당뇨병성 신병증에서 족세포의 역할

연세대학교 의과대학 내과학교실

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Podocyte Biology in Diabetic Nephropathy

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〈요 약〉

족세포 (podocyte)는 메산지움 세포, 내피세포와 더불어 사구체 내에 존재하는 고도로 분화된 세포로, 일차 돌기로부터 뻗은 족돌기 (foot process)를 이용하여 요강 측면의 사구체 기저막 (glomerular basement membrane) 위에 놓여 있게 된다. 하나의 족세포에서 기원한 족돌기는 서로 이웃하는 족세포의 족돌기와 깍지낀 손 형태를 이루면서 30-40 nm의 여과 세극 (filtration slit)을 구성하게 되며, 여과 세극에는 세극막 (slit diaphragm)이 존재한다. 이러한 족세포는 사구체 기저막의 구성 성분 중 하나인 제 4형 collagen을 합성할 뿐만 아니라 족돌기 사이에 존재하는 세극막이 사구체 기저막과 더불어 사구체 여과 장벽 역할을 하는 것으로 알려져 있기 때문에, 족세포와 단백뇨 사이에 밀접한 관련이 있을 것으로 생각되어져 오다가 1998년에 핀란드형 선천성 신증후군이 세극막 구성 성분의 하나인 nephrin을 코딩하는 유전자인 NPHS1의 돌연변이에 의한 것이라는 연구 결과가 발표되면서 사실로 밝혀졌다. 당뇨병성 신병증은 병리학적으로는 사구체 및 세뇨관 비후, 사구체 기저막의 비후, 세포외 기질의 축적 등이 특징적이면서 임상적으로는 단백뇨가 특징적인 소견이기 때문에, 사구체 기저막 합성 및 단백뇨와 밀접한 관련이 있는 족세포가 당뇨병성 신병증의 병태생리에 중요한 역할을 할 것이라는 가정 하에 수많은 연구가 이루어졌으며, 현재에도 진행 중이다. 당뇨병에 의한 신장 내 변화 중 족세포와 관련이 있는 것들로는 1) 사구체 비후 (족세포 비후), 2) 사구체 기저막의 비후, 3) 사구체경화증, 4) 족세포 수의 감소, 그리고 5) 족돌기 융합 등이 있다. 족세포 비후는 고혈당, angiotensin II, 그리고 기계적 스트레스 (mechanical stress) 등에 의한 세포주기 조절 단백의 하나인 cyclin-dependent kinase 억제제의 발현 증가에 의하여 발생하는 것으로 알려져 있으며, 사구체 기저막의 비후는 당뇨 조건 하에서 제 4형 collagen, 그 중에서도 특히 α3 chain의 합성 증가와 관련이 있는 것으로 보고되고 있다. 또한 사구체경화증은 족세포의 탈락에 의한 사구체 기저막의 노출, 노출된 기저막과 Bownman's capsule의 접촉, 그리고 이어지는 synechiae의 형성이 병인으로 되어 있으며, 세포사멸, integrin의 변화에 의한 족세포 탈락, 그리고 세포증식의 결여 등으로 인하여 족세포의 수가 감소되는 것으로 알려져 있다. 마지막으로 족돌기 융합은 당뇨병성 신병증을 포함한 단백뇨를 동반하는 대부분의 사구체 질환에서 발견되는 소견으로, 1) 세극막 관련 단백의 변화 2) 비정상적인 족세포-사구체 기저막 사이의 상호작용 3) actin 세포골격 (cytoskeleton)의 재배열, 그리고 4) 음전하 (negative charge)의 변화 등이 족세포 융합과 밀접하게 연관되어 있는 것으로 보고되고 있다.

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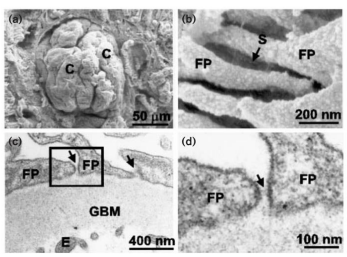
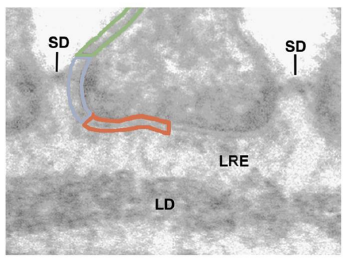
Cells in Kidney

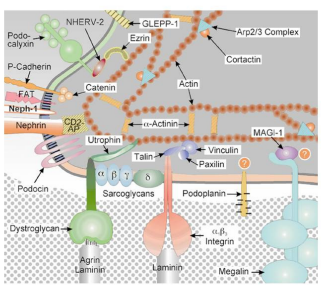
- **Glomerular cells**
  - Epithelial cells
    - Visceral epithelial cells (Podocytes)
    - Parietal epithelial cells
  - Endothelial cells
  - Mesangial cells
- **Tubular epithelial cells**
- **Others**



Scanning (a, b) and Transmission (c, d) Electron Micrograph of a Human Glomerulus



Podocyte Foot Process, Showing the Apical Domain, Slit Diaphragm, and Basal Domain



Molecular Anatomy of the Podocyte Foot Process, Showing the Apical Domain, Slit Diaphragm, and Basal Domain

Diabetic Nephropathy

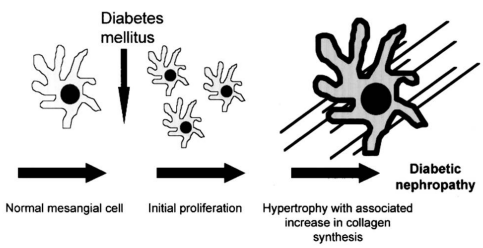
- Glomerular and tubular hypertrophy
- Extracellular matrix (ECM) accumulation
  - Mesangial matrix deposition
  - Thickening of GBM and TBM
- Glomerulosclerosis
- Tubulointerstitial inflammation and fibrosis
- Podocytopenia
- Foot process widening

Glomerular Hypertrophy

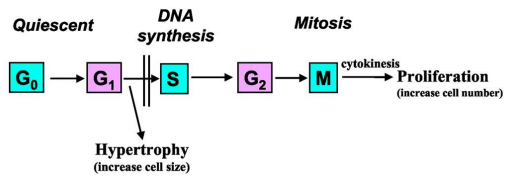
- Hypertrophy of glomerular cells
  - Mesangial cells
  - Podocytes
  - Endothelial cells (?)
- Increased capillary diameter
- Accumulation of ECM

Hypertrophy

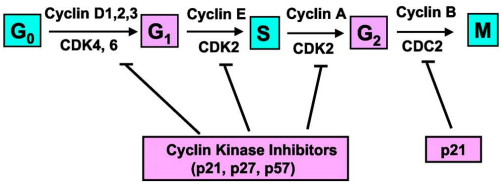
- **Growth factors/Cytokines**
  TGF-β, PDGF-B, IGF-1, HGF
- **Chemokines**
  IL-8, MCP-1
- **Vasoactive substances**
  Ang II, ET-1
  Prostanoids



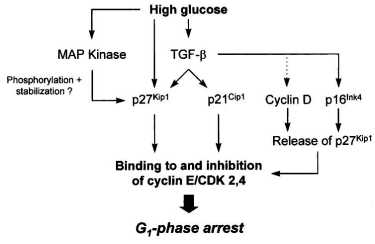
Mesangial Cells in Diabetic Nephropathy



Phases of the Cell Cycle



Cell Cycle Regulatory Proteins

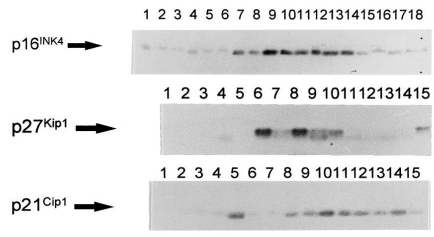


Role of CKIs in HG-Induced G1 Phase Arrest

Podocyte Hypertrophy (I)

❖ **Wolf G, et al (Diabetologia, 1999)**

BBdp rat, an autoimmune model of type I DM
Sacrificed at 3 weeks after onset of DM
Glomerular isolation by sieving
Immortalized mouse podocytes
Increased $p16^{INK4}$, $p27^{Kip1}$, and $p21^{Cip1}$ protein expression in glomeruli from diabetic *BBdp* rats assessed by Western blot
Enalapril treatment for 3 weeks reduced $p16^{INK4}$ and $p27^{Kip1}$ but not $p21^{Cip1}$ expression

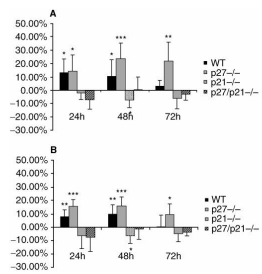


Western Blot for $p16^{INK4}$, $p27^{Kip1}$, and $p21^{Cip1}$ Protein in Glomeruli from Control, DM, and DM+Enalapril Rats

Podocyte Hypertrophy (II)

❖ **Petermann AT, et al (Kidney Int, 2005)**

Primary cultured mouse podocytes
Mechanical stress
Reduced cell cycle progression in wild-type and single p27-/- podocytes but not in single p21-/- and double p21/p27-/- podocytes
Prevention of stretch-induced hypertrophy by specific blocker of ERK1/2 or Akt
No effect of p38 MAPK inhibitor on stretch-induced hypertrophy

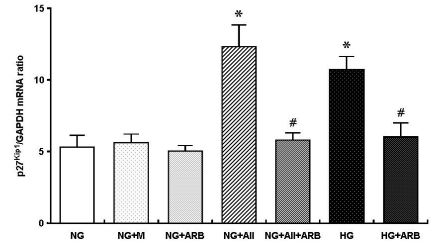


The Increase in Cell Volume of Stretched Podocytes in G0/G1 Phase (A) or S Phase (B) Assessed by FACScan

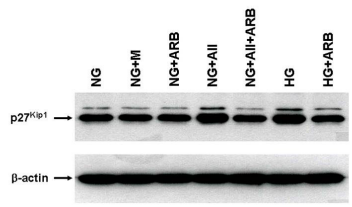
Podocyte Hypertrophy (III)

❖ **Xu Z-G, et al (Kidney Int, 2005)**

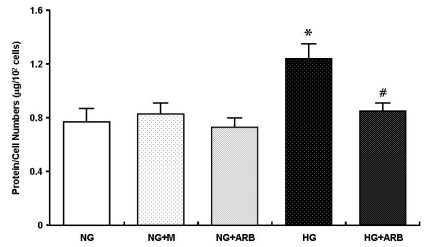
Streptozotocin rat
Sacrificed at 3 months after STZ injection
Glomerular isolation by sieving
Immortalized mouse podocytes
Increased $p27^{Kip1}$ mRNA and protein expression in DM glomeruli and HG-stimulated podocytes assessed by RT-PRC and Western blot
Glomerular hypertrophy in DM rats
Podocyte hypertrophy in HG-stimulated cells



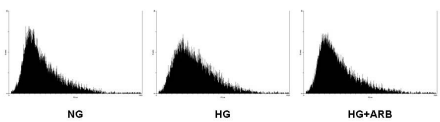
$p27^{Kip1}$/GAPDH mRNA Ratio of Podocytes Assessed by Competitive RT-PCR



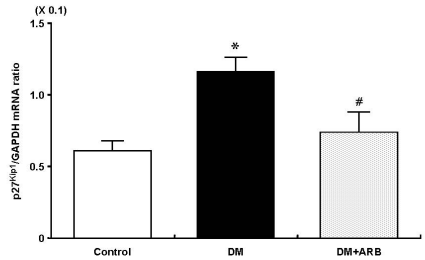
p27Kip1 Protein Expression in Podocytes
Assessed by Western blot



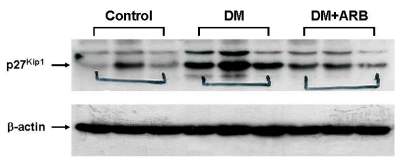
Assessment of Cellular Hypertrophy in Podocytes
by Protein/Cell Numbers



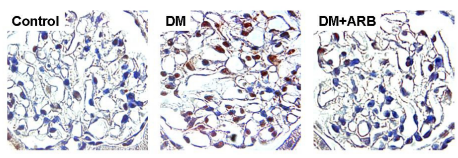
Assessment of Cellular Hypertrophy in Podocytes
by FACScan Flow Cytometer



Glomerular p27Kip1/GAPDH mRNA Ratio
Assessed by Competitive RT-PCR



Glomerular p27Kip1 Protein Expression
Assessed by Western blot



Glomerular p27Kip1 Protein Expression
Assessed by Immunohistochemistry

Thickening of GBM

- Increase in
  - Collagen α3(IV)
  - Collagen α4(IV)
  - Collagen α5(IV)
- Decrease in
  - Heparan sulfate proteoglycans
  - Collagen α1(IV) and α2(IV)

Table 1. Glomerular matrix proteins in diabetic nephropathy (DN)

Protein	Comment	Reference
Mesangium		
collagen I	Only detected in late glomerulosclerosis. May bind decorin and TGF-β.	22, 23, 24
collagen III	Only detected in late glomerulosclerosis.	25, 26, 27
collagen IV	α1(IV), α2(IV) chains expressed in normal mesangium, increased in DN.	28, 29, 30, 31
collagen V	Minor component in normal mesangium. Increased in DN.	25, 32, 33
collagen VI	Present in normal mesangium. Same distribution as α1(IV) in normal mesangium. Reports of increase in DN not substantiated in type I "fast track" DN, using quantitative immunogold EM.	30, 32–35
fibronectin	Present in normal mesangium. Increased in DN. Oncofetal, ED-A, and ED-B isoforms expressed in glomerulosclerosis.	32, 36, 37, 38
laminin	Minor component in normal mesangium. Report of increase in diffuse mesangial expansion not confirmed.	25, 32
SLR proteoglycans	Includes decorin, biglycan, lumican, fibromodulin; mRNAs for all overexpressed in DN, but proteins barely detected, except in advanced glomerulosclerosis.	23, 24
GBM		
collagen IV	α3(IV), α4(IV) chains present normally, increased in DN. α1(IV), α2(IV), minor components normally decreased in DN.	28, 31, 39–41
entactin	Present normally. Increased in DN.	42
laminin	Present normally, may be increased in early DN, but generally reported to decrease.	37, 43–45
heparan sulfate proteoglycan	Present normally. Decreased in DN.	44–48

Factors Affecting Collagen Synthesis of Podocytes

- High glucose
  - ↑ Collagen α1(IV), α3(IV), and α5(IV)
- Angiotensin II
  - ↑ Collagen α3(IV)
- TGF-β
  - ↑ Collagen α3(IV)
  - ↓ Collagen α1(IV) and α5(IV)

Glomerulosclerosis

- May start from podocytopenia
  - Detachment of podocyte from GBM
  - → Denudation of GBM
  - → Contact of GBM with Bowman's capsule
  - → Synechiae formation
  - → Glomerulosclerosis

Causes of Podocytopenia

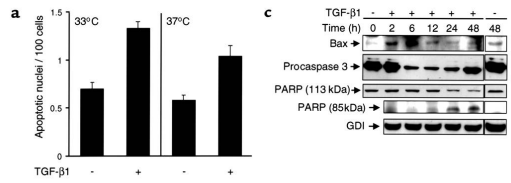
- Apoptosis
- Detachment
- Lack of proliferation

Apoptosis (I)

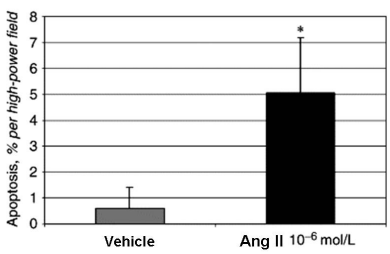
- **Apoptosis factors**
  - Serum deprivation
  - ROS, Nitric oxide
  - Fas ligation
  - TNF-α, IL-1α
  - Radiation, Cytotoxic drugs
  - Shear stress
- **Survival factors**
  - IGF-1
  - Normal ECM

Apoptosis (II)

- High glucose (?)
- TGF-β
  - Transgenic mice
  - Augmented in the absence of p21 and p27
- Angiotensin II
  - Dose- and time-dependent
  - Via subtype I and II receptor
  - Partly TGF-β-Smad pathway dependent



Effect of TGF-β on Apoptosis Quantitated by TUNEL Assay (a) and Immunoblotting (c)



Effect of Vehicle and Angiotensin II (10^{-6} M) on Apoptosis of Cultured Podocytes

Detachment

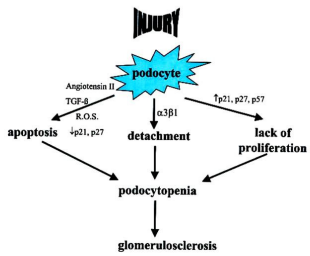
- Podocytes in urine
  - Diabetic patients
  - Streptozotocin-induced diabetes model
- Detached podocytes
  - Viable or only apoptotic
- Mechanism (?)
  - Defect in specific integrins ($\alpha 3\beta 1$ or DG)

Lack of Proliferation

- Results in a decrease in podocyte number
- Podocyte proliferation
  - Depends on its state of differentiation
  - Govern by cell cycle regulatory proteins
    - : Cyclins, CDKs, CDK-inhibitors
  - During glomerulogenesis
    - : Proliferate
      Absent p27 and p57
  - During S-phase of kidney development
    - : Differentiate and become quiescent
      Increase in p27 and p57 expression

Lack of Proliferation (II)

- Intraglomerular hypertension
  - Common pathway to glomerular scarring
  - Mechanical stretch on glomerular cells
- Mechanical stretch on podocytes
  - Decreased levels of cyclins D, A and B1 and cdc2
  - Increased level of CDK-inhibitors
    - An early increase in p21
    - An increase in p27 at 24 hours
    - A delayed increase in p57 at 72 hours
  - Proliferation of p21 –/– podocytes exposed to stretch



Causes of Podocytopenia

Foot Process Widening (I)

- Cell body and primary processes
  - Microtubules
  - Intermediate filaments
  - Microtubule-associated proteins (-2, -3, and -4)
- Foot processes
  - Actin
  - Myosin-II
  - Actin-associated proteins
    - α-actinin-4
    - Synaptopodin

Foot Process Widening (II)

- Effacement, fusion, retraction, simplification
- Not a fusion of neighboring cells
- Gradual simplification of the inter-digitating process → Formation of a cell that looks flat and elongated
- Widening and shortening of the foot processes of each podocyte → Decrease in total slit length
- Initiated by changes in the podocyte's cytoskeleton
- Energy-dependent active phenomenon

Causes of Podocyte Effacement

Mechanism underlying effacement	Mediator of effect
Changes in slit diaphragm (SD) proteins	Nephrin Podocin FAT CD2AP Neph1
Abnormal podocyte-GBM interaction	Integrins Integrin-linked kinase SPARC
Actin cytoskeleton reorganization	Rho GTPases α-actinin-4 Synaptopodin CDK5
Changes in negative charge	Podocalyxin GLEPP

Changes in SD Proteins

- SD proteins are not only structural proteins but also signals-regulating proteins involved in podocyte's polarity, survival, and cytoskeleton organization
- Nephrin complexes with podocin, FAT-1, CD2AP, and Neph1
- ZO-1 associates with cortical actin cytoskeleton
- Densin binds to α-actinin-4

Abnormal Interaction with GBM (I)

- Anchorage of podocytes to the GBM is mediated by α3β1-integrin complex
- Integrin heterodimers are linked to the contractile structure of the podocyte cytoskeleton, containing actin, myosin, α-actinin, talin, and vinculin
- Antibody-mediated blockade of glomerular integrins results in podocyte detachment
- α3-integrin KO mice are born with a congenital nephrotic syndrome phenotype

Abnormal Interaction with GBM (II)

- Integrins activate intracellular signaling pathways, even though they lack intrinsic enzymatic activity
- Integrin ligation recruits or activates signaling molecules to the cytoplasmic tails of integrins
- β1 integrins bind to talin, α-actinin, tensin, focal adhesion kinase (FAK), and integrin-linked kinase (ILK)
- ILK is involved in integrin-mediated cell-matrix interactions and in cell phenotype regulation

Cytoskeleton Reorganization (I)

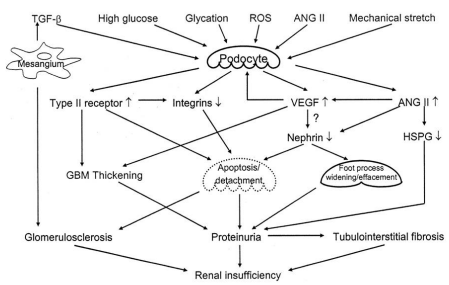
- Actin-associated proteins
  - Synaptopodin
  - α-actinin-4
- Factors involved in the maintenance of cytoskeleton
  - Small GTPases (RhoA, Rac1, and Cdc42)
  - Small heat shock protein 27 (HSP27)
- Synaptopodin is induces stress fibers by competitive blocking of Smurf1-mediated ubiquitination of RhoA in podocytes

Cytoskeleton Reorganization (II)

- Rac1 and Cdc42 promote cell motility through the formation of lamellipodia and filopodia, respectively.
- RhoA promotes the formation of contractile actin-myosin-containing stress fibers in the cell body and at the rear.
- HSP25, the mouse homolog of the corresponding human protein, HSP27, is known to be involved in maintaining cell shape by binding to and stabilizing actin cytoskeletal filaments.

Apical Membrane Proteins

- In addition to prevent proteinuria by acting as a size barrier, podocytes also act as a negative charge barrier like the GBM to prevent the passage of anion proteins
- Anionic apical proteins
  - Podocalyxin
  - Podoplanin
- Involved in the maintenance of podocyte shape by linking to the actin cytoskeleton



Mechanisms of Podocyte Injury Leading to DN