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Hepatocyte Growth Factor: A Regulator of Extracellular Matrix Genes in Mouse Mesangial Cells

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ABSTRACT. The potential role of hepatocyte growth factor (HGF) in regulating extracellular matrix in mouse mesangial cells (MMC) was evaluated. Functional HGF receptors were detected in MMC by HGF-induced extracellular acidification, a response that was inhibited by the HGF inhibitor HGF/NK2, a splice variant expressing the N-terminal domain through the second kringle domain. HGF also increased fibronectin and collagen α1 (IV) mRNA levels in these cells; the increases were associated with a concentration-dependent increase in transcriptional activity of the collagen α1 (IV) gene. HGF also stimulated fibronectin and collagen α1 (IV) mRNA levels in primary rabbit proximal tubule epithelial cells. To evaluate the potential consequences of chronic elevation of HGF on renal function, HGF was administered continuously for 18 days to normal and diabetic C57BLKS/J lepr^{db} mice. In the diabetic mice, HGF reduced creatinine clearance and increased microalbuminuria, indicating that chronic exposure to HGF impairs renal function. Thus, chronically elevated HGF may contribute to the progression of chronic renal disease in diabetes by decreasing the glomerular filtration rate and possibly promoting the accumulation of extracellular matrix. BIOCHEM PHARMACOL **59**;7:847–853, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. fibronectin; collagen; diabetes; kidney; c-met; microalbuminuria

HGF‖ is a heterodimeric molecule derived from a prepro-precursor of 728 amino acids, which is processed proteolytically to form mature HGF. HGF stimulates the growth of renal epithelial cells (mitogen), enhances the motility of epithelial cells (motogen), and induces renal epithelial tubule formation (morphogen) by acting on the tyrosine kinase receptor c-met [1–3]. HGF is also involved in renal remodeling following injury. Thus, HGF and its receptor c-met are increased in the kidney following nephrectomy or ischemia [4], and acute administration of HGF improves renal function following $HgCl_2$ administration or ischemia [5, 6]. There is also evidence that expression of the HGF receptor c-met is elevated in the kidneys of rats with streptozotocin-induced diabetes [7]. Although serum HGF levels are elevated in patients with chronic renal disease [8], the effect of chronically elevated HGF levels on renal function is unknown.

Glomerular hypertrophy and fibrosis occur during renal remodeling in many chronic renal diseases. These events are associated with glomerular basement membrane expansion, proliferation of mesangial and epithelial cells, and the accumulation of collagen and fibronectin [9]. The role of HGF in glomerular extracellular matrix expansion, however, is not known. Initial studies have suggested that HGF mRNA is found in mesangial cells, whereas the mRNA for the receptor c-met is found in epithelial cells. Correspondingly, HGF stimulates mitogenesis in epithelial cells but not in mesangial cells [10, 11]. Recent data, however, indicate that glomerular mesangial cells also express c-met mRNA [11–13]. Moreover, transgenic mice overexpressing HGF in the kidney and serum demonstrate prominent tubular cystic disease, progressive glomerulosclerosis, and premature renal disease [14]. Therefore, the present study was designed (i) to determine the presence of functional HGF receptors on mesangial cells, (ii) to evaluate the effects of HGF on fibronectin and collagen mRNA levels in MMC and rabbit proximal tubular epithelial cells, and (iii) to study the effect of chronic elevation of HGF on renal function.

MATERIALS AND METHODS

Cell Culture

MMC cultures were established from glomeruli obtained from the kidneys of 8- to 10-week-old SJL/J (H-2^s) mice [15]; the cultures were grown in DMEM containing 2 mM

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‖ *Abbreviations*: HGF, hepatocyte growth factor; HGF/NK2, splice variant of hepatocyte growth factor containing the amino terminal end through the second kringle domain; MMC, mouse mesangial cells; EGF, epidermal growth factor; PAF, platelet-activating factor; TGF-β1, transforming growth factor-β1; MMC-SV40, mouse mesangial cells immortalized with SV40 antigen; DMEM, Dulbecco's modified Eagleís medium; and FBS, fetal bovine serum.

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L-glutamine and 180 mg/dL of glucose and supplemented with 10% FBS and 100 U/mL of penicillin/streptomycin at 37° in 5% CO_2. Cells were subcultured by rinsing with PBS, and then incubating with 0.05% trypsin supplemented with 20 mM EDTA. MMC transformed with noncapsid-forming SV-40 virus to establish a permanent cell line are designated as MMC-SV40 [15]. These cells exhibit many features of differentiated mesangial cells [15]. A stable transfection was performed on the MMC-SV40 with a reporter construct, HB35, which expresses luciferase driven by a "minigene" comprising the 5′ flanking and first intron regions of the murine collagen α1 (IV) gene [16]. The stable transformants are designated as MMC-COL. These cells exhibited patterns of growth and protein synthesis in response to elevated glucose concentration similar to those of MMC-SV40 [17]. When the MMC were transferred to SmithKline Beecham Pharmaceuticals, the passage number was designated arbitrarily as passage 1. Experiments reported here used cells from passages 10–15. MMC-SV40 were subcultured into 150-cm^2 flasks and incubated for 3–4 days in the growth medium described above. The medium was changed to DMEM containing 2 mM L-glutamine and 100 mg/dL of glucose and supplemented with 3% FBS, 100 U/mL of penicillin, and 100 μg/mL of streptomycin for 48 hr before the addition of HGF. Cells were treated with HGF (Collaborative Biomedical Products) and/or RO-32–0432 (synthesized by A. Krog, SmithKline Beecham). Rabbit renal proximal tubule epithelial cells were established by microsieving renal cortex through a mesh [18]. Cells were grown in 50:50 DMEM and Ham's F12 medium as described previously [18]. Cells were serum-starved for 24 hr prior to the administration of HGF (50 ng/mL) for 16 hr and then were harvested for RNA extraction.

Cloning and Expression of HGF/NK2

A DNA fragment encoding the NK2 domain of HGF was isolated from a human kidney medulla cDNA library by polymerase chain reaction (PCR) amplification. The primers used were specific to the 5′ end of HGF (forward primer 5′ GTC GAC GCG GCC GCC TTT CAC CCA GGC ATC TCC 3′) and to the 3′ end of the second kringle domain corresponding to residue 288 (reverse primer 5′ TCG CGA AGC TTT CTA GAT TAG CAT GTT TTA ATT GCA CAG TAC 3′). The resulting 952-bp fragment contained the nucleotide sequence from position -53 to +864 with respect to the HGF cDNA (Accession No. M29145). The PCR product was cloned into the baculovirus transfer vector pFastBac (Life Technologies, Inc.). The recombinant virus preparation, the infection, and the expression of NK2 protein using Sf9 (*Spodoptera frugiperda*) insect cells in Sf900 SFM were performed as described by the manufacturer. The presence of secreted HGF/NK2 recombinant protein in culture medium was analyzed and confirmed by western immunoblotting with 1000-fold diluted anti-HGF AS No. 388 (a peptide antiserum against FLPSSYRGKDLQENY) at room temperature for 1 hr. Then the blot was incubated with 1000-fold diluted goat anti-rabbit IgG conjugated with horseradish peroxidase in blocking buffer for 1 hr at room temperature. The immunoreactive protein bands were visualized using an enhanced chemiluminescence (ECL) kit (Amersham) as described by the manufacturer.

Microphysiometry

The cytosensor microphysiometer is based on a pH-sensitive silicon sensor, which is part of a microvolume flow chamber in which cells are immobilized [19]. MMC were subcultured with trypsin from 150-cm^2 flasks into capsule cups (Molecular Devices) having a polycarbonate membrane of 3-μm pore size at a density of 300,000 cells per cup. Cells were allowed to attach for 24 hr in the medium specified for each cell type. After spacer rings and insert cups were fitted into the capsule cups, the assembled units were transferred to the sensor chamber and perfused at 100 μL/min with bicarbonate-free RPMI 1640 medium (Molecular Devices) in the microphysiometer at 37°. Acidification rate was measured as change in pH over time, which was determined when the pumps were turned off for 30 sec at 2-min intervals. Cells were treated with human recombinant HGF (Collaborative Biomedical Products) in the presence or absence of the recombinant HGF inhibitor HGF/NK2, which is the truncated amino-terminal domain through the second kringle domain of mature HGF [20].

Northern Blot

Total RNA was extracted from cells by guanidinium thiocyanate denaturation and acidified phenol-chloroform extraction [21]. Total RNA (10 μg/lane) was fractionated on 0.2 M formaldehyde 1% agarose gels and transferred to nylon membranes (Nylon-1, BRL) in 4x standard saline citrate. Equivalent loading and transfer were verified by methylene blue staining. Random-primed [^{32}P]DNA probes for fibronectin were made that recognize a mRNA at 7.6 kb. The fibronectin clone was obtained from the American Type Culture Collection. Hybridizations were performed with 10^6 cpm/mL in 50% formamide, 750 mM NaCl, 50 mM NaH_2PO_4 (pH 7.6), 5 mM EDTA, 2% SDS, 5x Denhardt's solution, 100 μg/mL of salmon DNA, at 42° for 16 hr. Blots were washed with a final stringency of 0.1x standard saline citrate and 0.1% SDS at 65°. Membranes were exposed to a PhosphorImaging plate, and bands were quantitated with ImageQuant software (Molecular Dynamics).

Luciferase Assay

MMC-COL cells were subcultured in 24-well plates at 25,000 cells per well in growth medium for 48 hr. Then cells were incubated in DMEM with 3% FBS for an additional 48 hr before HGF was added. Cells were lysed 24

hr after HGF exposure using 500 μL of a buffer containing 0.1 mM potassium phosphate and 1 mM dithiothreitol, pH 7.2, with 1% Triton X-100 (for luciferase activity), or were trypsinized (for cell number). The lysed cells were centrifuged, and 100 μL of the supernatant was added in duplicate to wells of a 96-well microtiter plate. Light emission was measured directly at room temperature using a Microlumat LB96P luminometer (Wallac) and integrated over a 20-sec period following the automated injection of 100 μL of a luciferin reaction mixture containing a stock buffer of 0.1 mM potassium phosphate, 10 mM ATP, 20 mM $MgCl_2$, and 1 mM dithiothreitol, pH 7.2, and freshly added 0.8 mg/mL of D-luciferin (Boehringer Mannheim). Luciferase activity was expressed as relative light units (RLU) per number of cells as determined from similarly treated sister wells, which were trypsinized and counted (Coulter Electronic LTD).

Renal Function

Five-month-old lean and obese db mice (C57BLKS/J-m+/+Lepr^{db}; Jackson Laboratory) were anesthetized with 5 mg/kg of xylazine and 25 mg/kg of ketamine and implanted with osmotic pumps (Alza). Pumps contained either vehicle (0.35 M NaCl, 0.01 M phosphate, pH 7.3, 625 μg/mL of human serum albumin) or human recombinant HGF (Becton Dickinson Labware) at 14.6 ng/μL and delivered fluid at 0.45 μL/hr. Eighteen days after implantations, 24-hr urine was collected in metabolic cages, after which mice were killed with 100 mg/kg pentobarbital, and blood was collected by cardiac puncture. Urine and plasma creatinine and urine urea were measured by Synchron Clinical System AS 8 (Beckman). Microalbuminuria was determined by competitive ELISA [22] (Exocell Inc.). All animals were treated in accordance with the NIH guidelines for use of animals in research. The appropriate institutional animal care committee approved all procedures.

RESULTS

Effect of the HGF Inhibitor HGF/NK2 on HGF-Induced Acidification of MMC

Because the acidification rate data (change in voltage potential over time) are dependent on seeding density and seeding uniformity, acidification rates were normalized during the pretreatment period. We had shown previously that HGF increased extracellular acidification in human and mouse glomerular mesangial cells [11]. HGF-induced acidification in MMC-SV40, therefore, was evaluated in the presence of the competitive HGF inhibitor HGF/NK2 [20] to determine if MMC have a functional HGF receptor (c-met). MMC-SV40 were pretreated with baculovirus-expressed HGF/NK2 (Fig. 1) for 30 min prior to HGF (50 ng/mL) or EGF (30 ng/mL) treatment. HGF/NK2 inhibited HGF- but not EGF-induced acidification of MMC-SV40 in a concentration-dependent fashion (Fig. 2). The IC_{50} value of HGF/NK2 for inhibition of HGF-induced acidification was 63 ± 23 ng/mL.

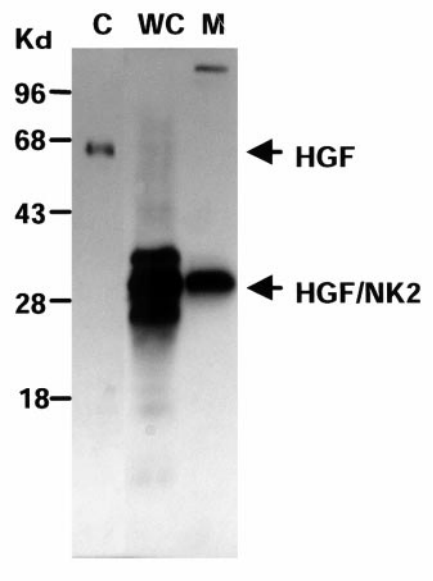

FIG. 1. Western blot for HGF/NK2 expression in an Sf9 cell line. 34-kDa mature HGF/NK2 protein was detected with anti-HGF both in whole cell lysate (WC) and in culture medium (M). Full-length human recombinant HGF (Collaborative Biomedical Products) was detected in lane 1 (C, control). Purification was performed once.

Effect of HGF on Extracellular Matrix Protein Gene Expression and Collagen α1 (IV) Promoter Activity

Fibronectin mRNA levels were evaluated in MMC pretreated with PAF, TGF-β1, and HGF. In addition to the expected increases in fibronectin mRNA after PAF and TGF-β1, HGF also increased fibronectin mRNA (Fig. 3). HGF increased fibronectin mRNA in a concentration-dependent fashion (Fig. 4). A time course revealed that HGF increased fibronectin mRNA levels up to 16 hr,

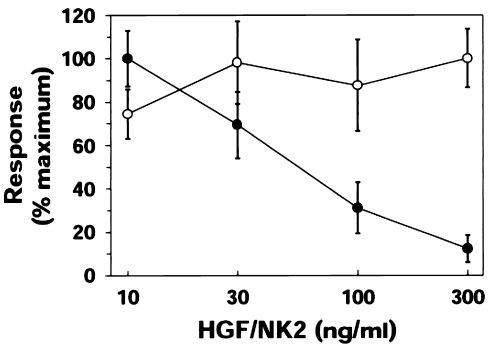

FIG. 2. Effect of HGF/NK2 on HGF- and EGF-induced acidification of MMC-SV40. Extracellular acidification was measured as the change in voltage potential over time that was detected by a pH-sensitive detector. Data are expressed as a percentage of the maximum acidification response to either 50 ng/mL of HGF (filled circles) or 30 ng/mL of EGF (open circles; means ± SEM, N = 3).

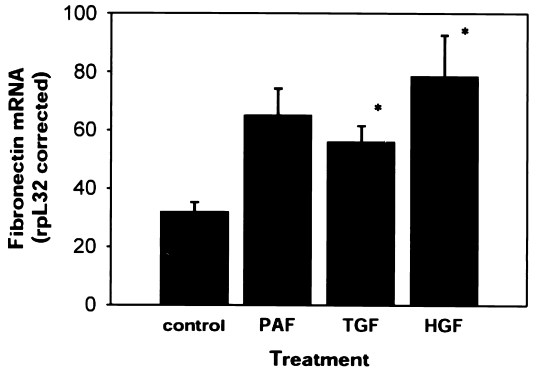

FIG. 3. Effect of PAF, TGF-β1, or HGF on relative fibronectin mRNA levels corrected with ribosomal protein L32 (rpL32) mRNA levels in MMC. The bar graph shows means ± SEM (N = 4 for all conditions except for PAF, where mean ± range, N = 2, is shown). Key: (*) $P < 0.01$ vs control by *t*-test.

whereas collagen α1 (IV) mRNA was increased 2.8-fold at 6 hr after HGF dosing (Fig. 5). The activity of the collagen α1 (IV) promoter following HGF receptor stimulation was evaluated by activation of a luciferase reporter gene in MMC transfected with the collagen promoter construct. HGF activated the collagen promoter construct in MMC in a concentration-dependent fashion, as indicated by a concentration-dependent increase in luciferase activity (Fig. 6). Because c-met is also expressed in epithelial cells, primary rabbit renal proximal tubule epithelial cells were also treated with HGF (50 ng/mL) for 16 hr. Fibronectin and collagen α1 (IV) mRNA were increased 5.8- and 2.9-fold, respectively, whereas thrombospondin mRNA was unchanged after HGF treatment (Fig. 7). Thus, HGF increased expression of markers for extracellular matrix in primary as well as in transformed cells.

Effect of Continuous HGF Administration to Diabetic Mice

To determine if chronic elevation of HGF affects renal function, HGF was administered continuously to the db

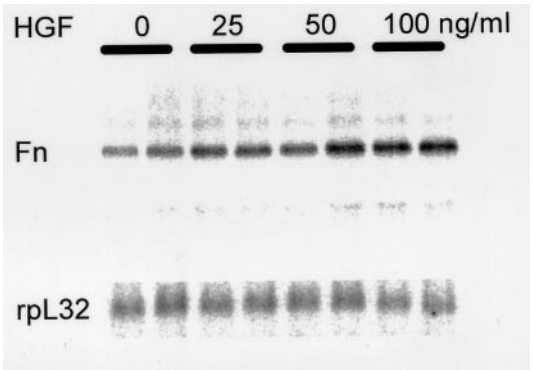

FIG. 4. Effect of increasing concentrations of HGF on fibronectin (Fn) and ribosomal protein L32 (rpL32) mRNA levels in MMC as determined by northern blot. One experiment with duplicate culture flasks was performed.

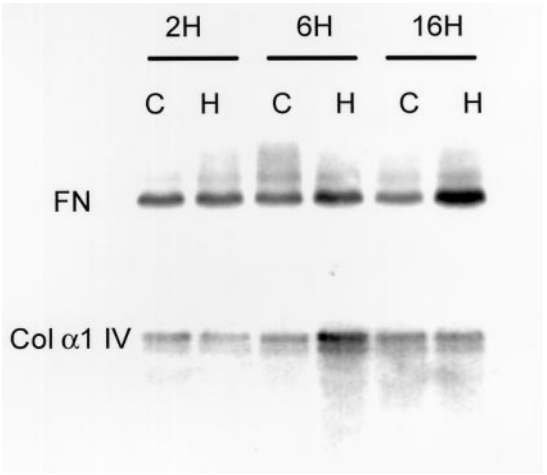

FIG. 5. Composite northern blot for fibronectin (FN) and collagen α1 (IV) (Col α1 IV) mRNA. MMC were treated with vehicle (C) or HGF (H, 50 ng/mL) for 2, 6, and 16 hr. One experiment was performed.

mouse, which develops a renal pathology similar to human diabetics [22]. HGF was given s.c. to lean and obese db mice for 18 days by an Alzet mini-osmotic pump with an estimated rate of 160 ng/day. Obese mice had a 10-fold increase in urine flow and a nearly 2-fold increase in plasma creatinine levels (Table 1). Chronic HGF administration significantly decreased creatinine clearance in obese mice, suggesting that continuous administration of HGF reduced the glomerular filtration rate (Table 1). Moreover, HGF also increased albumin excretion in obese mice (Table 1), suggesting that HGF might reduce renal function in animals with compromised kidneys. Kidneys from these mice were also examined for histological changes and fibronectin mRNA levels. Whereas the obese mice had higher expression of fibronectin mRNA, the additional treatment with HGF had no additional effect on fibronectin expression or glomerular and tubular histology (not shown). It is likely that longer exposure to HGF is required to affect these parameters as seen in the HGF transgenic mice [14].

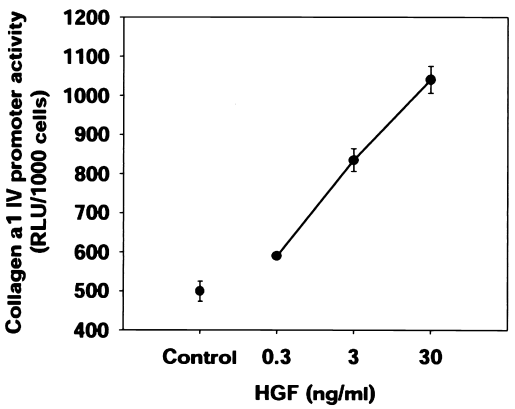

FIG. 6. Collagen α1 (IV) promoter activity in MMC-COL in response to increasing concentrations of HGF. Data show relative light units (RLU) per 1000 cells. Values are means ± SEM of quadruplicates.

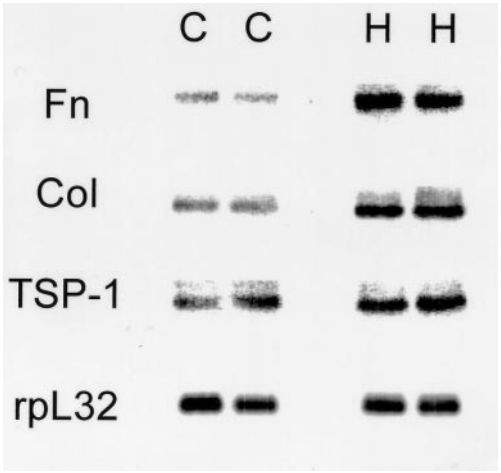

FIG. 7. Northern blot analysis of fibronectin (Fn), collagen α1 (IV) (Col), thrombospondin-1 (TSP-1), and rpL32 mRNA from primary rabbit renal proximal tubule epithelial cells (C, control; H, 50 ng/mL of HGF, N = 2).

DISCUSSION

Chronic renal disease involves the progressive loss of functional renal mass, accompanied by compensatory growth and remodeling. The molecular and cellular events that take place during chronic renal disease include release of growth factors, proliferation of glomerular mesangial cells, and expansion of extracellular matrix [23–25]. Whereas several protein factors have been implicated in stimulating extracellular matrix production during chronic renal failure, this is the first demonstration that HGF can increase gene expression of extracellular matrix genes in renal cells. Specifically, HGF increased the levels of fibronectin and collagen α1 (IV) mRNA in MMC and rabbit proximal tubule epithelial cells. The effect on collagen included transcriptional activation because in addition to increasing collagen α1 (IV) mRNA within 6 hr, HGF increased the activity of the collagen α1 (IV) mRNA promoter. HGF transduces its signal through several factors including phosphatidylinositol 3-kinase and the transcription factor STAT3 [26, 27], which may regulate collagen mRNA synthesis directly, or indirectly through known activators of collagen transcription, protein kinase C and c-fos [17]. The exact transcriptional factors that mediate HGF regulation of collagen remain to be determined. However, the above findings are in contrast to the effect of HGF on extracellular fibronectin immunoreactivity in MMC immortalized with the temperature-selective mutant of SV40 T antigen [13]. While it is possible that transformation of cells could disrupt normal HGF regulation of extracellular matrix, we show that HGF also increased fibronectin and collagen α1 (IV) mRNA in primary rabbit renal proximal tubule epithelial cells. Thus, HGF may contribute to renal remodeling by regulating the expression of extracellular matrix genes.

Persistent proteinuria and progressive loss of renal function without ischemia mark diabetic nephropathy. The db/db mouse is a genetically diabetic animal model characterized by obesity and hyperglycemia associated with insulin resistance [28, 29]. A recent study shows that progression of renal disease as measured by renal functions, such as creatinine clearance and albumin excretion, in db/db mice is very similar to the human diabetic condition leading to renal failure [22]. While the expression of c-met in the db/db mouse has not been examined, tubulointerstitial foci immunopositive for c-met were observed in streptozotocin-induced diabetic rats [7], indicating that the HGF axis is activated in diabetic nephropathy. In the present study, continuous elevation of HGF in the db/db mouse resulted in decreased creatinine clearance and increased microalbuminuria. However, HGF had a slight effect on body weight, which could influence the production of creatinine and urea. Nevertheless, albuminuria excretion still increased, which suggests decreased tubular function. Thus, prolonged activation of the HGF axis can lead to reduced renal function.

In support of the hypothesis that chronic elevation of HGF results in renal disease, transgenic mice, which overexpress HGF in the kidney and serum, have prominent

TABLE 1. Effect of chronic HGF on renal function

	Lean/Vehicle	Lean/HGF	Obese/Vehicle	Obese/HGF
Urine Flow (μL/min/100 g)	2.2 ± 0.6	1.4 ± 0.3	18.7 ± 3.3*	21.3 ± 1.8*
Plasma Cr (mg/dL)	0.40 ± 0.00	0.38 ± 0.02	0.58 ± 0.02*	0.64 ± 0.02*
Cr excretion (mg/100 g)	1.7 ± 0.3	1.3 ± 0.4	4.7 ± 1.7*	2.6 ± 0.2*
Cr clearance (μL/min/100 g)	400 ± 91	283 ± 68	454 ± 95	287 ± 24†
Urea excretion (mmol/100 g)	4.0 ± 0.8	3.0 ± 0.7	2.7 ± 0.4	2.1 ± 0.6
Albuminuria (μg/day/g)	0.60 ± 0.27	0.84 ± 0.20	5.11 ± 1.95*	13.29 ± 2.30†
Body weight (g)	33.0 ± 0.4	32.2 ± 0.6	45.8 ± 1.4*	41.9 ± 2.5*

Numbers are mean ± SEM. (N = 5–6 animals per group.
*$P < 0.05$ vs respective Lean group, †$P < 0.05$ vs respective vehicle-treated group (*t*-test).

tubular cystic disease and progressive glomerulosclerosis [14]. These animals show tubular epithelial hyperplasia with cystic lesions as early as 2 weeks after birth, with an increase in mesangial matrix by 1 month of age. Segmental or global glomerulosclerosis develops in older animals. Together, these studies suggest that HGF may stimulate c-met-responsive mesangial expansion and glomerular and tubulointerstitial fibrosis.

It should be noted, however, that HGF accelerates recovery of renal function after acute injury due to $HgCl_2$ or ischemic acute tubular necrosis [5, 6, 30]. In this context, HGF is a beneficial factor and aids in the restoration of normal renal function after an acute insult. Whether the chronic elevation of serum HGF observed in patients with renal insufficiency [8] is a compensatory response of the damaged kidney or contributes to further renal damage remains to be determined. In a recent study with a new strain of mice (ICGN) that spontaneously develops a nephrotic syndrome, large doses of HGF (500 μg/kg per day for 28 days) were able to prevent elevation in serum creatinine and microalbuminuria [31]. At least in that strain of mice, pharmacological doses of HGF are able to slow down the progression of chronic renal disease. Whether chronic elevation of HGF in animals with different genetic backgrounds has different effects on renal function awaits results from additional studies. Furthermore, experiments using HGF antagonists in various chronic renal failure models will clarify the role of HGF in progressive renal disease.

In summary, this study demonstrated that MMC have functional HGF receptors and are able to respond to HGF with increased expression of extracellular matrix genes such as fibronectin and collagen α1 (IV). The effect of HGF on fibronectin and collagen was not limited to mesangial cells and was also seen in primary proximal tubule epithelial cells. These results suggest that HGF may play a role in the extracellular matrix expansion in progressive renal disease. HGF also decreased creatinine clearance and increased microalbuminuria in diabetic mice, suggesting that chronic elevation of HGF may contribute to the reduction in renal function in diabetes. Because transgenic mice for HGF display renal fibrosis and c-met immunoreactivity was increased within tubulointerstitial foci in a model of diabetic nephropathy, chronic elevation of HGF likely contributes to renal tubular remodeling that results in renal tubular hyperplasia, interstitial fibrosis, and renal cystic disease. Therefore, further studies are warranted to evaluate the effect of HGF inhibition on the progression of diabetic nephropathy and chronic renal disease.

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References

1. Montesano R, Matsumoto K, Nakamura T and Orci L, Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor. *Cell* **67:** 901–908, 1991.
2. Santos OF, Moura LA, Rosen EM and Nigam SK, Modulation of HGF-induced tubulogenesis and branching by multiple phosphorylation mechanisms. *Dev Biol* **159:** 535–548, 1993.
3. Cantley LG, Barros EJ, Gandhi M, Rauchman M and Nigam SK, Regulation of mitogenesis, motogenesis, and tubulogenesis by hepatocyte growth factor in renal collecting duct cells. *Am J Physiol* **267:** F271–F280, 1994.
4. Joannidis M, Spokes K, Nakamura T, Faletto D and Cantley LG, Regional expression of hepatocyte growth factor/c-met in experimental renal hypertrophy and hyperplasia. *Am J Physiol* **267:** F231–F236, 1994.
5. Kawaida K, Matsumoto K, Shimazu H and Nakamura T, Hepatocyte growth factor prevents acute renal failure and accelerates renal regeneration in mice. *Proc Natl Acad Sci USA* **91:** 4357–4361, 1994.
6. Miller SB, Martin DR, Kissane J and Hammerman MR, Hepatocyte growth factor accelerates recovery from acute ischemic renal injury in rats. *Am J Physiol* **266:** F129–F134, 1994.
7. Liu Y, Tolbert EM, Sun AM and Dworkin LD, *In vivo* and *in vitro* evidence for increased expression of HGF receptor in kidney of diabetic rat. *Am J Physiol* **271:** F1202–F1210, 1996.
8. Sugimura K, Kim T, Goto T, Kasai S, Takemoto Y, Matsuda J, Yoshimoto M, Yamagami S and Kishimoto T, Serum hepatocyte growth factor levels in patients with chronic renal failure. *Nephron* **70:** 324–328, 1995.
9. Floege J, Alpers CE, Burns MW, Pritz P, Gordon K, Couser WG and Johnson RJ, Glomerular cells, extracellular matrix accumulation, and the development of glomerulosclerosis in the remnant kidney model. *Lab Invest* **66:** 485–497, 1992.
10. Ishibashi K, Sasaki S, Sakamoto H, Nakamura Y, Hata T, Nakamura T and Marumo F, Hepatocyte growth factor is a paracrine factor for renal epithelial cells: Stimulation of DNA synthesis and Na,K-ATPase activity. *Biochem Biophys Res Commun* **182:** 960–965, 1992.
11. Laping NJ, Olson BA, DeWolf RE, Albrightson CR, Fredrickson T, King C, Chirivella M, Ziyadeh FN and Nambi P, Activation of glomerular mesangial cells by hepatocyte growth factor through tyrosine kinase and protein kinase C. *Biochem Pharmacol* **55:** 227–234, 1998.
12. Liu Y, Tolbert EM, Sun AM and Dworkin LD, Primary structure of rat HGF receptor and induced expression in glomerular mesangial cells. *Am J Physiol* **271:** F679–F688, 1996.
13. Kolatsi-Joannou M, Woolf AS, Hardman P, White SJ, Gordge M and Henderson RM, The hepatocyte growth factor/scatter factor (HGF/SF) receptor, met, transduces a morphogenetic signal in renal glomerular fibromuscular mesangial cells. *J Cell Sci* **108:** 3703–3714, 1995.
14. Takayama H, LaRochelle WJ, Sabnis SG, Otsuka T and Merlino G, Renal tubular hyperplasia, polycystic disease, and glomerulosclerosis in transgenic mice overexpressing hepatocyte growth factor/scatter factor. *Lab Invest* **77:** 131–138, 1997.
15. Wolf G, Haberstroh U and Neilson EG, Angiotensin II stimulates the proliferation and biosynthesis of type I collagen in cultured murine mesangial cells. *Am J Pathol* **140:** 95–107, 1992.
16. Kuncio GS, Alvarez R, Li S, Killen PD and Neilson EG, Transforming growth factor-β modulation of the α1(IV)

collagen gene in murine proximal tubular cells. *Am J Physiol* **271:** F120–F125, 1996.
17. Fumo P, Kuncio GS and Ziyadeh FN, PKC and high glucose stimulate collagen α_1(IV) transcriptional activity in a reporter mesangial cell line. *Am J Physiol* **267:** F632–F638, 1994.
18. Sakhrani LM, Badie-Dezfooly B, Trizna W, Mikhail N, Lowe AG, Taub M and Fine LG, Transport and metabolism of glucose by renal proximal tubular cells in primary culture. *Am J Physiol* **246:** F757–F764, 1984.
19. McConnel HM, Owicki JC, Parce JW, Miller DL, Baxter GT, Wada HG and Pitchford S, The cytosensor microphysiometer: Biological application of silicon technology. *Science* **257:** 1906–1912, 1992.
20. Chan AML, Rubin JS, Bottaro DP, Hirschfield DW, Chedid M and Aaronson SA, Identification of a competitive HGF antagonist encoded by an alternative transcript. *Science* **254:** 1382–1385, 1991.
21. Chomczynski P and Sacchi N, Single-step method for RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162:** 156–159, 1987.
22. Cohen MP, Clements RS, Hud E, Cohen JA and Ziyadeh FN, Evolution of renal function abnormalities in the db/db mouse that parallels the development of human diabetic nephropathy. *Exp Nephrol* **4:** 166–171, 1996.
23. Klahr S, Schreiner G and Ichigawa I, The progression of renal disease. *N Engl J Med* **318:** 1657–1666, 1988.
24. Striker LJ, Doi T, Elliot S and Striker GE, The contributions of mesangial cells to progressive glomerulosclerosis. In: *Seminars in Nephrology* (Ed. Klahr S), pp. 318–328. W.B. Saunders, Philadelphia, 1989.
25. Ebihara I, Suzuki S, Nakamura T, Fukui M, Yaguchi Y, Tomino Y and Koide H, Extracellular matrix component mRNA expression in glomeruli in experimental focal glomerulosclerosis. *J Am Soc Nephrol* **3:** 1387–1397, 1993.
26. Cantley LG and Cantley LC, Signal transduction by the hepatocyte growth factor receptor, c- met. Activation of the phosphatidylinositol 3-kinase. *J Am Soc Nephrol* **5:** 1872–1881, 1995.
27. Boccaccio C, Ando M, Tamagnone L, Bardelli A, Michieli P, Battistini C and Comoglio PM, Induction of epithelial tubules by growth factor HGF depends on the STAT pathway. *Nature* **391:** 285–288, 1998.
28. Like AA, Lavine RL, Poffenbarger PL and Chick DL, Studies in the diabetic mutant mouse. VI. Evolution of glomerular lesions and associated proteinuria. *Am J Pathol* **66:** 193–204, 1972.
29. Hummel KP, Dickie MM and Coleman DL, Diabetes, a new mutation in the mouse. *Science* **153:** 1127–1128, 1966.
30. Igawa T, Matsumoto K, Kanda S, Saito Y and Nakamura T, Hepatocyte growth factor may function as a renotropic factor for regeneration in rats with acute renal injury. *Am J Physiol* **265:** F61–F69, 1993.
31. Mizuno S, Kurosawa T, Matsumoto K, Mizuno-Horikawa Y, Okamoto M and Nakamura T, Hepatocyte growth factor prevents renal fibrosis and dysfunction in a mouse model of chronic renal disease. *J Clin Invest* **101:** 1827–1834, 1998.