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Notch Pathway Is Involved in High Glucose-Induced Apoptosis in Podocytes Via Bcl-2 and p53 Pathways

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ABSTRACT

Recent studies have shown that Notch pathway plays a key role in the pathogenesis of diabetic nephropathy (DN), however, the exact mechanisms remain elusive. Here we demonstrated that high glucose (HG) upregulated Notch pathway in podocytes accompanied with the alteration of Bcl-2 and p53 pathways, subsequently leading to podocytes apoptosis. Inhibition of Notch pathway by chemical inhibitor or specific short hairpin RNA (shRNA) vector in podocytes prevented Bcl-2- and p53-dependent cell apoptosis. These findings suggest that Notch pathway mediates HG-induced podocytes apoptosis via Bcl-2 and p53 pathways. J. Cell. Biochem. 114: 1029–1038, 2013.

KEY WORDS: HIGH GLUCOSE; NOTCH PATHWAY; PODOCYTES; BCL-2; P53; APOPTOSIS

Diabetic nephropathy (DN) is one of the most common complications of diabetes and has become the most cause of end-stage renal disease. The pathogenesis of DN is very complicated and there are many signal pathways involved in the pathogenesis of DN, including Notch pathway [Shi et al., 2008; Liu, 2010]. The family of Notch pathway plays an important role in cells differentiation, acting primarily to determine and regulate cell survival [Mertens et al., 2008; Cook and Figg, 2010; Ji et al., 2011]. In mammal there are four receptors (Notch1–Notch4) and five ligands including Jagged1, Jagged2, Delta-like (Dll) 1, Dll3, and Dll4 [Samon et al., 2008]. The ligand is present on the surface of cells and the corresponding Notch receptor is present on the surface of the adjacent cells. The binding of a ligand and receptor induces a conformational change of the Notch receptor. This allows an extracellular metalloprotease to cleave the receptor, which then allows the γ-secretase-mediated protease to release the Notch intracellular domain (NICD). Then, NICD travels into the nucleus where it activates the transcription of downstream genes such as Hes and Hey genes [McCright, 2003].

It is believed that the apoptosis of podocytes, a type of glomerular epithelial cell, can lead to the development of DN [Butt and Riaz, 2010; Piwkowska et al., 2011]. Since podocytes are unable to divide, apoptosis of the cells will result in proteinuria, accumulation of extracellular matrical components and glomerulosclerosis. Recent studies found activation of Notch pathway in apoptotic podocytes of diabetic patients and animal models [Niranjan et al., 2008]. However, the definite molecular mechanisms involved in Notch pathway-induced podocytes apoptosis in DN have not been fully elucidated. Notch pathway possibly induced podocytes apoptosis through regulation of some apoptotic pathways. Bcl-2 and p53 protein family are the common protein in researches on relevant gene of cells apoptosis [Culmsee and Plesnila, 2006; Chen et al., 2011]. The anti-apoptotic effect of Bcl-2 protein is based on its ability to bind Bcl-2-associated X protein (Bax) protein in the heterodimer form, and thus to block the forming of Bax/Bax proapoptotic homodimers. Upregulation of the ratio of Bax/Bcl-2 and cleaved caspase-3 could induce cell apoptosis [Lau et al., 2012; Liu et al., 2012]. The tumor suppressor p53 plays a central role in inducing cell cycle arrest or apoptosis in response to various injury [Yang et al., 2006; Miyachi et al., 2009].

In some previous studies, it has been revealed that high glucose (HG), an underlying factor that affects normal cells' physical

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metabolism and function in diabetes patients, can induce apoptosis in renal mesangial cells and tubular cells via Bcl-2 and p53 pathways [Dang et al., 2010; Lim et al., 2011; Kim et al., 2012a]. Here we hypothesize Notch pathway may mediate HG-induced podocytes apoptosis through activation of Bcl-2 and p53 pathways. Therefore, to test this hypothesis, HG-induced podocytes were chosen to detect Notch pathway, Bcl-2 pathway, p53 pathway, and cells apoptotic rate. Subsequently, we suppressed Notch pathway using chemical inhibitor or specific short hairpin RNA (shRNA) vector to investigate the effect of Notch pathway on Bcl-2 pathway, p53 pathway, and podocytes apoptosis under HG condition, and further explored the mechanism of podocytes apoptosis.

MATERIALS AND METHODS

CELL LINE AND REAGENTS

Conditionally immortalized mouse podocytes were obtained from cell resource center, Peking Union Medical College, Beijing, China. DMEM-F12 medium was from Gibco BRL (Grand Island, NY). D-Glucose and GSI (γ-secretase inhibitor) were obtained from Sigma (St.-Louis, MO). γ-Interferon was obtained from Peprotech (Rochy Hill, NJ). Rabbit polyclonal antibodies against Jagged1 and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit polyclonal antibodies for full-length Notch1 (FL Notch1), NICD1, Hes1, and Hey1 were purchased from Abcam Technology (Cambridge, MA). Rabbit polyclonal antibodies against Bax, Bcl-2, p-p53 (Ser-37), and p53 were obtained from Proteintech (Chicago, IL). Rabbit anti-cleaved Caspase-3 polyclonal antibody was obtained from Cell Signaling Technology (Danvers, MA). Lipofectamine 2000 and TRIzol reagent were obtained from Invitrogen Life Technologies (Carlsbad, CA). The real-time PCR system and fluorometric TUNEL system were obtained from Promega (Madison, WI).

CELL CULTURE

In conditionally immortalized mouse podocytes cell line, a temperature-sensitive SV40 large T-cell antigen (tsA58 Tag) is controlled by a g-interferon inducible H-2K^b promoter. Podocytes were firstly cultured in DMEM-F12 medium supplemented with 10% fetal bovine serum (FBS) and 10 U/ml γ-interferon (growth permissive conditions) in 33°C 5% CO_2 atmosphere to induce proliferation, then incubated in DMEM-F12 medium supplemented with 10% FBS and deprived of γ-interferon (growth restrictive conditions) in 37°C 5% CO_2 atmosphere for 14 days to induce quiescence and the differentiated phenotype, as previously described [Mundel et al., 1997]. Podocytes were grown to 75–85% confluence under growth restrictive conditions and growth-arrested in serum-free DMEM-F12 for 24 h to synchronize the cell growth. After this time period, the media were changed to fresh serum-free media containing normal glucose (NG, 5.5 mmol/L) and HG (30 mmol/L) at indicated time points. Transient transfections of podocytes with sh-Jagged1 vector, sh-Notch1 vector, or negative control sh-Scramble vector (Jingsai Co, Wuhan, PRC) were performed with Lipofectamine 2000 according to the manufacturer's instructions. Cells of HG plus GSI group need pretreatment with GSI (1 μmol/L) for 30 min.

WESTERN BLOTTING

Whole cells were harvested by scraping in lysis buffer. The protein concentration was assessed by Coomassie Protein Assay Reagent (Sigma–Aldrich). Forty micrograms of protein/lane was loaded and separated by electrophoresis, transferred to PVDF membranes. The membranes were blocked with 5% dry milk and incubated overnight at 4°C with primary antibodies. After washing, the membrane was incubated with goat anti-rabbit IgG horseradish peroxidase conjugate (Proteintech, Chicago, IL, 1:5,000). Proteins in western blot were quantified following acquisition and analysis of the image using the software of a UVP Image Station Lab works 4.5 (Upland, CA). Proteins expression was quantified by comparison with internal-control β-actin.

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). Results were analyzed using the relative standard curve method of analysis/ΔC_t method of analysis.

TUNEL

Immerse slides in 4% formaldehyde in phosphate-buffered saline (PBS) at 4°C and then in 0.2% Triton X-100 in PBS for 10 min at room temperature. Add 100 μl Equilibration Buffer at room temperature for 10 min. Add 50 μl of TdT reaction mix to the cells for 60 min at 37°C. Immerse slides in 2× SSC for 15 min. Add propidium iodide (PI) to stain all cells. Detect localized green fluorescence of apoptotic cells in a red background by fluorescence microscopy. For quantification of TUNEL-positive (apoptotic) 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

Apoptotic cells in different groups were determined using an Annexin V/PI apoptosis detection kit according to manufacturer's protocol (MultiSciences Biotech, Hangzhou, PRC). 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, Becman Coulter). This test discriminates intact cells (Annexin V^-/PI^-), early apoptotic cells (Annexin V^+/PI^-), and late apoptotic cells (Annexin V^+/PI^+).

STATISTICAL ANALYSIS

All data presented as bar graphs were the means ± standard deviation (SD) of at least three independent experiments and analyzed with SPSS 15.0 for Windows. Statistical analysis was performed by one-way analysis of variance (ANOVA). The results were considered statistically significant at $P < 0.05$.

RESULTS

HG ACTIVATES NOTCH PATHWAY IN PODOCYTES

To determine the effect of HG on activation of the Notch pathway, we examined Jagged1, FL Notch1, NICD1, Hes1, and Hey1 protein by Western blot (Fig. 1A,B). The protein expression of Jagged1 and NICD1 began to increase at 12 h after the stimulation of HG, reached the peak at 48 h and slightly decreased at 72 h. FL Notch1 protein expression at 12–72 h within HG stimulation was twofold greater than that of the 0 h of HG stimulation. The Hes1 protein significantly increased in podocytes stimulated by HG for 24 h, continuously increased up to 48 h, and slightly decreased at 72 h. The increased Hey1 protein expression was confirmed after 12 and 24 h of stimulation with HG separately, and gradually decreased with prolonged stimulation. HG-induced time-dependant mRNA levels of Jagged1, Notch1, Hes1, and Hey1 were evaluated by real-time PCR analysis (Fig. 1C). The effects of HG on mRNA levels of Jagged1, Notch1, and Hes1 were revealed at 12 h and peaked at 48 h. HG also induced Hey1 mRNA expression and peaked at 24 h. In addition, no differences of Jagged1, FL Notch1, NICD1, Hes1, and Hey1 expression were found in podocytes cultured under NG conditions among different time points (data not shown).

HG ACTIVATES BCL-2 AND P53 PATHWAYS

As shown in Figure 1D–F, we examined Bax, Bcl-2, cleaved Caspase-3, p-p53 (Ser-37), and p53 protein levels in HG-induced podocytes by Western blot. HG notably increased protein level of Bax in time-dependent manner and peaked at 72 h. HG stimulation decreased Bcl-2 protein level in time-dependent manner and the cells stimulated by HG for 72 h showed the minimum expression. The expression of the HG-induced cleaved Caspase-3 significantly increased at 12 h, peaked at 24 h, and gradually decreased with prolonged HG stimulation. HG stimulation increased p-p53 (Ser-37) protein levels in time-dependent manner and the maximum expressions were at 72 h after stimulation of HG. However, there was no difference of total p53 expression among all time spots in HG-induced podocytes. In addition, no differences of Bax, Bcl-2, cleaved Caspase-3, p-p53 (Ser-37), and p53 protein levels were found in podocytes cultured under NG conditions among different time points (data not shown).

KNOCKDOWN OF NOTCH PATHWAY INHIBITS BCL-2 AND P53 PATHWAYS IN HG-INDUCED PODOCYTES

Compared with the cells of the NG group, the protein levels of Jagged1, FL Notch1, NICD1, Hes1, and Hey1 significantly increased in HG group for 48 h. The transfection with sh-Jagged1 or sh-Notch1 vector respectively decreased HG-induced the protein overexpression of Jagged1, Notch1, NICD1, Hes1, and Hey1 in podocytes (Fig. 2A,B). Real-time PCR showed the similar changes of Jagged1, Notch1, Hes1, and Hey1 mRNA after transfection (Fig. 2C). To investigate the effect of HG-induced activation of Notch pathway on Bcl-2 and p53 pathways, podocytes were transfected respectively with sh-Jagged1 vector, sh-Notch1 vector, or negative control sh-Scramble vector (Fig. 2D–F). Compared with the cells treated with HG, Bax, and cleaved Caspase-3 protein levels significantly decreased in podocytes transfected with sh-Jagged1 or sh-Notch1 vector, reversely Bcl-2 protein level increased. In addition, HG induced an obviously increased p-p53 (Ser-37) protein expression in podocytes, which was significantly inhibited by transfection with sh-Jagged1 or sh-Notch1 vector. No change of total p53 protein was found between podocytes transfected with shRNA vector and untransfected podocytes stimulated with HG.

GSI INHIBITS BCL-2 AND P53 PATHWAYS IN HG-INDUCED PODOCYTES

As shown in Figure 3A,B, the protein levels of NICD1, Hes1, and Hey1 were markedly higher in podocytes stimulated with HG for 48 h than the cells treated with NG and were dramatically reduced in response to GSI. Real-time PCR showed the similar changes of Hes1 and Hey1 mRNA after treatment with GSI (Fig. 3C). Western blot and real-time PCR analyses revealed that GSI did not inhibit Jagged1 and FL Notch1 overexpression induced by HG. As illustrated in Figure 3D–F, incubation with HG resulted in a significant up-regulation in Bax and cleaved Caspase-3 expression and down-regulation in Bcl-2 expression compared with NG medium-cultured cells. However, the alternations of Bax, cleaved Caspase-3, and Bcl-2 protein levels in HG-induced podocytes were reversed by addition of GSI in HG culture medium. Compared with cells of NG group, Western blot revealed that exposure of podocytes to HG led to a notable increase in p-p53 (Ser-37) protein expression and treatment with GSI impaired p-p53 (Ser-37) protein levels in HG culture medium. The expression of total p53 did not vary between NG- and HG-treated podocytes and was not affected by treatment with GSI.

GSI AND KNOCKDOWN OF NOTCH PATHWAY INHIBIT HG-INDUCED PODOCYTES APOPTOSIS

The representative image of TUNEL staining showed positive nuclear staining in podocytes (Fig. 4). Compared with the NG stimulation, green fluorescence in HG-induced apoptotic cells for 48 h were more easily observed by fluorescence microscopy. These apoptotic cells disappeared after transfection with sh-Jagged1 or sh-Notch1 vector and treatment of GSI. By flow cytometry (Fig. 5), apoptosis rate of podocytes stimulated with HG for 48 h than the cells treated with NG, which was efficiently inhibited by GSI, sh-Jagged1, or sh-Notch1 vector.

DISCUSSION

The results of these experiments demonstrate that the HG-induced Notch pathway activation changes Bcl-2 and p53 apoptotic pathways levels and induces podocytes apoptosis. Notch pathway inhibitor and knockdown of Jagged1 and Notch1 prevented HG-induced Bcl-2 and p53 apoptotic pathways activation and podocytes apoptosis.

There are many investigations have showed Notch pathways are involved in apoptosis, cell arrest as well as proliferation. As suggested by Waters et al. [2008], Notch pathway may also represent a correlative pathway to podocytes injury of glomerular disease. Since podocytes are unable to divide, podocytes apoptosis might be responsible for the decreased podocytes number of renal glomerulus, resulting in glomerulosclerosis. Lin et al. [2010] had already

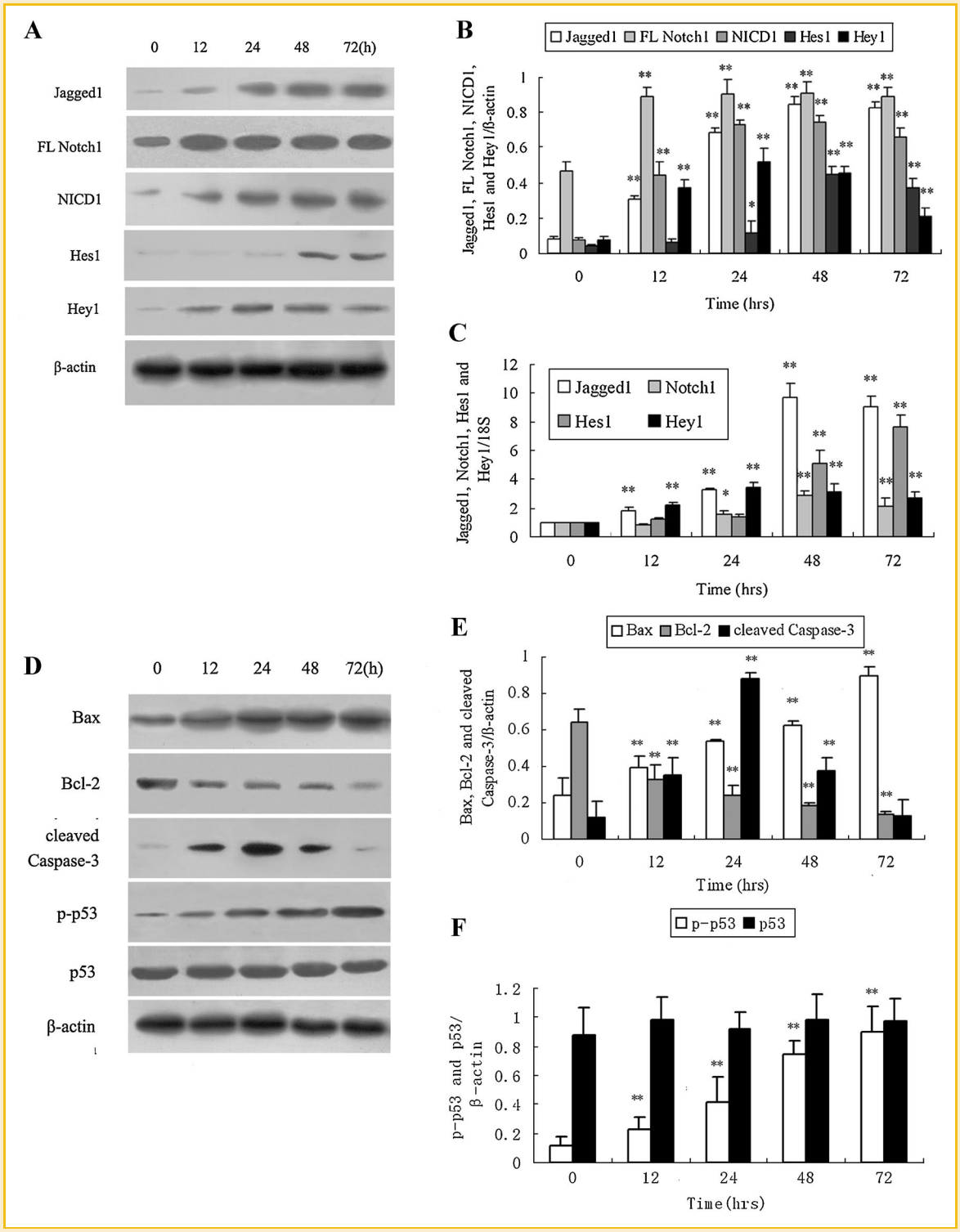

Fig. 1. Time course of the activation of Notch, Bcl-2, and p53 pathways by HG in podocytes. Podocytes were incubated with HG (30 mM) at the indicated times (0–72 h). A: The protein expression of Jagged1, FL Notch1, NICD1, Hes1, and Hey1 was analyzed by Western blot. B: The protein levels of Jagged1, FL Notch1, NICD1, Hes1, and Hey1 were quantified by densitometry. C: The mRNA expression of Jagged1, Notch1, Hes1, and Hey1 was detected by real-time PCR analysis. D: The protein levels of Bax, Bcl-2, cleaved Caspase-3, p-p53, and p53 were analyzed by Western blot. E: The levels of Bax, Bcl-2, and cleaved Caspase-3 were quantified by densitometry. F: The levels of p-p53 and p53 were quantified by densitometry. Values are expressed as means ± SD, n = 6. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control (0 h).

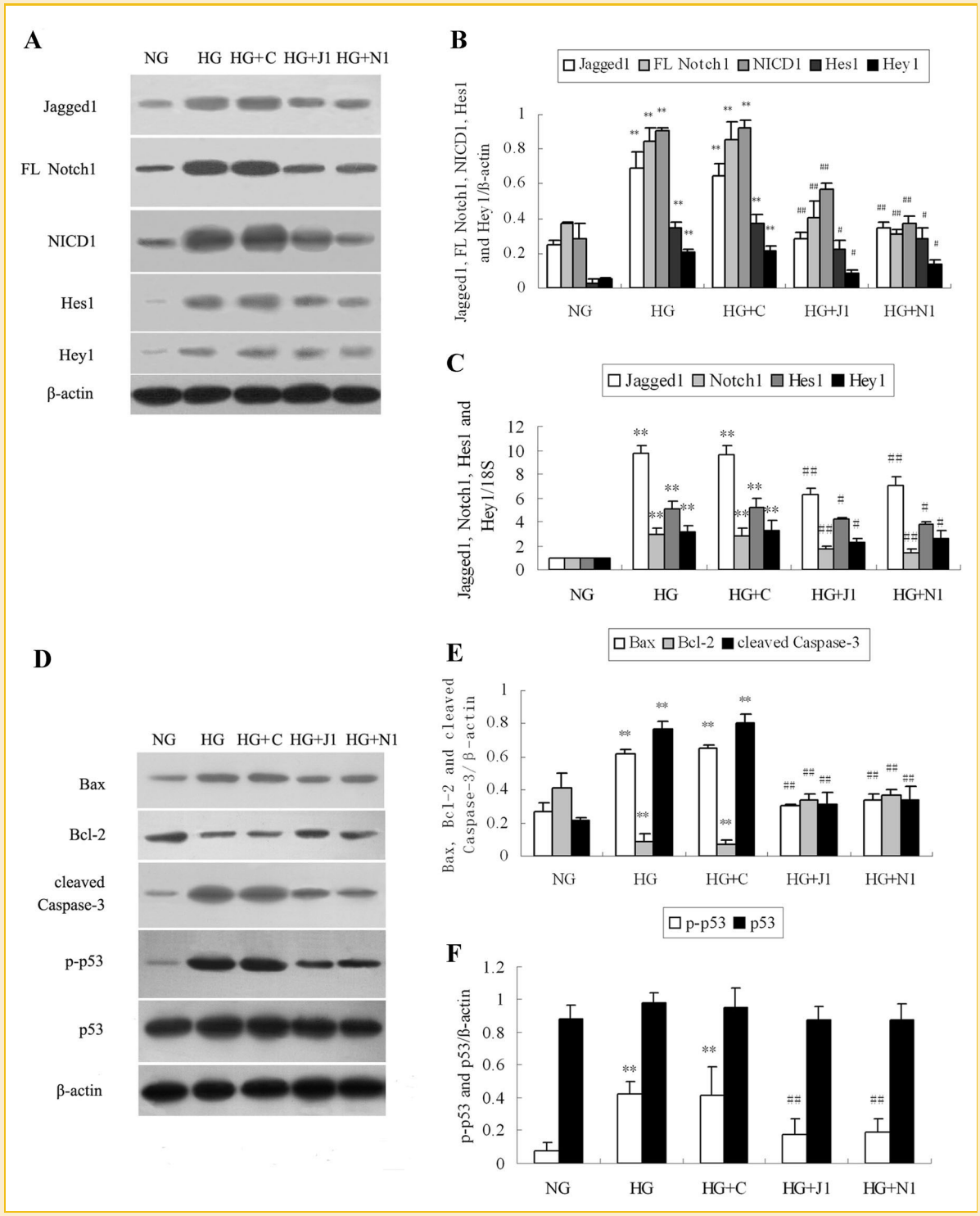

Fig. 2. Effects of sh-Jagged1 or sh-Notch1 vector on HG-induced expression of Notch, Bcl-2, and p53 pathways in podocytes. A: The protein expression of Jagged1, FL Notch1, NICD1, Hes1, and Hey1 was analyzed by Western blot. B: The protein levels of Jagged1, FL Notch1, NICD1, Hes1, and Hey1 were quantified by densitometry. C: The mRNA levels of Jagged1, Notch1, Hes1, and Hey1 were analyzed by real-time PCR. D: The protein levels of Bax, Bcl-2, cleaved Caspase-3, p-p53, and p53 were analyzed by Western blot. E: The levels of Bax, Bcl-2, and cleaved Caspase-3 were quantified by densitometry. F: The levels of p-p53 and p53 were quantified by densitometry. NG: podocytes incubated with 5.5 mM D-glucose; HG: podocytes incubated with 30 mM D-glucose; HG + C: podocytes transfected with negative control sh-Scramble vector and incubated with 30 mM D-glucose; HG + J1: podocytes transfected with sh-Jagged1 vector and incubated with 30 mM D-glucose; HG + N1: podocytes transfected with sh-Notch1 vector and incubated with 30 mM D-glucose. Values are expressed as means ± SD, n = 6. $^{**}P < 0.01$ versus NG, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus HG + C.

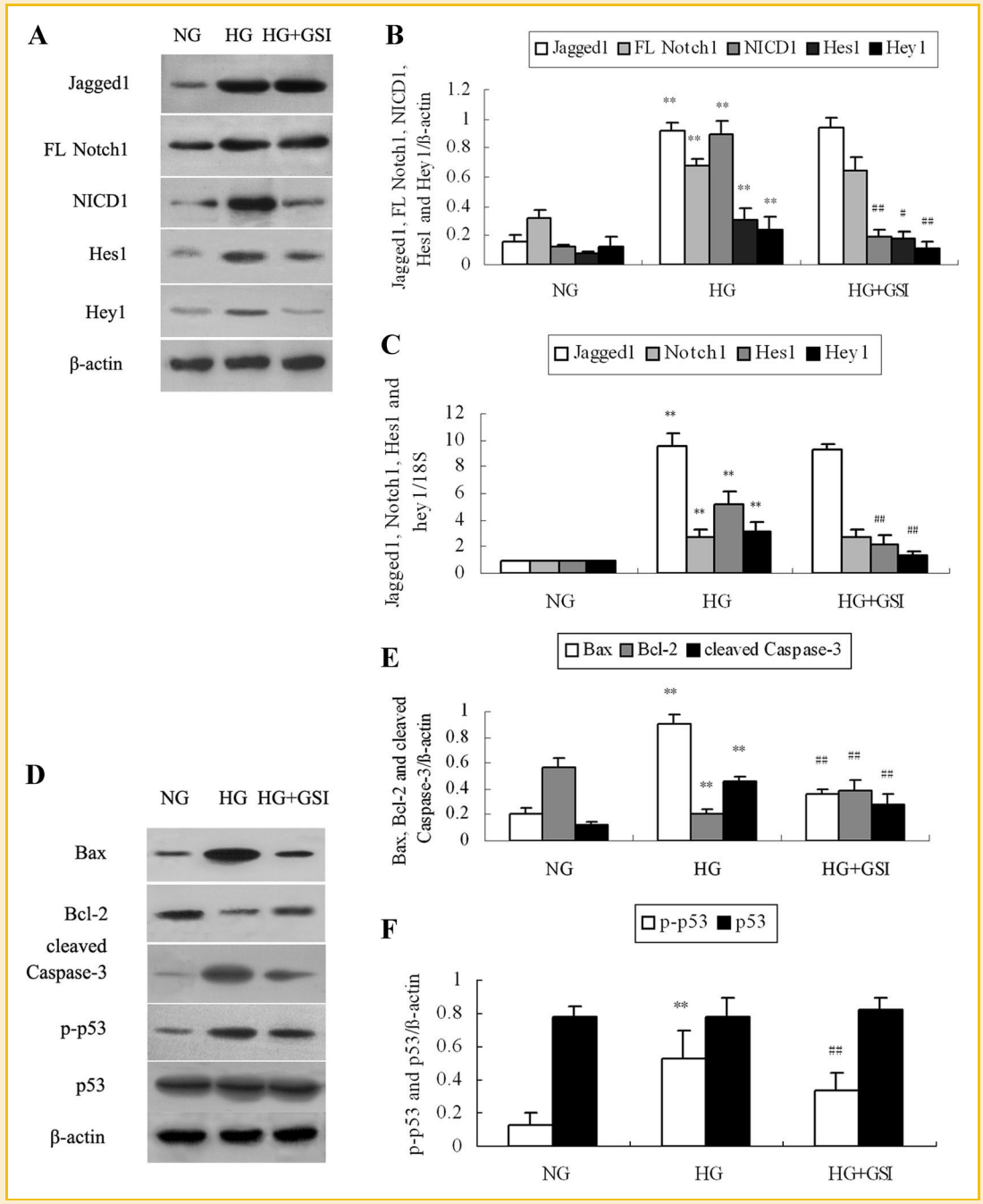

Fig. 3. Effects of GSI on HG-induced expression of Notch, Bcl-2, and p53 pathways in podocytes. A: The protein expression of Jagged1, FL Notch1, NICD1, Hes1, and Hey1 was analyzed by Western blot. B: The protein levels of Jagged1, FL Notch1, NICD1, Hes1, and Hey1 were quantified by densitometry. C: The mRNA levels of Jagged1, Notch1, Hes1, and Hey1 were analyzed by real-time PCR. D: The protein levels of Bax, Bcl-2, cleaved Caspase-3, p-p53, and p53 were analyzed by Western blot. E: The levels of Bax, Bcl-2, and Caspase-3 were quantified by densitometry. F: The levels of p-p53 and p53 were quantified by densitometry. NG: podocytes incubated with 5.5 mM D-glucose; HG: podocytes incubated with 30 mM D-glucose; HG + GSI: podocytes treated with 1 μM GSI and incubated with 30 mM D-glucose. Values are expressed as means ± SD, n = 6. $^{**}P < 0.01$ versus NG, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus HG.

demonstrated that Notch signaling was significantly activated in HG-treated podocytes and diabetic animals, accompanied with VEGF over-expression, nephrin repression, and apoptosis. We also found activation of Notch pathway in a time-dependent manner and the maximum expression of Notch pathway was at 24 or 48 h after stimulation of high glucose in podocytes. The Notch1 ligand Jagged1 and Notch1 seem to be the modulatory targets of HG in podocytes. Overexpression and binding of Jagged1 and Notch1 were likely to be responsible for Notch1 cleavage and activation. NICD1, the activated form of Notch1, travels into the nucleus where it activate the Hes1 and Hey1 genes in HG-cultured podocytes [Graziani et al., 2008; Kennard et al., 2008]. The activation of Notch

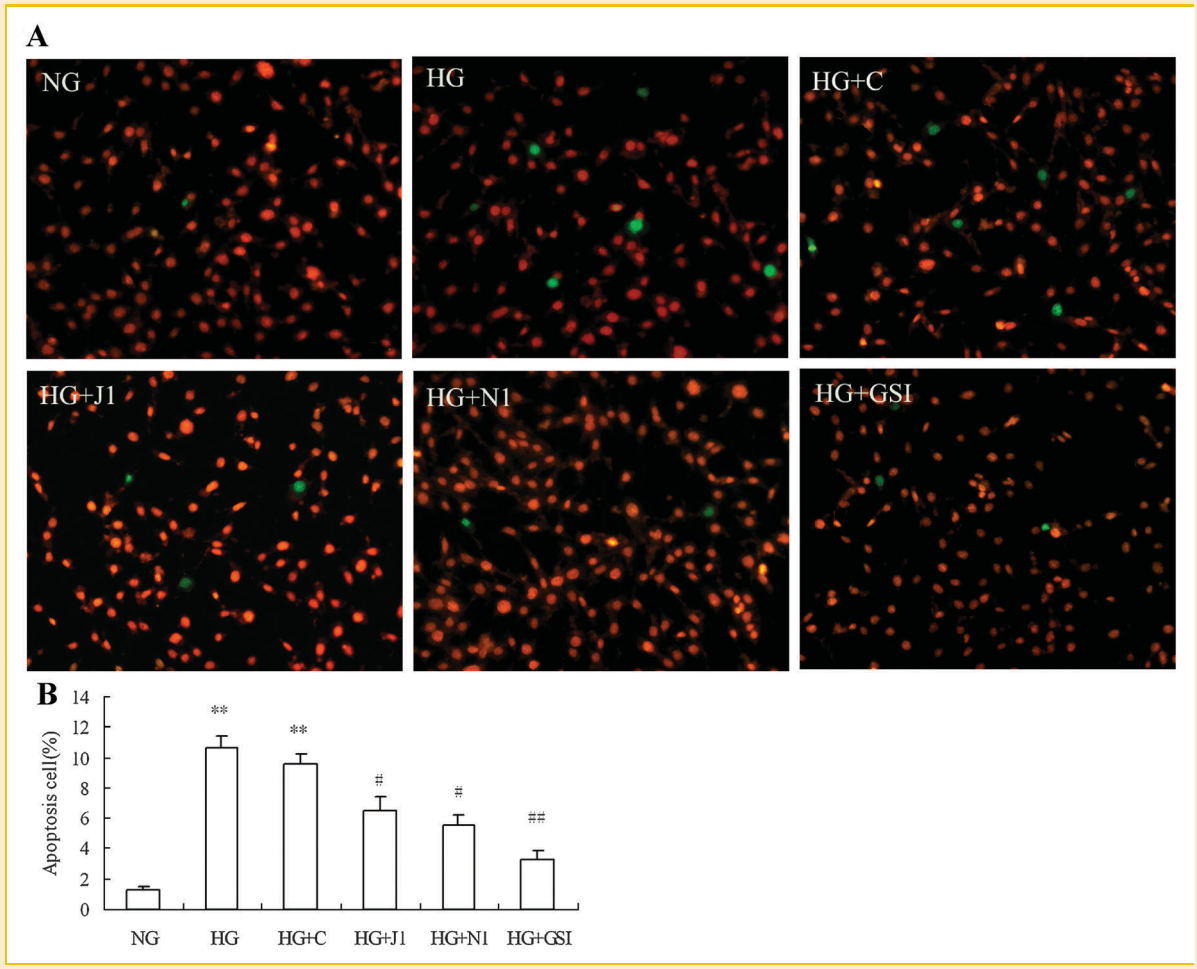

Fig. 4. Effects of Notch pathway shRNA vector and GSI on HG-induced podocytes apoptosis for 48 h. A: TUNEL staining showed green fluorescence of apoptotic podocytes (at 200× magnification). B: TUNEL-positive cells were counted out of a total of more than 200 cells over six random fields. The results were expressed as apoptosis cell (%). NG: podocytes incubated with 5.5 mM D-glucose; HG: podocytes incubated with 30 mM D-glucose; HG + C: podocytes transfected with negative control sh-Scramble vector and incubated with 30 mM D-glucose; HG + J1: podocytes transfected with sh-Jagged1 vector and incubated with 30 mM D-glucose; HG + N1: podocytes transfected with sh-Notch1 vector and incubated with 30 mM D-glucose. HG + GSI: podocytes treated with 1 μM GSI and incubated with 30 mM D-glucose. Values are expressed as means ± SD, n = 6. $^{**}P < 0.01$ versus NG, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus HG + C.

pathway by HG can be suppressed by Jagged1 or Notch1 shRNA plasmid. Western blot and real-time PCR confirmed that knockdown of Jagged1 or Notch1 reduced Jagged1, Notch1 and downstream genes (Hes1 and Hey1) protein and mRNA overexpression in podocytes cultured with high glucose medium. GSI, 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 [McCright, 2003]. We found that HG-induced activation of Notch pathway was inhibited by GSI in podocytes.

It is demonstrated that Bcl-2 and p53 is related to apoptosis in diabetic animals or cellular study. Under diabetic condition, Bcl-2 protein expression was decreased, whereas cleaved caspase-3 protein expression was increased, and apoptotic cells were also significantly increased in diabetic glomeruli and in high glucose-stimulated mesangial cells [Jung et al., 2008]. Sun et al. [2008] had already demonstrated that high glucose led to decreased Bcl-2 expression, reduced GTPase Ras-proximate-1 (Rap1b) activity, and increased levels of both Bax and GTPase activating protein in a proximal tubular cell line. These changes were accompanied by increased DNA fragmentation, decreased high molecular weight mitochondrial DNA, altered mitochondrial morphology and function, disrupted Bcl-2–Bax and Bcl-2–Rap1b interactions, and reduced cell survival. Many investigations have showed that p53-dependent apoptosis resulting in suppressed apurinic/apyrimidinic endonuclease (APE) might be an underlying mechanism of streptozotocin-induced diabetic rats kidneys [Kim et al., 2012b]. Similarly, Huang et al. [2008] showed that triterpenoid was potent in stimulating p53-mediated cell cycle arrest, leading to apoptosis via activation of the caspase signaling pathway in HepG2 cells. We found that HG activated Notch pathway accompanied with the higher ratio of Bax/Bcl-2 and p53 phosphorylation. The activation of NICD1 in a time-dependent manner up-regulated Hes1 and Hey1 expression, promoted Bax and p-p53 levels, inhibited Bcl-2 protein

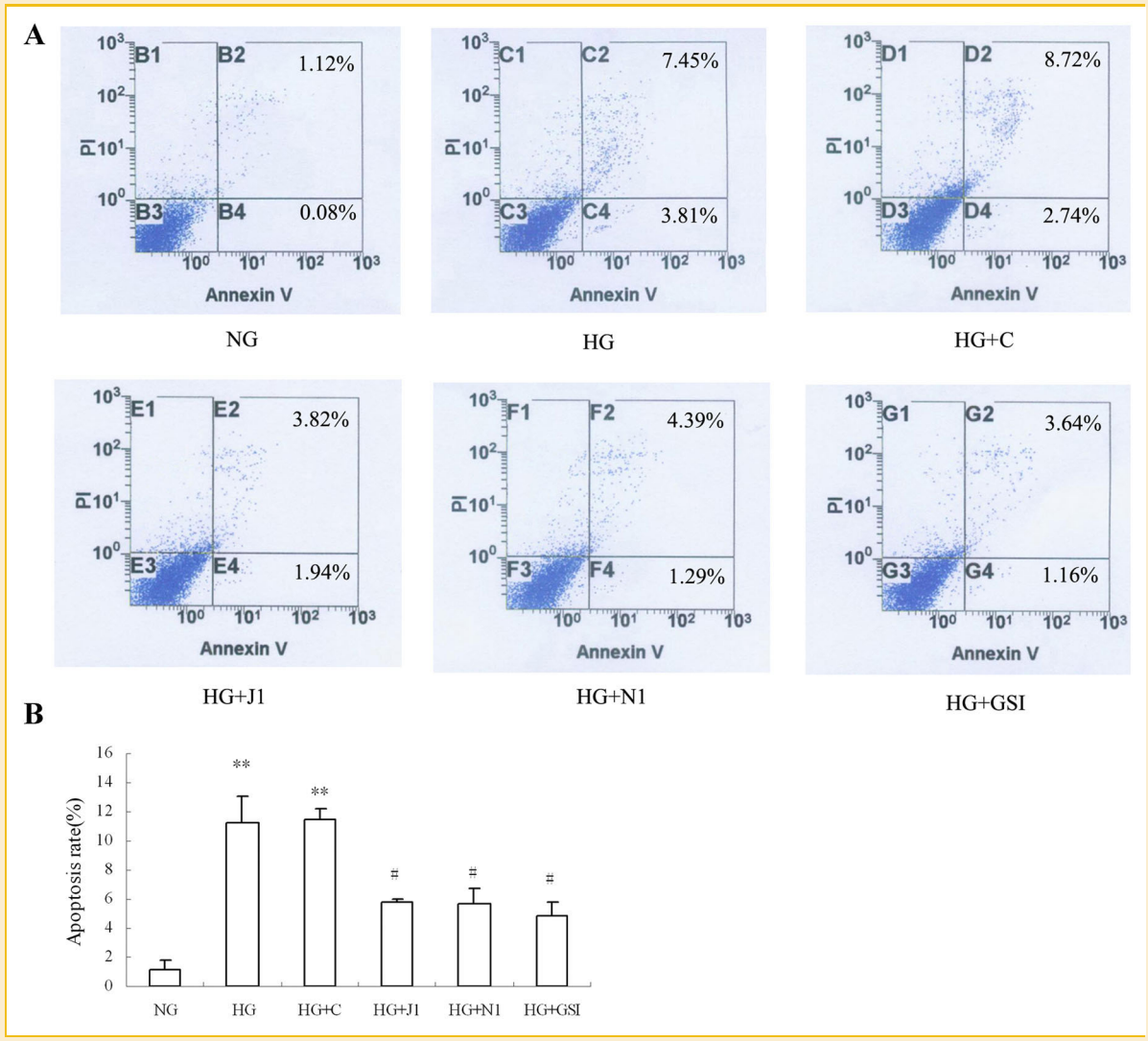

Fig. 5. Effects of Notch pathway shRNA vector and GSI on HG-induced podocytes apoptosis were detected by flow cytometry for 48 h. A: Podocytes were stained with Annexin V/PI for flow cytometry analysis. Apoptotic cells were divided into two stages: early apoptotic (Annexin V^+/PI^-) and late apoptotic (Annexin V^+/PI^+) cells. B: The total apoptosis rates examined using flow cytometry, including the early and late apoptosis rate, were quantified and are shown using a histogram. NG: podocytes incubated with 5.5 mM D-glucose; HG: podocytes incubated with 30 mM D-glucose; HG + C: podocytes transfected with negative control sh-Scramble vector and incubated with 30 mM D-glucose; HG + J1: podocytes transfected with sh-Jagged1 vector and incubated with 30 mM D-glucose; HG + N1: podocytes transfected with sh-Notch1 vector and incubated with 30 mM D-glucose. HG + GSI: podocytes treated with 1 μM GSI and incubated with 30 mM D-glucose. Values are expressed as means ± SD, n = 6. $^{**}P < 0.01$ versus NG, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus HG + C.

expression as well as an associated up-regulation of Caspase-3 in podocytes cultured with high glucose medium. Some studies found that Hes1 and Hey1 could also activate Bcl-2 and p53 pathways inducing cells apoptosis in epithelial and glioma cells [Niimi et al., 2007; Purow et al., 2008].

Then, we further used the chemical inhibitor GSI or knockdown of Notch pathway by shRNA vector to investigate whether Notch pathway mediated podocytes apoptosis through regulation of Bcl-2 and p53 pathways in HG medium. Knockdown of Notch pathway could suppress Bcl-2 and p53 apoptotic pathways and prevent podocytes apoptosis. Similarly HG-induced activation of Bcl-2 and p53 apoptotic pathways were also reversed by GSI, which inhibited γ-secretase-mediated proteolytic cleavage of Notch. Compared with the NG stimulation, HG-induced apoptotic cells were more easily observed by fluorescence microscopy and flow cytometry. These apoptotic cells decreased after transfection with Jagged1 or Notch1 shRNA plasmid and treatment of GSI. Cheng and Kopan [2005] observed that activation of Notch was essential for podocyte formation when S-shaped bodies were forming in the developing mouse kidney. After Notch pathway was block by secretase inhibitor, fewer renal epithelial structures were observed, with a severe deficiency in glomerular podocytes. Notch pathway proteins are not only required in podocytes during the development of nephron segments, but they seem to play an important role in podocytes differentiation in glomerular disease [El-Dahr et al., 2008; Surendran et al., 2010]. Liu et al. [2012] suggested that nestin, which

is dependent on Cdk5 regulation, plays a cytoprotective role in HG-induced podocyte apoptosis. Here we have demonstrated that HG-induced activation of Notch pathway in podocytes was sufficient to induce cells apoptosis via Bcl-2 and p53 pathways. Podocytes apoptosis can lead to decreased podocyte density and cause the development of DN [Niranjan et al., 2008]. Inhibition of the Notch pathway could be used as a potential strategy for the treatment of DN.

In summary, our data demonstrate that Notch pathway mediated HG-induced podocytes apoptosis via Bcl-2 and p53 pathways. We can speculate that the blockade of Notch pathway activation in the kidney may be a potential therapy to DN.

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