

[127] Soza-Ried C, Bleul CC, Schorpp M, Boehm T. Maintenance of thymic epithelial phenotype requires extrinsic signals in mouse and zebrafish. Journal of Immunology. 2008;**181**:5272-5277

[128] Metz A, Knochel S, Buchler P, Koster M, Knochel W. Structural and functional analysis of the BMP-4 promoter in early embryos of Xenopus laevis. Mechanisms of Development. 1998;**74**:29-39

[129] Swann JB, Krauth B, Happe C, Boehm T. Cooperative interaction of BMP signalling and Foxn1 gene dosage determines the size of the functionally active thymic epithelial compartment. Scientific Reports. 2017;**7**:8492. DOI: 10.1038/s41598-017-09213-1

[130] Heinonen KM, Vanegas JR, Brochu S, Shan J, Vainio SJ, Perreault C. Wnt4 regulates thymic cellularity through the expansion of thymic epithelial cells and early thymic progenitors. Blood. 2011;**118**:5163-5173. DOI: 10.1182/blood-2011-04-350553

[131] Ma D, Wei Y, Liu F. Regulatory mechanisms of thymus and T cell development. Developmental and Comparative Immunology. 2013;**39**:91-102. DOI: 10.1016/j.dci.2011.12.013

[132] Osada M, Jardine L, Misir R, Andl T, Millar SE, Pezzano M. DKK1 mediated inhibition of Wnt signaling in postnatal mice leads to loss of TEC progenitors and thymic degeneration. PLoS One. 2010;**5**:e9062. DOI: 10.1371/journal.pone.0009062

[133] Swann JB, Happe C, Boehm T. Elevated levels of Wnt signaling disrupt thymus morphogenesis and function. Scientific Reports. 2017;**7**:785. DOI: 10.1038/s41598-017-00842-0

[134] Zuklys S, Gill J, Keller MP, Hauri-Hohl M, Zhanybekova S, Balciunaite G, et al. Stabilized beta-catenin in thymic epithelial cells blocks thymus development and function. Journal of Immunology. 2009;**182**:2997-3007. DOI: 10.4049/jimmunol.0713723

[135] Dean CH, Miller LA, Smith AN, Dufort D, Lang RA, Niswander LA. Canonical Wnt signaling negatively regulates branching morphogenesis of the lung and lacrimal gland. Developmental Biology. 2005;**286**:270-286. DOI: 10.1016/j.ydbio.2005.07.034

[136] Niida A, Hiroko T, Kasai M, Furukawa Y, Nakamura Y, Suzuki Y, et al. DKK1, a negative regulator of Wnt signaling, is a target of the beta-catenin/TCF pathway. Oncogene. 2004;**23**:8520-8526. DOI: 10.1038/sj.onc.1207892

[137] Mulroy T, McMahon JA, Burakoff SJ, McMahon AP, Sen J. Wnt-1 and Wnt-4 regulate thymic cellularity. European Journal of Immunology. 2002;**32**:967-971. DOI: 10.1002/1521-4141(200204)32:4<967::Aid-immu967>3.0.Co;2-6

[138] Hamazaki Y, Fujita H, Kobayashi T, Choi Y, Scott HS, Matsumoto M, et al. Medullary thymic epithelial cells expressing Aire represent a unique lineage derived from cells expressing claudin. Nature Immunology. 2007;**8**:304-311. DOI: 10.1038/ni1438

[139] Munoz JJ, Cejalvo T, Tobajas E, Fanlo L, Cortes A, Zapata AG. 3D immunofluorescence analysis of early thymic morphogenesis and medulla development. Histology and Histopathology. 2015;**30**:589-599. DOI: 10.14670/HH-30.589

[140] Tucker AS. Salivary gland development. Seminars in Cell & Developmental Biology. 2007;**18**:237-244. DOI: 10.1016/j.semcdb.2007.01.006

[141] Ginda WJ, Jaroszewski J, Warchol JB, Brelinska R. Three dimensional analysis of thymic medulla. Folia Morphologica. 1994;**53**:157-164

[142] Masuda K, Itoi M, Amagai T, Minato N, Katsura Y, Kawamoto H. Thymic anlage is colonized by progenitors restricted to T, NK, and dendritic cell lineages. Journal of Immunology. 2005;**174**:2525-2532

[143] Vroegindeweij E, Crobach S, Itoi M, Satoh R, Zuklys S, Happe C, et al. Thymic cysts originate from Foxn1 positive thymic medullary epithelium. Molecular Immunology. 2010;**47**:1106-1113. DOI: 10.1016/j.molimm.2009.10.034

[144] Dooley J, Erickson M, Farr AG. An organized medullary epithelial structure in the normal thymus expresses molecules of respiratory epithelium and resembles the epithelial thymic rudiment of nude mice. Journal of Immunology. 2005;**175**:4331-4337

[145] Nowell CS, Bredenkamp N, Tetelin S, Jin X, Tischner C, Vaidya H, et al. Foxn1 regulates lineage progression in cortical and medullary thymic epithelial cells but is dispensable for medullary sublineage divergence. PLoS Genetics. 2011;**7**:e1002348. DOI: 10.1371/journal.pgen.1002348

[146] Guo J, Rahman M, Cheng L, Zhang S, Tvinnereim A, Su DM. Morphogenesis and maintenance of the 3D thymic medulla and prevention of nude skin phenotype require FoxN1 in pre- and post-natal K14 epithelium. Journal of Molecular Medicine (Berlin). 2011;**89**:263-277. DOI: 10.1007/s00109-010-0700-8

[147] Barsanti M, Lim JM, Hun ML, Lister N, Wong K, Hammett MV, et al. A novel Foxn1(eGFP/+) mouse model identifies Bmp4-induced maintenance of Foxn1 expression and thymic epithelial progenitor populations. European Journal of Immunology. 2017;**47**:291-304. DOI: 10.1002/eji.201646553

IntechOpen