Troglitazone ameliorates high glucose-induced EMT and dysfunction of SGLTs through PI3K/Akt, GSK-3β, Snail1, and β-catenin in renal proximal tubule cells

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High glucose (HG) itself has been reported to be associated with most HG-induced effects and functional and structural changes of renal proximal tubular cells (PTCs) (15, 27, 50). One of the potential mechanisms of HG-induced renal dysfunction is the epithelial-mesenchymal transition (EMT), which contributes to the generation of renal fibrosis (54). EMT is characterized by the loss of epithelial features and the gain of mesenchymal features. Tubular EMT is an orchestrated, highly regulated process that consists of four key steps: 1) loss of epithelial cell adhesion, 2) de novo α-smooth muscle actin (α-SMA) expression and actin reorganization, 3) disruption of tubular basement membrane, and 4) enhanced cell migration and invasion (23, 38, 45). EMT is a dynamic and complex process that may require the participation of growth factors or cytokines and integration of multiple signal pathways at different stages. Transforming growth factor-β1 (TGF-β1) is a key mediator of EMT, principally via the activation of the smad signaling pathway (16, 29). The loss of epithelial markers and acquisition of mesenchymal features are achieved through a well-orchestrated transcription program that involves three families of transcription factors, the Snail (Snail1, Snail2), ZEB (ZEB1, SIP1/ZEB2), and basic helix-loop-helix (bHLH) (E12/E47, Twist, Ids) families (24, 35, 38). After delocalization from the membranous complex with E-cadherin, the accumulation of cytoplasmic β-catenin and its subsequent nuclear translocation is also a key event of EMT (5, 17, 38).

Understanding the mechanism of EMT is essential in establishing novel therapeutic strategies for the prevention or arrest of progressive renal failure. In strategies to disrupt any one of the EMT steps, peroxisome proliferator-activated receptor-γ (PPARγ) agonists have shown promise. In the kidney, PPARγ, one of the nuclear hormone receptor superfamilies, is expressed in different cells including the proximal tubules, but the highest concentration can be found in the collecting duct cells (13, 21, 55). As PPARγ agonists, thiazolidinediones (TZDs) regulate cell growth, inflammation, lipid metabolism, and insulin sensitivity (22). In recent years, several clinical studies have demonstrated beneficial effects in patients with type 2 diabetes treated with PPARγ agonists. Based on these observations, we questioned whether treatment with a PPARγ agonist could regulate HG-induced EMT and membrane transport function. However, there are few reports that HG induces EMT except for recent studies with streptozotocin (STZ)-induced diabetic rats (9, 29) and with human peritoneal mesothelial cells (62). Moreover, although the molecular regulation of EMT has been extensively studied in renal cells, the mechanisms linking EMT to cellular transport dysfunction in renal PTCs have not been reported.

For our cell model, we chose primary cultured renal PTCs, because they exhibit a number of functions that typify differentiated renal PTCs in vivo: a polarized morphology, distinctive proximal tubule transport, and various hormone responses (12). Moreover, previous uptake studies with 14C-labeled α-methyl-d-glucopyranoside (α-MG) indicated that these cells stably maintain the activity of their transport system in culture (40, 42). Thus the outcomes of membrane transport studies...
with these PTCs could be compared directly with the original renal tissue. In addition, PTCs in hormonally defined, serum-free culture conditions provide a valuable in vitro model for examining the effect of HG on the renal Na+-glucose cotransporters (SGLTs). Here, we investigate whether treatment with the PPARY agonist troglitazone or alteration of EMT-mediated signaling pathways can prevent or reverse HG-induced EMT and SGLT dysfunction in primary cultured renal PTCs.

**MATERIALS AND METHODS**

**Animals and materials.** New Zealand White male rabbits (1.5–2.0 kg) were purchased from Daehan Experimental Animals (Chungju, Korea). All animal management procedures followed the standard operation protocols of Seoul National University. The Institutional Review Board at Chonnam National University approved the research proposal and the relevant experimental procedures, including those for animal care. In addition, all authors were Doctors of Veterinary Medicine with licenses granted from the Ministry of Agriculture and Forestry of Republic of Korea. Class IV collagenase, d-glucose, l-glucose, mannitol, hydrogen peroxide (H2O2), TGF-β1, fenofibrate, L-165,041, N-acetylcyesteine (NAC), LY 294002, FITC-conjugated anti-rabbit IgG, FITC-conjugated anti-mouse IgM, α-SMA, FITC-conjugated α-SMA, LiCl, and monoclonal α-actin were obtained from Sigma-Aldrich (St. Louis, MO). Soybean trypsin inhibitor and DMEM/F-12 medium were purchased from GibCO BRL (Grand Island, NY). Akt inhibitor and GW 9662 were acquired from Calbiochem (La Jolla, CA). Troglitazone was supplied by Biomol (Plymouth Meeting, PA). α-MG was purchased from DuPont/NEN (Boston, MA). Goat anti-rabbit IgG, goat anti-mouse IgG, E-cadherin, Na+/H+ exchangers NHE1 and NHE3, phosphatidylinositol 3-kinase (PI3K), Akt (Ser 473), Snail, β-catenin, and lamin A/C antibodies were acquired from Santa Cruz Biotechnology (Santa Cruz, CA). Phosphoglycerogen synthase kinase-3β (GSK-3β; Ser 9) antibody was obtained from Cell Signaling Technology (Danvers, MA). Rabbit anti-SGLT1 was supplied by Chemicon (Temecula, CA), and rabbit anti-SGLT2 was from Alpha Diagnostic (San Antonio, TX). Lissocinat was obtained from National Diagnostics (Parsippany, NJ). All other reagents were of the highest purity commercially available.

**Cell preparation and culture conditions.** Primary rabbit renal PTC cultures were prepared as previously described (12). The PTCs were grown in DMEM/F-12 medium supplemented with 15 mM HEPES and 20 mM sodium bicarbonate (pH 7.4). Three other growth supplements (5 μg/ml insulin, 5 μg/ml transferrin, and 5 × 10−8 M hydrocortisone) were added immediately before the medium was used. The kidneys from a rabbit were perfused through the renal artery. In addition, PTCs in hormonally defined, serum-free culture conditions provide a valuable in vitro model for examining the effect of HG on the renal Na+-glucose cotransporters (SGLTs). Here, we investigate whether treatment with the PPARY agonist troglitazone or alteration of EMT-mediated signaling pathways can prevent or reverse HG-induced EMT and SGLT dysfunction in primary cultured renal PTCs.

**Fig. 1. Effect of high glucose (HG) on epithelial-mesenchymal transition (EMT) and membrane transport function.** A and B: proximal tubule cells (PTCs) were treated with HG (25 mM) for 0–72 h (A) or HG, H2O2 (10−4 M), or transforming growth factor (TGF)-β1 (5 ng/ml) for 72 h (B). Protein was extracted and blotted with antibodies against E-cadherin, vimentin, α-smooth muscle actin (α-SMA), or β-actin. Each example shown is representative of 3 independent experiments. Values are means ± SE of 3 experiments for each condition determined from densitometry relative to β-actin. *P < 0.05 vs. control. C: cells were treated with HG, H2O2, or TGF-β1 for 72 h. Na+/H+ exchanger (NHE1 and NHE3) and Na+–glucose cotransporters (SGLT1 and SGLT2) protein expression levels were determined by Western blotting of the plasma membrane protein fraction. Each example shown is representative of 3 independent experiments. Values are means ± SE of 3 experiments for each condition determined from densitometry relative to β-actin. D: cells were treated with 25 mM d-glucose, 25 mM mannitol, 25 mM l-glucose, H2O2, or TGF-β1 for 72 h. Then, the α-methyl-d-glucopyranoside (α-MG) uptake was measured. Values are means ± SE of 3 independent experiments from triplicate dishes. *P < 0.05 vs. control.
EFFECT OF TROGLITAZONE ON HG-INDUCED EMT AND α-MG UPTAKE

A

HG 0 12 24 48 72 (hr)

E-cadherin

Vimentin

α-SMA

β-actin

Relative Optical Density (% of Control)

B

HG 0 + + +
H₂O₂ − − + −
TGFβ1 − − − +

E-cadherin

Vimentin

α-SMA

β-actin

Relative Optical Density (% of Control)

C

HG − + − −
H₂O₂ − − + −
TGFβ1 − − − +

NHE1

NHE3

β-actin

SGLT1

SGLT2

Relative Optical Density (% of Control)

D

14C-α-MG uptake (pmol/min)

Control D-glucose Mannitol L-glucose H₂O₂ TGFβ1

Relative Optical Density (% of Control)

Control HG H₂O₂ TGFβ1
oxidized to the highly fluorescent 2',7'-dichlorofluorescin (DCF). ROS generation was detected as a result of the oxidation of DCFH (excitation, 488 nm; emission, 515–540 nm) using a Fluoview 1000 fluorescent microscope (Olympus). The effect of DCF photooxidation was minimized by collecting the fluorescent image with a single rapid scan (line average, 4; total scan time, 5.2 s) and identical parameters, such as contrast and brightness, for all samples.

Preparation of cytosolic and membrane fractions. After confluent cultures were treated under the indicated conditions, the media was removed and the cells were washed twice with ice-cold PBS, scraped, harvested by microcentrifugation, and resuspended in buffer A [137 mM NaCl, 8.1 mM Na2HPO4, 2.7 mM KCl, 1.5 mM KH2PO4, 2.5 mM EDTA, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin (pH 7.5)]. The resuspended cells were then lysed mechanically on ice by trituration with a 21.1-gauge needle. To prepare the cytosolic and particulate fractions, the lysates were centrifuged at 1,000 g for 10 min at 4°C, after which the supernatants were centrifuged at 100,000 g for 1 h at 4°C. The particulate fraction, which contained the membranes, was washed twice and resuspended in buffer A containing 1% (vol/vol) Triton X-100. The protein levels in each fraction were quantified using the Bradford procedure (8).

Nuclear and nonnuclear protein fractionation. Cells were lysed in hypotonic buffer (20 mM HEPES, pH 7, 10 mM KCl, 2 mM MgCl2, 0.5% NP-40, 1 mM sodium orthovanadate) containing a cocktail of protease inhibitors. Lysates were homogenized gently (30 strikes in a 30-ml polypropylene tube) with a Teflon-glass homogenizer and centrifuged at 13,000 g for 10 min at 4°C, after which the pellets were discarded and the supernatant was centrifuged at 105,000 g for 1 h at 4°C. The supernatant, containing the nuclear fraction, was cleared by centrifugation at 12,000 g for 5 min and conserved at −70°C. The pellets, containing the nuclear fraction, were washed three times in hypotonic buffer. After homogenization in hypertonic buffer (hypotonic buffer containing 0.5 M NaCl and a cocktail of protease inhibitors), they were centrifuged at 12,000 g for 10 min, and the supernatants were conserved at −70°C.

Western blot analysis. Cell homogenates (30 μg protein) were separated by 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes. The blots were then washed with water, blocked for 1 h with 5% skim milk powder in TBST (10 mM Tris·HCl, pH 7.6, 150 mM NaCl, 0.05% Tween 20), and incubated with primary polyclonal antibody at the dilutions recommended by the suppliers. The specificity of the SGLT1 or SGLT2 antibody was confirmed using control peptides. Because of its low molecular weight (<3 kDa), the control peptide was not suitable for Western blot analysis, and so was used for ELISA or antibody blocking to confirm the specificity of the antibody. The membrane was washed and the primary antibodies were detected using goat anti-rabbit-IgG conjugated to horseradish peroxidase. Immunoreactive bands were visualized in an enhanced chemiluminescence procedure (Amersham Pharmacia Biotech, Buckinghamshire, UK).

RNA isolation and RT-PCR. Total RNA was extracted from PTCs with STAT60 (a monophasic solution of phenol and guanidine isothiocyanate; Tel-Test, Friendswood, TX). RT was conducted with 3 μg RNA using an AccuPower RT PreMix RT system kit (Bioneer, Daejeon, Republic of Korea) with oligo(dT)18 primers. Five micro-liters of the product was amplified using a PCR PreMix kit (Bioneer, Daejeon, Republic of Korea) with primers used were 5'-TTCTTCTGCGCTACTGCTGCG-3' and 5'-GGAGTCCGCAGTCTTACGAG-3' for PPARγ, 5'-ACCTGCAGATGGGCTGCTAC-3' and 5'-GTCTCTGTAGCTGGATGAC-3' for SGLT1, 5'-AAGAGCTGCCAACATGTTG-3' and 5'-TTCCATAGTGGAAGC-3' for NHE1, 5'-CGGCTAGTGTCCATAC-3' and 5'-GCTTTAGTGCTGATAA-3' for Sip1, and 5'-GGAGTCCGCGATCTTACAG-3' and 5'-TCTG-GAGACCTGTTAGAGG-3' for Twist. To control for the individual amounts of cDNA, RT-PCR was performed in parallel using β-actin primers. PCR products were visualized with ethidium bromide staining.

**RESULTS**

Effect of HG on EMT and membrane transport function. To elucidate the mechanism of EMT by HG, we monitored for expressions of E-cadherin, vimentin, and α-SMA. Exposure of PTCs to HG (25 mM D-glucose) for 12−72 h decreased protein expression of the epithelial cell marker E-cadherin and increased the expression of the mesenchymal markers vimentin and α-SMA (Fig. 1A). Mannitol or L-glucose (25 mM) did not change the expression of any of these markers, which suggested not high osmolality but HG per se induced EMT of PTCs (Supplemental Figure; all supplementary material for this article is accessible on the journal web site). In addition to HG, H2O2 (10−4 M) or TGF-β1 (5 ng/ml) as EMT inducers decreased E-cadherin expression and increased vimentin and α-SMA (Fig. 1B). Mannitol or L-glucose (25 mM) did not change the expression of any of these markers, suggesting not high osmolality but HG per se induced EMT of PTCs. As shown in Fig. 1, C or D, HG, H2O2, or TGF-β1 decreased NHE1, NHE3, SGLT1, and SGLT2 expression and α-MG uptake. On the other hand, 25 mM mannitol or L-glucose had no effect on α-MG (Fig. 1D). These results indicated that HG disrupted membrane transporters which were providing a clue to elucidate the relationship between EMT and membrane transport dysfunction in PTCs.

Involvement of ROS and PI3K/Akt pathways in HG-induced EMT. To determine the mechanism of the HG-induced EMT process, we monitored for intracellular ROS levels using DCF. Figure 2A shows that HG increased the DCF-sensitive cellular ROS level, but a 30-min pretreatment with the antioxidant NAC (10−3 M) blocked this increase in ROS. Consequently, antioxidants ascorbic acid (10−3 M) and NAC decreased HG-induced activation of PI3K and Akt (Fig. 2B). In experiments to examine HG-induced EMT protein expression, pretreatment with ascorbic acid, NAC, LY294002 (a PI3K inhibitor; 10−6 M), or an Akt inhibitor (10−6 M) abolished the HG-induced decrease in E-cadherin and increase in vimentin or α-SMA expression (Fig. 2, C and D).
Fig. 2. Involvement of reactive oxygen species (ROS) and phosphatidylinositol 3-kinase (PI3K)/Akt pathways in HG-induced EMT. A: 2',7'-dichlorofluorescein (DCF)-sensitive cellular ROS was measured by confocal microscopy. Control (a), HG (25 mM) treatment for 30 min (b), H2O2 (10^{-4} M) treatment for 30 min (c), and pretreatment with NAC (10^{-5} M) for 30 min before HG treatment for 30 min (d). Each is a representative picture of 5 independent experiments. B and C: cells were pretreated with ascorbic acid (10^{-3} M) or N-acetylcysteine (NAC) for 30 min before the HG incubation for 1 (B) or 72 h (C). The protein was extracted and blotted with antibodies against PI3K, Akt (Ser 473), or β-actin (B) and against E-cadherin, vimentin, α-SMA, or β-actin (C). D: cells were pretreated with LY294002 (PI3K inhibitor, 10^{-6} M) or Akt inhibitor (10^{-6} M) for 30 min before HG treatment for 72 h. Then, protein expression of E-cadherin, vimentin, or α-SMA was monitored by Western blotting. Each example shown is representative of 3 independent experiments. Values are means ± SE of 3 experiments for each condition determined from densitometry relative to β-actin. *P < 0.05 vs. control. **P < 0.05 vs. HG alone.
Fig. 3. Involvement of glycogen synthase kinase (GSK)-3β and Snail1 in HG-induced EMT. A: PTCs were pretreated with LY294002 or Akt inhibitor for 30 min before HG treatment for 1 h. Phosphorylation of GSK-3β was monitored by Western blotting. B: after incubation of HG for 72 h, Snail1, Sip1, and Twist gene expression levels were analyzed by RT-PCR. C: cells were treated with HG or a GSK-3β inhibitor (LiCl, 40 mM). Protein was then extracted and blotted with antibodies against p-GSK-3β, GSK-3β, Snail1, β-catenin, or β-actin. D: cells were transfected with either a SMARTpool of Snail1 small interfering (si) RNAs (100 nmol/l) or a nontargeting control siRNA (100 nmol/l) using LipofectAMINE 2000, before HG treatment for 72 h. Protein expressions of E-cadherin, vimentin, or α-SMA were monitored by Western blotting. Each example shown is representative of 3 independent experiments. Values are means ± SE of 3 experiments for each condition determined from densitometry relative to β-actin. *P < 0.05 vs. control. **P < 0.05 vs. HG alone.
Involvement of GSK-3β, Snail, and β-catenin in HG-induced EMT. We next sought to determine whether the HG-induced EMT process involved GSK-3β, Snail, or β-catenin. Inhibition of PI3K or Akt reversed HG-induced inactivation (phosphorylation) of GSK-3β (Fig. 3A). We used RT-PCR to detect PTC expression of EMT-regulatory genes such as Snail1, Sip1, and Twist to determine whether these genes were involved in the HG-induced EMT. As shown in Fig. 3B, HG increased Snail1 mRNA expression rather than sip1 or twist mRNA. HG or LiCl (a GSK-3β inhibitor, 40 mM) increased GSK-3β phosphorylation, and Snail or β-catenin activation (Fig. 3C). In experiments to examine the effect of Snail on HG-induced EMT, Snail1-specific siRNA transfection reversed the HG effects on E-cadherin, vimentin, or α-SMA expression (Fig. 3D). To further examine the involvement of GSK-3β or β-catenin, cells were lysed and the cytosol/nuclear fraction was used for Western blotting with antibodies specific for β-catenin or lamin A/C (a nuclear antigen). As shown in Fig. 4A, HG or LiCl increased the β-catenin level in the nuclear fraction. In accordance with the observed Western blotting, β-catenin accumulated in the nucleus in immunofluorescence staining when cells were treated with HG or LiCl (Fig. 4B). Moreover, β-catenin-specific siRNA significantly blocked the HG-induced decrease in E-cadherin expression and increased vimen-

![Fig. 4. Involvement of GSK-3β and β-catenin in HG-induced EMT. A and B: PTCs were treated with HG or LiCl (40 mM). Nuclear translocation of β-catenin was determined in the cytosol/nuclear fraction (A). Also, for confocal microscopy of immunofluorescence-stained samples, cells were fixed and stained with a primary antibody against β-catenin (B). C: cells were transfected with either a SMARTpool of β-catenin siRNAs (100 nmol/l) or a nontargeting control siRNA (100 nmol/l) using LipofectAMINE 2000 before HG treatment for 72 h. Protein expressions of E-cadherin, vimentin, or α-SMA were monitored by Western blotting. Each example shown is representative of 3 independent experiments. Values are means ± SE of 3 experiments for each condition determined from densitometry relative to β-actin. *P < 0.05 vs. control. **P < 0.05 vs. HG alone.](http://ajprenal.physiology.org/)

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**EFFECT OF TROGLITAZONE ON HG-INDUCED EMT AND α-MG UPTAKE**

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Fig. 5. Involvement of Peroxisome proliferator-activated receptor-γ (PPARγ) activation in HG-induced membrane transport dysfunction. 

A: after PTCs were treated with HG for 72 h, PPARα, PPARβ/δ, and PPARγ gene expression levels were analyzed by RT-PCR and real-time RT-PCR. B and C: cells were pretreated with a PPARα agonist (fenofibrate, 2.5 × 10⁻⁵ M), PPARβ/δ agonist (L-165,041, 10⁻⁵ M), and PPARγ agonist (troglitazone, 10⁻⁶ M). Protein was extracted and blotted with antibodies against SGLT1, SGLT2, or β-actin (B). Also, α-MG uptake was measured (C). D: cells were treated with HG, PPARγ siRNA, or GW9662 (PPARγ antagonist, 10⁻⁶ M) for 72 h. Protein expressions of SGLTs were monitored by Western blotting. Each example shown is representative of 3 independent experiments from triplicate dishes. Values are means ± SE of 3 experiments for each condition determined from densitometry relative to β-actin. *P < 0.05 vs. control. **P < 0.05 vs. HG alone.
Fig. 6. Effect of troglitazone on HG-induced EMT. PTCs were treated with HG and/or troglitazone (10^{-6} M). A and B: protein was extracted and blotted with antibodies against PI3K, Akt, or β-actin (A) and p-GSK3β, GSK3β, Snail1, β-catenin, or β-actin (B). C: nuclear translocation of β-catenin was determined with the cytosol/nuclear fraction. D and E: protein was extracted and blotted with antibodies against E-cadherin, vimentin, α-SMA, or β-actin (D). Each example shown is representative of 3 independent experiments. Values are means ± SE of 3 experiments for each condition determined from densitometry relative to β-actin. Also, for confocal microscopy of immunofluorescence-stained samples, cells were fixed and stained with a primary antibody against E-cadherin, vimentin, and α-SMA (E). *P < 0.05 vs. control, **P < 0.05 vs. HG alone.
tin and α-SMA expression (Fig. 4C). These results indicate that GSK-3β regulated Snail1 and β-catenin activity, which plays important roles in the HG-induced EMT process.

**Effect of PPAR agonists on HG-induced EMT and membrane transport dysfunction.** Because we previously observed that PPARγ agonists increase SGLT-mediated glucose uptake (42), we hypothesized that PPAR activation might be involved in the HG-induced EMT process and membrane transport dysfunction. As shown in Fig. 5A, PTCs expressed PPARα, PPARβ/δ, and PPARγ mRNA. Moreover, the results of real-time RT-PCR showed that the treatment with HG significantly decreased the mRNA level of PPARγ, whereas those of other isoforms of PPARs remained unchanged, suggesting that PTCs have their own protective system against hyperglycemia. PTCs were treated with a PPAR agonist (fenofibrate, 2.5 × 10⁻⁵ M), PPARβ/δ agonist (t-165,041, 10⁻⁵ M), and PPARγ agonist (troglitazone, 10⁻⁶ M). As shown in Fig. 5, B and C, troglitazone significantly reversed HG-induced reduction of SGLT1 or SGLT2 expression and α-MG uptake. Moreover, to confirm these results, we treated cells with PPARγ siRNA and GW9662 (PPARγ antagonist; 10⁻⁶ M; Fig. 5D). As expected, both PPARγ siRNA and GW9662 decreased the expression of SGLTs as did HG, suggesting that the HG-induced decrease in PPARγ plays a role in downregulation of SGLT expression. Therefore, subsequently, PTCs were treated with troglitazone to examine the HG-induced EMT signaling pathways. As shown in Fig. 6, A–C, troglitazone reversed protein activities of PI3K/Akt, GSK-3β, Snail1, or β-catenin in response to HG. Moreover, troglitazone blocked the HG effects of decreased E-cadherin expression and increased vimentin and α-SMA expression by Western blotting (Fig. 6D) and immunofluorescence staining (Fig. 6E). These data indicated that troglitazone ameliorates the HG-induced EMT process, which may cause membrane transport dysfunction in PTCs.

**Relationship among HG-induced EMT signaling pathways and SGLT protein expression and α-MG uptake.** In experiments to elucidate the relationship between HG-induced EMT and membrane transport dysfunction in PTCs, pretreatment with LY294002, Akt inhibitor, Snail siRNA, β-catenin siRNA, and troglitazone restored HG-induced reductions in SGLT protein expression (Fig. 7A) and α-MG uptake (Fig. 7B). These observations suggested that, in addition to the PPARγ agonist, inhibition of EMT-related signaling pathways such as PI3K, Akt, Snail1, or β-catenin is important in the recovery of HG-induced reductions in glucose uptake.

**DISCUSSION**

This study presents evidence that the HG-induced EMT process might cause SGLT dysfunction, which can be inhibited by the PPARγ agonist troglitazone in primary cultured renal PTCs. Thus we took advantage of PPARγ activation by its agonist as one of the protective mechanisms to ameliorate EMT-induced membrane transport dysfunction by the HG condition. Given the pathogenic role of EMT in the development and progression of chronic kidney disease, attention has turned to finding novel ways to inhibit or even reverse this process. The fact that EMT is a complicated process provides us with a wide spectrum of opportunities for therapeutic intervention. Thus we tried to explore novel therapeutic approaches for restoration of renal membrane transport function in PTCs. PPARγ agonists ameliorate renal fibrotic lesions through inhibition of extracellular matrix (ECM) production in both diabetic nephropathy and nondiabetic chronic kidney diseases (34, 43, 57, 59). In addition, PPARγ agonists can attenuate the increase in activator protein-1 (AP-1), TGF-β1, and the downstream production of the ECM exposed to HG.
The postulated mechanisms are multifactorial, involving the inhibition of cell growth, reduction of matrix, and cytokine production (31). Although PPARγ agonists have antidiabetic and antifibrotic effects, their inhibitory effect on EMT in vivo was not known. However, in in vitro studies, PPARγ agonists have reversion effects on EMT in anaplastic thyroid cancer cells (1) and retinal pigment epithelial cells (11). Moreover, there are controversial data on expression of PPAR isotypes in different tissues in diabetes. PPARγ levels are increased in the cortical tubules of diabetic mice (49), whereas PPARγ mRNA expression is decreased in the renal cortex or endothelium of STZ-induced diabetic rats (32, 58) and in patients with type 2 diabetes (53). Thus the diabetes-induced reduction in PPARγ mRNA levels may impair protective effects of PPARγ activation in the diabetic kidney. Although the effect of PPARγ agonists on glucose homeostasis was not fully explored, we previously demonstrated that PPARγ agonists [trogilitazone and 15-deoxy-Δ12,14-prostaglandin J2 (PGJ2)] increased in glucose uptake (42). Based on the previous studies, we suggest that the PPARγ agonist troglitazone can ameliorate HG-induced EMT process as well as restoring the decreased glucose uptake in PTCs.

Alterations in transporter function may occur as a consequence of hyperglycemia because proteins and lipids are also subject to oxidant injury (25, 26). The present study also shows that activation of PI3K and Akt by ROS generation also mediated HG-induced EMT. Similarly, Akt inhibition induces the mesenchymal-to-epithelial reverting transition in oral squamous cell carcinoma (30). Furthermore, inhibition of either PI3K or Akt activity causes a decrease in GSK-3β phosphorylation attenuated TGF-β1-mediated EMT in rat kidney epithelial cells (33). Especially, activity of GSK-3β is necessary for the maintenance of epithelial architecture, and inhibition of GSK-3β activity or expression results in a bona fide EMT (3). Concomitantly, our present results demonstrate that activation of PI3K and Akt in response to HG triggers Ser-9 phosphorylation of GSK-3β. Because phosphorylation and functional inactivation of GSK-3β stabilize cytoplasmic β-catenin as well as Snail (63), we hypothesized that GSK-3β may be a regulator of EMT through regulation of Snail and β-catenin in PTCs. The Snail family has been intensively explored and has been shown to play key roles in EMT, with the expression of Snail proteins appearing to be inversely correlated with E-cadherin expression (4, 24, 61). In addition to Snail, nuclear accumulation of β-catenin is another typical change during EMT (38, 46, 47). Similarly, both HG and GSK-3β inhibition by LiCl increased Snail expression levels and β-catenin nucleus translocation, and inhibition of Snail1 or β-catenin with specific siRNA blocked HG-induced EMT. Recently, Snail could functionally interact with β-catenin to increase Wnt-dependent target gene expression, but Snail does not affect the stability, phosphorylation, or nuclear localization of β-catenin (56). Taken together, we considered that HG-induced EMT is mediated by ROS, PI3K/Akt activation, and that GSK-3β inactivation causes the increase in transcriptional activity of Snail or β-catenin in PTCs.

As a potential mechanism to inhibit HG-induced EMT, the PPARγ agonist troglitazone inhibits PI3K and Akt activities,
consistent with previous results (6, 10, 18, 19, 39). Among the research about the effect of PPARγ agonists on GSK-3β and β-catenin activation, troglitazone stimulates the degradation of β-catenin, depending on GSK-3β activity during adipogenesis (44). In addition, the antiproliferative effect of rosiglitazone is mediated by inhibition of ERK and activation of GSK-3β, but not through inhibition of Akt (36). A recent study also showed that the PPARγ agonist pravastatin and pioglitazone interfere with the expression of surrogate markers of transdifferentiation of tubular cells, suggesting that these agents might decrease EMT (60). In this study, it is conceivable that the PPARγ agonist troglitazone inhibited PI3K/Akt activation, which was in turn likely to enhance GSK-3β activity and inhibit Snail1 expression and β-catenin nuclear translocation for transcription of EMT genes. Therefore, it is possible that activation of PPARγ by troglitazone leads to the renal-protective effect and to the reversal of EMT in the HG condition.

The present data shed new light on the important mechanism by which HG-induced EMT may give rise to membrane transport dysfunction in PTCs. To investigate membrane transport dysfunction of PTCs, we examined the expression of SGLTs and NHEs according to previous studies about distribution and functional integrity of membrane transporters in PTCs (14, 20). Especially, NHE3 predominately expressed in the renal proximal tubules is localized along the apical border and is responsible for the bulk reabsorption of sodium, fluid, and bicarbonate (2, 7). Our previous review (41) pointed out that some studies have yielded conflicting results on the effects of diabetes on SGLT-mediated glucose transport. Studies have reported both increased (51) and decreased (28) glucose transport mediated by SGLT in diabetes. Taken together, we surmise that HG-induced EMT causing the physiological alteration of cellular phenotype consequently results in membrane transport dysfunction in PTCs. As shown in the hypothetical model presented in Fig. 8, the signaling mechanisms underlie how troglitazone ameliorates HG-induced EMT and the reduction of SGLT expression. Therefore, these data may have implications for understanding the complex regulation of the EMT process and for recovery of cellular function from diabetic nephropathy. However, many important questions remain to be explored in future research into diabetic therapy.

In conclusion, pretreatment with the PPARγ agonist troglitazone inhibits the HG-induced EMT process and protects from consequent membrane transport dysfunction through PI3K/Akt, GSK-3β, Snail1, and β-catenin in primary cultured renal PTCs.

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DISCLOSURES
No conflicts of interest are declared by the authors.

REFERENCES
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