Original Research

Glucagon-like peptide-1 receptor pathway inhibits extracellular matrix production by mesangial cells through store-operated Ca^{2+} channel

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Impact statement

Diabetic kidney disease continues to be a major challenge to health care system in the world. There are no known therapies currently available that can cure the disease. The present study provided compelling evidence that activation of GLP-1R inhibited extracellular matrix protein production by glomerular mesangial cells. We further showed that the beneficial effect of GLP-1R was attributed to upregulation of store-operated Ca^{2+} channel function. Therefore, we identified a novel mechanism contributing to the renal protective effects of GLP-1R pathway. Activation of GLP-1R pathway and/or store-operated Ca^{2+} channel signaling in MCs could be an option for patients with diabetic kidney disease.

Abstract

Glomerular mesangial cell is the major source of mesangial matrix. Our previous study demonstrated that store-operated Ca^{2+} channel signaling suppressed extracellular matrix protein production by mesangial cells. Recent studies demonstrated that glucagon-like peptide-1 receptor (GLP-1R) pathway had renoprotective effects. However, the underlying mechanism(s) remains unclear. The present study was aimed to determine if activation of GLP-1R decreased extracellular matrix protein production by mesangial cells through upregulation of store-operated Ca^{2+} function. Experiments were conducted in cultured human mesangial cells. Liraglutide and exendin 9–39 were used to activate and inhibit GLP-1R, respectively. Store-operated Ca^{2+} function was estimated by evaluating the SOC-mediated Ca^{2+} entry (SOCE). We found that liraglutide treatment reduced high glucose-stimulated production of fibronectin and collagen IV. The inhibitory effects of liraglutide were not observed in the presence of exendin 9–39. Exendin-4, another GLP-1R agonist also blunted high glucose-stimulated fibronectin and collagen IV production. Treatment of human mesangial cells with liraglutide for 24 h significantly attenuated the high glucose-induced reduction of Orai1 protein. Consistently, Ca^{2+} imaging experiments showed that the inhibition of high glucose on SOCE was significantly attenuated by liraglutide. However, in the presence of exendin 9–39, liraglutide failed to reverse the high glucose effect. Furthermore, liraglutide effects on fibronectin and collagen IV protein abundance were significantly attenuated by GSK-7975A, a selective blocker of store-operated Ca^{2+}. Taken together, our findings suggest that GLP-1R signaling inhibited high glucose-induced extracellular matrix protein production in mesangial cells by restoring store-operated Ca^{2+} function.

Keywords: Glucagon-like peptide-1 receptor, store-operated calcium entry, mesangial cells, extracellular matrix, diabetic kidney disease, liraglutide

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Introduction

About 40% of diabetic patients are affected by diabetic kidney disease (DKD). The early features of DKD include expansion of glomerular extracellular matrix (ECM), which, if not controlled, will develop to glomerulosclerosis and renal insufficiency, and eventually renal failure.[1,2] However, there are no known therapies currently available that can cure the progressive lesion of glomerular histology and loss of renal function in DKD. New and effective treatments would, therefore, be a significant advance.

Glomerular mesangial cells (MCs) are a major cell population in glomerulus and also a major source of glomerular matrix proteins. Dysfunction of MCs is firmly associated with the development of DKD.[3] MCs are the major source of mesangial matrix and over production of ECM proteins by MCs leads to glomerular injury in DKD.[4] Thus, exploration of molecular pathways inhibiting ECM production by MCs would help find therapeutic strategies for patients with DKD.

Ca^{2+} signaling is an important mechanism for regulating MC function.[5,6] Among diverse Ca^{2+} signal pathways, store-operated Ca^{2+} channel (SOC) signaling is tightly associated with many physiological and pathological processes in MCs.[6] SOC is defined as the channel which opens as the Ca^{2+} inside the endoplasmic reticulum (ER) is depleted.[7] STIM1[8,9] and Orai1[10–12] are two essential molecules in the signaling pathway of SOC. STIM1 in the ER membrane gates SOC by sensing intraluminal Ca^{2+} concentration of the ER. Orai1 is the plasma membrane protein and constitutes SOC channel itself.[13,14] Over past 20 years, published studies have revealed that SOC contributes to MC Ca^{2+} signaling stimulated by many hormones and growth factors.[15–19] Recently, we demonstrated that SOC signaling suppressed ECM protein production by MCs through the Smad pathways,[20–22] suggesting an anti-fibrotic function of SOC. However, the regulation of SOC in MCs still remains unclear.

Glucagon-like peptide-1 (GLP-1), mainly produced by the intestinal L cells, has multiple physiological actions, including stimulation of insulin secretion and regulation of glucose metabolism.[23] Several agonists of GLP-1 receptor (GLP-1R) have recently developed for patients with type 2 diabetes to improve glycemic control.[24,25] GLP-1R was also expressed in kidney cells, including MCs and renal tubular cells.[26–28] Increased GLP-1R signaling in kidney had renoprotective effects in diabetic animals.[29–33] For instance, GLP-1R signaling was reported to ameliorate inflammatory response and oxidative stress in MCs.[30,34] Upregulation of GLP-1R signaling delayed the DKD progression in male *db/db* mice.[35] However, whether GLP-1R pathway regulates ECM production by MCs and whether SOC mediates the GLP-1R effects are not known. The present study was aimed to determine if activation of GLP-1R pathway decreased MC-derived ECM protein production by upregulating SOC function.

Material and methods

MC culture

Human MCs (HMCs) (Sciencell Research Laboratories, catalog no. 4200) were cultured as described previously.[22] In all experiments except for Ca^{2+} imaging, when HMCs reached ~80% confluence, the cells were treated with 0.5% FBS overnight to stop growth before various treatments. Cells continued to be incubated with 0.5% FBS medium during treatments. HMCs only with sub-passage 4–9 were used for study.

Western blot

The protein extracts from HMCs were run in 10% SDS-PAGE. Proteins were transferred to PVDF membranes. The primary antibodies used in the present study include fibronectin (FN) (cat. no. F3648, Sigma-Aldrich, RRID: AB_476976), collagen IV (Col IV) (cat. no. ab135802, Abcam, RRID:AB_2773062), Orai1 (cat. no. O8264, Sigma-Aldrich, RRID:AB_1078883), GLP-1R (cat. no. ab186051, Abcam, RRID:AB_2731993), and α-tubulin (no. sc-5286, Santa Cruz Biotechnology, RRID:AB_628411). The AlphaEaseFC imaging system (Alpha Innotech, San Leandro, CA) was used to capture target protein bands which were semi-quantitated using the integrated density value (IDV). The content of target proteins was normalized to α-tubulin on the same blot.

Fluorescence assay of $[Ca^{2+}]_i$

Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was estimated using dual-excitation wavelength fluorescence of fura 2 as previously described.[36] Cells were loaded with 2 μM fura-2-AM (Invitrogen, Grand Island, NY) for 50 min at room temperature in dark followed by fura-2-free physiological saline solution for 20 min. The excitation/emission wavelengths were 340 and 380-nm (alternately)/510-nm. The NIS Elements AR software was used to calculate $[Ca^{2+}]_i$. Calibrations were performed at each experiment using 5 μM ionomycin (for high $[Ca^{2+}]_i$) and 5 mM EGTA (for low $[Ca^{2+}]_i$). Cells were initially bathed in physiological saline solution containing 1 mM Ca^{2+}. The concentrations of $CaCl_2$ in the Ca^{2+}-free saline solution and 2 mM Ca^{2+} saline solution were 0 and 2 mM, respectively. The glucose (D-glucose) concentrations in the saline solutions were 5.6 mM for normal glucose (NG) group and 25 mM for HG group, respectively.

Materials

FBS was purchased from Life Technologies (Gibco BRL, Grand Island). Liraglutide (Cat no: HY-P0014), D-Mannitol (Cat no: HY-N0378), Exendin 9–39 (Cat no: HY-P0264), and GSK-7975A (Cat no: HY-12507) were purchased from MedChemExpress. Cyclopiazonic acid (CPA) was purchased from Alomone Labs (Cat no: C-750 and Lot no: C750CS1025). Exendin-4 was purchased from TopScience (Cat no: T-3967).

Statistical analyses

All summary data were expressed as means ± SEM. The differences among multiple groups were analyzed using one-way repeated-measures ANOVA followed by Student–Newman–Keuls test. Comparison of difference between two groups was performed using the unpaired Student *t*-test. The statistically significant level was set at $P < 0.05$. All statistical tests were performed using SigmaStat (Jandel Scientific, San Rafael, CA).

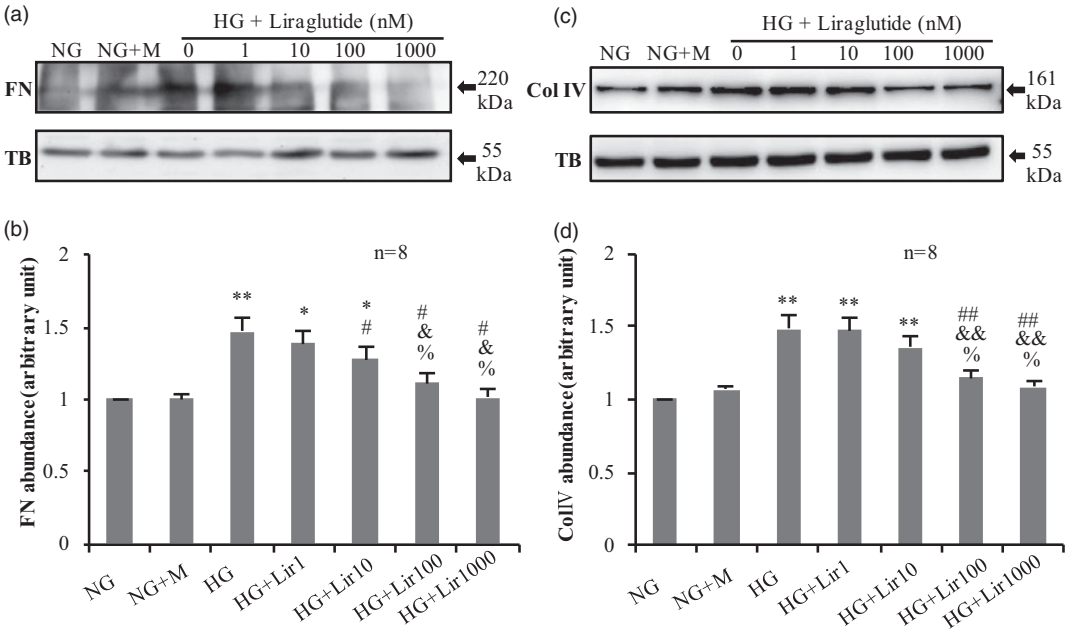

Figure 1. Liraglutide reduced HG-induced increases of FN and Col IV protein content in cultured HMCs.
Western blot showing the effects of liraglutide at different concentrations on FN and Col IV protein expression. HMCs were incubated in media containing 5.6 mM D-glucose (NG) or 5.6 mM D-glucose + 20 mM mannitol (NG+M) or 25 mM D-glucose (HG) with or without different concentrations of liraglutide for 2 days. (a) and (c) Representative immunoblots. TB: α-tubulin, loading control. (b) and (d) Summary data. HG+Lir1: HG+liraglutide (1 nM); HG+Lir10: HG+liraglutide (10 nM); HG+Lir100: HG+liraglutide (100 nM); HG+Lir1000: HG+liraglutide (1000 nM). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with both NG and NG+M groups. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus HG group. $^{\&}P < 0.05$, $^{\&\&}P < 0.01$, versus HG+Lir1 group. $^{\%}P < 0.05$ versus HG+Lir10 group. "$n = 8$": No. of independent experiments.

Results

GLP-1R agonist, liraglutide reduced HG-induced ECM proteins in HMCs

HG is known to stimulate production of ECM by MCs during the development of DKD.[37] The previous study from our group also showed that HG treatment significantly increased protein abundance of both FN and Col IV.[22] In the present study, we examined if liraglutide, an agonist of GLP-1R altered the HG-induced fibrotic response in HMCs. As shown in Figure 1, HG exposure for two days significantly increased both FN and Col IV protein contents. These HG effects were blunted by liraglutide with various degrees at concentrations from 1 to 1000 nM. The inhibitory effects of liraglutide showed a dose-dependent manner for FN and Col IV (Figure 1).

Activation of GLP-1R pathway contributed to the inhibitory effects of liraglutide

To verify if the inhibitory effects of liraglutide on the HG-stimulated ECM protein production by MCs were mediated by GLP-1R, we conducted three lines of experiments. First, we examined if another GLP-1R agonist, exendin-4 could recapitulate liraglutide responses. In agreement with liraglutide's effects, exendin-4 (100 nM) significantly inhibited HG-stimulated production of FN and Col IV by HMCs (Figure 2(a) to (d)). We then examined the influence of chronic treatment (two days) of liraglutide on the amount of GLP-1R in HMCs. As shown in Figure 2(e) and (f), incubation with HG for two days significantly decreased protein content of GLP-1R. In correspondence to its inhibitory effects on FN and Col IV, liraglutide significantly blunted the HG-induced decrease in GLP-1R abundance in a trend of dose-dependent manner. Lastly, we examined if exendin 9–39, a potent and selective GLP-1R antagonist could block the liraglutide effects. Similar to the data shown in Figure 1, treatment of cultured HMCs with 100 nM liraglutide for two days significantly reduced HG-induced production of FN and Col IV. However, in the presence of exendin 9–39, these inhibitory effects of liraglutide were significantly reduced (Figure 3). Taken together, these results indicate that upregulation of GLP-1R pathway inhibited ECM protein production by MCs.

Activation of GLP-1R blunted HG-induced decrease of Orai1 protein abundance

Orai1 is the channel protein of SOC in many cell types,[13,14] including MCs.[36,38] We have previously demonstrated that HG incubation for a time period from 8 h to 24 h decreased content of Orai1 protein in HMCs.[38] In the present study, we treated HMCs with HG for 24 h with and without liraglutide at different concentrations. As shown in Figure 4, liraglutide significantly attenuated the HG-induced reduction of Orai1 protein and the liraglutide effect was dose dependent. A concentration as low as 10 nM was sufficient to reverse the HG-effect and completely abolished the HG response at a concentration of >100 nM.

Liraglutide protected SOC function in HG-treated HMCs

We have previously demonstrated that Orai1 mediated Ca^{2+} entry through SOC (SOCE) in MCs and HG treatment

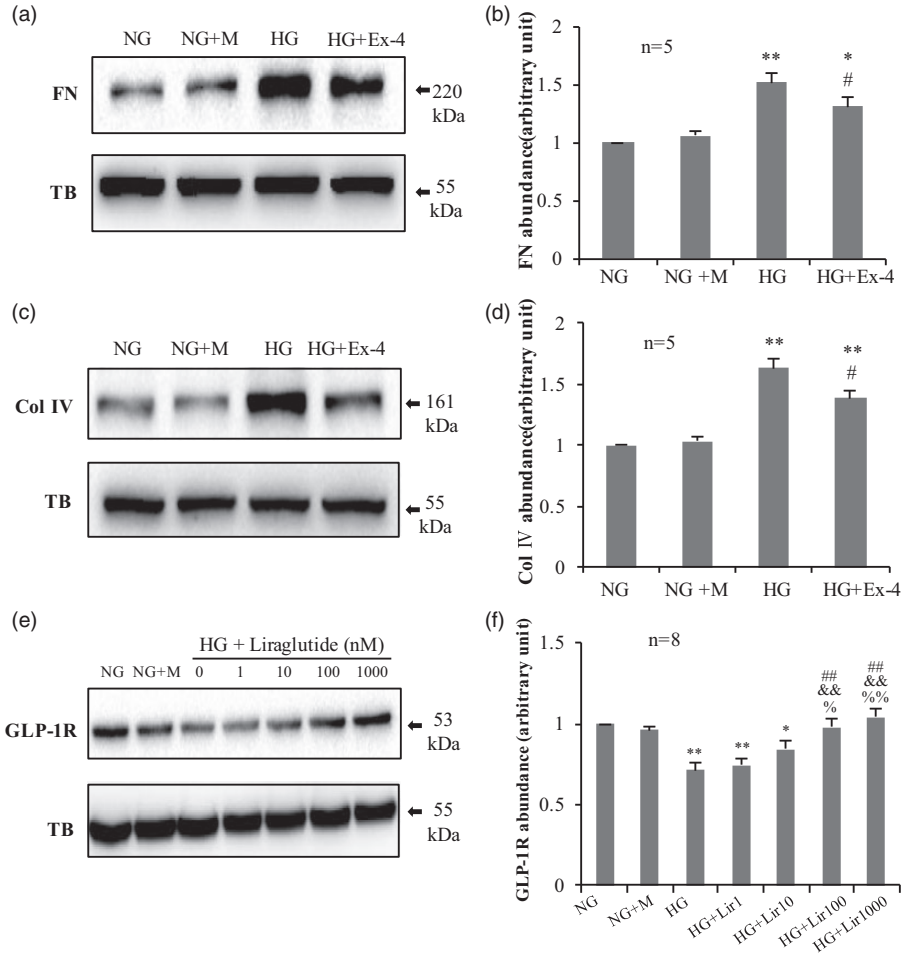

Figure 2. Exendin-4 inhibited HG-stimulated ECM production and liraglutide blunted HG-reduced abundance of GLP-1R in HMCs.
Western blot showing exendin-4 effect on FN and Col IV production (a–d) and liraglutide effect on GLP-1R protein abundance (e and f) in HMCs. (a) and (c) Representative blots. HMCs were incubated with medium containing NG or NG+M or HG in the presence or absence of 100 nM exendin-4 for two days. Ex-4: Exendin-4. TB: α-tubulin. (b) and (d) Summary data from experiments are shown in (a) and (c), respectively. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with both NG and NG+M groups. $^{\#}P < 0.05$ versus HG group. "$n = 5$": No. of independent experiments. (e) Representative blot. HMCs were cultured in NG or NG+M or HG with or without liraglutide at different concentrations for two days. (f) Summary data from experiments shown in (e). HG+Lir1: HG+liraglutide (1 nM); HG+Lir10: HG+liraglutide (10 nM); HG+Lir100: HG+liraglutide (100 nM); HG+Lir1000: HG+liraglutide (1000 nM). $^{*}P < 0.05$, $^{**}P < 0.01$, compared with both NG and NG+M groups. $^{\#\#}P < 0.01$ versus HG group. $^{\&\&}P < 0.01$ versus HG+Lir1 group. $^{\%}P<0.05$, $^{\%\%}P < 0.01$ versus HG+Lir10 group. "$n = 8$": No. of independent experiments.

for one day significantly decreased the Ca^{2+} influx by reducing Orai1 protein abundance.[36,38] Because liraglutide prevented HG-induced decrease of Orai1 protein (Figure 4), we reasoned that the attenuated SOCE by HG could be reversed by liraglutide treatment. Ca^{2+} imaging assay was conducted to assess SOC function in response to liraglutide treatment. We assessed SOCE response using the classical Ca^{2+} re-admission approach described previously.[36,38] CPA, a selective inhibitor of the ER Ca^{2+} ATPase was used to activate SOC. Consistent with our previous study,[38] SOCE response was significantly attenuated in the cells exposed to HG compared to the cells cultured in NG medium. Importantly, the inhibitory effect of HG was blunted by liraglutide treatment (100 nM) (Figure 5). These fura-2 data suggest that liraglutide could reserve SOC function under deleterious HG environment.

GLP-1R mediated liraglutide effect on Orai1 protein

We have shown biochemical and functional data that liraglutide treatment positively regulated SOC protein Orai1 expression (Figure 4) and SOC function (Figure 5). We next determined whether liraglutide effects were GLP-1R dependent. In agreement with the results presented in Figure 4, treatment of cultured HMCs with 100 nM liraglutide for one day significantly attenuated HG-induced reduction of Orai1 protein content (Figure 6). However, in the presence of exendin 9–39, a potent and selective GLP-1R antagonist, liraglutide failed to reverse the HG effect (Figure 6). These results suggest that liraglutide suppressed HG-induced Orai1 protein reduction via GLP-1R.

GLP-1R activation antagonized HG-induced ECM production by activation of SOC in HMCs

We previously demonstrated that SOCE signaling in MCs was anti-fibrotic.[20–22] In the present study, we revealed that liraglutide blunted HG-stimulated production of FN and Col IV in cultured HMCs (Figure 1). We also showed that activation of GLP-1R increased content of SOC channel protein, Orai1 and importantly SOCE (Figures 4 and 5). We thereby reasoned that SOC could be an action site

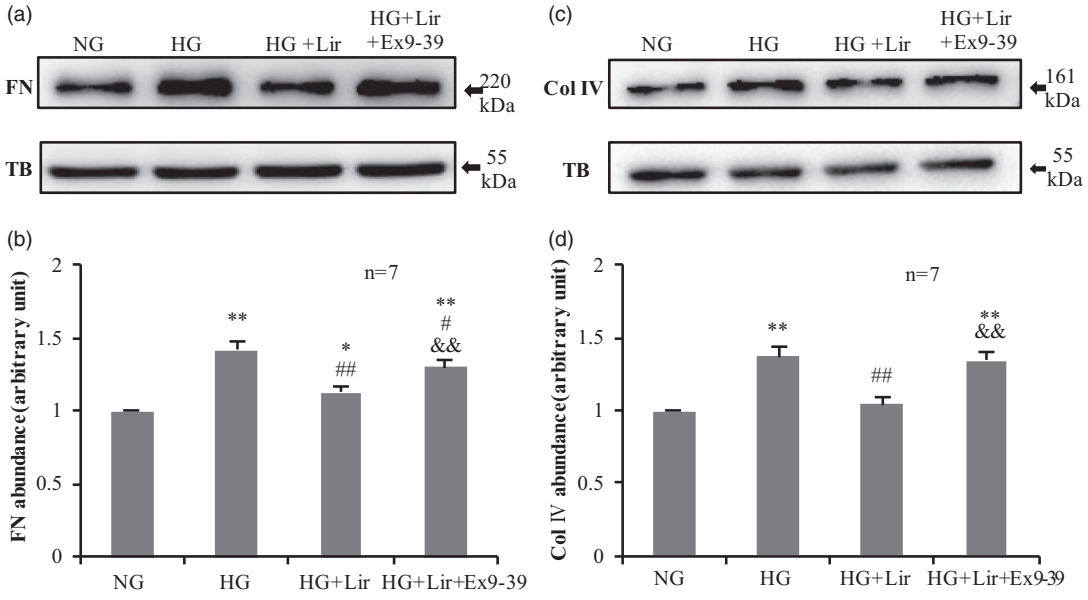

Figure 3. Exendin 9–39 attenuated liraglutide inhibition on HG-induced ECM protein production.
Western blot showing involvement of GLP-1R in liraglutide effects on production of FN (a and b) and Col IV (c and d). HMCs were cultured in 0.5% FBS NG medium or HG medium with 100 nM liraglutide with or without 1000 nM exendin 9–39 for two days. Lir: liraglutide, Ex9–39: exendin 9–39, TB: α-tubulin. (a) and (c) Representative blots. (b) and (d) Summary data. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with NG group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus HG group. $^{\&\&}P < 0.01$ versus HG+Lir group. "$n = 7$": No. of independent experiments.

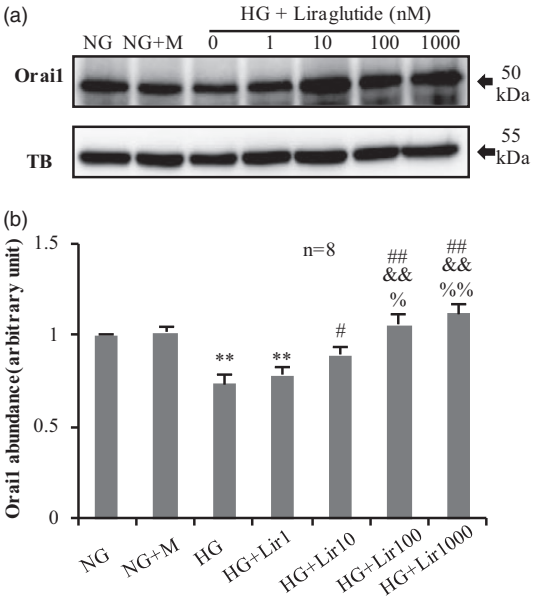

Figure 4. Liraglutide attenuated HG-induced decrease of Orai1 protein abundance in HMCs.
Western blot showing liraglutide effect on HG-induced decrease of Orai1 protein abundance in HMCs. Growth-arrested HMCs were incubated with NG or HG medium with and without liraglutide at different concentrations for 24 h. (a) Representative blots. TB: α-tubulin. (b) Summary graph. NG+M: NG+20 mM α-mannitol; HG+Lir1: HG+liraglutide (1 nM); HG+Lir10: HG+liraglutide (10 nM); HG+Lir100: HG+liraglutide (100 nM); HG+Lir1000: HG+liraglutide (1000 nM). $^{**}P < 0.01$ compared with both NG and NG+M groups. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus HG group. $^{\&\&}P < 0.01$ versus HG+Lir1 group. $^{\%}P < 0.05$, $^{\%\%}P < 0.01$ versus HG+Lir10 group. "$n = 8$": No. of independent experiments.

downstream GLP-1R in the cascade of GLP-1R/ECM inhibition in HMCs. To test this hypothesis, we used GSK-7975A, a recently identified selective SOC inhibitor by directly acting on the pore region of the channel[39] to inhibit SOC function. As shown in Figure 7, activation of GLP-1R by liraglutide (100 nM for two days) markedly decreased HG-induced FN and Col IV protein abundance. However, these responses were significantly abolished by GSK-7975A (10 μM). These data suggest that the inhibition on ECM protein production by GLP-1R activation was mediated by SOC in HMCs.

Discussion

DKD is the major cause of chronic kidney disease, which, if not controlled, will eventually develop to renal failure. With a continuous rise in the prevalence and incidence of DKD, finding an effective therapeutic strategy with minimal adverse effects is a great challenge to healthcare system. The current therapy for DKD, such as glycemic control and blood pressure control, improves renal function only modestly.[40] Recently, GLP-1 analogues and GLP-1R agonists were developed and used in clinic for diabetic patients. More recently, in addition to lowering blood glucose, GLP-1 analogues were found to have additional renoprotection.[41] For instance, activation of GLP-1R was shown to ameliorate renal injury and improve renal function in male *db/db* mice.[35] Recently, GLP-1R was found in kidney cells, including MCs and an increase in GLP-1R level in kidney had renal protection in diabetic animals.[29–33] Activation of GLP-1R inhibited HG-stimulated expression of TGF-β1 and connective tissue growth factor in HMCs.[42] Furthermore, GLP-1 was shown to attenuate advanced glycation end products-induced inflammation and oxidative stress in MCs.[30,34]Although the renal protection of GLP-1R pathway is evident, the underlying mechanisms for its beneficial effects are not clear. Our results provided evidence that SOC mediated inhibitory effects of GLP-1R pathway

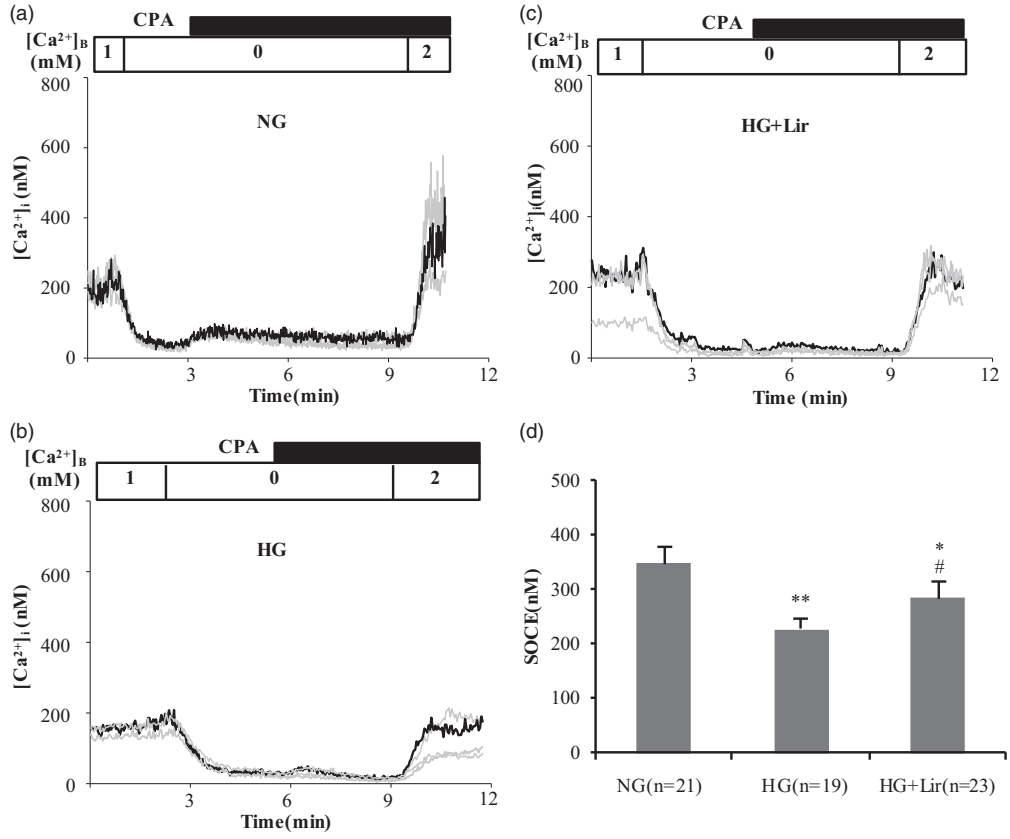

Figure 5. Liraglutide attenuated HG-induced decrease of SOCE.
Fura 2 fluorescence-indicated intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in MCs. Growth-arrested HMCs were incubated with NG, HG or HG with Liraglutide (100 nM) for 24 h. (a), (b) and (c) show original Ca^{2+} imaging traces from MCs incubated with NG medium, HG medium, and HG medium with liraglutide, respectively. In each panel, four original traces from four individual cells were superimposed and the trace showing representative response was indicated by a bold line. Lir: liraglutide. $[Ca^{2+}]_B$: Ca^{2+} concentration in bathing solution, indicated by the numbers in the top boxes of each graph. SOCE was defined as the difference in $[Ca^{2+}]_i$ between Ca^{2+}-free and 2 mM Ca^{2+} bathing solution. (d) Summary data, showing SOCE in each group. The numbers of cells analyzed in NG, HG and HG+Lir groups were 21, 19, and 23, respectively. $^{*}P < 0.05$, $^{**}P < 0.01$ versus NG group; $^{\#}P < 0.05$, versus HG group.

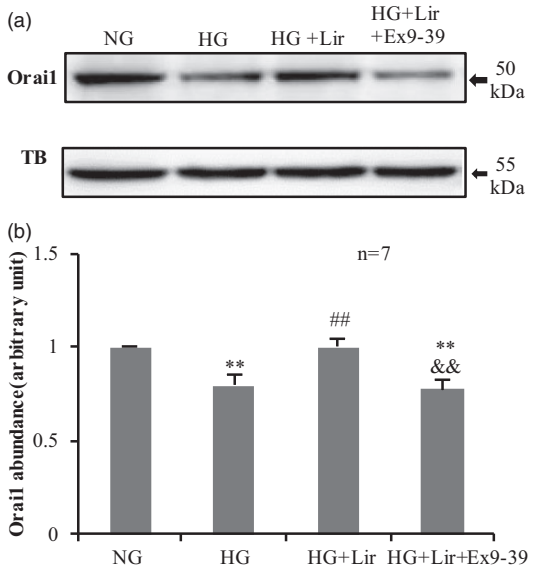

Figure 6. Liraglutide effect on Orai1 was GLP-1R dependent.
Growth-arrested HMCs were either in 0.5% FBS NG medium or in HG medium in the presence or absence of 100 nM liraglutide with and without 1000 nM exendin 9–39 for one day. Lir: liraglutide, Ex9–39: exendin 9–39. α-tubulin (TB) was used as the loading control. (a) Representative blots. (b) Summary data. $^{**}P < 0.01$, compared with NG. $^{\#\#}P < 0.01$, versus HG. $^{\&\&}P < 0.01$ versus HG+Lir. "n=7": No. of independent experiments.

on ECM production by MCs. Our findings suggest that activation of anti-fibrotic SOC signaling in MCs could be a downstream mechanism underlying GLP-1R-induced renoprotection in diabetic kidney.

The ubiquitous SOC is involved in multiple functions in both excitable and non-excitable cells.[7] In addition to MCs, many other GLP-1R present cells, such as renal proximal tubule cells, pancreatic islet β-cells, hepatocytes, neurons, also contain SOC.[7,43] Whether the GLP-1R/SOC cascade found in the MCs in the present study can be extended to GLP-1 associated responses in other type of cells is not known. In steatotic hepatocytes, activation of GLP-1R by exendin-4 reversed the inhibition of lipid on SOCE.[44] However, in the renal proximal tubular cells, effects of GLP-1R and SOC signaling are opposite. The former is protective[45] and the latter is deleterious.[43] Therefore, upregulation of SOC signaling may not be a general mechanism mediating effects of activation of GLP-1R pathway in different cell types.

How GLP-1R activation upregulated Orai1 protein abundance and SOC function is not known from this study. GLP-1R is Gαs-protein coupled receptor, activation of which initiates the cascade of cyclic AMP (cAMP)-protein kinase A (PKA).[42,46] We speculate that the GLP-1R effects on Orai1 and SOCE observed in this

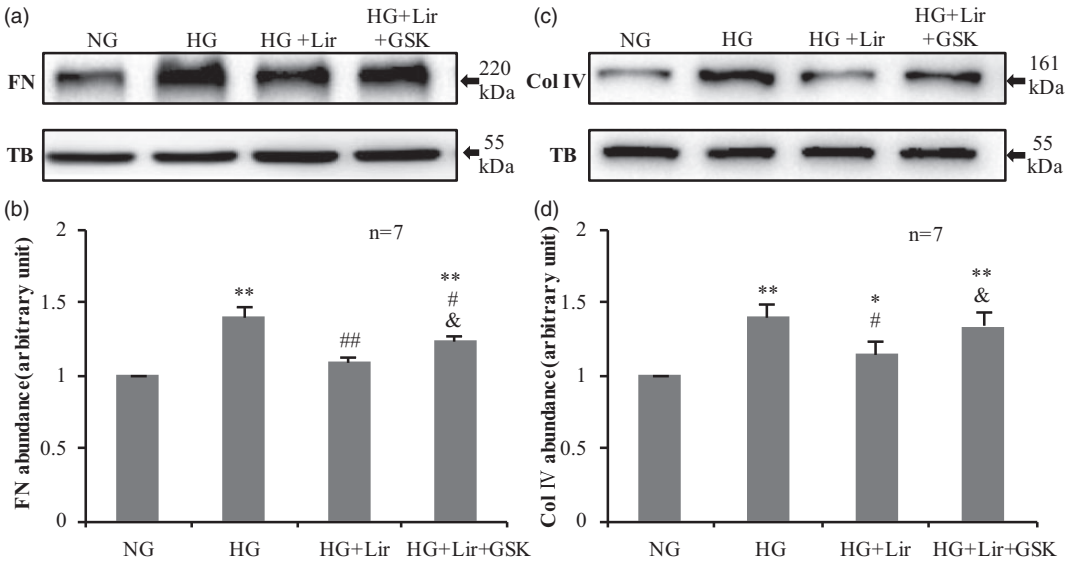

Figure 7. SOC mediated liraglutide effect on FN and Col IV protein abundance in HMCs.
Western blot. Liraglutide effect of reducing HG-induced increase of FN (a and b) or Col IV (c and d) protein abundance in HMCs was abolished by GSK-7975A. HMCs were either in 0.5% FBS NG medium or HG medium with or without liraglutide (100 nM) or GSK-7975A (10 μM) for two days. Lir: liraglutide, GSK: GSK-7975A. (a) and (c) Representative blots. (b) and (d) Summary graph. $^{*}P < 0.05$, $^{**}P < 0.01$ versus NG; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus HG; $^{\&}P<0.05$ versus HG+Lir. "n=7": No. of independent experiments.

study were attributed to the Orai1 expression-modulated protein kinases and/or transcription factors associated with GLP-1R pathway. For instance, the serum-and-glucocorticoid-inducible-kinase-1 (SGK1) was reported to increase Orai1 protein content and enhance SOCE in many cell types.[47–50] SGK1 has been demonstrated to be activated by cAMP-PKA,[51,52]a pathway downstream of GLP-1R activation.[42,44,46]

Another interesting finding is that liraglutide significantly reversed HG-induced decrease of GLP-1R protein abundance in HMCs. It is known that liraglutide functions as a GLP-1R agonist and stimulates GLP-1 signaling via receptor binding, followed by activation of the cAMP-PKA pathway.[42,44,46] We found that liraglutide treatment for two days also increased amount of GLP-1R in MCs. Therefore, liraglutide may upregulate GLP-1R signaling pathway through both acute (activation of GLP-1R) and chronic (increasing the number of GLP-1R) mechanisms. In agreement with our observation, one study demonstrated that in cultured HK-2 cells, liraglutide treatment for 72 h significantly enhanced GLP-1R mRNA expression.[53] However, whether the findings on liraglutide can be extended to other GLP-1R agonists, such as exendin-4 is not known and further study is needed.

Previous studies by others demonstrated that the GLP-1 effects involved both GLP-1R-dependent and independent pathways.[54,55] Our findings study suggest that the inhibition of ECM proteins by GLP-1 analogue (liraglutide) in kidney MCs was through GLP-1R pathway because blockade of GLP-1R by exendin-3 (9–39) significantly abolished the anti-fibrotic effects of liraglutide. GLP-1R signaling pathway involves cAMP production and PKA activation.[46] This mechanistic pathway also exists in MCs. For instance, Li *et al*.[42] reported that inhibition of exendin-4 on proliferation of HMCs was attributed to adenylate cyclase

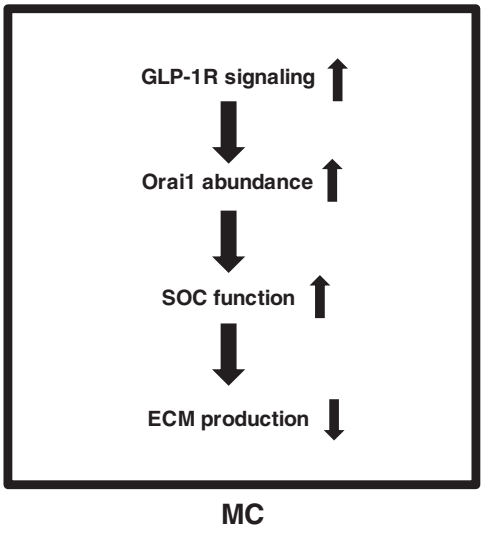

Figure 8. The diagram illustrating the pathway of GLP-1R signaling in negative regulation of ECM production by MCs.

activation. The present study showed that SOC was also involved in the GLP-1R signaling in MCs. It would be interesting to further study if the GLP-1R/cAMP and GLP-1R/SOCE are two independent pathways (in parallel) or in a common pathway (in serial) in future.

One limitation of this study is lack of animal data to validate our *in vitro* findings. Decrease of glomerular ECM protein production by activation of GLP-1R pathway has been reported in animal model of DKD.[35] Whether the renoprotective effects of GLP-1R signaling can be blocked by *in vivo* inhibition of SOC selectively in MCs will provide valuable information. However, it is technically difficult to genetically *in vivo* manipulate SOC signaling specifically in

MCs because the cells do not have the cell type specific promoter. Recently, Davis *et al.*[56] established the targeted nanoparticle/siRNA *in vivo* delivery system which delivered siRNAs into MCs with high selectivity in mice. This novel approach provides a possibility to downregulate SOC function specifically in MCs in an animal model of DKD.

In summary, the results from this study suggest that activation of GLP-1R inhibited ECM protein production in MCs by increasing Orai1 protein abundance and upregulating SOC function. Therefore, we identified a novel mechanism underlying the renal protective effects of GLP-1R pathway. This mechanistic pathway is illustrated in Figure 8. GLP-1R agonists have been used in clinic to treat patients with diabetes mellitus. However, their side effects are noticeable. Understanding the molecular mechanisms underlying the general anti-diabetic effects and the organ/cell type specific protection of the GLP-1R pathway can help design innovative therapeutic strategies for diabetic patients with various complications. Our study indicates that upregulation of GLP-1R/SOCE signaling in MCs could be an option for patients with DKD.

Authors' contributions: LJH, TTL, SC and PYS conducted the experiments, LJH and PWW performed statistical analysis and drafted the manuscript. RM revised the manuscript. PWW and LYY conceived and designed the study, and revised the manuscript, accordingly prior to submission. All authors have read and agreed with the final content of this manuscript.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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