Differential Expression of the Intermediate Filament Protein Nestin during Renal Development and Its Localization in Adult Podocytes

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Nestin, an intermediate filament protein, is widely used as stem cell marker. Nestin has been shown to interact with other cytoskeleton proteins, suggesting a role in regulating cellular cytoskeletal structure. These studies examined renal nestin localization and developmental expression in mice. In developing kidney, anti-nestin antibody revealed strong immunoreactivity in vascular cleft of the S-shaped body and vascular tuft of capillary loop-stage glomerulus. The nestin-positive structures also were labeled by endothelial cell markers FLK1 and CD31 in immature glomeruli. Nestin was not detected in epithelial cells of immature glomeruli. In contrast, in mature glomerular, nestin immunoreactivity was observed only outside laminin-positive glomerular basement membrane, and co-localized with nephrin, consistent with podocyte nestin expression. In adult kidney, podocytes were the only cells that exhibited persistent nestin expression. Nestin was not detected in ureteric bud and its derivatives throughout renal development. Cell lineage studies, using a nestin promoter–driven Cre mouse and a ROSA26 reporter mouse, showed a strong β-galactosidase activity in intermediate mesoderm in an embryonic day 10 embryo and all of the structures except those that were derived from ureteric bud in embryonic kidney through adult kidney. These studies show that nestin is expressed in progenitors of glomerular endothelial cells and renal progenitors that are derived from metanephric mesenchyme. In the adult kidney, nestin expression is restricted to differentiated podocytes, suggesting that nestin could play an important role in maintaining the structural integrity of the podocytes.


Nestin is a cytoskeleton-associated class VI intermediate filament protein (1). The functional significance of nestin expression has not been fully elucidated (2). Recent studies demonstrate that nestin can interact with the three major components of cytoskeleton (microfilaments, intermediate filaments, and microtubules), suggesting an important role in coordinating changes in cell dynamics, including cell division (3). Nestin is first identified in mitotically active central and peripheral nervous system progenitor cells that give rise to both neurons and glia during early neurogenesis (4–6). Subsequently, nestin is detected in progenitor cells of non-neuronal tissues, including muscle (7,8), heart (7), pancreas (9–11), and skin follicle (12). Nestin expression also has been reported in adult tissues, such as skin and central nervous system, but is restricted mainly to areas of regeneration (6,12,13). Owing to its characteristic expression pattern, nestin generally is considered a marker of stem cells or progenitor cells (1,6,12).

The kidney is characterized by substantial heterogeneity of cell types (14). Developmentally, the kidney is derived from two primordial structures: The ureteric bud and the metanephric mesenchyme (15). The epithelial cells in the collecting duct are derived from ureteric bud, and the remainder of the nephron derives from metanephric mesenchyme, which differentiates into more than 26 distinct cell types (14). Determining whether nestin marks particular progenitor cells during kidney development could be important for understanding renal development and the damage–repair process in the diseased kidney. In addition, because nestin is a cytoskeletal intermediate filament protein, which contains binding domains for microtubule and microfilament actin, it also may be involved in maintenance of the integrity of the cytoskeletal structure of renal cells, including the glomerular podocyte (16). Disorganization or abnormal expression of podocyte cytoskeleton proteins has been suggested to cause podocyte foot process effacement and proteinuria (17,18). A recent study that used Cre recombinase LacZ reporter suggested activity of nestin promoter–driven Cre (NesCre) in some cells within the kidney (19). To determine the potential role of nestin in the glomerular podocyte and in kidney development, our studies examined expression of nestin in developing and adult kidney.

Materials and Methods

Animals

C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were used to examine nestin distribution in developing and adult kidney. For cell
B-Gal Staining

Kidneys from ROSA26R/NesCre double-transgenic mice were fixed in 4% paraformaldehyde and 0.25% glutaraldehyde in PBS for 2 h at 4°C, and tissue sections were cut with a vibratome into 200-μm slices. For detection of β-gal activity, these slices were bathed twice in permeabilization solution (2 mM MgCl₂, 0.01% sodium deoxycholate, and 0.02% Nonidet P-40 in PBS) for 30 min and then stained with 1 mg/ml x-galactosidase (Sigma, St. Louis, MO) in staining solution (2 mM MgCl₂, 5 mM potassium ferricyanide, potassium ferrocyanide, and 20 mM Tris [pH 7.4] in PBS) at room temperature in the dark for 48 h. Tissues were washed, dehydrated, and embedded in paraffin using standard procedures. Serial 5-μm sections were cut and examined by light microscopy.

Immunohistochemistry

Mouse kidneys were fixed with 4% paraformaldehyde and embeded in paraffin. Four-micrometer sections were cut and blocked with 10% donkey serum/PBS for 30 min at room temperature. Then, sections were incubated with the following primary antibodies: (1) Anti-nestin (1:1000, 556309; BD Pharmingen, San Diego, CA), (2) anti-Tamm-Horsfall protein (THP) antibody (1:2500; Organon-Technika, Durham, NC), (3) anti-aquaporin-2 (AQP2) antibody (anti-rat AQP2 IgG no. 2; Alpha Diagnostic International, San Antonio, TX), (3) anti-thiazide-sensitive NaCl co-transporter (TSC) antibody (provided by Dr. Mark Knepper, National Institutes of Health, Bethesda, MD), and (4) anti-AQP1 antibody (1:500, AB3065; Chemicon International, Temecula, CA). After 60 min of incubation, the sections were washed and incubated with biotinylated anti-IgG secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA) for 60 min. Biotin was identified by using streptavidin coupled to horseradish peroxidase and was visualized with diaminobenzidine (Vectastain ABC kit; Vector Laboratories, Burlingame, CA). Sections were viewed and imaged with a Zeiss Axioskop and spot-cam digital camera (Diagnostic Instruments, Sterling Heights, MI).

Immunofluorescence

Kidney tissues were fixed with 4% paraformaldehyde and incubated in 30% sucrose overnight. Alternatively, kidney tissues were snap-frozen in a dry ice-acetone bath. Cryostat sections (5 μm) were fixed in acetone for 10 min at −20°C (only for snap-frozen sections) and were blocked with 10% normal donkey serum for 20 min. A blocking buffer from M.O.M kit (Vector Laboratories) was used when the primary antibody was mAb. Sections then were incubated with primary antibodies for 60 min. After washing, the sections were incubated in Cy2- or Cy3-conjugated anti-IgG secondary antibody (Jackson Immunoresearch Laboratories) for 30 min. Sections were viewed and imaged with a Zeiss Axioskop and spot-cam digital camera (Diagnostic Instruments) or confocal microscopy (Zeiss LSM510). The primary antibodies that were used for immunofluorescent studies included anti-nestin antibody (1:200; Pharmingen, San Diego, CA), nephrin (1:50, sc19000; Santa Cruz Biotechnology, Santa Cruz, CA), anti-CD31 antibody (1:100, clone MECl3.3; Pharmingen), anti-FKl1 antibody (1:50; Pharmingen), laminin (1:40, AB2034; Chemicon International), and Cited1 (1:250, RB9219; Lab Vision Corp., Fremont, CA).

In Situ Hybridization

In situ hybridization was performed as described previously (20). A 200-bp nestin cDNA fragment from the 3’ untranslated region of mouse nestin was obtained from an EST clone (6417060; Invitrogen, Carlsbad, CA) and subcloned into pBluscript SKII. Sense and antisense riboprobes were transcribed in the presence of [α-35S]UTP. The kidneys were fixed with 4% paraformaldehyde and embedded in paraffin. Sections (7 μm) were cut and hybridized to 50 to 55°C for approximately 18 h. After hybridization, sections were washed at 50°C in 50% formamide, 2× SSC, and 100 mM β-mercaptoethanol for 60 min; treated with RNase A (10 mg/ml, 37°C, 30 min); and followed by washes in 19 mM Tris, 5 mM EDTA, 500 mM NaCl (37°C), 2× SSC (50°C), and 0.1’ SSC (50°C). Slides were dehydrated with ethanol that contained 300 mM ammonium acetate. Photomicrographs were taken from slides that were dipped in K5 emulsion (Ilford Ltd., Knutsford, Cheshire, UK) that was diluted 1:1 with 2% glycerol/water and exposed for 7 d at 4°C. After development in Kodak D-19, slides were counterstained with hematoxylin. Photomicrographs were taken with a Zeiss Axioskop microscope using bright-field optics.

Immunoblot

Nestin immunoblots were performed on tissue lysates from the kidney of mice with different ages. Protein concentration was determined using bicinchoninic acid protein assay (Sigma). Twenty micrograms of protein extract was separated on a 10% SDS-PAGE. Protein was transferred to a polyvinylidene difluoride membrane at 22 V overnight at 4°C. The membrane was washed three times with TBST (50 mM Tris [pH 7.5], 150 mM NaCl, and 0.05% Tween 20) and then incubated in blocking buffer (150 mM NaCl, 0.05% Tween 20) and then incubated in blocking buffer (150 mM NaCl, 50 mM Tris, 0.05% Tween 20, and 5% Carnation nonfat dry milk [pH 7.5]) for 1 h at room temperature. The membrane then was incubated with an anti-nestin antibody (1:500; Pharmingen) overnight at 4°C. After three washes, the membrane was incubated with a horseradish peroxidase–conjugated secondary antibody (1:20,000; Jackson Immunoresearch Laboratories) for 1 h at room temperature followed by three 15-min washings. Antibody labeling was visualized with ECL (Amersham Biosciences, England, UK).

Nestin Silencing by siRNA

An immortalized murine podocyte cell line was provided by Dr. Mundel (21). The cells were cultured at 37°C for 7 d to allow for differentiation. The cells then were transfected with nestin siRNA vector or control vector using SuperFect (Qiagen, Valencia, CA). Nestin siRNA was synthesized according to the mouse nestin cDNA sequence GGAAGTGACTAGTGAGACA. The siRNA cassette was subcloned into an expression vector driven by an U6 promoter. The vector also contains a cytomegalovirus-driven enhanced green fluorescent protein (eGFP) so that transfection can be monitored by examining eGFP under fluorescence microscope. In differentiated podocyte, the transfection efficiency of nestin siRNA was 15 to 20%. To determine the effect of nestin silencing on podocyte process formation, we examined the cells that were transfected with nestin siRNA or control vector that contained nonhomologous oligo, and the cells with the projections that were longer than maximal length of the cell body (designated as cells with process) in siRNA or control vector–transfected cells were counted, respectively. The percentage of cells with processes was compared between nestin siRNA–transfected and control vector–transfected cells.
Results

Nestin Is Selectively Expressed in the Glomerular Podocyte of Adult Kidney

Strong glomerular nestin immunoreactivity was observed by immunohistochemistry in adult mouse (Figure 1, A and B). No nestin immunoreactivity was detected in other parts of the kidney (Figure 1). In situ hybridization confirmed restriction of nestin mRNA to the glomerulus in adult kidney (Figure 1, C and D). Co-staining experiments showed nestin localized outside laminin-positive glomerular basement membrane (Figure 1E) and co-localization of nestin immunoreactivity with the podocyte-specific marker nephrin (Figure 1F). Therefore, in the adult kidney, nestin expression predominates in the glomerular podocytes.

To determine the potential role of nestin in maintaining their characteristic shape, we examined the effect of nestin knockdown on cultured podocytes. In differentiated podocytes, nestin antibody revealed filament-like staining in cell body and processes. Nestin siRNA markedly reduced or abolished nestin expression as determined by immunofluorescence (Figure 2A). More important, siRNA nestin knockdown was associated with significantly reduced process formation. In cells transfected with nestin siRNA, the percentage of cells with processes was significantly lower than cells transfected with control vector (77 ± 6.3% versus 16 ± 4.6%; from three independent experiments; \( P < 0.01 \); Figure 2B).

Nestin Expression in the Developing Kidney

Because nestin has been reported to be expressed in progenitor cells in a variety of tissues, including nervous system and skin, expression of nestin in embryonic kidney was examined. In the developing kidney, nestin immunoreactivity was detected as early as embryonic day 11.5 (E11.5; Figure 3A). In the E11.5 metanephric kidney, nestin-positive cells were distributed throughout the intermediate mesoderm at the site of early ureteric bud branching. Comparison with expression of Cited1, a marker of “cap” mesenchyme (22), showed nestin expression

Figure 1. Expression of nestin in adult kidney determined by immunohistochemistry and in situ hybridization. (A) Nestin immunoreactivity in cortex. (B) Nestin immunoreactivity in glomerulus. (C) Nestin mRNA expression in the kidney (dark field). (D) Nestin mRNA in glomerulus (bright field). (E) Co-localization of nestin (red) and laminin (green) in adult glomerulus. (F) Co-localization of nestin (red) and nephrin (green) in glomerulus. Magnifications: \( \times 10 \) in A and C; \( \times 100 \) in B and D.

Figure 2. Effect of nestin silencing on process formation on cultured murine podocytes. Immortalized murine podocytes were cultured. Nestin siRNA or control vectors (both vectors also contain cytomegalovirus-driven enhanced green fluorescent protein [eGFP]) were transfected into cultured podocytes. (A) Nestin expression determined by immunofluorescence (red). Cells that are transfected with nestin siRNA or control vector can be identified by eGFP (green). (B) Percentage of cells with processes (from three independent experiments). * \( P < 0.01 \); \( n = 3 \).
in a subset of cited1-positive cells at this time point, and it is excluded from the ureteric bud (Figure 3). In the E15.5 kidney, nestin was still detected in a small number of cited1-positive cells in condensed mesenchyme, but the number was reduced. In contrast, in the E15.5 kidney, anti-nestin antibody revealed strong nestin immunoreactivity in the vascular cleft of the S-shaped body (Figure 4A) and vascular tuft of immature glomeruli of capillary loop stage (Figure 4B). Co-immunostaining with CD31, an endothelial marker, confirmed nestin expression in capillaries of immature glomeruli (Figure 5A). Endothelial expression was supported further by immunofluorescence showing co-localization of nestin with Flk1, another endothelial marker (Figure 5B). In contrast, in mature glomeruli, nestin was not detected in either Flk1- or CD31-positive cells (Figure 5, C and D). Instead, strong nestin immunoreactivity was observed in glomerular visceral epithelial cells (Figure 4C). On postnatal day 0.5 (p0.5), glomerular podocytes that were positive for nestin expression were localized mainly in the juxtamedullary region. Thereafter, podocyte nestin expression increased dramatically, and podocytes became the only cells where nestin was detected in adult kidney (Figure 1, A and C).

Nestin Is Transiently Expressed in Immature Proximal Tubule Epithelial Cells of Newborn Kidney

In the embryonic kidney, nestin expression was absent from tubular epithelial cells. However, in the newborn kidney (p0.5), nestin immunoreactivity was observed in some tubules. These nestin-positive cells were labeled by the proximal tubule marker lectin lotus tetragonolobus agglutinin (Figure 6C) but not AQP2, Dolichos biflorus agglutinin, or THP antibodies, suggesting that these nestin-positive cells were proximal tubule cells, rather than collecting ducts or thick ascending limb (Figure 6, A and B). It is interesting that the intensity of nestin staining seemed to be inversely correlated with the intensity of AQP1 (Figure 6D), suggesting that nestin-positive proximal tubule cells may represent a population of relatively immature cells. Two days after birth, nestin protein disappeared from tubule epithelial cells of the normal kidney (Figure 6, E and F). Immunoblot studies confirmed a small amount of nestin immunoreactive protein in early embryonic kidney, and nestin protein expression dramatically increased on E19.9 and reached peak 48 h after birth (Figure 7).

NesCre-Expressing Cells Contribute to Metanephric Mesenchyme of Developing Kidney

To examine further whether nestin was expressed in the renal progenitor cells and whether nestin-positive cells contributed to a specific cell population in adult kidney during development, we conducted cell lineage studies using a mouse that was transgenic for Cre-recombinase driven by the nestin promoter intercrossed with a ROSA26 LacZ reporter mouse. In E10 whole-mount embryo, β-gal activity was detected as a stripe along intermediate mesoderm (Figure 8A) but extending caudally and forward throughout embryo by E11.5 (Figure 8B). However, sections from metanephric kidney at E15.5 showed that β-gal staining was distributed only in metanephric mesenchymal compartment and excluded from ureteric bud structures (Figure 8C). In adult kidney, β-gal activity was detected in
glomeruli and a subset of the tubules (Figure 9). Co-labeling with anti-AQP1 antibody, anti-THP antibody, anti-TSC antibody, and anti-AQP2 antibody (Figure 9) indicated that β-gal-positive tubules are proximal tubules, thick ascending limb, and distal convoluted tubules. In contrast, no β-gal activity was detected in AQP2-positive collecting ducts (Figure 9E). These results indicated that NesCre had induced recombination in all of the structures derived from metanephric mesenchyme. In contrast, Cre immunohistochemistry showed immunoreactivity only in periphery of the glomerulus in the adult mouse (Figure 9F), consistent with endogenous nestin expression, suggesting that the recombination in the Cre-negative segments occurred at an earlier stage of kidney development.

Discussion

Our studies demonstrated that during renal development, nestin-positive progenitor cells contribute to metanephric mesenchyme but not to structures that are derived from ureteric bud. Nestin also is transiently expressed in precursors of glomerular endothelial cells. These results are consistent with the studies of other tissues, suggesting that nestin is expressed in precursor cells of some specific cell lineages. Most notably, these studies also demonstrated that nestin is selectively expressed in differentiated podocytes of the adult kidney, suggesting a special role of nestin in this epithelial cell.

Although nestin expression also has been reported in mature adult tissues, it usually is restricted to areas of regeneration (6). Our studies now demonstrate that nestin is expressed in the terminally differentiated podocyte. Podocytes are highly specialized cells with a complex cyto-architecture (16,23). Their most prominent features are interdigitated foot processes (16,24). These foot processes are bridged by the slit diaphragm, which plays a major role in establishing selective permeability of the glomerular filtration barrier (25). A well-developed cytoskeleton accounts for the unique shape of the cells and the maintenance of the foot processes. In the cell body and the primary foot processes, microtubules and intermediate filaments, such as vimentin and desmin, have been reported to predominate, whereas microfilaments, in addition to a thin meshwork of actin filaments beneath the cell membrane, are densely packed in foot processes (25,26). The abundant and well-organized cytoskeletal proteins within podocyte also are believed to be critical for counterbalancing the mechanical stretch and stress on these cells, thereby preventing outward ballooning of the vessel and preserving the normal architecture of the glomerular tuft (17,27–30). The cytoskeleton also may play an important role in transduction signaling via mechanical force (31).

As is typical for intermediate filament proteins, nestin is characterized by an α-helical central “rod” domain that con-

Figure 5. Nestin expression in immature and mature glomeruli. Nestin expression was determined by immunofluorescent staining. The distribution of nestin within the glomerulus was examined by co-staining with markers for endothelium (CD31, Flk1). (A and B) Glomeruli from an E15.5 kidney. (C and D) Glomeruli from postnatal day 7 (p7) kidneys.
contains repeated hydrophobic heptad motifs (1), but unlike other intermediate filament proteins, nestin contains a short N-terminus and an unusually long C-terminus (1). Nestin is unable to self-assemble (3), most likely because of its very short N-terminus (a domain necessary for intermediate filament assembly), but nestin can interact with other intermediate filament proteins to form heterodimers and mixed polymers (32,33). Nestin also can interact with microtubules and microfilaments through its characteristic long C-terminus (1). These features may point to a potential role of nestin in coordinating or regulating cellular structural protein organization. Nestin has been reported to participate in assembly of vimentin that also is expressed in the podocyte (16). Because there is no nestin-selective inhibitor and nestin-deficient animal models available, studies to define the in vivo function of nestin expression remain technically unapproachable. However, our cell culture studies showed that nestin silencing using siRNA was associated with significantly reduced cell process formation, supporting an important role of nestin in maintaining the special structure of the podocytes. The mechanism by which nestin is involved in process formation remains explored.

Two decades ago, nestin was first identified in a population of proliferating neuronal cells, which were recognized as neuronal stem cells (1,4). Later, nestin also was detected in precursors of non-neuronal cells, including smooth muscle cells, vascular endothelial cells, and pancreatic islet cells (7–12). On the basis of these studies, nestin has been used widely as a marker of stem cells.

In these studies, differential developmental nestin expression and cell lineage experiments in the kidney suggest that nestin may be expressed renal progenitor cells that contribute to metanephric mesenchyme–derived structures of the adult kidney. A NesCre transgenic mouse and a ROSA26 reporter mouse were used for the cell lineage study. ROSA26 reporter mice were mated with nestin promoter–driven Cre (NesCre) mice. β-Gal activity was determined as described in Materials and Methods. (A) Whole mount of 10-d embryo. (B) Whole mount of E11.5 embryo. (C) Embryonic kidney from 15.5-d embryo. Pink color is eosin counterstain. H, hind limb; F, forelimb.
intermediate mesoderm and its derivatives of metanephric mesenchyme. In contrast, β-gal activity was not detected in Wolffian duct/ureteric bud–derived collecting ducts. These studies suggest that in the intermediate mesoderm, a population of cells that exhibit active nestin transcriptional activity contribute to metanephric mesenchyme–associated lineages but not the ureteric bud derivatives. Nevertheless, immunoreactive nestin protein was detected only in a subpopulation of cells within the condensed mesenchyme and was not expressed in the epithelial cells of the developing kidney, indicating that nestin was transiently expressed in early renal progenitor cells within the intermediate mesoderm.

LacZ expression also was detected in the mature glomerular capillary in the cell lineage experiment. In the endothelial lineage, nestin immunoreactivity first was observed in Flk1-positive cells adjacent to the ureteric bud and subsequently in vascular clefts of the S-shaped body and throughout the vascular tuft in capillary loop stage of glomerular development. In contrast, no nestin was detected in mature glomerular endothelial cells, similarly suggesting transient nestin protein expression in these endothelial progenitor cells of the developing kidney. These results are consistent with recent studies showing contribution of nestin-positive cells to endothelial lineage in central nervous system and skin (34–36).

In the p0.5 to p2 kidney, nestin expression was detected in a subpopulation of epithelial cells in cortical tubules. These nestin-positive tubules were co-labeled by lectin lotus tetragonolobus agglutinin and anti-AQP1 antibody but not by anti-AQP2 and

Figure 9. Nestin-expressing cell lineages in the adult kidney. The ROSA26 reporter mice were mated with NesCre mice. The NesCre-induced gene recombination was determined by β-gal reporter activity (stained in blue). (A) Co-staining with proximal tubule marker AQP1. (B) Co-staining with distal convoluted tubule marker thiazide-sensitive co-transporter TSC. (C and D) Co-staining with THP, a marker of thick ascending limb, in the cortex (C) and the medulla (D). (E) Co-staining with collecting duct marker AQP2. (F) Co-staining with anti-Cre recombinase antibody.
anti-THP antibodies, suggesting that these nestin expressing cells are proximal tubules. Because the intensity of nestin staining is negatively correlated with AQP1, a functional molecule predominantly in proximal tubules (37), these nestin-expressing cells seem to be immature proximal tubule cells. The specific role of nestin expression in progenitor cells and transcriptional regulation of nestin in these cells are not known (6). Because nestin is a cytoskeletal protein, it may be involved in cell migration and proliferation, characteristic of progenitor cells (6).

The mechanism by which nestin expression is controlled during renal development is not known. Studies have shown that the intron 2 of the nestin gene is required for embryonic nestin expression in the progenitor cells of the central nervous system. Intron 1 is associated with nestin expression in the somites (5). In intron 2, consensus sequences for several transcription factors have been identified, including RXR, RAR, TR, COUP-TF, and Brn-2 (38,39). The role of these transcription factors in the transcriptional regulation of nestin expression remains to be characterized. Whether a similar transcriptional mechanism is involved in nestin expression in renal progenitor cells remains to be explored. In contrast to nestin expression in progenitor cells, which disappears when cells become differentiated, nestin in podocytes does not appear until the podocytes become differentiated, suggesting that a different mechanism is involved. Several transcription factors have been described to be associated with podocyte differentiation, including WT1, POD1, maf-1, and Lamx1b (27). It is interesting that the intron 1 of murine nestin gene contains an 8-nucleotide consensus sequence (CGTGGGTG). More important, in the developing glomerulus, WT1 expression is restricted in the differentiating and mature podocyte from capillary loop onward (27), right before nestin is expressed. It is conceivable that WT1 may be involved in transcriptional regulation of nestin expression in the podocytes. However, more studies are required to elucidate the mechanism by which renal nestin expression is regulated.

Conclusion

Our studies characterized the renal expression of nestin, demonstrating that nestin is transiently expressed in glomerular endothelial cells and metanephric mesenchyme progenitors. Nestin also is expressed in mature and differentiated podocytes, where it may play an important role in maintaining normal function of this specialized epithelial cell. This study also suggests that the NesCre mice can be used to induce a recombination of a floxed gene specifically in metanephric mesenchyme–derived structures in the kidney. While this paper was in review, another paper about nestin expression in rat podocytes was published (40). This work supports our findings, suggesting a potential role of nestin in glomerular podocytes.

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References