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Structural links between the renal stem/progenitor cell niche and the organ capsule

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Abstract A special feature of the renal stem/progenitor cell niche is its always close neighborhood to the capsule during organ development. To explore this link, neonatal kidney was investigated by histochemistry and transmission electron microscopy. For adequate contrasting, fixation of specimens was performed by glutaraldehyde including tannic acid. The immunohistochemical data illustrate that renal stem/progenitor cells are not distributed randomly but are positioned specially to the capsule. Epithelial stem/progenitor cells are found to be enclosed by the basal lamina at a collecting duct (CD) ampulla tip. Only few layers of mesenchymal cells are detected between epithelial cells and the capsule. Most impressive, numerous microfibers reacting with soybean agglutinin, anti-collagen I and III originate from the basal lamina at a CD ampulla tip and line between mesenchymal stem/progenitor cells to the inner side of the capsule. This specific arrangement holds together both types of stem/progenitor cells in a cage and fastens the niche as a whole at the capsule. Electron microscopy further illustrates that the stem/progenitor cell niche is in contact with a tunnel system widely spreading between atypical smooth muscle cells at the inner side of the capsule. It seems probable that stem/progenitor cells are supplied here by interstitial fluid.

Keywords Kidney · Organ capsule · Stem/progenitor cell niche · Extracellular matrix · Electron microscopy · Tannic acid · Microfibers

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Introduction

When physiological functions are lost during an acute or chronic kidney disease, dialysis or transplantation of a donor organ are available therapeutic options (Li et al. 2013; Knoll 2013; Axelrod 2013; Watson et al. 2013). However, due to the lack of donors, certain limitations in dialysis and economical reasons, since several years the implantation of stem/progenitor cells is under debate as a possible alternative therapy to regenerate renal parenchyma (Caldas et al. 2011; Yokote and Yokoo 2012; Rak-Raszewska et al. 2012; McCampbell and Wingert 2012). Although this concept sounds convincing by the first view, the present data point out that intense research has still to be performed so that a series of biomedical obstacles can be removed (Iwatani and Imai 2013; Fanni et al. 2012; Sedrakyan et al. 2012). One of the unsolved problems is an up-to-date ineffective implantation technique at the site of kidney damage. Due to a harmful environment including inflammation, degraded extracellular matrix, toxic metabolites, inadequate nutrition and low oxygen, the survival and consequently seeding of stem/progenitor cells are limited (Burst et al. 2013; O'Brien and McMahon 2013). Thus, the strategy for the future is for example, to create for implanted stem/progenitor cells a stimulating artificial interstitium (Minuth et al. 2013). To learn about structural stability, critical physiological and biochemical signals sustaining cellular functions, one of the possibilities is to investigate the environment of the stem/progenitor cell niche (Little 2011; Hanein and Horwitz 2012; Wagers 2012; Brizzi et al. 2012; Pasquier and Rafii 2013).

Development of parenchyma takes place exclusively in the cortex corticis of the growing kidney and consequently in close vicinity to the inner side of the organ capsule (Iino et al. 2001; Faa et al. 2012; Piludu et al. 2012; Carroll and Das 2013; Yokote and Yokoo 2013). It may surprise,

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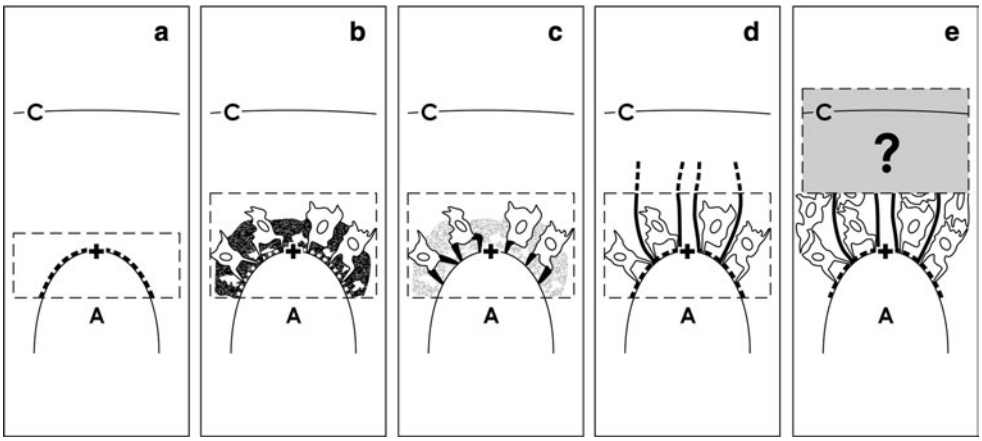

Fig. 1 Schematic illustration informs about stepwise exploration of structural links detected between the renal stem/progenitor cell niche and the organ capsule (*C*). **a** Epithelial stem/progenitor cells are covered at the CD ampulla (*A*) tip by a unique basal lamina (Strehl et al. 1997, 1999; Strehl and Minuth 2001). **b** Epithelial and mesenchymal stem/progenitor cells are separated by a special interstitial interface containing heterogeneously composed extracellular matrix (Minuth et al. 2011; Minuth and Denk 2012a, 2013). **c** Individual cell projections from mesenchymal cells cross the interstitial interface to penetrate the basal lamina at the CD ampulla to contact epithelial stem/progenitor cells via tunneling nanotubes (Minuth and Denk 2012b). **d** Microfibers labeled by SBA and anti-collagens II and IV originate from the basal aspect of a CD ampulla to line through the interstitial interface and the group of mesenchymal stem/progenitor cells (Schumacher et al. 2002a, b, 2003). **e** In the present investigation, structural links between the renal stem/progenitor cell niche and the capsule were analyzed

although at this special site capillary precursors are present, an intact microvascular system with circulating blood cells was not observed (Kloth et al. 1994, 1997).

Within the niche, epithelial and mesenchymal stem/progenitor cells are present. Epithelial cells are enclosed within an ureteric bud-derived CD ampulla tip, while mesenchymal cells are forming the surrounding cap condensate (Little and McMahon 2012; Chai et al. 2013). A reciprocal exchange of morphogenetic molecules between them leads to a condensation of part of mesenchymal cells resulting in a comma-shaped body as first sign of nephron formation.

Epithelial stem/progenitor cells occur within a CD ampulla tip that is covered by an individual basal lamina containing, for example, P_{CD} Amp I (Strehl et al. 1997, 1999; Strehl and Minuth 2001) and tissue transglutaminase (Tgase2) (Fig. 1a) (Schumacher et al. 2005). Between epithelial and mesenchymal stem/progenitor cells, an impressive interstitial interface is developed (Fig. 1b) (Minuth et al. 2011; Minuth and Denk 2012a). Improved contrasting of specimens by cupromeronic blue, ruthenium red and tannic acid demonstrates by the use of transmission electron microscopy that the extracellular matrix within the interstitial interface is not homogeneously composed but exhibits unique microheterogeneous features (Minuth and Denk 2012a, 2013). Further on, single projections from mesenchymal cells are crossing together with filigree strands of extracellular matrix the interstitial interface, penetrate the basal lamina to contact via tunneling nanotubes epithelial stem/progenitor cells (Fig. 1c) (Minuth and Denk 2012b). In an opposite direction, microfibers labeled by Soybean Agglutinin(SBA), anti-collagen II and IV rise out of the basal lamina and cross the interstitial interface (Fig. 1d) (Schumacher et al. 2002a, b, 2003). However, the way of microfibers spanning through the interstitium and the contact between the niche and the inner side of the capsule has not previously been adequately investigated (Fig. 1e).

An often largely disregarded feature of the renal stem/progenitor cell niche is their exact orientation throughout development of parenchyma. Mesenchymal stem/progenitor cells are persistently in contact with the inner side of the organ capsule, while epithelial stem/progenitor cells are orientated toward the medulla. They are connected in the neck of an ampulla with an elongating collecting duct.

Previous investigations further demonstrated that the capsule is not a simple envelop but an astonishingly structured tissue consisting at the outer side of a tunica fibrosa and at the inner side of a tunica muscularis (Möllendorf 1930). However, cells contained in the tunica muscularis are different as compared to typical smooth muscle cells (Bulger 1973; Kobayashi 1978). It may surprise, but recently it was shown that within the organ capsule, numerous stem/progenitor cells are harbored (Park et al. 2010).

Since actual data about the structural relationship between mesenchymal stem/progenitor cells and the inner side of the organ capsule are lacking, the present morphological investigation was performed. To explore microfibers spanning between the basal aspect of a CD ampulla and the organ capsule, histochemical experiments were made and

their spatial distribution was analyzed by confocal laser scanning microscopy. To obtain insights in ultrastructural features, transmission electron microscopy in combination with an improved contrasting technique with tannic acid was used. The present results show structural elements of extracellular matrix fastening the renal stem/progenitor cell niche with the inner side of the organ capsule. In the complimentary space between matrix and cells, an astonishingly spreading tunnel system for the exchange of interstitial fluid is present.

Materials and methods

Tissue preparation

To investigate structural elements between the renal stem/progenitor cell niche and the organ capsule, 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 surgically removed to process them for light and electron microscopy.

Histochemical labeling

Isolated kidneys were surrounded by TissueTek (OCT™Compound, Sakura Finetek, Zoeterwoude, The Netherlands) and frozen at −80 °C. To analyze cell biological features, 20-µm-thick cryosections were made, fixed in ice-cold ethanol, washed several times with phosphate-buffered saline (PBS) and incubated for 30 min with blocking solution (PBS, pH 7.5, 10 % horse serum from GIBCO/Invitrogen, Karlsruhe, Germany; 1 % bovine serum albumin from Serva, Heidelberg, Germany). For soybean agglutinin-labeling (SBA, Vector, Burlingame, USA), the samples were exposed to fluorescein isothiocyanate (FITC)-conjugated lectin diluted 1:1,000 in blocking solution for 45 min.

For immunohistochemical label of epithelial basal lamina, anti-agrin (clone 6D4, Developmental Studies Hybridoma Bank (DSHB), Iowa, USA) was 1:20 diluted in blocking solution, while anti-laminin γ1 (clone 3E10, Santa Cruz Biotechnology, Santa Cruz, USA) was used undiluted. Monoclonal anti-collagen I (Southern Biotech, Birmingham, USA) and polyclonal anti-collagen III (Southern Biotech, USA) had a concentration of 1:20, whereas monoclonal anti-collagen III (clone III-53, Calbiochem, Schwalbach, Germany) was diluted 1:250 in blocking solution. The antibodies were applied for 1 h. After washing with 1 % BSA in PBS, the specimens were incubated for 45 min with goat anti-rat-IgG-rhodamine, donkey anti-goat-IgG-fluorescein isothiocyanate, respectively, donkey anti-mouse-IgG-fluorescein isothiocyanate (Jackson Immunoresearch Laboratories, West Grove, USA) diluted 1:50 in PBS containing 1 % BSA.

Further individual samples labeled with anti-collagen III (clone III-53) were co-incubated with 4′,6-diamino-2-phenylindole dihydrochloride (DAPI, Sigma-Aldrich Chemie, München, Germany) in a concentration of 1:10,000 in PBS.

Following several washes with PBS, the sections were embedded with Slow Fade Light Antifade Kit (Molecular Probes, Eugene, USA) and then analyzed using either an Axioskop 2 plus microscope (Zeiss, Oberkochen, Germany) or a CM12 confocal laser scanning microscope (Zeiss). Fluorescence images were taken with a digital camera at a standard exposure time and thereafter processed with Corel DRAW Graphic Suite X5 (Corel Corporation, Ottawa, Canada).

Transmission electron microscopy

In the present investigation, specimens were analyzed after conventional fixation in glutaraldehyde (GA) and after improved contrasting by GA solution including tannic acid as it was earlier demonstrated (Minuth et al. 2011; Minuth and Denk 2013; Hasko and Richardson 1988; Rothenburger et al. 2002).

For fixation of tissue, the following solutions were used:

1. Specimens for control: 5 % GA (Serva) buffered with 0.15 M sodium cacodylate, pH 7.4.
2. Specimens for improved contrast with tannic acid: 5 % GA buffered with 0.15 M sodium cacodylate, pH 7.4 + 1 % tannic acid (Sigma-Aldrich Chemie).

The period of primary fixation was for 1 day at room temperature. After several washes with 0.15 M sodium cacodylate, the samples were postfixed in the same buffer but additionally containing 1 % osmium tetroxide (Science Services, München, Germany). Then, the tissue was washed with sodium cacodylate buffer and dehydrated in graded series of ethanols.

Finally, the specimens were embedded in Epon (Fluka, Taufkirchen, Germany), which was polymerized at 60 °C for 48 h. Semithin and ultrathin sections were made with a diamond knife on an ultramicrotome EM UC6 (Leica, Wetzlar, Germany).

Semithin sections were labeled with Richardson staining and analyzed with a Leica DM 750 microscope (Leica). Images were taken with a digital camera and thereafter processed with Corel DRAW Graphic Suite X5 (Corel Corporation).

Ultrathin sections were collected onto slot grids coated with 1.5 % Pioloform (Plano, Wetzlar, Germany) and contrasted using 2 % uranyl acetate and lead citrate as earlier described (Minuth et al. 2011; Minuth and Denk 2013).

Analysis of them was performed at 80 kV using an EM 902 transmission electron microscope (Zeiss).

Amount of analyzed specimens

A total of 54 exactly orientated renal stem cell niches were analyzed for the present study. Performed experiments are in accordance with the Animal Ethics Committee, University of Regensburg, Regensburg, Germany.

Definition of cells contained in the renal stem/progenitor cell niche

In this paper, the embryonic cortex of human fetal and neonatal rabbit kidney was described. In consequence, the nomenclature of previously published papers was used (Minuth et al. 2011; Minuth and Denk 2013).

Results

Development of renal parenchyma depends on an exact temporal–spatial formation of nephrons initiated by numerous reciprocal morphogenetic molecular interactions within the stem/progenitor cell niche (Faa et al. 2012; Carroll and Das 2013; Chai et al. 2013). Beginning with the organ anlage and up to the end of the neonatal phase, the niches are not randomly distributed but are always found in close neighborhood to the expanding organ capsule.

Light microscopy of the renal stem/progenitor cell niche

To obtain a comparable view to both the stem/progenitor cell niche and the neighboring organ capsule, each kidney was first divided between both poles (Fig. 2a). Following this strategy, the parenchyma is orientated along the

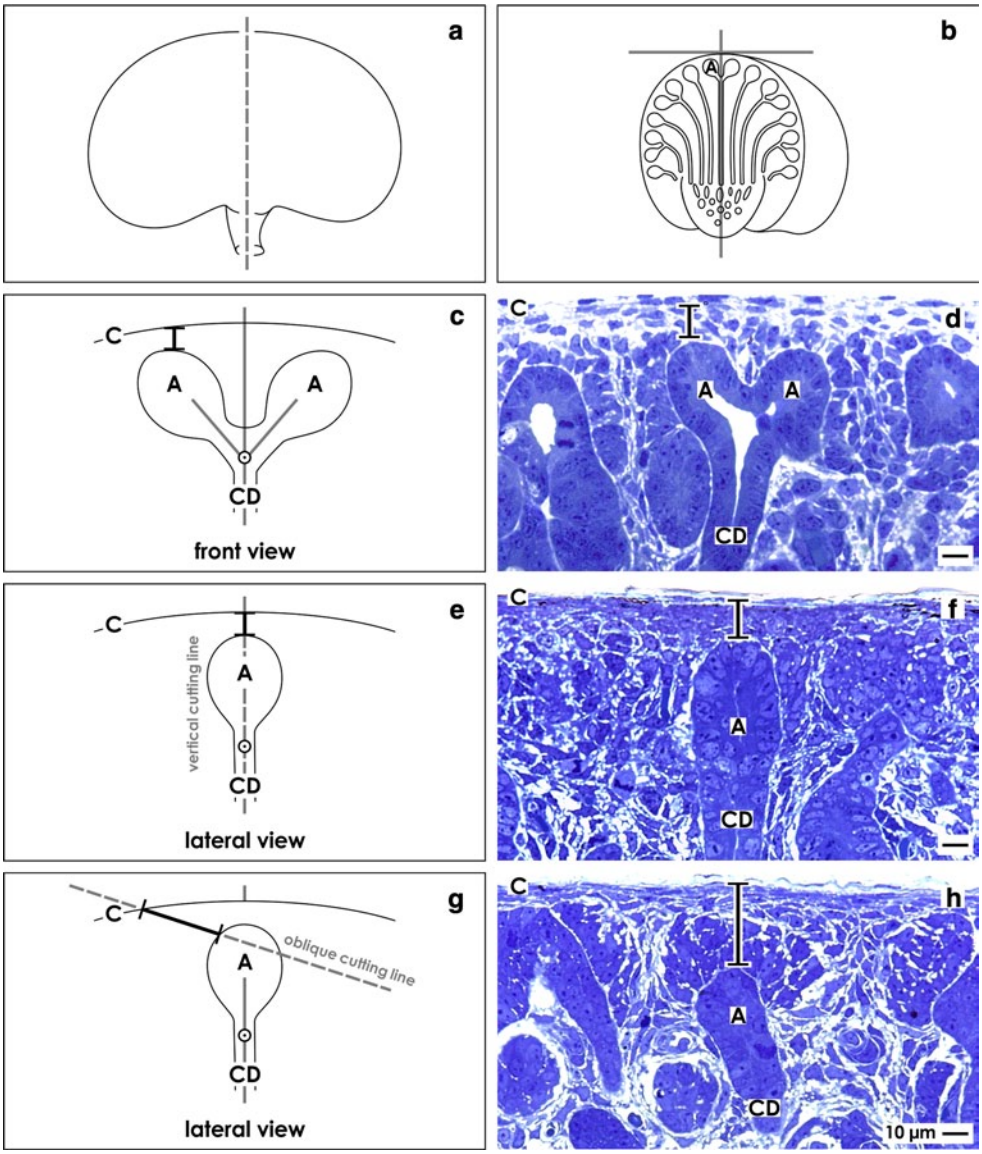

Fig. 2 Exact orientation of developing kidney is a need to obtain comparable views to the stem/progenitor cell niche. **a** After fixation, the kidney is first cut between both poles. **b** As a result, the section plane shows straightly lining collecting ducts ending beyond the organ capsule as an ureteric bud-derived ampulla (*A*). **c**, **d** When an orientated section is analyzed, the basal aspect of a dichotomously arborizing collecting duct (*CD*) ampulla shows a distance of 14–16 µm to the outer side of the organ capsule. In this case, mesenchymal stem/progenitor cells show two cell layers. **e**, **f** The same distance and the same number of mesenchymal cell layers are registered, when a *CD* ampulla is seen in a *lateral view*. **g**, **h** In contrast, when a section is not orientated along lining collecting ducts, an oblique cutting plane is obtained. In this case, the distance between a *CD* ampulla and the capsule is more than 20 µm so that yet multiple layers of mesenchymal cells can be seen as the cap condensate

straight cortico-medullary course of lining collecting ducts (CD; Fig. 2b). Now, a correct view to the renal stem/progenitor cell niche in relation to the covering organ capsule (C) is possible (Fig. 2c). As a consequence, all of the here demonstrated micrographs show this perspective so that comparisons between different experimental series become possible.

Light microscopy shows on a semithin section that epithelial stem/progenitor cells are enclosed within dichotomously dividing tips of a ureteric bud-derived CD ampulla (A; Fig. 2d), while mesenchymal stem/progenitor cells form the surrounding cap condensate. Astonishingly, both stem/progenitor cell populations do not stand closely together but are separated by a striking interstitial interface. In a lateral view, a single CD ampulla tip is illustrated (Fig. 2e, f). The individual distance of the basal aspect of a CD ampulla to the outer side of the organ capsule is as well in front as in lateral views between 14 and 16 µm (Fig. 2c–f).

An example illustrates that incorrect orientation of a sample is leading to an oblique cutting line and a shifting in perspective (Fig. 2g, h). Such a peripheric section through the CD ampulla tip demonstrates that yet the distance between its basal aspect and the outer side of the organ capsule is more than 20 µm. Astonishingly, only by an oblique cutting, the extension of the multilayered cap condensate containing mesenchymal stem/progenitor cells is visible (Fig. 2h). In contrast, on an exact vertical section can be seen that the mesenchymal cap condensate consists of only two layers of cells (Fig. 2d, f).

Histochemical view to structural links between the niche and capsule

A surface view in light microscopy further illustrates that epithelial and mesenchymal stem/progenitor cells do not contact each other but are separated by an interstitial interface lining in parallel to the basal lamina of a CD ampulla tip (Fig. 2d, f, h). The aim of next experiments was to investigate by histochemistry and laser scanning microscopy on orientated sections extracellular matrix molecules occurring between epithelial, mesenchymal stem/progenitor cells and the organ capsule.

Agrin label is detected in form of a punctuate pattern bordering the basal aspect at the CD ampulla tip (Fig. 3a). Only a faint punctual label of agrin is found between neighboring mesenchymal stem/progenitor cells. Label for laminin γ1 is seen as a punctuate pattern at the basal aspect and within the lumen of a CD ampulla tip (Fig. 3b). Interestingly, parts of the inner side of the organ capsule show an intense but punctual label, while between mesenchymal stem/progenitor cells only faint label for laminin γ1 is observed.

Strong label for SBA is recognized along the basal aspect of a CD ampulla (Fig. 3c). In addition, microfibers labeled by SBA are lining from this basal aspect to the inner side of the organ capsule. Similar to SBA, label intense reaction for collagen I was detected along the basal aspect of a CD ampulla tip (Fig. 3d). Further intensively labeled microfibers originate from the basal aspect of a CD ampulla tip and to the organ capsule. Label for collagen III was investigated as well by a polyclonal (Fig. 3e) as by a monoclonal (Fig. 3f) antibody. Both antibodies exhibit intense label at the basal aspect of a CD ampulla tip and on numerous microfibers spanning toward the organ capsule. Thus, as well label for agrin (Fig. 3a) as laminin γ1 (Fig. 3b) illustrates that the basal aspect of a CD ampulla tip is a demarcation line between epithelial and mesenchymal stem/progenitor cells. In contrast, label for SBA (Fig. 3c), collagen I (Fig. 3d) and collagen III (Fig. 3e, f) shows that intensively labeled microfibers originate from the basal aspect of a CD ampulla tip and line to the inner side of the organ capsule. These results demonstrate that structural elements of the renal stem/progenitor cell niche are linked with the inner side of the organ capsule.

Co-label for DAPI and collagen III illustrates that microfibers are not found within epithelial stem/progenitor cells of a CD ampulla tip, but line exclusively between labeled nuclei of mesenchymal stem progenitor cells to end at the inner side of the organ capsule (Fig. 3g, h). Thus, the present results clearly depict that renal stem/progenitor cells are not accidentally clustering but are contained in an unexpectedly structured cage consisting of numerous microfibers. These appear as structural elements spanning between the basal aspect of a CD ampulla tip, the group of mesenchymal stem/progenitor cells up to the inner side of the organ capsule.

Focus to the contact between the niche and capsule

In a first set of experiments, specimens were traditionally fixed with glutaraldehyde (GA) and analyzed on orientated sections by transmission electron microscopy (Fig. 4a). The outer side of the organ capsule contains flat fibroblasts but does not show high amounts of collagen fibers. Screening of the space between the middle and the inner side of the organ capsule and the basal aspect of a CD ampulla tip shows that this special area is heterogenously composed and can roughly be divided into different zones. Zones I–III are belonging to the genuine organ capsule, while zone IV harbors mesenchymal stem/progenitor cells. Zone V represents the interstitial interface separating epithelial from mesenchymal stem/progenitor cells.

Higher magnification of specimens fixed in GA illustrates that the organ capsule can be split in a tunica fibrosa and a tunica muscularis (Fig. 4b). Thus, zone I shows the

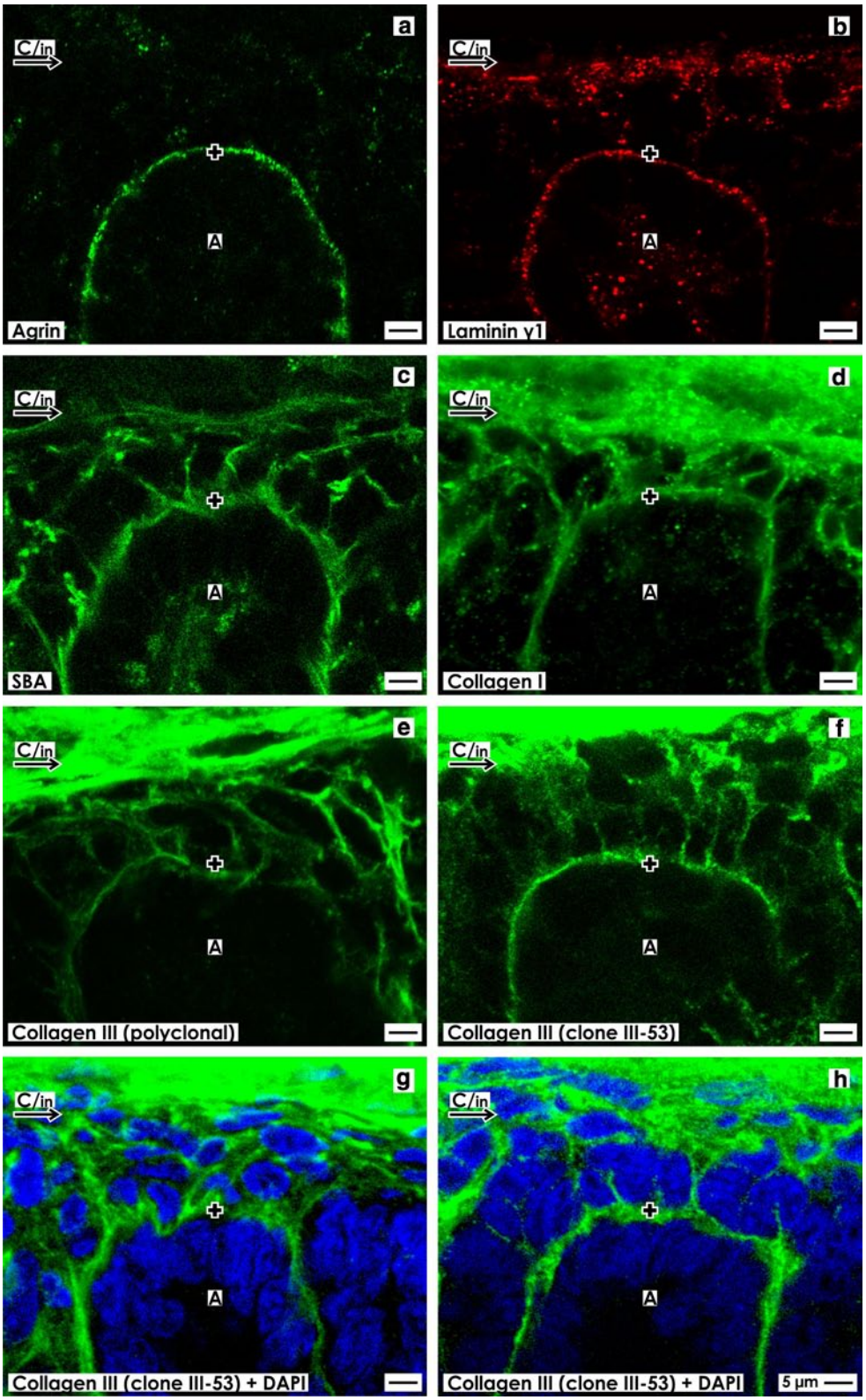

Fig. 3 Fluorescence microscopy on orientated sections of embryonic parenchyma to analyze structural links between the renal stem/progenitor cell niche and the inner side of the organ capsule (*C/in*). In each micrograph, the basal aspect of a CD ampulla (*A*) is labeled by a (*cross*). Label for **a** *agrin* and **b** *laminin γ1* illustrates the presence of a basal lamina at the tip of a CD ampulla. Label for **c** SBA, **d** *collagen I* and **e**, **f** *collagen III* shows that microfibers originate from the basal lamina of a CD ampulla to line to the inner side of the organ capsule. **g**, **h** Co-label for collagen III and DAPI illustrates that the microfibers are lining between mesenchymal stem/progenitor cells before they reach the inner side of the organ capsule

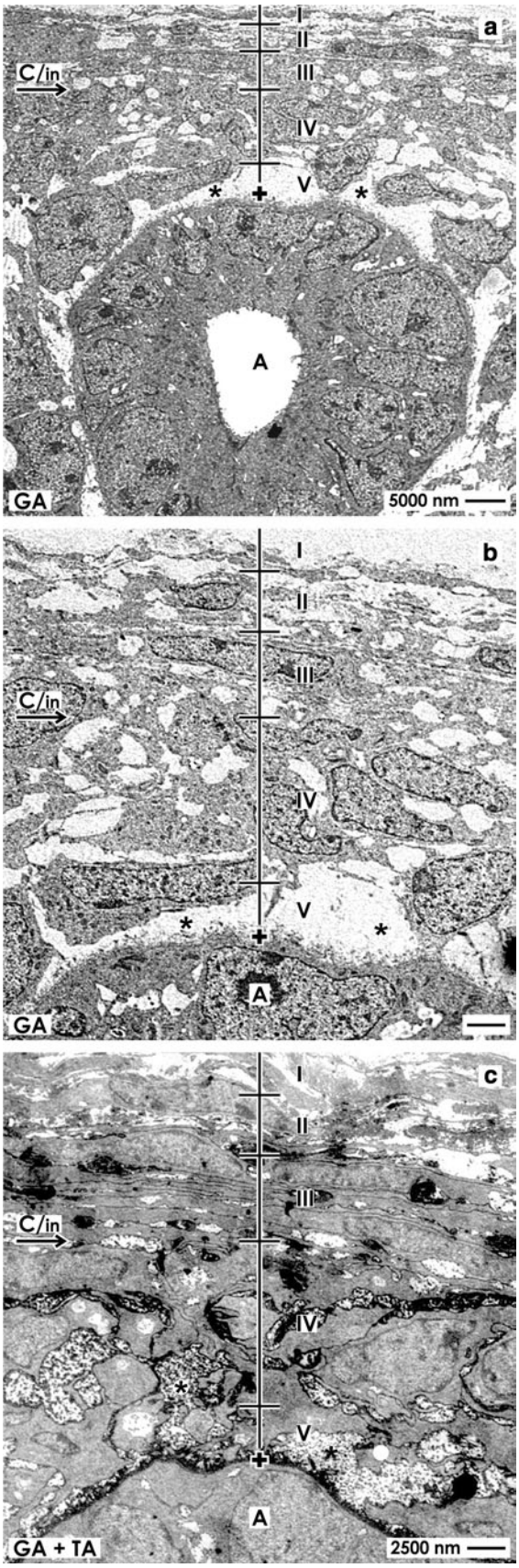

Fig. 4 Transmission electron microscopy of the renal stem/progenitor cell niche and the organ capsule of specimens fixed with glutaraldehyde (*GA*) or GA including tannic acid. **a** Low magnification of specimens fixed by traditional GA depicts that epithelial stem/progenitor cells are enclosed by the basal lamina (*cross*) at a CD ampulla (*A*) tip. Mesenchymal stem/progenitor cells are separated from epithelial cells by a bright interstitial interface. **b** Higher magnification shows that the interstitium between mesenchymal stem/progenitor cells is bright. **c** In contrast, specimens fixed by GA including tannic acid show that the basal lamina at a CD ampulla, the interstitial interface (*asterisk*) and single structures at the inner side of the organ capsule (*C/in*) are darkly labeled. Further on, the overviews exhibit that the space between the outer side of the organ capsule and the basal lamina at a CD ampulla tip can be divided into five zones (*I–V*). *Zones I* represents the outer side of the tunica fibrosa, *zone II* shows the inner side of the tunica fibrosa, while *zone III* belongs to the tunica muscularis. *Zone IV* harbors mesenchymal stem/progenitor cells, while *zone V* shows the interstitial interface around the basal aspect of a CD ampulla

outer side of the tunica fibrosa. Here, flat fibroblasts with even flat nuclei and extravagant cell projections are found. Zone II demarks the inner side of the tunica fibrosa. In this layer, the volume of cells is increased as compared to zone I. The nuclei of these cells are oval, but their endings are asymetrically formed. Zone III depicts the tunica muscularis and belongs to the inner side of the genuine organ capsule. As compared to zones I and II, the volume of cells and nuclei is here enlarged. The nuclei show round endings on both sides. Further long cell projections are interdigitating with each other. In contrast, in zone IV, cells become polymorph and their volume is increased as compared to zones I–III. The here contained cells represent mesenchymal stem/progenitor cells. Via numerous filigree projections, they are connected with each other. Between the cells, an extended but bright interstitial space can be observed. Zone V shows the interstitial interface, where single projections from mesenchymal stem/progenitor cells cross the interstitial interface to contact the basal lamina at a CD ampulla tip. The interstitial space between the organ capsule and the CD ampulla tip appears at all sites bright but more or less unremarkable (Fig. 4a, b).

Fixation of specimens by glutaraldehyde including tannic acid

In contrast, when fixation of specimens was performed by GA including tannic acid (Fig. 4c), a completely different view is obtained as compared to traditional fixation with GA (Fig. 4a, b). By a first screening, it is recognized that the impregnation by tannic acid unpacks structures on cells and extracellular matrix, which were not earlier observed. For detailed investigation, specimens were analyzed under high (Fig. 5) and very high magnification (Fig. 6).

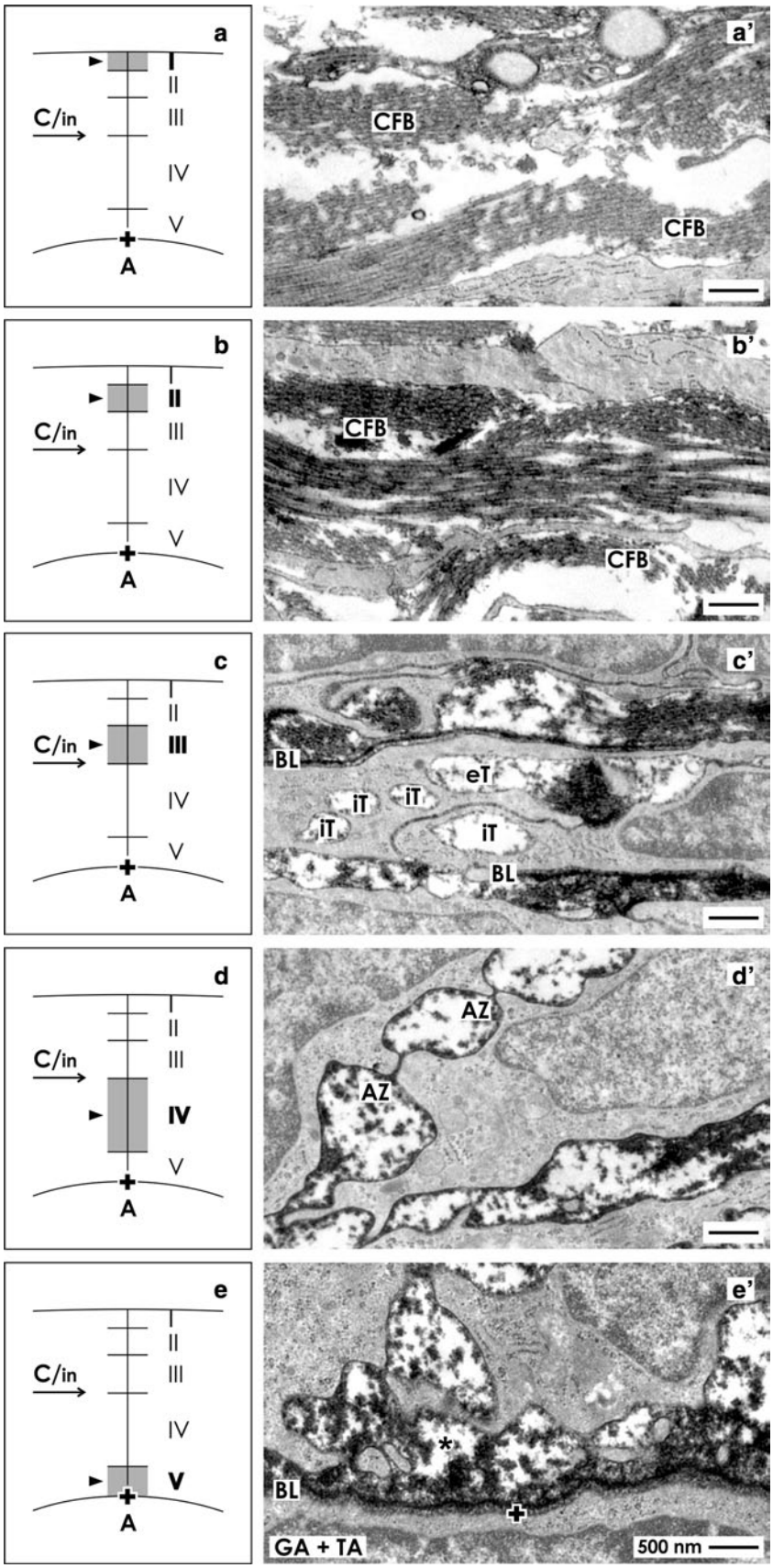

Fig. 5 Transmission electron microscopy shows structural features between the renal stem/progenitor cell niche and the organ capsule after fixation of specimens in GA including tannic acid. **a** In *zone I* (**a**′), flat fibroblasts and collagen fiber bundles (*CFB*) are found, which are not labeled by tannic acid. **b** In contrast, in *zone II* (**b**′), collagen fiber bundles occur, which are intensively labeled by tannic acid. **c** *Zone III* (**c**′) shows cells that exhibit numerous projections interdigitating with each other. Further, at this site, intra- (*iT*) and extra-cellular tunnels (*eT*) are developed. Part of cells is covered by an intense basal lamina (*BL*). The here contained cell type resembles earlier described atypical smooth muscle cells. **d** In *zone IV* (**d**′), cells occur, which stand in distinct distance to each other. Short cell protrusions form specific adhesion zones (*AZ*) between each other. These mesenchymal stem/progenitor cells can be further recognized by an intense glycocalix. **e** In *zone V* (**e**′), typical features of the interstitial interface (*asterisk*) can be seen separating mesenchymal from epithelial stem/progenitor cells. Cell projections from mesenchymal cells and tiny strands of extracellular matrix cross the interface to contact the basal lamina at a CD ampulla tip. *C/in* inner side of the organ capsule, *cross* marks the basal aspect of a CD ampulla

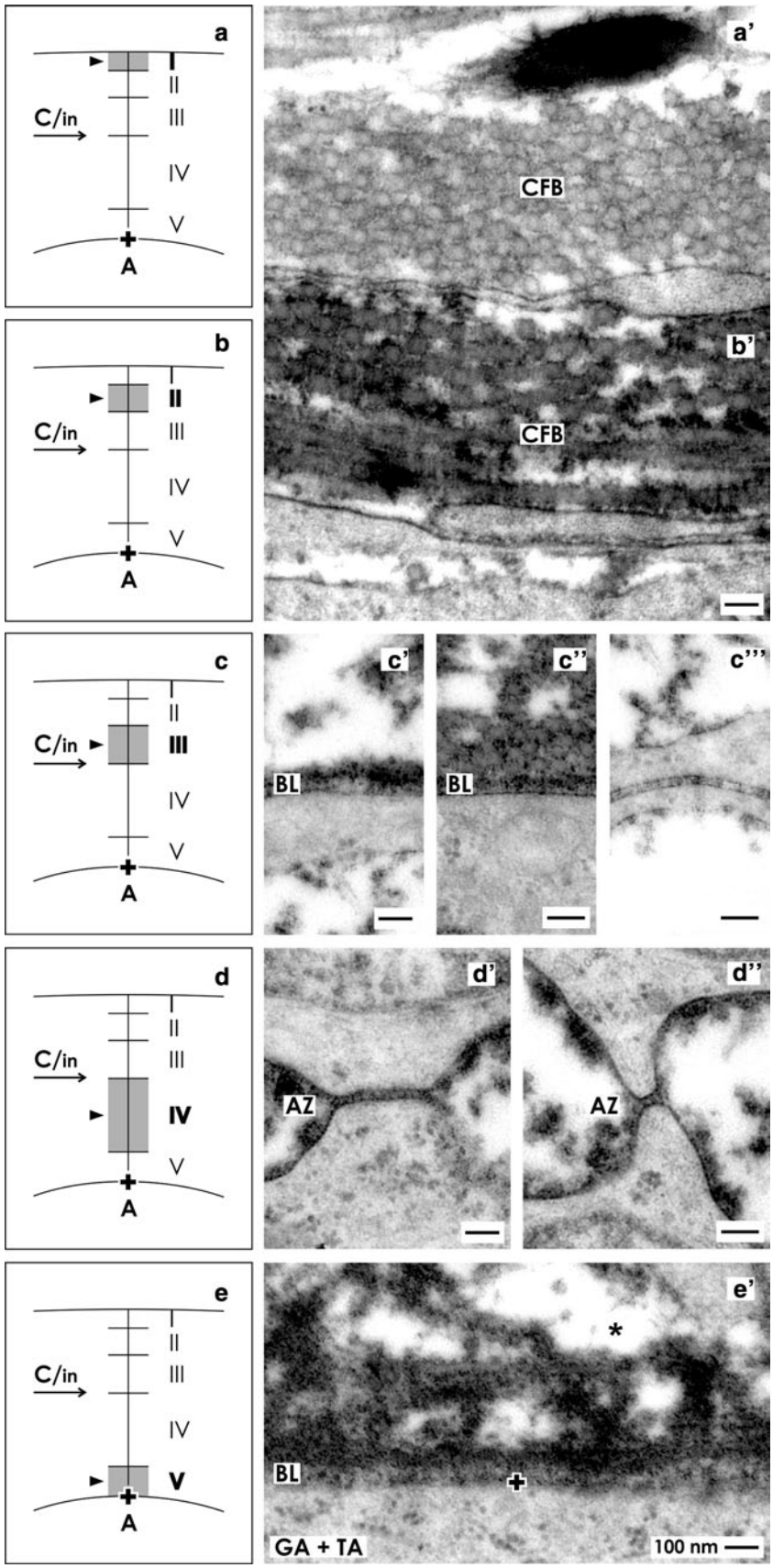

Fig. 6 Structural features between the renal stem/progenitor cell niche and the organ capsule. Specimens were fixed in GA including tannic acid and analyzed by transmission electron microscopy under high magnification. **a** Typical for *zone I* and **b** *II* are numerous collagen fiber bundles (*CFB*). Most impressive, on the same section can be seen that in *zone I* (**a**′) the tunica fibrosa shows bright collagen fiber bundles, while in *zone II* (**b**′) darkly labeled collagen fiber bundles are observed. **c** Typical for the tunica muscularis in *zone III* are atypical smooth muscle cells. **c**′ On many sites, these cells are covered by a basal lamina (*BL*). **c**″ In some cases, collagen fiber bundles are attached to the basal lamina. **c**‴ Further cell projections with a faint glycocalix are frequently observed contacting each other. **d** Typical for *zone IV* is the occurrence of mesenchymal stem/progenitor cells. **d**′ and **d**″ They exhibit short cell projections forming with neighboring cells adhesion zones (*AZ*). The cells are covered by a dense but irregular glycocalix. Between the cells, a surprisingly wide interstitium is present. **e** Typical for *zone V* is the interstitial interface (*asterisk*) separating mesenchymal an epithelial stem/progenitor cells. **e**′ It can be recognized by an intense label on the basal lamina (*BL*) of a CD ampulla enclosing epithelial stem/progenitor cells. Within the interstitial space, single cell projections from mesenchymal cells and filigree strands of extracellular matrix are noticeable. *C/in* inner side of the organ capsule, *cross* marks the basal aspect of a CD ampulla

Focus was first directed to the outer layer of the organ capsule. Zone I represents the outer side of the tunica fibrosa (Figs. 5a, 6a). Here, numerous collagen fiber bundles (CFB) are present (Figs. 5a′, 6a′). The micrograph can be further seen that collagen fiber bundles are bright. Cross-sections reveal that each of these fibers shows a punctuate pattern on its surface labeled by tannic acid. Between the collagen fiber bundles, single fibroblasts with long but tiny cell projections are present.

Zone II illustrates the inner side of the tunica fibrosa (Figs. 5b, 6b), where single fibroblasts with numerous and extended projections can be seen (Figs. 5b′, 6b′). In close neighborhood of them, numerous collagen fiber bundles are present. However, as compared to collagen fiber bundles in zone I (Figs. 5a′, 6a′), the here contained ones are darkly labeled by tannic acid. This distinct difference can be demonstrated as well on separate sections (Fig. 5a′, b′) as on the same section (Fig. 6a′, b′).

Zone III (Figs. 5c, 6c) belongs to the tunica muscularis and consequently to the inner side of the organ capsule. Here, elongated cells are found, whose numerous projections interdigitate with each other. In earlier investigations, they were described as atypical smooth muscle cells (Fig. 5c′) (Bulger 1973; Kobayashi 1978). The cell body of these cells is covered by a basal lamina (BL), which is intensively label by tannic acid (Fig. 5c′). It consists of a clearly recognizable lamina rara and lamina densa (Fig. 6c′). Part of the numerous cell projections is showing on all sides the basal lamina, while in other cases, only one side is covered. On special sites, the basal lamina is in contact with intensively labeled collagen fiber bundles (Fig. 6c″). In some cases, contacting cell projections do not have a covering basal lamina but exhibit a tiny glycocalyx (Fig. 6c‴). Regarding the numerous cell projections, it appears that the space between belongs to an extended intra- (iT) respectively extracellular tunnel (eT) system probably involved in the exchange of interstitial fluid (Fig. 5c′).

Zone IV (Figs. 5d, 6d) is the layer, where mesenchymal stem/progenitor cells are localized. These cells show an intense but not consistently developed glycocalyx (Figs. 5d′, 6d′, d″). In irregular distances, unique adhesion zones (AZ) between neighboring cells are formed. It cannot be recognized if the adhesion zones are focal or if they are extended in length by microfolds of the plasma membrane. It appears that between connected cell projections, a remarkable extracellular tunnel system is present (Fig. 6d′, d″).

Zone V (Figs. 5e, 6e) represents the interstitial interface between mesenchymal and epithelial stem/progenitor cells. This site is bordered by a concrete basal lamina at the CD ampulla tip enclosing epithelial stem/progenitor cells (Figs. 5e′, 6e′). Individual projections of mesenchymal stem/progenitor cells cross here together with tiny strands of extracellular matrix the interstitial interface to contact the basal lamina at a CD ampulla tip as it was described earlier (Minuth and Denk 2012b).

Discussion

Recent publications have shown that the renal stem/progenitor cell niche is not an accidental accumulation of stem/progenitor cells but exhibits an unexpected complex microarchitecture (Minuth et al. 2011; Minuth and Denk 2012a, b, 2013; Carroll and Das 2013). Since microvessels are not developed at this site, provision with nutrition and oxygen is restricted. Consequently, contained cells appear both to be survival artists and performance specialists fulfilling pleiotropic tasks to maintain stemness, proliferation and differentiation (Faa et al. 2012; Carroll and Das 2013).

Coherence of the renal stem/progenitor cell niche

A special feature is that throughout kidney growth, the niche stays in the outer cortex and in close contact with the inner side of the organ capsule (Minuth and Denk 2012b). Laser scanning microscopy illustrates that the basal lamina covering epithelial stem/progenitor cells at the CD ampulla tip reflects a demarcation line, which can be visualized by agrin (Fig. 3a) or laminin γ1 (Fig. 3b). At this site, microfibers originate, which are labeled by SBA (Fig. 3c), anti-collagen I (Fig. 3d) and anti-collagen III (Fig. 3e, f). Co-label with DAPI further illustrates that microfibers cross the interstitial interface, line through the group of mesenchymal cells to end at the inner side of the capsule (Fig. 3g, h). This result shows that microfibers exists forming between the basal aspect of a CD ampulla, the group of mesenchymal cells and the inner side of organ capsule a spatial fiber cage (Fig. 7a).

Complex histoarchitecture between the niche and capsule

To obtain more information, transmission electron microscopy was performed (Figs. 4, 5, 6). Generally, fixation is performed by traditional glutaraldehyde (GA) solution. Screening from the outer side of the capsule toward a CD ampulla elucidates that the interstitial space between cells looks inconspicuously (Fig. 4a, b).

In contrast, when fixation of specimens is performed in GA containing tannic acid, extracellular matrix is unpacked and new structural details within the interstitium and on cell surfaces become visible (Fig. 4c). According to these new data, the space between the renal stem/progenitor cell niche and the outer side of the organ capsule can be split into five different zones (Figs. 4, 5, 6, 7):

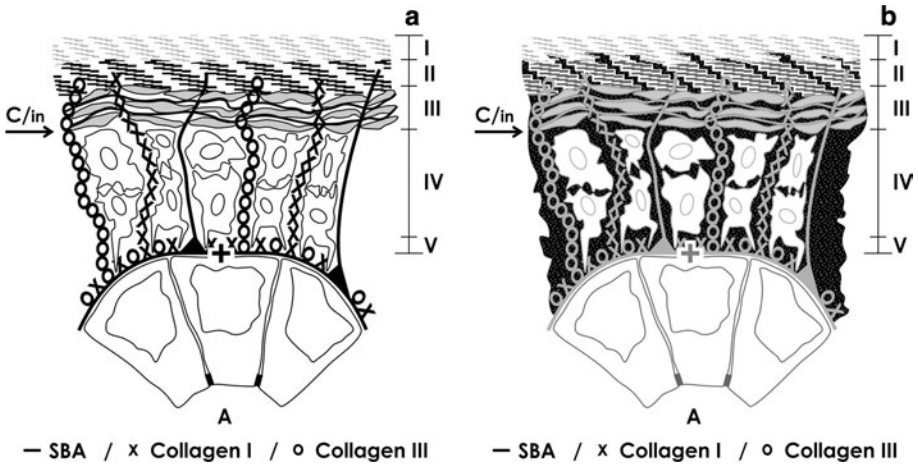

Fig. 7 Schematic illustration depicts structural features between the renal stem/progenitor cell niche and the organ capsule. **a** According to present findings, this area can be divided into five zones. *Zone I* is the outer side of the tunica fibrosa containing flat fibroblasts and bright collagen fiber bundles. *Zone II* represents the inner side of the tunica fibrosa. At this site, collagen fiber bundles are detected that are intensively labeled by tannic acid. In *zone III*, atypical smooth muscle cells are contained. This layer is the inner border of the organ capsule (*C/in*). In *zone IV*, two layers of mesenchymal stem/progenitor cells are found. *Zone V* represents the interstitial interface separating mesenchymal from epithelial stem/progenitor cells enclosed within a CD ampulla (*A*). The basal lamina at an ampulla tip is labeled by a *cross*. Histochemistry reveals that microfibers labeled by SBA, anti-collagen I and III originate at the basal lamina of a CD ampulla to cross the interstitial interface, to line through the group of mesenchymal stem/progenitor cells and atypical smooth muscle cells to end between the light and darkly labeled collagen fiber bundles of the capsule. **b** Electron microscopy further demonstrates that beside filigree extracellular matrix, a widely spreading space (colored in *black*) for interstitial fluid exists obviously providing contained cells with nutrition and respiratory gas

- Zone I: Outer side of the tunica fibrosa.
- Zone II: Inner side of the tunica fibrosa.
- Zone III: Tunica muscularis with atypical smooth muscle cells.
- Zone IV: Layer of mesenchymal stem/progenitor cells.
- Zone V: Interstitial interface around the basal aspect of a CD ampulla tip containing epithelial stem/progenitor cells.

Electron microscopy further illuminates that tannic acid label is not homogeneously dispersed but very individually distributed. On the same section can be seen that collagen fiber bundles in zone I (Fig. 6a) do not exhibit label (Fig. 6a′), while bundles in zone II (Fig. 6b) show intense label (Fig. 6b′). In between, bright areas of the interstitium and non-labeled projections from single cells can be seen. These differences in label speak for a high quality of fixation by glutaraldehyde in combination with tannic acid.

Most interesting is zone III reflecting the tunica muscularis (Figs. 5c, 6c). In this layer, earlier described atypical smooth muscle cells are contained (Figs. 5c′, 6c′–c‴) (Bulger 1973; Kobayashi 1978). Although cells lack abundance of contractile microfilaments, they can be recognized by a consistently developed basal lamina (Figs. 5c′, 6c′). On distinct sites, tannic acid-positive collagen fibers are attached to the basal lamina (Fig. 6c″). Further numerous cell projections are present contacting each other. In some of the cases, cell projections are not covered by a basal lamina but exhibit a faint glycocalix (Fig. 6c‴). The numerous interdigitating cell projections form a complex tunnel system, which may direct the flow of interstitial fluid providing cells in the niche with nutrition, respiratory gas and morphogenetic molecules.

Zone IV (Figs. 5d, 6d) can be easily recognized by an increased volume of cells and a wide but unlabeled interstitial space between. Each of the contained mesenchymal stem/progenitor cells is covered by an irregular glycocalyx (Figs. 5d′, 6d′, d″). Further, short but broad cell projections from neighboring cells contact each other to form a clearly recognizable and structured adhesion zone. However, it cannot be recognized, if cells form here atypical tight junctions or even gap junctions.

Zone V (Figs. 5e, 6e) represents the interstitial interface of the renal stem/progenitor cell niche. At this site, individual projections of mesenchymal stem/progenitor cells cross together with tiny strands of extracellular matrix the interface to contact the basal lamina at a CD ampulla tip (Figs. 5e′, 6e′). Tannic acid label shows within the basal lamina a punctuate lamina rara and a dark ribbon comprising the lamina densa and lamina fibroreticularis (Minuth and Denk 2012a).

In conclusion, the results demonstrate that the renal stem/progenitor cell niche exhibits a special microenvironment and a structural link with the organ capsule (Fig. 7).

In this scenario, epithelial stem/progenitor cells are enclosed by the basal lamina at the CD ampulla tip, while mesenchymal cells are harbored in a cage consisting of microfibers labeled by earlier detected SBA, anti-collagen II and IV (Schumacher et al. 2003) and presently detected anti-collagen I and III (Fig. 3d–f). The fibers are spanning from the basal aspect of a CD ampulla tip, cross the interstitial interface and the group of mesenchymal stem/progenitor cells to end at the inner side of the organ capsule (Fig. 7a). This specific arrangement declares the permanent proximity of the renal stem/progenitor cell niche to the capsule throughout organ development. It appears further that the renal stem/progenitor cell niche is in contact with an extended tunnel system (Fig. 7b) spreading between atypical smooth muscle cells (Fig. 5c′; Zone III) and mesenchymal stem/progenitor cells (Fig. 5d′; Zone IV). These tunnels may be connected to special vessels within the organ capsule as it was earlier described for the human kidney (Hammersen and Staubesand 1961). It might be the spring providing the renal stem/progenitor cell niche at the side of mesenchymal cells with interstitial fluid distributing necessary nutrition, respiratory gas and morphogenetic molecules.

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