Histochem Cell Biol (2011) 136:321–334
DOI 10.1007/s00418-011-0851-x

ORIGINAL PAPER

Peculiarities of the extracellular matrix in the interstitium of the renal stem/progenitor cell niche

Will W. Minuth · Lucia Denk · Christian Miess · Anne Glashauser

Accepted: 21 July 2011 / Published online: 6 August 2011

Abstract The development of the nephron is piloted by interactions between epithelial and surrounding mesenchymal stem/progenitor cells. Data show that an astonishingly wide interstitial space separates both kinds of stem/progenitor cells. A simple contrasting procedure was applied to visualize features that keep renal epithelial and mesenchymal stem/progenitor cells in distance. The kidney of neonatal rabbits was fixed in solutions containing glutaraldehyde (GA) in combination with alcian blue, lanthanum, ruthenium red, or tannic acid. To obtain a comparable view to the renal stem/progenitor cell niche, the tissue was exactly orientated along the axis of collecting ducts. Fixation with GA or in combination with alcian blue or lanthanum revealed an inconspicuous interstitial space. In contrast, fixation with GA containing ruthenium red exhibits strands of extracellular matrix lining from epithelial stem/progenitor cells through the interstitium up to the surface of mesenchymal stem/progenitor cells. Fixation with GA containing tannic acid shows that the basal lamina of epithelial stem/progenitor cells, the adjacent interstitial space and also the surface of mesenchymal stem/progenitor cells are connected over a net of extracellular matrix. The applied technique appears to be a suitable method to illuminate the interstitium in stem/progenitor cell niches of specialized tissues, the microenvironment of tumors and extension of degeneration.

Keywords Kidney · Stem/progenitor cell niche · Extracellular matrix · Interstitium · Electron microscopy

W. W. Minuth (✉) · L. Denk · C. Miess · A. Glashauser
Department of Molecular and Cellular Anatomy, University of Regensburg, University Street 31, 93053 Regensburg, Germany
e-mail: will.minuth@vkl.uni-regensburg.de

Introduction

An increasing number of patients with acute or chronic renal failure makes it necessary to search for other therapies than renal dialysis or transplantation (Perin et al. 2008; Ross et al. 2009; Benigni et al. 2010; Burst et al. 2010). The focus of actual research is directed to the implantation of different kinds of stem/progenitor cells for the repair of diseased parenchyma (Al-Awqati and Oliver 2006; Humphreys et al. 2006; Lusis et al. 2010). In this regard the interstitium plays an essential role, since stem/progenitor cells migrate along the interstitial space to the site of damage, where they start with regeneration (Stroo et al. 2009; Asanuma et al. 2010). The repair will proceed if local inflammation is suppressed and if the correct cellular and extracellular signals stimulate implanted cells to develop into functional renal parenchyma (Iwatani and Imai 2010; Rodríguez-Iturbe and García García 2010). Despite several efforts, a milestone of therapeutic success is up to date not in sight. However, the limiting factors could be surmounted by learning from the microenvironment within the renal stem/progenitor cell niche. Since this site exhibits an optimal surrounding for the physiological development of nephrons, the idea is to create an adapted environment for the repair of parenchyma before, during and after the implantation of stem/progenitor cells so that the regeneration is substantially promoted (Scadden 2006; Minuth et al. 2010a, b).

Following this strategy, several developmental aspects have to be considered, since the generation of nephrons is a complex process. During the growth of the organ, nephrons are induced in consecutive waves within the stem/progenitor cell niche (Georgas et al. 2009). This process starts after the dichotomous arborization at the tip of a collecting duct ampulla (Davies 2002). Two very different types

of stem/progenitor cells initiate nephron development (Vigneau et al. 2006; Nishinakamura 2008; Shaw et al. 2010). Epithelial stem/progenitor cells are found within the ureter bud derived tip of the collecting duct (CD) ampulla. In contrast, the basal aspect of each CD ampulla tip is surrounded by the cap condensate containing nephrogenic mesenchymal stem/progenitor cells (Sariola 2002; Ikeya et al. 2010). Exchange of morphogenetic information including Pax2, Six1, Wnt9b, Ret, GDNF or BMP results finally in a recruitment of only few mesenchymal stem/progenitor cells at the lateral edge of the cap condensate to form the pretubular aggregate (Sariola 2002) and subsequently the comma- and S-shaped body as first visible signs of nephron development (Dressler 2009; Georgas et al. 2009; Michos 2009, Oxburgh et al. 2011).

New nephrons are generated exclusively at the tip of a CD ampulla that is always found in the cortex corticis of the developing kidney. For a physiological development, this process needs on the one hand an always correct spatial arborization of the CD ampulla and on the other hand an exact recruitment of a group of surrounding mesenchymal stem/progenitor cells. The accurate spatial orientation of both cell types may depend on soybean agglutinin (SBA)-positive micro-fibers lining from the basal aspect of a CD ampulla tip through the mesenchymal stem/progenitor cells toward the organ capsule (Schumacher et al. 2005). The unique distribution of these micro-fibers leads to the assumption that the tip of a CD ampulla is connected to the organ capsule by strings, which act as a vectorial plumbline. A structural connection over micro-fibers would also explain that the tip of a CD ampulla exhibits always a constant distance of 20 μm to the organ capsule, although multiple dichotomous branchings occur (Schumacher et al. 2002a).

Immunohistochemical and electron microscopical data further exhibit that around the tip of a CD ampulla a very special basal lamina is established, which differs completely from other tubules (Strehl et al. 1999; Schumacher et al. 2002a, 2005). Moreover, light and electron microscopical analysis show that a wide interstitial space is found around the tip of a CD ampulla spatially separating the epithelial stem/progenitor cells from the surrounding mesenchymal stem/progenitor cells. This striking interstitial space is not restricted to the developing rabbit kidney (Debiec et al. 1998; Strehl and Minuth 2001a), but is also observed in mice (Saxén and Lehtonen 1987; Ekblom and Weller 1991; Chan et al. 2010, rat (Barasch et al. 1999; Plisov et al. 2001) and human kidneys (Schumacher et al. 2002a, b). In addition, individual proteins are facing the interstitial space of the renal stem/progenitor cell niche. At the basal lamina of a CD ampulla tip, intense expression of osteopontin (Hudkins et al. 1999; Schumacher et al. 2002b), P_{CD}Amp1 (Strehl et al. 1997), laminin α1 (Schéele et al. 2007), galectin 3 (Bullock et al. 2001), nephronectin (Brandenberger 2001), collagen type IV (Mounier et al. 1986) and hyaluronan (Pohl et al. 2000) was described.

The conspicuous interstitium separating epithelial stem/progenitor cells from neighboring mesenchymal stem/progenitor cells shows remarkable morphological features (Strehl et al. 1999; Schumacher et al. 2005). Except collagen type IV, collagen types I, II, III, VI and IX are present (Schumacher et al. 2003; Minuth and Schumacher 2003). Since at this site a variety of morphogenetic signals is exchanged during the induction of nephrons, one would expect that an especially close connection between both types of stem/progenitor cells exists (Dressler 2009). However, the morphological data illuminate that the contrary is true. The unsolved question is if a special extracellular matrix is present in the wide interstitial space.

The spatial separation of epithelial and mesenchymal cells by a wide interstitium within the renal stem/progenitor cell niche is conspicuous. It can be caused on the one hand by masked extracellular matrix as it is known from connective tissue (Charbonneau et al. 2010). On the other hand, the wide interstitial space may harbor an up to date unknown individual environment supporting recruitment and development of stem/progenitor cells. Thus, to elucidate individual morphological features of the interstitium within the renal stem/progenitor cell niche, the present investigation was performed. For light and electron microscopy, a simple fixation protocol with glutaraldehyde (GA) in combination with lanthanum, alcian blue, ruthenium red and tannic acid was applied as earlier described (Hasko and Richardson 1988). This simple technique illuminates for the first time in the renal stem/progenitor cell niche that conspicuous strands of extracellular matrix are lining through the interstitial space. Surprisingly, the newly detected arrangement of extracellular matrix connects on the one hand epithelial and mesenchymal stem/progenitor cells but keeps them on the other hand in distinct position. The documented construction reflects a unique spatial micro architecture of the interstitium within the renal stem/progenitor cell niche.

Materials and methods

Tissue preparation

One-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 process them for light and electron microscopy.

Scanning electron microscopy

Performing scanning electron microscopy (SEM) tissue specimens were fixed in 2% glutaraldehyde (Serva, Heidelberg, Germany) buffered with PBS. Afterward the kidneys were cut from the capsule toward the medulla along a precisely orientated cortico-medullary axis (Fig. 1). After rinsing in PBS, dehydratation was performed in a graded series of ethanols; then the specimens were transferred in acetone and critical point dried with CO_2 to sputter coat with gold (Polaron E 5100, Watford, GB). Examination was performed in a scanning electron microscope DSM 940 A (Zeiss, Oberkochen, Germany) as earlier described (Blattmann et al. 2008).

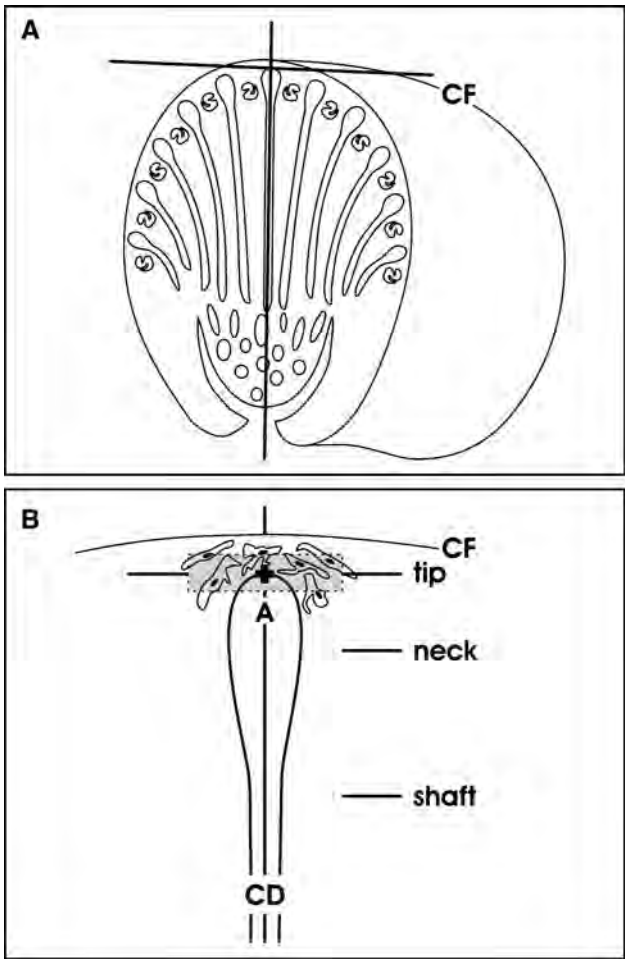


Fig. 1 Accurate orientation of tissue for histological sectioning. Vertical view to the renal stem/progenitor cell niche beyond the organ capsule (CF) is obtained by cutting in parallel to the lumen of lining collecting ducts (CD) (**a**). Illustration shows the exact site of the renal stem/progenitor cell niche (**b**). For identification, the basal lamina at the tip of a CD ampulla (A) is marked by a *cross* (+). The *line* within CD shows the longitudinal axis

Transmission electron microscopy

In the present investigation a protocol of fixation was applied, which was developed years ago for the investigation of the adult mouse tectorial membrane matrix (Hasko and Richardson 1988). Without any modification, the above-mentioned technique was applied on embryonic parenchyma to visualize specific features of the renal stem/progenitor cell niche. Specimens were fixed in following solutions for transmission electron microscopy:

Series 1: 5% glutaraldehyde (Serva) buffered with 0.15 M sodium cacodylate, pH 7.4
Series 2: 5% glutaraldehyde followed by postfixation with 0.1% lanthanum III chloride hydrate (Fluka, Taufkirchen, Germany)
Series 3: 5% glutaraldehyde with 1% alcian blue (Serva, Heidelberg, Germany)
Series 4: 5% glutaraldehyde with 1% alcian blue followed by postfixation with 0.1% lanthanum III chloride hydrate
Series 5: 5% glutaraldehyde with 0.5% ruthenium red (Fluka)
Series 6: 5% glutaraldehyde with 1% tannic acid (Sigma, Taufkirchen, Germany)

The period of primary fixation was for 1 day at room temperature. After several washes with 0.15 M sodium cacodylate, the specimens were postfixed in the same buffer but 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), which was polymerized at 60°C for 48 h. Semithin and ultrathin sections were performed with a diamond knife on an ultramicrotome EM UC6 (Leica GmbH, Wetzlar, Germany). Sections were collected onto grids (200 mesh) and contrasted using 2% uranyl acetate and lead citrate as earlier described (Minuth et al. 2009). Sections were examined at 80 kV using an EM 902 transmission electron microscope (Zeiss).

Amount of analyzed specimens

A total of 43 exactly orientated renal stem cell niches were analyzed for the present study. All of the specimens were analyzed at least in triplicates. Performed experiments are in accordance with the Animal Ethics Committee, University of Regensburg, Regensburg, Germany.

Definition of cells within the renal stem/progenitor cell niche

In this paper, the embryonic part of the growing kidney was described. In consequence, the nomenclature of previously

published papers was used (Strehl et al. 1997, 1999; Schumacher et al. 2002a; Nishinakamura 2008).

Results

Vertical view to the renal stem/progenitor cell niche

During the phase of induction, reciprocal morphogenetic interactions between the CD ampulla and the surrounding mesenchymal stem/progenitor cells are resulting in the formation of a nephron. In consequence, the interstitial space between both types of stem/progenitor cells is of outmost relevance for the exchange of morphogenetic signals.

To analyze this important site, a tissue block was orientated along the cortico-medullary axis and in parallel to the lumen of lining collecting ducts (CD) to obtain always a correct view to this site in histological sections (Fig. 1a). In the present investigation, all of the micrographs show this perspective so that comparisons between the different experimental series can be performed. For identification, the basal lamina at the tip of a CD ampulla is marked by a cross on each micrograph (see cross in Fig. 1b).

Scanning electron microscopy

For orientation, analysis was started by SEM. A surface view depicts that the CD ampulla is completely covered by a basal lamina (Fig. 2). At the tip of the CD ampulla a group of mesenchymal stem/progenitor cells is recognized, which sends out long cellular protrusions (see arrow in Fig. 2), contacting either neighboring cells or the lamina fibroreticularis of the CD ampulla. At the top and at the lateral side of the CD ampulla, mesenchymal stem/progenitor cells are separated from the basal lamina by a wide interstitial space (see asterisk in Fig. 2). A contact between mesenchymal stem/progenitor cells and the basal lamina of the CD ampulla occurs but is scarce. In irregular distance, thin cellular protrusions from mesenchymal stem/progenitor cells line through the interstitial space and contact the lamina fibroreticularis at the surface of the CD ampulla. Presence of extracellular matrix in form of fibers (see arrow head in Fig. 2) is rare and can be occasionally detected along the neck of the CD ampulla.

Light microscopy

Intact development of renal parenchyma depends on the one hand on reciprocal morphogenetic interactions (Dressler 2009; Michos 2009) and on the other hand on the exact spatial orientation of cells within the renal stem/progenitor cell niche (Nigam and Shah 2009). The site of

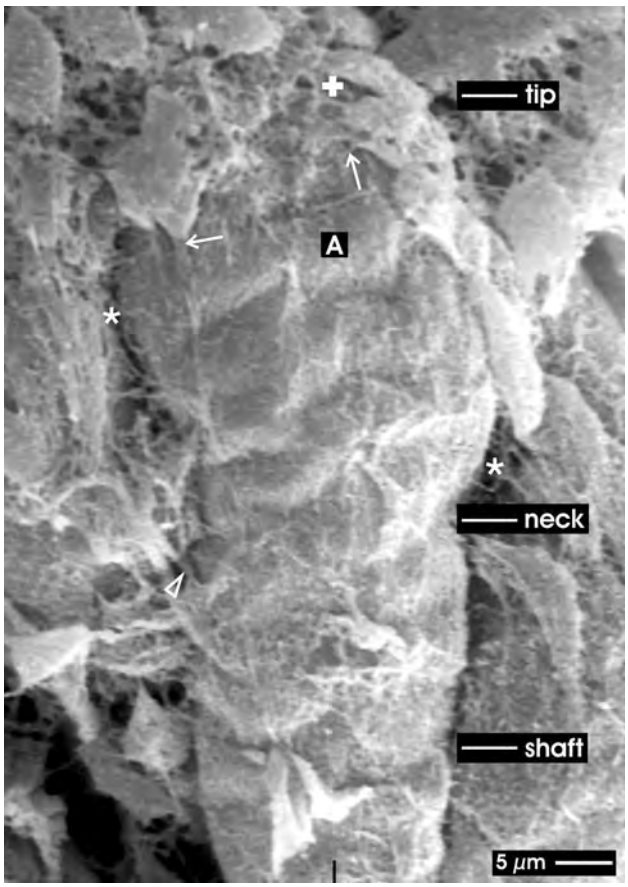


Fig. 2 SEM of the renal stem/progenitor cell niche. The CD ampulla (A) is completely covered by a basal lamina. At the tip, mesenchymal stem/progenitor cells are seen sending out cellular protrusions to contact either neighboring cells or the lamina fibroreticularis of the CD ampulla (*arrow*). At the tip, a wide interstitial space is present (*asterisk*). Extracellular matrix can be occasionally detected along the neck of the CD ampulla (*arrow head*)

nephron induction can be visualized on a semithin section made from the outer cortex of the neonatal kidney. Surprisingly, each ureter bud derived tip of a CD ampulla that contains epithelial stem/progenitor cells is found in an average distance of 20 µm beneath the organ capsule (Fig. 3a). This distance is maintained whether or not a CD ampulla is in the process of branching (Schumacher et al. 2002a, 2005). The section further reveals that the lumen of a CD ampulla is always vertically orientated to the organ capsule. Furthermore, underneath the organ capsule a thin layer of mesenchymal stem/progenitor cells is present, which is separated by an astonishingly wide interstitial space from the basal aspect of the CD ampulla.

Transmission electron microscopy

It is barely imaginable that the apparent, interstitial space between the CD ampulla and the surrounding mesenchymal stem/progenitor cells is simply a reservoir with fluid

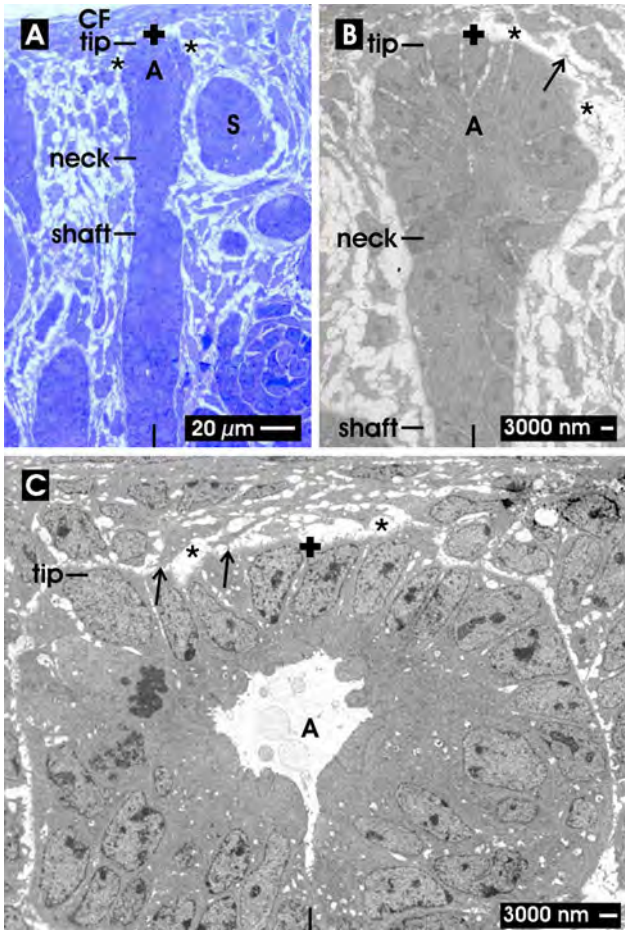


Fig. 3 Light microscopy and TEM of the renal stem/progenitor cell niche. A semithin section of the outer cortex of the neonatal kidney depicts the tip of a CD ampulla (A, +) containing epithelial stem/progenitor cells beyond the organ capsule (CF). A thin layer of mesenchymal stem/progenitor cells is found between the CD ampulla and the organ capsule. They are separated by an astonishingly wide interstitial space from the basal aspect of a CD ampulla (*asterisk*) (**a**). Surface view (**b**) and higher magnification (**c**) of an ultrathin section in TEM after fixation in GA show a conspicuous interstitial space (*asterisk*) separating epithelial stem/progenitor cells within the CD ampulla from the surrounding mesenchymal stem/progenitor cells. It is obvious that only single protrusions (*arrow*) from mesenchymal stem/progenitor cells contact the basal lamina

containing morphogenic factors, nutrition and respiratory gas. However, up to date sound morphological information dealing with the interface between epithelial and mesenchymal stem/progenitor cells is hardly available. In consequence, TEM was performed to analyze the morphological peculiarities of the interstitium within the renal stem/progenitor cell niche. Series with conventional GA fixation was compared with series using GA in combination with lanthanum, alcian blue, ruthenium red or tannic acid to visualize interstitial structures.

Fixation with GA

For control, specimens were fixed in GA in a first set of experiments. A surface view of an ultrathin section shows the interstitial space separating epithelial stem/progenitor cells within the CD ampulla from the surrounding mesenchymal stem/progenitor cells (see asterisk in Fig. 3b). The surface view further depicts that the mesenchymal stem/progenitor cells send out thin cellular protrusions toward the basal lamina of the CD ampulla. As seen in higher magnification, it is obvious that only single protrusions from mesenchymal stem/progenitor cells are crossing the wide interstitial space to contact the basal lamina of the CD ampulla (see arrow in Fig. 3c).

Fixation of specimens in GA further depicts that a consistently developed basal lamina covers epithelial stem/progenitor cells within the tip of the CD ampulla (Fig. 4a). The basal lamina consists of a clearly visible lamina rara (L.r.), a lamina densa (L.d.) and an extended lamina fibroreticularis (L.f.) (Fig. 4b) as earlier described (Strehl et al. 1999). Mesenchymal stem/progenitor cells are seen in the neighborhood of the CD ampulla. Astonishingly, these cells keep a remarkable distance to the CD ampulla (see asterisk in Fig. 4). However, in irregular distance, long cellular protrusions (see arrow in Fig. 4) are searching contact to the lamina fibroreticularis. The arrangement of cellular protrusions argues for an interstitial space that is well preserved by fixation. In so far, the micrographs reflect the natural situation and cannot be ascribed to an artifact due to fixation.

Using low magnification, the interstitial space between the CD ampulla and the surrounding mesenchymal stem/progenitor cells appears to be free of extracellular matrix fibers (Fig. 4a). However, increasing the magnification reveals that single fibers consisting of extracellular matrix (see arrow head in Fig. 4) are lining from the tip of the CD ampulla through the wide interstitial space toward the mesenchymal stem/progenitor cells (Fig. 4b). The space between protrusions from mesenchymal stem/progenitor cells and tiny fibers consisting of extracellular matrix appears as a big reservoir for transport of interstitial fluid.

Fixation with GA containing lanthanum

In the second series, GA containing lanthanum was used for fixation. Low magnification illustrates the basal side of epithelial stem/progenitor cells within the tip of the CD ampulla (Fig. 4c). Comparable to series with pure GA fixation (Fig. 4a), it can be recognized that a consistently developed basal lamina covers the tip. It is obvious that the mesenchymal stem/progenitor cells stay in distance to the CD ampulla. However, their long cellular protrusions line

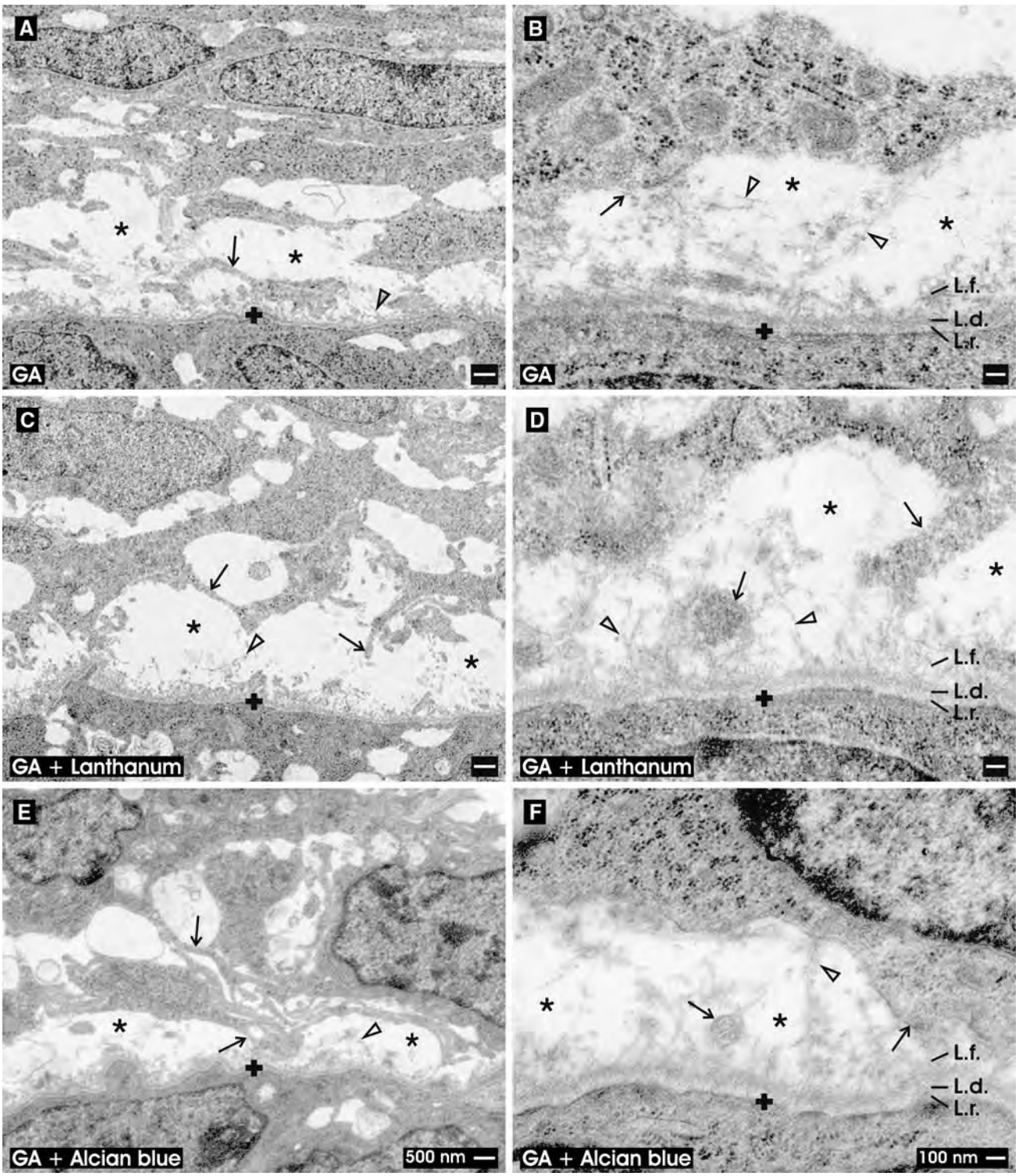


Fig. 4 TEM illustrates in low (**a**) and high magnification (**b**) the interstitium within the renal stem/progenitor cell niche after fixation in GA. Low (**c**) and high magnification (**d**) after fixation with GA containing lanthanum. Low (**e**) and high magnification (**f**) after fixation with GA containing *alcian blue*. In these series, it is recognized that a consistently developed basal lamina covers epithelial stem/progenitor cells within the tip of the CD ampulla (+). The basal lamina consists of a clearly visible lamina rara (L.r.), a lamina densa (L.d.) and an extended lamina fibroreticularis (L.f.) (**b**, **d**, **f**). Although mesenchymal stem/progenitor cells are separated from the CD ampulla by a wide interstitium (*asterisk*) long cellular protrusion contact the lamina fibroreticularis (*arrow*). Only single fibers consisting of extracellular matrix (*arrow head*) line through the interstitial space toward the mesenchymal stem/progenitor cells

through the wide interstitial space toward the lamina fibroreticularis at the tip.

Higher magnification reveals that the basal lamina of the CD ampulla exhibits a lamina rara, a lamina densa and an extended lamina fibroreticularis (Fig. 4d). Most interestingly, in longitudinal and vertical view can be seen that cellular protrusions from mesenchymal stem/progenitor cells span through the interstitial space to contact tiny extracellular matrix fibers at the lamina fibroreticularis at the tip of the CD ampulla. Parallel to this, it is also recognized that tiny fibers consisting of extracellular matrix are spanning between the lamina fibroreticularis of the CD ampulla tip toward the surface of surrounding mesenchymal stem/progenitor cells.

Fixation with GA containing alcian blue

In the third experimental series, fixation was performed by GA containing alcian blue. TEM shows in low magnification the basal aspect of epithelial stem/progenitor cells within the tip of the CD ampulla (Fig. 4e). A clearly recognizable basal lamina borders the wide interstitial space at the tip of the CD ampulla. Mesenchymal stem/progenitor cells are found in distance to the basal lamina. Also in this series, long cellular protrusions from mesenchymal stem/progenitor cells span through the interstitial space to contact the lamina fibroreticularis. As seen in previous series, the interstitial space between the fibers appears as a big reservoir for fluid. In higher magnification, it further can be seen that the basal lamina at the tip of the CD ampulla exhibits a clearly visible lamina rara, a lamina densa and an extended lamina fibroreticularis (Fig. 4f). It is obvious that single tiny fibers consisting of extracellular matrix are spanning from the lamina fibroreticularis through the interstitial space toward the surface of mesenchymal stem/progenitor cells.

Fixation with GA containing alcian blue and lanthanum

In the fourth series, fixation was made by GA containing alcian blue and lanthanum. TEM shows in low magnification the basal aspect of epithelial stem/progenitor cells at the tip of the CD ampulla (Fig. 5a). Further it is seen that mesenchymal stem/progenitor cells send out long cellular protrusions (see arrow in Fig. 5) toward the lamina fibroreticularis. Tiny fibers consisting of extracellular matrix (see arrow head in Fig. 5) line from the lamina fibroreticularis through the interstitial space (see asterisk in Fig. 5) to the neighboring mesenchymal stem/progenitor cells.

Higher magnification illuminates at the basal lamina a clearly recognizable lamina rara, a lamina densa and an intensely labeled lamina fibroreticularis (Fig. 5b). Both cellular protrusions from mesenchymal stem/progenitor cells and fibers consisting of extracellular matrix are spanning through the interstitial space. It appears that fixation of specimens with GA containing alcian blue and lanthanum exhibits in the interstitial space a slightly increased amount of extracellular matrix in form of fibers as observed in series with GA (Fig. 4a, b), GA and lanthanum (Fig. 4c, d) or GA and alcian blue (Fig. 4e, f).

Fixation with GA containing ruthenium red

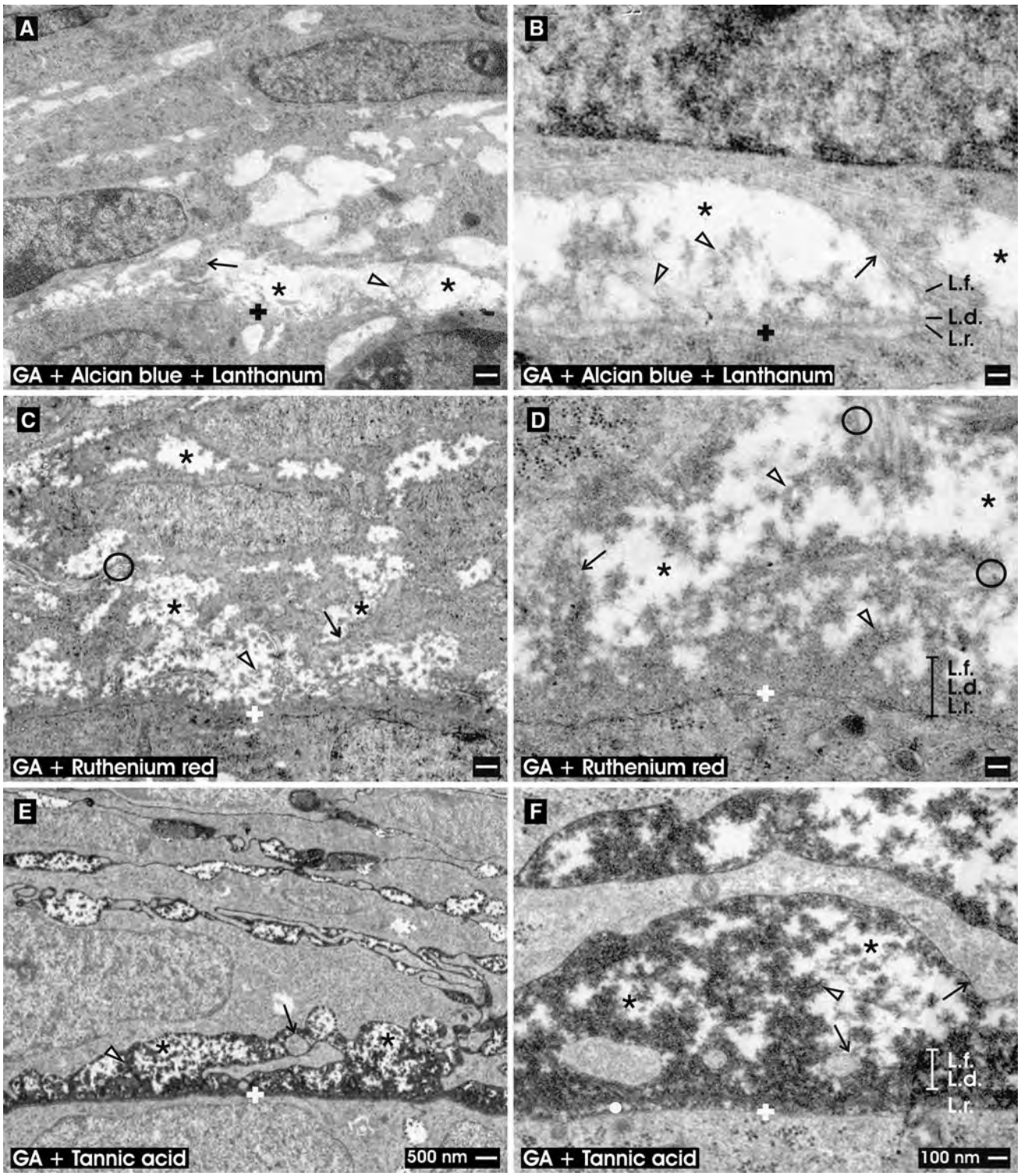
In the fifth series of experiments, specimen was fixed by GA containing ruthenium red. In low magnification, the tip of the CD ampulla can be recognized (Fig. 5c). Most interestingly, this kind of fixation shows that the basal lamina bordering the interstitial space appears to be completely changed as compared to previous series. The three-laminar structure is not visible anymore. Instead, a single broad band of intensive label surrounds the basal aspect of the CD ampulla. In addition, cellular protrusions of the mesenchymal stem/progenitor cells exhibit a roughly punctuate pattern on their surface. It can be recognized that single cellular protrusions line through the interstitial space up to the lamina fibroreticularis at the tip of the CD ampulla.

Higher magnification depicts that the basal lamina of the CD ampulla does not show the typical three-laminar structure consisting of a lamina rara, a lamina densa and the intense lamina fibroreticularis anymore (Fig. 5d). Instead, the basal lamina is recognized as a broad band covering the tip of the CD ampulla. At the site of the lamina fibroreticularis, roughly structured bundles of extracellular matrix are protruding into the interstitial space. In addition, using this kind of fixation, bundles of translucent fibers (see circle in Fig. 5c, d) become visible lining through the interstitial space. They are covered by extracellular matrix labeled by ruthenium red. Since the translucent fibers do not exhibit a repeating period, they cannot be ascribed to a certain type of collagen.

Further, it is visible that the complete surface of mesenchymal stem/progenitor cells is covered by a dense and roughly structured coat labeled by ruthenium red. However, the distribution of ruthenium red does not exhibit a typical glycocalix label, since not only the surface of cells but also adjacent extracellular matrix within the interstitial space and the basal lamina of the neighboring CD ampulla are contrasted. In consequence, not the complete interstitial space but only part of it is labeled by ruthenium red (Figs. 5e, f, 7; asterisk). This result speaks in favor for a stain-specific label and not for a background signal.

Fixation with GA containing tannic acid

In the sixth series, fixation was performed by GA containing tannic acid. Low magnification shows the basal



aspect at the tip of the CD ampulla (Fig. 5e). Astonishingly, the complete basal lamina is covered by an electron-dense coat as detected after fixation with GA containing ruthenium red (Fig. 5c). The intensively stained pattern lines discontinuously from the basal lamina of the CD ampulla through the interstitial space toward cell protrusions and the surface of neighboring mesenchymal stem/progenitor cells.

Higher magnification depicts that tannic acid labels the complete basal lamina at the tip of the CD ampulla so

◀ **Fig. 5** TEM shows in low (**a**) and high magnification (**b**) the interstitium within the renal stem/progenitor cell niche after fixation with GA containing *alcian blue* and lanthanum. Low (**c**) and high magnification (**d**) after fixation with GA containing *ruthenium red*. Low (**e**) and high magnification (**f**) after fixation with GA containing tannic acid. A consistently developed basal lamina covers epithelial stem/progenitor cells within the tip of the CD ampulla (+). The basal lamina consists of a lamina rara (L.r.), lamina densa (L.d.) and extended lamina fibroreticularis (L.f.) (**b**, **d**, **f**). Mesenchymal stem/progenitor cells are separated from the CD ampulla by a wide interstitium (*asterisk*). Cellular protrusions contact the lamina fibroreticularis (*arrow*). Single fibers consisting of extracellular matrix (*arrow head*) line through the interstitium toward the mesenchymal stem/progenitor cells (**a**, **b**). Fixation with GA containing ruthenium red shows a broad band at the CD ampulla. Mesenchymal stem/progenitor cells exhibit a roughly punctuate pattern. Their cellular protrusions line through the interstitial space up to the lamina fibroreticularis of the CD ampulla. Translucent fibers are lining through the interstitial space (*circle*) (**c**, **d**). Tannic acid labels bundles of extracellular matrix (*arrow head*) within the interstitium (*asterisk*), cellular protrusions and the cell surface of mesenchymal stem/progenitor cells (*arrow*) (**e**). Fixation with tannic acid indicates at the basal lamina a discontinuously labeled lamina rara (L.r.; *white dot*). The lamina densa and lamina fibroreticularis appear as a common band surrounding the CD ampulla (**f**)

much, that a discontinuously contrasted lamina rara can be detected (see white dot in Fig. 5f), while the lamina densa and lamina fibroreticularis appear as a broad band (Fig. 5f). Most interestingly, it can be seen that tannic acid labels to a high-degree roughly structured bundles of extracellular matrix within the interstitial space. Further more, the cellular protrusions and the complete cell surface of neighboring mesenchymal stem/progenitor cells are labeled. Fixation with GA containing tannic acid shows that an unexpected large amount of extracellular matrix is present within the interstitium around the tip of the CD ampulla spanning up to the surface of neighboring mesenchymal stem/progenitor cells. However, not the complete interstitial space but only part of it is labeled by tannic acid (Fig. 5f; asterisk). This result speaks in favor for a stain-specific label and not for a background signal.

Site-specific reaction of tannic acid label

An important question is if the label after fixation in GA containing tannic acid is restricted to the embryonic part of the interstitium including the renal stem/progenitor cell niche or if the complete interstitium is contrasted. In consequence, the region between the tip (see cross in Fig. 6) and neck of the CD ampulla was analyzed (Fig. 6a). At the tip of the CD ampulla, intense label of tannic acid is found within the basal lamina, in between the adjacent interstitial space (see asterisk in Fig. 6b–e) and on the complete surface of neighboring mesenchymal stem/progenitor cells (Fig. 6b). At the upper lateral side of the CD ampulla, intensity of label is decreased at the basal lamina and within the interstitial space, while the surface of interstitial cells does not show label for tannic acid (Fig. 6c). At the lower lateral side of the CD ampulla, the basal lamina and single contacting protrusions (see arrow in Fig. 6b–e) of interstitial cells show label for tannic acid (Fig. 6d). The surface of mesenchymal stem/progenitor cells is free of label. Finally, at the neck of the CD ampulla, label for tannic acid is found neither at the basal lamina nor within the interstitial space or at the surface of interstitial cells (Fig. 6e). Moreover, at the tip of the CD ampulla, an astonishingly wide interstitial space is detected (Fig. 6b), while at the neck of the CD ampulla the interstitium reduces so that interstitial cells stand in much closer contact to the basal lamina (Fig. 6e). Thus, label for tannic acid is most prominent at the area of the renal stem/progenitor cell niche, while label decreases in the area of maturing S-shaped bodies and is lacking at the neck of the CD ampulla. The same result was obtained with ruthenium red label (not shown). Both results are paralleled by a high expression of P_{CD} Amp1 and osteopontin at the tip of the CD ampulla, while low and lack of label was found at the lateral side and the neck (Schumacher et al. 2002b).

Finally, it was reported that microfibers are lining from the basal aspect of the CD ampulla through the interstitial space to the cap condensate harboring mesenchymal stem/progenitor cells (Schumacher et al. 2002a, b, 2003, 2005). Such fibers can be recognized in TEM as well in longitudinal as in oblique course spanning through the interstitial space (Fig. 7). Single micro-fibers are lacking ruthenium red or tannic acid (not shown label). However, when the micro-fibers form bundles, the outer surface is covered by an intense ruthenium red (Fig. 7; circle) respectively tannic acid positive coat.

In conclusion, SEM (Fig. 2), light microscopy (Fig. 3a) and TEM (Figs. 3b, c, 4, 5, 6, 7) reveal that epithelial stem/progenitor cells within the CD ampulla and the surrounding mesenchymal stem/progenitor cells are separated by an astonishingly structured interstitial space. Mesenchymal stem/progenitor cells within the cap condensate send out long cellular protrusions into the wide interstitial space to contact discontinuously the lamina fibroreticularis at the tip of the CD ampulla. Performing fixation with GA in combination with ruthenium red (Figs. 5c, d, 7) or tannic acid (Fig. 5e, f) further reveals that the interstitial space contains an unexpected amount of extracellular matrix. It is most astonishingly that the extracellular matrix is not restricted to the lamina fibroreticularis but extends through the interstitium up to cellular protrusions and the complete surface of neighboring mesenchymal stem/progenitor cells.

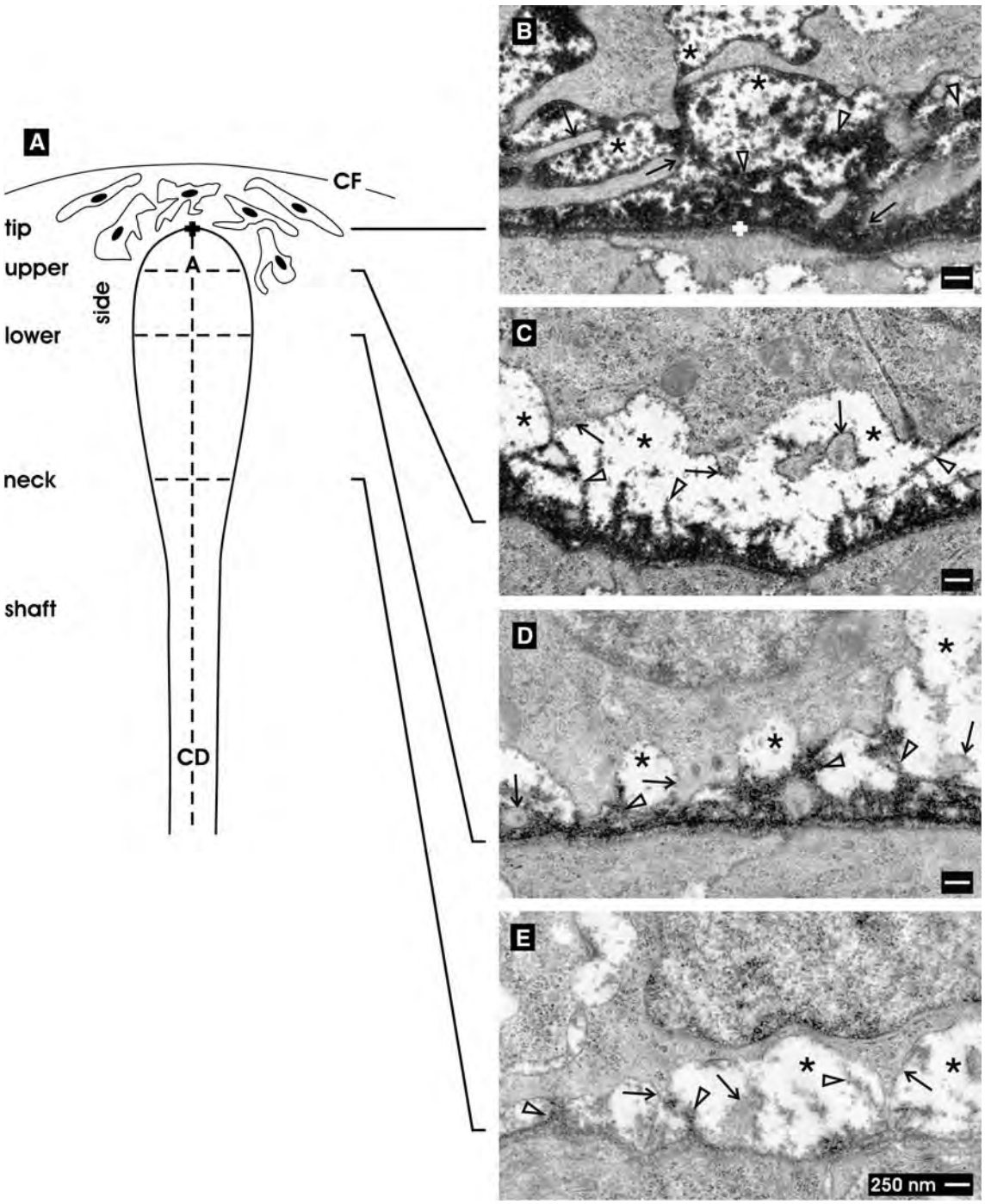


Fig. 6 Distribution of tannic acid label in the embryonic cortex of neonatal kidney. Schematic illustration depicts floors of sections (**a**). TEM at the tip of the CD ampulla (A, +) depicts intense label of tannic acid at the basal lamina, the adjacent interstitial space and on the complete surface of neighboring mesenchymal stem/progenitor cells (**b**). At the upper lateral side, intensity of label at the basal lamina and within the interstitial space is decreased. Interstitial cells do not show label for tannic acid at their surface (**c**). At the lower lateral side, the basal lamina and single contacting protrusions of interstitial cells show label for tannic acid. The surface of mesenchymal stem/progenitor cells does not exhibit label (**d**). At the neck of the CD ampulla, apparent label for tannic acid is found neither at the basal lamina nor within the interstitial space or at the surface of interstitial cells (**e**). Organ capsule (CF), interstitial space (*asterisk*), cellular protrusion from mesenchymal stem/progenitor cells (*arrow*), fibers consisting of extracellular matrix (*arrow head*)

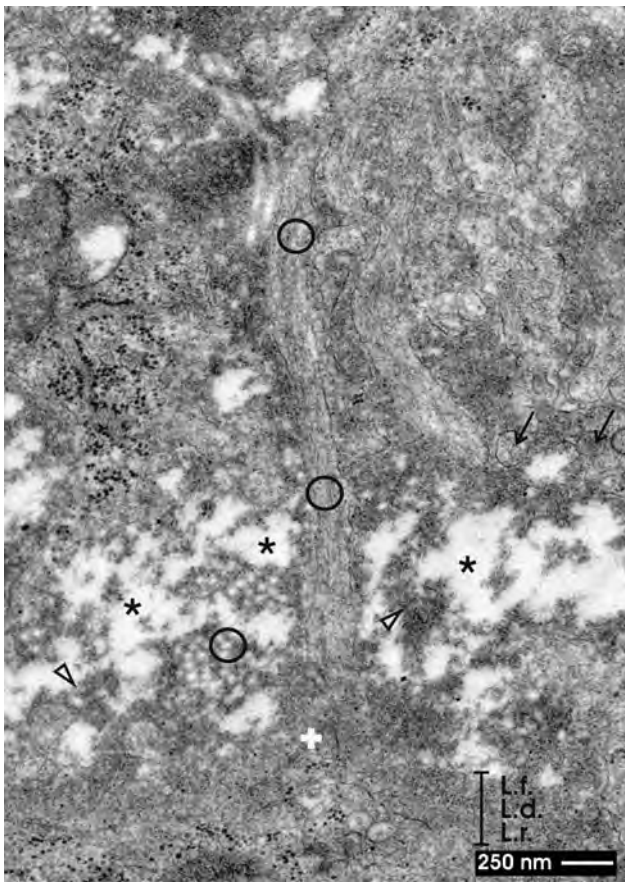


Fig. 7 TEM depicts the interstitium within the renal stem/progenitor cell niche after fixation with GA containing *ruthenium red*. A consistently developed basal lamina containing a lamina rara (L.r.), lamina densa (L.d.) and lamina fibroreticularis (L.f.) covers as a broad *ruthenium red band* epithelial stem/progenitor cells within the tip of the CD ampulla (+). Micro-fibers (*circle*) are lining in longitudinal and oblique course from the CD ampulla through the interstitial space. Single micro-fibers are lacking *ruthenium red label*. However, when bundles are formed, the outer surface illustrates intense *ruthenium red label*. Interstitial space (*asterisk*), extracellular matrix labeled by *ruthenium red* (*arrow head*), cellular protrusions from mesenchymal cells (*arrow*)

Discussion

Renal interstitium

The interstitium is an important compartment not only for the adult but also for the embryonic and growing kidney. By light and electron microscopy, it can be recognized as a narrow slit between the basal lamina of tubules and the outer surface of blood vessels (Lemley and Kriz 1991; Kaissling and Le Hir 2008). Although looking inconspicuously from a morphological sight, the interstitial space is of great physiological importance (Grande and López-Novoa 2009; Tanaka and Nangaku 2010). Structural elements consisting of mainly collagen type III sustain as endoskeletonal elements the three-dimensional structure of the organ (Fleischmajer et al. 1992). In the complementary interstitial space, fluid is crossing between collagen fibers, tubules and blood vessels to provide the parenchyma with nutrition, hormones, morphogenetic factors and respiratory gas. In the diseased kidney, inflammatory cells infiltrate the interstitium. The resulting increase of interstitial cells and synthesized extracellular matrix causes obstructive nephropathy and fibrosis (Grande et al. 2010). During this process, tubule cells loose epithelial features and convert to fibroblast-like cells (Guarino et al. 2009). The epithelial-mesenchymal transition (EMT) is paralleled by the expression of fibroblast-specific protein-1(FSB1) (Kim et al. 2010), heat shock proteins and α-smooth muscle actin (Abe et al. 2000; Lim et al. 2009; Vidyasagar et al. 2008).

Information about the origin of interstitial cells is barely available. It has been suggested that they develop from mesenchymal cells within the cap condensate that fails to be converted to nephron epithelia (Ekblom and Weller 1991; Ekblom 1992; Cullen-McEwen et al. 2005). During organ growth, abundant interstitial cells are connected via cellular processes to form a widely distributed network. This arrangement results in the characteristic "rungs of ladder" appearance of interstitial cells found between tubules and blood vessels (Maric et al. 1997). During further development, the interstitial space in the cortex decreases in conjunction with the spatial extension and maturation of superficial nephrons (Sundelin and Bohman 1990).

Interstitium within the renal stem/progenitor cell niche

Sound knowledge about the interstitium within the renal stem/progenitor cell niche lacks. It is the peculiar site, where epithelial stem/progenitor cells within the tip of the CD ampulla face mesenchymal stem/progenitor cells. On the one hand, a specific microenvironment supports stem/progenitor cells as long as the kidney develops. On the other hand, spatial and temporal processes such as dichotomous branching of the CD ampulla and reciprocal interactions between both types of stem/progenitor cells must lead to the recruitment of individual mesenchymal stem/progenitor cells to induce the next generation of nephrons. During this step, the interstitial space between the epithelial and mesenchymal stem/progenitor cells is of special importance for communication, exchange of morphogenetic information and spatial orientation (Dressler 2009). Up to date, it is an unsolved question if the morphogenetic factors are exchanged exclusively by diffusion or if also cellular contacts are involved. Parallel to the process of nephron induction, vascular precursor cells migrate through the interstitium toward the developing S-shaped body to form a net of capillaries. The site of the renal stem/progenitor cell niche lacks intact vessels (Kloth et al. 1997).

Interstitial peculiarities within the renal stem/progenitor cell niche

In previous and present experiments it was shown that an astonishingly structured interstitial space separates epithelial and mesenchymal stem/progenitor cells (Strehl et al. 1999; Strehl and Minuth 2001b; Miess et al. 2010) (Figs. 2, 3, 4, 5). Moreover, TEM demonstrates that cellular protrusions from mesenchymal stem/progenitor cells line through the interstitial space to contact the lamina fibroreticularis at the tip of the CD ampulla (Figs. 4a, c, e, 5a, c, e). The morphology and orientation of cellular protrusions look fully intact. This important result demonstrates that the interstitial space is not caused by a fixation artifact.

The presented data further reveal that conventional fixation with GA (Figs. 3b, c, 4a, b) or in combination with lanthanum (Fig. 4c, d) or alcian blue (Fig. 4e, f) or both together (Fig. 5a, b) shows that astonishingly few fibers consisting of extracellular matrix span through the interstitial space between epithelial and mesenchymal stem/progenitor cells. In contrast, application of ruthenium red (Fig. 5c, d) or tannic acid (Fig. 5e, f) to the fixation solution demonstrates that the interstitial space within the renal stem/progenitor cell niche contains an unexpectedly high amount of extracellular matrix. It is most concentrated at the tip of the CD ampulla (Fig. 6b), decreases along the lateral side (Fig. 6c, d) and is completely lost between the neck and shaft (Fig. 6 e). This clearly recognizable gradient suggests that the ruthenium red (Figs. 5c, d, 7) respectively tannic acid (Figs. 5e, f, 6) label is restricted to the embryonic zone containing the renal stem/progenitor cell niche.

Most interestingly, the extracellular matrix contrasted by ruthenium red (Fig. 5c, d) and tannic acid (Fig. 5e, f) is strongly associated to all of the three layers of the basal lamina at the tip of the CD ampulla. In addition, the electron-dense material is lining from the lamina fibroreticularis in form of striking bundles through the interstitial space up to the surface of mesenchymal stem/progenitor cells. Contrasting the specimens with GA containing ruthenium red further reveals that the striking extracellular matrix is crossing the interstitial space in parallel with cellular protrusions and translucent fibers (Fig. 5d). It is obvious that the extracellular matrix is covering the complete cell surface of mesenchymal stem/progenitor cells including their cellular protrusions. TEM further illustrates that the extracellular matrix contrasted by ruthenium red (Fig. 5c, d) or tannic acid (Fig. 5e, f) consists of roughly organized bundles. It is obvious that the extracellular matrix detected in the interstitium of the renal stem/progenitor cell niche is connecting to an unexpectedly high degree both epithelial and mesenchymal stem/progenitor cells. The complementary space between the ruthenium red and tannic acid-positive material is free of any recognizable structures. In comparison with specimens fixed in GA (Figs. 3b, c, 4a, b) it appears that this "reduced interstitial space" non-labeled by ruthenium red (Fig. 5c, d) or tannic acid (Fig. 5e, f) is the path, where fluid is crossing to provide the surrounding cells with morphogenic factors, nutrition and respiratory gas. In each case, the interstitial space of the renal stem/progenitor cell niche contains more structural features as earlier believed. Up to date, only speculations can be made about the molecular composition of the special extracellular matrix and their tasks. So far, the extracellular matrix may stabilize the spatial orientation of stem/progenitor cells within the renal stem/progenitor cell niche.

Conclusions

For the first time it has been demonstrated that the interstitial space within the renal stem/progenitor cell niche shows morphological peculiarities in form of abundant extracellular matrix labeled by ruthenium red (Fig. 5c, d) and tannic acid (Fig. 5e, f). It is obvious that cellular contacts between epithelial and mesenchymal stem/progenitor cells are scarce. Instead, the interstitial space is discontinuously bridged by ruthenium red (Fig. 5c, d) and tannic acid-labeled material (Fig. 5e, f). If the newly detected extracellular matrix represents an environment supporting primary development of stem/progenitor cells or if morphogenetic factors for differentiation are contained, remains to be investigated in next future.

References

Abe K, Ozono Y, Miyazaki M, Koji T, Shioshita K, Furusu A, Tsukasaki S, Matsuya F, Hosokawa N, Harada T, Taguchi T, Nagata K, Kohno S (2000) Interstitial expression of heat shock protein 47 and alpha-smooth muscle actin in renal allograft failure. Nephrol Dial Transplant 15(4):529–535

Al-Awqati Q, Oliver JA (2006) The kidney papilla is a stem cells niche. Stem Cell Rev 2(3):181–184

Asanuma H, Meldrum DR, Meldrum KK (2010) Therapeutic applications of mesenchymal stem cells to repair kidney injury. J Urol 184(1):26–33

Barasch J, Yang J, Qiao J, Tempst P, Erdjument-Bromage H, Leung W, Oliver JA (1999) Tissue inhibitor of metalloproteinase-2 stimulates mesenchymal growth and regulates epithelial branching during morphogenesis of the rat metanephros. J Clin Invest 103(9):1299–1307

Benigni A, Morigi M, Remuzzi G (2010) Kidney regeneration. Lancet 375(9722):1310–1317

Blattmann A, Denk L, Strehl R, Castrop H, Minuth WW (2008) The formation of pores in the basal lamina of regenerated renal tubules. Biomaterials 29(18):2749–2756

Brandenberger R (2001) Identification and characterization of a novel extracellular matrix protein nephronectin that is associated with

integrin alpha8beta1 in the embryonic kidney. J Cell Biol 154(2):447–458
Bullock SL, Johnson TM, Bao Q, Hughes RC, Winyard PJ, Woolf AS (2001) Galectin-3 modulates ureteric bud branching in organ culture of the developing mouse kidney. J Am Soc Nephrol 12(3):515–523
Burst VR, Gillis M, Pütsch F, Herzog R, Fischer JH, Heid P, Müller-Ehmsen J, Schenk K, Fries JWU, Baldamus CA, Benzing T (2010) Poor cell survival limits the beneficial impact of mesenchymal stem cell transplantation on acute kidney injury. Nephron Exp Nephrol 114(3):e107–e116
Chan SK, Riley PR, Price KL, McElduff F, Winyard PJ, Welham SJM, Woolf AS, Long DA (2010) Corticosteroid-induced kidney dysmorphogenesis is associated with deregulated expression of known cystogenic molecules, as well as Indian hedgehog. Am J Physiol Renal Physiol 29(2):F346–F356
Charbonneau NL, Jordan CD, Keene DR, Lee-Arteage S, Dietz HC, Rifkin DB, Ramirez F, Sakai LY (2010) Microfibril structure masks fibrillin-2 in postnatal tissues. J Biol Chem 285(26): 20242–20251
Cullen-McEwen LA, Caruana G, Bertram JF (2005) The where, what and why of the developing renal stroma. Nephron Exp Nephrol 99(1):e1–e8
Davies JA (2002) Morphogenesis of the metanephric kidney. ScientificWorldJournal 28(2):1937–1950
Debiec H, Christensen EI, Ronco PM (1998) The cell adhesion molecule L1 is developmentally regulated in the renal epithelium and is involved in kidney branching morphogenesis. J Cell Biol 143(7):2067–2079
Dressler GR (2009) Advances in early kidney specification, development and patterning. Development 136(23):3863–3874
Ekblom P (1992) Renal development. In The kidney: Physiology and pathophysiology, Raven Press Ltd., pp 475–501
Ekblom P, Weller A (1991) Ontogeny of tubulointerstitial cells. Kidney Int 39(3):394–400
Fleischmajer R, Jacobs L, Perlish JS, Katchen B, Schwartz E, Timpl R (1992) Immunochemical analysis of human kidney reticulin. Am J Pathol 140(5):1225–1235
Georgas K, Rumballe B, Valerius MT, Chiu HS, Thiagarajan RD, Lesieur E, Aronow BJ, Brunskill EW, Combes AN, Tang D, Taylor D, Grimmond SM, Potter SS, McMahon AP, Little MH (2009) Analysis of early nephron patterning reveals a role for distal RV proliferation in fusion to the ureteric tip via a cap mesenchyme-derived connecting segment. Dev Biol 332(2): 273–286
Grande MT, López-Novoa JM (2009) Fibroblast activation and myofibroblast generation in obstructive nephropathy. Nat Rev Nephrol 5(6):319–328
Grande MT, Pérez-Barriocanal F, López-Novoa JM (2010) Role of inflammation in túbulo-interstitial damage associated to obstructive nephropathy. J Inflamm (Lond) 22(7):19
Guarino M, Tosoni A, Nebuloni M (2009) Direct contribution of epithelium to organ fibrosis: epithelial-mesenchymal transition. Hum Pathol 40(10):1365–1376
Hasko JA, Richardson GP (1988) The ultrastructural organization and properties of the mouse tectorial membrane matrix. Hear Res 35(1):21–38
Hudkins KL, Giachelli CM, Cui Y, Couser WG, Johnson RJ, Alpers CE (1999) Osteopontin expression in fetal and mature human kidney. J Am Soc Nephrol 10(3):444–457
Humphreys BD, Duffield JS, Bonventre JV (2006) Renal stem cells in recovery from acute kidney injury. Minerva Urol Nefrol 58(4):13–21
Ikeya M, Fukushima K, Kawada M, Onishi S, Furuta Y, Yonemura S, Kitamura T, Nosaka T, Sasai Y (2010) Cv2, functioning as a pro-BMP factor via twisted gastrulation, is required for early development of nephron precursors. Dev Biol 337(2):405–414
Iwatani H, Imai E (2010) Kidney repair using stem cells: myth or reality as a therapeutic option? J Nephrol 2:143–146
Kaissling B, Le Hir M (2008) The renal cortical interstitium: morphological and functional aspects. Histochem Cell Biol 23(2):247–262
Kim S, Min W-K, Chun S, Lee W, Chung H-J, Choi SJ, Yang SE, Yang YS, Yoo J-I (2010) Protein expression profiles during osteogenic differentiation of mesenchymal stem cells derived from human umbilical cord blood. Tohoku J Exp Med 221(2):141–150
Kloth S, Ebenbeck C, Monzer J, de Vries U, Minuth WW (1997) Three-dimensional organization of the developing vasculature of the kidney. Cell Tissue Res 287(1):193–201
Lemley KV, Kriz W (1991) Anatomy of the renal interstitium. Kidney Int 39(3):370–381
Lim BJ, Hong SW, Jeong HJ (2009) Renal tubular expression of Toll-like receptor 4 in cyclosporine nephrotoxicity. APMIS 117(8): 583–591
Lusis M, Li J, Ineson J, Christensen ME, Rice A, Little MH (2010) Isolation of clonogenic, long-term self renewing embryonic renal stem cells. Stem Cell Res 5(1):23–39
Maric C, Ryan GB, Alcorn D (1997) Embryonic and postnatal development of the rat renal interstitium. Anat Embryol (Berl) 195(6):503–514
Michos O (2009) Kidney development: from ureteric bud formation to branching morphogenesis. Curr Opin Genet Dev 19(5): 484–490
Miess C, Glashauser A, Denk L, de Vries U, Minuth WW (2010) The interface between generating renal tubules and a polyester fleece in comparison to the interstitium of the developing kidney. Ann Biomed Eng 38(6):2197–2209
Minuth WW, Schumacher K (2003) From the renal stem cell niche to functional tubule. Med Klin 98(SupllI):31–35
Minuth WW, Denk L, Meese C, Rachel R, Roessger A (2009) Ultrastructural insights in the interface between generated renal tubules and a polyester interstitium. Langmuir 25(8):4621–4627
Minuth WW, Denk L, Glashauser A (2010a) Towards a guided regeneration of renal tubules at a polyester interstitium. Materials 3(4):2369–2392
Minuth WW, Denk L, Glashauser A (2010b) Cell and drug delivery therapeutics for controlled renal parenchyma regeneration. Adv Drug Deliv Rev 62(7–8):841–854
Mounier F, Foidart JM, Gubler MC (1986) Distribution of extracellular matrix glycoproteins during normal development of human kidney. An immunohistochemical study. Lab Invest 54(4): 394–401
Nigam SK, Shah MM (2009) How does the ureteric bud branch? J Am Soc Nephrol 20(7):1465–1469
Nishinakamura R (2008) Stem cells in the embryonic kidney. Kidney Int 73:913–917
Oxburgh L, Brown AC, Fetting J, Hill B (2011) BMP signalling in the nephron progenitor niche. Pediatr Nephrol. doi:10.1007/s00467-011-1819-8
Perin L, Giuliani S, Sedrakyan S, DA Sacco S, De Filippo RE (2008) Stem cell and regenerative science applications in the development of bioengineering of renal tissue. Pediatr Res 63(5): 467–471
Plisov SY, Yoshino K, Dove LF, Higinbotham KG, Rubin JS, Perantoni AO (2001) TGF beta 2, LIF and FGF2 cooperate to induce nephrogenesis. Development 128(7):1045–1057
Pohl M, Sakurai H, Stuart RO, Nigam SK (2000) Role of hyaluronan and CD44 in in vitro branching morphogenesis of ureteric bud cells. Dev Biol 224(2):312–325

Rodríguez-Iturbe B, García García G (2010) The role of tubulointerstitial inflammation in the progression of chronic renal failure. Nephron Clinical Practice 116(2):c81–c88

Ross EA, Williams MJ, Hamazaki T, Terada N, Clapp WL, Adin C, Ellison GW, Jorgensen M, Batich CD (2009) Embryonic stem cells proliferate and differentiate when seeded into kidney scaffolds. J Am Soc Nephrol 20(11):2338–2347

Sariola H (2002) Nephron induction revisited: from caps to condensates. Curr Opin Nephrol Hypertens 11(1):17–21

Saxén L, Lehtonen E (1987) Embryonic kidney in organ culture. Differentiation 36(1):2–11

Scadden DT (2006) The stem-cell niche as an entity of action. Nature 441(7097):1075–1079

Schéele S, Nyström A, Durbeej M, Talts JF, Ekblom M, Ekblom P (2007) Laminin isoforms in development and disease. J Mol Med 85(8):825–836

Schumacher K, Strehl R, de Vries U, Groene HJ, Minuth WW (2002a) SBA-positive fibers between the CD ampulla, mesenchyme, and renal capsule. J Am Soc Nephrol 13(10):2446–2453

Schumacher K, Strehl R, Minuth WW (2002b) Detection of glycosylated sites in embryonic rabbit kidney by lectin histochemistry. Histochem Cell Biol 118:79–87

Schumacher K, Strehl R, Minuth WW (2003) Characterization of micro-fibers at the interface between the renal collecting duct ampulla and the cap condensate. Nephron Exp Nephrol 95:e43–e54

Schumacher K, Klar J, Wagner C, Minuth WW (2005) Temporal-spatial co-localisation of tissue transglutaminase (Tgase2) and matrix metalloproteinase-9 (MMP-9) with SBA-positive microfibres in the embryonic kidney cortex. Cell Tissue Res 319(3):491–500

Shaw L, Johnson PA, Kimber SJ (2010) Gene expression profiling of the developing mouse kidney and embryo. In Vitro Cell Dev Biol Anim 46(2):155–165

Strehl R, Minuth WW (2001a) Nephron induction—the epithelial mesenchymal interface revisited. Pediatr Nephrol 16(1):38–40

Strehl R, Minuth WW (2001b) Partial identification of the mab (CD) Amp1 antigen at the epithelial-mesenchymal interface in the developing kidney. Histochem Cell Biol 116(5):389–396

Strehl R, Kloth S, Aigner J, Steiner P, Minuth WW (1997) PCDAmp1, a new antigen at the interface of the embryonic collecting duct epithelium and the nephrogenic mesenchyme. Kidney Int 52(6):1469–1477

Strehl R, Trautner V, Kloth S, Minuth WW (1999) Existence of a dense reticular meshwork surrounding the nephron inducer in neonatal rabbit kidney. Cell Tissue Res 298(3):539–548

Stroo I, Stokman G, Teske GJD, Florquin S, Leemans JC (2009) Haematopoietic stem cell migration to the ischemic damaged kidney is not altered by manipulating the SDF-1/CXCR4-axis. Nephrol Dial Transplant 24(7):2082–2088

Sundelin B, Bohman SO (1990) Postnatal development of the interstitial tissue of the rat kidney. Anat Embryol (Berl) 182(4):307–317

Tanaka T, Nangaku M (2010) The role of hypoxia, increased oxygen consumption, and hypoxia-inducible factor-1 alpha in progression of chronic kidney disease. Curr Opin Nephrol Hypertens 19(1):43–50

Vidyasagar A, Reese S, Acun Z, Hullett D, Djamali A (2008) HSP27 is involved in the pathogenesis of kidney tubulointerstitial fibrosis. Am J Physiol Renal Physiol 295(3):F707–F716

Vigneau C, Zheng F, Polgar K, Wilson PD, Striker G (2006) Stem cells and kidney injury. Curr Opin Nephrol Hypertens 15(3):238–244