Hepatocyte growth factor exerts beneficial effects on mice with type II diabetes-induced chronic renal failure via the NF-κB pathway

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Abstract. Type II diabetes is associated with a low quality of life and chronic renal failure, and is often accompanied by varying degrees of chronic renal failure. Chronic renal failure is considered one of the most important factors that aggravates diabetes and contributes to renal insufficiency in patients with diabetes though cellular fibrosis. It has previously been reported that hepatocyte growth factor (HGF) serves extensive biological roles, and is a multifunctional antifibrotic factor that is involved in kidney development, acute injury and regeneration. The present study aimed to investigate whether HGF exerts beneficial effects on type II diabetes-induced chronic renal failure in a mouse model. Plasma concentration levels of HGF, tumor necrosis factor (TNF)-α, monocyte chemoattractant protein (MCP)-1, interleukin (IL)-1 and IL-6 were analyzed prior to and following treatment with HGF. Blood urea nitrogen, plasma creatinine concentrations, and electrolyte, total serum protein, parathyroid hormone and C-reactive protein levels were analyzed by ELISA. The mechanism underlying the effects of the HGF-mediated signaling pathway was also investigated in mice with type II diabetes-induced chronic renal failure. Histological analysis was used to determine the therapeutic effects of HGF on mice with type II diabetes-induced chronic renal failure. The results indicated that HGF exhibited lower plasma concentrations in mice with type II diabetes-induced chronic renal failure compared with in healthy mice. In addition, treatment with HGF relieved chronic renal failure via inhibition of inflammation. The results indicated that TNF-α, MCP-1 and IL-1 serum concentration levels were downregulated following treatment with HGF. Conversely, IL-6 and vascular endothelial growth factor concentration was increased in the HGF-treated mice compared with in the control mice. The results also demonstrated that HGF treatment downregulated the expression of nuclear factor (NF)-κB molecules, and target molecules C-C motif chemokine ligand (Ccl)2, Ccl5, intercellular adhesion molecule 1 and TNF-α. The present study demonstrated that HGF markedly improved renal failure induced by type II diabetes in a mouse model; histological analyses revealed that renal cell injury was improved following treatment with HGF. In conclusion, these results suggested that HGF may exert beneficial effects on type II diabetes-induced chronic renal failure via regulation of the NF-κB signaling pathway.

Introduction

Chronic renal failure is a syndrome associated with serious metabolic disorders and refers to damage caused by various types of chronic kidney disease (1). Inflammation is one of the most common characteristics of patients with type II diabetes, and is associated with dysfunctional urinary albumin excretion, endothelial function and cellular metabolism (3). In addition, hypertension is often present in patients with type II diabetes, and the onset of hypertension is a frequent focus of clinical investigations (4–6). A previous study reported that inhibition of the renin-angiotensin system was able to markedly decrease blood pressure by reducing vascular inflammation (7). However, long-term treatment with antihypertensive drugs may lead to a decline of renal function and may even cause chronic renal failure in patients with type II diabetes (8).

The main pathogeneses associated with chronic renal failure include glomerulonephritis, interstitial nephritis, high blood pressure, diabetes and nephritis (9). At present, the prevalence of diabetes-associated chronic renal failure is increasing, which may be due to the marked rapid increase in patients with diabetes worldwide (10,11). A previous study noted that patients with diabetes and end-stage renal failure exhibited prolonged tissue healing of critical limb ischemia (12). In addition, chronic renal failure demands specific, continuous and varied care, which may present burden for family caregivers (13). Therefore, it is vital to investigate the association between chronic renal failure and type II diabetes in order to explore novel strategies for the diagnosis and treatment of patients with chronic renal failure.

Hepatocyte growth factor (HGF) is produced by mesenchymal cells during organ injury (14). HGF exerts
extensive biological activities and serves as a multifunctional antifibrotic factor that has a critical role in kidney development, acute injury and regeneration. HGF is activated by proteolytic cleavage at the site of injury, thus resulting in generation of the biological HGF protein (15). A previous study reported that serum levels of HGF were correlated with quality of life in patients undergoing hemodialysis (16). Furthermore, biologically active HGF is able to suppress fibrosis, and a molecular basis for HGF-mediated regression of renal fibrosis has previously been elaborated on (17,18). Therefore, HGF may be regarded as a local acute phase protein associated with chronic renal failure.

The present study aimed to investigate whether the expression and function of HGF were decreased and associated with inflammation in mice with type II diabetes-induced chronic renal failure. The results demonstrated that treatment with HGF markedly relieved chronic renal failure by decreasing the inflammatory response. Notably, the results revealed that HGF may improve chronic renal failure via the nuclear factor (NF)-κB signaling pathway. These preclinical data may provide important information for doctors and clinicians in the treatment of patients with type II diabetes-induced chronic renal failure.

Materials and methods

Ethics statement. The present study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of Southern Medical University (Guangzhou, China). The study was approved by the ethics committee of Zhujiang Hospital, Southern Medical University. All surgical procedures and methods of euthanasia were conducted to minimize suffering.

Type II diabetes-induced chronic renal failure mouse model. HLA-A2 mice with type II diabetes-induced renal failure (age, 16-18 weeks; n=120) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). All mice were fed under pathogen-free conditions and were given free access to food and water, and housed in a temperature-controlled facility at 23±1˚C and relative humidity of 50±5% with a 12‑h light cycle. The mice were divided into three groups (n=30/group), which received treatment with HGF (10 mg/kg), aldosterone (10 mg/kg) or the same dose of PBS using RNAeasy Mini kit (Qiagen, Inc., Valencia, CA, USA). Subsequently, 1 µg total RNA was transcribed into cDNA using a RT kit (Qiagen, Inc.) according to manufacturer's protocol and quality was confirmed by electrophoresis. cDNA (10 ng) was then subjected to qPCR (Bio‑Rad Laboratories, Inc., Hercules, CA, USA) using the SYBR‑Green Master Mix system. All forward and reverse primers were synthesized by Invitrogen (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and are detailed in Table I. Thermocycling conditions were as follows: Pre-denaturation at 95°C for 120 sec, denaturation at 95°C for 30 sec and annealing at 57°C for 10 sec for 45 cycles. Relative mRNA expression levels were determined according to the 2−ΔΔCq method (21). The results are expressed as n-fold compared with the control (β‑actin).

Reverse transcription‑quantitative polymerase chain reaction (RT‑qPCR). Total RNA was extracted from renal cells prior to (healthy mice) or following treatment with HGF (10 mg/kg), aldosterone (10 mg/kg) or the same dose of PBS using RNAeasy Mini kit (Qiagen, Inc., Valencia, CA, USA). Subsequently, 1 µg total RNA was transcribed into cDNA using a RT kit (Qiagen, Inc.) according to manufacturer's protocol and quality was confirmed by electrophoresis. cDNA (10 ng) was then subjected to qPCR (Bio‑Rad Laboratories, Inc., Hercules, CA, USA) using the SYBR‑Green Master Mix system. All forward and reverse primers were synthesized by Invitrogen (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and are detailed in Table I. Thermocycling conditions were as follows: Pre-denaturation at 95°C for 120 sec, denaturation at 95°C for 30 sec and annealing at 57°C for 10 sec for 45 cycles. Relative mRNA expression levels were determined according to the 2−ΔΔCq method (21). The results are expressed as n-fold compared with the control (β‑actin).

Western blot analysis. Renal cells from experimental mice were homogenized in lysis buffer containing protease-inhibitor (Sigma‑Aldrich; Merck KGaA) and were centrifuged at 6,000 x g at 4°C for 10 min. The supernatant was then used to analyze protein expression. To detect proteins of interest, Protein concentration was measured by a BCA kit method (Thermo Fisher Scientific, Inc.). Protein samples (20 µg/lane) were resolved by 15% SDS‑PAGE and then transferred onto polyvinylidene fluoride membranes (Merck KGaA) according to the manufacturer's protocol. For western blotting, primary antibodies: HGF (ab83760; 1:2,000), aldosterone (10 mg/kg) or PBS. Serum was collected from 10 ml blood using centrifugation at 6,000 x g for 10 min at 4°C. Serum and urine samples were collected to analyze inflammatory factors and biochemical indexes. Renal cells also underwent immunohistochemical staining and western blotting.
antibodies (ab150077; 1:2,000; Abcam) for 24 h at 4˚C. Blots were visualized using a chemiluminescence detection system (Pierce™ Fast Western Blot kits, SuperSignal™ West Femto; Thermo Fisher Scientific, Inc.).

NF-κB activity. Renal cells (1x10⁶ cells/well) were seeded in 6-well plates for 12 h at 37˚C. NF-κB-luc plasmids (Qiagen, Inc.) containing the response element were transfected into the renal cells, following replacement with MEM medium (Invitrogen; Thermo Fisher Scientific, Inc.) 12 h after transfection, the cells were then treated with various concentrations of apigenin. Cell lysate (NF-κB) was collected on a 6-well white plate after 48 h, and luciferase activity was detected using a luciferase reporter assay as described previously (22).

Immunohistochemical staining. Immunohistochemical staining was performed according to the avidin-biotin-peroxidase technique. Tissues were fixed with 10% formaldehyde for 2 h at 37˚C, then embedded in paraffin and sectioned at 4 μm. Epitope retrieval using Epitope Retrieval kit (cat. no. GTX30934; GenTex, Inc., Irvine, CA, USA) was performed for further analysis. The paraffin-embedded sections were incubated with hydrogen peroxide (3%) for 10-15 min at 37˚C, and were then blocked with regular blocking solution (5% skimmed milk) for 10-15 min at 37˚C. Finally, the sections were incubated with anti-HGF (ab83760; 1:1,000), anti-CD3 (ab16669; 1:1,000), anti-kidney injury molecule (KM)-1 (ab47634; 1:1,000), anti-IL-18 (ab52914; 1:1,000), anti-cluster of differentiation (CD)86 (ab119857; 1:1,000; all from Abcam) and anti-CD3 (1:5,000; Bio-Rad Laboratories, Inc.) for 1 h at 37˚C. For hematoxylin and eosin (H&E) assay, tissue sections (4 μm) were stained with H&E for 2 h at 37˚C. Then 6 random fields of view were observed under a fluorescent microscope (Olympus Corporation, Tokyo, Japan) at magnification, x40.

TUNEL assay. For analysis the apoptosis of renal cells in experimental mice after treatment with HGF, aldosterone or PBS, the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) assay (Biotool) were used to detect TUNEL-positive cells. The procedures were performed by previous study (23). Finally, hippocampal neuron cells images were captured with a ZEISS LSM 510 confocal microscope at 488 nm.

Statistical analysis. All data are presented as the mean ± standard error of the mean of triplicate experiments. Comparisons between multiple groups were made by one-way analysis of variance followed by Dunnett’s t test. P<0.05 was considered to indicate a statistically significant difference.

Results

HGF expression, blood glucose, blood lipid levels and body weight in mice with chronic renal failure. In order to investigate the role of HGF in chronic renal failure, the present study analyzed the expression levels of HGF in renal cells from mice with chronic renal failure, and compared them with those in healthy mice. As shown in Fig. 1A, the expression levels of HGF were downregulated compared with the healthy mice. However, treatment with HGF upregulated HGF expression, as determined by western blotting. Furthermore, protein expression of HGF was decreased in chronic renal failure mice compared with healthy mice (Fig. 1B). Blood lipid levels were improved in mice with type II diabetes-induced chronic renal failure treated with HGF compared with those treated with PBS (Fig. 1C). The present study also aimed to determine the therapeutic effects of HGF on body weight in a mouse model of type II diabetes-induced chronic renal failure. The results indicated that HGF markedly inhibited body weight compared with those in the aldosterone-treated and control mice on day 30 (Fig. 1D). Collectively, these data suggested that HGF was downregulated in a mouse model of type II diabetes-induced chronic renal failure, whereas restoration of HGF exerted beneficial effects on blood lipid levels and body weight in mice with type II diabetes-induced chronic renal failure.

Effects of HGF on inflammatory factor expression in renal cells from experimental mice. The present study further analyzed the effects of HGF on inflammatory factor expression in renal cells from experimental mice. As presented in Fig. 2A, the results

Table I. Sequences of primers used in the present study.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Reverse</th>
<th>Sequence</th>
<th>Forward</th>
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<tr>
<td>p53</td>
<td>5'-TTAAGCTTTCTTGATGCTGGAGC-3'</td>
<td>5'-ATGTTGGCCATGAACCTGTGG-3'</td>
<td></td>
</tr>
<tr>
<td>Bid</td>
<td>5'-CGACAGGTGTTAACGATCTCC-3'</td>
<td>3'-AGCGAGATGTTGTCATGAC-3'</td>
<td></td>
</tr>
<tr>
<td>Ccl2</td>
<td>5'-CCCCAGTCACCTGCTGTGTA-3'</td>
<td>5'-TGGAAATCCTGAACCACACTTC-3'</td>
<td></td>
</tr>
<tr>
<td>Ccl5</td>
<td>5'-TGTAACATACGCGCTACAGCTCCA-3'</td>
<td>5'-ATGCACTGTTCCTGCTGCTTA-3'</td>
<td></td>
</tr>
<tr>
<td>Icam-1</td>
<td>5'-GCCCTTGCTCTGTGAGTG-3'</td>
<td>5'-CCTAGCAATGGAGCGCAAGC-3'</td>
<td></td>
</tr>
<tr>
<td>TNFα</td>
<td>5'-ATGTGTGTAAGAACACCTTCA-3'</td>
<td>5'-AGGACCTGGGAGTAGATGA-3'</td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>5'-CCTTCCTGGCATGGAGTCTCT-3'</td>
<td>5'-GGAGCAATGATCTTGTATCTTC-3'</td>
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Bid, BH3 interacting-domain death agonist; Ccl, C-C motif chemokine ligand; Icam-1, intercellular adhesion molecule 1; TNF, tumor necrosis factor.
indicated that TNF-α plasma concentration levels were decreased following treatment with HGF compared with aldosterone. In addition, MCP-1 levels were downregulated in HGF-treated mice (Fig. 2B). IL-1 concentration levels were also decreased in mice with chronic renal failure following HGF treatment (Fig. 2C). Notably, HGF expression was recovered to normal levels following HGF treatment (Fig. 2D). Furthermore, the concentration levels of IL-6 and VEGF were increased in renal cells from experimental mice following HGF treatment, which may contribute to improved functioning of renal cells (Fig. 2E and F). These results suggested that treatment with HGF was beneficial for the treatment of mice with chronic renal failure.

**Effects of HGF on the NF-κB signaling pathway.** A previous study demonstrated that NF-κB serves an essential regulatory role in inflammation, via its control of the expression of numerous genes involved in cellular activity (24). Therefore, the present study analyzed the association between HGF and the NF-κB signaling pathway in mice with type II diabetes-induced chronic renal failure. Treatment with HGF blocked the nuclear import of activated NF-κB (p65) in renal cells from experimental mice (Fig. 3A). In addition, HGF treatment markedly suppressed activation of NF-κB in renal cells from mice on day 60 (Fig. 3B). The expression levels of IκBα, IκBβ and IκB were downregulated in renal cells from mice treated with HGF (Fig. 3C). In addition, HGF treatment markedly suppressed activation of NF-κB in renal cells from mice on day 60 (Fig. 3B). The expression levels of IκBα, IκBβ and IκB were downregulated in renal cells from mice treated with HGF (Fig. 3C). Furthermore, the present study indicated that HGF treatment decreased the expression levels of proinflammatory genes involved in the NF-κB signaling pathway, including C-C motif chemokine ligand (Ccl)2, Ccl5, intercellular adhesion molecule 1 (Icam1) and TNF-α, as determined by RT-qPCR (Fig. 3D). The present data also demonstrated that p53 and BH3 interacting-domain death agonist expression levels were decreased in renal cells following HGF treatment (Fig. 3E). Notably, the number of TUNEL-positive renal cells was markedly decreased following treatment with HGF (Fig. 3F). These results indicated that HGF may regulate the physiological functions of renal cells by controlling the NF-κB signaling pathway.

**Effects of HGF on histopathological alterations of renal cells from mice with type II diabetes-induced chronic renal failure.** To further determine the beneficial effects of HGF on the morphology and function of renal cells, histopathological alterations were analyzed by H&E and immunohistochemical staining of renal cells. As shown in Fig. 4A, cellular morphology was improved following treatment with HGF, presenting a pebble-like shape that is typical renal cell morphology. In addition, KM-1 and IL-18 levels were downregulated following HGF treatment (Fig. 4B), and the number of immune cells was decreased in renal sections obtained from HGF-treated mice (Fig. 4C). Furthermore, reduced CD86 expression was detected in HGF-treated mice, whereas CD86 expression was much higher in the control group (Fig. 4D). These data indicated that the physiology of renal cells was improved in HGF-treated experimental mice.

**Biochemical analysis of the therapeutic effects of HGF in mice with type II diabetes-induced chronic renal failure.** Following detection of the histopathological alterations of renal cells from mice with type II diabetes-induced chronic renal failure, the plasma biochemical indexes of mice were determined following treatment with HGF. As shown in Fig. 5A, blood
urea nitrogen levels were decreased following HGF treatment. Creatinine plasma concentration levels also were downregulated in HGF-treated mice (Fig. 5B). In addition, electrolyte concentration was increased in renal cells obtained from the HGF group (Fig. 5C). Serum total protein content was also recovered to normal levels in HGF-treated mice (Fig. 5D). Furthermore, parathyroid hormone levels were upregulated and C-reactive protein (CRP) expression levels were downregulated in HGF-treated mice (Fig. 5E and F). Taken together, these data suggested that HGF not only improves renal cell histopathology, but also regulates the biochemical indexes of mice with type II diabetes-induced chronic renal failure.

**Discussion**

Previous studies have demonstrated that chronic renal failure may induce the formation of complex symptoms and reduce quality of life in patients (25,26). In addition, patients with
chronic renal failure frequently develop uremia, resulting in glomerular cell dysfunction and loss of the biological activity of kidney cells (27). Dialysis is the most effective treatment for multiple organ failure and deterioration of the quality of the life. In addition, the pathogenesis of chronic renal failure is genetic and multifactorial (28,29). A previous study indicated that age has an important role in survival, limitation of physical activity and quality of life in patients with chronic renal failure (30). The present study investigated whether the expression and restoration of HGF affects inflammation, histopathological alterations and biochemical indexes in a mouse model of type II diabetes-induced chronic renal failure.

Inflammation is one of the most common characteristics of patients with type II diabetes (31). Temelkova-Kurktschiev et al (32)
reported that subclinical inflammation was upregulated in patients with type II diabetes, thus indicating that inflammatory responses may be associated with the occurrence, degree and prognosis of diabetes. Varughese and Lip reported that inflammation was associated with hypertension and urinary albumin excretion in patients with type II diabetes (3). In addition, a previous study demonstrated that vascular inflammation serves an important role in drug-induced rapid and persistent reduction of urinary albumin excretion, endothelial function and inflammation in patients with hypertension and type II diabetes (2). Furthermore, Bitar et al. reported that inflammation was associated with the degree of pathogenesis in aortic tissues from aged patients with type II diabetes via the phosphatidylinositol 3-kinase/protein kinase B-dependent signaling pathway (33). In the present study, the expression levels of inflammatory factors, including IL-6, IL-1, TNF-α, MCP-1 and VEGF, were detected in mice with type II diabetes-induced chronic renal failure. The results indicated that these inflammatory factors were downregulated in response to HGF, and the NF-κB signaling pathway was involved in HGF-mediated improvement of type II diabetes-induced chronic renal failure.

The present study indicated that treatment with HGF may regulate biochemical metabolism of renal cells via inactivation of the NF-κB signaling pathway. A previous study reported that NF-κB-induced oxidative stress contributes to mitochondrial and cardiac dysfunction in type II diabetes (34). In addition, receptor activator of NF-κB ligand exerted beneficial effects for patients with chronic renal failure via inhibition of the NF-κB signaling pathway (35). Biochemical indexes, including blood urea nitrogen, plasma creatinine concentration, electrolyte, serum protein, parathyroid hormone and CRP levels are crucial for normal kidney function. In the present study, these biochemical indexes were analyzed in mice with type II diabetes-induced chronic renal failure following treatment with HGF (36). The results revealed that HGF may be considered an efficient drug for the treatment of type II diabetes-induced chronic renal failure.

Although a previous study reported that inflammation is associated with hypertension in patients with type II diabetes, correlations between inflammatory factors and type II diabetes have not been clinically analyzed (37). To determine the association between HGF and serum inflammatory factors, the present preclinical study analyzed mice with type II diabetes-induced chronic renal failure. The results indicated that HGF treatment decreased the accumulation of immune cells in renal tissues in mice with type II diabetes-induced chronic renal failure. In conclusion, based on these preclinical data, serum concentration levels of IL-1, TNF-α and MCP-1 may be increased in mice with type II diabetes-induced chronic renal failure. However, HGF treatment was revealed to decrease the expression of inflammatory factors in mice with type II diabetes-induced chronic renal failure. In addition, HGF was downregulated in mice with chronic renal failure, whereas restoration of HGF exerted beneficial effects on blood lipid levels and body weight in mice with type II diabetes-induced chronic renal failure. Furthermore, HGF regulated the physiological function and biochemical indexes of renal cells via regulation of the NF-κB signaling pathway. Overall, the present study researched the function of HGF in a mouse model of type II diabetes-induced chronic renal failure; however, the role of HGF in patients with renal failure induced by type II diabetes requires further study.

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Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

GC and XT performed the experiments and JZ designed the study. All the authors have read and approved the manuscript and have approved this submission.

Ethics approval and consent to participate

The present study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of Southern Medical University (Guangzhou, China). The study was approved by the ethics committee of Zhujiang Hospital, Southern Medical University. All surgical procedures and methods of euthanasia were conducted to minimize suffering.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References


