Contents lists available at ScienceDirect

Cellular Signalling

journal homepage: www.elsevier.com/locate/cellsig

IQGAP1 regulates actin cytoskeleton organization in podocytes through interaction with nephrin

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ARTICLE INFO

Article history:
Received 13 January 2015
Accepted 27 January 2015
Available online xxxx

Keywords:
Actin cytoskeleton
IQ domain GTPase-activating protein 1
Nephrin
Podocyte

ABSTRACT

Increasing data has shown that the cytoskeletal reorganization of podocytes is involved in the onset of proteinuria and the progression of glomerular disease. Nephrin behaves as a signal sensor of the slit diaphragm to transmit cytoskeletal signals to maintain the unique structure of podocytes. However, the nephrin signaling cascade deserves further study. IQGAP1 is a scaffolding protein with the ability to regulate cytoskeletal organization. It is hypothesized that IQGAP1 contributes to actin reorganization in podocytes through interaction with nephrin. IQGAP1 expression and IQGAP1-nephrin colocalization in glomeruli were progressively decreased and then gradually recovered in line with the development of foot process fusion and proteinuria in puromycin aminonucleoside-injected rats. In cultured human podocytes, puromycin aminonucleoside-induced disruption of F-actin and disorders of migration and spreading were aggravated by IQGAP1 siRNA, and these effects were partially restored by a wild-type IQGAP1 plasmid. Furthermore, the cytoskeletal disorganization stimulated by cytochalasin D in COS7 cells was recovered by cotransfection with wild-type IQGAP1 and nephrin plasmids but was not recovered either by single transfection of the wild-type IQGAP1 plasmid or by cotransfection of mutant IQGAP1 [△1443(S → A)] and wild-type nephrin plasmids. Co-immunoprecipitation analysis using lysates of COS7 cells overexpressing nephrin and each derivative-domain molecule of IQGAP1 demonstrated that the poly-proline binding domain and RasGAP domain in the carboxyl terminus of IQGAP1 are the target modules that interact with nephrin. Collectively, these findings showed that activated IQGAP1, as an intracellular partner of nephrin, is involved in actin cytoskeleton organization and functional regulation of podocytes.

1. Introduction

Podocytes are terminally differentiated epithelial cells that are essential components of the glomerular filtration barrier, and their injury or depletion plays a pivotal role in the onset of proteinuria and the progression of glomerular diseases [1–6]. Phenotypic alterations in podocytes were detected in a variety of human and experimental glomerular diseases, and foot process (FP) fusion is a unique ultrapathological characteristic of podocyte injury [6–8]. Therefore, discovery of the molecular mechanism of FP fusion could be a crucial step in establishing an effective therapy for podocytopathy.

Previous studies have indicated that cytoskeletal reorganization is the common final pathway of FP fusion [9,10]. The highly ordered parallel contractile actin filament bundles in FP are transformed into disordered, short, branched ones under pathological conditions. Moreover, the elaborate actin cytoskeleton network links the three unique areas of FPs (the apical domain, the slit diaphragm [SD] domain and the basal domain) into structurally and functionally complete units.

A series of molecules are well known to localize to the SD domain of FPs associated with the actin cytoskeleton through scaffold/adaptor proteins, functioning as essential regulators of FP structure and dynamics [11]. Nephrin, the first-described SD protein of podocytes, behaves as a signaling hub to activate several signaling pathways essential for cytoskeletal assembly and cell survival. Collectively, the PI3K-AKT/Rac1, Nck2/N-WASP/Arp2/3, and CD2AP/CapZ/F-actin pathways have been reported to be recruited by nephrin to regulate cytoskeletal dynamics [12–14].

IQ domain GTPase-activating protein 1 (IQGAP1) is a newly discovered scaffolding protein that is also found in the SD region of podocytes [15,16] and contains several functional domains that link multiple signaling molecules, including Rac1/Cdc42, Arp2/3, CLIP-170, E-cadherin, and EGFR. Following interaction with these proteins, IQGAP1

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http://dx.doi.org/10.1016/j.cellsig.2015.01.015
0898-6568/

participates in cytoskeletal regulation, ultimately triggering diverse cellular behaviors, such as adhesion, migration, and polarization [17].

Our previous study demonstrated that IQGAP1 had a linear distribution along the capillary loops of glomeruli *in vivo*. Furthermore, IQGAP1 and nephrin colocalized in podocytes [18]. These findings raised our interest regarding whether IQGAP1 contributes to cytoskeletal regulation in podocytes, and whether the function of IQGAP1 in podocytes is realized through interaction with nephrin. In the present study, we used a puromycin aminonucleoside (PAN) nephrosis rat model and PAN-stimulated human podocytes to evaluate the role of IQGAP1 in podocyte cytoskeletal reorganization.

2. Material and methods

2.1. Animals

All experimental procedures were approved by the ethics committee for animal experiments of Wuhan University. Twenty-four male SPF Wistar rats (140 to 160 g) were supplied by the Hubei Research Center of Experimental Animals and raised in a temperature- and humidity-controlled laminar flow room under a 12 h light/12 h dark cycle with free access to tap water and standard rat chow. The PAN model was established [19] by a single intraperitoneal injection of PAN (15 mg/100 g body weight, Sigma-Aldrich, USA). The 24-h urinary protein was measured on days 0, 4, 7, and 28 after injection. Animals were sacrificed under 10% chloral hydrate anesthesia on day 0, 4, 7, or 28. Kidneys were perfused with the phosphatase inhibitor vanadate before harvest. Part of the kidney was used for the isolation of glomeruli. The cortex of the rest of the kidney was separated and fixed in 4% phosphate-buffered paraformaldehyde, glutaraldehyde, or tissue-freezing medium for renal pathological and immunofluorescence analysis.

Glomeruli were isolated by the differential sieving method [20,21]. The renal cortex was separated and successively passed through three stainless steel sieves with pore sizes of 177 μm (80 mesh), 125 μm (120 mesh), and 74 μm (200 mesh). D-Hank's buffer containing vanadate was used for the continuous rinsing of debris. Glomeruli collected from the last sieve were harvested and used for Western blotting analysis.

Transmission electron microscopy was used to evaluate the ultrastructural changes of podocytes. Briefly, 2.5% glutaraldehyde-fixed 1-mm^3 blocks of renal cortices were postfixed with 1% osmic acid, dehydrated in graded ethanol, embedded in EPON, sectioned, stained with uranyl acetate and lead citrate, and then observed and recorded with a Hitachi H-600 transmission electron microscope (Hitachi, Japan). The mean FP width was defined as the total length of the glomerular basement membrane (GBM) divided by the total number of FPs. The FP fusion rate was presented as the length of FP fusion divided by the total length of the GBM.

2.2. Cell culture

Conditionally immortalized human podocytes were provided by Dr. Moin A. Saleem (Academic Renal Unit, Southmead Hospital, Bristol, UK) and cultured in RPMI 1640 medium (HyClone, USA) containing 10% fetal bovine serum (Gibco, USA), 1× penicillin–streptomycin, and 1 mM L-glutamine in a 5% CO_2 incubator. During the proliferation phase, 1× insulin, transferrin, and selenium (ITS) (Invitrogen, USA) was added to the medium, and the cells were subcultured at 33 °C. These cells were cultured in a 37 °C incubator with ITS-free medium for 7 days to induce differentiation.

COS7 cells were purchased from the Cell Resource Center of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, and cultured in a 37 °C incubator in DMEM (Corning, USA) containing 10% fetal bovine serum (Gibco, USA). G418 (Sigma-Aldrich, USA) was used to select stably transfected nephrin cell lines (NCOS7). When the cells had grown to approximately 60% confluence, 20 μg/mL cytochalasin D (CyD, Enzo Life Sciences, Switzerland) was added to the medium for 30 min to depolymerize the actin cytoskeleton [22].

2.3. Plasmid construction and transfection

The full-length expression plasmids of nephrin (pcDNA3.1-NPHS1) and IQGAP1 (pCAN-myc-IQGAP1) were kindly provided by Dr. Lawrence B Holzman (University of Michigan Medical School, MI, USA) and Dr. Jon Erickson (Cornell University, NY, USA), respectively. The △1443(S → A) IQGAP1 mutant plasmid was constructed by mutating the serine to alanine to inhibit IQGAP1 phosphorylation. The pEGFP-C1 eukaryotic expression vector was purchased from Clontech (USA) and reformed to carry a Myc tag (pEGFP-C1-Myc). The Myc primers used in PCR amplification were as follows: sense: 5′-TCTGAAGAGGATCTGTCCGGACTCAGATCTC-3′ and anti-sense: 5′-CAGATCCTCTTCAGAGATGAGTTTCTGCTCCTTGTACAGCTCGTCC-3′. The calponin homology domain (CHD), the IQGAP-specific repeat motif (IR), the poly-proline binding domain (WW), the calmodulin-binding motif (IQ), the RasGTPase-activating protein-related domain (GRD), and the RasGAP domain in the carboxyl terminus (RGCT) of IQGAP1 were then subcloned into pEGFP-C1-Myc. Detailed information for the plasmids is shown in Table 1.

For plasmid transfection, 6×10^5 cells were seeded into a 100-mm dish and incubated with transfection complexes containing 5 μg relevant plasmid and 15 μL X-tremeGENE HP DNA Transfection Reagent (Roche, Germany) under growth conditions for 72 h.

2.4. Transfection of IQGAP1 siRNA

Transfection of podocytes with IQGAP1 siRNA was performed according to the HiPerFect Transfection Reagent Handbook (QIAGEN, Germany). Briefly, 3×10^6 cells were seeded into a 100-mm dish and incubated with transfection complexes containing 5 nM IQGAP1 siRNA (or scrambled siRNA) and 40 μL HiPerFect transfection reagent under growth conditions for 48 h.

Table 1
Detailed IQGAP1 plasmid information.

Plasmids	Amino acids	Primers
△1443(S → A)	1443	Sense: 5′-ATGAAAAAGTCAAAA**GC**TGTAAAGGAAG-3′ Anti-sense: 5′-**C**TTTTGACTTTTTCATCTTGTCAGG-3′
CHD	44–159	Sense: 5′-CCGCTCGAGCTCTTTGTCATTTGGAAGA AGC-3′ Anti-sense: 5′-CGGGGTACCTTACAGGTACAAACTGAGT GCATG-3′
IR	266–642	Sense: 5′-CCGCTCGAGCTAAGCAGGACAAAATGACAA ATGC-3′ Anti-sense: 5′-CGGGGTACCTTATGTTTTGCCAACATCA CCACTTTC-3′
WW	643–744	Sense: 5′-CCGCTCGAGCTCTGAGTGCCCTTCGCTCC-3′ Anti-sense: 5′-CGGGGTACCTTAGGCCAGCCACAGCTGT TC-3′
IQ	745–864	Sense: 5′-CCGCTCGAGCTAATGAAGGCCTGATCACCA GG-3′ Anti-sense: 5′-CGGGGTACCTTAAGGATCCTCAGCATTG ATGAGAG-3′
GRD	1004–1237	Sense: 5′-CCGCTCGAGCTTCTGTAATCTTCACACTCTAC AAC-3′ Anti-sense: 5′-CGGGGTACCTTAGGAAGCAGCATGCTGA AG-3′
RGCT	1563–1657	Sense: 5′-CCGCTCGAGCTTATACAGCAGCAAGACTAC ATG-3′ Anti-sense: 5′-CGGGGTACCTTACTTCCCGTAGAACTTT TTGTTGAG-3′

Please cite this article as: Y. Liu, et al., IQGAP1 regulates actin cytoskeleton organization in podocytes through interaction with nephrin, Cell. Signal. (2015), http://dx.doi.org/10.1016/j.cellsig.2015.01.015