Podocyte Cell Biology Update

-Understanding the Formation of Podocyte Structure in Health and Disease

HIDETAKE KURIHARA *

*Department of Anatomy and Life Structure, Juntendo University Graduate School of Medicine, Tokyo, Japan

The podocyte possesses a unique morphology characterized by a large cell body and numerous foot processes, which enwrap the glomerular capillary. The intercellular space between foot processes is kept wide open to pass through the glomerular filtrate and connected with the slit diaphragms. It has been believed that the slit diaphragm plays an important role in permeability barrier function, because the lack of the components of slit diaphragm causes massive proteinuria. The podocyte is derived from the polarized epithelial cell mass converted from the undifferentiated metanephric mesenchyme at the first stage of glomerular development. The dramatic structural and functional transformation of the podocyte is occurred during differentiation of the kidney. The morphological changes of podocytes seen under the pathological condition are tightly associated with the pathogenesis of proteinuria. The organization and maintenance of the unique cell shape is achieved by the novel cytoskeletal elements. Especially, regulation of actin filaments in foot processes is important for maintaining integrity of the cytoskeleton and organization of the slit diaphragm. Recently, we have focused on two myosins which are actin-binding motor proteins and expressed in podocyte. In addition, we have investigated a new cell signaling system at the slit diaphragm. In this lecture, I review our recent progress in the function of those molecules in health and disease.

Key words: cell signaling, myosin, cell process formation, cell-cell junction

SIRP α -CD47 system functions as an emergency signal in renal glomerulus

Renal glomerulus consists with glomerular endothelial cells, podocytes and mesangial cells. Those cells cooperate with each other for the glomerular filtration. However, the intercellular signaling molecules among those cells have not so far been clarified. Perfusion of kidney with protamine sulfate (PS) induces foot process fusion and tyrosine phosphorylation of slit diaphragm molecules (ZO-1, nephrin and NEPH-1), suggesting that tyrosine phosphorylation is an indicator of the early event on the morphological changes in podocyte¹⁾. Although src kinase is involved in this event, the molecules working for dephosphorylation are not fully determined. SIRP α is a type I transmembrane glycopro-

tein, which has three immunoglobulin-like domain in the extracellular region and two SH2 binding motif in the cytoplasm. This molecule functions as a scaffold to many proteins, especially SHP2-tyrosine phosphatase to the plasma membrane. We found that SIRP α is concentrated at the slit diaphragm region of podocyte²⁾. SIRP α is heavily tyrosine-phosphorylated under the normal condition. On the other hand, PS-perfusion induced a dramatic decrease of tyrosine phosphorylation of SIRP α . The slit diaphragm components, nephrin and Neph1, assemble a signaling complex in a tyrosine phosphorylation-dependent manner, and regulate the unique actin cytoskeleton of podocytes. We analyzed the expression of SIRP α in developing kidneys, in kidneys from CNS patients and in proteinuric rat models. The possibility that SIRP α interacts with

Hidetake Kurihara

Department of Anatomy and Life Structure, Juntendo University Graduate School of Medicine, Tokyo, Japan

2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan

TEL: +81-3-3813-3111 E-mail: hidetake@juntendo.ac.jp

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Figure-1 SIRP α -CD47 system in renal glomerulus. Schematic drawing of SIRP α and CD47 in normal (left) and Thy1.1 nephritic glomerulus (right) is shown. SIRP α is expressed on podocyte cell membrane, whereas CD47, a ligand of SIRP α , is expressed on mesangial cell membrane. SHP-2-tyrosine phosphatase is bound to the cytoplasmic region of SIRP α . When mesangiolysis is occurred, CD47 is released and binds to SIRP α . As a result, dephosphorylation of SIRP α is occurred and SHP-2 is released.

slit diaphragm proteins was also investigated. SIRP α was concentrated at the slit diaphragm junction during the maturation of intercellular junctions. In the glomeruli of CNS patients carrying mutations in *NPHS1*, where slit diaphragm formation is disrupted, the expression of SIRP α as well as Neph1 and nephrin was significantly decreased, indicating that SIRP α is closely associated with the nephrin complex. Indeed, SIRP α formed hetero-oligomers with nephrin in cultured cells and in glomeruli. Furthermore, the cytoplasmic domain of SIRP α was highly phosphorylated in normal glomeruli, and its phosphorylation was dramatically decreased upon podocyte injury *in vivo*. Thus, SIRP α interacts with nephrin at the slit diaphragm, and its phosphorylation is dynamically regulated in proteinuric states³. More recently, the role of SIRP α expression in podocytes was examined by using knockin mice (C57BL / 6 background) expressing mutant SIRP α that lacks a cytoplasmic region (SIRP α -mutant mice) and lose the SHP-2 binding ability. Electron microscopic examination showed the morphological changes of podocytes (irregular major processes and flattened foot processes) in the SIRP α -mutant mice. In addition, massive albuminuria with focal glomerulosclerosis was induced in adult SIRP α -mutant mice⁴. These data demonstrate that SIRP α is involved in the regulation of podocyte structure and function at the slit diaphragm region via SHP-2 under both

physiological and pathological conditions.

CD47, a ligand for SIRP α , is also expressed in glomerulus, but its distribution is distinct from that of SIRP α . CD47 is located along the plasma membrane of mesangial cells, but not podocytes. It is known that the cytoplasmic domain of SIRP α is dephosphorylated, when CD47 is bound to the extracellular domain of SIRP α . In normal glomerulus, mesangial cells cannot attach directly to podocytes. However, when mesangial cells are injured by Thy1.1 monoclonal antibody injection, the phosphorylation of SIRP α is dramatically decreased because of the interaction between SIRP α and CD47 (Figure-1). The complex of SIRP α and CD47 are incorporated in podocytes after mesangiolysis. The data suggest that SIRP α -CD47 system plays a role in the cell-cell communication in diseased glomerulus.

Myosin IIA regulates the cellular architecture of podocyte

Podocytes are characterized by a large cell body and numerous foot processes. Cell body and primary processes contain microtubules and intermediate filaments, while foot processes have only actin filaments⁵. Cytoskeletal organization in podocyte is a key factor for maintaining its cell shape. Especially, actin cytoskeleton may play a crucial role in the regulation of foot process structure,

Figure-2 Localization of myosin IIA in cultured rat podocyte. Immortalized rat podocyte cells show the long cell processes like *in vivo* podocyte (left). Myosin IIA and vimentin intermediate filament are located in cell body and primary processes, while secondary processes elongated from primary long processes have only actin filaments (right).

because the reorganization of actin-binding proteins causes the effacement or retraction of foot processes⁶. Point mutations in MYH9, the gene encoding nonmuscle myosin heavy chain II-A (NMHCII-A), underlie autosomal dominant syndromes in humans. The human abnormalities manifest as macrothrombocytopenia, granulocyte inclusions, progressive proteinuric renal disease, cataracts, and sensorineural deafness. These syndromes, now referred to as MYH9-related diseases, were formerly called May-Hegglin, Fechtner, Sebastian, and Epstein syndromes. However, the roles of myosin IIA in kidney remains uncertain. Our study showed that podocytes specifically express myosin IIA, but not myosin IIB. Immunoelectron microscopy of myosin IIA demonstrated that myosin IIA is distributed in the cytoplasm of cell body and primary processes. Specialized localization is seen on the actin arc shown at the boundary between cell body and foot processes. The signals for myosin IIA were dramatically decreased in PAN nephrotic kidney, in which the morphological changes of podocytes including the effacement of foot processes were seen⁷. In addition, myosin IIA was dephosphorylated under the nephrotic condition. We next examined the role of a cytoskeletal motor protein, myosin IIA in cell process formation by using immortalized rat podocyte cell lines which we have originally established. These cell lines express a number of cytoskeletal proteins seen *in vivo*

podocyte including alpha-actinin4, vimentin and nestin intermediate filaments. Cytochalasin D treatment induces the rearrangement of vimentin and nestin intermediate filaments and cell process formation through the membrane retraction. Cell processes induced by cytochalasin D treatment contain bundled intermediate filaments never seen in the resting cells. Myosin IIA is located at the cell margin with actin filaments in resting cells, but the main signals for myosin IIA are colocalized with vimentin intermediate filaments after stimulation (Figure-2). Myosin IIA is also seen at the base between neighboring cell processes with actin filaments, suggesting that the membrane retraction is achieved by the mechanical force of actomyosin. Treatment of blebbistatin, myosin II inhibitor reduced the morphological change of cultured podocytes induced by cytochalasin D. Myosin IIA silencing using siRNA is associated with significantly reduced cell process formation and inhibition of intermediate filament bundling. In conclusion, myosin IIA is expressed in podocyte and could play an important role in maintaining the structural integrity of podocytes.

Glomerular permeability regulator myosin 1e modulates junctional actin organization during cell-cell contact formation between podocytes

Myosin 1e (Myo1e) is a member of the myosin superfamily of actin-dependent motor proteins that

Figure-3 Role of myosin 1e in cell-cell junction. Myosin 1e is localized at the cell junction via the multiple domain interaction as TH1 with the plasma membrane and lipids and TH2 domain to junctional molecule. Motor domain of myosin 1e interacts with actin filaments and SH3 domain interacts with ZO-1. The molecular complex stabilize the cell-cell junction.

is expressed in many cell types in humans and mice. Like other members of the myosin superfamily, myo1e contains an N-terminal motor domain responsible for actin binding and ATPase activity, a neck domain that includes a light chain-binding IQ motif, and a tail domain that may determine cargo binding properties and intracellular localization. Myo1e tail domain consists of three tail homology (TH) regions: TH1 domain that binds acidic phospholipids, proline-rich TH2 domain, and SH3 domain, also known as TH3, which binds to proline-rich proteins.

In the kidney, myo1e expression is particularly high in podocytes. Myo1e knockout mice exhibit defects in renal filtration that can be attributed to podocyte dysfunction, and mutations in the MYO1E gene are associated with glomerular disease in humans. The precise functional role of myo1e in podocytes is unknown. We have used biochemical and microscopic approaches to determine whether myo1e is associated with the slit diaphragm complexes in podocytes. Myo1e was consistently enriched in the slit diaphragm fraction during subcellular fractionation of renal glomeruli and colocalized with the slit diaphragm markers. Live cell imaging studies showed that myo1e was recruited to the newly formed cell-cell junctions in cultured podocytes, where it colocalized with the actin filament cables aligned with the nascent contacts. Myo1e-null podocytes expressing FSGS-associated myo1e mutant (A159P) did not

efficiently assemble actin cables along new cell-cell junctions. ZO-1 is the first protein shown to be specifically associated with both slit diaphragm and tight junction^{8) 9)}. We have mapped domains in myo1e that were critical for its localization to cell-cell junctions and determined that the SH3 domain of myo1e tail interacts with ZO-1¹⁰⁾. These findings suggest that myo1e represents a component of the slit diaphragm complex and may contribute to regulating junctional integrity in podocytes (Figure-3).

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