Hepatocyte Growth Factor Attenuates Transforming Growth Factor-β-Angiotensin II Crosstalk Through Inhibition of the PTEN/Akt Pathway

Kazuma Iekushi, Yoshiaki Taniyama, Hiroshi Kusunoki, Junya Azuma, Fumihiro Sanada, Keita Okayama, Nobutaka Koibuchi, Masaaki Iwabayashi, Hiromi Rakugi, Ryuichi Morishita

Abstract—Both angiotensin II (Ang II) and transforming growth factor (TGF)-β1 are thought to be involved in the progression of chronic kidney disease. In contrast, hepatocyte growth factor (HGF) counteracts the actions of Ang II and TGF-β1. Therefore, in this study, we investigated the molecular mechanisms of how HGF antagonizes the Ang II-TGF-β axis in renal cells. In cultured human mesangial cells, TGF-β1 increased angiotensin type 1 receptor (AT1R) mRNA, mainly dependent on the Akt/phosphatidylinositol 3-kinase signaling pathway. Furthermore, TGF-β1 decreased the expression and phosphatase activity of phosphatase and tensin homolog, deleted on chromosome 10 (PTEN), a negative regulator of the phosphatidylinositol 3-kinase/Akt pathway. These data revealed positive feedback of the Ang II-TGF-β pathway, because Ang II increased TGF-β expression. In contrast, HGF significantly attenuated the increase in AT1R gene expression, and inhibited the decrease in PTEN induced by TGF-β1. Of importance, a PTEN-specific inhibitor significantly attenuated the reduction in TGF-β1–induced AT1R expression by HGF. These data suggest that HGF attenuated TGF-β1–induced AT1R expression through the PTEN/Akt pathway. To investigate this hypothesis, we performed in vivo experiments in mice with increased circulating levels of HGF produced by transgenically expressing HGF under control of a cardiac-specific transgene (HGF-Tg). In HGF-Tg mice, renal injury and fibrosis were significantly decreased, associated with reduction in AT1R expression and increase in PTEN after Ang II infusion, as compared with control mice. Moreover, these renal protective effects were abrogated by a neutralizing antibody against HGF. Thus, the present study demonstrated that HGF counteracts the vicious cycle of Ang II-TGF-β1-AT1R, mediating the inhibition of PTEN. (Hypertension. 2011;58:00-00.) • Online Data Supplement

Key Words: fibrosis ■ TGF-β HGF ■ CKD ■ PTEN

Maladaptive activation of the renin-angiotensin system (RAS) has been shown to play a pivotal role in the pathogenesis of chronic kidney disease (CKD). Angiotensin II (Ang II), the major bioactive peptide of RAS, is a potent systemic vasoconstrictor and modulator of the renal circulation. Ang II has important nonhemodynamic effects that have been implicated in the pathogenesis of CKD by promoting mesangial cell hypertrophy and proliferation, increasing extracellular matrix synthesis1 and promoting inflammation.2 The biological responses to Ang II are mediated by 2 plasma membrane receptors, Ang II type 1 receptor (AT1R) and Ang II type 2 receptor.3 Most of the known pathophysiological effects of Ang II are mediated by AT1R. In both clinical and experimental studies, angiotensin-converting enzyme (ACE) inhibitors and Ang II type 1 receptor blockers (ARBs) clearly revealed renoprotective effects. In contrast, transforming growth factor-β (TGF-β) is also well known to be a key mediator in the progression of CKD. TGF-β1 has been linked mainly to fibrinogenesis in some animal models. One of the striking effects of TGF-β1 is stimulation of AT1R expression, whereas the proatherosclerotic action of Ang II is mediated, at least in part, by TGF-β1. Thus, the positive feedback loop of Ang II and TGF-β1 might result in amplification of their profibrotic effects in renal cells.

In contrast, hepatocyte growth factor (HGF) is a pleiotropic factor that plays an essential role in the regulation of cell proliferation, survival, and differentiation in a variety of organs. Specifically, HGF prevented renal interstitial fibrosis in several animal models of CKD. Recent studies suggest that HGF counteracts the action of TGF-β1 in different types of kidney cells, resulting in inhibition of TGF-β–mediated myofibroblastic activation of glomerular mesangial cells and interstitial fibroblasts and blockade of tubular epithelial-to-mesenchymal transition. In general, it is believed that HGF
antagonizes the profibrotic actions of TGF-β1 by intercepting Smad signal transduction in different kidney cells and the injured kidney.4 In addition, our group demonstrated recently that HGF promoted apoptosis of myofibroblasts, an important source of TGF-β1.5 However, the molecular mechanisms of inhibition of the Ang II-TGFβ pathway by HGF have not been fully clarified. Thus, in this study, we investigated how HGF counteracts the positive feedback loop between Ang II and TGF-β1 that results in amplification of their profibrotic effects in the kidney.

Materials and Methods
Please see the online Data Supplement (http://hyper.ahajournals.org) for information on the primary antibodies and reagents used in the study.

In Vitro Experiments
Human mesangial cells were cultured in mesangial cell basic culture medium plus 5% FBS. The cells were seeded to 70% to 80% confluence in complete medium and then changed to serum-free medium. Recombinant human TGF-β1 was added to the cultures at a final concentration of 1 ng/mL.

Western Blot Analysis and Quantitative Real-Time PCR
Preparation of protein extract and Western blotting were performed as described previously.6 Total RNA was isolated as described previously.6 In each experiment, mouse β-actin RNA was amplified as a reference standard.

In Vivo Experiments
We generated mice (C57BL6 background) with cardiac-specific (α-major histocompatibility complex–driven) overexpression of HGF in which serum HGF derived from the heart was significantly increased.7 We administrated Ang II (0.7 mg/kg per day) by using osmotic minipump (DURECT Corporation, Cupertino, CA) to HGF transgenic (Tg) mice and C57BL6N mice (littermates) aged 8 to 10 weeks for 4 weeks. Blood pressure was measured by tail-cuff plethysmography. Mice were divided into 4 groups: (1) wild-type control mice treated with Ang II; (2) wild-type control mice treated with Ang II and hydralazine (30 mg/kg per day); (3) HGF-Tg mice treated with Ang II and hydralazine neutralizing antibody (200 μg/wk); (4) HGF-Tg mice treated with Ang II and HGF neutralizing antibody (200 μg/wk). Mice were euthanized at 4 weeks after Ang II infusion. All of the procedures were performed in accordance with the Osaka University School of Medicine Institutional Animal Committee.

Statistical Analysis
Values are expressed as mean±SE. ANOVA and t test followed by Bonferroni adjustment for multiple comparisons were used for comparisons of >2 groups. A P value <0.05 was considered to indicate significance of difference of mean values.

Results
Role of Phosphatase and Tensin Homolog, Deleted on Chromosome 10 (PTEN) in Inhibition of AT1R Expression by HGF
To elucidate the molecular mechanisms of renal injury by Ang II, we first tested the effect of TGF-β1 on AT1R mRNA expression in human mesangial cells, because our previous study demonstrated that Ang II stimulated TGF-β1 mRNA expression in human mesangial cells.7 As shown in Figure S1 (available in the online Data Supplement), TGF-β1 significantly stimulated the expression of AT1R mRNA in a time-dependent manner from 1 to 24 hours (P<0.01). In contrast, Ang II stimulation alone did not change AT1R mRNA expression (Figure 1A). Of importance, pretreatment with HGF significantly attenuated the increase in AT1R mRNA expression induced by TGF-β1 (Figure 1A). Our previous study demonstrated that pretreatment with HGF significantly decreased the increase in TGF-β1 expression induced by Ang II; HGF attenuated the signal through the positive loop of the Ang II-TGF-β1-AT1R axis. Although the main pathway of TGF-β1 is the Smad pathway, recent studies revealed other pathways, such as phosphatidylinositol 3-kinase (PI3K), extracellular signal–regulated kinase (ERK) 1/2 inhibitor (2×10−5 M); PD, PD98059, extracellular signal–regulated kinase (ERK) 1/2 inhibitor (2×10−5 M); SB, SB203580, p38 inhibitor (2×10−5 M); SP, SP600125, c-Jun N-terminal kinase (JNK) inhibitor (1×10−5 M).

Figure 1. Effect of hepatocyte growth factor (HGF) on angiotensin (Ang) II type 1 receptor (AT1R) mRNA expression in vitro. A, AT1R mRNA expression induced by transforming growth factor (TGF)-β1 with Ang II and HGF. Expression of AT1R was determined by quantitative PCR using β-actin as an internal control. Data represent mean±SEM. †P<0.05 vs control, †P<0.05 vs TGF-β1. B, AT1R mRNA expression induced by TGF-β1 with pharmacological inhibitors of signaling pathways. Expression of AT1R was determined by quantitative PCR using β-actin as an internal control. Data represent mean±SEM. †P<0.05 vs non-treated, †P<0.05 vs TGF-β1. LY indicates LY294002, phosphatidylinositol 3-kinase (PI3K) inhibitor (1×10−5 M); PD, PD98059, extracellular signal–regulated kinase (ERK) 1/2 inhibitor (2×10−5 M); SB, SB203580, p38 inhibitor (2×10−5 M); SP, SP600125, c-Jun N-terminal kinase (JNK) inhibitor (1×10−5 M).
extracellular signal–regulated kinase 1/2 (by PD98059) and p38 (by SB203580) did not affect it, indicating that the increase in \(\text{AT}_1\beta\) expression induced by \(\text{TGF}-\beta_1\) might be mainly through the PI3K-Akt signaling pathway.

Therefore, we focused on the HGF-PI3K/Akt pathway. \(\text{TGF}-\beta_1\) significantly increased the phosphorylation of Akt, which was maximal at 15 minutes (data not shown). However, pretreatment with HGF significantly attenuated Akt phosphorylation stimulated by \(\text{TGF}-\beta_1\) (Figure 2A and 2B; \(P<0.01\)). Because PI3K action is regulated by the tumor suppressor protein, phosphatase and tensin homolog, deleted on chromosome 10 (PTEN), by dephosphorylation of phosphatidylinositol (PI) 3,4,5-trisphosphate,10,11 we next focused on the role of PTEN. Interestingly, as shown in Figure S2A, \(\text{TGF}-\beta_1\) significantly decreased the expression of PTEN in a time-dependent manner, whereas HGF markedly attenuated the decrease in PTEN expression induced by \(\text{TGF}-\beta_1\) (Figure S2B; \(P<0.01\)). Of importance, a PTEN inhibitor (bpV(OH)pic) significantly inhibited the attenuation of \(\text{TGF}-\beta_1\)–induced Akt activation by HGF (Figure 2A and 2B; \(P<0.01\)). To elucidate the role of PTEN in the increase in \(\text{AT}_1\beta\) expression induced by \(\text{TGF}-\beta_1\), we further studied the effects of a PTEN inhibitor on \(\text{AT}_1\beta\) expression. Of importance, as shown in Figure 2C, the addition of a PTEN inhibitor significantly prevented the decrease in \(\text{TGF}-\beta_1\)–induced \(\text{AT}_1\beta\) expression by HGF (\(P<0.01\)). Furthermore, to determine by which mechanism HGF controls PTEN synthesis, luciferase reporter constructs encoding the PTEN promoter were used. \(\text{TGF}-\beta_1\) significantly reduced PTEN reporter gene activity, whereas this effect was blocked by HGF treatment (Figure S3), suggesting that HGF regulated \(\text{TGF}-\beta_1\)–induced PTEN transcriptional downregulation. These data indicated that HGF inhibited \(\text{TGF}-\beta_1\)–induced \(\text{AT}_1\beta\) expression via the Akt-PTEN pathway.

Overexpression of HGF Inhibited Renal Damage Induced by Ang II Infusion in HGF-Tg Mice

To further confirm the in vitro findings, we used an in vivo model of Ang II infusion into mice, which exhibited severe renal damage. Our present data confirmed renal injury, such as fibrosis, by Ang II infusion (Figure 3). Because HGF is a renal protective growth factor, we used transgenic mice with cardiac-specific overexpression of human HGF (HGF-Tg). These HGF-Tg mice showed a significant increase in the serum human HGF concentration of \(\sim3\)-fold as compared with control littermates.7 As expected, HGF-Tg mice exhibited a much lower degree of renal injury, as assessed by diameter of the glomerulus, and glomerular injury index (Figure 3A through 3C, \(P<0.01\), respectively). Similarly, HGF-Tg mice exhibited significantly less renal fibrosis as compared with control (Figure 3D and 3E, \(P<0.01\)). Although there was no significant difference in systolic blood pressure (SBP) between HGF-Tg and wild-type mice at baseline (SBP: 100.1 ± 2.8 versus 104.8 ± 5.8 mm Hg, respectively), SBP was significantly lower in HGF-Tg mice than in control littermates after 3 weeks of Ang II infusion (Figure S4; SBP: 104.2 ± 3.1 versus 120.1 ± 1.0 mm Hg, respectively; \(P<0.05\)). Thus, to examine whether the prevention of renal fibrosis in HGF-Tg mice could be attributed to the reduction in BP, we further examined the effects of hydrazine, an antihypertensive drug, on fibrosis. Although treatment with hydralazine significantly decreased SBP and DBP as compared with vehicle treatment (Figure S4; SBP: 120.1 ± 1.8 versus 107.3 ± 4.1 mm Hg; DBP: 79.2 ± 4.7 versus 69.7 ± 4.2 mm Hg; \(P<0.05\), respectively), there were no significant differences in the diameter of glomeruli and glomerular injury index between control and hydralazine-treated animals (Figure 3). These data indicate that reductions in blood pressure alone may not be sufficient to account for the renal protective effects of HGF. To further explore the role of HGF in renal protection, we administered a neutralizing antibody against HGF to HGF-Tg mice treated with Ang II. At baseline, there was no difference in SBP between those with or without neutralizing antibody against HGF in HGF-Tg mice (100.1 ± 2.8 versus 99.8 ± 1.8 mm Hg, respectively). As shown in Figure 3, the renal protective effect in HGF-Tg mice was significantly inhibited by neutralizing anti-HGF antibody (Figure 3A through 3C; \(P<0.01\)). Similarly, renal fibrosis was significantly inhibited in HGF-Tg mice as compared with control, whereas administration of neutralizing anti-HGF antibody attenuated the renal protective action in HGF-Tg mice (Figure 3D and 3E; \(P<0.01\)).
Interestingly, Ang II infusion into control mice significantly increased the expression of renal AT1R mRNA as determined by quantitative PCR analyses (Figure 4A; \( P < 0.05 \)). In contrast, renal AT1R mRNA induced by Ang II infusion was significantly lower in HGF-Tg mice (Figure 4A; \( P < 0.05 \)). Because our previous report demonstrated that TGF-\( \beta \) mRNA expression was significantly lower in HGF-Tg mice as compared with control,\(^5\) we hypothesized that HGF might affect PTEN expression and Akt phosphorylation in the kidney, because PTEN attenuated Akt phosphorylation. As shown in Figure 4B, Ang II infusion to control mice induced a marked decrease in the level of PTEN and an increase in the level of Akt phosphorylation. However, in HGF-Tg mice treated with Ang II, PTEN gradually increased and Akt phosphorylation decreased. The contribution of HGF was confirmed by the observation that treatment with neutralizing antibody against HGF significantly decreased the increase in PTEN expression (Figure 5A and 5B; \( P < 0.05 \)). Importantly, neutralizing anti-HGF antibody significantly increased AT1R expression induced by Ang II, whereas AT1R expression induced by HGF was significantly lower in HGF-Tg mice as compared with control (Figure 5C; \( P < 0.05 \)).

Discussion

Mesangial cells are the major cells involved in the development of CKD. Ang II regulates mesangial cell hypertrophy and increases the expression and synthesis of ECM. Indeed, it is well known that blockade of the activation of RAS by ACE inhibitors and ARBs preserved or improved renal function in numerous clinical studies. Another important player related to CKD is TGF-\( \beta \), which is mainly involved in the progression of glomerulosclerosis. Thus, researchers tried to elucidate the relation between Ang II and TGF-\( \beta \). In mesangial cells, Ang II increased TGF-\( \beta \) mRNA expression, whereas a neutralizing antibody against TGF-\( \beta \) significantly reduced ECM production induced by Ang II.\(^12\) Furthermore, previous reports showed that ARBs inhibited the expression of TGF-\( \beta \).\(^13–15\) The present study demonstrated that TGF-\( \beta \) increased the expression of AT1R, suggesting positive feedback of Ang II-TGF-\( \beta \) by which Ang II increased TGF-\( \beta \), the induced TGF-\( \beta \) expression stimulated AT1R expression, and upregulation of AT1R enhanced Ang II signaling (Figure S5). To clarify this complicated pathway, we focused on PTEN, because PTEN protein is a phosphatidylinositol phosphate phosphatase specific for the 3-position ring. Although PTEN can dephosphorylate PI3P, Pl(3,4)P2, and Pl(3,4,5)P3, it is
likely that PI(3,4,5)P3 level in the plasma membrane
decreases, which, in turn, regulates Akt kinase. Furthermore,
a change in expression and activation of PTEN is involved in
the pathophysiology of various diseases, such as pulmonary
fibrosis.16 Moreover, PTEN acts as a negative regulator of
myofibroblast, a major source of TGF-β1. The present study
clearly demonstrated that the missing link in the Ang II–
TGF-β pathway is PTEN/Akt.

Interestingly, one of the most well-known factors antago-
nizing TGF-β is HGF. Currently, HGF is reported to coun-
teract the profibrotic action of TGF-β1 by intercepting Smad
signaling through different mechanisms in various types of
kidney cells.4 For example, in human mesangial cells, HGF
signaling stabilizes the Smad transcriptional corepressor
TGF-β-induced factor homeobox 1 (TGIF) against degrada-
tion. Accumulated TGIF binds to Smads and sequesters their
ability to initiate the transcription of TGF-β1 target genes.17
In addition, our recent study demonstrated that HGF induced
apoptosis of myofibroblasts, associated with an increase in
MMPs expression and a decrease in ECM, potentially be-
cause of inhibition of TGF-β1 signaling.5,18,19 In addition to
these known mechanisms, the present study suggests a novel
pathway through the PI3K-Akt/PTEN pathway. HGF plays the
role of antiapoptotic effect, mediating the activation of Akt/
PI3K pathway.20 However, our present data showed that
pretreatment with HGF decreased TGF-β1-induced Akt
phosphorylation. In addition, some groups, other than ours
have reported that HGF has antiapoptotic effects on endo-
thelial cells.21,22 On the other hand, HGF induced apoptotic cell
death in some distinct types of cells, including transformed or
neoplastic cells23 and myofibroblasts.5,18,19 It might be a
reason that HGF biologically regulates cell survival in a cell
type–dependent manner. Furthermore, our recent study re-
ported that HGF promotes oxidative stress in cultured endo-
thelial cells but inhibits Ang II–induced excess oxidative stress
through an epidermal growth factor receptor degradation
mechanism.24 These results suggest that it might be
different from the expression levels of c-Met, because local
HGF is thought to be commonly decreased on pathological
conditions. However, further studies are necessary to reveal
the role of the c-Met/HGF system.

Here, we demonstrated that HGF inhibited the increase in
Ang II expression by TGF-β1 by inhibition of phosphoryla-
tion of Akt through upregulation of PTEN. Not only in vitro
experiments but also in vivo studies using HGF-Tg mice
demonstrated that HGF inhibited the increase in Ang II
expression by Ang II through the PI3K-Akt/PTEN pathway.
From the clinical view points, it is noteworthy that ACE
inhibitors and ARBs significantly increased local HGF ex-
pression in the heart, kidney, and other organs in various
models.25–27 Our present data demonstrated that reductions in
blood pressure alone may not be sufficient to account for the
renal protective effects of HGF. Because ARBs and ACE
inhibitors, which are known to increase local HGF expres-
sion, have pleiotropic effects beyond blood pressure control,28
the increase in local HGF expression by ARBs or ACE
inhibitors might contribute to those pleiotropic effects. In
fact, we confirmed that telmisartan, one of the ARBs that
have the characteristics of a partial agonist of peroxisome
proliferator-activated receptors, induced HGF expression in
rat mesangial cells (data not shown).

Overall, the present study revealed novel molecular mech-
anism of the inhibition of the Ang II-TGF-β-positive feed-
back system by HGF, which was related to the PI3K-Akt/
PTEN pathway. The increase in local HGF by blockade of
RAS might contribute to the clinical benefits of ARBs and
ACE inhibitors in CKD. A clinical trial of recombinant HGF
to prevent renal injury is now underway. Further development
of pharmacotherapy to stimulate HGF, together with RAS
inhibition, might improve CKD.

Perspectives
CKD is an independent risk factor for cardiovascular events
and outcomes. Conversely, cardiovascular diseases are asso-
associated independently with CKD. Recently, the concept of the cardiorenal syndrome has been spreading. Fortunately, the importance of the cardiorenal syndrome has recently been realized, and researchers are looking at both the cause and treatment.\(^{29}\) Currently, therapy for chronic renal fibrosis is quite limited, despite the introduction of ACE inhibitors and ARBs into standard medical therapy. Ang II blockade alone is not sufficient to prevent CKD. Importantly, combined therapy with HGF and ARB would result in a synergistic, marked attenuation of renal fibrosis.\(^{30}\) Furthermore, our group and others showed an antifibrotic effect of HGF on not only renal fibrosis but also cardiac fibrosis.\(^{25,31}\) Activation of the HGF system might thus be a new potential therapy to prevent the cardiorenal syndrome.

**Sources of Funding**

This work was partially supported by the Osaka Kidney Foundation, a Grant-in-Aid from the Organization for Pharmaceutical Safety and Research, a Grant-in-Aid from the Ministry of Public Health and Welfare, a Grant-in-Aid from Japan Promotion of Science, and through special coordination funds of the Ministry of Education, Culture, Sports, Science, and Technology of the Japanese Government.

**Disclosures**

None.

**References**

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Hypertension. published online June 13, 2011;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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http://hyper.ahajournals.org/content/early/2011/06/13/HYPERTENSIONAHA.111.173013

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Hepatocyte Growth Factor Attenuates TGF-β- Angiotensin II Crosstalk through Inhibition of PTEN/Akt Pathway

Kazuma Iekushi, M.D., Ph.D.1,2, Yoshiaki Taniyama, M.D., Ph.D.1,2, Hiroshi Kusunoki M.D.1,2, Junya Azuma, M.D., Ph.D.1,2, Fumihiro Sanada, M.D. Ph.D.1, Keita Okayama M.D.1, Nobutaka Koibuchi, Ph.D.3, Masaaki Iwabayashi1, Hiromi Rakugi, M.D., Ph.D.2, Ryuichi Morishita, M.D., Ph.D.1

1) Department of Clinical Gene Therapy, 2) Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, 3) Department of Advanced Clinical Science and Therapeutics, Graduate School of Medicine, the University of Tokyo,

Supplementary Materials and Methods

Materials

Human renal mesangial cells (HMC) and mesangial cell growth medium (MsBM) were purchased from Cambrex (Walkersville, MD). Antibody to anti-α-tubulin and Ang II were purchased from Sigma (St Louis, MO), and anti-phospho Akt antibodies and PTEN antibodies were purchased from Cell Signaling (Boston, MA). Antibodies to Akt1/2 (N-19) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Recombinant human TGF-β1 was purchased from R&D Systems (Minneapolis, MN). Recombinant human HGF protein was purchased from Peprotech EC (London, UK). Phosphatidylinositol 3-kinase inhibitor (LY-294002), mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2 inhibitor (PD-98059), p38 mitogen-activated protein kinase inhibitor (SB-203580), and c-Jun N-terminal kinase inhibitor (SP-600125) were purchased from Calbiochem (San Diego, CA). PTEN specific inhibitor (bpV(HO pic) was purchased from Enzo Life Sciences (Farmingdale, NY).

Evaluation of glomerular injury and expansion

Renal sections embedded in paraffin (5 μm thick) were stained with periodic acid-Schiff and examined with a light microscope. Glomerular injury scores were graded as 0: 0 to 10%; 1+: 10 to 25%; 2+: 26 to 50%; 3+: 51 to 75%; 4+: 75 to 100%. Glomerulosclerotic expansion was scored on a scale of 0 to 4 as described by Raij et al.2 These were then analyzed visually under a light microscope by two investigators.
blinded to treatment.

To evaluate of renal interstital fibrosis, kidney sections (4-5 sections, 5 μm thick, per kidney) were stained with Masson's trichrome stain and analyzed visually under a light microscope by two investigators blinded to treatment. Masson's Trichrome-stained images were subsequently quantified using NIH ImageJ software. Sirius red staining was employed. Sections of kidney 5 μm thick were placed on slides, deparaffined and incubated with a saturated solution of picric acid containing 0.1 % Sirius red. Sirius red-stained images were also subsequently quantified using NIH ImageJ software.

**PTEN promoter-luciferase assay**

The PTEN vector was kindly provided by Prof. Mak TW\(^3\). For measuring luciferase activity, human mesangial cells were grown in 12-well plates. 0.2 ug Luciferase plasmid was co-transfected with 0.01 ug pGL4 Renilla plasmid (Promega) as control for the transfection efficiency using lipofectamine 2000 (invitrogen) according to the manufacturer’s protocol. The activity of Luciferase and Renilla was assessed with the Dual-Luciferase Reporter Assay System (Promega).

**Supplementary references**


**Figure S1**

Time course of AT1R mRNA expression induced by TGF-β1.

AT1R mRNA expression was determined by quantitative PCR using b-actin as an internal control. Data represent mean ± SEM, *: P<0.05; non-treated vs. TGF-b1-treated.
Figure S2
Time course of PTEN expression induced by TGF-β1 and effect of HGF.

(A) Immunoblotting analysis of PTEN expression after treatment with TGF-β1 in vitro (1 ng/ml for 1 to 3 hr). (B) Immunoblotting analysis of PTEN expression after treatment with TGF-β1 (1 ng/ml for 1 to 3 hr) and HGF (40 ng/ml) in vitro.
PTEN reporter gene activity after treatment with TGF-β1 (1 ng/ml for 1 hr) and/or HGF (40 ng/ml) in vitro. *P<0.05; vs no treatment group,
**Figure S4**

the evaluation of Ang II-induced blood pressure after 3 weeks in vivo.

Blood pressure was measured by the tail cuff method. Hyd represent hydrazine-treated mice. *P<0.05; vs control with Ang II
Figure S5
The model for vicious cycle of Ang II and TGF-β1.

First, Ang II increased TGF-β1 expression, and then TGF-β1 induced AT1R expression. Up-regulation of AT1R enhanced Ang II signaling cascade, resulting in the acceleration of renal fibrosis. HGF antagonizes TGF-β1 induced AT1R expression through the inhibition of PTEN.