Insulin in podocyte podiatry

Diabetic nephropathy, a major complication of diabetes mellitus, is the leading cause of end-stage renal disease worldwide and recently it has been recognized as a high risk for the development of cardiovascular disease. Clinical features of diabetic nephropathy are development of albuminuria followed by persistent proteinuria and, later, reduction of glomerular filtration rate. Chronic hyperglycemia has been shown to activate various pathways in the kidney cells, both directly and through gene transcription, to induce reactive oxygen species, transforming growth factor-β, the renin–angiotensin–aldosterone system (RAAS) and advanced glycation end-products, leading collectively to glomerular injury. Accordingly, clinical studies, such as the Diabetes Control and Complications Trial (DCCT) in patients with type 1 diabetes and UK Prospective Diabetes Study (UKPDS) in patients with type 2 diabetes, have shown that strict control of hyperglycemia can slow the progression of diabetic nephropathy.

Over the past few decades, mesangial cells and the glomerular basement membrane have tended to be the focus of many diabetic kidney disease studies. Although glomerular hypertrophy, mesangial matrix expansion and glomerular basement membrane thickening are classical hallmarks of diabetic glomerular lesions, loss of podocyte structure and function has also been described as one of the early features of diabetic nephropathy in both patients with type 1 and type 2 diabetes, and represents an independent predictor of disease progression in patients at high risk for diabetic nephropathy[1]. The notion that abnormal glucose metabolism plays an essential role in podocyte damage has been emerging, but remains to be established.

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In contrast, albumin loss in the urine also occurs in insulin-resistant metabolic syndrome. This often occurs in patients with a normal level of blood glucose and blood pressure, suggesting a non-hyperglycemic and non-hemodynamic pathway of glomerular dysfunction, and as well a causative role of insulin resistance in the onset of albuminuria.

With a setting of cell type-specific deletion of insulin receptor in mice, Welsh *et al.*[2] showed the loss of insulin action in podocytes leads to glomerular lesions resembling the renal complication of human diabetes, even in the absence of hyperglycemia.

Podocytes are highly differentiated neuron-like epithelial cells with a limited capacity for cell division and replacement. Podocytes, covering the glomerular capillaries with the foot process, function to support and maintain the glomerular filtration barrier. Emerging studies of diabetic patients and animal models show that the onset of albuminuria, which might reflect a disruption of the filtration barrier, is most closely associated with podocyte injury. Welsh *et al.*[2] generated two podocyte-specific insulin receptor knockout mice (podIRKO) using the nephrin and podocin promoters-driven Cre recombinase mice. Both of the podIRKO mice were born with normal Mendelian frequency and initially appeared entirely normal, including having normal kidney structure and being normoglycemic. At 5 weeks-of-age, however, they started to develop albuminuria and shortening of the podocyte foot process. At 8 weeks-of-age, severe albuminuria, along with effacement of the podocyte foot process, higher incidence of podocyte apoptosis, thickening of glomerular basement membrane and glomerular matrix expansion – all histological features typical of human diabetic nephropathy – were present. These mice also showed mild worsening of kidney function. However, mesangial hypercellularity was not observed.

Podocytes express all the elements of the insulin signaling cascade as well as glucose transporters, such as GLUT-4, thus are capable of response to insulin[3]. By comparing glomeruli of wild-type and podIRKO mice treated with insulin, Welsh *et al.*[2] showed that insulin signals predominantly in the podocytes through the mitogen-activated protein kinases (MAPK) 42, MAPK 44 and phosphatidylinositol 3-kinase (PI3K)/Akt pathways. They also found that insulin directly, rapidly and specifically reorganizes the actin cytoskeleton of normal human podocytes maintained in culture, resulting in retraction of cellular processes and increased cellular motility and migration. Insulin also induces transient changes in the monolayer permeability of the podocytes[2]. Although the direct evidence is lacking, these observations might show that insulin supports podocytes in the dynamic physiological response to local demand for the maintenance of glomerular filtration barrier. Such remodeling of the actin cytoskeleton seemed to be correlated to an activation of RhoA and an inhibition of the cell division cycle (CDC) 42 by insulin (Figure 1). Of great interest, the earlier study showed the presence of insulin receptors by binding assays in isolated rat glomeruli, most possibly on the surface of the epithelium and endothelium, and showed an insight that insulin might underlie the alteration of glomerular ultrafiltration through, for example, modulation of glomerular cAMP concentrations[4].

Impaired insulin action in peripheral tissues, such as skeletal muscle, fat and liver, comprises a major cause of abnormal glucose metabolism in type 2 diabetes mellitus. Recently, not only these primary target organs, but also the cardiovascular system and the kidney have been shown to be responsive to insulin, and insulin resistance in these tissues has been recognized to be associated with macrovascular and microvascular complications in diabetes. Particularly in respect to the kidney, a panel of studies has

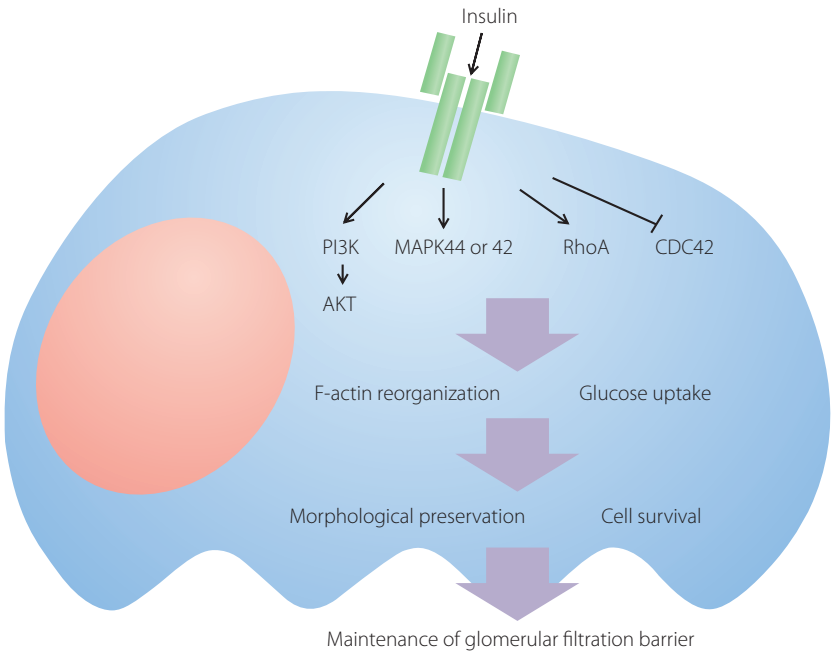

Figure 1 | Insulin signaling is critical for podocyte function. Insulin signals through the insulin receptor to phosphorylate Akt, mitogen-activated protein kinase (MAPK) 44 and 42, activate Ras homolog gene family member A (RhoA) and inhibit cell division cycle (CDC) 42 in glomerular podocytes. This signaling results in the reorganization of the actin cytoskeleton and the morphological preservation of the podocyte. Insulin also induces rapid glucose uptake by podocytes[3]. Welsh *et al.* showed that podocyte-specific deletion of insulin receptors in mice resulted in the development of effacement of the foot process, podocyte loss, glomerular basement membrane thickening and albuminuria. These malfunctions of podocytes seemed to be associated with the disruption of intracellular signals through Akt and MAPK 44 or 42[2].

shown that insulin resistance correlates with the onset of microalbuminuria in patients with type 2 diabetes[5]. Interestingly, it has recently been shown that podocytes isolated from diabetic *db/db* mice are unable to respond to insulin, which might correlate with increased apoptosis of podocytes, even when isolated from mice before the onset of microalbuminuria[6]. In addition, clinical trials comparing the effect of insulin sensitizers, such as thiazolidine, to hypoglycemic agents or insulin on the renal outcome showed that the improvement of insulin sensitivity resulted in the reduction of urinary albumin excretion and in renal protection[7]. These observations show that insulin resistance *per se* is responsible for the development of albuminuria in diabetes.

At this time, there might be an argument that the podIRKO mice model by Welsh *et al.*[2] might not be equivalent to the insulin resistance model and rather resembles the disease phenotype of insulin deficiency. Diabetic nephropathy affects patients with both type 1 and type 2 diabetes; as yet, a precise mechanism in which similar histopathological features develop in distinct types of diabetes is elusive. In this regard, there have been reports supporting the presence of insulin resistance in type 1 diabetes and such insulin resistance is considered to contribute to the development of microalbuminuria in patients with type 1 diabetes[8]. However, it is still to be established if podocytes are insulin resistant; even systemic surrogate markers of insulin resistance are found in those patients. In this line, the models by Welsh *et al.*[2] might explain the process of developing microalbuminuria in type 1 diabetes. Consistently, clinical observations showed that genetic mutations of the insulin receptor, although not always the case, cause kidney disease resembling diabetic nephropathy[9].

There are questions requiring more detailed investigation. How the loss of actin cytoskeleton remodeling in podocytes of podIRKO mice leads to increased apoptosis of podocytes? What is the essential role of insulin in podocytes – preservation of cellular structure or regulation of glucose uptake to maintain the energy metabolism? The contribution of upregulated IGF-1 receptor mRNA in compensation of insulin receptor deletion to the phenotype of podIRKO is also not clear[2]. However, it is apparent that the paper by Welsh *et al.*[2] would raise intriguing discussions. Although impaired insulin signaling in podocytes causes albuminuria, the same signal defects in endothelial cells might cause vasculature damage, which might partially explain the widely accepted correlation of microalbuminuria with increased cardiovascular disease risk. Development of insulin analogs that specifically augment, for example, cytoskeletal reorganization in podocytes, might open a new window for a treatment of diabetic nephropathy. It also provides a rational opportunity to re-evaluate insulin-sensitizing drugs use, dyslipidemia treatment and pathophysiology of adipokines in the management of diabetic complications. Obviously, it is anticipated that strategies to preserve insulin action in podocytes might translate into a decrease in the risk of diabetic nephropathy and other glomerular diseases.

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