Hepatocyte Growth Factor Antagonizes the Profibrotic Action of TGF-β1 in Mesangial Cells by Stabilizing Smad Transcriptional Corepressor TGIF

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Abstract. Mesangial cell activation is a predominant pathologic feature of diabetic nephropathy that precedes the accumulation of extracellular matrix leading to glomerulosclerosis. For understanding the potential mechanism by which hepatocyte growth factor (HGF) ameliorates diabetic nephropathy, the effects of HGF on mesangial cell activation induced by TGF-β1 were investigated. Western blot analysis and immunostaining revealed that HGF suppressed α-smooth muscle actin expression induced by TGF-β1 in cultured rat and human mesangial cells. HGF also inhibited TGF-β1–mediated fibronectin and type I collagen expression. Such action of HGF was dependent on the activation of extracellular signal–regulated kinase-1 and -2 but not on Akt and p38 mitogen-activated protein kinase. HGF did not affect TGF-β1–mediated Smad2 phosphorylation and its nuclear translocation. However, it rapidly upregulated Smad transcriptional corepressor TG-interacting factor (TGIF) abundance in mesangial cells, which was primarily mediated by stabilizing its protein from degradation. Ectopic expression of TGIF markedly suppressed Smad-mediated activation of TGF-β1–responsive promoter activity and completely blocked TGF-β1–induced α-smooth muscle actin expression. In vivo, TGIF expression was dramatically downregulated in the glomeruli of diabetic kidneys, and delivery of exogenous HGF induced TGIF expression. These results suggest that HGF specifically antagonizes the profibrotic action of TGF-β1 in mesangial cells by stabilizing Smad transcriptional corepressor TGIF.

Mesangial cell activation, characterized by an induction of α-smooth muscle actin (α-SMA) expression and matrix overproduction, is considered as a predominant feature of diabetic nephropathy (1). Extensive studies indicate that activated mesangial cells are the major effector cells responsible for a relentless expression of interstitial matrix components such as fibronectin and type I collagen (2), which not only directly results in mesangial expansion leading to glomerulosclerosis but also is fundamentally linked to endothelial and podocyte damage within the glomeruli, as well as to secondary tubulointerstitial injury. Hence, mesangial cell activation is often regarded as a central event that plays a critical role in the onset and progression of diabetic nephropathy.

Numerous factors regulate the phenotypic transformation of mesangial cells from quiescent to matrix-overproducing, activated state (2–4). Of the many factors identified, TGF-β1 is the most potent cytokine that induces mesangial activation both in vitro and in vivo (5–8). The importance of TGF-β1 in diabetic nephropathy is also illustrated by the fact that many metabolic, hormonal, and hemodynamic factors in diabetic milieu all converge on a downstream pathway to upregulate the expression of TGF-β1 and its receptors in the kidney (9,10). In vitro, TGF-β1 has been demonstrated to stimulate mesangial cell activation and to induce matrix expression in many studies (2,6). Accordingly, blockade of TGF-β1’s action with neutralizing antibody has been proved to be effective in ameliorating diabetic nephropathy in animals (11).

Whereas many factors with positive influence on mesangial cell activation have been described, relatively little is known about the factors that can suppress the process of mesangial activation. Recent studies demonstrate that hepatocyte growth factor (HGF) is a potent antifibrotic cytokine that prevents α-SMA–positive myofibroblast activation from interstitial fibroblasts and blocks mesenchymal transdifferentiation from tubular epithelial cells induced by TGF-β1 (12–15). In addition, emerging evidence indicates that HGF is also capable of preventing α-SMA expression in renal glomeruli and mitigating diabetic nephropathy in vivo (16,17). These observations led us to hypothesize that HGF may be an endogenous negative regulator that specifically suppresses mesangial cell activation under pathologic conditions.

In the present study, we show that HGF specifically blocks mesangial cell activation induced by TGF-β1 in vitro. Furthermore, we demonstrate that HGF antagonizes the profibrotic action of TGF-β1 by upregulating Smad transcriptional corepressor TG-interacting factor (TGIF), which in turn suppresses Smad-mediated gene transcription. Such induction of TGIF expression by HGF is largely mediated by promoting the
corepressor’s stability, rather than by inducing its gene expression. Moreover, HGF restores TGIF protein expression in the glomeruli of diabetic kidney in vivo. These findings unravel the mechanism underlying the interaction between profibrotic TGF-β1 and antifibrotic HGF in mesangial cells and provide a cellular and molecular basis for HGF’s acting as a therapeutic agent for combating diabetic nephropathy.

Materials and Methods

Cell Culture and Treatment

Rat glomerular mesangial cells were provided by Dr. C. Wu (University of Pittsburgh, Pittsburgh, PA) and described previously (18). Human renal mesangial cells were purchased from the ScienCell Research Laboratories (San Diego, CA). These cells are characterized by the manufacturer using morphologic appearances and immunofluorescent method with various antibodies, including anti–Thy-1 and fibronectin. Cells were cultured in DMEM-F12 medium supplemented with 10% FBS (Invitrogen, Carlsbad, CA). After serum starvation for 16 h, cells were treated with growth factors for various periods of time as indicated. Recombinant human TGF-β1 was purchased from R&D Systems (Minneapolis, MN). Recombinant human HGF protein was provided by Genentech (South San Francisco, CA). Chemical inhibitors PD98059, wortmannin, and SC68376 were purchased from Calbiochem (La Jolla, CA). The cells were collected at different time points after treatments for various analyses.

Western Blot Analysis

Detection of protein expression by Western blot analyses was performed according to the procedures described previously (13). The primary antibodies used were as follow: anti–α-SMA (clone 1A4) and anti-extracellular signal–regulated kinase-1 and -2 (Erk-1/2) (Sigma, St. Louis, MO); antibodies against phospho-specific Erk-1/2, phospho-specific and total Akt, phospho-specific and total p38 mitogen-activated protein kinase (MAPK), phospho-specific and total c-Jun N-terminal kinase (Cell Signaling Technology, Beverly, MA); anti–phospho-specific Smad-2 (Upstate, Charlotteville, VA); anti-TGF (sc-17800), anti–c-Ski (sc-9140), anti-SnoN (sc-9595), anti-Sp1 (sc-420), and anti-actin (sc-1616) antibodies (Santa Cruz Biotechnology, Santa Cruz, CA); antifibronectin (clone 10; BD Biosciences Pharmingen, San Jose, CA); and anti–type 1 collagen (Calbiochem). Affinity-purified secondary antibodies were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA).

Immunofluorescence Staining

Indirect immunofluorescence staining was performed using an established procedure (13,19). Cells were double stained with 4',6-diamidino-2-phenylindole, HCl to visualize the nuclei. Kidney cryosections were stained with antibodies against TGFIF (sc-9825; Santa Cruz) and human HGF using the Vector MOM immunodetection kit by the protocol specified by the manufacturer (Vector Laboratories, Burlingame, CA). Monoclonal antibody against human HGF (clone H-14) was prepared by our laboratory and described elsewhere (20). This antibody specifically recognizes human HGF but does not cross-react with mouse or rat HGF protein. Stained cells were mounted with antifade mounting medium (Vector Laboratories) and viewed with a Nikon Eclipse E600 Epi-fluorescence microscope equipped with a digital camera (Melville, NY).

Nuclear Protein Extraction

Mesangial cells were subjected to various treatments with different growth factors for 30 min except where otherwise indicated. For HGF blockage of TGF-β1 signaling, cells were treated with 40 ng/ml HGF 30 min before addition of 2 ng/ml TGF-β1. Cell nuclei were isolated by procedures described elsewhere (21). After being collected by centrifugation, the nuclei were lysed with SDS sample buffer and subjected to Western blot analysis as described above.

Northern Blot Analysis

Northern blot analysis for gene expression was carried out by routine procedures, as described previously (22). Briefly, samples of 20 μg of total RNA were electrophoresed on 1.0% formaldehydeagarose gels and then transferred to GeneScreen plus nylon membrane (DuPont, Boston, MA) by capillary blotting. The membrane was hybridized with 32P-labeled TGF IF cDNA probe. After autoradiography, the membrane was stripped and rehybridized with rat glyceraldehyde-3-phosphate dehydrogenase probe to ensure equal loading of each lane.

Immunoprecipitation

Immunoprecipitation was carried out according to a procedure established previously (21). Briefly, mesangial cell lysates were incubated overnight at 4°C with 1 μg of anti-TGIF, followed by precipitation with 20 μl of protein A/G Plus-agarose for 3 h at 4°C. The immunoprecipitates were boiled for 5 min in SDS sample buffer. The resulting precipitated complexes were separated on SDS-polyacrylamide gels and blotted with anti-Smad4 antibody.

DNA Transfection and Luciferase Assay

The reporter construct p3TP-Lux, Smad2 and Smad3 expression vectors, and TGFIF expression vector (pHA-TGIF) were provided by Dr. J. Massague (Memorial Sloan-Kettering Cancer Center, New York, NY) (23–25). Mesangial cells were transfected with pTP-Lux (1.0 μg), with or without Smad2 (0.5 μg) and/or Smad3 (0.5 μg) expression vectors, and pHA-TGIF (0.5 μg) expression vector. A fixed amount (0.1 μg) of internal control reporter Renilla reniformis luciferase driven under thymidine kinase (TK) promoter (pRL-TK; Promega, Madison, WI) was also co-transfected for normalizing the transfection efficiency. After transfection with Lipofectamine 2000 reagent (Invitrogen), the cells were incubated for an additional 48 h in the absence or presence of 2 ng/ml TGF-β1. Luciferase assay was performed using the Dual Luciferase Assay System kit essentially according to the manufacturer’s protocols (Promega). Relative luciferase activity of each construct (arbitrary unit) was reported as fold induction after normalizing for transfection efficiency. All experiments were repeated at least three times to assume reproducibility.

Animals

The mouse uninephrectomized diabetic model and HGF treatment were described previously (16,26,27). A fraction of kidney was pooled from 6 to 8 animals in each group. Renal glomeruli were isolated by differential sieving technique, as reported elsewhere (28). Isolated glomeruli were lysed in SDS sample buffer and subjected to Western blot analysis for TGFIF and actin abundance, respectively. Kidney cryosections were stained for TGFIF and human HGF by using double immunofluorescence staining techniques.

Statistical Analyses

Statistical analysis of the data was performed using SigmaStat software (Jandel Scientific, San Rafael CA). Comparison between groups was made using one-way ANOVA followed by Student-Newman-Kuels test (29). P < 0.05 was considered significant.
Results

HGF Blocks Mesangial Cell Activation and Matrix Overproduction Induced by TGF-β1

Figure 1 shows that TGF-β1 induced α-SMA expression in cultured rat and human mesangial cells in a time-dependent manner. Maximal expression of α-SMA was found at 48 and 72 h after treatment with 2 ng/ml TGF-β1 in rat and human mesangial cells, respectively (Figure 1, A and C). Induction of α-SMA expression by TGF-β1 was also dose-dependent (data not shown). Of interest, simultaneous incubation of TGF-β1 with HGF significantly suppressed α-SMA expression in mesangial cells at different time points (Figure 1). Dose-dependent studies revealed that HGF at the concentration of 20 ng/ml (0.21 nM) largely abolished the α-SMA expression induced by 2 ng/ml TGF-β1 (0.16 nM; Figure 1B). Possibly because of its human origin, recombinant HGF seemed to be more effective in human mesangial cells (Figure 1C) than in their rat counterparts (Figure 1A). Of note, treatment with HGF alone at various concentrations did not affect α-SMA expression in mesangial cells (Figure 1B).

Similar results were obtained by using an immunofluorescence staining. As shown in Figure 1F, treatment of rat mesangial cells with 2 ng/ml TGF-β1 for 48 h markedly induced α-SMA expression. There were abundant α-SMA-positive fibrils in the cytoplasm of mesangial cells. However, HGF essentially abrogated TGF-β1-mediated α-SMA-positive fibril formation (Figure 1G).

Because one of the consequences of mesangial cell activation is to overproduce extracellular matrix, we next examined the effects of HGF on TGF-β1–mediated induction of matrix components in mesangial cells. As shown in Figure 1, TGF-β1 induced both fibronectin and type I collagen expression (Figure 1, I and L), and HGF effectively abolished their expression and assembly (Figure 1, J and M).

Blockage of Mesangial Cell Activation by HGF Is Dependent on Erk-MAPK Pathway

To unravel the mechanism underlying HGF inhibition of mesangial cell activation, we investigated the signaling events that are important for HGF abrogation of TGF-β1–mediated α-SMA expression. Treatment of mesangial cells with HGF activated several distinct signal pathways (Figure 2). Erk-1/2, members of the MAPK family, were markedly activated in mesangial cells upon HGF stimulation (Figure 2A). Such activation of Erk-1/2 as illustrated by their phosphorylation could be completely blocked by PD98059, a specific Erk-1/2 upstream kinase Mek1 inhibitor. HGF also induced p38 MAPK phosphorylation in mesangial cells (Figure 2C), whereas c-Jun N-terminal kinase was not activated (data not shown). In addition, HGF induced Akt phosphorylation in mesangial cells, which could be blocked by wortmannin, a specific inhibitor of the Akt upstream phosphoinositide 3-kinase (Figure 2B).

We next examined the impact of blocking specific HGF signal transduction pathway on TGF-β1–mediated mesangial cell activation. As shown in Figure 2, blockade of either p38 MAPK activation by SC68376 or Akt phosphorylation by wortmannin did not affect HGF-mediated suppression of α-SMA expression. However, blockade of Erk-1/2 activation by PD98059 restored TGF-β1–induced α-SMA expression.

HGF Does Not Inhibit Smad Activation and Nuclear Translocation

To elucidate how HGF counteracts TGF-β1 in mesangial cells, we investigated the potential interplay between the signals triggered by HGF and TGF-β1. To this end, we first examined the effects of HGF on Smad2 phosphorylation, a critical step in TGF-β1–mediated signal transduction pathway. As illustrated in Figure 3A, TGF-β1 induced Smad2 phosphorylation in mesangial cells. However, pretreatment with HGF did not affect Smad2 phosphorylation.

We further examined the subsequent nuclear translocation of activated Smad2, because earlier studies showed that this signaling event of TGF-β1 is subjected to regulation by HGF in renal interstitial fibroblasts (12). However, we found that HGF did not intercept Smad2 nuclear translocation, as there was no difference in phosphorylated Smad2 accumulation in the nuclei in the presence or absence of HGF (Figure 3B). Consistently, immunofluorescence staining showed that TGF-β1 induced Smad2 phosphorylation and nuclear accumulation (Figure 3D) and that HGF did not attenuate activated Smad2 nuclear accumulation (Figure 3E).

HGF Specifically Increases TGIF Abundance by Promoting Its Stability in Mesangial Cells

We next assessed the effects of HGF on the expression of Smad transcriptional corepressors in mesangial cells. As presented in Figure 4A, HGF induced TGIF protein abundance in rat mesangial cells, which peaked as early as 1 h after HGF stimulation. Under the same conditions, HGF failed to affect significantly the expression of SnoN and Ski in mesangial cells, two other members of Smad transcriptional corepressor family (Figure 4, B and C). HGF also upregulated the TGIF protein level in human mesangial cells, and the TGIF protein level started to increase at 0.5 h after HGF treatment and was sustained at least to 24 h (Figure 4D).

The mRNA levels of TGIF after HGF treatment in rat mesangial cells were examined by Northern blot analysis. In contrast to TGIF protein, the steady-state level of TGIF mRNA was not significantly altered at the different time points after HGF treatment (Figure 4, E and F). This suggests that HGF induction of TGIF in mesangial cells is primarily mediated by stabilizing TGIF protein from degradation, rather than by increasing gene expression.

Figure 4G shows that HGF induction of TGIF protein abundance was dependent on Erk-1/2 activation. Preincubation of mesangial cells with PD98059 completely abolished HGF-mediated TGIF induction, suggesting that Erk-1/2 activation is required for mediating TGIF stabilization. Of note, HGF was capable of inducing TGIF expression even in the presence of TGF-β1 (Figure 4G).
Figure 1. Hepatocyte growth factor (HGF) blocks mesangial cell activation and fibronectin and type I collagen expression induced by TGF-β1. (A through D) Western blot analyses demonstrate that HGF inhibited α-smooth muscle actin (α-SMA) expression induced by TGF-β1 in rat (A, B, and D) and human (C) mesangial cells. Mesangial cells were incubated without or with HGF and/or TGF-β1 at the concentrations as indicated for various periods of time (A and C) or at different doses of HGF for 48 h (B), respectively. Whole-cell lysates were immunoblotted with antibodies against α-SMA and actin, respectively. (D) Graphical presentation of the relative α-SMA abundance in A after quantitative determination. (E through M) Immunofluorescence staining for α-SMA (E through G), fibronectin (H through J), and type I collagen (K through M) in mesangial cells after various treatments. Rat mesangial cells were treated without (E, H, and K) or with TGF-β1 at 2 ng/ml (F, I, and L), or TGF-β1 plus 40 ng/ml of HGF (G, J, and M) for 48 h. Scale bar = 20 μm.
TGIF Forms Complexes with Smad and Blocks Smad-Mediated Gene Transcription

Figure 5, A and B, shows that the TGIF protein induced by HGF was predominantly localized in the nuclei, as demonstrated by immunofluorescence staining. Thus, TGIF clearly co-localizes with activated Smad after TGF-β1 stimulation (Figure 3D). This prompted us to examine a potential interaction between TGIF and Smad in mesangial cells after co-incubation with HGF and TGF-β1. To this end, mesangial cell lysates were immunoprecipitated with anti-TGIF antibody, followed by immunoblotting with Smad4. As shown in Figure 5C, Smad4 was detected in the immunoprecipitates of the cells treated with HGF and TGF-β1. Of note, treatment of mesangial cells with TGF-β1 alone resulted in no TGIF/Smad4 complex. Likewise, no TGIF/Smad complex formation was evident when the cells were preincubated with PD98059 to abolish TGIF induction (Figure 5C).

To investigate the functional significance of increased TGIF expression on TGF-β1 signaling, we examined the effects of TGIF on Smad-mediated gene transcription by transient transfection of TGIF. Rat mesangial cells were transfected with p3TP-Lux, a TGF-β1-responsive luciferase reporter plasmid that has been widely used in examining TGF-β1 responsiveness (23,24). As shown in Figure 5D, TGF-β1 significantly increased the
luciferase reporter activity of p3TP-Lux. Co-transfection with TGIF repressed both basal and TGF-β1–induced luciferase activity, suggesting that increased TGIF can abrogate endogenous Smad-mediated gene transcription in mesangial cells.

When Smad were overexpressed in mesangial cells after transient transfection of both Smad2 and Smad3 expression vectors, luciferase activity of p3TP-Lux reporter was increased in the absence or presence of TGF-β1 (Figure 5D), suggesting that Smad play an important role in mediating the promoter activity. Finally, ectopic expression of TGIF also suppressed the gene transcription activated by overexpressed Smad2/3 in mesangial cells with or without TGF-β1 stimulation (Figure 5D). Similar results were obtained when the cells were co-transfected with Smad3 (Figure 5E).

Ectopic Expression of TGIF Abolishes TGF-β1–Initiated Mesangial Cell Activation

To investigate further the potential role of TGIF in mediating the antifibrotic action of HGF, we examined the effect of TGIF on TGF-β1–induced mesangial cell activation. As shown in Figure 6A, transient transfection of mesangial cells with pHA-TGIF resulted in robust TGIF expression. Figure 6B shows the results of Western blot analysis for α-SMA expression in mesangial cells at different time points after TGF-β1 treatment. In rat mesangial cells transfected with empty vector pcDNA3, TGF-β1 induced α-SMA expression in a time-dependent manner. However, forced expression of TGIF by transfection completely abolished TGF-β1–induced α-SMA expression.
HGF Induces TGIF Protein Expression in Diabetic Nephropathy In Vivo

To establish the relevance of these in vitro studies to the in vivo situation, we examined the TGIF protein in diabetic nephropathy in mice. Western blot analyses showed that TGIF was expressed in the glomeruli of normal kidney (Figure 7). However, the level of glomerular TGIF protein was markedly reduced in the diabetic state. Quantitative measurement of TGIF abundance in the isolated glomeruli showed that TGIF protein expression was suppressed by ~70% in diabetic glomeruli at 12 wk after streptozotocin injection (Figure 7, A and B). Delivery of exogenous HGF via naked plasmid vector induced TGIF protein expression, leading to almost complete restoration of the TGIF level in the glomeruli of diabetic kidney (Figure 7).

To confirm further the induction of TGIF by HGF in vivo, we performed double immunofluorescence staining to examine the localization of exogenous HGF and TGIF in the diabetic kidney. In diabetic mice that received injections of human HGF plasmid, virtually all renal glomeruli were positive for exogenous HGF and TGIF protein expression (Figure 7, F and G). A clear co-localization (yellow) of human HGF (green) and TGIF (red) was evident in glomerular mesangial area when the images were merged (Figure 7H). However, in diabetic mice that received an injection of empty vector pcDNA3, no human HGF was found in renal glomeruli (Figure 7C), and the glomerular TGIF staining was essentially negative (Figure 7D). These results suggest that exogenous HGF can upregulate TGIF protein expression in the glomeruli in vivo as well. Of note, such induction of TGIF expression by exogenous HGF was associated with the attenuation of mesangial activation and matrix deposition, inhibition of glomerular hypertrophy, and mitigation of diabetic nephropathy (16).

Discussion

Phenotypic transformation of mesangial cells from the quiescent to the activated state, accompanied by accumulation of extracellular matrix in the mesangium, is a hallmark in most, if
not all types of primary glomerular diseases, including diabetic nephropathy observed in animal models and humans (30,31). In this sense, strategies to control mesangial cell activation may provide an effective means for therapeutic intervention of diabetic nephropathy. In this study, we have provided evidence that HGF, a potent antifibrotic cytokine that has been previously shown to suppress myofibroblast activation from interstitial fibroblasts and blocks mesenchymal transdifferentiation from tubular epithelial cells (12,13), is capable of suppressing mesangial cell activation induced by TGF-β1 in vitro. Furthermore, we show that HGF counteracts TGF-β1’s activity by upregulating Smad transcriptional corepressor TGIF, a functional antagonist that specifically represses TGF-β1–mediated Smad signaling (32). Such findings are consistent with in vivo observations demonstrating that HGF blocks mesangial activation and glomerular sclerotic lesions in diabetic kidney (16,17). This study provides novel insights into understanding the mechanism by which HGF antagonizes the profibrotic action of TGF-β1 in mesangial cells. Our results further establish a cellular and molecular basis for the therapeutic application of exogenous HGF in mitigating diabetic renal insufficiency.

It has been widely recognized that hyperactive TGF-β1 signaling plays a central role in mesangial cell activation and development of diabetic nephropathy (6,9,33). TGF-β1 expression is markedly induced in the glomeruli of diabetic kidney (6,8,16). The signal of TGF-β1 is transduced through its type I and type II serine/threonine kinase receptors (7,34,35) and tightly controlled by multiple negative regulatory mechanisms at different levels (36). In extracellular compartments, decorin, a proteoglycan associated with matrix components, binds to active TGF-β1 and prevents it from engaging with its receptors (37). In the cytoplasm, inhibitory Smad7 competes with receptor-regulated Smad to bind to activated type I receptor, thereby attenuating receptor-regulated Smad activation (38,39), or recruits GADD34-PP1c complex to dephosphorylate and inactivate TGF-β type I receptor (40). Once inside the nuclei, activated Smad may bind to Smad transcriptional corepressors by forming transcriptionally inactive complexes (32). Thus, the abundance of corepressors in a given cell may determine an ultimate cellular response after TGF-β1 stimulation. In view of the fact that Smad phosphorylation and nuclear translocation are intact after HGF treatment (Figure 3), the inhibitory action of HGF on TGF-β1 signal is unlikely to operate at a prenuclear
TGIF is a short-lived protein that undergoes rapid degradation (25), and its stabilization by HGF leads to an overall and rapid build-up in the steady-state level in mesangial cells (Figure 4). The increased TGIF, via forming complexes with activated Smad, sequesters the ability of Smad to initiate the transcription of TGF-β1–responsive genes and overrides the profibrotic action of TGF-β1 in mesangial cells (Figures 5 and 6). In addition to binding to activated Smad to make them transcriptionally inactive (32), TGIF may exert its repressive activity by other mechanisms. It has been shown that TGIF can directly bind to DNA via TGIF binding site (43) or interact with other transcriptional repressors such as mSin3 and recruits it to TGF-β–activated Smad complexes (44). Further studies are needed to clarify whether a single or multiple mechanisms are involved in mediating TGIF repression of TGF-β1–activated gene expression in mesangial cells.

Because TGIF is expressed in the glomeruli of normal kidney (Figure 7), profibrotic TGF-β1 signaling is presumably confined by TGIF under normal conditions in vivo. That TGIF expression is reduced in the diabetic glomeruli suggests that TGF-β1 signal is profoundly amplified, as a result of the loss of one key negative regulatory mechanism. It should be noted that this occurs concurrently with an increased TGF-β1 expression in the diabetic kidney. Hence, the profibrotic TGF-β1 signal is virtually transduced without negative control in the kidney in the diabetic state. In this regard, restoration of TGIF expression in the diabetic glomeruli by HGF would reinstate the negative controlling mechanism governing TGF-β1’s action in vivo (Figure 7). Indeed, HGF-mediated restoration of TGIF expression is associated with inhibition of mesangial activation and attenuation of diabetic nephropathy in mice (16).

It is worthwhile to point out that the interplay between HGF and TGF-β1 signal transduction pathways occurs in different renal cells in a dissimilar way. Studies show that HGF is able to counteract TGF-β1 activity in diverse types of kidney cell, including mesangial cells, interstitial fibroblasts, and tubular epithelial cells (12,13). Whereas HGF stabilizes TGIF in mesangial cells, it does not affect this corepressor abundance in interstitial fibroblasts (12) and tubular epithelial cells (unpublished data). This suggests that HGF-mediated stabilization of TGIF protein is cell content dependent. Earlier studies showed that HGF also antagonizes TGF-β1 actions in interstitial fibroblasts, which is evidently mediated by intercepting activated Smad2/3 nuclear translocation (12). Such signal interception would prevent the Smad from direct access to the cis-acting DNA element for Smad binding, thereby incapacitating Smad-mediated transcription of TGF-β1–responsive genes (12,45). Regardless of the mechanisms involved, that HGF is capable of counteracting profibrotic actions of TGF-β1 in various kidney cells by diverse strategies underscores a remarkable capacity of HGF as an endogenous antifibrotic factor in the pathogenesis of chronic renal fibrosis.

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References

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**Figure 7.** HGF induces TGIF protein expression in the glomeruli of diabetic mice. (A) Western blot analysis shows the TGIF protein levels in the isolated glomeruli of various groups. Renal glomeruli were isolated by differential sieving technique from the kidneys of normal, diabetic, and diabetic treated with HGF groups. For each group, a fraction of kidney was pooled from 6 to 8 animals. Glomerular lysates were immunoblotted with antibodies against TGIF and actin, respectively. (B) Graphical presentation of relative TGIF abundance after normalization with actin in various groups. (C through H) Immunofluorescence staining shows the co-localization of TGIF and exogenous HGF in the glomeruli of diabetic kidneys after administration of human HGF gene via naked plasmid vector. (C through E) Diabetic kidney treated with pcDNA3. (F through H) Diabetic kidney treated with pcMV-HGF. (C and F) Staining for human HGF (green). (D and G) Staining for TGIF (red). (E and H) Merge of HGF and TGIF. Inserts in E and H are enlarged glomeruli from the boxed areas. Broken circles outline the glomeruli, which were identified from overexposed images. Scale bar = 100 μm.


