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# Regulation of Nephrin Phosphorylation in Diabetes and Chronic Kidney Injury

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**Abstract**

Diabetes is the leading cause of microalbuminuria and end-stage renal failure in industrial countries. Disruption of the filtration barrier, seen in almost all nephrotic diseases and diabetes, is the result of the loss or effacement of the podocyte foot process, notably damage of proteins within the slit diaphragm such as nephrin. For many years, nephrin has been viewed as a structural component of the slit diaphragm. It is now well recognized that nephrin contains several tyrosine residues in its cytoplasmic domain, which influences the development of glomerular injury. In this review, we propose an overview of nephrin signaling pathways in kidney injury.

**Keywords**

Nephrin • Nephropathy phosphatases • Podocyte • SHP-1

## Abbreviations

| | |
|---|---|
| DN | diabetic nephropathy |
| FP | foot process |
| NF-B | nuclear factor-kappa B |
| LPS | lipopolysaccharides |
| PAN | puromycin aminonucleoside |
| PI3K | phosphatidylinositol 3-kinase |
| PS | protamin sulfate |
| SD | slit diaphragm |
| SHP-1 | Src homology 2 domain phosphatase 1 |

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People living with diabetes are at high risk of developing complications affecting both the macrovascular and microvascular systems. Complications of the large vessels include coronary artery diseases, atherosclerosis and peripheral arterial disease, while complications disturbing small vessels include neuropathy, retinopathy and nephropathy, the latter being the focus of this review. Diabetic nephropathy is the leading cause of end-stage renal disease in the world and is linked with both high economic cost and morbidity (Collins et al. 2007). Unfortunately, there are limited ways to prevent the progression of the disease, which can ultimately result in kidney failure. The two main contributors to the disease are chronic hyperglycemia and hypertension (The Writing Team for the Diabetes Control and Complications Trial/

Epidemiology of Diabetes Interventions and Complications Research Group 2002; Epstein and Sowers 1992).

## 1 Diabetic Nephropathy

The first clinical manifestation of diabetic nephropathy (DN) is albuminuria, which is defined by abnormal high levels of the protein albumin in the urine. At the early stage, patients with DN will exhibit elevated glomerular filtration rate. As the disease progress, albuminuria will rise and the glomerular filtration rate will decline. Eventually, DN will evolve to end-stage renal disease, with very low glomerular filtration rate levels ($<15$ mL/min/1.73m$^2$) in which dialysis or kidney transplant will be required to prevent death (Dronavalli et al. 2008). Histopathological studies from the kidney cortex revealed that one of the first structures affected by DN is the glomerulus. The glomerulus is composed of three main cell types (mesangial cells, endothelial cells and podocytes). Diabetes-induced glomerulus dysfunction is characterized by the expansion of the mesangium, extracellular matrix deposition, thickening of the basal membrane, and dedifferentiation and cell death of the podocytes. Loss of the podocytes is believed to be one of the strongest predictors of DN progression (Meyer et al. 1999). The importance of podocytes in preserving renal functions in DN was highlighted by morphometric observations from kidney biopsies of diabetic patients which showed a significant reduction in numbers of podocytes in patients with short duration of diabetes before the apparition of microalbuminuria or other markers of the disease (Meyer et al. 1999; Pagtalunan et al. 1997; White et al. 2002). It is clear now that the mechanisms of diabetes-induced complications of the glomerular dysfunction are very complex and involve crosstalk between the mesangial cells, the endothelial cells and the podocytes (Kriz et al. 1998; Siddiqi and Advani 2013; Schlondorff and Banas 2009). In this review, the focus will be on the podocytes.

## 2 The Podocytes

Podocytes are highly specialized epithelial cells found in the kidney glomerulus that participate in blood filtration by creating a physical barrier around the blood capillaries. They are composed of three distinct morphological structures: the cellular body, the main process and the foot process (FP). Main processes of the podocytes are cytoplasmic projections that emerge from their cellular body. They further divide into smaller projections called FP that create a zipper-like structure with another FP of the neighboring podocytes. This highly organized zipper-like structure is responsible for filtering molecules and proteins found in the blood by their molecular sizes (Pavenstädt et al. 2003; Karnovsky and Ryan 1975). The FP expresses various integrins, notably the α3β1 integrin, that anchors the podocyte to the glomerular basement membrane (Sachs and Sonnenberg 2013). The integrity of the podocyte FP is very dependent on a structure found between adjacent FP called the slit diaphragm (SD). The SD can be best described as a multiprotein complex that regulates podocyte homeostasis and function (Reiser et al. 2000a; Verma et al. 2016). The essential role of the SD on podocyte ability to filtrate the blood was highlighted in various kidney diseases. The most striking example is the mutation of the protein nephrin which causes the earliest and most severe nephrotic syndrome (Greka and Mundel 2012).

## 3 Nephrin

The discovery of nephrin in 1998 was made by identifying the genetic mutation responsible for the congenital nephrotic syndrome of the Finnish type (NPHS1), which is one of the most severe forms of nephrotic syndrome (Kestilä et al.

1998). This finding underlined the critical role of nephrin in the maturation and function of the glomerulus, and marked a new era in glomerular disease research. Nephrin is a transmembrane protein of the *immunoglobulin-like* family. Its extracellular domain is composed of 8 *igG-like* domains and a single *fibronectin type 3* domain. It was first believed that nephrin was only expressed in the kidney, but recent studies confirmed its expression in the pancreatic β-cells playing a role in glucose-stimulated insulin release and cell survival signaling (Fornoni et al. 2010; Kapodistria et al. 2015; Jeon et al. 2012). Nephrin expression in the kidney is specific to the podocytes and is located in the SD (Fig. 1). Moreover, nephrin can be found in a detergent resistant cellular membrane compartment called lipid raft. Knockout mouse studies showed that the extracellular domain of nephrin interacts with nephrin and NEPH1 of the adjacent podocytes to link neighboring foot process together in a highly organized manner (Wartiovaara et al. 2004; Khoshnoodi et al. 2003).

## 3.1 Nephrin Phosphorylation

Sequencing of the intracellular domain of nephrin across various species revealed multiple tyrosine residues, which are evolutionally conserved suggesting that this portion of nephrin may have functional importance. Of them, many tyrosine residues are recognized by proteins that have SH2 domains. Previous data have demonstrated that the SRC-family kinase FYN had the highest affinity for nephrin and was the most potent at increasing nephrin tyrosine phosphorylation (Li et al. 2004; Verma et al. 2003). Since then, multiple studies discovered that phosphorylation of these tyrosine residues is critical to the regulation of various intracellular pathways in the podocytes. Those pathways include remodeling of the actin cytoskeleton, activation of the PI3 kinase/Akt pathway, calcium signaling and endocytosis of nephrin (Patrakka and Tryggvason 2007; Jones et al. 2009; Li et al. 2015; Quack et al. 2011) (Fig. 2).

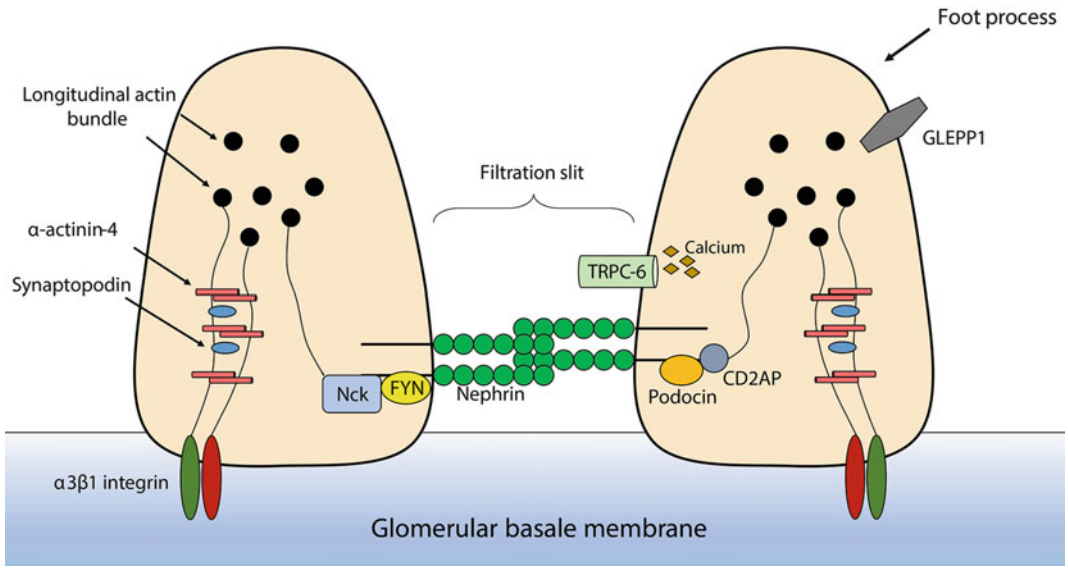


**Fig. 1** Representative image of the foot process of the podocytes

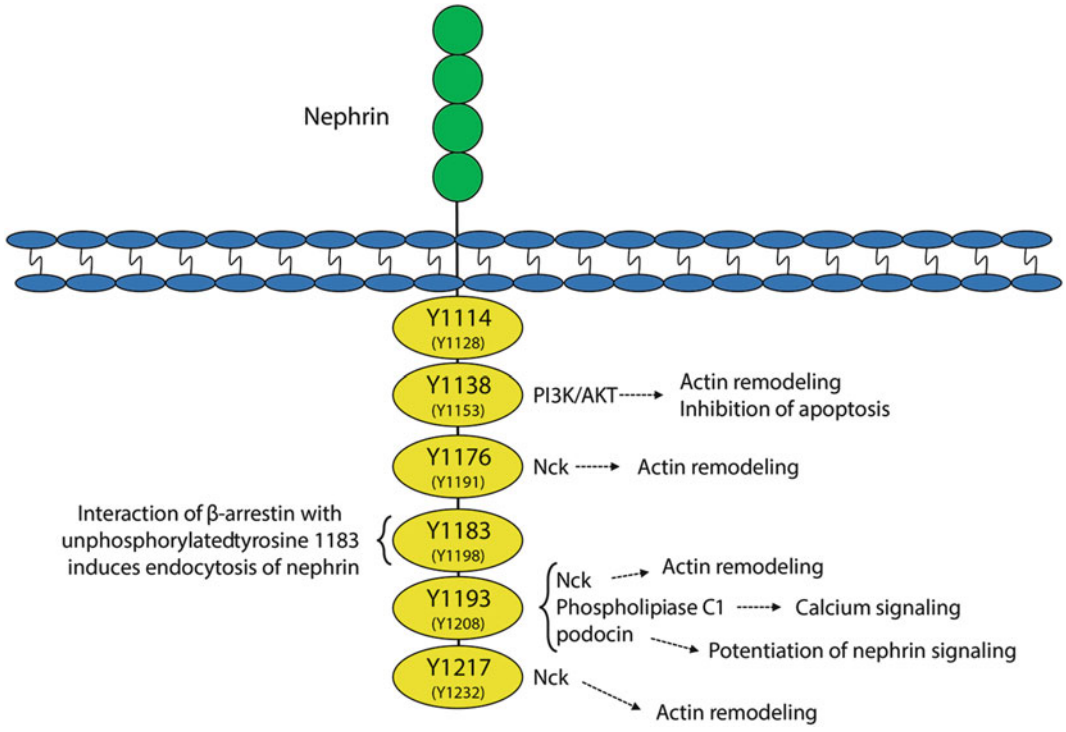


**Fig. 2** Tyrosine residues in the cytoplasmic compartment of nephrin

## 3.2 Actin Remodeling

In 2006, two important studies by Pawson and Holzmann showed the crucial role of nephrin tyrosine phosphorylation in the regulation of actin remodeling in the podocytes. This remodeling required interaction of nephrin with both NCK1 and NCK2, two adaptor proteins containing three SH3 domains and a single SH2 domain. Both studies reported that triggering phosphorylation of tyrosine residues (Y1176, Y1193 and Y1208) of nephrin enhanced its interaction with NCK1 and NCK2, resulting in the formation of actin tails (Jones et al. 2006; Verma et al. 2006a). The role of the interaction of nephrin with NCK in maintaining kidney function was first highlighted using the NCK1 deficient mice and the podocyte specific NCK2 knockout mice, which developed glomerulosclerosis and podocyte FP fusion (Jones et al. 2009). These observations were further confirmed in a study by Dr. Jones' laboratory, who has generated mice with mutations in the tyrosine residues responsible of nephrin interaction with NCK. Mice expressing nephrin mutated at tyrosine 1176, 1193 and 1217 showed progressive proteinuria with aging, which was accompanied with podocyte foot process effacement, dilated capillary loops and thickening of the basal membrane. Moreover, these mice were more susceptible to heparin and lipopolysaccharides (LPS) induced kidney injury (New et al. 2016). This study clearly demonstrated that nephrin-mediated regulation of the actin cytoskeleton is required in both keeping a healthy glomerulus and during the recovery process after kidney injury.

## 3.3 Regulation of Calcium

The importance of calcium regulation in the podocytes was emphasized when gain-of-function mutation of the transient receptor potential cation channel 6, or TRPC6, was linked to focal segmental glomerulosclerosis, a glomerular

disease which ultimately leads to end-stage renal disease (Reiser et al. 2005; Winn et al. 2005). Calcium signaling in the podocytes is linked to the remodeling of the actin cytoskeleton, which is essential in maintaining a healthy filtration barrier (Greka and Mundel 2011). A previous study showed that phosphorylation of the tyrosine 1193 of nephrin promoted its interaction with phospholipase C-γ1 and triggered calcium mobilization (Harita et al. 2009). It was found that nephrin is implicated in the inhibition of the activity of TRPC6, but in a non-tyrosine-dependent mechanism (Kanda et al. 2011).

### 3.4 PI3K/Akt Pathway

The AKT family of proteins (Akt1, Akt2 and Akt3) are serine/threonine kinases that are involved in multiple cellular pathways including cell survival, proliferation, migration and actin remodeling. The phosphoinositide 3-kinase (PI3K) is composed of a catalytic subunit called p110 and a regulatory subunit called p85. Activation of Akt is made by a complex signaling cascade which starts with activated receptor tyrosine kinases, such as the platelet-derived growth factor receptor (PDGF) and the insulin receptor (IR). Upon activation, receptor tyrosine kinase activates PI3K through direct interaction or with a scaffolding protein such as the insulin receptor subtrat-1 (IRS-1). Activated PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) to form phosphatidylinositol-3,4,5-triphosphate (PIP3). Then, Akt translocates to the membrane to be stimulated by both PIP3 and PDK1 (Manning and Cantley 2007). In podocytes, Akt2, and not Akt1, is critical for podocyte survival and function in various kidney diseases (Canaud et al. 2013). Interestingly, Akt2 is the major isoform activated upon insulin stimulation (Kim et al. 2000; Cho 2001; Garofalo et al. 2003).

The regulation and activation of the PI3K/Akt pathways by nephrin was initially associated with nephrin interaction with CD2AP and podocin, which are two proteins implicated in podocyte function found in the SD (Huber et al. 2003). Overexpression of nephrin in podocytes increased basal Akt phosphorylation, which is associated with an increased interaction of p85 subunit with nephrin on phosphorylated tyrosine residue 1138. The increased interaction between both proteins resulted in the reduction of actin stress fiber formation in cultured podocytes (Zhu et al. 2008).

## 4 Nephrin Phosphorylation in the Development of Mature Glomerulus

The first evidence that nephrin phosphorylation could be involved in the maturation of the glomerulus came from immunofluorescence experiments indicating that nephrin phosphorylation was greatly induced in the development of the glomerulus in mice (Verma et al. 2006b). Interestingly, the same group could not detect nephrin phosphorylation in the mature glomerulus, which contradicts results from other studies (Jones et al. 2009; Denhez et al. 2015; Verma et al. 2015). One possible explanation is the sensitivity of the antibodies used in these studies. Furthermore, mice expressing the triple mutant of nephrin did not exhibit abnormal filtration barrier and maturation of the glomerulus, which strongly suggests that nephrin-mediated actin regulation is not required for the maturation of the glomerulus, or that other pathways can compensate for the loss of nephrin mediated actin remodeling. Nonetheless, these results do not rule out that other nephrin tyrosine residues could be essential for the development of the glomerulus (New et al. 2016). As described above, the fundamental role of nephrin phosphorylation in the mature glomerulus was underlined by the presence of proteinuria and glomerular abnormalities in mice lacking of nephrin tyrosine residues related to actin regulation. Unfortunately, there is no indication that nephrin tyrosine residues involved in other cellular pathways such as calcium signaling or PI3K activation could play a role in maintaining kidney function in the mature glomerulus.

## 5 Nephrin Expression in Various Models of Glomerular Injury

The notion that the disruption of nephrin expression could lead to glomerular damage came from the observation that patients with congenital nephrotic syndrome of the Finnish type possessed mutations on the gene coding for nephrin (Hulkko et al. 2014). Subsequent studies revealed that the vast majority of these mutations contributed to impaired trafficking of nephrin to the cellular membrane. Downregulation of nephrin expression was observed in a number of glomerular disease, including lupus nephritis and DN (Doublier et al. 2003; Perysinaki et al. 2011), whereas some studies reported that expression of nephrin was not affected in proteinuric kidney disease. Patrakka and collaborators observed that the mRNA and protein expression of nephrin, using immunohistochemistry and in situ hybridization, was similar between pediatric minimal change nephrosis patients and controls (Patrakka et al. 2001). Quack and collaborator showed that high glucose level exposure in cultured podocytes activated PKC-α activity causing the phosphorylation on nephrin threonine residues 1120 and 1125 (Quack et al. 2011). These phosphorylation sites subsequently increased nephrin interaction with β-arrestin2 promoting nephrin endocytosis. The authors reported that the interaction of PKC-α with nephrin was dependant of the adaptor protein PICK1. Moreover, deletion of PKC-α in diabetic mice prevented the loss of nephrin expression and development of DN (Menne et al. 2006). Other than weakening of the structural integrity of the SD, loss of nephrin expression has been shown to promote cellular pathways contributing to glomerular injury. Hussain and collaborators demonstrated that nephrin deficiency activated the nuclear factor-kappa B (NF-κB), a transcription factor known to be upregulated in various glomerular diseases (Hussain et al. 2009; Zheng et al. 2006; Fujihara et al. 2007; Takano et al. 2001). The authors reported that cultured podocytes expressing a truncated mutant of nephrin exhibited elevated levels of NF-κB transcriptional activity and a shift of aPKC-ζ expression from the nucleus to the cytoplasm, which induced the phosphorylation of the cytoplasmic IκB causing its dissociation with NF-κB and subsequently its translocation to the nucleus (Hussain et al. 2009). More importantly, the rescue of nephrin expression prevented the increased NF-κB activation, suggesting that loss of nephrin expression could be a driving factor in NF-κB dependant glomerular diseases.

## 6 Nephrin Phosphorylation in Various Models of Acute Kidney Injury

Multiple studies sought to find if nephrin tyrosine phosphorylation was affected in various types of kidney injury. Typical mouse models of acute kidney injury include the puromycin aminonucleoside (PAN) nephropathy, the protamine sulfate (PS), and the LPS treatment. In these models, both PAN nephropathy and LPS treatment reduced nephrin tyrosine 1176/1193 phosphorylation levels (Jones et al. 2009; Zhu et al. 2008). It is believed that PAN nephropathy mimics minimal-change nephropathy in humans. Both diseases are characterized by almost no visible glomerular change on light microscopy but the major changes occurred in the structural integrity of the podocyte FP (Ryan and Karnovsky 1975; Löwenborg et al. 2000). Multiple studies that evaluated nephrin tyrosine phosphorylation in PAN models reported a stark decrease in its phosphorylation levels, in which the lowest expression of nephrin phosphorylation was observed when proteinuria reached its highest level (Jones et al. 2009; Zhu et al. 2008; Li et al. 2006). Moreover, human glomerulonephritis treatment with glucocorticoids exerted its actions by increasing the phosphorylation of nephrin (Ohashi et al. 2011). In contrast, the rat Heymann nephritis and PS-induced damage to the kidney significantly increased nephrin phosphorylation at tyrosine 1176/1193, while reperfusion of the kidney with heparin sulfate, which reversed podocyte FP spreading by PS, restored

nephrin phosphorylation to baseline levels. (Li et al. 2004; Verma et al. 2006a; New et al. 2016). In addition, another study found that preventing the increased phosphorylation levels of nephrin following PS treatment abrogated the podocyte FP effacement (Verma et al. 2015). On the other hand, mice expressing nephrin mutated at tyrosine 1176, 1193 and 1217 did not exhibit reduction in FP effacement in reperfused kidney with heparin sulfate suggesting that nephrin mediated remodeling of the actin cytoskeleton is required to restore the integrity of the FP in this model (New et al. 2016). Taken together, these data suggest that the homeostasis of the podocyte structure and its FP integrity by nephrin is a fine balance that can be disrupted by tipping it toward both sides.

## 7 Regulation of Tyrosine Phosphorylation of Nephrin

Ample evidence from *in vitro* studies showed that FYN and SRC kinases have the highest affinity for nephrin and are the most potent at increasing nephrin phosphorylation (Li et al. 2004; Verma et al. 2003; Jones et al. 2006). FYN was found to mostly phosphorylate nephrin tyrosine 1176, 1993 and 1217, which as we discussed above are required for nephrin interaction with NCK adaptor proteins. It is now widely accepted that regulation of cellular signaling mediated by phosphorylation is a fine balance between the activity of kinases and phosphatases. A change in either part of this balance can lead to various consequences, including diseases like cancer, diabetes and rheumatoid arthritis (Hendriks et al. 2013; Echwald et al. 2002; Hinks et al. 2006). Since nephrin phosphorylation is required for a healthy glomerulus, a better understanding of cellular mechanisms that contribute to the inhibition of nephrin-mediated intracellular pathways could be of therapeutic interest. One of the potential mechanisms is the increase interactions of nephrin with tyrosine phosphatases, which can dephosphorylate tyrosine residues of nephrin, leading to the inhibition of its cellular signaling pathways. Glomerular epithelial protein 1 (GLEPP1) is the most well-characterized tyrosine phosphatase in podocytes and is highly expressed on the apical membrane of the cells (Thomas et al. 1994; Wang et al. 2000). GLEPP1 deficient mice showed abnormal podocyte morphology and elevated blood pressure after uninephrectomy (Wharram et al. 2000). In cultured mouse podocytes, expression profile of selected phosphatases was detected by quantitative PCR and demonstrated that PTP1B, Src homology-2 domain phosphatase-2 (SHP-2), PTP-proline, glutamate, serine and threonine sequences (PTP-PEST), and PTPD2 were expressed in podocytes (Reiser et al. 2000b).

### 7.1 PTP1B

The first evidence that phosphatases could regulate nephrin phosphorylation was shown in a study from Dr. Takano's group. They found that PTP1B could bind to nephrin and dephosphorylate tyrosine 1193 and 1217 (Fig. 3). Moreover, inhibition or overexpression of PTP1B in podocytes drastically altered their actin cytoskeleton and remodeling, further reinforcing the idea that phosphatases can regulate nephrin-mediated cellular signaling actions (Aoudjit et al. 2011). In addition, the same group reported that inhibition of PTP1B significantly improved albuminuria and proteinuria following kidney injury in mice treated with PAN or adryamicin treatment (Kumagai et al. 2014).

### 7.2 SHP-1

Recently, our laboratory reported that Src homology 2 domain phosphatase 1 (SHP-1) can bind nephrin and reduce its tyrosine phosphorylation levels (Denhez et al. 2015). SHP-1 is part of the *non-receptor-like* family of tyrosine phosphatases. SHP-1 contains two SH2 domains (N-SH2 and C-SH2) which control both its activation and interaction with its targets. In its non-active state, the N-terminal SH2 domain

binds to the phosphatase catalytic domain of SHP-1 (Fig. 3). Binding of the C-terminal SH2 domain with the phosphorylated tyrosine of a targeted substrate disrupts the interaction of the N-SH2 domain with the phosphatase domain liberating the catalytic phosphatase domain to induce dephosphorylation of the tyrosine residues of its substrate. Activation of SHP-1 can also be enhanced by the phosphorylation of its tyrosine 536 and 546 residues (Wang et al. 2011; Uchida et al. 1994; Yoshida et al. 1999; Neel et al. 2003). SHP-1 is a negative modulator of multiple signaling pathways linked to growth factors, which includes platelet-derived growth factor, vascular endothelial growth factor and insulin signaling (Neel et al. 2003; Wu et al. 2003). Results from both our group and other laboratories found that hyperglycemia and diabetes increased the expression of SHP-1 in various cell types causing cellular growth factor inhibition (Geraldes et al. 2009; Mima et al. 2012; Drapeau et al. 2013; Lizotte et al. 2016). In podocytes, hyperglycemia induced a persistent increase of SHP-1 expression, due to epigenetic modification in the SHP-1 promoter (Lizotte et al. 2016) leading to insulin signaling resistance, podocyte dysfunction and cell death, and DN. This resulted in blunted Akt and ERK activation upon insulin stimulation in podocytes, both of which are associated with pro survival functions of insulin (Drapeau et al. 2013; Lizotte et al. 2016).

## 7.3 SHP-1 and Nephrin Tyrosine Phosphorylation

Results from our laboratory demonstrated that overexpression of SHP-1 significantly reduced phosphorylation of tyrosine 1176, 1993 and 1217 residues of nephrin suggesting that SHP-1 could be a negative modulator of nephrin-mediated actin remodeling. Moreover, using cultured human podocytes, our group showed that cells exposed to high levels of glucose significantly exhibited elevated SHP-1 expression, which was associated with reduced nephrin tyrosine phosphorylation. In addition, overexpression of the dominant negative form of SHP-1 prevented high glucose levels-induced inhibition of nephrin phosphorylation in DN (Denhez et al. 2015).

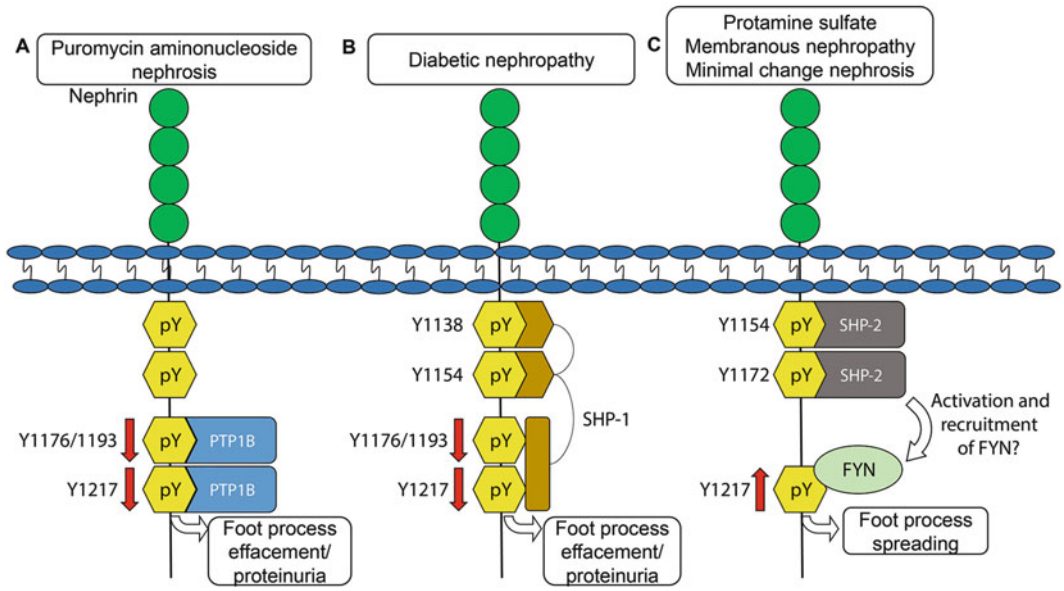


**Fig. 3** Protein tyrosine phosphatase interaction and mechanism of action with nephrin tyrosine phosphorylation

### 7.4 SHP-2

Whereas SHP-1 is considered as a negative regulator of cell proliferation and dephosphorylated receptors of growth factors, SHP-2, on the other hand, is generally viewed as a positive signal transducer. Functional deletion of the *Shp-2* gene in mice induced death of the embryos at mid-gestation (Saxton et al. 1997). Both cells isolated from the SHP-2 null mice and expressing the catalytically inactive cysteine-to-serine mutant of SHP-2 exhibited decreased signal transduction activation upon growth factors and cytokine stimulation (Feng 1999; Neel et al. 2003). A recent study observed an enhanced nephrin phosphorylation activation on tyrosine 1191 and 1208 residues in cells that expressed both the CD16-nephrin chimera and SHP-2 (Verma et al. 2015). The same group also reported that the phosphorylation of tyrosine 542 of SHP-2 is increased in both minimal-change nephrosis and membranous nephropathy.

## 8 The Podocyte and Nephrin Phosphorylation in DN

Multiple studies showed that podocytes are the first cell affected by diabetes and disruption of the structural integrity of the SD could be a potential site that hyperglycemia causes podocyte injury (Ellis et al. 1987; Torbjörnsdotter et al. 2005; Steffes et al. 2001). There are multiple hypotheses to explain the dysfunction of podocytes in DN that contributes to reduced podocyte density in the glomerulus. Interestingly, in both rodent models and human kidney samples, it has been found that nephrin expression is significantly reduced by diabetes (Doublier et al. 2003; Aaltonen et al. 2001). The dysfunction of the podocyte SD in DN raises the question of whether nephrin tyrosine phosphorylation could be modulated. Few studies in the literature evaluated the phosphorylation levels of nephrin in rodent models of diabetes. Of those, opposite results have been reported. One study has observed an increase in tyrosine phosphorylation of nephrin in early DN (Dessapt-Baradez et al. 2014) whereas we and others have demonstrated a decrease in tyrosine phosphorylation levels (Denhez et al. 2015; Batchu et al. 2016; Mima et al. 2012). One possible explanation for these differences could be the model used to study kidney complications in a context of diabetes. The study that observed an increase in nephrin phosphorylation used the streptozotocin-induced diabetic mouse model, which can produce renal morphology variability in response to hyperglycemia (Gurley et al. 2006). Moreover, no difference in the podocyte FP width was shown in streptozotocin mice compared to non-diabetic mice. In contrast, we and other groups found that podocyte cell death and FP effacement occurred in Akita mice (Drapeau et al. 2013; Susztak et al. 2006). Duration of diabetes could be another explanation for these discrepancies. Increase nephrin tyrosine phosphorylation was observed at 13 weeks of diabetes, while our group reported that nephrin tyrosine phosphorylation was decreased after 20 weeks of diabetes. This raises the possibility that the mechanism leading to decreased nephrin tyrosine phosphorylation only occurs after prolonged chronic hyperglycemia.

## 9 Conclusion

The discovery of nephrin greatly improved our understanding of the podocyte function and revealed the central role of this protein in maintaining the structure of the podocyte slit diaphragm. Further studies showed that its intracellular domain is involved in the regulation of multiple signaling pathways that are required to preserve the foot process architecture in mature glomerulus. These findings raised the possibility that the disruption of the homeostasis of nephrin phosphorylation may participate in the pathogenesis and the progression of various kidney diseases. Particularly in DN, podocyte dysfunction could be a critical factor in disease progression. We and others reported that tyrosine phosphorylation of nephrin is reduced by hyperglycemia and diabetes, which may contribute to

DN development and progression. A better understanding of the mechanisms leading to the reduction of nephrin phosphorylation and its consequences in DN may provide new therapeutic strategies to prevent the progression of chronic kidney disease.

**Compliance with Ethical Standards** We do not have any conflicts of interest. Also, there is no animal or human participants in the study.

**Conflicts of Interest** The authors declare that they have no conflicts of interest.

**Ethical Approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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