Review

What is the functional background of filigree extracellular matrix and cell-cell connections at the interface of the renal stem/progenitor cell niche?

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Abstract

Development of a nephron is induced by a reciprocal exchange of morphogenetic proteins between epithelial and mesenchymal cells within the renal stem/progenitor cell niche. For sustaining concentration of diffusing proteins high, it is believed that an intimate contact exists between involved cells. However, actual morphological data show that both types of stem/progenitor cell bodies are separated by an interface. To analyze details of this arrangement, neonatal rabbit kidneys were fixed in traditional glutaraldehyde (GA) solution for transmission electron microscopy. For an enhanced contrast fixation of samples was performed in GA solution including either cupromeronic blue, ruthenium red or tannic acid. To record always the same perspective, embedded blocks of parenchyma were cut in orientated vertical and transverse planes to the lumen of lining collecting duct tubules. Screening of samples fixed by GA solution demonstrates a constant separation of stem/progenitor cell bodies by an unobstrusively looking interface. In contrast, improved fixation of specimens in GA solution including cupromeronic blue, ruthenium red or tannic acid unveils between them earlier not visible filigree extracellular matrix. Further projections of mesenchymal cells covered by

this matrix cross the interface to contact epithelial cells. The end of a projection does not dangle but is mounted by a special plug connection. At this site the plasma membranes of mesenchymal and epithelial cells are connected via tunneling nanotubes. Regarding this unique arrangement the principal question is to what extent illustrated extracellular matrix and cell-cell connections are involved in the exchange of morphogenetic proteins during induction of a nephron.

Keywords

Neonatal kidney, stem/progenitor cell niche, extracellular matrix, cell-cell connection, tunneling nanotubes, morphogenetic proteins.

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How to cite

Introduction

Acute and chronic kidney diseases are an essential problem to public health. Beside conventional therapies such as hemodialysis and transplantation, there is growing interest to find a secure therapy for the repair of damaged renal parenchyma by implantation of stem/progenitor cells [1]. In this coherence the renal stem/progenitor cell niche as a source of renal parenchyma gains increasing importance in research. Until a few years ago this site was still regarded as an incidental aggregation of stem/progenitor cells. However, actual research illustrates that contained stem/progenitor cells are accommodated in a structural environment so that the earlier view cannot be longer maintained [2]. A further new dimension is that contained cells do not live in the niche as hermites but form complex cell-cell connections for communication. Frequently the impression is created that the niche in the developing kidney [3] persists in a similar form in adult parenchyma [4]. However, one needs to accept that they concern two different topics.

The niche in the course of kidney development

Beginning with the organ anlage and up to the neonatal period the renal parenchyma radially extends by a spatiotemporal program [5] and by a cell biological process called branching morphogenesis [6]. During this period a more or less constant number of nephrons is developing. When the organ has reached its final size, also the formation of nephrons is terminated by an unknown mechanism.

Within each niche cells derived from two different tissues are contained: cells of the metanephric mesenchyme (MES) and epithelial (EPI) cells of the ureteric bud (UB) [7]. The arrangement of stem/progenitor cells is noteworthy [8]. During the phase of organ anlage epithelial stem/progenitor cells are included in the UB, while during subsequent radial growth of parenchyma they stay within the arborizing tip of a bud-derived collecting duct (CD) ampulla (A) (**Fig. 1a**) [9]. Mesenchymal stem/progenitor cells are grouped around the tip of each CD ampulla so that they are exposed to the basal aspect of epithelial cells (**Fig. 1b**) [10]. In this form the niche persists from the organ anlage until extension of parenchyma is terminated. Surprisingly, throughout development of the kidney the niche as an ensemble is not randomly distributed but stays always in close neighborhood to the inner side of the organ capsule [11].

Primary steps in nephron development

Formation of a nephron starts, when the dichotomous branching of an UB-derived tip of a CD ampulla is completing [12]. At that time epithelial and mesenchymal stem/progenitor cells stand in the correct position for a reciprocal exchange of morphogenetic proteins such as glial-derived neurotrophic factor (GDNF), hepatocyte growth factor (HGF), epidermal growth factor receptor ligands (EGF, HBEGF, TGFα), WNT family members, bone morphogenetic proteins (BMPs), TGFβ, fibroblast growth factors (FGFs) and leukemia inhibitory factor (LIF) [13-16]. As the result few mesenchymal cells are elected first to condensate and then to transform into epithelial cells forming in turn a renal vesicle as initial sign of a nephron [17].

Structural links between niche and capsule

Over time it became apparent that the renal niche is not a random accumulation of stem/

progenitor cells but accommodates cells within a surprisingly structured extracellular matrix [18-20]. Although epithelial stem/progenitor cells are strongly involved in the exchange of morphogenetic proteins, they do not stand naked but are covered by a consistently developed basal lamina [21]. In their vicinity numerous microfibers occur consisting of collagen type I, II, III and IV [11, 22]. Further individual microfibers labeled by Soybean agglutinin (SBA) originate at the basal lamina and line through the layer of mesenchymal stem/progenitor cells to end at the inner side of the organ capsule [23]. Thus, both types of stem/progenitor cells are included in an earlier not presumed framework of microfibers.

Gap between epithelial and mesenchymal cells

Experiences over years have shown that incidental histological sections do not provide a reliable view to the niche. For that reason blocks of embryonic parenchyma must be exactly orientated along the lumen of lining CD tubules for embedding and histological cutting (**Fig. 1a**) [2]. When such a section is analyzed by optical microscopy, the distance between a CD ampulla and the capsule is 14 to 16 μm, while incorrectly orientated (oblique) ones show more than 20 μm. Further specimens of human or rabbit fetal parenchyma exhibit that epithelial and mesenchymal stem/progenitor cell bodies are not in direct contact but stand at a distance between 1 to 2 μm (**Fig. 1b**) [24, 25]. Frequently it was argued that the gap between both types of stem/progenitor cells is an artifact and that it is caused by poor tissue preparation. However, regarding frozen sections, sections of paraffine- or Epon-embedded embryonic parenchyma, one has to accept that the gap between epithelial and mesenchymal stem/progenitor cell bodies is constant.

Microstructure of the interface

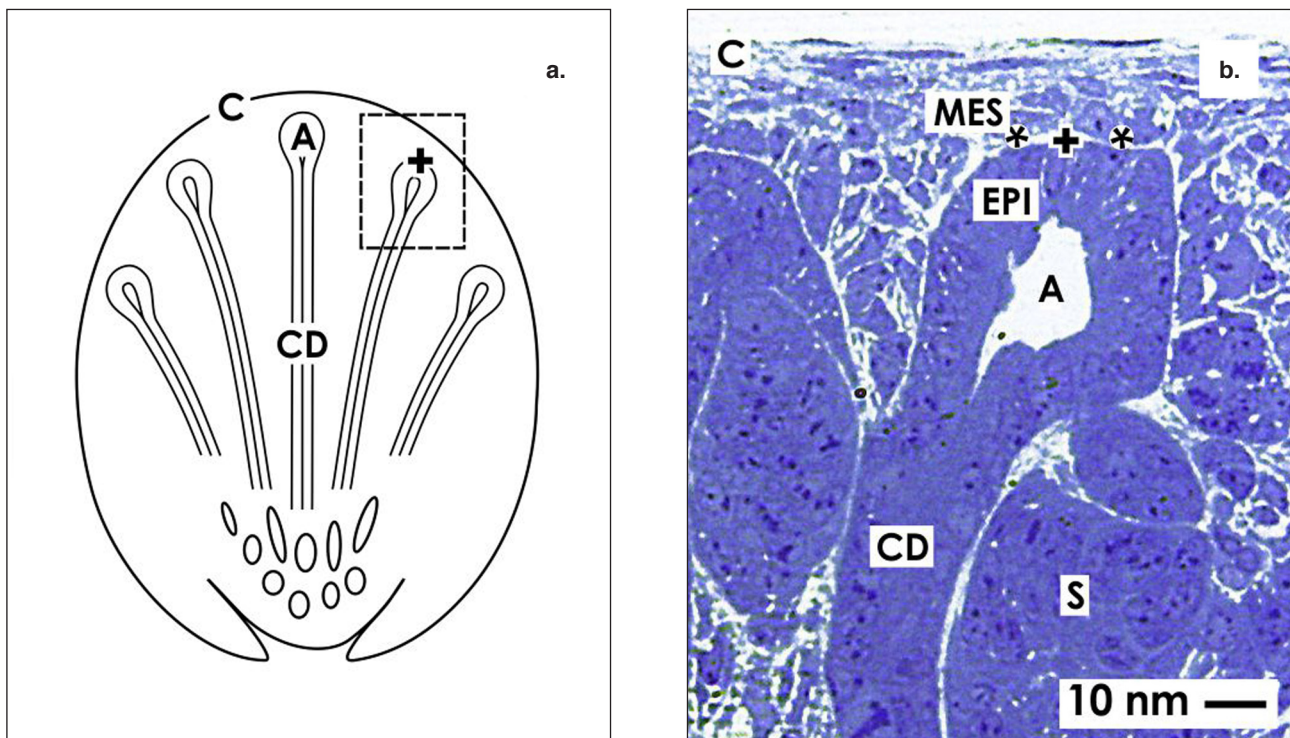In transmission electron microscopy the gap between epithelial and mesenchymal cell bodies

Figure 1. Accurate orientation of parenchyma is a must for a reproducible view to the renal stem/progenitor cell niche. (**a**) A neonatal kidney is cut after fixation in the middle between both poles for embedding. The section plane is now in parallel to the lumen of lining collecting ducts (CD) and perpendicular to the organ capsule (C). Yet comparable perspectives of stem/progenitor cell niches (marked area) can be analyzed in the outer cortex. (**b**) Optical microscopy shows that epithelial (EPI) stem/progenitor cells are integrated in the tip of a CD ampulla (A), while few layers of mesenchymal (MES) stem/progenitor cells surround them. Further mesenchymal cells are separated from epithelial cells by an interface (asterisk). The basal aspect of epithelial stem/progenitor cells at a CD ampulla (A) tip is labeled by a cross (+). S marks a maturing S-shaped body.

within the renal niche looks unremarkable by the first view. When samples were fixed in traditional glutaraldehyde (GA) solution, it is recorded that epithelial cells are enclosed at the tip of a CD ampulla and are covered by a basal lamina consisting of a lamina rara (L.r.), lamina densa (L.d.) and lamina fibroreticularis (L.f.) (**Fig. 2a**). The body of a mesenchymal cell does not touch but has a distance between 1 and 2 μm to the basal lamina of an opposite epithelial cell. Interestingly, projections of mesenchymal cells cross the interface to contact the basal lamina of an epithelial cell. Occasionally, tiny microfibers originate at the lamina fibroreticularis and line through the interface to contact a mesenchymal cell.

Although looking unobstrusive, all of the elaborated data illustrate that the interface between epithelial and mesenchymal stem/progenitor cells is real. It seems that it is not caused by hydraulic forces of interstitial fluid but must be based on masked extracellular matrix. Since fixation of specimens by traditional GA solution was not able to visualize suspected structural details, improved fixation and contrasting for transmission electron microscopy

was performed [26]. As a result, specimens fixed in GA solution including 0.1% cupromeronic blue now demonstrate that numerous braces of proteoglycans connect the basal lamina of epithelial cells with the surface of mesenchymal cell projections (**Fig. 2b**). When specimens were fixed in GA solution including 0.5% ruthenium red (**Fig. 2c**) or 1% tannic acid (**Fig. 2d**) an intense but filigree extracellular matrix becomes visible covering the basal lamina of epithelial cells and contacting projections of mesenchymal cells.

To summarize, special fixation in GA solution including cupromeronic blue, ruthenium red or tannic acid unveils earlier not visible textural extracellular matrix within the interface, on the basal lamina of epithelial cells and on the surface of mesenchymal cell projections. It is obvious that detected extracellular matrix acts as a spacer and causes the interface between mesenchymal and epithelial stem/progenitor cell bodies.

Mesenchymal cells contact epithelial cells

Electron microscopy futher illustrates that projections of mesenchymal cells cross the interface
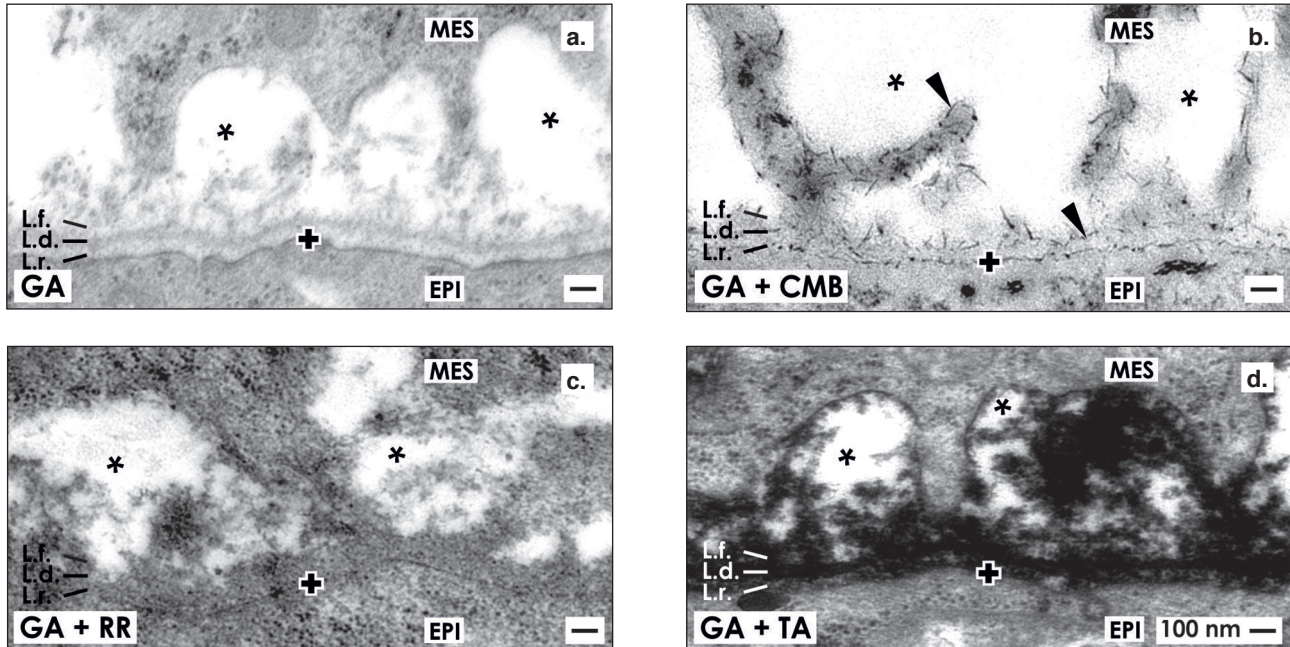

Figure 2. Transmission electron microscopy of the renal stem/progenitor cell niche elucidates that an interface (asterisk) is present between mesenchymal (MES) and epithelial (EPI) stem/progenitor cells. (**a**) Specimens fixed by conventional GA solution demonstrate that epithelial cells are covered by a basal lamina consisting of a lamina rara (L.r.), lamina densa (L.d.) and lamina fibroreticularis (L.f.). Projections of mesenchymal cells cross the interface to touch the basal lamina of epithelial cells. Within the interface only few extracellular matrix is recognized. (**b**) Samples fixed by GA solution including cupromeronic blue (CMB) show numerous braces of proteoglycans that are contained in the basal lamina and on the surface of mesenchymal cell projections (arrow head). (**c**) Specimens fixed by GA solution including ruthenium red (RR) or (**d**) tannic acid (TA) illuminate earlier non visible extracellular matrix within the interface. The basal plasma membrane of epithelial stem/progenitor cells at a CD ampulla (A) tip is labeled by a cross (+).

to contact epithelial cells. Of special interest is whether the end of a mesenchymal cell projection only touches or even establishes a functional connection between the two types of stem/progenitor cells [27].

Fixation of specimens in traditional GA solution suggests that the end of a mesenchymal cell projection is only touching the lamina fibroreticularis at the tip of a CD ampulla (**Fig. 2a, 3a**). In contrast, fixation in GA solution including cupromeronic blue clearly shows that numerous braces of proteoglycans originate at the basal lamina of epithelial cells to form a cover around a contacting mesenchymal cell projection (**Fig. 2b**, **3b**). Fixation of specimens by GA solution including ruthenium red (**Fig. 2c**, **3c**) or tannic acid (**Fig. 2d**, **3d**) illustrates that a mesenchymal cell projection is wrapped by a striking coat of extracellular matrix forming in turn a sleeve. This unexpected construction points

out that obviously a long lasting connection exists between a contacting mesenchymal cell projection and an epithelial cell.

Improved fixation of specimens by GA solution including cupromeronic blue (**Fig. 3b**), ruthenium red (**Fig. 3c**) or tannic acid (**Fig. 3d**) further elucidates that the end of a mesenchymal cell projection penetrates the lamina fibroreticularis, the lamina densa and lamina rara of epithelial cells. A striking features is that it is conducted during its passage in a special sleeve of extracellular matrix. High enlargement in the electron microscope also depicts that the distance between the end of a mesenchymal cell projection and the plasma membrane of an epithelial cell stays constant and has an average length of 167 nm (**Fig. 3**). Finally, tunneling nanotubes are recognized at this site. They cross the basal lamina and penetrate the basal plasma membrane of an epithelial cell (**Fig.**
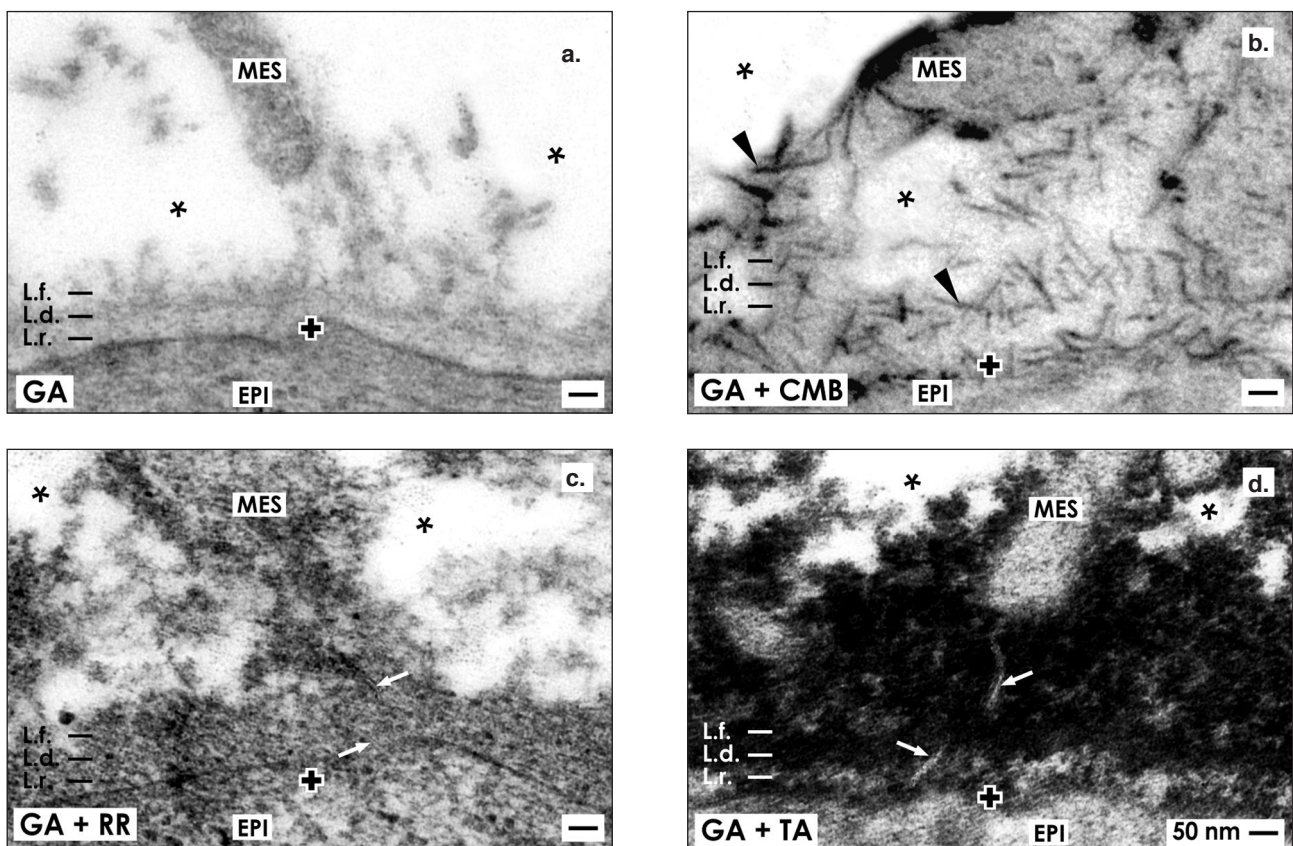

Figure 3. Transmission electron microscopy illustrates the contact between mesenchymal (MES) cell projections and epithelial (EPI) cells in the renal niche. (**a**) Specimens fixed by conventional GA solution suggest that a mesenchymal cell projection is only touching the basal lamina of epithelial cells via microfibers. (**b**) In contrast, samples fixed by GA solution including cupromeronic blue (CMB) illustrate that numerous braces of proteoglycans (arrow head) link the end of a projection with the basal lamina of epithelial cells. (**c**) Specimens fixed by GA solution including ruthenium red (RR) or (**d**) tannic acid (TA) demonstrate that the end of a mesenchymal cell projection is embedded in a special sleeve of extracellular matrix. The end of a projection is connected via tunneling nanotubes (arrow) with the plasma membrane of an epithelial cell. The basal plasma membrane of epithelial cells is marked by a cross (+).

3c,d). These morphological findings exhibit that a functional plug-in connection exists between mesenchymal and epithelial cells.

Cell-cell connections encounter functionality

Earlier and present morphological data show that the body of a mesenchymal and an epithelial stem/progenitor cell is separated by an interface (**Fig. 2**). Further projections of mesenchymal cells cross it, penetrate the basal lamina to contact via tunneling nanotubes the basal plasma membrane of epithelial cells (**Fig. 3c,d**). Transfering these results to a physiological understanding, it seems likely that at the end of a mesenchymal cell projection integrin α8β1 is localized, which contacts nephronectin as receptor at the basal lamina of an epithelial cell as it was earlier described [28-30]. Moreover, the micro-tubule-dependent motor protein kinesin KIF26B was shown in mesenchymal cell projections possibly involved in regulating attraction of cells, signal transduction or developmental patterning [31, 32]. However, recently performed immunohistochemistry with antibodies reacting against mentioned proteins and analysis by confocal laser scanning fluorescence microscopy in our laboratory did not show clear evidence so that this issue cannot be ascertained.

A surprising result was that tunneling nanotubes connect a mesenchymal cell projection with the basal plasma membrane of an epithelial stem/progenitor cell (**Fig. 3c,d**) [2]. In previous investigations it was shown that tunneling nanotubes can be generally involved in a variety of physiological functions such as intercellular transfer of organelles, membrane compounds and cytoplasmic molecules [33, 34]. However, comparable functions of tunneling nanotubes were not described for the embryonic kidney respectively niche, but were investigated on cell cultures [35, 36]. For that reason more details about the number, construction, co-localization with other proteins and transport features of tunneling nanotubes within the renal niche have to be elaborated in future. A basic problem is further that suitable antibodies reacting on specific sites on tunneling nanotubes are commercially not available. Consequently, labeling experiments with appropriate antibodies for electron microscopy are still waiting to be done.

It could be argued that the here presented data are limited to neonatal rabbit kidney (**Fig. 1b**, **2**, **3**) [2]. However, screening earlier literature homologous data were elaborated 40 years ago on embryonic mouse kidney [37]. In contrast to our experiments at that time microscopical analysis was performed at the stage of organ anlage, when an inducing ureter bud has branched only once. First of all it was observed that mesenchymal cells are separated from the tip of an ureter bud by an earlier called interspace. Further cytoplasmic processes were recognized that cross this interspace [37] (see **Fig. 2**). Moreover, high enlargement in electron microscopy revealed that mesenchymal and epithelial cells are connected via projections within the interspace [37] (see **Fig. 3**).

Simulation of an interface

Consciously or unconsciously, an artificial interface was created 60 years ago by performing transfilter experiments for induction of tubules in mouse metanephrogenic mesenchyme [38]. In those culture experiments a filter was applied as a substitute for the interface. To investigate development of tubules, isolated mesenchyme was mounted on the one side, while spinal cord as an inducer tissue was placed on the other side of the filter [39, 40]. By this culture set up it was demonstrated that success of induction depends on thickness, porosity and pore size of the inserted filter. Surprisingly, a transfilter contact between the interacting cells is established within one hour provided that cytoplasmic processes emerge through the interposed filter. Then an unexpected long lag period of 16 to 24 hours is needed for completion of induction. Hence, these findings supplement actual results and point out that interacting cells keep a distance but communicate via projections during successful induction of a nephron.

Challenge for the future

As shown in numerous publications, development of a nephron depends on a reciprocal exchange of morphogenetic proteins between mesenchymal and epithelial stem/progenitor cells [13, 15]. In this coherence it is believed that involved proteins are exchanged by diffusion and that both types of stem/progenitor cells have an intimate contact (**Fig. 4a** and **Fig. 4b**) [41]. Under such a condition the route of diffusion is short and their concentration stays high, since loss by dilution in the interstitial space is unattended small. However, earlier [37] and the actual [2] elaborated morphological data contradict this assumption.

- Transmission electron microscopy demonstrates that mesenchymal and epithelial stem/progenitor

cell bodies are separated by a striking interface with a thickness of 1 to 2 μm (**Fig. 2, 3**).

- Contrasting of specimens by cupromeronic blue, ruthenium red or tannic acid illustrates that within the interface abundant filigree extracellular matrix is present (**Fig. 2b-d, 3b-d**).
- Projections of mesenchymal cells cross the interface to contact epithelial cells (**Fig. 2a-d, 3a-d**). Their surface is covered by numerous braces of proteoglycans (**Fig. 2b, 3b**) and other kind of filigree extracellular matrix (**Fig. 2c-d, 3c-d**).
- The end of a mesenchymal cell projection is embedded in a special sleeve. Tunneling nanotubes are integrated here to establish a connection between mesenchymal and epithelial cells (**Fig. 3c,d**).

Thus, the spatial separation of mesenchymal and epithelial stem/progenitor cell bodies, in-between a structured interface, crossing cell projections, cell-cell connections and tunneling nanotubes converge in a new working hypothesis (**Fig. 4c,d**). It is suggested that not all but only a part of morphogenetic proteins is exchanged by diffusion. It is further assumed that diffusing proteins are confronted by extracellular matrix within the interface. Here it is decided whether they are accessible for the target cell or whether they are bound, stored and delivered on demand. For the other part of morphogenetic proteins it is assumed that they are transported in tunneling nanotubes from an epithelial to a mesenchymal stem/progenitor cell (**Fig. 4c**) or vice versa from a mesenchymal to an epithelial cell (**Fig. 4d**).

A challenge for the near future is to verify this hypothesis. The start could be to inject fluorescent tracer molecules either in mesenchymal or epithelial stem/progenitor cell bodies of isolated niches. By this technique in combination with confocal laser fluorescence microscopy it can be visualized, whether a transport system in tunneling nanotubes exists shuttling morphogenetic proteins between mesenchymal and epithelial stem/progenitor cells.

Conclusions

Since decades it is believed that the induction of a nephron is based on a simple diffusion process of morphogenetic proteins between mesenchymal and epithelial stem/progenitor cells. However, detection of an interface between both cell types, filigree extracellular matrix, crossing cell projections and cell-cell connections including tunneling nanotubes

now illustrate that the microenvironment within the renal stem/progenitor cell niche is more complex than it was earlier assumed. Especially the presence of tunneling nanotubes between both cell types
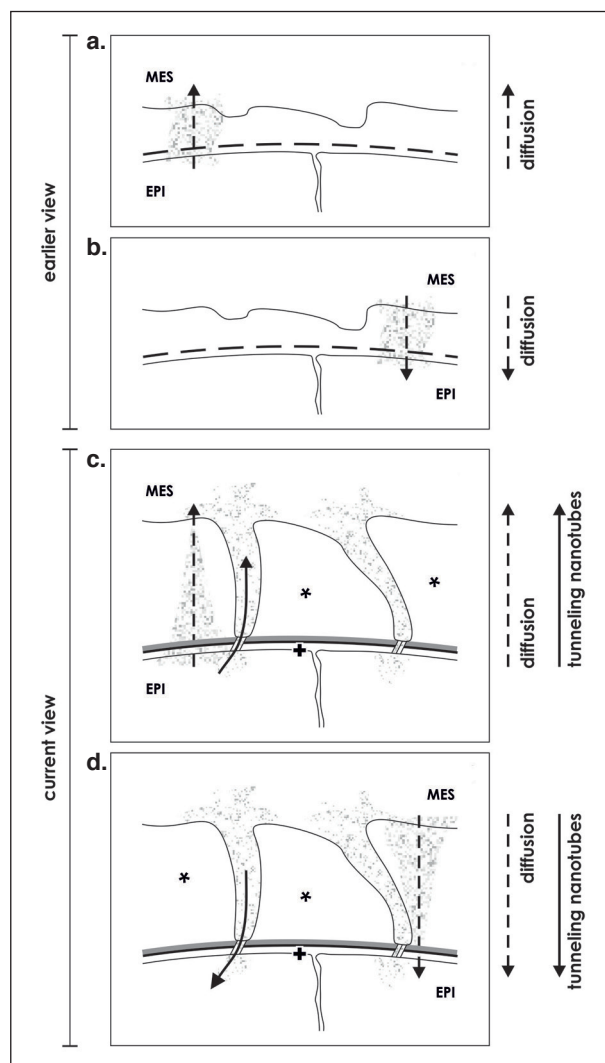

Figure 4. Schematic illustration informs about exchange of morphogenetic proteins within the renal stem/progenitor cell niche in an (**a,b**) earlier and (**c,d**) current view. (**a,b**) It was assumed that stem/progenitor cells stay close together and that all morphogenetic proteins are transmitted from (**a**) an epithelial (EPI) to a mesenchymal (MES) or (**b**) from a mesenchymal to an epithelial cell by diffusion (dashed arrow). (**c,d**) In contrast, the current view is that both types of mesenchymal and epithelial cells are separated by an interface (asterisk) including a basal lamina and abundant extracellular matrix. Further mesenchymal cell projections cross the interface to establish a connection with epithelial cells. On that situation it is speculated that only a part of morphogenetic proteins is exchanged by diffusion (dashed arrow) from (**c**) an epithelial to a mesenchymal cell or vice versa from (**d**) a mesenchymal to an epithelial cell. The other part is transported by tunneling nanotubes (solid arrow) from (**c**) an epithelial to a mesenchymal cell or vice versa from (**d**) a mesenchymal to an epithelial cell. The basal aspect of epithelial cells is marked by a cross (+).

make it likely that beside diffusion also an active transport system is involved in the exchange of morphogenetic proteins. For that reason it is time to thoroughly investigate the exchange of related proteins and the communication between involved cells by actual cell biological techniques. Taking further into consideration the recently discovered microenvironment within the niche, it is worthwhile to think about its biomedical simulation, when stem/progenitor cells are implanted into the harmful environment within diseased renal parenchyma. There are good reasons to suppose that implantation of cells in combination with an artificial interstitium mimicking the niche environment can improve survival, seeding and regeneration.

Acknowledgements

Declaration of interest

The Authors declare that there is no conflict of interest.

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