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NEPHROLOGY - REVIEW

From ureteric bud to the first glomeruli: genes, mediators, kidney alterations

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Abstract The development of the mammalian kidney is a complex and in part unknown process which requires interactions between pluripotential/stem cells, undifferentiated mesenchymal cells, epithelial and mesenchymal components, eventually leading to the coordinate development of multiple different specialized epithelial, endothelial and stromal cell types within the kidney architectural complexity. We will describe the embryology and molecular nephrogenetic mechanisms, a fascinating traffic of cells and tissues which takes place in five stages: (1) ureteric bud (UB) development; (2) cap mesenchyme formation; (3) mesenchymal–epithelial transition (MET); (4) glomerulogenesis and tubulogenesis; (5) interstitial cell development. In particular, we will analyze the multiple cell types involved in these dramatic events as characters moving between different worlds, from the mesenchymal to the epithelial world and back, and will start to define the multiple factors that propel these cells during their travels throughout the developing kidney. Moreover, according with the hypothesis of renal perinatal programing, we will present the results reached in the fields of immunohistochemistry and molecular biology, by means of which we can explain how a loss or excess of molecular factors governing nephrogenesis may cause the onset of pathologies of different gravity, in some cases leading to a chronic kidney disease at different times from birth.

Keywords Human kidney · Human nephrogenesis · Renal development · Embryology · Ureteric bud · Mesenchimal epithelial transition · Glomeruli

Introduction

Human nephrogenesis is a process based on the complex interaction of epithelial and mesenchymal factors requiring development of different cell types at the same time. In recent years, the innovations introduced in the fields of immunohistochemistry and molecular biology have led to the acquisition of further knowledge and the revealing of the presence of different elements capable of modulating the stages in the nephrogenetic process [1].

The objective of this review is to correlate the different kidney alterations that may take place within the nephrogenetic context with kidney diseases in the pediatric age.

Embryology and molecular nephrogenetic mechanisms

The development of the metanephros, the primordium of the permanent kidney, takes place in five stages: (1) *ureteric bud* (UB) development; (2) *cap mesenchyme* formation; (3) mesenchymal–epithelial transition (MET); (4) glomerulogenesis and tubulogenesis; (5) interstitial cell development [2]. We will describe the first four stages.

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1. *Ureteric bud* development

The GDNF/c-RET/Wnt system

The UB develops by gemmation of the distal portion of the mesonephric duct starting from the 28th day of embryonic life. Following its growth in the cranial direction, at approximately the 32nd day it comes into contact with the mesenchyme of the metanephric blastema [3–5], thus forming the permanent kidney collector system [3, 6]. The main signal pathway involved in this process is represented by the GDNF/c-RET/WntI system [1], the alteration of which may lead to branching disorders [7] and/or development of the UB [8], up to renal agenesis [9] (Fig. 1). Knowledge of the factors that determine the entity of the nephronic mass at birth is still scanty, but it is thought that certain variations of the RET gene are associated with renal hypoplasia in a number of neonates [10]. A key role in the GDNF/c-RET/Wnt signal pathway is played by the SOX genes expressed at the extremities of the UB in the process of formation; experimental models with double SOX8/9 mutation presented hypoplasia and renal agenesis [11]. The activity of the GDNF/c-RET/Wnt system is controlled by several inhibitory factors: the first is represented by the SPRYI gene, which through inhibition of ERK phosphorylation (kinase regulated by extracellular signals), determines negative feedback on the GDNF, thus limiting UB development to a single site. In mice, its loss causes defects in renal development with the appearance of supernumerary ureteric buds along the caudal portion of Wolff's duct [12]. Another factor capable of inhibiting UB extroflection in culture is represented by the BMP4 belonging to the TGF-β family; since it is expressed at the level of the mesenchyme surrounding Wolff's duct, its mutation may lead to the development of ectopic buds [13]. Finally, the class 3 semaphorins (Sema3a) perform an inhibitory action on GDNF and VEGF-A (growth factor of the vascular endothelium) and are involved in the podocyte differentiation process. The deletion of Sema3a is the cause of an excess of endothelial cells but if it is hyperexpressed during kidney development it leads to apoptosis of the glomerule endothelium cells, alterations in podocyte development, absence of the filtration diaphragm and congenital proteinuria [14].

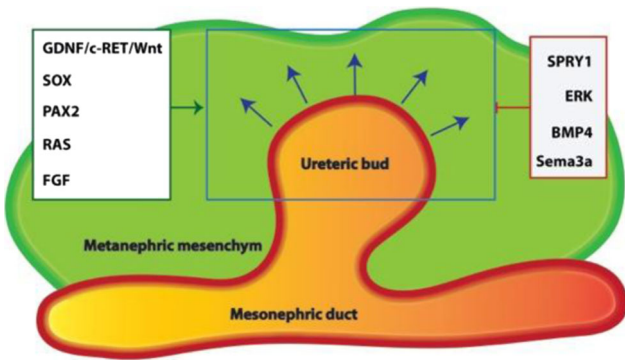

Fig. 1 Main mediators involved in changing the fate of metanephric mesenchyme

It has recently been demonstrated that alterations of β-catenin, which is an integral part of the regulatory pathway starting from WNT, may influence RET expression; its inactivation at the level of the cells that create Wolff's duct and the UB may lead to renal agenesis caused by blocking the branching of the UB itself [15].

PAX2 and the renal-coloboma syndrome

Among the stimulating factors, we find transcriptional PAX2 and PAX8 [16]; in particular, PAX2 plays a key role not only in renal development, but also in the regeneration of nephrons in some experimental models. Studies conducted on the adult zebrafish have demonstrated that following kidney damage induced by the administration of gentamicin there is coalescence of PAX2-positive renal stem cells in mesenchymal clusters from which derive renal vesicles and primitive nephrons in succession [17]. An altered expression of PAX2 is observed in different conditions, among which a maternal diet characterized by a reduced protein intake [18], following exposure to dexamethasone [19] and after accelerated postnatal growth [20]. PAX2 gene mutations favor increased apoptosis of the cells located at the extremity of the UB, with consequent alterations [21].

In humans, PAX2 gene mutations (*de novo* or hereditary) are at the base of a rare dominant autosomal disorder characterized by renal hypoplasia with or without renal insufficiency and abnormalities of the optical nerve, the renal-coloboma syndrome. The phenotypical characteristics of the syndrome have been correlated with a condition of haploinsufficiency of the protein normally produced by the PAX2 gene. The most frequent mutations are those of exon 2 of the PAX2 gene and these lead to the synthesis of a truncated protein that loses its normal capacity to bind DNA [22]. The main renal alterations found in studies on children and/or families with the renal-coloboma syndrome are represented by renal hypoplasia (60 % of patients), renal dysplasia, oligomeganephronia (a rare congenital or sporadic anomaly presenting bilateral renal hypoplasia with a reduced number of nephrons of larger size) and multicystic dysplastic kidney, the latter found for the first time in a family with a new heterozygote deletion of ten pairs of bases in exon 2 of the PAX2 gene [23–25]. Such

alterations may possibly be associated with vesicoureteral reflux (25 % of patients), neurosensorial deafness, skin and joint anomalies (20 % of cases) and the central nervous system. Noteworthy phenotypical variations have been observed even among members of the same family who share the same mutation [26]. In their 2007 report, Benetti et al. reported the first clinical case of PAX2 gene deletion not associated with coloboma in humans. The case in question was a six-year-old girl born at 35 weeks after a pregnancy complicated by a condition of oligohydramnios (weight at birth 2,500 g). At birth, a moderate chronic renal insufficiency in the first days of life was diagnosed with high creatinine and azotemia levels. The renal ultrasound scan performed at 5 months revealed the presence of a hypoplastic right kidney and a diffuse hyperechogenic left kidney with reduced corticomedullary differentiation in absence of vesicoureteral reflux. The authors suggest a dominant role of the PAX2 gene mutation and thus the condition of haploinsufficiency of the protein in the genesis of the ocular anomalies of the renal-coloboma syndrome; the absence of such alterations is supposedly connected to a complete deletion of the gene [22].

RAS and CAKUT

Another system that appears to be involved in UB development is the renin–angiotensin system (RAS). According to Yosypiv's studies, angiotensin II (ATII) appears to be capable of favoring UB branching by means of a stimulatory action on the ATII receptor either directly or through PAX2 activation. Instead, stimulation of the ATI receptor inhibits activity of the SPRYI gene which in turn exerts a stimulatory effect on the GDNF/c-RET/Wnt11 signal pathway [5].

Mutations of the RAS gene favor anomalies in the renal collector system. In particular, gene mutations of each of the single components of RAS or the use of ACE-blockers have been associated with a series of congenital anomalies indicated by the acronym *Congenital Anomalies of the Kidney and Urinary Tract* (CAKUT), which are considered among the main causes of renal insufficiency in infancy [5]. CAKUTs include a spectrum of malformations of the kidney and the ureteropelvic and ureterovesicle junction; they are the cause of more than 50 % of the abdominal masses found in neonates and involve 0.5 % of all pregnancies [27]. Studies conducted on two different cohorts (21 American Caucasians + 23 German Caucasians presenting CAKUT versus 31 American Caucasians + 24 German Caucasians not affected) showed that most of the study population presented a functionally significant mutation of intron 1 of the ATII receptor (MIM 300034) and that there was a significant association between the incidence of CAKUT and the mutation which compromised the efficiency of the splicing of the RNAm of ATII. Such studies also demonstrated that at the base of the development of the anomaly there was a retarded apoptosis of the undifferentiated mesenchymal cells surrounding the urinary tract in the key stages of the nephrogenetic process, from the UB to the development of the kidney and ureter [28].

RAS executes a fundamental function in the development of ureter peristalsis; its alteration may result in the onset of hydronephrosis [29]. Deletions of the ATII receptor have been associated with obstructions of the ureteropelvic or ureterovesicle junction in murine models [28]. The fibroblast growth factor (FGF) is another factor involved in the first stages of nephrogenesis and in particular in UB branching [30]. Studies on knockout mice for genes that encode for FGF1 and FGF2 receptors revealed important alterations in morphogenesis, among which cystic alterations, the impossibility of the UB to grow and branch, up to renal agenesis [31].

Metalloproteinases and actin-depolymerizing proteins

Among the factors that induce UB branching we once again find matrix metalloproteinases, in particular MMP9, which stops the developing collector tubules from invading the surrounding metanephric blastema [32]; knockout mice for the gene that encodes MMP9 have shown a delay in embryonic renal maturation and an increased ex vivo apoptosis, responsible for a 30 % reduction in the number of nephrons, 20 % in the weight of the adult kidney and alterations in renal morphology at 12 months [33].

Besides MMP, the two actin-depolymerizing proteins cofilin 1 and dextrin come into play to favor UB branching; the simultaneous loss of both these proteins blocks ureter branching and alters the organization of cell migration [34].

2. Mesenchyme formation

Together with progressive UB subdivision, the mesenchymal cells of the metanephric blastema organize to form aggregates around the UB branches to become cap mesenchyme cells [35].

The main factors involved in the process of differentiating cap mesenchyme cells are represented by the PAX2 and Wnt4 genes [1].

The sall1 proteins, renal hypodysplasia and the Townes–Brockes syndrome

It is thought that at the periphery of the nephrogenic zone there is the development of a population of stem cells, starting from the cap mesenchyme [36]; such cells are characterized by the expression of sall1 [37] and Six 2 [38].

The sall1 protein is fundamental in attracting the UB to the metanephric mesenchyme; mice deprived of this gene developed fatal renal agenesis [39].

In a multicenter study, on a cohort of 100 children from 99 reciprocally unrelated families presenting renal hypodysplasia (pathology characterized by a reduced number of nephrons, reduced renal volume and disorganized tissue architecture) and 2-4 stage chronic renal insufficiency, in one patient with bilateral renal hypoplasia in absence of extra-renal symptoms a frameshift mutation into heterozygosis of the sall1 gene was observed; according to the authors, this is the first case of the pathogenetic mutation of sall1 associated with an isolated renal form [40].

Moreover, sall1 gene mutations are thought to be at the base of pathogenesis of Townes–Brocks syndrome, a rare dominant autosomal disease characterized by the association of anal defects (imperforate anus), supernumerary thumbs, malformations of the outer ear and different degrees of deafness [41, 42]. In many cases, cardiac and renal malformations have been found: the latter have sometimes been the cause of renal insufficiency [43, 44]. Kohlase et al. found the presence of sall1 gene modifications in 9 out of 23 families with Townes–Brocks syndrome or similar phenotypes; the consequence of each of the single mutations found was the production of a truncated protein without its *double zinc finger* (DZF) motifs [45].

The cap mesenchyme cells that express the Six2 and Osr-1 proteins originate the main components of the cell population of the metanephros [46]. Osr-1 is capable of blocking the formation of the endoderm starting from the mesoderm; the loss of Osr-1 in zebrafish embryos determined the loss of stem cells of a specific segment of the renal anterior portion in favor of an increase in the number of angioblasts in the same region: the consequence was altered mesoderm development with an excess of endoderm and a favored vascular development [47]. Loss of the Six2 function causes a premature and ectopic epithelial mesenchymal differentiation with loss of the mesenchymal stem cells [48].

The WT1 gene, focal segmental glomerulosclerosis and diffuse mesangial sclerosis

A fundamental role in kidney development is played by the WT1 gene (Wilms Tumor 1), which regulates the expression of different critical genes; it partly behaves as an anti-apoptotic factor, thus favoring the development of the metanephric mesenchyme [49]. The WT1 gene contains ten exons and encodes a zinc finger protein that binds the DNA and which plays a fundamental role in renal and gonadic development [50–52]. WT1 exercises a fundamental action both in the MET process and in podocyte maturation [53]. Among the WT1 targets we find the BMP-7 gene, the deletion of which may lead to loss of the stem cell population with consequent oligonephronia [54] podocalyxin [55], nephrine [56] and PAX2 [57].

WT1 gene mutations are the cause of certain forms of focal segmental glomerulosclerosis (GSFS) [58], as well as diffuse mesangial sclerosis (Denys–Drash syndrome) [59] and steroid-resistant GSFS (Frasier syndrome) [60].

Different degrees of loss of the WT1 gene in the podocytes have been found in specific forms of GSFS. Barisoni et al. assessed the expression of differentiation markers (such as WT1, CALLA, C3b receptor, GLEPP-1, podocalyxin and synaptopodin) and proliferation (Ki-67) of podocytes in 28 cases taken from the records of the kidney pathology laboratory of the Columbia Presbyterian Medical Center, which included ten cases of idiopathic GSFS of the collapsing variant, eight cases of nephropathy associated with HIV infections, five cases of nephropathy with minimum lesions and five cases of membranous glomerulopathy. The kidneys of fetuses and healthy adults were used as controls. While in minimal change nephropathy and membranous glomerulopathy, all the podocyte markers were present at normal levels, in patients with collapsing idiopathic GSFS and HIV-associated nephropathy, there was the disappearance of all markers in all the collapsing glomeruli and the synaptopodin in 16 % of the uncollapsed glomeruli [61].

A multicenter study was performed by Orloff et al. on adult patients with a clinical history, suggesting idiopathic GSFS or associated with HIV who were compared to HIV-1-positive African-American controls who had not developed GSFS after at least eight years of exposure; a second group of controls was composed of African-American blood donors selected randomly. The authors found that certain single-nucleotide polymorphisms (SNP) of the WT1 gene were significantly associated with the development of a GSFS. Furthermore, a concomitant HIV infection interacted with these SNPs to influence the final phenotype of the disease [62].

Denys–Drash syndrome is a rare disease characterized by the association of diffuse mesangial sclerosis (the cause of massive proteinuria and a nephrotic syndrome resistant to corticosteroids and immunosuppressors), male pseudohermaphroditism and Wilms tumor (nephroblastoma), which represents the first clinical manifestation in affected patients. Evolution toward renal insufficiency is observed between the first and second year of life. In a study of encoding exons of the WT1 gene for the germinal line, Pelletier et al. found in ten independent cases of Denys–Drash syndrome dot-like mutations of the WT1 gene (9 in exon 9, the rest in exon 8), which concern the DNA recognition sequence. In the two families studied, such mutations were found to onset *de novo*, with a consequent low risk of occurrence between brothers/sisters [59].

Besides Denys–Drash syndrome, another rare disease characterized by the association of male pseudohermaphroditism and progressive glomerulopathy is Frasier syndrome, in which affected patients present a high risk of gonadoblastoma [63–65]. The nephropathy consists of aspecific glomerular lesions associated with focal and segmental glomerular sclerosis; its onset is in infancy and is associated with proteinuria and the nephrotic syndrome, with progressive evolution to terminal renal insufficiency in adolescence or adulthood [66]. Barbaux et al. [60] demonstrated that the mutations causing Frasier syndrome are located in intron 9, an alternative splicing site, and lead to the loss or haploinsufficiency of the WT1 + KTS isoform, a transition factor.

Another highly expressed form in cap mesenchyme cells is the FGF8 gene, thought to play a role in inducing the development of nephrons and in survival of the stem cell population [67].

3. Mesenchymal–epithelial transition (MET)

The mesenchymal cell aggregates that form around UB branches are induced to differentiate into epithelial cells, thus originating the renal vescicole [1].

The factor that performs a key role in first inducing the MET is Wnt9b (Fig. 2); its activation leads to the secretion of two proteins, Wnt4 and FGF8, the genes of which are both expressed at the level of pretubular aggregates [68]; according to what has been demonstrated by the studies of Karner et al. [69], Wnt9b contributes to the formation of renal vesicles as well as to the development and maintaining of the diameter of renal tubules, perhaps by guiding cell orientation; mutations of this gene have led to the development of renal cysts in mice. Moreover, immunohistochemical studies have shown that MUC1 (mucin associated with the cell surface) probably plays an important role in the formation of renal vesicles starting from the cap mesenchyme; such a function may be connected with a protective role that MUC1 may have as concerns pluripotent stem cells, thus modifying the destiny of the cells [70].

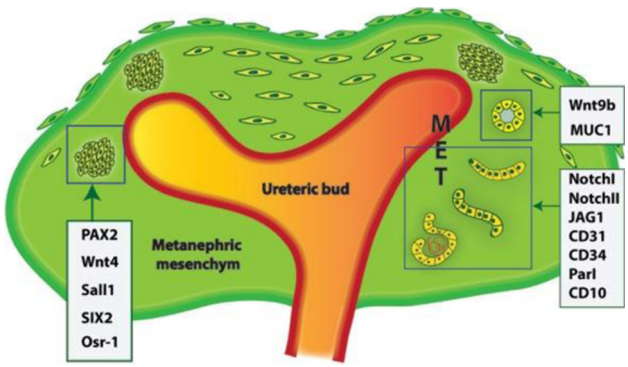

Fig. 2 Main mediators involved in MET

4. Glomerulogenesis and tubulogenesis

The development of the glomeruli and tubules of the permanent kidney can be subdivided into four more stages: (1) renal vesicle formation; (2) transformation of the renal vesicles into comma-shaped bodies and then into *S-shaped bodies*; (3) development of the renal vascular system; (4) progressive development of the nephron and differentiation of the renal interstice [71].

Naruse et al. [72] and Thony et al. [73], subdivided glomerule maturation into three stages: stage V (renal vesicles), S (*comma bodies* and *S-shaped bodies*), and C (*capillary loop*), seen within renal histological sections stained with hematoxylin and eosin (H&E).

In renal histological sections, it is possible to assess the presence of active glomerulogenesis through the search for basophilic *S-shaped bodies* located immediately below the renal cap [74]. At the end of nephrogenesis, the glomerulogenetic zone disappears and is replaced by connective tissue and some renal tubules at the passage between the cap and the last generation of glomeruli [75].

Each of the stages in the development of the glomeruli and tubules takes place through the action of different factors, among which the genes NotchI and NotchII (stage II); inactivation of NotchII in murine models has a slight effect on the number of nephrons, but if associated with loss of NotchI, the nephronic patrimony, renal function and survival are strongly compromised [76].

In humans, mutations of the JAG1 gene (ligand of the notch signal pathway) [77, 78] and the NotchII gene [79] have been found in patients affected by Alagille syndrome of type 1 and type 2, respectively, a dominant autosomal disease characterized by chronic cholestasis caused by bile duct paucity, defects of the heart, skeleton and eyes, characteristic facies and in some cases (40–70 %) associated with renal dysplasia with small kidneys, especially in type 2 [80].

In some cases, renal tubular acidosis is the picture most frequently associated with different degrees of renal insufficiency [81, 82]. In a study conducted on 11 children presenting Alagille syndrome and negative for JAG1 gene mutations, Mc Daniell et al. found mutations of the notch II gene in two cases, both with severe renal manifestations: in the first patient, who died of cardiorespiratory arrest at the age of 2 years, small kidneys with bilateral cysts, renal tubular acidosis and renal insufficiency were found; in the second patient, who at the time of the study was awaiting a kidney transplant, renal dysplasia and tubular acidosis were found. Moreover, renal alterations were also found in members of the patients' families: the mother of the first

patient presented dysplastic kidneys and proteinuria which required kidney transplantation due to severe renal insufficiency; the mother and grandmother of the second patient were diagnosed with sub-nephrotic proteinuria with microscopic hematuria and severe chronic renal insufficiency which required peritoneal dialysis, respectively. NotchII gene mutations were found in the three affected family members [79].

Other molecular factors involved in the glomerulogenesis and tubulogenesis processes are vascular markers such as CD31 and CD34 (stage III) [83] and finally the ParI gene [84] and the glycoprotein CD10 (stage IV) [85]. Faa et al. [85] demonstrated the presence of CD10-positive cells in the metanephric mesenchyme starting from the eleventh week of gestation; its expression was high in the mesenchymal–epithelial transition, in the stage of podocyte development and that of the Bowman capsule cells and proximal tubules, while it was suppressed in the first stages of renal organogenesis, undergoing what is described as a "double switch" in its gene expression. In human carcinogenesis, the loss of CD10 following a process of methylation may favor neoplastic development and progression [86]; indeed, the CD10 glycoprotein is expressed in most of the cells of the renal carcinoma [86, 87]. According to Faa et al. [88], renal carcinogenesis may partly mimic glomerulogenesis: inactivation of CD10 through methylation may be the cause of the first stage of glomerulogenesis while its expression (through demethylation) may regulate the differentiation of the epithelial cells of the proximal nephron.

Conclusions

Present-day knowledge on the complexity of the nephrogenetic process is the result of many studies performed over the years on experimental animals. The close interaction of the factors that act to modulate nephrogenesis explains why a perturbation of the homeostasis at the base of the process is responsible for a chain phenomenon that leads progressively to alterations in renal development. In the last ten years, the results produced have become integrated with the results reached in the fields of immunohistochemistry and molecular biology, by means of which we can explain how a loss or excess of molecular factors governing nephrogenesis may cause the onset of pathologies of different gravity, in some cases leading to a CKD or ERSD at different times from birth.

Differences in the duration and origins of the nephrogenetic process observed in the different species suggest that the molecular and morphogenetic mechanisms may also be different [1]. Today, we can state that further studies involving humans are required to understand whether or not the alterations observed in experimental animals can be reproduced in humans and thus prevent the appearance of serious renal pathologies in children and/or adults in the future.

Conflict of interest None.

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