CURRENT OPINION

Protein phosphatases and podocyte function

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Purpose of review
Deregulation of protecting factor signaling actions in podocytes has emerged as an alternative pathway of podocyte injury mechanisms. Here, we review recent knowledge that highlighted how podocyte protecting factors are modulated by protein phosphatases.

Recent findings
Protein tyrosine kinases and phosphatases participate in many, if not all, aspects of cellular function by turning on or off multiple signaling cascades and podocytes are no exception. Modulation of tyrosine residue phosphorylation of podocyte factors such as nephrin, vascular endothelial growth factor, insulin receptors and substrates has been shown to promote podocyte damage and cell death that contributed to multiple glomerular diseases. Protein phosphatase activity can cause either an increase [Src homology 2 domain-containing phosphatase 2 (SHP-2)] or a decrease [Protein tyrosine phosphatase1B (PTP1B), SHP-1 and SH2 domain–containing 5′-inositol phosphatase 2 (SHIP2)] in nephrin tyrosine phosphorylation depending on which podocyte injury model was used. Insulin resistance is closely linked to the development and progression of renal disease. Expression of PTP1B, SHP-1, phosphatase and tensin homolog and SHIP2 are potential mechanisms of podocytes insulin resistance in diabetic kidney disease.

Summary
Tight regulation of protein phosphatases is critical to maintain cell homeostasis and may offer new perceptive targets to restore protecting factor actions in order to prevent podocyte dysfunction and glomerular diseases.

Keywords
insulin, nephrin, phosphatase, podocytes, tyrosine phosphorylation

INTRODUCTION

The glomerulus is composed of three main cell types forming the filtration layer, namely the endothelial cells at the inside of the capillary, mesangial cells and podocytes on the outside of the capillary.

Podocytes cell-bodies 'float' in the urinary space and extend cellular projections, called primary processes, toward the capillary loops. At the capillary glomerular basement membrane (GBM) surface, these projections divide further into terminal foot processes. The neighboring foot processes derived from different cells are connected to each other by a continuous membrane-like structure called the slit diaphragm. As the slit diaphragm is the final barrier that prevents protein leakage into the urinary space, it is not surprising to observe that, independently of the underlying disease, the early events of glomerulopathies are characterized by alterations in the molecular composition of the slit diaphragm. These alterations, although reversible at an early stage, may lead to podocyte dysfunction, detachment from the GBM and ultimately in podocyte cell death. Several endogenous factors have been characterized to confer podocyte protection against toxic effects of stress and inflammatory signals. The main focus of this review is to overview how these protecting factors are deregulated by protein phosphatases contributing to podocyte dysfunction and glomerular disease.

OVERVIEW OF PROTECTING FACTORS IN PODOCYTES

Nephrin

Among all components of the slit diaphragm, nephrin plays a critical role in maintaining glomerular

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KEY POINTS

- Different models of podocyte injury can either increase or decrease nephrin tyrosine phosphorylation of which tyrosine phosphatases play a key role.
- Impaired insulin signaling in podocytes of both type 1 and type 2 diabetes mouse models is attributed to protein phosphatase PTP1B, SHP-1, PTEN and SHIP2 activity.
- Persistent podocyte expression of SHP-1 due to epigenetic changes could be a potential mechanism for the glycemic memory phenomenon as seeing diabetes kidney disease.
- Distinctive VEGF isoforms can have detrimental or beneficial effects on podocyte foot process effacement and glomerular diseases.

permeability. The inactivation of the nephrin gene in mice resulted in severe proteinuria and foot process effacement [1]. The extracellular domain of nephrin aligns with nephrin molecules from the neighboring foot process in an antiparallel mode. The cytoplasmic tail of nephrin contains several tyrosine residues that are conserved from zebrafish to humans. The tyrosine phosphorylation of nephrin is regulated by the Src-family kinase Fyn and genetic inactivation of both Fyn and Lyn caused proteinuria in mice [2]. Notably, several phosphorylated tyrosine residues are located within motifs that could provide a docking site for SH2 domain-containing kinases and adaptor proteins including Nck [3–5,6▪▪], CD2-associated protein (CD2AP) [7] and phosphoinositol-3 kinase (PI3K)/Akt. These adaptor proteins regulate nephrin-induced actin remodeling and cell survival [4,8,9]. Therefore, nephrin appears to be an essential factor not only of podocyte remodeling but also of podocyte function and survival. Not surprisingly, decreased nephrin expression and signaling have been observed early in many podocyte injury models [6▪▪]. More recently, a study established a new role of nephrin as cell nutrient-sensing modulator in podocytes independently of insulin [10▪▪].

Insulin

Insulin has been shown to be essential for normal glomerular filtration. Of all three cell types in the glomeruli, podocytes are the one with the higher levels of both insulin receptor and insulin receptor subtract (IRS)-1 expression. Coward *et al*. [11] showed that podocytes are insulin-responsive cells. The loss of insulin-stimulated Akt phosphorylation has been reported in podocytes of both type 1 [12] and type 2 diabetes mouse models [13]. The inability to signal through Akt2 was associated with increased podocyte death susceptibility [14]. Therefore, podocyte insulin sensitivity appears to be crucial for their function. Strategies to enhance insulin response in podocytes, like treatment with adiponectin [15], leptin [16] and PPAR family (alpha, delta, gamma) agonist [17,18], have all shown beneficial effects in preventing glomerular damage and podocyte dysfunction. More evidence for the critical role of insulin in podocyte function came from mice with podocyte-specific deletion of the insulin receptor [19]. In these animals, albuminuria developed, along with effacement of the podocyte foot process, apoptosis, thickening of the GBM and increased glomerulosclerosis. In addition, studies have reported that the expression levels of the insulin receptor and IRS1 were decreased in the glomeruli of insulin-resistant and diabetic rats [20,21]. Recent data demonstrated that high glucose levels impaired IRS-1 activation favoring the phosphorylation of the serine residues (that inhibits IRS-1 activity) rather than tyrosine phosphorylation [22▪]. IRS-2 has also been shown to participate in sensitizing the podocytes to insulin. Podocytes depleted of IRS-2 were insulin resistant in respect to Akt stimulation, glucose transporter type 4-mediated glucose uptake, cytoskeleton remodeling and cell motility [23]. Moreover, insulin regulates podocyte function by modulation of vascular endothelial growth factor-A (VEGF-A) production both *in vitro* and *in vivo* [24]. There is also an important cross-talk between nephrin and insulin. Inhibition of nephrin translocation to plasma membrane rendered podocytes unresponsive to insulin, an effect that was rescued by restoring nephrin membrane expression [25]. In addition to these functions, insulin signaling may also modify podocyte contractility by regulating the calcium ion influx via the coordinated actions of large conductance Ca2+-activated K+ (BK) channels and the cation channel, transient receptor potential cation channel, subfamily C, member 6 through a calcineurin-dependent pathway [26,27].

Vascular endothelial growth factor

Podocytes are the main VEGF producer cells, with VEGF-A being the most abundant isoform [28]. In order to ensure a good formation of the glomeruli, tight regulation of VEGF expression by podocytes is crucial, as both podocyte-specific deletion and overexpression of VEGF during development lead to dramatic and distinct glomerular phenotypes [28–32]. Although the paracrine effect of podocyte-produced VEGF on endothelial cells is well described, an autocrine pathway for VEGF-A in

podocytes remains highly controversial. Mouse podocytes *in vivo* express negligible VEGFR2 and podocyte-derived VEGFR2 did not contribute to the diseases observed when there is too much or too little glomerular VEGF [28]. However, other studies demonstrated that VEGFR1, VEGFR2, VEGFR3 and neuropilin-1 are important receptors for VEGF responsiveness in podocytes [33–36]. In addition, VEGF may modulate local complement proteins that could protect the glomerular microvasculature against complement-mediated injury [37▪]. VEGF-A also regulates slit diaphragm signaling and podocyte shape by inducing podocin upregulation, podocin–CD2AP interaction and nephrin phosphorylation in cultured podocytes [36,38]. Although this association was not demonstrated *in vivo*, these findings strongly implicated VEGFR2 signaling in podocytes as nephrin expression in the kidney is limited to this cell type. The necessity to have a good amount of VEGF was also highlighted by the observation that deletion of the Flt1 gene, which codes for VEGFR-1 and its soluble form (sFLT1), is lethal. Apart from inhibition of VEGF, sFlt1 can regulate podocyte cell morphology and glomerular barrier function [39]. Other VEGF-A isoforms, notably VEGF-A_{165b}, have been recently shown to protect glomerular changes in the diabetic condition [40,41▪]. Further, VEGF-B can modulate fatty acid (FA) transport proteins to promote FA uptake into cells. Recent data showed that VEGF-B-induced lipid accumulation in podocytes caused insulin resistance [42▪▪]. Finally, VEGF-C through activation of VEGFR3 may provide protection against the cytotoxic effect of serum starvation [43].

REGULATION OF PROTEIN PHOSPHATASES IN PODOCYTES

As described above, survival factors such as nephrin, insulin and VEGF trigger signaling pathways that influence podocyte function and survival. Nephrin and receptors of insulin and VEGF are mainly regulated by a balance between tyrosine phosphorylation and dephosphorylation. Any disruption in the equilibrium between protein tyrosine kinase activity and protein tyrosine phosphatase (PTP) activity will promote abnormal cell proliferation or death, thereby resulting in various pathophysiological abnormalities. Evidence that PTP plays an important role in podocyte homeostasis was shown by treating podocytes with a nonspecific PTP inhibitor that provoked drastic morphological alterations in the actin cytoskeleton network [44]. Initial expression profile of PTP by qPCR in a mouse podocyte cell line indicated that the protein tyrosine phosphatase 1B (PTP1B), Src homology-2 domain phosphatase-2, PTP-proline, glutamate, serine and threonine sequences (PTP-PEST) and PTP36 were expressed in these cells [44]. Since then, other PTPs have been implicated in podocyte function.

Glomerular epithelial protein 1

The glomerular epithelial protein 1 (GLEPP1) is a receptor membrane PTP expressed on the apical cell membrane of the podocyte foot process. GLEPP1 is the most abundant tyrosine phosphatase in podocytes that plays a role in controlling cellular signaling, actin cytoskeleton remodeling and permeability. Disruption of tyrosine phosphorylation of nephrin can alter its association with other proteins at the membrane. Deletion or reduction of GLEPP1 expression has been observed in podocyte injury models causing foot process effacement as well as in various proteinuric nephropathies, such as primary focal segmental glomerulosclerosis (FSGS), severe immunoglobulin A nephropathy and childhood-onset nephrotic syndrome [45–47]. GLEPP1-deficient mice displayed podocytes with amoeboid shape rather than the typical octopoid shape with blunted and widened foot process, reduced total slit diaphragm length and increased blood pressure after uninephrectomy [48]. In a model of podocyte damage [puromycin aminonucleoside (PAN)], downregulation of GLEPP1 preceded the onset of proteinuria [49] and inhibition of GLEPP1 with specific antibodies raised albumin permeability in both rat and rabbit glomeruli, supporting GLEPP1's critical role in preserving the glomerular permeability [50].

Protein tyrosine phosphatase 1B

PTP1B is a major regulator of insulin and leptin signaling [51,52]. By dephosphorylating the insulin receptor and IRS-1, PTP1B negatively regulates downstream insulin signaling and participates in insulin resistance. In contrast, PTP1B-deficient mice exhibited elevated insulin sensitivity and were resistance to obesity [51]. Thus, it is not surprising that PTP1B was considered as an attractive antidiabetic target to treat type 2 diabetes. In the kidney, Dr Takano's group showed that PTP1B is implicated in podocyte physiology. PTP1B was upregulated in injured podocytes using the rat model of PAN, adriamycin or lipopolysaccharide (LPS) [53,54]. Elevated levels of PTP1B directly dephosphorylated nephrin, leading to deregulation of the actin cytoskeleton structure [53], foot process effacement and proteinuria [54]. In contrast, PTP1B null mice with anti-GBM glomerulonephritis displayed less podocyte loss, reduced endoplasmic reticulum

dysfunction and proteinuria [55]. The same group has also generated podocyte-specific PTP1B knockout mice and podocyte-specific PTP1B transgenic mice to further investigate the role of PTP1B in podocyte biology [54]. They showed that PTP1B overexpression exacerbated LPS-induced podocyte injury by activating focal adhesion kinase (FAK) through dephosphorylation of Src-family kinases (causing its activation). Increased activity of FAK was associated with podocyte motility *in vitro* and proteinuria *in vivo*. Apart from FAK activation, the authors investigated the possibility that the absence of PTP1B could preserve nephrin tyrosine phosphorylation [54]. PTP1B-deficient mice exhibited elevated nephrin phospho-tyrosine residues (Y1232) involved in the interaction of nephrin with Nck. In contrast, phosphorylation of Y1232 of nephrin was decreased in podocyte-specific PTP1B transgenic mice. PTP1B can also dephosphorylate an array of receptor tyrosine kinases. Thus, it is possible that upregulation of PTP1B in podocyte damage models deactivates, directly or indirectly, insulin receptor, IRS1 and insulin-like growth factor receptor. In a rat model of fructose-induced insulin resistance, PTP1B expression was upregulated and associated with reduction of insulin receptor and IRS1 tyrosine phosphorylation [56]. Caveolin-1, a critical regulator of insulin receptor expression, is highly expressed in podocytes. PTP1B overexpression decreased tyrosine residue 14 (Y14) phosphorylation of caveolin-1 in podocytes [56] suggesting that podocytes exposed to high levels of fructose displayed insulin signaling impairment through increased expression of PTP1B.

Src homology region 2 domain-containing phosphatase-1

The Src homology region 2 domain-containing phosphatase-1 (SHP-1) is a cytoplasmic tyrosine phosphatase that regulates a variety of cellular processes including cell growth, differentiation, mitotic cycle and oncogenic transformation. Our group and others have demonstrated that SHP-1 is expressed in podocytes and is increased in type 1 diabetic glomeruli and in immortalized mouse cultured podocytes exposed to high glucose levels [12,57,58]. Diabetes-induced elevated SHP-1 expression in podocytes was a consequence of protein kinase C δ isoform activation [57]. The upregulation of SHP-1 expression and activity was associated with inhibition of VEGF-A actions, including phosphorylation of VEGFR2. In addition, SHP-1 directly interacted with the insulin receptor-β, which reduced insulin signaling pathway and increased podocyte cell death. More recently, increased expression of SHP-1 in diabetes persisted despite normoglycemia for several months in renal glomeruli due to epigenetic modifications in the SHP1 promoter region [59▪▪], suggesting that SHP-1 could be a potential explanation of the glycemic memory effect observed in some diabetic patients. Apart from insulin receptor and VEGFR, SHP-1 also binds to specific tyrosine residues of nephrin. Diabetes-induced reduction of nephrin phosphorylation was caused by SHP1 interaction with nephrin [60]. Taken together, these studies have demonstrated that elevated SHP-1 expression in diabetic kidney could be an important regulator of protecting factors, which may contribute to the progression of diabetic nephropathy.

Src homology region 2 domain-containing phosphatase-2

Src homology 2 domain-containing phosphatase 2 (SHP-2) is a ubiquitously expressed nontransmembrane phosphatase essential during development as the SHP-2 null mice are embryonically lethal [61]. In contrast to SHP-1 that deactivates receptor tyrosine kinases, SHP-2 is required for full activation of most of the receptor tyrosine kinase actions, suggesting that SHP-2 plays a significant role in maintaining many facets of cell signaling. Podocyte-specific SHP-2-deficient mice did not develop foot process spreading after injury using protamine sulfate or nephrotoxic serum models [62]. In this article, the author showed that, like SHP-1, SHP-2 bounds with nephrin tyrosine residues 1174 and 1172. However, in contrast to SHP-1, the absence of SHP-2 in podocytes prevented protamine sulfate-induced nephrin phosphorylation, suggesting that SHP-2 is needed for nephrin phosphorylation in response to specific podocyte damage.

Interestingly, the authors noted that increased SHP-2 activity in the kidney was found in patients with minimal-change nephrosis and membranous nephropathy but not with FSGS. More recently, a study reported that podocyte-specific SHP-2 deletion attenuated LPS-induced proteinuria by decreasing renal nuclear factor-kappa B and mitogen-activated protein kinase (MAPK) pathways [63▪]. These observations identified SHP-2 as a significant contributor of podocyte damage following an inflammatory response.

Phosphatase and tensin homolog

Phosphatase and tensin homolog (PTEN) is a dual-function lipid and protein phosphatase implicated in cell growth, migration and metabolism. PTEN prevents PI3K pathways by dephosphorylating

phosphatidylinositol-3,4,5-triphosphate (PIP3) into phosphatidylinositol-3,4-bisphosphate (PIP2), which results in the prevention of Akt activation. PTEN modulates cytoskeleton dynamics raising the idea that PTEN acts in podocyte actin remodeling in response to stressful condition such as diabetes. In cultured podocytes, PTEN expression was either not changed or increased by high glucose levels depending on the exposure time [12,64]. In contrast, another study established that PTEN levels were decreased in podocytes of *db/db* mice and patients with diabetic nephropathy [65]. Giving that the PI3K/Akt pathway is important for podocyte function, deletion of PTEN could be viewed as beneficial. However, podocyte-specific PTEN null mice exhibited an increase in albuminuria as compared to control littermates fed with high-fat diet [65]. Pathological examination detected early glomerulosclerosis, interrupted nephrin immunostaining and extensive foot process effacement in podocyte-specific PTEN-deficient mice maintained on a high-fat diet. The author suggested that the loss of PTEN in podocytes did not favor insulin signaling due to its localization. Insulin receptor is mainly located in the cell body, whereas PTEN has been visualized in the foot process. Another potential explanation is that deletion of PTEN in podocytes caused swelling of the glomerular endothelial cells reinforcing the need to better understand the crosstalk communication between podocytes and glomerular endothelial cells.

Lipid phosphatase SH2 domain–containing 5′-inositol phosphatase 2

Lipid phosphatase SH2 domain-containing 5′-inositol phosphatase 2 (SHIP2) downregulates the PI3K/Akt-mediated signaling pathway, caused by insulin and other growth factors, by hydrolyzing PIP3 to PIP2. SHIP2 overexpression in rodents had a negative feedback on glucose tolerance [66], whereas

SHIP2 knockout mice were resistant to high-fat diet-induced obesity and insulin resistance [67]. Overexpression of SHIP2 was able to reduce Akt phosphorylation and promoted cell injury in podocytes [68]. In addition, previous study by Fornoni's group established that a podocyte cell line derived from *db/db* mice was incapable to activate Akt upon insulin stimulation due to upregulation of SHIP2 in podocytes [13]. Another study corroborated this finding by reporting SHIP2 upregulation in the glomeruli of obese Zucker rats, a model of insulin resistance. Data from this study also suggested that SHIP2 was not regulated by hyperglycemia as obese Zucker rats did not exhibit significant elevated blood glucose levels nor did high glucose-level exposure altered SHIP2 expression in cultured podocytes [68].

In addition to insulin, SHIP2 interacted with c-Abl to reduce nephrin-stimulated Akt under angiotensin II stimulation [69▪]. SHIP2 is also involved in nephrin's capacity to assemble a protein complex (filamin and lamellipodin) in a tyrosine phosphorylation-dependent manner giving nephrin's ability to initiate the generation of the actin filament and regulate the architecture of the actin network [70].

Serine/threonine phosphatases

The protein phosphatase-1 (PP1) and protein phosphatase-2A (PP2A) are known as serine/threonine phosphatases which are implicated in various cellular processes such as cell cycle regulation, metabolism and insulin resistance. It has been previously demonstrated that the PP1 activity was increased in podocytes exposed to advanced glycation end products as well as in the kidney of type 2 diabetic mice (*db/db*) [71]. The suppression of PP1 endogenous inhibitor by advanced glycation end-products led to PP1 activation causing cell cycle arrest and cellular hypertrophy of the podocytes. In hepatocytes, hyperactivity of PP2A is involved in the development of insulin resistance. A previous study indicated that PP2A was activated by free FA-induced insulin resistance resulting in human podocyte cell death [72]. However, how PP2A deregulates specific proteins of the insulin signaling cascade remains to be determined. As serine/threonine phosphorylation stabilized synaptopodin [73▪], it would be interesting to investigate if serine/threonine phosphatases may participate to synaptopodin degradation resulting in a damaged podocyte actin cytoskeleton network.

CONCLUSION

The necessity to halt or even reverse glomerular diseases is critically needed to decrease the major burden of chronic kidney diseases. Therapeutically, modulating protein phosphatase remains challenging because of a lack of specific inhibitors. However, this approach may provide better outcomes to preserve podocyte function.

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Conflicts of interest

There are no conflicts of interest.

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