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Cell and molecular biology of kidney development

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Abstract

Abnormalities of kidney and urinary tract development are the most common cause of end-stage kidney failure (ESKD) in childhood in the U.S. (NAPRTCS 2006). Over the past 20 years, the advent of mutant and transgenic mice and manipulation of gene expression in other animal models has resulted in major advances in identification of the cellular and molecular mechanisms that direct kidney morphogenesis, providing insights into the pathophysiology of renal and urologic anomalies. This review focuses on the molecular mechanisms that define kidney progenitor cell populations, induce nephron formation within the metanephric mesenchyme, initiate and organize ureteric bud branching, and participate in terminal differentiation of the nephron. Highlighted are common signaling pathways that function at multiple stages during kidney development, including signaling via Wnts, bone morphogenic proteins (BMPs), fibroblast growth factor (FGF), sonic hedgehog (shh), GDNF/Ret, and *notch* pathways. Also emphasized are the roles of transcription factors *Odd1, Eya1, Pax2, Lim1* and *WT-1* in directing renal development. Areas requiring future investigation include the factors which modulate signaling pathways to provide temporal and site specific effects. The evolution of our understanding of the cellular and molecular mechanisms of kidney development may provide methods for improved diagnosis of renal anomalies and, hopefully, targets for intervention for this common cause of childhood ESKD.

Keywords

genes; kidney development; metanephric mesenchyme; ureteric bud; progenitor; nephron; differentiation

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Abnormalities of kidney and urinary tract development, including aplasia/dysplasia, vesicoureteral reflux (VUR) and obstructive uropathies are the most common cause of renal failure in childhood in the U.S., comprising 31% of children with end-stage kidney disease (NAPRTCS 2006). Over the past 20 years, the advent of manipulation of gene expression in mice and other animal models has resulted in major advances in identification of the cellular and molecular mechanisms that direct kidney morphogenesis, providing insight into the pathogenesis of these anomalies. Yet many discoveries remain to be made, and questions are still unanswered.

The original studies of kidney development involved descriptive studies of the morphologic changes, including seminal work by Edith Potter on human fetal kidneys.[1,2] There are two embryonic kidney precursors, the pronephros and mesonephros. Remarkably, these precursors take form and then involute and yet are necessary for development of the definitive kidney, as

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interruption of their development results in renal agenesis. The pronephros, composed of simple tubules that empty into a pronephric duct, originates from nephrogenic cords of intermediate mesoderm. Subsequently, as the pronephros begins to regress, the mesonephros arises at its caudal end and matures into well developed nephrons with vascular glomeruli connected to proximal and distal tubules that drain into the mesonephric duct (also known as the Wolffian duct). The mesonephros will ultimately fuse with the cloaca, and contributes to formation of the urinary bladder. The last embryonic kidney, the metanephros, is formed as the ureteric bud branches out of the caudal end of the Wollfian duct (Figure 1). Reciprocal interactions between the ureteric bud and the metanephric mesenchyme result in nephron induction, and a subset of cells within the mesenchyme coalesce (forming "condensates") and develop an epithelial phenotype (known as a mesenchymal-epithelial transition). The ureteric bud branches in a highly reproducible manner, and nephrons are induced at each ureteric bud tip. These branches will form the collecting system, including collecting ducts, renal pelvis, ureter, and bladder trigone. At the same time, the epithelial cells undergo a stereotyped sequence of morphologic changes, starting as a sphere of cells (the vesicle), becoming a comma, and then an S-shape body. Three segments of the S-shape body emerge, oriented with the distal segment adjacent to the ureteric bud tips: the proximal segment differentiates into glomerular epithelial cell (podocytes), the mid-section forms the proximal tubule and loop of Henle, and the distal segment becomes the distal tubule and joins with the ureteric bud branches.

Vascular development in the kidney occurs concurrent with glomerular development. There is evidence that the vasculature may arise from both progenitor cells within the metanephric mesenchyme (angiogenesis) as well as penetration of developing mesenchyme by existing vessels (via vasculogenesis)[3,4]. Endothelial cells migrate into the vascular cleft of the S-shape body and differentiate to form the fenestrated glomerular endothelium.[4] While neurogenic factors are expressed in the developing kidney and have been shown to play a role in ureteric bud branching,[5] little is known about the factors which induce kidney innervation, although a recent description of renal nerves may provide a platform to investigate which genes are involved[6].

Human kidney development begins as early as the third week of embryonic development, with formation of the pronephros, followed by the mesonephros at 4 weeks and the metanephros at 5 weeks gestation. The first glomeruli appear at nine weeks in humans, and nephrogenesis is complete by 36 weeks gestation. In mice, in whom gestation lasts approximately 21 days, the ureteric bud forms from the Wollfian duct at embryonic day 9 (E9), nephron induction begins at E10.5, the first glomeruli appear at E14.5, and nephrogenesis continues after birth for two weeks.

The central question of renal development is how does one "make" a kidney. To answer this, investigators have used genetic manipulation in a variety of animal models to dissect out the cellular and molecular signals which define the morphologic transitions. This review focuses on major signaling pathways and transcription factors that have been identified which coordinate cell fate determination, migration, proliferation and differentiation required for kidney development. It is notable that the same signaling pathways and transcription factors may play distinct roles depending on the spatial and temporal context; for example, *Wnt/β-catenin* signaling plays different roles in the metanephric mesenchyme and ureteric bud, while *Pax2* signaling functions early as a determinant of nephron progenitors and late in terminal differentiation of specific nephron segments.[7-11] Thus, relatively few molecular mechanisms are capable of directing a diverse sequence of events during kidney organogenesis; the current challenge is to identify the molecular modifiers that provide the temporal and cell specific effects.

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What genes define kidney progenitor cells?

One of the earliest genetic markers of kidney progenitor cells in both chickens and mice is the transcription factor *Odd skipped related1* (*Odd1* or *Osr1*).[12,13] Chickens, like mammals, form 3 embryonic kidneys; their rapid development and accessibility of developing embryos have made them a useful model for studying gene expression profiles. *Osr1* expression in developing chick kidneys localizes to intermediate mesoderm and then to the mesenchyme surrounding the mesonephros[13]. Recently, genetic fate mapping has clarified further the gene expression patterns of *Osr1*; inducible genetic labeling of *Osr-1* expressing cells demonstrated that early in development (before E9.5) *Osr-1* cells are multipotent, and contribute to nephron and collecting duct epithelia and the cortical interstitium; however, later in development *Osr-1* transcription becomes gradually restricted to the developing cap mesenchyme (the mesenchyme at the tip of the ureteric bud which undergoes active nephrogenesis).[14] Gene deletion of *Odd1(Osr1)* in mice results in failure to form a metanephric mesenchyme[12]. The function of *Odd1(Osr1)* was further examined in zebrafish. Zebrafish are a useful model for studying the genetic determinants of intermediate mesoderm cell fate, for while they develop solely a pronephric stucture, their development occurs rapidly (within 2 days), visibly (embryos are translucent), and genes can be manipulated with morpholinos in a temporal and spatial specific manner.[15] In the zebrafish model, *Odd1(Osr1)* acted as a transcriptional repressor and directed cell fate towards kidney rather than vascular phenotype; loss of *Odd1(Osr1)* function resulted in either complete absence of a pronephric structure, or increased vascular cells at the expense of renal progenitor cells.[16,17] In mice, *Odd1* expression was required for expression of other transcription factors, including *Eya1*, *Pax2*, *Six2*, *Sall1*, and *GDNF*, indicating that *Odd1* acts upstream of these pathways[12].

It was shown by tissue recombination experiments that a subpopulation of cells within the mesonephros and metanephric mesenchyme formed renal tubules in the presence of inductive signals, suggesting that this subset of cells may express specific genes that define them as nephron progenitors[18]. Several transcription factors are expressed in these nephron progenitors, including LIM-class homeodomain transcription factor *Lim1*, *Pax2*, *Eya1*, homeobox family members *Six 1, 2*, and *4*, *Sall1* and *WT-1*. These factors are required for nephron development, and loss of their function in the developing metanephric mesenchyme results in either renal agenesis or hypoplasia. There is an apparent hierarchy amongst the transcription factors, and several physically interact, resulting in complex regulation of DNA binding and transcriptional activation versus repression (figure 2). For example, *Eya1* is capable of binding both *Pax2* and *Six1*: the *Eya1-Pax2* complex upregulates *Six2* and *GDNF*, while the binding of *Eya1* to *Six1* turns *Six1* from a repressor to an activator and upregulates *Pax2*, resulting in a positive feedback loop.[19,20] Together, *Six1-Eya1-Pax2* act synergistically and upregulate *GDNF*.[21,22] Both *Six1* and *Six4* are required for *GDNF* expression, and *Six1* induces transcription of *Sall1*.[20,23,24] *Sall1* deletion in the metanephric mesenchyme results in renal agenesis, not because of a mesenchymal cell autonomous role for *Sall1* (*Sall1*$^{-/-}$ mesenchyme can be induced in vitro), but rather because *Sall1* is required to allow invasion of the ureteric bud into the metanephric mesenchyme that provides the signals for ongoing nephrogenesis[25]. Interestingly, *Six2* upregulates *GDNF* and appears to maintain the metanephric mesenchyme in a dedifferentiated state; this is important because differentiation of the metanephric mesenchyme results in the cessation of *GDNF* stimulated ureteric bud branching (see below)[26]. Thus, *Six2* maintains the population of renal progenitor cells required for nephron formation[27]. The homeobox transcription factor *Lim1* is a downstream target of *Pax2*; gene deletion of *Lim1* in the metanephric mesenchyme halts development at the renal vesicle stage and its own targets include *Notch* patterning genes.[10,28,29] The gene mutated in Wilms tumor, *WT-1*, is a transcription factor with functions both early and late in nephron development; it is required in metanephric mesenchyme but also plays later roles in podocyte differentiation.[30] In the metanephric mesenchyme, *Pax-8* may activate *WT-1* expression[31].

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WT-1 is required to for metanephric mesenchymal survival, but the mechanism is unclear: it has been proposed that *WT-1* contributes to maintaining *Pax-2* expression via stimulation of *VEGF-A*, as *Flk1* signaling upregulates *Pax2* in metanephric mesenchyme, yet the metanephric mesenchyme of *WT-1*$^{-/-}$ mutant embryos express *Pax2*.[32,33]

Remarkably, gene mutations in several of these transcription factors have been identified in syndromes characterized by renal hypodysplasia, including *Sall1* (Townes-Brocks syndrome), *Eya1-Six1* (branchial-oto-renal syndrome), and *Pax2* (renal-coloboma syndrome).[34] A mutation was also identified in a related transcription factor, *Sall4*, in Okihiro syndrome; *Sall1* was found to be required for proper localization of *Sall4*, indicating that the *Sall1* phenotype (Townes-Brocks) may be mediated in part by loss of *Sall4* function.[35]

Stromal and vascular progenitors in the metanephric mesenchyme

The cells of the metanephric mesenschyme cells which do not coalesece to form the developing nephron are generally held to form the stroma and the renal capsule. *FoxD1* was recently identified as marking the progenitors of the renal capsule. Remarkably, loss of *FoxD1* function resulted in fused pelvic kidneys that lost their radial organization, suggesting that *FoxD1* signaling contributes to patterning by defining the boundaries that limit nephron induction to appropriate zones within the metanephric mesenchyme[36].

Vascular progenitors within the metanephric mesenchyme or angioblasts, have been identified by their expression of the *VEGF-A* receptor, *VEGFR2* (*Flk1)*; *VEGF-A* acts as a chemo-attractant to these vascular progenitors, directing cell migration towards the developing nephron.[37,38] Recently, a subpopulation of stromal cells that are *FoxD1* negative have been shown to express *C-kit*, a receptor for stem cell factor (*SCF*); *SCF* secreted from the ureteric bud may expand this cell population[39]. These *C-kit* positive cells appear to be a source of vascular progenitors, as a subset also express *Flk1*.

Notably, while multiple progenitors cell populations have been identified within metanephic mesenchyme, there is a relative paucity of data regarding genes that define ureteric bud progenitors within the Wollfian duct, as the focus has been on molecular signals which organize ureteric budding and branching; further investigation will be required to identify the genes which define ureteric bud progenitors.

Molecular patterning in the metanephric mesenchyme and ureteric bud

There are conserved pathways for spatial organization of morphogenesis that contribute to kidney patterning. The homeobox (*Hox*) genes (1-13) are a family of transcription factors involved in body segmentation. *Hox11* is expressed in the intermediate mesoderm and loss of function results in kidney agenesis as a result of failure to form a ureteric bud[21]. Studies of 4 *Hox11* paralogues (*Hoxa11-Hoxd11)* demonstrated functional redundancy between *Hoxa11* and *Hoxad11*, with dual loss of *Hoxa11* and *Hoxad11* recapitulating the phenotype of complete loss of *Hox11*. *Hoxa11* and *Hoxd11* are expressed in a restricted region within intermediate mesoderm, thereby controlling development of the dorso-ventral renal axis.[40,41] *Hox11* paralogues are required for *Six2* and *GDNF* expression in the metanephric mesenchyme, indicating a mechanism by which they may affect the position of ureteric bud outgrowth (see below).[40] Of note, the restricted expression of another *Hox* gene, *HoxB7*, to the branching ureteric bud has proved useful for visualization of the branching both in vivo and in vitro with the *HoxB7-GFP* mouse and for generating ureteric bud lineage specific transgenic mice.[42,43]

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What are the molecular signals that induce the ureteric bud to arise from the Wollfian duct?

The ureteric bud emerges as a single outgrowth from a stereotyped site of the Wollfian duct caudal to the hindlimb. *GDNF* signaling via its receptor *Ret* is the major trophic factor for ureteric budding: $GDNF^{-/-}$ and $Ret^{-/-}$ mutants fail to form a ureteric bud and die perinatally with agenesis of both kidneys and ureters.[5,44-49] As indicated above, *GDNF* expression in the metanephric mesenchyme is upregulated by multiple transcription factors (*Eya1, Pax2,* and *Six 1,2* and *4*). *GDNF* is a secreted growth factor, and its receptor, *Ret* is a protooncogene and tyrosine kinase receptor expressed in the Wollfian duct, with the highest expression level at the site of ureteric bud outgrowth and later at the tips of the ureteric bud.[49] Vitamin A (or retinoids) are required for *Ret* expression.[50] Signaling via *Ret* stimulates cell proliferation (via *PI-3K/AKT* and *ERK* pathways) and migration, resulting in invasion of the ureteric bud into the metanephric mesenchyme[51-53]. Mutations in *Ret* are associated with renal tumors, but also have been recently identified in fetuses with renal agenesis.[54,55]

Several other genes contribute to specifying the unique origin of the ureteric bud. *Sprouty*, a tyrosine kinase inhibitor, acts to modulate sensitivity of *Ret* cells to *GDNF* signals, and is required for a unique origin of the ureteric bud[56]. Gene deletion of *FoxC1* resulted in multiple ureteric buds, leading to duplicated ureters with abnormal bladder insertion and hydronephrosis[57]. *FoxC1* was determined to restrict *GDNF* expression within the intermediate mesoderm. A chemo-repellent ligand-receptor pair involved in neural guidance, *Slit2-Robo2,* also functions to limit the expression of *GDNF*, and gene deletion of either *Slit* or *Robo* resulted in multiple, rather than a single, ureteric bud.[58] The relevance of these genes to human kidney disease was confirmed by finding of mutations in *FOXC1* and *ROBO2* associated with congenital anomalies of the kidney and urologic tract (CAKUT) and vesicoureteral reflux (VUR), respectively (VUR is held to result from abnormal insertion of the ureter into the bladder).[59,60]

What are the molecular signals that contribute to ureteric bud branching and patterning?

The ureteric bud outgrowth undergoes serial branching, defining the kidney architecture. Nephron are induced at the tips of the branching bud, so that ureteric bud branching also determines nephron number. *GDNF* is an important stimulant of ureteric bud branching; remarkably, localization of its receptor *Ret*, initially expressed throughout the ureteric bud, becomes restricted to a tip region of the ureteric bud.[61,62] This appears to regulate sites of cell proliferation and branching. Overexpression of *Ret* in ureteric bud resulted in small, cystic kidneys and VUR[63]. While *GDNF* is a major trophic factor for bud branching, its effects are modulated by several other growth factors as well as inhibitors of bud branching. Bud branching is stimulated by *Angiotensin-II, VEGF, Protein kinase X* and inhibited by *transforming growth factor β (TGF-β)* and *Semaphorin3a*.[64] *Angiotensin II* activates both a*ngiotensin receptors type 1* and *2* on the ureteric bud to stimulate branching and is also required for elongation of the collecting duct. *Renin-angiotensin* system (*RAS*) blockade results in renal agenesis, and mutations in the *RAS* have also been identified in renal tubular dysgenesis and congenital obstructive uropathy.[65,66] Interactions between cells and the extracellular matrix contribute to both ureteric bud branching and nephron induction (see below). Gene deletion of *Glypican3*, a cell surface heparin sulfate, stimulates early excessive ureteric bud branching and later in development induces apoptosis and loss of medullary collecting ducts.[67,68] Other pathways (Wnts, sonic hedgehog (shh), bone morphogenic proteins (BMPs) and *fibroblast growth factors (FGFs))* which control ureteric bud branching are discussed separately below, emphasizing the central role of these signaling mechanisms in renal development.

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What are the molecular signals that result in nephron induction?

Reciprocal interactions between the ureteric bud and the metanephric mesenchyme are required for nephron induction with transformation of mesenchyme to an epithelial cell phenotype. Signaling via *Wnts* provide major molecular signals of this transition. Early studies identified that the metanephric mesenchyme could be induced to form nephron epithelia by multiple tissues in vitro, including spinal cord; spinal cords secrete several *Wnts*. In vivo, the tips of the ureteric bud are the source of signals for induction of nephrons: *Wnt9b* is produced by ureteric tip cells and stimulates *Wnt-4* expression in the metanephric mesenchyme. *Wnt4* is require by the metanephric mesenchyme for differentiation into nephron epithelia in vivo. [69] Other *Wnts*, including *Wnt 1,3,7* and *11* can replicate *Wnt4* induction of metanephric mesenchyme in vitro.[70] *Wnt-4* expression is stimulated by the transcription factor *Pax-2*.[71] As indicated above, the extracellular matrix may contribute to *Wnt4* induction, as inhibition of glycosaminoglycans prohibits epithelial transformation in vitro.[72] Furthermore, loss of *heparan sulfate 2-sulfotransferase (HS2ST)* expression in vivo inhibits aggregation of the metanephric mesenchyme and results in renal agenesis.[73]

Growth factors also are capable of inducing nephrons, and may augment epithelial induction, including *fibroblast growth factors*, *FGF2* and *FGF7, leukemia inhibitory factor (LI*F) and *TGFβ2*. The contribution of *TGFβ* signaling contribution is highlighted by the fact that deletion of its intracellular effector *Smad4* from mesenchyme impaired nephron induction. The transcription factor *Lim1* is also required for nephron induction, and may act to upregulate other genes involved in nephron segmentation and differentiation such at the *Notch* pathways.[28]

A few molecular signaling pathways coordinate multiple aspects of renal development

Common molecular signaling pathways with conserved roles in cell fate determination, proliferation, migration and differentiation during tissue morphognesis regulate kidney organogenesis. These pathways have distinct site and time specific effects. Thus, signaling by canonical *Wnt/β-catenin* and *sonic hedgehog* (*shh*) pathways, as well as by *bone morphogenic proteins (BMP)* and *fibroblast growth factors* (both members of the *TGF-β* signaling superfamily) contribute both to nephrogenesis within the metanephric mesenchyme and to ureteric bud branching (figure 3).

Canonical Wnt/β-catenin signaling

As discussed above, *Wnt* signals play key roles in induction of the mesenchymal-epithelial transformation during nephrogenesis. *Wnts* are ligands for transmembrane frizzled receptors[74]. Binding of *Wnts* to *Frizzleds* and co-receptors *LRP 5/6* recruits *Disheveled* to the cell membrane. The activated *Disheveled* inhibits *glycogen synthase kinase (GSK-3β)* a serine threonine kinase that phosphorylates *β-catenin* and marks it for degradation. Thus, canonical signaling by *Wnts* results in stabilization of *β-catenin*, which translocates to the nucleus to initiate transcription of downstream mediators, including cell proliferation factors *CyclinD1* and *C-myc*. Canonical *Wnt* signaling plays a role in induction of the metanephric mesenchyme and in ureteric bud branching.[7,8,69] The tissue specific roles of *β-catenin* were elegantly demonstrated by gene deletion in the metanephric mesenchyme and ureteric bud, respectively, by breeding mice with *Cre-recombinase* under control of tissue specific promoters with mice with *β-catenin* flanked by *lox-P* sites, allowing for recombination in the presence of the *Cre-recombinase*. Gene deletion in the ureteric bud lineage (*HoxB7-Cre*) resulted in renal agenesis and hypoplasia secondary to impaired ureteric bud branching; this appeared to be a result of expression of premature differentiation with failure to maintain the cap mesenchyme in the

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precursor necessary state.[7,8,75] Inhibition of canonical *Wnt* signaling by *Dickkopf1* (an inhibitor of *LRP 5/6*) in vitro also impaired ureteric bud branching.[11] Gene deletion of *β-catenin* in the nephron progenitors (*Six2-Cre*) prevented formation of the renal vesicle and tubulogenesis, resulting in hypoplastic kidneys.[8] Congruent with this finding, competitive inhibition of *Wnt* signaling by *secreted Frizzled Related Proteins (sFRP)* impairs nephron differentiation in vitro (*sFRP* can compete with membrane bound *Frizzled* receptors for their shared ligand, *Wnt*).[76]

Sonic hedgehog (Shh) signaling

Sonic hedgehog is an inhibitory ligand for *Patched* receptor, which constituitively inhibits *Smoothened.*[77] On binding of *Hedgehog*, *Smoothened* becomes activated, inhibits the processing of full length Gli3 to a shorter protein that represses gene transcription, and stimulates translocation of *Gli 1* and *2* to the nucleus where they activate transcription of multiple downstream effectors, including patterning genes such as *Pax2* and *Sall1* and cell cycle regulators, *cyclin D1* and *n-myc*. In the absence of hedgehog binding, the repressor *Gli-3* is instead dominant. Truncating mutations in *Gli-3* that activate its repressor functions are associated with Pallister-Hall Syndrome and renal hypo/dysplasia.[78,79] Mutations in *Shh* are associated with a VACTERYL-like syndrome in mice, with midline defects and hypoplastic kidneys.[80] Deletion of *Shh* from the ureteric bud lineage using the HoxB7-Cre resulted in hypoplastic kidneys with hydronephrosis and hydroureter, associated with decreased proliferation of ureteral mesenchyme and impaired the ureteral smooth muscle differention.

The TGFβ Superfamily: Bone Morphogenic Proteins (BMPs)

BMPs, *activin* and *growth/differentiation factor (GDF)* are all members of the *TGFβ* superfamily, which act as ligands for transmembrane serine-threonine kinase receptors, the *activin-like receptor kinases (Alks)*.[81] Binding of *BMP* can be facilitated by extracellular activators *(Krim/KCP)* and inhibited by extracellular inhibitors such as *Gremlin*, *Chordin* and *Noggin*. On binding of their ligands, *Alks* dimerize and phosphorylate their mediators, the *Smad* proteins, which are also involved in *TGFβ* signaling. BMPs signal via activating *Smads 1,5* and *8* while TGFβ signals vial *Smads 2* and *3*. Activating *Smads* translocate to the nucleus and stimulate transcription of metallomatrix proteases (MMPs), extracellular matrix proteins (ECM), Pai1, and other modulators of cell adhesion and differentiation. *BMPs 4* and *7* are highly expressed in both the ureteric bud and cap mesenephric mesenchyme. Tight regulation of these proteins appears to be critical, as demonstrated by the finding that both complete loss of *BMP7* expression and loss of inhibition of *BMP* by *gremlin* results in renal agenesis.[82] This phenotype may in part result from effects on ureteric bud branching. In vitro, low levels of *BMPs 4* and *7* may stimulate bud branching, while at high levels they inhibit bud branching, in part by activation of inhibitory *Smad1*.[83] In vivo, gene deletion of the *BMP* receptor *Alk3* from ureteric bud cell causes early increased and abnormal ureteric bud branching, resulting in fewer branches at later stages, indicating that dysregulation of bud branching can impair renal development.[84] Another *BMP* expressed in the kidney is *BMP2*. *BMP2* gene deletion is embryonic lethal; it inhibits ureteric bud branching in vitro and in vivo.[83,85]

There appears to be some functional redundancy between *BMP4* and *7*, as expression of *BMP4* under the control of the *BMP7* promotor rescues the null mutant phenotype.[86] However, recent studies have identified podocyte specific roles for *BMP4* and *7* during development. Podocyte *BMP4* is required for glomerular capillary formation, while podocyte *BMP7* is required for proximal tubular cell proliferation and growth during nephrogenesis; gene deletion of either *BMP* from developing podocytes resulted in a hypodysplastic renal phenotype.[87,88]

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Several other ligands in the *TGFβ* superfamily may contribute to specification of the site of ureteric bud outgrowth. *GDF11, BMP4* and *Activin A* are endogenous inhibitors that restrict the site of outgrowth of the ureteric bud to one location.[89,90,91]

Fibroblast Growth Factors (FGFs)

The family of *FGFs* bind the receptor tyrosine kinases (*FGFR1* and *2*) ;*FGF* binding stimulates homodimerization and phosphorylation of the receptor, leading to recruitment of the *Grb2* adaptor and activation of *Ras GTP* proteins and *ERK* activation and cell proliferation. Binding by *FGF* to *FGFR* is facilitated by heparin sulfate proteoglycans.[92] *FGFs* are critical mitogens in development of multiple organs, and deletion of *FGF* ligands and receptors are embryonic lethal. In vitro, *FGF2* can promote condensation, induce *WT-1* expression and inhibit apoptosis in metanephric mesenchyme.[93,94] Studies of *FGF* in zebrafish demonstrated an early requirement of *FGF* in the intermediate mesoderm, with a late requirement for *FGF8* in condensation of the metanephric mesenchyme.[62] *FGFs* also function in the ureteric bud, and deletion of *FGF7* and *10*, and their receptor isoform *FGFR2-IIIb* results in decreased ureteric bud branching and smaller kidneys with decreased nephron number.[95]

Conditional deletion mutants have further defined lineage specific roles for *FGFs*. [95] In the metanephric mesenchyme, loss of *FGF8* interrupts nephron development, with failure to express important mediators of nephron epithelial development, such as *Wnt4* and *Lim1*.[96] Deletion of the receptors *FGFR1* and *2* in metanephric mesenchyme resulted in renal aplasia; remarkably this was also associated with decreased ureteric bud branching, providing further evidence that *FGFs* function in reciprocal signaling between metanephric mesenchyme and ureteric bud.[97] The *FGF* receptors site-specific roles were further dissected in vivo: gene deletion of *FGFR2* in mesenchymal stromal cells (by *Pax-3-cre*) resulted in formation of more than one ureteric bud, leading to duplicated collecting systems with ectopic ureteral insertion and hydroureter. Meanwhile, loss of *FGFR1* expression in these mesenchymal cells had no phenotypic consequences. This indicates that signaling via mesenchymal *FGFR2* defines the site of ureteric bud outgrowth, either secondary to effects of *FGFR2* on the metanephric mesenchyme or possibly as a direct effect of stromal cells on the ureteric bud.[98] In comparison, gene deletion of *FGFR2* from ureteric bud by conditional targeting with *Hox-B7-cre* resulted in thin UB stalks, decreased branching, decreased nephrons and increased stroma. Remarkably, these *FGFR* effects were independent of changes in *GDNF, Ret, Sprouty, Slit/Robo,* and *BMP-4* expression level.[98]

What are the factors that contribute to overall renal patterning and nephron number?

Serial branching of the ureteric tree with nephron induction at the ureteric tips generates the pattern of adult renal architecture and determines nephron number. It has been demonstrated that the above signaling pathways converge and modulate each other, provide complex regulation of overall renal patterning (figure 4). *Shh, Wnt* and *BMP* pathways can interact, with *GSK-3β* stimulating degradation of *Gli* effectors and *Shh* up-regulating *BMP4* in the ureteric bud, providing evidence for complex modulation of signaling pathways.[77] *Shh* signaling may also inhibit *FGF* signaling. *Sprouty* has also been shown to coordinate *Wnt11, FGF7* and *GDNF* expression and *sprouty* can inhibit the intracellular signaling of both *FGF* and *GDNF* pathways.[99]

The signals which terminate bud branching and regulate cell proliferation and apoptosis during kidney development are not fully understood. Tight control of apoptotic rate is critical during renal development, as evidence by renal dyplasia in salt stressed *kallikrein/bradykinin B2 receptor* deficient mice.[100] Increased epithelial cell apoptosis and decreased ureteric bud

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branching was induced via a *p53* dependent pathway resulting in renal dysplasia.[100] Inappropriate cell proliferation may also contribute to the pathogenesis of renal tumors and cysts.[101] The tumor suppressor protein *von Hippel-Lindau* gene (*pVHL*) downregulates the *hypoxia induced factor* (*HIF 2α*), which is upregulated in cysts and renal carcimomas from patients with *VHL*. In support of this pathophysiologic mechanism of cyst formation, specific gene deletion of *pVHL* from the proximal tubule resulted in cyst formation, composed of dedifferentiated cells with an increased rate of cell proliferation. *Bcl-2* and *cut related homeobox* (*cux-1*) also contribute to regulation of apoptosis during development.[102] Aberrant tubular flow as a result of impaired ciliary function is also thought to contribute to cell proliferation and cyst formation. Mutations in ciliary genes have been associated with cystic kidney diseases including *polycystin 1* and *2* (*PKD1* and *2*, mutated in autosomal dominant polycystic kidney disease), *polyductin (Pkhd1), nephronopthisis* (*NPHS 1-6*), and the *oral facial digital syndrome* gene (*ODS1*). Signaling via growth factors may also contribute to cyst formation. Kidney specific deletion of *hepatocyte nuclear factor-1β* led to renal failure and cyst formation, associated with decreased *Pkhd1* expression[103]. *EGF* and *VEGF* may also play a role; blockade of *VEGFR2* also led to cysts.[104]

What are the molecular mechanisms that control terminal differentiation the nephron segments?

Notch genes encode single transmembrane proteins that mediate short-range signaling between cells by their ligands *delta* and *jagged*. Binding of their ligands stimulates *Notch* receptor cleavage by a proteolyte enzyme, *γ-secretase*, releasing the *cleaved intracellular notch* portion. The *cleaved intracellular notch* translocates to the nucleus and forms a complex with *Cbf1/Rbp-J DNA* binding proteins, whereupon it activates transcription of downstream targets. *Notch* signaling defines the podocyte and proximal tubular cell fates during S-shape segmentation.[105] By using cultured kidney explants removed at various stages of nephron development, time sensitivity to *Notch* signaling could be established. Early explants (E12.5) exposed to *Notch* inhibitor failed to form proximal tubules and podocytes; whereas podocyte development in late explants (E14.5) was preserved. In vivo studies demonstrated *Notch2*, but not *Notch1*, was required for segmentation and differentiation of the proximal tubular segments and podocytes.

The signaling mechanisms by which podocytes differentiate are incompletely understood and are an area of active investigation; they will not be reviewed in depth here. In brief, *Pax-2* is required for aggregation of the metanephic mesenchyme. Subsequent differentiation of epithelial cells into podocytes requires *WT-1* induced downregulation of *Pax-2*.[106] It is also known that *WT-1* induces transcription characteristic of differentiated podocytes, ie. the slit diaphragm protein, *nephrin*.[107] Gene deletion studies have demonstrated that several proteins are involved in podocyte differentiation and form foot processes, including *Lmx1b, podocalyxin, pod1, kreisler* and *GLEPP1*. The proteins that contribute to forming the slit diaphragms that link adjacent foot processes, including *podocin, nephrin, CD2AP* are also critical for normal differentiated podocyte structure. The GBM is secreted by podocytes and endothelial cell and is composed in part by *collagen* and *laminin* chains which undergo a switch during development. The GBM provides a framework for podocyte development, and can provide intracellular signals via binding of *laminin* components to *integrins*. It is notable that glomerular development is disturbed in *integrin deficient* mice.[108] Signaling via growth factors contribute to migration of mesangial and endothelial cells during glomerular development, with *PDGFR-β* and *VEGF* required for mesangial and endothelial cell migration, respectively. The stimulus of low oxygen tension of the developing kidney may induce expression of *Hypoxic inducible factor (HIF)* and lead to expression of *VEGF*; in addition, podocyte *WT1* also upregulates *VEGF* expression. Endothelial cell migration is further modulated by *angiopoeitin-Tie2* and *angiotensin II* signaling pathways.

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What genes control maturation of the ureteric bud into the ureter and bladder?

Even less is known about the factors involved in terminal differentiation of the ureteric bud. Notably, *Tbx18* is required for development of ureteral mesenchyme, and loss of *Tbx18* function results in phenotype similar to the clinical syndrome of megaureter, with abnormal peristalsis of the ureteric musculature. *Shh* and another protein, *teashirt*, which is upregulated by *BMP4*, have both been recently shown to play a role in ureteral smooth muscle differentiation.[109]

In summary, complex interactions between a relative few signaling pathways regulate the multiple steps of renal development. The ongoing challenge is to identify the modulating factors which provide time and site specific specificity to the actions of these signaling pathways. The evolution of our understanding of the cellular and molecular mechanisms of kidney development may provide methods for improved diagnosis of renal anomalies and, hopefully, targets for intervention for this common cause of childhood ESKD.

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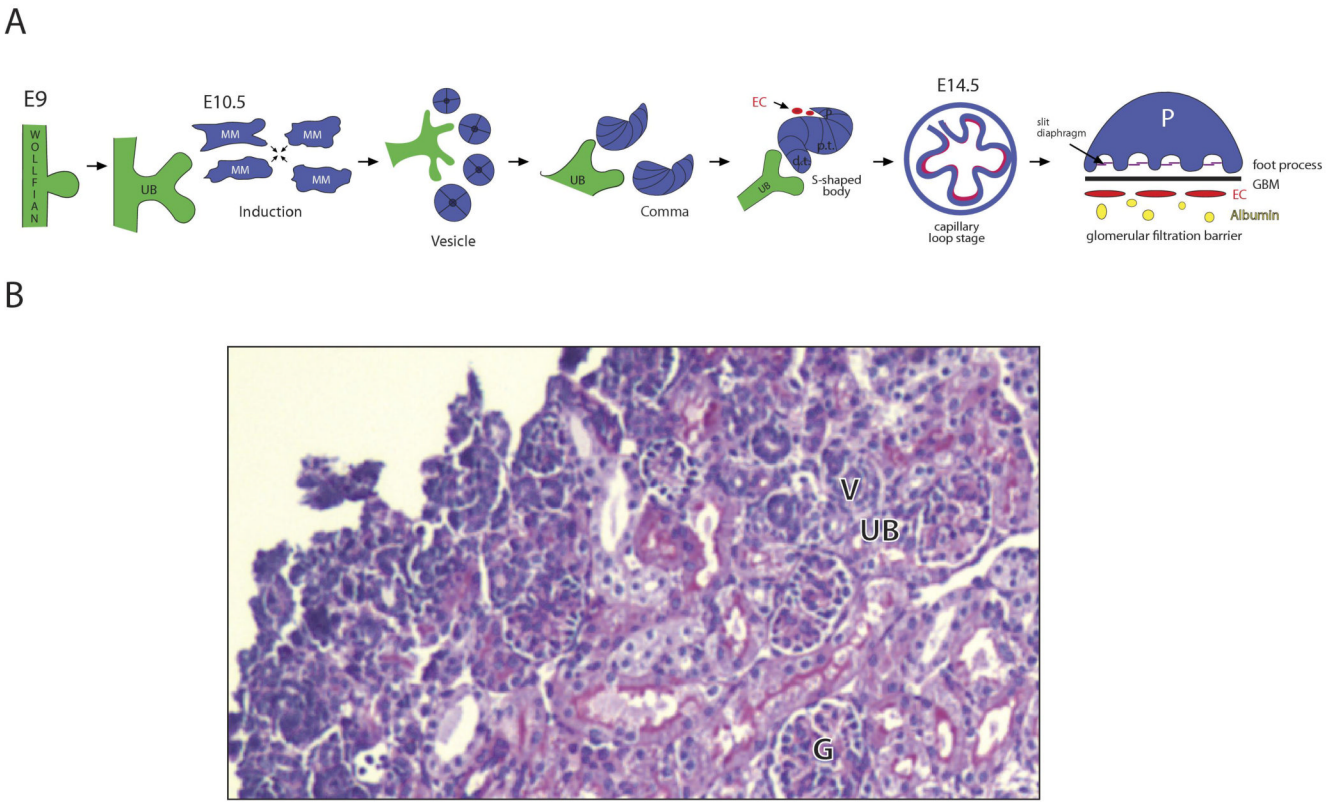

Figure 1.

(A) schema of metanephric kidney development: The ureteric bud (UB) arises from the Wolfian duct (part of the mesonephros) at embryonic day 9 (E9) in mice. Nephrons are induced at tips of the ureteric bud branches; the serial branching of the ureteric bud establishes the radial structure of the kidney architecture. Wnt signals provide the major signal for induction, stimulating aggregation of metanephric mesenchymal (MM) cells and transformation to an epithelial cell phenotype (MET). The epithelial cells form a vesicle, then comma and S-shaped body. Notch2 defines cell fate of the proximal segments of the S-shaped body, the future podocytes and proximal tubule, while the distal segment form the distal tubule. By E14.5, the first glomeruli are formed, with podocytes with foot processes, slit diaphragms, glomerular basement membrane (GBM) and a fenestrated endothelium (EC). (B) PAS of newborn mouse kidney exhibiting developing nephrons (ureteric bud (UB) and renal vesicles (V)) in the outer nephrogenic cortex, and a mature glomeruli (G) in the deep cortex.

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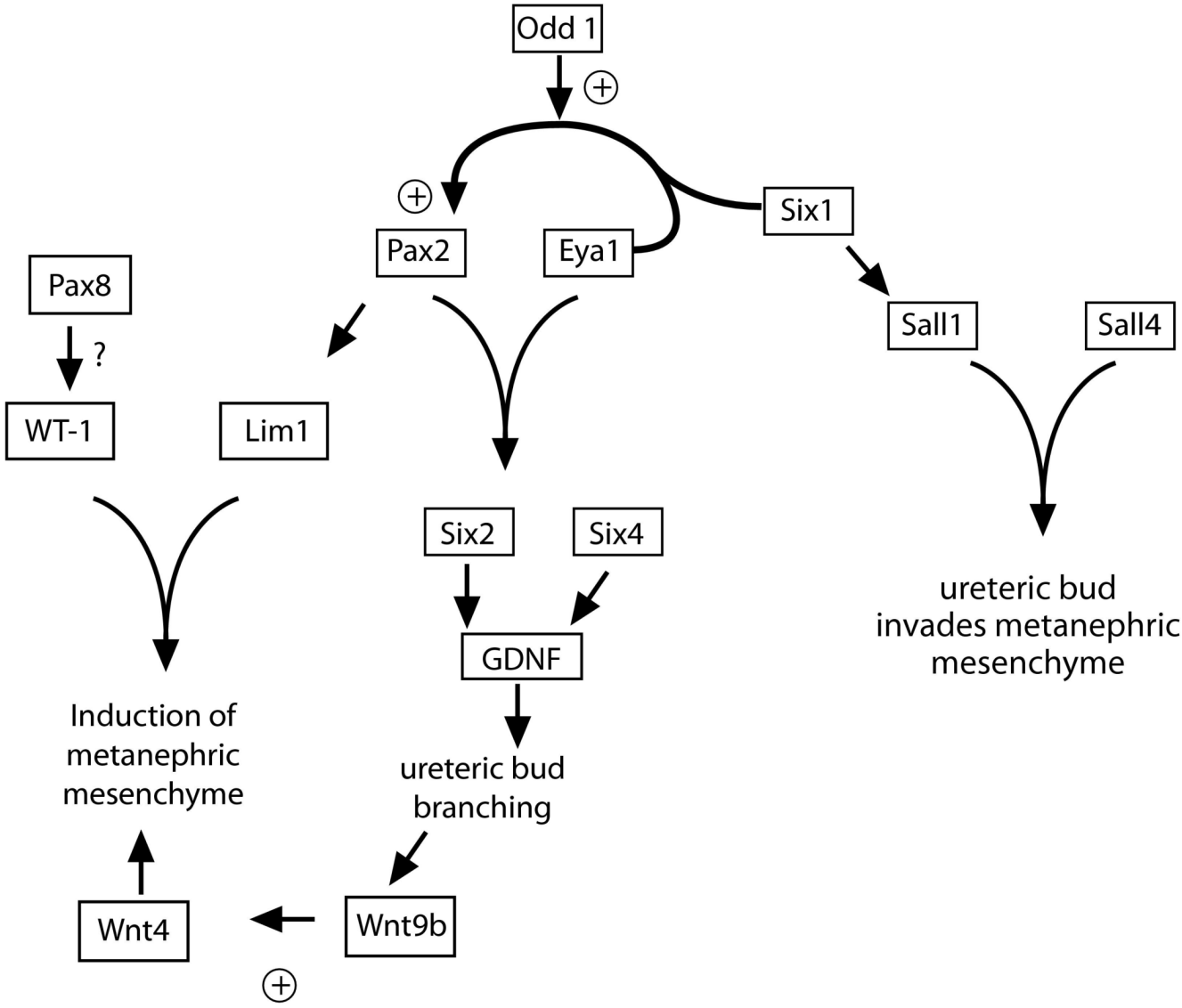

Figure 2.
Hierarchy of transcription factors in nephron progenitor cells

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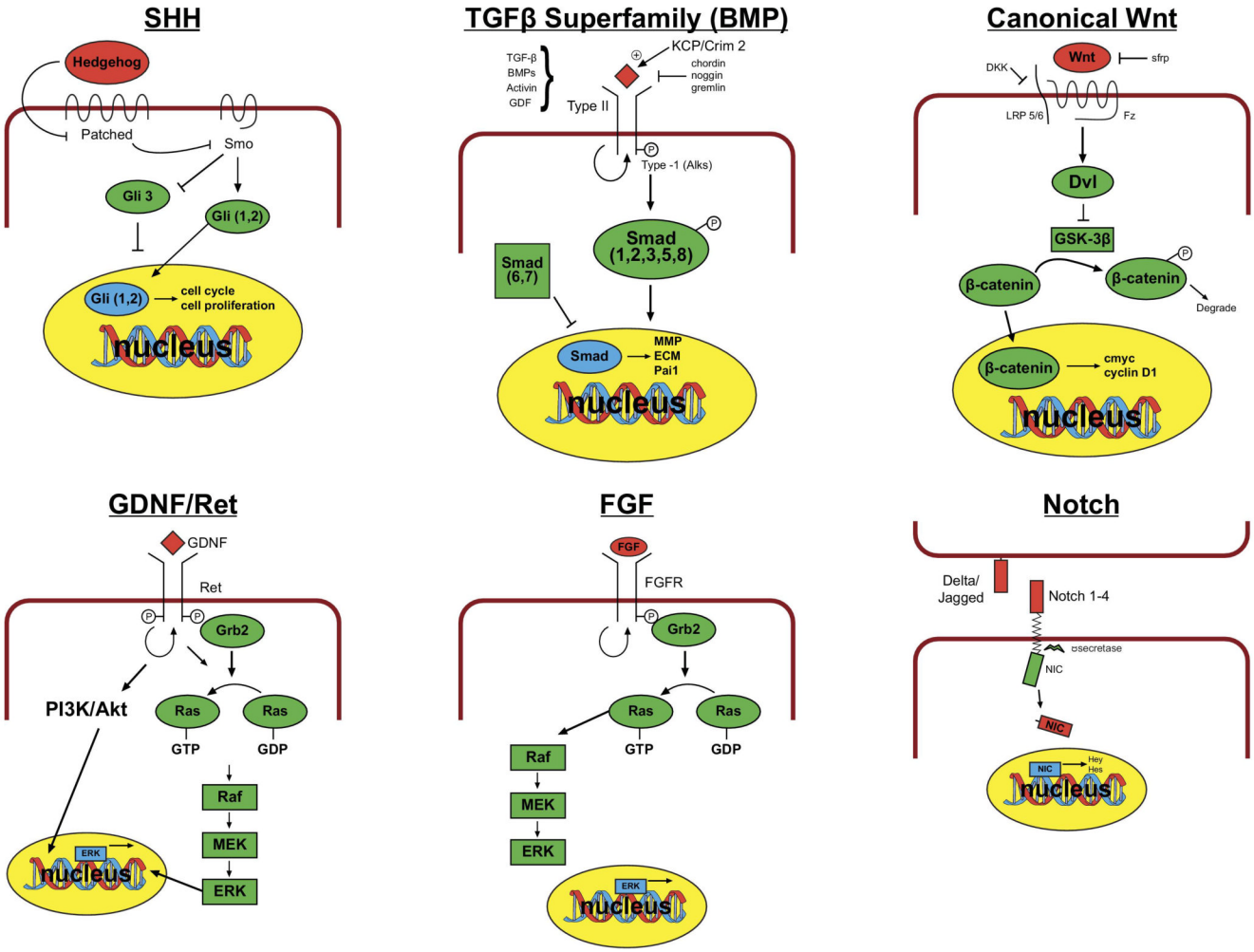

Figure 3.
Schema of major signaling pathways involved in renal development (for space and clarity, not all factors in each pathway shown)

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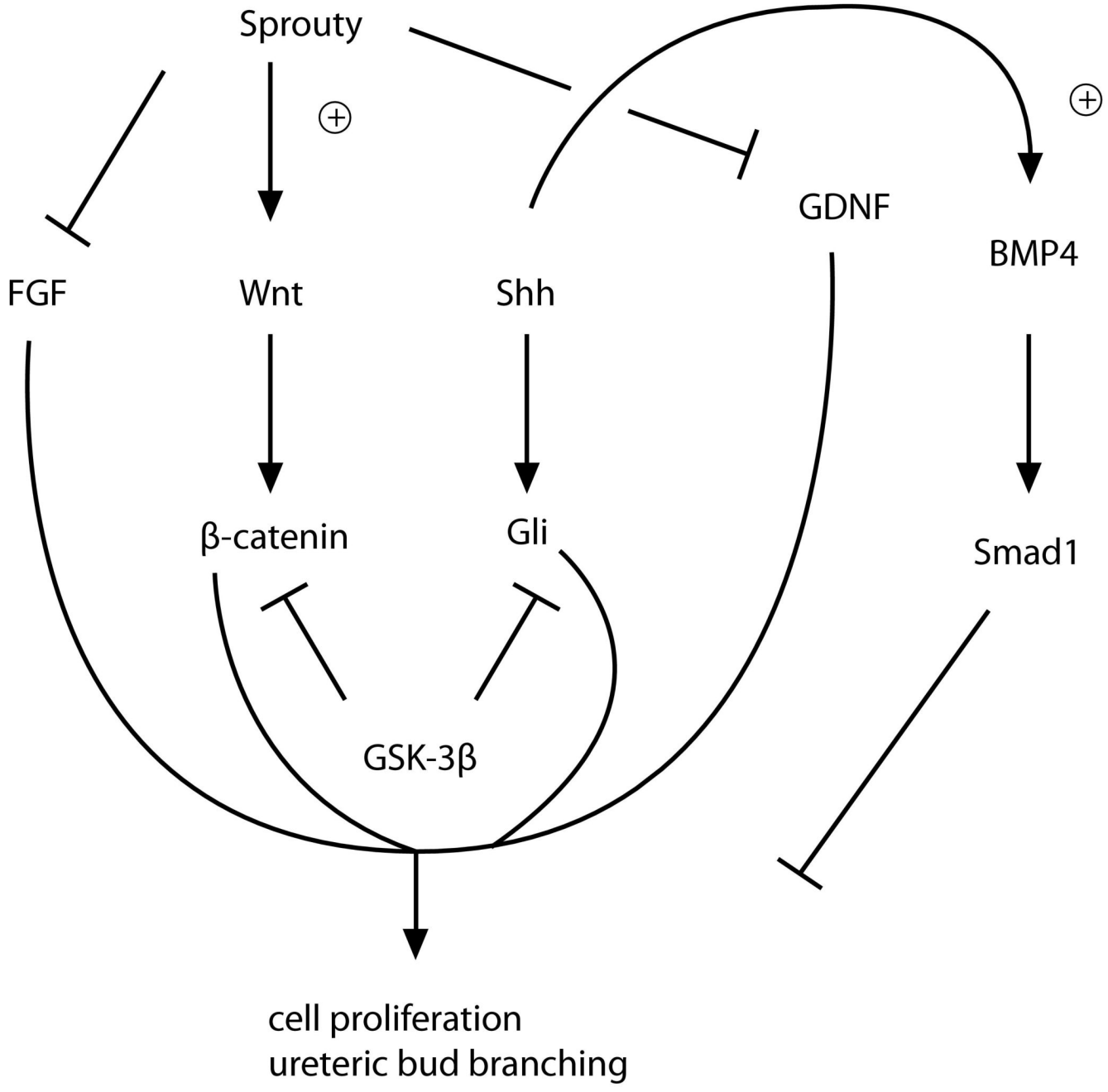

Figure 4.
Crosstalk between sonic hedgehog (Shh), bone morphogenic protein (BMP), fibroblast growth factor (FGF) and GDNF/ret signaling pathways modulates ureteric bud branching

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Table 1

Genes expressed by progenitor cell populations

Renal Progenitor Cell Populations	
Pronephros	
Iroquois[110]	Required for pronephros
Kohtalo/trap230[111]	Required for pronephros
XTRAP-gamma[112]	Required for pronephros
Odd1	Pronephros vs. vascular cell fate
Metanephric Mesenchyme Progenitors	
Eya1	Branchio-oto-renal syndrome
Six 1,2,4	Renal hypodysplasia[113]
Pax2	Renal-coloboma syndrome
Sall1	Townes-B rocke/ Okihiro syndrome
Lim1	Stimulate ureteric bud cell differentiation?
Emx2	
WT-1	
Stroma	
foxD1 (Bf2)	
Pax3	Pax3 expression is suppressed in nephrogenic precursors by W T-1 and remains persistently expressed in stroma.
Vascular Progenitors	
VEGFR2 (Flk1)	
ckit	

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Table 2

Factors involved in ureteric bud outgrowth and branching

Ureteric Bud (UB): Origin and Branching	
FoxC1	Single origin of UB; mutated in CAKUT
Slit2/Robo2	Single origin of UB; mutated in VUR
GDNF/c-Ret	UB formation/ branching
Retinoic acid receptors (RARαβγ)	Maintains c-ret expression in UB tips
FGFR2	Single origin of UB(in mesenchyme);branching
BMP2 & glypican3, BMP 4/7, Alk3, Gremlin, smad1	Patterning of ureteric bud branching
Sonic Hedgehog (Shh) & Gli effectors	
Wnt 11/ β-catenin	
Activin	
GDF11	
Sprouty	
Angiotensin II/ angiotensin receptors 1 & 2	Stimulate ureteric bud branching
VEGF	
Protein kinase X	
Semaphorin 3a	Inhibit ureteric bud branching
TGFβ	

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Table 3

Factors involved in induction and differentiation of metanephric mesenchyme

Metanephric Mesenchyme: Induction / Mesenchymal-Epithelial Transition (MET)	
Wnt 4 (MM)/ Wnt 9b (UB) [Wnts 1,3,7,11] β-catenin	Induction
BMP4/7	Induction
FGF2	Aggregation
Leukemia inhibitory factor (LIF)	Induction
TGFβ2/ Smad4	
FGF 7,8/ FGFR1/2	
Pax 2/8	Epithelial differentiation
WT-1/Lim1/Pax 2/8	Epithelial differentiation
Lim1	Epithelial differentiation

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Table 4

Factors involved in differentiation of nephron segments and glomerular differentiation

Nephron Differentiation and Glomerular Development	
Segmentation	
Notch2	Proximal tubular and podocyte cell fate
Podocyte BMP7	Proximal tubular cell proliferation
Podocyte differentiation	
WT-1	
Pax-2	
Lmx1b	
Pod-1(Tcf21)	
GLEPP1	
lim1	
Pod1	
Kreisler (Mafb)	
Podocalyxin	
FAT1	
Slit diaphragm proteins	
Nephrin	
Neph 1,2,3	
Podocin	
CD2AP	
GBM	
s-laminin/ laminin beta2	
Alpha 3 integrin	
Alpha 8 integrin	
dystroglycan	
laminin	
entactin	
fibronectin	
Type IV collagen	
heparan sulfate proteoglycan	
vitronectin	
Glomerular capillaries	
VEGF/ VEGFR2 (Flk)	
Podocyte BMP4/ Id1	
Eph family tyrosine kinases ELK/LERK	
Angiopoietin II/ Tie2	
Mesangial cells	
Pdgfβ/ Pdgfβ receptor	
Stromal cells	
foxC1/C2	