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# Development of the Human Kidney: Morphological Events

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## Introduction

The human kidney is a very strange organ, often acting on impulses that remain hidden, often reacting to endogenous or external stimuli with behaviors not completely comprehensible for us. Kidney development appears peculiar, even at panoramic view. Who is the mature human kidney? Why two kidneys? One was not enough, like for heart or liver? Why three kidneys during human development? One was not enough, like for the vast majority of organs? Why so many cell types participating to development of the mature kidney? Why so strict interrelationships between

the mesenchymal and the epithelial world during kidney development?

Few cell types were not enough, like for liver, for suprarenal glands, for lungs, and for the vast majority of human organs? To these old questions, science has in recent years brought powerful tools and reams of data: in particular, genetics and molecular biology have opened a big window onto human kidney development [1]. Thanks to these new findings, a new morphological interpretation of human nephrogenesis is emerging, in which the process of epithelial–mesenchymal transition appears as the new main actor of kidney development [2]. But, the more we learn about kidney evolution and development, the more complicated the story becomes, even on morphological grounds [3]. New findings on the immunoreactivity of the pluripotential cells that give rise to the nephrogenic area located under the renal capsule are evidencing a previously unrecognized complexity of the nephrogenic zone, revealing the pres-

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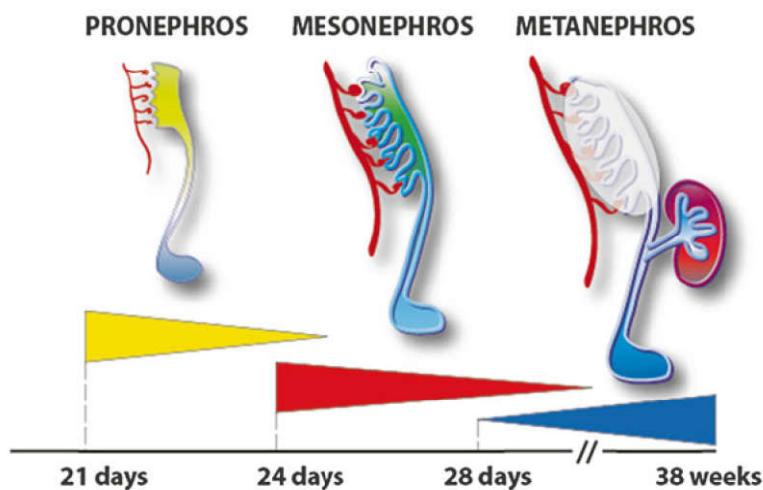
ence of new cell types or, alternatively, of new differential stages of the renal progenitors during their trip towards the epithelial structures of the mature kidney, including glomeruli and tubules [4].

Here we will report the main known morphological steps of human nephrogenesis, with a particular attention to the process of epithelial-mesenchymal transition, the complex process originating with an indifferentiated metanephric mesenchymal cell and ending with the origin of the mature proximal nephron, and with its fusion with the collecting tubule. We will try to communicate our present view on the sequence of morphological events regulating human kidney development, and will analyze the multiple cell types until now known to be involved as characters of renal development, defining the known factors that propel these cells during their differentiation from a mesenchymal cell towards the multiple complex epithelial structures of the mature kidney. Sure that what we are here reporting is only a part of the story and that, in the next future, the application of immunohistochemistry and of molecular biology to the study of the developing human kidney will add new data, including new cell types or new differential stages of previously known renal cells, to the complex picture of the human nephrogenesis, with possible relevant consequences on the growth of regenerative renal medicine [5].

## Morphological Sequence of Events in Kidney Development

### Pronephros

Pronephros represents the first of three pairs of embryonic excretory organs, including mesonephros and metanephros, which appear in sequence during human embryogenesis. It appears early during the fourth week of human gestation, around the 21st–22nd day of gestation, and represents the simplest and early rudimentary kidney form, an obligatory precursor of the adult kidney. Pronephros is a much simpler organ than the mesonephric or metanephric kidney, consisting of three main components: (1) one blood filtering glomus/glomerulus; (2) the pronephric tubules, which connect glomus with the pronephric duct; (3) the pronephric duct, which transports the wastes to the cloaca. The filtration unit, represented by a capillary tuft, projects into a cavity defined nephrocoel, which is in communication with the coelom. The nephrocoel is joined through multiple pronephric tubules to the pronephric duct, which will give rise to the Wolffian duct, the structure along which the mesonephros and the metanephros will develop (Fig. 1.1). The epithelial cell of the pronephric tubules shows the first differentiation that will evolve into one of the most complex cells of our body: the proximal



**Fig. 1.1** Pronephros, mesonephros, and metanephros development in sequence during human gestation

tubular cells of the mature kidney. The epithelial cell of the pronephric tubules is ciliated: the co-ordinated movements of cilia move fluid towards the pronephric duct, experimenting some selective absorbing activity on the fluid secreted by the glomus. In the pronephric tubule, development of the epithelial cells occurs in concert with the organ function, being regulated and dependent on the nephron fluid flow. Migration of fully differentiated epithelial cells, epithelial proliferation, and differentiation, the main factors responsible for the elongation of pronephric tubules, are induced by nephron fluid flow, which orchestrates epithelial cell movements during tubulogenesis [3].

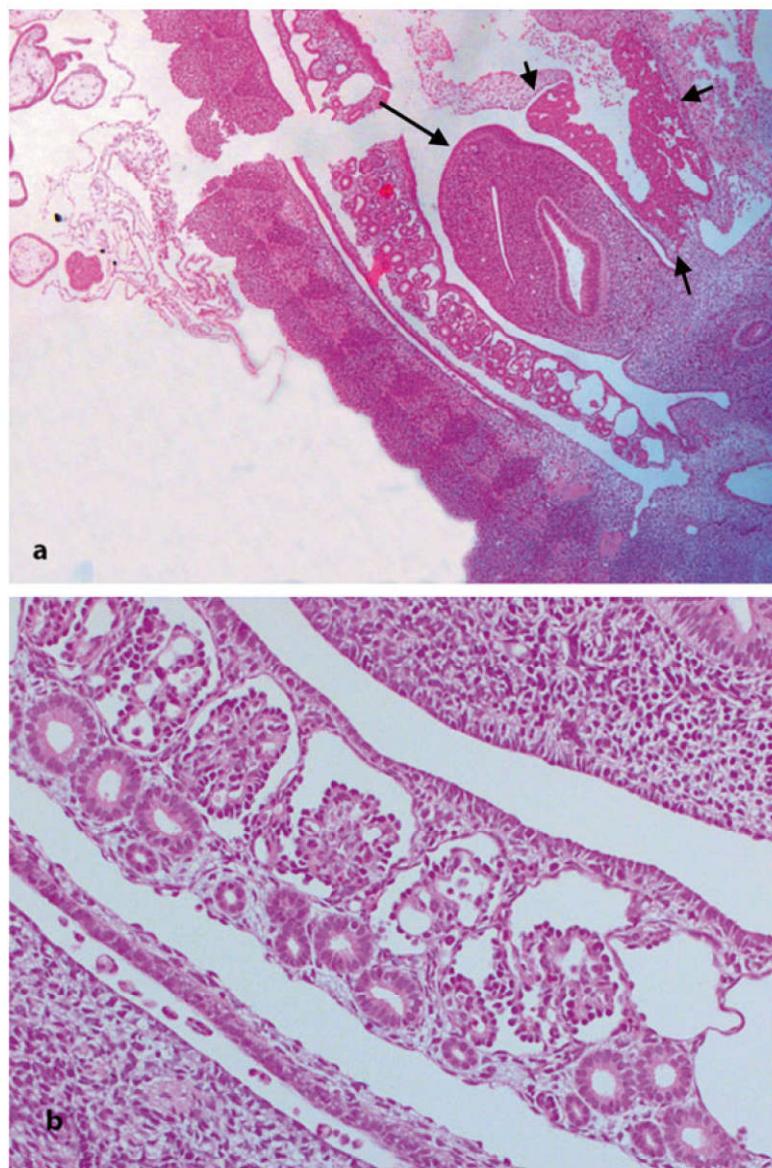
Despite its simplicity, the genetic programs regulating building of pronephros are evolutionarily conserved in all vertebrates. Patterning of pronephros appears as the result of the first approach of the human embryo to the construction of a so complex organ such as the mature kidney. For reaching the goal of building the metanephric kidney, embryo operates modelling renal architecture across development via a series of attempts, the pronephros representing the first attempt. In the pronephros project, the main functional structures of the mature kidney are present: the filtering unity, represented by one glomerulus, the collecting system, represented by the pronephric tubules, and the urinary excretory system, represented by the pronephric duct. The life of pronephros is very short: around the 25th day of gestation, it regresses and, at the same time, mesonephros begins to develop (Fig. 1.2).

## Mesonephros

The mesonephros represents a much more complex project, as compared to pronephros, containing the majority of cell types and showing very similar stages of glomerular and tubular differentiation schemes observed in the metanephros [1]. For these reasons, historically the mammalian mesonephros has represented one of the most utilized system for the study of kidney formation, complementary to the study of the developing metanephros, and its analysis yielded fundamental information about the mechanisms underlying

nephrogenesis. The mesonephros develops via the reciprocal interactions between the Wolffian duct (WD), which represents the evolution of the pronephric duct and the mesonephric mesenchyme. Multiple, buds emerge from the WD and invade the mass of the mesonephric mesenchyme, inducing the mesonephric pluripotential mesenchymal cells to differentiate into epithelial cells through a process of mesenchymal–epithelial transition, in response to inductive signals from buds emerging from WD (Fig. 1.3). This process gives rise to the origin of a series of renal vesicles, that differentiate into S-shaped structures, which originate a variable number (up to 40) of glomeruli and proximal mesonephric tubules, which fuse with the WD. These simple rudimentary but functioning nephrons represent the first functioning filtration units, connected among them by the Wolffian duct, into which the mesonephric nephrons secrete the first urine. Mesonephroi appear as well developed excretory organs, consisting of a restricted number of glomeruli and tubuli, which function as “interim kidneys” for approximately four weeks. Mesonephron develop in a cranial-caudal wave, reaching the highest level of development around the 33rd day of gestation: at this time point, the highest number of functioning mesonephric nephrons is achieved, the mesonephroi representing the most prominent organ in the future abdominal region of the human embryo (Fig. 1.2a, b) [3]. At this gestational age, two events occur: (1) mesonephric nephrons initiate their regression, following a crano-caudal wave paralleling the developmental wave; (2) the caudal region of the Wolffian duct gives rise to the ureteric bud that starts its invasion of the metanephric mesenchyme, giving rise to the origin of the metanephros.

For some weeks, from the 5th to the 9th week of gestation, both the mesonephros and the metanephros coexist in the human embryo, mesonephros progressively regressing and the metanephros enlarging. The process of involution of the mesonephros ends in different ways in the two sexes: in males mesonephric tubules form the efferent tubules of the testis; on the contrary, in females mesonephric nephrons regress completely, around the 10th week of gestation.

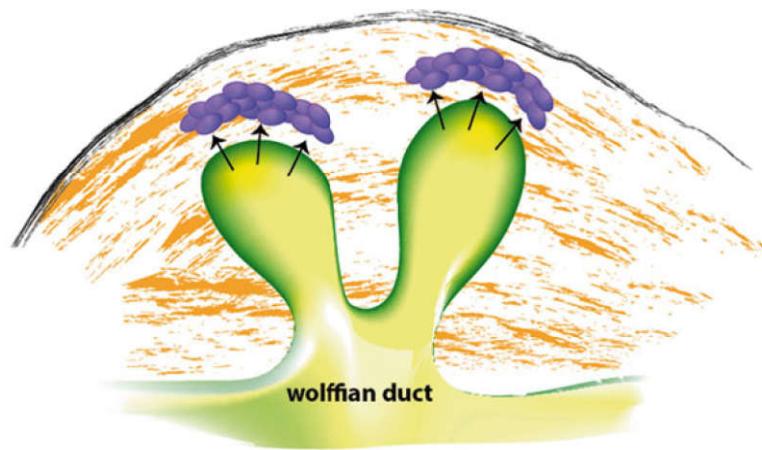


**Fig. 1.2** Mesonephros in a human embryo. (a) A panoramic view showing mesonephros developing in a cranial–caudal wave, dorsal to the gut (arrow) and to the liver (arrowheads). (b) At higher power, mesonephric nephrons are formed by a rudimentary glomerulus from which a tubule emerges

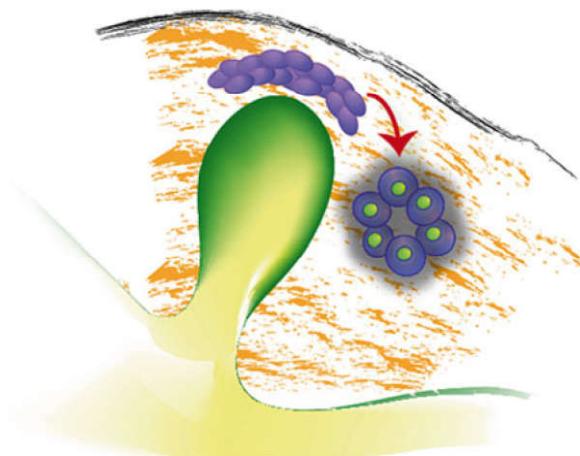
### Metanephros

The metanephric kidney develops via a series of reciprocal interactions between, the ureteric bud and the metanephric mesenchyme, both originating from the intermediate mesoderm. The metanephroi begin to develop early during the fifth week of gestation, and start its function during the

ninth week with urine formation. The primary ureteric bud originates at the posterior end of the Wolffian duct as a solid aggregate of the epithelial cells that proliferate, migrate, and progressively invade the surrounding metanephric mesenchyme. The permanent human kidney develops through reciprocal interactions between these two precursor tissue cells: the epithelial cells originating



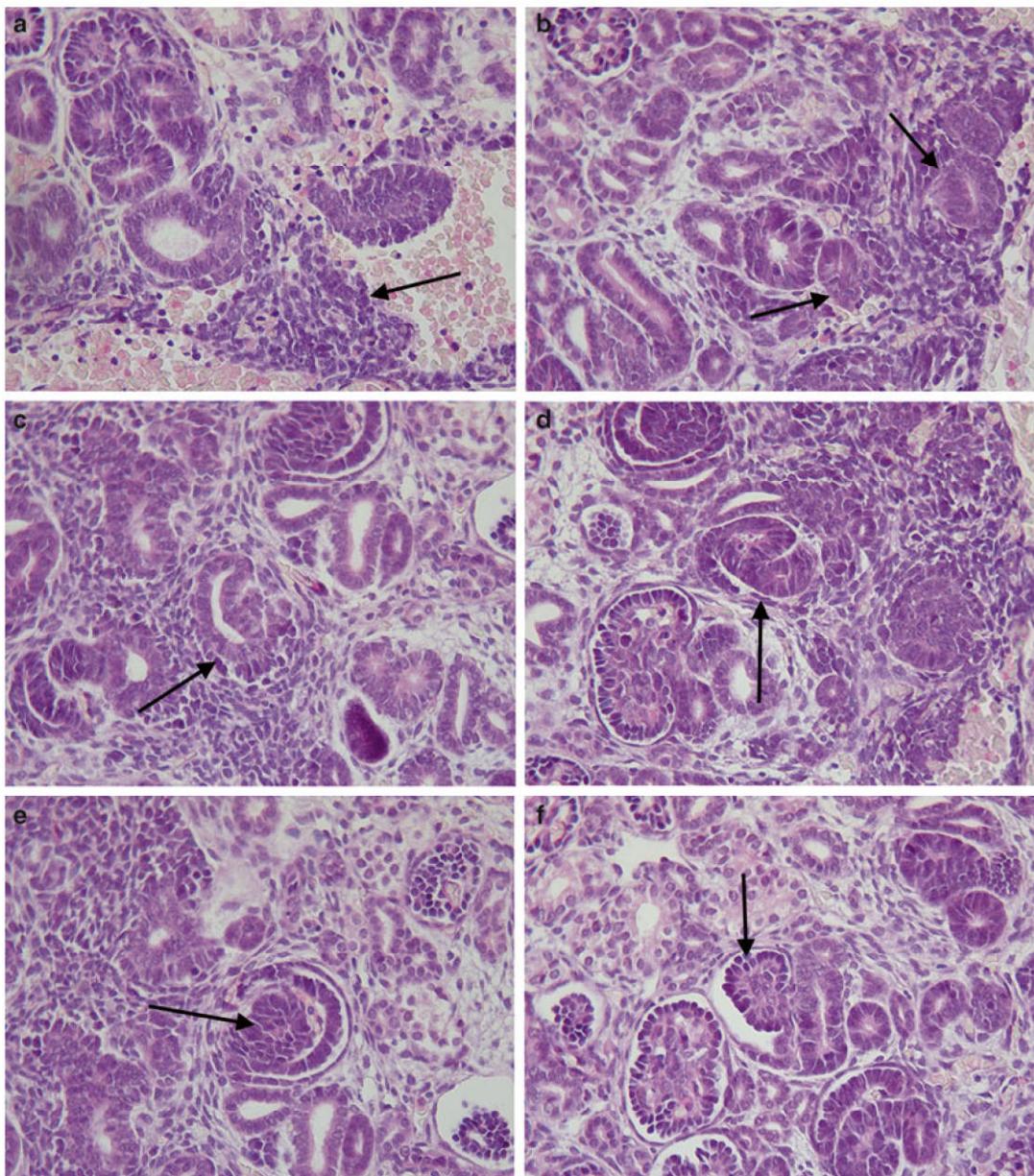
**Fig. 1.3** Branching ureteric bud tip cells induce metanephric mesenchyme to condense and differentiate giving rise to the cap mesenchyme



**Fig. 1.4** Mesenchymal–epithelial transition of cap mesenchymal cells originates renal vesicles

from the Wolffian duct, and the mesenchymal cells of the metanephric mesenchyme, a mass of mesenchymal cells originating from the intermediate mesoderm which are programmed to make renal progenitors only in response to the inductive signals coming from the branching tips of the ureteric bud. Epithelial cords originating from the ureteric bud, branch into the metanephric mesenchyme, giving rise to the ureteric tree via branching morphogenesis and reaching its periphery, inducing nephron formation at each of its tips. Metanephric mesenchymal progenitor cells condense and aggregate around the tips of epithelial branches, transforming into the cap

mesenchymal cells (Fig. 1.3). The pluripotential scarcely differentiated cap mesenchymal cells progressively undergo a process of mesenchymal-to-epithelial transition, which will form most of the epithelial cells of the mature nephrons. The epithelial cells of the tips of the ureteric tree induce cap mesenchymal cells to differentiate into pretubular aggregates, roundish groups of condensed cells which develop in their center a lumen, giving rise to the renal vesicles, the first simple epithelial structure and the precursor of each developing nephron. The renal vesicle is a simple tubule formed by small adherent cells polarized around a central lumen (Fig. 1.4) [1].



**Fig. 1.5** Different steps of human nephrogenesis. (a) A cap aggregate ( $\uparrow$ ) gives rise to a renal vesical, to tubules, and to a rudimentary glomerulus (*top left*). (b) Renal vesicle ( $\uparrow$ ). (c) Comma-shaped body ( $\uparrow$ ). (d) S-shaped body ( $\uparrow$ ). (e) Vascular precursor cells ( $\uparrow$ ). (f) Glomeruli ( $\uparrow$ ) in the early phases of development

The transition from the renal vesicle towards the mature proximal nephron requires multiple sequential processes of segmentation and patterning (Fig. 1.5a–f). This process may be subdivided into four stages: stage I, in which the renal vesicle originates; stage II, in which two sequential segmentation events give rise first to the comma-body and subsequently to the S-shaped body; stage III,

also defined as the capillary loop stage, in which angioblasts originate the vascular tuft; stage IV, characterized by the differentiation of the proximal tubule, elongation of the Henle loop, differentiation of the distal tubule, and differentiation of all the cell types that characterize the mature human kidney. The first stage is characterized by the differentiation of the solid pretubular aggregates,

formed by pluripotential cap mesenchymal cells, into roundish epithelial structures with a central lumen, the renal vesicles. This process, known as mesenchymal–epithelial transition, occurs in many developmental processes and is a multi-step process beginning with non-polarized cells embedded in the extracellular matrix and producing well-polarized and adhesive epithelial cells. The following sequential steps have been outlined: (a) cytoskeletal reorganization to actively drive cell adhesion, (b) acquisition of polarity markers, (c) expression of intercellular adhesion mediated by cadherins and adherens junctions, (d) basal membrane assembly. These steps are not all necessarily present in a given example of MET but, as a whole, they characterize the MET process. The second stage of differentiation is characterized by two sequential segmentations occurring in the renal vesicle: the first gives rise to the comma-shaped body, and the second originates the S-shaped body [3]. At the stage of comma-body, the developing nephron may be subdivided into a proximal and a distal segment, both characterized by the expressions of intercellular adhesion molecules such as E-cadherin. A second segmentation of the comma-body originates the S-shaped body, which is organized into three segments, proximal, medial, and distal. These three segments are, at this point, committed to originate different segments of the developing nephron: the cells of the proximal segment further differentiate to form the parietal epithelial cells of the Bowman capsule and develop into the precursors of podocytes; cells of the median segment of the S-shaped body differentiate into the proximal tubules; cells of the distal segment give rise to the distal tubules and drive the process of fusion with the collecting tubules [6]. The third stage is characterized by rapid developmental changes in the renal vasculature. The development of renal arteries and veins, with the origin of afferent and efferent arteries, the appearance of the glomerular capillary tufts in strict contact with the Bowman capsule, the differentiation of the endothelial cells of the corpuscle represent the most important changes in the developing nephron in this stage. Moreover, at the border between the median and the distal segment of the S-shaped body, the primitive loop of Henle originates, initiating its migra-

tion into the inner medulla that will be completed only later, during the fourth stage.

Stage IV is characterized by the differentiation and proliferation of the principal cell types that will give rise to the evolution of the mature renal corpuscles, by the differentiation of the different interstitial cells that characterized the cortical, the medullary, and the perihilar zones, and by the development of the juxtaglomerular complex. Inside the glomerulus, two main cell types undergo differentiation: podocytes and mesangial cells.

Podocytes originate from the inner layer of the proximal part of the S-shaped body. In immature glomeruli, they appear as roundish cells, characterized by a voluminous nucleus with dense chromatin and by a scant cytoplasm. These podocyte precursors envelop the developing renal corpuscle, and delimitate its borders, giving rise to a nest-like structure. During migration of the developing glomerulus from the subcortical zones towards the deep cortex, podocyte precursors develop an arborized structure within the glomerulus, progressively embracing the capillary structures with their foot-processes attached to the glomerular basal membrane. Finally, podocytes and glomerular capillaries undergo structural fusion, paralleled by slit diaphragms development among foot-processes, allowing filtration to occur.

Conflicting data have been reported on the origin of mesangial cells over the years. An origin from the multipotent self-renewing nephron progenitor population of the cap mesenchyme has been proposed by some authors [7].

Another hypothesis supporting the origin of the mesangium from the bone marrow has been proposed: according to this hypothesis, mesangial cell precursors might migrate into the developing glomerulus from outside, deriving from a hematopoietic stem cell [8].

The interstitial cells of the mature kidney take their origin from the cap mesenchymal progenitor cells through the non-nephron lineage, which parallels and intersects with the nephron lineage. A number of subcomponents of the renal interstitium progressively differentiate from the first actors in the non-nephron lineage, including angioblasts, the renal interstitial cells, putatively mesangium, pericytes, cells of the renal capsule, fibroblasts, muscle cells, and

resident macrophages [9]. Ontogeny of intrinsic innervations throughout the developing human kidney represents a new field of research. An abundance of adrenergic nerve fibers arise around the 20th week of gestation in the cortex in close proximity to arterioles and arteries, and in the medulla close to tubular cells [10].

The ureteric tree inside the metanephric mesenchyme will subsequently originate the collecting system, including collecting ducts, calyces, and the renal pelvis, whereas the part of the ureteric bud that does not enter the metanephric mesenchyme gives rise to the ureter and to the bladder trigone [11].

The sequential steps of kidney development here reported have been frequently reported in the literature as typical of all mammals. Recent studies have shown that renal development on pigs shows peculiar differences as compared to humans, suggesting that caution should be taken when comparing data on kidney development in experimental animals to human nephrogenesis, in health and disease [12].

### **The Newborn Kidney: A Checklist for a Developmental-Morphological Approach**

The morphological study of the newborn kidney, and in particular of the premature human kidney, shows some relevant peculiarities, when compared to the study of the adult kidney. The neonatal kidney is often characterized by the presence of ongoing nephrogenesis, with multipotent stem cells giving rise to new nephron through the process of mesenchymal-epithelial transition and to new interstitial cells which cooperate with newly formed epithelial cells to originate new mature nephrons. In simple words, whereas the adult kidney is characterized by the presence of stable structures, the neonatal kidney is mainly characterized by developing and traveling immature structures. On the basis of these relevant differences, the morphological analysis of the newborn kidney necessitates a peculiar approach, focused on identifying the main cell types participating to nephrogenesis and their different differentiation

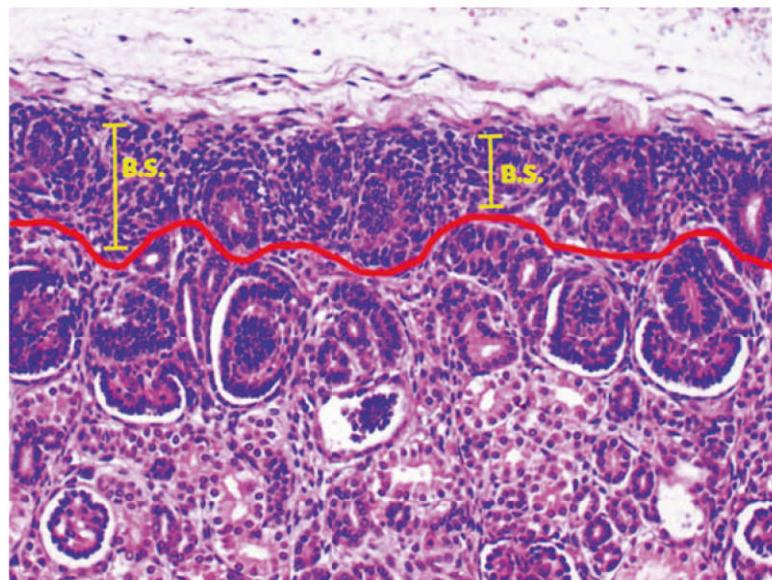
levels. In order to simplify this morphological approach, mainly based on the knowledge that the neonatal kidney is a developing organ, we will try to give an answer to some questions.

#### **Is Nephrogenesis Active in this Kidney?**

The presence of ongoing nephrogenesis is characterized by the presence, in the subcapsular region, of pluripotential stem cells and of all the structures representing the multiple steps of mesenchymal-epithelial transition (Fig. 1.6). The pluripotential renal cells appear as small cells, with a roundish or elongated nucleus and a scant cytoplasm. The scarcity of the cytoplasm is at the basis, in H&E-stained sections, of the “blue” appearance of the nephrogenic areas located in close proximity of the renal capsule. Whereas the less differentiated pluripotential cells are not strictly aggregated, groups of these cells located in close proximity to the tips of the ureteric buds give rise to nest-like agglomerates, which are characterized by development of adhesion structures among them. The epithelialization of the cap mesenchymal aggregates is evidenced by the appearance of a central lumen. The number of the renal vesicles observed in the subcapsular area gives to the observer a semiquantitative indication regarding how many nephron are going to develop, each renal vesicle expressing one possible new nephron. The frequency of renal vesicles, of comma-shaped and of S-shaped structures, all taken together, may well represent the activity of the nephrogenic process in a neonatal kidney.

#### **Did the Ureteric Bud Proliferate Correctly into the Metanephric Mesenchyme?**

To give an answer to this apparently complex question, one should clearly evidence the collecting tubules, i.e., the component of the distal nephron that originates from the ureteric bud. In a normally developing kidney, ureteric bud tips may be observed in close proximity to the subcapsular nephrogenic zone, in strict contact with cap



**Fig. 1.6** The blue strip (BS) represented by stem/progenitor cells located in close proximity to the renal capsule

mesenchymal cells, nephrons originating upon induction by ureteric bud cells of the metanephric mesenchyme. Recently, a simple trick for identifying the ureteric bud tree in the neonatal kidney has been reported. An old histochemical method, the PAS stain has been recently reported to clearly evidence collecting tubules, which are characterized by high amounts of glycogen, appearing as coarse PAS-positive granules scattered throughout the cytoplasm of tubular cells [13].

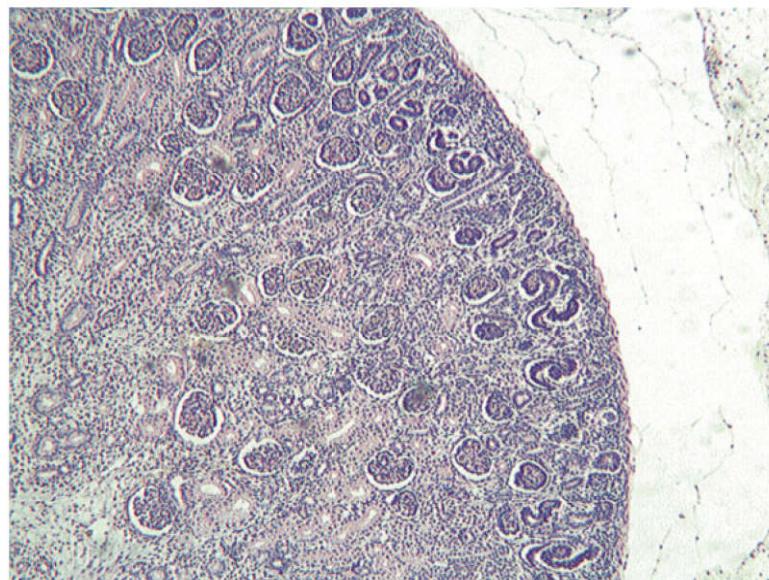
### Which Is the Nephron Burden of this Kidney?

This question regards the past nephrogenic activity in a newborn kidney. It can be easily determined by the count of the previously developed glomeruli and by the evaluation of their developmental stage. As for the quantitation of nephron number, a simple method has been developed, defined the “radial glomerular count” [14]. The radial glomerular count is based on the number of glomeruli detected along a straight line extending from the capsule and ending in the deepest cortex. The count should be made in well-oriented kidney sections, showing a well-defined corticomedullary boundary and complete renal pyramids (Fig. 1.7).

The final count may be obtained by counting glomeruli in four different locations and averaging them. This semiquantitative datum may be considered as representative of the efficacy of the previous nephrogenesis during gestation.

### Which Is the Nephrogenic Potential of the Kidney?

The multipotent metanephric mesenchymal cells located under the renal capsule represent the nephron progenitor population of a neonatal kidney, capable to give rise to all segments of new nephrons, except the collecting tubules. These scarcely differentiated multipotent cells appear as a hematoxylinophilic (blue) strip at H&E-stained kidney sections, located in close proximity to the renal capsule. The width of the nephrogenic zone, i.e., the width of the blue strip, has been recently suggested to represent the residual nephrogenic potential of each neonatal kidney [15]. Measurement of the amount of pluripotential renal cells in a neonatal kidney could be considered as a simple and effective new tool for the evaluation of the residual potential nephrogenesis. The absence of the blue strip in a preterm newborn might indicate the early cessation of



**Fig. 1.7** The radial count in a fetal human kidney

nephrogenesis, following maternal or postnatal pharmacological treatments as recently demonstrated in baboons [16]. Alternatively, a reduction of the blue strip in a newborn kidney, as compared to the width normally expected at a certain gestational age, might suggest a modification of the complex factors regulating nephrogenesis in humans. The absence of the blue strip indicates that the glomerulogenic zone disappeared, and that no potential nephrogenesis could go on in that kidney.

- Interstitial hemorrhages
- Tubular casts
- Tubular cell apoptosis
- Tubular necrosis
- Calcium deposits
- Increased glomerular matrix
- Glomerulosclerosis
- Cystic dilatation of the Bowman capsule
- Endothelial damage in renal vessels

## Conclusions

According to our experience in the morphological interpretation of the newborn human kidney, our opinion is that morphology maintains a major role in the study of renal development. The recent acquisitions on genetic programming regulating nephrogenesis, the identification of progenitor/stem cells, the new correlations between signaling and morphogenesis in the neonatal kidney, taken together all these data allow morphologists to come back to H&E-stained renal section for a new interpretation. Moreover, the application of immunohistochemistry to the study of the developing human kidney may help researchers and

### Are Signs of Renal Injury Present?

The following elementary lesions should be checked in any neonatal kidney. The vast majority of renal lesions may be easily detected in H&E-stained sections. Periodic acid–schiff (PAS) method may be considered a simple ancillary stain, able to evidence the basal membranes inside the glomerular tuft. The following pathological changes should be checked:

- Vacuolization of the proximal tubular epithelium
- Interstitial edema

pathologists to better identify the multiple cell types involved in human nephrogenesis, evidencing their specific morphological modifications and, eventually, allowing their identification in routine histological sections.

Our experience of pathologists involved in the interpretation of neonatal kidneys and in the discussion of morphological data at the microscope with the neonatologist induces us to state that every kidney is morphologically different from the next. The marked interindividual variability in renal maturation in preterm infants, regarding the number of nephrons developed as well as the number of pluripotential/stem cells responsible for glomerulogenesis in the postnatal period, makes the interpretation of every neonatal kidney complex and difficult. Only the correlation with the clinical history, with pharmacological treatments of the mother and/or of the newborn in the postnatal period may allow a correct interpretation, correlating hypoxia or other pathological events with the peculiar behavior of nephrogenesis in a single case. A training in the interpretation of the neonatal kidney is absolutely recommended, even for expert renal pathologists involved in the study of the adult kidney. The interpretation of the different cell types involved in human nephrogenesis may easily lead to errors, given the complexity of the histological picture of the fetal kidney. The acquisition of the different steps of the process of mesenchymal–epithelial transition is fundamental, to build a morphological bridge between the elongated mesenchymal cells and the roundish adherent epithelial cells that originate the renal vesicles and the other epithelial structures of the proximal nephron.

Finally, a fascinating world may be found into each histological section of a neonatal kidney, and many answers may be given to the neonatology regarding the influence of multiple factors on the evolution of nephrogenesis during the intrauterine life. A question-based approach is mandatory, in order to give a functional significance to morphological changes and, more important, to give good answers to the questions of clinicians.

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