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REVIEW

Key features of the nephrogenic zone in the fetal human kidney—hardly known but relevant for the detection of first traces impairing nephrogenesis

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Abstract

In healthy newborn babies, nephrogenesis proceeds unnoticed until birth. With start of the perinatal period, morphogenetic activity in the renal outer cortex consisting of an inner maturation zone and an outer nephrogenic zone is downregulated by unknown signals. One of the results is that the entire nephrogenic zone as well as the contained progenitor cells and niches disintegrate. In contrast, a too early inactivation of the nephrogenic zone takes place in the kidneys of preterm and low birth weight babies. Although they are born in a period of active nephrogenesis, pathological findings show that they evolve to a high incidence oligonephropathy. However, very few data exist about cell biological changes that are evoked by harming, further most of causing molecules, exact cell targets, and related molecular pathways are not identified. Although impairment of nephrogenesis was the subject of research in animal species, there is only limited information available pertaining to the pathological traces in the nephrogenic zone of the human fetal kidney. In this situation, the lack of basic morphological data is particularly aggravating. Surprisingly, there are not even ultrastructural investigations available. Since concrete information is lacking also in relevant textbooks, the current contribution likes to present key features of the nephrogenic zone in the fetal human kidney. Simultaneously, it is a call to explore systematically a hardly known area.

Keywords Fetal human kidney · Preterm infants · Low birth weight babies · Impaired nephrogenesis · Renal capsule · Nephrogenic zone · Niche

Recording of literature shows that quite different extra- and intrauterine influences can cause the impairment of nephrogenesis in preterm and low birth weight babies (Stritzke et al. 2017). The first influencer mentioned is restricted nutrition, particularly, low protein or micronutrient intake and poor antenatal perfusion combined with a lack of oxygen (Woods et al. 2001; Buchholz et al. 2016). Inflammatory cytokines, reactive oxygen species, and antiangiogenic factors are also suspected to harm nephrogenesis (Sutherland et al. 2014; Nguyen et al. 2015). To date, also the role of drugs administered to preterm and low birth weight babies is unknown (Schreuder et al. 2014; Girardi et al. 2015). From many of these drugs, it was not explored, whether expected therapeutic benefits are associated with adverse toxic effects on renal progenitor cells.

In fact, about 10% of babies are born preterm respectively with a low birth weight (Luyckx 2017). Seen from a temporal perspective, the kidneys of those babies are still in a period of an active nephrogenesis (Abitbol et al. 2016). There is now evidence that delivery of preterm and low birth weight babies is interfering with this process. Clinically registered oligonephropathy is estimated to effect between 8 and 24% of these babies (Kandasamy et al. 2012). Pathological data further illustrates that in those cases, up to 18% morphologically abnormal glomeruli are present (Gubhaju et al. 2009). Analyzed specimens exhibited a dilated Bowman's space and a shrunken glomerular tuft (Sutherland et al. 2011). Current literature also shows that prematurity leads not only to damage of currently developing glomeruli but also to a decrease in their total number (Callaway et al. 2018).

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Harming evokes cell biological changes

The mentioned sites of pathological changes in a fetal kidney indicate that the outer cortex of a fetal kidney is affected. Per definition, the last generations of developing nephrons in the maturation zone and the anlage of nephrons in the overlaying nephrogenic zone are the targets of described noxae (Li et al. 2014). More concrete data for the fetal human kidney is missing, but animal models shed some light on physiological and cell biological changes taking place during the impairment of nephrogenesis (Menendez-Castro et al. 2018). For example, malnutrition produces murine neonates with a 40% reduced body weight (Barnett et al. 2017). Renal blood perfusion is thereby reduced by 37%, and cells in renal parenchyme exhibit a 2.2-fold increased rate of apoptosis and 76% decreased $Six2^+$ progenitor cells in the nephrogenic zone that are basically needed for nephron induction and its anlage. Moreover, a down-regulation of locally synthesized morphogens Wnt9b and Fgf8 essential for nephron formation is observed. As a result, the renal vesicles were 64% less developed and there were 32% fewer nephrons registered than in the controls. A further study illustrates that maternal food restriction is leading to an up-regulation of mRNA for morphogenic molecules such as WT1, FGF2, and BMP7, whereas Pax2, GDNF, FGF7, BMP4, Wnt4, and Wnt11 mRNAs were downregulated (Abdel-Hakeem et al. 2008). Another investigation concludes that the maternal nutrient restriction inhibits ureteric bud branching but does not affect the duration of nephrogenesis (Awazu and Hida 2015). Although malnutrition is a major cause, it is not the exclusive cause interfering with nephrogenesis. Site-specific changes in oxygen delivery (Buchholz et al. 2016; Gerl et al. 2017), changes in morphogen synthesis, secretion, and transport (Minuth 2017), altered deposition of extracellular matrix (Harvey 2012), and disrupted cell-to-cell interactions (Combes et al. 2015) within the nephrogenic zone and on the site of niches are also suspected to influence continuation of nephrogenesis.

Nephrogenesis is a chain with multiple links

Although it is often not considered, nephrogenesis is a complex chain of very different cell biological processes. At the start, it comprises recruitment of progenitor cells, spatial arrangement, arise of competence, and lastly, induction in the niche. As a result, the first anlage of the nephron is in form of a pretubular aggregate. Then, a mesenchymal to epithelial transformation (MET) into a polarized renal vesicle takes place. It is followed by the formation of the comma-shaped body. These initial stages develop close to one another but they are separated by basal laminae. One of them is enveloping the nephron anlage, while the other covers the CD ampullae. In the succeeding stage of the S-shaped body the glomerulus and the different tubular segments of the later nephron are formed (Rosenblum 2008). During this phase, the presumptive connecting tubule in the S-shaped body establishes a connection with the lateral aspect of the CD ampulla. Disturbance of this connection may lead to an increase of pressure in the ultrafiltrate that evokes a formation of glomeruli with a dilated Bowman's space (Sutherland et al. 2011). When the different links in the chain of nephrogenesis are taken into account, it becomes obvious that suspected noxae can interact in the beginning, middle, and also at the end of the described links. What remains unclear is, whether noxae, which are quite different in molecular composition, have a common tissue target or whether each of them interacts with a specific link in the chain (Barnett et al. 2017).

All of the before-mentioned noxae have something in common, namely, that phenomenologically in the outer cortex arrest of nephron formation in the fetal human kidney is observed. Screening of specimens further shows that harming leads here to a decrease in the total number of glomeruli (Callaway et al. 2018) and a rise of abnormal glomeruli (Gubhaju et al. 2009; Sutherland et al. 2011). It indicates that secondary links in the chain of nephrogenesis, such as the formation of a glomerulus in the S-shaped body and its succeeding development, must be affected. However, the before-mentioned decrease in the number of $Six2^+$ progenitor cells and less-developed renal vesicles in the nephrogenic zone are clear hints that noxae can also harm primary links in the chain of nephrogenesis such as recruitment of progenitor cells and anlage of the nephron (Barnett et al. 2017).

Harming meets natural cessation of nephrogenesis

When the impairment of nephrogenesis is analyzed from a pathological point of view, the focus is directed to the total number of glomeruli and the arise of atypical glomeruli (Gubhaju et al. 2009; Sutherland et al. 2011; Callaway et al. 2018). Although marred glomerulogenesis is an important reference, it is only one of other even important and parallel lining processes during the arise, differentiation, and maturation of renal parenchyma (Rosenblum 2008). Those include, for example, vessel formation (Molema and Aird 2012), arise of the interstitium (Bruno et al. 2014) or areal expansion of the capsule in a developing kidney (Minuth 2018a).

An often overlooked and rarely investigated phase in kidney development is its prenatal period. At the end of the fetal kidney development and at the time of birth as well, the anlage as individual setting of the nephron number is completed by natural cessation of nephrogenesis (Rumballe et al. 2011). Through an unknown molecular signaling, the nephrogenic zone and contained progenitor cell niches disappear (Oxburgh et al. 2017). In regard to the kidneys of preterm

and low birth weight babies, a similar but too early termination of the entire developmental program takes place. However, what happens with the nephrogenic zone in this situation, it is not known.

Thus, the actual problem is that on the one hand concrete data, dealing with natural cessation of nephrogenesis including the disappearance of the nephrogenic zone in healthy newborns is missing and on the other hand, data dealing with its too early inactivation in preterm and low birth weight babies is not available. The only known fact is that natural cessation of nephrogenesis is accompanied by the disappearance of the nephrogenic zone (Rumballe et al. 2011).

Renal growth forces expansion of the nephrogenic zone

The nephrogenic zone of a fetal human kidney extends as a narrow strip along the entire inner side of the renal capsule (Fig. 1) (Minuth 2018b). At first glance, this area appears to be rather inconspicuous. However, one has to remember that not only the entire nephrogenic potential in form of progenitor cells but also the cell biological machinery for production of the nephron anlage up to the late S-shaped body are contained here, while maturing nephrons are contained in the underlying maturation zone. As such, the nephrogenic zone including the ends of CD tubules must be seen as the regulating source of the renal cortical parenchyma during fetal development. Due to its special position, new nephrons are produced here step-by-step by apposition resulting in a radial extension of parenchyma.

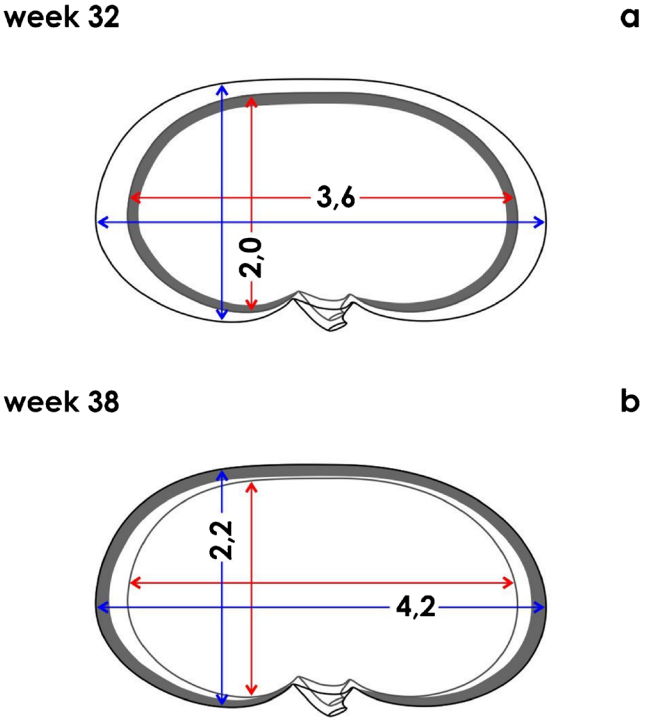


Fig. 1 Schematic illustration shows growth parameters of a fetal human kidney between gestation weeks **a** 32 and **b** 38 according to Kandasamy et al. 2013 and Brennan and Kandasamy 2017. During this period, the length of a kidney increases from 3.6 to 4.2 cm and the width from 2 to 2.2 cm. The increase of 7.1 cm^3 in kidney volume forces an increase in surface. It includes an areal expansion of the capsule and underlying nephrogenic zone indicated as gray seam in an order of 30%

Apposition of new nephrons in the outer cortex results in growth of the fetal kidney. This process has consequences for the nephrogenic zone, which covers the entire organ. To give an example, earlier published data shows that between gestation weeks 32 and 38 the length of the kidney increases from 3.6 cm (Fig. 1a) to 4.2 cm (Fig. 1b) and the width from 2 cm (Fig. 1a) to 2.2 cm (Fig. 1b) (Kandasamy et al. 2013; Brennan and Kandasamy 2017). During this period, the total renal volume (TRV) enlarges from 14.5 to 21.6 cm^3. One must realize that with the rise of the volume, not only the renal capsule but also the underlying nephrogenic zone extends in the same dimension.

The actual problem is that data dealing with measurements of the kidney surface is not available in literature. However, data of kidney length, width, and total renal volume can be used to calculate the surface area of a model sphere and the surface of a model cuboid (Minuth 2018a). Following this approach, the sphere reflects the model with the minimal surface area, while the cuboid represents the model with the maximal surface area. As a logical consequence, the increase in surface area of a fetal kidney must be between these two models. Calculations of the surface area (S) of a model sphere $S = 4 \cdot \pi \cdot r^2$ (r/radius) and a cuboid body $S = 2x(LxW + WxH + LxH)$; (H/Length, W/Width, H/Height) can be made for example by these equations found in the coaching program Frustfrei Lernen: https://www.frustfrei-lernen.de/mathematik/geometrie-volumen-oberflaeche-fass-ku-gel.html. The results of calculations exhibit that the surface area of a model sphere enlarges from 28.7 to 37.5 cm^2, while the surface of a cuboid increases from 36.9 to 48.4 cm^2. It is surprising in so far, since expansion of the surface area in the sphere is 30.6%, while it is with 31.1% nearly the same in the cuboid. Paraphrased, between weeks 32 and 38, the areal expansion of the capsule and in parallel, the underlying nephrogenic zone increase by an astonishing 30%.

Meant here is the areal respectively horizontal expansion along the renal capsule, not meant is the width of the nephrogenic zone. To date, it is unknown, whether the contained cell biological machinery maintaining stemness, triggering branching morphogenesis, induction, anlage, and initial development of nephrons up to the late S-shaped body stage is increasing during this period in the same relation. In other words, it is unclear, whether the nephrogenic zone maintains its structural and functional compactness during this period.

Repeatable microscopic perspectives

As mentioned, the nephrogenic zone extends along the entire surface of a fetal human kidney (Fig. 1). Due to its exposed position, one has to prevent damage of the kidney surface during isolation and histological preparation (Fig. 2) (Minuth and Denk 2015). Consequently, one should hold a fetal kidney only on its hilum and should avoid touching the renal capsule with fine forceps.

Histological sections cut incidentally for microscopic analysis do not show comparable views onto the nephrogenic zone. To obtain repeatable perspectives, blocks of parenchyma have to be orientated before embedding and during histological cutting as it was earlier described (Fig. 2) (Minuth and Denk 2015). Following this advice, a fixed kidney is cut from the capsule towards the papilla of a lobus (Fig. 2a). Then, the medulla is separated from the cortex (Fig. 2b). Embedding of the tissue block is performed so that the axis of collecting duct tubules is perpendicular to the capsule. After trimming the lateral sides of the block, sections with comparable perspective can be produced by a microtome (Fig. 2c). Following this approach, repeatable perspectives of the nephrogenic zone with contained CD tubules, branching sites, CD ampullae, nephrogenic mesenchyme, stages of nephron anlage up to the late s-shaped body, and the renal capsule can be produced.

For the illustrations and morphometric measures shown here, a human kidney of gestational age between weeks 16 and 18 was selected from the stock of preparations used for the Course of Microscopic Anatomy for Medical Students at the University of Regensburg. According to routine methods, specimens were fixed in paraformaldehyde solution and embedded in paraffin wax. Then, sections of 5 μm thickness were produced and stained with hematoxylin-eosin solution for analysis by optical microscopy.

Images of histological sections were taken with a digital camera and processed with CorelDRAW X7 (Corel Corporation, Munich, Germany). To obtain information about morphological coordinates and metric parameters, recordings were analyzed with the same program.

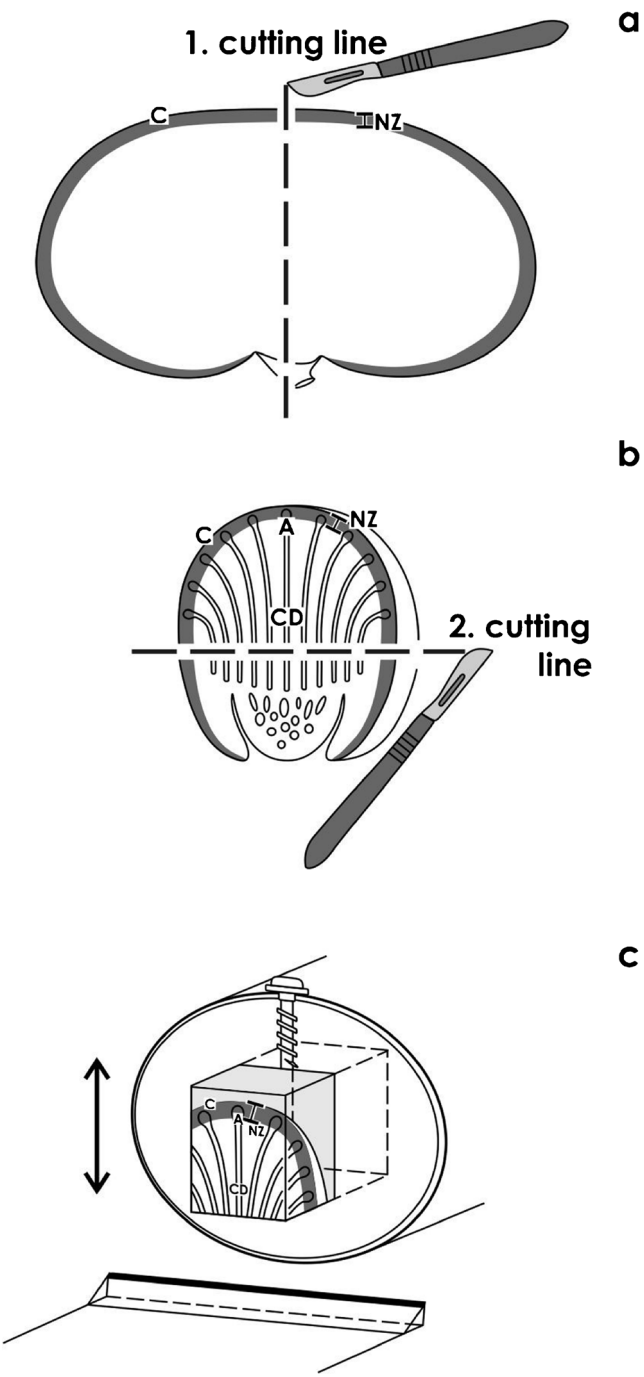


Fig. 2 Schematic illustration shows embedding of fetal human kidney for sectioning of the nephrogenic zone (NZ) indicated as gray seam. **a** First, a fixed kidney is cut by a vertical section from the renal capsule (C) towards the papilla of a lobus. **b** Then, the medulla is separated by a transversal section from the cortex. **c** Embedding for sectioning is made so that collecting duct (CD) tubules line perpendicular to the capsule. By trimming the lateral sides of the block, sections are made that show CD tubules, branching sites, CD ampullae (A), nephrogenic mesenchyme, and renal capsule in comparable perspectives

Definition of borders

In sections stained by Hematoxylin eosin, the nephrogenic zone appears as a slightly pronounced seam (Fanni et al. 2015). To analyze contained morphological structures, only histological sections are shown that are orientated along the lumen of the lining collecting duct (CD) tubules (Figs. 2 and 3). A first look onto those sections exhibits that the entire outer border of the nephrogenic zone is defined by the renal capsule. Further out, the adipose capsule is found.

Previously performed microscopic analysis shows that in gestational controls, the width of the nephrogenic zone is not more than 150 μm, while in the group of preterm babies, it is with 100 μm significantly smaller (Sutherland et al. 2011). Comparable metric dimensions of the nephrogenic zone were

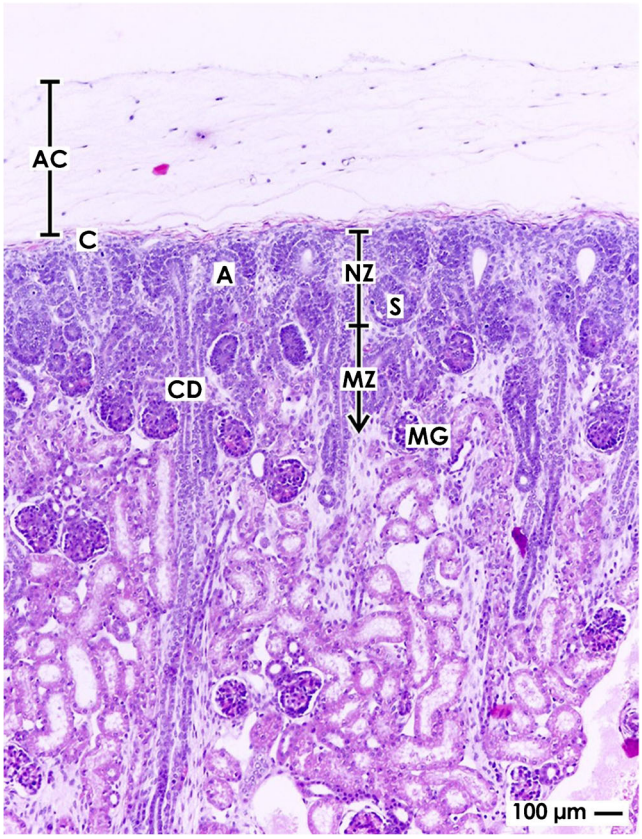


Fig. 3 View onto a fetal human kidney by optical microscopy. The section lines in parallel to the lumen of collecting duct (CD) tubules. The adipose capsule (AC) and the renal capsule (C) cover the organ. Below can be seen the nephrogenic zone (NZ) and the maturation zone (MZ). In the nephrogenic zone, the endings of CD tubules show branching and arise of CD ampullae (A). Nephrogenic mesenchymal progenitor cells occur between the tip of a CD ampulla and the inner side of the capsule. Restricted to the nephrogenic zone, at the lateral aspect of a CD ampulla, the anlage and initial stages of nephron formation such as pretubular aggregate, renal vesicle, and comma- or S-shaped body (S) occur. In contrast, in the maturation zone further developed stages of nephron formation such as maturing of glomeruli (MG), development and differentiation of nephron segments takes place

shown in recently performed morphological investigations (Ryan et al. 2017; Minuth 2018b). However, the inner border was not exactly defined. A proposal was to draw a horizontal line along the branching sites of CD tubules and the proximal (medulla-orientated) end of a late S-shaped body as an inner border. Different stages of maturing glomeruli and developing nephron segments occur below the inner border of the nephrogenic zone besides differentiating collecting duct tubules. Due to contained structures, this area represents the maturation zone of the outer cortex in a fetal human kidney.

Nephrogenic niche

A series of data reflecting physiological functions of the nephrogenic zone is available from experiments on animals (Barnett et al. 2017). Recently published literature further exhibits that the immunohistochemical profile between animal and human species shows similarities but also unexplained inequalities (Morizane and Bonventre 2017; Lindström et al. 2018a; Lindström et al. 2018b; Lindström et al. 2018c). It is perhaps surprising, but information dealing with examinations by the optical microscope is scarcely available. Ultrastructural data of the nephrogenic zone and contained niches in the neonatal kidney was published, however, for the fetal human kidney comparable investigations are lacking (Minuth and Denk 2015).

In commonly used laboratory animals such as mice, rat, and rabbit as well as in human, the metanephros develops by branching morphogenesis and multiple interaction between the metanephric mesenchyme and the ureteric bud (Mari and Winyard 2015; Nagalakshimi and Yu 2015). However, what is often not considered is that a human polypapillary kidney is more complexly built than the monopapillary kidney of an animal model. For this reason, the term “ureteric bud” does not match the facts, when it is used in conjunction with the nephrogenic zone and niches contained there in the outer cortex of a fetal human kidney.

For a better understanding, after anlage of a human kidney and its pelvic formation, the ureteric bud epithelium forms Ducts of Bellini. During starting development of the inner cortex they transmit into collecting duct (CD) tubules. Then, in the late fetal period, CD tubules elongate in the outer cortex straight but fan-like towards the capsule. Each of them shows within the nephrogenic zone at its end bifid branching (Fig. 4) (Al-Awqati and Goldberg 1998). One can see in the illustrations that the ureteric bud-derived branches of a CD tubule are orientated towards the capsule. Finally, an established branch dilates to form a CD ampulla consisting of a neck, conus, head, and tip (Minuth 2018a, b). Within a CD ampulla, epithelial progenitor cells are contained, which exhibit a cuboidal shape. Electron microscopy in neonatal rabbit kidney revealed that their apical side is bordering the lumen, while their basal aspect is covered by a striking basal lamina pointing to the most inner layer of nephrogenic mesenchymal progenitor cells (Minuth and Denk 2016).

When a histological section of the nephrogenic zone in the fetal human kidney is analyzed, one must realize that it reflects only a brief snapshot of multiple processes occurring there during nephrogenesis. Above all, one thinks about morphogenic interactions occurring during arise of a single nephron by a niche at the tip of a CD ampulla (Fig. 4). Nephrogenic mesenchymal cells are present in close proximity. In the optical microscope, one can further see that the tip of a CD ampulla is separated from the bodies of neighboring mesenchymal progenitor cells by an interface (Minuth and Denk 2016). Interestingly, one can also recognize that only two layers of mesenchymal progenitor cells occur between the tip of a CD ampulla and the inner side of the capsule. As will be later

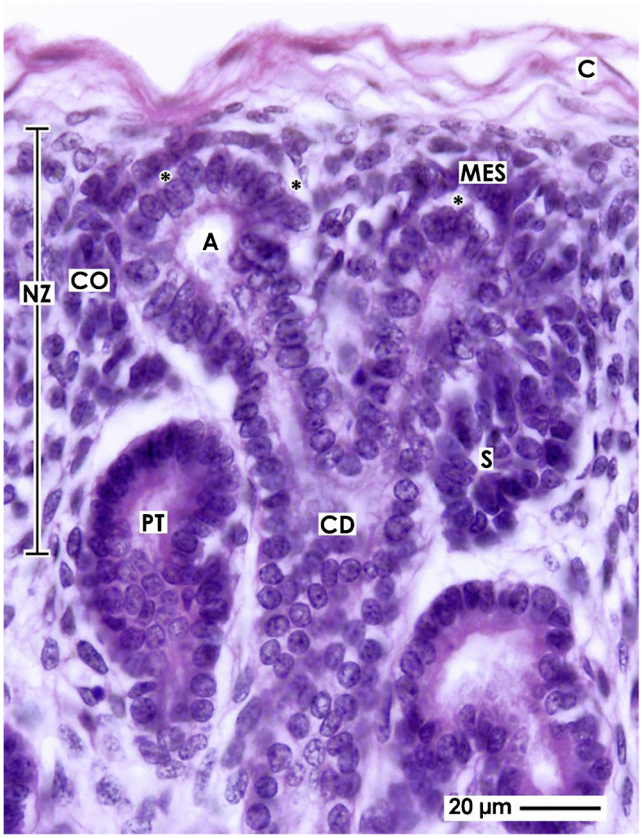


Fig. 4 The nephrogenic zone (NZ) and the renal capsule (C) in fetal human kidney illustrated by optical microscopy. At the end of a collecting duct (CD) tubule bifid branching including arise of CD ampullae (A) is seen. Only two layers of nephrogenic mesenchymal progenitor cells (MES) occur between the tip of CD ampulla (A) containing epithelial progenitor cells and the renal capsule. An interface (asterisks) separates epithelial from mesenchymal progenitor cell bodies and points to the center of a nephrogenic niche. At the left side of a CD ampulla induced mesenchymal cells show aggregation to establish first a pretubular aggregate, a renal vesicle and then a comma-shaped body (CO). At the right side of CD tubule branching, an S-shaped body (S) is visible. Below the branching site of the CD tubule, the beginning maturation zone can be seen. Here, for example, a developing proximal tubule (PT) is visible

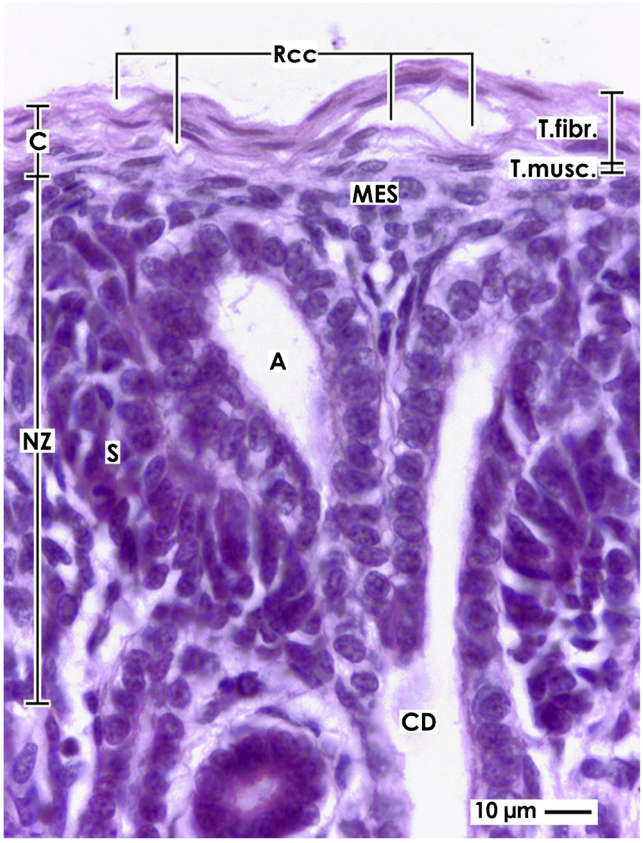


Fig. 5 View onto the renal capsule (C) covering the nephrogenic zone by optical microscopy. The renal capsule consists of a Tunica fibrosa (T.fib) with several Strata and a Rete capillare capsulare (Rcc) that is needed as vasa private for the own supply of nutrition and respiratory gas. Below, one can see the Tunica muscularis (T.musc), which represents the inner layer of the capsule. It meets the outer border of the nephrogenic zone and especially the outer layer of mesenchymal (MES) progenitor cells along the entire surface of the fetal kidney. Collecting duct (CD) tubule, CD ampulla (A), transition from a comma-shaped to S-shaped body (S)

discussed, a multilayered "cap mesenchyme" is in this perspective not visible (Figs. 4, 5, and 6).

Animal models have shown that the arrangement of the epithelial progenitor cells at the tip of a CD ampulla and the facing nephrogenic mesenchymal ($GDNF^+/Six2^+/CITED1^+$) stem cells define the center of an individual niche enabling induction and subsequent anlage and the formation of a single nephron (Combes et al. 2015; Oxburgh et al. 2017). As mentioned, investigations on a neonatal rabbit kidney revealed that the spatial separation between the tip of a CD ampulla containing epithelial progenitor cells and the inner layer of nephrogenic mesenchymal progenitor cells is not accidental, but is due to a structured interface that was intensively investigated by ultrastructural techniques (Minuth and Denk 2015; Minuth and Denk 2016). It contains a textured extracellular matrix and shows projections of mesenchymal progenitor cells, which contact the basal aspect of epithelial progenitor cells at the tip of a CD ampulla via tunneling nanotubes. This configuration enables cell-to-cell communication including directed transport of morphogens. Functions of tunneling nanotubes relevant for biomedicine were recently reviewed, while related data for the nephrogenic niches in the fetal human kidney is lacking (Vignais et al. 2017).

CD ampulla and mesenchymal progenitor cells must meet

As described for animal species, the tip of a CD ampulla and facing nephrogenic ($GDNF^+/Six2^+/CITED1^+$) mesenchymal progenitor cells define the center of a niche (Combes et al. 2015; Oxburgh et al. 2017). In a fetal human kidney, one finds the same tissue arrangement (Fig. 4), however, one of the peculiarities for human niches and involved mesenchymal

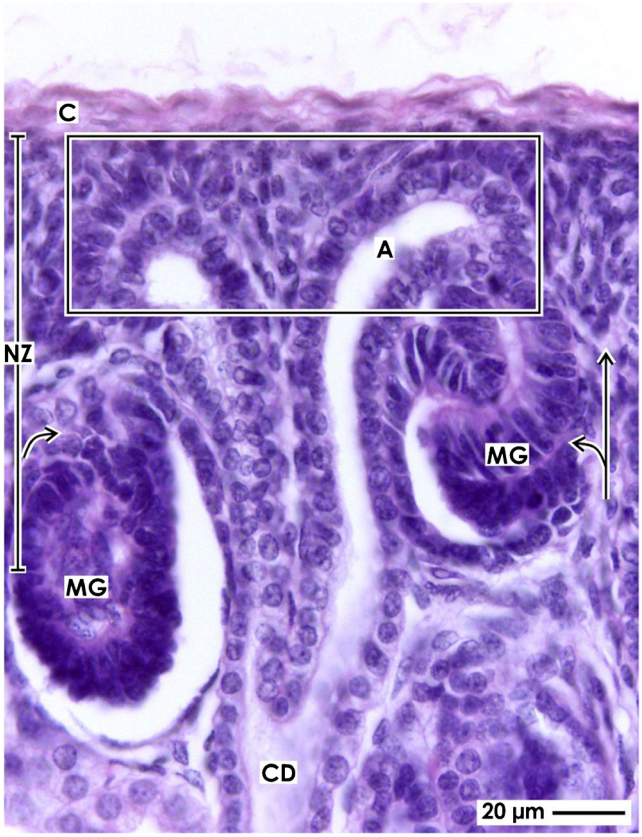


Fig. 6 Details of the vessel system in the nephrogenic zone (NZ) of the fetal human kidney shown by optical microscopy. One of the interlobular arteries (cortical radiate arteries) ascends straight through the inner, mid, and then outer cortex towards the renal capsule (C). At intervals, it gives rise to the afferent arterioles, each of which supplies a glomerulus (Molema and Aird 2012). In the maturation zone, developing arterioles (short arrows) establish a connection with maturing glomeruli (MG). In the overlying nephrogenic zone, the distal part of an interlobular artery (cortical radiate artery) lines straight as a perforating radiate artery (long arrow) into the Rete capillare capsulare (Rcc) of the renal capsule. The area at the lateral aspect of CD ampullae (A) does not show intact capillaries, but spreading endothelial cells. The niche district (framed) at a CD ampulla tip and facing mesenchymal (MES) progenitor cells is avascular

progenitor cells is that for example, Six1 was identified as a Six2 target (O'Brien et al. 2016; Lindström et al. 2018a, b, c).

Before the induction by morphogens initiates the anlage of a nephron, the tip of a CD ampulla and nephrogenic mesenchymal progenitor cells must precisely meet. Movement of the involved cells within an individual niche is adjusted by the secreted Bone Morphogenic Protein (BMP) antagonist Cerberus homologue1 (Cer1), Ret protooncogene (Ret), and ETS translocation variant4 (Etv4) (Riccio et al. 2016; Chi et al. 2011). By molecular positioning the tip of a CD ampulla, only the inner layer of nephrogenic mesenchymal progenitor cells finds close vicinity to epithelial progenitor cells. While approaching, some of these mesenchymal cells acquire competence so that they can respond to involved morphogens. This operation is directed by protocadherin (cadherin family member14: FAT4/dachsous cadherin related1: Dchs1) signaling (Mao et al. 2015). Through time-lapse imaging, it was shown that during approaching, mesenchymal progenitor cells attach and detach from the CD ampulla tip (Combes et al. 2016).

After correct approaching, epithelial progenitor cells in the tip of a CD ampulla stand exactly vis-à-vis of $GDNF^+/Six2^+/CITED1^+$ mesenchymal progenitor cells (Combes et al. 2015; Da Sacco et al. 2017). Then nephron induction takes place by a reciprocal exchange of morphogens between positioned epithelial and nephrogenic mesenchymal cells. When it is successful, a group of induced mesenchymal cells separates, condensates, and forms at the upper lateral aspect of a CD ampulla a pretubular aggregate as the first morphological sign of nephron formation.

Constant and variable coordinates

What kind of link in the chain is damaged during impairment of nephrogenesis in preterm and low birth weight babies is unknown. To date, there is also no data that has been recorded regarding pathological alterations. Consequently, one must first familiarize themselves with basic morphological coordinates (Minuth 2018a, b). To give a short insight, the mean horizontal distance between the heads of the neighboring CD ampullae is 68 µm, while the mean horizontal distance between the tips of neighboring CD ampullae is 161 µm. The mean vertical distance between the branching site of a CD ampulla and the inner side of the capsule is 106 µm. Between the tip of a CD ampulla and the inner side of the capsule is a mean vertical distance of 32 µm. The mean length of a CD ampulla is 85 µm, while its mean diameter is 71 µm. The mean vertical thickness of the mesenchymal cell layer between the inner side of the capsule and the tip of a CD ampulla is 26 µm. The mean diameter of a CD tubule is 34 µm. The mean horizontal diameter of a bifid CD tubule branch is 120 µm. More details including tables and images are given in cited literature.

To obtain information about alignment of niches along the renal capsule, some morphometric recordings have to be more precisely studied. Preliminary data exhibits that the tip of a CD ampulla and the inner side of the capsule can have a vertical distance between 16 and 41 µm (Minuth 2018a, b). The calculated mean of 32 µm signals that the tip of a CD ampulla keeps a relatively close vertical position below the inner side of the capsule (Figs. 4 and 5).

However, regarding the horizontal (lateral) alignment of niches below the capsule, calculation of a mean distracts and leads to incorrect interpretations (Minuth 2018a, b). For example, the horizontal distance between the heads of neighboring CD ampullae can show not only a minimal distance of 34 µm, but also a maximal distance of 104 µm. The horizontal

distance from a CD ampulla tip to the next can exhibit a minimum of 107 μm and a maximum of 211 μm. Thus, a minimal horizontal distance of a CD ampulla to the next mirrors how many CD ampullae respectively niches can stand side by side in the nephrogenic zone per metric unit. One can further assume that a close horizontal alignment signals a rich prospective nephron endowment. In contrast, a wide horizontal distance shows that the number of niches has decreased and in turn, that the nephron endowment per metric unit will be reduced. This information may be of most importance for future pathological evaluation, when an index for prospective nephron endowment is searched.

Moreover, a wide horizontal distance between neighboring CD ampullae can be a mirror for a decreased ureteric bud respectively CD tubule branching and a minor activity of nephrogenesis in this particular district (Awazu and Hida 2015). It may be caused by a branching disparity, which can arise by mutual suppression of branching in the cortical end of a CD tubule caused by intrinsic and locally operative mechanisms (Lefevre et al. 2017). A molecule involved in this process is the prorenin receptor, which controls branching morphogenesis via Wnt/β-catenin signaling (Song et al. 2017a, 2018).

An increase in the horizontal distance between CD ampullae may also arise by an areal expansion of the capsule and in parallel by the underlying nephrogenic zone. In this connection, one has to bear in mind that each tip of a CD ampulla is fastened via microfibers at the inner side of the capsule like a puppet on strings (Minuth and Denk 2014). Consequently, an active areal expansion of the capsule would inevitably pull apart tissue compounds of the nephrogenic zone in a horizontal direction. This process results in an increase in the horizontal distance between neighboring CD ampullae. However, much more systematic morphometric measurements, confocal immunohistochemistry, and a close look at pathological specimens are necessary to confirm or refuse this hypothesis.

Initial nephron formation in the shadow of a CD ampulla

When the anlage of a nephron is starting, related morphological alterations are exclusively observed at the outer border of the nephrogenic zone. Morphological sign of nephron anlage is first seen at the tip and then at the upper lateral aspect of a CD ampulla as a separation and then aggregation of the few induced nephrogenic mesenchymal progenitor cells (Fig. 4). This process leads to a pretubular aggregate, which develops through a mesenchymal to epithelial transition into a renal vesicle near the head of a CD ampulla. It can be recognized by arise of polarized cells and a developing basal lamina. Stages of advanced nephron formation such as comma- and S-shaped bodies do not occur at the head but on the lateral aspect of a CD ampulla. Due to a covering basal lamina, these stages are still spatially separated from the related CD ampulla. At the deep lateral aspect (neck) of a CD ampulla and in close proximity to its related differentiating CD tubule, the early S-shaped body exhibits at his proximal part the anlage of the glomerulus and at its distal part, the later tubule system. In the late S-shaped body, a junction is formed with the CD ampulla head via the presumptive connecting tubule. As mentioned, the proximal pole of the late S-shaped body marks the border between the nephrogenic zone and the underlying maturation zone (Fig. 3) (Kloth et al. 1993).

The renal capsule covers all

Generally, the renal capsule was considered as a fibrous envelope that protects the developing organ against inappropriate exogenous signals. Furthermore, one associates that it consists of a Tunica fibrosa with several Strata and a Rete capillare capsulare, which fulfills as Vasa privata the necessary supply for personal nutrition and respiratory gas (Fig. 5). The Tunica muscularis received a relatively small amount of attention, although it contacts as most inner layer of the renal capsule the underlying nephrogenic zone. It contains atypical smooth muscle cells that show similarities to both smooth muscle cells and fibroblasts (Kobayashi 1978; Minuth and Denk 2014). Further, it is not widely known, but the described atypical smooth muscle cells exhibit projections. Some of them are covered by a basal lamina, while others show a faint glycocalix. Most interestingly, these cell projections establish with neighboring cells numerous cell-to-cell contacts. The extracellular space between contacting cell projections belongs to a complex tunnel system. Although not yet physiologically investigated, one could speculate that it directs the flow of interstitial fluid from the capsule towards the nephrogenic zone.

Growth forces expansion of the renal capsule

As illustrated before, during gestation weeks 32 and 38, the total renal volume (TRV) exhibits an increase of 7.1 cm^3 (Fig. 1) (Kandasamy et al. 2013; Brennan and Kandasamy 2017). This change leads to an areal expansion of the renal capsule and the underlying nephrogenic zone in an order of 30% (Minuth 2018a). In parallel, elongation of developing CD tubules including iterative branching, formation of CD ampullae and exact positioning of niches below the renal capsule require space (Fig. 4). Considering that both developmental processes occur not locally but synchronized along the entire surface of a fetal human kidney, it is obvious that an increase in kidney volume needs coordination for areal expansion of the renal capsule as well as for the nephrogenic zone. In this

context, one must realize that each bifid branching of a CD tubule requires a mean areal horizontal claim of about 120 μm (Minuth 2018a). Therefore, the factual problem is that a more or less non-elastic fibrous renal capsule covers the nephrogenic zone (Figs. 3, 4, and 5). A resulting question is whether successive branching, formation of CD ampullae, positioning of niches, and complement of mesenchymal progenitor cells in sum produce tension of the capsule or whether a turgor of growing parenchyma and interstitial fluid provoke its passive and permanent stretching. An alternative consideration is that an actively expanding renal capsule provides the necessary space.

Interactions between the renal capsule and nephrogenic zone

Previous and current investigations exhibit that the actual available number of mesenchymal progenitor cells in the nephrogenic zone is determined by CD tubule branching and thereby influences prospective nephron endowment (Cebrian et al. 2014; Muthukrishnan et al. 2018). One primarily thinks about progenitor cells that occur exclusively in the nephrogenic zone. However, there is now clear evidence that not only the nephrogenic zone but also the contacting renal capsule represent physiological frameworks for progenitor cells (Rowan et al. 2017). Earlier investigations showed the pool of integrated mesenchymal progenitor cells, their development, and that also initial steps of nephrogenesis are controlled here (Hatini et al. 1996). A further set of experiments revealed that a discrete cell population in the capsule expresses progenitor cell markers Foxd1, Raldh2, and Sfrp1 (Levinson et al. 2005). Another investigation demonstrated that renal capsule cells also exhibit labels for CD29, vimentin, Sca1, and nestin (Park et al. 2010). Most interestingly, progenitor cells in the capsule of mice kidneys have the ability to migrate towards the underlying nephrogenic zone with the rate of 30 μm/h. Illustrated progenitor cells in the capsule of human kidney are not randomly distributed, but show a special patterning and occur predominantly in close proximity to capillaries (Leuning et al. 2017). An actual investigation further exhibits that cells in the capsule show strong CD 34 and partial nestin and CD 105 immunoreaction (Rusu et al. 2018).

Pathological findings suggest that in the renal capsule of preterm babies, the amount of progenitor cells varies from one case to the next and that their amount does not seem to be exclusively related to gestational age (Fanni et al. 2015). A further investigation exhibited that by renal capsule cells, the developmental modulator thymosine beta-4 (Tβ4) is expressed (Nemolato et al. 2014). Further on, not only nephrogenic mesenchymal progenitor cells in the nephrogenic zone but also stromal cells in the capsule were detected to synthesize morphogen GDNF that is normally directing branching morphogenesis (Magella et al. 2018). Surprisingly, also β-catenin is expressed in stroma including the renal capsule. It modulates morphogen Wnt9b signaling, which in turn drives nephron induction (Boivin et al. 2015). On the down side, growth factor Midkine expressed in this area promotes expansion of nephrogenic mesenchyme, but at the same time, it suppresses expansion of the stromal compartment and branching morphogenesis (Qiu et al. 2004).

Regarding the growth of a fetal human kidney and areal expansion of the renal capsule including the nephrogenic zone during late gestation, it is worth having a closer look to the before-mentioned Foxd1 cell lineage and correlated downstream functions. For example, ablation of Foxd1-derived stromal cells illustrates a mispatterning in the nephrogenic zone in the form of altered mesenchyme, areas devoid of nephron progenitors, ureteric branching defects, and aberrant vessel formation (Hum et al. 2014; Phua et al. 2015). Further intact prolyl-4-hydroxylation of the hypoxia-inducible factor regulated by prolyl-4-hydroxylase domain (PHD) dioxygenases PHD1, PHD2, and PHD3 is essential in Foxd1 lineage cells for normal nephrogenesis (Kobayashi et al. 2017). Further removal of hedgehog intracellular effector smoothened (Smo-defiencient mutants) in the cortical stroma produces an atypical renal capsule, an expansion of nephron progenitors, and a decrease in nephron number via block of epithelialization (Rowan et al. 2018). Finally, cross communication between Foxd1 and the renin-angiotensin system (RAS) is a prerequisite for intact CD tubule branching morphogenesis (Song et al. 2017b). In this respect and due to its overall presence in the renal capsule, Foxd1 could belong to a monitoring system, which controls continuity of nephrogenesis not only on the site of a single niche, but along the entire nephrogenic zone of a fetal human kidney.

Blood vessels lining towards the nephrogenic zone

Not only pathological evaluation, but also therapeutic concepts and in particular site-specific administration of eligible drugs for prolongation of nephrogenesis in preterm and low birth weight babies require an exact knowledge of blood supply in the outer cortex of the fetal human kidney.

To give some physiological background, measurements revealed that under normal physiological conditions, an adult kidney extracts only 10–20% of the oxygen delivered to it (Evans et al. 2011). Further investigations show that the partial pressure of oxygen in the cortex, corticomedullary junction, and the outer medulla is with 11.9, 4.1, and 7.9 mmHg quite different (Whitehouse et al. 2006). However, respiratory gas supply for a kidney is still more complex, since not only delivery but also oxygen extraction and cellular demand must be considered (O'Connor 2006). The actual problem is that for

the outer cortex and especially for nephrogenic zones in the fetal human kidney, basic physiological data is lacking and awaits investigation by adequate methods such as intravital phosphorenscence lifetime imaging or light sheet fluorescence microscopy (Hirakawa et al. 2018; Isaacson et al. 2018).

To illustrate vessels lining towards the nephrogenic zone, one has to start at the border between the medulla and the inner cortex of a fetal human kidney. In a renal lobus, an arcuate artery lines into interlobular arteries (cortical radiate arteries), which ascend straight through the inner, mid, and outer cortex towards the renal capsule (Fig. 6). At intervals, they give rise to the afferent arterioles, each of which supplies a glomerulus (Molema and Aird 2012). Then, in the height of the maturation zone and further along in the nephrogenic zone, the distal part of an interlobular artery (cortical radiate artery) lines as a perforating radiate artery into the Rete capillare capsulare of the renal capsule (Fig. 5). Furthermore, not in the nephrogenic zone of the fetal kidney but later in the postnatal kidney efferent vessels of glomeruli in the outer cortex may develop a Ramus capsularis (capsular branch), which reaches capillaries in the renal capsule (Jastrow 2018).

The nephrogenic zone represents a bradytrophic district

As it was shown before, the outer cortex of a fetal human kidney during the late phase of gestation consists of an inner maturation zone and an outer nephrogenic zone (Fig. 3). Both are crossed by perforating radiate arteries, which line radially to the renal capsule (Fig. 6). The horizontal spaces between them in the nephrogenic zone show only developing but not perfused vessels. In contrast, in the underlying maturation zone besides developing capillaries, established vessels and initial perfusion with erythrocytes can be observed in the area of further matured glomeruli.

To obtain an idea about the special physiological situation of the nephrogenic zone, it is necessary to have a closer look into its incomplete vessel system. Earlier performed histochemistry with *Ulex europaeus* I lectin on human fetal kidney exhibits that in the area of nephron anlagen not intact capillaries but only strands of spreading endothelial cells exist (Holthöfer 1987). Neonatal rabbit kidney illuminates after immunohistochemical label that capillaries line from cortical radiate arteries towards the cortex cortices. Only strands of endothelial cells are seen at the lateral aspect of a CD ampulla and on the lower cleft of S-shaped bodies, where the presumptive glomerular tuft arises (Kloth et al. 1997). In the fetal human kidney, forming vessels are recognized on the lower cleft of S-shaped bodies as well (Fig. 6). Actual immunohistochemical data with the endothelium marker anti-CD 31 exhibits that endothelial cell strands occur at the branching site of a CD tubule and at the lateral aspect of a CD ampulla (Gerosa et al. 2017). However, at the niche site including the tip of a CD ampulla and within the layer of facing nephrogenic mesenchymal progenitor cells, spreading endothelial cells are not present (Munro et al. 2017a). Also, expression of endothelial nitric oxide synthase was registered in the kidney of rat only on S-shaped bodies, but not within the mesenchymal progenitor cell layers (Han et al. 2005).

Of special importance is the interaction between the developing vessel system and nephrogenesis. In homology to the ureteric bud, it appears most probable that Wingless Int-1 (Wnt7b) protein expressed by epithelial progenitor cells at the lateral aspect of CD ampullae activates canonical Wnt signaling in the surrounding interstitial cells to establish capillaries (Roker et al. 2017). Other investigations on developing mice kidneys exhibit that forming vessels in the nephrogenic zone remain un-perfused, although oxygenation is able to drive nephron progenitor cell differentiation (Rymer et al. 2014; Hemker et al. 2016).

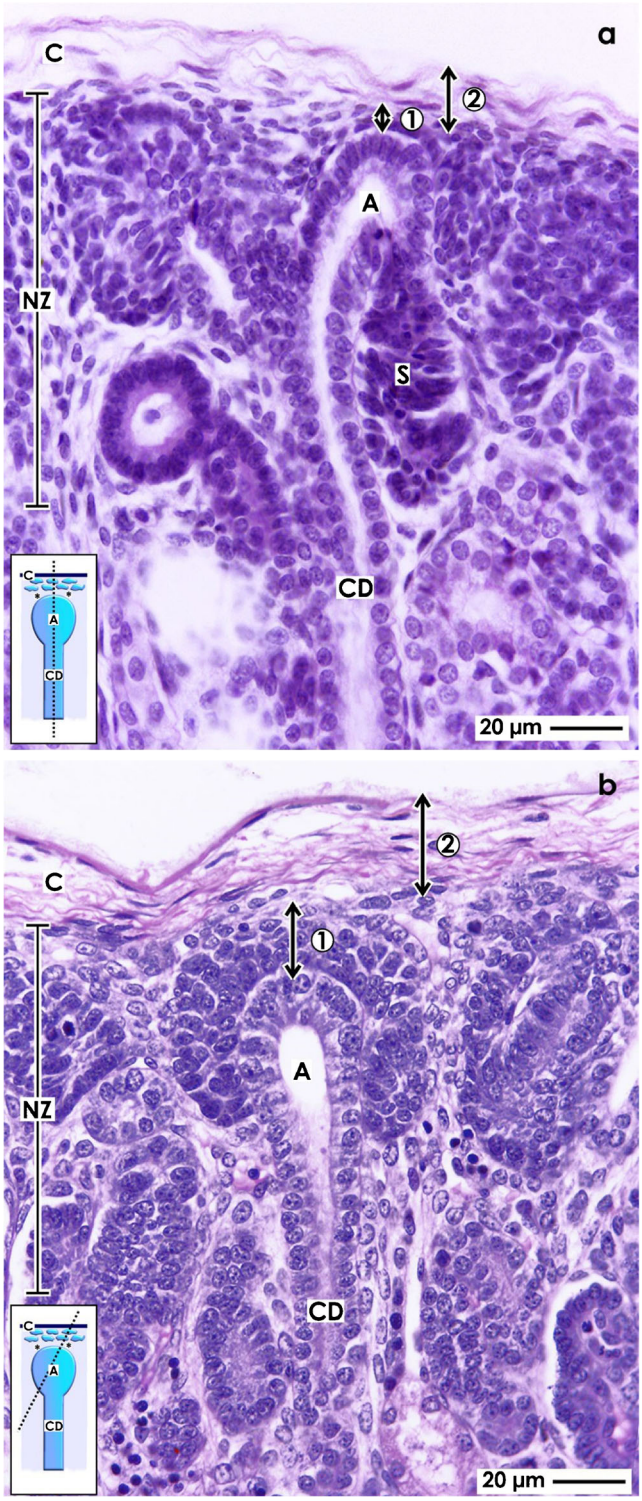
Finally, via perforating radiate arteries, the nephrogenic zone is connected with the Rete capillare capsulare of the renal capsule. In the lateral spaces between perforating radiate arteries developing but non perfused capillaries are seen. The district of niches that occur here is avascular. The question is, how these are provided with nutrition and respiratory gas. One possibility could be that capillaries within the renal capsule produce interstitial fluid that is then transported in the Tunica muscularis by the previously described tunnel system along the outer side of the nephrogenic zone (Fig. 5) (Minuth and Denk 2014). One could imagine that alternate contraction and relaxation of smooth muscle cells in sum leads to transport of fluid in the extracellular tunnel system contained here.

Hence, vascular supply including perfusion with erythrocytes in the maturation zone is in part developed, while it is incomplete in the nephrogenic zone. The district of niches is avascular (Munro et al. 2017b). One can conclude that the nephrogenic zone belongs to a bradytrophic area with unknown interstitial fluid composition.

"Cap mesenchyme"—fact or wrong interpretation?

Pathologists mostly make diagnosis on sections lining accidentally through a tissue. Daily experiences show that this procedure leads to reliable statements. However, for the fetal human kidney and especially for the nephrogenic zone that it contains, accidentally cut sections can result in misleading interpretations.

Sections orientated according to the earlier described method (Fig. 2) show only two layers of mesenchymal cells between the tip of a CD ampulla and the inner side of the renal capsule in the fetal human kidney (Figs. 3, 4, 5, 6, and 7a). The same cell configuration was also documented for the neonatal



◀ **Fig. 7** Appearance of a cap mesenchyme in human fetal kidney—fact or wrong interpretation? **a** A section of a fetal human kidney orientated along the lumen of CD tubules (CD) shows that the distance between the inner side of the renal capsule (C) and the tip of a CD ampulla (A) is 9.9 μm (1), while the distance between the outer side and inner side of the renal capsule is 17 μm (2). One can recognize that in the orientated specimen, only two layers of nephrogenic mesenchymal cells are visible at the tip of a CD ampulla. **b** In contrast, when a section with an oblique perspective is regarded, the mentioned parameters are changing. Although a CD ampulla is still visible, the CD tubule below its branching site disappears due to leaving the optical plain. Yet, the distance between the inner side of the renal capsule and the tip of the CD ampulla is 19.9 μm (1). The distance between the outer and inner side of the renal capsule is 26.6 μm (2). Only in this oblique perspective several layers of nephrogenic mesenchymal cells resembling a "cap mesenchyme" are seen at the tip of a CD ampulla. Inlets depict direction of cutting line

rabbit kidney (Minuth and Denk 2016). In contrast, for other animal models, it was described that a multilayered "cap mesenchyme" occurs at the tip of a CD ampulla (Combes et al. 2016; Munro et al. 2017b; Song et al. 2018). For these reasons, it was analyzed, whether a "cap mesenchyme" is lacking in the nephrogenic zone of the fetal human kidney or whether its appearance is based on an incorrect interpretation.

To give a concrete example, sections orientated along the lumen of lining CD tubules show in this perspective that the distance between the inner side of the renal capsule and the tip of a CD ampulla is 9.9 μm. Furthermore, the distance between the outer side and inner side of the renal capsule is 17 μm (Fig. 7a). One can see that in this perspective, only two layers of nephrogenic mesenchymal progenitor cells are visible at the tip of a CD ampulla. In contrast, when a section of the same specimen but in an oblique perspective is analyzed, the registered parameters are altered. In the oblique perspective a CD ampulla is seen, but the related CD tubule below the branching site is no longer visible, since it leaves the optical plain. Yet, the distance between the inner side of the renal capsule and the tip of the CD ampulla is 19.9 μm. The distance between the outer and inner side of the renal capsule is 26.6 μm (Fig. 7b). Most importantly, several layers of mesenchymal progenitor cells are only visible in this oblique perspective suggesting presence of a "cap mesenchyme" at the tip of a CD ampulla. Finally, comparing both perspectives, one can recognize that nephrogenic mesenchymal progenitor cells at the tip of a CD ampulla must have a more or less flat but relatively widespread outer shape.

Therapeutic challenge

Most of the substances, receptors, and related molecular pathways involved during the impairment of nephrogenesis in the nephrogenic zone of preterm and low birth weight babies are not yet identified. In this situation, it is obvious that most of the questions dealing with a biomedical therapy cannot be answered. Nevertheless, it is necessary to develop concepts for a site-specific therapeutic approach that buffers harming influences and/or that are able to prolong nephrogenesis in preterm and low birth weight babies (Hendry et al. 2011; Fanos et al. 2015). One primarily thinks about a therapeutic application of site-specific

morphogens or growth factors that control multiple links in the chain of nephrogenesis (Benedetto et al. 2014; Oxburgh et al. 2017; Wang et al. 2018). However, in this context, one must also consider that such applied molecules act site-specific, may have a nephrotoxic influence and may evoke unwished ectopic effects (Saifudeen et al. 2009; Nishita et al. 2014; Chung et al. 2017).

A completely underestimated problem is the application of a drug prolonging nephrogenesis. First of all, application of an eligible drug must be adapted to the incomplete vessel system occurring in the nephrogenic zone. In parallel, reliable information about spatial distribution, binding on extracellular matrix and bioavailability of a drug in the outer cortex of the fetal human kidney must be generated. Due to the lack of vessels, investigations dealing with long distance diffusion of an administered drug must also be performed. Finally, an unsolved problem is not only the administration of a drug but also its bioavailability along the entire surface of a fetal kidney.

A medication that stimulates nephrogenesis by a smart drug delivery system via the renal capsule seems plausible (Tran and Damaser 2015; Saboktakin and Tabatabaei 2015). However, the release of drugs must be adapted to less considered physiological, structural, and functional features of the nephrogenic zone and covering renal capsule (Minuth 2018a, b, c). Moreover, a therapeutic approach should not focus only on nephron induction and initial stages of nephron formation but it must also consider the molecular processes dealing with areal expansion of the capsule and the nephrogenic zone including exact positioning of niches in a fetal human kidney during late gestation (Minuth 2018c). In sum, data of site-specific physiology, synthesis, secretion, and transport of locally operating morphogens maintaining stemness, patterning of niches and triggering of nephron induction for animal species is available, but for the fetal human kidney, they are missing.

Conclusion

Impaired nephrogenesis in preterm and low birth weight babies disturbs developmental processes in the outer cortex of the fetal kidney. The resulting, too early termination of nephron formation and kidney growth consequently leads to oligonephropathy later in life. It is obvious that in this situation, a therapeutic approach is urgently required. However, molecular causes, harmed pathways and the exact site of damage in the nephrogenic and maturation zones are not known. To facilitate the admittance to this rarely investigated complex, data of relevant literature was collected and supplemented with data from my earlier laboratory at the Institute of Anatomy, University of Regensburg. The resulting take-home messages are:

- Earlier, it was believed that noxae causing impaired nephrogenesis interfere with maturation of glomeruli in the maturation zone. However, actual data points out that not only secondary but also primary steps of nephrogenesis such as anlage and early nephron formation taking place in the nephrogenic zone are affected.
- New data forces to take a close look not only at harmed initial nephron formation but also at less considered upstreaming processes. This involves areal expansion of the renal capsule and the underlying nephrogenic zone.
- Increase in the surface area of a fetal kidney during the late phase of gestation is remarkable. For example, between gestation weeks 32 and 38 as well, the capsule as the nephrogenic zone exhibits an areal expansion in the order of 30%.
- An areal expansion holds cell biological risks, since propagation of progenitor cells, extension of parenchyma, and development of blood vessels run through a sensitive developmental period.
- Due to the current lack of knowledge, it must be worked out in how far the processes of renal capsule and nephrogenic zone expansion interacts with nephron formation and whether they are affected by intra- or extrauterine noxae.
- First morphometric recordings exhibit that the distribution of niches in the nephrogenic zone can vary. It appears that a small horizontal (lateral) distance indicates regular patterning, while a wide distance points to a minor prospective endowment of nephrons.
- Recently published data implies to focus future research on less-noticed structural and functional links between capsule, nephrogenic zone, and niches. There is strong evidence that continuation of nephrogenesis depends on intact links.
- The Foxd1-dependent signals are of special interest in this context. A plausible working hypothesis is that disturbance of those signals interacting with capsule synthesis and/or expansion of the nephrogenic zone provokes a too early termination of nephrogenesis along the entire surface of the fetal human kidney.

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References

Abdel-Hakeem AK, Henry TQ, Magee TR, Desal M, Ross MG, Mansano RZ, Torday JS, Nast CC (2008) Mechanisms of impaired nephrogenesis with fetal growth restriction: altered renal transcription and growth factor expression. Am J Obstet Gynecol 199(3): 252.e1–252.e7

Abitbol CL, DeFreitas MJ, Strauss J (2016) Assessment of kidney function in preterm infants: lifelong implications. Pediatr Nephrol 31(12):2213–2222

Al-Awqati O, Goldberg MR (1998) Architectural patterens in branching morphogenesis in the kidney. Kidney Int 54(6):1832–1842

Awazu M, Hida M (2015) Maternal nutrition restriction inhibits ureteric bud branching but does not affect the duration of nephrogenesis in rats. Pediatr Res 77(5):633–639

Barnett C, Nnoli O, Abdulmahdi W, Nesi L, Shen M, Zullo JA, Payne DL, Azar T, Dwivedi P, Syed K, Gromis J, Lipphardt M, Jules E, Maranda EL, Patel A, Rabadi MM, Ratcliff BB (2017) Low birth weight is associated with impaired murine kidney development and function. Pediatr Res 82(2):340–348

Benedetto A, Accetta G, Fujita Y, Charras G (2014) Spatiotemporal control of gene expression using microfluids. Lab Chip 14(7):1336–1347

Boivin FJ, Sarin S, Lim J Javidan A, Svajger B, Khalili H, Bridgewater D (2015) Stromally expressed β-catenin modulates Wnt9b signaling in the ureteric epithelium. PLoS One 10(3):e0120347

Brennan S, Kandasamy Y (2017) Ultrasound imaging of the renal parenchyma of premature neonates for the assessment of renal growth and glomerulomegaly. Ultrasound Med Biol 43(11):2546–2549

Bruno S, Chiabotto G, Camussi G (2014) Consise review: different mesenchymal stromal/stem cell populations reside in the adult kidney. Stem Cells Transl Med 3(12):1451–1455

Buchholz B, Schley G, Eckhardt KU (2016) The impact of hypoxia on nephrogenesis. Curr Opin Nephrol Hypertens 25(3):180–186

Callaway DA, McGill-Vargas LL, Quinn A, Jordan JL, Winter LA, Anzueto D, Dick EJ, Blanco CL (2018) Prematurity disrupts glomeruli development while prematurity and hyperglycemia lead to altered nephron maturation and increased oxidative stress in newborn baboos. Pediatr Res 83(3):702–711

Cebrian C, Asai N, D'gati V, Costantini F (2014) The number of fetal nephron progenitor cells limits ureteric branching and adult nephron endowment. Cell Rep 7(1):127–137

Chi L, Saarela U, Railo A, Prunskaite-Hyyryläinen R, Skovorodkin I et al (2011) A sectreted BMP antagonist, CER1, fine tunes the spatial organization of the ureteric bud tree during mouse kidney development. PLoS One 6(11):e27676

Chung E, Deacon P, Park JS (2017) Notch is required for the formation of all nephron segments and primes nephron progenitors for differentiation. Development 144(24):4530–4539

Combes AN, Davies JA, Little MH (2015) Cell-cell interactions driving kidney morphogenesis. Curr Top Dev Biol 112:467–508

Combes AN, Lefevre JG, Wilson S, Hamilton NA, Little MH (2016) Cap mesenchyme cell swarming during kidney development is influenced by attraction, repulsion, and adhesion to the ureteric tip. Dev Biol 418(2):297–306

Da Sacco S, Thornton ME, Petrosyan A, Lavarreda-Pearce M, Sedrakyan S et al (2017) Direct isolation and characterization of human nephron progenitors. Stem Cells Transl Med 6(2):419–433

Evans RG, Goddard D, Eppel GA, O'Connor PM (2011) Factors that render the kisney susceptible to tissue hypoxia in hypoxemia. Am J Phys Regul Integr Comp Phys 300(4):R931–R940

Fanni D, Sanna A, Gerosa C, Puddu M, Faa G, Fanos V (2015) Each niche has an actor: multiple stem cell niches in the preterm kidney. Ital J Pediatr 41:78

Fanos V, Castagnola M, Faa G (2015) Prolonging nephrogenesis in preterm infants: a new approach for prevention of kidney disease in adulthood? Iran J Kidney Dis 9(3):180–185

Gerl K, Steppan D, Fuchs M, Wagner C, William C, Kurtz A, Kurt B (2017) Activation of hypoxia signaling in stromal progenitors impairs kidney development. Am J Pathol 187(7):1496–1511

Gerosa C, Fanni D, Faa A, Van Eyken P, Ravarino A, Fanos V, Faa G (2017) Low vascularization of the nephrogenic zone of the fetal kidney suggests a major role for hypoxia in human nephrogenesis. Int Urol Nephrol 49(9):1621–1616

Girardi A, Raschi E, Galletti S, Poluzzi E, Faldella G, Allegaert K, De Ponti F (2015) Drug-induced renal damage in preterm neonates: state of the art and methods for early detection. Drug Saf 38(6):535–551

Gubhaju L, Sutherland MR, Yoder BA, Zulli A, Bertram JF, Black MJ (2009) Is nephrogenesis affected by preterm birth? Studies in a non-human primate model. Am J Physiol Ren Physiol 297(6):F1668–F1677

Han KH, Lim JM, Kim MY, Kim H, Madsen KM et al (2005) Expression of endothelial nitric oxide synthase in developing rat kidney. Am J Physiol Ren Physiol 288(4):F694–F702

Harvey SJ (2012) Models for studies of proteoglycans in kidney pathophysiology. Methods Mol Biol 836:259–284

Hatini V, Huh SO, Herzlinger D, Soares VC, Lai E (1996) Essential role of stromal mesenchyme in kidney morphogenesis revealed by targeted disruption of winged Helix transcription factor BF-2. Genes Dev 10(12):1467–1478

Hemker SL, Sims-Lucas S, Ho J (2016) Role of hypoxia during nephrogenesis. Pediatr Nephrol 31(10):1571–1577

Hendry C, Rumballe B, Moritz K, Little MH (2011) Defining and redefining the nephron progenitor population. Pediatr Nephrol 26(9):1395–1406

Hirakawa Y, Mizukami K, Yoshihara T, Takahashi I, Khulan P, Honda T, Mimura I, Tanaka T, Tobita S, Nangaku M (2018) Intravital phophrence lifetime imaging of the renal cortex accurately measures renal hypoxia. Kidney Int 93(6):1483–1489

Holthöfer H (1987) Vascularization of the embryonic kidney. Detection of endothelial cells with Ulex europaeus I lectin. Cell Differ 20(1):27–31

Hum S, Rymer C, Schaefer C, Bushnell D, Sims-Lucas S (2014) Ablation of the renal stroma defines its critical role in nephron progenitor and vasculature patterning. PLoS One 9(2):e88400

Isaacson D, Shen J, McCreedy D, Calvert M, McDevitt T, Cunha G, Baskin L (2018) Lightsheet fluorescence microscopy of branching human kidney. Kidney Int 93(2):525

Jastrow H (2018) Anatomisches Institut, Johannes Gutenberg Universität, Mainz: Gefässe der Niere: http://www.drjastrow.de/WAI/EM/EMNiere.html

Kandasamy Y, Smith R, Wright IM (2012) Oligonephropathy of prematurity. Am J Perinatol 29(2):115–120

Kandasamy Y, Smith R, Wright IM, Lumbers ER (2013) Extra-uterine renal growth in preterm infants: oligonephropathy and prematurity. Pediatr Nephrol 28(9):1791–1796

Kloth S, Aigner J, Brandt E, Moll R, Minuth WW (1993) Histochemical markers reveal an unexpected heterogeneous composition of the renal embryonic collecting duct epithelium. Kidney Int 44(3):527–536

Kloth S, Ebenbeck C, Monzer J, de Vries U, Minuth WW (1997) Three-dimensional organization of the developing vasculature of the kidney. Cell Tissue Res 287(1):193–201

Kobayashi K (1978) Fine structure of the mammalian renal capsule: the atypical smooth muscle cell and its functional meaning. Cell Tissue Res 195(3):381–394

Kobayashi H, Liu J, Urrutia AA, Burmakin M, Ishii K, Rajan M, Davidoff O, Saifudeen Z, Haase VH (2017) Hypoxia-inducible factor prolyl-4-hydroxylation in FOXD1 lineage cells is essential for normal kidney development. Kidney Int 92(6):1370–1383

Lefevre J, Short KM, Lamberton TO, Michos O, Graf D, Smyth IM, Hamilton NA (2017) Branching morphogenesis in the developing kidney is governed by rules that pattern the ureteric tree. Development 144(23):4377–4385

Leuning DG, Engelse MA, Lievers E, Bijkerk R, Reinders MEJ, de Boer HC, van Kooten C, Rabelink TJ (2017) The human kidney capsule contains a functionally distinct mesenchymal stromal cell population. PLoS One 12(12):e0187118

Levinson RS, Batourina E, Choi C, Vorontchiikhina M, Kitajewski J, Mendelsohn CL (2005) Fox1-dependent signals control cellularity

in the renal capsule, a structure required for normal renal development. Development 132(3):529–539
Li W, Hartwig S, Rosenblum ND (2014) Developmental origins and functions of stromal cells in the normal and diseased mammalian kidney. Dev Dyn 243:853–863
Lindström NO, Guo J, Kim AD, Tran T, Guo Q, De Sena Brandine G, Ransick A, Parvez RK, Thornton ME, Baskin L, Grubbs B, McMahon JA, Smith AD, McMahon AP (2018a) Conserved and different features of mesenchymal progenitor cell types within the cortical nephrogenic niche of the human and mouse kidney. J Am Soc Nephrol 29(3):806–824
Lindström NO, Tran T, Guo J, Rutledge E, Parvez RK, Thornton ME, Grubbs B, McMahon JA, McMahon AP (2018b) Conserved and different molecular and anatomic features of human and mouse nephron patterning. J Am Soc Nephrol 29(3):825–840
Lindström NO, McMahon JA, Guo J, Tran T, Rutledge E, Parvez RK, Saribekyan G, Schuler RE, Liao C, Kim AD, Abdelhalim A, Ruffins SW, Thornton ME, Baskin L, Kesselmann C, McMahon AP (2018c) Conserved and different features of human and mouse kidney organogenesis. J Am Soc Nephrol 29(3):785–805
Luyckx VA (2017) Preterm birth and its impact on renal health. Semin Nephrol 37(4):311–319
Magella B, Adam M, Potter AS, Venkatasubramanian M, Chetal K, Hay SB, Salomonis N, Potter SS (2018) Cross-platform single cell analysis of kidney development shows stromal cells express GDNF. Dev Biol 434(1):36–47
Mao Y, Francis-West P, Irvine KD (2015) Fat4/Dchs1 signaling between stromal and cap mesenchyme cells influences nephrogenesis and ureteric bud branching. Development 142(15):2574–2585
Mari C, Winyard P (2015) Concise review: understanding the renal progenitor cell niche in vivo to recapitulate nephrogenesis in vitro. Stem Cells Transl Med 4:1463–1471
Menendez-Castro C, Nitz D, Cordasic N, Jordan J, Bäuerle T, Fahlbusch FB, Rascher W, Hilgers KF, Hartner A (2018) Neonatal nephron loss during active nephrogenesis – detrimental impact with long-term renal consequences. Sci Rep 8(1):4542
Minuth WW (2017) Concepts for a therapeutic prolongation of nephrogenesis in preterm and low birth weight babies must correspond to structural-functional properties in the nephrogenic zone. Mol Cell Pediatr 4(1):12
Minuth WW (2018a) Structural and functional links between capsule and nephrogenic zone in fetal human kidney. J Pediatric Neonatal Individualized Medicine (JPNIM) (in press)
Minuth WW (2018b) Reading first coordinates from the nephrogenic zone in human fetal kidney. Nephron 138(2):137–146
Minuth WW (2018c) Action plan for prolongation of nephrogenesis in preterm and growth restricted babies: explore ultrastructure of the nephrogenic zone, identify a molecular target, select a viable drug and find a path for administration. Drug Res 68(1):5–16
Minuth WW, Denk L (2014) Structural links between the renal stem/progenitor cell niche and the organ capsule. Histochem Cell Biol 141(5):458–471
Minuth WW, Denk L (2015) Advanced fixation for transmission electron microscopy unveils special extracellular matrix within the renal stem/progenitor cell niche. Methods Mol Biol 12:21–37
Minuth WW, Denk L (2016) What is the functional background of filigree extracellular matrix and cell-cell connections at the interface of the renal stem/progenitor cell niche? J Pediatric and Neonatal Individualized Medicine (JPNIM) 5(1):e50115
Molema G, Aird WC (2012) Vascular heterogeneity in the kidney. Semin Nephrol 32(2):145–155
Morizane R, Bonventre JY (2017) Kidney organoids: a translational journey. Trends Mol Med 23(3):246–263
Munro DAD, Hohenstein P, Davies JA (2017a) Cycles of vascular plexus formation within the nephrogenic zone of the developing mouse kidney. Sci Rep 7(1):3273
Munro DAD, Hohenstein P, Coate TM, Davies JA (2017b) Refuting the hypothesis that semaphorin-3f/neuropilin-2 exclude blood vessels from the cap mesenchyme in the developing kidney. Dev Dyn 246(12):1047–1056
Muthukrishnan SD, Ryzhov S, Karolak M, Oxburgh L (2018) Nephron progenitor cell death elicits a limited compensatory response associated with interstitial expansion in neonatal kidney. Dis Model Mech 11(1). https://doi.org/10.1242/dmm.030544
Nagalakshimi VK, Yu J (2015) The ureteric bud epithelium: morphogenesis and roles in metanephric kidney patterning. Mol Reprod Dev 82:151–166
Nemolato S, Cabras T, Messana I, Gerosa C, Faa G, Castanola M (2014) Do betathymosins play a role in human nephrogenesis? In: Faa G, Fanos V (eds) Kidney development in renal pathology. Current Clinical Pathology, Humana Press - Springer, New York, pp 81–93
Nguyen MU, Wallace MJ, Pepe S, Menheniott TR, Moss TJ, Burgner D (2015) Perinatal inflammation: a common factor in the early origins of cardiovascular disease? Clin Sci (Lond) 129(8):769–784
Nishita M, Qiao S, Miyamoto M, Okinaka Y, Yamada M, Hashimoto R, Iijama K, Otani H, Hartmann C, Nishinakamura R, Minami Y (2014) Role of Wnt5a-Ror2 signaling in morphogenesis of the metanephric mesenchyme during ureteric budding. Mol Cell Biol 34(16):3096–3105
O'Brien LL, Guo Q, Lee Y, Tran T, Benazet JD, Whitney PH, Valouev A, McMahon AP (2016) Differential regulation of mouse and human nephron progenitors by the six family of transcriptional regulators. Development 143(4):595–608
O'Connor PM (2006) Renal oxygen delivery: mating delivery to meatbolic demand. Clin Exp Pharmacol Physiol 33(10):961–967
Oxburgh L, Muthukrishnan SD, Brown A (2017) Growth factor regulation in the nephrogenic zone of the developing kidney. Results Probl Cell Differ 60:137–164
Park HC, Yasuda K, Kuo MC, Ni J, Ratliff B, Chander P, Goligorsky MS (2010) Renal capsule as stem cell niche. Am J Physiol Renal Physiol 298(5):F1254–F1262
Phua YL, Chu JY, Marrone AK, Bodnar AJ, Sims-Lucas S, Ho J (2015) Renal stromal miRNAs are required for normal nephrogenesis and glomerular mesangial survival. Physiol Rep 3(10). https://doi.org/10.14814/phy2.e12537
Qiu L, Hyink DP, Gans WH, Amsler K, Wilson PD, Burrow CR (2004) Midkine promotes selective expansion of the nephrogenic mesenchyme during kidney organogenesis. Organogenesis 1(1):14–21
Riccio P, Cebrian C, Zong H, Hippenmeyer S, Constantini F (2016) Ret and Etv promote directed movements of progenitor cells during renal branching morphogenesis. PLoS Biol 14(6):e1002382
Roker LA, Nemri K, Yu J (2017) Wnt7b signaling from the ureteric bud epithelium regulates medullary capillary development. J Am Soc Nephrol 28(1):250–259
Rosenblum ND (2008) Developmental biology of the human kidney. Semin Fetal Neonatal Med 13:125–132
Rowan CJ, Sheybani-Deloui S, Rosenblum ND (2017) Origin and function of the renal stroma in health and disease. Results Probl Cell Differ 60:2015–2229
Rowan CJ, Li W, Martiirosyan H, erwood S, Hu D, Kim YK, sheybani-Deloui S, Mulder J, Blake J, chen L, Rosenblum ND (2018) Hedgehog-GLI signaling in Foxd1-positive stromal cells promotes murine nephrogenesis via TGFβ signaling. Development 145(13). https://doi.org/10.1242/dev.159947
Rumballe BA, Georgas KM, Combes AN, Ju AL, Gilbert T, Little MH (2011) Nephron formation adopts a novel spatial topology at cessation of neohrogenesis. Dev Biol 360(1):110–122
Rusu MC, Mogoanta L, Pop F, Dobra MA (2018) Molecular phenotypes in the human kidney: myoid stromal cells/telocytes and myoepithelial cells. Ann Anat 218:95–104
Ryan D, Sutherland MR, Flores TJ, Kent AL, Dahlstrom JE, Puelles VG, Bertram JF, McMahon AP, Little MH, Moore L, Black MJ (2017)

Development of the human fetal kidney from mid to late gestation in male and female infants. EBioMedicine 27:275–283
Rymer C, Paredes J, Halt K, Schaefer C, Wiersch J et al (2014) Renal blood flow and oxygenation drive nephron progenitor differentiation. Am J Physiol Ren Physiol 307(3):F337–F345
Saboktakin MR, Tabatabaei RM (2015) Supramolecular hydrogels as drug delivery systems. Int J Biol Macromol 75:426–436
Saifudeen Z, Dipp S, Stefkova J, Yao X, Lookabaugh S, El-Dahr SS (2009) P53 regulates metanephroc development. J Am Soc Nephrol 20(11):2328–2337
Schreuder MF, Bueters RR, Allegaert K (2014) The interplay between drugs and the kidney in premature neonates. Pediatr Nephrol 29(11): 2083–2091
Song R, Janssen A, Li Y, El-Dahr S, Yosypiv IV (2017a) Prorenin receptor controls renal branching morphogenesis via Wnt/β-catenin signaling. Am J Physiol Ren Physiol 312(3):F407–F417
Song R, Lopez MLSS, Yosypiv IV (2017b) Foxd1 is an upstream regulator of the renin-angiotensin system during metanephric kidney development. Pediatr Res 82(5):855–862
Song R, Kidd L, Janssen A, Yosypiv IV (2018) Conditional ablation of the prorenin receptor in nephron progenitor cells results in developmental programming of hypertension. Physiolol Rep 6(7):e13644
Stritzke A, Thomas S, Amin H, Fusch C, Lodha A (2017) Renal consequences of preterm birth. Mol Cell Pediatr 4(1):2
Sutherland MR, Gubhaju L, Moore L, Kent AL, Dahlstrom JE, Horne RSC, Hoy WE, Bertram JF, Black MJ (2011) Accelerated maturation and abnormal morphology in the preterm neonatal kidney. J Am Soc Nephrol 22:1365–1374
Sutherland MR, Bertagnolli M, Lucaszewski MA, Huyard F, Yzydorczyk C, Luu TM, Nuyt AM (2014) Preterm birth and hypertension risk: the oxidative stress paradigm. Hypertension 63(1):12–18
Tran C, Damaser MS (2015) Stem cells as drug delivery methods: application of stem cell secretome for regeneration. Adv Drug Deliv Rev 82-83:1–11
Vignais ML, Caicedo A, Brondello JM, Jorgensen C (2017) Cell connections by tunneling nanotubes: effects of mitochondrial trafficking on target metabolism, homeostasis, and response to therapy. Stem Cells Int. https://doi.org/10.1155/2017/6917941
Wang J, Wang F, Wang Z, Li S, Chen L, Liu C, Sund D (2018) Protective effect of GDNF-engineered amniotic fluid-derived stem cells on the renal ischaemia reperfusion injury in vitro. Cell Prolif 51(2):e12400
Whitehouse T, Stotz M, Taylor V, Stidwill R, Singer M (2006) Tissue oxygen and hemodynamics in renal medulla, cortex, and corticomedullary junction during hemorrhage-reperfusion. Am J Phys 291(3):F647–F653
Woods LL, Ingelfinger JR, Nyengaard JR, Rasch R (2001) Maternal protein restriction suppresses the newborn renin-angiotensin system and programs adult hypertension in rats. Pediatr Res 49(4):460–467