Development of the kidney medulla

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The mature renal medulla, the inner part of the kidney, consists of the medullary collecting ducts, loops of Henle, vasa recta and the interstitium. The unique spatial arrangement of these components is essential for the regulation of urine concentration and other specialized kidney functions. Thus, the proper and timely assembly of medulla constituents is a crucial morphogenetic event leading to the formation of a functioning metanephric kidney. Mechanisms that direct renal medulla formation are poorly understood. This review describes the current understanding of the key molecular and cellular mechanisms underlying morphological aspects of medulla formation. Given that hypoplasia of the renal medulla is a common manifestation of congenital obstructive nephropathy and other types of congenital anomalies of the kidney and urinary tract (CAKUT), better understanding of how disruptions in medulla formation are linked to CAKUT will enable improved diagnosis, treatment and prevention of CAKUT and their associated morbidity.

Introduction

Congenital hydronephrosis, defined as increased diameter of the renal pelvis, is the most frequently identifiable anomaly by prenatal ultrasound with an overall prevalence of 0.5 to 1%.[1,2] Moreover, congenital obstructive nephropathy, frequently accompanied by hydronephrosis, is a common cause of renal failure in children.[3] In turn, hydronephrosis is frequently associated with effacement of the renal medulla, the inner zone of the kidney.[4] Small medulla may accompany renal hypodysplasia, a form of congenital anomaly of the kidney and urinary tract (CAKUT) characterized by reduced number of ureteric bud (UB) branches, presence of undifferentiated mesenchymal and stromal cells, cysts and cartilage, which is observed in 0.027% of fetuses by ultrasonography.[5] For example, renal medullary hypodysplasia is observed in patients with Simpson-Golabi-Behmel (OMIM# 312870) and Beckwith-Wiedeman (OMIM# 130650) syndromes.[6,7] Although hypoplastic renal medulla may result from backward pressure to the renal parenchyma due to urinary stasis,[8]

emerging evidence indicates that it may be also due to an intrinsic defect in medullary morphogenesis.[9]

Major Steps in Kidney Organogenesis

On the fifth week of gestation in humans (embryonic day E10.5 in mice), the caudal portion of the nephric duct forms an epithelial outgrowth called the UB, which invades the metanephric mesenchyme.[10,11] UB branches repeatedly within the mesenchyme to form the renal collecting system (the ureter, pelvis, calyces and collecting ducts).[10,11] Proximal ureter subsequently translocates from the nephric duct to fuse with the bladder which originates from the urogenital sinus.[12] As distal UBs branch, the mesenchymal cells condense around the UB tips to form nephrons (the glomerulus, proximal and distal tubules and loop of Henle). Kidney vascularization is synchronized with nephrogenesis and occurs by vasculogenesis (formation of new blood vessels from endothelial cell precursors) and angiogenesis (sprouting of new capillaries from preexisting blood vessels).[13,14] The stromal mesenchyme or stroma is a complex mixture of cell types which surround nascent UBs and nephrons, do not differentiate into nephrons or the UBs/collecting ducts and give rise to renal interstitium.[15]

Brief Overview of Morphology and Function of Mature Renal Medulla

The medulla of the adult kidney has a modified cone shape with a broad base adjacent to renal cortex and the narrow apex termed papilla. The mature renal medulla consists of the medullary collecting ducts, loops of Henle, vasa recta (straight capillaries) and the interstitium (lipid-laden interstitial cells, lymphocyte-like cells and pericytes).[16] The main function of the medulla is to regulate concentration of the urine. The urine flows from the collecting ducts into the renal calyces and pelvis, which undergoes unidirectional peristaltic movements to allow drainage of the urine into the downstream ureter and bladder.[8] In addition, the renal papilla is a niche for adult kidney stem cells which may play a role in repair after ischemic kidney injury.[17,18]
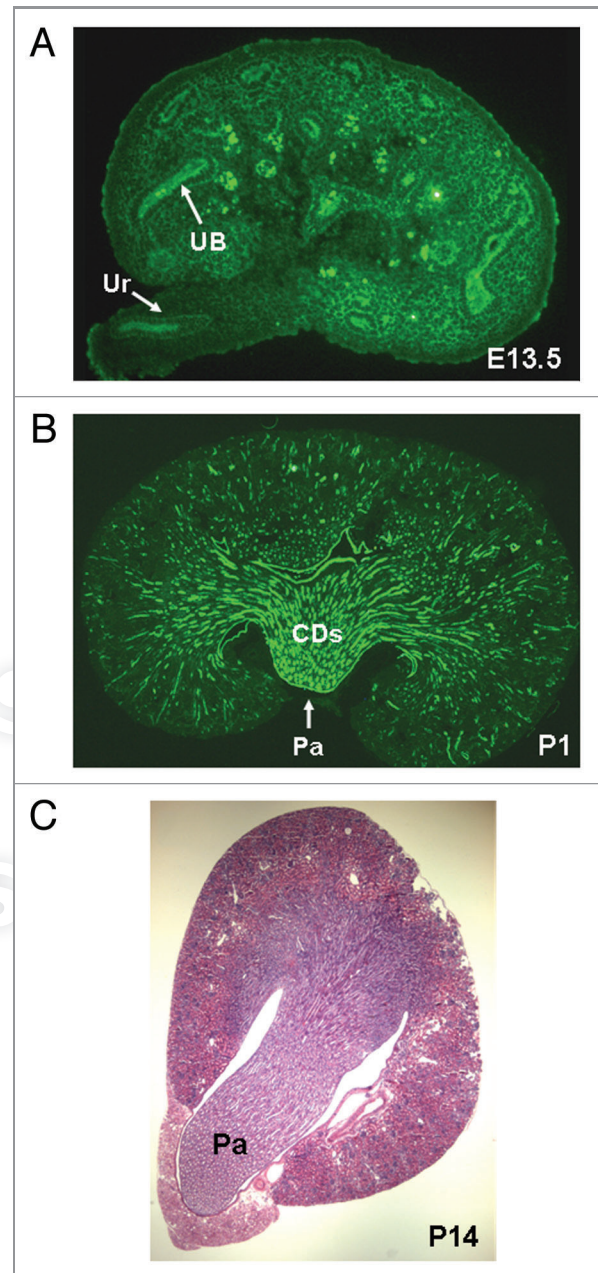
Developmental Aspects of Medullary Morphogenesis

Development of collecting ducts. *Morphologic development of collecting ducts.* Collecting ducts and other components of the renal collecting system (calyces, pelvis and the ureter) are formed

during kidney development as a result of sequential branching, elongation and patterning (gradual acquisition of structure characteristic of mature organ) of the UB, driven by reciprocal inductive interactions with the metanephric mesenchyme and stroma.[10,19] In the mouse, renal medulla becomes identifiable morphologically on E15.5, expands longitudinally (along the corticomedullary axis), increases in length 4.5-fold from E15.5 to birth (mouse gestation period is 20 d) and represents 30% of total kidney volume at birth (**Fig. 1**).[20] The papilla grows through proliferation of collecting duct cells in a distal-to-proximal fashion, with cell proliferation arresting in the distal papilla before E17.5, a time when extensive proliferative growth continues in the proximal papilla.[21] In the human kidney, once UB branching is completed by 20–22 weeks of gestation, subsequent morphogenesis of collecting ducts occurs by extension and patterning of peripheral branch segments.[19] Initial generations of UB branches undergo remodeling (change in structure or form) by dilatation and growth to form the ureter, renal pelvis and calyces.[11] The 6th–8th generations of UB branches give rise to the long unbranched outer medullary collecting ducts in mice.[20] While significant longitudinal elongation of the medullary collecting ducts accompanies renal medulla formation,[20] the mechanisms by which linear arrays of inner medullary collecting ducts converge centrally to form the papilla and how many collecting ducts enter the tip of the papilla remains unknown.

Genetic control of collecting duct morphogenesis. Recent genetic studies in mice demonstrate that medullary hypoplasia may be due, in part, to an intrinsic defect in medullary morphogenesis normally driven by longitudinal elongation of UB-derived collecting ducts (**Table 1**). Global deletion of cell surface heparan sulfate proteoglycan glypican3 (*Gpc3*), expressed in the UB, in mice causes renal medullary cystic dysplasia due to increased apoptosis of medullary collecting duct cells.[22,23] Global genetic inactivation of α*3 integrin*, expressed in the UB and their derivative collecting ducts, in mice results in hypoplastic medulla due to decreased branching of the medullary collecting ducts.[24] Genetic removal of estrogen-related receptor gamma (*Esrrg*), expressed in the UB and UB-derived collecting ducts, in mice results in medullary hypoplasia without detectable morphological changes in the renal pelvis or the ureter.[25] Targeted removal of bone morphogenic protein (BMP) receptor *Alk3* in the UB reduces the number of UB branches and medullary collecting ducts resulting in medullary hypoplasia.[26] UB-specific inactivation of the fibroblast growth factor receptor (*FGFR*) in mice decreases UB branching and leads to abnormally shaped and hydro-nephrotic kidneys.[27] Deletion of the epidermal growth factor receptor (*EGFR*) in the UB in mice, normally expressed in the UB trunk domain, does not appear to alter UB branching, but results in moderate dilation of the medullary collecting ducts, thin medulla and reduced urine-concentrating ability.[28] Absence of *Wnt7b*, a secreted protein which is also normally expressed in UB trunk, is accompanied by dilated collecting ducts that have reduced number of branch points and leads to a failure of medullary development.[9] These changes in *Wnt7b* mutants are likely due to an abnormal plane of collecting duct cell division where cell division occurs orthogonal to the longitudinal axis of

the collecting ducts. In contrast, inactivation of *Dickkopf1* (*Dkk1*), a secreted Wnt antagonist, in the UB lineage in mice leads to overgrowth of the whole papilla resulting from enhanced proliferation of the collecting duct and loop of Henle cells.[29] This effect of absent *Dkk1* may be due to induction of Wnt7b signaling from the collecting duct cells to the stroma.[29] The

Figure 1. Renal medulla development in the mouse. (A) Whole mount immunohistochemistry of E13.5 kidney. Ureteric buds (UB) and their derivatives (ureter, Ur) are visualized with anti-pancytokeratin antibody (green). Medulla is not identifiable morphologically at this stage. (B) Longitudinal section through a newborn (postnatal day P1) kidney. Medulla and papilla (Pa) are identifiable morphologically. UB-derived collecting ducts (CDs) are visualized with anti-pancytokeratin antibody (green). (C) Transverse section through P14 kidney stained with hematoxylin and eosin. Long papilla (Pa) is present at this stage.

Table 1. Gene mutations leading to defects in morphogenesis of renal medulla in mice

Gene	Medullary phenotype	Mechanisms	References
Metanephric mesenchyme			
Cited1^{-/-}	Hypoplastic medulla hydronephrosis	Increased apoptosis in the medulla	46
Stroma			
Foxd1^{-/-}	Small, fused low-set kidneys	Reduced UB branching	54
FGF7^{-/-}	Thin small papilla	Reduced UB branching	62
BMP4^{-/-}	Medullary hypodysplasia hydronephrosis	Reduced UB branching	57
RARα/β₂[-/-]	Small kidneys	Reduced UB branching	63
Pod1^{-/-}	Renal hypodysplasia	Reduced UB branching	64
Pbx-1^{-/-}	Renal hypodysplasia	Reduced UB branching	66
AGT^{-/-}	Thin medulla atrophic papilla hydronephrosis	Reduced UB branching	81, 82
ACE^{-/-}	Atrophic papilla Hydronephrosis	Unknown	83
AT$_2$R[-/-]	Hydronephrosis	Reduced UB branching	74, 84
Collecting duct			
Alk3[UB-/-]	Hypoplastic medulla	Reduced UB branching decreased number of medullary collecting ducts	26
Pax8[Cre]/Dkk1[loxP/loxP]	Overgrowth of papilla hydronephrosis	Increased proliferation of collecting duct and LOH cells	29
Gpc3^{-/-}	Large initially and small later kidneys	Decreased number of medullary collecting ducts	22
FGFR2[UB-/-]	Abnormally shaped kidneys, hydronephrosis	Reduced UB branching	27
EGFR[UB-/-]	Thin medulla, dilated medullary collecting ducts	Increased apoptosis of papillary collecting duct cells	28
Esrrg^{-/-}	Hypoplastic medulla	Reduced UB branching	25
Wnt7b[UB-/-]	Absent medulla, dilated medullary collecting ducts	Aberrant division of collecting duct cells orthogonal to longitudinal axis of collecting ducts	9
Loop of Henle			
Adamts1-/-	Thin medulla hydronephrosis	Expansion of interstitial matrix	34
Brn1^{-/-}	Decreased kidney size, reduced number of LOH, retarded elongation of LOH	Reduced proliferation of LOH cells, expanded area of apoptotic cells throughout primitive LOH bend	35
Urinary tract obstruction			
Rarb2[Cre]/Smo[loxP/-]	Blunted medulla hydronephrosis	Abnormal ureteral peristalsis, reduced number of ureteral pacemaker cells	77
AT$_1$R[-/-]	Atrophic papilla hydronephrosis, reduced length of medullary capillaries, impaired organization of vasa recta bundles	Decreased ureteral peristalsis, reduced expression of angiogenic growth factors, decreased proliferation of vasa recta cells	39, 41, 78
Pax3[Cre]/Cnb1[loxP/loxP]	Hydronephrosis	Aberrant ureteral peristalsis	76
Sox17/18[-/-]	Atrophy of outer medulla	Reduced neovascularization in kidney outer medulla vasa recta	43

findings that ureter and renal pelvis are comparable between Wnt7b-null and wild-type kidneys[9] indicate that medullary hypoplasia may occur in the presence of normally structured pelvis and ureter. Moreover, the findings that selective removal of β-catenin, a key mediator of canonical Wnt signaling, from the interstitium phenocopies the medullary hypoplasia of Wnt7b mutants underscores the importance of cross-talk between nascent collecting ducts and stroma in medulla formation.[9] Together, these findings demonstrate that defects in UB branching and collecting duct growth may be causally linked to medullary hypodysplasia in mice.

Development of the loop of Henle (LOH). *Anatomical development of the LOH.* The LOH is the anatomical basis for the countercurrent multiplier system that maintains the osmotic gradient in the renal medulla. Two types of LOH are distinguished in the mature kidney: short and long. Short loops make a turn in the outer medulla and long loops in the inner medulla. The outer medulla contains thick descending and ascending (TAL) limbs and descending thin limbs.[16] The inner medulla contains descending and ascending thin limbs. Long loops loop back at successive levels in the medulla and only few penetrate into the tip of the papilla.[16] In the adult rat kidney, about 1,500 of 10,000 loops that enter the inner medulla reach the second half of the inner medulla and only about 250 enter the last millimeter of the papilla.[16] In the newborn rat kidney, renal medulla is not separated into an outer and inner zone, lacks ascending thin limbs and has the structural composition characteristic of the mature inner stripe of the outer medulla.[30]

During the first 2 weeks of the postnatal life (when nephrogenesis continues in the rat), immature TALs in the renal papilla are transformed into thin limbs by apoptotic deletion of cells in thick limbs and the growth of the loops of Henle occurs mainly at the corticomedullary junction.[21,30,31] Low levels of circulating glucocorticoids promote proliferation of TAL cells and elongation of the outer medulla during postnatal development in the rat.[32]

Genetic control of LOH morphogenesis. Recent studies showed that several genes are essential in the formation of the LOH (**Table 1**). Studies in Xenopus demonstrated that Iroquois (Irx) 3 is necessary for intermediate tubule formation.[33] Intermediate tubule progenitors give rise to epithelia of LOH in mammals. Generation of conditional *Irx3*-mutant mice will clarify the role of Irx3 in LOH development in mammals. In the developing mouse kidney, Adamts-1 (disintegrin and metalloproteinase with thrombospondin motifs) expression is specifically detected in the LOH.[34] *Adamts-1*-null mice display thin medulla and hydronephrosis in the absence of morphological alterations in the renal pelvis or ureter.[34] Given that Adamts-1 is a secreted zinc metalloproteinase, it is conceivable that it plays a role in the remodeling of the ECM in medullary interstitium to facilitate elongation of maturing LOH. Mice deficient in POU transcription factor *Brn1*, expressed in the TAL, exhibit decreased number and length of LOH, enhanced apoptosis of LOH cells and reduced expression of TAL-specific genes.[35] Thus, Brn1 is required for growth and differentiation of the loop of Henle. One of the mechanisms by which Brn1 promotes elongation of the LOH may involve suppression of cell apoptosis in primitive loop. To this end, emerging evidence demonstrates that defects in growth and elongation of the developing LOH may account for medullary hypodysplasia. While growth, elongation and remodeling of LOH can be explained by segment-specific cell proliferation, apoptosis and differentiation, the mechanisms directing bending and turning of the loop remain to be elucidated. Other challenges include identification of molecular networks that regulate morphogenesis of LOH and their cross-talk among the loop, stroma/interstitium, collecting ducts and vasculature.

Development of medullary vasculature. *Anatomical development of vasa recta.* Vasa recta are straight capillaries that branch off the efferent arterioles of juxtamedullary nephrons, enter the medulla and surround the LOH. These vessels play an important role in the maintenance of mass balance and osmotic gradient in the medulla by returning the NaCl and water reabsorbed in the LOH and medullary collecting tubule to the systemic circulation. The vasa recta function as countercurrent exchangers to delay the washout of NaCl and urea and prevent the excess accumulation of water in the inner medulla.[36] In the developing rat kidney, vasa recta bundles grow centrally into the medulla around the previously developed collecting ducts.[37]

Factors that control angiogenesis of vasa recta. Angiogenic factors. Angiogenic factors not only direct developmental angiogenesis, but provide fundamental cues for cell fate specification, embryo patterning, organ differentiation and postnatal tissue remodeling.[38] Recent data demonstrate that impaired development of medullary microcirculation may be causally linked to medullary

hypoplasia observed in *AT1R*-mutant mice.[39,40] Treatment of newborn rats with the specific AT1R antagonist reduces length, volume and surface area of capillaries in the renal medulla, and inhibits cell proliferation and organization of the developing vasa recta bundles.[41] These changes are accompanied by decreased expression of angiogenic growth factors such as vascular endothelial growth factor (VEGF), angiopoietins 1 and 2, Flk1/Flt1 and Tie1 in the medulla, and lead to decreased renal blood flow later in life. Moreover, administration of AT1R antagonist during pregnancy in humans results in atrophy of the renal medulla and poorly developed vasa recta in the fetal kidney.[42] Double-mutant mice deficient in high mobility group (HMG) box transcription factor genes *Sox17/Sox18*, show reduced neovascularization in the outer medulla vasa recta on postnatal day 7.[43] Notably, *AT1R* or *Sox17* mutations are causative factors in CAKUT in humans.[44,45] Whether structural maldevelopment of the medulla affects vasa recta formation or defects in vascular development account for decreased size of the medulla remains to be determined. Regardless, aberrant medullary microcirculation may have a major role in how fetal reprogramming results in postnatal diseases such as hypertension and progressive kidney disease.

Embryonic blood flow and oxygenation. The role of embryonic blood supply in the development of medullary vasculature in vivo and the mechanisms by which aberrant blood flow in the embryonic kidney may affect medulla formation are poorly understood. It is plausible that the inflow of blood into the renal medulla, with the resulting increase in oxygen tension, may regulate both the development of vasa recta and medullary morphogenesis. This possibility is supported by the findings that mice deficient in *Cited1,* a transcriptional co-factor expressed in the induced metanephric mesenchyme of the embryonic kidney, have renal medullary dysplasia that does not result from defects in UB branching or lower urinary tract obstruction, but may be due to intrauterine hypoxia resulting from placental insufficiency.[46] Hypoxia-inducible factors (HIF) HIF-1 and HIF-2 are transcription factors induced in hypoxia to alleviate hypoxic stress, in part by promoting neovascularization.[47] HIF-1 and HIF-2 mRNAs are highly expressed in the medulla, and particularly in the medullary collecting ducts, of the newborn mouse kidney in vivo.[48] In addition, VEGF mRNA is detected in the outer medullary collecting ducts dissected from the developing rat kidney on P14.[41] Moreover, the expression of HIF-1, HIF-2 and VEGF, a crucial regulator of vascular development, is induced in embryonic kidneys maintained in hypoxic organ cultures.[48] These data suggest that HIF stabilization by hypoxia may be critical for VEGF production and kidney vascular development. Potential role for HIF-1 in the regulation of medulla growth is supported by the findings that administration of dominant-negative HIF-1 induces severe damage in the medulla of normal adult rats.[49]

Recent studies using in utero intracardiac injection of vascular tracer to living mouse embryos demonstrate that perfusion of embryonic pancreas correlates with pancreatic cell differentiation in the region of the blood flow in vivo.[50] Given that the regions adjacent to perfused vessels have more oxidized thiols, the authors propose that enhanced oxygen delivery after the inflow of blood is a likely permissive factor for initiating cell differentiation.[50] Thus,

vascular flow, rather than vascular endothelium alone, may provide specific signals necessary for regional organ differentiation and complete vessel morphogenesis (e.g., development of anastomoses between the main vessels that carry blood flow and small vessels that do not have blood flow) during early embryogenesis. In accord with this hypothesis, cardiac-specific deletion of a key angiogenic factor *angiopoietin 1* in mice recapitulates kidney vascular defects observed in global *angiopoietin 1* knockout.[51] Application of in utero intracardiac injection of vascular tracer to living embryos in model organisms to measure maturation of the embryonic medullary perfusion may provide correlative evidence that enhanced blood flow underlies normal morphogenesis of the renal medulla.

Development of interstitium. *Anatomical development of medullary interstitium.* The medullary interstitium of the adult kidney consists of lipid-laden interstitial cells, lymphocyte-like cells, pericytes and extracellular matrix (ECM), and provides a structural supportive framework around collecting ducts, loops of Henle and vasa recta.[16,52] Other possible functions of mature medullary interstitial cells include production of ECM and prostaglandins.[16] The interstitium of the adult kidney originates from the renal stroma of the developing metanephros.[15,53] During early metanephric development, fibroblast-like spindle-shaped stromal cells are detected around Pax2-positive cell of the cap mesenchyme and later on around nascent UBs and nephrons.[54] These stromal cells specifically express forkhead box transcription factor Foxd1.[54] Later in kidney development, stroma is divided into two distinct populations: cortical and medullary. Cortical stroma is characterized by the expression of Foxd1, retinoic acid (RA) receptors (RAR) α and β2, and retinaldehyde dehydrogenase 2, an enzyme required for most fetal RA synthesis. Medullary stroma expresses fibroblast growth factor 7 (FGF7), basic helix-loop-helix transcription factor Pod1 and bone morphogenic protein 4 (BMP4).[55-57] Whether molecular differences between these two stromal populations imply a lineage relationship is currently unknown. While medullary stromal cells eventually differentiate into mature interstitial cells, pericytes or neurons,[58] it is conceivable that a subpopulation of stromal cells that do not differentiate into other cell types may undergo apoptosis during late gestation to free space for LOH as they extend toward the tip of the papilla.[59] Whether apoptosis of medullary stromal cells indeed promotes elongation of LOH or collecting ducts during later stages of kidney development warrants further investigations.

Developmental functions of the metanephric stroma. Besides forming adult interstitium, stroma modulates glomerulogenesis and UB branching in the developing metanephros.[54,60,61] The contribution of stroma to morphogenesis of renal medulla is demonstrated by aberrant medullary development in mice that are deficient in genes expressed exclusively in the stroma (**Table 1**). Kidneys of *Foxd1*-null mice have a reduced number of UB branches and ectopic mesenchymal condensates in the medulla.[54] Genetic inactivation of *FGF7* in mice causes a decrease in the size of the UB compartment and in total kidney size.[62] *BMP4* mutants manifest hypoplastic-dysplastic kidneys, duplex kidneys with bifid ureters and hydronephrosis.[57] Combined absence of RA receptors RAR α and β2 or retinaldehyde dehydrogenase 2 causes formation of small kidneys with reduced number of UB branches.[61,63] In *Pod1*-null mice, fibroblast-like spindle-shaped stromal cells are absent and renal medulla does not form.[64] Similar phenotype is observed in mice that lack a homeodomain transcription factor *Pbx1*.[65] Notably, Pod1 expressed in the stromal, but not in the metanephric, mesenchyme is critical for the proper UB and glomerular development.[64] Mutations in cell cycle regulatory gene *p57*[KIP2], expressed in embryonic renal stroma, in mice leads to medullary hypodysplasia, a phenotype very similar to that of patients with Beckwith-Wiedemann syndrome (OMIM# 130650).[66] Simpson-Golabi-Behmel syndrome (OMIM# 312870), caused by mutations of heparin sulfate proteoglycan *GPC3*, leads to dysplastic renal medulla secondary to increased proliferation of UB cells followed by enhanced apoptosis of medullary collecting ducts.[7] These observations suggest that balance of cell proliferation and apoptosis is essential during morphogenesis of the renal medulla. Collectively, these findings indicate that aberrant stromal signaling is causally linked to medullary hypodysplasia.

Development of renal neuronal cells. *Anatomical development of renal nerves.* Despite recognition of the important role of the renal sympathetic nerve activity in the regulation of renin secretion, sodium reabsorption and vascular tone,[67] our knowledge of the role of the renal neuronal cells during metanephric development is still rudimentary. Sympathetic nerves grow into the embryonic kidney following the track of blood vessels.[68,69] Terminal axon arborization to allow innervation of multiple target cells also parallels vessel sprouting. Nerve fibers are first detected in the fetal rat kidney on the outer aspect of the main branches of the renal artery at E17.[69] The density of nerve fibers increases progressively with age and extends to afferent and efferent arterioles. On postnatal day 15 of life in the rat, nerve fibers are also detected in the medulla where vasa recta are developing.[68] The human fetal kidney, including renal medulla, is richly innervated during the 2nd and 3rd trimesters when collecting duct patterning and nephrogenesis continue.[70] In the mature rabbit kidney, neuropeptide Y-positive neurons are detected in the medulla where they regulate renal medullary perfusion.[71]

Factors that control development of renal nerves. The guidance of axons is directed by guidance molecules such as semaphorin 3A, Slits and their Robo receptors, Ephrins and their Eph receptors and others.[69] In the rat kidney, neurofilament-, L1 neural cell adhesion protein- and neurotrophin 3 nerve growth factor-positive neuronal precursor cells are present early in embryonic development.[72] Functionally, neurotrophin 3 promotes survival and induces differentiation of embryonic renal neuronal cells.[72] Of interest, L1 is expressed in the collecting ducts but not in the renal stroma. Expression of neurofilament in stromal cells raises the possibility that they may derive from the neural crest rather than from the mesoderm. In the central nervous system (CNS), AT2R promotes neurite outgrowth and elongation.[73] Since AT2R is expressed in the medullary stroma of the embryonic mouse kidney,[74] AT2R may influence nerve growth in the forming renal medulla. In addition to a potential role in the regulation of medulla morphogenesis per se, aberrant innervation of the renal medulla may be causally linked to development of programmed hypertension in the offspring in later life.[75]

Impact of lower urinary tract obstruction on structural development of the renal medulla. Murine models of renal hypodysplasia demonstrate that blunting of renal papilla and medulla may result from anatomical or functional obstruction of the lower urinary tract at the level of the renal pelvis or the ureter. Targeted deletion of calcineurin b1 (*Cnb1*) or hedgehog effector smoothened (*Smo*) in periureteral mesenchyme in mice causes nonobstructive hydronephrosis, hydroureter and medullary hypoplasia not associated with abnormal collecting duct morphogenesis.[76,77] Thus, medullary hypoplasia observed in these mice may result from backward pressure to the renal parenchyma due to urinary stasis. The mechanisms that have been implicated in functional ureteral obstruction in these mice include defective ureteral peristalsis (failure of ureteral peristalsis to propagate in a sequential proximal-distal direction), reduced number of ureteral pacemaker cells and decreased proliferation of periureteral mesenchymal cells.[76,77] Although medullary hypoplasia and hydronephrosis observed in angiotensin (Ang) II AT1 receptor (*AT1R*)-deficient mice may result from impaired ureteral peristalsis due to hypoplastic ureteral smooth muscle layer,[78] our previous studies suggest that aberrant branching morphogenesis of the UB may also contribute to medullary defects observed in these mutants.[79] The possibility that medullary hypoplasia may be due in some cases to an intrinsic defect in medullary morphogenesis, rather than to urinary tract obstruction, is supported by the findings that *Wnt7b*- and *Adamts 1/4*- or *Esrrg*-null mice exhibit hypoplastic renal medulla at birth in the absence of structural abnormalities of the lower urinary tract.[9,25,34] Of interest, renal tubular dysgenesis, a congenital and frequently fatal human disease due to mutations in *angiotensinogen*, *renin*, *angiotensin-converting enzyme* or *AT1R* genes is characterized by collapsed medullary collecting ducts and abundant interstitial fibrosis that occur in the absence of papillary hypoplasia or hydronephrosis.[80] The reasons for occurance of papillary hypoplasia and hydronephrosis in mice, but not in humans, with *AT1R* gene mutations remain to be determined. Defining embryonic origin and lineage of renal pacemaker cells and the mechanisms that regulate differentiation of these cells could lead to the discovery of new treatments for congenital functional obstruction of the urinary tract.

Conclusions and Perspectives

Although the renal medulla is essential for normal renal physiology, more work is needed to develop an integrated mechanistic understanding of its morphogenesis. A novel emerging paradigm suggests that the cross-talk among renal stroma, collecting ducts, LOH and vasculature is essential in spatiotemporal development of the renal medulla. Understanding the molecular cues for progenitor differentiation, directional elongation, patterning and remodeling, blood flow and blood-borne oxygen delivery, developmental transport physiology and collecting system contractility are fundamental biologic questions with applicability to multiple renal and extrarenal disease processes. Defining the mechanisms that direct morphogenesis of medulla offers opportunity to understand pathogenesis of CAKUT, identify biomarkers of risk and to conceive new preventive and therapeutic strategies. Future studies investigating the effect of spatiotemporal compartment/cell type-specific inactivation and epigenetic imprinting of the genes that govern morphogenesis of the medulla, identification of the association of known genetic mutations linked to medullary hypodysplasia with human CAKUT will provide essential information regarding the mechanisms whereby critical signaling networks direct formation of the medulla.

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