Essential Role of Integrin-Linked Kinase in Podocyte Biology: Bridging the Integrin and Slit Diaphragm Signaling

Chunsun Dai,* Donna B. Stolz,† Sheldon I. Bastacky,* Rene St.-Arnaud,‡ Chuanyue Wu,* Shoukat Dedhar,§ and Youhua Liu*

Departments of *Pathology and †Cell Biology and Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; ‡Genetics Unit, Shriners Hospital for Children, Montreal, Quebec, Canada; and §BC Cancer Research Centre, Vancouver, British Columbia, Canada

Integrin-linked kinase (ILK) has been implicated in the pathogenesis of proteinuria and congenital nephrotic syndrome. However, the function of ILK in glomerular podocyte in a physiologic setting remains unknown. In this study, a mouse model was generated in which ILK gene was selectively disrupted in podocytes by using the Cre-LoxP system. Podocyte-specific ablation of ILK resulted in heavy albuminuria, glomerulosclerosis, and kidney failure, which led to animal death beginning at 10 wk of age. Podocyte detachment and apoptosis were not observed at 4 wk of age, when albuminuria became prominent, indicating that they are not the initial cause of proteinuria. Electron microscopy revealed an early foot process effacement, as well as morphologic abnormality, in ILK-deficient podocytes. ILK deficiency caused an aberrant distribution of nephrin and α-actinin-4 in podocytes, whereas the localization of podocin and synaptopodin remained relatively intact. Co-immunoprecipitation demonstrated that ILK physically interacted with nephrin to form a ternary complex, and α-actinin-4 participated in ILK/nephrin complex formation. Therefore, ILK plays an essential role in specifying nephrin and α-actinin-4 distribution and in maintaining the slit diaphragm integrity and podocyte architecture. These results also illustrate that the integrin and slit diaphragm signals in podocytes are intrinsically coupled through an ILK-dependent mechanism.

glomerular podocyte in a physiologic setting is completely unknown. In this study, we demonstrated that selective ablation of ILK in podocytes caused an aberrant distribution of the SD protein nephrin, as well as an early foot process effacement and morphologic abnormality. These lesions resulted in heavy albuminuria, which inevitably led to podocyte detachment and apoptosis, glomerulosclerosis, kidney failure, and animal death. Furthermore, we found that ILK physically interacted with nephrin to form a ternary complex. These findings indicate that ILK is essential for podocyte biology through bridging the integrin and SD signaling.

Materials and Methods
Mice and Genotyping
The ILK floxed mice, in which LoxP sites were inserted downstream from exons 4 and 12 at the ILK locus through homologous recombination, was described previously (24). Transgenic mice that expressed Cre recombinase under the control of a 2.5-kb fragment of the human podocin promoter (2.5P-Cre mice) were reported elsewhere (25). By mating ILK floxed mice with podocin-Cre transgenic mice, mice that were heterozygous for the ILK floxed allele were generated (genotype ILKfl/fl, Cre). These mice were cross-bred to inactivate both ILK alleles by Cre-mediated excision, thereby creating conditional knockout mice in which ILK gene was specifically disrupted in glomerular podocytes (genotype ILKfl/fl, Cre). The breeding protocol also generated heterozygous littersmates (genotype ILKfl/fl, Cre), as well as wild-type and several control groups with different genotype (ILKfl/fl, Cre), referred to as controls). A routine PCR protocol was used for genotyping of tail DNA samples with following primer pairs: Cre transgene, 5'-AGGTGAGAGAGCCACTTACG-3' and 5'-CTAATCGCCATCTTCCAGCAGG-3', which generated a 411-bp fragment; and ILK genotyping, 5'-CAAGGAATAAGGTGAGCTTCAAGA-3' and 5'-AAGGTGCTGAGGCTGAGA-3', which yielded 1.3- and 1.1-kb bands for the floxed and wild-type alleles, respectively. The presence of excision products was confirmed by PCR analysis of glomerular DNA using the following primer pair: 5'-CCAGGTGCGCATCGTA-3' and 5'-CAAGGAATAAGGTGAGCTTCAGA-3', which gave rise to a 230-bp fragment. All animals were born normally at the expected Mendelian frequency. All control mice displayed normal phenotype. Animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh.

Urine Albumin, Total Protein, and Creatinine Assay
Urine total protein levels were determined using a bichrominic acid-based protein assay kit (Sigma, St. Louis, MO) with BSA as a standard. Urine albumin was measured by using a mouse Albumin ELISA Quantitation kit, according to the manufacturer’s protocol (Bethyl Laboratories, Inc., Montgomery, TX). Serum and urine creatinine expression in isolated glomeruli was analyzed by Western blot analysis and immunohistochemistry. Glomerular size. The tubules were examined for dilation by proteinuria, which inevitably led to podocyte detachment and apoptosis, glomerulosclerosis, kidney failure, and animal death. Furthermore, we found that ILK physically interacted with nephrin to form a ternary complex. These findings indicate that ILK is essential for podocyte biology through bridging the integrin and SD signaling.

Histology and Immunohistochemical Staining
Kidney sections were prepared at 2 μm thickness by a routine procedure. Sections were stained with hematoxylin-eosin, periodic acid-Schiff, methenamine silver-trichrome, and Masson-trichrome by standard protocol. The histologic sections were examined by a renal pathologist. The glomeruli were assessed for segmental glomerulosclerosis and global glomerulosclerosis, mesangial matrix expansion, and glomerular size. The tubules were examined for dilation by proteinaceous fluid and tubular atrophy. The interstitium was assessed for fibrosis and mononuclear inflammation. Immunohistochemical staining for WT-1 was performed using a routine protocol as described previously (28). For quantitative determination of podocyte numbers, the WT-1-positive cells were counted in at least 10 randomly chosen glomeruli under high power (×400) in each mouse. The averages of podocyte numbers per glomerulus were calculated on the basis of individual values, which were determined on four mice per group.

Terminal Deoxynucleotidyl Transferase–Mediated dUTP Nick-End Labeling Staining
Apoptotic cell death was determined by using terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) staining with DeadEnd Fluorometric Apoptosis Detection System (Promega, Madison, WI), as previously reported (26). The slide was double stained with anti–WT-1 antibody (Santa Cruz Biotechnology) for the identification of podocytes. Apoptosis in glomeruli and tubulointerstitial area was counted in all glomeruli and at least 10 randomly chosen nonoverlapping high-power (×400) fields for each mouse, four mice per group (n = 4). Apoptotic podocytes (both TUNEL and WT-1 positive) were expressed as apoptotic cells per field. For a better view of kidney morphology, another set of the paraffin-embedded kidney sections were stained with DeadEnd Colorimetric TUNEL System (Promega).

Electron Microscopy
Electron microscopy of kidney samples was carried out by routine procedures as described previously (29). Briefly, mouse kidneys were perfusion fixed with 2.5% glutaraldehyde in PBS by left cardiac ventricular injection and postfixed in aqueous 1% OsO4. Specimens were dehydrated through an ethanol series, infiltrated in a 1:1 mixture of propylene oxide-Polybed 812 epoxy resin (Polysciences, Warrington, PA), and then embedded. Ultrathin sections were stained with 2% uranyl acetate, followed by 1% lead citrate. Sections were observed and photographed using a JEOL JEM 1210 transmission electron micro-
scope (TEM; JEOL, Peabody, MA). For scanning electron microscopy (SEM), kidneys were perfusion fixed in 2.5% glutaraldehyde in PBS as for TEM, dissected lengthwise, then postfixed in 1% OsO₄ (30). After dehydration through an ethanol series, kidney samples were critical point-dried, mounted on aluminum stubs, sputtered with gold coat, and examined under JEOL 6335F field emission gun scanning electron microscope.

**Immunofluorescence Staining and Confocal Microscopy**

Kidney cryosections were fixed with 3.7% paraformaldehyde for 15 min at room temperature, except that methanol was used for sections that were stained with ILK. After blocking with 10% donkey serum for 30 min, the slides were immunostained with primary antibodies against ILK, nephrin, α-actinin-4, podocin (Santa Cruz Biotechnology), and synaptopodin (Progen), respectively. For ILK and α-actinin-4 staining, the Vector M.O.M. immunodetection kit (Vector Laboratories, Burlingame, CA) was used. As a negative control, the primary antibody was replaced with nonimmune IgG, and no staining occurred. Slides were viewed under a Leica TCS-SL confocal microscope. For determination of the disruptive nephrin and α-actinin-4 distribution, a semiquantitative scoring method was used. Score 0 represents no lesion, whereas 1, 2, 3, and 4 represent the disruptive redistribution of nephrin and α-actinin-4, involving <25%, 25 to 50%, 50 to 75%, and >75% of the glomerular tuft area, respectively. At least 10 randomly chosen glomeruli were evaluated for each mouse, and an average composite score was calculated. Four mice from each group were analyzed.

**Immunoprecipitation**

Immunoprecipitation was carried out by using an established method (31). Briefly, isolated glomeruli were lysed on ice in 1 ml of non-denaturing lysis buffer that contained 1% Triton X-100, 0.01 M Tris-HCl (pH 8.0), 0.14 M NaCl, 0.025% NaN₃, 1% protease inhibitors cocktail, and 1% phosphatase inhibitors cocktail I and II (Sigma). After preclearing with normal IgG, glomerular lysates (0.5 mg of protein) were incubated overnight at 4°C with 4 μg of anti-ILK (Upstate) and anti-nephrin (Santa Cruz Biotechnology), respectively, followed by precipitation with 30 μl of protein A/G Plus-Agarose for 1 h at 4°C. The precipitated complexes were separated on SDS-polyacrylamide gels and blotted with various antibodies as indicated.

**Statistical Analyses**

Statistical analysis of the data was performed by using SigmaStat software (Jandel Scientific, San Rafael, CA). Comparison between groups was made using one-way ANOVA, followed by the Student-Newman-Keuls test. P < 0.05 was considered significant.

**Results**

**Podocyte-Specific Disruption of ILK Gene in Mice**

We generated a mouse model in which ILK gene was selectively disrupted in glomerular podocytes by mating ILK floxed mice with podocin-Cre transgenic mice (Figure 1). Mice with podocyte-specific disruption of ILK (podo-ILK⁻/⁻), as well as wild-type and several control groups, were confirmed by PCR analysis of tail genomic DNA (Figure 1B). PCR analysis of the glomerular DNA that was isolated from the podo-ILK⁻/⁻ mice displayed a 230-bp band that corresponded to the excised floxed ILK allele, indicating the Cre-mediated deletion of the ILK gene (Figure 1C). Figure 1D shows the glomerular ILK protein levels in podo-ILK⁻/⁻ mice and their wild-type littermates. Comparing with the controls, ILK protein level in the

![Figure 1](https://example.com/figure1.png)

Figure 1. Podocyte-specific ablation of the integrin-linked kinase (ILK) in mice. (A) Diagram illustrates the strategy of generating podocyte-specific ILK knockout mice. Shown is the deletion of exons 5 through 12 in the event of recombination of the ILK gene. (B) Representative picture shows PCR analysis of the genomic DNA from tail clippings. The PCR bands of wild-type (1.1 kb), floxed (1.3 kb), and Cre (411 bp) are indicated. Genotypes of representative litters are indicated. fl, ILK floxed; Cre, Cre recombinase. (C) PCR analysis shows the Cre-excised band (230 bp) in the glomeruli of podo-ILK⁻/⁻ mice. (D) Western blot analysis of ILK protein expression in whole glomeruli (pooled from eight mice per group) that were isolated from the control and podo-ILK⁻/⁻ mice. Glomerular lysates were immunoblotted with antibodies against ILK and α-tubulin, respectively. (E) Body weights of the podo-ILK⁻/⁻ mice and their control littermates at different time points after birth. **P < 0.01 versus controls (n = 8). (F) Representative picture shows the phenotypic appearance of podo-ILK⁻/⁻ mouse and its control littermate at 10 wk after birth.
isolated glomeruli in podo-ILK−/− mice was reduced by approximately 40% (Figure 1D), which is consistent with the fact that podocytes only constitute a fraction of total glomerular cell population. Podocyte-specific ablation of ILK in podo-ILK−/− mice also was confirmed by immunofluorescence staining (Figure 8; see the ILK Physically Interacts with Nephrin and α-Actinin-4 section).

We observed no difference in the body weights in podo-ILK−/− mice at 2 wk of age when compared with the control littermates (Figure 1E). However, significant reduction in body weight was found at 10 wk of age in podo-ILK−/− mice (Figure 1, E and F). This growth retardation likely resulted from the severe morbidity characterized by heavy proteinuria that developed in podo-ILK−/− mice (see below). Podo-ILK−/− mice began to die at 10 wk. The majority of podo-ILK−/− mice died of renal failure at approximately 3 to 4 mo, although a few of them survived beyond 6 mo of age.

Podocyte-Specific Ablation of ILK Leads to Heavy Proteinuria, Glomerulosclerosis, and Kidney Failure

We next determined the urine albumin levels at different time points in podo-ILK−/− mice and their control littermates. As shown in Figure 2A, urine albumin levels were dramatically elevated at 4 wk in podo-ILK−/− mice and reached to >7 mg/mg creatinine, whereas no or little urine albumin was detected at 2 wk. Albuminuria progressively deteriorated at 10 wk in podo-ILK−/− mice. Similar results were obtained when urine total protein was measured (Figure 2B). Consistent with an elevated albumin, urine protein also was significantly increased at 4 and 10 wk in podo-ILK−/− mice, respectively. Separation of urine samples by SDS-PAGE revealed that albumin was the predominant constituent of urine proteins (Figure 2C).

In accordance with heavy albuminuria and proteinuria, kidney function in podo-ILK−/− mice was severely impaired in an age-dependent manner. An elevation of serum creatinine levels was observed at 10 wk in podo-ILK−/− mice, compared with their control littermates (Figure 2D). However, little difference in serum creatinine levels was found at 4 wk between podo-ILK−/− mice and controls (Figure 2D), suggesting that the development of albuminuria and proteinuria precedes kidney dysfunction in podo-ILK−/− mice.

Figure 3 shows the representative micrographs of kidney sections at different time points. Kidney morphology was normal at 2 wk after birth in podo-ILK−/− mice (data not shown). At 4 wk, kidney histology remained essentially normal, when compared with the control littermates (Figure 3, A and B). However, dramatic morphologic abnormality was observed at 10 wk in the podo-ILK−/− kidney, which was characterized...
by primary glomerular lesions, including an enlargement of glomeruli in size, moderate to marked mesangial expansion, and segmental glomerulosclerosis to global sclerosis (Figure 3, C, F, and I). There was widespread tubular dilation distended by proteinaceous fluid and cellular debris. The interstitium contained a mild, patchy mononuclear inflammatory infiltrate, and interstitial fibrosis generally was mild (Figure 3).

Podocyte Detachment and Apoptosis Are Not the Initial Causes of Albuminuria

Because ILK plays an important role in mediating cell–matrix adhesion and cell survival (32), we reasoned that ILK deficiency may lead to podocyte detachment and apoptosis, thereby causing proteinuria and kidney dysfunction. To test this hypothesis, we first examined the podocyte abundance in podo-ILK−/− kidney at different time points. As shown in Figure 4, podocytes in the glomeruli were readily identifiable by the nuclear staining for transcription factor WT-1. Quantitative determination revealed that there was no difference in podocyte number per glomeruli between podo-ILK−/− mice and control littermates at 2 and 4 wk, respectively (Figure 4E), suggesting a lack of podocyte depletion at the cellular level when albuminuria became prominent (Figure 2). This finding was supported further by measuring WT-1 abundance from the isolated glomeruli using Western blot analysis (Figure 4F). However, podocyte depletion was predominant at 10 wk in podo-ILK−/− kidney (Figure 4D). The number of podocytes per glomeruli was decreased by approximately 70%, compared with the controls. WT-1-positive podocytes were obviously detached from the GBM and easily found in the lumens of the dilated tubules. Some tubular epithelial cells occasionally were stained positively for WT-1, possibly because they endocytosed the debris or apoptotic bodies of the dead podocytes (Figure 4D).

We also assessed the podocyte apoptosis in podo-ILK−/− kidney. As illustrated in Figure 5, no apoptosis was found at 2 and 4 wk, respectively, in the kidney with podocyte-specific deficiency of ILK. At 10 wk, little or no podocyte apoptosis was found in situ in the glomeruli of podo-ILK−/− kidney (Figure 5, G through I), even though podocyte depletion was eminent at this time point (Figure 4E). Substantial apoptosis was observed in the tubulointerstitial area of the kidney at 10 wk in podo-ILK−/− mice (Figure 5, J through M). Co-staining with TUNEL and WT-1 displayed that significant podocytes were positive for TUNEL staining in the lumens of tubules at this stage, suggesting that detached podocytes underwent apoptotic death. Intriguingly, there were WT-1-positive podocytes that were negative for TUNEL staining, indicating that these detached podocytes were still alive in the tubular lumens. Some apoptotic cells lacked WT-1 protein, indicating their origin of tubular epithelium (Figure 5L). Similar results were obtained when kidney tissues were stained by using a colormetric apo-
Figure 4. Podocyte depletion is a late event secondary to the development of albuminuria in mice that lack ILK in podocyte. (A through D) Representative micrographs show the podocyte abundance at 2 (B), 4 (C), and 10 wk (D) in the glomeruli of podo-ILK−/− mice and their control littermates at 4 wk (A). Kidney sections were stained with antibody against WT-1. Arrow denotes the glomerulus with a reduced podocyte number at 10 wk in podo-ILK−/− mice (D). Arrowhead indicates the WT-1–positive podocytes in the lumen of renal tubules. Bar = 25 μm. (E) Quantitative determination of the glomerular podocyte numbers at different time points in podo-ILK−/− mice and their control littermates. **P < 0.01 versus controls (n = 4). (F) Western blot analysis demonstrates a comparable glomerular WT-1 protein level at 4 wk in podo-ILK−/− mice and their control littermates. Whole glomerular lysates (pooled from eight mice) were immunoblotted with antibodies against WT-1 and α-tubulin, respectively.
fected by ablation of ILK in podocytes (Figure 7, I through L), suggesting the specificity of the interplay among ILK, nephrin, and β1/α-actinin-4.

ILK Physically Interacts with Nephrin and α-Actinin-4

The finding that ILK deficiency resulted in nephrin redistribution prompted us to explore their intrinsic connection. Double immunofluorescence staining exhibited a co-localization of ILK and nephrin in glomerular podocytes in vivo. In normal glomeruli, ILK localization within the podocytes displayed a linear pattern along the GBM (Figure 8A), similar to that of nephrin (Figure 8B). Merging of the TUNEL and WT-1 staining images is presented (C, F, I, and L). (A through C) Control littermates at 4 wk. (D through F) Podo-ILK−/− mice at 4 wk. (G through I) Podo-ILK−/− mice at 10 wk. (J through L) Tubulointerstitial area of podo-ILK−/− mice at 10 wk. Arrow indicates an apoptotic tubular cell with negative staining for WT-1, whereas arrowhead denotes WT-1-positive podocyte with negative TUNEL staining. Bar = 30 μm. (M) Quantitative determination of apoptotic podocytes in tubulointerstitial area at 4 and 10 wk in podo-ILK−/− mice and their control littermates. **P < 0.01 versus controls (n = 4). (N through P) Representative micrographs show apoptotic cells in the tubular lumens detected by colorimetric TUNEL staining at 2 (N), 4 (O), and 10 wk (P) in podo-ILK−/− mice. Arrows indicate glomeruli. Bar = 30 μm.

We examined the potential interaction between ILK and nephrin in the glomeruli by co-immunoprecipitation. As shown in Figure 8, G and H, nephrin was readily detected in the complex that was precipitated by anti-ILK antibody. As a negative control, no nephrin was detected when the glomerular lysates were precipitated with normal IgG (Figure 8G, lane 2). Likewise, podocyte-specific ablation of ILK abolished the interaction between ILK and nephrin (Figure 8K). These results suggest that ILK physically interacts with the SD protein nephrin in vivo.

We next investigated the potential involvement of α-actinin-4 in the formation of ILK/nephrin complex, because ILK ablation also disturbs its localization. As shown in Figure 8, I and J, α-actinin-4 also was detected easily in the complex that was precipitated by either anti-ILK or anti-nephrin antibodies. Ab-
lation of ILK in podocytes largely abolished the interaction between ILK and α-actinin-4 (Figure 8J). The weak band of α-actinin-4 that was detected in the immunocomplexes likely was caused by the contamination of other cell types such as tubular cells in the glomerular preparation from the podo-ILK+/− mice (Figure 8J). Double immunofluorescence staining also revealed that α-actinin-4 co-localized with nephrin and ILK in glomerular podocytes in vivo (data not shown). Therefore, α-actinin-4 participates in the ILK/nephrin complex formation and perhaps functions as an adaptor protein bridging ILK and nephrin (Figure 8L).

Discussion

The SD of podocyte foot processes is a pivotal component of the glomerular filtration barrier, and disruption of its integrity represents a key event in the development of proteinuria and nephrotic syndrome in a variety of inherited and acquired glomerular diseases (1,2,33). Previous studies largely focused on elucidating the role of various SD proteins such as nephrin and podocin in the SD function and glomerular filter integrity (5,6,8,10). Not surprising, mutations or deletion of these SD proteins lead to the development of congenital nephrotic syndrome. In this study, we found that selective ablation of ILK in podocyte causes heavy proteinuria, podocyte detachment and apoptosis, glomerulosclerosis, and kidney failure. The underlying mechanism of these pathologic manifestations seems to be attributable to a disturbance of nephrin localization and foot process effacement. More important, we have uncovered that ILK can physically interact with nephrin, which provides direct evidence that the integrin signaling is intrinsically linked to SD function in podocytes. These findings not only underscore an essential role of ILK in podocyte biology in normal physiologic setting but also suggest that a dysregulation of ILK abundance and/or activity potentially may play a critical role in the development of proteinuria and podocyte dysfunctions. Because the integrin/ILK signaling is regulated by many extracellular cues, such as matrix components, high glucose, growth factors, and cytokines, the intrinsic connection of ILK with nephrin that was unraveled in this study offers important insights into understanding the pathogenesis of proteinuria in many common glomerular diseases, such as diabetic nephropathy. Therefore, our study may have wide implications in comprehending how alterations in the extracellular environment lead to defects in SD function and impairment of glomerular filtration.

The finding that ILK binds to nephrin defines a novel, physiologically important interacting partner for ILK. By interacting with the cytoplasmic domains of β-integrins and nephrin, ILK virtually acts as an adaptor or scaffolding protein that bridges and integrates the integrin and the SD signals. Physical interaction between ILK and nephrin is clearly evident in the glomeruli of normal kidney in vivo, as demonstrated by co-immunoprecipitation (Figure 8). Whether ILK binds to nephrin directly or via additional adaptor proteins, however, remains uncertain. In this aspect, it is interesting to note that α-actinin-4
was involved in the ILK/nephrin complex formation, suggesting the presence of a multicomponent ternary complex in which ILK likely is a central element. We propose that the coupling of ILK to nephrin likely is mediated by additional proteins such as α-actinin-4 (Figure 8L). Further studies are warranted in this area to characterize fully the molecular details of the ILK/nephrin interaction in podocyte.

Given the physical interaction between ILK and nephrin, it is not difficult to understand that ablation of ILK causes a redistribution of nephrin, resulting in a diffused pattern throughout the podocytes in podo-ILK−/− mice. In the absence of ILK, nephrin would lose its anchoring sites for proper trafficking and localization to the SD. This underscores that ILK may be essential for specifying nephrin distribution in podocytes, possibly by acting as an anchoring apparatus. It is worthwhile to point out that in many glomerular diseases, such as diabetic nephropathy, redistribution and reduction of nephrin are predominant pathologic features in podocytes (34–36). Although the exact mechanism remains elusive, dysregulation of ILK abundance and/or activity, which is common in chronic kidney diseases, may play a significant role in causing nephrin redistribution in these conditions.

Another striking feature in ILK-deficient podocyte is the aberrant distribution of α-actinin-4, an actin cross-linking protein that binds to actin filaments (37,38). Genetic mutations in α-actinin-4 gene have been shown to cause an autosomal dominant focal segmental glomerulosclerosis in patients (39). In normal glomeruli, the pattern of α-actinin-4 distribution essentially resembles that of SD proteins such as nephrin and podocin, consistent with its close association with the SD. Similar to nephrin, selective ablation of ILK in podocyte causes α-actinin-4 redistribution, indicating that ILK also directs α-actinin-4 localization. This notion is corroborated further by α-actinin-4’s binding to ILK and nephrin (Figure 8). The complex interactions among ILK, nephrin, and α-actinin-4 virtually connect the integrin and the SD with the actin cytoskeleton and integrate both extracellular and intracellular signals from multiple directions.

Our study also strengthens the notion that the disorganization of actin cytoskeleton may play a critical role in the develop-
opment of podocyte foot process effacement and structural abnormality. In ILK-deficient podocyte, α-actinin-4 forms aggregated dots (Figure 7F). Such mislocalization of α-actinin-4 certainly would change the actin cytoskeleton dynamics (40) and may cause the collapse of the actin meshwork underneath or near the SD of the foot processes. Because actin cytoskeleton defines cell shape and morphology, altered cytoskeletal structure of the foot processes will lead to disappearance of the SD structures and development of an “effaced” phenotype, as demonstrated by electron microscopy studies (Figure 6). This speculation also is supported by the observations that mutations or deletion of α-actinin-4 gene cause proteinuria and focal segmental glomerulosclerosis in both animal models and patients (39,41,42). Because no alteration in α-actinin-4 level was seen in podocytes that lacked ILK, this suggests that not only the quality (mutations) and the quantity (deletion or induction)

Figure 8. ILK physically interacts with nephrin and α-actinin-4 in vivo. (A through F) Co-localization of ILK and nephrin in glomerular podocyte. Representative micrographs show the staining of ILK (A and D), nephrin (B and E), and co-localization (C and F) in the control (A through C) and podo-ILK−/− mice (D through F). Arrowheads indicate the co-localization of ILK and nephrin in podocyte in normal mice. Arrows denote ILK-deficient podocyte in which nephrin distribution was disturbed in podo-ILK−/− mice. Bar = 12 μm. (G and H) Co-immunoprecipitation demonstrates the ILK/nephrin complex formation. Glomerular lysates (pooled from eight mice) were immunoprecipitated with anti-ILK or normal IgG (G) and anti-ILK or anti-nephrin (H), followed by immunoblotting with anti-nephrin. (I) Co-immunoprecipitation demonstrates the presence of α-actinin-4 in the ILK/nephrin complex in vivo. Immunocomplexes that were precipitated with anti-ILK or anti-nephrin were immunoblotted with anti–α-actinin-4 antibody. (J and K) Ablation of ILK in podocyte abolished ILK/α-actinin/nephrin interaction. Glomerular lysates from either podo-ILK−/− (ko) or control littermates (wt) were immunoprecipitated with anti-ILK and immunoblotted with either anti–α-actinin-4 (J) or anti-nephrin (K). (L) Schematic model illustrates that ILK bridges the integrin and slit diaphragm signaling. ILK functions as an adaptor protein that physically and functionally associates with slit diaphragm protein nephrin. α-Actinin-4 participates in the ILK/nephrin complex formation. Ablation of ILK disrupts ILK/nephrin complex formation and leads to an aberrant distribution of nephrin and α-actinin-4, thereby inducing podocyte foot process effacement and morphologic alterations. That is followed by albuminuria, podocyte detachment and apoptosis, glomerulosclerosis, and kidney failure.
of α-actinin-4 but also its location are of extreme importance in maintaining the integrity of the podocyte architecture. Furthermore, massive microvilli-like structures developed in ILK-deficient podocytes, illustrating that the submembranous actin skeleton architecture is profoundly changed.

ILK has been implicated in the regulation of cell adhesion and cell survival (43,44). However, podocyte detachment and apoptosis seem not to be the initial cause of proteinuria in the podo-ILK−/− mice, because the podocyte numbers were preserved at the time point when severe albuminuria became eminent. Nevertheless, we cannot exclude the possibility that podocyte depletion via cell detachment and apoptosis in the podo-ILK−/− mice may deteriorate the proteinuria and accelerate the kidney dysfunction. Along this line, it is conceivable that podocyte loss from 4 wk onward may play a critical role in the development of glomerulosclerosis and kidney failure that eventually lead to animal death in podo-ILK−/− mice.

Conclusion

We have shown herein that ILK plays an essential role in podocyte biology in vivo. Through interacting with nephrin and α-actinin-4, ILK builds a multicomponent complex that is essential for the maintenance of the podocyte function and glomerular filter integrity. Our findings have established that the cell–matrix integrin signaling and the cell–cell adhesion SD merular filter integrity. Our findings have established that the essential for the maintenance of the podocyte function and glomerular permselectivity.

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