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# Morphogenesis and Molecular Mechanisms Involved in Human Kidney Development

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The development of the human kidney is a complex process that requires interactions between epithelial and mesenchymal cells, eventually leading to the coordinated growth and differentiation of multiple highly specialized stromal, vascular, and epithelial cell types. The application of molecular biology and immunocytochemistry to the study of cell types involved in renal morphogenesis is leading to a better understanding of nephrogenesis, which requires a fine balance of many factors that can be disturbed by various prenatal events in humans. The aim of this paper is to review human kidney organogenesis, with particular emphasis on the sequence of morphological events, on the immunohistochemical peculiarities of nephron progenitor populations and on the molecular pathways regulating the process of mesenchymal to epithelial transition. Kidney development can be subdivided into five steps: (i) the primary ureteric bud (UB); (ii) the cap mesenchyme; (iii) the mesenchymal–epithelial transition; (iv) glomerulogenesis and tubulogenesis; (v) the interstitial cells. Complex correlations between morphological and molecular events from the origin of the UB and its branching to the metanephric mesenchyme, ending with the maturation of nephrons, have been reported in different animals, including mammals. Marked differences, observed among different species in the origin and the duration of nephrogenesis, suggest that morphological and molecular events may be different in different animal species and mammals. Further studies must be carried out in humans to verify at the morphological, immunohistochemical, and molecular levels if the outcome in humans parallels that previously described in other species.

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Early kidney development, given its complexity, has become a paradigm for the fascinating phenomenon of mesenchymal-to-epithelial transition, for branching morphogenesis, and, in a general sense, for human organogenesis (Dressler, 2008). The development of the human kidney is a complex process that requires interactions between epithelial and mesenchymal cells, eventually leading to the coordinated development of multiple highly specialized stromal, vascular, and epithelial cell types, a peculiar feature of the kidney architectural and functional complexity (Gruenwald and Popper, 1940; Dressler, 2006). Overall, kidney morphogenesis appears as a self-regulating process whereby kidney function, i.e., nephron fluid flow, orchestrates multiple, mutually dependent cellular processes within the developing nephron, including epithelial cell movements during tubulogenesis (Drummond and Vasilyev, 2010).

In recent years, the application of new techniques such as molecular biology and immunocytochemistry to the study of cell types involved in renal morphogenesis has represented a key challenge in nephrology, leading to better understanding of kidney development during prenatal and postnatal life. Nephrogenesis requires a fine balance of many factors that can be disturbed by various prenatal events in humans as well as in animal models (Rodríguez et al., 2004; Mühle et al., 2010). Recent studies have highlighted multiple factors that play a role in the epigenetic modulation of kidney development, including maternal diet, stress and hypertension, drugs administered to the mother or to the newborn (namely antibiotics, non-steroid anti-inflammatory drugs), prematurity, low birth weight, and intrauterine growth restriction. All these factors may lead to a disturbance of nephrogenesis, resulting in low nephron numbers at birth, which may represent the main factor favoring the development of hypertension and, eventually, of end stage renal disease in childhood or adulthood (Puddu et al., 2009; Fanos et al., 2010).

The clinical importance of nephron mass is well recognized in the literature: clinical surrogates for low nephron numbers and susceptibility to hypertension and renal disease in humans have been presented in recent studies (Luyckx and Brenner, 2010; Bertram et al., 2011; Table 1).

A marked interindividual variability in renal maturation has recently been observed by our group in autopsies of preterm infants: this should in all likelihood represent a major risk factor of progressive renal disease in adulthood (Faa et al., 2010).

To investigate in depth the mechanisms of renal development, genetic manipulation in a variety of animal models has been used to discover the cellular and molecular signals that define the morphological transitions (Reidy et al., 2009). It must be emphasized that limitations on extrapolation from animal studies should be taken into account particularly in rodents, in which the achievement of nephrogenesis covers the lactation

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TABLE 1. Clinical surrogates for low nephron number and susceptibility to hypertension and renal disease in humans (from Luyckx and Brenner, 2010; mod)

| |
|---|
| 1 Low birth weight; ELBW; VLBW |
| 2 Preterm birth |
| 3 Short stature |
| 4 Reduced kidney volume (ultrasound) |
| 5 Low kidney mass (scintigraphy) |
| 6 Glomerulomegaly (biopsy) |
| 7 Gene polymorphisms: PAX2, RET |
| 8 IDM |

LBW, low birth weight; ELBW, extremely low birth weight; VLBW, very low birth weight; IDM, infant diabetic mother.

period, whereas it normally ends before birth at 35–36 weeks of gestation in humans (Symonds et al., 2009).

The aim of this paper is to review human kidney organogenesis, with particular emphasis on the sequence of morphological events which, starting from the indifferentiated intermediate mesenchymal cells and from their cross-talking with the elongating and branching ureteric bud (UB), eventually give origin to the complexity of the mature human kidney. In particular, we shall focus on morphological and immunohistochemical peculiarities of nephron progenitor populations and on the molecular pathways regulating the process of mesenchymal-to-epithelial transition, the main development responsible for the origin of the nephron, the functional unit of the kidney.

Progress in our understanding of the cellular and molecular mechanisms of kidney development may provide methods for improved diagnosis of renal anomalies and, hopefully, targets for prevention and treatment of childhood kidney disease (Reidy et al., 2009).

## Historical Notes

The first studies on the sequence of events regulating human nephrogenesis go back to 1842, when Bowman (1842) first described the "invagination" of the capillary tuft inside the developing nephron, originating the immature glomerulus and the capsule. The early phases of renal development in the human embryo were better defined by Golgi (1889), who first described the "S-shaped" bodies as one of the early structures that originate the glomerular capsule. According to Golgi C., the contemporary development of blood vessels and "S-shaped" tubules is at the basis of glomerulogenesis. The intimal and complex interactions between epithelial cells and the capillary tuft give rise, according to Golgi, to the mature tuft. Herring (1900) first surmized that the nephron might originate from a nest of undifferentiated mesenchymal-like cells and not from the branching UB. In addition, he identified the "comma" bodies as the precursors of the "S-shaped" bodies, and described the multiple steps of the differentiation of the cells lining the glomerular capsule, eventually differentiating into parietal cells and mature podocytes.

Zimmermann (1933) revealed the intimate relationships among the endothelial cells, the basal membranes, and the invaginated capsular epithelial cells, and named as mesangial cells the fibroblast-like cells interposed between the capillaries. Noel and Pigeaud (1932) hypothesized that the tuft may originate from a nodule of metanephric blastema in contact with an S-shaped tube. According to the latter authors, the tuft and the glomerular capsule should be considered, at their origin, two separate entities, which subsequently fuse, thus originating the mature glomerulus. Montaldo and Murri (1958) studied the relationship between the glomerular capillaries, the epithelial cells surrounding the glomerular capillaries and the mesangial cells at their basis. Montaldo also hypothesized that basal membranes might originate from the synthetic activity of both epithelial and mesangial cells. Hall (1956) first studied the ultrastructure of the glomerulus in the developing kidney of the rat and described the differentiation of the inner cells of the capsule toward mature podocytes. Moreover, he revealed the complex structure of the basement membrane and its subdivision into inner and outer lamina rara and lamina densa. Kurtz (1958) applied electron microscopy to the study of human fetal kidneys and showed that the glomerulus originates from a nest of mesenchymal cells which differentiate into podocytes and endothelial cells, both contributing to the synthesis of the basal membrane. Benedetti and Marinozzi (1958) contributed to better knowledge of the mesangial cells during kidney development. They first hypothesized a common origin for mesangial and endothelial cells and suggested that mesangial cells might function as pericytes, regulating the caliber of glomerular capillaries. The hypothesis of Benedetti and Marinozzi was confirmed by Farquhar and Palade (1962) who first gave functional evidence of the existence of a "third" cell type in the renal glomerulus, the mesangium. In her PhD thesis, Lazorthes-Herland (1961) supported Herring's theory and clearly demonstrated that the entire glomerulus originates from the same mesenchymal progenitors.

## Stages of Kidney Development and Branching

The mature kidney of mammals is the final product of three embryonic excretory organs, i.e., the pronephros, the mesonephros, and the metanephros (O'Rahilly and Muecke, 1972). The metanephros takes origin from the UB, a branching epithelial tube originating from the Wolffian duct, and the mesenchymal cells which originate from the intermediate mesenchyme (Poladia et al., 2006) and are programmed to make epithelial precursors (Stark et al., 1994) in response to inductive signals from the UB (Schmidt-Ott et al., 2005; Dressler, 2008). The nephron and the collecting system have different developmental histories: the former arises from mesenchymal cells, which undergo mesenchymal–epithelial transition, whereas the latter forms from the reiterated branching of the UB (Constantini and Kopan, 2010; Rumballe et al., 2010).

The analysis of kidney development will be subdivided into five steps: (i) the primary UB; (ii) the cap mesenchyme; (iii) the mesenchymal–epithelial transition; (iv) glomerulogenesis and tubulogenesis; (v) the interstitial cells.

## The Primary Ureteric Bud

The complex process of human nephrogenesis develops from the primary nephric duct, also known as the common mesonephric duct, or Wolffian duct, which represents the first epithelial component of the human urogenital system. The primary UB originates at the posterior end of the Wolffian duct as a solid aggregate of epithelial cells that proliferate, migrate, and progressively invade the surrounding metanephric mesenchyme (Watanabe and Costantini, 2004; Fig. 1).

Preliminary data we have obtained indicate that MUC 1 is able to discriminate between committed and uncommitted cap mesenchymal cells (Locci, unpublished data).

In humans, the development of the UB from the Wolffian duct begins at the 28th day of gestation (Yosypiv, 2008). It branches in a highly reproducible manner, and the nephron formation is induced at each of its tips. These branches will form the collecting system, including collecting ducts, renal pelvis, ureter, and bladder trigone (Reidy and Rosenblum, 2009). Numerous factors expressed in a specific spatial and temporal pattern play a role in the UB origin, elongation, and branching (Constantini, 2006). Multiple gene regulatory networks have been reported to act either as inducers (c-Ret, ETv4, ETv5, GDNF, SOX8, SOX9, Wnt11, Angiotensin II, FGFR1, FGFR2,

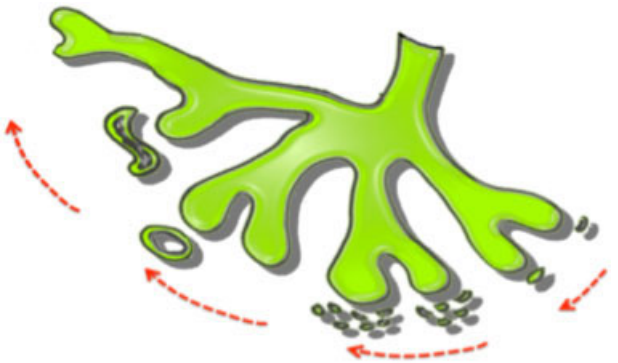

**Fig. 1. Schematic representation of interactions between branching UB tips and the metanephric mesenchyme.**

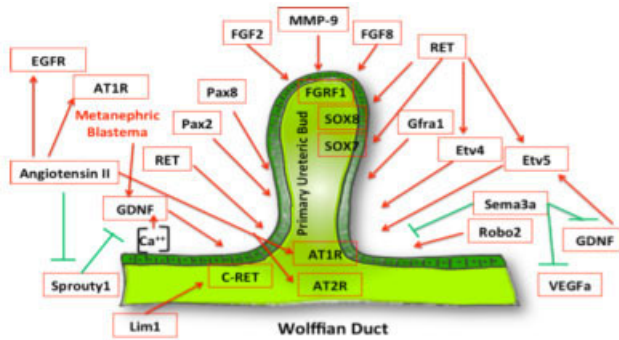


**Fig. 2. Main molecular pathways involved in the origin of the primary UB from the Wolffian duct.**

FGF8, p53, MMP-9, Cofilin1, Destrin, AT1R, AT2R, and PAX2), or inhibitors (Spry1, class 3 semaphorins, Robo2, Slit2, BMP4, FoxC1, and FoxC2; Yosypiv, 2008).

The GDNF/c-Ret/Wnt1 pathway is considered the major positive regulator of UB development. The c-RET signaling pathway plays multiple crucial roles during human kidney development. Its earliest role is evident in the Wolffian duct, where it promotes cell movements that give rise to the first UB tips. The correct outgrowth from the primary UB domain is essential for further kidney development, as shown by the perinatal lethality due to bilateral renal agenesis in RET knock-out mice (Jain, 2009). A further role of RET in kidney morphogenesis is well evidenced by the fact that kidneys developing in the absence of GDNF/RET display severe branching abnormalities (Michos et al., 2010).

A significant role within the c-RET signaling pathway is played by SOX genes, a family of important developmental regulators, which are expressed at the tips of the growing UB, where they direct epithelial branching. Their relevance is highlighted by the occurrence of severe kidney defects, ranging from renal hypoplasia to renal agenesis, in SOX8/9 double mutants (Schedl et al., 2010). Lastly, a balanced action of RET tyrosine kinase on distal ureter maturation controls the fine tuning of the ureter/bladder connection (Uetani et al., 2009).

The glial cell-line-derived neurotrophic factor (GDNF) is secreted by the metanephric mesenchyme under control of the transcription factor PAX2 (Brophy et al., 2001; Majumdar et al., 2003; Constantini, 2006), and interacts with the c-Ret tyrosine kinase receptor expressed on the UB tip cells to induce branching (Pachnis et al., 1993). The activity of the GDNF/c-Ret/Wnt1 pathway is inhibited by Spry1, a critical regulator of GDNF/RET-mediated kidney induction (Basson et al., 2005). GDNF has been shown to drive arborization in culture cells and, when applied near the Wolffian duct, to induce the production of supernumerary UBs (Sainio et al., 1997). A number of genes whose expression is induced in the UB by GDNF has been identified, including two ETS transcription factors, Etv4 and Etv5: these factors are required for Wolffian duct cell movements that give rise to the UB, as well as for later UB growth and branching (Lu et al., 2009).

The role of GDNF in early kidney development has been evidenced in knock-out mice, where the lack of GDNF blocks the development of the UB (Pichel et al., 1996; Sanchez et al., 1996; Moritz et al., 2008).

PAX2 and PAX8 expression is necessary for morphogenesis and guidance of the primary nephric duct in the early phases of kidney development (Grote et al., 2006; Fig. 2) and protects nephron progenitor cells during nephrogenesis (Benetti et al., 2007). Several data suggest that PAX2 is also involved in nephron regeneration in some animals, such studies on the adult zebrafish. Following gentamicin-induced renal injury, renal stem cells have been shown to coalesce to form mesenchymal clusters that express PAX2, and then epithelialize into renal vesicles, which give rise to primitive nephrons that fuse with pre-existing renal tubules (Hopkins et al., 2010). Again, aminoglycosides are able to inhibit UB branching in the fetus, when administered antenatally, thus inducing at birth a reduced number of nephrons (Gilbert et al., 1996).

Moreover, human adult kidney tubule cells (HK2s) in vitro, when treated with the histone deacetylase inhibitor valproic acid, showed a functional conversion to renal progenitors, and underwent an epithelial-mesenchymal transition, by means of the re-expression of high PAX2 levels (Hopkins et al., 2010). Studies on PAX genes in kidney development suggest that decreased PAX2 protein levels, due to its mutation, cause an excessive amount of apoptosis in the UB tips, followed by loss or paucity in UBs (Ostrom et al., 2000; Eccles et al., 2002; Cohen et al., 2007). The PAX2 gene is normally expressed in the mesonephric duct, in UB cells, in the condensing cap mesenchyme and in early epithelial structures derived from the process of mesenchymal–epithelial transition, and is quickly downregulated as the tubular epithelium matures (Dressler et al., 1990). An altered expression of PAX2 in the fetal kidney has been reported following a maternal low protein diet (Welham et al., 2005), after exposure to dexamethasone (Singh et al., 2007) and as a consequence of accelerated neonatal growth (Simeoni et al., 2011).

The involvement of the renin-angiotensin system (RAS) has been proposed in the regulation of the development of the UB (Yosipiv, 2004; Song et al., 2010a,b). According to this hypothesis, angiotensin II produced by stromal cells stimulates UB branching, upregulating PAX2 via AT2R. Moreover, angiotensin II, acting via the AT1R, may down-regulate the Spry1 expression (Yosypiv, 2008). The RAS gene mutations determine a spectrum of anomalies of the renal collecting system in humans and in mice, due to defects in UB branching (Yosypiv, 2008; Song et al., 2010b).

Class 3 semaphorins are involved in UB branching. They are a family of guidance proteins involved in branching morphogenesis of epithelial structures. Semaphorin3a (Sema3a) is expressed in UB cells throughout kidney development, and participates in patterning the UB tree as an essential and negative regulator of UB branching. Its inhibitory effects on UB growth and branching involve its ability to downregulate GDNF signaling, to inhibit the vascular

endothelial growth factor-A (VEGF-A), and to decrease the activity of the Akt survival pathway (Reidy et al., 2009).

The fibroblast growth factor (FGF) was also found to be fundamental for early kidney formation and critical for ureteric branching (Fig. 2). The role of FGF in nephrogenesis was first suggested by its expression in the rat metanephric mesenchyme as an inducer of early events in renal development, which maintain the survival of metanephric mesenchymal cells (Perantoni et al., 1995) through the interaction between FGF2 and BMP7, a member of the bone morphogenic protein family (Dudley et al., 1999). FGF receptors (FGFRs) are expressed in the UB cells: they are critical mitogens in the development of multiple organs, and deletion of FGF ligands and receptors are embryonic lethal. The crucial role of FGF signaling in kidney development was better explained by showing that conditional knockout of FGFR1 and FGFR2 in the rat led to severe dysgenetic effects, cystic alterations, inability of the UB to elongate or branch and renal agenesia (Poladia et al., 2006). Conditional deletion of murine FGFR1 and 2 in metanephric mesenchyme leads to renal agenesis, with unbranched UBs (Hains et al., 2008).

Recently, a role for p53 has been hypothesized in the regulation of metanephric development on the ground of a spectrum of congenital anomalies of the kidney and urinary tract, including UB ectopia and delayed branching of the UB in p53 knockout embryos (Saifudeen et al., 2009).

Matrix metalloproteinases, particularly MMP-9, are produced in the early stages of renal morphogenesis and are required to enable developing collecting tubules to invade the surrounding metanephric mesenchyme (Lelongt et al., 1997).

Development, growth, and branching of the UB requires many cellular changes, including migration and changes in shape that involve the actin cytoskeleton (Short et al., 2010). Recent studies have clearly shown the role of actin depolymerizing factors, Cofilin1, and Destrin, in normal growth and branching of the UB. The simultaneous lack of both genes in mouse embryos resulted in the arrest of branching morphogenesis at an early stage, with disruption of normal epithelial organization of the UB cells and defects in cell migration (Kuure et al., 2010).

Similarly to the kidney, a reduced arborization of the developing structures during a pathological process is observed in other organs, such as the brain and the lung (Fanos, 2011 in press).

## The Cap Mesenchyme

While epithelial cords originating from the UB are branching into the metanephric mesenchyme, some metanephric mesenchymal cells, including self-renewing progenitor cells (Boyle et al., 2008), condensate and aggregate around the tips of epithelial branches, transforming themselves into the cap mesenchymal cells (Sariola, 2002; Rosenblum, 2008). Cap mesenchyme progressively undergoes mesenchymal-to-epithelial transition (Stark et al., 1994; Kispert et al., 1998), which will form most of the epithelia of the nephron (Carroll et al., 2005; Fig. 3a,b).

The molecular phenotype of renal progenitor cells has been identified by comparing the expression pattern of the metanephric mesenchyme to that of the adjacent intermediate mesoderm in the mouse at embryonic day 10. Challen et al. (2004) identified CD24 as an early metanephric mesenchyme-specific marker. Recent immunohistochemical studies on stem cell markers in the human fetal kidney evidenced the adhesion molecule NCAM exclusively localized in the metanephric mesenchymal cells and in its nephron progenitors derivative, whereas EpCAM, an epithelial surface marker absent from the metanephric mesenchyme, increased its expression along nephron differentiation (Metsuyanim et al., 2009). Moreover, the intermediate mesoderm is characterized by the expression of few other molecular markers, including the Lim-type homeobox gene, Lhx1 (Barnes et al., 1994; Dressler, 2009). A transcription factor complex, including Eya1 and Six1, is required for the initial specification of the metanephric mesenchyme from the intermediate mesoderm, at the caudal end of the urogenital ridge (Li et al., 2003), as shown by the abnormal apoptosis of organ primordia and kidney absence (Xu et al., 1999).

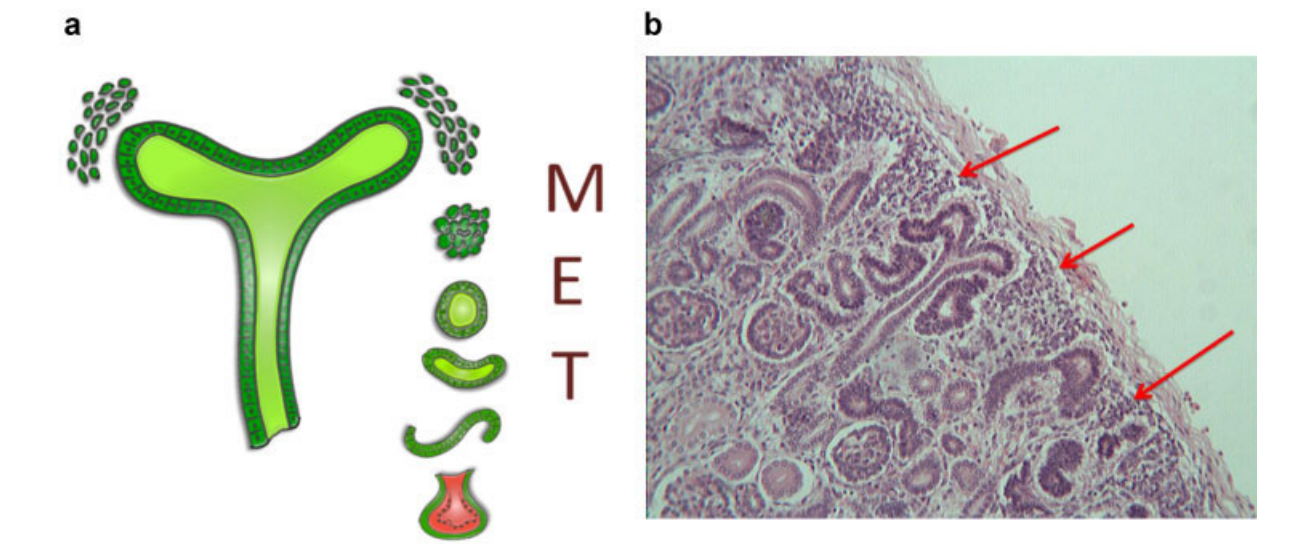


**Fig. 3. a: Schematic representation of the process of mesenchymal–epithelial transition (MET): cap mesenchymal cells origin PTA, renal vesicles, comma-bodies, S-shaped bodies and, eventually, glomeruli. b: Nephrogenesis in human fetal kidney (11 weeks of gestation): arrows indicate the metanephric mesenchyme giving rise to cap mesenchymal cells around the tips of Y-shaped UB branches.**

Two gene products are mainly involved in the process of metanephric mesenchyme differentiation toward the cap mesenchyme, PAX2 and Wnt4. PAX2 is probably the most important, being highly expressed at the tip of the UB, in the surrounding condensing mesenchyme and in the renal vesicle (Batchelder et al., 2009). It probably plays a double, and in some way, an opposite role. Indeed, while it induces the differentiation of the metanephric mesenchyme toward the nephron lineage, giving rise to the cap mesenchymal cells, it blocks the differentiation of the progenitor cells toward the non-nephron lineage, thus downregulating the generation of the nephrogenic interstitium (Fig. 4). According to this interpretation, PAX2 might be fundamental in fixing the epithelial phenotype within the metanephric mesenchyme, thus imprinting an epithelial fate by the activation of epithelia-specific genes and by the repression of non-epithelial ones, in such a way establishing a developmental boundary between the nephron and non-nephron lineages (Tamimi et al., 2008).

The condensed cap mesenchyme is hypothesized to generate a population of stem progenitor cells at the periphery of the nephrogenic zone in the developing kidney (Bard et al., 1994; Plecineanu et al., 2010; Boyle et al., 2008). Multipotent renal progenitor cells in the embryonic kidney strongly expressing Sall1 (Fig. 5) are able to form colonies (Osafune et al., 2006). Sall1-positive progenitor cells have been shown to partially reconstitute a three-dimensional kidney structure in an organ culture setting (Nishinakamura, 2008). These stem-like cells are also marked by the expression of Six2 (Kobayashi et al., 2008), a transcription factor responsible for maintaining cells in a progenitor state and for inhibiting their differentiation (Self et al., 2006).

The Six2-positive and Osr1-expressing cap mesenchymal cells surrounding the UB tips represent the nephron progenitor population of the metanephros (Kreidberg, 2010; Fig. 5) and give rise to all segments of the uriniferous tubule with the exception of the collecting ducts. Functional inactivation of the homeobox gene Six2 results in premature and ectopic differentiation of mesenchymal cells into epithelia, followed by depletion of the progenitor cell population within the metanephric mesenchyme. Failure to renew the mesenchymal cells results in severe renal hypoplasia (Self et al., 2006). The cessation of nephrogenesis, occurring at 35–36 weeks in humans or postnatal week 1 in rodents, arises from the terminal commitment of this progenitor field to nephron formation. It follows that, in humans, no new nephrons arise after birth, hence no postnatal renal stem cell is capable of true nephron differentiation. This may have outstanding relevance in clinical practice.

The zinc finger protein Sall1 is essential for UB attraction toward the mesenchyme: it is strongly expressed in the metanephric mesenchyme by multipotent nephron progenitors which form colonies and originate the cap mesenchyme, upon Wnt4 stimulation (Nishinakamura, 2003). The essential role of Sall1 in kidney nephrogenesis has been shown in deficient Sall1 mice, which die in the perinatal period with kidney agenesia (Nishinakamura and Takasato, 2005).

Recently, Ho et al. (2010) showed that MicroRNAs (miRs) miR-10a, miR-106b[no virgola] and miR-17-5p promote the survival of nephron progenitors cap mesenchymal cells by downregulating the expression of the pro-apoptotic protein Bim. MiRs promote survival of nephron progenitors, thus determining nephron endowment during kidney development by increasing the nephron number at birth. Podocyte specific deletion of dicer, the most important miRNA-processing enzyme, has been shown to result in severe alterations of cytoskeletal dynamics (Harvey et al., 2008), leading to rapid glomerular and tubular injury (Ho et al., 2008) and eventually inducing glomerulosclerosis (Shi et al., 2008).

WT1 has been proposed as a master control gene that regulates the expression of a large number of genes that have a critical role in kidney development (Kreidberg, 2010), as shown by the occurrence of renal agenesis in mouse embryos when it was lacking (Kreidberg et al., 1993). The WT1 expression increases in the cap nephrogenic progenitors and promotes differentiation toward the epithelial phenotype. It encodes a zinc finger protein that acts both as an RNA binding protein and as a transcription factor that binds to several target genes, including members of the bone morphogenic protein family (Bmp 4, Smad 4) and Hedgehog (Smo, Hhat) pathways, as well as PAX2, VEGF-A, and Sall1 (Kreidberg and Hartwig, 2008; Hartwig et al., 2010). Recent studies have identified a new set of WT1 target genes suggesting a broad role for WT1 in the epigenetic regulation of gene expression during kidney development (Kreidberg, 2010).

Recent data from our group confirm in the human kidney the relevant role played by WT1 during nephrogenesis. Its

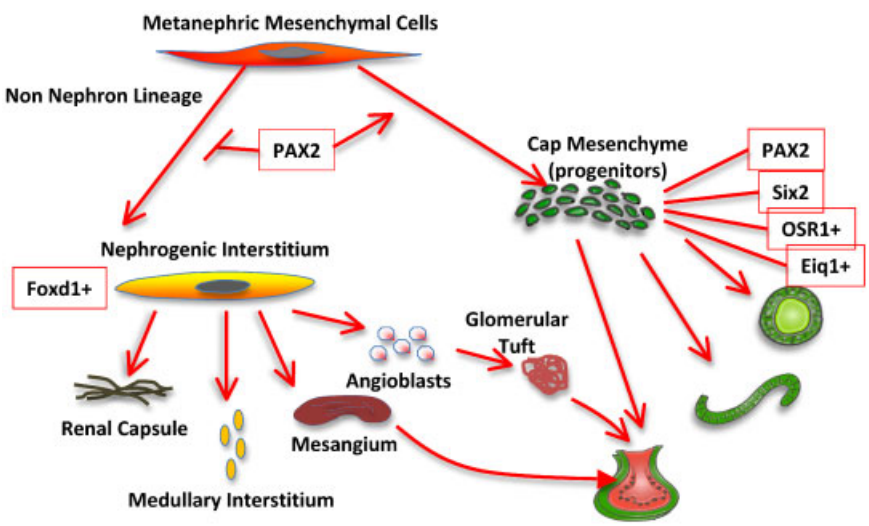


**Fig. 4. Differentation of the metanephric mesenchyme.**

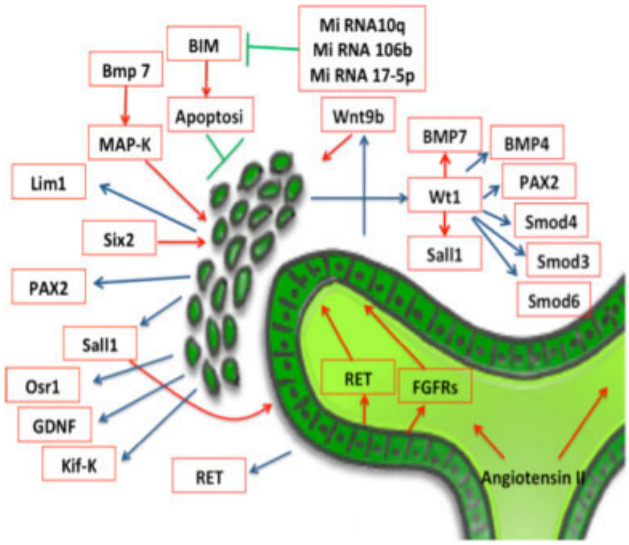


**Fig. 5. Schematic representation of the main molecular pathways involved in the interaction between cap mesenchyme and the UB tip cells.**

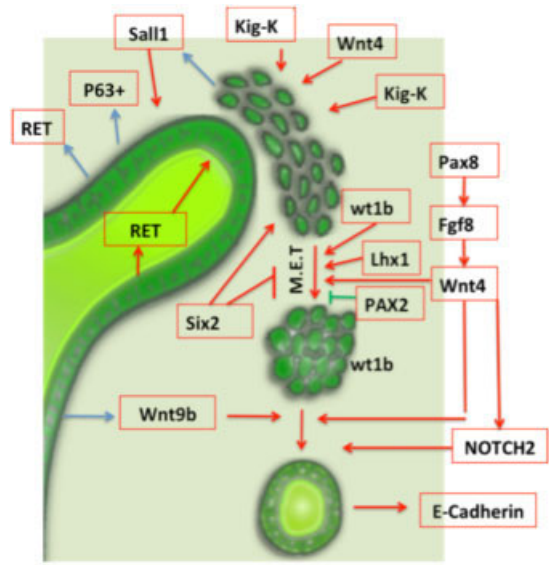


**Fig. 6. Main molecular pathways regulating the early phases of mesenchymal–epithelial transition.**

expression pattern suggests a main role in the regulation of the process of mesenchymal–epithelial transition and in maturation of podocytes (Fanni et al., 2011).

Among the aforementioned target genes for WT1, an important role has been assigned to Bmp-7 (Gai et al., 2009), which has been reported to be highly expressed during early embryonic kidney development in the renal progenitor cells (Dudley et al., 1995; Luo et al., 1995), in the UB cells and, eventually, in mature podocytes (Kazama et al., 2008). The role of Bmp-7 in nephron growth and differentiation and, in particular, in kidney stem cell survival, has been better clarified in the Bmp-7 null embryos which showed poorly developed kidneys characterized by oligonephronia, mainly due to the premature loss of the progenitor population (Dudley et al., 1995; Luo et al., 1995).

A member of the FGF family, FGF8, is highly expressed in the cap mesenchyme condensating in the pre-tubular aggregates (PTA) around each branch of the UB, and may play an important role in nephron induction and maintenance of the renal progenitor population in the cap mesenchyme (Perantoni et al., 2005). FGF8 is also required for cell survival at distinct stages of nephrogenesis and for regulation of gene expression in nascent nephrons (Grieshammer et al., 2005).

## Mesenchymal-Epithelial Transition

The complex process of transition from spindle-shaped mesenchymal cells toward epithelial cells with adherens junctions, better known as mesenchymal–epithelial transition, occurs in the cap mesenchymal cells, the self-renewing progenitor cells located around the UB tips. Epithelial differentiation involves morphological changes, from a comparatively disordered mesenchyme to an organized simple epithelium (Fig. 6). Morphological changes in progenitor cells are paralleled by the activation of a large number of "epithelial" genes, including genes encoding for cytokeratins, desmosomal components, adherens and tight junctions, basement-membrane collagens, laminin types, and, conversely, by the inactivation of "mesenchymal" genes, including those encoding for vimentin and collagen (Kispert et al., 1998; Carroll et al., 2005). This complex process likely occurs in a defined order, controlled by the new transcription factors activated at this time (Davies et al., 1999).

A critical phase during nephrogenesis is the transition between the aggregation process, which gives rise to the cap mesenchyme and the pre-tubular clustering (Fig. 7), and the epithelial transformation. During the progression from the cap mesenchyme toward the renal vesicle (Fig. 8), marked changes have been described as concerns gene expression. In particular, Six2 and Cited2 showed a decreased expression, whereas Lhx1 and the FGF8 expression was found to progressively increase (Brunskill et al., 2008). Wnt-4 has the ability to induce the mesenchymal-to-epithelial transformation of the PTA of cap mesenchymal cells to become the renal vesicles (Kispert et al.,

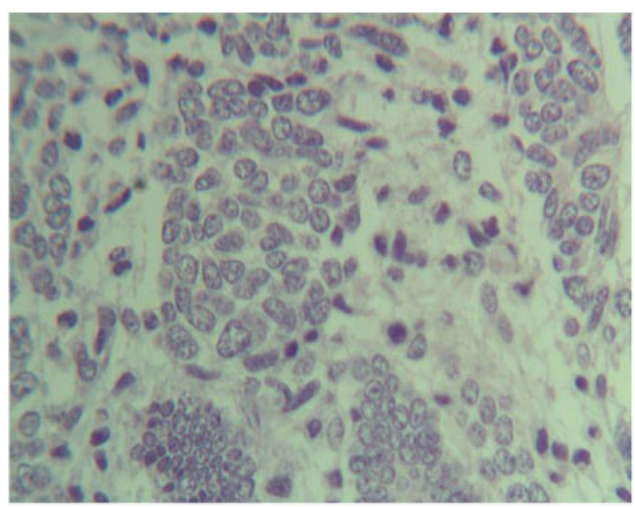

**Fig. 7. Nephrogenesis in human fetal kidney (11 weeks of gestation): PTAs.**

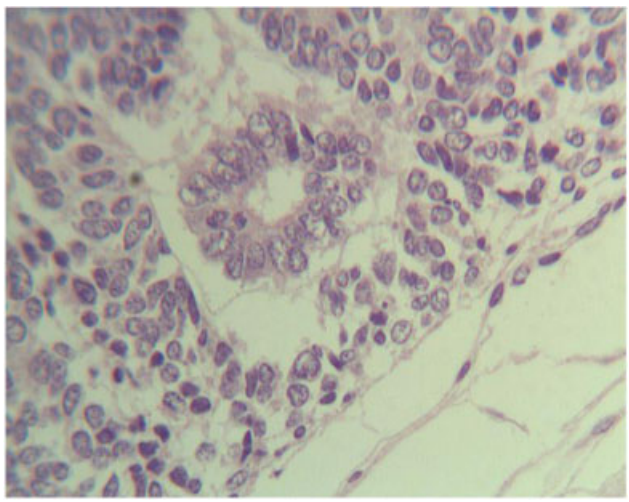

**Fig. 8. Nephrogenesis in human fetal kidney (11 weeks of gestation): renal vesicle.**

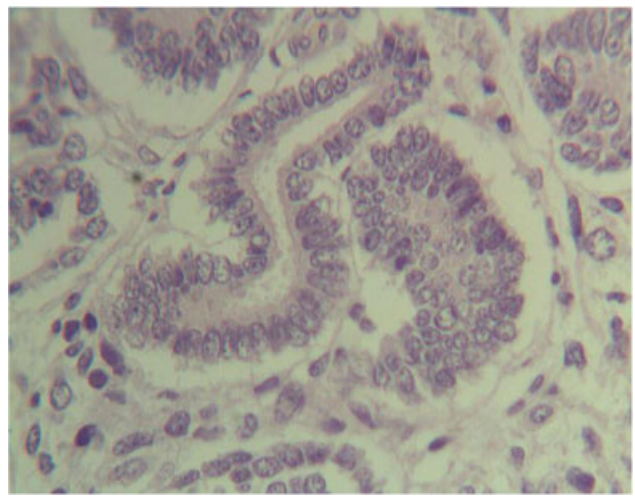

**Fig. 10. Nephrogenesis in human fetal kidney (11 weeks of gestation): S-shaped body.**

1998). During the process of aggregation, cap mesenchymal cells express increasing amounts of the signaling molecule Wnt-4, in an autocrine positive feedback loop, so that Wnt-4 signals drive Wnt-4 expression further, thus regulating the progressive epithelial transformation of cap mesenchymal cells (Stark et al., 1994; Fig. 6). Cap mesenchymal cells respond to Wnt-4 signaling by differentiating into the renal vesicle, a simple tubule undergoing extensive growth, segmentation, and differentiation that will end with the origin of the mature proximal nephron (Figs. 8–10; Park et al., 2007). This process was confirmed by the failure to generate epithelial renal vesicles in Wnt-4 mutants (McMahon et al., 2008). The Wnt4 expression is closely related to the FGF8 expression, since inactivation of FGF8 in early mesoderm results in the absence of Wnt-4 (Perantoni et al., 2005).

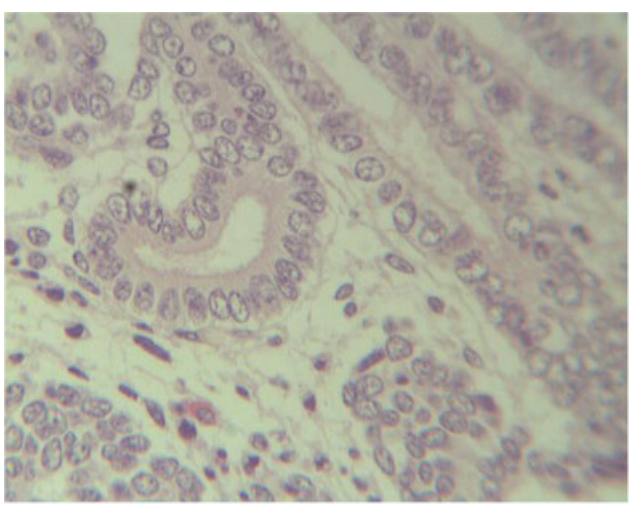

**Fig. 9. Nephrogenesis in human fetal kidney (11 weeks of gestation): Comma shaped body.**

Mesenchymal–epithelial transition occurs under epigenetic control: cap progenitor cells exhibit a bivalent histone methylation signature, characteristic of pluripotent cells, while further stages of the epithelial differentiation are marked by epigenetic modifications, including loss of repressive marks and gain of new activation marks on the PAX2 promoters (El-Dahr et al., 2010).

Very little research has focused on the relationship between differentiation of the cap mesenchymal cells during kidney development and the conditions the fetus encounters in the uterus, including the kind of nutrition, pollutants, drugs, and infections the mother is exposed to, and fetal hypoxia. Recent research indicates that the major effect of fetal hypoxia is represented by a block in the process of the epithelial-to-mesenchymal transition occurring in the cap mesenchyme, mediated by the down-regulation of Wnt-4 (Wilkinson et al., 2010). An hypoxia-induced block in the differentiation of cap mesenchyme may lead to a lesser degree of UB branching and failure to develop nephron structures, ending in a reduction in nephron number and kidney size.

## Glomerulogenesis and Tubulogenesis

Most nephrons in human kidneys are endowed with 36 weeks of gestation (Ho et al., 2008). Their number ranges from 300,000 up to 1,800,000 per kidney, due to both genetic and environmental causes occurring during pregnancy and largely not fully defined (Georgas et al., 2009).

Several genes are involved in regulating the complex process of glomerulogenesis. Sema3a is an essential negative regulator of endothelial cell survival in developing glomeruli, and plays a crucial role in podocyte differentiation (Reidy et al., 2009). Indeed, Sema3a deletion resulted in trouble in renal vascular patterning, with an excess of endothelial cells, whereas its overexpression resulted in glomerular hypoplasia, characterized by glomerular endothelial cell apoptosis, delayed and abnormal podocyte foot process development, complete absence of slit diaphragms, and congenital proteinuria.

The renal vesicle may be considered the first epithelial structure originating from the cap mesenchyme (Fig. 8). Formation of a mature nephron requires segmentation and patterning of the renal vesicle, followed by fusion with the

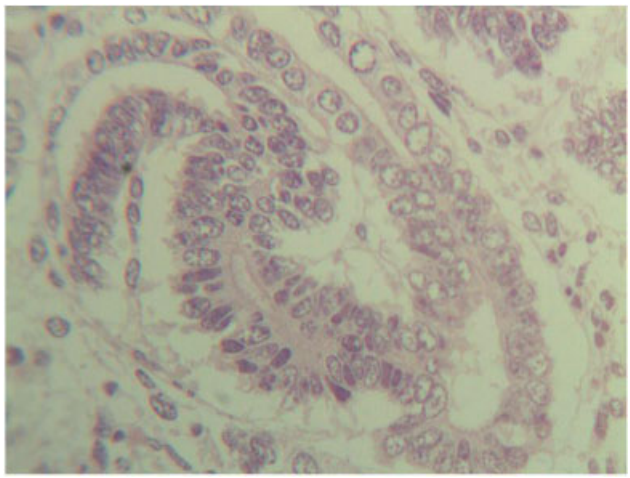

**Fig. 11. Nephrogenesis in human fetal kidney (11 weeks of gestation): arrow indicates the fusion between the distal compartment of a S-shaped body and a collecting tubule originating from a branch of the UB.**

ureteric component of the fetal kidney (Fig. 11) to form a patent contiguous uriniferous tubule (Georgas et al., 2009).

Four stages of nephron development have been defined: Stage I, corresponding to the appearance of the renal vesicle (Fig. 8); stage II, characterized by the transformation of the renal vesicle into the comma-shaped body (Fig. 9) and then into the S-shaped bodies (Fig. 10); stage III, also defined as the capillary loop stage (Fig. 11); stage IV, or the maturing nephron stage (Little et al., 2007; Fig. 12). The first stage is characterized by the progression of the mesenchymal–epithelial transition, with transformation of the solid pretubular aggregates of mesenchymal cells into roundish epithelial structures with a central lumen, the renal vesicles. At the molecular level, eight markers have been identified as specific of the cells of the distal renal vesicle, i.e., Dkk1, Papss2, Greb1, Dll1, Pcsk9, Lhx1, Bmp2, and Pou3f3, whereas two others, Tmem100 and Wt1, were strongly expressed in the proximal renal vesicle, probably reflecting the differential developmental programs of the proximal and distal cell populations in which renal vesicle cells may be subdivided at this stage of nephron differentiation (Georgas et al., 2009). In the second stage, clear segmentations of the renal vesicle give rise to the "comma-shaped body" and to the "S-shaped body." At the stage of Comma-shaped body, the developing nephron may be subdivided into a proximal and a distal segment, characterized by the expression of intercellular adhesion molecules such as Cadherin-6 and E-cadherin. Recent data from our group clearly show the expression in the Comma- and s-shaped bodies of thymosin Beta 10, a member of beta thymosins, a family of peptides which play essential roles in many cellular functions (Gerosa et al., 2010).

Notch1, in concert with Notch2, has been shown to contribute to segmentation of the Comma-shaped body and to the origin of the S-shaped body (Surendran et al., 2010), which has been demonstrated to be organized into three segments, proximal, medial, and distal: the cells of the proximal segment further differentiate to form the parietal (Bowman capsule) and visceral (podocytes) epithelium of the glomerulus; those of the median segment give rise to the proximal tubules, whereas those of the distal segment drive the process of fusion with the collecting tubules (Georgas et al., 2009; Fig. 13). The third stage, the capillary loop stage, is characterized by the appearance and development in the renal cortex of the primitive loop of Henle. Only during the fourth stage, following the subdivision of the medulla into outer and inner medulla, does the loop of Henle develop its medullary portion. During stage III, the renal vasculature undergoes rapid developmental changes, including the appearance of the glomerular capillary system, the differentiation of the presumptive endothelium of the renal corpuscle and the development of renal arteries and veins. Recent immunohistochemical studies, carried out in kidneys of human fetuses, clearly showed the absence of vascular markers such as CD31 and CD34 in primitive nephrons and their appearance, during the third stage, in the endothelial cells of the mature glomerular tufts (Fonseca Ferraz et al., 2008).

Stage IV is characterized by the final differentiation of the main components of the renal corpuscles and the tubular segments, including proximal tubules, the loop of Henle, distal tubules, and by development of the juxtaglomerular complex, macula densa, mesangium, and part of the afferent arterioles. During this stage, the renal interstitium differentiates into different components, including cortical, medullary, perihilar, and nephrogenic zone interstitia (Little et al., 2007).

Podocytes evolve from columnar epithelial cells and develop an arborized structure within the glomerulus, with their foot processes attached to the glomerular basal membrane. Par1, a member of the Partitioning defective protein family, is required for normal differentiation of podocytes by establishing and maintaining a polarized podocyte structure (Reidy and Tufro, 2011). Recently, a role in podocyte development has been proposed for CD10, which appeared to be highly expressed in the undifferentiated metanephric mesenchyme and, during differentiation, in precursors of podocytes and of Bowman capsule cells (Faa et al., 2011). Mesangial cells, which constitute approximately 30–40% of the glomerular cell population have been proposed to originate from the cap mesenchymal cells which originate the non-nephron lineage and are characterized by the expression of Foxd1 (Kobayashi et al., 2008). Over the years, numerous studies focused on determining the origin of mesangial cells have been carried out: although all the data suggest that mesangial cells migrate, together with endothelial cells, into the glomerulus from the outside, their origin could not be conclusively determined (Holthofer et al., 1995). A single hematopoietic stem cell has been demonstrated to differentiate into glomerular mesangial cells, thus supporting the hypothesis of the hematopoietic origin of the mesangium (Masuya et al., 2003), probably deriving from the granulocyte/macrophage lineage (Abe et al., 2005). The scarcity of studies on the mechanisms that differentiate cell types along the axis of the nephrogenic interstitium, including mesangial cells, may in large part be due to the fact that specific molecular and immunohistochemical markers distinguishing these early decision events are unavailable (Dressler, 2009).

Regional differentiation and maturation of the tubules is accompanied by a number of morphological changes, paralleled by changes in the activity of multiple transcription factors. Some components that have been shown to play a key role in the earlier stages of kidney development, e.g., PAX2, are progressively downregulated and disappear before tubule maturation (Dressler and Douglass, 1992). Regional differentiation and maturation of the renal tubules are accompanied by the increase in activity of multiple transcription factors, including WT-1 and PAX8.

This design of sequential steps in nephron development has been generally considered typical of all mammals. Recently, a paper by our group showed that piglet kidneys are characterized during nephrogenesis by peculiar morphological events, with marked differences compared with humans (Gerosa et al., 2011). Such information should be taken into account when experimental data in piglets are extrapolated to humans, especially for clinical purposes.

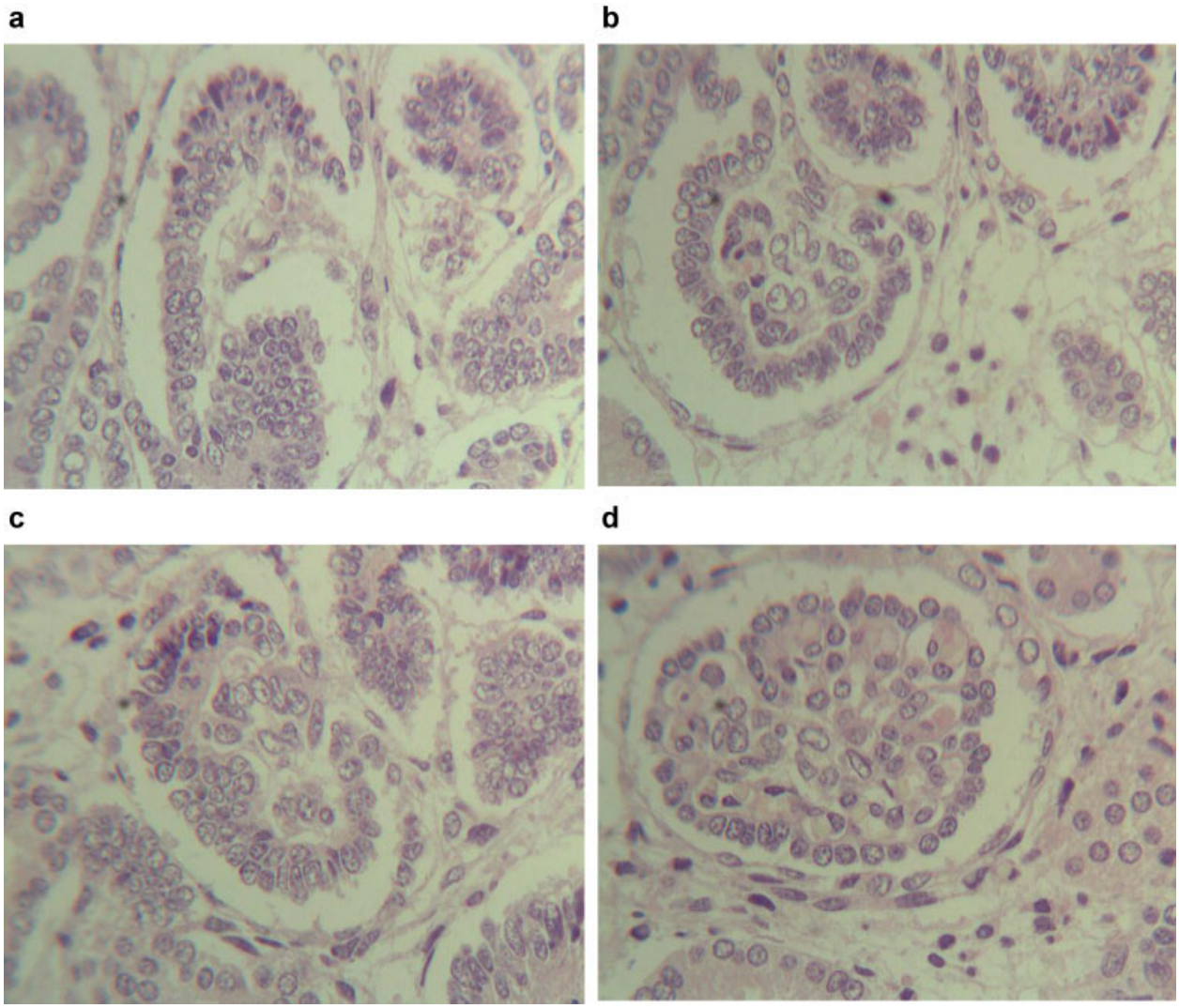


**Fig. 12. Different phases of glomerulogenesis: (a) angioblasts (arrow) start to interact with the prossimal segment of a S-shape body; (b and c) differentiation between visceral and parietal epithelial cells parallels differentiation of vascular and mesangial cells; (d) advances stages of glomerular morphogenesis.**

## The Interstitial Cells

Besides the Six2-positive cells of the cap mesenchyme (see above in The Cap Mesenchyme section), metanephric mesenchymal cells give origin to the Foxd1-positive cells, the first actors in the non-nephron lineage and a self-renewing progenitor population for the medullary interstitium, the renal capsule, putatively mesangium, and pericytes of the kidney (Kobayashi et al., 2008). The interstitial cell fate should be repressed by PAX2 which probably represents a developmental boundary between the nephron and non-nephron lineages, maintaining and favoring the nephron lineage. A number of subcomponents of the renal interstitium are defined early during kidney development, including cells located at the periphery of the developing organ that give rise to the renal capsule, fibroblasts, and resident macrophages (Little et al., 2007).

The gene encoding the T-box transcription factor Tbx18 is expressed in undifferentiated mesenchymal cells surrounding the distal ureter stalk, which are able to differentiate into periureteral muscle fibers. The ureteric mesenchyme derives from a distinct cell population that is separated early in kidney development from the other mesenchymal cells of the renal system (Kispert et al., 1998). Several genes, with specific expression in the periureteric mesenchyme, have been shown to depend upon Tbx18: among them, SOX9 is required for correct differentiation of the ureteric smooth muscle layer (Kispert et al., 1998).

Angioblast mesenchyme induction during early kidney development is mediated by VEGF-A, which appears to act on the angioblast population under WT1 control (Gao et al., 2005).

Recently, HOX10 genes have been shown to play a critical role in the developing of the stromal cell compartment and in regulating patterning, differentiation, and integration of different stromal cell types. HOX10 mutant kidneys did not form a renal capsule, they exhibited decreased nephrogenesis, and developed hydronephrosis (Wellik, 2010).

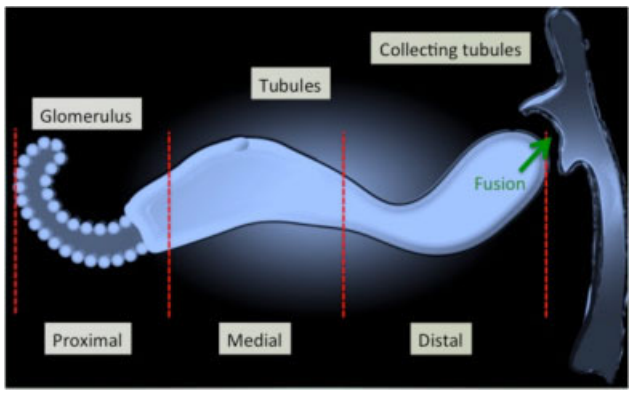


**Fig. 13. Organization of the S-shaped body intothreesegments: proximal, medial, and distal.**

## Conclusions

The picture illustrated herein, which deals with the hypothesized sequence of morpho-molecular changes occurring during kidney development, reflects up- and down-regulation of genes expressed in the UB epithelial cells, as well as in the metanephric mesenchyme and in stromal cells, during renal development. This complex picture clearly shows that mutations or altered epigenetic modulation in genes expressed during nephrogenesis may compromise ureteric elongation and branching and, subsequently, the process of mesenchymal–epithelial transition. As a consequence, even subtle changes in the complex reciprocal interactions between all these cell types may have severe consequences on the ultimate development of the human kidney. Complex correlations between morphological and molecular events starting with the origin of the UB and its branching into the metanephric mesenchyme, and ending with the maturation of nephrons, have been reported in different animal species. However, more studies must be carried out on humans, to verify if the outcome in humans parallels that previously described in other species at the morphological, immunohistochemical, and molecular levels. Marked differences observed among different species in the origin and duration of nephrogenesis suggest that morphological and molecular events may be different. Future contributions are needed to better understand the nature of events culminating in the formation of the mature metanephros in humans. In particular, knowledge concerning the intimate mechanisms underlying patterning of nephron precursors of distinct kidney must improve in the next few years to link top basic research to clinical settings, namely for prevention of acute kidney injury and end-stage renal disease.

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## Literature Cited

Abe T, Fleming PA, Masuya M, Minamiguchi H, Ebihara Y, Drake CJ, Ogawaa M. 2005. Granulocyte/macrophage origin of glomerular mesangial cells. Int J Hematol 82:115–118.
Bard JBL, McConnell JE, Davies JA. 1994. Towards a genetic basis for kidney development. Mech Dev 48:3–11.
Barnes JD, Crosby JL, Jones CM, Wright CV, Hogan BL. 1994. Embryonic expression of Lim1, the mouse homolog of Xenopus Xlim1, suggests a role in lateral mesoderm differentiation and neurogenesis. Dev Biol 161:168–178.
Basson MA, Akbulut S, Watson-Johnson J, Simon R, Carroll TJ, Shakya R, Gross I, Martin GR, Lufkin T, McMahon AP, Wilson PD, Constantini FD, Mason IJ, Licht JD. 2005. Sprouty 1 is a critical regulator of GDNF/RET-mediated kidney induction. Dev Cell 8:229–239.
Batchelder CA, Chang C, Lee I, Matsell DG, Yoder MC, Tarantal AF. 2009. Renal ontogeny in the Rhesus monkey (Macaca mulatta) and directed differentiation of human embryonic stem cells towards kidney precursors. Differentiation 78:45–56.
Benedetti EL, Marinozzi V. 1958. Sulla fine struttura del tessuto intercapillare nel glomerulo di Malpighi. Rass Fisiopat Clin 30:1–10.
Benetti E, Artifoni L, Salviati L, Pinello L, Perrotta S, Zuffardi O, Zacchello G, Murer L. 2007. Renal hypoplasia without optic coloboma associated with PAX2 gene deletion. Nephrol Dial Transplant 22:2076–2078.
Bertram JF, Douglas-Denton RN, Diouf B, Hughson MD, Hoy WE. 2011. Human nephron number: implications for health and disease. Pediatr Nephrol 26:1529–1533.
Bowman W. 1842. On the structure and use of the malpighian bodies of the kidney with observations on circulation through that gland. Philos Trans R Soc Lond 57:132.
Boyle S, Misfeldt A, Chandler KJ, Deal KK, Southard-Smith EM, Mortlock DP, Baldwin HS, de Caestecker M. 2008. Fate mapping using Cited1-CreERT2 mice demonstrates that the cap mesenchyme contains self-renewing progenitor cells and gives rise exclusively to nephronic epithelia. Dev Biol 313:234–245.
Brophy PD, Ostrom L, Lang KM, Dressler GR. 2001. Regulation of ureteric bud outgrowth by Pax2-dependent activation of the glial derived neurotrophic factor gene. Development 128:4747–4756.
Brunskill EW, Aronow BJ, Georgas K, Rumballe B, Valerius MT, Aronow J, Kaimal V, Jegga AG, Grimmond S, McMahon AP, Patterson LT, Little MH, Potter SS. 2008. Atlas of gene expression in the developing kidney at microanatomic resolution. Dev Cell 15:781–791.
Carroll TJ, Park JS, Hayashi S, Majumdar A, McMahon AP. 2005. Wnt9b plays a central role in the regulation of mesenchymal to epithelial transitions underlying organogenesis of the mammalian urogenital system. Dev Cell 9:283–292.
Challen GA, Martinez G, Davis MJ, Taylor DF, Crowe M, Teasdale RD, Grimmond SM, Little MH. 2004. Identifying the molecular phenotype of renal progenitor cells. J Am Soc Nephrol 15:2344–2357.
Cohen T, Loutochin O, Amin M, Capolicchio JP, Goodyer P, Jednak R. 2007. PAX2 is reactivated in urinary tract obstruction and partially protects collecting duct cells from programmed cell death. Am J Physiol Renal Physiol 292 F:1267–1273.
Constantini F. 2006. Renal branching morphogenesis: Concepts, questions and recent advances. Differentiation 74:402–421.
Constantini F, Kopan R. 2010. Patterning a complex organ: Branching morphogenesis and nephron segmentation in kidney development. Dev Cell 18:698–712.
Davies JA, Perera A, Walker C. 1999. Mechanisms of epithelial development and neoplasia in the metanephric kidney. Int J Dev Biol 43:473–478.
Dressler GR. 2006. The cellular basis of kidney development. Annu Rev Cell Dev Biol 22:509–529.
Dressler GR. 2008. Epigenetics, development, and the kidney. J Am Soc Nephrol 19:2060–2067.
Dressler GR. 2009. Advances in early kidney specification, development and patterning. Development 136:3863–3874.
Dressler GR, Douglass EC. 1992. Pax-2 is a DNA-binding protein expressed in embryonic kidney and Wilms tumor. Proc Natl Acad Sci USA 89:1179–1183.
Dressler GR, Deutsch U, Chowdhury K, Nornes HO, Gruss P. 1990. Pax2, a new murine paired-box containing gene and its expression in the developing excretory system. Development 109:787–795.
Drummond I, Vasilyev A. 2010. Epithelial cell movements during tubulogenesis. 11th International Workshop on Developmental Nephrology, New York, Oral Presentation Abstract O-44, p 47.
Dudley AT, Lyons KM, Robertson FJ. 1995. A requirement for bone morphogenic protein-7 during development of the mammarian kidney and eye. Genes Dev 9:2795–2807.
Dudley AT, Godin RE, Robertson EJ. 1999. Interaction between FGF and BMP signaling pathways regulates development of metanephric mesenchyme. Genes Dev 13:1601–1613.
Eccles MR, He S, Legge M, Kumar R, Fox J, Zhou C, French M, Tsai RWS. 2002. PAX genes in development and disease: The role of PAX2 in urogenital tract development. Int J Dev Biol 46:535–544.
El-Dahr S, McLoughlin N, Wang F. 2010. Epigenetic control of nephron differentiation. 11th International Workshop on Developmental Nephrology, New York, Oral Presentation Abstract O-42, p 45.
Faa G, Gerosa C, Fanni D, Nemolato S, Locci A, Cabras T, Marinelli V, Puddu M, Zaffanello M, Monga G, Fanos V. 2010. Marked interindividual variability in renal maturation of preterm infants: Lessons from autopsy. J Matern Fetal Neonatal Med 23:129–133.
Faa G, Gerosa C, Fanni D, Nemolato S, Marinelli V, Locci A, Senes G, Mais V, Van Eyken P, Iacovidou N, Monga G, Fanos V. 2011. CD10 in the developing human kidney: Immunoreactivity and possible role in renal embryogenesis. J Matern Fetal Neonatal Med (in press).
Fanni D, Fanos V, Monga G, Gerosa C, Locci A, Nemolato S, Van Eyken P, Faa G. 2011. Expression of WT1 during normal human kidney development. J Matern Fetal Neonatal Med (Suppl 2): 45–48.
Fanos V, Puddu M, Reali A, Atzei A, Zaffanello M. 2010. Perinatal nutrient restriction reduces nephron endowment increasing renal morbidity in adulthood: a review. Early Hum Dev 1:37–42.
Fanos V. 2011. Cells, the tree, medicines and the tailor. Curr Pharm Design (in press).
Farquhar MG, Palade GE. 1962. Functional evidence for the existence of a third cell type in the renal glomerulus. Phagocytosis and filtration residues by a distinctive "third" cell. J Cell Biol 13:55–87.
Fonseca Ferraz ML, Dos Santos AM, Cavellani CL, Rossi RC, Correa RR, Dos Reis MA, de Paula Antunes Teixeira V, da Cunha Castro EC. 2008. Histochemical and immunohistochemical study of the glomerular development in human fetuses. Pediatr Nephrol 23:257–262.
Gai Z, Zhou G, Itoh S, Morimoto Y, Tanishima H, Hatamura I, Uetani K, Ito M, Muragaki Y. 2009. Trps1 functions downstream of Bmp7 in kidney development. J Am Soc Nephrol 20:2403–2411.
Gao X, Chen X, Taglienti M, Rumballe B, Little MH, Kreidberg JA. 2005. Angioblast mesenchyme induction of early kidney development in mediated by WT1 and Vegfa. Development 132:5437–5449.
Georgas K, Rumballe B, Valerius MT, Chiu HS, Thiagarajan RD, Lesieur E, Aronow BJ, Brunskill EW, Combes AN, Tang D, Taylor D, Grimmond SM, Potter SS, McMahon AP, Little MH. 2009. Analysis of early nephron patterning reveals a role for distal RV

proliferation in fusion to the ureteric tip via a cap mesenchyme-derived connecting segment. Dev Biol 332:273–286.
Gerosa C, Fanni D, Nemolato S, Locci A, Marinelli V, Cabras T, Messana I, Castagnola M, Monga G, Fanos V, Faa G. 2010. Thymosin beta-10 expression in developing human kidney. J Matern Fetal Neonatal Med 3:125–128.
Gerosa C, Fanos V, Fanni D, Nemolato S, Locci A, Xanthos T, Papalois A, Faa G, Iacovidou N. 2011. Toward nephrogenesis in the pig kidney: the composite tubulo-glomerular nodule. J Matern Fetal Neonatal Med (Suppl 2): 53–55.
Gilbert T, Cibert C, Moreau E, Géraud G, Merlet-Bénichou C. 1996. Early defect in branching morphogenesis of the ureteric bud in induced nephron deficit. Kidney Int 50: 783–795.
Golgi C. 1889. Annotazioni intorno all'Istologia dei reni dell'uomo e di altri mammiferi e sull'istogenesi dei canalicoli oriniferi". Rend R Acad Lincei 5:545–557.
Grieshammer U, Cebrian C, Hagan R, Meyers F, Herzlinger D, Martin GR. 2005. FGF8 is required for cell survival at distinct stages of nephrogenesis and for regulation of gene expression in nascent nephrons. Development 132:3847–3857.
Grote D, Souabni A, Busslinger M, Bouchard M. 2006. Pax 2/8-regulated Gata 3 expression is necessary for morphogenesis and guidance of the nephric duct in the developing kidney. Development 133:53–61.
Gruenwald P, Popper H. 1940. The histogenesis and physiology of the renal glomerulus in early postnatal life. J Urol 43:452–466.
Hains D, Sims-Lucas S, Kish K, Saha M, McHugh K, Bates CM. 2008. Role of fibroblast growth factor receptor 2 in kidney mesenchyme. Pediatr Res 64:592–598.
Hall BV. 1956. Observations on the organization development and ultra microscopic structure of the capillaries of the renal glomerulus. Am J Pathol 32:646–647.
Hartwig S, Ho J, Pandey P, MacIsaac K, Taglienti M, Xiang M, Alterovitz G, Ramoni M, Fraenkel E, Kreidberg A. 2010. Genomic characterization of Wilms' tumor suppressor 1 targets in nephron progenitor cells during kidney development. Development 137:1189–1203.
Harvey SJ, Jarad G, Cunningham J, Goldberg S, Schermer B, Harfe BD, McManus MT, Benzing T, Miner JH. 2008. Podocyte-specific deletion of dicer alters cytoskeletal dynamics and causes glomerular disease. J Am Soc Nephrol 19:2150–2158.
Herring R. 1900. The development of the malpighian bodies of the kidney and its relation to pathological changes which occur in them. J Path Bacteriol 6:459–474.
Ho J, Ng KH, Rosen S, Dostal A, Gregory RI, Kreidberg JA. 2008. Podocyte-specific loss of functional microRNAs leads to rapid glomerular and tubular injury. J Am Soc Nephrol 19:2159–2169.
Ho J, Pandey P, Schatton T, Khalid M, Frank M, Hartwig S, Kreidberg J. 2010. The pro-apoptotic protein BIM is a microRNA target affecting the maintenance of kidney progenitor cells. 11th International Workshop on Developmental Nephrology New York, Oral Presentation Abstract P-5, page 56.
Holthofer H, Sainio K, Miettinen A. 1995. The glomerular mesangium: Studies of its developmental origin and markers in vivo and in vitro. APMIS 103:354–366.
Hopkins C, Ineson J, Little M. 2010. Functional conversion of the human kidney epithelial cells to renal progenitors requires more than generalized epigenetic destabilization. 11th International Workshop on Developmental Nephrology, New York, Oral Presentation Abstract P-6, p 57.
Jain S. 2009. The many faces of RET dysfunction in kidney. Organogenesis 5:177–190.
Kazama I, Mahoney Z, Miner HJ, Graf D, Economides AN, Kreidberg JA. 2008. Podocyte-derived BMP-7 is critical for nephron development. J Am Soc Nephrol 19:2181–2191.
Kispert A, Vainio S, McMahon AP. 1998. Wnt-4 is a mesenchymal signal for epithelial transformation of metanephric mesenchyme in the developing kidney. Development 123:4223–4234.
Kobayashi A, Valerius MT, Mugford JW, Carroll TJ, Self M, Oliver G, McMahon AP. 2008. Six2 defines and regulates a multipotent self-renewing nephron progenitor population throughout mammalian kidney development. Cell Stem Cell 3:169–181.
Kreidberg JA. 2010. WT1 and kidney progenitor cells. Organogenesis 6:61–70.
Kreidberg JA, Hartwig S. 2008. Wilms' tumor 1: A riddle wrapped in a mystery inside a kidney. Kidney Int 74:411–412.
Kreidberg JA, Sariola H, Loring JM, Maeda M, Pelletier J, Housman D, Jaenisch R. 1993. WT1 is required for early kidney development. Cell 74:679–691.
Kurtz SM. 1958. The electron microscopy of the developing human renal glomerulus. Exp Cell Res 14:355–367.
Kuure S, Cebrian C, Machingo Q, Lu BC, Chi X, Hyink D, D'Agati V, Gurniak C, Witke W, Costantini F. 2010. Actin depolymerizing factors Cofilin1 and Destrin are required for ureteric bud branching morphogenesis. PloS Genet 6:e1001176.
Lazorthes-Herland A. 1961. Etude embryologique du glomérule rénal. Thesis at the University of Toulouse 1-50.
Lelongt B, Trugnan G, Murphy G, Ronco PM. 1997. Matrix metalloproteinases MMP2 and MMP9 are produced in early stages of kidney morphogenesis but only MMP9 is required for renal organogenesis in vitro. J Cell Biol 136:1363–1373.
Li X, Oghi KA, Zhang J, Krones A, Bush KT, Glass CK, Nigam SK, Aggarwal AK, Maas R, Rose DW, Rosenfeld MG. 2003. Eya protein phosphatase activity regulates Six1-Dach-Eya transcriptional effects in mammalian organogenesis. Nature 426:247–254.
Little MH, Brennan J, Georgas K, Davies JA, Davidson DR, Baldock RA, Beverdam A, Bertram JF, Capel B, Chiu HS, Clements D, Cullen-McEwen L, Fleming J, Gilbert T, Houghton D, Kaufman MH, Kleymenova E, Koopman PA, Lewis AG, McMahon AP, Mendelsohn CL, Mitchell EK, Rumballe BA, Sweeney DE, Valerius MT, Yamada G, Yang Y, Yu J. 2007. A high-resolution anatomical ontology of the developing murine genitourinary tract. Gene Expr Patterns 7:680–699.
Lu BC, Cebrian C, Chi X, Kuure S, Kuo R, Bates CM, Arber S, Hassell J, MacNeil L, Hoshi M, Jain S, Asai N, Takahashi M, Schmidt-Ott KM, Barasch J, D'Agati V, Costantini F. 2009. Etv4 and Etv5 are required downstream of GDNF and Ret for kidney branching morphogenesis. Nat Genet 41:1295–1302.
Luo G, Hofmann C, Bronckers AL, Sohocki M, Bradley A, Karsenty G. 1995. BMP-7 is an inducer of nephrogenesis and is also required for eye development and skeletal patterning. Genes Dev 9:2808–2820.
Luyckx VA, Brenner BM. 2010. The clinical importance of nephron mass. J Am Soc Nephrol 21:898–910.
Majumdar A, Vainio S, Kispert A, McMahon AP. 2003. Wnt11 and Ret/Gdnf pathways cooperate in regulating ureteric branching during metanephric kidney development. Development 130:3175–3185.
Masuya M, Drake CJ, Fleming PA, Reilly CM, Zeng H, Hill WD, Martin-Studdard A, Hess DC, Ogawa M. 2003. Hematopoietic origin of glomerular mesangial cells. Blood 101:2215–2218.
McMahon AP, Aronow BJ, Davidson DR, Davies JA, Gaido KW, Grimmond S, Lessard JL, Little MH, Potter SS, Wilder EL, Zhang P. GUDMAP project. 2008. GUDMAP: The genitourinary developmental molecular anatomy project. J Am Soc Nephrol 19:667–671.
Metsuyanim S, Harari-Steinberg O, Buzhor E, Omer D, Pode-Shakked N, Ben-Hur H, Schneider D, Dekel B. 2009. Expression of stem cell markers in the human fetal kidney. PloS One 4:1–15.
Michos O, Cebrian C, Hyink D, Grieshamer U, Williams L, D'Agati V, Licht JD, Martin GR, Costantini F. 2010. Kidney development in the absence of Gdnf and Spry1 requires Fgf10. PloS Genet 6:e1000809.
Montaldo G, Murri C. 1958. Rilievi sullo sviluppo del glomerulo renale in particolare sulla formazione della parete del capillare glomico, nell'uomo. Biol Lat 11:148–161.
Moritz KM, Wintour EM, Black MJ, Bertram JF, Caruana G. 2008. Factors influencing mammalian kidney development: implications for health in adult life. Adv Anat Embryol Cell Biol 196:1–78.
Mühle A, Mühle C, Amann K, Dotsch J, Nusken K-D, Boltze J, Schneider H. 2010. No juvenile arterial hypertension in sheep multiples despite reduced nephron numbers. Pediatr Nephrol 25:1653–1661.
Nishinakamura R. 2003. Kidney development conserved over species: Essential role of Sall1. Semin Cell Dev Biol 14:241–247.
Nishinakamura R. 2008. Stem cells in the embryonic kidney. Kidney Int 73:913–917.
Nishinakamura R, Takasato M. 2005. Essential roles of Sall1 in kidney development. Kidney Int 68:1948–1950.
Noel R, Pigeaud M. 1932. Sur le développement du flocule glomérulaire dans le rein foetal. Bull Histol Appl Physiol Pathol Tech Microsc 9:302–312.
O'Rahilly R, Muecke EC. 1972. The timing and sequence of events in the development of the human urinary system during the embryonic period proper. Z Anat Entw-Gesch 138:99–109.
Osafune K, Takasato M, Kispert A, Asashima M, Nishinakamura R. 2006. Identification of multipotent progenitors in the embryonic mouse kidney by a novel colony-forming assay. Development 133:151–161.
Ostrom L, Tang MJ, Gruss P, Dressler GR. 2000. Reduced Pax2 gene dosage increases apoptosis and slows the progression of renal cystic disease. Dev Biol 219:250–258.
Pachnis V, Mankoo B, Constantini F. 1993. Expression of the c-ret proto-oncogene during mouse embryogenesis. Development 119:1005–1017.
Park S, Valerius MT, McMahon AP. 2007. Wnt/β-catenin signaling regulates nephron induction during mouse kidney development. Development 134:2533–2539.
Perantoni AO, Dove LF, Karavanova I. 1995. Basic fibroblast growth factor can mediate the early inductive events in renal development. Proc Natl Acad Sci USA 92:4696–4700.
Perantoni AO, Timofeeva O, Naillat F, Richman C, Pajni-Underwood S, Wilson C, Vainio S, Dove LF, Lewandoski M. 2005. Inactivation of FGF8 in early mesoderm reveals an essential role in kidney development. Development 132:3859–3871.
Pichel GJ, Shen L, Sheng HZ, Granholm A-C, Drago J, Grinberg A, Lee EJ, Huang SP, Saarma M, Hoffer BJ, Sariola H, Westphal H. 1996. Defects in enteric innervation and kidney development in mice lacking GDNF. Nature 382:73–76.
Plecineanu O, Harari-Steinberg O, Dekel B. 2010. Concise review: Kidney stem/progenitor cells: differentiate, sort out or reprogram? Stem Cell 28:1649–1660.
Poladia DP, Kish K, Kutay B, Hains D, Kegg H, Zhao H, Bates CM. 2006. Role of fibroblast growth factor receptors 1 and 2 in the metanephric mesenchyme. Dev Biol 291:325–339.
Puddu M, Fanos V, Podda F, Zaffanello M. 2009. The kidney from prenatal to adult life: Perinatal programming and reduction of number of nephrons during development. Am J Nephrol 30:162–170.
Reidy KJ, Rosenblum ND. 2009. Cell and molecular biology of kidney development. Semin Nephrol 29:321–337.
Reidy K, Tufro A. 2011. Semaphorins in kidney development and disease: modulators of ureteric bud branching, vascular morphogenesis, and podocyte-endothelial crosstalk. Pediatr Nephrol 26:1407–1412.
Reidy KJ, Villegas G, Teichman J, Veron D, Shen W, Jimenez J, Thomas D, Tufro A. 2009. Semaphorin3a regulates endothelial cell number and podocyte differentiation during glomerular development. Development 136:3979–3989.
Rodríguez MM, Gómez AH, Abitbol CL, Chandar JJ, Duara S, Zilleruelo GE. 2004. Histomorphometric analysis of postnatal glomerulogenesis in extremely preterm infants. Pediatr Dev Pathol 7:17–25.
Rosenblum ND. 2008. Developmental biology of the human kidney. Semin Fetal Neonatal Med 13:125–132.
Rumballe B, Georgas K, Wilkinson L, Little M. 2010. Molecular anatomy of the kidney: What have we learned from gene expression and functional genomics? Pediatr Nephrol 25:1005–1016.
Saifudeen Z, Dipp S, Stefkova J, Yao X, Lookabaugh S, El-Dahr SS. 2009. P53 regulates metanephric development. J Am Soc Nephrol 20:2328–2337.
Sainio K, Suvanto P, Davies JA, Wartiovaara J, Wartiovaara K, Saarma M, Arumae U, Meng X, Lindhal M, Pachnis V, Sariola H. 1997. Glial cell-line derived neurotrophic factor is required for bud initiation for ureteric epithelium. Development 124:4077–4087.
Sanchez MP, Silos SI, Frisen J, He B, Lira SA, Barbacid M. 1996. Renal agenesis and the absence of enteric neurons in mice lacking GDNF. Nature 382:70–73.
Sariola H. 2002. Nephron induction revisited: From caps to condensates. Curr Opin Nephrol Hypertens 11:17–21.
Schedl A, Reginensi A, Clarkson M, Lu B. 2010. Role of SOX genes during kidney development. 11th International Workshop on Developmental Nephrology, New York, Oral Presentation Abstract O-30.
Schmidt-Ott KM, Yang J, Chen X, Wang H, Paragas N, Mori K, Li JY, Lu B, Costantini F, Schiffer M, Bottinger E, Barasch J. 2005. Novel regulators of kidney development from the tips of the ureteric bud. Am Soc Nephrol 16:1993–2002.
Self M, Lagutin OV, Bowling B, Hendrix J, Cai Y, Dressler GR, Oliver G. 2006. Six2 is required for suppression of nephrogenesis and progenitor renewal in the developing kidney. EMBO J 25:5214–5228.
Shi S, Yu L, Chiu C, Sun Y, Chen J, Khitrov G, Merkenschlager M, Holzman LB, Zhang W, Mundel P, Bottinger EP. 2008. Podocyte-selective deletion of dicer induces proteinuria and glomerulosclerosis. J Am Soc Nephrol 19:2159–2169.
Short KM, Hodson MJ, Smyth IM. 2010. Tomographic quantification of branching morphogenesis and renal development. Kidney Int 77:1132–1139.
Simeoni U, Ligi I, Buffat C, Boubred F. 2011. Adverse consequences of accelerated neonatal growth: Cardiovascular and renal issues. Pediatr Nephrol 26:493–508.
Singh RR, Moritz KM, Bertram JF, Cullen-McEwen LA. 2007. Effects of dexamethasone exposure on rat metanephric development: In vitro and in vivo studies. Am J Physiol Renal Physiol 293:F548–F554.
Song R, Spera M, Garrett C, Yosypiv IV. 2010. Angiotensin II-induced activation of c-Ret signaling is critical in ureteric bud branching morphogenesis. Mech Dev 127:21–27.
Song R, Van Buren T, Yosypiv IV. 2010. Histone deacetylases are critical regulators of the renin-angiotensin system during ureteric bud branching morphogenesis. Pediatr Res 67:573–578.

Stark K, Vainio S, Vassileva G, McMahon AP. 1994. Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. Nature 372:679–683.
Surendran K, Boyle S, Barak H, Kim M, Stromberski C, McCright B, Kopan R. 2010. The contribution of NOTCH1 to nephron segmentation in the developing kidney is revealed in a sensitized NOTCH2 background and can be augmented by reducing mint dosage. Dev Biol 337:386–395.
Symonds ME, Stephenson T, Budge H. 2009. Early determinants of cardiovascular disease: The role of early diet in later blood pressure control. Am J Clin Nutr 89: 1518S–1522S.
Tamimi Y, Ekuere U, Laughton N, Grundy P. 2008. WNT5A is regulated by PAX2 and may be involved in blastemal predominant Wilms tumorigenesis. Neoplasia 10:1470–1480.
Uetani N, Bertozzi K, Chagnon MJ, Hendriks W, Tremblay ML, Bouchard M. 2009. Maturation of ureter-bladder connection in mice is controlled by LAR family receptor protein tyrosine phosphatases. J Clin Invest 119:924–935.
Watanabe T, Costantini F. 2004. Real-time analysis of ureteric bud branching morphogenesis in vitro. Dev Biol 271:98–108.
Welham SJ, Riley PR, Wade A, Hubank M, Woolf AS. 2005. Maternal diet programs embryonic kidney gene expression. Physiol Genomics 22:48–56.
Wellik D. 2010. HOX genes are required for the differentiation and integration of kidney cortical stroma cells. 11th International Workshop on Developmental Nephrology, New York, Oral Presentation Abstract O-14, page 24.
Wilkinson L, Chiu H, Rumballe B, Georgas K, Ju A, Moritz K, Little M. 2010. The effect of hypoxia on the development of the kidney. 11th International Workshop on Developmental Nephrology, New York, Oral Presentation Abstract P-29, page 83.
Xu PX, Adams J, Peters H, Brown MC, Heancy S, Maas R. 1999. Eya1-deficient mice lack ears and kidneys and show abnormal apoptosis of organ primordia. Nat Cancer 23:113–117.
Yosipiv IV. 2004. Hypothesis: A novel role for the renin-angiotensis system in ureteric bud branching. Organogenesis 1:26–32.
Yosypiv IV. 2008. A new role for the renin-angiotensin system in the development of the ureteric bud and renal collecting system. Keio J Med 57:184–189.
Zimmermann K. 1933. Uber den Ban des glomerulus der Saugerniere; Weite e Mitteilungen. Ztschr Mikr Anat Forsch 32:176–278.