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Rat nephrin modulates cell morphology via the adaptor protein Nck

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Abstract

Nephrin is a transmembrane molecule essential for morphology and function of kidney podocytes. We and others reported previously that the cytoplasmic domain of human and mouse nephrin interacts with the adaptor protein, Nck, in a tyrosine phosphorylation-dependent manner. In the current study, we characterized the interaction of rat nephrin with Nck and further addressed its impact on cell morphology. Rat nephrin expressed in Cos-1 cells co-immunoprecipitated with Nck in a manner dependent on the phosphorylation of Y1204 and Y1228. Nephrin from normal rat glomeruli was also tyrosine phosphorylated and associated with Nck. Overexpression of rat nephrin in HEK293T cells induced morphological changes resembling process formation, which became more distinct when the extracellular domain of nephrin was cross-linked by antibodies. The morphological changes were attenuated by expression of dominant negative constructs of Nck. In the rat model of podocyte injury and proteinuria, nephrin tyrosine phosphorylation and nephrin–Nck interaction were both reduced significantly. Taken together, we propose that Nck couples nephrin to the actin cytoskeleton in glomerular podocytes and contributes to the maintenance of normal morphology and function of podocytes.

Keywords: Podocytes; Nephrin; Nck; Tyrosine phosphorylation; Cell morphology; Proteinuria; Puromycin aminonucleoside nephrosis

Visceral glomerular epithelial cells, also known as podocytes, are highly differentiated cells, which surround glomerular capillaries of the kidney and have an important role in the maintenance of glomerular permselectivity. Podocytes project primary processes from the cell body, which further branch into numerous finger like processes called ''foot processes''. Interdigitating adjacent foot processes of podocytes are connected by the structure called the slit diaphragm [1]. The slit diaphragm is believed to play a central role in regulating the actin cytoskeleton in foot processes, thereby contributing to the maintenance of their intricate morphologies [2]. Nephrin is a transmembrane protein of 180 kDa, which belongs to the Ig superfamily. In addition to its role as a structural protein in the slit diaphragm, nephrin serves as a scaffold of a number of proteins including podocin and CD2AP, and transmits signals from the slit diaphragm into the cells [2]. The cytoplasmic domain of nephrin contains a series of conserved tyrosine-based motifs, which upon phosphorylation can bind to cytoplasmic targets such as phosphoinositide 3-kinase [3], the Src-family kinase, Fyn [4], and the adaptor protein, Nck [5,6].

Nck1/α and Nck2/β comprise a family of adaptor proteins (hereafter called Nck) with three SH3 domains followed by a carboxy-terminal SH2 domain [7]. Nck binds to a variety of molecules at phosphotyrosine-containing motifs via the SH2 domain and recruits other molecules via the SH3 domains. The Nck SH2 domain prefers to bind to the phosphotyrosine–aspartate–glutamate–proline/aspartate/valine (pYDEP/D/V) consensus motif, although some redundancies are known [8]. Tyrosine phosphoproteins known to associate directly with the SH2 domain of Nck include receptor tyrosine kinases, as well as adaptor molecules such as Dok1 and Dok2 [7]. SH3 domains are

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known to bind to proline-rich motifs. The list of molecules that bind to the SH3 domains of Nck is long, including focal adhesion kinase (FAK), p21-activated kinase (PAK), WASP (Wiskott–Aldrich syndrome protein), and more ubiquitous neural (N)-WASP. Notably, most proteins that interact with Nck via its SH3 domains are involved in actin cytoskeletal regulation [7]. Nck1$^{-/-}$and Nck2$^{-/-}$ mice do not show obvious abnormalities, while double null mutation is embryonic lethal, indicating that the function of the two isoforms could be redundant [9].

We [5] and others [6] reported recently that the cytoplasmic domain of human and mouse nephrin interacts with Nck in a tyrosine-dependent manner. Nephrin–Nck interaction led to localized actin polymerization (forming ''actin tails'') [5,6], while podocyte-specific gene deletion of *Nck1* and *Nck2* led to abnormal foot process development and congenital proteinuria [5], suggesting that nephrin–Nck interaction is critical for the normal development of podocyte foot processes. The current study confirmed tyrosine phosphorylation-dependent nephrin–Nck interaction with rat nephrin and further demonstrated morphological changes of cells as a consequence of actin reorganization induced by nephrin–Nck interaction. In addition, we showed that tyrosine phosphorylation of nephrin, as well as nephrin–Nck interaction, was diminished in a rat model of podocyte injury and proteinuria, suggesting their important role in the maintenance of glomerular permselectivity in the adult kidney.

Materials and methods

Materials. Tissue culture media and lipofectamine 2000 were from Invitrogen-Life Technologies (Burlington, ON). Reagents for molecular biology were from New England BioLabs (Mississauga, ON). Protease inhibitor cocktail was from Roche Diagnostics (Laval, QC). Mouse monoclonal anti-Nck antibody and anti-phosphotyrosine antibody, PY69, were from BD Biosciences (Mississauga, ON). FITC-labeled anti-Myc and anti-HA antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Rhodamine–phalloidin was from Molecular Probes (Eugene, OR). FITC-goat anti-mouse IgG and anti-Src [pY^{418}] phospho-specific antibodies were from Biosource International (Camarillo, CA). Src phosphorylated at Y^{418} is known to be active. Since the region surrounding Y418 is highly conserved in all of the related Src-family kinases, the phospho-specific Src antibody cross-reacts with the other members of the family, such as Fyn and Yes. Rabbit anti-Nck (which recognizes both Nck1 and 2) and anti-nephrin antibodies were described previously [10,11]. Fibronectin, puromycin aminonucleoside, and other standard biochemicals were from Sigma–Aldrich (St. Louis, MO).

Plasmids. Construction of rat nephrin and its tyrosine mutants was reported previously [10]. The plasmids for Nck1, GST–Nck, GST–Nck-SH2, GST–Nck-SH3s, HA–Nck-SH3-M3, and Fyn were described previously [10,12,13]. For construction of Myc–Nck-SH2, human Nck1 cDNA corresponding to amino acids 282-377 was amplified by PCR and cloned into pcDNA3.1-Myc (Invitrogen) using *Bam*HI and *Eco*RI sites. Sequence was confirmed by automated sequencing.

Transient transfection, immunoprecipitation, immunoblotting, pull-down assay. Transient transfection was performed in Cos-1 cells or HEK293T cells as described previously using lipofectamine 2000 [10]. Cells or glomeruli were lysed in buffer containing 1% Triton X-100 and immunoprecipitation and immunoblotting were performed as described previously [10]. For immunoprecipitation and immunoblotting of Nck, rabbit anti-Nck antibody and mouse monoclonal anti-Nck antibody were used, respectively. Pull-down assays were performed using Nck1 or its subdomains conjugated with GST as described previously [12].

Cross-linking of nephrin. HEK293T cells were stably transfected with rat nephrin (HEK293T-nephrin or HEK-nephrin in short) and plated on glass coverslips coated with fibronectin (0.05 mg/ml). Cells were incubated with mouse monoclonal mAb5-1-6 antibody (5 μg/ml), which recognizes the extracellular domain of rat nephrin [14], for 60 min at 4 °C in buffer containing 145 mM NaCl, 5 mM KCl, 0.5 mM $MgSO_4$, 0.5 mM $CaCl_2$, 1 mM $NaHPO_4$, 5 mM glucose, and 20 mM Hepes, pH 7.4 (measurement buffer). Cells were washed and incubated with goat anti-mouse IgG (10 μg/ml, with or without FITC) for 60 min (for morphological studies) or 10 min (for tyrosine phosphorylation) at 37 °C in measurement buffer. For morphological studies, cells were fixed with 3% formaldehyde, permeabilized with 0.5% Triton X-100, blocked with 3% BSA, and stained with rhodamine–phalloidin (0.04 μg/ml) plus/minus appropriate secondary antibodies. Cells were examined with a confocal microscope (Olympus, Fluoview FV1000).

Induction of puromycin aminonucleoside nephrosis (PAN) nephrosis and isolation of rat glomeruli. PAN was induced with a single intravenous injection of puromycin aminonucleoside (50 mg/kg body weight) in male Sprague–Dawley rats (150–175 g, Charles River, St. Constant, QC), as described previously [15]. Rats were sacrificed on day 7, when significant proteinuria was observed. Isolation of rat glomeruli was performed by differential sieving and lysates were prepared in buffer containing 1% TritonX-100, as described previously [10]. Studies were approved by the Animal Care Committee at McGill University.

Results

The cytoplasmic domain of rat nephrin associates with Nck in a tyrosine phosphorylation-dependent manner

The cytoplasmic domain of nephrin consists of ~150 amino acids and has a series of tyrosine-containing motifs. Using rat nephrin cDNA, we demonstrated previously that some of the tyrosine residues are phosphorylated by the Src-family kinase Fyn, leading to augmented interaction between nephrin and podocin [10]. We hypothesized that SH2 containing protein(s) may interact with phosphotyrosine containing motifs in the cytoplasmic domain of nephrin. In search of such molecules, we analyzed the protein sequence of the cytoplasmic domain of nephrin by Motif scan analysis (http://scansite.mit.edu). This program identifies potential interacting proteins for various protein motifs in a given molecule. SH2 containing proteins such as Src-family kinases (Src, Fyn, Fgr), as well as the p85 subunit of PI3K, were predicted to bind to nephrin, consistent with the previous reports [3,4]. In addition, it was predicted that the adaptor molecule Nck would bind to nephrin (Fig. 1A). There were three potential Nck binding sites in rat nephrin and five in mouse and human by a low stringency analysis and indeed, we and others reported recently that human [5] and mouse [6] nephrin interact with Nck in a tyrosine phosphorylation-dependent manner. In the current paper, we first characterized the interaction of Nck with rat nephrin.

When rat nephrin was transfected in Cos-1 cells with Fyn, nephrin was strongly tyrosine phosphorylated, while in the absence of Fyn, there was negligible phosphorylation, consistent with our previous report [10] (Fig. 1B). In the presence of Fyn, nephrin co-immunoprecipitated with Nck (Fig. 1B). In the absence of Fyn, there was only a

A

Rat	Mouse	Human
1127-YEES	1128-YEES	1114-YEES
	1191-YDEV	1176-YDEV
1204-YDEV	1208-YDEV	1193-YDEV
	1225-YEDP	1210-YQDP
1228-YDQV	1232-YDQV	1217-YDQV

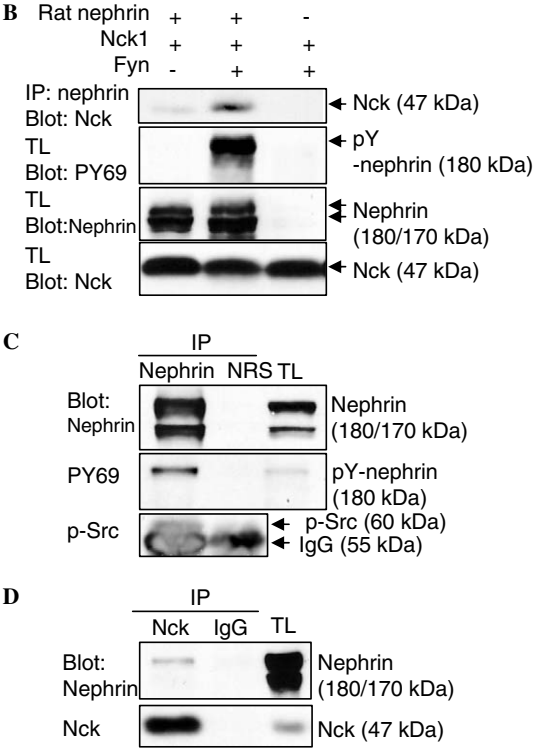

Fig. 1. Nephrin and Nck interact in a tyrosine phosphorylation-dependent manner. (A) Predicted binding sites for Nck1 in the cytoplasmic domain of rat, mouse, and human nephrin by Motif scan analysis. (B) Cos-1 cells were transiently transfected with the indicated plasmids. Cell lysates were immunoprecipitated with anti-nephrin antibody and blotted for Nck. Total lysates (TL) were blotted for phosphotyrosine (Py69), nephrin, and Nck. (C,D) Normal rat glomerular lysates were immunoprecipitated with anti-nephrin antibody and blotted for phosphotyrosine (Py69) and phospho-Src (Y418) (C), or immunoprecipitated with anti-Nck antibody and blotted for nephrin (D). NRS, normal rabbit serum (control antibody).

minor co-immunoprecipitation, indicating that nephrin–Nck interaction is mainly dependent on tyrosine phosphorylation. When normal rat glomerular lysates were immunoprecipitated for nephrin, nephrin was clearly tyrosine phosphorylated, consistent with our previous report [10] (Fig. 1C). In addition, nephrin co-immunoprecipitated with phospho-Src (Y418, this antibody recognized the active form of Src-family kinases), suggesting that Src-family kinases likely interact with and contribute to tyrosine phosphorylation of nephrin in vivo (Fig. 1C). On SDS–PAGE, rat nephrin runs as a doublet, presumably because of differential glycosylations, and phosphorylated nephrin consistently corresponded to the upper band (~180 kDa. Fig. 1B and C). In normal rat glomerular lysates, Nck co-immunoprecipitated with the upper band of nephrin, suggesting that tyrosine phosphorylated nephrin interacts with Nck in vivo (Fig. 1D).

Identification of the nephrin-interacting domain(s) of Nck

We next studied which domain(s) of Nck interact with nephrin, using pull-down assays with GST-fusion proteins of Nck1 or its subdomains. When Cos-1 cells were transfected with rat nephrin and cell lysates were subjected to pull-down assay, nephrin was clearly pulled down by GST–Nck (containing full-length Nck1) and GST–Nck-SH2 (containing the SH2 domain of Nck1) in a Fyn-dependent manner (Fig. 2A, top panel). Only after a longer exposure, we also observed that GST–Nck-SH3s (containing three SH3 domains of Nck1) pulled down nephrin (Fig. 2A, second panel), however, this weak interaction was not affected by Fyn. GST–Nck and GST–Nck-SH2 also pulled down nephrin from normal rat glomerular lysates (Fig. 2D). Taken together, these results indicate that nephrin–Nck interaction occurs predominantly between the SH2 domain of Nck and phosphotyrosine containing motif(s) of the cytoplasmic domain of nephrin in vitro and in vivo. In addition, there appears to be a minor interaction via the SH3 domains of Nck, which is independent of tyrosine phosphorylation.

Identification of the tyrosine residues of rat nephrin responsible for molecular interaction with Nck

By Motif scan, three predicted binding sites for Nck1-SH2 in rat nephrin were, in the order of likelihood, Y1204, Y1228, and Y1127. Our previous study with mutagenesis indicated that Y1127 is not a preferred substrate for Src-family kinases [10], thus we focused on Y1204 and Y1228. We first used pull-down assay to map the binding sites for Nck. When Y1204 and Y1228 were mutated to phenylalanine individually, there was no obvious change in the interaction of nephrin to GST–Nck or GST–Nck-SH2 (Fig. 2B). However, when two tyrosine residues were mutated simultaneously, interaction to GST–Nck and GST–Nck-SH2 was virtually abolished (Fig. 2B). We next expressed rat nephrin and its mutants transiently in HEK293T cells and immunoprecipitated endogenous Nck with rabbit anti-Nck antibody. Wild-type nephrin co-immunoprecipitated with Nck in the presence of Fyn, which was abolished with the Y1204/1228F double mutant (Fig. 2C), similar to the results of pull-down assays. However, unlike the pull-down assay, single mutants of Y1204 or Y1208 also showed markedly reduced interaction with Nck, suggesting that each tyrosine residue contributes significantly to the interaction with Nck at the physiological expression level of Nck. In particular, Nck interaction of Y1228F was not distinguishable from the double mutant, implying that Y1228F may be a preferred Nck binding site in vivo. In human and mouse nephrin, in addition to the two tyrosine residues corresponding to rat Y1204 and Y1228 (Y1193/1217 in human and Y1208/Y1232 in mouse), third residue (Y1176 in human and Y1191 in mouse) also contributed to Nck binding ([5,6] and data not shown).

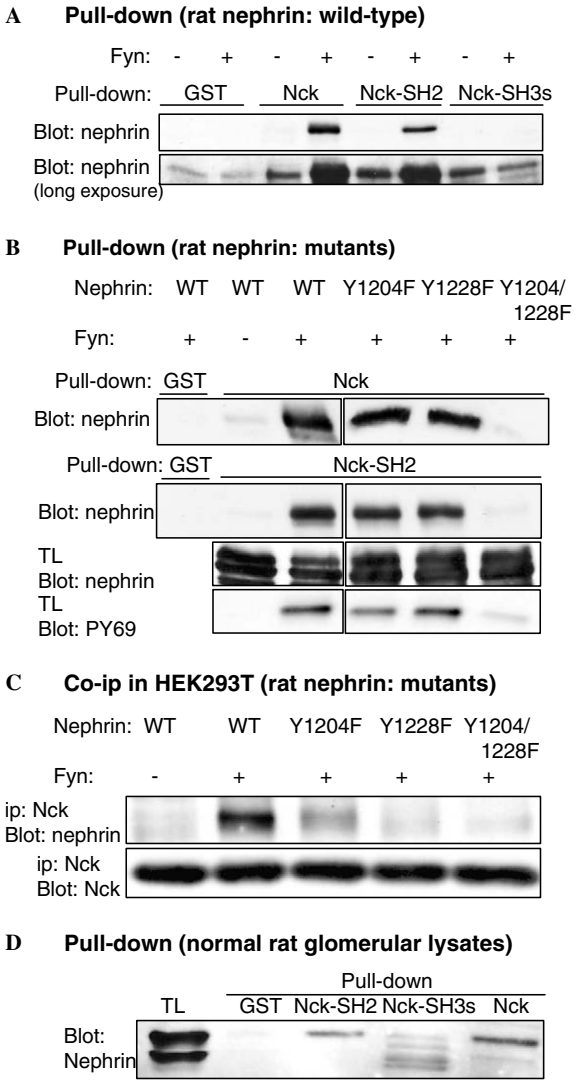

Fig. 2. Mapping of the sites responsible for nephrin–Nck interaction. (A) Cos-1 cells were transiently transfected with wild-type rat nephrin with or without Fyn. Cell lysates were subjected to pull-down assay using GST alone, GST–Nck (containing full-length Nck1), GST–Nck-SH2 (containing the SH2 domain of Nck1 alone), and GST–Nck-SH3s (containing three SH3 domains of Nck1). Precipitates were blotted for nephrin. Short (top panel) and long (bottom panel) exposure are shown. (B) Wild-type rat nephrin and its mutants were expressed in Cos-1 cells and pull-down assay was performed using GST–Nck and GST–Nck-SH2 as in (A). (C) Wild-type rat nephrin and its mutants were expressed in HEK293T cells. Endogenous Nck was immunoprecipitated with anti-Nck antibody and precipitates were blotted for nephrin and Nck. (D) Normal rat glomerular lysates were subjected to pull-down assay as in (A). Identities of the multiple bands seen with GST–Nck-SH3s pull-down are not known, however some of them may represent tyrosine phosphorylation-independent interaction of Nck with nephrin.

Cross-linking of the extracellular domain of nephrin causes actin reorganization and cell morphological changes in a Nck-dependent manner

Nck is a known regulator of actin re-organization [7] and it was shown previously that aggregation of Nck at the plasma membrane leads to localized actin polymerization (formation of "actin tails") [5,6,16]. To further address the functional consequence of nephrin–Nck interaction on the actin cytoskeleton and cell morphology, we stably expressed rat nephrin in HEK293T cells. HEK293T cells express many of the slit diaphragm proteins including podocin, FAT, and P-cadherin, and were successfully used to study homophilic interaction of nephrin previously [17]. Untransfected HEK293T cells showed relatively round morphology with distinct corti-

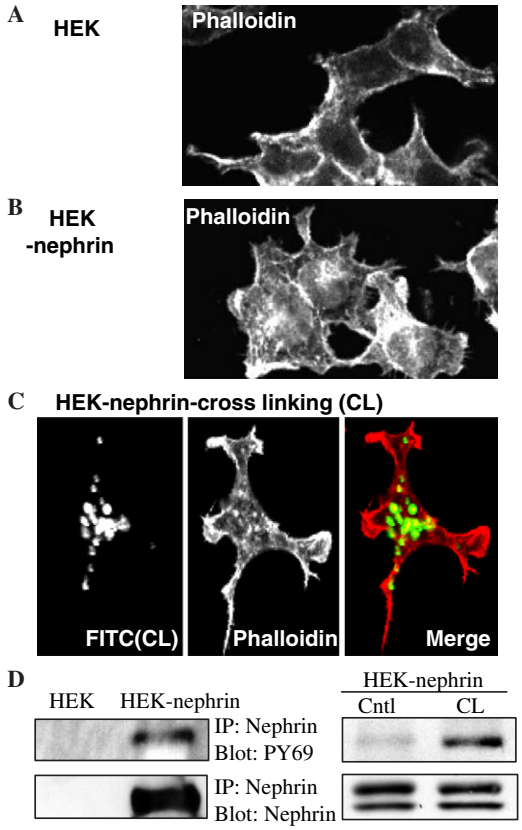

Fig. 3. Nephrin expression and its cross-linking induce morphological changes in HEK293T cells. Untransfected HEK293T cells showed relatively round morphology with distinct cortical F-actin and few cell protrusions (A, HEK). When HEK293T cells were stably transfected with rat nephrin, many cells demonstrated more cell protrusions and appeared more "spikey", as compared with untransfected cells (B, HEK-nephrin). When nephrin was cross-linked with antibodies in HEK-nephrin (Materials and methods), cells showed punctuated cytosolic F-actin, co-localizing at the site of nephrin cross-linking. Morphological changes were further augmented and many cells showed elongated cell shape with prominent cell protrusions (C, HEK-nephrin-cross-linking). Site of nephrin cross-linking is labeled with FITC (green) and phalloidin is labeled with rhodamine (red)). Transfection of HEK293T cells with vector alone, with or without cross-linking treatment, did not induce discernible morphological changes (not shown). (D) Cell lysates of HEK293T or HEK-nephrin cells with or without antibody-mediated cross-linking were immunoprecipitated with anti-nephrin antibody and blotted for phosphotyrosine or nephrin. Nephrin is tyrosine phosphorylated in unstimulated HEK-nephrin cells, which is further augmented by cross-linking. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

cal F-actin and few protrusions (Fig. 3A). When HEK293T cells were stably transfected with rat nephrin (HEK293T-nephrin or HEK-nephrin in short), nephrin was weakly but clearly tyrosine phosphorylated, likely induced by a high expression level of nephrin and homotypic binding of nephrin molecules from adjacent cells (Fig. 3D). Many HEK-nephrin cells demonstrated more cell protrusions and appeared more "spikey", as compared with untransfected cells (Fig. 3B). When nephrin was cross-linked with antibodies in HEK-nephrin (see Materials and methods), tyrosine phosphorylation of nephrin was augmented further (Fig. 3D), consistent with the previous report [18]. Cells showed punctuated cytosolic F-actin, co-localizing at the site of nephrin cross-linking and cell protrusions became more prominent, as compared with non-cross-linked HEK-nephrin. Many cells showed elongated cell shapes with prominent protrusions, resembling process formation, as shown in Fig. 3C. Transfection of HEK293T cells with vector alone or cross-linking treatment of vector transfected cells did not cause any discernible morphological changes, as compared with untransfected cells (not shown).

To study if the changes induced by nephrin expression and cross-linking are mediated by Nck, we first attempted a gene knock-down of Nck1 (Nck2 expression in HEK293T cells is negligible) using a commercially available siRNA. However, the knock-down efficiency was not satisfactory, thus we next utilized two Nck mutants. Myc–Nck-SH2 contains only the SH2 domain of Nck and HA-Nck-SH3-M3 has all three SH3 domains mutated. Both mutants would bind to and occupy the phosphotyrosine containing motifs in nephrin but are not able to recruit the downstream effector molecules via the SH3 domains, thereby blocking the signaling cascade induced by nephrin–Nck interaction. When HEK-nephrin was transfected with GFP alone and nephrin was cross-linked (GFP control), cells showed elongated morphology and prominent protrusions, consistent with the previous results (Fig. 4, top). When HEK-nephrin cells were transfected with Nck-SH2 and nephrin was cross-linked, Nck-SH2 localized mainly at the plasma membrane and transfected cells showed smoother cell contour, more distinct cortical F-actin, and fewer protrusions, as compared with GFP control (Fig. 4, middle). When Nck-SH3-M3 was transfected, it localized both at the plasma membrane and in the cytoplasm. Nck-SH3-M3 transfected cells were also generally less elongated and had fewer protrusions, as compared with GFP control (Fig. 4, bottom). The expression level of Nck-SH2 was much lower than Nck-SH3-M3, however, its dominant negative effect appeared to be more potent than Nck-SH3-M3. We have observed similar differences of the two dominant-negative constructs in other experimental systems (not shown). Although the reason is unclear, Nck-SH3-M3 may still interact with the other intracellular molecules, thereby activating some of the signaling cascades. Taken together, these results suggest that nephrin tyrosine phosphorylation-mediated morphological changes are, at least in part, mediated by Nck.

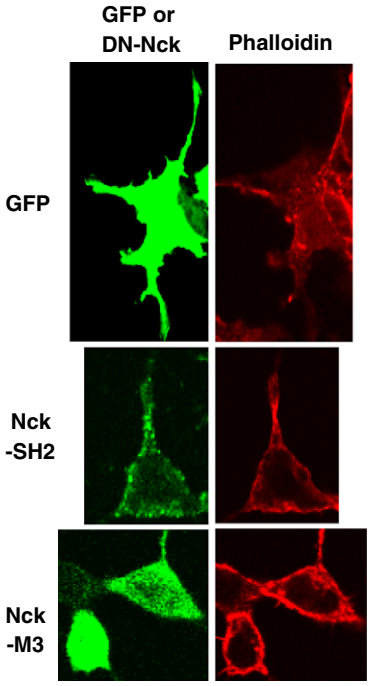

Fig. 4. Morphological changes induced by nephrin cross-linking are attenuated by dominant-negative Ncks. HEK293T-nephrin cells were transiently transfected with GFP alone, Myc–Nck-SH2, or HA–Nck-SH3-M3 (for the construct information, see Results) and nephrin was cross-linked with antibodies for all experiments. Although cross-linking is not visualized, preliminary experiments confirmed >90% efficiency of cross-linking. Expression of transfected plasmids was detected by GFP or FITC-labeled anti-Myc/anti-HA antibodies. F-actin was visualized with rhodamine–phalloidin. GFP did not affect prominent cell protrusions, while Nck-SH2 and, to a lesser extent, Nck-SH3-M3 attenuated them.

Nephrin–Nck interaction is diminished in the rat model of PAN

To study the potential role of nephrin–Nck interaction in the maintenance of glomerular permselectivity in vivo, we next utilized PAN, a well-established rat model of podocyte injury and proteinuria [19]. Seven days after a single intravenous injection of puromycin aminonucleoside, heavy proteinuria was observed (control, 5 ± 1 mg/day; PAN, 162 ± 26 mg/day; $p < 0.01$, $N = 4$ rats each). Nephrin tyrosine phosphorylation, as well as nephrin–Nck co-immunoprecipitation, was decreased by ~57% and ~46%, respectively, in rats with PAN, as compared with control rats (Fig. 5). Expression of Nck and nephrin in total glomerular lysates in rats with PAN was not significantly different, as compared with control rats (nephrin, control: 100 ± 4, PAN: 164 ± 46; Nck, control: 100 ± 3, PAN: 118 ± 13; arbitrary units, $N = 8$ rats per group). Therefore, the decrease in nephrin–Nck co-immunoprecipitation likely represents diminished interaction between these two molecules, rather than decreased glomerular expression of nephrin or Nck in PAN.

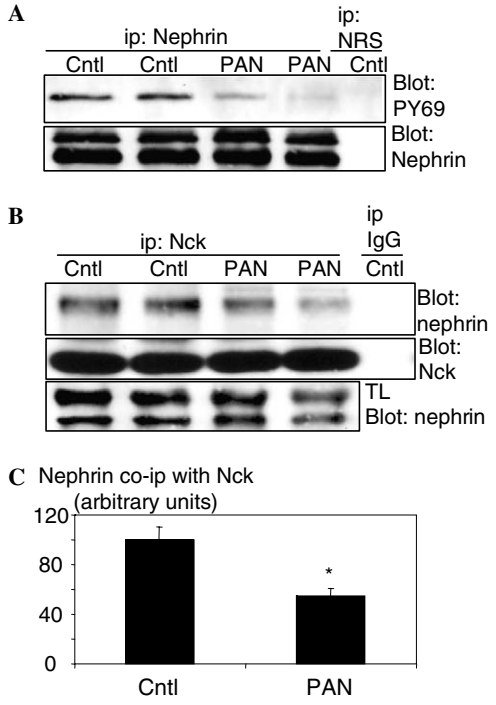

Fig. 5. Nephrin–Nck interaction is decreased in PAN. PAN was induced as in Materials and methods. Glomerular lysates from rats with PAN (day 7) and control rats were immunoprecipitated for nephrin (A) or Nck (B) and precipitates were blotted for phosphotyrosine (A) or nephrin (B). (C) Densitometric analysis of nephrin co-immunoprecipiated with Nck is shown. Error bars are SEM. $^{*}p < 0.05$ vs. Cntl (by Student's t-test), $N = 6$ rats for each group. Note that expression of nephrin and Nck is not different between PAN and control (see Results).

Discussion

There are three major novel findings in the current study; (1) in addition to human and mouse nephrin, we confirmed that rat nephrin also interacts with Nck predominantly in a tyrosine phosphorylation-dependent manner. Two tyrosine residues in rat nephrin critical for the Nck interaction were conserved among the three species, supporting that this important machinery is conserved through evolutions. (2) We demonstrated for the first time that nephrin expression and tyrosine phosphorylation lead to cell morphological changes, which resemble process formation, an important feature of differentiated podocytes in vivo. (3) We have shown that nephrin–Nck interaction is disturbed in the animal model of podocyte injury in the adult kidney.

Recent advances in podocyte biology highlight the pivotal role of the actin cytoskeleton in the maintenance of normal podocyte morphology and function [1]. It is of great interest that a number of proteins, which interact with the intracellular domain of nephrin, are known for their role in the regulation of the actin cytoskeleton; CD2AP has an actin-binding site and is known to regulate actin reorganization [20]; PI3K has versatile intracellular actions, including regulation of actin polymerization via ADP-ribosylating factor 6 (ARF6) or Rac [21]; IQGAP1 is an effector protein of small GTPases Rac1 and Cdc42 [22]. Another slit diaphragm protein, FAT1 was also shown to regulate actin dynamics via Ena/VASP proteins and FAT1 knockout mice showed deficiency in podocyte foot process development [23,24]. Taken together with the current results, it is not unreasonable to hypothesize that transmembrane proteins at the slit diaphragm, such as nephrin and FAT1, serve as the signaling scaffold, which coordinates actin dynamics in podocyte foot processes. Current results are of particular interest because tyrosine phosphorylation of nephrin and its subsequent interaction with Nck appeared to facilitate process formation of the cells, which is important for the function of podocytes in vivo. These morphological changes were not noted in the previous reports, most likely because of different cell types used (mouse embryonic fibroblasts in [5] and NIH3T3 in [6]). Whether similar morphological changes could be induced in various podocyte cell lines is currently under investigations. As well, downstream effector(s) responsible for process formation are yet to be determined.

We reported previously that podocyte specific gene deletion of Nck1 and Nck2 leads to abnormal podocyte foot process development and heavy proteinuria [5]. However, these results did not allow us to determine whether Nck is critical only for the development of normal podocytes or it is also important in the maintenance of normal podocyte function/glomerular permselectivity in the adult kidney. In the current study, we have shown that podocyte injury in rats induced by puromycin aminonucleoside led to decreased tyrosine phosphorylation of nephrin and nephrin–Nck interaction (Fig. 5). These results strongly suggest that tyrosine phosphorylation-dependent nephrin–Nck interaction has an important role not only in podocyte development but also in the maintenance of podocyte morphology/function in the adult kidney. Its disruption may contribute to impaired glomerular permselectivity in kidney diseases in adults.

Acknowledgments

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