Origin of Parietal Podocytes in Atubular Glomeruli Mapped by Lineage Tracing

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ABSTRACT

Parietal podocytes are fully differentiated podocytes lining Bowman’s capsule where normally only parietal epithelial cells (PECs) are found. Parietal podocytes form throughout life and are regularly observed in human biopsies, particularly in atubular glomeruli of diseased kidneys; however, the origin of parietal podocytes is unresolved. To assess the capacity of PECs to transdifferentiate into parietal podocytes, we developed and characterized a novel method for creating atubular glomeruli by electrocoagulation of the renal cortex in mice. Electrocoagulation produced multiple atubular glomeruli containing PECs as well as parietal podocytes that projected from the vascular pole and lined Bowman’s capsule. Notably, induction of cell death was evident in some PECs. In contrast, Bowman’s capsules of control animals and normal glomeruli of electrocoagulated kidneys rarely contained podocytes. PECs and podocytes were traced by inducible and irreversible genetic tagging using triple transgenic mice (PEC- or Pod-rtTA/LC1/R26R). Examination of serial cryosections indicated that visceral podocytes migrated onto Bowman’s capsule via the vascular stalk; direct transdifferentiation from PECs to podocytes was not observed. Similar results were obtained in a unilateral ureter obstruction model and in human diseased kidney biopsies, in which overlap of PEC- or podocyte-specific antibody staining indicative of gradual differentiation did not occur. These results suggest that induction of atubular glomeruli leads to ablation of PECs and subsequent migration of visceral podocytes onto Bowman’s capsule, rather than transdifferentiation from PECs to parietal podocytes.


Multiple studies have reported the presence of podocytes on Bowman’s capsule in normal1 and diseased kidneys, close to the vascular pole in particular.2–5 These unique cells were named parietal podocytes and are defined as cells with features of differentiated podocytes lining the inner aspect of Bowman’s capsule (Figure 1A). Using an extended panel of antibodies, Bariety et al. showed that parietal podocytes expressed the same marker proteins as visceral podocytes.1 On electron microscopic images, parietal podocytes mostly form interdigitating foot processes3,6,7 and recruit periglomerular capillaries.8 In patients with membranous GN, parietal podocytes behaved similar to visceral podocytes in that they formed subepithelial deposits on the parietal basement membrane identical to those on the glomerular basement membrane (GBM).5

The number of parietal podocytes on Bowman’s capsule may vary significantly. Published data

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indicate that their number increases throughout life. In normal kidneys, parietal podocytes are rare, whereas their frequency and number is increased in transplant nephrectomies and in diseased kidneys. In atubular glomeruli or glomerular cysts, parietal podocytes can often be found on Bowman’s capsule—in many cases, covering its entire circumference. Atubular glomeruli and glomerular cysts are not uncommon even in chronology of tubular loss (Figure 1, C–F). Focal disruption of the tubular integrity, tubular necrosis, and infiltrating inflammatory cells were observed. Renal tissue distant from the coagulation site appeared unaffected. Seven days after coagulation, dilated tubules and interstitial infiltrating cells were found throughout the kidneys (Figure 1E). These pathologic findings were observed not only in the vicinity of the

Figure 1. Characterization of the early events in the coagulation model. (A) Schematic of a parietal podocyte on Bowman’s capsule forming foot processes (arrow) and recruiting periglomerular capillaries (asterisk). (B and B’) Schematic of the electrocoagulation model. Along the lateral margin of the kidney, a necrotic area is visible after coagulation (arrowheads, right kidney). (C) Immediately after coagulation, a triangular area of necrosis is observed, reaching from the renal cortex into the medulla. (D) Higher magnification of the interface between the coagulated renal tissue (arrow) and remaining parenchyma shows several necrotic tubules 3 days after coagulation (arrowheads) with inflammatory demarcation. (E) After 1 week, multiple tubules are dilated (arrowhead). (F) After 3 weeks, glomeruli with obliterated urinary poles (arrow) and atrophic tubular remnants (arrowheads) are observed. C–F show periodic acid–Schiff stainings of paraffin sections. (G) Immunohistologic double staining shows that cells obstructing the tubular outlet are SSeCKS-positive parietal cells (arrow) and that Bowman’s capsule is lined by parietal podocytes projecting from the vascular pole (arrowheads). (H–I) TUNEL assays consistently show positive nuclear staining in PECs (approximately 1–2 positive nuclei per 100 glomeruli) 1–3 weeks after electrocoagulation (G, arrow). (H) No nuclear staining is observed in healthy controls.

RESULTS

Glomerular Detubularization by Electrocoagulation

Because parietal podocytes were reported to occur predominantly in atublar glomeruli (i.e., in glomeruli that had been disconnected from their tubule), we first developed a novel model to induce atubular glomeruli in adult mice. In this model, short electrocoagulation pulses were applied via a thin needle inserted in different locations into the cortex of the right kidney (Figure 1, B and B’). Subsequent light microscopic examinations at different time points thereafter documented the classic
coagulation but also in areas more distant to the primary damage. Three weeks after coagulation, multiple atubular glomeruli were apparent (Figure 1F), in which the tubuloglomerular junction was obstructed by a cellular mass that consisted predominantly of SSeCKS-positive parietal cells (Figure 1G, see below). Terminal deoxynucleotidyl transferase–mediated digoxigenin-deoxyuridine nick-end labeling (TUNEL)–positive nuclei could be detected in PECs after 1, 2, and 3 weeks (1–2 TUNEL-positive PECs in 100 random glomerular cross-sections per animal) (Figure 1, H and I). This effect could not have been a consequence of doxycycline administration because TUNEL-positive PECs were not observed in a previous study in control mice.27

Characterization of the Coagulation Model to Induce Atubular Glomeruli
For a detailed characterization of the novel model of detubularization by electrocoagulation, a total of 80 mice were treated under different conditions. Atubular glomeruli developed in all mice independent of genetic background (Sv129 or C57Bl/6), sex, or age of the mice at coagulation (3 weeks versus 20 weeks of age) (Figure 2, A–E and G–J). Serial sections revealed that the urinary poles of all glomerular cysts were obstructed and had degenerated. In addition, cystic dilation of atubular glomeruli (“large glomerular cysts”) was observed particularly in Sv129 mice (Figure 2, A–C and K). The larger cysts reached a diameter of 500–750 μm (Figure 2B). Sv129 mice coagulated at a younger age and those analyzed 6 months after coagulation showed the highest percentage of dilated large cystic glomeruli (Figure 2K).

Within the larger cysts, glomerular cross-sections contained more proliferating cells on Bowman’s capsule (Figure 2L). Because the glomerular tufts were not always obvious in the examined tissue sections and to analyze whether these cysts represent glomerular or tubular cysts, double immunofluorescence staining was performed with synaptopodin, a podocyte marker, and SSeCKS, a protein expressed by PECs18,29 (Figure 3A). All large cysts, with or without a glomerular tuft, showed similar SSeCKS immunostaining of the cells lining the cyst, indicating the presence of PECs. Therefore, the large cysts were glomerular cysts (Figure 3B).

Parietal Podocytes in Atubular Glomeruli
Synaptopodin immunostaining revealed synaptopodin-positive cells lining Bowman’s capsule of atubular (cystic) glomeruli (Figure 3C, arrows). PEC marker SSeCKS was absent along segments of Bowman’s capsule covered by synaptopodin-positive cells (Figure 3, C’ and C”, arrows). In atubular (cystic) glomeruli, the transition from podocytes to PECs was shifted away from the vascular pole (Figure 3, C’ and C”, arrowheads).

Parietal podocytes identified by the expression of synaptopodin were observed predominantly in the coagulated kidneys and were a rare finding in control kidneys. Within the coagulated kidneys, parietal podocytes were localized exclusively within atubular (cystic) glomeruli regardless of the glomerular size, but were not observed in normal glomeruli of the same kidney (Figure 4, A and B). Parietal podocytes occurred in approximately 40%–45% of all glomerular cross-sections (counting all glomeruli, tubular or atubular) irrespective of genetic background, time since coagulation, sex, or age at intervention (Figure 4, C–F).

By transmission electron microscopy, parietal podocytes exhibited typical podocyte features such as large lobulated nuclei and interdigitating foot processes (Figure 5), regardless of the glomerular size. Not all parietal podocytes were fully differentiated and some showed a flattened appearance and partial foot process effacement, particularly in the unilateral ureter obstruction (UUO) model (see below). A potential explanation for this difference might be that parietal podocytes were analyzed after 6 months in the coagulation model versus 3 weeks in the UUO model. Occasionally, podocytes projected foot processes onto Bowman’s capsule as well as onto the glomerular tuft (Figure 5C). This was also observed in normal human kidney (Supplemental Figure 1).

Tracing Podocytes or PECs in Atubular Glomeruli
The origin of parietal podocytes was determined by lineage tracing.25,30–32 For this purpose, podocytes or PECs were irreversibly genetically tagged for 14 days using doxycycline including a wash-out phase of at least 7 days in transgenic Pod-rtTA/LC1/R26R30 or PEC-rtTA/LC1/R26R25 mice, respectively. After induction of atubular glomeruli by coagulation, animals were euthanized after 2 or 6 months and serial kidney sections were stained either for the genetic tag (β-galactosidase [β-gal]) (Figure 6, A1, A3, B1) or nephrin (Figure 6, A2, A4, B2), which is expressed exclusively by differentiated podocytes in the kidney.

Tracing of podocytes in atubular glomeruli revealed the appearance of genetically tagged podocytes on Bowman’s capsule (Figure 6, A1–A4). In cells lining Bowman’s capsule, nephrin was expressed exclusively by genetically tagged podocytes. In normal glomeruli, no β-gal or nephrin expression was observed on Bowman’s capsule (not shown). Genetically labeled PECs were detected exclusively on Bowman’s capsule in both normal and atubular glomeruli. In atubular glomeruli, genetically tagged PECs were found next to parietal podocytes (Figure 6, B1 and B2). In summary, these findings show that parietal podocytes originate from differentiated visceral podocytes migrating onto the Bowman’s capsule. By transmission electron microscopy using Bluo-Gal staining (forming an insoluble intracellular black precipitate in cells expressing β-gal), capsules of atubular glomeruli were shown to contain genetically tagged podocytes with a typical podocyte phenotype, confirming our results (Figure 6C).

Formation of Parietal Podocytes in Atubular Glomeruli in the UUO Model
We next assessed the UUO model, which results in synchronized formation of atubular glomeruli in the entire kidney.33 After UUO, the glomerular architecture remains largely
Figure 2. Characterization of the coagulation model. (A) Atubular glomerular cysts (arrowheads) and adjacent intact renal parenchyma (interface highlighted with arrows). (B) Higher magnification of a large glomerular cyst with a characteristic hypotropic tuft (arrowhead). The urinary pole is obstructed by cells and extracellular matrix (arrow). (C) Normal tubules are located immediately adjacent to atrophic, protein-filled tubules (arrow). A–C show periodic acid–Schiff stainings of kidneys from 6-month-old Sv129 mice, electrocoagulated at 3

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*Figure 2 continues...*
preserved. Three weeks after UUO, marked tubular damage, interstitial cellular infiltration, and formation of atubular glomeruli with obstruction of the urinary pole by a cellular layer were observed (Figure 7, A and B, arrowheads). Staining for the podocyte markers synaptopodin and nephrin revealed parietal podocytes on Bowman’s capsule (Figure 7, C–G). Serial cryosections stained for β-gal or nephrin in the triple transgenic Pod-rtTA/LC1/R26R mice confirmed migration of visceral podocytes as the origin of parietal podocytes (Figure 7, E–E’). Again, TUNEL-positive nuclei were observed in PECs 7 days after UUO indicating apoptosis or necrosis of these cells (Figure 7H).

**Atubular Glomeruli in Human Kidney**

First, the normal expression pattern of synaptopodin, claudin-1, was examined in normal kidneys, one of which was a kidney from an 18-year-old healthy individual (who died in a car accident). Synaptopodin and claudin-1 did not colocalize and were expressed exclusively by cells on the glomerular tuft or on Bowman’s capsule, respectively (Figure 8, A–A’). Twelve human kidneys were evaluated for atubular glomeruli on serial sections (four and eight kidneys with or without renal pathologies, respectively). In atubular glomeruli, podocyte marker synaptopodin was expressed by cells lining Bowman’s capsule, indicating the presence of parietal podocytes, close to the vascular pole in particular (Figures 8, C–E” and 9). PEC marker claudin-1 was not detected, indicating that PECs were absent (Figure 8, B–B”). Interestingly, no overlap or colocalization of podocyte marker synaptopodin and PEC marker claudin-1 was observed in atubular glomeruli lined partially by parietal podocytes. In approximately 15% of all glomeruli of diseased kidneys, the entire Bowman’s capsule was lined by synaptopodin-positive parietal podocytes (Figure 9). In these glomeruli, claudin-1 expression was not detectable (Figure 8, C–C”). Costaining for the marker synaptopodin and the PEC marker CD133 also did not show any overlap (Figure 8, D–E”). As a side finding, approximately 30%–40% of all glomeruli show few parietal podocytes (located mostly close to the vascular pole) even in healthy human kidneys (Figure 9). This finding suggests that the boundary between podocytes and parietal cells may shift somewhat (a similar finding is also shown in Supplemental Figure 1).

**DISCUSSION**

As outlined in the introduction, atubular glomeruli and glomerular cysts can be observed in a wide range of renal diseases. This study sheds light on the pathogenesis: Tubular injury resulting in permanent loss of the affected tubule may be a major mechanism by which atubular glomeruli are formed in human kidneys. Thus, the frequency of atubular glomeruli may indicate the sum of tubular losses within an individual kidney.

In this study, we first established a new model to induce atubular glomeruli in adult kidneys in experimental mice. A systematic characterization showed that this induced the formation of large glomerular cysts in some animals. Formation of larger glomerular cysts was determined by the age at disease induction, the length of the postoperative observation period until euthanasia, and the genetic background. The results of this study are in good agreement with previous observations that fetal urinary obstruction results in glomerular cyst formation. In addition, it has been shown that glomerular cysts can be induced by high-dose corticosteroid injections into newborn rabbits. In this study, we show that formation of glomerular cysts is also feasible in adult animals and that glomerular cysts can result from renal pathologies acquired in adult life.

What triggers the cystic enlargement of atubular glomeruli? We previously described the Pax8-Cre/β-catenin^{fl}^{fl} mouse in which Bowman’s capsules are lined by fully differentiated parietal podocytes and not by PECs. We showed that inactivation of β-catenin during renal development causes glomerular epithelial cell precursors on Bowman’s capsule to differentiate toward the podocyte lineage. Of note, these glomeruli formed large cysts but were not atubular. These findings argue against the notion that blocked filtration and subsequently raised intraglomerular pressures mediate dilation of atubular glomeruli into cysts. Rather, the presence of parietal podocytes on Bowman’s capsule appears to be a critical prerequisite for glomerular cyst formation.

The second major and surprising finding of this study is that parietal podocytes originate from visceral podocytes migrating from the vascular pole onto Bowman’s capsule (summarized in Figure 10). Using irreversible genetic tagging, it became possible to trace podocytes or PECs after induction of atubular cysts and observe the proportion of cells with or without parietal podocytes.
glomeruli for the first time. Three possible explanations for the origin of parietal podocytes have been discussed in the literature over the years: (1) evagination of the capillary tuft, due to high intraglomerular pressures; (2) direct transdifferentiation of PECs toward a podocyte phenotype; or (3) migration of podocytes from the tuft. The first explanation of evagination involves unfolding or spreading of the glomerular tuft along the Bowman’s capsule due to the enlargement of the Bowman’s space. By this process, it is inferred that segments of the glomerular tuft become an integral part of Bowman’s capsule. This mechanism would be consistent with the finding that the largest glomerular cysts usually contain the smallest and most rudimentary tuft remnants, consistent with previous studies.

Our study cannot exclude evagination as one of the processes leading to the parietal podocytes in the glomerular cysts. Nonetheless, in the “normal sized” atubular glomeruli that were analyzed in both models, parietal podocytes were also present on Bowman’s capsule despite the lack of cystic glomerular enlargement. The fact that the size of the glomerular cyst is inversely correlated with the size of the glomerular tuft is also consistent with the third explanation that visceral podocytes migrate onto Bowman’s capsule. In the largest cysts, there might be an increased demand of podocytes to cover the inner aspect of Bowman’s capsule. As a consequence, more podocytes will be recruited away from the glomerular tuft onto Bowman’s capsule. Finally, as already outlined above, glomerular cyst formation does not require increased intraglomerular pressures.

The second explanation is that PECs have the capacity to transdifferentiate into podocytes on Bowman’s capsule. Podocytes are an essential part of the glomerular filtration barrier but cannot regenerate themselves. Loss of podocytes beyond a certain threshold is sufficient to trigger glomerulosclerosis. We recently showed that late in renal development, a fraction of podocytes are recruited from cells on Bowman’s capsule (presumptive PECs), offering the perspective that this mechanism might still be preserved in adults. However, the lineage tracing experiments of this study in adult mice showed no evidence for transdifferentiation of PECs to a podocyte phenotype in atubular glomeruli, ruling out the second explanation in this specific situation. This is consistent with lineage tracing studies of two research groups (including ourselves) in adult mice during aging or

Figure 3. Cysts induced by electrocoagulation are glomerular cysts. (A) In normal glomeruli, synaptopodin (A, arrow) and SSeCKS (A’, arrow) are specifically detected in podocytes and PECs, respectively. The boundary from synaptopodin-positive podocytes and SSeCKS-positive PECs is at the VP (arrow). (B) Overview kidney section from a 6-month-old Sv129 mouse coagulated at 3 weeks of age containing a normal segment and a segment containing atubular and cystic glomeruli costained for synaptopodin and SSeCKS. Both cysts with (arrows) and without (asterisks) a visible glomerular tuft show expression of the PEC marker SSeCKS, indicating that all represent glomerular cysts. (C) Synaptopodin expression is observed on Bowman’s capsule in multiple atubular glomerular cysts (arrows). These parietal podocytes are negative for SSeCKS (C’, arrows). In these glomeruli, the boundary between podocytes and PECs shifts from the vascular stalk onto Bowman’s capsule (3C”, arrowheads). VP, vascular pole; UP, urinary pole.
after induction of progressive glomerular hypertrophy. Under these circumstances, as in this study, no recruitment of podocytes from parietal cells was observed (Berger et al. and Wanner et al., unpublished data). However, we did confirm our earlier findings that podocytes are recruited from cells of Bowman’s capsule in newborn mice after the capillary loop stage.25 These results indicate that some cells on Bowman’s capsule differentiate directly into podocytes, likely forming a small reserve of podocytes (10% at the most) that are recruited onto the glomerular tuft as the glomeruli undergo physiologic hypertrophy during adolescence.

It was recently proposed that in normal glomeruli of human kidneys, parietal podocytes may form close to the vascular pole as a result of progressive differentiation of CD133- and CD24-positive PECs acting as a potential regenerative cell population for visceral podocytes.26,44 The hypothesis describing differentiation of PECs into new podocytes is based to a large extent on the observation of a gradient in the expression of differentiation markers. Specifically, toward the vascular pole, CD133- and CD24-positive PECs were described to coexpress the presumptive podocyte marker proteins podocalyxin, nestin, and WT-1. Further along, mature podocytes progressively lost the expression of the PEC “progenitor” markers CD133 and CD24, resulting in a gradient of differentiating cells. These observations were all done in human kidneys. In this study, we analyzed the expression of the PEC markers claudin-1 and CD133 and the podocyte marker synaptopodin in the human kidneys. In our hands, the transition from parietal podocytes to PECs was abrupt, supporting that PECs and parietal podocytes are distinct cell populations. Of note, because the origin of parietal podocytes was analyzed in this study by lineage tracing in cystic and/or atubular glomeruli exclusively, these results do not rule out the previously

Figure 4. Parietal podocytes in atubular glomeruli. (A and B) Bowman’s capsules of atubular glomeruli are lined by synaptopodin-positive parietal podocytes regardless of the cyst diameter. (C–F) Influence of genetic background (38 Sv129 mice versus 36 C57BL/6 mice) (C), postoperative observation time until euthanasia (30 mice at 2 months versus 44 mice at 6 months) (D), sex of the mice (39 female mice versus 35 male mice) (E), or age at coagulation (37 mice at 3 weeks versus 37 mice at 20 weeks) (F) on the frequency of atubular glomeruli with parietal podocytes. The y-axis shows the percentage of glomeruli with synaptopodin-positive cells on Bowman’s capsule versus all glomeruli on a renal cross-section, with 50 glomeruli per mouse analyzed. *P<0.05; **P<0.01; ***P<0.001.

Figure 5. Ultrastructure of atubular glomeruli. (A) Transmission electron microscopic images of an atubular glomerulus forming a glomerular cyst. Parietal podocyte differentiation varies along Bowman’s capsule, with areas of partial foot process effacement (arrow) between areas with interdigitating foot processes (arrowheads). (B) At higher magnification, a parietal podocyte forms interdigitating foot processes (arrowhead), resides on a thin GBM-like basement membrane, and recruits a periglomerular capillary indicating vascular endothelial growth factor expression (arrow). (C) A podocyte adheres to the glomerular tuft as well as to Bowman’s capsule forming foot processes on both sides (arrowheads).
postulated regeneration of podocytes by PECs under physiologic conditions.\textsuperscript{25,26}

The major finding of this study is that visceral podocytes may migrate from the glomerular tuft onto Bowman’s capsule. This concept is very surprising and unexpected. Thus far, podocytes have only been proven to migrate onto Bowman’s capsule in the context of an acute inflammatory nephritis such as rapidly progressive GN (RPGN).\textsuperscript{31,45–47} In RPGN, podocytes dedifferentiate and migrate via direct adhesions onto Bowman’s capsule. There they contribute to the formation of cellular crescents by proliferation together with activated PECs. In this study, we show for the first time that fully differentiated podocytes may also leave the glomerular tuft. In contrast to RPGN, the exodus of podocytes occurred \textit{via} the vascular stalk. In this study, the boundary between podocytes and PECs may shift significantly from the vascular stalk onto Bowman’s capsule. In normal glomeruli of human kidneys, parietal podocytes could often be observed close to the vascular pole and in continuity to the visceral podocytes, suggesting that the boundary between the two cell types may shift gradually over time. Isolated patches of labeled parietal podocytes were rarely observed,\textsuperscript{1} supporting the observation that the vascular stalk is the major route of differentiated podocytes onto Bowman’s capsule.

Progressive loss of PECs in atubular glomeruli could be a potential trigger for differentiated podocytes to migrate onto Bowman’s capsule. Sporadic TUNEL-positive nuclei were consistently observed in PECs. PECs are ciliated cells, similar to tubular epithelial cells, whereas podocytes do not form a primary cilium.\textsuperscript{6,48} It has been proposed that tubular cells require the urinary flow, which is sensed by the primary cilium, for survival.\textsuperscript{49–51} Tubular cells—as well as PECs, as suggested by our findings—may become apoptotic/necrotic after interruption of the urinary flow by electrocoagulation or UUO. We propose that the decreasing number of PECs on Bowman’s capsule might represent the stimulus for visceral podocytes to migrate from the capillary tuft and populate Bowman’s capsule. Both cell populations are in direct contact so that migration of podocytes might be mediated by “disinhibited” lateral contact inhibition (similar to the wound healing assay in \textit{in vitro} systems).

In summary, this study revealed a surprising and unexpected finding in atubular glomeruli, which is opposite to what was previously generally believed. Differentiated podocytes may migrate from the glomerular tuft \textit{via} the vascular pole onto Bowman’s capsule.

**CONCISE METHODS**

**Transgenic Mice**

PEC-rtTA/LC1/R26R and POD-rtTA/LC1/R26R transgenic mice were previously described.\textsuperscript{25,38,52} Animals were housed under standard
specific pathogen-free conditions, including a 12-hour light cycle. All animal procedures were approved by the local authorities (LANUV NRW, AZ 8.87-51.04.20.09.345).

**Doxycycline Treatment**
The PEC-rtTA and the POD-rtTA animals received doxycycline hydrochloride *via* the drinking water *ad libitum* for a total of 14 days (5% sucrose, 0.5 mg doxycycline/ml, protected from light), which was exchanged every 2 days. To be sure that the mice were free from doxycycline during the experimental procedures, the mice received normal drinking water *ad libitum* for at least 7 days.

**Electrocoagulation Model**
Twelve 3-week-old Sv129 mice (equal sex distribution) underwent the following procedure. Mice were anesthetized with ketamine-xylazine (100 mg/ml ketamine and 20 mg/ml xylazine in normal saline 0.9%; 0.1 ml per 10 g of body weight) and positioned on a counter electrode. Afterward mice were shaved and disinfected and a laparotomy was performed. The intestine was mobilized and the right renal capsule was removed. A thin needle was then injected in the renal margin into a depth of approximately 3–4 mm, and an electrical current was subsequently applied along the needle using an electro-surgical unit. This was repeated five times in regular distances. The left kidney remained unaltered. After the intervention, the mice were treated with buprenorphine for 3 days (0.05–0.1 mg/kg subcutaneously). Mice were euthanized after 3, 7, 14, and 21 days and renal tissue was preserved for microscopic examination. For characterization of the model, 3-week-old Sv129 and Bl6 mice (*n*=20 each, equal sex distribution) and 20-week-old Sv129 and Bl6 mice (*n*=20 each, equal sex distribution) were subjected to electrocoagulation as described above (Charles River Laboratories International Inc., Wilmington, MA). Mice were euthanized as shown in Table 1.

Six-week-old POD-rtTA/LC1/R26R (*n*=12), LC1, R26R transgenic mice a complete UUO of the right kidney was performed. Animals were anesthetized as mentioned above, and the ureter was exposed by a median laparotomy and then ligated with 8-0 nylon. One third of the animals (*n*=4) were euthanized after 1, 2, and 3 weeks, respectively. One third of the animals (*n*=4) were euthanized after 1, 2, and 3 weeks, respectively.

**Perfusion Fixation**
Mice were anesthetized (ketamine/xylazine) and perfused with 3% paraformaldehyde in PBS (pH 7.4) via the left ventricle for 3 minutes. Pieces of both kidneys were snap-frozen in Tissue-Tek (Miles Inc., Iowa City, IA), postfixed in 3% buffered formalin for 24 hours, and embedded in paraffin.

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**Figure 7.** Lineage tracing of parietal podocytes in the UUO model. (A) Typical morphologic changes are observed 3 weeks after UUO on sections stained with periodic acid-Schiff. These include degeneration of renal medulla (black arrows), dilated proximal tubules with protein casts (white arrows), and atubular glomeruli (arrowheads). (B) Degeneration of the glomerulotubular junction. A thin basement membrane with a layer of cubic cells (arrowheads) has disconnected the proximal tubule (arrow). (C–D’) Three weeks after UUO, the Bowman’s capsule of the atubular glomeruli is lined by synaptopodin- and nephrin-positive parietal podocytes (arrows). (E and E’) Serial cryosections of a Pod-rtTA/LC1/R26R mouse show colocalization of β-gal and nephrin staining in parietal podocytes 3 weeks after UUO (arrows). (F) Statistical analysis of the percentage of glomeruli with synaptopodin-positive cells on Bowman’s capsule 1–3 weeks after UUO (12 mice total, 50 glomeruli per time point). Using ANOVA and Bonferroni analysis, no statistically significant differences are observed between experimental animals over time. (G) Negative control for nephrin immunostaining using irrelevant guinea pig antiserum as a primary antibody shows no reactivity. (H) Seven days after UUO, nuclear TUNEL staining is observed in PECs (arrows; SSeCKS, TUNEL, Hoechst triple staining). Nonspecific green fluorescence arises from erythrocytes in glomerular and tubulointerstitial capillaries. ***P<0.001.
**Light Microscopy**

Four-micrometer paraffin sections were stained with periodic acid–Schiff. β-gal expression was visualized using enzymatic X-gal staining. For this purpose, 4- to 6-μm cryosections were incubated overnight at 37°C in a humidified atmosphere in staining solution (1 mg/ml X-gal, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 2 mM MgCl₂ in PBS pH 7.8). The next day, samples were stained with eosin, washed in tap water, and mounted (Immuno-Mount; Thermo Scientific, Waltham, MA).

Immunofluorescence and immunohistochemical stainings were performed on renal paraffin and cryosections. For the single and double staining, the paraffin sections were rehydrated in PBS and subjected to microwave heating (3 × 5 minutes at 600 W with Antigen unmasking solution; Vector Laboratories, Burlingame, CA). Sections were incubated with the following primary antibodies: rabbit anti-claudin-1 (1:50, RB-9209-P1; Thermo Fisher), goat anti-synaptopodin (1:100, sc-50459; Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti-synaptopodin (1:50, 65294; Progen, Brisbane, Australia), rabbit anti-SSeCKS (1:100; Irwin H. Gelman Laboratory, Roswell Park Cancer Institute, Buffalo, NY), rabbit anti-Ki67 (1:100, ab166667; Abcam, Cambridge, UK), and guinea pig anti-nephrin (1:400, GP-N2; Progen). The following secondary antibodies were used for immunofluorescence: DyLight 488 anti-rabbit (1:200, 711-485-152; Dianova, New Paltz, NY), DyLight 549 anti-mouse (1:200, 715-505-151; Dianova), goat anti-rabbit Cy-3 (1:200, 711-165-152; Dianova), and donkey anti-rabbit Alexa Fluor 647, 1:200, 711-605-152, Dianova). Slides were counterstained with Hoechst 33258 (Molecular Probes, Darmstadt, Germany) to stain nuclei. The following secondary antibodies were used for immunohistochemistry: biotinylated anti-guinea pig...

**Figure 8.** Podocyte and PEC marker expression in humans. (A–A′′) Immunofluorescence double stainings of an apparently normal kidney of an 18-year-old old healthy man show no colocalization of the podocyte marker synaptopodin and the PEC marker claudin-1 on Bowman’s capsule. (B–E′′) Sporadic atubular glomeruli in human kidneys. Synaptopodin-positive parietal podocytes (arrowhead in B) does not show costaining for the PEC marker claudin-1 in atubular glomeruli (arrowheads in B′ and B′′). A sharp border between synaptopodin and claudin-1-stained cells is always observed in atubular glomeruli (B′′, arrowhead). Atubular glomeruli in which the entire Bowman’s capsule stained for synaptopodin are negative for claudin-1 (C–C′′, arrows). (D–E′′) Similarly, no costaining for synaptopodin (podocytes) and CD133 (PECs) is observed in glomeruli in which Bowman’s capsule is totally (D) or partially (E) lined by synaptopodin-positive parietal podocytes.

**Table 1.** Characteristics of the study mice

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**Figure 9.** Statistical analysis of the proportion of Bowman’s capsules covered by synaptopodin-positive cells (presumptive parietal podocytes). More than half of the glomeruli do not show parietal podocytes. In a significant portion of glomeruli (approximately 30%–40%), few parietal podocytes are detected (mostly close to the vascular pole, indicating that minor shifts of the podocyte to parietal cell interface occur in human kidneys over time). Bowman’s capsules populated more than one third by parietal podocytes occur predominantly in diseased kidneys and represent atubular glomeruli. Four and eight total kidney specimens with or without renal pathologies, including all 1404 random cross-sections of individual glomeruli, are analyzed.
IgG (1:300, BA-7000; Vector Labs, Burlingame, CA) and biotinylated anti-rabbit IgG (1:300, BA-1000; Vector Labs). The Vectastain ABC kit (Vector Labs) and the Vector Peroxidase Substrate kit (SK-4100; Vector Labs). For nucleus staining, slides were counterstained using Mayer’s hemalaun solution (Merck, Darmstadt, Germany). The Apoptosis Detection Kit S7111 was used (Chemicon International Inc, Temecula, CA) for TUNEL staining. Sections were evaluated with a Leica DMRX microscope (Leica, Wetzlar, Germany). Images were collected with Diskus (Diskus Kameras, Koenigswinter, Germany) and prepared for presentation with Adobe Photoshop CS5 and Illustrator CS5 software (Adobe Systems, Mountain View, CA).

Transmission Electron Microscopy
For transmission electron microscopy, 2×2 mm pieces of the kidneys were either postfixed in Karnovsky fixative, processed, and embedded into Epon using standard procedures, or they were fixed in 3% glutaraldehyde solution for 1 hour, subsequently incubated in Bluo-Gal solution at 37°C overnight, and were then treated as mentioned above.

Human Renal Tissue
Human renal tissues were sourced from the database of the Institute of Pathology in Aachen and one unused transplant kidney from the School of Physiology and Pharmacology, University of Bristol (Bristol, UK). Use of these tissues was approved by the local ethics committee in both countries. We used specimens of kidneys with recurrent pyelonephritides because tubular destruction in this disease often leads to atubular glomeruli. The details on each specimen are listed in Tables 2 and 3.

Evaluation of Glomerular Cysts
At least 50 glomeruli of each experimental animal were evaluated on random transverse sections through the kidney. Cavities with a diameter >100 μm and without a glomerular tuft were counted only when the capsule was typical for an atubular glomerulus. Glomeruli with an obvious Bowman’s space and a diameter of >100 μm and <150 μm were categorized as small glomerular cysts. Glomeruli with a diameter >150 μm were categorized as large glomerular cysts.

Serial Block Face Scanning Electron Microscopy of Human Glomeruli
An unused transplant kidney was perfused with transport solution (Soltran) and transported on ice. Renal arteries were cannulated and perfused (100 mmHg hydrostatic pressure) with a mammalian HEPES Ringer solution containing Ficoll 400 as an oncotic balance (25 mmHg colloid osmotic pressure). This was replaced with a glutaraldehyde fixative solution at the same pressures. Kidney tissues were fixed and loaded with heavy metals en bloc using osmium teroxide, osmium ferrocyanide, thiocarbohydrazide, osmium tetroxide, uranyl acetate, and lead aspartate, and then dehydrated and embedded in a hard formulation resin. Blocks of tissue were trimmed to one glomerulus and this was mounted in a Serial Block Face Scanning Electron Microscope set to take 500 sections at 150-nm thick (total 75-μm depth).

Figure 10. Schematic for the origin of parietal podocytes. In atubular glomeruli, the urinary outlet is disconnected from the tubule (orange) by a layer of epithelial cells (blue, PECs and/or proximal tubular cells) forming their own basement membrane (arrowhead) and becomes obstructed. PECs (blue) are progressively lost and visceral podocytes (green) migrate from the capillary tuft onto Bowman’s capsule via the vascular stalk to become parietal podocytes (arrow).
and image the block face at each section level. Image stacks were manipulated using ImageJ software (National Institutes of Health, Bethesda, MD).

**Evaluation of Synaptopodin Staining**
Fifty glomeruli of each experimental animal on random transverse sections through the kidney were analyzed. All of those glomeruli (also those without a cut glomerular tuft) with a synaptopodin positively stained Bowman’s capsule were put into relation to the sum of analyzed glomeruli.

**Statistical Analyses**
For the electrocoagulation or UUO model in experimental mice, 7400 glomeruli were analyzed; 700 glomeruli were analyzed in serial sections through the kidney were analyzed. All of those glomeruli (also those without a cut glomerular tuft) with a synaptopodin positively stained Bowman’s capsule were put into relation to the sum of analyzed glomeruli.

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**DISCLOSURES**
None.

**REFERENCES**