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Insulin signaling: implications for podocyte biology in diabetic kidney disease

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Abstract

Purpose of review—Several key elements of the insulin signaling cascade contribute to podocyte function and survival. While it was initially thought that the consequences of altered insulin signaling to podocyte function was strictly related to altered glucose uptake, it has become clear that upstream signaling events involved in cell survival, lipid metabolism or nutrient sensing and modulated by insulin are strong independent contributors to podocyte function.

Recent findings—Akt2, the major isoform of Akt activated following cellular insulin stimulation, protects against the progression of renal disease in nephron-deficient mice, and podocyte-specific deletion of Akt2 results in a more rapid progression of experimental glomerular disease. In diabetes, podocyte mammalian target of rapamycin activation clearly contributes to podocyte injury and regulated autophagy. Furthermore, podocyte-specific glucose transporter type 4 (GLUT4) deficiency protects podocytes by preventing mammalian target of rapamycin signaling independently of glucose uptake. Finally, intracellular lipids have been recently recognized as major modulators of podocyte insulin signaling and as a new therapeutic target.

Summary—The identification of new contributors to podocyte insulin signaling is of extreme translational value as it may lead to new drug development strategies for diabetic kidney disease, as well as for other proteinuric kidney diseases.

Keywords

Akt; GLUT4; insulin signaling; intracellular lipids; mammalian target of rapamycin

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Conflicts of interest

A.F. holds patent application numbers US13/879,892, PCT/US11/56272: 'Assays, methods and kits for predicting renal disease and personalized treatment strategies'; PCT/US2012/062594, 'Soluble urokinase receptor (suPAR) in diabetic kidney disease'; PCT/US13/36484, 'Method of using cyclodextrin'. A.F. is a consultant for Hoffman-La Roche, Abbvie and Mesoblast.

INTRODUCTION

Diabetic kidney disease (DKD) is the most common cause of end-stage renal disease (ESRD). Multifactorial intervention trials targeting glycemic control, blood pressure and lifestyle interventions have been demonstrated to slow but not halt the progression of DKD in both type 1 [1] and type 2 diabetes [2]. Therefore, additional determinants of disease progression that can lead to novel drug development are yet to be discovered. The prevalence of DKD has increased in parallel with the increased prevalence of diabetes, and despite higher usage rates of glucose-lowering medications and antagonists of the renin–aldosterone system, the prevalence of DKD among those with diabetes has remained unchanged [3]. Chronic kidney disease has been linked to increased mortality [4,5], and consequently early interventions to treat DKD are important.

Characteristic histopathologic findings of DKD often precede the development of albuminuria, may occur in the setting of normoalbuminuric DKD and may predict the progressive nature of DKD [6–10], challenging the concept that albuminuria *per se* may cause progressive DKD. Among them, glomerular and tubular basement membrane (GBM) thickening, mesangial expansion, glomerular and arteriolar hyalinosis, and the loss of podocytes (podocytopenia) have been extensively studied in longitudinal cohorts of patients with either type 1 or type 2 diabetes [10]. While it has been thought that hyperglycemia and hemodynamic factors are the main contributors to those lesions, several additional circulating factors have been demonstrated to affect podocyte function in DKD. As we have extensively reviewed elsewhere the nature of potential circulating factors contributing to DKD [11,12], this review will focus on the role of insulin-stimulated pathways in the pathogenesis of DKD, with a focus on the mechanisms by which insulin signaling affects podocyte function in DKD.

OVERVIEW OF PODOCYTE INJURY IN DIABETIC KIDNEY DISEASE

Albuminuria (which will no longer be discriminated here as microalbuminuria or macroalbuminuria, based on the 2014 recommendations from the American Diabetes Association) is the first clinical manifestation of DKD and inversely correlates with the number of podocytes in experimental animal models and in humans with DKD [6–9,13–15]. Podocytopenia has, therefore, been recognized as a key event in the development of DKD in patients with diabetes [6,9,14]. Other than podocyte number, other measures of podocyte structure, such as the fraction of peripheral GBM covered by intact podocytes, correlate with the course of clinical DKD [8]. These data overall suggest that podocyte injury is an important feature of DKD.

Podocytes are specialized, differentiated cells of the kidney glomerulus, which consist of a cell body, major processes and foot processes, derived from major processes. Podocyte foot processes are heavily regulated by their actin cytoskeleton and are formed on the urinary side of the glomerular basement membrane, where they interdigitate with foot processes from the neighboring cells. The resulting filtration slits are bridged by the slit diaphragm, a structure composed of a variety of molecules that play an important and often podocyte-specific role in the selective permeability of the glomerular filtration barrier [16–18].

Integrity of the glomerular filtration barrier is the key in preventing the loss of protein into the urine (proteinuria) and mutations in genes coding for slit diaphragm proteins cause proteinuria-associated congenital nephropathies [19–23].

CLINICAL EVIDENCE FOR INSULIN SIGNALING INVOLVEMENT IN DIABETIC KIDNEY DISEASE

Multiple studies have shown that insulin resistance correlates with the development of albuminuria in patients with both type 1 or type 2 diabetes [24–27], their siblings [28,29] and in those without diabetes [30]. Furthermore, impaired insulin sensitivity in diabetic patients is associated with altered renal cell glucose metabolism that may directly contribute to progressive kidney damage independently of hyperglycemia [27]. The evidence that those patients with a genetic mutation of the insulin receptor may develop a renal disease that resembles DKD [31] underlines the importance of proper insulin signaling in the glomerular structure–function relationship and provides the rationale for interventions that target insulin receptor signaling. The fact that insulin-sensitizing agents of the class of thiazolidinediones (TZD) may reduce proteinuria of both diabetic and nondiabetic origin [32,33] independently of their ability to control hyperglycemia strongly supports this possibility. However, because TZDs may negatively affect cardiovascular outcome [34,35], other means to improve insulin sensitivity are needed.

OVERVIEW OF CELLULAR INSULIN SIGNALING

The primary function of insulin as a metabolic hormone is to regulate systemic glucose levels in the classical insulin-responsive tissues such as skeletal muscle, liver and adipose tissue. Apart from this function of insulin, which occurs primarily in the postprandial state and which is clearly linked to increased glucose uptake, lipid raft-mediated autocrine insulin signaling through two different isoforms of insulin receptor (A and B) has been shown to protect pancreatic beta cell survival and function in a way that is independent of glucose uptake [36]. In particular, autocrine insulin signaling through insulin receptor isoform A (IRA) leads to p70S6K dependent insulin transcription while insulin signaling through insulin receptor isoform B (IRB) augments Akt-dependent glucokinase transcription [37]. It is therefore possible that even in the presence of an equal amount of insulin, a modulation of the relative amount of IRA and IRB would affect their ability to form homodimers or heterodimers among themselves or with the insulin-like growth factor I receptor (IGF-1R), thus affecting downstream signaling [38]. Furthermore, insulin also has the capacity to exert an array of cellular responses, thus linking insulin signaling with other cellular signaling networks.

Receptor auto-phosphorylation on key tyrosine residues resulting from ligand binding leads to the recruitment and activation of insulin receptor substrate (IRS) proteins, of which IRS1–4 is the best documented. Phosphorylation of the IRS proteins marks another key point of signal divergence and regulation, and influences the formation of downstream complexes involved in a large variety of metabolic pathways, including glucose uptake, sodium transport, fatty acid synthesis, glycogen synthesis, gluconeogenesis, apoptosis, protein translation and transcription, as recently reviewed [12].

PODOCYTE INSULIN SIGNALING

Ever since it was demonstrated that insulin infusion can induce an acute transient increase in the albumin excretion rate despite euglycemia [39], the possibility of a direct effect of insulin signaling in glomerular cells has been addressed. While it was initially thought that the effect of insulin on albuminuria was primarily hemodynamic in nature and affected the tone of afferent and efferent arterioles, subsequent studies, performed by ourselves and others, were key in determining that glomerular cells are indeed insulin-sensitive. Within the kidney glomerulus, podocytes express several glucose transporters (1, 2, 3, 4 and 8) that are activated in diabetes [40–43,44^{*}]. Podocytes also express all the elements of the insulin-signaling cascade, such as functional IRS1 and insulin receptor, and are capable of increasing their glucose uptake when they are stimulated with insulin through glucose transporters, primarily glucose transporter type 4 (GLUT4) [42]. The relevance of podocyte insulin signaling to the pathogenesis of DKD was suggested by early findings that podocytes isolated from diabetic db/db mice are unable to phosphorylate AKT in response to insulin and are unable to translocate GLUT4 to the plasma membrane after insulin stimulation [44^{*}], even when isolated from mice at the time of onset of albuminuria [45]. A similar impairment is observed in glomeruli isolated from diabetic rats [46].

A link between insulin signaling and actin remodeling was suggested by the fact that nephrin – a key slit diaphragm protein expressed in podocytes – directly affects insulin signaling via a modulation of glucose transporters vesicle trafficking at the plasma membrane [47]. Apart from nephrin, other key molecules can modulate insulin signaling in podocytes. Among them, the lipid phosphatase SHIP2 [48] and septin 7 [49] may have an important role in linking insulin signaling to the actin cytoskeleton in podocytes. The direct effect of insulin on podocyte vascular endothelial growth factor production [50] supports a role of insulin as an important endocrine modulator of podocyte function. Furthermore, insulin may control podocyte depolarization and contractility [51,52]. Although podocytes are not excitable cells, such contractility may be regulated by calcium ion influx via the coordinated action of large conductance Ca^{+} -activated K^{+} channels and the cation channel, transient receptor potential cation channel 6 [51,52]. Recent study in support of such mechanism shows that high concentrations of insulin increase albumin permeability of both isolated rat glomeruli and podocyte monolayers in the culture [53] and that insulin induces oxidative stress in podocytes. These observations, coupled with those of increased albumin permeability of glomeruli isolated from Zucker obese rats, suggest a mechanism by which insulin may regulate filtration barrier permeability, which may be dysregulated in disease.

The physiological importance of proper insulin receptor expression and function in podocyte was proven with the development of podocyte-specific insulin receptor knockout mice. These mice develop a DKD-like phenotype in the absence of hyperglycemia [54], suggesting an important role for insulin signaling in podocyte function that does not require hyperglycemia [55]. If and how the insulin ability to modulate actin cytoskeleton and podocyte function occurs via a modulation of glucose transporter-mediated glucose uptake, or simply via the modulation of key signaling events through AKT and mammalian target of rapamycin (mTOR), remains to be established. Studies with podocyte-specific modulation

of GLUTs expression, as well as AKT and mTOR expression, were therefore undertaken to understand their relative contribution as modulator of podocyte function and survival.

Traditionally, the final step in insulin action is physiological modulation of glucose uptake and metabolism [56]. Thus, disrupting glucose uptake by facilitative glucose transporters (GLUTs) might negatively affect podocytes, similarly to what is observed in insulin receptor-deficient podocytes. The first GLUT transporter to be studied was GLUT1. While overexpression of GLUT1 in mesangial cells leads to a phenotype resembling DKD [57] and is associated with an up-regulation of mTOR [58], this was not the case for podocytes, where podocyte-specific overexpression of GLUT1 prevents mesangial expansion [59]. While these data suggested the interesting concept of cell type-specific functions of GLUTs, they also suggested that increased podocyte glucose uptake may be the mechanism responsible for the preservation of glomerular structure and function in insulin receptor-sufficient glomeruli. Furthermore, glucose uptake and metabolism may also affect nutrient sensing pathways independently of insulin signaling [60]. In particular, the AMP-activated protein kinase (AMPK) [61] and the mTOR pathways [62,63] are key direct modulators of podocyte function that can be affected by intracellular glucose.

Because GLUT4 is the most insulin-sensitive glucose transporter and is susceptible to translocation upon insulin stimulation, a large body of literature has analyzed the role of the actin cytoskeleton in GLUT4 trafficking. In fat and muscle cells, activation of both a phosphatidylinositol-4,5 bisphosphate 3-kinase (PI3-K)-dependent and a PI3-K-independent/caveolin-dependent pathway involving the protooncogene c-Cbl, the Cbl-associated protein (CAP) and the Rho GTPase TC10, is required for GLUT4 translocation to the plasma membrane [64]. The possibility that GLUT4 *per se* may regulate actin remodeling is suggested by the ability of GLUT4 in adipocytes to bind aldolase, which is known to interact with actin [65]. Therefore, a role of GLUT4 as a modulator of podocyte function through a glucose uptake-dependent or independent mechanism had to be studied and has been recently described. GLUT4-null mice are growth-retarded and exhibit decreased longevity, but do not develop diabetes [66], suggesting the possibility of a new, yet unexplored, function of GLUT4 that is independent from its metabolic function.

Mice with a podocyte-specific deletion of GLUT4 (G4 knockout) do not develop albuminuria despite having larger and fewer podocytes than wild-type mice [44[¶]]. Glomeruli from G4 knockout mice are indeed protected from diabetes-induced hypertrophy, mesangial expansion and albuminuria, and fail to activate the mTOR pathway. In order to investigate if the protection observed in G4 knockout mice was due to a failure to activate mTOR, three independent in-vivo experiments were performed. G4 knockout mice did not develop lipopolysaccharide-induced albuminuria, which requires mTOR activation. In contrast, G4 knockout mice, as well as wild-type mice treated with the mTOR inhibitor rapamycin, developed worse adriamycin-induced nephropathy than wild-type mice, consistent with the fact that adriamycin toxicity is augmented by mTOR inhibition. Additional studies are needed to investigate if the function of GLUT4 in podocytes is independent of insulin signaling. In this respect, it would be interesting to determine if podocyte-specific deletion of GLUT4 is sufficient to restore the glomerular phenotype of mice with a podocyte-specific deletion of the insulin receptor. These findings suggest that the link between insulin

signaling and preservation of cell function and survival is likely unrelated to insulin-dependent glucose uptake, and could be linked to the ability of insulin to modulate upstream signaling events. In this respect, a recent study by Canaud *et al.* [67^{***}] has shown that Akt2 – the major isoform of Akt activated following cellular insulin stimulation [68] – may be a key molecule in podocyte survival and protect against the progression of renal disease in nephron-deficient mice. Podocyte Akt2 is activated following glomerular stress both in mice and in patients with a variety of glomerular diseases, and podocyte-specific deletion of Akt2 results in a more rapid progression of experimental glomerular disease. These data indicate that podocyte Akt2 expression and activation are essential for the preservation of podocyte function and survival. As IGF-I [69] and IGF-II [70] are major modulators of AKT and may affect podocyte function and survival, further investigation in this area is also needed.

EXTRINSIC MODULATORS OF PODOCYTE INSULIN SENSITIVITY

There is now accumulating evidence that a number of circulating factors that are associated with diabetes and the metabolic syndrome can directly affect cellular insulin sensitivity of the podocyte. These include circulating factors associated with obesity such as adipokines; factors linked to renal inflammation including the pattern recognition receptors of inflammatory states that transmit inflammatory signals to the podocyte rendering it insulin-resistant, and hyperglycemia, which has been shown to inhibit cellular insulin signaling at a number of points in the insulin signaling cascade. This has recently been reviewed in depth [12]; so it will not be repeated here.

CELLULAR CHOLESTEROL AS MAJOR DETERMINANT OF ALTERED PODOCYTE INSULIN SIGNALING IN DIABETIC KIDNEY DISEASE

Cholesterol is a critical component of the eukaryotic plasma membrane (PM) that regulates fluidity, stiffness, molecular transport, and lipid raft formation – all processes that require interaction between the PM and the actin cytoskeleton [71]. Most membrane proteins are found in cholesterol-enriched regions that require actin anchors for maintaining their composition, and a reciprocal regulatory interaction between the cytoskeleton and lipid-rich invaginations of the PM, named caveolae, occurs [72,73]. In prostatic adenocarcinoma cells, regulation of RhoA, Src and caveolin-1 by PM cholesterol results in actin cytoskeleton remodeling [74]. Caveolin-1, the major constituent of caveolae, is highly expressed in podocytes [75], where it binds slit diaphragm proteins such as nephrin and CD2AP. In fact, caveolin-1 acts as a scaffolding protein for the modulation of several signaling pathways [76], such as insulin receptor [77,78]. Furthermore, a direct binding of cholesterol to the slit diaphragm protein podocin has been described [79]. While PM lipid and caveolin may play an important role in granting proper localization of slit diaphragm proteins [80], accumulation of cellular cholesterol may negatively affect cell function and cause cell senescence [81,82]. While it has been demonstrated that oxidized low-density lipoprotein (LDL) [83,84] and free fatty acids affect podocyte function [85–87], more recent data have demonstrated that pathological accumulation of cellular cholesterol can also occur in podocytes exposed to inflammatory cytokines [88]. A strong link between PM cholesterol and insulin signaling has been established. A physiological concentration of PM cholesterol is essential for proper insulin signaling and GLUT4 translocation via a caveolin/Cbl-

associated protein (CAP)/TC10 pathway, which is essential but not sufficient to cause GLUT4 translocation as it requires concomitant activation of the PI3K-AKT pathway [77]. PM cholesterol influences insulin receptor signaling at different levels: it influences insulin receptor A-type or B-type signaling [89,90], it binds together with the insulin receptor to caveolin, a major determinant of insulin receptor endocytosis and function [91,92], and it modulates GLUT4 translocation [93]. Under pathological conditions, however, altered cholesterol distribution may cause cellular inflammation and insulin resistance as described in adipocytes [94]. Similarly, an increase in the absolute amount of unesterified cholesterol can cause cell toxicity in macrophages [95]. Therefore, cholesterol depletion under physiological or pathological conditions can have the opposite effects. This has been clearly demonstrated in normal hepatocytes versus Niemann–Pick hepatocytes, where cholesterol depletion causes insulin resistance in the former and insulin sensitivity in the latter [90]. The latter seems to be the case for human podocytes, where exposure to the sera of patients with established DKD causes resistance to insulin in association with increased cellular cholesterol content, and where cholesterol depletion with cyclodextrin causes restoration of the ability of insulin to phosphorylate Akt and cell survival [96].

CONCLUSION

Ten years have passed since the discovery that podocytes are insulin-sensitive cells. Since then, many studies relevant to this field have been performed and have shed new light on the complex mechanisms by which insulin may affect podocyte function in health and disease. Future studies will be needed to define if there are any complex downstream effectors of insulin signaling directly linked to podocyte function and survival that can be pharmacologically targeted.

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KEY POINTS

- Several elements of the insulin-signaling cascade modulate the function of podocytes.
- Both systemic and local lipids and lipid-related proteins are being identified as major modulators of insulin signaling.
- The identification of new pathways linked to insulin signaling in podocytes may uncover new therapeutic targets for DKD.