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REVIEW

The Genetics of Childhood Disease and Development: A Series of Review Articles

This review is the eighth in this series. Drs. Hu and Rosenblum describe the genetic control of branching morphogenesis and the central role of epithelial-mesenchyme interaction. The review contains descriptions of animal models and human disorders resulting from specific genetic defects. Branching morphogenesis is a fundamental process in human developmental biology and this review describes the process and its control in many organs of the body, including kidney, pancreas, lung, breast and salivary glands.

Alvin Zipursky
Editor-in-Chief

Genetic Regulation of Branching Morphogenesis: Lessons Learned from Loss-of-Function Phenotypes

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ABSTRACT

Branching morphogenesis, defined as growth and branching of epithelial tubules during embryogenesis, is a fundamental feature of renal, lung, mammary gland, submandibular gland, and pancreatic morphogenesis in mammals. Disruption of branching morphogenesis has been demonstrated to result in maldevelopment of some of these organs. Genetic studies performed in affected humans and mutant mice have implicated transcription factors, secreted growth factors, and cell surface signaling molecules as critical regulators of branching morphogenesis. These factors function within networks that appear to exert tight control over the number and location of branches. This review summarizes current knowledge regarding the molecular control of branching morphogenesis *in vivo* with particular emphasis on the genetic contribution to perturbed branching morphogenesis in mice and humans. **(*Pediatr Res* 54: 433–438, 2003)**

Abbreviations
FGF, fibroblast growth factor
BMP, bone morphogenetic protein
ALK, activin-like kinase
SHH, sonic hedgehog
EGF, epidermal growth factor
FGFR, fibroblast growth factor receptor
EGFR, epidermal growth factor receptor

OVERVIEW

Branching morphogenesis, defined as growth and branching of epithelial tubules during embryogenesis, is fundamental to the formation of several mammalian tissues including the kidney, lung, salivary gland, mammary gland, and pancreas. During branching morphogenesis, a treelike (*e.g.* kidney) or bushlike (*e.g.* salivary gland) tubular network is generated via mesenchymal-epithelial tissue interactions mediated, in part, by the coordinated actions of a complex network of gene products. The expression pattern of a large number and variety of genes has been demonstrated in branching organs. Although the structural and functional properties of many of these gene products, defined in *in vitro* systems, suggest that they may serve important roles during branching morphogenesis, a

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Table 1. *Human gene mutations exhibiting defects in branching morphogenesis*

Primary disease	Gene	Phenotype
Apert syndrome	*FGFR2*	Hydronephrosis
Beckwith-Wiedemann syndrome	$p57^{KIP2}$	Renal medullary dysplasia
Branchio-Oto-Renal (BOR) syndrome	*EYA1*	Unilateral or bilateral renal agenesis, dysplasia, or hypoplasia; collecting system anomalies
CAKUT	*AGTR2*	Renal dysplasia, hypoplasia; collecting system anomalies
Renal-Coloboma syndrome	*PAX2*	Unilateral renal agenesis, hypoplasia; vesicoureteral reflux
Simpson-Golabi Behmel syndrome	*GPC3*	Renal medullary dysplasia
Townes-Brocks syndrome	*SALL1*	Renal hypoplasia, dysplasia; vesicoureteral reflux
Ulnar-mammary syndrome	*TBX3*	Mammary gland aplasia or hypoplasia

critical function has been demonstrated for only a subset of these genes in experimental models *in vivo*. A much smaller group of genes has been implicated in the genesis of organ maldevelopment in humans. Here, we highlight mechanisms that are common among branching organs. Next, we summarize major morphologic events and highlight advances in the developmental genetics of mammalian branching morphogenesis in kidney, lung, mammary and salivary glands, and pancreas. The limitations of space preclude analysis of the voluminous results arising from studies that do not include tests of function *in vivo*. Rather, we focus on genes and gene networks elucidated via the study of loss-of-function mutations *in vivo*. Knowledge derived from the study of dysplastic kidney tissues is more extensive than that derived from other organs. However, this information, in the context of that derived from lung, glandular tissues, and pancreas, suggests that different organs use shared biologic mechanisms to build branched epithelial structures.

COMMON THEMES DURING BRANCHING MORPHOGENESIS

Branching morphogenesis is dependent on interactions between epithelial tissues such as the ureteric bud (in the kidney) or the lung bud and embryonic mesenchyme. The identity of this mesenchyme is specific to a particular organ. Evidence derived from several different embryonic organs demonstrates that signals derived from the mesenchyme instruct the epithelium to grow and branch (see specific examples below). These signals are mediated by secreted growth factors that signal, most commonly, to tyrosine kinase or serine/threonine kinase cell surface receptors expressed on the surface of epithelial cells. The rather invariant spatial organization of mature organs including the lung and kidney suggests that branching morphogenesis is a tightly regulated process. Consistent with this concept, expression of growth factors is regulated both positively and negatively by molecules including transcription factors. Further, an expanding body of evidence suggests that growth factors of different classes act to either stimulate or inhibit branching morphogenesis, providing a means to control the number and spatial organization of epithelial tubules. Although several classes of growth factors are involved, members of the FGF family act in many tissues to positively regulate branching morphogenesis. In contrast, emerging evidence in the kidney and lung suggests that members of the BMP family limit branching.

BRANCHING MORPHOGENESIS DURING KIDNEY DEVELOPMENT

Formation of the kidney is initiated at 5 wk gestation in the human when the ureteric duct is induced to undergo lateral outgrowth from the Wolffian duct and to invade the adjacent metanephric mesenchyme. The ureteric bud then undergoes repetitive bifid branching events, so termed because each event consists of expansion of the advancing ureteric bud branch at its leading tip, division of the ampulla resulting in formation of new branches, and elongation of the newly formed branches. Beginning with the 10th–11th branch generation, the pattern of branching becomes terminal bifid. During branching morphogenesis, the 65,000 collecting ducts, so formed, become specified as cortical and medullary collecting ducts, a process that is essential to the function of the mature kidney. During the latter stages of kidney development, tubular segments formed from the first five generations of ureteric bud branching undergo remodeling to form the pelvis and calyces [reviewed by Piscione and Rosenblum (1)].

Molecular Control of Renal Branching Morphogenesis

Ureteric bud outgrowth. Outgrowth of the ureteric bud from the Wolffian duct is dependent on genes expressed in either the ureteric bud or metanephric blastema or, simultaneously, in both these tissues. Before the onset of ureteric bud outgrowth, the transcription factors *Pax2* (2) and *Lim1* (3), the nuclear protein *Formin* (4), and the tyrosine kinase receptor *Ret* (5) are expressed in the Wolffian duct whereas *Pax2*, the transcription factors *Sall1* (6) and *Eya1* (7), and the RET ligand and peptide growth factor glial-derived neurotrophic factor (*Gdnf)* (8) are expressed in the metanephric blastema. Homozygous deletion of any of these genes in mice causes failure of ureteric bud outgrowth and bilateral renal agenesis or severe renal dysgenesis with variable penetrance depending on the gene involved. Identical phenotypes have been observed in mice deficient in $\alpha 8$ integrin (9) and heparan sulfate 2-sulfotransferase (10), demonstrating a critical role for integrins and heparan sulfate in mediating interactions between the ureteric bud and the metanephric blastema. In humans, mutations in *EYA1*, *SALL1*, and *PAX2* cause renal maldevelopment ranging in severity from agenesis to dilatation of the ureter (Table 1).

During kidney development, the site of ureteric bud outgrowth is invariant, and the number of outgrowths is limited to one. Studies of genetic interactions *in vivo* have identified

genes that function upstream of *Gdnf* to limit or promote its expression, thereby controlling ureteric bud outgrowth. *Pax2* is required for *Gdnf* expression because *Gdnf* is not expressed in the presumptive metanephric mesenchyme in *Pax2*-null mice (11). The recent identification of PAX2 binding sites in the 5' untranslated region of *Gdnf* suggests that PAX2 directly activates transcription of *Gdnf* (11). Absence of *Gdnf* expression in the metanephric mesenchyme of $Eya1^{-/-}$ mutant kidneys suggests that *Eya* also controls *Gdnf* (7). *Foxc1* (also known as *Mf1*), a forkhead/winged helix transcription factor, is expressed in a similar metanephric domain to *Gdnf* during embryonic development (12). Homozygous *Foxc1*-null mutant mice exhibit renal abnormalities consisting of ureteric duplication, hydroureter, and ectopic ureteric buds, suggesting that *Foxc1* negatively controls the domain of *Gdnf* expression (12). Indeed, anterior expansion in the spatial expression domain of *Eya1* and *Gdnf* in *Foxc1*-null mice is consistent with this concept. Thus, PAX2 and EYA1 working in opposition to FOXC1 may control the number and site of ureteric bud outgrowths.

Ureteric bud and collecting duct branching. The invariant number and spatial pattern of collecting ducts in the mature kidney suggests that branching morphogenesis is tightly regulated. Members of the glial-derived neurotrophic factor and FGF families of growth factors and their cognate receptors stimulate ureteric bud branching. A role for *Gdnf* and *Ret* was suggested by the finding of decreased ureteric bud branching in the small number of $Gdnf^{-/-}$ and $Ret^{-/-}$ mice that escape renal agenesis. Further support in favor of a stimulatory role for RET was provided via the study of RARα and RARβ2, members of the retinoic acid receptor family of transcription factors, which are expressed in stromal cells surrounding *Ret*-expressing ureteric bud branch tips (13). $Rar\alpha^{-/-};Rar\beta2^{-/-}$ double mutant mice have small kidneys characterized by a decreased number of ureteric bud branches associated with diminished expression of *Ret*. Transgenic overexpression of *Ret* in these mice rescues branching morphogenesis (14). At least two members of the FGF family of signaling peptides stimulate collecting duct morphogenesis in mice. Homozygous null mutations in the *Fgf7* gene result in a reduced number of ureteric bud branches and underdevelopment of the renal papilla (15). Mice with a homozygous null mutation in *Fgf10* also have kidneys that are smaller than wild-type and exhibit a decreased number of medullary collecting ducts, medullary dysplasia, and dilatation of the renal pelvis (16).

Recent results suggest that BMP signaling via their ALK receptors inhibits branching morphogenesis. Targeted overexpression of ALK3 in the ureteric bud lineage decreases branching morphogenesis and is associated with decreased nephron formation (17). Inasmuch as ureteric bud and collecting duct cells express RET, FGFR, and ALK receptors, the studies cited above suggest that integration of signals from these diverse and opposing pathways by ureteric bud and collecting duct cells controls branching behavior.

Corticomedullary patterning. Specification of the cortical and medullary domains of the renal collecting duct system (also known as corticomedullary patterning) is a relatively late event during kidney development. Inactivation of at least four soluble growth factor genes (*Fgf7* and *Fgf10*), one proteoglycan gene [glypican-3 (*Gpc3*)], and one cell cycle regulatory gene ($p57^{KIP2}$) causes distinct defects in medullary collecting duct morphogenesis and suggests that these genes may function in patterning of the collecting duct system. For example, the kidneys of *Fgf7*-null mice are characterized by marked underdevelopment of the papilla, the innermost portion of the medulla (15). The cellular response of FGFs, and other peptide growth factors, may be modulated through interactions with sulfated glycosaminoglycan side chains of cell surface proteoglycans (18). Recent genetic and experimental evidence in humans and mice has demonstrated that glypican-3, a glycosylphosphatidylinositol–linked cell surface heparan sulfate proteoglycan, is required for normal patterning of the medulla (19, 20). The pathogenesis of renal medullary dysplasia in the *Gpc3* mutant background may be related to the effects of glypican-3 on cell proliferation and apoptosis and signaling by inhibitory BMPs (20). A role for control of the cell cycle in the pathogenesis of medullary renal dysplasia is further supported by the finding of medullary renal dysplasia in mice and humans (Beckwith-Wiedemann syndrome) with inactivating mutations in $p57^{KIP2}$, a cell cycle regulatory gene that encodes a cyclin-dependent kinase inhibitor (21).

Formation of the calyces and pelvis. Patterning of the collecting system to form the calyces and pelvis is controlled by members of the BMP family and by the angiotensin signaling axis. The expression profiles of *Bmp4* and *Bmp5* mRNA in mesenchymal cells lining the ureter and medullary collecting ducts in the region of the developing renal pelvis are compatible with functions in the development of these anatomic structures. A subset of mice with spontaneous and engineered mutations in *Bmp4* and *Bmp5* demonstrates dilatation of the ureters and collecting system (ureterohydronephrosis) and ureteral bifurcation (22, 23). Mutations in the genes encoding components of the renin-angiotensin axis, best known for their role in controlling renal hemodynamics, also cause abnormalities in the development of the renal calyces and pelvis. Mice that are homozygous null for angiotensin receptor-1 (*Agtr1*) demonstrate progressive widening of the calyx and atrophy of the papillae and underlying medulla (22). The underlying defect appears to be a decrease in proliferation of the smooth muscle cell layer lining the pelvis, resulting in decreased thickness of this layer in the proximal ureter. Mutational inactivation of *Agtr2* results in a range of anomalies including vesicoureteral reflux, a duplex kidney, renal ectopia, ureteropelvic junction stenosis, ureterovesical junction stenosis, renal dysplasia, renal hypoplasia, multicystic dysplastic kidney and renal agenesis (23). Null mice demonstrate a decreased rate of apoptosis of the cells around the ureter, suggesting that *Agtr2* plays a role in modeling of the ureter. Taken together, these studies highlight the role of smooth muscle patterning in the formation of the terminal components of the renal collecting system.

BRANCHING MORPHOGENESIS DURING LUNG DEVELOPMENT

The lungs originate from the laryngotracheal groove in the endodermal epithelium lining the floor of the primitive embry-

onic anterior pharynx. At 3–4 wk of gestation in the human, the groove separates dorsoventrally from the primitive esophagus to form the tracheal rudiment that gives rise to two primary bronchial buds. This initial branching event is followed by 23 generations of branches via invasion into the surrounding mesenchyme and bifid branching. The lobular development of the mammalian lung is asymmetric, resulting in a larger number of lobes in the right lung (four in mouse; three in human) than in the left (one in mouse; two in human) [reviewed by Mendelsohn *et al.* (24) and Motoyama *et al.* (25)].

Molecular Control of Lung Branching Morphogenesis

Lung bud outgrowth. The generation of branched airways is dependent on separation of the primitive trachea from the primitive esophagus, lung bud outgrowth, and establishment of a left-right axis. *Nkx2.1*, a homeodomain transcriptional regulator, SHH, a secreted growth factor, and the α and β2 retinoic acid receptors control separation of the primitive trachea from the esophagus. Deficiency of these gene products results in failure of esophagotracheal septation and complete failure of branching morphogenesis (24, 26, 27). Further evidence in favor of a critical role for SHH in lung bud outgrowth is suggested by the aplastic lung phenotype of mice deficient in both *Gli2* and *Gli3*, transcriptional factors that act downstream of SHH (27). Lung bud outgrowth is also controlled by *Hnf-3β,* a transcription factor, because failure of outgrowth of the trachea from the primitive foregut endoderm occurs in *Hnf-3β*$^{-/-}$ mice (28). The spatial and temporal expression and phenotype of mice with mutations in genes encoding secreted peptides including *Lefty-1* and *-2*, and *nodal*, and *Pitx-2,* a transcription factor, demonstrate that these factors control asymmetric lobar lung development (left-right). For example, mutational inactivation of murine *Lefty-1* results in pulmonary isomerism (single lobes bilaterally) (29). Interestingly, expression of *Lefty-1* may be controlled by retinoic acid (27), suggesting a critical role for retinoic acid as observed in the embryonic kidney.

Lung branching morphogenesis. Growth and branching of respiratory airways is controlled by mesenchymal-epithelial tissue interactions. These interactions are mediated by secreted growth factors that act to stimulate or inhibit these processes. Stimulatory factors include EGF and FGFs. A stimulatory role for EGF was demonstrated by the reduction in branching morphogenesis observed in EGFR-null mice (30). However, FGFs are considered to be the most critical stimulators. FGF9 is expressed in respiratory epithelium and stimulates mesenchymal cell proliferation. *Fgf9*$^{-/-}$ mice are characterized by a decrease in the amount of mesenchyme, decreased mesenchymal cell proliferation, and impaired airway branching (31). Conversely, FGF10 is expressed within mesenchyme at prospective sites of bud formation. *Fgf10*$^{-/-}$ mice are characterized by bilateral lung agenesis (32, 33). The lung aplasia observed in mice that overexpress a dominant negative FGFR further emphasizes the importance of the FGF pathway.

Studies of genetic interactions *in vivo* suggest that *Fgf10* acts within a complex pathway consisting of stimulatory and inhibitory factors. The spatial expression of *Fgf10* is negatively controlled by *Shh* because mutational inactivation results in loss of this restricted *Fgf10* expression. Consistent with a role for SHH in limiting the expression of the protubulogenic FGF10, *Shh* overexpression down-regulates *Fgf10* expression. A second regulatory mechanism that may serve to tightly regulate FGF-10 actions is FGF-10–dependent induction of *Bmp4* at branch tips. The finding that BMP4 inhibits lung branching morphogenesis *in vitro* suggests that *Fgf10* autoregulates its stimulatory activity by inducing the expression of an inhibitory BMP (34).

BRANCHING MORPHOGENESIS DURING MAMMARY GLAND DEVELOPMENT

Embryonic mammary gland branching morphogenesis is initiated by invagination of an epithelial bud derived from an ectodermal thickening, the primordial nipple, during the sixth week of gestation in the human (35). The bud becomes surrounded by a specialized layer of fibroblasts derived from mesenchyme and is induced to undergo branching (36, 37). Production of androgens in the male fetus results in destruction of the bud. In contrast, in the female fetus the bud elongates and branches, forming 15–20 branching epithelial ducts by the end of gestation. A second phase of sex hormone–dependent branching morphogenesis occurs in females during puberty. Regulation of branching during this phase is beyond the scope of this review.

Molecular Control of Mammary Gland Branching Morphogenesis

In the mammary gland, branching morphogenesis is dependent on mesenchymal-epithelial interactions. The transcription factors *Tbx3* and *Lef1* and the secreted protein *PTHrP* are expressed in the epithelial bud whereas *Fgf10* and the PTHrP receptor, *PTHR1*, are expressed in the mesenchyme. Inactivation of *Tbx3*, a T-box type transcription factor mutated in the ulnar-mammary syndrome (Table 1), is associated with mammary aplasia or severe hypoplasia, suggesting a critical role during the early stages of branching morphogenesis (38). Mutational inactivation of murine *Lef1*, a transcriptional effector in the WNT/β-catenin signaling pathway, abrogates formation of mammary epithelial buds (39). A similar phenotype was observed in *Fgf10*$^{-/-}$ mice (40). The additional finding of extensive epithelial apoptosis in these mice demonstrated an essential role for FGF10 in epithelial cell survival, perhaps by controlling the integrity of the mesenchyme in signaling to the adjacent epithelium. PTHrP is a growth factor related structurally to PTH. PTHrP signals in a paracrine manner via PTHR1 expressed in mesenchyme. Mutational inactivation of either *PTHrP* or *PTHR1* results in failure of androgen-mediated destruction of buds in the male and commencement of ductal branching morphogenesis in the female. Together, these studies begin to generate a hierarchy of molecules that control cell specification, epithelial survival, and branching in the mammary gland.

BRANCHING MORPHOGENESIS DURING SUBMANDIBULAR GLAND DEVELOPMENT

In the human, the major salivary glands are ectodermal derivatives, originating in the floor of the mouth as epithelial buds between approximately the sixth and eighth weeks of gestation. All three of these glands arise from the first pharyngeal pouch. Morphogenesis of the parotid gland occurs first. This is followed by formation of the submandibular gland and finally by the sublingual gland at approximately 8 wk gestation. Submandibular organogenesis begins as a thickening of the primitive oral cavity epithelium adjacent to the developing tongue. This thickened epithelium forms a bud that grows into the first branchial (mandibular) arch mesenchyme. With continued epithelial proliferation, the submandibular gland primordium becomes a solid, elongated epithelial stalk that forms lateral and interactive bifid branches at the distal ends of successive epithelial buds. These branches and buds are remodeled via epithelial cell apoptosis to form luminal tubules [reviewed by Jaskoll *et al.* (41)].

Molecular Control of Submandibular Gland Branching Morphogenesis

Members of the *Pax, Fgf, Egf,* and *Bmp* families are expressed in a spatial and temporal pattern consistent with roles in submandibular development (41). Informative phenotypes arising from mutational inactivation of some of these genes have begun to define their functions. The spatial expression of *Pax6* is restricted to mesenchyme during the early bud stage. Disorganization of mesenchyme and hypoplasia in $Pax6^{-/-}$ mice indicates that *Pax6* controls crucial morphogenetic events at this stage (41). FGFR2IIIc, an FGFRIII splice variant, is hypothesized to bind FGF8, expressed in early epithelial buds and later in both epithelium and mesenchyme. The finding of decreased branching morphogenesis and hypoplasia in mice heterozygous for the partial loss-of-function FGFR2IIIc mutant, FGFRIIIcΔ, demonstrates the importance of FGF signaling during branching morphogenesis. EGFR is able to bind transforming growth factor-α and EGF. Transforming growth factor-α and EGFR, but not EGF, are expressed in epithelium during the initial budding and pseudoglandular stages. EGF is expressed subsequently during the formation of mature ducts and terminal end buds. The decreased number of terminal end buds observed in $Egfr^{-/-}$ mice suggests that EGF/EGFR signaling is nonredundant at this stage. BMP7 is expressed in epithelium at the pseudoglandular stage. $Bmp7^{-/-}$ mice are characterized by disorganized mesenchyme and decreased branching (41), demonstrating a critical role for BMP7 in branching morphogenesis. Taken together, these studies demonstrate that signaling molecules critical to the development of other branching organs are also required for branching morphogenesis in the submandibular gland.

BRANCHING MORPHOGENESIS DURING PANCREAS DEVELOPMENT

The pancreas is derived from endodermal cells of the upper duodenal region of the foregut. In the human, the pancreatic primordia first appear at the fifth week of gestation in the form of epithelial buds from dorsal and ventral evaginations of the primitive foregut. The buds undergo lateral and iterative bifid branching to form a complex branched structure.

Molecular Control of Branching Morphogenesis in the Pancreas

The specification of cells that are destined to become pancreas appears to be dependent on insulin-promoter-factor 1(IPF1, also termed PDX1) (42). Mutational inactivation of the gene encoding this homeodomain protein results in agenesis of pancreatic tissue and neonatal death. FGF10, expressed in the mesenchyme, regulates epithelial cell proliferation and, therefore, the size of the epithelial progenitor cell pool marked by PDX (43). In contrast, the hedgehog pathway appears to limit early bud formation. In mice, Indian hedgehog, a member of the hedgehog family of secreted morphogens, regulates ventral bud branching. Approximately 40% of mice deficient in Indian hedgehog display annular pancreas, a malformation in which a band of pancreatic tissue completely encircles the duodenum associated with formation of a second ventral bud (44). Signaling via the EGFR stimulates pancreatic branching morphogenesis. $Egfr^{-/-}$ mice demonstrate an approximately 50% decrease in the size of the embryonic pancreas and decreased ductal branching. This defect may be caused, in part, by altered modification of the extracellular matrix required for initiation and growth of new branches because $Egfr^{-/-}$ mice elaborate less matrix metalloproteinase in the pancreas compared with their wild-type littermates (45).

CONCLUSIONS

Several major themes emerge from the analysis of branching morphogenesis in mammalian organs. First, branching is controlled by mesenchymal-epithelial interactions mediated by a complex network of gene products that function in pathways that extend from the cell nucleus to the cell surface. Second, these pathways function to stimulate or inhibit branching, thus exerting regulatory control needed during organogenesis. Third, the number of human disorders, involving defective branching morphogenesis, for which a molecular basis has been identified is far smaller than the number predicted by the number and diversity of identified phenotypes. Discovery of the molecular basis for these disorders has been limited by the small size of affected kindreds, the inaccessibility of the respective organs, and limitations in discriminating pathologic abnormalities caused by abnormal organogenesis from those as a result of secondary injury occurring after birth. Advances in genomics and developmental genetics are providing new opportunities to expand our understanding of the molecular control of normal branching morphogenesis and the contribution of disrupted branching morphogenesis to human disease.

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