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Kidney development: from ureteric bud formation to branching morphogenesis

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Summary

Epithelial branching morphogenesis is critical to formation of various organs such as the vasculature, mammary glands, lungs and kidneys in vertebrate embryos. One fascinating aspect of branching morphogenesis is to understand how a simple epithelial tube grows by reiterative branching to form a complex epithelial tree structure. Recent studies combining mouse genetics and chimeric analysis with live imaging have uncovered the molecular networks and interactions that govern kidney branching morphogenesis. This review focuses on ureteric bud formation and epithelial branching during kidney development. The invasion of the metanephric mesenchyme by the ureteric bud is a fundamental step towards establishing the cyto-architecture of the kidney and determining the number of nephrons, which form the filtration units of the adult kidney.

Introduction

Embryonic development of the vertebrate urogenital system requires progressive formation of three types of kidneys by the intermediate mesoderm: the pronephros, the mesonephros and the metanephros. In amniotes like mammals, the pronephros is a vestigial structure rapidly replaced by the mesonephros, which functions during embryonic development. The mesonephros will degenerate or differentiate into part of the reproductive system in the male [1]. In mouse, the Wolffian Duct (WD) grows rostro-caudally along the embryonic axis. As it elongates caudally it first induces the pronephros, then the mesonephros and finally the metanephros at the level of the midhind limb [1]. Metanephric kidney development is initiated around midgestation (E10.5) when the caudal part of the Wolffian duct (WD) starts to swell and an epithelial thickening called the ureteric bud (UB) forms and elongates to invade the adjacent metanephric mesenchyme (mm). Interactions between the metanephric mesenchyme and the tip of the UB lead to the establishment of complex epithelial-mesenchymal (e-m) signaling interactions [1–4]. These interactions are critical to initiation of outgrowth and repetitive branching of the UB that will generate the definitive renal collecting system and induce formation of renal vesicles. The renal vesicles form by a mesenchyme to epithelium conversion and are the precursors of the nephrons [1,3,4]. Nephrons are the basic functional units of the kidney and are essential for regulating the water content and removing soluble waste substances by filtering the blood [4].

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Conflict of interest

The author has no conflict of interests.

Understanding the molecular mechanisms underlying UB formation and branching morphogenesis is very important with respect to gaining insight into the etiology of human congenital malformations and diseases. Indeed, urinary tract malformations are among the most common birth defects in humans and represent almost 1% of all birth defects [5–7]. Outgrowth and correct positioning of the UB is critical as anomalies in this process account for the majority of all developmental defects affecting kidneys [7]. Moreover, the number of nephrons is primarily determined by the amount of branches generated during metanephric kidney morphogenesis. Therefore, defects in metanephric kidney branching morphogenesis may predispose people to future, chronic renal disease [4]. This review will focus on recent studies exploring the role of Ret/GDNF signaling during UB induction and the control of branching morphogenesis.

The molecular networks controlling ureteric bud formation

Kidney development is controlled by e-m signaling interactions that are mediated in large parts by the Ret/GDNF signaling pathway [2,8]. Indeed, genetic inactivation of *Gdnf* or its receptor complex results in complete renal agenesis or severe reduction in kidney size in mouse embryos [9–14]. *Gdnf* is expressed by the metanephric mesenchyme and signals through a receptor complex consisting of the receptor tyrosine kinase (RTK) Ret and the co-receptor GRF α , both expressed initially by the WD epithelium and later by the tips of the UB [13,15]. Upon stimulation by GDNF, Ret activates several downstream effectors pathways including the ERK MAPK, PI3Kinase/Akt and PLC pathways [2].

Recently, several groups have shown that β -catenin, a key component of canonical WNT signal transduction [16], is involved in the regulation of *Ret* expression. Using a specific Cre recombinase transgenic mouse strain, it was shown that targeted inactivation of β -catenin specifically in the epithelial cells forming the WD and UB results in either aberrant UB budding along the entire WD [17] or rapid cessation of branching morphogenesis, which causes renal agenesis [18]. These different effects after inactivating β -catenin in the epithelium are a likely consequence of incomplete mosaic inactivation, which results in an epithelium consisting of both wild type and mutant cells. Interestingly, it was shown that *Ret* expression is lost from β -catenin deficient WD cells, which also resulted in premature activation of genes normally expressed by differentiated epithelial cells such as the water channel proteins or the tight junction protein ZO1 [17]. On the contrary, cells that continue to express β -catenin also retain *Ret* expression, which reveals the cell autonomous effects of β -catenin. In summary, WNT/ β -catenin appears important regulators of *Ret* expression in WD/UB cells and seems to maintain the epithelium in an undifferentiated stage that allows progression of morphogenesis. However, the nature of the involved Wnt ligand remains unknown as none of the *Wnt* loss-of-function mutations reproduces the kidney phenotypes of β -catenin deficient mouse embryos [19–22].

Another important transcriptional regulator involved in the control of *Ret* expression is Gata3, a Zn-finger transcription factor. Genetic inactivation of *Gata3* results in formation of ectopic buds, renal agenesis and/or severe hypoplasia with associated loss of *Ret* expression [23•]. The observed phenotypes are strikingly similar to the ones observed upon conditional deletion of β -catenin from the WD epithelium (see above) and [17]. Indeed, *Gata3* expression is down-regulated in β -catenin mutant WD epithelial cells, while β -catenin expression is retained in the WD epithelium of *Gata3* deficient mouse embryos. In conclusion, these data suggest that Gata3 acts downstream of β -catenin in activation of *Ret* expression and prevents precocious differentiation of the WD epithelium, thereby allowing continued morphogenesis [17,18,23•].

The first step in UB formation is the swelling of the caudal part of the WD at the prospective budding site. It has been shown that caudal WD cells have a greater proliferative index than WD cells located more rostrally [24]. From mainly this observation, it was hypothesized that

the swelling of the WD was due to locally increased cell proliferation. However a recent study established that UB formation is mainly initiated as a consequence of directed cell movements within the WD epithelium rather than altered proliferation rates [25••]. Although *Ret* is expressed all along the entire WD epithelium prior to localized induction of the swelling, *Ret* activity is clearly heterogeneous as judged by using the pERK antigen as readout of *Ret*-mediated signal transduction. In response to such localized stimulation by GDNF induced *Ret* signal transduction, WD cells compete for inclusion in the forming primary ureteric bud based on their *Ret* activity levels. WD cells with high *Ret* signal transduction will out-compete cells with lower activity and move towards the region that will swell to form the ureteric bud. At the end of this process, the caudal WD epithelium is composed of two compartments: the swelling region that is composed of cells with high *Ret* activity and the adjacent region, which is mostly composed of cells with low or no *Ret* activity. The *Ret* positive cells included in the swelling then go on to form the UB that elongates toward the source of GDNF [25••] (Figure 1). This study begins to reveal the molecular and cellular mechanisms that underlie formation of the UB and shows that an essential step in this process involves cell competition based on *Ret* activity levels.

However, it is important to note that during this process the entire WD expresses *Ret* [15] and several studies using mouse embryos lacking particular genes [26–28••] in combination with *in vitro* kidney culture experiments [29,30•] have established that the caudal WD has the capability to form UB along its entire length. Therefore, additional levels of regulation must restrict formation of the ureteric bud to one single site.

There are different ways to inhibit UB formation by the WD such as inhibiting *Ret* activity by intra-cellular inhibitors directly in the epithelium or production of mesenchymal-derived signaling inhibitors that exert their effects on the epithelium. With respect to the latter, BMP4, a member of the TGF β -super family, is expressed by the mesenchyme surrounding the WD and was shown to inhibit UB outgrowth in culture. Addition of recombinant BMP4 to kidney explants in culture inhibits UB outgrowth [31] and mouse embryo heterozygous for a loss-of-function *Bmp4* mutation display ectopic buds at low frequency [32]. Interestingly, Gremlin1, a secreted BMP antagonist, is also dynamically expressed in a restricted mesenchymal region around the future budding site. Moreover, mouse embryos lacking *Gremlin1* display complete renal agenesis due to a failure of the UB to invade the metanephric mesenchyme [30•,33]. Initially, all genes required for initiation of UB outgrowth are normally expressed and a UB forms in *Gremlin1* deficient mouse embryos albeit its shape being rather abnormal. However, initiation of its outgrowth is blocked resulting in down-regulation of *Gdnf* expression in the adjacent mesenchyme. This block results in massive mesenchymal cell death due to the failure to induce the e-m signaling interaction between the UB and the metanephric mesenchyme [33]. Interestingly, reduction of the *Bmp4* gene dosage completely restores UB outgrowth and epithelial branching in *Gremlin1* deficient mouse embryos in agreement with the proposal that high BMP activity represses UB outgrowth and branching morphogenesis [30•] (Figure 2). These results place Gremlin1 in a critical position during induction of kidney branching morphogenesis as its activity in the mesenchyme enables the *Ret* positive epithelial tip, i.e. the UB, to invade the metanephric mesenchyme and initiate branching morphogenesis.

Furthermore, Sprouty1 plays an important function in inhibition of *Ret*-mediated signal transduction directly in the WD epithelium. Sprouty1 is an intra-cellular inhibitor of RTK signaling. Although, the exact mechanism by which Sprouty1 acts is not clear, it was shown that Sprouty1 inhibits ERK signal transduction [34]. Inactivation of *Sprouty1* results in aberrant formation of extra buds along the caudal WD in mouse embryos [28••]. Interestingly, reduction of the *Gdnf* gene dosage in *Sprouty1* deficient mouse embryos inhibits the formation of additional buds [28••]. Taken together, these results indicate that Sprouty1 inhibits *Ret* activity probably via inhibition of ERK phosphorylation, which contributes to restricting UB formation

to one single site. In summary, UB formation requires cell movements within the WD epithelium that are mediated by high Ret activity. Therefore, tight regulation of Ret-mediated signal transduction is critical to normal metanephric kidney development.

Branching morphogenesis and formation of the UB tree

During invasion of the metanephric mesenchyme, the UB generates “tip-trunk” identities and tip cells that function as a signaling center under control of Ret activity [29]. In fact, most proliferation during epithelial branching occurs in the tip region, which expresses genes regulating branching morphogenesis [35]. At this stage, tip cells engage in rapid expansion, which initiates outgrowth of the UB and is followed by bifurcation of its tip [36] (Figure 3).

During branching, GDNF continues to play a pivotal role and its importance has been analyzed in detail. For example, only 50% of all kidneys develop normally in *Gdnf* heterozygote mice, the others display branching defects ranging from reduced kidney size to complete renal aplasia [2]. In contrast, UB tips are aberrantly enlarged and abnormal branching patterns in association with increased *Gdnf* expression are detected in developing kidneys of *Sprouty1* deficient mouse embryos [37]. Interestingly, genetic reduction of *Gdnf* rescues most of the branching defects observed in *Sprouty1* deficient kidneys. Moreover, the hypomorphic phenotype (smaller kidneys) observed in *Gdnf* heterozygous mice is also rescued by reducing the *Sprouty1* gene dosage [37]. These experiments point to an important aspect of signaling during kidney branching morphogenesis, namely that is not a particular signal that is decisive, but the balance between activating (GDNF) and inhibitory (*Sprouty1*) signals received by cells.

During branching morphogenesis, other RTK signaling pathways also participate in regulating branching of the UB. FGF signaling via FGFR2 plays a significant role as epithelial branching is reduced in mouse embryos lacking *Fgfr2* in the WD/UB epithelium [38]. Similarly, branching morphogenesis is also reduced in *Fgf7* and *Fgf10* deficient mouse embryos [39, 40]. Moreover, HGF/Met and EGF/EGFR were recently shown to participate in branching morphogenesis [41•]. Although, inhibition of these growth factors in kidney rudiment cultures inhibits UB branching [42–44], their genetic inactivation does not impair branching morphogenesis significantly [45–47]. However, *Met* deficient mouse embryos are characterized by increased *Egfr* expression during kidney development [41•]. Interestingly, the generation of the *Met-EGFR* double mutant causes more severe branching defects than the one observed in single mutant embryos. These results indicate that Met and EGFR interact synergistically during kidney epithelial branching morphogenesis. In conclusion, this study points to compensatory effects among different growth factors during kidney morphogenesis. Such compensatory mechanisms could explain why the addition of recombinant factors to kidney rudiments in culture causes more significant alterations than expected from the rather subtle phenotypes following their genetic inactivation in mouse embryos (see above) [41•]. Last but not least, these studies reveal that other RTK signaling pathways are required for normal kidney epithelial branching morphogenesis albeit Ret/GDNF signaling being the key regulatory pathway. These pathways may act in parallel or sequentially to Ret/GDNF signaling but additional analysis is required to identify the relevant interactions and underlying networks that orchestrate branching morphogenesis.

For example, the functions of Wnt signaling during initiation (see above) and progression of branching morphogenesis remain largely elusive. To date only Wnt11 is known to be directly required for normal branching morphogenesis. Mice deficient for *Wnt11* display reduced kidney sizes, which correlates with reduced Ret/GDNF signaling [22]. In *Wnt11* deficient mouse embryos, *Gdnf* expression is reduced in the mesenchyme and conversely, *Wnt11* expression is down-regulated in *Ret* deficient kidney rudiments [22]. It was concluded that GDNF activates *Wnt11* expression in the *Ret* expressing UB tip cells and that Wnt11 in turn

maintains and reinforces *Gdnf* expression in the adjacent metanephric mesenchyme [22]. Therefore, *Wnt11* is part of a positive feedback loop that potentiates GDNF signaling to enable progression of epithelial branching morphogenesis (Figure 2).

An important aspect of UB branching morphogenesis is specification of the branching pattern over space and time. GDNF has long been thought to be the factor that determines where the next branches will be generated [2,48]. However, several attempts to verify this hypothesis have failed to provide conclusive insights and it remains unclear if and how GDNF would exert its function as a chemo-attractant that would direct UB tip outgrowth toward the GDNF source [49]. In this respect, the specific restriction of the expression of e.g. *Ret* and *Wnt11* to the tip of the UB might have significant implications in terms of regulating the epithelial branching pattern. Another way to control the branching pattern could be imposed by external forces independent of Ret/GDNF signaling. Interestingly, *Bmp4* and *Gremlin1* remain expressed in a complementary pattern during progression of branching. *Bmp4* is expressed along the ureter and UB stalks and *Gremlin1* in the metanephric mesenchyme surrounding the UB tips ([30] and unpublished). At this stage, BMP4 is required for differentiation of the ureter formed by the proximal part of the UB stalk that does not invade the metanephric mesenchyme [50]. Moreover, addition of BMP4 to kidney rudiments in culture inhibits branching morphogenesis [31]. Conversely, supplementing kidney rudiments in culture with Gremlin1 causes aberrant extra budding from the WD and distal UB stalk and perturbs the regular epithelial branching pattern [30]. In agreement, mouse embryos lacking the BMP4 receptor *Alk3* display an initial increase in branching, which is followed by a marked reduction resulting again in abnormal branching [51]. Therefore, the Gremlin1/BMP pathway may be part of the genetic network that regulates the branching pattern in time and space.

Conclusions

Although much is still to be learnt with respect to the molecular and cellular mechanisms that underlie establishment of the UB epithelial branching network, the development of genetic tools such as mouse mutants that allow generation and analysis of kidney development in chimeric embryos has begun to facilitate these studies. Moreover, the results obtained from such clonal analysis in combination with 3D imaging of branching morphogenesis make the developing kidney an attractive model system to identify and study the basic developmental mechanisms and networks that govern epithelial branching morphogenesis in vertebrate embryos. The analysis of different epithelial branching modes is not only important to uncover the extent of evolutionary conservation of the underlying mechanisms, but is necessary to understand how possibly rather similar principles are used to generate organ specific branching patterns.

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Figure 1. Cell movements and heterogeneous Ret signaling during ureteric bud formation

Diagram illustrating rearrangement of Ret “positive” (blue) and Ret “negative” cells (green). Gray ovals represent the metanephric mesenchyme. Initially Ret “positive” cells are dispersed along the WD (A) and start to move (yellow arrows) to form the primary ureteric bud (UB) tip domain (B). As the UB grows out, these cells “lead” to form the distal tip, while Ret “negative” cells follow (C, red arrows). Reproduced with permission from [25]

Figure 2. Molecular interactions of the signals and antagonists participating in kidney epithelial branching morphogenesis

The Ret/GDNF pathway is restricted to the tip of the developing UB epithelium to insure progression of branching morphogenesis. Correct activity of Ret is assured by positive (Wnt11) and negative (Sprouty1) feedback signaling. Other RTK signal pathways such as FGF, EGF and HGF also positively contribute to this feedback loop. Along the ureteric stalk, BMP4 signaling exerts an inhibitory effect on branching morphogenesis. Inhibition of BMP4 by Gremlin 1 in the mesenchyme surrounding the UB tip region could enable branching morphogenesis.

Figure 3. *In vivo* visualization of UB branching morphogenesis

(A) A metanephric kidney rudiment (E12.5) cultured on a Transwell filter at the air/surface interface at 37°C for 48 hours. The epithelium is marked by GFP expression using the HoxB7-mVenus transgene [52]. (B) The epithelial branching tree visualized in an E15.0 kidney by confocal microscopy to reveal the complex 3D architecture of the UB tree.