Interplay between the Notch and PI3K/Akt pathways in high glucose-induced podocyte apoptosis

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Submitted 4 January 2013; accepted in final form 16 October 2013

Wang X, Yao M, Liu S, Hao J, Liu Q, Gao F. Interplay between the Notch and PI3K/Akt pathways in high glucose-induced podocyte apoptosis. Am J Physiol Renal Physiol 306: F205–F213, 2014. First published November 13, 2013; doi:10.1152/ajprenal.90005.2013.—Podocyte apoptosis contributes to the pathogenesis of diabetic nephropathy (DN). However, the mechanisms that mediate high glucose (HG)-induced podocyte apoptosis remain poorly understood. Conditionally immortalized mouse podocytes were cultured in HG medium. A chemical inhibitor or a specific short-hairpin RNA (shRNA) vector was used to inhibit the activation of the Notch pathway and the PI3K/Akt pathway in HG-treated podocytes. Western blotting and real-time PCR were used to evaluate the levels of Notch, PI3K/Akt, and apoptotic pathway signaling. The apoptosis rate of HG-treated podocytes was assessed by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling and annexin V/propidium iodide staining. In HG-treated podocytes, PI3K/Akt pathway activation prevented podocyte apoptosis in the early stage of HG stimulation and Notch pathway-induced podocyte apoptosis in the late stage of HG stimulation. The inhibition of the Notch pathway or the activation of the PI3K/Akt pathway prevented cell apoptosis in HG-treated podocytes. These findings suggest that the Notch and PI3K/Akt pathways may mediate HG-induced podocyte apoptosis.

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Diabetic nephropathy (DN) is one of the most common complications of diabetes and has become the most common cause of end-stage renal disease. The pathogenesis of DN is notably complicated. It is believed that the apoptosis of podocytes, a type of glomerular epithelial cell, can lead to the development of DN (3, 26). Because podocytes are unable to divide, apoptosis of these cells will result in proteinuria, the accumulation of extracellular matrix components, and glomerulosclerosis.

Several previous studies have reported that high glucose (HG), an underlying factor that affects the physical metabolism and function of normal cells in diabetic patients, can induce apoptosis in renal mesangial cells and tubular cells (6, 14, 17). However, the mechanisms that contribute to hyperglycemia-induced podocyte apoptosis are not completely understood. Recently, several studies have determined that certain signaling pathways are related to podocyte apoptosis. Notch family pathways play an important role in cell differentiation, acting primarily to determine and regulate cell survival (5, 12, 23). In mammals, there are four Notch receptors (Notch1–Notch4).

The ligand is present on the surface of cells, and the corresponding Notch receptor exists on the surface of the adjacent cells. The binding of ligand and receptor induces a conformational change in the Notch receptor. This binding allows an extracellular metalloprotease to cleave the receptor, which subsequently allows the γ-secretase-mediated protease to release the Notch intracellular domain (NICD). Next, the NICD travels into the nucleus, where it activates the transcription of downstream genes (22). Notch and its ligand are direct transcriptional targets of HG (18). Niranjan et al. (25) found that Notch1 and Notch2 are activated in the podocytes of diabetic patients and experimental animal models; however, Notch3 and Notch4 expression was unaltered. In cultured podocytes, HG alone activated Notch1 along with VEGF overexpression, nephrin repression, and apoptosis (18).

The phosphatidylinositol 3-kinases (PI3K) have been linked to an extraordinarily diverse group of cellular functions via the activation of protein kinase B, also known as Akt. These cellular functions include, for example, cell growth, proliferation, differentiation, motility, and survival (29). A variety of reports have demonstrated in various cell types that the constitutive activation of Akt signaling is sufficient to block cell death induced by a variety of apoptotic stimuli and that the transduction of dominant-negative Akt inhibits growth factor-induced cell survival (21, 30). The downregulation of Akt activation during long-term hyperglycemia contributes to enhanced p38 MAPK activation and renal proximal tubular cell apoptosis (28). In PAN nephropathy rats, 1,25(OH)2D3 significantly prevented the loss of nephrin, foot process retraction, and podocyte apoptosis by stimulating Akt phosphorylation and suppressing the transforming growth factor (TGF)-β/Smad-signaling pathway (33). Several researchers reported that the relationship between the Notch and PI3K/Akt pathways is relevant to the regulation of cell apoptosis in breast and colon cancer (7, 8, 16). However, the role of Notch and PI3K/Akt pathways in podocyte apoptosis during DN is unknown.

In this study, we hypothesize that HG activates apoptotic genes and induces podocyte apoptosis via the Notch and PI3K/Akt pathways. To test this hypothesis, HG-induced mouse podocytes were chosen to detect Notch1, NIDC1, Akt, phospho-Akt, and cellular apoptosis. Considering the relationship between the Notch pathway and the PI3K/Akt pathway, we suppressed the Notch or PI3K/Akt pathway using a chemical inhibitor or a short-hairpin (sh) RNA vector to investigate their effect on apoptosis in HG-treated podocytes at various time points. In addition, we further explored the mechanism of podocyte apoptosis.
MATERIALS AND METHODS

Cell culture and treatment groups. Conditionally immortalized mouse podocyte cells were obtained from the cell resource center at Peking Union Medical College (Beijing). Cells were firstly cultured in RPMI 1640 (GIBCO, BRL) medium supplemented with 10% FBS and 10 U/ml γ-interferon (Peprotech) at 33°C in a 5% CO2 atmosphere to induce quiescence and were incubated in RPMI 1640 medium supplemented with 10% FBS and deprived of γ-interferon at 37°C and a 5% CO2 atmosphere for 10–14 days to induce quiescence and the differentiated phenotype, as previously described (19). Podocytes were grown to 75–85% confluence and growth-arrested in serum-free RPMI 1640 for 24 h to synchronize cell growth. After this time period, the media were changed to fresh serum-free media containing normal glucose (NG; 5.5 mmol/l), NG plus mannitol (24.4 mmol/l), and HG (30 mmol/l) at the indicated time points. Transient transfections of podocytes with the sh-Notch1 vector, the negative control sh-Scramble vector (Jingsai, Wuhan, China), and the sh-Akt vector (generously provided by Dr. Myung-Haing Cho, Seoul National University, Seoul, Korea) were performed with Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions, after the cells were differentiated in a 37°C atmosphere. GSI (Sigma, St. Louis, MO), a Notch pathway inhibitor, inhibits γ-secretase-mediated proteolytic cleavage of Notch, which, in turn, reduces the release of the NICD from the plasma membrane and its subsequent translocation into the nucleus (22). LY294002 (Promega, Madison, WI) is a chemical inhibitor of the PI3K/Akt pathway that can inhibit the phosphorylation of Akt (10). Cells in the HG plus GSI group and the HG plus LY294002 group required pretreatment with GSI (1 μmol/l) or LY294002 (20 μmol/l) for 30 min.

Western blot analysis. Whole cells were harvested by scraping the culture dishes in lysis buffer. Nuclear proteins were extracted using a nuclear and cytoplasm protein extraction kit (KeyGen Biotech, Nanjing, China). The protein concentration was assessed using Coomassie blue reagent (Sigma-Aldrich). A total of 40 μg of protein/lane was loaded and separated by electrophoresis and then transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5% dry milk and incubated overnight at 4°C with rabbit anti-Notch1 (1:200; Abcam, Cambridge, MA); NICD1 (1:200; Abcam); Akt (1:1,000; Epitomics, Burlingame, CA); p-Akt (Ser473; 1:1,000; Epitomics); Bas (1:200; Proteintech, Chicago, IL); Bcl-2 (1:1,000; Proteintech); p-p53 (Ser37; 1:2,000; Signalway, Pearlrand, TX); p53 (1:500; Proteintech); NF-κB (p65; 1:400; Proteintech); β-actin (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA); and histone H1 (1:200, Santa Cruz Biotechnology) polyclonal antibodies. After washing, the membrane was incubated with a goat anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody. Proteins in the Western blot were quantified following the acquisition and analysis of the image using UVP Image Station Lab Works software, version 4.5. Protein expression was quantified by comparison with one of the internal controls, β-actin, or histone H1.

Real-time PCR. Total RNA was extracted from podocytes, and complementary DNA was synthesized through a reverse transcription reaction. Real-time PCR amplification was performed on an ABI 7500 real-time PCR system with the following conditions: 95°C for 30 s and 40 cycles of amplification (95°C for 5 s, 60°C for 30 s). The nucleotide sequences of the primers were as follows: Notch1, 5'-GTA TGC AGG TAA ACC CAA ACG A-3' and 5'-GCA TCC TCC TTG TTT ACG A-3'; Akt, 5'-TGC ATT GCC GAG TGA AAT TC-3' and 5'-GCA TCC GAG AAA CAA AAC ATC A-3'; and 18S, 5'-CGC TAG AGG TGA TTA CTG AAT TC-3' and 5'-CCA GTG GAC ACG GTT TAC GAT G-3'. The results were analyzed using the relative standard curve method of analysis/ΔCt method of analysis, and 18S rRNA was used as the internal control.

Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling. Slides were immersed in 4% formaldehyde in PBS at 4°C and in 0.2% Triton X-100 in PBS for 10 min at room temperature. Equilibration buffer (100 μl) was then added at room temperature for 10 min followed by the addition of 50 μl of TdT reaction mix to the cells for 60 min at 37°C. The slides were then immersed in 2× SSC for 15 min. Propidium iodide (PI) was used to stain all cells. Apoptotic cells were detected as localized green fluorescence in a red background by fluorescence microscopy. For the quantification of

Fig. 1. Time course of Notch1 intracellular domain (NICD1), Notch1, p-Akt, and Akt expression upon stimulation by high glucose (HG) in podocytes. Podocytes were incubated with HG (30 mM) at the indicated times (0–48 h). A: expression of NICD1, Notch1, p-Akt, and Akt was analyzed by Western blot analysis. B: levels of NICD1, Notch1, p-Akt, and Akt were quantified by densitometry. C: mRNA levels of Notch1 and Akt were analyzed by real-time PCR. HG augmented the expression of NICD1 and p-Akt in a time-dependent manner and reached their peaks at 48 and 24 h, respectively. Values are means ± SD (n = 6). **p < 0.01 vs. control (0 h).

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terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling ([TUNEL]-positive; apoptotic) of cells, a minimum of 200 cells were counted per group, and the percentage of the positively labeled cells was calculated.

**Annexin V/PI staining assay.** The number of apoptotic cells in the different groups was determined using an annexin V/PI apoptosis detection kit according to the manufacturer’s protocol (MultiSciences Biotech, Hangzhou, China). Briefly, the cell pellet was resuspended in 1× binding buffer followed by incubation with 5 ml of annexin V (conjugated with FITC) and 10 ml of PI in the dark for 5 min. Cell fluorescence was then analyzed using a flow cytometer (Epics-XLII, Beckman Coulter). This test discriminates between intact cells (annexin V−/PI−), early apoptotic cells (annexin V+/PI−), and late apoptotic cells (annexin V+/PI+).

**Statistical analysis.** Data presented in bar graphs are means ± SD of at least three independent experiments. Statistical analysis was performed by one-way ANOVA. The results were considered to be statistically significant at **P** < 0.05.

**RESULTS**

HG upregulates the expression of NICD1 and phospho-Akt in podocytes. To determine the effect of HG on the activation of the Notch and PI3K/Akt pathways in podocytes, we examined NICD1, Notch1, total Akt, and phospho-Akt protein expression by Western blot analysis (Fig. 1, A and B). The expression of NICD1 and Notch1 protein began to increase at 6 h after stimulation with HG and reached their peak at 48 h (**P** < 0.01). HG augmented the expression of phospho-Akt in a time-dependent manner. The maximum expression of phospho-Akt was as early as 24 h, and then the expression declined (**P** < 0.01). However, there was no difference in total Akt expression among all the different time points in HG-induced podocytes (**P** > 0.05). The HG-induced time-dependent mRNA level of Notch1 was evaluated by real-time PCR analysis (**P** < 0.01) (Fig. 1C). There was no difference in Akt mRNA expression among all the time points in HG-induced podocytes (**P** > 0.05). In addition, no differences in Notch1, NICD1, phospho-Akt, and total Akt expression were found in the cultured podocytes under conditions of NG or NG plus mannitol at all time points tested (**P** > 0.05) (data not shown).

**The sh-Notch1 vector and GSI upregulate HG-induced phospho-Akt expression in podocytes.** To investigate the effect of Notch pathway signaling on the PI3K/Akt pathway in HG-induced podocytes, cells were transfected with the sh-Notch1 vector or were treated with GSI (Fig. 2). NICD1 and Notch1 protein levels were markedly higher in podocytes stimulated with HG for 48 h than the cells treated with NG (**P** < 0.01). Cells that were transfected with the sh-Notch1 vector or were treated with GSI decreased NICD1 protein expression in podocytes than the cells treated with HG (**P** < 0.01). Compared with the cells treated with HG, only cells transfected with the sh-Notch1 vector reduced Notch1 protein levels (**P** < 0.01). HG induced the overexpression of phospho-Akt in podocytes, which was clearly increased by the sh-Notch1 vector or GSI treatment (**P** < 0.01). Compared with the cells of the NG group, Notch1 mRNA significantly increased in the HG group for 48 h. Transfection with the sh-Notch1 vector decreased the HG-induced mRNA overexpression of Notch1 in podocytes (**P** < 0.01). No change in Akt protein and mRNA expression

![Fig. 2. Effects of a short-hairpin (sh)-Notch1 vector and GSI on the HG-induced expression of NICD1, Notch1, p-Akt, and Akt in podocytes at 48 h. A: protein expression of NICD1, Notch1, p-Akt, and Akt was analyzed by Western blotting. B: levels of NICD1, Notch1, p-Akt, and Akt were quantified by densitometry. C: mRNA levels of Notch1 and Akt were analyzed by real-time PCR. The overexpression of NICD1 in HG-induced podocytes was significantly decreased by the sh-Notch1 vector or GSI. HG induced the overexpression of p-Akt protein in podocytes, which was clearly increased by the sh-Notch1 vector or GSI. Values are means ± SD (n = 6). **P** < 0.01 vs. normal glucose (NG). ##P** < 0.01 vs. HG+sh-Scramble.](http://ajprenal.physiology.org/)
was determined in the cultured podocytes of the different groups \((P > 0.05)\).

The sh-Notch1 vector and GSI inhibit HG-induced podocyte apoptosis. As shown in Fig. 3, Bcl-2 expression at 48 h in the HG-stimulated group was significantly lower than that of the NG group \((P < 0.01)\). In contrast, the levels of Bax, p-p53, and NF-κB (p65) in HG-induced podocytes at 48 h were significantly increased compared with cells in the NG group \((P < 0.01)\). Compared with the cells treated with HG, Bax, p-p53, and NF-κB (p65) protein levels were significantly decreased in cells transfected with the sh-Notch1 vector or pretreated with GSI \((P < 0.05, P < 0.01)\), whereas the Bcl-2 level was increased \((P < 0.01)\). No change in p53 protein expression was found in the cultured podocytes of the different groups \((P > 0.05)\). Furthermore, HG enhanced the apoptosis rate of podocytes at 48 h, and apoptosis was efficiently inhibited by transfection with the sh-Notch1 vector or treatment with GSI as measured by TUNEL and flow cytometry \((P < 0.05, P < 0.01)\) (Fig. 4).

The sh-Akt vector and LY294002 upregulate HG-induced NICD1 expression in podocytes. To investigate the effect of the PI3K/Akt pathway on the Notch pathway in HG-induced podocytes, cells were transfected, respectively, with the sh-Akt vector or treated with LY294002 (Fig. 5). HG treatment induced the overexpression of phospho-Akt protein in podocytes for 24 h, which was clearly decreased by the sh-Akt vector or LY294002 \((P < 0.01)\). Podocytes transfected with the sh-Akt vector had reduced total Akt levels compared with the cells stimulated with HG \((P < 0.01)\). Compared with the NG group, NICD1 and Notch1 protein levels significantly increased in the HG group at 24 h \((P < 0.01)\). After cells were transfected with the sh-Akt vector or were treated with LY294002, NICD1 and Notch1 protein expression in podocytes was increased compared with cells treated with HG \((P < 0.01)\). Compared with the cells of the NG group, Notch1 mRNA was significantly increased in the HG group for 24 h. Transfection with the sh-Notch1 vector increased the HG-induced mRNA overexpression of Notch1 in podocytes \((P < 0.01)\). Akt mRNA expression was inhibited in the cultured podocytes transfected with the sh-Notch1 vector compared with cells in the HG group \((P < 0.01)\).

The sh-Akt vector and LY294002 induce HG-induced podocyte apoptosis. As shown in Fig. 6, the HG treatment of podocytes decreased the Bcl-2 protein level compared with cells grown in NG medium for 24 h \((P < 0.01)\). The levels of Bax, p-p53, and NF-κB (p65) in HG-induced podocytes at 24 h were significantly higher than in NG group cells \((P < 0.01)\). Compared with the cells treated with HG, Bax, p-p53, and NF-κB (p65) protein levels significantly increased in cells transfected with the sh-Akt vector or treated with LY294002 \((P < 0.01)\), whereas the Bcl-2 level decreased \((P < 0.05)\). No change in p53 protein expression was found in the cultured podocytes of the different groups \((P > 0.05)\). Furthermore, podocytes transfected with the sh-Akt vector or treated with

Fig. 3. Effects of the sh-Notch1 vector and GSI on the HG-induced protein expression of Bax, Bcl-2, p-p53, p53, and NF-κB (p65) in podocytes at 48 h. A: protein levels of Bax, Bcl-2, p-p53, p53, and NF-κB (p65) were analyzed by Western blotting. B: levels of Bax, Bcl-2, p-p53, and p53 were quantified by densitometry. C: the level of NF-κB (p65) was quantified by densitometry. HG decreased Bcl-2 and increased Bax, p-p53, and NF-κB (p65) protein levels. Compared with the cells treated with HG, Bax, p-p53, and NF-κB (p65) protein levels significantly decreased in cells transfected with the sh-Notch1 vector or treated with GSI, whereas the Bcl-2 level increased. Values are means ± SD \((n = 6)\). **\(P < 0.01\) vs. NG. #\(P < 0.05\), ##\(P < 0.01\) vs. HG+sh-Scramble.

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LY294002 exhibited an enhanced HG-induced apoptosis rate at 24 h, as determined by TUNEL and flow cytometry (P < 0.05, P < 0.01) (Fig. 7).

DISCUSSION

The results of these experiments demonstrate that HG activates Notch and PI3K/Akt pathways in podocytes at different time points relative to one another. Therefore, we investigated the relationship between the Notch and PI3K/Akt pathways on HG-induced podocyte apoptosis.

First, we explored the question of whether HG can activate the Notch and PI3K/Akt pathways in podocytes. The Notch pathway is an evolutionarily conserved local cell signaling mechanism that participates in a variety of cellular processes and is important in glomerular development (1, 4). As suggested by Waters et al. (31), the Notch pathway may represent...
Fig. 5. Effects of the sh-Akt vector and LY294002 on HG-induced expression of p-Akt, Akt, NICD1, and Notch1 in podocytes at 24 h. A: protein expression of p-Akt, Akt, NICD1, and Notch1 was analyzed by Western blot analysis. B: levels of p-Akt, Akt, NICD1, and Notch1 were quantified by densitometry. C: mRNA levels of Akt and Notch1 were analyzed by real-time PCR. In HG-induced podocytes, p-Akt overexpression was clearly decreased by the sh-Akt vector or LY294002. NICD1 protein levels increased in the HG group, and NICD1 protein expression in podocytes transfected with the sh-Akt vector or LY294002 were further increased compared with the cells treated with HG. Values are means ± SD (n = 6). **P < 0.01 vs. NG. ##P < 0.01 vs. HG/sh-Scramble.

Fig. 6. Effects of the sh-Akt vector and LY294002 on the HG-induced expression of Bax, Bcl-2, p-p53, p53 and NF-κB (p65) in podocytes at 24 h. A: the protein levels of Bax, Bcl-2, p-p53, p53 and NF-κB (p65) were analyzed by Western blot. B: The levels of Bax, Bcl-2, p-p53 and p53 were quantified by densitometry. C: The level of NF-κB (p65) was quantified by densitometry. HG stimulation decreased Bcl-2 and increased Bax, p-p53 and NF-κB (p65) protein levels. Compared with the cells treated with HG, Bax, p-p53 and NF-κB (p65) protein levels significantly increased in cells transfected with the sh-Akt vector or LY294002, whereas the Bcl-2 level decreased. Data represent the means ± SD (n = 6). **P < 0.01 vs. NG. #P < 0.05, ##P < 0.01 vs. HG/sh-Scramble.
a correlative pathway to the podocyte injuries observed during glomerular disease. In this study, we determined that the levels of Notch1 and NICD1, the activated form of Notch, increased in a time-dependent manner in HG-induced podocytes and peaked at 48 h. The expression of Notch1 and NICD1 clearly increased in the later phase of the HG treatment condition. NICD1 travels into the nucleus, where it activates the Hes1 and Hey1 genes by a similar mechanism that has been described previously (9, 13). Several studies have found that Hes1 and Hey1 can activate apoptotic pathways in epithelial and glioma cells (24, 27). Our results also showed that the total Akt (phospho-Akt and non-phospho-Akt) level did not change
appreciably in podocytes stimulated by HG at all time points. However, the phospho-Akt level was significantly increased in cells cultured with HG medium. The regulation of expression occurred in a time-dependent manner, and the maximum expression of phospho-Akt occurred at 24 h after stimulation with HG. Because Akt phosphorylation is a notably rapid process, the phospho-Akt level was upregulated in the early stage of HG stimulation at 6–24 h. These data suggest that HG is a positive regulator of Akt phosphorylation in podocytes. These results are similar to those of several previous studies that revealed that HG affects the PI3K/Akt pathway in renal mesangial cells (30) and human renal proximal tubular cells (10). The PI3K/Akt signaling pathway plays a central role in modulating cell proliferation, survival, and motility. Recently, Bridgewater et al. (2) showed a direct protective effect of IGF-1, via the activation of PI3K/Akt, on apoptosis in human fetal podocytes.

Next, we investigated the effect of the Notch and PI3K/Akt pathways on HG-induced podocyte apoptosis. The activation of the Notch pathway by HG can be suppressed by transfection with the sh-Notch1 vector or a Notch pathway inhibitor (GSI), which was confirmed by Western blot analysis and real-time PCR. We found that the knockdown of Notch1 and NICD1 expression resulted in increased p-Akt and Bcl-2 levels and inhibited several proapoptotic targets such as Bax, p-p53, and NF-κB. The knockdown also protected podocytes from apoptosis under HG conditions at 48 h. The Akt phosphorylation level was also enhanced by the addition of the Notch pathway inhibitor (GSI) in podocytes under HG conditions at 48 h, which promoted cell survival. These findings indicated that the activation of the Notch pathway suppressed p-Akt levels and resulted in podocyte apoptosis in the late phase of HG conditions. Following Notch1 activation, the MAPK and PI3K/Akt pathway activity reversed Notch1-induced growth inhibition (20). The PI3K/Akt signaling axis is a dominant survival pathway and prevents cell death in many cancers. In human breast cancer cells, wogonin-induced apoptosis was accompanied by a significant decrease in Bcl-2 and survivin and an increase in Bax and p53 and is correlated with the blockade of the PI3K/Akt/survivin signaling pathways (11). In metastatic prostate cancer cells, DAB2IP protein, a potent growth inhibitor, can suppress the PI3K/Akt pathway and enhance apoptosis-stimulated kinase (ASK1) activation, leading to cell apoptosis. The loss of DAB2IP expression resulted in PI3K/Akt activation and ASK1-JNK inactivation, leading to accelerated cell growth in vivo (34). In this study, we have demonstrated that the activation of the Notch pathway in podocytes in the late phase of HG treatment (at 48 h) was sufficient to induce apoptosis via the inhibition of the PI3K/Akt pathway. As podocytes are unable to replicate, apoptosis can lead to decreased podocyte density and cause the development of DN.

Our study indicated that activation of the PI3K/Akt pathway protected podocytes against HG-induced apoptosis in the early stage of HG treatment. Kim et al. (15) demonstrated that HG stimulates podocyte hypertrophy through the reactive oxygen species (ROS)-dependent activation of ERK1/2 and Akt/PKB. Enhanced ROS generation accounted for the additive effects of HG and ANG II. Akt phosphorylation induced by HG at 24 h in podocytes can be suppressed by transfection with a sh-Akt vector or treatment with the chemical LY294002. The inhibition of p-Akt expression raised NICD1 levels, activated several proapoptotic targets, such as Bax, p-p53, and NF-κB, inhibited Bcl-2 levels, and resulted in podocyte apoptosis. The PI3K/Akt pathway mediates the survival of podocytes, and the activation of the PI3K/Akt pathway prevents podocyte apoptosis via inhibiting the Notch pathway in the early stage of HG stimulation. In podocytes, HG initially results in Akt phosphorylation, which increases podocyte survival. Prolonged exposure to HG leads to Notch1 activation, which suppresses Akt activity and leads to increased podocyte apoptosis. The interaction of the Notch and PI3K/Akt pathways affects several apoptotic targets, such as Bax, p-p53, and the NF-κB pathway, and determines the fate of HG-stimulated podocytes.

In summary, our data demonstrated that the PI3K/Akt pathway prevented podocyte apoptosis by inhibiting the Notch pathway in the early stage of HG stimulation. In addition, we determined that the Notch pathway induced podocyte apoptosis by inhibiting the PI3K/Akt pathway in the late stage of HG stimulation. We can speculate that the balance of the Notch and PI3K/Akt pathways in the podocytes is notably important in the context of DN.

ACKNOWLEDGMENTS

We thank Dr. Myung-Haing Cho for providing us with the sh-Akt vector.

GRANTS

This study was supported by the National Natural Science Foundation (81000301 and 81100517), the Department of Education of the Hebei Province of China (20111168), and the Department of Health of the Hebei Province of China (20130229).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: X.-M.W. and Q.-J.L. performed experiments; X.-M.W. and Q.-J.L. drafted manuscript; M.Y. analyzed data; M.Y. interpreted results of experiments; S.-X.L. prepared figures; J.H. edited and revised manuscript; F.G. provided conception and design of research.

REFERENCES


