Podocyte biology in diabetic nephropathy

JJ Li[1,2], SJ Kwak[2], DS Jung[2], J-J Kim[2], T-H Yoo[2], D-R Ryu[3], SH Han[2], HY Choi[2], JE Lee[2], SJ Moon[2], DK Kim[2], DS Han[2] and S-W Kang[2]

[1]Department of Internal Medicine, Nephrology and Dialysis Unit, The Affiliated Hospital, YanBian University Medical College, JiLin, China; [2]Department of Internal Medicine, College of Medicine, Brain Korea 21, Yonsei University, Seoul, Korea and [3]Department of Internal Medicine, College of Medicine, Ewha Woman's University, Seoul, Korea

Glomerular visceral epithelial cells, namely podocytes, are highly specialized cells and give rise to primary processes, secondary processes, and finally foot processes. The foot processes of neighboring podocytes interdigitate, leaving between them filtration slits. These are bridged by an extracellular substance, known as the slit diaphragm, which plays a major role in establishing size-selective barrier to protein loss. Furthermore, podocytes are known to synthesize matrix molecules to the glomerular basement membrane (GBM), including type IV collagen, laminin, entactin, and agrin. Because diabetic nephropathy is clinically characterized by proteinuria and pathologically by glomerular hypertrophy and GBM thickening with foot process effacement, podocytes have been the focus in the field of research on diabetic nephropathy. As a result, many investigations have demonstrated that the diabetic milieu *per se*, hemodynamic changes, and local growth factors such as transforming growth factor-β and angiotensin II, which are considered mediators in the pathogenesis of diabetic nephropathy, induce directly and/or indirectly hypertrophy, apoptosis, and structural changes, and increase type IV collagen synthesis in podocytes. This review explores some of the structural and functional changes of podocytes under diabetic conditions and their role in the development and progression of diabetic nephropathy.

Kidney International (2007) **72,** S36–S42; doi:10.1038/sj.ki.5002384

KEYWORDS: podocyte; diabetic nephropathy; hypertrophy; podocytopenia; glomerulosclerosis; foot process effacement

Correspondence: *S-W Kang, Yonsei University College of Medicine, Department of Internal Medicine, 134 Shinchon-Dong, Seodaemoon-Gu, Seoul 120-752, Korea. E-mail: kswkidney@yumc.yonsei.ac.kr*

Podocytes are terminally differentiated and highly specialized cells.[1] They line the urinary side of the glomerular basement membrane (GBM) and function as a fine filter contributing ultimate size-selectivity, permitting permeability to molecules smaller than albumin.[2] Recent studies have shown that podocyte injury plays a role in the pathogenesis of various glomerular diseases,[3] including diabetic nephropathy, the leading cause of end-stage renal disease in many countries.[4,5] Among the characteristic findings of diabetic nephropathy, podocytes are involved in the development of glomerular hypertrophy, podocytopenia, glomerulosclerosis, and foot process effacement.[6] In this review, podocyte structural and functional changes under diabetic conditions are discussed.

GLOMERULAR HYPERTROPHY

Kidney size is typically increased in diabetes, even at the time of diagnosis in human.[7] Although this is primarily due to glomerular and tubular hypertrophy, inflammatory cell infiltration, extracellular matrix (ECM) accumulation, and hemodynamic factors also contribute.[8] In addition, some low-grade proliferation of glomerular cells is present in the early stage of diabetic nephropathy.[9]

Mesangial cell culture experiments under high glucose conditions, and *in vivo* studies of various diabetic models, suggest a biphasic growth response.[9] Initially, self-limited proliferation occurs, followed by cell-cycle arrest and hypertrophy; the latter hypertrophic mechanism requires the combined effects of mitogen-induced entry into the cell cycle, and subsequent arrest at G1/S interphase. Cell-cycle entry and progression through the cell cycle depend on the expression and activation of a series of protein kinases, termed cyclin-dependent kinases (CDKs), and their regulatory subunits, cyclins.[10] Some cyclin/CDK complexes act only at specific phases of the cell cycle, whereas others are more widely distributed. For G1 phase, cyclins D, E, and A play important roles.[11] Cyclin D is activated early in G1 phase, forms complexes with CDK4/CDK6, and regulates G1-phase progression. In late G1 phase, cyclins E and A are activated, and complexes with CDK2 are essential for G1/S transition. For cell growth, cyclin D mediates both hyperplastic and hypertrophic responses, whereas cyclin E determines whether the growth pattern will be hyperplastic or hypertrophic.[12]

The kinase activity of cyclin/CDK complexes is negatively regulated by small-sized proteins called CDK inhibitors.[13] Two subfamilies of CDK inhibitors are defined in terms of structural homology and the cyclin/CDK complexes they inhibit. The INK family (p15, p16, p18, and p19) inhibits G1-phase CDKs, whereas the Cip/Kip family (p21, p27, and p57) contains CDK-binding domains and inhibits CDKs active in G1 or S phase.[14,15] In cultured rat mesangial cells, high glucose, in the absence of other exogenous growth factors, was found to induce $p27^{Kip1}$ protein expression, but not mRNA expression.[16] This high glucose-stimulated expression of $p27^{Kip1}$ involved the activation of protein kinase C and depended partly on the induction of transforming growth factor-β (TGF-β).[16] Furthermore, $p27^{Kip1}$ antisense oligonucleotides treatment prevented glucose-induced mesangial cell hypertrophy, and resulted in a proliferative phenotype.[16] Glomerular $p27^{Kip1}$ protein expression, but not mRNA expression, was increased in diabetic db/db mice,[17] a model of type II diabetes, and in murine streptozotocin type I diabetes.[18] $p21^{Cip1}$ protein was also enhanced in 3- and 9-day streptozotocin-induced diabetic glomeruli assessed by immunohistochemical staining, whereas the expression of $p57^{Kip2}$, another member of the Cip/Kip family, did not change.[19]

In contrast to mesangial cells, mature podocytes do not actively synthesize DNA nor proliferate under normal conditions due to high levels of CDK inhibitor expression.[1] However, proliferation of podocytes is observed in certain glomerular diseases, such as HIV nephropathy,[20] collapsing glomerulopathy,[21] and the cellular variant of focal segmental glomerulosclerosis,[22] along with decreased $p27^{Kip1}$, $p57^{Kip2}$, and cyclin D1 expression. Under diabetic conditions, podocytes also undergo hypertrophic processes like mesangial cells, resulting in increased cell size.[23,24] Petermann *et al.*[23] demonstrated that mechanical stretch, an *in vitro* condition of increased intraglomerular capillary pressure, reduced cell-cycle progression and induced hypertrophy in wild-type and single p27−/− podocytes, whereas hypertrophy was not induced in single p21−/− and double p21/p27−/− podocytes. They also observed that stretch-induced hypertrophy was prevented by specifically blocking extracellular signal-regulated kinase 1/2 or Akt, but not by p38 mitogen-activated protein kinase (MAPK) inhibitor. Furthermore, we previously showed that high glucose also induced podocyte hypertrophy, which was ameliorated by angiotensin type I receptor blocker, thereby suggesting the involvement of angiotensin II in glucose-induced podocyte hypertrophy.[24] Most recently, increased angiotensin II levels were found in high glucose-treated podocytes, along with enhanced angiotensinogen and angiotensin II type I receptor expression, suggesting local renin-angiotensin system activation by high glucose *per se*.[25]

In addition to hypertrophy of glomerular cells, ECM accumulation contributes to glomerular hypertrophy.[8] Mesangial matrix expansion and GBM thickening are attributed to increased accumulation of normally existing ECM protein and/or deposition of proteins not present in normal tissue. Mesangial matrix accumulation in diabetic nephropathy is primarily due to abnormal ECM metabolism in mesangial cells, whereas GBM thickening is a consequence of changes in ECM metabolism mainly in podocytes and endothelial cells.[26] Normal GBM is composed of type IV collagen, laminin, fibronectin, entactin, and proteoglycans. Among type IV collagen, α3, α4, and α5 chains are predominant, whereas α1 and α2 chains exist in small amounts.[26] In diabetic nephropathy, there is increased expression and accumulation of α3 and α4 chains of type IV collagen,[27–29] whereas α1 and α2 chains are decreased.[29] On the other hand, heparan sulfate proteoglycan content is reduced and associated with disease progression and proteinuria in diabetic nephropathy.[30] Because podocytes not only produce GBM components, but also secret matrix-degrading proteinases, they are partly responsible for GBM thickening in diabetic nephropathy.[1] Iglesias-de la Cruz *et al.*[31] demonstrated that high glucose directly increased collagen α1(IV) and α5(IV) mRNA expression and stimulated α1(IV), α3(IV), and α5(IV) protein production in cultured mouse podocytes. Furthermore, high glucose-induced increase in collagen α3(IV) protein was mediated by TGF-β. We have also shown that collagen α5(IV) mRNA and protein expression were increased in mouse podocytes exposed to high glucose, which was partly dependent on the 12-lipoxygenase and p38 MAPK pathway.[32] In contrast, Bai *et al.*[33] observed a decrease in collagen α5(IV) protein levels in high glucose-conditioned media. Although the reasons for this divergence in type IV collagen expression are unclear, differences in experiment samples (i.e., cell lysates vs conditioned media) and different duration of high glucose stimulation may all contribute. In addition to high glucose, angiotensin II, an important mediator in the pathogenesis of diabetic nephropathy, induces collagen α3(IV) expression in cultured mouse podocytes.[34] On the other hand, the effects of TGF-β1, another considerable mediator in the pathogenesis of diabetic nephropathy, on type IV collagen expression are somewhat different from high glucose or angiotensin II. It has been reported to decrease collagen α1(IV) and α5(IV) mRNA and protein expression, while to increase collagen α3(IV) protein expression.[31] As high glucose does not increase TGF-β1 expression in podocytes and both have disparate effects on collagen α1(IV) and α5(IV) expression, the TGF-β1 system does not seem to mediate all of the effects of high glucose on collagen IV expression in podocytes.

ECM accumulation is the result of an imbalance between the synthesis and the degradation of ECM components. Therefore, increased ECM synthesis and decreased production of matrix-degrading proteinases, matrix metalloproteinases, play an important role in the pathogenesis of diabetic nephropathy.[35,36] Even though podocytes have the capacity to produce matrix metalloproteinase 9,[1] there have been limited studies to investigate the pathogenesis of GBM thickening in diabetic nephropathy in regards to ECM degradation. A recent study by Bai *et al.*[33] showed that high glucose