c-Abl Contributes to Glucose-Promoted Apoptosis via p53 Signaling Pathway in Podocytes

Yiqiong Ma, Qian Yang, Xinghua Chen, Wei Liang, Zhilong Ren,
Guohua Ding*

Division of Nephrology, Renmin Hospital of Wuhan University, Wuhan,
China

*Corresponding author, Guohua Ding, MD, PhD.

Division of Nephrology

Renmin Hospital of Wuhan University

238 Jiefang Road, Wuhan, Hubei 430060 (China)

Fax: +86 27 88042292

E-Mail: ghxding@gmail.com

ABSTRACT

Aim: To investigate the role of the non-receptor tyrosine kinase c-Abl in high glucose-induced podocyte injury and its possible signal transduction pathway.

Methods: Sixteen C57BL/6 mice were randomly assigned to a group with diabetes and a normal control group. Subsequently, differentiated mouse podocytes were exposed to high-glucose conditions, and podocyte apoptosis was then assessed by flow cytometry and Hoechst 33258 staining. Western blot and immunofluorescence assay were used to measure c-Abl expression. Co-immunoprecipitation assay was used and c-Abl siRNA was applied to evaluate the interaction between c-Abl and p53.

Results: High glucose promotes podocyte apoptosis. The c-Abl expression in podocytes was increased after exposure to high glucose, stimulating the p53 signaling pathway. Conversely, treatment with c-Abl siRNA restored high glucose-promoted podocyte apoptosis and resulted in the reduction of p53 expression.

Conclusion: c-Abl contributes to high glucose-induced podocyte apoptosis via p53 signaling pathway.

Key words:

Podocytes; High glucose; c-Abl; Apoptosis

1. Introduction

Podocytes are terminally differentiated cells that contribute to maintaining the integrity of the glomerular basement membrane [1]. Recent studies have shown that podocyte injury is involved in several glomerular diseases [2-5]. Diabetic nephropathy (DN) is considered the leading cause of end-stage renal disease in Western countries, and podocytes have been confirmed to play a vital role in the development of the pathological changes that characterize DN, including podocytopenia, hypertrophy, glomerulosclerosis, and apoptosis [6].

c-Abl, which belongs to the Abl family, contains SH2 and SH3 structural domains of tyrosine kinase for specific binding. A 140 kDa protein, c-Abl is considered as a Src-related non-receptor tyrosine kinase conserved during evolution but ubiquitously expressed in many tissues, including kidney [7-9]. In recent studies, c-Abl is confirmed to have multiple biological functions. It also has a correlation with aggressive tumors [10] and plays an important role in inflammatory diseases [11]. In addition, many studies point toward the role of c-Abl in the signal transduction pathways that are involved in apoptotic signaling and cytoskeletal rearrangement [12-15]. As a tumor suppressor, p53 plays a key role in many biological processes. The best-understood function of p53 is supporting cell survival and promoting cell death [16, 17]. Accumulating

evidence demonstrates that p53 signaling contributes to the progression of several kidney diseases [18]. It is also implicated in the pathogenesis of DN [19]; p53 expression is increased and activated in the glomeruli of mice with type 1 and type 2 diabetes mellitus [20, 21]. Our previous study indicated that c-Abl up-regulation promotes podocyte apoptosis upon exposure to angiotensin II [22]. However, the function of c-Abl in high glucose-induced podocyte injury remains to be clarified. The present study further investigates the role of c-Abl in podocyte injury under high-glucose conditions.

2. Subjects

In this study, mice with diabetes were used to investigate the effects of hyperglycemia on the c-Abl expression in glomeruli. Furthermore, conditionally immortalized murine podocytes were cultured in a high-glucose environment to elucidate the role of c-Abl in podocyte apoptosis and the underlying signaling pathway.

3. Materials and Methods

3.1. Animals

The experimental procedures and protocols were approved by the Ethical Committee for the Experimental Use of Animals of Renmin Hospital of Wuhan University in Wuhan, China. Sixteen male C57BL/6 mice were supplied by the Research Center of Medical Experimental Animals of Wuhan University. A combination of high-fat diet (HFD) and low-dose streptozotocin (STZ) was used to induce type 2 diabetes in mouse model [23, 24]. The method was performed as described previously [25]. Briefly, the mice were randomly assigned to the group with diabetes on a purified high-fat diet consisting of 24% total fat, 24% protein, and 48% carbohydrate (45% calories from fat; D12541, Research Diets) and the control group on a normal chow diet. The animals were housed under a 12 h light-dark cycle and a temperature of 22 °C. Eight-week-old experimental mice were each intraperitoneally injected with a single dose of STZ (50 mg/kg). Age-matched control mice received an equal volume of 0.1M citrate buffer (pH 4.5). Blood glucose in the blood from tail vein was measured. Mice with blood glucose levels over 16 mmol/ L were considered as having diabetes. At 12 weeks post-STZ injection, mice were individually placed into metabolic cages for 24 h and urine was collected for subsequent analysis. Urinary albumin concentration was

measured using a mouse albumin ELISA quantification kit (Bethyl Laboratories, USA). Then the mice were sacrificed and the kidneys were harvested. The cortices of the kidneys were isolated and fixed in 4% phosphate-buffered paraformaldehyde for renal pathological analysis.

3.2. Cell culture and treatment

Conditionally immortalized murine podocytes were kindly provided by Dr. Peter Mundel (Mount Sinai School of Medicine, New York, USA). The cells were grown at 33 °C in RPMI 1640 medium (HyClone, USA) containing 10% heat-inactivated fetal calf serum (Gibco, USA), 100 U/ mL penicillin G, 100 µg/ mL streptomycin, and 10 U/ mL recombinant murine interferon-γ (IFN-γ, Sigma-Aldrich, USA) in the presence of 5% CO₂. Proliferating cells were transferred to an incubator at 37 °C in IFN-γ-free medium for 7-14 days to induce differentiation. Differentiated podocytes were cultured for 24 h in a medium containing 5 mM glucose (normal glucose) for control group, 5 mM glucose + 25 mM mannitol for the hypertonic control group, and 30 mM glucose (high glucose) for the experimental group.

3.3. c-Abl siRNA transfection

Before transfection, 2×10^5 cells per well were seeded in 6-well plates. Subsequently, 10 nmol/ L c-Abl siRNA (QIAGEN, Germany) was

applied to each well. The siRNA transfection was carried out using HiPerfect Transfection Reagent (QIAGEN, Germany) according to the manufacturer's instructions. Transfected Cells were incubated at 37 °C for 72 h.

3.4. Western blot

The cells were rinsed with ice-cold phosphate-buffered saline (PBS) and lysed in RIPA buffer (150 mM sodium chloride; 1.0% Triton X-100; 0.5% sodium deoxycholate; 0.1% sodium dodecyl sulfate; 50 mM Tris, pH 8.0) mixed with protease inhibitors (P8340, Sigma-Aldrich) for 30 min on ice. Samples were collected and centrifuged at 12,000 rpm for 10 min at 4 °C. Supernatant protein was boiled in loading buffer at 100 °C for 5 min. The protein samples were subjected to SDS-PAGE and transferred to nitrocellulose membranes (GE Healthcare, USA). After blocking with milk, membranes were incubated with primary antibodies (c-Abl rabbit polyclonal antibody, 1:200, Santa Cruz Biotechnology, USA; p53 mouse polyclonal antibody, 1:100, Santa Cruz Biotechnology; β -actin mouse monoclonal antibody, control, 1:1,000, Santa Cruz Biotechnology) overnight at 4 °C. IRDye 800CW conjugated purified goat anti-mouse IgG (1:5,000, LI-COR Biosciences, USA) was used as a secondary antibody. The bands were visualized with an Odyssey infrared imaging system (LI-COR Biosciences).

3.5. Co-immunoprecipitation

Protein lysates were mixed with p53 mouse polyclonal antibody (Santa Cruz Biotechnology) and incubated at 4 °C over night. Then, the supernatants loaded with protein A + G agarose were applied for 3 h at 4 °C. After the centrifuged sediments were collected, bound proteins were eluted by boiling in loading buffer at 95 °C–100 °C for 5 min. Then, the protein samples were analyzed by Western blot for c-Abl and p53.

3.6. Apoptosis assays

Hoechst 33258 (Sigma-Aldrich) staining was employed to assess podocyte apoptosis *in vitro*. The prepared cells were fixed in 4% paraformaldehyde at room temperature for 10 min, and then stained with Hoechst 33258 for 5 min at room temperature. Subsequently, cells were rinsed thrice with ice-cold PBS. Podocytes were observed under a fluorescence microscope (DP72, Olympus, Japan), and all microscopic images were recorded.

Flow cytometry was also performed to evaluate podocyte apoptosis *in vitro*. Fluorescein isothiocyanate (FITC)-conjugated annexin V and PI (KGA108, KeyGen Biotech, China) were used to identify apoptotic cells according to the manufacturer's instructions. Cells in the upper-right and

lower-right quadrants were classified as apoptotic.

3.7. Immunofluorescence assay

The cell-climbing films were fixed with 4% paraformaldehyde for 30 min at 4 °C and stained with c-Abl antibody (1:100, Santa Cruz Biotechnology) overnight at 4 °C. The films were then subjected to FITC-conjugated IgG at 37 °C for 45 min. All microscopic images were recorded using a 12.8- megapixel camera (DP72, Olympus, Japan).

3.8. Immunohistochemistry

The c-Abl expression in the kidney tissue was visualized by immunostaining. The paraffin-embedded sections (3 µm thick) were deparaffinized, treated with 3 % H₂O₂ for 30 min at room temperature, and then autoclaved in citrate buffer (0.01 mol/ L, pH 6.0) for 10 min for antigen retrieval. After blocking with 5% bovine serum albumin, the sections were incubated with c-Abl primary antibody (1:100, Abbiotec, USA) overnight at 4 °C. Subsequently, they were incubated with biotin-conjugated secondary antibody and avidin-biotin peroxidase complex (Dako, USA) for 30 min. After DAB (Dako) staining, sections were stained with haematoxylin. The data were analyzed using Image-Pro Plus 5.10 software (Media Cybernetics, USA).

3.9. Statistical analyses

All values were presented as mean $\pm$ SD and analyzed with SPSS 17.0.

Differences in mean values were tested using student's t test or one-way ANOVA test. Differences are statistically if $p < 0.05$.

4. Results

4.1 Effect of hyperglycemia on podocyte structure

Table 1 demonstrates that the blood glucose levels and urinary albumin excretion of the mice on a HFD were elevated. The kidneys of the mice with diabetes and those of the control mice were obtained to study the ultrastructure of glomeruli by electron microscopy. Representative electron microphotographs of podocytes in the two groups are shown in Fig. 1A. Podocytes foot processes (FPs) widening and effacement were evident in the group with diabetes. The cumulative data shown in Fig. 1B indicated that the mean FP (foot process) width of the group with diabetes significantly increased.

4.2 Effect of hyperglycemia on c-Abl expression in vivo

Our previous study demonstrated the expression of c-Abl in glomeruli. To test the pattern of the expression of c-Abl under hyperglycemic conditions, we established the mouse model of diabetes. The renal cortical sections of the mice were assessed by immunohistochemistry staining, and a significant increase of c-Abl expression was found in the

glomeruli of mice with diabetes (Figs. 2A and B). Western blot was employed to analyze c-Abl expression quantitatively and consequently confirm such an increase, and the increased c-Abl expression was detected in the glomeruli of the mice with diabetes (Fig. 2C).

4.3 Effect of high glucose on c-Abl expression and distribution in cultured podocytes

The increased expression of c-Abl was also detected in podocytes exposed to high glucose *in vitro*. As shown in Fig. 3A, the level of c-Abl expression dramatically increased after 24 h in high-glucose (30 mM) medium, and the increased expression of c-Abl was also detected in cells exposed to tapered concentrations of glucose (Fig. 3B). In addition, the immunofluorescence assays indicated that the amount of c-Abl fluorescence in the nucleus dramatically increased in high glucose-treated cells. The results suggested that the high glucose-promoted expression and distribution of c-Abl might be involved in the biological changes in podocytes in diabetes.

4.4 Effect of c-Abl on high glucose-induced apoptosis

To evaluate the role of c-Abl in high glucose-induced podocyte apoptosis, we used c-Abl siRNA to suppress the expression of c-Abl (Fig. 4). The results (Figs. 4A and B) indicated that c-Abl-specific siRNA reduced the

c-Abl expression in cultured podocytes treated with high glucose. In addition, the amount of c-Abl fluorescence in the nucleus was also reduced (Fig. 4A). Hoechst 33258 staining and flow cytometry were used to assess podocyte apoptosis (Fig. 4C and D). As shown in Figs. 3C and D, high-glucose exposure significantly enhanced podocyte apoptosis. Reversely, the down-regulation of c-Abl with siRNA prevented podocyte apoptosis caused by high glucose. These findings indicated that c-Abl was involved in the signaling pathways in podocyte injury promoted by high glucose.

4.5 Effect of high glucose on c-Abl-p53 interaction

A well-known tumor suppressor, p53 is also involved in the process of apoptosis. Podocytes were treated with a high concentration (30 mM) of glucose for 24 h. PBS- and mannitol-treated groups were used as the normal and hypertonic control groups, respectively. The expression of p53 protein was markedly up-regulated in high glucose-treated cells (Fig. 4B). Conversely, the knock-down of c-Abl by specific siRNA diminished the high-glucose-induced up-regulation of p53 (Fig. 4B). This result indicated that the expression of p53 was up-regulated, and c-Abl was essential for this up-regulation under high-glucose conditions.

Furthermore, we tested the interaction between endogenous c-Abl and p53 in podocytes with the use of co-immunoprecipitation. c-Abl was able

to interact with p53 under normal conditions (Fig. 5). High-glucose treatment promoted the interaction of c-Abl with p53, which was diminished by c-Abl siRNA (Fig. 5).

5. Discussion

This study reveals the important role of c-Abl in the process of podocyte apoptosis, which is a common pathological phenomenon in a range of glomerular diseases, including DN. The typical clinical presentation of DN is the onset of urinary albumin excretion and the progression of renal dysfunction, which is pivotally attributed to podocyte dysfunction. Our previous study demonstrated the expression of c-Abl in podocytes and indicated that c-Abl up-regulation promoted podocyte apoptosis upon exposure to angiotensin II [22].

As a non-receptor tyrosine kinase, c-Abl contains several domains that are essential for target protein binding. The core of c-Abl is composed of tandem SH3 and SH2 domains, which bind to a substrate. The C-terminal region of c-Abl contains an actin-binding domain, which specifies the location of F-actin, and nuclear localization signals, which drive the nuclear entry of c-Abl. Furthermore, several PxxP motifs for SH3 domains interactions and DNA-binding motifs provide additional location cues [7, 26]. Several cellular behaviors are believed to be triggered by c-Abl, including actin rearrangement, immune response, and apoptosis

[26-28]. Recent studies [29, 30] have put forward the vital role of c-Abl in the pathogenesis of nerve diseases. Neural cells and podocytes are highly dynamic cells with dendritic spines or foot processes and share similar structures and functions. However, the role of c-Abl in the biology functions of podocytes remains elusive. Our results indicated that high-glucose treatment increased the expression of c-Abl in glomeruli and cultured podocytes and that the knockdown of c-Abl prevented the high glucose-induced apoptosis of podocytes. A previous study [11] reported that c-Abl plays an important role in prion-induced neuronal apoptosis. In addition, Chung et al. [31] indicated that up-regulation of c-Abl contributes to the Kr28-induced apoptosis of PC-3 human prostate carcinoma cells. Collectively, these results suggest that the up-regulation of c-Abl is a pro-apoptosis signaling in response to high-glucose conditions.

Our study further confirms that p53 is involved in the process of podocyte apoptosis. Notably, after high glucose treatment, c-Abl clusters in the nucleus and interacts with p53. More interestingly, previous studies reported that the C-terminus of c-Abl contains a p53-binding domain [7] and that c-Abl-induced apoptosis is partially executed in a p53-dependent manner [32, 33]. As a major tumor suppressor, p53 also plays a vital role in the progression of kidney diseases [34,35]. It has been reported to

mediate high glucose-induced podocyte apoptosis [36]. As a nuclear transcription factor, p53 has the ability to mediate the expression of a large number of genes such as p21, Bax, p53INP1, Bid, and Puma [17, 37, 38]. Furthermore, p53 can be activated by a number of cellular stressors, including DNA damage, hypoxia, and oncogene activation [39]. Our results show that high glucose increases the expression of p53 in cultured podocytes, which display a considerable number of apoptotic cells. Moreover, the co-immunoprecipitation results confirm that high glucose stimulation promotes p53-c-Abl interaction, which was abolished by the transfection with c-Abl siRNA. Thus, consistent with the results of previous studies, our results demonstrate that the c-Abl-p53 pathway contributes to high glucose-induced podocytes apoptosis [32, 33]. However, the specific binding domains and other signaling pathways that contribute to c-Abl-associated podocytes apoptosis need to be further investigated. Mokhtari et al. [8] reported that imatinib mesylate, a 2-phenylaminopyrimidine-based ATP-competitive inhibitor of the Abl tyrosine kinase family, could stimulate ERK and Akt phosphorylation in a c-Abl-dependent manner. In addition, our previous study demonstrated that the ERK and Akt pathway play vital roles in the progression of kidney diseases [40, 41]. Thus, c-Abl possibly regulates ERK and Akt phosphorylation during the progression of DN, and further research need to be undertaken to confirm this premise.

In summary, the present study shows that high glucose directly promotes podocyte apoptosis and up-regulates c-Abl in podocytes *in vivo and in vitro*. c-Abl contributes to high glucose-induced podocyte apoptosis via the transcription factor p53. Thus, the c-Abl-p53 signaling pathway may be a new candidate for therapeutic targets to prevent podocyte injury during the progression of DN.

Acknowledgements

This study was supported by the grants from the National Science Foundation of China (81270762 to G.D. and 81470912 to W.L.) and the Natural Science Foundation of Hubei Province of China (2015CFB347 to X.C.).

References

- [1]. Shankland SJ. The podocyte's response to injury: Role in proteinuria and glomerulosclerosis. *Kidney International* 2006; 69: 2131-2147.
- [2]. Nangaku M, Shankland SJ, Couser WG. Cellular Response to Injury in Membranous Nephropathy. *Journal of the American Society of Nephrology* 2005; 16: 1195-1204.
- [3]. Mundel P, Reiser J. Proteinuria: an enzymatic disease of the podocyte? *Kidney International* 2009; 77: 571-580.
- [4]. Zhang C, Hu JJ, Xia M, Boini KM, Brimson C, Li PL. Redox signaling via lipid raft clustering in homocysteine-induced injury of podocytes. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 2010; 1803: 482-491.
- [5]. Campbell KN, Raij L, Mundel P. Role of angiotensin II in the development of nephropathy and podocytopathy of diabetes. *Curr Diabetes Rev* 2011; 7: 3-7.
- [6]. Wolf G, Chen S, Ziyadeh FN. From the periphery of the glomerular capillary wall toward the center of disease: podocyte injury comes of age in diabetic nephropathy. *Diabetes* 2005; 54: 1626-34.
- [7]. Colicelli J. ABL Tyrosine Kinases: Evolution of Function, Regulation, and Specificity. *Science Signaling* 2010; 3: re6. doi: 10.1126/scisignal.3139re6.
- [8]. Mokhtari D, Al-Amin A, Turpaev K, Li T, Idevall-Hagren O, Li J, et al. Imatinib mesilate-induced phosphatidylinositol 3-kinase signalling and improved survival in insulin-producing cells: role of Src homology 2-containing inositol 5' -phosphatase interaction with c-Abl. *Diabetologia* 2013; 56: 1327-1338.
- [9]. Liu W, Wu J, Xiao L, Bai Y, Qu A, Zheng Z, et al. Regulation of Neuronal Cell Death by c-Abl-Hippo/MST2 Signaling Pathway. *PLoS One* 2012; 7: e36562.

- [10]. Koos B, Jeibmann A, Lünenbürger H, Mertsch S, Nupponen NN, Roselli A, et al. The tyrosine kinase c-Abl promotes proliferation and is expressed in atypical teratoid and malignant rhabdoid tumors. *Cancer* 2010; 116: 5075-5081.
- [11]. Jackson RC, Radivoyevitch T. Modelling c-Abl Signalling in Activated Neutrophils: the Anti-inflammatory Effect of Seliciclib. *Biodiscovery* 2013; 7: 4.
- [12]. Estrada LD, Zanlungo SM, Alvarez AR. c-Abl tyrosine kinase signaling: a new player in AD tau pathology. *Curr Alzheimer Res* 2011; 8: 643-51.
- [13]. Fred RG, Boddeti SK, Lundberg M, Welsh N. Imatinib mesylate stimulates low-density lipoprotein receptor-related protein 1-mediated ERK phosphorylation in insulin-producing cells. *Clinical Science* 2015; 128: 17-28.
- [14]. Cleary RA, Wang R, Waqar O, Singer HA, Tang DD. Role of c-Abl tyrosine kinase in smooth muscle cell migration. *Am J Physiol Cell Physiol* 2014; 306: C753-61.
- [15]. Karuppagounder SS, Brahmachari S, Lee Y, Dawson VL, Dawson TM, Ko HS. The c-Abl inhibitor, Nilotinib, protects dopaminergic neurons in a preclinical animal model of Parkinson's disease. *Scientific Reports* 2014; 4: 4874.
- [16]. Jacson JG, Post SM, Lozano G. Regulation of tissue- and stimulus- specific cell fate decisions by p53 *in vivo*. *Journal of Pathology* 2011; 223: 127-136.
- [17]. Megyeri K, Orosz L, Kemeny L. Vesicular stomatitis virus infection triggers apoptosis associated with decreased Δ Np63 α and increased Bax levels in the immortalized HaCaT keratinocyte cell line. *Biomedicine & Pharmacotherapy* 2007; 61: 254-260.
- [18]. Skirnisdottir I, Seidal T. The apoptosis regulators p53, bax and PUMA: Relationship and impact on outcome in early stage (FIGO I-II) ovarian carcinoma after post-surgical taxane-based treatment. *Oncology Reports* 2012; 27: 741-747
- [19]. Chen YW, Chenier I, Chang SY, Tran S, Ingelfinger JR, Zhang SL. High glucose promotes nascent nephron apoptosis via NF- κ B and p53 pathways. *Am J Physiol Renal Physiol* 2011; 300: F147-F156.
- [20]. Carvajal LA, Manfredi JJ. Another fork in the road-life or death decisions by the tumour suppressor p53. *European Molecular Biology Organization* 2013; 14:

414-421.

- [21]. Deshpande SD, Putta S, Wang M, Lai JY, Bitzer M, Nelson RG, et al. Transforming Growth Factor- β -Induced Cross Talk Between p53 and a MicroRNA in the Pathogenesis of Diabetic Nephropathy. *Diabetes* 2013; 62: 3151-3162.
- [22]. Chen X, Ren Z, Liang W, Zha D, Liu Y, Chen C, et al. c-Abl mediates angiotensin II-induced apoptosis in podocytes. *Journal of Molecular Histology* 2013; 44: 597-608.
- [23]. Mu J, Woods J, Zhou YP, Roy RS, Li ZH, Zychband E, et al. Chronic Inhibition of Dipeptidyl Peptidase-4 With a Sitagliptin Analog Preserves Pancreatic β -Cell Mass and Function in a Rodent Model of Type 2 Diabetes. *Diabetes* 2006; 55: 1695-1704.
- [24]. Liu TY, Shi CX, Gao R, Sun HJ, Xiong XQ, Ding L, et al. Irisin inhibits hepatic gluconeogenesis and increases glycogen synthesis via PI3K/Akt pathway in type 2 diabetic mice and hepatocytes. *Clin Sci* 2015;129: 839-50
- [25]. Shao ML, Lu XM, Cong WT, Xing X, Tan Y, Li YQ, et al. Multiple Low-Dose Radiation Prevents Type 2 Diabetes-Induced Renal Damage through Attenuation of Dyslipidemia and Insulin Resistance and Subsequent Renal Inflammation and Oxidative Stress. *Plos One* 2014; 9: e92574
- [26]. Tegtmeyer N, Backert S. Role of Abl and Src family kinases in actin-cytoskeletal rearrangements induced by the *Helicobacter pylori* CagA protein. *European Journal of Cell Biology* 2011; 90: 880-890.
- [27]. Dudek SM, Chiang ET, Camp SM, Guo Y, Zhao J, Brown ME, et al., Abl Tyrosine Kinase Phosphorylates Nonmuscle Myosin Light Chain Kinase to Regulate Endothelial Barrier Function. *Mol Biol Cell* 2010; 21: 4042-56.
- [28]. Iqbal S, Zhang S, Driss A, Liu ZR, Kim HR, Wang Y, et al. PDGF Upregulates Mcl-1 Through Activation of β -Catenin and HIF-1 α -Dependent Signaling in Human Prostate Cancer Cells. *PLoS ONE* 2012; 7: e30764.
- [29]. Jia DY, Du ZH, Liu SM, Liu HJ, Wang FW, Ling EA, et al. c-Abl is involved in high glucose-induced apoptosis in embryonic E12.5 cortical neural progenitor cells from the mouse brain. *Journal of Neurochemistry* 2008; 106: 1720-30.

- [30]. Pan B, Yang L, Wang J, Wang Y, Wang J, Zhou X, et al. c-Abl tyrosine kinase mediates neurotoxic prion peptide-induced neuronal apoptosis via regulating mitochondrial homeostasis. *Molecular Neurobiology* 2014; 49: 1102-1116.
- [31]. Chung KS, Han G, Kim BK, Kim HM, Yang JS, Ahn J, et al. A novel antitumor piperazine alkyl compound causes apoptosis by inducing RhoB expression via ROS-mediated c-Abl/p38 MAPK signaling. *Cancer Chemotherapy and Pharmacology* 2013; 72: 1315-1324.
- [32]. Wang X, Zeng L, Wang J, Chau JF, Lai KP, Jia D, et al. A positive role for c-Abl in Atm and Atr activation in DNA damage response. *Cell Death and Differentiation* 2010; 18: 5-15.
- [33]. Furlan A, Stagni V, Hussain A, Richelme S, Conti F, Prodosmo A, et al. Abl interconnects oncogenic Met and p53 core pathways in cancer cells. *Cell Death and Differentiation* 2011; 18: 1608-1616.
- [34]. Vashistha H, Meggs L. Diabetic nephropathy: lessons from the mouse. *The Ochsner journal* 2013; 13: 140-146.
- [35]. Zhou LL, Cao W, Xie C, Tian J, Zhou Z, Zhou Q, et al., The receptor of advanced glycation end products plays a central role in advanced oxidation protein products-induced podocyte apoptosis. *Kidney International* 2012; 82: 759-770.
- [36]. Gao F, Yao M, Shi Y, Hao J, Ren Y, Liu Q, et al. Notch pathway is involved in high glucose-induced apoptosis in podocytes via Bcl-2 and p53 pathways. *Journal of Cellular Biochemistry* 2013; 114: 1029-1038.
- [37]. Zhou Y, Zhang E, Berggreen C, Jing X, Osmark P, Lang S, et al. Survival of pancreatic beta cells is partly controlled by a TCF7L2-p53-p53INP1-dependent pathway. *Hum Mol Genet* 2012; 21: 196-207.
- [38]. Skírnisdóttir I, Seidal T. The apoptosis regulators p53, bax and PUMA: Relationship and impact on outcome in early stage (FIGO I-II) ovarian carcinoma after post-surgical taxane-based treatment. *Oncol Rep* 2012; 27:741-7.
- [39]. Freed-Pastor WA, Prives C. Mutant p53: one name, many proteins. *Genes Development* 2012; 26: 1268-1286.
- [40]. Chen C, Liang W, Jia J, van Goor H, Singhal PC, Ding G. Aldosterone Induces

Apoptosis in Rat Podocytes: Role of PI3-K/Akt and p38MAPK Signaling Pathways.
Nephron Experimental Nephrology 2009; 113: e26-34.

[41]. Liu Y, Liang W, Yang Q, Ren Z, Chen X, Zha D, et al. IQGAP1 Mediates Angiotensin II-Induced Apoptosis of Podocytes via the ERK1/2 MAPK Signaling Pathway. American Journal of Nephrology 2013; 38: 430-444

Figure 1:

Effect of hyperglycemia on podocyte structure. A: Representative transmission electron microscopy images of glomerular capillary loops in two groups. Original magnification $\times 2000$, scale, 1100nm. B: Quantitative analysis of the FP (foot process) width (n=8, n represents the number of mice in each group). *P<0.05 relative to control.

Figure 2:

Effect of hyperglycemia on c-Abl expression *in vivo*. A: Light microscopy evaluation of glomeruli c-Abl expression with immunohistochemistry staining in different groups. Original magnification $\times 400$, scale, 30 μm . (a1-a3, b1-b3): Representative microscopy images of glomeruli in normal and diabetes mice respectively. B: Quantitative analysis of glomeruli c-Abl expression integrated optical density (n=8, n represents the number of mice in each group). C: Western blot analysis of glomeruli c-Abl in different groups and quantitative analysis (n=8, n represents the number of mice in each group). *P<0.05 relative to control.

Figure 3:

Expression and distribution of c-Abl in the cultured podocytes under the high-glucose treatment. A and B: Representative immunofluorescence results of c-Abl distribution in cultured podocytes with medium

containing 30 mM glucose at various time points and representative western blot and quantitation of these results (n=3, n represents the number of repeat times). Original magnification $\times 400$, *scale*, 20 μm . C and D: Representative immunofluorescence results of c-Abl distribution in cultured podocytes exposed to different concentrations of glucose for 24 h and representative western blotting and quantitation of these results (n=3, n represents the number of repeat times). Original magnification $\times 400$, *scale*, 20 μm . *P<0.05 compared with cells for 0 h. #P<0.05 compared with cells exposed to normal glucose.

Figure 4:

Effect of high glucose on podocytes apoptosis. A. Representative immunofluorescence results of c-Abl distribution in cultured podocytes with different medium. Original magnification $\times 400$, *scale*, 20 μm . (a): 5 mM glucose for 24 h. (b): 5 mM glucose + 25 mM mannitol for 24 h. (c): 30 mM glucose for 24 h. (d): 30 mM glucose + c-Abl siRNA for 24 h. B. Western blot detection of c-Abl and p53 expression in cultured podocytes with different medium and quantitation of these results (n=3, n represents the number of repeat times). (NG): 5 mM glucose for 24 h. (MA): 5 mM glucose + 25 mM mannitol for 24 h. (HG): 30 mM glucose for 24 h. (HG + siRNA): 30 mM glucose + c-Abl siRNA for 24 h. *P<0.05 compared with cells exposed to normal glucose. #P<0.05 compared with cells exposed to high glucose. C and D: Representative Hoechst 33258 staining

of apoptotic podocytes (original magnification $\times 400$, scale, $20\ \mu m$) and flow cytometry analysis of apoptosis in cultured podocytes with different medium and quantitation of these results (n=3, n represents the number of repeat times). (a): 5 mM glucose for 24 h. (b): 5 mM glucose + 25 mM mannitol for 24 h. (c): 30 mM glucose for 24 h. (d): 30 mM glucose + c-Abl siRNA for 24 h. *P<0.05 compared with cells exposed to normal glucose. #P<0.05 compared with cells exposed to high glucose.

Figure 5:

Effect of high glucose on the interaction between c-Abl and p53 *in vitro*. Co-immunoprecipitation result of the interaction between c-Abl and p53 in cultured podocytes with different medium and quantitation of these results (n=3, n represents the number of repeat times). (a): 5 mM glucose for 24 h. (b): 5 mM glucose + 25 mM mannitol for 24 h. (c): 30 mM glucose for 24 h. (d): 30 mM glucose + c-Abl siRNA for 24 h. *P<0.05 compared with cells exposed to normal glucose. #P<0.05 compared with cells exposed to high glucose.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Table 1

Biochemical values of each group at the end of 12 week ($\bar{x}\pm s$)

Group	N	BG (mmol/L)	KW/BW (mg/g)	UAE (ug/24h)
Control	8	5.63 ± 0.67	7.92 ± 0.37	68.42 ± 12.33
Diabetic	8	23.49 ± 2.82*	12.08 ± 0.54*	895.61 ± 104.28*

BG: blood glucose; KW: kidney weight; BW: body weight;

UAE: urinary albumin excretion.

* $p<0.05$ compared with control group.