博士論文の要旨及び審査結果の要旨

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博士論文名	Synbindin downregulation participates in slit diaphragm dysfunction. （シンビンディンの発現低下がスリット膜の機能障害に関与している）
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博士論文の要旨

Background: The glomerular visceral epithelial cell (podocyte) is a highly differentiated cell, characterized by multiple interdigitating foot processes, covering the outer side of the glomerular basement membrane (GBM). The neighboring foot processes are connected by a slit diaphragm, which is a key structure regulating the barrier function of the glomerular capillary wall. It is now accepted that the dysfunction of the slit diaphragm is involved in the development of proteinuria in several glomerular diseases. However, the precise molecular composition involved in the interaction between foot processes and GBM, and the molecular structure of the slit diaphragm are not well understood yet. Synbindin, originally identified as a neuronal cytoplasmic molecule, which plays a role in spine formation in nerve cells, was found in glomeruli. cDNA subtractive hybridization technique showed the mRNA expression of synbindin in glomeruli was down-regulated in puromycin aminonucleoside (PAN) nephropathy, a mimic of minimal-change nephrotic syndrome. These observations prompted the applicant to investigate synbindin function in podocyte.

Methods: The expression of synbindin in podocyte was analyzed in normal rats and two types of rat nephrotic models, anti-nephrin antibody (ANA)-induced nephropathy a pure slit diaphragm injury model, and PAN nephropathy by immunohistochemical analysis with an antibody designed and produced in rabbits immunized against 16 amino acid of rat synbindin sequence and real-time RT-PCR techniques. Developmental expression of synbindin was analyzed in neonatal rat kidney by immunohistochemical analysis. To elucidate the function of synbindin, a gene silencing study with human cultured podocytes was performed. Real-time RT-PCR and rhodamine phalloidin staining was performed with synbindin knockdown cells.

Results: Synbindin was mainly expressed at slit diaphragm area of podocytes. Synbindin appears together with nephrin, a key molecule of slit diaphragm, in presumptive podocyte and generally

was colocalized with nephrin in maturing podocyte. In both nephrotic models, decreased mRNA expression and the altered staining of synbindin were already detected at the early phase when proteinuria and the altered staining of nephrin were not detected yet. Synbindin staining was clearly reduced when severe proteinuria was observed. The remained synbindin and nephrin signal were clearly dissociated in ANA nephropathy. In PAN nephropathy, remained nephrin was colocalized with synbindin. When the cultured podocytes were treated with siRNA for synbindin, the cell changed to be round-shape, and filamentous actin structure was clearly altered. The expression of ephrin-B1, a transmembrane protein at slit diaphragm, was clearly lowered, and synaptic vesicle-associated protein 2B (SV2B), a glycosylated synaptic vesicle membrane protein involved in the formation and maintenance of the slit diaphragm, was upregulated in the synbindin knockdown cells.

Discussion and conclusions: Synbindin expression in normal glomerular podocyte and its colocalization with nephrin and ephrin-B1, as well as developmental expression pattern implied that synbindin is associated with slit diaphragm. Alterations of synbindin expression in ANA nephropathy model, that resulted from the rearrangement of slit diaphragm molecules caused by stimulation to nephrin, and PAN nephropathy were detected before the onset of proteinuria, suggesting that synbindin has an etiological role in the initiation event of podocyte injury, which leads to proteinuria. Cytoskeletal alteration of synbindin siRNA treated human cultured podocytes implied that synbindin participates in the formation and/or the maintenance of processes with dense F-actin structure. Altered mRNA expression of Ephrin-B1 and SV2B suggests that these molecules are associated with synbindin. Based on previous reports and the findings obtained in this study, the applicant proposes that synbindin may participate in proper ephrin-B1 expression and upregulation of SV2B is a negative feedback mechanism compensating the function of synbindin. In conclusion, synbindin participates in maintaining foot processes and slit diaphragm as a downstream molecule of SV2B-mediated vesicle transport. Synbindin downregulation participates in slit diaphragm dysfunction. Synbindin could be an early marker to detect podocyte injury.

審査結果の要旨

多くの糸球体疾患におけるタンパク尿は腎糸球体上皮細胞（ポドサイト）の細胞間接着装置であるスリット膜のバリア機能の低下により発症すると考えられているが、スリット膜の分子構造、スリット膜のバリア機能低下機序は十分に解明されていない。本研究は、高度のタンパク尿を示す疾患であるネフローゼ症候群のモデルラット腎材料を用いた cDNA-Subtraction Assay で発現が低下している分子として同定されたSynbindinに着目し、腎での局在、機能の検討を行った。

Synbindinに対する特異抗体を作成し、腎での発現、局在、ネフローゼ症候群モデルでの発現変化を免疫組織化学的手法を用いて検討した。Synbindinは、糸球体血管壁に沿ったパターンで観察され、各種糸球体細胞マーカーとの二重染色で主にポドサイトに発現していること、スリット膜分子であるネフリンの近傍に局在していることが示された。ネフローゼ症候群モデルでの検討で、Synbindinは、病態誘導初期から発現が低下し、高度の蛋白尿を示す時期には発現低下が顕著になることを観察した。次に培養ポドサイトを用いた検討を行い、siRNAを用いてSynbindin発現をノックダウンした細胞では、細胞骨格の染色性、細胞の形態が変化していること、スリット膜関連分子であるSV2Bの発現が上昇し、エフリンB1の発現が低下

することを観察した。これらの結果は、Synbindin は、SV2B、エフリンB1 の関連分子で、スリット膜構成分子の１つであること、スリット膜の機能維持、ポドサイトの細胞形態の維持に重要な役割を果たしていること、Synbindin はポドサイト傷害を検知する早期マーカーとして有用であることを示していると考えられる。

本研究は、Synbindin のスリット膜における機能を解明し、スリット膜の分子構造の解明、タンパク尿発症機序の解明に寄与した点に、学位論文としての価値を認める。