Hepatocyte growth factor in kidney fibrosis: therapeutic potential and mechanisms of action

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HGF is a multifunctional polypeptide, originally discovered in late 1980s as a unique protein that promotes hepatocyte proliferation and liver regeneration (43, 54, 55). The mature form of HGF is a heparin-binding, heterodimeric glycoprotein consisting of a 69-kDa α-chain and a 34-kDa β-chain held together by a disulfide bond. Extensive studies demonstrate that HGF regulates such diverse cellular processes as cell survival, proliferation, migration, and differentiation (43, 54, 55, 75). These biological actions are mediated by a single c-met receptor, the product of a c-met protooncogene, which is a member of the receptor tyrosine kinase superfamily. In simple terms, HGF binding triggers the activation of the c-met receptor through tyrosine autophosphorylation. This results in recruitment of intracellular signaling molecules and initiation of signal transduction cascades that lead to specific cellular actions (Fig. 1) (43, 54, 65, 75).

Although earlier studies implicated HGF in embryonic development and in promoting tissue regeneration after acute injury (for a review, see Refs. 49, 54, and 55), evidence is now emerging that HGF is also an intrinsic antifibrotic factor that plays a critical role in preventing tissue fibrosis in various animal models. Over the past several years, progress has been made in identifying the cellular targets of HGF and in unraveling the molecular mechanisms that underlie its actions in the setting of chronic kidney diseases (CKD) (24, 58, 59, 80). This article attempts to provide a concise review on the therapeutic potential of HGF in chronic renal fibrosis. Emphasis is placed...
on recent advances in our understanding of the cellular and molecular basis of HGF actions.

**HGF: A POTENT ANTIFIBROTIC FACTOR IN VIVO**

After chronic injury, endogenous HGF expression is transiently induced in most animal models of CKD (47, 61). To establish the relevance of HGF expression in the pathogenesis of CKD, two groups independently demonstrated that blocking HGF signaling with neutralizing antibody markedly promoted the onset and progression of tissue damage (47, 61). Administration of either HGF protein or its gene can also inhibit renal fibrotic lesions and kidney dysfunctions in remnant kidney after 5% nephrectomy (18, 28, 73). A beneficial effect of HGF has been reported in animal models of aristolochic acid nephropathy and cyclosporine A nephropathy, as well as in progressive anti-Thy-1.1 glomerulonephritis (57, 62, 64).

HGF not only prevents the onset and progression of CKD but also exhibits therapeutic efficacy even when tissue injury is already established. We have shown that at 3 days after UUO, the obstructed kidneys display interstitial fibrotic lesions with characteristic features of established renal fibrosis, as manifested by myofibroblastic activation, interstitial matrix deposition, and TGF-β upregulation. Beginning at this time point, administration of exogenous HGF into mice markedly suppresses the progression of renal interstitial fibrosis. HGF significantly inhibits the expression of renal α-smooth muscle actin (α-SMA), interstitial matrix components, TGF-β1, and its type I receptor (81). In another study, Mizuno and Nakamura (61) showed that administration of HGF to the mice with established diabetic nephropathy blocked the progression of fibrotic lesions and renal dysfunction. Similarly, HGF has been shown to ameliorate renal dysfunction in an established injury after 5% nephrectomy (18). These studies are clinically relevant, because by the time patients are diagnosed with chronic renal insufficiency, under most circumstances their kidneys are already displaying various degrees of renal fibrosis.

HGF can act synergistically with other therapeutic agents to inhibit renal interstitial fibrosis. In obstructive nephropathy, a combination of HGF gene therapy with inhibition of the renin-angiotensin system has been shown to produce synergistic effects that lead to dramatic attenuation of renal tubulointerstitial fibrosis (77). The combined therapy with human HGF gene and losartan, an ANG II type I receptor blocker, preserved renal mass and gross morphology and almost completely abolished α-SMA induction in obstructed kidneys. This regimen also synergistically inhibited the accumulation of interstitial matrix components and suppressed renal expression of both TGF-β1 and its type I receptor.

Despite many studies showing a favorable effect of HGF in CKD (Table 1), controversy exists regarding the role of HGF in kidney structure and function. For instance, among the

![Fig. 1. Simplified model depicting major hepatocyte growth factor (HGF) signal pathways in kidney cells. On HGF binding, the c-met receptor undergoes autophosphorylation in the tyrosine kinase domain, resulting in the recruitment of various intercellular signal transducers containing the Src homology domain, including the p85/p110 subunits of phosphoinositide 3-kinase (PI-3K), the Grb2/Sos complex, phospholipase C-γ (PLC-γ), and the multiadaptor protein Gab-1. This leads to diverse cellular responses, such as cell survival, proliferation, migration, and tubulogenesis (for a review, see Refs. 10, 54, and 75).](image)

<table>
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<tr>
<th>CKD Model</th>
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<tr>
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<td>57</td>
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<td>Progressive glomerulonephritis</td>
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HGF, hepatocyte growth factor; CKD, chronic kidney disease.
various HGF transgenic mouse lines that have been generated from different laboratories (2, 21, 25, 36, 71), one strain of transgenic mice expressing HGF, under the control of ubiquitous metallothionein promoter, developed progressive renal disease, as characterized by tubular hyperplasia, glomerulosclerosis, and polycystic lesions (71). In addition, inappropriate expression of HGF in these animals also caused malignant transformation and tumor formation (72). However, it remains uncertain whether the phenotype of this particular strain of transgenic mice is relevant to the actions of HGF in CKD, because no correlation was found between kidney pathology and HGF levels (71). Furthermore, transgenic mice engineered to specifically overexpress HGF in renal proximal tubules display normal kidney morphology and function, with no renal tumors, cysts, or glomerular abnormalities (21).

An early report showed that administration of HGF into genetically obese db diabetic mice reduced creatinine clearance and increased microalbuminuria, indicating that chronic exposure to HGF impairs renal function in this model (37). This appears contradictory to several recent studies showing advantageous effects of HGF in diabetic nephropathy (11, 15, 61). The reasons behind these inconsistent observations are unknown; however, possible explanations include the differences in species, strain, or genetic backgrounds (such as the specific gene mutation associated with db mice) as well as variations in the duration, dose, route, and particular preparation of recombinant HGF that was utilized.

**HGF AND TGF-β1: YIN AND YANG OF THE TISSUE FIBROTIC SIGNAL**

In vitro studies have found that HGF specifically counteracts many profibrotic actions of TGF-β1, suggesting that the balance between HGF and TGF-β1 may play a decisive role in the pathogenesis of chronic renal fibrosis (22, 59). In a broad sense, HGF and TGF-β1 function as the Yin and Yang of the tissue fibrotic signals that elicit opposite actions. Therefore, disturbance of the delicate balance between HGF and TGF-β1 activities will eventually cause disastrous consequences.

Both HGF and TGF-β1 are initially induced after tissue injury, insinuating that both are necessary and important for the initial wound-healing response and tissue repair (Fig. 2A) (47, 61). If the injurious stimulus is transient, such as in the situation after an acute insult, HGF signaling will predominate and prevail, resulting in tissue repair and regeneration, perhaps leading to complete recovery. In this scenario, the expression of TGF-β1 is not only important for matrix remodeling during the reparative phase but also may be crucial for negative modulation of HGF activity after recovery. On the other hand, if the injury is chronic in nature, TGF-β1 expression progressively increases throughout the entire course of injury (82), whereas HGF levels initially increase but gradually decline, possibly due to inhibition by TGF-β1. Thus the net effect after chronic injury is to shift the balance between TGF-β1 and HGF, favoring the profibrotic TGF-β1. Along this line, the duration of injury largely determines the ratio of TGF-β1/HGF and thereby the ultimate outcome of tissue responses.

Hyperactive TGF-β1 signaling in the diseased kidney can be attributed to many mechanisms. Induction of TGF-β1 expression is only one of them. Studies indicate that the posttranslational activation of TGF-β1 protein and its release from latent complexes within the pericellular compartment is enhanced in pathological conditions (4, 6, 16). Also, different isoforms of TGF-β (TGF-β1, -β2, and -β3) are increased in the diseased kidney; they all exhibit fibrogenic effects on renal cells and reciprocally stimulate the expression of one another (86). In addition, the receptors for TGF-β are induced in disease states (82). Apart from these, recent studies have revealed a dramatic downregulation of the Smad transcriptional corepressors SnoN and Ski in fibrotic kidneys (83), suggesting a diminished negative controlling mechanism for Smad signaling. Such a multitude of regulation at different levels, i.e., expression, activation, receptors and corepressors, is likely to instigate an amplification of TGF-β1 signaling, which will enormously shift the balance of TGF-β1/HGF activities in the fibrotic kidney.

Unbalanced TGF-β1 activity initiates various profibrotic actions in injured tissue, such as induction of myofibroblastic activation and promotion of apoptosis (Fig. 2B). TGF-β1 is
known to stimulate renal interstitial fibroblasts and glomerular mesangial cells to undergo myofibroblastic activation (12, 68, 78), as characterized by the induction of α-SMA expression and overproduction of ECM components. TGF-β1 also induces tubular epithelial-to-mesenchymal transition (EMT) (20, 82), a phenotypic conversion that plays a critical role in generating matrix-producing effector cells. In addition, TGF-β1 is able to induce or potentiate kidney cells to undergo apoptosis (14), leading to podocyte depletion, capillary loss, and tubular atrophy. Such broad actions of TGF-β1 will eventually lead to tissue fibrosis. In contrast, HGF can directly counteract the actions of TGF-β1, thereby leading to tissue repair (Fig. 2B). In this context, it is conceivable that any defects in HGF activation would have ruinous consequences. Consistent with this view, a recent study showed that mice lacking the urokinase plasminogen activator receptor (uPAR−/−) displayed impairment of the conversion of single-chain, pro-HGF into the active, two-chains form, which may at least partially account for the worsened renal fibrosis after obstructive injury (87).

The opposite effects of HGF and TGF-β1 are also reflected by their reciprocal regulation of each other. In vitro evidence has confirmed that TGF-β1 suppresses HGF expression in fibroblasts and mesangial cells (85), supporting an intrinsic connection between HGF and TGF-β1. Similarly, HGF is known to abolish TGF-β1 induction in vitro and in diseased kidneys in vivo (24, 60, 80).

Given the predominance of TGF-β1 signaling in the diseased kidney, it is likely that various strategies to influence the balance between TGF-β1 and HGF would be effective in ameliorating tissue fibrosis (Fig. 2C). Indeed, inhibition of TGF-β1 expression and/or activity through antisense technology or neutralizing antibodies is beneficial for reducing fibrotic lesions (29, 41, 51, 90). Conversely, supplementation with exogenous HGF prevents the progression of chronic renal fibrosis in diverse animal models (Table 1). Of interest, the beneficial action of several therapeutic agents may be related to their influence on the balance between TGF-β1 and HGF. For instance, vitamin D analogs are known to exert renoprotective activities (52, 69), and these actions are likely mediated by inducing HGF gene expression (39). Similarly, estrogen-mediated upregulation of HGF expression may explain its favorable role in slowing the progression of renal fibrosis and dysfunction (27, 45, 46). ANG II has been shown to induce TGF-β1 and inhibit HGF expression (5, 85); hence, the efficacy of anti-ANG II therapy in the clinical setting (9, 38) may be attributable, at least in part, to its regulation of TGF-β1 and HGF. Therefore, various therapeutic strategies for chronic fibrotic diseases seem to converge on a common pathway leading to restoration of the delicate balance between profibrotic TGF-β1 and antifibrotic HGF (Fig. 2C).

ROLE OF HGF IN KIDNEY CELLS: MULTIPLE TARGETS AND DIVERSE ACTIONS

Recent studies have shown that HGF elicits diverse cellular activities on multiple targets in the adult kidney, which has shed light on understanding the cellular mechanisms that underlie HGF-mediated inhibition of renal fibrosis. Although HGF is primarily produced in nonepithelial cells such as endothelial cells, mesangial cells, and fibroblasts, its c-met receptor is constitutively expressed in virtually every type of kidney cell (88). Such widespread expression of c-met is primarily controlled by the ubiquitous Sp family of transcription factors (88, 89). This insinuates that HGF may elicit various biological activities in a broad spectrum of target cells within the kidney. Consistent with this speculation, HGF is known to induce protein kinase B/Akt phosphorylation in all kidney cells tested, including glomerular mesangial cells, podocytes, proximal tubular cells, collecting duct epithelial cells, and interstitial fibroblasts (88).

Figure 3 summarizes the major cellular events in the fibrotic kidney, wherein TGF-β1 and HGF elicit opposite actions. Upregulation of TGF-β1 signaling is considered to be a convergent pathway after chronic renal injury, irrespective of the initial etiologies (8, 91). TGF-β1 induces renal fibrosis by promoting podocyte, endothelial, and tubular epithelial cell apoptosis, by activating mesangial cells and interstitial fibroblasts to produce massive amounts of matrix components, and by initiating mesenchymal transition of tubular epithelial cells. These actions of TGF-β1 lead to glomerulosclerosis and tubulointerstitial fibrosis, and, ultimately, to end-stage renal failure (8). Thus as highlighted by Bottinger and Bitzer (8) in a recent review, apoptosis and myofibroblastic activation represent two major cellular themes in the pathogenesis of renal fibrosis. Remarkably, HGF exerts its antifibrotic actions by precisely targeting these cellular processes that are essential for renal fibrogenesis (Fig. 3).

HGF Suppresses Myofibroblastic Activation and Matrix Overproduction

The progression of CKD is characterized by persistent accumulation of ECM, leading to widespread tissue fibrosis. In glomeruli, activated mesangial cells represent the principal matrix-producing cells that are responsible for excess ECM accumulation and deposition. In tubulointerstitium, α-SMA-positive myofibroblasts are considered to be the major effector cells that play a key role in interstitial fibrosis (Fig. 3) (8, 19). Studies indicate that HGF specifically blocks both mesangial and myofibroblastic activation in diseased kidneys (78, 80). This likely provides a mechanistic insight into understanding how HGF can prevent progressive renal fibrosis in a wide variety of animal models.

Mesangial cell activation is a predominant pathological feature of many glomerular diseases, including diabetic nephropathy. Although numerous factors may be involved in mesangial activation, TGF-β1 is certainly a major player in this process. In vitro studies illustrate that HGF suppresses α-SMA expression induced by TGF-β1 in cultured rat and human mesangial cells in a time- and dose-dependent manner (12). HGF also inhibits TGF-β1-mediated expression of fibronectin and type I collagen. Mizuno and Nakamura (61) have recently shown that HGF can abolish the α-SMA and type IV collagen expression induced by a high concentration of glucose in cultured human mesangial cells.

HGF also suppresses myofibroblastic activation from renal interstitial fibroblasts (78). TGF-β1 is capable of inducing normal rat renal interstitial fibroblasts (NRK-49F) to undergo phenotypic activation, as demonstrated by α-SMA expression, F-actin reorganization, and matrix protein overexpression (78). Simultaneous incubation with HGF abolishes TGF-β1-medi-
HGF Inhibition of Renal Fibrosis

HGF Blocks Tubular Epithelial-To-Mesenchymal Transition

Matrix-producing fibroblasts may originate from tubular epithelial cells via EMT under pathological conditions (31, 42). The observation that more than one-third of the interstitial fibroblasts are derived from tubular epithelium highlights the pathological importance of this cellular phenotypic conversion during renal fibrogenesis (30). In vitro, TGF-β induces tubular EMT (20, 66, 82), which is likely mediated by integrin-linked kinase (ILK) (40). We have found that in cultured proximal tubular epithelial cells, HGF completely abolishes TGF-β-induced ILK induction and blocks EMT (40, 80). This suggests that HGF plays a critical role in maintaining the differentiation status of renal epithelia. In harmony with this observation, blocking the action of HGF by a neutralizing antibody induces α-SMA expression in renal tubular epithelial cells (47). Tubular expression of α-SMA implies a phenotypic transition from tubular epithelial cells to myofibroblasts. Thus both exogenous and endogenous HGF are essential for preserving tubular epithelial cell phenotype through blocking EMT in the fibrotic kidney.

It should be pointed out that not all of the antifibrotic effects of HGF can be explained by its antagonistic effects on TGF-β. An earlier study reported by Wang and Hirschberg (76) indicates that HGF can downregulate the steady-state mRNA level of type III (α1) collagen in tubular epithelial cells, indicating a direct influence of HGF on matrix gene expression.

HGF Inhibits Apoptosis

One of the pathological features in renal fibrosis is cell apoptosis, which leads to podocyte depletion, capillary collapse, and tubular atrophy, depending on specific disease model (Fig. 3). As a potent survival factor, HGF can protect various types of kidney cells from apoptosis (17, 23, 48). Through this mode of action, HGF is able to preserve normal kidney structure and function under pathological conditions.

The cytoprotective actions of HGF on tubular epithelial cells had been demonstrated several years ago. We and others showed that exogenous HGF can prevent cultured tubular epithelial cells from apoptosis triggered by various death cues, such as deprivation of survival factors or addition of cisplatin (48, 84). In vivo, exogenous HGF inhibits tubular epithelial cell apoptosis that is often seen after chronic injury (24, 77). Similarly, preconditioning kidney with exogenous HGF almost completely prevents tubular epithelial cell death and kidney dysfunction after acute renal failure induced by folic acid (13). These results underscore a dramatic cytoprotective potential of HGF for tubular epithelial cells. This prosurvival activity of HGF is apparently mediated by dual mechanisms involving two distinct Bcl-2 family proteins (44). HGF triggers rapid phosphorylation of Akt kinase in tubular epithelial cells via a phosphoinositide 3-kinase-dependent pathway. Akt activation, in turn, induces Bad phosphorylation, thereby inactivating this
proapoptotic protein. Meanwhile, HGF can induce expression of the antiapoptotic Bcl-xL protein in a delayed fashion (44). Studies from Gao et al. (24) show that HGF induces prosurvival Bcl-2 expression in obstructive nephropathy.

Podocyte depletion is a characteristic feature of many chronic glomerular diseases, such as diabetic nephropathy. TGF-β1 is one of the triggers that initiates podocyte apoptosis (67). Studies indicate that HGF is also a potent survival factor for cultured podocytes. Fornoni et al. (23) reported that HGF, but not insulin-like growth factor-I, protected podocytes from cyclosporine A-induced apoptosis. This action of HGF in podocytes is also related to the phosphorylation and activation of Akt kinase. The potential role of HGF in protection of endothelial cells from apoptosis has also been demonstrated (63). Such action is presumably critical for prevention of capillary loss in the fibrotic kidney. In light of the apparent activation of the prosurvival Akt kinase after HGF stimulation in all kidney cells tested (88), it is reasonable to assume that HGF is able to protect all kidney cells against apoptosis in disease states.

**HGF Modulates Cell Proliferation**

As a regenerative factor, HGF is capable of inducing kidney cell proliferation and differentiation, thereby promoting injury-initiated repair and regeneration. The ability of HGF to stimulate tubular epithelial cell proliferation during acute renal failure is widely recognized (13, 32, 56). The mitogenic effect of HGF on tubular epithelial cells is also assumed to be beneficial in CKD, since tubular atrophy is a characteristic feature of fibrotic kidneys. Under pathologic conditions, cell proliferation induced by HGF should lead to preservation and/or restoration of tubular structural integrity. Apart from tubular epithelial cells, HGF is also shown to stimulate endothelial cell growth and accelerate glomerular capillary repair in experimental progressive glomerulonephritis (62). In a rat model of glomerulonephritis induced by anti-Thy-1.1 antibody, administration of HGF promoted glomerular capillary regeneration with marked endothelial cell proliferation. Consistently, almost all of the glomeruli showed a normal structure and improved renal function in this model (62).

HGF modulates renal cell proliferation in different ways, depending on the particular type of cell and cellular context. For instance, HGF does not increase DNA synthesis in cultured mesangial cells under basal conditions, and it actually inhibits PDGF-induced mesangial cell growth. Accordingly, administration of HGF to rats with anti-Thy 1.1 nephritis results in a selective suppression of activated mesangial cell proliferation (3).

**HGF Abolishes TGF-β1 Induction**

HGF-mediated inhibition of TGF-β1 expression has consistently been confirmed in many studies utilizing assorted models of CKD, which may provide an explanation for its therapeutic efficacy. Under basal conditions, HGF has no significant effects on TGF-β1 expression in either tubular epithelial or mesangial cells; however, it abolishes TGF-β1 induction after injurious stimulation in vitro (61, 79). Recent studies using human mesangial cells have also demonstrated that HGF can suppress TGF-β1 expression induced by a high concentration of glucose (61). Thus it is apparently clear that HGF specifically blocks the overexpression of TGF-β1 under pathological conditions but it has little effect on basal TGF-β1 expression. Accordingly, long-term expression of exogenous HGF via naked plasmid injection does not suppress renal TGF-β1 expression in normal mice (15). As TGF-β1 is capable of inducing its own expression by a positive autocrine loop, it seems plausible to hypothesize that inhibition of TGF-β1 expression by HGF is indirect, i.e., via disruption of TGF-β1 autocrine loop formation, rather than by suppression of TGF-β1 expression per se. However, irrespective of the mechanism(s), down-regulation of TGF-β1 by HGF underlines its therapeutic effectiveness in inhibiting major profibrotic signaling.

**HGF Accelerates Matrix Degradation**

In view of its antifibrotic potential in established renal fibrosis (18, 61, 81), HGF should be able to accelerate matrix catabolism by modulating matrix degradation pathways, thereby reducing the net matrix accumulation in the diseased kidney (26, 47). Indeed, a report by Gong et al. (26) indicates that HGF increases matrix catabolism by modulating both the plasminogen activator/plasmin and matrix metalloproteinases proteolytic pathways. Incubation of tubular epithelial cells with HGF abolishes TGF-β1-induced expression of the type I plasminogen activator inhibitor (PAI-1) and tissue inhibitor of matrix metalloproteinase. Such actions of HGF would theoretically lead to the upregulation of plasminogen activator/plasmin and matrix metalloproteinase activities, thereby accelerating matrix degradation in the fibrotic kidney.

**INTERCEPTION OF TGF-β1/Smad SIGNALING BY HGF: MANY PATHS TO THE SAME END**

As described above, the antifibrotic effects of HGF are primarily mediated by its ability to counteract the profibrotic actions of TGF-β1 (Fig. 3); however, until recently it remained poorly understood as to the mechanism behind the action of HGF in kidney cells. New studies provide significant insights into understanding the molecular mechanisms by which HGF antagonizes TGF-β1 in the setting of CKD. It is well known that TGF-β1 mediates its biological effects from the cell surface to the nucleus through the type I and type II serine/threonine kinase receptors and their downstream signaling effectors, known as Smads (8, 53, 74). On TGF-β1 stimulation, Smad-2 and -3 are phosphorylated on serine residues in their COOH termini by the type I receptor. This phosphorylation of Smad-2/3 induces an association with the common partner Smad-4, followed by translocation into the nuclei, where they control the transcription of TGF-β1-responsive genes by binding to the cognate Smad-binding element in the promoter region (53, 70) (Fig. 4). We have recently shown that HGF can specifically intercept TGF-β1-initiated Smad signaling by using diverse mechanisms in different types of kidney cells (Fig. 4).

**HGF Blocks Smad Nuclear Translocation in Interstitial Fibroblasts**

To decipher the mechanism that HGF employs to inhibit TGF-β1-mediated myofibroblastic activation, the potential interplay of HGF and TGF-β1/Smad signaling was examined in rat renal interstitial fibroblasts (NRK-49F) (78). It appears
clear that HGF inhibits neither Smad-2/3 phosphorylation nor their association with Smad-4. Furthermore, it does not significantly affect expression of the inhibitory Smad-6 and -7 or the cellular abundance of Smad transcriptional corepressors. However, HGF markedly attenuates the translocation and accumulation of activated Smad-2/3 to the nuclei (Fig. 4A) (78). This action of HGF is dependent on Erk-1 and -2 (Erk-1/2) phosphorylation and activation. Inhibition of Erk-1/2 activation restores TGF-β1-mediated Smad-2/3 nuclear accumulation and myofibroblastic activation. Consistent with this finding, earlier studies reported that either Ras signaling or EGF induced cytoplasmic retention of Smad-2/3 after stimulation with TGF-β1 in mink lung epithelial cells (35). Erk-1/2 kinases are known to induce phosphorylation of Smad-2 and -3 in the linker region that couples the DNA-binding domain and the transcriptional activation domain (35). This Erk-mediated phosphorylation prevents Smad-2/3 from undergoing nuclear translocation, precluding their access to the promoter region of TGF-β1-responsive genes. Therefore, Smad signal transduction is effectively intercepted on receipt of opposing regulatory inputs from HGF signaling (Fig. 4A). This provides a molecular illumination of the counterbalanced regulation of Smad signaling by HGF in interstitial fibroblasts and possibly explains HGF-mediated suppression of interstitial myofibroblastic activation in the kidneys.

Other actions of HGF may also contribute to its inhibitory effect on TGF-β1 in interstitial fibroblasts (34). Kobayashi et al. (34) showed that HGF induced expression of both decorin mRNA and protein in renal fibroblasts in an Erk-1/2-dependent fashion. As decorin can bind to active TGF-β1 and sequester its actions (4, 7), this pathway may also be relevant for HGF inhibition of TGF-β1 signaling in renal interstitial fibroblasts. However, de novo induction of decorin requires a long period of incubation, ranging from hours to days (34); thus this mechanism does not readily explain the immediate interplay between HGF and TGF-β1/Smad signaling.

**HGF Stabilizes Smad Corepressor TGIF in Glomerular Mesangial Cells**

HGF also inhibits TGF-β1-mediated mesangial cell activation, as demonstrated by the suppression of TGF-β1-induced α-SMA and type I collagen expression (12). This action of HGF is dependent on the activation of Erk-1/2, but not on Akt and p38 MAP kinase. In sharp contrast to fibroblasts, HGF does not affect TGF-β1-mediated Smad-2/3 nuclear translocation in mesangial cells. Instead, it rapidly induces expression of the Smad transcriptional corepressor TGIF (Fig. 4B) (12). As HGF does not modulate the abundance of TGIF mRNA, the induction of TGIF by HGF is primarily mediated by stabilization of its protein from degradation. Stabilization of TGIF has also been reported in other cell type by EGF signaling via the Ras/Mek/Erk pathway (50). Erk kinase is reportedly able to stimulate the phosphorylation of TGIF at two Erk kinase sites, the induction of TGIF by HGF is primarily mediated by stabilization of its protein from degradation. Stabilization of TGIF has also been reported in other cell type by EGF signaling via the Ras/Mek/Erk pathway (50). Erk kinase is reportedly able to stimulate the phosphorylation of TGIF at two Erk kinase sites, leading to its stabilization and the formation of Smad/TGIF complexes in response to TGF-β1 (50). In agreement with this, we found that in mesangial cells, forced expression of TGIF markedly suppresses the Smad-mediated activation of TGF-β-responsive promoter activities and completely blocks the TGF-β1-induced expression of α-SMA. In vivo, TGIF expression is dramatically downregulated in the glomeruli of diabetic kidneys, and exogenous HGF restores its expression. Hence, in
mesangial cells it appears that HGF specifically antagonizes the profibrotic action of TGF-β1 by stabilizing the Smad transcriptional corepressor TGIF (Fig. 4B) rather than by acting via attenuation of Smad-2/3 nuclear translocation as occurs in fibroblasts (Fig. 4A).

**HGF Induces Smad Corepressor SnoN Expression in Tubular Epithelial Cells**

A different mechanism is apparently implicated in the HGF-mediated antagonism of TGF-β1 in tubular epithelial cells (Fig. 4C). HGF blocks tubular EMT induced by TGF-β1 (80), which may account for a dramatic preservation of tubular epithelium after obstructive injury. In vitro studies have shown that disruption of TGF-β1 signaling, rather than modulation of its expression, plays a critical role in mediating HGF activity in these cells. However, HGF does not affect TGF-β1-induced Smad2/3 nuclear translocation, as exemplified in fibroblasts. Furthermore, unlike in mesangial cells, HGF fails to modulate TGIF expression. Instead, HGF specifically induces the expression of another Smad transcriptional corepressor, SnoN, at both the mRNA and protein levels (Fig. 4C) (79). This observation indicates that HGF is able to upregulate SnoN gene expression, rather than by influencing its protein stability. SnoN physically interacts with activated Smad-2 by forming a transcriptionally inactive complex and hence overrides the profibrotic action of TGF-β1. In vivo, SnoN is markedly downregulated in the fibrotic kidney (83) and HGF can restore SnoN protein abundance in obstructive nephropathy. Therefore, it is likely that HGF blocks EMT by antagonizing the action of TGF-β1 via upregulation of the Smad transcriptional corepressor SnoN (Fig. 4C). These findings uncover a novel mode of interaction between the signals activated by the HGF receptor tyrosine kinase and the TGF-β receptor serine/threonine kinases.

Through various mechanisms as depicted above (Fig. 4), HGF signaling converges on a common pathway that blocks Smad-mediated gene transcription in different types of kidney cells, despite initially diverging at various points in the signal circuit. At this stage, it remains unknown why such diverse responses operate in different kidney cells after stimulation with TGF-β1 and HGF. A logical explanation would be the unique genetic makeup and environment that comprises each type of target cell and defines the signal specificities, routes, and consequences of the interplay between HGF and TGF-β1. Clearly, further studies are warranted in this area to identify the cellular niche that accounts for the signal divergence of HGF in different types of kidney cells. Nevertheless, regardless of the molecular mechanisms involved, the fact that HGF precisely blocks the TGF-β1/Smad signaling axis in different renal cells underscores its potential for amelioration of chronic fibrotic diseases.

**CONCLUSION**

HGF has emerged as an intrinsic factor that protects kidney tissues from the development of fibrotic lesions after chronic injury. To a large extent, the reciprocal balance of TGF-β1 and HGF plays a critical role in determining whether the injured tissues undergo recovery or fibrogenesis. Whereas TGF-β1 is already widely recognized to play a causative role in the pathogenesis of renal fibrosis after diverse types of insult, it is becoming increasingly obvious that HGF represents a principal antifibrotic factor that counteracts the actions of TGF-β1. Through diverse mechanisms, HGF signaling in different types of kidney cells converges on a common pathway that effectively intercepts TGF-β1/Smad signal transduction. In this context, it is plausible to speculate that therapeutic strategies such as supplementation of exogenous HGF or induction of endogenous HGF expression would likely provide an effective means for the treatment of a wide range of chronic fibrotic disorders.

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