

REVIEW

A New Role for the Renin-angiotensin System in the Development of the Ureteric Bud and Renal Collecting System

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Abstract. The renin-angiotensin system (RAS) plays a critical role in kidney development. Mutations in the genes encoding components of the RAS or pharmacological inhibition of RAS in mice or humans cause a spectrum of congenital abnormalities of the kidney and urinary tract (CAKUT). The observed defects include renal vascular abnormalities, abnormal glomerulogenesis, renal papillary hypoplasia, hydronephrosis, aberrant ureteric bud (UB) budding, duplicated collecting system and renal tubular dysgenesis. Little is known about the potential role of Ang II and its receptors in the morphogenesis of the UB and renal collecting system. This review emphasizes a novel role for the RAS in the development of the UB, collecting ducts and renal medulla. We observe that UB and surrounding stroma express angiotensinogen and Ang II AT₁R receptors (AT₁R) *in vivo*. Ang II stimulates UB cell branching in collagen gel cultures *in vitro* and induces UB morphogenesis in intact whole embryonic metanephroi grown *ex vivo*. In contrast, treatment of metanephroi with the AT₁R antagonist candesartan inhibits UB branching. In addition, Ang II induces tyrosine phosphorylation of the epidermal growth factor receptor (EGFR) in UB cells. Furthermore, Ang II-stimulated UB morphogenesis is abrogated by inhibition of EGFR tyrosine kinase activity. In summary: 1) Ang II, acting via the AT₁R, stimulates UB branching; 2) This process depends on tyrosine phosphorylation of the EGFR. Together, these data indicate that cooperation of AT₁R and EGFR signaling performs essential functions during renal collecting system development via control of UB branching morphogenesis. (Keio J Med 57 (4) : 184–189, December 2008)

Key words: kidney development, renin-angiotensin, metanephros, ureteric bud

Introduction

Congenital abnormalities of the kidney and urinary tract (CAKUT) are the major cause of renal failure in childhood.¹ Many CAKUT have a genetic basis and are associated with hereditary human syndromes.² The inheritance pattern of some CAKUT (autosomal-dominant or recessive polycystic kidney disease) is well known.³ In other nonsyndromic CAKUT (obstructive uropathy, vesico-ureteral reflux, kidney aplasia, duplex kidneys), the hereditary mechanisms remain to be determined. In this respect, recent discovery of a number of genes involved in coordination of metanephric development pro-

vided a further impulse to explore in depth the pathogenesis of the CAKUT.

Overview of Metanephric Kidney Development

The development of the metanephric kidney begins when the nephric duct (ND) gives rise to ureteric bud (UB) on embryonic (E) day E10.5 in mice and E28 in humans (Fig. 1). The initial events controlling UB induction are regulated by numerous transcription factors and signaling molecules that are expressed in a specific spatial and temporal patterns.⁴ Signals from the mesenchyme induce the UB to originate from the ND, invade

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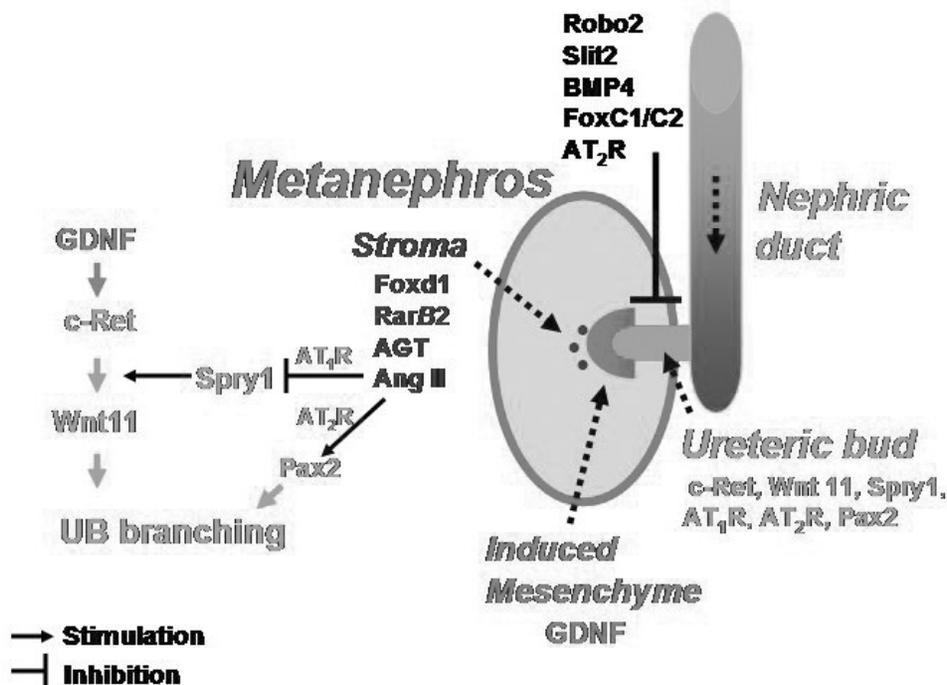


Fig. 1 Gene regulatory networks in kidney development. The nephric duct is formed from the intermediate mesoderm and extends caudally. On E10.5 in mice and E28 in humans, nephric duct forms an outgrowth, the ureteric bud (UB), which invades the mesenchyme. UB induces mesenchyme to undergo patterning into two compartments: induced (metanephrogenic) mesenchyme and stroma. Metanephrogenic and stromal mesenchymal compartments interact reciprocally with the UB to form the metanephros. Multiple gene regulatory networks have been shown to regulate UB elongation, branching and nephron formation. *Robo2*, *Slit2*, *BMP4*, *FoxC1/C2* and *AT₂R* act to restrict *GDNF* expression anteriorly and specify correct site of UB outgrowth from the nephric duct. *GDNF/c-Ret/Wnt11* pathway is the major positive regulator of UB development. Angiotensinogen (AGT) and Ang II are novel stromal factors that regulate UB morphogenesis. Ang II, acting via the *AT₁R*, inhibits *Spry1* expression and thereby releases inhibition of signaling via the *GDNF/c-Ret/Wnt11* pathway. Acting via the *AT₂R*, Ang II upregulates *Pax2*. This stimulates UB branching.

the mesenchyme and then branch repeatedly by a process called branching morphogenesis (Fig. 2). Initial generations of UB branches will be remodeled into the ureter and collecting system. Subsequent generations of UB branches will differentiate into collecting ducts. Collecting ducts will subsequently undergo patterning to contribute importantly to the renal papilla and medulla. Each UB tip is capable of inducing the adjacent metanephrogenic mesenchyme (MM) to undergo mesenchymal-to-epithelial transition (MET) and form nephrons (from the glomerulus to the distal tubule).¹² Therefore, UB branching morphogenesis is critical in determining total nephron number, proper kidney size and structure.

The *GDNF/c-Ret/Wnt11* signaling pathway is a major positive regulator of UB induction and branching in the metanephros (Fig. 1).⁵ Glial-derived neurotrophic factor (*GDNF*) is released from the MM and interacts with the *c-Ret* tyrosine kinase receptor expressed in the UB tip cells to induce branching.⁶ *GDNF/c-Ret* and *Wnt11* cooperate to maintain continued UB branching.^{6,7} The activity of the *GDNF-c-Ret-Wnt11* pathway is inhibited by *Spry1*.⁸ The correct site of initial UB induction is speci-

fied by restricting *GDNF* expression within the specified domain of the mesenchyme by *Robo2/Slit2/FoxC1/FoxC2*.^{9–11} Interestingly, mutations in the genes expressed in the stromal mesenchyme compromise both UB branching and MET.^{13,14} Therefore, even subtle aberrations in reciprocal interactions among the UB, MM and stroma have profound effect on the ultimate development of the kidney and appearance of CAKUT.

Role of the Renin-angiotensin System in Metanephric Kidney Development

The developing metanephric kidney expresses all the components of the renin-angiotensin system (RAS).^{15–17} The activity of the intrarenal RAS is high during fetal and neonatal life and declines during postnatal maturation. Renin mRNA and angiotensin (Ang) II levels are remarkably higher, respectively, in newborn than adult kidneys.^{15,18} *AT₁R* (*AT₁R*) and *AT₂R* (*AT₂R*) receptors are abundantly expressed in the nephrogenic area.¹⁹ The ontogenic expression of *AT₁R* and *AT₂R* in the metanephros differs. *AT₂R* is expressed earlier than *AT₁R*, peaks during

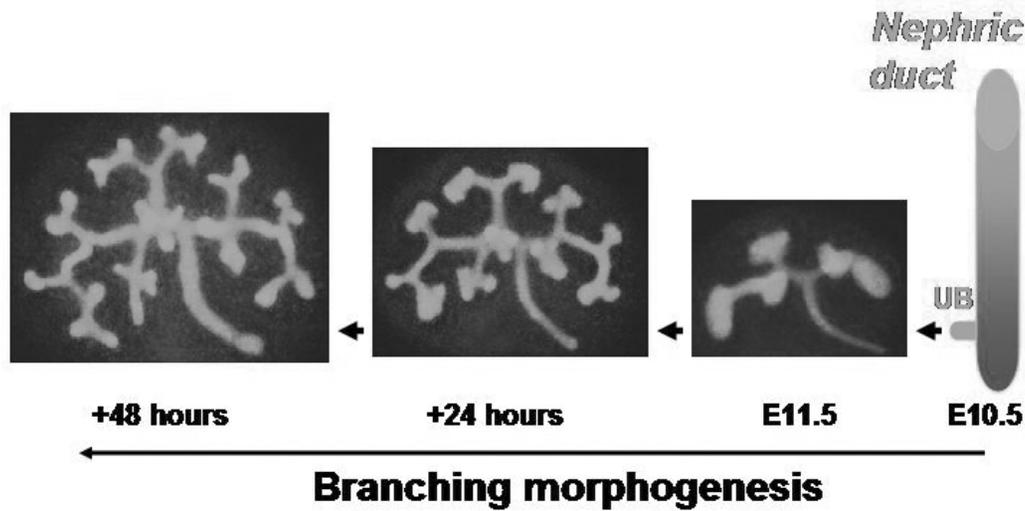


Fig. 2 Ureteric bud branching morphogenesis in the mouse. Ureteric bud (UB) emerges from the nephric duct on embryonic (E) day E10.5. Metanephros isolated on E11.5 from *Hoxb7*-GFP transgenic mouse embryo and grown *ex vivo* for 48 hours. *Hoxb7* promoter directs expression of green fluorescent protein (GFP) to the UB. The number of branching structures increases progressively with time.

fetal life and rapidly declines postnatally.^{19,20} AT_1R expression increases during gestation, peaks perinatally and declines gradually thereafter.²⁰ Spatially, AT_1R is present in glomeruli, in distal and proximal tubules, whereas AT_2R is expressed in mesenchymal cells adjacent to the UB stalk.

Genetic inactivation of the RAS components in mice or pharmacologic antagonism of the RAS cause a spectrum of abnormalities in the development of the ureter, renal pelvis and papilla.^{21–27} Angiotensinogen, angiotensin-converting enzyme (ACE) or AT_1R -deficient mice manifest hydronephrosis, hypoplastic medulla and papilla. Functionally, ACE and AT_1R null animals also have a reduced ability to concentrate urine.^{24,25} Elegant studies from Dr. Ichikawa's laboratory have suggested that antagonism of AT_1R signaling in ureteral smooth muscle cells impairs smooth muscle development in the ureter as well as its peristalsis.²⁸ Treatment with ACE inhibitors or AT_1R antagonists during gestation or postnatal metanephrogenesis leads to renal tissue dysplasia, a decrease in the number and size of glomeruli, renal arteries and delay in glomerulogenesis.^{29–31} Use of ACE inhibitors and AT_1R blockers in humans cause oligohydramnios and anuria.^{32,33} Therefore, these drugs should not be used by pregnant women.

AT_2R is expressed between the ND and the metanephrogenic mesenchyme anterior to the normal branching site in wild-type mice on E11.^{34,35} AT_2R -mutant mice demonstrate a decrease in the rate of apoptosis of mesenchymal cells around the nascent UB.³⁵ Mutations in the

AT_2R gene in mice are associated with increased incidence of duplex ureters and vesicoureteral reflux.³⁴ Thus, it is conceivable that AT_2R may mark mesenchymal cells destined to undergo apoptosis and that absence of timely apoptosis of these cells may cause an aberrant UB budding and lead to duplicated ureters. In addition, AT_2R may act as restricting signal to limit anterior expansion of the *GDNF* domain (Fig. 1).

Mutations in the genes encoding for angiotensinogen, renin, ACE or AT_1R in humans lead to renal tubular dysgenesis (RTD).^{36,37} In RTD, renal cortex exhibits a paucity of proximal tubules. In the medulla, collapsed collecting ducts and abundant interstitial fibrosis are observed.³⁷ Importantly, RTD in humans is characterized by perinatal death due to anuria combined with pulmonary hypoplasia. The more severe outcome in humans than in mice without the functional RAS may be due to the temporal difference in completion of nephrogenesis. In mice, nephrogenesis is completed 2 weeks after birth, whereas in humans- at 38 weeks of gestation. Collectively, the observed abnormalities in UB/medullary development imply that UB-derived structures are targets for Ang II actions during metanephric development in both mice and humans.

Role of the Renin-angiotensin System in Ureteric Bud Morphogenesis

To examine the importance of the RAS in UB development, we recently tested the hypothesis that Ang II, act-

ing via AT₁R, stimulates UB branching morphogenesis. Immunohistochemistry demonstrated that angiotensinogen and AT₁R are present in both UBs and the stromal mesenchyme on embryonic (E) day E12 in the mouse and that their expression increases progressively from E12 to E16.³⁸ Importantly, AT₁R immunoreactivity was present on both luminal and basolateral aspects of UB branches.³⁸ Since juxtaglomerular renin-producing cells originate from the mesenchyme on E11-E12, at a time when UB branching is just beginning,³⁹ Ang II may be generated locally in the mesenchyme to act in a paracrine fashion on the adjacent AT₁R-expressing UBs to regulate branching. AT₁R expressed in the mesenchyme may mediate mesenchymal Ang II signaling to stimulate UB branching. Since fibroblast growth factor (FGF) 7 is expressed in the stromal mesenchyme, and that Ang II increases FGF7 mRNA levels in luteal cells,⁴⁰ it is conceivable that Ang II may regulate UB branching via induction of FGF7 in the stroma.

Given that cultured UB-derived cells (UB cells) maintain expression of AT₁R mRNA and protein, we next investigated the direct role of Ang II and AT₁R in UB branching morphogenesis in UB cells.³⁸ Because utilization of UB cell culture model allows to avoid confounding influence of the mesenchyme, it is more relevant to define the direct role of Ang II in UB development. Ang II (10⁻⁵ M) increased the number and complexity of UB cells processes grown in three-dimensional collagen matrix gels. Ang II-induced increase in UB cell branching was prevented by AT₁R antagonist candesartan (10⁻⁶ M). These data demonstrate that Ang II stimulates cell process formation and branching cord extension in UB cells *in vitro* via activation of the AT₁R.

We further tested the role of Ang II in UB branching morphogenesis in the intact metanephric kidney where mesenchymal-epithelial interactions are intact. To determine the role of Ang II in early stages of UB development in the whole metanephros, we utilized embryonic kidneys obtained from Hoxb7-GFP transgenic mice which express green fluorescent protein (GFP) exclusively in the UB.⁴¹ Quantitative analysis of UB branching morphogenesis demonstrated that exogenous Ang II increased the number of UB tips and branch points compared to control.⁴² To examine the role of endogenous Ang II and AT₁R in UB branching in the metanephros, we utilized the AT₁R antagonist candesartan. Treatment of E11.5 metanephroi with candesartan (10⁻⁶ M) decreased the number of UB tips and branch points compared to control. These findings demonstrate that Ang II, acting via the AT₁R, stimulates UB branching morphogenesis in the intact metanephros.

The findings of angiotensinogen and renin in the stroma, and of AT₁R in the UB epithelia during active UB branching *in vivo*, together with AT₁R-mediated increase in UB branching *in vitro* suggest that angiotensinogen

and Ang II may represent novel stromal factors that regulate UB branching morphogenesis (Fig. 1). It will be crucial to delineate the effects of the AT₁R null mutation on expression of stromal factors necessary for proper UB branching (Foxd1, FGF7, Wnt2b, pbx, p57 and retinoic acid receptors). This will allow better understanding of the mechanisms regulating the development of the renal medulla and papilla.

Collectively, our data suggest that papillary hypoplasia in RAS-deficient mice is due, in part, to an intrinsic defect in UB branching and aberrant development of the renal medulla. Indeed, Ang II may regulate multiple steps of renal collecting system development. During early metanephric development, Ang II is formed in the stromal mesenchyme and acts on AT₁R and AT₂R located on UBs to stimulate branching (Fig. 1). Later in metanephric development, Ang II stimulates patterning of UB-derived collecting ducts, pelvis and ureter.

Given that stimulation of the epidermal growth factor receptor (EGFR) induces branching morphogenesis in murine inner medullary collecting duct (IMCD3) cells^{43, 44} and that renal papilla of angiotensinogen- and AT₁R-deficient mice exhibits reduced EGF mRNA levels,⁴⁵ we next investigated the contribution of EGFR activation to Ang II-induced UB branching. Ang II increased tyrosine phosphorylation of EGFR in UB cells.⁴² Inhibition of EGFR tyrosine kinase activity abrogated Ang II-induced cell process formation in UB cells and UB branching in the intact metanephros.⁴² These findings indicate that cooperation of AT₁R and EGFR signaling promotes the development of the renal collecting system.

Ang II can stimulate UB branching directly or through interaction with other factors present in the metanephrogenic mesenchyme, stroma or the UB that are known to regulate UB morphogenesis (Fig. 1). For example, Ang II may stimulate *Pax2* present in the UB and thereby enhance UB branching. This possibility is supported by the ability of Ang II to increase *Pax2* expression in metanephric kidney.⁴⁶ Since *GDNF/c-Ret/Wnt11* signaling plays a major role in promoting UB morphogenesis, we recently examined the role of this pathway in Ang II-induced UB branching. Our preliminary findings demonstrate that Ang II enhances *GDNF*, *c-Ret* and *Wnt11* expression in the UB tip cells and indicate that activation of this pathway by Ang II plays a critical role in Ang II-mediated signaling to stimulate UB development.⁴⁷ Since *Spry1* is a negative regulator of the *GDNF/c-Ret/Wnt11* pathway, we tested the hypothesis that Ang II inhibits *Spry1* gene expression. Our preliminary data indicate that Ang II downregulates *Spry1* expression in the UB in the intact embryonic kidneys grown *ex vivo*.⁴⁷ Furthermore, downregulation of *Spry1* expression is prevented by antagonism of the AT₁R. Therefore, Ang II may stimulate the *GDNF/c-Ret/Wnt11* pathway indirectly

via repression of *Spry1* (Fig. 1).

The role of AT₂R in UB branching is beginning to emerge. AT₂R-mutant mice exhibit ectopic ureteral budding and duplicated collecting systems.³⁴ This suggests that AT₂R inhibits aberrant UB budding. It is conceivable that unopposed stimulation of AT₂R in AT₁R-mutant mice may hinder UB branching. Our preliminary data demonstrate that AT₂R is expressed in UB branches during mouse nephrogenesis *in vivo*⁴⁸ and that AT₂R antagonist PD123319 inhibits Ang II-induced cell process formation in UB cells cultured in three-dimensional collagen matrix gels.⁴⁹ The ultimate effect of Ang II on UB branching may depend on the balance between AT₁R- and AT₂R-mediated actions.

The signaling events linking Ang II receptors to UB branching morphogenesis remain to be determined. Several signaling pathways, including MAP kinase and PI3K/Akt, have been shown to mediate the effects of AT₁R on renal cell proliferation.⁴³ Thus, one of the possible mechanisms leading to Ang II-stimulated tyrosine kinase signaling may involve EGFR- or c-Ret-mediated stimulation of MAP kinase and PI3K/Akt pathways.^{4,43} AT₂R-dependent mesenchymal apoptosis³⁵ may result from activation of MAP kinase phosphatase 1 leading to inactivation of ERK1/2.⁵⁰ AT₂R-dependent activation of Pax2 in whole metanephroi and mesenchymal cells is mediated by JAK2/STAT pathway²³.

In addition to stimulating UB morphogenesis, paracrine distal nephron RAS may regulate the expression of renal function genes, control terminal differentiation of the collecting duct cells and promote acquisition of their mature physiological function. These possibilities are supported by the following observations: 1) Ang II, acting via AT₁R, regulates urinary concentrating ability;²⁵ 2) In the adult animal, Ang II acts via basolateral AT₁R to stimulate luminal alkalization in rabbit cortical collecting duct;⁵¹ 3) In rat collecting duct, Ang II regulates H⁺-ATPase and basolateral K channel activities.^{52,53}

Perspectives

Future studies investigating the effect of UB- and stromal mesenchyme-specific inactivation of angiotensinogen, AT₁R and AT₂R on urinary system phenotype and the expression of the critical gene networks known to regulate UB branching, collecting duct/medullary/renal collecting system/ureteral patterning will provide essential information regarding the nature of events culminating in the formation of the mature metanephros.

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