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Original Paper

GSK-3β and Vitamin D Receptor are Involved in β-Catenin and Snail Signaling in High Glucose-Induced Epithelial-Mesenchymal Transition of Mouse Podocytes

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Key Words
Diabetic nephropathy • Podocytes • Epithelial-mesenchymal transition • Glycogen synthase kinase 3β • β-catenin • Snail

Abstract
Background: Epithelial-mesenchymal transition (EMT) is recognized to play an important role in diabetic nephropathy (DN). ***Objective:*** To analyze the roles of glycogen synthase kinase 3β (GSK-3β), β-catenin and Snail signaling in high glucose (HG)-induced mouse podocytes EMT. ***Methods:*** Differentiated podocytes were divided into: the normal glucose group (NG: glucose 5.6mM), the HG groups (12.5HG: 12.5mM; 25HG: 25mM; and 50HG: 50mM of glucose), and the osmotic control group (NG+M: glucose 5.6mM and mannitol 44.4mM). GSK-3β, β-catenin and Snail were assessed using semi-quantitative RT-PCR, western blot and immunofluorescence. β-catenin and Snail pathways were assessed after down-regulating GSK-3β expression using an inhibitor (LiCl) or a small-interfering RNA (siRNA). ***Results:*** HG increased GSK-3β, β-catenin and Snail expressions, and promoted EMT, as shown by decreased nephrin expression (epithelial marker), and increased α-SMA expression (mesenchymal marker). GSK-3β inhibitor and GSK-3β siRNA decreased β-catenin and Snail expressions, and reversed HG-induced EMT. Immunofluorescence showed that GSK-3β and β-catenin did not completely overlap; β-catenin was transferred to the nucleus in the 25HG group. VDR seems to be involved in HG-induced β-catenin nuclear translocation. ***Conclusion:*** Down-regulating GSK-3β expression decreased β-catenin and Snail expression and reversed HG-induced podocytes EMT. Thus, modulating GSK-3β might be a target to slow or prevent DN.

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Introduction

Diabetic nephropathy (DN) is one of the most common microvascular complications in diabetes, and has become the leading cause of end-stage renal diseases (ESRD) [1]. DN is characterized by structural and functional changes in the kidney. The major clinical manifestations are proteinuria, hematuria and progressive chronic kidney diseases [2]. These complications may be slowed or prevented using the appropriate therapy [2]. Nevertheless, ESRD still affect 15 cases per 1000 patient-years [3].

Podocytes are located in the glomerular basement membrane, and are highly differentiated epithelial cells, forming the outermost layers of the glomerular filtration barrier. Recent studies showed that podocyte injury plays an important role in DN and in proteinuria [4], and accumulating evidences suggest that DN is more a "podocyte disease" [5]. Epithelial-mesenchymal transition (EMT) is a pathological phenomenon by which normal epithelial cells lose their characteristics, such as cell polarity and adhesion, and gain characteristics of mesenchymal cells, such as migration and invasion [6]. EMT is essential to normal embryogenesis and wound healing, but is also involved in organ fibrosis, metastases and DN [7]. Some studies support the concept that high glucose (HG) environment, such as the one observed in diabetes, could be involved in podocytes EMT [8-10]. Indeed, podocytes' proteins, such as nephrin and zonula occludens-1, are lost in the HG environment, while mesenchymal cell proteins such as α-SMA and fibronectin are expressed, leading to podocytes' dysfunction and proteinuria [11-14].

Glycogen synthase kinase 3β (GSK-3β) is a serine/threonine phosphate kinase involved in glucose synthesis, and plays an important role in variety of cell activities and functions, such as proliferation, migration, inflammation, immune response, glucose regulation and apoptosis [15]. Wnt/β-catenin is now recognized as one of the cell signaling pathways involved in EMT [16]. In addition, the Snail pathway affects EMT by modulating the expression of the downstream β-catenin [17]. GSK-3β plays an important role in the β-catenin pathway [18]. Vitamin D receptor (VDR) is involved in organ fibrosis through changes in β-catenin expression and intracellular localization [19].

GSK-3β is a key regulator of numerous signaling pathways [20], but the exact relationship between GSK-3β, β-catenin, Snail and VDR in podocytes' EMT under HG conditions is also still unclear. Thus, the present study was to analyze the role of GSK-3β, β-catenin, Snail and VDR in HG-induced podocytes' EMT. Results from the present study could provide new knowledge on DN pathogenesis and new ways to prevent or treat it.

Materials and Methods

Cell culture

Conditional immortalized mouse podocytes were provided by Professor Nie Jing (Southern Medical University, Guangzhou, China). Podocytes were cultured according to the method by Mundel et al. [21]. Undifferentiated podocytes were cultured in RPMI1640 medium (GIBCO, USA) with 10% fetal bovine serum (FBS) (GIBCO), 5.6mmol/L of glucose (Dingguo Changsheng Biotech Co., Ltd., Beijing, China), and 10U/ml of recombinant mice interferon-γ (Sangon Biotech, Shanghai, China), in an incubator at 33°C in 5% CO_2. After differentiation, podocytes were cultures at 37°C in RPMI1640 medium without recombinant mice interferon-γ. Podocytes were cultured for 12-14 days before being ready for the next experiments.

Five groups of podocytes were created: the normal glucose group (NG: 5.6mM of glucose), the high glucose groups (12.5HG: 12.5mM; 25HG: 25mM; and 50HG: 50mM of glucose), and the osmotic control group (NG+M: 5.6mM of glucose and 44.4mM of mannitol (Dingguo Changsheng Biotech Co., Ltd.), which had an osmotic pressure comparable with the 25HG group). Experiments also used water-soluble LiCl (Dingguo Changsheng Biotech Co., Ltd.), a GSK-3β inhibitor, a final concentration of 10mM of LiCl. Finally, a small interference RNA (siRNA) against GSK-3β (Genechem, Shanghai, China) was also used in this study.

Table 1. Primers and PCR reaction conditions. *Note*: GAPDH: glyceraldehydes 3-phosphate dehydrogenase; GSK-3β: glycogen synthase kinase 3β; VDR: vitamin D receptor

Primers	Size	Annealing temperature (°C)	Cycle number		Sequences
GAPDH	238bp	55	35	Forward	5'-CCTGCACCACCAACTGCTTAGC-3'
				Reverse	5'-CCAGTGAGCTTCCCGTTCAGC-3'
GSK-3β	152bp	60	35	Forward	5'-TTCAGGCCGCTGCTTCACCG-3'
				Reverse	5'-GTGCTGGTCTTTCCCGCGCA-3'
β-atenin	509bp	60	40	Forward	5'-GCCAAGTGGGTGGTATAGAGG-3'
				Reverse	5'-TTCAATGGGAGAATAAAGCAG-3'
Snail	150bp	56	35	Forward	5'-AGCCCAACTATAGCGAGCTG-3'
				Reverse	5'-CCAGGAGAGAGTCCCAGATG-3'
VDR	325bp	57	35	Forward	5'-GGTAGAGGGGGCAGGTTAGA-3'
				Reverse	5'-CAGTGTGGCTGCATTCCCTA-3'
nephrin	200bp	55	35	Forward	5'-CCCAGGTACACAGAGCACAA-3'
				Reverse	5'-CTCACGCTCACAACCTTCAG-3'
α-SMA	489bp	51	35	Forward	5'-TACTGCCGAGCGTGAGA-3'
				Reverse	5'-CTCACGCTCACAACCTTCAG-3'

siRNA transfection

The sequence of the siRNA against GSK-3β was 5'-CCA CTC AAG AAC TGT CAA GTA-3'), based on the GSK-3β full-length mouse gene (GeneBank NO. NM 019827.6). The sequence of the scramble siRNA was 5'-UUC UCC GAA CGU GUC ACG UTT-3'. GSK-3β and scramble siRNA were transfected into differentiated podocytes using Lipofectamine 2000 (Invitrogen Inc., Carlsbad, CA, USA).

Semi-quantitative RT-PCR

Total RNA was extracted from each group using Trizol (Invitrogen Inc., Carlsbad, CA, USA), according to the manufacturer's instructions. RNA purity was determined using absorbance at 260 and 280 nm (A260/280). RNA integrity was verified by formaldehyde-denaturing gel electrophoresis. cDNA was synthesized using 2μg of RNA and the First Strand cDNA Synthesis Kit (Fermentas, Burlington, Canada) using the M-MuLV Reverse Transcriptase and random primers. The sequences of specific primers were shown in Table 1. cDNA (1μl) was mixed with the forward and reverse primers (each 0.5μl), 2×PCR Master Mix (12.5μl) (Life Technologies Co., Grand Island, NY, USA) and deionized water (for total volume of 25μl), and reaction was performed in a thermocycler (BD Diagnostics, Sparks, MD, USA). PCR products were separated on 2.0% agarose gel electrophoresis. The Image J image software (National Institutes of Health, USA) was used to quantify the grayscale value.

Western blot

Total proteins were obtained from each group using the RIPA lysis buffer (Dingguo Changsheng Biotech Co., Ltd.). Proteins were quantified using a BCA protein assay kit (Dingguo Changsheng Biotech Co., Ltd.). Proteins (15-20μg) were separated using 10% SDS-PAGE, and were transferred on PVDF membranes (Roche, Mannheim, Germany). Non-specific sites were blocked using 5% powdered milk diluted in TBS with 0.05% Tween 20 (TBST) for 1h. Primary antibody was incubated overnight at 4°C. After washing the membranes, the secondary antibody was incubated at 37°C for 2h. An ECL chemiluminescence reagent kit (CoWin Biotech, Beijing, China) was used to reveal the bands. Blots were analyzed using the ImageJ software. Antibodies were: mouse polyclonal antibody against GAPDH (Shanghai Kang Cheng biological engineering Co., Ltd., China), mouse polyclonal antibody against β-catenin, antibody against Snail (Abcam, Cambridge, MA, USA), mouse polyclonal antibody against VDR (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and horseradish peroxidase-labeled goat anti-mouse secondary antibody (Dingguo Changsheng Biotech Co., Ltd.).

Immunofluorescence

Podocytes were stimulated by 25mM of glucose for 36h, and were fixed in 4°C 4% paraformaldehyde for 20 min, 0.6% Tween-20 for 15min, and blocked with 5% BSA blocking solution for 20 min at room temperature. Cells were exposed overnight to antibodies against β-catenin, GSK-3β (Abcam, Cambridge, MA, USA) and VDR (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Cells were washed three times in PBS. Cells were revealed using the Alexa Fluor 488 goat anti-rabbit IgG (H+L) (green) and the Alexa Fluor 594 donkey anti-mouse IgG (H+L) (red) (Invitrogen Inc., Carlsbad, CA, USA) at 37°C for 1 h. Fluorescence was observed using a fluorescence microscope (Olympus, CX31-32RFL, Japan).

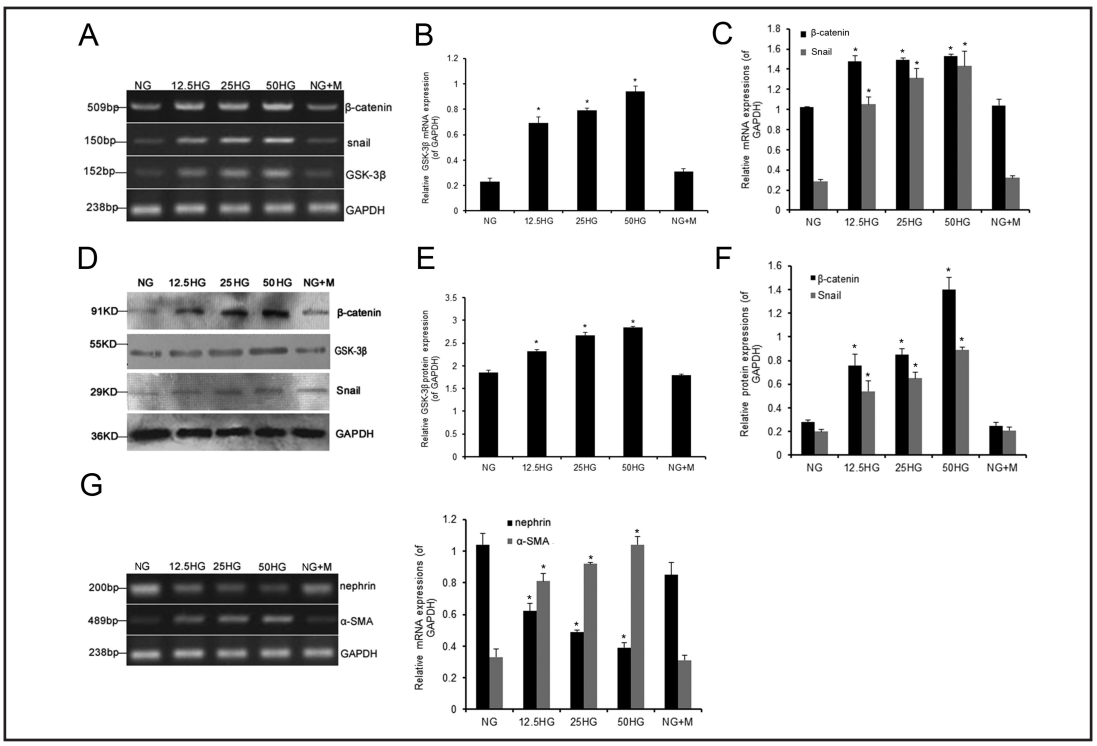

Fig. 1. Changes in GSK-3β, β-catenin, Snail, α-SMA and nephrin expressions under different glucose concentrations. The differentiated podocytes were cultured in the normal glucose group (NG: 5.6mM of glucose), the high glucose groups (12.5HG: 12.5mM; 25HG: 25mM; and 50HG: 50mM of glucose), and the osmotic control group (NG+M: 5.6mM of glucose and 44.4mM of mannitol, which had an osmotic pressure comparable with the 25HG group) for 36 hours. (A) GSK-3β, β-catenin and Snail mRNA expressions were determined by Semi-quantitative RT-PCR; (B) Relative mRNA expression of GSK-3β; (C) Relative mRNA expressions of β-catenin and Snail, GAPDH was used as an internal control; (D) GSK-3β, β-catenin and Snail protein expressions were determined by western blot; (E) Relative protein expression of GSK-3β; (F) Relative protein expressions of β-catenin and Snail, Protein expression was normalized to GAPDH. (G) Relative mRNA expressions of α-SMA and nephrin. The data represents the means ± standard error of the mean (SEM) of three independent experiments. **P*<0.05, ***P*<0.01 *vs.* NG group.

Statistical analysis

All data were obtained from three independent experiments, and are expressed as mean ± standard error of the mean (SEM). SPSS17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Statistical significance was evaluated by one-way analysis of variance (ANOVA) with Student-Newman-Keuls (SNK) test for post hoc analysis. P-values <0.05 were considered statistically significant.

Results

HG stimulation up-regulated GSK-3β, β-catenin and Snail expression and promoted EMT in the differentiated podocytes

Using differentiated podocytes cultured in the normal glucose group (NG: 5.6mM of glucose), the high glucose groups (12.5HG: 12.5mM; 25HG: 25mM; and 50HG: 50mM of glucose), and the osmotic control group (NG+M: 5.6mM of glucose and 44.4mM of mannitol) for 36 hours, RT-PCR and Western blot experiments showed that with increasing glucose levels, the expressions of GSK-3β, β-catenin and Snail mRNA and protein increased (all P<0.05), and there was no significant difference between NG and NG+M group (Fig. 1A-1F).

To confirm HG promoting podocytes' EMT, the expressions of the epithelial marker, nephrin, and of the mesenchymal marker, α-SMA, were determined by RT-PCR. Compared

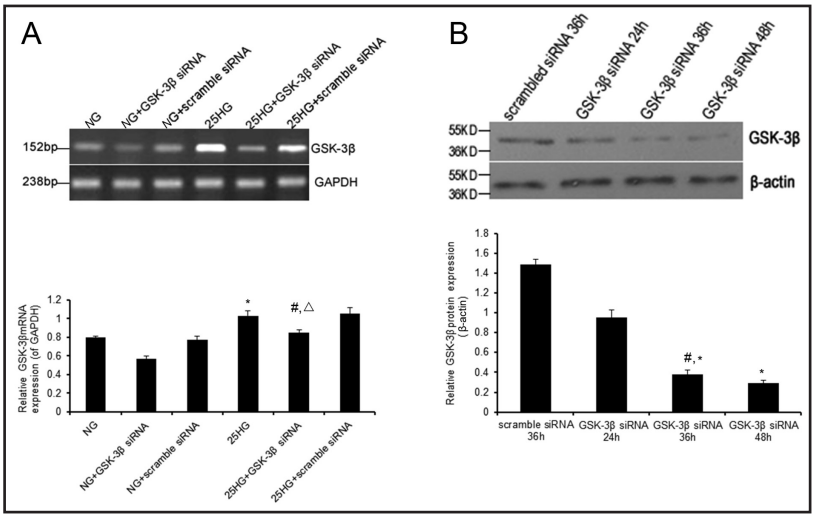

Fig. 2. The effects of GSK-3β siRNA on the mRNA and protein expressions of GSK-3β. (A) Semi-quantitative RT-PCR showing mRNA expression of GSK-3β, GAPDH was used as an internal control; **P*<0.05 *vs.* NG group; #*P*<0.05 *vs.* 25HG group; Δ*P*<0.05 25HG+GSK-3β siRNA *vs.* 25HG+scramble siRNA; (B) Western blot showing protein expression of GSK-3β. Protein expression was normalized to β-actin. **P*<0.05 *vs.* GSK-3β siRNA 24h; #*P*<0.05, ##*P*<0.01 GSK-3βsiRNA 36h *vs.* scrambled siRNA 36h. The data represents the means ± SEM of three independent experiments.

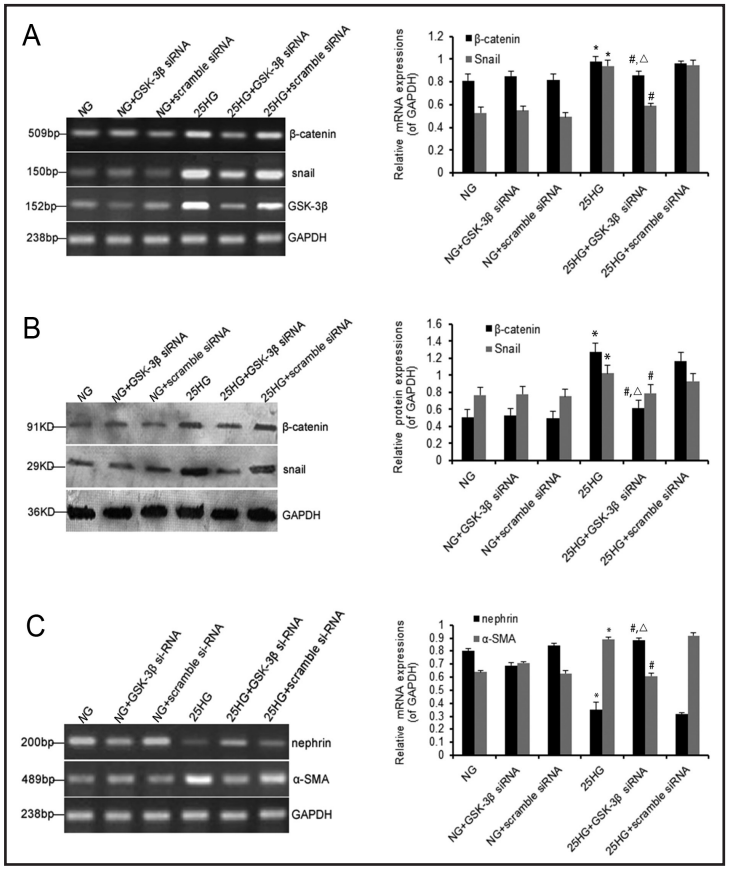

Fig. 3. The effects of GSK-3β siRNA on the expressions of β-catenin, Snail, α-SMA and nephrin. NG and 25HG podocytes were transfected with GSK-3β siRNA or scramble siRNA for 36h. (A) Relative mRNA expressions of β-catenin and Snail were determined by semi-quantitative RT-PCR, GAPDH was used as an internal control; (B) Relative protein expressions of β-catenin and Snail were determined by western blot. Protein expression was normalized to GAPDH; (C) Relative mRNA expressions of α-SMA and nephrin were determined by semi-quantitative RT-PCR. The data represents the means ± SEM of three independent experiments. **P*<0.05 *vs.* NG group; #*P*<0.05 *vs.* 25HG group; Δ*P*<0.05 25HG+ GSK-3β siRNA *vs.* 25HG+scramble siRNA.

with the NG group, HG (50mM) down-regulated the expression of nephrin mRNA by 62.5%, and increased the expression of α-SMA mRNA by 68.3%, and osmotic pressure didn't affect the podocytes' EMT (Fig. 1G).

GSK-3β siRNA decreases β-catenin and Snail expressions and reverses HG-induced EMT in the differentiated podocytes

Compared with the scramble siRNA, GSK-3β siRNA effectively decreased GSK-3β mRNA expressions by 28.8% and 17.5% in NG and 25HG podocytes, respectively (Fig. 2A),

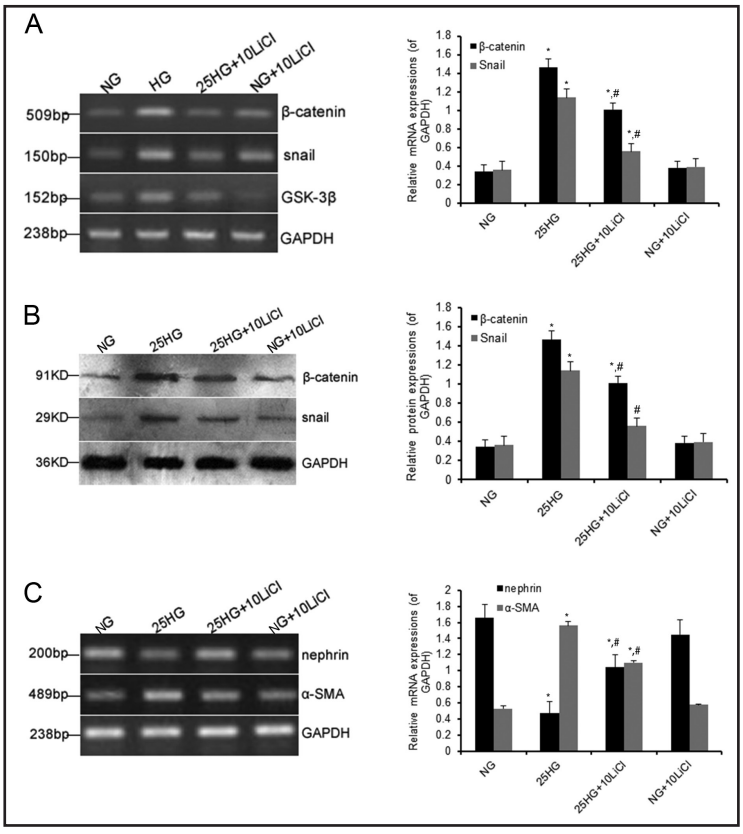

Fig. 4. The effects of GSK-3β inhibitor (LiCl) on the expressions of β-catenin, Snail, α-SMA and nephrin. Podocytes from the NG and 25HG groups were treated with GSK-3β inhibitor LiCl (10mM) for 36h. (A) Relative mRNA expressions of β-catenin and Snail were determined by semi-quantitative RT-PCR, GAPDH was used as an internal control; (B) Relative protein expressions of β-catenin and Snail were determined by western blot. Protein expression was normalized to GAPDH; (C) Relative mRNA expressions ofα-SMA and nephrin were determined by semi-quantitative RT-PCR. The data represents the means ± SEM of three independent experiments. **P*<0.05 *vs.* NG group; #*P*<0.05 25HG + 10LiCl group *vs.* 25HG group.

and also decreased GSK-3β protein expressions (Fig. 2B).

NG and 25HG podocytes were transfected with GSK-3β siRNA or scramble siRNA for 36h. β-catenin and Snail mRNA and protein expression levels were detected. As shown in Figures 3A and 3B, β–catenin and Snail mRNA and protein levels were increased in the 25HG group (all *P*<0.05). However, expressions of β-catenin and Snail mRNA (β-catenin: 0.86±0.03 *vs.* 0.96±0.02; Snail: 0.59±0.02 *vs.* 0.95±0.04; both *P*<0.05) and protein (β-catenin: 0.62±0.09 *vs.* 1.17±0.10; Snail: 0.79±0.10 *vs.* 0.93±0.09; both *P*<0.05) in the 25HG group transfected with GSK-3β siRNA were decreased compared with the 25HG group transfected with scramble siRNA.

Figure 3C showed that GSK-3β siRNA attenuated HG induced-podocytes' EMT, as shown by an increased nephrin mRNA expression (0.88±0.02 *vs.* 0.32±0.01, *P*<0.05), and a decreased α-SMA mRNA expression (0.61±0.02 *vs.* 0.92±0.02, *P*<0.05) in the 25HG group transfected with GSK-3β siRNA, compared with the 25HG group transfected with scramble siRNA.

GSK-3β inhibitor LiCl decreases β-catenin and Snail expressions and reverses HG-induced EMT in the differentiated podocytes

Podocytes from the NG and 25HG groups were treated with GSK-3β inhibitor LiCl (10mM) for 36h, and β-catenin and Snail mRNA and protein expression levels were detected. As shown in Figures 4A and 4B, β-catenin and Snail mRNA and protein were increased in the 25HG group (all *P*<0.05). After treatment with LiCl, expression of β-catenin and Snail mRNA and protein in the 25HG group were decreased compared with the untreated group, but remained higher than in the NG group (all *P*<0.05).

Figure 4C showed that LiCl attenuated HG induced-podocytes' EMT, as shown by an increased nephrin mRNA expression (1.04±0.16 *vs.* 0.47±0.15, *P*<0.05), and a decreased α-SMA mRNA expression (1.10±0.02 *vs.* 1.56±0.05, *P*<0.05) in the 25HG group treated with LiCl, compared with the 25HG group.

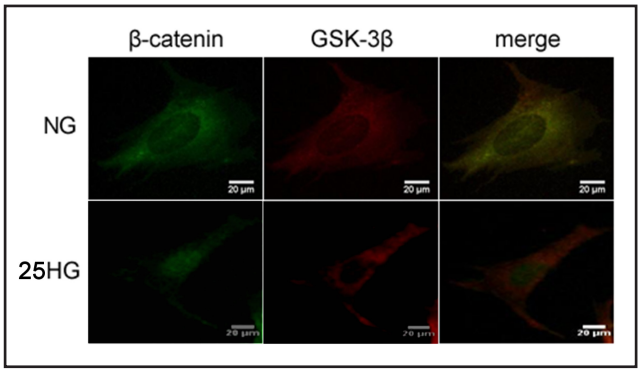

Fig. 5. GSK-3β and β-catenin immunofluorescence in NG and 25HG podocytes (×400).HG environment increased the expression of GSK-3β and β-catenin compared with the NG group. In the HG group, β-catenin was localized in the nucleus. In the HG group, GSK-3β and β-catenin did not completely overlap. Green: Alexa Fluor 488; Red: Alexa Fluor 594.

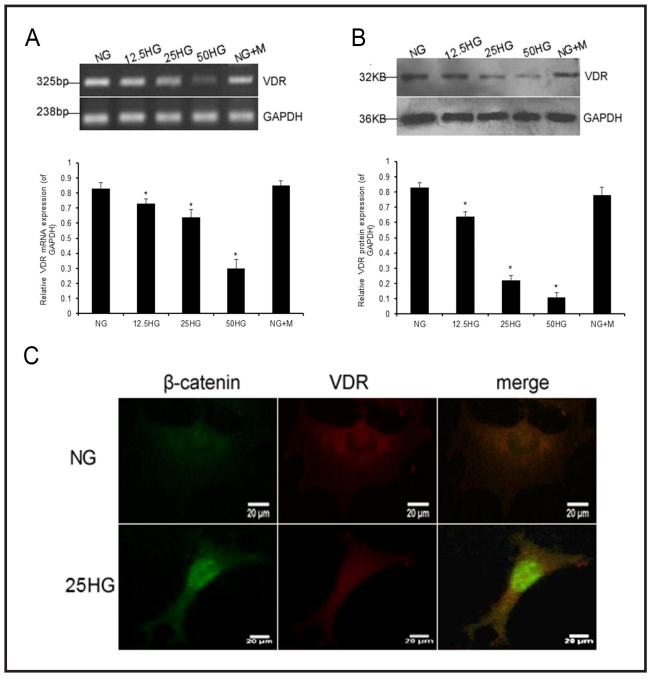

Fig. 6. Changes in VDR mRNA and protein expressions under different glucose concentrations. (A) Relative mRNA expression of VDR was determined by semi-quantitative RT-PCR, GAPDH was used as an internal control; (B) Relative protein expression of VDR was determined by western blot. Protein expression was normalized to GAPDH. The data represents the means ±SEM of three independent experiments. **P*<0.05 *vs.* NG group; (C) VDR and β-catenin immunofluorescence in NG and 25HG podocytes (×400).β-catenin and VDR overlapped in both the 25HG and NG groups. Green: Alexa Fluor 488; Red: Alexa Fluor 594.

HG environment changes the cellular localization of GSK-3β and β-catenin in the differentiated podocytes

Podocytes from the NG and 25HG groups were treated with fluorescence-labeled antibodies against GSK-3β and β-catenin. As shown in Figure 5, GSK-3β and β-catenin expression were increased in the 25HG group. In addition, in the 25HG group, β-catenin was transferred to the nucleus. Immunofluorescence showed that GSK-3β and β-catenin did not completely overlap in the HG group compared with the NG group. We speculated that β-catenin may was not completely influenced by GSK-3β in high glocose environment of podocytes, and that there may be other unknown pathways regulateing β-catenin's expression and intracellular localization.

HG stimulation down-regulated VDR expression in the differentiated podocytes

Using differentiated podocytes cultured in different glucose concentration for 36 hours, RT-PCR and western blot experiments showed that VDR expression was decreased with increasing concentrations of glucose (all *P*<0.05), and there was no significant difference between NG and NG+M group (Fig. 6A and 6B).

Podocytes from the NG and 25HG groups were treated with fluorescence-labeled antibodies against VDR and β-catenin. As shown in Figure 6C, VDR and β-catenin overlapped in the 25HG and NG groups, and β-catenin was transferred to the nucleus in the 25HG group.

Experiments showed that the podocytes' EMT process in HG environment may also involve VDR regulating β-catenin's intracellular localization.

Discussion

GSK-3β is necessary to maintain epithelial cells' structure [22]. TGFβ/Smad, ILK and Wnt/β-catenin are recognized to participate in the signaling pathways leading to cell transdifferentiation [7, 23, 24], GSK-3β plays an important role in these three pathways by providing a connecting link between them. Thus, changes in GSK-3β activity will affect the expression of differentiation factors, thus affecting the EMT process. Studies have found that GSK-3β participate in renal EMT [25]. In the present study, down-regulating GSK-3β expression decreased β-catenin and Snail expression and reversed HG-induced EMT of podocytes.

Wnt signaling pathway is mainly involved in the regulation of cell differentiation, especially in malignant tumor cells, affecting the invasion and mobility of tumor cell [26]. β-catenin is a multifunctional protein, and is a key factor involved in classic Wnt signaling [27]. Under normal circumstances, free β-catenin is phosphorylated and degraded by the proteasome. When Wnt signaling is activated, most β-catenin is unphosphorylated, leading to β-catenin accumulation in the cytoplasm, entry into the nucleus in combination with T cells nuclear factor/lymphatic enhancement factors (TCF/LEF), and modulation of the expression of target genes involved in a variety of physiological mechanisms [23]. Snail gene is activated by the Wnt/β-catenin pathway [28]. High expression of Snail can decrease the expression of E-cadherin, reducing the adhesion between epithelial cells, and participating in EMT [29]. On the other hand, high expression of Snail interact with β-catenin, further activating Wnt signaling and activating target genes [28], leading to EMT. Studies showed that GSK-3β can modulate Snail activity [24, 30].

GSK-3β participates in podocytes' EMT in the HG environment [24]. However, whether GSK-3β regulates Wnt/β-catenin signaling and its effects on podocytes' EMT under HG were still unclear. The present study showed that with increasing glucose levels, GSK-3β expression increased, affecting the expression of β-catenin and Snail. When down-regulation of GSK-3β expression, the expression of β-catenin and Snail decreased. According to our results, we speculated that GSK-3β may regulate the expression of β-catenin and Snail to influence podocytes' EMT in the HG environment.

It was usually believed that GSK-3β could phosphorylate β-catenin, resulting in proteasome degradation [23, 27]. However, in recent years, studies on GSK-3β and Wnt/β-catenin signaling in tumor cells suggested that inhibition of GSK-3β could both inhibit or promote Wnt/β-catenin signaling [31]. GSK-3β has two ways to modulate β-catenin, revealing that GSK-3β can negatively regulate Wnt/β-catenin signaling through degradation of β-catenin (the traditional view), but can also positively control expression of β-catenin by an even more complex regulatory mechanism [32, 33]. Thus, we speculated that HG-induced EMT is not entirely mediated by the classical GSK-3β pathway, but that other factors are involved. Thus, using an indirect immunofluorescence method, we observed that the localization of GSK-3β and β-catenin in the cell nucleus did not fully overlap in the HG group compared with the NG group, confirming our inference that β-catenin expression is not entirely modulated by GSK-3β through the classical Wnt/β-catenin pathway.

Therefore, we hypothesized that the influence of the HG environment on β-catenin in podocytes may not only be through GSK-3β, but also from other pathways influencing β-catenin's expression and intracellular localization. Studies in breast and colon cancer cells found that VDR, β-catenin and Snail are interrelated [34]. Studies showed that vitamin D and VDR may help to alleviate EMT, and that nucleus β-catenin could be reduced [35, 36]. When VDR is activated, it will compete with β-catenin for combination with TCF-4, thus inhibiting the activity of β-catenin in colon cancer [36]. In a mouse model of unilateral ureteral obstruction, VDR was related to the degree of renal fibrosis, and was involved in

EMT, together with β-catenin [19]. Consequently, in addition to the classical GSK-3β/Wnt/β-catenin pathway, we consider that HG-induced podocytes' EMT might also involve VDR. Therefore, the present study provides preliminary results on the relation between VDR, GSK-3β and β-catenin in the HG environment in podocytes.

In conclusion, HG environment increased GSK-3β, β-catenin and Snail expressions, and promoted EMT in differentiated podocytes. Down-regulating GSK-3β expression decreased β-catenin and Snail expression and reversed HG-induced EMT of podocytes. VDR seems to be involved in HG-induced β-catenin nuclear translocation. Thus, results from the present study provide refinements about DN pathogenesis, as well as clues for new treatment targets.

Acknowledgements

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Conflicts of Interest

The authors declare that they have no conflict of interest.

References

1 Cowie CC, Port FK, Wolfe RA, Savage PJ, Moll PP, Hawthorne VM: Disparities in incidence of diabetic end-stage renal disease according to race and type of diabetes. N Engl J Med 1989;321:1074-1079.
2 de Boer IH, Rue TC, Cleary PA, Lachin JM, Molitch ME, Steffes MW, Sun W, Zinman B, Brunzell JD, White NH, Danis RP, Davis MD, Hainsworth D, Hubbard LD, Nathan DM: Long-term renal outcomes of patients with type 1 diabetes mellitus and microalbuminuria: An analysis of the diabetes control and complications trial/epidemiology of diabetes interventions and complications cohort. Arch Intern Med 2011;171:412-420.
3 Pavkov ME, Knowler WC, Bennett PH, Looker HC, Krakoff J, Nelson RG: Increasing incidence of proteinuria and declining incidence of end-stage renal disease in diabetic pima indians. Kidney Int 2006;70:1840-1846.
4 Widmann TJ, Dahmann C: Wingless signaling and the control of cell shape in drosophila wing imaginal discs. Dev Biol 2009;334:161-173.
5 Kim JJ, Li JJ, Jung DS, Kwak SJ, Ryu DR, Yoo TH, Han SH, Choi HY, Kim HJ, Han DS, Kang SW: Differential expression of nephrin according to glomerular size in early diabetic kidney disease. J Am Soc Nephrol 2007;18:2303-2310.
6 Thiery JP, Sleeman JP: Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 2006;7:131-142.
7 Kong D, Li Y, Wang Z, Sarkar FH: Cancer stem cells and epithelial-to-mesenchymal transition (emt)-phenotypic cells: Are they cousins or twins? Cancers (Basel) 2011;3:716-729.
8 Lv Z, Hu M, Zhen J, Lin J, Wang Q, Wang R: Rac1/pak1 signaling promotes epithelial-mesenchymal transition of podocytes in vitro via triggering beta-catenin transcriptional activity under high glucose conditions. Int J Biochem Cell Biol 2013;45:255-264.
9 Wang C, Liu X, Ke Z, Tang Y, Li CC, Li CM, Ye Z, Zhang J, Lou T: Mesangial medium from iga nephropathy patients induces podocyte epithelial-to-mesenchymal transition through activation of the phosphatidyl inositol-3-kinase/akt signaling pathway. Cell Physiol Biochem 2012;29:743-752.
10 Boini KM, Xia M, Xiong J, Li C, Payne LP, Li PL: Implication of cd38 gene in podocyte epithelial-to-mesenchymal transition and glomerular sclerosis. J Cell Mol Med 2012;16:1674-1685.
11 Rincon-Choles H, Vasylyeva TL, Pergola PE, Bhandari B, Bhandari K, Zhang JH, Wang W, Gorin Y, Barnes JL, Abboud HE: Zo-1 expression and phosphorylation in diabetic nephropathy. Diabetes 2006;55:894-900.
12 Xu ZG, Ryu DR, Yoo TH, Jung DS, Kim JJ, Kim HJ, Choi HY, Kim JS, Adler SG, Natarajan R, Han DS, Kang SW: P-cadherin is decreased in diabetic glomeruli and in glucose-stimulated podocytes in vivo and in vitro studies. Nephrol Dial Transplant 2005;20:524-531.
13 Tesar V, Zima T: Recent progress in the pathogenesis of nephrotic proteinuria. Crit Rev Clin Lab Sci 2008;45:139-220.

14 Jefferson JA, Shankland SJ, Pichler RH: Proteinuria in diabetic kidney disease: A mechanistic viewpoint. Kidney Int 2008;74:22-36.
15 Jope RS, Yuskaitis CJ, Beurel E: Glycogen synthase kinase-3 (gsk3): Inflammation, diseases, and therapeutics. Neurochem Res 2007;32:577-595.
16 Shapiro IM, Cheng AW, Flytzanis NC, Balsamo M, Condeelis JS, Oktay MH, Burge CB, Gertler FB: An emt-driven alternative splicing program occurs in human breast cancer and modulates cellular phenotype. PLoS Genet 2011;7:e1002218.
17 Min S, Xiaoyan X, Fanghui P, Yamei W, Xiaoli Y, Feng W: The glioma-associated oncogene homolog 1 promotes epithelial-mesenchymal transition in human esophageal squamous cell cancer by inhibiting e-cadherin via snail. Cancer Gene Ther 2013;20:379-385.
18 Naves MA, Requiao-Moura LR, Soares MF, Silva-Junior JA, Mastroianni-Kirsztajn G, Teixeira VP: Podocyte wnt/ss-catenin pathway is activated by integrin-linked kinase in clinical and experimental focal segmental glomerulosclerosis. J Nephrol 2012;25:401-409.
19 Xiong M, Gong J, Liu Y, Xiang R, Tan X: Loss of vitamin d receptor in chronic kidney disease: A potential mechanism linking inflammation to epithelial-to-mesenchymal transition. Am J Physiol Renal Physiol 2012;303:F1107-1115.
20 Doble BW, Woodgett JR: Gsk-3: Tricks of the trade for a multi-tasking kinase. J Cell Sci 2003;116:1175-1186.
21 Mundel P, Reiser J, Zuniga Mejia Borja A, Pavenstadt H, Davidson GR, Kriz W, Zeller R: Rearrangements of the cytoskeleton and cell contacts induce process formation during differentiation of conditionally immortalized mouse podocyte cell lines. Exp Cell Res 1997;236:248-258.
22 Doble BW, Woodgett JR: Role of glycogen synthase kinase-3 in cell fate and epithelial-mesenchymal transitions. Cells Tissues Organs 2007;185:73-84.
23 Zerlin M, Julius MA, Kitajewski J: Wnt/frizzled signaling in angiogenesis. Angiogenesis 2008;11:63-69.
24 Zhou BP, Deng J, Xia W, Xu J, Li YM, Gunduz M, Hung MC: Dual regulation of snail by gsk-3beta-mediated phosphorylation in control of epithelial-mesenchymal transition. Nat Cell Biol 2004;6:931-940.
25 Liu Y: Epithelial to mesenchymal transition in renal fibrogenesis: Pathologic significance, molecular mechanism, and therapeutic intervention. J Am Soc Nephrol 2004;15:1-12.
26 Rappa G, Mercapide J, Anzanello F, Le TT, Johlfs MG, Fiscus RR, Wilsch-Brauninger M, Corbeil D, Lorico A: Wnt interaction and extracellular release of prominin-1/cd133 in human malignant melanoma cells. Exp Cell Res 2013;319:810-819.
27 Rao TP, Kuhl M: An updated overview on wnt signaling pathways: A prelude for more. Circ Res 2010;106:1798-1806.
28 Stemmer V, de Craene B, Berx G, Behrens J: Snail promotes wnt target gene expression and interacts with beta-catenin. Oncogene 2008;27:5075-5080.
29 Peinado H, Ballestar E, Esteller M, Cano A: Snail mediates e-cadherin repression by the recruitment of the sin3a/histone deacetylase 1 (hdac1)/hdac2 complex. Mol Cell Biol 2004;24:306-319.
30 Dominguez D, Montserrat-Sentis B, Virgos-Soler A, Guaita S, Grueso J, Porta M, Puig I, Baulida J, Franci C, Garcia de Herreros A: Phosphorylation regulates the subcellular location and activity of the snail transcriptional repressor. Mol Cell Biol 2003;23:5078-5089.
31 Dirnberger D, Seuwen K: Signaling of human frizzled receptors to the mating pathway in yeast. PLoS One 2007;2:e954.
32 Zeng X, Huang H, Tamai K, Zhang X, Harada Y, Yokota C, Almeida K, Wang J, Doble B, Woodgett J, Wynshaw-Boris A, Hsieh JC, He X: Initiation of wnt signaling: Control of wnt coreceptor lrp6 phosphorylation/activation via frizzled, dishevelled and axin functions. Development 2008;135:367-375.
33 Zeng X, Tamai K, Doble B, Li S, Huang H, Habas R, Okamura H, Woodgett J, He X: A dual-kinase mechanism for wnt co-receptor phosphorylation and activation. Nature 2005;438:873-877.
34 Palmer HG, Larriba MJ, Garcia JM, Ordonez-Moran P, Pena C, Peiro S, Puig I, Rodriguez R, de la Fuente R, Bernad A, Pollan M, Bonilla F, Gamallo C, de Herreros AG, Munoz A: The transcription factor snail represses vitamin d receptor expression and responsiveness in human colon cancer. Nat Med 2004;10:917-919.
35 Fretz JA, Zella LA, Kim S, Shevde NK, Pike JW: 1,25-dihydroxyvitamin d3 regulates the expression of low-density lipoprotein receptor-related protein 5 via deoxyribonucleic acid sequence elements located downstream of the start site of transcription. Mol Endocrinol 2006;20:2215-2230.
36 Palmer HG, Gonzalez-Sancho JM, Espada J, Berciano MT, Puig I, Baulida J, Quintanilla M, Cano A, de Herreros AG, Lafarga M, Munoz A: Vitamin d(3) promotes the differentiation of colon carcinoma cells by the induction of e-cadherin and the inhibition of beta-catenin signaling. J Cell Biol 2001;154:369-387.