Wnt/β-Catenin Signaling Modulates Survival of High Glucose–Stressed Mesangial Cells

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Glomerulosclerosis and diabetic nephropathy are attributable to high glucose induction of mesangial cell apoptosis. Whereas Wnt signaling has been found to regulate renal morphogenesis and pathogenesis, the biologic role of Wnt/β-catenin signaling in controlling high glucose–induced mesangial cell apoptosis is not well defined. Herein is reported that Wnt/β-catenin signaling is required for protecting glomerular mesangial cells from high glucose–mediated cell apoptosis. High glucose downregulated Wnt4 and Wnt5a expression and the subsequent nuclear translocation of β-catenin, whereas it increased glycogen synthase kinase-3β (GSK-3β) and caspase-3 activities and apoptosis of glomerular mesangial cells. Suppression of GSK-3β activation or increase in nuclear β-catenin by transfection of Wnt4 or Wnt5a or stable β-catenin (S33Y) reversed Akt activation and reduced the high glucose–mediated caspase-3 cleavage and cell apoptosis. Pharmacologic inhibition of GSK-3β by recombinant Wnt5a or bromoindirubin-3′-oxime or LiCl increased Akt phosphorylation and β-catenin translocation and abrogated high glucose–mediated proapoptotic activities. Exogenous bromoindirubin-3′-oxime treatment reduced phospho-Ser389-GSK-3β and β-catenin expression and apoptosis of cells adjacent to glomeruli in diabetic kidneys and attenuated urinary protein secretion in diabetic rats. Taken together, mesangial cells responded to high glucose by impairing that canonical Wnt pathway to increase proapoptotic activities. Sustaining Wnt/β-catenin signaling is beneficial for promoting survival of mesangial cells that are exposed to high glucose stress.


Advanced stages of glomerulosclerosis are characterized by loss of cellular components (1) and accumulation of extracellular matrix around glomerular mesangial cells (2,3). Increased mesangial cell apoptosis has been found to mediate the pathogenesis of glomerular sclerosis and crescentic glomerulonephritis (4,5) and correlate with proteinuria in remnant kidney (6,7). Hyperglycemia has been shown to induce renal glomerulosclerosis in patients with diabetes (8). Increased mesangial cell apoptosis correlates with extracellular matrix accumulation in hyperglycemia-induced segmentation of glomerular tuft (9). High glucose through provoking proapoptotic signaling or sensitivity to TGF-β1 increases apoptosis of mesangial cells (10,11). Whereas high glucose–induced mesangial cell apoptosis contributes to the development of glomerulosclerosis and diabetic nephropathy, the molecular mechanism by which high glucose promotes mesangial cell apoptosis is not well defined.

Canonical Wnt proteins via inactivation of glycogen synthase kinase-3β (GSK-3β) and β-catenin translocation into the nucleus increase Wnt responsive gene transcription (12). Wnt signaling molecules act as potent regulators for renal tissue morphogenesis and pathogenesis. Wnt4 regulates mesenchymal-to-epithelial transition during nephrogenesis (13) and controls cell-cycle progression during tissue regeneration after acute renal failure (14). Transplantation of fibroblasts that express Wnt4 proteins under the renal capsule induces lesions with tubular epithelial destruction in mice (15). Elevated β-catenin expression correlates with renal dysplasia and increased collecting duct cysts in ALK3 transgenic mice (16). Transgenic mice with overexpression of β-catenin display severe polycystic lesions in glomeruli, proximal and distal tubules, and collecting ducts (17). Decreased β-catenin is associated with excess TGF-β1 synthesis and dysfunction of peritoneal mesothelial cells in the presence of high glucose (18). We recently found that TGF-β1 was involved in reactive oxygen radical–mediated fibronectin accumulation of high glucose–stressed mesangial cells and early renal injuries in diabetic rats (19).

GSK-3β signaling regulates many biologic processes, including cell death, cell survival, and transcriptional regulation of several cell types (20,21). Modulation of GSK-3β activity has been reported to control glucose metabolism of renal cells (22).
and hypertonic-induced apoptosis of renal medullary interstitial cells (23). Whereas previous studies have suggested that Wnt signaling molecules are important for modulating renal cell function, the biologic role of Wnt/β-catenin signaling pathway in high glucose–stressed glomerular mesangial cells is not well defined. We hypothesized that Wnt signaling may be involved in regulating the fate of mesangial cells that are exposed to high-glucose conditions. The purposes of this study were to investigate whether Wnt/β-catenin signaling in mesangial cells is altered in the presence of high glucose and whether modulation of Wnt/β-catenin signaling controls high glucose–induced apoptosis in mesangial cells and in a rodent model of diabetic nephropathy.

Materials and Methods

Cell Cultures

Rat mesangial cells and mouse SV40 MES-13 glomerular mesangial cells (American Type Culture Collection, Manassas, VA) were cultured, respectively, in DMEM and 10% FBS (Life Technologies, Gaithersburg, MD) or a mixture of DMEM and Ham’s F12 medium (3:1; vol/vol), 5% FBS; and 14 mM HEPES in a 5% CO₂, 37°C incubator for 6 d and harvested by trypsinization for further studies. Cell viability was determined using trypan blue exclusion.

High Glucose Treatment

Cells (1 × 10⁶ cells/well, six-well plate) were cultured in basal medium (5 mM d-glucose) with or without 35 mM d-glucose for 96 h. Cell cultures that were exposed to 35 mM mannitol were used as osmolar control. In some experiments, cells were co-cultured in high glucose with 250 ng/ml recombinant Wnt5a (R&D Systems, Minneapolis, MN) or pretreated with 10 mM LiCl or 10 mM pyruvate to inhibit glycolysis. In some experiments, cells were co-cultured in high glucose with 250 ng/ml recombinant Wnt5a (R&D Systems, Minneapolis, MN) or pretreated with 10 mM LiCl or 10 mM pyruvate to inhibit glycolysis. In some experiments, cells were co-cultured in high glucose with 250 ng/ml recombinant Wnt5a (R&D Systems, Minneapolis, MN) or pretreated with 10 mM LiCl or 10 mM pyruvate to inhibit glycolysis. In some experiments, cells were co-cultured in high glucose with 250 ng/ml recombinant Wnt5a (R&D Systems, Minneapolis, MN) or pretreated with 10 mM LiCl or 10 mM pyruvate to inhibit glycolysis. In some experiments, cells were co-cultured in high glucose with 250 ng/ml recombinant Wnt5a (R&D Systems, Minneapolis, MN) or pretreated with 10 mM LiCl or 10 mM pyruvate to inhibit glycolysis. In some experiments, cells were co-cultured in high glucose with 250 ng/ml recombinant Wnt5a (R&D Systems, Minneapolis, MN) or pretreated with 10 mM LiCl or 10 mM pyruvate to inhibit glycolysis. In some experiments, cells were co-cultured in high glucose with 250 ng/ml recombinant Wnt5a (R&D Systems, Minneapolis, MN) or pretreated with 10 mM LiCl or 10 mM pyruvate to inhibit glycolysis.

Cell proliferation was measured using a Cell Proliferation Kit (Boehringer Mannheim GmbH, Mannheim, Germany). Briefly, cells (2 × 10⁶ cells/well, 96-well plate) with or without high glucose in the presence of Wnt5a or Wnt5a and 10 mM LiCl were plated to reach 60 to 80% confluence and transfected using FuGENE 6 transfection reagent (Roche Diagnostic Corp., Indianapolis, IN). Cells that were stably transfected with the plasmids were selected in medium that contained 600 μg/ml G418 (Life Technologies, Gaithersburg, MD).

Cell Growth

Cell proliferation was measured using a Cell Proliferation Kit (Boehringer Mannheim GmbH, Mannheim, Germany). Briefly, cells (2 × 10⁶ cells/well, 96-well plate) with or without high glucose in the presence of Wnt5a or Wnt5a and 10 mM LiCl were plated to reach 60 to 80% confluence and transfected using FuGENE 6 transfection reagent (Roche Diagnostic Corp., Indianapolis, IN). Cells that were stably transfected with the plasmids were selected in medium that contained 600 μg/ml G418 (Life Technologies, Gaithersburg, MD).

Real-Time PCR

Total RNA was extracted and purified from 10⁶ cells using QIAzol reagent (Qiagen, Valencia, CA). Total RNA (1 μg) was reverse-transcribed into cDNA. Twenty-five microliters of PCR mixture that contained cDNA template equivalent to 20 ng of total RNA, 2.5 μM each forward and reverse primer, and 2× iQ SYBR Green Supermix was amplified using the iCycler iQ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA) with an initial melt at 95°C for 5 min followed by 40 cycles at 94°C for 15 s, 52°C for 20 s, and 72°C for 30 s using the following primer oligonucleotide sequences followed by PCR amplification: Wnt1 (forward 5'-ATG TGA CCC AGG ATG TTC G-3'; 162 bp expected), Wnt4 (forward 5'-GCC AGC CAC TAA AGG AGA AG-3', reverse 5'-GCC CTA AGG GTT GCT GC-3'; 215 bp expected), Wnt5a (forward 5'-AGC CGA GAG ACA GCC TTC AC-3', reverse 5'-TCC TGC GAC CTG CTTCATG-3'; 289 bp expected), and β-adrenoceptors (forward 5'-GCC CTA AGG GAA GT-3', reverse 5'-CGT CAC CCG AGT CCA TCA-3'; 168 bp expected). The number of amplification steps required to reach an arbitrary intensity threshold (Ct) was computed. The relative gene expression levels were presented as 2^-ΔΔCt, where ΔΔCt = Ct_target - Ct_vehicle. Fold change for the treatment was defined as the relative expression, compared with the vehicle and was calculated as 2^-ΔΔCt, where ΔΔCt = ΔCt_treatment - ΔCt_vehicle (19).

Western Blotting

Membrane, cytosolic, and nuclear extracts of cell cultures were prepared as described previously (19). Aliquots of cytosolic or nuclear extracts (100 μg) were subjected to Western blot assay. The designated proteins on the blots were probed by antibodies against GSK-3β, phospho-Ser²-GSK-3β, phospho-Ser³⁸-Akt, β-catenin, caspase-3, cleaved caspase-3, and phospho–poly(ADP-ribose) polymerase (phospho-PARP) (Cell Signaling Technology, Beverly, MA), followed by horseradish peroxidase–conjugated IgG as the secondary antibody and visualized with chemiluminescence agents. Protein band intensity on each blot from three repeated experiments was quantified by scan densitometry. The fold of increase was calculated by dividing the band intensity from the high glucose–stressed sample by that of the respective control sample.

Wnt4, Wnt5a, and β-Catenin cDNA Transfection

cDNA encoding Wnt4 or Wnt5a or stable (S33Y) β-catenin or wild-type β-catenin (24) were ligated and cloned, respectively, into pUSE (Upstate Biotechnology, Lake Placid, NY) and pC1-neo vectors. Cells (5 × 10⁵ cells/well, in six-well plate) were plated to reach 60 to 80% confluence and transfected using FuGENE 6 transfection reagent (Roche Diagnostic Corp., Indianapolis, IN). Cells that were stably transfected with the plasmids were selected in medium that contained 600 μg/ml G418 (Life Technologies, Gaithersburg, MD).

Terminal Deoxynucleotidyl Transferase–Mediated Deoxyuridine Triphosphate-Biotin Nick End-Labeling

Trypsinized and floating cells that were cultured in high glucose with or without Wnt signaling modulators were pooled, spun (1 × 10⁶ cells) onto glass slides, and fixed in 70% methanol, and 70% methanol for investigation of cell apoptosis using in situ cell death detection kits (Roche Diagnostics, Mannheim, Germany). Specimens that were pretreated with 50 U/ml DNase I (Sigma Chemical, St. Louis, MO) or incubated in reaction buffer without terminal deoxynucleotidyl transferase were used as positive or negative controls. Terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate-biotin nick end-labeling (TUNEL)-stained cells were recognized using fast red as substrates.

Streptozotocin-Induced Diabetes

Four-month-old male Wistar rats were caged in pairs and maintained on rodent diet and water ad libitum. Diabetes in rats was induced as described previously (19). Briefly, diabetes was induced by a single intraperitoneal injection of 50 mg/kg streptozotocin (Sigma Chemical). One week after injection, blood glucose was measured from tails. Rats with blood glucose >300 mg/dl, defined as successful induction of diabetes, were used for succeeding experiments. For equalization of blood glucose levels in all diabetic rats, intermittent-acting insulin was administered subcutaneously once a day until the rats were killed.
Blood glucose levels were measured every day just before insulin injections. The dose of insulin was adjusted to reach the target blood glucose level of 200 to 250 mg/dl. All studies were approved by the Institutional Animal Care and Use Committee of the hospital.

**Exogenous BIO Treatment**

Diabetic rats were given BIO subcutaneously (n = 6; 200 µg/kg per d) or vehicle (n = 6; 200 µl of corn oil) for 28 consecutive days. Six rats without streptozotocin injections were used as normal controls. At day 28, urine was collected using metabolic cage systems, and urinary protein and creatinine levels were measured using respective assay kits (Sigma-Aldrich, St. Louis, MO). Rats were killed with an overdose of pentobarbital sodium, and kidneys were harvested for immunohistochemical analysis. After perfusion with PBS, fresh kidney tissues were ground with a mortar and pestle under liquid nitrogen; lysed with ice-cold PBS that contained 1% NP-40, 0.1% SDS, 0.5% sodium deoxycholate, 100 µg/ml PMSF, and 30 µg/ml aprotinin; and homogenized by ultrasonication. Aliquots of kidney tissue homogenate (50 µg) were subjected to assessment of Wnt4 and Wnt5a, phospho-Ser9-GSK-3β, and β-catenin expression using immunoblotting.

**Immunohistochemistry**

Kidneys were fixed in 4% PBS-buffered formaldehyde, embedded in paraffin, sliced longitudinally into 5-µm-thick sections, and subjected to immunohistochemical or TUNEL staining. Antibodies against Wnt4, Wnt5a, phospho-Ser9-GSK-3β and β-catenin were used for immunohistochemistry. Immunoreactivity in sections was demonstrated using a horseradish peroxidase–3,3’-diaminobenzidine kit (R&D Systems), followed by counterstaining with hematoxylin, dehydration, and mounting. Sections without primary antibodies were enrolled as negative controls for immunostaining. Six regions within renal glomeruli from three sections that were obtained from four rats were studied. Each region that contained positive immunostained cells were analyzed microscopically and quantitatively (Carl Zeiss, Gottingen, Germany). Three random images from each selected region then were taken, captured, and analyzed under ×400 magnification using image analysis software (Media Cybernetics, Silver Spring, MD). The percentage of positive immunolabeled cells and total cells in each area was counted. Renal mesangial and tubular cells were identified morphologically. Apoptosis of mesangial cell cultures was counted as the ratio of TUNEL-positive stained mesangial cell number and total cell number under ×200 magnification.

**Statistical Analyses**

All values were expressed as means ± SE calculated from at least three repeated experiments. Wilcoxon test was used to evaluate differences between the sample of interest and its respective control. For...
analysis of time course, a multiple range of ANOVA and post hoc tests were used. $P < 0.05$ was considered significant.

**Results**

**High Glucose–Induced Caspase-3 Activation and Cell Apoptosis**

We examined whether high glucose altered proliferation or apoptosis of mesangial cells. In comparison with the control groups, high glucose significantly reduced cell proliferation (Figure 1A) and promoted cell apoptosis (Figure 1B) by 48 h. We further examined whether high glucose–mediated cell apoptosis was linked to caspase-3 activation. Immunoblotting showed that high glucose significantly increased the levels of cleaved caspase-3 and activated PARP (Figure 1C). Inhibition of caspase-3 activity by Z-DEVD-FMK reduced the high glucose–mediated activation of caspase-3, PARP (Figure 1D), and cell apoptosis (Figure 1B). Furthermore, TUNEL staining indicated that high glucose induces DNA fragmentation, which was abrogated with caspase-3 inhibitor pretreatment (Figure 1E).

**High Glucose Downregulated Wnt/β-Catenin Signaling**

We investigated whether high-glucose stress could alter Wnt/β-catenin signaling. Real-time PCR showed that high glucose altered Wnt1, Wnt3a, Wnt4, and Wnt5a mRNA expression (Figure 2A). Of the Wnt expression, high glucose reduced Wnt4 and Wnt5a expression by $>50\%$ throughout the study period and was analyzed further. A reduction in phosphorylation of Ser$^\beta$ in GSK-3β is known to increase GSK-3β activity and attenuate β-catenin translocation into the nucleus. An antibody against phospho-Ser$^\beta$-GSK-3β was used to determine the level of GSK-3β activation (25). Immunoblotting showed that high glucose reduced Wnt4 and Wnt5a expression, which correlated with a reduction in phospho-Ser$^\beta$-GSK-3β levels and nuclear β-catenin expression (Figure 2B). These findings indicate that mesangial cells respond to high glucose by promoting GSK-3β activation and suppressing Wnt/β-catenin signaling.

**Wnt4, Wnt5a, and β-Catenin Signaling Is Required for Cell Survival**

We examined the biologic role of Wnt4, Wnt5a, and β-catenin in high glucose–mediated mesangial cell apoptosis. Transfection of mesangial cells with either Wnt4 or Wnt5a (Figure 3A) suppressed high glucose–mediated GSK-3β activity as evidenced by increases in phosphorylated Ser$^\beta$-GSK-3β and Akt activation and nuclear β-catenin accumulation (Figure 3B). Overexpression of Wnt4 or Wnt5a reduced the promoting effect of HG on caspase-3 and PARP activation (C), cell apoptosis (D), and increased cell growth (E). Mesangial cells were subjected to transfection with pUSE or Wnt4 or Wnt5a cDNA. Stably transfected cell cultures were subjected to HG stress for 48 h. Immunoblotting of total GSK-3β or nuclear caspase-3 showed equal loadings and transfer for all lanes. $P < 0.05$, *vehicle- and #HG-treated groups.
We investigated whether pharmacologic modulation of Wnt/\(\beta\)-catenin signaling could alter proapoptotic activities of mesangial cells that are exposed to high glucose. Recombinant Wnt5a or GSK-3\(\beta\) inhibitors BIO or LiCl suppressed high glucose–mediated activation of GSK-3\(\beta\) and restored nuclear \(\beta\)-catenin levels and phospho-Akt expression (Figure 5A). Wnt/GSK-3\(\beta\) modulators abrogated high-glucose induction of caspase-3, PARP activation (Figure 5B), and cell apoptosis (Figure 5C) and increased cell proliferation (Figure 5D).

**Figure 4.** Transfection of stable \(\beta\)-catenin (S33Y) increased nuclear \(\beta\)-catenin and phosphorylated Akt expression (A) and reduced the promoting effect of HG on caspase-3 and PARP activation (B), cell apoptosis (C), and reversed cell proliferation (D). Mesangial cells were subjected to transfection with wild-type or stable \(\beta\)-catenin cDNA. Stably transfected cell cultures were subjected to HG stress for 48 h. Immunoblotting of total caspase-3 and actin showed equal loadings and transfer for all lanes. *P < 0.05, vehicle and HG-treated groups.

**Figure 5.** Recombinant Wnt5a and (2′Z, 3′E)-6-bromoindirubin-3′-oxime (BIO) and LiCl increased the inhibitory phospho-Ser9-GSK-3\(\beta\), nuclear \(\beta\)-catenin, and phosphorylated Akt expression (A) and abrogated HG-induced caspase-3 and PARP activation (B) and cell apoptosis (C). (D) Wnt5a and GSK-3\(\beta\) inhibitors reversed proliferation of cell cultures. Cell cultures were co-cultured or pretreated with 250 ng/ml recombinant Wnt5a or 10 mM LiCl or 10 \(\mu\)M BIO for 48 h. Immunoblotting of total GSK-3\(\beta\) and caspase-3 showed equal loadings and transfer for all lanes. *P < 0.05 from the vehicle- and HG-treated groups.

Wnt Signaling Modulators Alleviated High Glucose–Mediated Apoptotic Signaling

We investigated whether pharmacologic modulation of Wnt/\(\beta\)-catenin signaling could alter proapoptotic activities of mesangial cells that are exposed to high glucose. Recombinant Wnt5a or GSK-3\(\beta\) inhibitors BIO or LiCl suppressed high glucose–mediated activation of GSK-3\(\beta\) and restored nuclear \(\beta\)-catenin levels and phospho-Akt expression (Figure 5A). Wnt/GSK-3\(\beta\) modulators abrogated high-glucose induction of caspase-3, PARP activation (Figure 5B), and cell apoptosis (Figure 5C) and increased cell proliferation (Figure 5D).

**BIO Alleviated Urinary Protein Secretion and Mesangial Cell Apoptosis in a Model of Diabetic Nephropathy**

We examined whether modulation of GSK-3\(\beta\) signaling by BIO could alter diabetes-induced glomerulopathy. In comparison with the normal group, diabetes significantly increased blood glucose and urinary protein excretion (Table 1). BIO treatment significantly reduced the promoting effect of diabetes on urinary protein secretion (Table 1). BIO treatment did not seem to alter blood glucose in diabetic rats throughout the study period.

In the diabetes group, mesangial cells in glomeruli and tubular cells surrounding glomeruli displayed intensive DNA fragmentation as demonstrated by positive TUNEL staining. Fewer mesangial cells and tubular cells adjacent to glomeruli of diabetic kidney with BIO treatment showed TUNEL staining (Figure 6A). Immunoblotting showed that diabetes decreased Wnt4, Wnt5a, phospho-Ser\(^9\)-GSK-3\(\beta\), and \(\beta\)-catenin expression in kidney tissue. BIO treatment reduced the suppressing effect of diabetes on phospho-Ser\(^9\)-GSK-3\(\beta\) and \(\beta\)-catenin expression but not Wnt4 or Wnt5a expression in the kidneys (Figure 6B).

In the absence of primary antibodies, no immunostaining was visible (Figures 7 and 8). Cells that were adjacent to glomeruli and showed positive Wnt4, Wnt5a, phospho-Ser\(^9\)-GSK-3\(\beta\), and \(\beta\)-catenin immunoeexpression exhibited brown color in cell periphery or cytoplasm. In the diabetes group, mesangial cells and tubular cells around glomeruli in the renal cortex expressed lower amounts of Wnt4, Wnt5a (Figure 7), phospho-Ser\(^9\)-GSK-3\(\beta\), and \(\beta\)-catenin (Figure 8) when compared with the normal group. In the BIO groups, mesangial cells and tubular cells expressed evident phospho-Ser\(^9\)-GSK-3\(\beta\) and \(\beta\)-catenin expression (Figure 8). We found that diabetes significantly promoted cell apoptosis that was associated with attenuated Wnt4, Wnt5a, phospho-Ser\(^9\)-GSK-3\(\beta\), and \(\beta\)-catenin immunoreactivities in renal glomeruli of diabetic rats. BIO treatment did not markedly affect Wnt4 and Wnt5a expression in renal tissue (Table 1).
Table 1. Biochemical characteristics and Wnt4, Wnt5a, phospho-GSK-3β, β-catenin, and TUNEL expression in renal glomerular mesangial cells of diabetic rats with or without BIO treatment

| Characteristic          | Normal          | Diabetes Vehicle | Diabetes BIO  
<table>
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<tbody>
<tr>
<td>Blood glucose</td>
<td>89.8 ± 7.6</td>
<td>212.3 ± 19.4^d</td>
<td>241.4 ± 21.6^d</td>
</tr>
<tr>
<td>Urinary protein secretion</td>
<td>0.7 ± 0.1</td>
<td>2.0 ± 0.4^d</td>
<td>0.8 ± 0.6^e</td>
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<tr>
<td>Immunohistochemistry</td>
<td></td>
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<tr>
<td>Wnt4</td>
<td>43.4 ± 1.8</td>
<td>14.5 ± 1.4^d</td>
<td>15.3 ± 1.9^d</td>
</tr>
<tr>
<td>Wnt5a</td>
<td>31.9 ± 3.5</td>
<td>14.1 ± 3.1^d</td>
<td>16.4 ± 2.6^d</td>
</tr>
<tr>
<td>Phospho-Ser^β-GSK-3β</td>
<td>24.8 ± 3.7</td>
<td>6.7 ± 1.1^d</td>
<td>18.5 ± 1.4^e</td>
</tr>
<tr>
<td>β-catenin</td>
<td>34.6 ± 3.9</td>
<td>11.0 ± 1.2^d</td>
<td>22.0 ± 0.9^e</td>
</tr>
<tr>
<td>TUNEL</td>
<td>7.0 ± 1.0</td>
<td>48.8 ± 2.3^d</td>
<td>16.1 ± 1.4^e</td>
</tr>
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^aBIO, (2’Z,3’E)-6-bromoindirubin-3’-oxime; GSK-3β, glycogen synthase kinase-3β; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end-labeling.

^bData are means ± SE calculated from six rats. Blood glucose (mg/dl) was measured from tails. Total urinary protein secretion (mg/mg creatinine) was assayed using urine protein kits and normalized with total creatinine level in urine.

^cData are means ± SE calculated from percentage of positive immunostained cells in glomeruli per high-power field in three regions from three sections of four rats.

P < 0.05, *normal and ^diabetic vehicle group.

Discussion

In this study, increased proapoptotic activities in mesangial cells followed high-glucose stress, which downregulated Wnt/β-catenin signaling. Whereas previous studies have demonstrated that high glucose–promoted mesangial cell apoptosis is attributable to increasing oxidative stress or altering growth factor expression (26–28), little research has been done to define the biologic role of Wnt/β-catenin in regulating the homeostasis of mesangial cells that are exposed to high glucose. Our findings provide the first indication that high-glucose induction of mesangial cell apoptosis was through activation of GSK-3β and subsequent destabilization of β-catenin–responsive cell survival activities. We suggest that it is the modulation of apoptosis–survival-regulatory molecules by altering canonical Wnt signal transduction pathway that bring about high-glucose promotion of mesangial cell apoptosis.

We found that mesangial cells that were exposed to high-glucose conditions responded by activating GSK-3β and subsequently promoted proapoptotic cascades. GSK-3β is reported to regulate apoptosis of renal medullary interstitial cells (23) and glucose intolerance in GSK-3β transgenic mice and diabetic animals (29,30). In our study, inhibiting GSK-3β activation by LiCl or BIO reduced high glucose–promoted caspase-3 and PARP phosphorylation and cell apoptosis, suggesting that GSK-3β–dependent signaling pathways are involved in mediating high-glucose stress to increase apoptotic programs of mesangial cells.

We noted that high glucose suppressed nuclear β-catenin translocation, Akt activation, and growth of mesangial cells. β-Catenin is involved in regulating apoptosis of renal cell carcinoma (31) and multiple kidney cysts (32). Increased phosphorylated β-catenin expression was noted in placenta vessels of patients with diabetes (33), suggesting that diabetes may destabilize β-catenin signaling to alter tissue function. We provide evidence that β-catenin signaling acts as a survival-stimulatory molecule for mesangial cells that are exposed to high glucose. These are based on the findings that increasing nuclear β-catenin accumulation reduced high glucose–promoted DNA fragmentation and reversed Akt phosphory-
ylation and proliferation of stable β-catenin–transfected cell cultures. To our knowledge, this is the first report that high glucose raised mesangial cell apoptosis that is regulated by attenuation of β-catenin signaling. Stabilization of β-catenin is required for sustaining survival of mesangial cells that are exposed to high-glucose stress.

We previously showed that alteration of Wnt signaling correlates with renal injury (34,35). In this study, we found that mesangial cells that were exposed to high-glucose conditions reduced Wnt expression. Restoring Wnt4 or Wnt5a expression by gene transfection or recombinant protein reduced high glucose–induced cell apoptosis. These findings suggest that Wnt4 and Wnt5a molecules are beneficial for promoting mesangial cell survival. Previous studies demonstrated that Wnt5a signals through a β-catenin–independent pathway or by inhibition of the canonical Wnt signaling pathway to regulate chondrocyte dedifferentiation (36,37). In our study, cell cultures that overexpressed Wnt4 or Wnt5a raised nuclear β-catenin accumulation to promote mesangial cell survival, suggesting that Wnt4 and Wnt5a initialized a β-catenin–responsive mechanism. We speculate the discrepancy that Wnt4 and Wnt5a regulation of β-catenin signaling depends on cell type and stimulation used.

To our knowledge, control of GSK-3β and β-catenin signaling in glomerular mesangial cells of the diabetic kidney in vivo has not been reported previously. This study provides the first evidence that glomerular mesangial cells and tubular cells in diabetic kidneys displayed weak Wnt level, phospho-Ser9-GSK-3β, and β-catenin expression. Impairing GSK-3β activation by exogenous BIO administration increased β-catenin signaling and alleviated diabetes-induced glomerular mesangial cell death and urinary protein secretion in diabetic rats. These phenomena in vivo are in line with those of cell culture models. We cannot exclude the possibility that high glucose may alter Wnt/β-catenin signaling in tubular cells. The role of Wnt signaling in regulating diabetes-stressed tubular cells needs to be explored in the future. We suggested that renal mesangial cells actively respond to high-glucose stress by altering Wnt/β-catenin signaling and subsequently promoted cell apoptosis. The role of mesangial cell apoptosis in proteinuria and diabetic glomerulosclerosis remains controversial. Previous studies suggested that apoptosis of resident glomerular mesangial cells is the earliest cellular lesion in the development diabetic nephropathy (38,39), and several bioactive molecules are involved in regulating apoptotic activities of high glucose–stressed mesangial cells (40,41). Our observation revealed that high glucose perturbed Wnt/β-catenin signaling and induced glomerular mesangial cell apoptosis and proteinuria. GSK-3β and β-catenin had a distinct role in modulating mesangial cell fate. We cannot exclude the possibilities that other Wnt-signaling molecules may be linked to high glucose–induced mesangial cell apoptosis. Further studies are needed to define the biologic role of these molecules in diabetic nephropathy.

Figure 7. Representative photographs of Wnt4 and Wnt5a immunostaining of glomeruli in diabetic kidneys with or without BIO treatment. At low-power field, cells that were located in diabetic kidney tissue with vehicle or BIO treatment displayed weak Wnt4 and Wnt5a expression when compared with the normal group. At high-power field, mesangial and tubular cells around glomeruli in diabetic kidneys with vehicle or BIO treatment expressed weak Wnt 4 and Wnt5a expression. Immunostained cells exhibited brown color in cell periphery and cytoplasm. Magnifications: ×100 and ×400.
Conclusion

Taken together, we have provided evidence that canonical Wnt signaling is involved in controlling high glucose–mediated mesangial cell death. Modulation of canonical Wnt/GSK-3β/β-catenin signal transduction is beneficial for enhancing mesangial cell survival in diabetic kidney.

Acknowledgments

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References


