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PTEN Inhibits High Glucose-Induced Phenotypic Transition in Podocytes

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ABSTRACT

Accumulating evidence has suggested that podocytes undergo epithelial–mesenchymal transition (EMT) in diabetic nephropathy (DN). However, the underlying mechanisms of EMT in podocyte are not well understood. PI3K/Akt pathway is involved in the progression of DN. In the present study, we demonstrated that PI3K/Akt pathway was activated in podocytes exposed to high glucose conditions, accompanied by down-regulation of the podocalyxin (PCX) and nephrin expression and up-regulation of the desmin and α-smooth muscle actin (α-SMA) expression. Inhibition of PI3K/Akt pathway by chemical LY294002 or Phosphase and tensin homology deleted on chromosome ten (PTEN) prevented the phenotypic transition. These findings indicate that PTEN/PI3K/Akt pathway mediates high glucose-induced phenotypic transition in podocytes. J. Cell. Biochem. 116: 1776–1784, 2015.

KEY WORDS: PODOCYTES; PTEN; PHENOTYPIC TRANSITION; HIGH GLUCOSE

Diabetic nephropathy (DN) is one of the frequent microvascular complications of diabetes and is also an important cause of end-stage renal disease. Proteinuria is the most common clinical manifestation of DN and is closely correlated with severity and progression of the disease. The pathological changes of DN include glomerular basement membrane thickening and mesangial proliferation, but these changes do not fully explain the reasons for proteinuria. In recent years, a growing number of studies have found that podocyte injury plays an important role in the pathogenesis of DN and concluded that such injury is a pivotal factor in the induction of proteinuria [Susztak et al., 2006; Eid et al., 2009, 2010; Kim et al., 2012; Weil et al., 2012].

Podocytes are terminally differentiated visceral epithelial cells that are located outside the glomerular capillaries where they constitute a major component of the glomerular filtration barrier [Mundel and Kriz, 1995; Pagtalunan et al., 1997]. Podocyte depletion is an important feature of DN, and a decreased number of podocytes is the strongest predictor of the progression of DN [Meyer et al., 1999; Pagtalunan et al., 1997]. The most plausible explanation for podocyte depletion is detachment from the glomerular basement membrane after cellular apoptosis. However, recent studies have proposed that the detached podocytes might be alive [Mundel, 2003; Appel et al., 2009; Skoberne et al., 2009]. Therefore, some researchers have proposed that injured podocytes might obtain motility that facilitates their detachment from the glomerular basement membrane. In this context, accumulating evidence suggests that podocytes might undergo an epithelial–mesenchymal transition (EMT) process in response to injurious stimuli [Kang et al., 2010; Li et al., 2008]. EMT renders podocytes motile, leading to their detachment from the GBM and thereby resulting in proteinuria. However, the mechanisms that mediate EMT in podocyte remain poorly understood.

Phosphoinositide 3 kinase (PI3K) mediates a diverse group of cellular functions via the activation of protein kinase B, also known as Akt; these functions include cell growth, proliferation, differentiation, apoptosis and migration [Eng, 2003; Saudemont and Colucci, 2009]. However, excessive activation of the PI3K/Akt

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pathway results in functional cell disorder. Phosphatase and tensin homology deleted on chromosome ten (PTEN) has phosphoesterase activity and antagonizes the activity of PI3K by dephosphorylating PIP3 to PIP2; therefore, PTEN is a negative regulator of the PI3K/Akt pathway [Tamguney and Stokoe, 2007]. It has been suggested that PI3K/Akt might mediate EMT in tubular epithelial cells that is induced by high glucose [Lee and Han, 2010]. However, the role of the PI3K/Akt pathway in podocyte injury remains controversial. In this study, we examined the roles of PTEN and the PI3K/Akt pathway in high glucose-induced podocyte phenotype transition.

MATERIALS AND METHODS

CELL CULTURE AND GROUP

Conditionally immortalized mouse podocytes were obtained from the cell resource center at Peking Union Medical College (Beijing, China). The cells were first cultured at 33°C in a humidified atmosphere of 5% CO_2 in DMEM-F12 medium (Gibco PRL, USA) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin and 10 U/ml interferon-γ to promote propagation and were then incubated at 37°C in a 5% CO_2 atmosphere in DMEM-F12 medium containing 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin and were deprived of interferon-γ for 10–14 days to promote differentiation as previously described [Liu et al., 2012]. Podocytes were incubated in serum-free medium for 24 h to synchronize cell growth after 75% confluence was achieved. (1) To investigate the effect of high glucose on podocyte EMT, podocytes were respectively stimulated by normal glucose medium (NG, 5.5 mM), high glucose medium (HG, 15 or 30 mM) and NG plus mannitol medium (9.5 or 24.5 mM) as an osmotic control. The cells were collected at 48 h after the simulation. (2) In order to determine the time-dependent effects of high glucose on the PI3K/Akt pathway, PTEN expression and the phenotypic transition of the podocytes, the cells were stimulated by the application of high glucose for 0, 3, 6, 12, 24, and 48 h. (3) To investigate the role of PI3K/Akt pathway in high glucose-induced podocyte EMT, the cells were pretreated with LY294002 (Promega, Madison, WI). In LY294002-treated group, the cells were randomly divided into the following five groups: NG, HG, and HG plus LY294002. The cells in the high glucose plus LY294002 groups were pretreatment with LY294002 for 30 min. (4) Given that the role of activation of the PI3K/Akt pathway in the insulin signaling, we investigated the effect of insulin on EMT. First, we detected the effect of different concentrations of insulin (1, 10 and 100 nM) on podocyte EMT. Then, LY294002 was used to explore the role of PI3K/Akt in insulin -induced podocyte EMT. At last, the cells were divided into five groups: NG, HG, NG plus insulin, and HG plus insulin to explore the interaction of insulin and high glucose in podocyte EMT.

TRANSIENT TRANSFECTION

(1) In the plasmid transfection experiment, the cells were randomly divided into the following four groups: NG, HG, HG plus control vector (pcDNA3.1-IRES-EGFP)-transfected group, and a high glucose plus the specific PTEN expression vector (pcDNA3.1-PTEN-IRES-EGFP)-transfected group. The vectors were constructed by Beijing Fungenome Technology Company (Beijing, China). Transient transfections were performed with Lipofectamine 2000 (Invitrogen, Carlsbad, CA). Briefly, the podocytes were transfected with 4.0 μg vector DNA with 10 μl Lipofectamine 2000 in 2 ml serum-free DMEM medium. At 6 h after transfection, the medium was replaced with normal DMEM-F12 medium with 10% FBS for 24 h. (2) In the RNA interference experiment, gene silencing of podocytes with PTEN, PI3K and Akt-target siRNA or the negative control siRNA (Santa Cruz, CA) were performed with Lipofectamine 2000 according to the manufacturer's protocol. Then, the cells were cultured for 48 h in high glucose medium to detect the activation of the PI3K/Akt pathway and the phenotypic transition of the podocytes.

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The podocytes were plated on cover slides in six-well plates. The cells were fixed with 75% ethanol for 30 min at room temperature and then permeabilized with 0.1% Triton X-100 at 37°C for 30 min. Next, the cells were incubated with two primary antibodies (PTEN + desmin or PTEN + α-SMA), which are derived from different species, at the same time overnight at 4°C. Monoclonal antibody against PTEN (1:100, Cascade Biosciences, Winchester, IN) is derived from mouse however polyclonal antibodies against desmin (1:100, Santa Cruz Biotechnology, Santa Cruz, CA) and α-SMA (1:250, Beijing Biosynthesis Biotechnology) are derived from rabbit. And on the following day, cells were incubated with FITC-conjugated goat anti-rabbit IgG and TRITC-goat anti-mouse IgG (1:100, Beijing Zhongshan Golden Bridge Biotechnology, Beijing, China) simultaneously at 37°C for 2 h. The slides were observed by fluorescence microscope (Olympus, Tokyo, Japan). Negative controls were obtained by replacing the specific antibodies with PBS.

WESTERN blot

Proteins extracted from the podocytes were separated on 10% SDS–PAGE gel and then transferred onto PVDF membranes. The membranes were blocked for 2 h at 37°C with 5% BSA and then incubated overnight at 4°C with primary antibodies against p-Akt (ser473, 1:100, Cell Signaling Technology, Beverly, MA), Akt (1:100, Cell Signaling Technology), PTEN (1:100, Cascade Biosciences), desmin (1:800), PCX (1:800, R&D Systems, Minneapolis, MN), nephrin (1:250, Beijing Biosynthesis Biotechnology, Beijing, China), α-SMA (1:250), β-actin (1:1000, Beijing Biosynthesis Biotechnology) and Histon-H1 (1:200, Santa Cruz Biotechnology). Subsequently, the membranes were washed with Tris-buffered saline containing 0.05% Tween 20 (TBST) and incubated with goat anti-rabbit IgG horseradish peroxidase-conjugated secondary antibodies. The protein expression levels were quantified using LabWorks4.5 software. The expression of p-Akt was normalized to that of Histon-H1, and the other proteins were normalized to β-actin.

PTEN ACTIVITY ASSAY

The supernatants were collected after the cells treated with different experimental conditions. The enzymatic activity of PTEN was measured using malachite green colorimetric assay. PTEN activity assay kit was purchased from Nanjing Senbeijia Biotechnology Company (Nanjing, China).

STATISTICAL ANALYSIS

The data were expressed as the mean ± SD and analyzed with one-way ANOVA. Student–Newman–Keuls test was used to determine statistically significant differences within and between groups. Statistical significance was set at $P < 0.05$.

RESULTS

INDUCTION OF PODOCYTE PHENOTYPE TRANSITION BY HIGH GLUCOSE

Compared with normal glucose-treated cells, high glucose-treated podocytes showed a significant down-regulation of PCX and nephrin and up-regulation of α-SMA and desmin. There was no difference in these proteins between normal glucose medium-cultured cells and normal glucose medium plus mannitol-cultured cells (Fig. 1). Time-kinetics experiment indicated that in the high glucose conditions, PCX and nephrin expression gradually decreased, while α-SMA and desmin expression gradually increased in a time-dependent manner (Fig. 2).

ROLE OF PI3K/AKT PATHWAY IN THE PODOCYTE PHENOTYPE TRANSITION INDUCED BY HIGH GLUCOSE

The Western blots revealed that in response to high glucose, the expression of p-Akt in podocytes was increased. The maximum expression of p-Akt occurred at 24 h after high glucose stimulation, and expression subsequently declined (Fig. 3a,b). The expression of the PTEN protein increased following high glucose stimulation, peaked at 3 h, and gradually decreased with prolonged stimulation

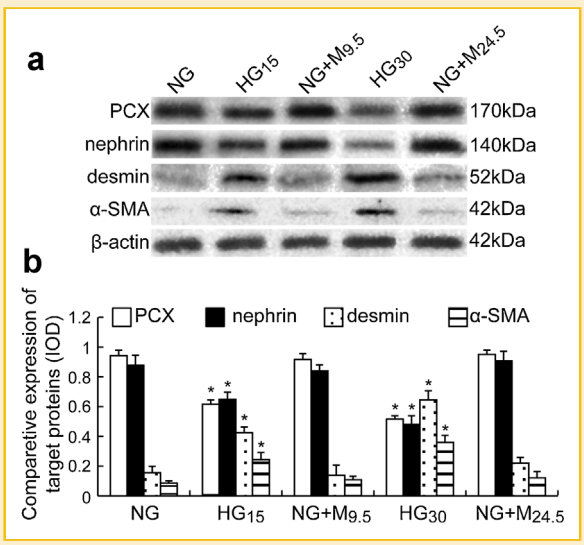

Fig. 1. High glucose induced decreases in podocalyxin (PCX) and nephrin protein levels and increases in desmin and α-smooth muscle actin (α-SMA) protein levels. a: Western blot analyses of the effects of high glucose on PCX, nephrin, desmin and α-SMA protein in podocytes. b: The levels of PCX, nephrin, desmin and α-SMA were quantified by densitometry (mean ± SD, n = 6). NG: Normal glucose (5.5 mM). HG15: High glucose (15 mM). NG + M9.5: Normal glucose plus mannitol (9.5 mM). HG30: High glucose (30 mM). NG + M24.5: Normal glucose plus mannitol (24.5 mM). $^*P < 0.05$ versus NG.

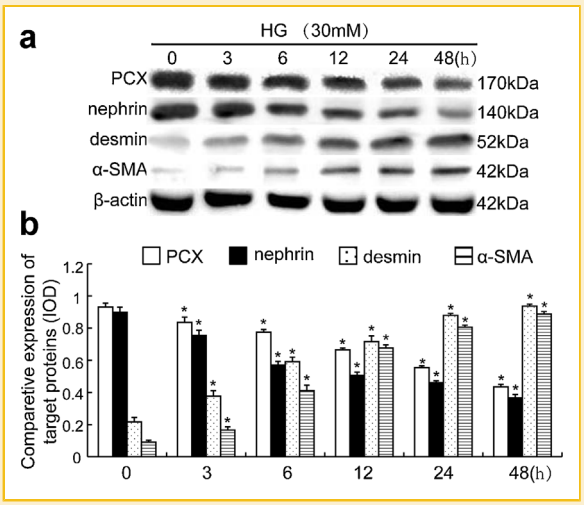

Fig. 2. Time course of the effects of HG on the expression of PCX, nephrin, desmin and α-SMA. The podocytes were incubated with HG (30 mM) for the indicated times (0–48 h). a: Western blot analyses of the time-dependent effects of HG on PCX, nephrin, desmin and α-SMA protein in podocytes. b: The levels of PCX, nephrin, desmin and α-SMA were quantified by densitometry (mean ± SD, n = 6). $^*P < 0.05$ versus control (0 h).

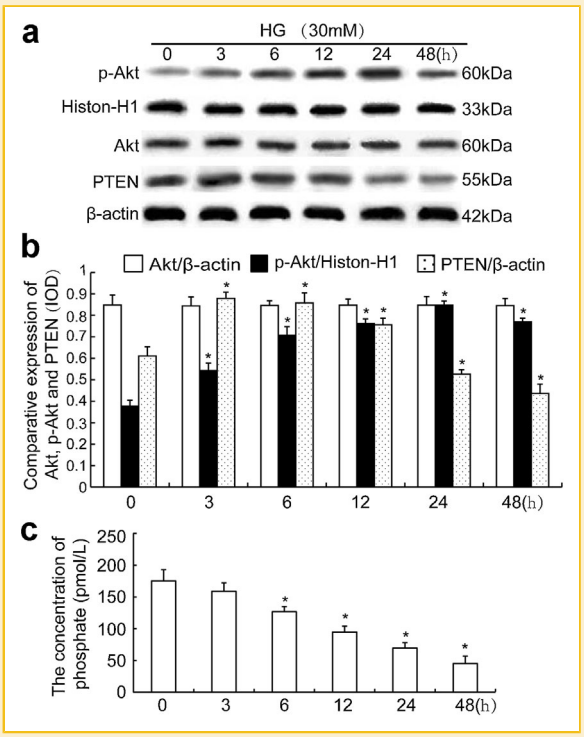

Fig. 3. Time courses of total Akt, p-Akt, and PTEN expression following the stimulation of podocytes with HG (30 mM). a,b: The expression of Akt, p-Akt, and PTEN was analyzed by Western blot assay and quantified by densitometry (mean ± SD, n = 6). c: Phosphate concentration released from PTEN-specific substrate was assessed using green reagent method. $^*P < 0.05$ versus control (0 h).

(Fig. 3a,b). Additionally, the PTEN activity was also decreased with the high glucose treatment (Fig. 3c).

To detect the effects of activation of the PI3K/Akt pathway on the podocyte phenotype transition, we added LY294002 (a classic inhibitor of the PI3K/Akt pathway) or transfected PI3K and Akt siRNA before high glucose stimulation. The podocytes were collected after stimulation with high glucose to detect the total Akt, p-Akt and phenotype transition-related proteins. The results revealed that the expressions of p-Akt, α-SMA and desmin decreased, while those of PCX and nephrin increased when LY294002 was introduced into the media (Fig. 4). Similar results were acquired when the cells were transfected with PI3K and Akt siRNA (Fig. 5). There was no effect of PI3K or Akt siRNA transfection on the expression of phenotype transition related-proteins in normal glucose-cultured podocytes. Moreover, the effects of LY294002 on the expression of p-Akt and the phenotype transition-related proteins were concentration dependent.

ROLE OF INSULIN IN THE PODOCYTE PHENOTYPE TRANSITION

Podocytes were treated with insulin at the concentration of 10 or 100 nM showed an up-regulation of desmin and α-SMA and down-regulation of PCX and nephrin. However, insulin at the concentration of 1 nM, which increased the expression of p-Akt more significantly, had no effect on the expression of phenotype

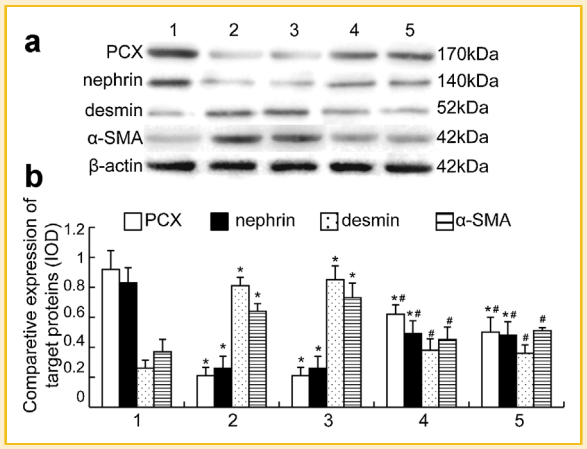

Fig. 5. The effect of PI3K or Akt knockdown on the expression of phenotype transition-related proteins in HG-treated podocytes. a: The expression of PCX, nephrin, desmin and α-SMA was analyzed by Western blot assay. b: The levels of PCX, nephrin, desmin and α-SMA were quantified by densitometry (mean ± SD, n = 6). (1) NG. (2) HG (30 mM). (3) HG + control siRNA. (4) HG + PI3K siRNA. (5) HG + Akt siRAN. $^{*}P < 0.05$ versus NG. $^{\#}P < 0.05$ versus HG + control siRNA.

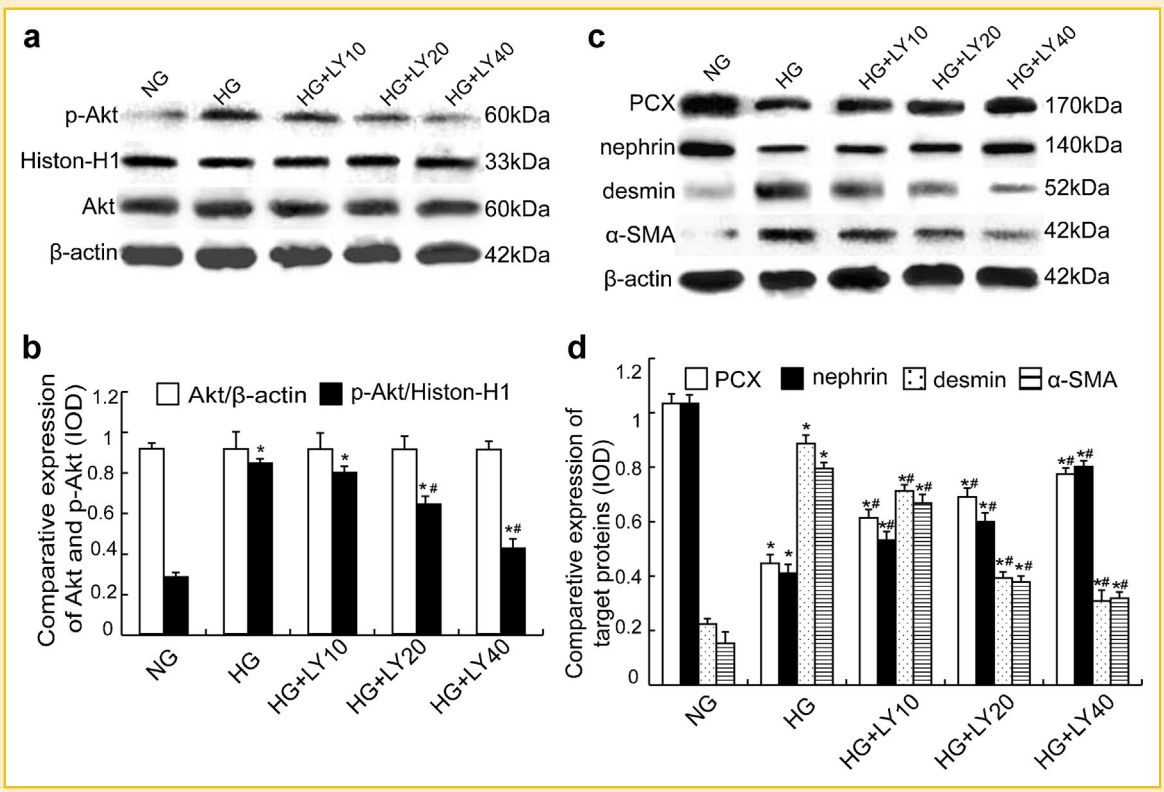

Fig. 4. LY294002 inhibits high glucose-induced expression of p-Akt and phenotype transition related-proteins. a,b: The expression of Akt and p-Akt was determined by Western blot assay and quantified by densitometry (mean ± SD, n = 6). c,d: The expression of the phenotype transition-related proteins was determined by Western blot assay and quantified by densitometry (mean ± SD, n = 6). NG: Normal glucose. HG: High glucose (30 mM). HG + LY10: HG plus LY294002 (10 μM). HG + LY20: HG plus LY294002 (20 μM). HG + LY40: HG plus LY294002 (40 μM). $^{*}P < 0.05$ versus NG. $^{\#}P < 0.05$ versus HG.

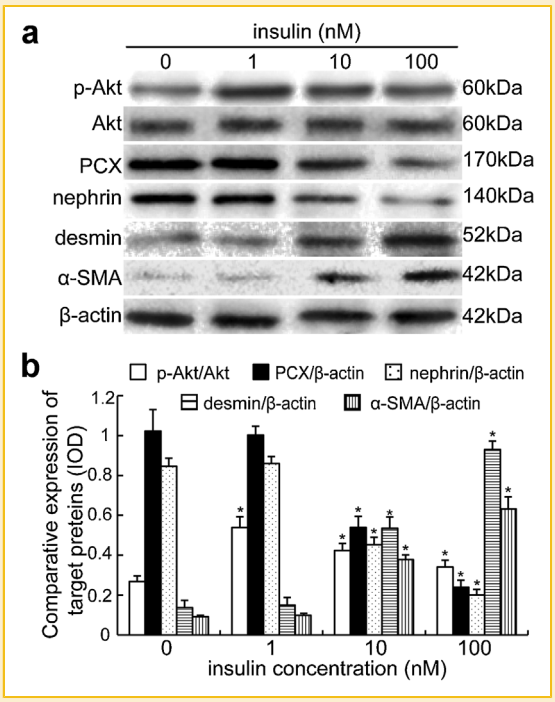

Fig. 6. The effects of insulin on the expression of p-Akt and phenotype transition related-proteins. The podocytes were incubated with different concentration of insulin (0, 1, 10, 100 nM). a: Western blot analyses of the effects of insulin on p-Akt, PCX, nephrin, desmin and α-SMA protein in podocytes. b: The levels of p-Akt, PCX, nephrin, desmin and α-SMA were quantified by densitometry (mean ± SD, n = 6). $^{*}P < 0.05$ versus 0 nM-insulin.

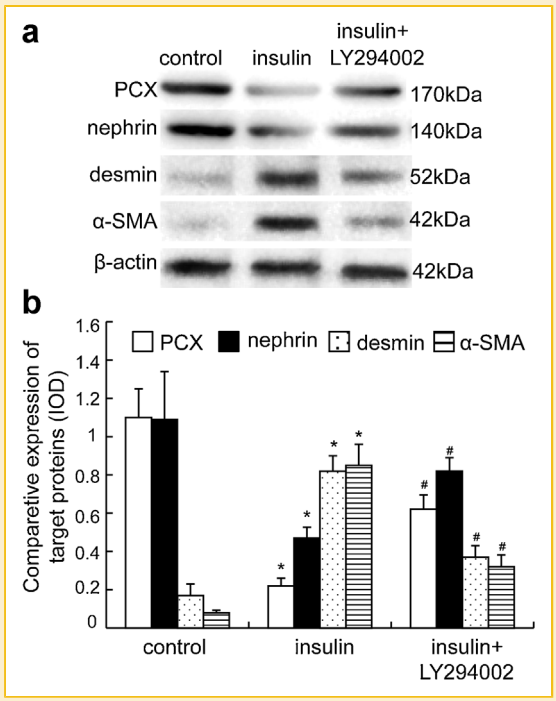

Fig. 7. LY294002 inhibits insulin (100 nM)-induced expression of phenotype transition related-proteins. a: The expression of PCX, nephrin, desmin and α-SMA was determined by Western blot assay. b: The levels of PCX, nephrin, desmin and α-SMA were quantified by densitometry (mean ± SD, n = 6). $^{*}P < 0.05$ versus control. $^{\#}P < 0.05$ versus insulin.

transition related-proteins (Fig. 6). Pretreatment with LY294002 could partially inhibit podocyte EMT induced by high insulin (100 nM) (Fig. 7).

THE INTERACTION OF INSULIN AND HIGH GLUCOSE IN PODOCYTE EMT

High glucose or insulin stimulation alone increased p-Akt, desmin and α-SMA and decreased PCX and nephrin in podocytes. However, the expression of these proteins was reversed when podocytes were treated with high glucose and insulin simultaneously (Fig. 8).

PTEN INHIBITED THE PHENOTYPIC TRANSITION OF PODOCYTES THAT WERE INDUCED BY HIGH GLUCOSE, AND THIS INHIBITION WAS ACCOMPANIED BY A DECREASE IN p-AKT

To investigate the role of PTEN in podocyte EMT, the cells were transfected with PTEN siRNA. The results showed that PTEN knockdown could induce the expression of desmin and α-SMA, while inhibit PCX and nephrin expression in podocytes (Fig. 9). To investigate the involvement of PTEN in high glucose-induced podocyte EMT, the cells were transfected with a specific PTEN vector. Transfection efficiency was defined by the percentage of EGFP-positive cells and the evidence was displayed in Figure 10a. Normal glucose medium-cultured podocytes exhibit baseline expression levels of PTEN and p-Akt. Notable decreases in the expression of

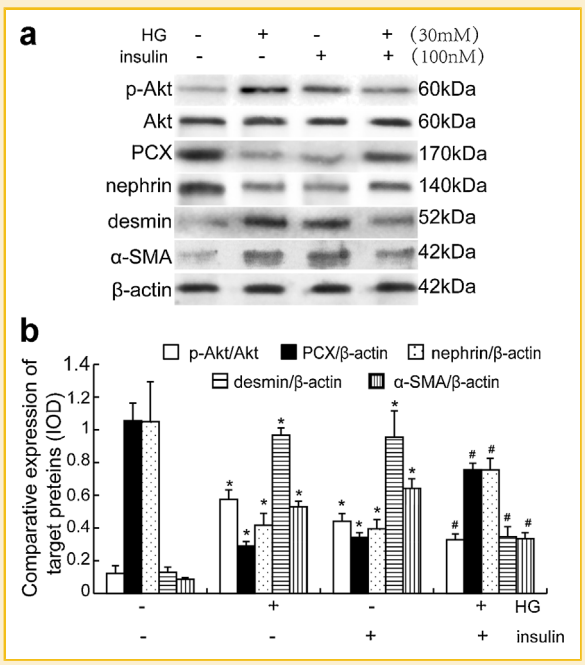

Fig. 8. The expression of p-Akt and phenotype transition-related proteins in the podocytes stimulated by HG and insulin. a: The expression of p-Akt, PCX, nephrin, desmin and α-SMA was analyzed by Western blot assay. b: The levels of p-Akt, PCX, nephrin, desmin and α-SMA were quantified by densitometry (mean ± SD, n = 6). $^{*}P < 0.05$ versus control. $^{\#}P < 0.05$ versus HG or insulin.

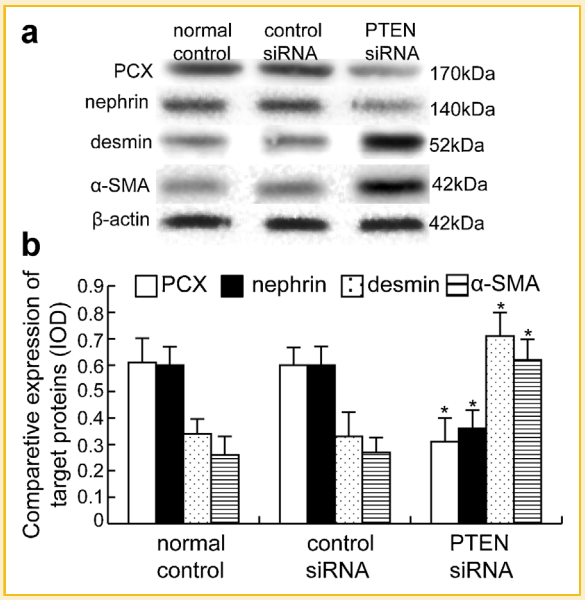

Fig. 9. The expression of phenotype transition-related proteins in the podocytes transfected with PTEN siRNA. Podocytes were cultured in normal glucose media. a: The expression of PCX, nephrin, desmin and α-SMA was analyzed by Western blot assay. b: The levels of PCX, nephrin, desmin and α-SMA were quantified by densitometry (mean ± SD, n = 6). $^{*}P < 0.05$ versus control siRNA.

PTEN and enhancements in the expression of p-Akt were seen in the untransfected cells that were stimulated with high glucose and the cells that were transfected with control vector exhibited. However, the podocytes that were transfected with specific PTEN vector up-regulated PTEN protein level (Fig. 10c,d) and activity (Fig. 10b), which was followed by a decrease in p-Akt (Fig. 10c,d). Additionally, the overexpression of PTEN significantly suppressed the synthesis of α-SMA and desmin and reversed the expression of PCX and nephrin that were induced by high glucose (Fig. 11).

DISCUSSION

Podocytes injury has been shown to play an important role in the proteinuria in DN. It is considered that podocyte depletion attributing to apoptosis is the initial cause in the genesis of proteinuria. However, resent studies have shown that the podocytes number had no significant change when there was an evident microalbuminuria in diabetic rats [Dai et al., 2004]. Additionally, podocytes detachment and apoptosis obviously lag behind the onset of proteinuria [Dai et al., 2006; El-Aouni et al., 2006]. Therefore, we can speculate that podocyte detachment and apoptosis may be the reason of macroalbuminuria in the advanced stage of chronic kidney disease. The early podocytes insult, which led to the onset of proteinuria, is uncertain. In this context, emergency evidence

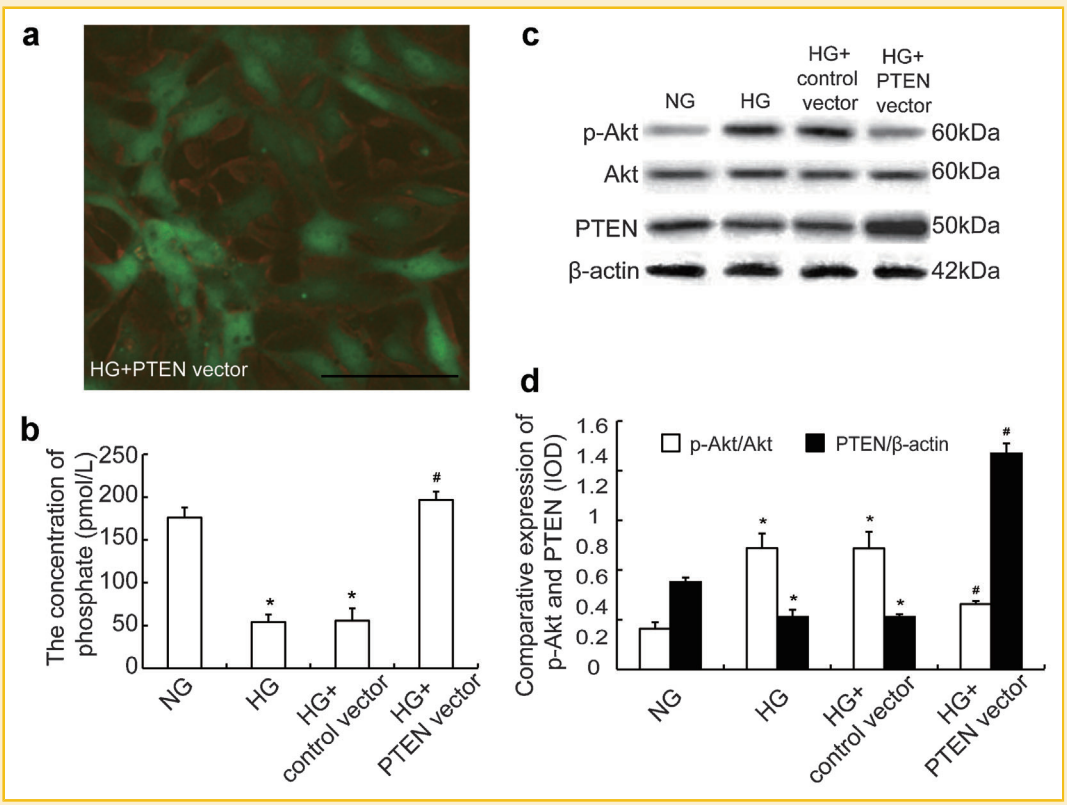

Fig. 10. The expression of PTEN and p-Akt in the specific PTEN vector-transfected podocytes. a: The evidence of transfection efficiency. b: Phosphate concentration released from PTEN-specific substrate was assessed using green reagent method. c,d: The expression of p-Akt and PTEN was analyzed by Western blot assay and the levels were quantified by densitometry (mean ± SD, n = 6). $^{*}P < 0.05$ versus NG. $^{\#}P < 0.05$ versus HG + control vector.

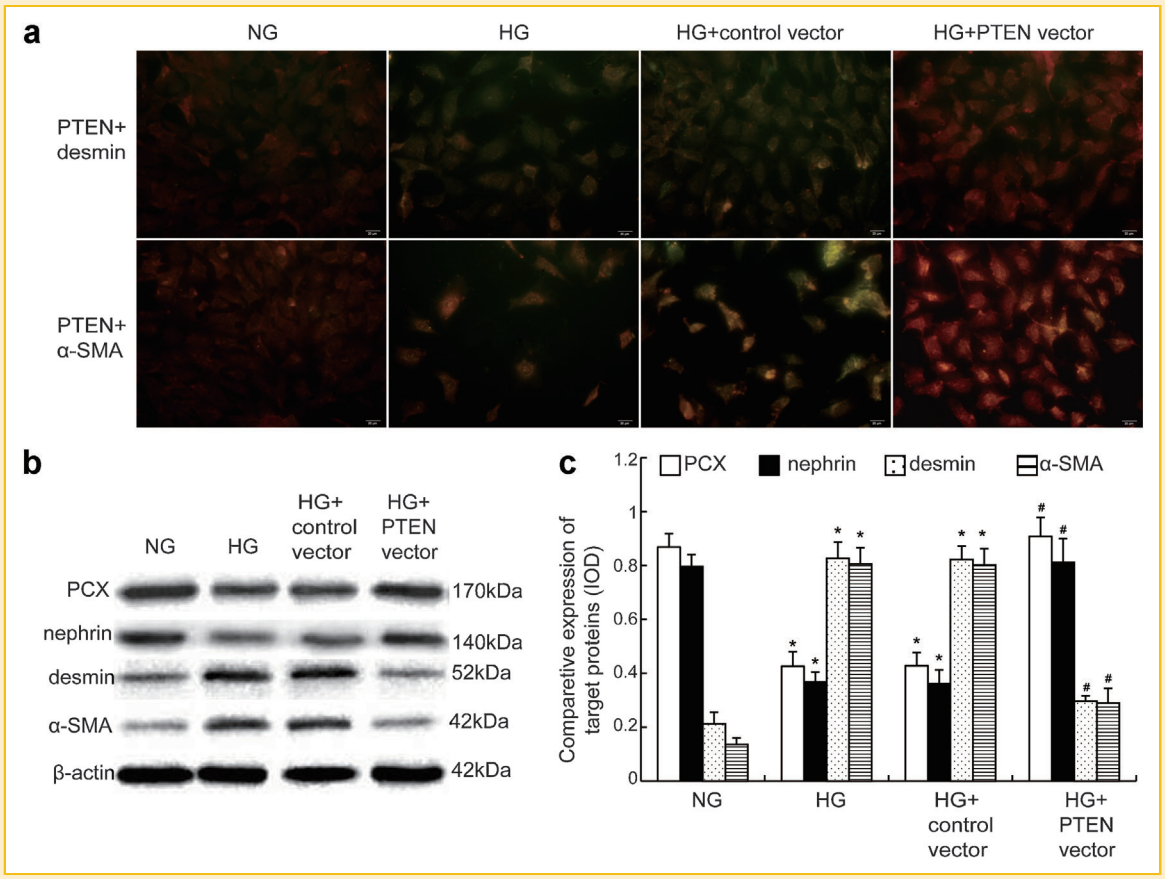

Fig. 11. The expression of the phenotype transition-related proteins in the specific PTEN vector-transfected podocytes. a: Double staining of PTEN and desmin or PTEN and α-SMA by immunofluorescence. Red fluorescence represent PTEN and green fluorescence represent desmin or α-SMA. b: The expression of PCX, nephrin, desmin and α-SMA was analyzed by Western blot assay. c: The levels of PCX, nephrin, desmin and α-SMA were quantified by densitometry (mean ± SD, n = 6). $^{*}P < 0.05$ versus NG. $^{\#}P < 0.05$ versus HG + control vector.

indicated that when exposed to specific pathological conditions, podocytes could undergo an EMT process, which may explain the genesis of proteinuria [Li et al., 2008; Herman-Edelstein et al., 2011; Wang et al., 2011; Lv et al., 2013]. In the literature, the phenotypic and morphological changes of diseased podocytes were considered as podocyte EMT. However, the concept of podocyte EMT remains ambiguous. Podocytes are not typical epithelial cells and they retain several mesenchymal features. More importantly, podocytes could only undergo a tendency of EMT after injury, and they fails to demonstrate the emblematic features of EMT. Therefore, some scholars suggest that the term podocyte disease transformation (PDT) is more appropriate than podocyte EMT [May et al., 2014]. Despite the dispute, the term podocyte EMT is still widely used than PDT. Furthermore, recent studies have proposed that EMT is engaged in podocyte depletion in DN [Yamaguchi et al., 2009].

To verify the phenotypic changes in podocytes following high glucose stimulation, we examined the expression of nephrin, PCX, desmin and α-SMA in podocytes. The results suggest that podocytes cease to express their own marker proteins and acquire the expression profile of myofibroblasts. The results of Mannitol control experiment showed that the osmotic pressure did not affect the podocyte phenotype, suggesting that podocyte EMT was resulted from high glucose itself rather than caused by osmotic pressure. However, in this study, there was no obvious morphological change associated with EMT in podocytes stimulated by high glucose. We speculate that the podocyte did not undergo a complete phenotypic transition, which results in the cell resembling typical fibroblasts.

To explore the mechanisms by which the phenotypic changes of podocytes were induced by high glucose, we investigated the role of the PI3K/Akt pathway in podocyte EMT. First, we confirmed that the PI3K/Akt signaling pathway is activated in podocytes following high glucose stimulation. The maximum expression of p-Akt appeared in podocyte incubated with high glucose for 24 h, and then the expression declined. However, there were different reports about the effect of high glucose on PI3K/Akt pathway [Drapeau et al., 2013; Rogacka et al., 2014]. We speculate that the effect of high glucose on the activation of PI3K/Akt pathway may be undulate. Such discrepancy might be originated from the high glucose stimulation time as it is different in these studies. Secondly, we blocked this pathway with LY294002 and found that LY294002 could inhibit the phenotypic changes of the podocytes. The effects of PI3K and Akt knockdown on the expression of PCX, nephrin, desmin and α-SMA

further confirmed the role of PI3K/Akt pathway activation in podocyte EMT. All of these results showed that high glucose induce podocyte EMT via the activation of the PI3K/Akt pathway. In addition, the expression level of p-Akt decreased, while the changes of phenotype transition-related protein were exacerbated in podocytes treated with high glucose for 48 h. It suggested that PI3K/Akt signaling pathway involved in the origination of podocyte EMT. Once obtaining the new phenotype, the cells will maintain this phenotype, which no longer depend on the activation of PI3K/Akt pathway.

Given that the PI3K/Akt pathway is one of the important signaling pathways in the insulin signaling, we also investigated the effect of insulin on podocyte EMT. The results showed that insulin activated the PI3K/Akt pathway, but the expression of p-Akt decreased along with the increase of insulin concentration. However, podocyte EMT could be induced by high-dose insulin (10 or 100 nM) rather than low-dose insulin (1 nM). Pretreatment with LY294002 verified that the PI3K/Akt pathway also mediates podocyte EMT induced by high insulin. But the activation of the PI3K/Akt pathway only involves in the podocyte EMT induced by pathological factor rather than physiological factor. Further, we detected the expression of phenotype transition related-proteins and p-Akt in podocytes stimulated by high glucose and insulin simultaneously. The results showed that high glucose and insulin play an antagonistic role in the activation of PI3K/Akt pathway and podocyte EMT. The effects of high glucose or insulin on p-Akt are dual. Both of them induce Tyr phosphorylation of insulin receptor substrate-1 (IRS-1), which activated the PI3K/Akt pathway. Meanwhile, they also induce Ser phosphorylation of IRS-1, which inhibit the expression of p-Akt [Tanti et al., 1994; Nikoulina et al., 2000]. Ser phosphorylation of IRS-1 was mainly induced by insulin-treatment for a long time [Dhand et al., 1994]. We speculated that in this study, high glucose or insulin stimulation alone activated the PI3K/Akt pathway via the Tyr phosphorylation of IRS-1. However, co-treatment with high glucose and insulin maybe down-regulate p-Akt expression via Ser phosphorylation of IRS-1. The different effects of high glucose and insulin on phosphorylation of IRS-1 maybe explain the antagonistic role of high glucose and insulin. Further investigation is needed to identify the mechanism of it.

Because the activation of PI3K/Akt signaling is required in podocyte EMT induced by high glucose, we reasoned that the negative regulation of this pathway should inhibit EMT. Phosphatase and tensin homology deleted on chromosome ten (PTEN) is a tumor-suppressing dual phosphatase that antagonizes the function of PI3K and negatively regulates AKT activity. In the present study, PTEN expression increased at 3–12 h after high glucose stimulation. Combined with the results of Drapeau and Rogacka [Drapeau et al., 2013; Rogacka et al., 2014], we speculate that the effect of high glucose on PTEN was instable. The expression of PTEN could be induced by high glucose for a short-term stimulus. However, with prolonged stimulation, the expression level of PTEN decreased. It is consistent with previous study, which reported that there was significant reduction in PTEN expression in the streptozotocin-induced diabetic glomeruli [Mahimainathan et al., 2006]. In addition, high glucose could also inhibit the phosphatase activity of PTEN. Therefore, we hypothesize that the down-regulation of PTEN activity led to the sustained activation of the PI3K/Akt pathway and ultimately induced podocyte EMT. Moreover, Wang's findings that PRL-3 down-regulates PTEN expression and signals through PI3K to promote EMT [Wang et al., 2007] increase the plausibility of this hypothesis. To verify the hypothesis, firstly, we confirmed that PTEN deprived podocyte itself might undergo EMT by PTEN knockdown. After that, the cells were transfected with PTEN vector and results suggested that overexpression of PTEN inhibited the podocyte EMT that was induced by high glucose and subsequently decreased the phosphorylation of Akt. In summary, our results indicate that the inhibitory effects of PTEN on high glucose-induced podocyte EMT, which might be mediated via the inhibition of the PI3K/Akt pathway.

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