



Site-specific expression of IQGAP1 in human nephrons

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Abstract

IQGAP1 is a multifunctional, 190-kDa scaffolding protein that plays an important role in the regulation of cell adhesion, migration, proliferation, differentiation, polarization and cytoskeletal remodeling. IQGAP1 is ubiquitously expressed in human organs and is highly expressed in the kidney. Currently, the site-specific expression of IQGAP1 in the human nephrons is unclear. We performed Western blotting analysis, immunohistochemistry and double-immunolabeling confocal microscopic analysis of IQGAP1 with specific biomarkers of each nephron segment to study the expression and distribution of IQGAP1 in human nephrons. We found that IQGAP1 was strongly expressed in human podocytes and glomerular endothelial cells, but weakly expressed in glomerular mesangial cells. In human renal tubules, IQGAP1 was strongly expressed in the collecting duct, moderately expressed in the proximal tubule, medullary loop, distal convoluted tubule and connecting tubule. IQGAP1 staining was much stronger in the apical membrane in the proximal tubule, thick descending limb and thick ascending limb of medullary loop and collecting duct. However, the expression of IQGAP1 was mainly in the basolateral membrane of the connecting tubule, and diffusely in the thin limb of medullary loop and distal convoluted tubule. The interaction between IQGAP1 and F-actin suggested that cytoskeleton regulation may be the underlying mechanism mediating the effect of IQGAP1 in human nephrons. To the best of our knowledge, this is the first report of specific expression and differential subcellular location of IQGAP1 in human nephrons. The site-specific expression pattern of IQGAP1 suggests that IQGAP1 may play diverse roles in various human nephron segments.

Keywords IQGAP1 · Human glomeruli · Human renal tubules · F-actin

Introduction

IQ domain GTPase-activating protein 1 (IQGAP1) is a multifunctional, 190-kDa scaffolding protein that was first cloned in 1994 in osteosarcoma; it is widely present in yeast, leeches and mammals (Weissbach et al. 1994). There are three isoforms of IQGAP proteins in eukaryotes, IQGAP1, IQGAP2 and IQGAP3. IQGAP1 is the most well-known member of the family, and it is highly conserved.

IQGAP1 consists of a calponin homology domain interacting with actin (Magill et al. 2016), a domain with two conserved tryptophan residues (WW domain) interacting with ERK1/2 (Cheung et al. 2013), an IQ-specific repeat motif (IQ domain) interacting with calmodulin and MEK1/2 (Pelikan-Conchaudron et al. 2011), a GAP-related domain (GRD domain) interacting with Rho GTPase, Cdc42 and Rac1 (Nouri et al. 2016), and a C-terminus domain interacting with APC, CLIP170 and E-cadherin (Fram et al. 2014; Swiech et al. 2011; Watanabe et al. 2004). By interacting with diverse protein kinases, signaling molecules and transporters, IQGAP1 plays important roles in the regulation of cell adhesion, migration, proliferation, differentiation, polarization and cytoskeletal remodeling.

IQGAP1 is ubiquitously expressed in various organs of the human body, including lung, pancreas, heart, brain and kidney (Weissbach et al. 1994); it participates in various physiological and pathological processes. By interacting with RhoA and p190A-RhoGAP, IQGAP1 contributes to the severity of asthma via controlling airway smooth muscle

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contractility in the lung (Bhattacharya et al. 2014). Kimura et al. reported that IQGAP1 in the pancreas interacts with GDP-bound Rab27a when it forms a complex with GTP-bound Cdc42 and regulates the endocytosis of insulin secretory membranes (Kimura et al. 2013). In the heart, IQGAP1 regulates ERK1/2 and AKT signalling and sustains functional remodelling upon pressure overload (Sbroglio et al. 2011). Furthermore, in the nervous system, several studies verified the vital role of IQGAP1 in neurite outgrowth, spine development, synaptic plasticity, memory formation and dendrite formation (Jausoro et al. 2013; Swiech et al. 2011).

In the kidney, IQGAP1 was first found in canine kidney II cells in 2001 (Fukata et al. 2001). Subsequently, the distribution and function of IQGAP1 in the kidney gradually attracted attention. Rigotherier et al. found that IQGAP1 expressed in podocyte processes and cell bodies, glomerular parietal epithelial cells and endothelial cells of normal human kidney sections (Rigotherier et al. 2012). The colocalization of IQGAP1 with nephrin, podocalyxin, β -catenin, F-actin, synaptopodin and MAGI-1 in podocytes suggested that IQGAP1 may be an integral component of the slit diaphragm (Rigotherier et al. 2012). Recent evidence demonstrated that IQGAP1 was strongly expressed in the macula densa, distal convoluted tubule (DCT) and collecting duct (CD) and was moderately expressed in the thick ascending limb (TAL) and proximal tubule (PT) in mouse renal tubule, suggesting that IQGAP1 may play several important roles in various renal functions (Lai et al. 2008). Unfortunately, there has not yet been a study reporting site-specific expression of IQGAP1 in various human nephron segments. The purpose of this study was to characterize the site-specific expression of IQGAP1 and preliminarily to investigate the possible functional mechanisms of IQGAP1 in various segments of the human nephron.

Materials and methods

Human renal samples

Renal tissue samples were obtained from the healthy kidney poles of patients who underwent nephrectomy for solitary renal carcinoma without other renal diseases. These investigations were approved by the Ethics Review Committee of Shandong Provincial Qianfoshan Hospital, Shandong University and were implemented according to the Declaration of Helsinki. All participants provided written informed consent.

Cell culture

Conditionally immortalized human podocytes were originally provided by Dr. Moin A. Saleem (Academic Renal

Unit, Southmead Hospital, Bristol, UK) and were cultured in RPMI 1640 medium (HyClone, USA) containing 10% fetal bovine serum (Gibco, USA) and $1 \times$ penicillin–streptomycin in a 5% CO₂ incubator. The cells were subcultured at 33 °C during the proliferation phase and were then cultured in a 37 °C incubator for 7 days to induce differentiation.

Human glomerular endothelial cells (HRGECs), glomerular mesangial cells (HRMCs) and proximal tubular epithelial cell line (HK-2) were provided by Dr. Yi Fan (Department of Pharmacology, Shandong University School of Medicine, Jinan, China) and were cultured as described (Wang et al. 2014).

siRNA transfection

IQGAP1 siRNA was synthesized by RIBOBIO Biotechnology Co., Ltd. (China) and transfected according to the manufacturer's instructions. In short, the differentiated podocytes were incubated with transfection complexes containing 5 μ L of siRNA and 12 μ L of riboFECTTM CP transfection reagent under growth conditions for 48 h.

Western blotting

Protein concentrations were measured using an enhanced BCA protein assay kit (Beyotime Biotechnology, China). Equal quantities of boiled protein in loading buffer were separated using 10% SDS–PAGE and were then electrophoretically transferred to polyvinylidene fluoride membranes (Millipore, USA). The membranes were incubated with primary antibodies [IQGAP1 rabbit polyclonal antibody (pAb), 1:200, Santa Cruz, USA; F-actin mouse monoclonal antibody (mAb), 1:200, Abcam, UK; β -actin mouse mAb, 1:1000, Santa Cruz, USA] overnight at 4 °C followed by incubation with a horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibody (1:10,000, Cell Signaling Technology, USA) respectively. Bands were visualized by ECL reagent (Santa Cruz, USA). The integrated optical density of each band was calculated using image J.

Immunohistochemistry

Paraffin-embedded sections were deparaffinised and treated with 3% H₂O₂ for 30 min at room temperature. Antigen retrieval for IQGAP1 was performed in high pressure Tris–EDTA (10 mM Tris–HCl, 1 mM EDTA, pH 9.0) for 10 min. Endogenous peroxidase was blocked with 5% bovine serum albumin in 10 mM phosphate-buffered saline (PBS, pH 7.4) for 30 min, and sections were incubated with IQGAP1 primary antibody (IQGAP1 rabbit pAb, 1:100, Santa Cruz, USA) overnight at 4 °C. Sections were washed in PBS, followed by incubation with a biotinylated anti-rabbit secondary antibody and an avidin–biotin

peroxidase complex (ZSBIO, China) for 30 min at 37 °C. After rinsing, the peroxidase activity was visualised by DAB (ZSBIO, China), and sections were counter-stained with haematoxylin.

Immunofluorescence

For histoimmunofluorescence analysis, frozen kidney sections (5- μ m thick) were blocked with 5% BSA for 30 min at 37 °C, incubated with IQGAP1 primary antibody (IQGAP1 rabbit pAb, 1:50, Santa Cruz, USA; or IQGAP1 mouse mAb, 1:50, Santa Cruz, USA) and one of the segment specific biomarkers of the nephron [synaptopodin goat pAb (1:100; Santa Cruz, USA) for podocytes; α -SMA mouse mAb (1:100; Boster, China) for HRMCs; CD31 mouse mAb (1:100; Biolegend, USA) for HRGECs; AQP1 goat pAb (1:50; Santa Cruz, USA) for PT; Tamm-Horsfall protein (THP) mouse mAb (1:100; Santa Cruz, USA) for medullary loop (ML), Calbindin D-28K mouse mAb (1:100; Santa Cruz, USA) for DCT; AQP3 goat pAb (1:100; Santa Cruz, USA) for CD] overnight at 4 °C. Fluorescent labeled secondary antibodies were subsequently used for multilabelling immunodetection.

For immunocytofluorescence analysis, cells grown on glass slides were fixed with 4% paraformaldehyde for 30 min at 4 °C and then incubated with IQGAP1 primary antibody (IQGAP1 rabbit pAb, 1:50, Santa Cruz, USA; or IQGAP1 mouse mAb, 1:50, Santa Cruz, USA) overnight at 4 °C. Fluorescent labeled secondary antibodies were subsequently used for immunodetection.

The fluorescent-labeled secondary antibodies included: FITC-conjugated goat anti-rabbit and anti-mouse IgG (1:100, ZSBIO, China); TRITC-conjugated goat anti-rabbit and anti-mouse IgG (1:100, ZSBIO, China); FITC-conjugated donkey anti-rabbit IgG (1:100, Santa Cruz, USA); and TRITC-conjugated donkey anti-goat IgG (1:100, Santa Cruz, USA).

Finally, all the samples were examined using a confocal fluorescence microscope (LSM780, Carl Zeiss, Germany) and were analyzed using the ZEN system 2012 (Carl Zeiss, Germany).

Coimmunoprecipitation

Total protein of cultured podocytes, HRGECs, HRMCs and HK-2 were extracted using lysis buffer (1 mM PMSF, 5 mM EDTA, 1.0% Triton X-100, 150 mM NaCl, 20 mM Tris, pH 7.5) containing a protease inhibitor cocktail (P8340, Sigma-Aldrich, USA). The samples were centrifuged at 13,000 \times g for 5 min at 4 °C. IQGAP1 rabbit pAb (2 μ g/500 μ g total protein; Santa Cruz, USA) was added to the supernatant and rotated overnight at 4 °C. Then, the mixture was loaded with protein A+G agarose (Beyotime Biotechnology, China) and

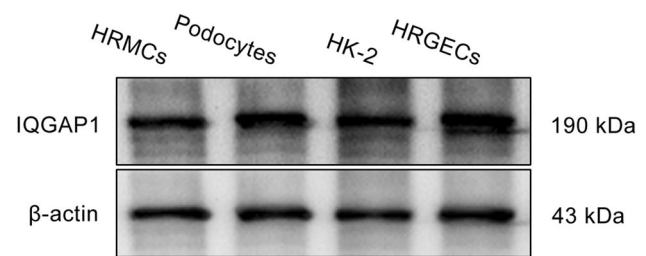


Fig. 1 The expression of IQGAP in human HRMCs, Podocytes, HK-2 and HRGECs. HRGECs showed the highest level of IQGAP1, followed by podocytes, HK-2 and HRMCs. HRMCs human glomerular mesangial cells, HK-2 proximal renal tubular epithelial cells, HRGECs human glomerular endothelial cells

Table 1 Expression of IQGAP1 in human glomeruli

Components	Intensity
Podocytes	Strong
Glomerular mesangial cells	Minimal
Glomerular endothelial cells	Strong

incubated for 3 h at 4 °C. The centrifuged sediment was saved and mixed with 1 \times LDS sample buffer. After boiling at 95 °C for 10 min, the samples were analyzed by Western blotting.

Results

The expression of IQGAP in human renal parenchymal cells

The expression levels of IQGAP1 in human renal parenchymal cells were tested by Western blotting. And IQGAP1 showed variable expression levels in different renal parenchymal cells. The order of expression level from the most to the least was HRGECs (1.36 ± 0.10), podocytes (1.12 ± 0.10), HK-2 (0.93 ± 0.14) and HRMCs (0.78 ± 0.12) (Fig. 1).

The distribution of IQGAP1 in the human renal nephron

The striking expression difference of IQGAP1 in various human renal parenchymal cells manifested by Western blotting analysis impelled us to further explore the site-specific expression and distribution of IQGAP1 in the human nephrons. In this study, we performed immunohistochemical and immunofluorescent staining. The results were summarized in Tables 1 and 2, shown in Figs. 2, 3, 4 and 5, and described as follows.

Table 2 Expression of IQGAP1 in human renal tubules

Components	Intensity	Location
Proximal tubule	Moderate	Apical > basolateral
Medullary loop		
Thick descending limb	Moderate	Apical > basolateral > cytosolic
Thin limb	Moderate	Diffuse
Thick ascending limb	Moderate	Apical > basolateral > cytosolic
Distal convoluted tubule	Moderate	Diffuse
Connecting tubule	Moderate	Basolateral > apical
Collecting duct		
Cortex	Strong	Apical and basolateral > cytosolic
Medulla	Strong	Apical

Glomeruli

The distribution of IQGAP1 in glomeruli was measured by immunohistochemistry (Fig. 2) and double-immunolabeling (Fig. 3) using antibodies against IQGAP1 and the specific biomarkers, synaptopodin, α -SMA and CD31 that identified podocytes, HRMCs and HRGECs, respectively. IQGAP1 was widely expressed in glomeruli and was strongly expressed in human podocytes and HRGECs. However, HRMCs showed weak IQGAP1 staining. Furthermore, the results were consistent with the expression level of IQGAP1 in human renal parenchymal cells detected by Western blotting (Fig. 1).

Proximal tubules

IQGAP1 was moderately expressed moderately in the PT with the apical membrane staining much stronger than that of the basolateral membrane (Figs. 2, 4).

Medullary loop

Similar to the PT, IQGAP1 was moderately expressed in the thick descending limb (TDL), the thin limb (TNL) and TAL of ML, with staining mainly in the cell membrane and with the apical membrane staining much stronger than that of the basolateral membrane in the TDL and TAL. However, the distribution of IQGAP1 was diffuse in the TNL. And interestingly, we found that the IQGAP1 staining intensity decreased as the ML moved from cortex to medulla without changing in the distribution pattern (Figs. 2, 4).

Distal convoluted tubule

Dual staining using antibodies against IQGAP1 and calbindin D-28K showed that IQGAP1 was moderately expressed in the DCT, and the intensity was not substantially different in the apical or basolateral membrane or in the cytosol (Fig. 5). And the result was consistent with the immunohistochemical staining (Fig. 2).

Connecting tubule

IQGAP1 was moderately expressed in the connecting tubule (CNT) with the basolateral membrane staining much stronger than that of the apical membrane (Fig. 2).

Collecting duct

In contrast to the structure above, IQGAP1 was strongly expressed in human cortex CD (cCD) and medulla CD (mCD) (Figs. 2, 5), however, the staining patterns in the cCD and mCD were different. In human cCD, IQGAP1 staining was seen diffusely, with strong expression in the apical and basolateral membrane, and slightly weak expression in

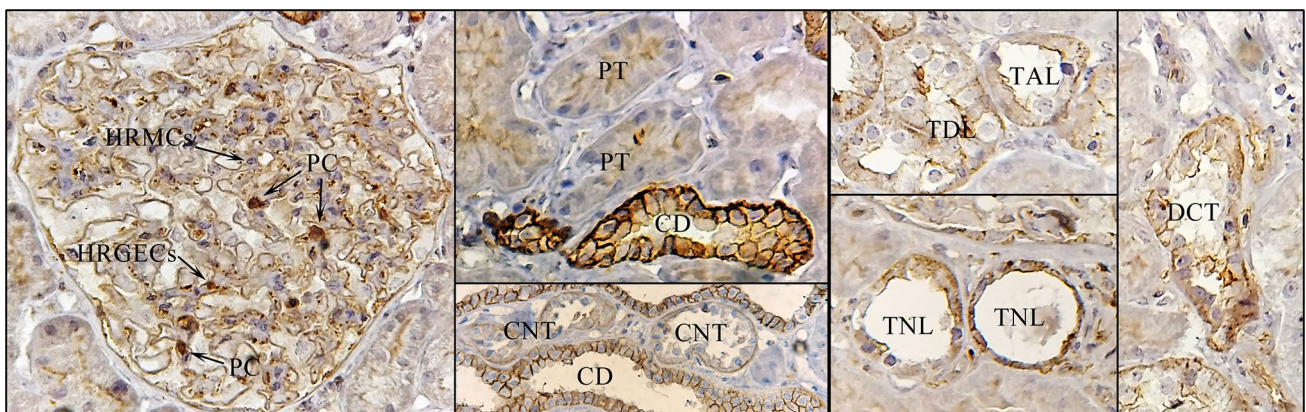


Fig. 2 The distribution of IQGAP1 in human nephrons by immunohistochemical staining. *PC* podocytes, *HRMCs* human glomerular mesangial cells, *HRGECs* human glomerular endothelial cells, *PT*

proximal tubule, *TDL* thick descending limb, *TNL* thin limb, *TAL* thick ascending limb, *DCT* distal convoluted tubule, *CNT* connecting tubule, *CD* collecting duct

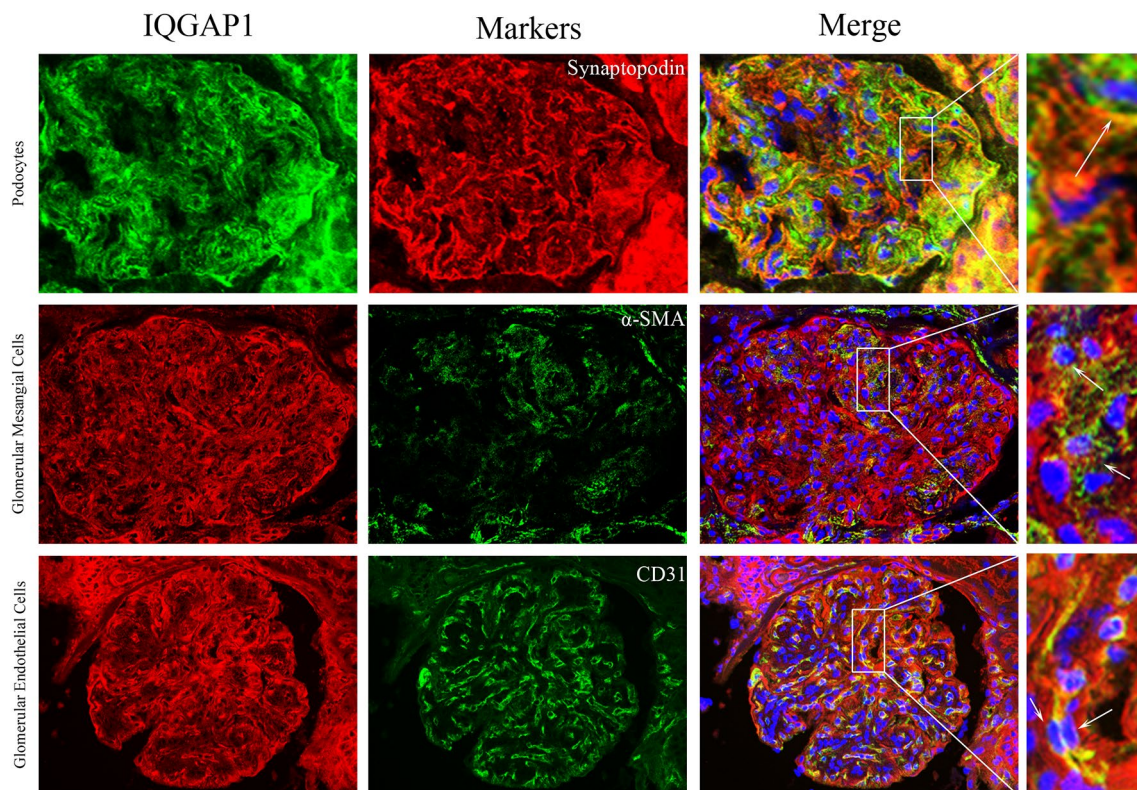


Fig. 3 The distribution of IQGAP1 in human glomeruli by immunofluorescent staining. The colocalization area of IQGAP1 and markers in the last column is the enlarged view of the boxed region. The

white arrows indicate the podocytes, glomerular mesangial cells and glomerular endothelial cells respectively

the cytosol (Fig. 5). Unlike the cCD, IQGAP1 was mainly expressed in the apical membrane in mCD, as shown in Fig. 5.

The interaction between IQGAP1 and F-actin

In view of the important cytoskeletal regulation of IQGAP1, we tested the interaction between IQGAP1 and F-actin in various human renal parenchymal cells by coimmunoprecipitation to explore the possible potential mechanisms for its role in various nephron segments. We were encouraged by the results that implied an interaction between IQGAP1 and the cytoskeleton system in various human renal parenchymal cells (Fig. 6).

The specificity of the anti-IQGAP1 antibodies

We performed Western blotting and Immunocytofluorescent staining in podocytes transfected with the specific IQGAP1 siRNA to confirm the specificity of the antibodies. As predicted in the Western blotting, there was only one band, with an estimated molecular mass of 190 kDa, consistent with IQGAP1, and the gray value of the band

was significantly decreased by IQGAP1 siRNA transfection ($P < 0.05$) (Fig. 7a).

The two anti-IQGAP1 antibodies used in the immunocytofluorescent staining could specifically identify the distribution of IQGAP1. In the normal podocytes, IQGAP1 expressed in the cytoplasm with strong fluorescence intensity, and IQGAP1 siRNA transfection significantly decreased the fluorescence intensity (Fig. 7b).

Discussion

To the best of our knowledge, our study is the first to report the immunolocalization of IQGAP1 in human nephron segments. We showed that IQGAP1 is abundantly expressed and its cellular localization is highly segment-specific in the human nephron according to Western blotting, immunohistochemical and immunofluorescent staining. The expression levels of IQGAP1 and its interaction with F-actin varied among various human renal parenchymal cells, indicating that IQGAP1 may play different roles among various nephron segments by regulating the cytoskeleton network.

On the whole, the cellular localization of IQGAP1 was highly segment-specific. As is shown above, human nephron

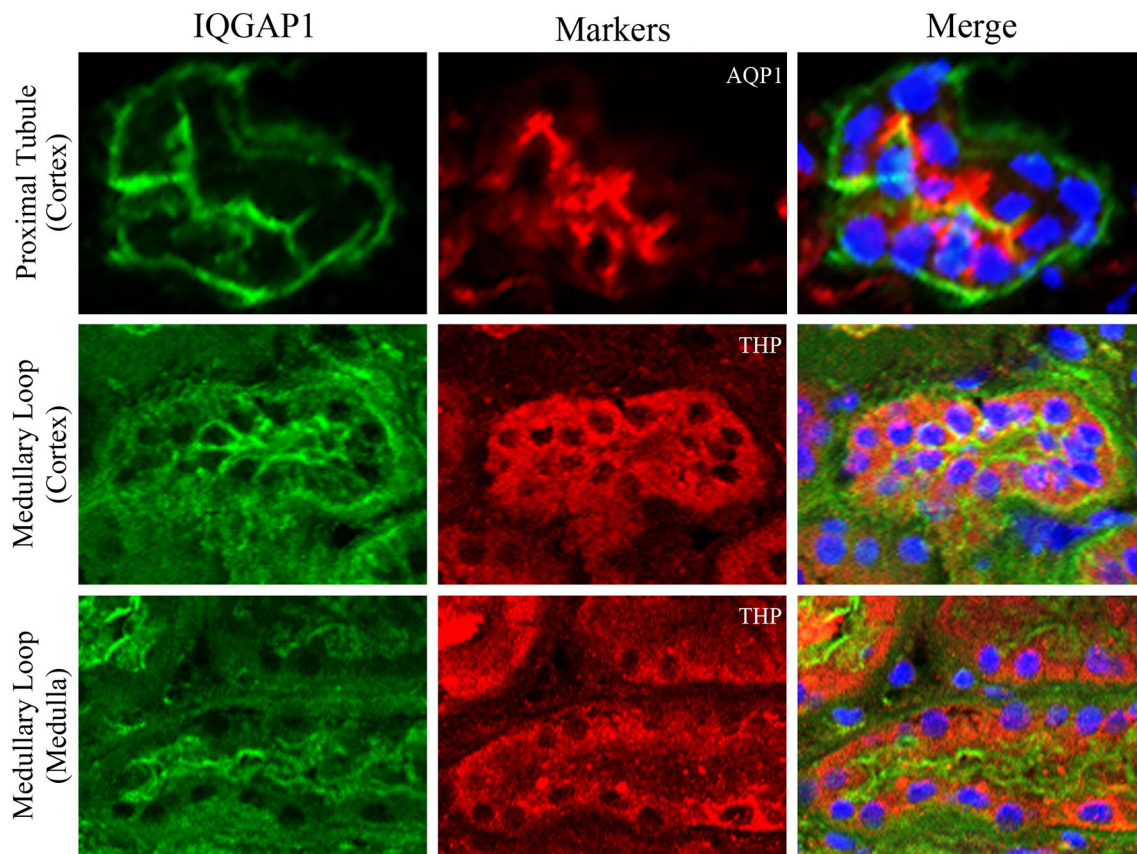


Fig. 4 The distribution of IQGAP1 in human proximal tubule, cortex medullary loop and medulla medullary loop by immunofluorescent staining

segments can be divided into three groups by intensity of IQGAP1 expression: (a) strong expression of IQGAP1: podocytes, HRGECs, cCD and mCD; (b) moderate intensity: PT, ML, DCT and CNT; and (c) minimal intensity: HRMCs. For the location of IQGAP1, there are four distinct patterns: (a) predominantly apical staining: PT, TDL, TAL and mCD; (b) predominantly apical and basolateral staining: cCD; (c) predominantly basolateral staining: CNT; and (d) diffuse staining: TNL and DCT. Renal epithelial cells are polarized cells, and the distinct subcellular distribution of IQGAP1 suggests that IQGAP1 may have specific functions in various nephron segments.

In human glomeruli, IQGAP1 was detected strongly expressed in the podocytes and HRGECs, and minimally expressed in the HRMCs. Podocytes were highly specialized and terminally differentiated epithelial cells that play an important role in maintaining the integrity of the glomerular filtration barrier. The structure of the podocyte is divided into the cell body, microtubule-driven membrane extensions termed primary processes, and actin-driven membrane extensions termed foot processes (FPs). Neighboring podocyte FPs are interdigitated to form the slit diaphragms (Grahammer et al. 2013). Rigotherier et al. reported that IQGAP1

was mainly expressed in podocyte processes and cell bodies and colocalized with nephrin and other components of the slit diaphragm complex that are involved in podocyte migration and permeability (Rigotherier et al. 2012). Our previous study also indicated that the interaction between IQGAP1 and nephrin contributed to modulate actin cytoskeleton and maintenance of the integrity of the slit diaphragm (Liu et al. 2015). Furthermore, another study by our team demonstrated that IQGAP1 participated in Ang II-induced podocytes apoptosis via the ERK1/2 MAPK signaling pathway (Liu et al. 2013). The strong fluorescence intensity of IQGAP1 in the present study also suggested its important role in podocyte function. Vascular endothelial growth factor (VEGF) affects nearly all aspects of blood vessel formation and function. VEGF-A is constitutively expressed in podocytes (Suyama et al. 2018) and its receptors VEGF receptor 1 (VEGFR1) and VEGF receptor 2 (VEGFR2) are mainly localized in glomerular endothelial cells (Robert et al. 2000). IQGAP1 binds to VEGFR2 and participates in VEGF-mediated endothelial cell migration and proliferation, necessary for the maintenance and repair of blood vessels (Yamaoka-Tojo et al. 2004). A subsequent study indicated that the IQGAP1/VEGFR2 interaction regulated angiogenesis (Li

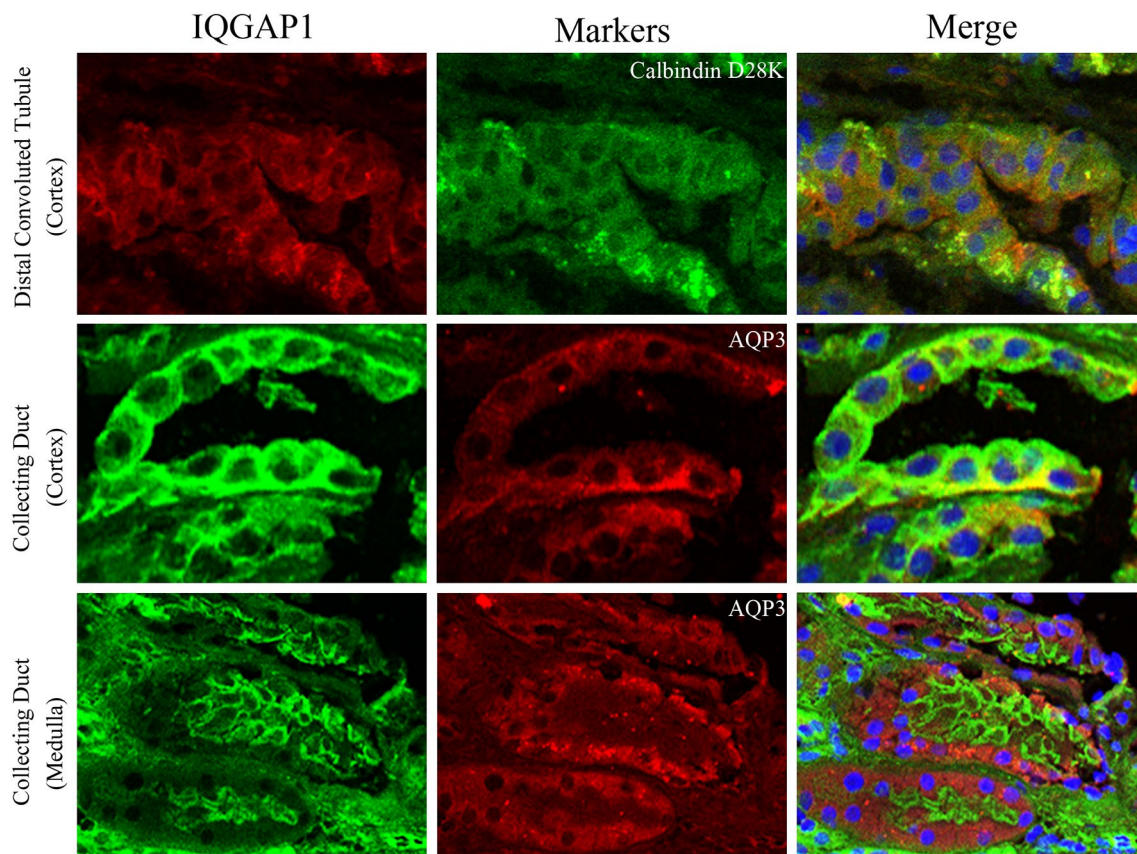


Fig. 5 The distribution of IQGAP1 in human distal convoluted tubule, cortex collecting duct and medulla collecting duct by immunofluorescent staining

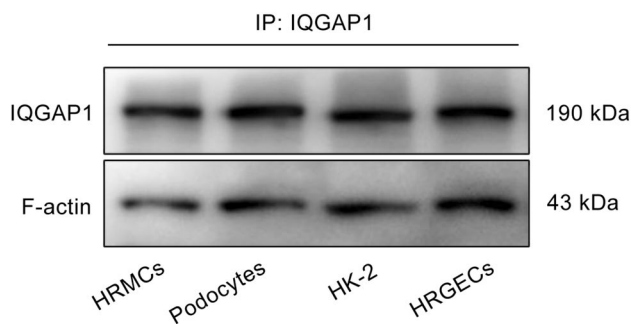


Fig. 6 The interaction between IQGAP1 and F-actin in human renal parenchymal cells by coimmunoprecipitation. *HRMCs* human glomerular mesangial cells, *HRGECs* human glomerular endothelial cells, *HK-2* proximal renal tubular epithelial cells

et al. 2018). Therefore, the strong expression of IQGAP1 in HRGECs suggests that IQGAP1 may play an important role in endothelial function. However, further in-depth research regarding the specific functional mechanism of IQGAP1 in human glomeruli remains needed.

Next, we looked at the human renal tubules. IQGAP1 is strongly expressed in the CD, hinting at a possible important

role of IQGAP1 in this segment. In the kidney aquaporin-2 (AQP2) was localized to apical membranes and subapical vesicles in cortical and medullary CD (Mobasheri et al. 2005). Reabsorption of water from the CD occurs when AQP2 is translocated to the apical plasma membrane of CD (Ikeda and Matsuzaki 2015). AQP2 phosphorylation and actin depolymerization play key roles in the translocation of AQP2 (Jung and Kwon 2016). PKA-anchoring proteins (AKAPs) can recruit PKA to AQP2-bearing vesicles, resulting in the phosphorylation of AQP2 (Nedvetsky et al. 2009). A recent study demonstrated that AKAP220 is an AQP2-binding protein that colocalizes with AQP2 in the inner medullary CD (Okutsu et al. 2008). Studies of Whiting et al. indicated that IQGAP1 interacted with AKAP220 to regulate actin polymerization and microtubule stability during membrane protrusion and cell migration, as well as managing apical actin networks that coordinate AQP2 location and renal water reabsorption (Logue et al. 2011; Whiting et al. 2016).

In addition, we found an interesting phenomenon: IQGAP1 staining was stronger in the apical membrane than in the basolateral membrane in almost all nephron segments. The establishment and maintenance of renal epithelial

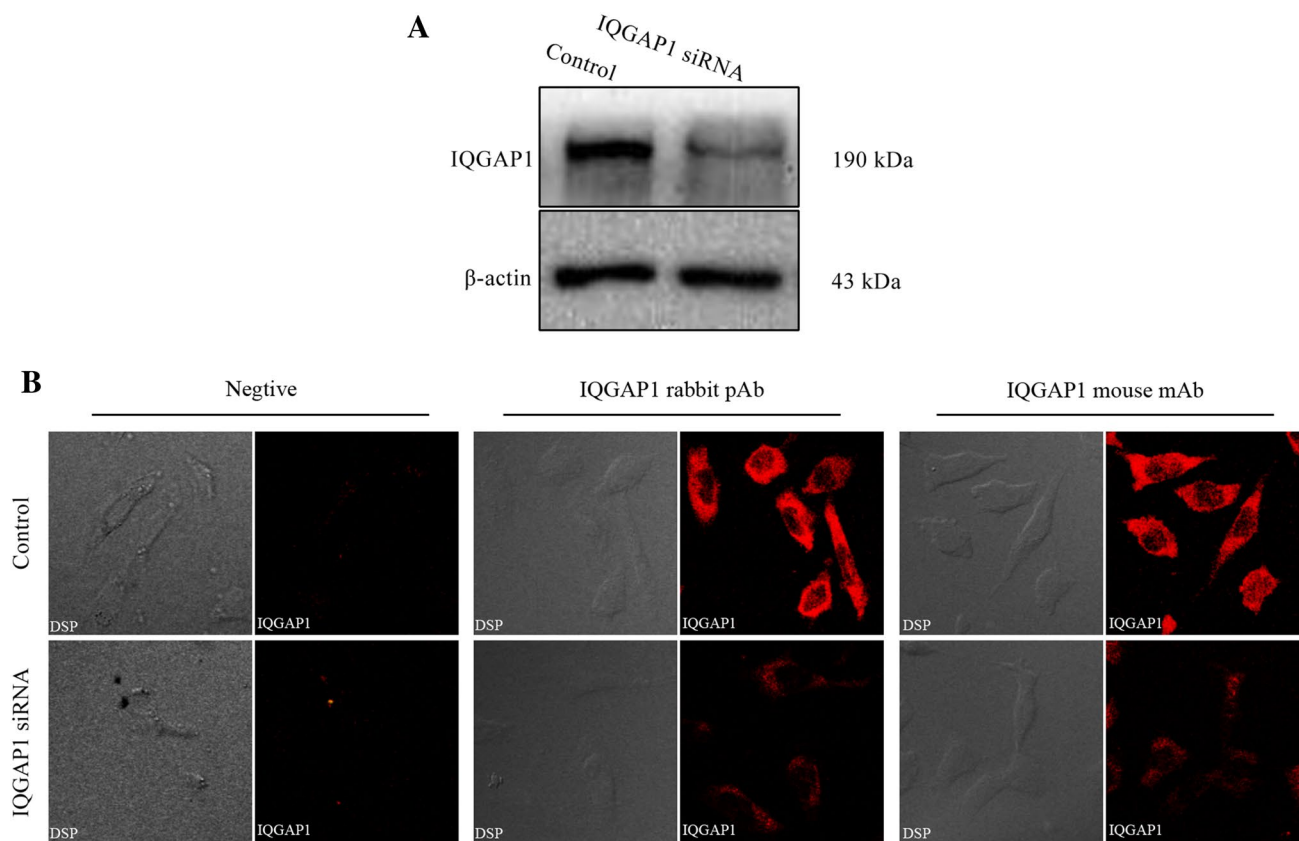


Fig. 7 The verification of the specificity of anti-IQGAP1 antibodies. **a** Western blotting analysis of the expression of IQGAP1 in podocytes transfected with IQGAP1 siRNA. **b** Immunofluorescent staining of IQGAP1 in podocytes transfected with IQGAP1 siRNA

polarity is essential for reabsorption and secretion in various segments of the nephron by the differential polarized insertion of channels, transporters and related proteins into apical membrane. For example, the sodium-coupled transporter, the amiloride-sensitive Na/H exchanger, carbonic anhydrase, AQP1 and AQP2 are located in the apical membrane of nephron segments. The distribution of IQGAP1 in the apical membrane maybe related to the reabsorption of ions, glucose and water molecules from tubular fluid. Renal reabsorption relies on the structural modification that controls the movement of water and solutes by transmembrane transport and intercellular passageway, including cytoplasmic scaffolding proteins, transmembrane proteins and signaling proteins (Szaszi and Amoozadeh 2014). In this process, the cytoskeleton is altered and IQGAP1 may participate in the trafficking of apical transporters by regulating the cytoskeleton network. For the various intensity of IQGAP1 expression, we suspected that it is associated with the varied activities of exchangers. Further studies are needed to investigate the specific mechanism.

The IQGAP1 and F-actin coimmunoprecipitation results indicated that IQGAP1 binds to F-actin in human renal parenchymal cells and the relation between IQGAP1 and

cytoskeleton system may be the underlying mechanism by which IQGAP1 exerts its different effects in various human nephron segments.

To the best of our knowledge, this is the first study to report the specific expression and differential subcellular location of IQGAP1 in human nephron segments. IQGAP1 is strongly expressed in podocytes, HRGECs and CD. The prominent apical membrane expression of IQGAP1 is also detected, possibly related to the renal reabsorption via cytoskeleton regulation. Nevertheless, the specific role of IQGAP1 in different human nephron segments and its potential mechanisms of action remain to be further studied.

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Compliance with ethical standards

Conflict of interest All the authors declare that there are no competing interests.

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