Suppressions of chronic glomerular injuries and TGF-β₁ production by HGF in attenuation of murine diabetic nephropathy

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Mizuno, Shinya, and Toshikazu Nakamura. Suppressions of chronic glomerular injuries and TGF-β₁ production by HGF in attenuation of murine diabetic nephropathy. Am J Physiol Renal Physiol 286: F134–F143, 2004. First published September 30, 2003; 10.1152/ajprenal.00199.2003.—Diabetic nephropathy is now the leading cause of end-stage renal diseases, and glomerular sclerotic injury is an initial event that provokes renal dysfunction during processes of diabetes-linked kidney disease. Growing evidence shows that transforming growth factor-β₁ (TGF-β₁) plays a key role in this process, especially in eliciting hypertrophy and matrix overaccumulation. Thus it is important to find a ligand system to antagonize the TGF-β₁-mediated pathogenesis under high-glucose conditions. Herein, we provide evidence that hepatocyte growth factor (HGF) targets mesangial cells, suppresses TGF-β₁ production, and minimizes glomerular sclerotic changes, using streptozotocin-induced diabetic mice. In our murine model, glomerular sclerogenesis (such as tuft area expansion and collagen deposition) progressed between 6 and 10 wk after the induction of hyperglycemia, during a natural course of diabetic disease. Glomerular HGF expression levels in the diabetic kidney transiently increased but then declined below a basal level, with induction of hyperglycemia, during a natural course of diabetic nephropathy. (43); and 3 importantly, neutralization of TGF-β₁ actions with a specific antibody suppresses glomerular hypertrophy as well as sclerosis in vivo (33, 47). Thus TGF-β₁ is now considered to be a key molecule that aggravates diabetic nephropathy (13, 34). To prevent TGF-β₁-mediated fibrogenesis under diabetic conditions, there may possibly be a self-protection mechanism in vivo, but such a defense system is not well understood.

Hepatocyte growth factor (HGF) was originally identified and cloned as a potent mitogen for mature hepatocytes (27, 28). HGF is a potent mitogen and morphogen for renal tubular epithelial cells (3, 21). Actually, HGF accelerates renal tubular repair after the onset of acute renal failure, with rapid recovery of tubular morphology and functions (14, 16). Of note, HGF has therapeutic effects on chronic renal failure linked with enhanced tubular regeneration, and tubulointerstitial fibrosis was inhibited (15, 23–25, 44, 45) even when renal function was impaired. These studies focused on HGF’s roles mainly related to tubular and tubulointerstitial lesions. We recently obtained evidence that HGF works on mesangial cells and then inhibits their proliferation in a rat model of acute glomerulonephritis (4). Nevertheless, it is still unclear whether HGF directly inhibits chronic mesangial injuries, an important cascade leading to renal dysfunction (29).

To elucidate a role of HGF during the onset of glomerular injuries, we focused on diabetic nephropathy, because glomerular sclerogenesis (including hypertrophy and matrix overdeposition) precedes the onset of tubulointerstitial fibrosis, and this time lag seems advantageous to determine whether HGF’s roles regarding glomeruli are direct or indirect. In this study when we used streptozotocin (STZ)-injected mice as a model of diabetic nephropathy, we found that HGF prevents chronic glomerular lesions, which may determine predisposition to

IN CHRONIC RENAL diseases, renal fibrosis (such as glomerulosclerosis and interstitial fibrosis) occurs to replace the loss of parenchymal nephrons (29), but this pathological condition eventually leads to intractable renal dysfunction and hemodialysis becomes necessary. Among chronic renal disorders, diabetic nephropathy is now worldwide one of the most common etiologies of hemodialysis (1, 19); for example, the annual cost is $900 billion in Japan, and diabetics reaching dialysis have a twofold excess mortality risk. Given that diabetic nephropathy is a major contributor to dialysis-related financial and medical problems, it is important to elucidate a mechanism(s) as to how diabetic nephropathy progresses (or is delayed) under high-glucose conditions. The initial attention of nephrologists was directed to glomerular components, as mesangial injuries are considered to be the first step in the manifestation of diabetic nephropathy (5, 31).

Several lines of evidence revealed critical roles of transforming growth factor-β₁ (TGF-β₁) during the progression of glomerular lesions in diabetic nephropathy: 1) TGF-β₁ expression is upregulated by glucose and enhances extracellular matrix (ECM) accumulation in mesangial cells (36, 48); 2) TGF-β₁ expression levels are markedly increased in mesangial areas in animals or in patients after the onset of diabetic nephropathy (43); and 3 importantly, neutralization of TGF-β₁ actions with a specific antibody suppresses glomerular hypertrophy as well as sclerosis in vivo (33, 47). Thus TGF-β₁ is now considered to be a key molecule that aggravates diabetic nephropathy (13, 34). To prevent TGF-β₁-mediated fibrogenesis under diabetic conditions, there may possibly be a self-protection mechanism in vivo, but such a defense system is not well understood.

Hepatocyte growth factor (HGF) was originally identified and cloned as a potent mitogen for mature hepatocytes (27, 28). HGF is a potent mitogen and morphogen for renal tubular epithelial cells (3, 21). Actually, HGF accelerates renal tubular repair after the onset of acute renal failure, with rapid recovery of tubular morphology and functions (14, 16). Of note, HGF has therapeutic effects on chronic renal failure linked with enhanced tubular regeneration, and tubulointerstitial fibrosis was inhibited (15, 23–25, 44, 45) even when renal function was impaired. These studies focused on HGF’s roles mainly related to tubular and tubulointerstitial lesions. We recently obtained evidence that HGF works on mesangial cells and then inhibits their proliferation in a rat model of acute glomerulonephritis (4). Nevertheless, it is still unclear whether HGF directly inhibits chronic mesangial injuries, an important cascade leading to renal dysfunction (29).

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albuminuria, tubulointerstitial fibrosis, and renal dysfunction. We describe herein physiological and therapeutic effects of HGF to suppress TGF-β1-induced pathological states in hyperglycemia-linked nephropathy.

MATERIALS AND METHODS

Animals. Eight-week-old female C57B/6Cr Slc mice (18–20 g; SLC, Hamamatsu, Japan) were used. We attempted to induce hyperglycemia in these mice, based on a reported method (8): after fasting these mice for 24 h, we injected STZ (Nacalai, Kyoto, Japan) in a dose of 120 mg/kg ip for the initial 2 days and in a dose of 80 mg/kg ip for the subsequent 2 days. About 70% of the mice manifested severe hyperglycemia (plasma glucose level >500 mg/dl) within 1 wk after the last injection of STZ, and 20% of the animals died of severe dehydration. The remaining mice had mild hyperglycemia and were removed from the present experiments.

Reagents. For HGF-neutralizing treatment, anti-HGF antibody was raised by immunizing rat HGF in normal rabbits. The anti-HGF IgG cross-reacts with mouse (but not human) HGF and accelerates renal fibrogenesis (24, 25). A variant type of recombinant human HGF (rh-HGF) was produced by Chinese hamster ovary cells, a cell line transfected with human HGF cDNA with a deletion of 5 amino acid residues in the first kringle domain (14–16, 23–25). The HGF protein was >98% pure.

Observations on the natural course of diabetic nephropathy. Twenty STZ-injected mice were housed under specific pathogen-free conditions and were fed a standard diet (MF, Oriental yeast, Tokyo, Japan). To analyze the natural course of renal phenotypes, mice were killed at 0, 2, 6, and 10 wk after the STZ treatment (each group includes 5 mice). At necropsy, they were anesthetized with pentobarbital sodium (50 mg/kg ip), and plasma and renal tissues were collected for biochemical or pathological analyses, as described below.

Anti-HGF antibody treatment. For the anti-HGF IgG treatment, another 12 STZ-injected mice were generated. Four weeks after the STZ injections, they were divided into two groups (based on clinical data as described below) and intraperitoneally injected with the rabbit anti-rat HGF IgG (250 µg/mouse; n = 6) or normal rabbit IgG (250 µg/mouse; n = 6) on alternate days over a period of 12 days. These mice were killed on day 14 after start of this treatment.

Administration of exogenous HGF. To evaluate the effect of rh-HGF on progression of diabetic nephropathy, 12 diabetic mice were prepared. These mice were found to be in an early stage of renal insufficiency when their blood urea nitrogen (BUN) levels reached near 40 mg/dl (e.g., 6 wk after the STZ treatments), and they were then divided into an HGF-injected group and a saline-injected group: in the rh-HGF-injected group (n = 6), mice were given 300 µg/kg –1.12 h –1 HGF sc daily for 28 days, whereas control mice (n = 6) received subcutaneous injections of an identical volume of saline.

Blood and tissue chemistry. Plasma glucose levels were determined using a kit (Glucose B test, Wako, Osaka, Japan). BUN levels were determined with the urease indophenol method, using a kit (Urea B test, Wako) (23). The plasma creatinine level was measured using a kit (Creatinine test, Wako) (23). In an experiment related to rh-HGF therapy, plasma was obtained from postorbital veins on weeks 6, 8.5, and 10 and subjected to the laboratory examinations. The urinary albumin levels were determined, using a kit (AG B test, Wako) (23, 24). Renal tissue extracts were prepared, as described (23–25). Renal HGF levels were determined in ELISA, using a kit (HGF EIA, Institute of Immunology, Tokyo, Japan). Renal TGF-β1 levels were determined, using an ELISA kit (Quantikine TGF-β1, R & D) (23–25). Renal monocyte chemotactic agent protein-1 (MCP-1) levels were determined using a sandwich ELISA system (Amersham–Pharmaccia, Little Chalfont, UK).

Histopathology. The left kidneys were excised and fixed in cold 70% ethanol. The transversally trimmed kidney tissues were submitted to a routine process for paraffin embedding. The sections were cut into 4-µm slices, dewaxed, and then stained with hematoxylin and eosin. The remaining sections were subjected to immunohistochemistry: goat IgG against mouse type IV collagen (1:400; Chemicon, Temecula, CA) was used for the primary reaction, together with a commercial kit (Vectastain Elite ABC, Vector Labs, Burlingame, CA) (23–25). To detect the expression of growth factors, rabbit IgG against rat HGF (1:1,000) [prepared in our laboratory (24, 25)] and rabbit IgG against porcine and human TGF-β1,2,3 (pan TGF-β1: 1:100) were used for the primary reactions, followed by the ABC technique mentioned above. To support the fibrogenic events in diabetic kidneys, other parameters such as fibronectin, type I collagen, α-smooth muscle actin (α-SMA; a marker for myofibroblasts), and Mac-1 (a marker for macrophages) were detected immunohistochemically as described (23–25). To detect a chemokine involved in macrophage influx, anti-mouse MCP-1 hamster IgG (BD Biosiences, San Jose, CA) was used, followed by the ABC technique as mentioned.

Renal morphometry. Glomerular sclerosis (characterized by mesangial expansion) was graded according to the extent of mesangial involvement on a scale of 0 to 4: 0, normal; 0.5, small focal area of the tubular injury; 1, involvement of over 10% of the cortex; 2, involvement up to 25% of the cortex; 3, involvement up to 50 to 75% of the cortex; and 4, extensive damage involving more than 75% of the glomeruli (23). To evaluate the glomerular tuft hypertrophy, glomerular size was determined by measuring the glomerular area on the same glomeruli, by means of a video microscope (VM-30, Olympus, Tokyo, Japan). The glomerular scores of collagen (IV/I), TGF-β1, α-SMA, and fibronectins were determined as described (23). The overall means of these parameters were calculated based on individual values (n = 6), which were determined in at least 30 glomeruli per mouse. Finally, the degree of tubulointerstitial lesions was evaluated based on interstitial Mac-1, α-SMA, and type IV collagen scores (24, 25). These semiquantitative analyses were all made in a blinded fashion.

In vitro study. To evaluate the direct effect of HGF on TGF-β1 production, we prepared an in vitro model of diabetic nephropathy, based on reported data (36). We used human normal mesangial cells (HNMC; Sanko, Chiba, Japan) to induce fibrogenic phenotypes: the culture was passaged in dishes supplemented with 10% fetal bovine serum containing MCDB-131 (GIBCO, Grand Island, NY). These cells were adjusted at a density of 3–10 cells/cm² in a 48-well plate that overnight and then the medium was replaced with a fresh serum-free MCDB-131, where D-glucose was pulsed at concentrations of 5.5 mM (=100 mg/dl, i.e., normal) or 33 mM glucose (=600 mg/dl, i.e., diabetic). After the medium change, rh-HGF was added to the culture systems in various doses (0–30 ng/ml) and TGF-β levels in the supernatants were determined, as evaluated. To determine the fibrogenesis on mesangial cells, type IV collagen and α-SMA expressions were evaluated in an immunoblot analysis for lysates of cultured cells, as described (4). We extracted mRNA from HNMC using an acid guanidinium thiocyanate-phenol chloroform method. To determine changes in TGF-β, at transcription levels, mRNA was reversed to cDNA and subjected to amplification with primers specific to TGF-β1 cDNA (sense vs. antisense primer): CCGCAAGGACCTCGGCTGGA vs. GGATTTGGCGTATTGGG vs. GGATTTGGCCTCTAAGGTCT. As an internal control, GAPDH was used (GGATTTGCCTGATGTTGCG vs. GGATTTGCGATGTTGCG).

Statistical analyses. All data are expressed as means ± SD. An unpaired two-tailed t-test was used to compare the means, and a value of P < 0.05 was considered to have statistical significance. Linear regression analysis was employed to evaluate the significance of the relationship between variables, using statistical computer software (Start-View 15.0, SAS Institute Tokyo, Japan) (24).
RESULTS

Changes in renal HGF levels during progression of diabetic nephropathy in mice. To determine the role of HGF during the pathogenesis of diabetic nephropathy, we first prepared an animal model in which renal dysfunction progresses under high-glucose conditions. In STZ-injected mice, blood glucose levels rapidly increased within 7 days after serial administrations of STZ to a threefold level over the control (Fig. 1A, left), concomitantly with persistent polyuria and glycouria. In the diabetic mice, BUN levels increased between 6 and 10 wk after the onset of hyperglycemia in association with persistent hyperglycemia (glucose levels >500 mg/dl; Fig. 1A, middle). Similarly, the mice showed significant increases in plasma creatinine levels during the experimental periods (0W: 0.36 ± 0.05 mg/dl; 6W: 0.52 ± 0.08 mg/dl; and 10W: 0.93 ± 0.12 mg/dl). Renal morphometry revealed that the glomerular sclerosis score increased, in association with elevated parameters of renal functions (Fig. 1A, right). In this model, renal HGF levels increased for up to 2 wk after the STZ injection to a twofold level over the pretreatment control (Fig. 1B, line graph). However, HGF levels reverted to near the basal level at 6 wk after the onset of hyperglycemia with reciprocal increases in BUN levels. In renal histochemistry, immunoreactive signals for HGF were evident in mesangial regions of the glomeruli in the mice especially at 2 wk after the initiation of diabetes. The glomerular HGF expression became faint following 6 wk (6W) of the STZ treatments (Fig. 1B). In this time point (i.e., 6W), interstitial lesions were very mild but became evident at 10 wk after the STZ challenge, with the increase in peritubular HGF expression (not shown).

Involvement of a decrease in HGF-positive glomerular cells in tuft sclerotic injuries. Measurement of endogenous HGF in whole renal tissues included glomerular and peritubular HGF and did not accurately reflect glomerular HGF changes. Thus we counted the number of HGF-positive cells in glomeruli, as described (4). The HGF-positive glomerular cells transiently increased 2 wk after STZ injections, followed by significant losses of intrinsic HGF, especially noted at 10 wk following the induction of diabetes (0W: 4.46 ± 0.57 vs. 10W: 2.15 ± 0.31 cells per glomerulus, P < 0.01; Fig. 2A, left). The degree of glomerular HGF expression negatively correlated with the glomerular collagen IV score during the progression of diabetic nephropathy in our mouse model (Fig. 2A, middle). Furthermore, there was an inverse correlation between the HGF-positive glomerular cell number and tuft area sizes (Fig. 2A, right). To elucidate the significance of the decreased HGF, we injected anti-rodent HGF-neutralizing IgG into the diabetic mice from 4 wk after STZ injections for 2 wk: in the HGF-neutralized mice, glomerular sclerogenic findings (such as type IV collagen deposition and tuft size expansion) were evident.
compared with those in the normal IgG-treated group (Fig. 2B). The glomerular type IV collagen score was 1.8-fold higher in the HGF-neutralized mice than in placebo-treated mice (1.71 ± 0.20 vs. 0.96 ± 0.17, P < 0.01). Furthermore, the size of the glomerular area in the mice significantly increased after the anti-HGF IgG treatment. Thus we hypothesized that a change in glomerular HGF expression may affect the initial pathogenesis of diabetic nephropathy.

**Effect of exogenous HGF administrations on diabetes-related conditions in mice.** To gain support for our hypothesis, supplement therapy with rh-HGF was given to the diabetic mice during a 4-wk period (from weeks 6 to 10 after STZ injections), because: 1) intrinsic HGF rapidly declined within this time and 2) in the earlier phase (i.e., 2W), the mice showed hydration and did not stably manifest renal dysfunction. Throughout the administration periods, rh-HGF did not alter the natural course of blood glucose levels, noted in diabetic mice treated with saline (Fig. 3A). We next checked BUN and creatinine levels in mice to estimate renal functions: in saline-injected diabetic mice, some glomeruli became hypertrophic, with a hyalynosis-like lobular nodule (Fig. 4A, left). In contrast, glomerular hypertrophy decreased in HGF-treated diabetic mice, with almost normal capillary morphology (Fig. 4A, middle). In the HGF-treated animals, glomerular tuft size was reduced to the level seen in the pretreatment group (e.g., 6W; Fig. 4A, right). Ratios of left kidney weight to heart weight in diabetic mice are pretreatment (6W) 1.98 ± 0.24, saline (10W) 2.52 ± 0.57, and rh-HGF (10W) 1.91 ± 0.21. A significant difference in kidney weight was seen between the saline- and HGF-injected animals (P < 0.01), thus demonstrating HGF’s role in preventing renal hypertrophy. We next examined mesangial ECM deposition (another feature of diabetic glomerulopathy): in the saline group, sclerotic changes (i.e., overdeposition of type IV collagen) were evident in some

![Fig. 2. Contribution of glomerular HGF to suppress tuft hypertrophy and matrix deposition in diabetic mice.](http://ajprenal.physiology.org/)

A. **left:** changes in glomerular HGF-positive cells in a natural course of diabetic nephropathy in STZ-injected mice. Data are means ± SD (n = 5). Statistical analysis: *P < 0.05 and **P < 0.01 compared with an initial HGF level (0W). **middle** and **right:** during the natural course of diabetes (0–10W), numbers of the HGF-positive glomerular cells were plotted to correlate with glomerular collagen score or tuft sizes (n = 20). B: acceleration of glomerular hypertrophy and collagen deposition in diabetic mice after anti-HGF IgG treatments. The anti-HGF IgG (250 μg/head −1·48 h · ip) was injected into mice from 4 wk after STZ injections for 2 wk. **Left:** microphotographs represent glomerular tuft findings, noted in normal IgG (used as placebo) or anti-HGF IgG groups (type IV collagen staining, ×330). **Right:** degree of sclerogenic changes was quantified through microscopic morphometry for glomerular collagen (IV) score and tuft size (means ± SD, n = 6). Statistical analysis = *P < 0.05 and **P < 0.01 compared with a normal IgG group.
glomeruli (Fig. 4B, left), occasionally with an increase in the size of glomerular tufts. Of note, HGF repressed collagen deposition in mesangial spaces during the 4 wk, with a significantly lowered score (Fig. 4B, right). HGF also suppressed glomerular deposition of type I collagen, fibronectin, and \( \alpha \)-SMA (Fig. 4C), which are all involved in initiation of glomerular sclerogenesis.

**Glomerular TGF-\( \beta \) expression and its modulation by HGF in diabetic mice.** As TGF-\( \beta \) is critical to elicit glomerular sclerosis in diabetic renal diseases (13, 33, 34), we asked whether HGF would alter glomerular TGF-\( \beta \) expression under diabetic conditions. Immunohistochemical examinations demonstrated TGF-\( \beta \)-positive areas in sclerotic regions in the saline group, while this pathological event was attenuated after rh-HGF treatment (Fig. 5A). The glomerular TGF-\( \beta \) score was significantly lower in the rh-HGF group than in the saline group (2.69 ± 0.41 vs. 1.27 ± 0.33, \( P < 0.01 \)). To gain support for the histological data, renal TGF-\( \beta \) levels were measured using an ELISA system (23): in diabetic mice, renal TGF-\( \beta \) levels increased 1.8-fold over the pretreated diabetic controls.
Consistent with the histochemical findings, HGF suppressed the increase in renal TGF-β1 concentrations in this animal model ($P < 0.01$).

**Inhibitory effect of HGF on TGF-β1 production, α-SMA, and type IV collagen accumulation in cultured mesangial cells.** Because mesangial cells are a major source of TGF-β1 in diabetic nephropathy (43, 48), we focused on a role for HGF in the TGF-β1-producing cells. Under a high-glucose condition (with 33 mM glucose), supernatant TGF-β1 levels increased to a 1.8-fold level over the physiological control (i.e., 5.5 mM glucose; Fig. 6A), being similar to documented data (36, 48). In this model, HGF dose dependently repressed sugar-induced increases in TGF-β1 levels, noted in the diabetic (33 mM glucose) but not nondiabetic (5.5 mM) cultures. Especially,
there was a significant difference in TGF-β1 levels between 33 mM glucose alone vs. the high glucose plus rh-HGF (30 ng/ml; \( P < 0.01 \)). Furthermore, high glucose (33 mM) increased the TGF-β1 mRNA expression levels, whereas HGF repressed the upregulation of TGF-β1 mRNA (Fig. 6B). We next investigated the effect of HGF on myofibroblast formation and ECM accumulation, based on α-SMA and type IV collagen expression, respectively. In the culture with high glucose, mesangial α-SMA and type IV collagen were detected as bands in the blot analysis (Fig. 6C). Supplementing the culture with HGF (30 ng/ml) led to suppressions of the α-SMA and collagen, evidence of the counteracting effects of HGF on TGF-β1-mediated sclerogenesis.

Prevention of albuminuria-related interstitial changes by HGF in diabetic kidneys. Glomerular injuries elicit urinary albumin excretion, while in turn albuminuria triggers peritubular inflammation and fibrogenesis, possibly via enhanced MCP-1 production (9, 35). In our model, urinary albumin levels gradually increased in the saline-injected group (Fig. 7A). By contrast, urinary albumin levels declined in HGF-treated mice, especially at 10 wk after STZ injections (\( P < 0.01 \)). In the HGF-treated mice, renal MCP-1 levels were reduced to 62% of the control group, with a significant difference (\( P < 0.05 \); Fig. 7B). In the control group, MCP-1 was widely noted in the proximal tubules, whereas tubular MCP-1 expression was limited in the HGF-treated mice (Fig. 7C). Concomitantly with the reduced MCP-1, the number of interstitial macrophage (judged as Mac-1-positive cells) was reduced in diabetic kidneys treated with HGF (Fig. 7C). Furthermore, interstitial α-SMA and type IV collagen accumulations were also attenuated in mice given HGF supplements (Fig. 7C). Consistently, there were significant differences in interstitial Mac-1, α-SMA, and type IV collagen scores between saline- and HGF-treated groups (Fig. 7D).

DISCUSSION

Diabetes is now the leading cause of end-stage renal disease in many developed countries and diabetic nephropathy has emerged as a silent epidemic worldwide (1, 19). This is the typical case in the United States, where diabetic nephropathy accounts for 42% of all new cases of end-stage renal disease as of 1997 (1). The physical and monetary costs for both patients and society are enormous, and these backgrounds stimulated basic research to elucidate a mechanism(s) related to progression of diabetic nephropathy regulated at a molecular level(s) (13, 34). Using cultures and animal models of diabetes, we provided evidence that HGF directly targets mesangial cells,
suppresses TGF-\(\beta_1\) production, and minimizes glomerular (and possibly peritubular) fibrosis, all contributing to prevention of renal dysfunction in diabetic nephropathy. This is the first report identifying a natural ligand to protect kidneys from pathological conditions related to diabetes.

Hyperglycemia and renal hypertrophy are key determinants of diabetic complications, including nephropathy in insulin-dependent (40) and -nondependent diabetes mellitus (41). It is of interest to note that glomerular HGF levels show an inverse correlation with the severity of tuft hypertrophy in the mouse model we used. Of note, anti-HGF IgG treatment led to a significant increase in the size of glomerular tufts. Inversely, supplements of rh-HGF almost completely not only arrested renal growth but also minimized glomerular tuft expansions, thereby revealing a role of mesangial HGF in inhibiting renal hypertrophy. A causal involvement of TGF-\(\beta_1\) in diabetic renal hypertrophy was demonstrated given that application of TGF-\(\beta\) antibodies attenuated the effect in experimental animals (33, 47). Therefore, we focused on renal TGF-\(\beta_1\) expression to explain anti-hypertrophic effects of HGF. In our culture system, we found that high-glucose-stimulated TGF-\(\beta_1\) induction was abolished by HGF. This effect was reproduced in vivo: rh-HGF therapy for diabetic mice led to a reduction of the TGF-\(\beta_1\)-positive mesangial areas. Thus one possible explanation is that HGF may inhibit tuft hypertrophy via suppression of TGF-\(\beta_1\) production. Another possibility is that HGF may reduce glomerular hyperfiltration [linked with glomerular hypertrophy (7, 29)], because urinary volume in the HGF-treated mice was reduced to 70% over control levels (data not shown).

In addition to tuft hypertrophy, glomerular sclerosis is a risk factor for renal dysfunction in subjects with diabetic nephropathy (5, 31). Overexcessive ECM in mesangial spaces can cause vascular capillary collapse (5, 29), leading to albuminuria and interstitial injuries are accelerated. To produce ECM proteins, mesangial cells acquire myofibroblast-like phenotypes (including \(\alpha\)-SMA fibers) (10) and TGF-\(\beta_1\) plays a central role in this process (6). After the onset of hyperglycemia, glomerular HGF levels are inversely proportional to the degree of mesangial sclerosis. Moreover, rh-HGF led to decreased TGF-\(\beta_1\) levels and attenuated sclerosis in the mice, suggesting that endogenous HGF is protective for the progression of diabetic glomerulopathy in the advanced stage. Our in vitro results suggest that antisclerogenic effects (such as attenuated myofibroblastosis and reduced ECM deposition) by HGF are, at least in part, directed toward mesangial cells.

We discuss other mechanisms of HGF-mediated outcomes in diabetic glomerulopathy. A loss of glomerular endothelial cells or podocytes may be critical for glomerular sclerosis to be manifest (17, 37). HGF is protective to endothelial cells and podocytes (11, 26), even under diabetic states. Thus protection of glomerular resident cells by HGF may involve attenuated glomerular injuries. We reported that HGF arrests mesangial overproliferation (4), an initial event that provokes sclerosis. HGF represses upregulation of connective tissue growth factor (15), a key cytokine needed for fibrosis to develop (32). Given that HGF induces ECM-degrading enzymes (such as matrix metalloproteinase-1/-9) (20, 30), HGF-induced matrix metalloproteinases likely contribute to attenuated fibrosis. HGF can decrease blood pressure in vivo (46), and this may be linked with suppressions of tuft hypertrophy by HGF. Such multifunctional activities by HGF would lead to attenuated glomerular injuries.

Clinical studies imply that tubulointerstitial lesions show the best correlation with renal failure in diabetic nephropathy (5, 12). Urinary albumin provokes MCP-1 upregulation, and then peritubular inflammation and fibrosis may develop (9, 35). Notably, HGF was shown to repress albuminuria in our model. Renal MCP-1 levels were reduced by HGF, such being the opposite of findings in vitro (42). Concomitantly, tubulointerstitial fibrogenic events (such as macrophage infiltration, myofibroblastosis, and ECM overdeposition) were suppressed in HGF-injected diabetic mice. Thus sequential mechanisms for attenuated renal dysfunction include 1) HGF protects from glomerular injuries in diabetic stress; 2) albumin excretion and in turn tubular MCP-1 expression are controlled; and 3) overall, onset of peritubular inflammation and fibrogenesis is avoided. On the other hand, we also consider direct effects of HGF toward tubular lesions, as reported (23–25).

Recently, Laping et al. (18) reported that a strain of mice (db/db) developed diabetic kidney disease under an HGF supplement protocol. This finding conflicts with our observations. In their study, however, very low doses of HGF (160 ng·kg\(^{-1}\)·day\(^{-1}\) = “1/3,000” of ours, sc) were used. Because the half-time of HGF in the circulation is within 10 min, at least several hundred micrograms of HGF are needed to produce and sustain physiological HGF levels (our unpublished

Fig. 8. Hypothetical model for molecular pathogenesis and therapy of renal fibrosis by HGF. Reciprocal balance between HGF and TGF-\(\beta_1\) is involved in determining the fates of chronic renal diseases. In an early stage of chronic renal disorders, HGF production is enhanced to suppress TGF-\(\beta_1\) production. In the HGF-dominant balance, renotrophic, protective, and antifibrotic events occur as a compensatory response. By contrast, TGF-\(\beta_1\) production is, in turn, upregulated in an advanced stage to prohibit HGF production. Under the TGF-\(\beta_1\)-dominant condition, a loss of parenchymal regeneration leads to accelerated renal fibrosis and dysfunction. To reverse the fibrosis-progressed balance, supplement therapy with HGF (or its gene) could be considered as a strategy for attenuating fibrosis, a common pathway leading to end-stage chronic renal failures [including diabetic nephropathy, nephrotic syndrome (23, 24), obstructive nephropathy (25, 44, 45), and chronic allograft nephropathy (2)].
data), especially in cases of systemic (subcutaneous or intramuscular) administrations. Of note, giving physiological doses of HGF (i.e., 600 μg·kg \(^{-1}\)·day \(^{-1}\)·sc) to db/lbd mice led to a trend of improvement of renal functions (Kaizhara M and Kuroda A, unpublished data). Although the way in which the very low dose of HGF has different effects awaits results from additional studies, the use of HGF at physiological doses seems to be safe and effective.

Finally, it is important to discuss a cause-and-result relationship between reciprocal expressions of HGF and TGF-β1 in the diabetic kidney. HGF represses TGF-β1 production, as shown herein and reported elsewhere (38, 39), and vice versa (22, 26). Thus a potential mechanism for regulating the balance involves TGF-β1 in an early stage of diabetic disease, HGF expression is enhanced to block TGF-β1 in an early stage of diabetic disease, and HGF production. Previous evidence implies that HGF is a hepatocyte growth factor, on endothelial damage in diabetes. Endocrinology 136: 660–667, 1995.


