

HGF as a renotrophic and anti-fibrotic regulator in chronic renal disease

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1. ABSTRACT

Hepatocyte growth factor (HGF) and Met/HGF receptor play roles in dynamic growth and morphogenesis during development and regeneration of organs, including the kidney. In the kidney, HGF targets different types of cells, while its biological actions depend on a target cell type. During the earlier stages of chronic renal failure, renal HGF expression increased, but in later stages HGF expression decreased, associated with manifestation of chronic renal failure. When anti-HGF IgG was administered into mice with chronic renal failure, renal dysfunction and fibrosis were accelerated, indicating a role of endogenous HGF to suppress chronic renal failure. For myofibroblasts, a key cell type in tissue fibrosis, HGF exerted biological activities, including (i) inhibition of growth, (ii) suppression of fibrogenic cytokine expression, and (iii) enhancement of matrix metalloproteinases involved in subsequent apoptosis. In models of glomerular and tubulo-interstitial fibrosis, administration of HGF or HGF gene therapy improved renal fibrosis and dysfunction. Since insufficient production of HGF is causative for renal fibrosis, supplementation with HGF represents a new approach to inhibit or improve chronic renal failure.

2. INTRODUCTION

Chronic renal failure (CRF) has been generally thought to be incurable, except through renal transplantation. The number of patients with CRF is now on the increase worldwide, due to the greater prevalence of diabetes, hypertension and hyperlipidemia, which may cause renal disease. Histopathologically, end-stage CRF is diagnosed based on evidence of renal fibrosis, such as glomerulosclerosis, atherosclerosis and tubulointerstitial fibrosis. All of these pathological processes are characterized by progressive loss of renal parenchymal cells with excessive deposition of extracellular matrix (ECM) proteins (1). In the clinical setting, prognosis for CRF patients correlates with the intensity and extent of fibrotic lesions (1, 2). Thus, regeneration-based therapies that prevent and possibly reverse the progression of renal fibrosis, a histological hallmark of end-stage CRF, are needed for CRF patients.

Many investigators had an initial interest in the molecular basis whereby renal fibrosis occurs and progresses under persistent renal injury (1-3). As a result of these pathological studies, transforming growth factor-beta

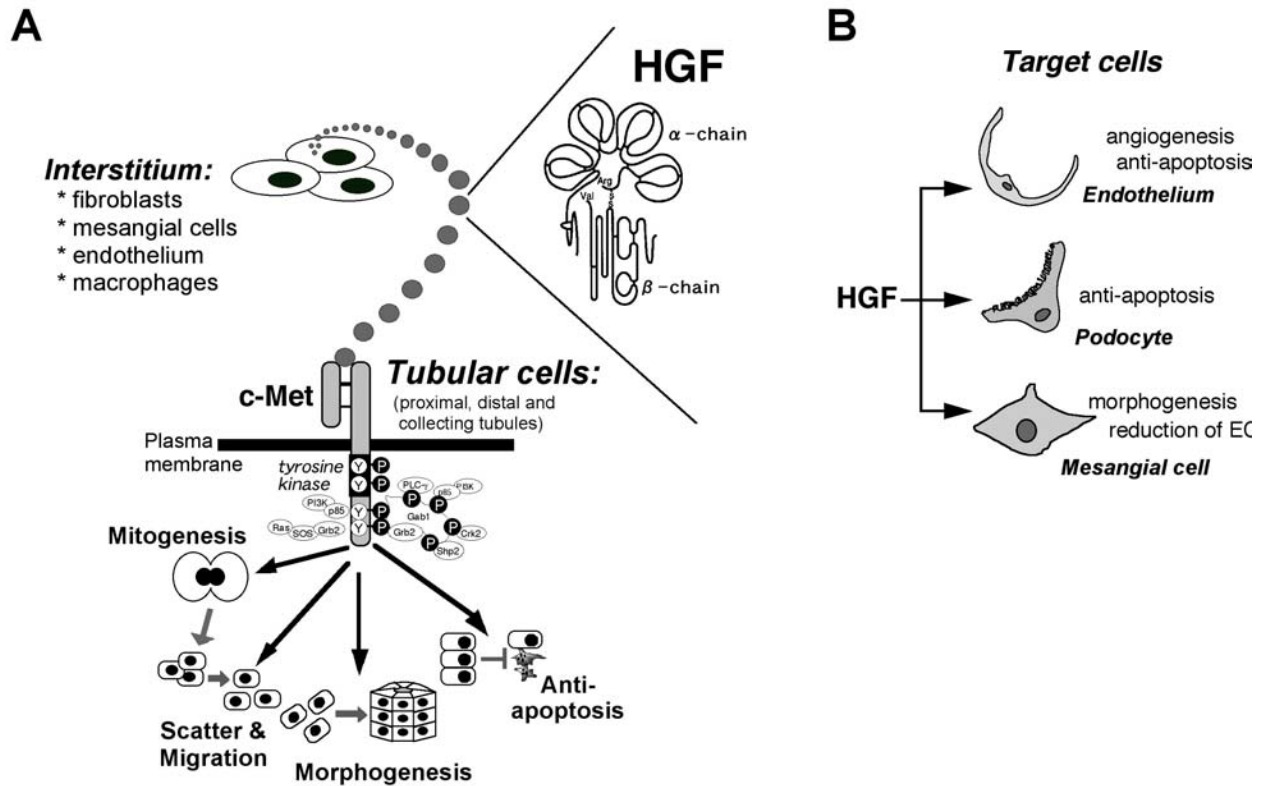


Figure 1. Biological effects of HGF on renal cells. (A) Functions of HGF on epithelial cells in renal tubules (including proximal, distal and collecting tubules). HGF is produced by interstitial cells (such as fibroblasts and mesangial cells), targets tubular epithelial cells, and exerts multiple activities through the Met receptor. (B) Multi-faced property of HGF on glomerular cells. For abbreviation see text.

(TGF- β) and angiotensin-II (Ang-II) were identified as pathological regulators that either trigger or accelerate renal fibrogenesis (2, 3). On the other hand, there is growing evidence obtained from animal models of CRF that progression of renal fibrosis is often reversible (4), indicating the presence of a “self-repair mechanism” that counteracts fibrosis. Progression and regression of renal fibrosis may be regulated by the balance between fibrogenic and regenerative cytokines. Thus, identification of the physiological regulator (s) for regression of renal fibrosis is critical for development of “cure-oriented” regeneration therapies. While one strategy to treat renal fibrosis is blocking of pathogenic factors (such as TGF- β), therapies based on supplementation with a regenerative factor (s) may more effectively treat fibrosis-related diseases.

Hepatocyte growth factor (HGF) is now recognized to be a pleiotropic factor that plays an imperative role in organ regeneration in response to tissue injury (5-7). In the kidney, HGF has mitogenic, morphogenic and reno-protective effects on resident renal cells (8-10). In 1998, we demonstrated for the first time that HGF reverses advanced renal fibrosis in a mouse model of CRF (11). Based on this initial and subsequent studies performed using different CRF models, we and other investigators proposed hypothesis that the decrease in

endogenous HGF expression is involved in the pathogenesis of renal fibrosis, providing the rationale for a treatment with HGF for CRF (12, 13). The present article reviews advances in understanding of the molecular and cellular mechanisms underlying inhibition and improvement of renal fibrosis by HGF.

3. BIOLOGICAL PROPERTY

HGF was originally identified and cloned as a potent mitogen for mature hepatocytes (14-16). HGF acts on various types of cells through the Met receptor tyrosine kinase, and elicits pleiotropic effects involved in embryogenesis and tissue repair (6-8) (Figure 1). In the kidney, HGF promotes renal regeneration by stimulating proliferation and tubulogenesis of resident epithelial cells (17-19) and by suppressing tubular cell death (20, 21). Administration of HGF accelerates recovery from acute renal failure in laboratory animals (22-24).

3.1. Effect on tubular cells

HGF stimulates DNA synthesis in cultured renal tubular cells (17). Importantly, HGF plays a role as a morphogenic factor that induces formation of branching tubules by Mardin-Darby canine kidney (MDCK) cells in collagen gel (i.e., 3-dimensional culture) (18). HGF stimulates scattering and migration of MDCK in monolayer

A decrease of HGF responsible for renal fibrosis

cultures (25). These mitogenic, motogenic and morphogenic functions of HGF are needed for reconstruction of renal tubules. In addition to these regenerative effects, HGF elicits an anti-apoptotic effect on renal tubular epithelium (20, 21), suggesting that HGF directly protects renal tubules from various stresses. HGF induces Na⁺-K⁺-ATPase in renal cells (26), by which a physiological role of HGF in the regulation of mineral/ion exchanges is considerable.

3.2. Biological activities for glomerular cells

Several lines of *in vitro* evidence suggest that HGF has direct effects on resident glomerular cells. Mesangial cells are thought to be the primary source of HGF in renal tissues (27), and HGF transduces morphogenic signals in renal mesangial cells in an autocrine manner (28). HGF protects mesangial cells from glucose-mediated injury (29) and H₂O₂-induced oxidative stress (30). Of interest, HGF has a potent anti-apoptotic effect on glomerular podocytes (31), the cells critical for prevention of proteinuria. HGF has mitogenic and anti-apoptotic effects on glomerular endothelial cells (27, 32).

3.3. Role in renal development

HGF is required for cellular proliferation, migration and morphogenesis during organogenesis, including the kidney (33). At the inception of the mouse metanephros at embryonic day 11, HGF was expressed in the mesenchyme, while Met was expressed in both the ureteric bud and the mesenchyme (34). In culture of metanephric rudiments, anti-HGF antibodies inhibited the differentiation of metanephric mesenchymal cells into the epithelial precursors of the nephron, increased cell death in mesenchyme, and perturbed branching morphogenesis of the ureteric bud (34, 35). The results imply an autocrine and/or paracrine role for HGF in the survival of the renal mesenchyme, mesenchymal-epithelial transition that occurs during nephrogenesis, and morphogenesis of renal tubules during the development. In *Xenopus* embryo, dominant-negative expression of mutant Met resulted in impaired development of the liver, intestine and kidney (36).

3.4. Renotrophic role in acute renal injuries

Renal enlargement occurs in remnant kidney after unilateral nephrectomy to compensate for the loss in the number of nephron. This phenomenon is called as "compensatory renal growth". Such a regenerative event occurs predominantly in cortico-medullary tubular cells at S3-segments. Humoral factors (i.e., "renotropine") are involved in renal growth after nephrectomy (37). In rats that underwent left nephrectomy, HGF mRNA and protein levels in the kidney and other intact organs (such as lungs and spleen) rapidly increased (38, 39), followed by hyperplasia in tubular cells. When recombinant HGF was administered into mice immediately after unilateral nephrectomy, tubular cell proliferation was enhanced (23), indicating HGF as a candidate to a "renotropine."

HGF participates in the protection and repair of renal tubules during the progression and recovery of acute renal failure. Using a model of post-ischemic acute renal failure, a role of endogenous HGF was examined (40, 41).

There was a two-waved increase in plasma HGF after renal ischemia-reperfusion in mice: one hour (i.e., before the onset of tubular destruction) and 24-36 hours (i.e., during the tubular cell proliferation) after renal ischemia-reperfusion. When neutralizing anti-HGF antibody was administered to these mice, apoptotic changes became evident in renal tubules (i.e., S3 segment), along with the acceleration of neutrophil infiltration (40). These findings indicate that the first peak in plasma HGF participates in the reduction of apoptotic and inflammatory events in renal tubules. In contrast, tubular cell proliferation was repressed when anti-HGF antibody was injected between 24 and 48 hours after renal ischemia-reperfusion. The inhibition of tubular cell proliferation led to the enhancements of renal hypoxia and dysfunction (41), hence indicating that the second peak in endogenous HGF is required for tubular reconstruction and for recovery from renal hypoxia and dysfunction.

Based on the renotrophic effects of HGF, the potential application of HGF as a treatment for acute renal failure was tested in various experimental models. Overall, HGF proved useful for inhibition of the onset of acute renal failure at the initial stage (i.e., as a preventive drug) and for acceleration of the recovery from renal dysfunction at the advanced stage of acute renal failure (reviewed in ref. 42).

4. ENDOGENOUS HGF, A REGULATOR TO SUPPRESS RENAL FIBROSIS

Plasma and urine HGF levels can be a sensitive indicator to predict the pathological status of patients leading to CRF (43-45). Blood HGF levels can be a marker for estimating a long-term outcome of grafted renal organs (46). Expression of HGF is altered during the progression of CRF in rats (47, 48). Collectively, progression of glomerular sclerosis or peri-tubular fibrosis is at least in part due to the insufficient levels of HGF.

4.1. Inhibition of glomerulosclerosis

Glomerulosclerosis is characterized by mesangial expansion, along with over-accumulation of ECM. In particular, glomerular fibrosis is a histological hallmark of diabetic nephropathy (49). Under hyperglycemic states, glomerular hypertension and hyperfiltration occur, followed by the fibrogenic responses such as transdifferentiation of mesangial cells into myofibroblasts and excessive deposition of ECM (49, 50). During this pathogenic sequence of events, TGF- β expression increases in fibrotic areas of glomeruli. Importantly, inhibition of TGF- β suppresses sclerotic changes of glomeruli without changing glucose levels (51, 52), hence demonstrating the key role of TGF- β 1 in diabetes-related glomerulopathy.

Plasma HGF levels are variable in patients with diabetes (53, 54). Liu *et al.* showed that high glucose levels increase renal expression of HGF and Met (55), while Morishita *et al.* reported that hyperglycemia induces reduction of the renal HGF expression via up-regulation of TGF- β 1 (56). Changes in the regulation of HGF suggested its possible involvement in diabetic nephropathy,

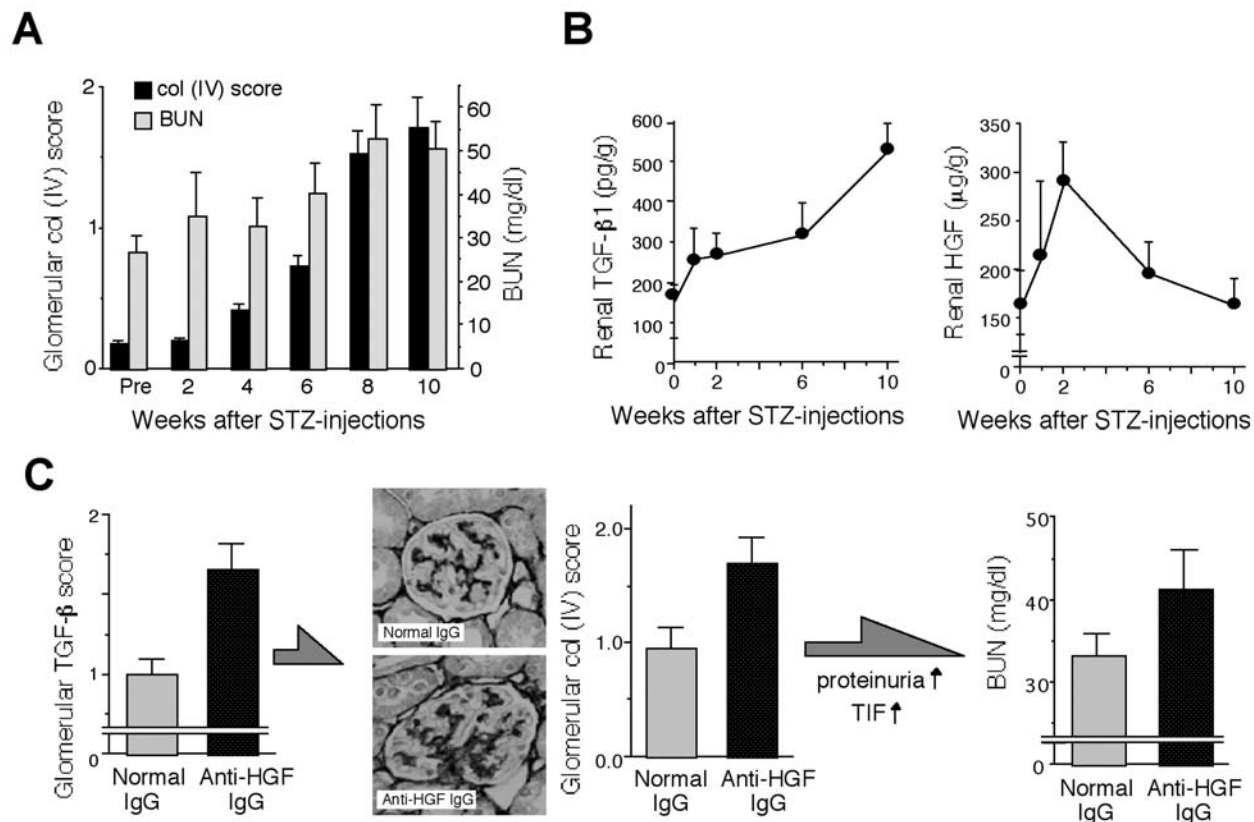


Figure 2. Key roles of endogenous HGF to suppress the diabetic nephropathy. (A) Changes in glomerular type IV collagen (IV) and BUN levels after the injection of STZ in mice. (B) Changes in TGF- β 1 and HGF levels in the kidney. (C) Acceleration of the diabetic nephropathy by anti-HGF IgG injections. Enhancement of glomerular TGF- β 1 expression by anti-HGF IgG led to glomerular fibrosis, and then renal dysfunction became evident.

whereas little was known about the physiological significance of HGF during hyperglycemia. We used an animal model of diabetic nephropathy to determine the role of HGF in the progression of chronic glomerular injury. Streptozotocin (STZ) causes insulin-dependent diabetes in mice through specific destruction of pancreatic beta-cells. In the mice given STZ-injections, blood glucose levels were elevated 3-fold compared with control mice, followed by over-deposition of type IV collagen in glomeruli and renal dysfunction between 6 and 10 weeks after the STZ-injections (Figure 2A). In these pathological processes, renal TGF- β 1 levels gradually increased (Figure 2B, left), indicating that glomerular fibrogenesis by TGF- β 1 triggers renal dysfunction. In contrast, renal HGF levels transiently increased at 2 weeks after STZ-injections, followed by a significant reduction in HGF, which was especially evident 10 weeks after STZ injections (Figure 2B, right). HGF was localized in mesangial cells and the degree of mesangial HGF expression negatively correlated with the glomerular collagen IV (12, 57).

To determine the role of the glomerular HGF, mice were injected with anti-HGF antibody for 2 weeks beginning 4 weeks after the STZ-injections. In the HGF-neutralized mice, glomerular sclerogenic findings (such as up-regulation of TGF- β 1 and type IV collagen) were

evident as compared with the control group (Figure 2C). Renal function was impaired in diabetic mice after the anti-HGF-treatment. These findings support two conclusions as follows: (i) up-regulation in HGF expression by glomerular cells is a physiological response designed to inhibit tuft sclerogenesis; and (ii) a failure to sustain sufficient levels of HGF leads to renal dysfunction during diabetes. Ang-II and TGF- β 1 levels increase in response to hyperglycemia (2, 3, 49, 52), and both factors reduce HGF gene expression (58, 59). Thus, decrease in HGF production is a mechanism by which glomerular sclerosis is accelerated during chronic hyperglycemia.

4.2. Suppression of tubulo-interstitial fibrosis

Regardless of the underlying cause, there is a strong correlation between renal failure and the degree of tubulo-interstitial fibrosis (TIF), rather than glomerulosclerosis (60, 61). Thus, prevention of renal dysfunction appears to depend on the ability to control TIF. Endogenous HGF plays a role in delaying or reversing the progression of TIF.

The homogenous ICGN mouse is a unique model of nephritic syndrome. In the ICGN mice with early pathology (~13 weeks old), renal function is conserved as long as tubular cell growth is maintained (Figure 3A),

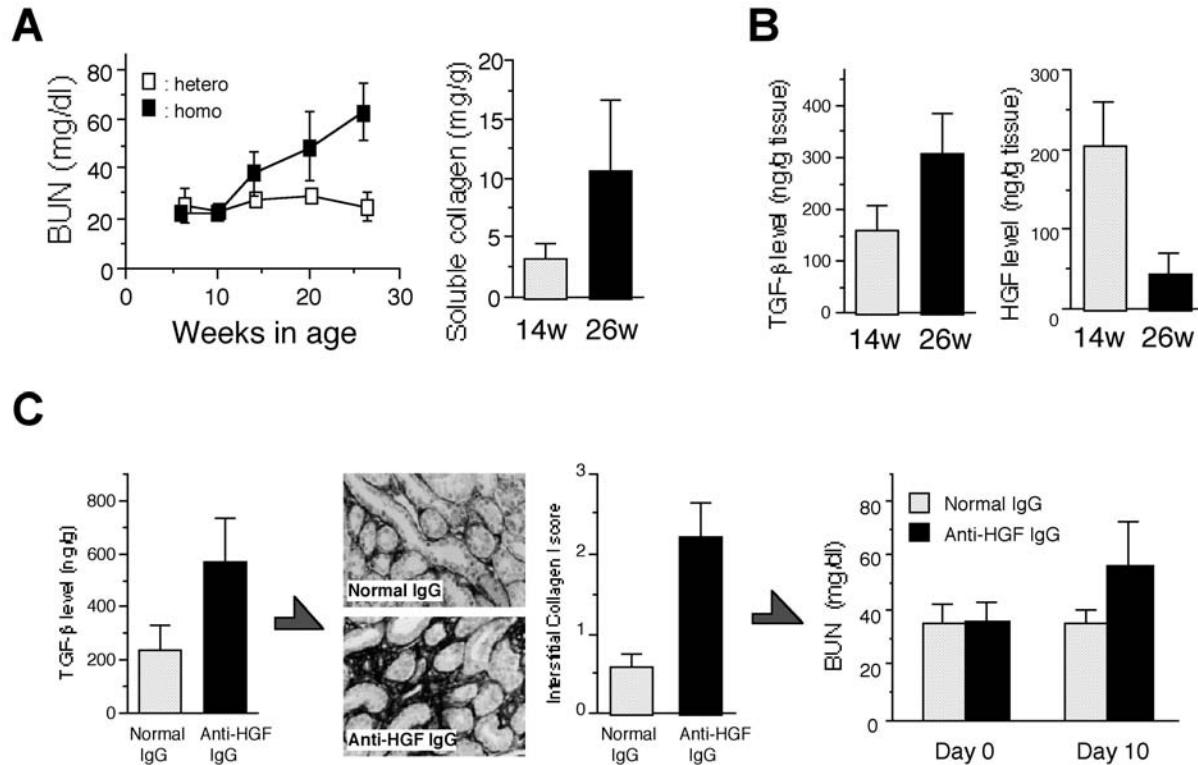


Figure 3. Importance of intrinsic HGF to inhibit the onset of TIF. (A) Changes in renal dysfunction and fibrosis, as evidenced by BUN and renal collagen levels, respectively. (B) Changes in renal HGF and TGF-beta1 levels during the progression of CRF in the ICGN mice. (C) Aggravation of pathological changes by administering an anti-HGF antibody. The 14-week-old ICGN mice were treated with anti-HGF antibody for 8 days and killed at 10 days after the anti-HGF treatment (63). Left: change in renal TGF-beta1 level; Middle: change in renal type-1 collagen level; and Right: change in BUN level.

regardless of severe glomerulosclerosis (62). Inversely, renal function is gradually impaired as tubular regeneration declines and pathological characteristics of TIF become evident (62). At 13 weeks after birth, renal HGF levels are greater than HGF than in normal mice. Elevated HGF was associated with enhancement of tubular regeneration. At 26 weeks after birth, renal HGF levels were decreased and this was associated with the decrease in tubular regeneration as well as the progression of TIF (Figure 3B) (63). When anti-HGF antibody was administered into 14-week-old ICGN mice (i.e., compensated phase), tubular cell proliferation was arrested, followed by an increase in renal TGF-beta1 levels, progressions of TIF, and renal dysfunction (Figure 3C) (63). These findings suggest that up-regulation of HGF production in injured areas is required for tubular regeneration, while down-regulation of HGF production (possibly regulated by TGF-beta1) may facilitate TIF and renal dysfunction.

The importance of HGF as an anti-fibrotic ligand was demonstrated in rodents that underwent surgical treatment of unilateral ureter obstruction (64, 65), which is a model to mimic obstructive nephropathy. Renal HGF levels transiently increased during the progression to nephropathy induced by unilateral ureter obstruction (64). There was a closed relationship not only between renal

HGF levels and tubular cell growth but also between renal TGF-beta1 levels and renal fibrosis. Treatment with anti-HGF antibody led to the suppression of tubular hyperplasia, up-regulation of TGF-beta1, and renal TIF (64). Conversely, administration of HGF (or HGF gene expression) enhanced tubular hyperplasia but suppressed TGF-beta1 expression and the onset of TIF (64, 66).

Sub-total ablation of the kidney (i.e., 5/6-nephrectomy) induces progressive proteinuria, glomerular and tubular fibrotic changes in rats. In this model, neutralization of HGF led to accelerations of morphological injury, proteinuria and the loss of renal function (67). Given that compensatory growth occurs in remnant nephron, suppression of tubular hyperplasia caused by inhibition of HGF-dependent tubular regeneration may explain the progression of TIF and renal dysfunctions. Subsequent studies indicated that suppression of inflammatory events (i.e., NF-kappaB activation, chemokine induction and infiltration of leukocytes) by endogenous HGF is required for delaying the progression of TIF and renal dysfunction (68, 69). These studies revealed that HGF is an intrinsic regulator to inhibit glomerular and peri-tubular inflammation and fibrosis (57, 63, 64, 67-69).

A transgenic mouse strain that over-express HGF under control of the methallothioneine promoter manifests

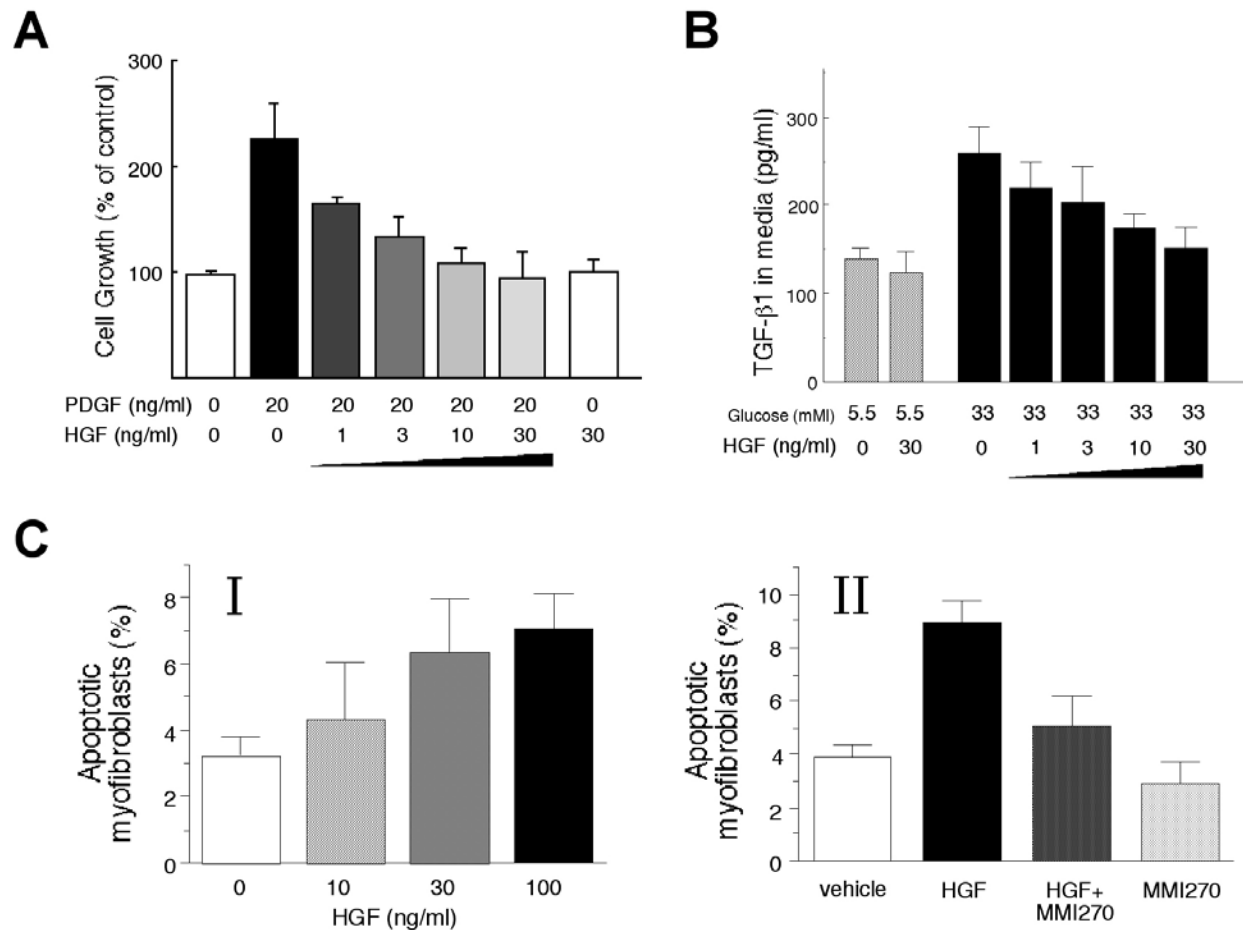


Figure 4. Anti-fibrotic effects of HGF on myofibroblasts. (A) Inhibitory effect of HGF on PDGF-mediated proliferation of mesangial cell-derived MyoFB. (B) Inhibition of TGF-beta1 production by HGF in mesangial cells under a hyperglycemic condition. (C) Enhancement of apoptotic death by HGF in a culture of lung fibroblast-derived MyoFB. (I) HGF dose-dependently enhanced apoptosis in MyoFB, caused by serum starvation. (II) HGF-induced apoptotic effect was diminished by an MMP-inhibitor (MMI270).

CRF with renal fibrosis (70). The reason why CRF develops as a result of transgenic HGF over-expression is unknown. In the transgenic mice, plasma HGF levels are extremely high (>50 ng/ml, i.e., 100-fold greater than the normal levels). Single-chain pro-HGF binds but does not activates Met. Thus, pro-HGF functions as an HGF-antagonist (71). Proteolytic processing of inactive single-chain HGF into two-chain active HGF occurs after activation of protease cascade for blood coagulation (72). Thus, it likely that large quantity of HGF in the transgenic mice of HGF exists in single-chain HGF, and thus susceptibility of two-chain active HGF to Met may be suppressed. Transgenic mice that express HGF under control of the keratin-14 promoter have a few ng/ml of blood HGF level and do not exhibit pathological changes characteristic of CRF (73).

5. ANTI-FIBROTIC MECHANISMS

During the scar formation, myofibroblasts (MyoFB) play a central role in excessive deposition of

ECM perhaps to compensate for defective functional cells (74, 75). In the 1990s, many investigators elucidated the molecular mechanisms responsible for the progression of MyoFB hyperplasia. Platelet-derived growth factor (PDGF) is a key ligand for growth of mesangial cell-derived MyoFB (76), while TGF-beta is critical for production of ECM by renal MyoFB (77). Indeed, forced expression of the PDGF or TGF-beta cDNA elicits fibrogenic changes in rat kidneys (78). HGF attenuates renal fibrogenesis, through its counteractive actions against PDGF- or TGF-beta-mediated signal transduction and fibrogenesis.

5.1. Inhibition of PDGF-mediated growth

Over-expression of PDGF in normal rat kidney leads to lesions similar to mesangial proliferative glomerulonephritis (78), while anti-PDGF antibody inhibits MyoFB overgrowth in a model of mesangial proliferative glomerulonephritis (76). HGF inhibited PDGF-dependent proliferation of mesangium-derived MyoFB in culture (Figure 4A), in association with the early inactivation of ERK-42/44 phosphorylation (79). Likewise, HGF

A decrease of HGF responsible for renal fibrosis

facilitated early dephosphorylation of ERK-42/44, which was associated with inhibition of the PDGF-dependent overgrowth of glomerular MyoFB in a model of mesangial proliferative glomerulonephritis. Thus, glomerular fibrosis was attenuated by HGF-treatment in this model (79). Moreover, HGF inhibited PDGF-induced growth of hepatic MyoFB and vascular smooth muscle cells in liver cirrhosis and pulmonary hypertension, respectively (80, 81).

5.2. Suppression of TGF-beta1 production

TGF-beta1 is a key player that elicits fibrogenic responses in many organs as follows: (i) up-regulation of ECM production; (ii) differentiation of interstitial cells to myofibroblasts; and (iii) induction of tissue inhibitor of matrix metalloprotease (82). Under pathological conditions, Ang-II is a key inducer of TGF-beta1 production (83), while blocking Ang-II/TGF-beta1 pathways by inhibitors of angiotensin-converting enzyme (ACE) (such as enalapril) or Ang-II receptor antagonists (such as losartan) produces an effect to slow the progression of renal fibrosis (84, 85). With regard to this, we reported that the Ang-II-mediated increase in TGF-beta1 production is suppressed by adding HGF in a culture of cardiac fibroblasts (86). Such an inhibitory effect of HGF on Ang-II-induced TGF-beta1 over-expression was also observed in culture of mesangial cells under hyperglycemic conditions (57). Of note, HGF diminished the high glucose-mediated increase in TGF-beta1 production by mesangial cells in a dose-dependent manner (Figure 4B). Since glucose-induced production of TGF-beta1 is mediated via an Ang-II pathway (87), HGF may directly antagonize the Ang-II/TGF-beta1 pathogenic pathway, which is a common pathway during renal fibrogenesis, especially under diabetic conditions (2, 3, 52).

5.3. Inhibition of TGF-beta-mediated fibrogenic signals

HGF suppresses not only TGF-beta production *per se* but also inhibits intra-cellular and extra-cellular TGF-beta signaling. Differentiation of resident fibroblasts into MyoFB was induced via TGF-beta signaling, while HGF suppressed TGF-beta-mediated conversion via interception of nuclear location of smad-2/3 (88). HGF down-regulates expression of TGF-beta-specific receptor during the progression of TIF in rats subjected to unilateral ureter obstruction (89). Interestingly, HGF induces decorin, which is known as the “natural TGF-beta inhibitor” in myofibroblasts (90). Decorin is a proteoglycan capable of inhibiting activation of TGF-beta and it blocks glomerular fibrogenic changes in a rat model of mesangial proliferative glomerulonephritis (82).

Connective tissue growth factor (CTGF) is the downstream molecule of TGF-beta1 that plays a significant role in TGF-beta-mediated ECM production. HGF reduced the renal expression of connective tissue growth factor and attenuated TIF in TGF-beta1-transgenic mice subjected to 5/6-nephrectomy, even though overexpression of TGF-beta1 continued in the transgenic mice (91). Thus, these results illustrate the mechanisms by which HGF inhibits TGF-beta-induced fibrogenic signal transduction.

5.4. Induction of MMPs and apoptosis

HGF is a potent inducer of proteolytic enzymes that degrade ECM proteins, including matrix

metalloproteinases (MMPs) (25, 67, 92-94), and this activity is likely to be involved in the resolution of liver cirrhosis by HGF (93). On the other hand, proliferation and survival of normal cells depend on anchoring to scaffold of ECM. These backgrounds suggest that HGF may change anchoring behavior of cells through the breakdown of ECM scaffold, thereby regulating cell growth and survival.

Met receptor is induced during phenotypic change of fibroblast-like cells into MyoFB, indicating that MyoFB are targets of HGF. More of importance, HGF enhances apoptotic death of lung MyoFB *in vitro* (Figure 4C) and induction of MMPs was involved in HGF-induced apoptosis (95). HGF induced MMP-9/-2 in MyoFB, and facilitated degradation of fibronectin and dephosphorylation of FAK (95). Since activation of integrin-linked intra-cellular signaling triggered by association between fibronectin and integrin is essential for fibroblast survival (96), HGF seems to induce anoikis-like cell death in MyoFB due to MMP-induced breakdown of ECM. In support of this scenario, MMP-specific inhibitors rescued HGF-induced apoptosis in MyoFB (Figure 4C).

Enhanced apoptosis of MyoFB by HGF was also noted in MyoFB derived from hepatic stellate cells or fibroblasts (80). The enhanced apoptosis of MyoFB by HGF was involved in “resolution” of liver cirrhosis and lung fibrosis (80, 95). Since not only MyoFB death but also ECM degradation participate in the recovery from fibrosis (97-100), even delayed treatment with HGF effectively resolved advanced fibrosis in kidney (11, 101), as well as in liver (93) and heart (86). Likewise, delayed treatment with HGF gene therapy inhibited the progression of chronic allograft nephropathy (102) and induced regression of advanced diabetic nephropathy (103). In humans, expression of Met in injured kidneys was reported to correlate with the degree of MyoFB formation (104). Blood HGF levels may be increased in patients suffering from CRF (43-45), via an endocrine-like pathway of HGF (39, 47), which compensate for the loss of local HGF production. These clinical findings suggest that possible HGF-Met signals in MyoFB may provide a direct mechanism by which renal fibrosis is delayed in patients with CRF.

5.5. Anti-inflammatory effect

Persistent inflammation triggers renal fibrogenesis (1-3), while HGF inhibits the inflammatory events. Macrophages are one of the major sources of TGF-beta1 in chronically injured kidneys (82), while HGF prevents the infiltration of macrophages in models of TIF (64, 68, 105). In this process, HGF inhibits expression of chemokines such as monocyte chemoattractant protein-1 and RANTES by renal tubules (57, 68, 106), and this may be mediated via the suppression of NF-kappaB activation by HGF (107). Anti-inflammatory effects of HGF on tubular cells may also participate in the down-regulation of TGF-beta1 through decreasing the numbers of infiltrating macrophages.

6. THERAPEUTIC APPROACH WITH HGF

Since insufficient HGF production is causative for renal fibrogenesis, possible therapeutic strategies to

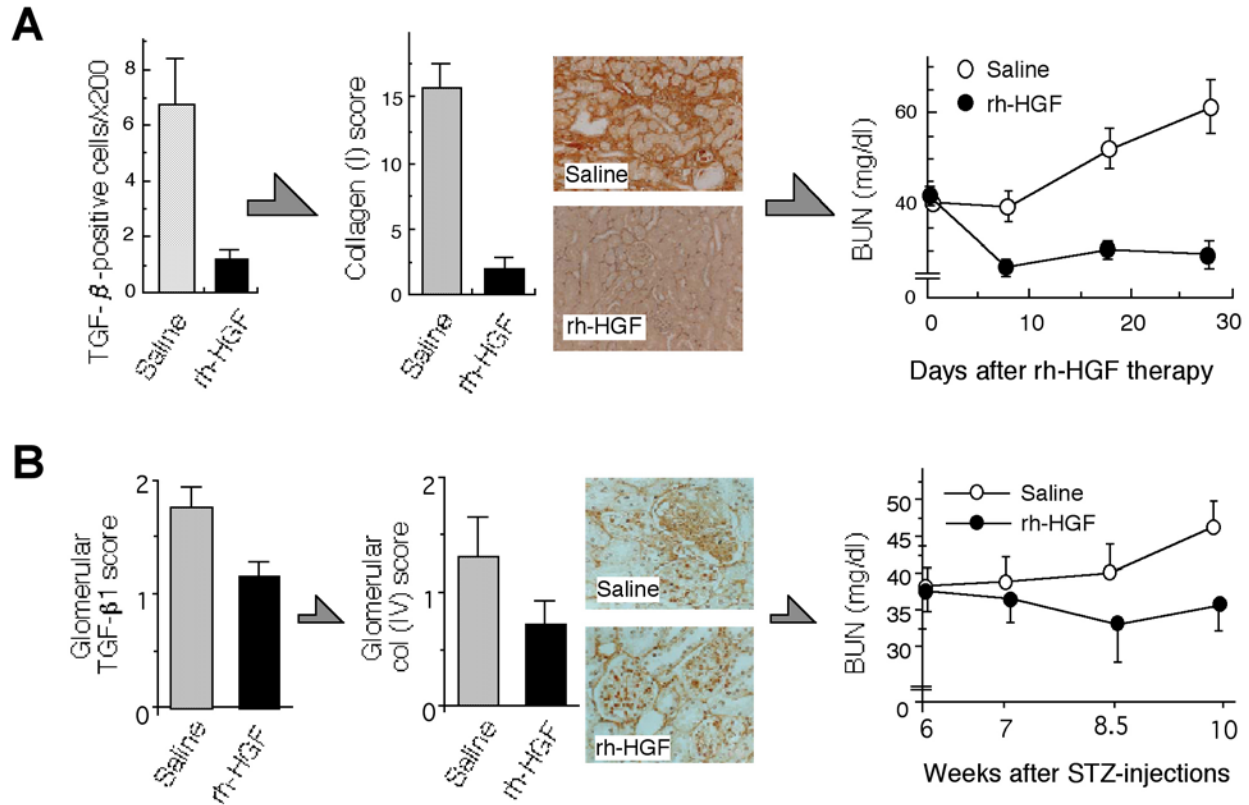


Figure 5. Therapeutic outcomes of HGF in mouse models of CRF. (A) Therapeutic effects of HGF on the ICGN mice, a model of nephritic syndrome. Changes in renal TGF-beta1 levels, TIF, and renal function by HGF-treatment (11, 63). (B) Improvement of glomerular sclerosis in the STZ-treated mice, a model of diabetic nephropathy (57). Suppression of glomerular TGF-beta1 expression by HGF led to the attenuation of glomerular sclerosis and then renal dysfunction was improved after the HGF supplement therapy.

compensating for the decrease in HGF production: (i) supplementation of HGF (or its gene) via systemic or local injections and (ii) restoration of lowered HGF production by either suppression of an HGF-suppressor (s) or by stimulation of HGF transcription pathways. Based on the experimental data, we expect that administration of HGF or enhancement of HGF production may be effective new strategies for cure-oriented treatment of CRF patients.

6.1. Administration of HGF or its gene

In the ICGN mice, renal dysfunction and fibrosis develops during 14 and 18 weeks of age, this is associated with the decrease in HGF expression (62, 63). When recombinant HGF was administered into 14-week-old ICGN mice for 4 weeks, renal TGF-beta1 production was suppressed, leading to the attenuation of TIF. Renal dysfunction was also improved in the HGF-treated mice (Figure 5A) (11, 63). The similar results were seen in other models of TIF: Administration of HGF (or HGF gene therapy) reduced the accumulations of MyoFB and ECM, associated with the decrease of TGF-beta levels in rats after unilateral ureter obstruction (64, 66, 101) or of renal allograft (102, 108).

Glomerular injury can accelerate the progression of TIF (52, 109). In a mouse model of type I diabetes,

glomerular HGF expression became faint during the progression of renal dysfunction (between 6 and 10 weeks after STZ injection) (57). When HGF was administered into mice during the period of decreased HGF expression, HGF suppressed TGF-beta expression and ECM deposition in glomeruli, leading to the improvement in TIF and renal dysfunction (Figure 5B). Similar improvement in diabetic nephropathy was observed in response to HGF gene therapy (103, 110). HGF also inhibited diabetic nephropathy in db/db mice, a model of type II diabetes (111), indicating potential use of HGF for treating diabetic nephropathy. Such anti-fibrotic effects of HGF on glomeruli were also seen in models of chronic allograft nephropathy (105) and mesangial proliferative glomerulonephritis (79).

6.2. Restoration of endogenous HGF expression

Another possible strategy to reduce renal fibrosis is to restore the lowered HGF production under chronic renal injuries. Angiotensin-II blockers decrease the local production of TGF-beta1 (112). Of note, ACE inhibitor repressed the glomerular fibrotic changes in the hypertensive SHR rats, and this was associated with the increase in HGF production (113). The combination of an angiotensin-II blocker and HGF had a synergic effect on the improvement of TIF in rats after unilateral ureter

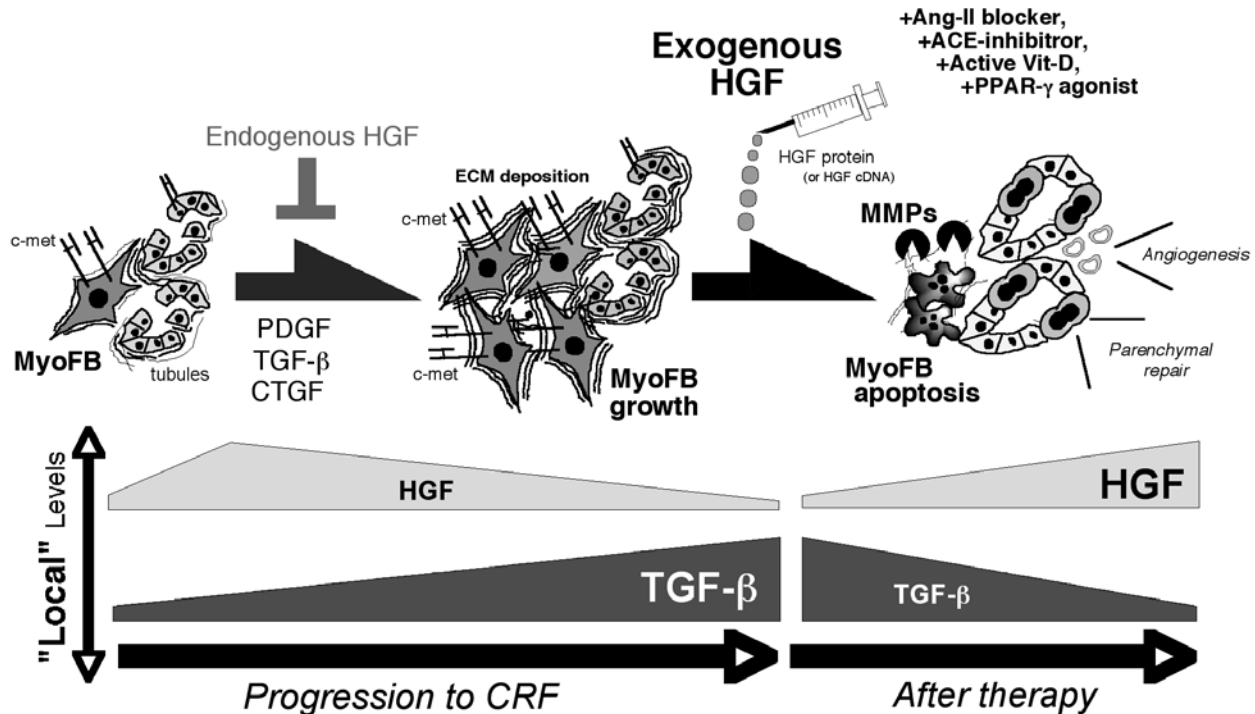


Figure 6. HGF supplement therapy, based on molecular pathogenesis of renal fibrosis. In response to persistent renal injury, HGF is produced by renal stroma (such as peri-tubular fibroblasts and mesangial cells). Interstitial MyoFB acquires Met expression, and endogenous HGF targets MyoFB in order to: (i) suppress TGF-beta expression, (ii) inhibit PDGF-mediated proliferation of MyoFB, and (iii) intercept the TGF-beta-induced (or CTGF-mediated) ECM accumulation. Under chronic injuries, Ang-II and TGF-beta levels gradually increase in injured site, and local HGF expression is suppressed. Under the HGF-insufficient condition, renal regenerative response is suppressed, leading to further progression of fibrosis. When HGF is supplemented under the pathological conditions, MMPs induced by HGF contribute to breakdown of ECM, while MyoFB is decreased via an anikis-like pathway (95). HGF down-regulates TGF-beta expression and enhances HGF expression (57, 63). During a shift from TGF-beta-dominant to HGF-dominant balance, tubular epithelial repair and angiogenesis could be accelerated. Eventually, renal fibrosis and dysfunction are improved. HGF-inducers (such as ACE-inhibitor, active vitamin-D and PPAR-gamma agonist) may be useful for stimulating endogenous HGF production.

obstruction (114). Because Ang-II and TGF-beta1 are suppressors for HGF gene expression (58, 59), the anti-fibrotic effects of ACE inhibitors, Ang-II antagonists, or decorin may be, in part, due to restoration of endogenous HGF production (115).

Active vitamin-D (1,25-(OH)₂D₃) is widely used in CRF patients to retard the progression of abnormality of bone metabolisms. Active vitamin-D increased HGF expression in culture of renal fibroblasts via transcriptional activation of HGF gene (116). Of interest, active vitamin-D blocked differentiation of renal fibroblasts to MyoFB, and this effect was abolished by anti-HGF antibody. Active vitamin-D reduced fibrotic changes in the kidney, associated with the enhancement of HGF production (116). Likewise, peroxisome proliferator-activated receptor (PPAR)-gamma agonist, a drug used for diabetic patients, up-regulated HGF mRNA expression and suppressed renal fibrosis in a rat model of renal fibrosis (117).

These therapeutic studies seem to support an etiological concept that inadequate HGF production appears to be, in part, responsible for development of renal

fibrosis, including glomerulosclerosis and peri-tubular fibrosis. Further studies should attempt to provide a rationale for therapeutic efficacy of HGF during progression of CRF. Given that endogenous HGF is delivered to injured renal tissues via an endocrine and paracrine pathways (38, 39, 47), both systemic and local injection of HGF (or its gene) would mimic the physiological response after tissue destruction. The experimental outcomes of HGF-treatment in animals are summarized in Table 1.

7. CONCLUSION AND PERSPECTIVE

Regardless of the primary etiology, tissue fibrosis occurs in tissues and organs, in response to chronic injury, via a common sequence of events as follows (74, 75, 82): (i) TGF-beta-induced differentiation of stromal cells to MyoFB; (ii) proliferation of MyoFB via PDGF-mediated signals; and (iii) production of ECM proteins via TGF-beta- and CTGF-mediated signals. Fibrotic change of the kidney and improvement of renal fibrosis from the aspect of HGF are described in Figure 6. Balance between HGF and TGF-beta can be an important determinant of renal fibrosis and

A decrease of HGF responsible for renal fibrosis

Table 1. Therapeutic effects of HGF-treatment on renal pathology and function in models of chronic renal disease

Target disease	Observed effects	Animal model	Ref. number	Delivery of HGF (protein or gene)
Diabetic nephropathy	Reduced GS ¹ , ECM degradation, Increased Na ⁺ K ⁺ -ATPase, Reduced albuminuria, Reduced podocyte injury, Restoration of dysfunction	STZ-treated rodents (type I DM ²) db/db mice (type II DM ²)	(57, 103, 110) (111)	Protein: sc ³ , im ⁴ , iv ⁵ Gene: im, local
Mesangial proliferative glomerulonephritis	Inhibition of mesangial cell over-growth, Suppressed GS, Endothelial repair	Anti-Thy-1 antibody	(79) (32)	Protein: sc, im, iv Gene: im, local
Obstructive nephropathy	Tubular repair & protection, Inhibited transdifferentiation of tubular cells, Resolution of fibrosis	Unilateral ureter obstruction	(64, 101) (65)	Protein: sc, im, iv Gene: im, local
Cyclosporine nephropathy	Tubular protection, Tubular repair, Suppression of GS/TIF	Repeated expose to cyclosporine	(122, 123)	Protein: sc, im, iv Gene: im, local
Herb tea nephropathy	Tubular protection, Tubular repair, Anti-fibrosis	Aristolochic acid	(124)	Protein: sc, im, iv Gene: im, local
Nephrotic syndrome	Tubular repair and protection, Resolution of fibrosis, Myofibroblast deletion, Restoration of dysfunction Prevention of albuminuria	Spontaneous model (ICGN mice)	(11, 63) (62)	Protein: sc, im, iv Gene: local, im
Renal hypertension	Compensatory growth, Tubular proliferation, Suppression of GS/TIF, Restoration of dysfunction	5/6-nephrectomy (rats or mice)	(68, 69, 91, 125)	Protein: sc, im, iv Gene: local, im
Chronic allograft nephropathy	Induction of immune tolerance, Prevention of GS/TIF,	Renal allograft (rats or pig)	(105) (102, 108)	Protein: sc, im, iv Gene: local, im

Abbreviations: ¹GS, glomerular sclerosis; ²DM, diabetic mellitus; ³sc, subcutaneous; ⁴im, intramuscular; ⁵iv, intravenous.

resolution from renal fibrosis. It should be emphasized that HGF attenuates not only renal fibrosis, but also liver cirrhosis (80, 93), lung fibrosis (81, 95), cardiomyopathy (86), and dermal sclerosis (118). Thus, the importance of counterbalance between HGF and TGF-beta, as noted during a process of renal fibrosis, may be extended to pathogenesis of other fibrotic disorders.

Nayari *et al.* (119) reported a pilot clinical study of recombinant human HGF for treatment of patients with chronic leg ulcers. Excellent or partial healing was seen in 8 out of 11 patients whose leg ulcers had been stable and resistant to conventional methods for between 1 and 14 years. In humans with liver disease, recombinant human HGF was safe for use, even if it was used at the maximum dose (i.e., 100 mg/man), with potential benefits such as decreases in serum bilirubin, ammonia and thromboplastin time as well as an increase in blood albumin levels (120). Based on angiogenic activity of HGF, a clinical study was conducted to investigate the safety and efficiency of HGF gene therapy in 6 patients with severe limb ischemia (121). In this study, naked plasmid DNA containing the human HGF gene was administered locally to the ischemic limb. The treatment proved to be safe, feasible and effective for the treatment of ischemic limbs. Phase-II and phase-III clinical trial of HGF gene therapy for the treatment of severe limb ischemia are in progress in the United States and have been completed in Japan, respectively. In the near future, clinical trials of recombinant HGF for renal diseases will

be begun to carefully evaluate safety and efficacy. It may not be a dream to treat patients suffering from CRF.

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Abbreviations: Ang-II: angiotensin-II; CRF: chronic renal failure; CTGF: connective tissue growth factor; ECM: extracellular matrix; HGF: hepatocyte growth factor; MMP: matrix metalloproteinase; MyoFB: myofibroblast; PDGF: platelet-derived growth factor; PPAR-gamma: peroxisome proliferator-activated receptor-gamma STZ: streptozotocin; TGF-beta: transforming growth factor-beta; TIF: tubulo-interstitial fibrosis

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