Delayed administration of hepatocyte growth factor reduces renal fibrosis in obstructive nephropathy

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Yang, Junwei, and Youhua Liu. Delayed administration of hepatocyte growth factor reduces renal fibrosis in obstructive nephropathy. Am J Physiol Renal Physiol 284: F349–F357, 2003; 10.1152/ajprenal.00154.2002.—Hepatocyte growth factor (HGF) is a renotropic protein that elicits antifibrogenic activity by preventing the activation of matrix-producing myofibroblast cells in animal models of chronic renal diseases. However, whether a delayed administration of HGF can still attenuate renal fibrosis remains uncertain. In this study, we examined the therapeutic potential of exogenous HGF on an established renal interstitial fibrosis induced by unilateral ureteral obstruction (UUO). Three days after UUO, the obstructed kidneys displayed interstitial fibrotic lesions with characteristic features of an established renal fibrosis, as manifested by myofibroblast activation, fibronectin overexpression, interstitial matrix deposition, and transforming growth factor-β1 upregulation. Beginning at this time point, administration of recombinant HGF into mice by intravenous injections for 11 days markedly suppressed the progression of renal interstitial fibrosis. HGF significantly suppressed renal α-smooth muscle actin expression, total kidney collagen contents, interstitial matrix components, such as fibronectin, and renal expression of transforming growth factor-β1 and its type I receptor. Compared with the starting point (3 days after UUO), HGF treatment largely blunted the progression of myofibroblast accumulation and collagen deposition but did not reverse it. Delayed administration of HGF also suppressed the myofibroblastic transdifferentiation from tubular epithelial cells in vitro, as demonstrated by a decline in α-smooth muscle actin and fibronectin expression. These results suggest that exogenous HGF exhibits potent therapeutic effects on retarding the progression of an established renal fibrosis.

c-met; renal fibrosis; ureteral obstruction; α-smooth muscle actin; myofibroblast

END-STAGE RENAL DISEASE (ESRD) presently affects a large number of patients worldwide, and the incidence of affected patients is increasing at a rate of 6–8% annually (4, 33). Once patients reach ESRD, mortality on dialysis is high. These data highlight the importance and urgency of developing effective therapeutic strategies for the treatment of ESRD. Importantly, most patients with chronic renal disease (CRD) are identified well before they reach end-stage renal failure; however, no presently available treatment is effective in halting the progressive loss of renal function.

Several pieces of evidence suggest that hepatocyte growth factor (HGF) is an excellent candidate for therapeutic use in the treatment of CRDs (20, 26, 40). HGF and c-met expression are increased, at least in the initial stage, in a variety of experimental animal models of CRD (21, 23, 28). Similarly, serum HGF levels are elevated in patients with end-stage renal failure (24). Both of these findings suggest that HGF may be part of the tissue-healing response. Moreover, administration of either recombinant HGF protein or its gene markedly prevents renal myofibroblast activation and interstitial fibrosis in numerous animal models of CRD (2, 27, 29, 42). Conversely, blockade of endogenous HGF signaling by a neutralizing antibody promotes the onset and progression of renal tissue fibrosis and kidney dysfunction in two distinct models of CRD (21, 28). Recently, we have demonstrated that HGF dramatically inhibits the activation of matrix-producing myofibroblasts by blocking epithelial-to-mesenchymal transition under pathological conditions (44). This suggests that HGF is capable of precisely targeting a critical event during renal fibrogenesis.

Despite several studies demonstrating an apparent efficacy of HGF in preventing renal fibrogenesis, it remains unclear whether HGF has a therapeutic role in established renal fibrosis. This unanswered question obviously has its profound clinical relevance, because most patients diagnosed with CRD already have different degrees of fibrotic lesions in their kidneys. In addition, contradicting data exist as to the role of HGF in progressive renal diseases (19, 41). For instance, it is reported that administration of HGF into genetically obese db diabetic mice actually reduces creatinine clearance and increases microalbuminuria (19). Clearly, more studies are needed in animal models to evaluate the therapeutic efficacy of exogenous HGF in an established renal fibrosis before clinical use of HGF can be considered for patients with chronic renal insufficiency.

In this study, we have examined the ability of HGF to suppress the progression of established renal fibrotic le-
sions in a mouse model induced by unilateral ureteral obstruction (UUO). Our results suggest that delayed administration of exogenous HGF is also effective in retarding the progression of an established renal fibrosis.

MATERIALS AND METHODS

Animal model. Male CD-1 mice (18–22 g) were purchased from Harlan-Sprague Dawley (Indianapolis, IN). They were housed in the animal facilities of the University of Pittsburgh Medical Center with free access to food and water. UUO was performed with an established procedure (6, 11, 42). Briefly, under general anesthesia, complete ureteral obstruction was performed by the double ligation of the left ureter with 4–0 silk after a midline abdominal incision. Sham-operated mice had their ureters exposed and manipulated but not ligated. In preliminary studies to establish the course of renal fibrogenesis in this model, four groups of mice (n = 4) were killed at days 1, 3, 7, and 14, respectively, after ureteral obstruction. One group of sham-operated mice (n = 4) was killed at day 3 after surgery. After preliminary studies on the kinetics of renal interstitial fibrosis after UUO in mice, four groups of animals were used in the experiments (Fig. 1). Groups 1 and 2 (n = 4) were sham-operated or UUO mice killed at 3 days after surgery. Eight additional mice underwent complete UUO. Three days later, these mice (n = 4) were randomly assigned into an HGF treatment (group 3) or vehicle control (group 4). Recombinant human HGF protein (kindly provided by Genentech, South San Francisco, CA) was administrated by intravenous injections via tail vein at 400 μg/kg body wt every 12 h for 11 days. Control mice were injected with the same volume of vehicle (PBS) in an identical manner. Mice were killed at day 14 after initial UUO, and the kidneys were removed. One part of the kidneys was fixed in 10% phosphate-buffered formalin for histological studies after being embedded in paraffin. Another part was immediately frozen in Tissue-Tek OCT compound for cryosection. The remaining kidneys were snap-frozen in liquid nitrogen and stored at −80°C for protein extractions.

Western blot analysis. Western blot analysis was carried out essentially according to the procedures described previously (22). Briefly, the kidney was homogenized in RIPA lysis buffer (1% Nonidet P-40, 0.1% SDS, 100 μg/ml phenylmethylsulfonyl fluoride, 0.5% sodium deoxycholate, 1 mM sodium orthovanadate, 2 μg/ml aprotinin, 2 μg/ml antipain, and 2 μg/ml leupeptin in PBS) on ice, and the supernatants were collected after centrifugation at 13,000 g at 4°C for 20 min. Protein concentration was determined by using a bicinchoninic acid protein assay kit (Sigma, St. Louis, MO), and tissue lysates were mixed with an equal amount of 2 x SDS loading buffer (100 mM Tris-HCl, 4% SDS, 20% glycerol, and 0.2% bromophenol blue). Samples were heated at 100°C for 5–10 min before loading and separated on precasted 10 or 5% SDS-polyacrylamide gels (Bio-Rad, Hercules, CA). The proteins were electrotransferred to a nitrocellulose membrane (Amersham, Arlington Heights, IL) in transfer buffer containing 48 mM Tris-HCl, 39 mM glycine, 0.037% SDS, and 20% methanol at 4°C for 1 h. Nonspecific binding to the membrane was blocked for 1 h at room temperature with 5% Carnation nonfat milk in Tris-buffered saline buffer (20 mM Tris-HCl, 150 mM NaCl, and 0.1% Tween 20). The membranes were then incubated for 16 h at 4°C with various primary antibodies in blocking buffer containing 5% milk. The mouse monoclonal anti-α-smooth muscle actin (α-SMA) antibody (clone 1A4) was purchased from Sigma-Aldrich (St. Louis, MO). An anti-α-SMA antibody was obtained from Transduction Laboratories (Lexington, KY). The polyclonal antibodies against transforming growth factor-β (TGF-β; TGF-β type I receptor (sc-398) and actin (sc-6160) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). After being washed extensively three times, the membranes were then incubated with horseradish peroxidase-conjugated secondary antibody (Bio-Rad) for 1 h at room temperature in 5% nonfat milk. The signals were visualized by an enhanced chemiluminescence system (ECL, Amersham). Quantitation was performed by measuring the intensity of the hybridization signals with the Image analysis program (National Institutes of Health).

Histology and immunohistochemical staining. Tissue sections from the mice were prepared at 4-μm thickness by a routine procedure. Sections were stained with hematoxylin/eosin for general histology. Immunohistochemical staining for α-SMA was performed by using the Vector MOM immunodetection kit (Vector Laboratories, Burlingame, CA), according to the procedures reported previously (44). To evaluate the extent of collagen deposition, Masson-Trichrome staining was performed according to the standard protocol by using a reagent kit purchased from Sigma.

Immunofluorescence microscopy. Kidney cryosections were prepared and fixed for 5 min in PBS containing 3% paraformaldehyde. After being blocked with 1% normal donkey serum in PBS for 30 min, the sections were incubated with primary antibodies against α-SMA, fibronectin, and collagen I, respectively, in PBS containing 1% BSA overnight at 4°C. Sections were then incubated for 1 h with fluorescein-conjugated secondary antibodies, at a dilution of 1:200 in PBS containing 1% BSA, before being washed extensively with PBS. As a negative control, the primary antibody was replaced with nonimmune IgG and no staining occurred. For double staining of α-SMA and proximal tubular marker, the slides were stained with fluorescein-conjugated lectin from *Tetragonolobus purpureas* (Sigma) after first being stained with α-SMA (44). Slides were mounted with antifade mounting media (Vector Laboratories) and viewed with an Eclipse E600 Epifluorescence microscope equipped with a digital camera (Nikon, Melville, NY).

Biochemical measurement of total kidney collagen content. For quantitative measurement of collagen deposition in the kidney, total tissue collagen content was determined by biochemical analysis of the hydroxyproline in the hydrolysates extracted from kidney samples, according to established pro-
procedures (16, 31). Briefly, accurately weighed portions of the obstructed kidneys were homogenized in distilled H₂O. The homogenates were hydrolyzed in 10 N HCl by incubation at 110°C for 18 h. The hydrolysates were dried by speed vacuum centrifugation over 3–5 h and redissolved in a buffer containing 0.2 M citric acid, 0.2 M glacial acetic acid, 0.4 M sodium acetate, and 0.85 M sodium hydroxide, pH 6.0. Hydroxyproline concentrations in the hydrolysates were chemically measured according to the techniques previously described (16, 31). Total collagen was calculated on the assumption that collagen contains 12.7% hydroxyproline by weight. The results of total tissue collagen content were expressed as micrograms of collagen per milligram of kidney weight.

**Determination of tissue TGF-β1 levels by enzyme-linked immunosorbent assay.** To measure renal TGF-β1 levels, kidneys from mice were homogenized in the extraction buffer containing 20 mM Tris·HCl, pH 7.5, 2 M NaCl, 0.1% Tween 80, 1 mM ethylenediamine tetraacetate, and 1 mM phenylmethylsulfonyl fluoride, and the supernatant was recovered after centrifugation at 19,000 g for 20 min at 4°C. Kidney tissue TGF-β1 level was determined by using the commercial Quantikine TGF-β1 ELISA kit in accordance with the protocol specified by the manufacturer (R&D Systems, Minneapolis, MN). This kit measures the abundance of active TGF-β1 protein that binds to its soluble type II receptor precoated onto a microplate. Total protein levels were determined by using a bicinchoninic acid protein assay kit as described above. The concentration of TGF-β1 in kidneys was expressed as picograms per milligram of total protein.

**Cell culture and treatment.** Human proximal tubular epithelial HKC cells (clone 8) were provided by Dr. L. Racusen (Johns Hopkins University, Baltimore, MD) and maintained in DMEM/F-12 medium supplemented with 10% FBS (Life Technologies, Grand Island, NY), as described previously (44). The HKC cells were seeded on six-well culture plates to 60–70% confluence in complete medium containing 10% FBS for 16 h and then changed to serum-free medium after being washed twice with medium. The cells were pretreated with recombinant human TGF-β1 (R&D Systems) at the concentration of 1 ng/ml for 48 h. Thereafter, the medium was changed, and the cells were continuously incubated with either TGF-β1 (1 ng/ml) alone or in combination with 50 ng/ml of recombinant human HGF (Genentech) for an additional 24 and 48 h, respectively. The cell lysates were subjected to Western blot analysis. For control experiments, the cells were incubated with vehicle (PBS).

**Statistical analysis.** Animals were randomly assigned to control and treatment groups. Data are expressed as means ± SE. Statistical analysis of the data was performed by using SigmaStat software (Jandel Scientific, San Rafael, CA). Comparison among groups was made with one-way ANOVA followed by the Student-Newman-Keuls test. A P value of <0.05 was considered to be significant.

**RESULTS**

Mice underwent complete UUO to generate a model of renal interstitial fibrosis characterized by myofibroblast activation, tubular atrophy, and interstitial matrix deposition (5, 17). Figure 2 shows the time course of fibronectin expression in the obstructed kidneys after the surgical operation. A more than 20-fold in-

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**Fig. 2.** Early onset of renal fibrotic lesions in obstructive nephropathy. A and B: time-dependent induction of fibronectin (FN) expression in kidneys after ureteral obstruction. Mice underwent UUO or sham operation for various durations (days) as indicated. A: representative Western blot analysis demonstrates an early induction of fibronectin expression in the obstructed kidneys after UUO. The blot was reprobed with actin to confirm equal loading. Two individual animals are represented at each time point. B: graphic presentation of the relative abundance of renal fibronectin after normalization with actin. Values (fold-induction relative to sham control) are means ± SE of 4 animals/group (n = 4). *P < 0.01 vs. sham. Representative micrographs show kidney morphology at 3 days after either sham operation (C) or UUO (D). Morphological injury manifested by tubular dilation and interstitial expansion was evident in the obstructed kidneys at 3 days after UUO. Bar = 20 μm. E: transforming growth factor (TGF)-β1 protein levels determined by ELISA in the kidneys at 3 days after UUO or sham operation. Values are means ± SE of 4 animals/group (n = 4). *P < 0.01 vs. sham.
duction of fibronectin expression was observed in the obstructed kidneys as early as 1 day after UUO. Although the renal fibronectin level increased progressively in accordance with the duration of ureteral obstruction, 3 days of obstruction appeared sufficient to induce marked accumulation of fibronectin and renal fibrosis in the obstructed kidneys. Further obstruction beyond this time point only resulted in a moderate increase in fibronectin expression (Fig. 2). Consistently, 3 days of obstruction began to induce significant morphological lesions and overexpression of TGF-β1 in the obstructed kidneys (Fig. 2). An approximately ninefold induction of the active TGF-β1 protein was detected in the kidneys at 3 days after ureteral obstruction (Fig. 2). These alterations are reminiscent of the characteristic features of an established renal fibrosis.

Figure 3 demonstrates the activation of α-SMA expression in the obstructed kidneys at 3 days after ureteral obstruction. Quantitative determination of Western blot analysis revealed that an ∼17-fold induction of α-SMA was found in the obstructed kidneys at as early as 3 days, suggesting a dramatic activation of myofibroblasts, the principal cells known to be responsible for accumulation and deposition of the interstitial matrix (9, 34, 36). This activation of renal myofibroblasts was independently confirmed by immunohistochemical staining for α-SMA (Fig. 3, C and D). Of note, α-SMA-positive cells were largely confined in the interstitial compartment at this stage.

After demonstrating that significant renal fibrosis was established at 3 days after UUO (Figs. 2 and 3), we next began to administer recombinant human HGF into mice at this time point to evaluate its therapeutic effects on established renal fibrosis. Figure 4 shows the α-SMA levels in the obstructed kidneys after intravenous injection of human HGF at 400 μg/kg body wt every 12 h for 11 days. Compared with the vehicle control, exogenous HGF markedly suppressed α-SMA expression by >70% in the obstructed kidneys. Immunofluorescence staining also demonstrated that the number of α-SMA-positive cells was dramatically decreased (Fig. 4, C and D). Of interest, double staining for α-SMA (red) and proximal tubular marker (green) revealed that in the obstructed kidneys of the control group, few cells retained the tubular marker, whereas α-SMA-positive cells were present in a widespread fashion. This suggests that at this advanced stage (14 days) of obstructive nephropathy, renal tubules already lost their characteristic epithelial marker, presumably due to epithelial-to-myofibroblast transition (EMT). Consistent with this, cells at the transitional stage with both α-SMA and tubular marker (yellow) were observable. However, administration of exogenous HGF largely preserved tubular marker and tubular structural integrity in the diseased kidneys (Fig. 4D), possibly by blocking tubular EMT as seen in the diseased kidneys.

Direct comparison of intrarenal α-SMA levels among different groups is also presented in Fig. 4. Delayed administration of HGF starting at day 3 after UUO almost completely blunted the progressive accumulation of α-SMA protein but did not reverse it. Quantitative determination revealed that similar levels of α-SMA were observed in the obstructed kidneys between the starting point (day 3 of UUO) and the end point (day 14 of UUO) of HGF treatment.

Along with the suppression of myofibroblastic activation, delayed administration of exogenous HGF also ameliorated the morphological lesions and extracellular matrix deposition seen in the obstructed kidneys. Figure 5, A and B, shows representative micrographs of the obstructed kidneys receiving either vehicle or

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**Fig. 3.** Induction of α-smooth muscle actin (α-SMA) expression in the obstructed kidneys. A: Western blot analysis shows the levels of α-SMA protein in the obstructed kidneys at 3 days after UUO or sham operation. Numbers (1–4) indicate 4 individual animals/group. B: Graphic presentation of the relative abundance of renal α-SMA after normalization with actin. Values (fold-induction relative to sham control) are means ± SE of 4 animals/group (n = 4). *P < 0.01 vs. sham. Representative micrographs show the immunohistochemical staining for α-SMA in the kidneys at 3 days after either sham operation (C) or UUO (D). Bar = 20 μm.
HGF protein. Kidneys with ureteral obstruction for 14 days displayed severe morphological lesions characterized by tubular dilation with epithelial atrophy, interstitial expansion with collagen accumulation and deposition (Fig. 5A). The interstitial space was clearly widened with some degree of hypercellularity, while the glomerular structure was generally well preserved. By contrast, kidneys with injections of exogenous HGF exhibited obvious attenuation of these morphological lesions with less fibrosis in the interstitium (Fig. 5B).

Figure 5C shows total kidney collagen contents from various samples determined by a quantitative, biochemical assay. Delayed administration of exogenous HGF markedly reduced total collagen deposition in the obstructed kidneys at day 14 after persistent injury. Direct comparison of collagen levels among different groups indicated that HGF largely blocked the progressive accumulation of renal total collagen after continuous, complete obstruction, although it did not reverse it. Accordingly, the expression of collagen I, a major component of the interstitial matrix, was also markedly suppressed by HGF in the obstructed kidneys, as demonstrated by immunofluorescence staining (data not shown). In addition, compared with that in the control group, exogenous HGF injections resulted in significant reduction of fibronectin expression in the kidneys. More than 48% repression of the fibronectin protein abundance was detected by Western blot analysis of whole kidney lysates (Fig. 5, D and E). Similar results were obtained by using an indirect immunofluorescence staining for fibronectin in the kidneys (not shown).

We further examined the expression of TGF-β1 and its type I receptor in the obstructed kidneys. As shown in Fig. 6, ureteral obstruction markedly induced the expression of TGF-β1 in the diseased kidneys, as detected by a specific ELISA. Administration of exogenous HGF considerably inhibited the induction of TGF-β1 expression (Fig. 6). Moreover, Western blot analysis exhibited that exogenous HGF also significantly downregulated the expression of TGF-β type I receptor in the obstructed kidneys (Fig. 6, B and C).

In an effort to provide mechanistic insight into understanding the actions of HGF on an established renal fibrosis, we investigated the effects of HGF on the phenotypes of newly transdifferentiated tubular epithelial cells by using an in vitro culture system. A treatment scheme was designed to mimic in vivo situations as described in this study (Figs. 1 and 7A). As shown in Fig. 7B, preincubation of renal proximal
tubular epithelial cells (HKC) with TGF-β1 for 48 h induced myofibroblastic transdifferentiation, as evidenced by de novo α-SMA expression as well as an induced fibronectin production. After this pretreatment, which is analogous to the onset of renal fibrosis in vivo (day 3 after UUO), HGF was added to the cultures with fresh medium in the continuous presence of TGF-β1. As shown in Fig. 7B, delayed administration of exogenous HGF suppressed α-SMA expression in HKC cells. In fact, the abundance of α-SMA in HKC cells after HGF treatment was below the preexisting level (lane 5 vs. lane 2, Fig. 7B). Accordingly, delayed administration of HGF also inhibited fibronectin expression in TGF-β1-pretreated HKC cells. These results suggest that delayed administration of HGF effectively suppresses, and possibly reverses, myofibroblastic transition of tubular epithelial cells.

DISCUSSION

When patients are diagnosed with chronic renal insufficiency, their kidneys may already display different degrees of renal fibrosis under most circumstances. Hence, a key to an effective therapy for patients with CRD is to develop a strategy that blocks the progression of an established renal fibrosis and dysfunction in a clinical setting. The purpose of this study was to test whether exogenous HGF, which has been shown to be preventive in retarding the onset of renal fibrosis and kidney dysfunction (27, 29, 42), also has therapeutic effects to ameliorate an established renal fibrosis. Our results demonstrate that HGF markedly suppresses renal myofibroblast activation and attenuates renal interstitial matrix deposition in the obstructed kidneys when given at 3 days after complete ureteral obstruction, a time point when significant renal fibrosis has clearly emerged. These observations suggest that the supplement of HGF not only may prevent the onset and progression of CRD as previously reported (27, 29, 42) but also may exhibit therapeutic effects on the diseased kidneys where tissue fibrosis is already established.

Although the pathological mechanism underlying chronic obstructive nephropathy is not completely elucidated, the fibrogenic process clearly plays a critical role in ultimately leading to permanent loss of the normal structural and functional integrity of the kidney (8, 18, 35). One of the intriguing observations in this study is that renal fibrogenesis initiates at a very early stage after ureteral obstruction and progresses rapidly in our mouse model. Unlike most forms of CRD models, such as remnant kidneys and diabetic nephropathy, that often take months to establish fibrotic lesions, obstructive nephropathy induced by complete, continuous ureteral obstruction perhaps is an exceptionally aggressive form of interstitial fibrogenesis. In accordance with this, an ~80-fold induction in fibronectin, 17-fold in α-SMA, and 9-fold in TGF-β1 expression are observed in the obstructed kidneys at 3 days after complete obstruction compared with the sham control group (Figs. 2 and 3). The kidneys obstructed for such a short duration display the characteristic features of chronic renal interstitial fibrosis often seen in diseased kidneys under various pathological conditions. Consistent with this, an earlier study shows that a significant upregulation of TGF-β1 and its type I receptor expression takes place in the kidneys as early as 1 day after obstruction (43). Thus major hallmarks for interstitial fibrotic lesions are already established in the obstructed kidneys at day 3 after complete obstruction. This notion is further supported by the fact that relief of obstruction after a short...
duration (days) does not completely reverse renal interstitial lesions (7, 30).

Although the therapeutic efficacy of exogenous HGF on established renal fibrosis is discernible, the mechanism underlying the therapeutic role of HGF remains largely unsolved. One clue for a potential mechanism comes from the observation that exogenous HGF inhibited the expression of both TGF-β1 and its type I receptor in the obstructed kidneys in vivo (Fig. 9). In light of the role of aberrant expression of TGF-β1 in the pathogenesis of many forms of CRDs (3, 25, 39), it is plausible to speculate that the inhibition of renal fibrosis may be mediated, at least in part, by the suppression of TGF-β1 axis expression in the obstructed kidneys. TGF-β1 is a well-documented profibrogenic factor that plays a determinant role in the pathological accumulation of extracellular matrix in normal tissues after various injurious insults (1). Studies indicate that overexpression of TGF-β1 in transgenic mice induced CRD with increased expression of fibrotic matrix protein. Conversely, suppression of TGF-β1 signaling either by truncated, soluble receptor or by an antisense approach significantly retards the progression of renal interstitial fibrosis in animals (13, 14). We previously demonstrated that the upregulation of both TGF-β1 and its type I receptor is an early event preceding the onset of significant renal fibrosis in the obstructed kidneys (43), supporting a causal relationship between overexpression of TGF-β1 axis and the development of renal interstitial fibrosis. Altogether, inhibition of TGF-β1 axis expression by exogenous HGF in vivo may play an important role in mediating its antifibrotic actions. However, it remains unclear whether the decreased TGF-β axis expression is due to a direct effect of HGF or a secondary consequence resulting from the antifibrotic action of HGF. Preliminary studies in our laboratory suggest that HGF does not directly inhibit TGF-β1 gene expression in cultured renal tubular cells (data not shown), implying that the antifibrogenic activities of HGF are probably mediated by a mechanism involving blockade of TGF-β1 signaling rather than via a direct inhibition of its expression.

The observation that HGF specifically blocks the activation of renal myofibroblasts in vivo may provide significant insights into the cellular mechanism underlying the amelioration of renal fibrotic lesions. Renal interstitial myofibroblast cells are α-SMA-positive, activated matrix-producing cells responsible for relentless accumulation and deposition of extracellular matrix in the interstitial compartments of diseased kidneys (12, 34, 37). Because myofibroblast cells are

**Fig. 6.** HGF suppresses the expression of TGF-β1 and its type I receptor in the obstructed kidneys. A: TGF-β1 protein levels determined by ELISA in the obstructed kidneys after injections of either HGF or vehicle. Values are means ± SE of 4 animals/group (n = 4). *P < 0.01 vs. vehicle. B: Western blot analysis shows the levels of TGF-β type I receptor (TβRI) protein in the obstructed kidneys after injections of either HGF or vehicle. The same blot was reprobed with actin. Numbers (1–4) indicate 4 individual animals/group. C: graphic presentation of the relative abundance of renal TGF-β type I receptor after normalization with actin. Values are means ± SE of 4 animals/group (n = 4). *P < 0.01 vs. vehicle.

**Fig. 7.** Delayed administration of HGF suppresses TGF-β1-induced α-SMA and fibronectin expression in tubular epithelial cells in vitro. A: diagram depicts treatment scheme. The in vitro experiments were designed to mimic HGF treatment scheme illustrated in Fig. 1. B: Western blot analysis shows the levels of α-SMA and fibronectin protein in renal tubular epithelial cells (HKC) after various treatments.
not present in normal kidneys, their origin under pathological conditions is uncertain. They are often presumed to derive from local resident interstitial fibroblasts. However, emerging evidence suggests that these cells may also come from tubular epithelial cells via a process known as epithelial-to-mesenchymal transition (10, 15, 32, 38, 45). It has been shown that TGF-β1 plays an essential role in initiating EMT that leads to the accumulation of matrix-producing myofibroblast cells (10, 43). Earlier studies from our laboratory show that HGF dramatically prevents myofibroblast activation from tubular epithelial cells triggered by TGF-β1 (44). Using an in vitro cell culture model system that recapitulates the in vivo treatment scheme, we show herein that delayed administration of HGF also suppresses, and possibly reverses, TGF-β1-induced α-SMA expression in tubular epithelial cells (Fig. 7). Such dramatic blockage of tubular EMT by HGF will undoubtedly lead to preservation of epithelial cell phenotypes and tubular structural integrity, thereby limiting the continuous supplies to the myofibroblast pool in obstructed kidneys after persistent injury. In view of the efficacy of HGF administered in a delayed fashion on blocking tubular EMT and renal fibrosis in obstructive nephropathy, it is reasonable to speculate that a supplement of HGF may hold promise as a novel therapeutic strategy for halting the progression of an established renal interstitial fibrosis in patients.

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