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Cell Projections and Extracellular Matrix Cross the Interstitial Interface within the Renal Stem/Progenitor Cell Niche: Accidental, Structural or Functional Cues?

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Key Words
Kidney · Stem/progenitor cell niche · Interstitium · Cell-cell contacts · Tunneling nanotubes

Abstract
Background: During nephron induction, morphogenetic molecules are reciprocally exchanged between epithelial and mesenchymal stem/progenitor cells within the renal stem/progenitor cell niche. That these molecules remain concentrated, it is assumed that both cell populations stand in close contact to each other. However, recently published data illustrate that epithelial and mesenchymal cells are separated by an astonishingly wide interstitial interface. ***Methods:*** To gain deeper morphological insights into the spatial distribution of mesenchymal and epithelial stem/progenitor cells, the embryonic zone of neonatal rabbit kidney was fixed either with glutaraldehyde (GA) or in a combination with cupromeronic blue, ruthenium red or tannic acid. Transmission electron microscopy was then performed on exactly orientated sections. ***Results:*** Conventional fixation with GA illustrates that epithelial and mesenchymal stem/progenitor cells are separated by a bright but inconspicuously looking interstitial interface. In contrast, fixation of specimens in GA containing cupromeronic blue, ruthenium red or tannic acid elucidates that part of the interstitial interface exhibits a special extracellular matrix extending like woven strands between mesenchymal and epithelial stem/progenitor cells. In parallel, filigree projections from mesenchymal stem/progenitor cells cross the interstitial interface to penetrate the basal lamina of epithelial cells. Fusion of the plasma membranes cannot be observed. Instead, touching mesenchymal cell projections form a cone at the contact site with tunneling nanotubes. ***Conclusions:*** The results demonstrate that the contact between mesenchymal and epithelial stem/progenitor cells does not form accidentally but physiologically and appears to belong to a suspected system involved in the exchange of morphogenetic information.

Introduction

For years the number of patients suffering from chronic kidney diseases has been increasing. A limited time period for dialysis and a permanent lack of donor organs available for transplantation are the driving forces to establish techniques for an effective implantation of stem/progenitor cells as an alternative therapeutic option [1]. Strategies for application of stem/progenitor cells are infusion over the blood vessel system [2], implantation by injections into parenchyma [3] or deposition under the organ capsule [4].

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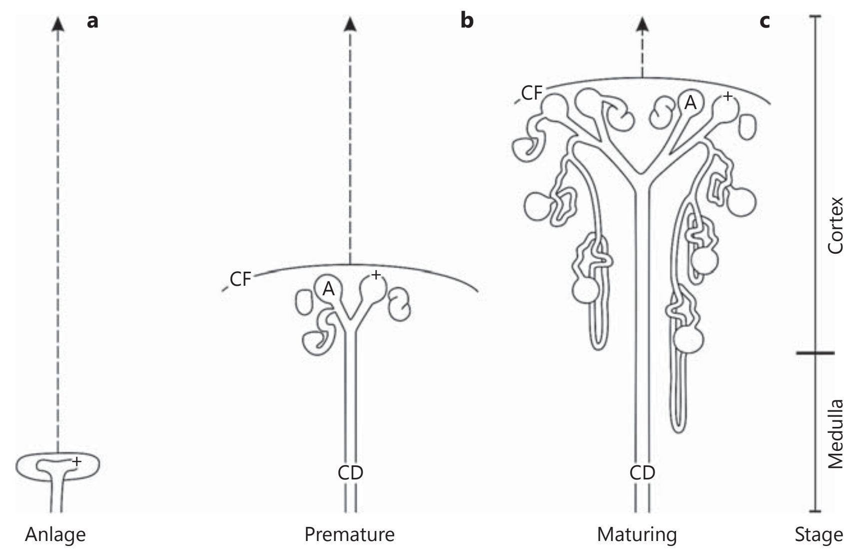

Fig. 1. Schematic illustration of the renal stem/progenitor cell niche shifting during development from the organ anlage to the outer cortex. **a** During kidney anlage the stem/progenitor cell niche is found between the invading ureteric bud and the surrounding nephrogenic mesenchyme. **b** During premature organ growth the stem/progenitor cell niche radially shifts due to permanent dichotomously dividing and successive elongation of the CD ampulla (A) in close contact to the neighboring organ capsule (CF). **c** When the final size of the maturing kidney is reached the last generation of nephron is induced by the CD ampulla in the outer cortex. The cross (+) marks the basal lamina at the tip of a CD ampulla.

However, despite intense trials over the last years the majority of presented results point out that treatment of renal diseases by the help of stem/progenitor cells has still not made a real breakthrough. Either survival of stem/progenitor cells is limited [5, 6] or an effective regeneration of parenchyma is missing due to environmental factors [7, 8]. In consideration of these facts the question arises which cell biological mechanisms are preventing the restoration of renal parenchyma [9]. Since revealing data are missing until now, the panel of speculations is broad. Possible reasons are that an appropriate type of stem/progenitor cells is not yet available, transfer from the beneficial culture environment into diseased parenchyma damages implanted cells or influences of interstitial fluid and degrading extracellular matrix (ECM) prevent regeneration [10].

Apparently a simple injection of stem/progenitor cells into diseased parenchyma does not sufficiently help. For that reason, considerations have to be made to support their survival after implantation so that stem cell properties for a repair are maintained. To gain insights into this complex question an excellent way is to focus on the microenvironment within the renal stem/progenitor cell niche. From the embryonic stage up to the neonatal period it perfectly guides the development of renal parenchyma.

The renal stem/progenitor cell niche can be recognized for the first time during the formation of the organ anlage (fig. 1a). Interestingly, it contains two different stem/progenitor cell populations [11, 12]. Epithelial stem/progenitor cells are localized within the invading ureteric bud. Its basal aspect is surrounded by metanephric mesenchymal stem/progenitor cells. The dichotomous branching and successive elongation of the ureteric bud-derived epithelium is piloting during further development the actual site of nephron formation leading in parallel to the collecting duct (CD) system found within the adult kidney.

Crucial for development of the renal parenchyma is the induction of the nephron. This essential process takes place exclusively at each tip portion of a ureteric bud-derived branch (fig. 1b). Since this portion is dilated, it was named CD ampulla [13]. Numerous reciprocal molecular interactions between epithelial stem/progenitor cells within the CD ampulla tip and surrounding cells of the metanephric mesenchyme belonging to the cap condensate lead to an aggregation of few elected cells. As a result a comma-shaped, a pine-cone and then an S-shaped body become visible as first morphological signs of nephron formation [14]. After induction, each tip of a CD ampulla divides again dichotomously so that in a close cooperation with the surrounding mesenchyme a successive generation of nephrons can be induced.

The complex temporospatial mechanisms illustrate that during kidney development, nephrons form step by step from the inner towards the outer cortex. As a consequence, the organ grows by an increase in parenchyma (fig. 1c). However, this specific development can only take place if epithelial and mesenchymal stem/progenitor cells stay strictly orientated to each other all the time. Thus, pushed by developing parenchyma, including successive branching and extension of the CD ampulla, the complete stem/progenitor cell niche radially shifts in direction to the outer cortex. During this long-lasting process it keeps constant and close contact to the extending organ capsule.

When the final size of the kidney is reached, the induction of nephrons is stopped by an unknown molecular mechanism and the stem/progenitor cell niche dissolves. During this process the ureteric bud-derived epithelium of the CD ampulla is integrated in the CD arcades, while the rest of the mesenchymal cap condensate seems to be incorporated in the organ capsule [15].

The specific arrangement of two different stem/progenitor cell populations and the close contact to the organ capsule throughout organ development suggest that the renal stem/progenitor cell niche is a piloting platform for the induction of nephrons and the spatial orientation of parenchyma. Actual data further demonstrate that epithelial and mesenchymal stem/progenitor cells are separated by a wide but individually composed interstitial interface [16]. However, cellular communication and transmission of morphogenetic signals across this interface remains widely unknown. To obtain more insight, the structural environment and in particular cellular projections crossing the interstitial interface within the renal stem/progenitor cell niche were investigated. Improved contrasting for electron microscopy demonstrates for the first time that projections from mesenchymal cells cross the interstitial interface to contact the plasma membrane of epithelial cells via special connecting cones.

Methods

Preparation of Embryonic Parenchyma

To analyze the interstitial interface of the renal stem/progenitor cell niche, 1-day-old male and female New Zealand rabbits (Seidl, Oberndorf, Germany) were anesthetized with ether and killed by cervical dislocation. Both kidneys were immediately removed to prepare them for light and electron microscopy.

Fixation of Tissue

For the current investigation, conventional fixation in glutaraldehyde (GA) and improved fixation were applied as introduced years ago for the analysis of ECM in mouse tectorial membrane [17] and proteoglycans in cardiovascular structures [18]. Techniques were performed without modifications to recognize masked ECM within the renal stem/progenitor cell niche. The following solutions were used for fixation of embryonic parenchyma in light and transmission electron microscopy (TEM): (1) specimens for control: 5% GA (Serva, Heidelberg, Germany) buffered with 0.15 M sodium cacodylate, pH 7.4; (2) series with cupromeronic blue: 5% GA buffered with 0.15 M sodium cacodylate, pH 7.4; specimens were then incubated in 0.1% cupromeronic blue (Santa Cruz Biotechnology Inc., Heidelberg, Germany) and 0.1 M magnesium chloride hexahydrate (Sigma, Taufkirchen, Germany) dissolved in sodium acetate buffer pH 5.6; counterstaining was performed with 0.5% sodium tungstate dehydrate (Sigma); (3) series with ruthenium red: 5% GA buffered with 0.15 M sodium cacodylate, pH 7.4 + 0.5% ruthenium red (Fluka, Taufkirchen, Germany), and (4) series with tannic acid: 5% GA buffered with 0.15 M sodium cacodylate, pH 7.4 + 1% tannic acid (Sigma).

Fixation was performed for 1 day at room temperature. Then, after several washes with 0.15 M sodium cacodylate, all samples except series containing cupromeronic blue were treated in the same buffer but containing additionally 1% osmium tetroxide (Science Services, Munich, Germany).

Orientation of the Renal Stem/Progenitor Cell Niche for Histology

To ensure a comparable view of renal stem/progenitor cell niches, it is essential to orientate the embryonic parenchyma. After fixation, an exact horizontal cut between both poles of the kidney was made (fig. 2a). The tissue block then has to be orientated along the lumen of the CD lining along the corticomedullary axis (fig. 2b). The same exact orientation makes a comparison between different experimental series possible. For a distinct identification of the same position, the zone between the plasma membrane and the lamina rara at the basal lamina of the CD ampulla tip reflects an important functional border centering the renal stem/progenitor cell niche. It is labeled by a cross on the individual micrographs. Further, the renal stem/progenitor cell niche contains mesenchymal as well as epithelial stem/progenitor cells separated by the interstitial interface. This border is labeled in related micrographs by an asterisk (fig. 2c).

Embedding, Sectioning and Electron Microscopy

Before embedding, specimens were washed with sodium cacodylate buffer and dehydrated in graded series of ethanols. Finally, the tissue samples were embedded in Epon (Fluka) and polymerized at 60 °C for 48 h. For analysis, semi- and ultrathin sections were prepared with a diamond knife on an ultramicrotome EM UC6 (Leica GmbH, Wetzlar, Germany). Ultrathin sections were collected onto grids (200 mesh) and contrasted using 2% uranyl acetate and lead citrate as earlier described [19]. The samples were then examined at 80 kV using an EM 902 transmission electron microscope (Zeiss, Oberkochen, Germany).

Number of Analyzed Specimens

A total of 41 renal stem cell niches were analyzed for the present work. All of the specimens were examined at least in triplicates. The performed experiments are in accordance with the Animal Ethics Committee, University of Regensburg, Regensburg, Germany.

Definition of the Renal Stem/Progenitor Cell Niche

In the present paper the embryonic part in the outer cortex of the neonatal rabbit kidney was described. The nomenclature of previously published papers was applied [12, 19].

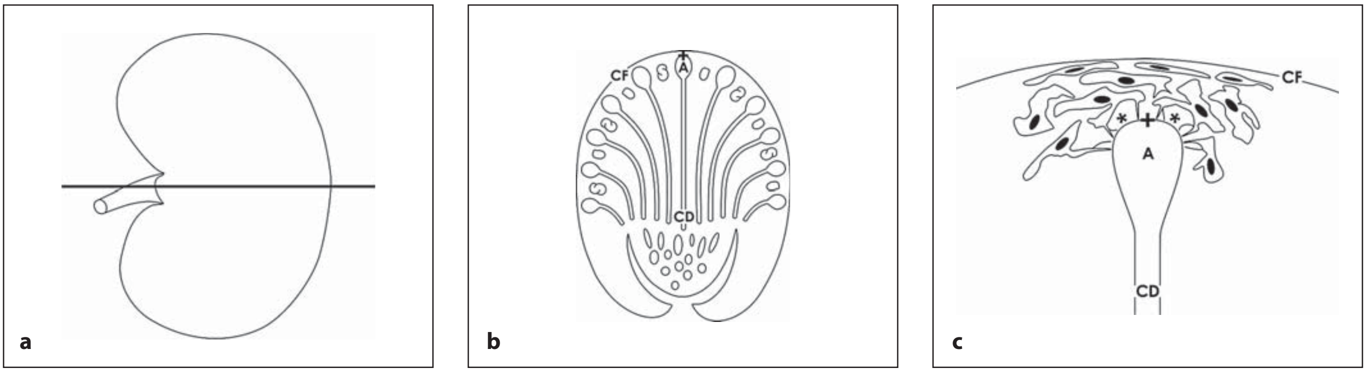

Fig. 2. Schematic illustration depicts orientation of embryonic renal parenchyma for light microscopy and TEM. **a** In a transversal section both poles were separated. **b** View to the section depicts the course of a CD ending in an ampulla (A) located beyond the CF. **c** Mesenchymal and epithelial stem/progenitor cells are separated by the interstitial interface (asterisk). The basal lamina of epithelial stem/progenitor cells is labeled by a cross (+).

Results

The actual morphological analysis was performed to investigate the microarchitecture, especially the ECM and cell projections crossing the interstitial interface between mesenchymal and epithelial cells within the renal stem/progenitor cell niche. For these experiments the embryonic parenchyma had to be orientated for sectioning so that comparable views to the stem/progenitor cell niche become possible (fig. 2). By a transversal section the kidney was divided between both poles (fig. 2a). As a consequence, the lumen of CD tubes line up to the organ capsule (fig. 2b). In the outer cortex a renal stem/progenitor cell niche can be recognized containing a CD ampulla with neighboring mesenchymal cells (fig. 2c).

Location of the Renal Stem/Progenitor Cell Niche

A semithin section through the outer cortex of neonatal rabbit kidney depicts under the light microscope three stem/progenitor cell niches (fig. 3a). At the top they are covered by the organ capsule [capsula fibrosa (CF)]. A few layers of mesenchymal stem/progenitor cells are found below the capsule which belong to the cap condensate. At the border of the cap condensate the tip of three CD ampullae are visible containing epithelial stem/progenitor cells. It can be recognized that the basal aspect of each CD ampulla is not in close contact with surrounding mesenchymal cells but is separated by a bright interstitial interface. It appears as a punctum fixum during organ development, since the interstitial interface is constantly found between 21 and 22 μm beyond the organ capsule.

Conventional View to the Renal Stem/Progenitor Cell Niche

TEM with low magnification of specimens fixed in GA illuminates the interface between epithelial and mesenchymal stem/progenitor cells. At the tips of a dichotomously dividing CD ampulla and in the recess between the interstitial interface, the separation of epithelial from mesenchymal stem/progenitor cells can be recognized (fig. 3b). Despite an intense reciprocal exchange of morphogenetic factors at this site, it is obvious that both stem/progenitor cell populations are not in close contact to each other. High magnifications reveal that mesenchymal stem/progenitor cells do not cluster, but stay in discrete distance. It is obvious that they send out numerous long cellular projections to each other (fig. 3c, d). In none of the cases could it be observed that the cell body of mesenchymal cells is in contact with the basal aspect at the tip of a CD ampulla. Instead the interstitial interface appears as a demarcation line to separate both cell populations. In addition, single cell projections originate from mesenchymal stem/progenitor cells and cross the interstitial interface to contact the lamina fibroreticularis at the outer surface of the CD ampulla.

Hidden Structures at the Interstitial Interface

High magnification of specimens in TEM depicts after conventional fixation in GA that the basal aspect of a CD ampulla tip containing epithelial stem/progenitor cells stays always separated to neighboring mesenchymal stem/progenitor cells (fig. 4a, 5a, a'). The interstitial interface can be recognized as a bright gap between both cell populations maintaining a minimal distance of at least

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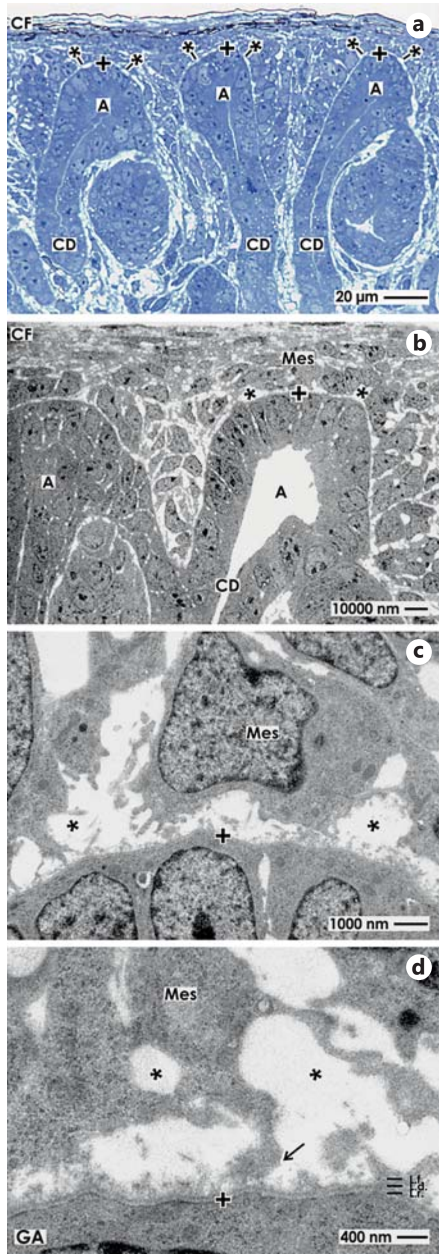

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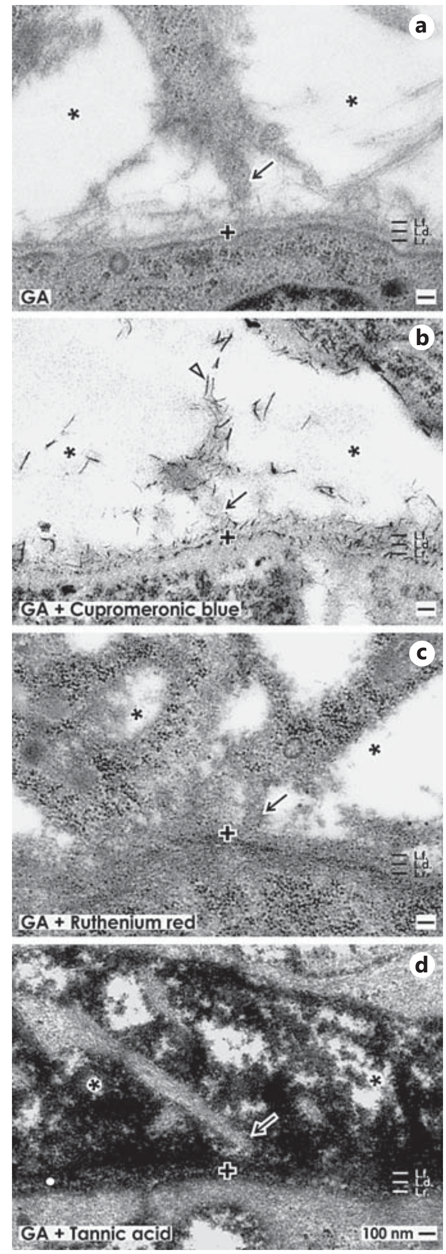

Fig. 3. Light microscopy and TEM of the renal stem/progenitor cell niche after conventional fixation in GA. **a** A semithin section demonstrates three renal stem/progenitor cell niches underneath the organ capsule (CF). Epithelial stem/progenitor cells are found within the tip of a ureteric bud-derived CD ampulla (A). A thin layer of nephrogenic mesenchymal stem/progenitor cells is found between the basal aspect of the CD ampulla and the organ capsule. The two cell populations are separated by a wide interstitial interface (asterisk). **b** TEM shows in low magnification a stem/progenitor cell niche. It comprises mesenchymal cells (Mes) underneath the organ capsule (CF), the interstitial interface (asterisk) and the basal aspect of a CD ampulla (A). **c**, **d** High magnification in TEM illustrates the basal lamina of a CD ampulla and neighboring mesenchymal stem/progenitor cells separated by the interstitial interface (asterisk). The basal lamina of epithelial stem/progenitor cells is labeled with a cross (+). L.f. = Lamina fibroreticularis; L.d. = lamina densa; L.r. = lamina rara of the basal lamina.

Fig. 4. TEM of the interstitial interface within the renal stem/progenitor cell niche after different fixation techniques. **a** Higher magnification of specimens after conventional fixation in GA shows a bright interstitial interface (asterisk) at the tip of a CD ampulla. Projections (arrow) are seen from mesenchymal stem/progenitor cells crossing the interstitial interface to the basal lamina of the CD ampulla. **b** Fixation in GA containing cupromeronic blue shows that projections (arrow) from mesenchymal cells are covered by proteoglycan braces (bright arrowhead). Endings of mesenchymal cell projections contact the basal lamina covering epithelial stem/progenitor cells. **c** Fixation in GA including ruthenium red depicts that mesenchymal cell projections (arrow) and strings of ECM line through the interstitial interface (asterisk) to contact the basal lamina at the CD ampulla. **d** Fixation in GA in combination with tannic acid illuminates that mesenchymal cell projections (arrow) and clouds of ECM cross the interstitial interface (asterisk) to contact the basal lamina at the CD ampulla. The basal lamina of epithelial stem/progenitor cells is labeled by a cross (+). L.f. = Lamina fibroreticularis; L.d. = lamina densa; L.r. = lamina rara of the basal lamina.

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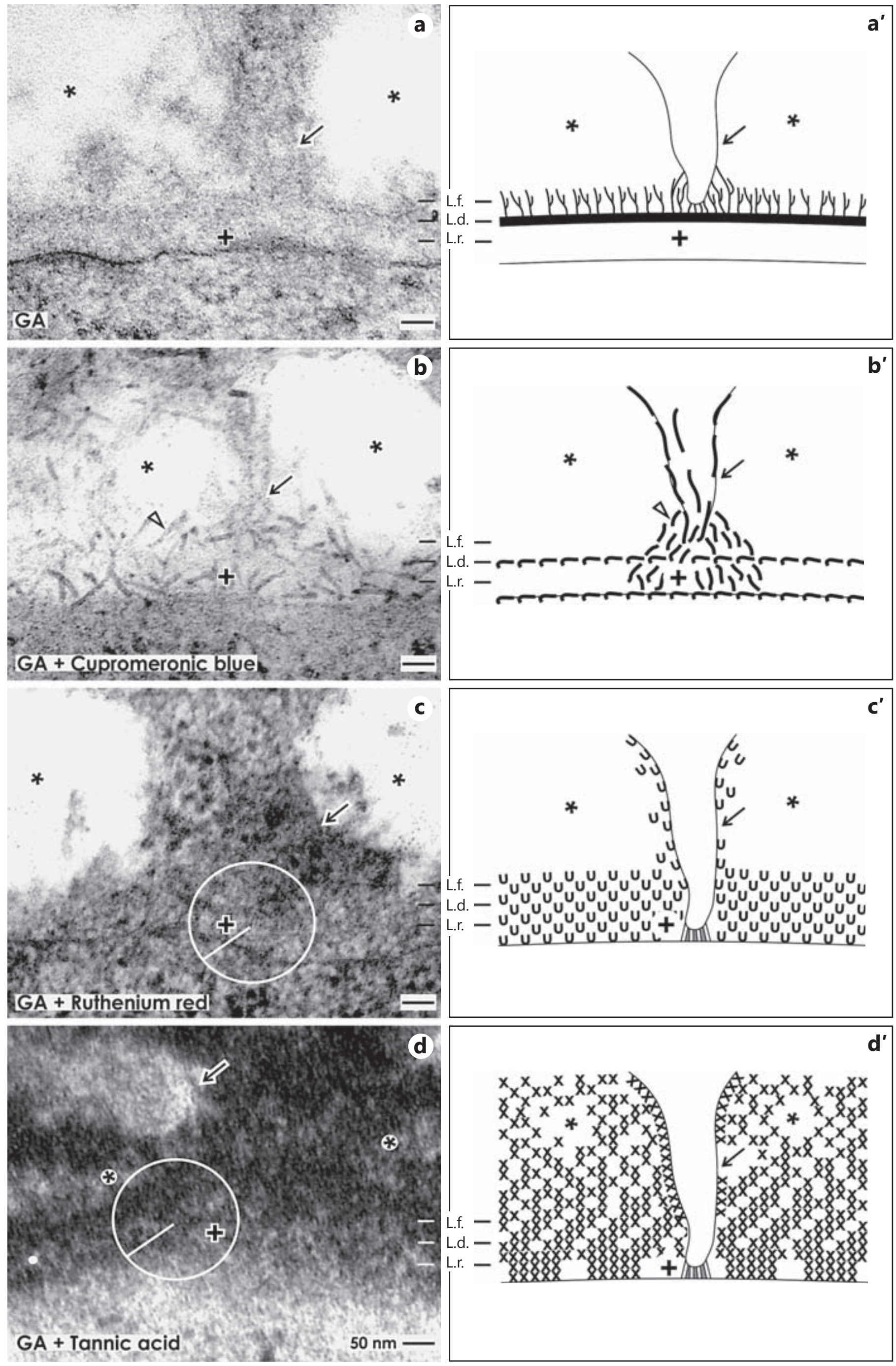

Fig. 5. High magnification in TEM (**a–d**) and schematic illustration (**a′–d′**) of the renal stem/progenitor cell niche after different kinds of fixation. **a**, **a′** Fixation in conventional GA illustrates that projections (arrow) from mesenchymal cells traverse the interstitial interface (asterisk) to contact the lamina fibroreticularis and lamina densa within the basal lamina of a CD ampulla. **b**, **b′** Fixation in GA containing cupromeronic blue shows that projections from mesenchymal cells (arrow) form a cone at the contact site, covered with proteoglycan braces (bright arrowhead). **c**, **c′** Fixation of specimens in GA containing ruthenium red depicts that mesenchymal cell projections (arrow) penetrate the basal lamina to contact the plasma membrane of epithelial stem/progenitor cells (circled area). **d**, **d′** Fixation of samples in GA containing tannic acid illuminates that projections from mesenchymal cells (arrow) penetrate the basal lamina to contact the plasma membrane of epithelial stem/progenitor cells (circled area). The basal lamina of epithelial stem/progenitor cells is labeled by a cross (+). L.f. = Lamina fibroreticularis; L.d. = lamina densa; L.r. = lamina rara of the basal lamina.

300 nm. Further, it can be seen that mesenchymal cells send out projections to contact the area of the lamina fibroreticularis at the CD ampulla tip. The filigree orientation of cellular projections crossing the interstitial interface is well preserved in all specimens and cannot be ascribed as an artifact.

The spatial separation of epithelial and mesenchymal stem/progenitor cells might be caused by masked ECM, which is not visible in tissue specimens after conventional fixation in GA. Consequently, alternative protocols for fixation and improved contrasting were applied.

To label proteoglycans, fixation of specimens was carried out in GA containing cupromeronic blue (fig. 4b, 5b, b'). TEM at the CD ampulla tip illustrates that after this fixation the typical three-laminar structure of the basal lamina was no longer visible. Instead, proteoglycan braces 50 nm in length were detected along the basal plasma membrane of epithelial stem/progenitor cells. They were found between the lamina densa and lamina fibroreticularis of the basal lamina. A staining within the lamina rara and lamina densa could not be detected. Further, it can be seen that mesenchymal stem/progenitor cell projections are covered by proteoglycan braces exhibiting 100 nm in length. Most interestingly, the endings of mesenchymal cell projections line up to the lamina densa within the basal lamina of the CD ampulla tip. At this site they form a cone as a smooth transition on the plasma membrane of epithelial stem/progenitor cells (fig. 5b, b').

To analyze more intensively the interstitial interface, specimens were fixed in GA including ruthenium red. The contrasting procedure exhibits that the staining is detected in the form of a broad band along the basal lamina at the CD ampulla tip (fig. 4c, 5c, c'). It can also be observed that mesenchymal cell projections are covered by a dense coat labeled by ruthenium red. A part of these projections crosses the interstitial interface to penetrate as tunneling nanotubes (TNT) the basal lamina at the CD ampulla tip to form a close contact to the plasma membrane of epithelial stem/progenitor cells (fig. 5c, c', circle).

In a third series of experiments, specimens were fixed in GA including tannic acid to elaborate the interstitial interface and the contact site between mesenchymal cell projections and epithelial cells more clearly (fig. 4d, 5d, d'). Labeling of tannic acid reveals that the complete basal aspect at the CD ampulla tip is covered by a dense coat. Interestingly, at the basal lamina a discontinuously labeled lamina rara is observed, while the lamina densa appears as a pronounced ribbon. Cell projections from mesenchymal stem/progenitor cells cross the interstitial interface. They are covered by a dense tannic acid coat. In parallel, numerous strands of ECM labeled by tannic acid span the interstitial space. Bright areas of the interstitium are visible between the strands. This result points out that the demonstrated profile for tannic acid label is specific, since an unspecific background signal can be excluded. Finally, the labeling also elucidates that the endings of mesenchymal cell projections penetrate via TNT all layers of the basal lamina at the CD ampulla tip to contact the plasma membrane of epithelial stem/progenitor cells (fig. 5d, d').

Contact Site between Mesenchymal and Epithelial Stem/Progenitor Cells

The contact site between mesenchymal cell projections and the basal lamina covering epithelial stem/progenitor cells within the CD ampulla tip shows interesting results. Specimens fixed in conventional GA demonstrate that mesenchymal cell projections end at the lamina fibroreticularis (fig. 5a). Fixation of specimens by GA including cupromeronic blue illustrates formation of a cone at the end of cell projections before an obviously indifferent confluence of both plasma membranes takes place (fig. 5b). However, fixation of tissue in GA containing either ruthenium red (fig. 5c, circle) or tannic acid (fig. 5d, circle) demonstrates that mesenchymal cell projections exhibiting an outer diameter between 100 and 250 nm penetrate, as TNT, the basal lamina at the CD ampulla tip to form a special contact zone at the plasma membrane of epithelial stem/progenitor cells. It remains unclear whether changes in fixation cause different sizes of detected nanotubes.

High magnification in TEM illuminates the contact zone between mesenchymal cell projections and epithelial stem/progenitor cells after fixation in GA containing ruthenium red (fig. 6a, circle) or tannic acid (fig. 6b, circle). In both cases it can be recognized that mesenchymal cell projections penetrate the basal lamina at the tip of the CD ampulla. Most interestingly, the plasma membranes between mesenchymal and epithelial stem/progenitor cells do not fuse with each other. Instead, numerous and parallel lining TNT cross between both cells. Specimens fixed in GA containing ruthenium red show TNT with a mean diameter of 4.1 nm (fig. 6a, circle), while in samples fixed in GA containing tannic acid a diameter of 3.5 nm was measured (fig. 6b, circle).

Discussion

The epithelial-mesenchymal interface of the renal stem/progenitor cell niche is recognized after fixation by GA as a bright and inconspicuously looking area (fig. 3c, d, 4a, 5a). In contrast, the present investigation elucidates that fixation of embryonic parenchyma in GA including cupromeronic blue illustrates braces of proteoglycans covering projections of mesenchymal stem/progenitor cells while crossing the interstitial interface (fig. 4b, 5b). Fixation with GA containing either ruthenium red (fig. 4c, 5c) or tannic acid (fig. 4d, 5d) further shows abundant ECM, which was not visible after conventional fixation with GA (fig. 3c, d, 4a, 5a). Although

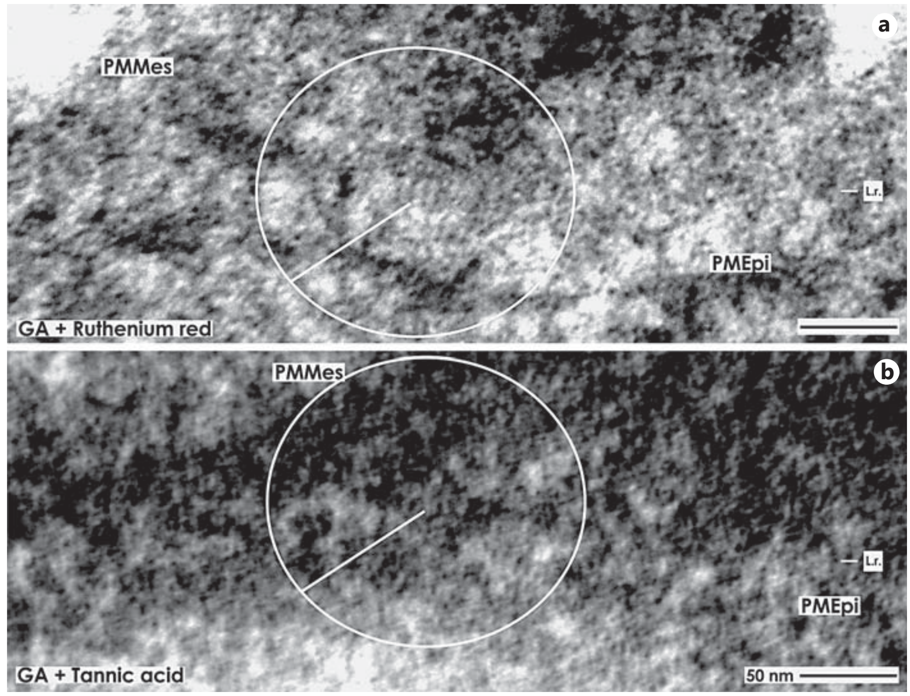

Fig. 6. High magnification in TEM illustrates TNT between mesenchymal and epithelial cells within the renal stem/progenitor cell niche. Related area is circled in figure 5. Fixation of specimens in GA containing either ruthenium red (**a**) or tannic acid (**b**) documents cell projections penetrating the basal lamina of a CD ampulla. At this position numerous parallel orientated TNT (top of pointer in circle) are found connecting the plasma membranes of mesenchymal (PMMes) and epithelial (PMEpi) stem/progenitor cells.

it cannot be defined at present which molecules are specifically labeled by cupromeronic blue, ruthenium red or tannic acid, the data elucidate a complex molecular composition and unexpected microarchitecture of the interstitial interface within the renal stem/progenitor cell niche.

During kidney development a program pilots the induction and consequently the formation of nephrons at the right time and in the right place. The site-specific process of induction is triggered by numerous reciprocal molecular interactions between the ureteric bud-derived epithelial stem/progenitor cells within the CD ampulla tip and the surrounding mesenchymal stem/progenitor cells found within the cap condensate [11, 20, 21]. It is assumed that the involved morphogenetic molecules are exchanged by diffusion. However, to keep these molecules concentrated one would expect that an always tight contact is maintained between epithelial and mesenchymal stem/progenitor cells. However, the actual data clearly exhibit that both cell populations are separated by an astonishingly wide interstitial interface seen after conventional fixation by GA (fig. 3c, d, 4a, 5a) but also after improved fixation by GA including either cupromeronic blue (fig. 4b, 5b), ruthenium red (fig. 4c, 5c) or tannic acid (fig. 4d, 5d).

In this study, special focus was pointed to projections of mesenchymal stem/progenitor cells crossing the interstitial interface. They show a varying thickness at their origin, whereas their endings show an outer diameter <100 nm as it was shown for expanding antlerogenic mesenchymal stem cells [22] and primary human renal epithelial cells [23]. In the course of cellular projections, gondolae within nanotubes were not found as it was described for cultured RT4 and T24 cells [24]. Thus, except gondolae, all of the demonstrated features show that projections of mesenchymal stem/progenitor cells have morphological similarities with TNT [25].

The endings of mesenchymal cell projections contact the basal lamina at the CD ampulla tip covering epithelial stem/progenitor cells (fig. 4, 5). In specimens fixed with conventional GA, only few mesenchymal cell projections were registered, which were touching the lamina fibroreticularis (fig. 3d, 4a, 5a). In contrast, fixation with GA including cupromeronic blue demonstrated that numerous cell projections penetrate all the layers of the basal lamina to end in the form of a cone at the plasma membrane of epithelial stem/progenitor cells (fig. 4b, 5b). High magnifications of specimens fixed by GA including ruthenium red (fig. 6a) or tannic acid (fig. 6b) illuminated that the plasma membranes of mesenchymal and epithelial stem/progenitor cells form a special contact at this site. Most interestingly, between cell projections of mesenchymal and epithelial cells, numerous parallel lining TNT were detected each exhibiting an average diameter between 3.5 and 4.1 nm. Regarding the exclusive occurrence at the interstitial interface [16], focusing on the presented results (fig. 6) or reflecting earlier findings made by Lehtonen [26], it appears most probable that illustrated mesenchymal cell projections are involved in the transmission of morphogenetic signals during nephron induction via TNT. Surprisingly, the ending of mesenchymal cell projections does not have any similarities with known hemidesmosomes [27], connexons [28] or focal adhesions [29]. Finally, analyzed mesenchymal cell projections are not identical with cytoplasmic processes found in earlier performed transfilter culture experiments [30, 31]. To induce tubulogenesis, isolated nephrogenic mesenchyme was kept in those cases with spinal cord and not with ureteric bud.

In conclusion, presented data illustrate that mesenchymal cell projections belong to regular structural cues crossing the interstitial interface within the renal stem/progenitor cell niche. These projections do not show classical fusion with the plasma membrane of epithelial stem/progenitor cells, but form specific TNT (fig. 6). As a consequence, investigations are in progress to elaborate if exchange of morphogenetic information occurs during nephron induction between mesenchymal and epithelial stem/progenitor cells not only by diffusion but also via illustrated projections.

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