Silencing of Hypoxia-Inducible Factor-1α Gene Attenuated Angiotensin II–Induced Renal Injury in Sprague-Dawley Rats

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Abstract—Although it has been shown that upregulation of hypoxia-inducible factor (HIF)-1α is protective in acute ischemic renal injury, long-term overactivation of HIF-1α is implicated to be injurious in chronic kidney diseases. Angiotensin II (Ang II) is a well-known pathogenic factor producing chronic renal injury and has also been shown to increase HIF-1α. However, the contribution of HIF-1α to Ang II–induced renal injury has not been evidenced. The present study tested the hypothesis that HIF-1α mediates Ang II–induced renal injury in Sprague-Dawley rats. Chronic renal injury was induced by Ang II infusion (200 ng/kg per minute) for 2 weeks in uninephrectomized rats. Transfection of vectors expressing HIF-1α small hairpin RNA into the kidneys knocked down HIF-1α gene expression by 70%, blocked Ang II–induced HIF-1α activation, and significantly attenuated Ang II–induced albuminuria, which was accompanied by inhibition of Ang II–induced vascular endothelial growth factor, a known glomerular permeability factor, in glomeruli. HIF-1α small hairpin RNA also significantly improved the glomerular morphological damage induced by Ang II. Furthermore, HIF-1α small hairpin RNA blocked Ang II–induced upregulation of collagen and α-smooth muscle actin in tubulointerstitial region. There was no difference in creatinine clearance and Ang II–induced increase in blood pressure. HIF-1α small hairpin RNA had no effect on Ang II–induced reduction in renal blood flow and hypoxia in the kidneys. These data suggested that overactivation of HIF-1α–mediated gene regulation in the kidney is a pathogenic pathway mediating Ang II–induced chronic renal injuries, and normalization of overactivated HIF-1α may be used as a treatment strategy for chronic kidney damages associated with excessive Ang II. **(*Hypertension*. 2011; 58:657-664.)**

Key Words: glomerular sclerosis ■ tubulointerstitial ■ fibrosis ■ albuminuria ■ renal blood flow

Hypoxia-inducible factor (HIF)-1α is a transcription factor that has been associated recently with the progression of chronic renal injuries.[1–4] HIF-1α is upregulated in different chronic kidney diseases.[1–3,5,6] HIF-1α is also shown to stimulate collagen accumulation by activating fibrogenic factors such as plasminogen activator inhibitor and tissue inhibitor of metalloproteinase.[6–9] Therefore, although upregulation of HIF-1α has been shown to be protective in acute ischemic injury,[5,10,11] there is evidence indicating that long-term activation of HIF-1α may be injurious in chronic kidney diseases.[2–5,12–15] Angiotensin II (Ang II), a major pathogenic factor producing renal injury in different chronic kidney diseases,[1,13,14,16,17] has been shown to stimulate HIF-1α accumulation.[18,19] However, the contribution of HIF-1α to Ang II–induced renal injury has not been determined. We showed recently that silencing of the HIF-1α gene blocked the profibrotic action of angiotensin II in cultured renal cells.[20] The present study was designed to test the hypothesis that HIF-1α accumulation by Ang II is a critical mediator in Ang II–induced renal injury.

We used HIF-1α small hairpin RNA (shRNA) to silence the gene expression of HIF-1α and evaluated the contributing role of HIF-1α in Ang II–induced renal injuries in animals chronically infused with Ang II for 2 weeks. To our knowledge, the present study provides the first direct evidence that HIF-1α–mediated gene regulation contributes to Ang II–induced renal injuries.

Materials and Methods

Animal

Experiments were performed in male Sprague-Dawley rats (250 to 350 g, Harlan, Madison, WI) with free access to food and water throughout the study. All of the animal procedures were approved by

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the Institutional Animal Care and Use Committee of the Virginia Commonwealth University.

Plasmids Expressing Rat HIF-1α shRNA

Predesigned rat HIF-1α small interfering RNA (siRNA) was purchased from Sigma-Aldrich. Sequences of HIF-1α siRNA were as follows: sense, GGA AAG AGA CUC AUA GAA A; antisense, UUU CUA UGA CUC UCU UUC C. After confirmation of effective knocking down of HIF-1α genes by these siRNA in cultured rat renal medullary interstitial cells, the sequences were constructed into a pRNA-CMV3.2 vector (Genscript, Piscataway, NJ) to produce shRNA. Vectors expressing scrambled shRNA used as control were purchased from Genscript. The effective gene silencing of renal HIF-1α by shRNA in vivo was also verified in preliminary experiments.

Transfection of DNA Into the Kidney

Rats were uninephrectomized 1 week before. Plasmids (50 μg) mixed in 25% of microbubble (Optison, GE HealthCare) in saline (0.6 mL) was injected into the remaining left kidney via renal artery followed by ultrasound irritation (Sonitron 2000, Rich-Mar), as described preciously by us and others.[21–26] Three groups of animals were included: vehicle infusion+control plasmids (control), Ang II infusion+control plasmids (Ang II), and Ang II infusion+HIF-1α shRNA plasmids (Ang II+HIF-1α shRNA).

Chronic Infusion of Ang II, Monitoring of Blood Pressure, Assay of Urinary Albumin, Measurement of Plasma and Urinary Creatinine, and Harvest of Kidney

Ang II (Sigma-Aldrich, 200 ng/kg per minute) was infused for 2 weeks using ALZET mini-osmotic pumps (model 2002) implanted intraperitoneally in the surgery above. Mean arterial blood pressures were recorded daily for 3 hours using a telemetry system (Data Sciences International) as we described previously.[27] On the last day of experiment, 24-hour urines were collected using metabolic cages. Urinary albumin concentrations were measured using a rat albumin ELISA kit (Bethyl Laboratories, Montgomery, TX). After urine collection, blood samples were collected and kidneys removed. Creatinine concentrations in plasma and urine were measured by Analysis Core Laboratory. The kidneys were cut longitudinally. Half of the kidney was fixed in 10% neutral buffered formalin and the other half dissected into cortex and medulla. A small piece of fresh cortex was used for isolation of glomeruli using differential sieving as described previously,[28,29] and the rest of the tissues were frozen in liquid N_2 and stored at −80°C.

Measurement of Renal Blood Flow Using Doppler Ultrasound

Animals were treated as described above. Before the end of experiment, rats were anesthetized with ketamine (80 mg/kg, IP) and xylazine (6 mg/kg, IP), and then renal artery blood flow velocity was measured by ultrasound imaging (Vevo 770 system, VisualSonics, Toronto, Ontario, Canada)[30–32] using pulse-wave Doppler mode with a dedicated 16-MHz probe. The average velocity of blood flow during 1 minute was determined by multiplying velocity time integral by heart rate.[33] Vascular resistance index was also calculated.

Detection of Hypoxia in the Kidneys Using Pimonidazole Staining

Renal tissue hypoxia was detected using a Hypoxyprobe-1 kit (HPI, Inc, Burlington, MA) following the manufacturer's instruction. Briefly, pimonidazole hydrochloride was injected (60 mg/kg IP) 2 hours before rats were euthanized. Immunostaining was performed as we described before[34] using antibody against pimonidazole (1:200, rabbit antisera from the same kit). The percentage of positive staining area was calculated using a computer program (Image-Pro Plus) as described previously.[35]

Morphological and Immunohistochemical Analysis

The fixed kidneys were paraffin embedded and cut into 4-μm sections. For morphological analysis, the tissue sections were stained with periodic acid-Schiff staining. Glomerular damage was morphologically evaluated by 2 independent examiners, who were blinded as to animal groups, and semiquantitatively scored based on the degree of glomerular damage as described previously.[36,37] In brief, a minimum of 20 glomeruli in each specimen was examined, and the severity of lesions were graded from 0 to 4 according to the percentage of glomerular involvement. Thus, 0=normal; 1=<25% of glomerular area involved; 2=25% to 50%; 3=50% to 75%; and 4=>75% of tuft area involved. The averaged scores from counted glomeruli were used as the glomerular damage index for each animal. Immunostaining was performed as we described previously[34] using antibodies against rat vascular endothelial growth factor (VEGF, monoclonal, Millipore, 1:300) and α-smooth muscle actin (SMA; rabbit polyclonal, Abcam, 1:200). Collagen I/III was stained using picrosirius red, and the percentage of positive staining area was calculated using a computer program (Image-Pro Plus) as described previously.[35]

RNA Extraction and Quantitative RT-PCR Analysis of HIF-1α mRNA Levels in Renal Cortex and VEGF mRNA Levels in Isolated Glomeruli

Total RNA from the renal cortex and isolated glomeruli was extracted using TRIzol solution (Life Technologies, Inc, Rockville MD) and then reverse transcribed (cDNA Synthesis kit, Bio-Rad, Hercules, CA). The reverse-transcribed products were amplified using TaqMan Gene Expression Assays kits (Applied Biosystems). The level of 18S ribosomal RNA was used as an endogenous control. The relative gene expressions were calculated in accordance with the ΔΔCt method. Relative mRNA levels were expressed by the values of $2^{-\Delta\Delta Ct}$.

Preparation of Tissue Homogenate and Nuclear Extracts and Western Blot Analyses for Protein Levels of HIF-1α, Collagen I/III, and α-SMA

Renal tissue homogenates, nuclear protein preparations, and Western blot analyses were performed as described previously.[34] Primary antibodies used in the present study included anti-rat HIF-1α (monoclonal, Novus Biologicals, 1:300 dilution), collagen I/III (rabbit polyclonal, Calbiochem, 1:300), and α-SMA (rabbit polyclonal, Abcam, 1:1000). The intensities of the blots were determined using an imaging analysis program (ImageJ, free download from http://rsbweb.nih.gov/ij/).

Statistics

Data are presented as mean±SE. The significance of differences in mean values within and between multiple groups was evaluated using an ANOVA followed by a Duncan multiple range test. Student *t* test was used to evaluate statistical significance of differences between 2 groups. $P<0.05$ was considered statistically significant.

Results

Effects of HIF-1α shRNA on Ang II–Induced Activation of HIF-1α

HIF-1α mRNA levels in renal cortex were knocked down by >70% in shRNA–treated rats (Figure 1A), indicating a successful silencing of HIF-1α gene expression in the kidneys. Renal HIF-1α protein levels were significantly increased in Ang II–infused rats, which is consistent with previous reports.[19] Intrarenal transfection of HIF-1α shRNA plasmids abolished Ang II–induced increase in HIF-1α proteins in both renal cortical and medullary areas (Figure 1B and 1C), which further confirmed successful knockdown of the HIF-1α gene in the kidneys.

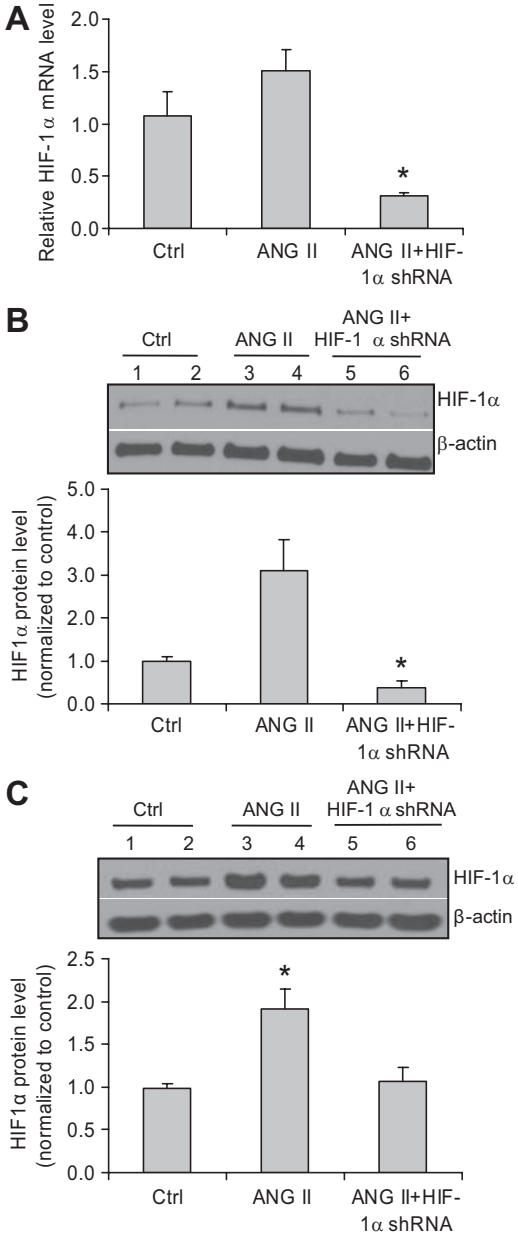

Figure 1. Effect of hypoxia-inducible factor (HIF)-1α small hairpin RNA (shRNA) on angiotensin II (Ang II)–induced activation of HIF-1α in the kidneys. **A**, HIF-1α mRNA levels in the renal cortex by real-time RT-PCR analysis. **B** (cortex) and **C** (medulla), representative enhanced chemiluminescence (ECL) gel documents of Western blot analyses depicting the protein levels of HIF-1α and summarized intensities of HIF-1α blots. *$P<0.05$ vs all other groups (n=6).

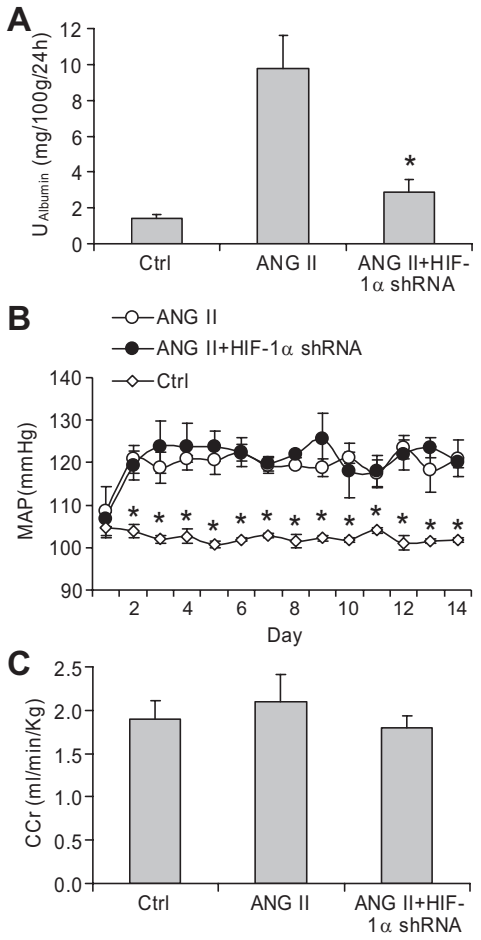

Figure 2. Effect of hypoxia-inducible factor (HIF)-1α small hairpin RNA (shRNA) on angiotensin II (Ang II)–induced increases in urinary albumin excretion and mean arterial pressure. **A**, Twenty-four–hour albumin excretion in urine. **B**, Mean arterial blood pressure (MAP). **C**, Creatinine clearance (CCr). *$P<0.05$ vs all other groups (n=6).

Effects of HIF-1α shRNA on Creatinine Clearance, Ang II–Induced Increases in Urinary Albumin Excretion, and Arterial Pressure

Ang II infusion caused considerable increases in urinary albumin levels, which were significantly attenuated in animals treated with HIF-1α shRNA (Figure 2A), suggesting that activation of HIF-1α is involved in Ang II–induced glomerular injury. There was no difference in Ang II–induced increases in mean arterial pressure between rats treated with Ang II+control plasmids and Ang II+HIF-1α shRNA plasmids (Figure 2B), indicating that the effect of HIF-1α shRNA was not through the alteration of blood pressure. There was no difference in creatinine clearances among the 3 animal groups (Figure 2C). There was also no statistical difference in animal body weight at the end of the experiment, although there was a tendency toward lower body weight in the Ang II group compared with the control group. The body weights were 332.8±21.6, 304.8±7.4, and 318.7±4.6 g in control, Ang II, and Ang II+HIF-1α shRNA groups, respectively.

Effects of HIF-1α shRNA on Ang II–Induced Histological Changes of Glomeruli

Morphological analysis showed that Ang II produced glomerular sclerotic damages as indicated by glomerular mesangial expansion with hypercellularity, capillary collapse, and fibrous deposition in glomeruli (Figure 3A). The glomerular damage index was substantially higher in Ang II–treated rats (Figure 3B). In HIF-1α shRNA-transfected rats, however, Ang II–induced glomerular damage was significantly diminished (Figure 3A and 3B). These results were consistent with the data in urinary albumin excretion and further suggested that activation of HIF-1α mediates Ang II–induced glomerular injury.

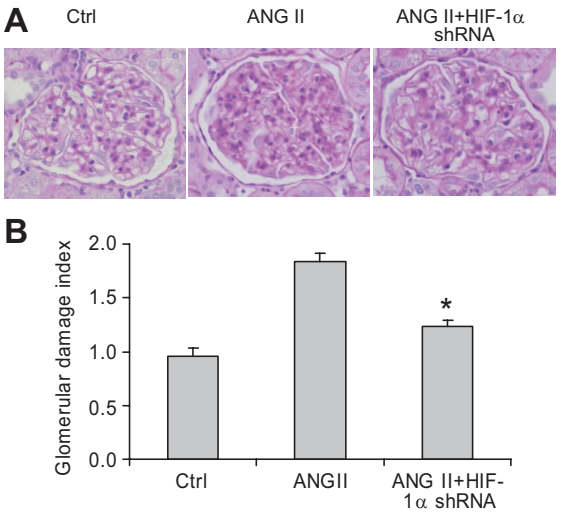

Figure 3. Effect of hypoxia-inducible factor (HIF)-1α small hairpin RNA (shRNA) on angiotensin II (Ang II)–induced morphological changes in the glomeruli. **A**, Representative photomicrographs showing glomerular structures (periodic acid-Schiff staining, ×400). **B**, Summarized glomerular damage index by semiquantitation of scores in different groups. *$P<0.05$ vs other 2 groups (n=6).

Effects of HIF-1α shRNA on Ang II–Induced Increase in VEGF in Glomeruli

Both immunostaining and real-time RT-PCR showed that Ang II significantly increased VEGF expression in the glomeruli and that HIF-1α shRNA blocked Ang II–induced increase in VEGF (Figure 4). These data indicated that HIF-1α–mediated activation of VEGF was involved in Ang II–induced glomerular injury.

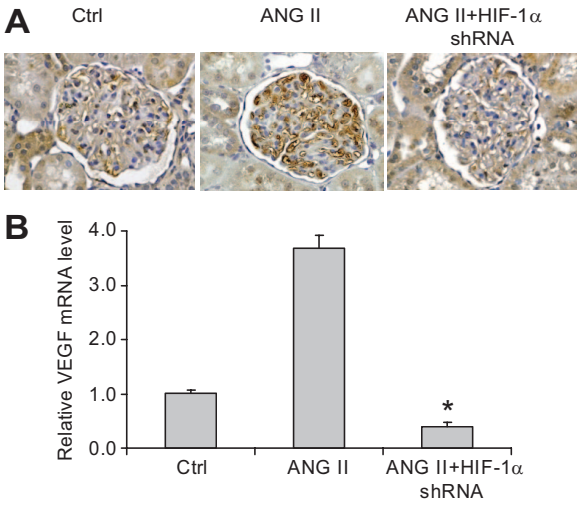

Figure 4. Effect of hypoxia-inducible factor (HIF)-1α small hairpin RNA (shRNA) on angiotensin II (Ang II)–induced increases of vascular endothelial growth factor (VEGF) in glomeruli. **A**, Representative photomicrographs showing immunostaining of VEGF in glomeruli (brown color). **B**, HIF-1α mRNA levels in isolated glomeruli by real-time RT-PCR analysis. *$P<0.05$ vs other 2 groups (n=6).

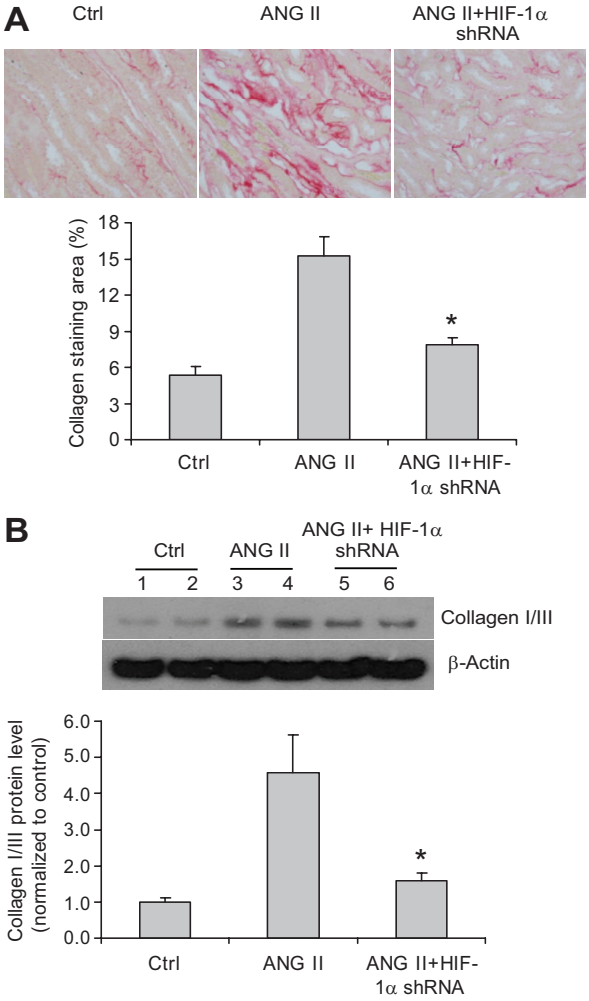

Figure 5. Effect of hypoxia-inducible factor (HIF)-1α small hairpin RNA (shRNA) on angiotensin II (Ang II)–induced increases of collagen I/III in the kidneys. **A**, Representative photomicrographs showing staining of collagens in outer medulla (red color) and calculated percentage of positively stained area. **B**, Representative enhanced chemiluminescence (ECL) gel documents of Western blot analyses depicting the protein levels of collagens in renal cortex and summarized intensities of collagen blots. *$P<0.05$ vs other 2 groups (n=6).

Effects of HIF-1α shRNA on Ang II–Induced Interstitial Injuries

The positive staining of collagens and α-SMA in the outer medulla was used as the index of interstitial injuries. Ang II infusion significantly increased the positive staining area of collagens and α-SMA (Figures 5A and 6A), which is consistent with previous reports.[38,39] In rats treated with HIF-1α shRNA, Ang II–induced increases in the positive staining of collagens were significantly attenuated (Figure 5A). Further quantitation of collagen I/III expression in the cortex by Western blot analyses also showed that Ang II increased the protein levels of collagens and that HIF-1α shRNA blocked the effect of Ang II on collagens (Figure 5B), both of which are consistent with the results of collagen staining (Figure 5A). The analysis of α-SMA expression showed the same pattern as that of collagens. Ang II increased the levels of α-SMA, and HIF-1α shRNA blocked the effect of Ang II on

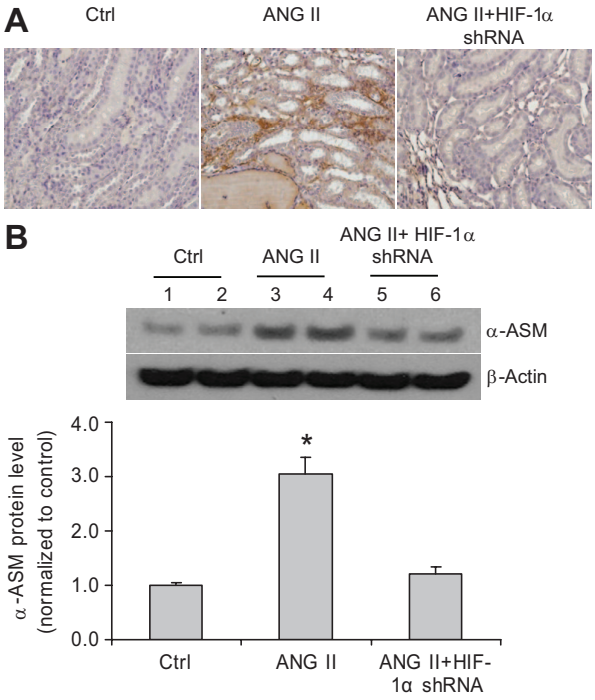

Figure 6. Effect of hypoxia-inducible factor (HIF)-1α small hairpin RNA (shRNA) on angiotensin II (Ang II)–induced increases of α-smooth muscle actin (SMA) in the kidneys. **A**, Representative photomicrographs showing staining of α-SMA in outer medulla (brown color). **B**, Representative enhanced chemiluminescence (ECL) gel documents of Western blot analyses depicting the protein levels of α-SMA in renal cortex and summarized intensities of α-SMA blots. *$P<0.05$ vs other 2 groups (n=6).

α-SMA, as shown by immunostaining and immunoblotting analyses of α-SMA (Figure 6). These data demonstrated that HIF-1α activation participated in the fibrotic effect of Ang II in the renal tubulointerstitial area.

Effects of HIF-1α shRNA on Ang II–Induced Ischemia/Hypoxia in the Kidneys

Ang II infusion significantly increased renal vascular resistance and reduced renal blood flow compared with control animals (Figure 7). There was no significant difference in renal vascular resistance and blood flow between animals treated with Ang II+HIF-1α shRNA and Ang II+control plasmids. Similarly, the areas of positive staining of hypoxia probe were largely increased in rats treated with Ang II as compared with control rats, which exhibited weak and less positive staining, whereas HIF-1α shRNA did not affect Ang II–induced changes in the staining of hypoxia probe (Figure 8). These data showed that Ang II caused ischemia/hypoxia in the kidneys, which was not influenced by HIF-1α shRNA.

Discussion

The present study showed that chronic infusion of Ang II increased HIF-1α levels in the kidneys, and gene silencing of HIF-1α in the kidneys significantly attenuated Ang II–induced elevation of urinary albumin excretion, glomerular morphological changes, increase of glomerular VEGF expression, upregulation of α-SMA, and collagen accumulation.

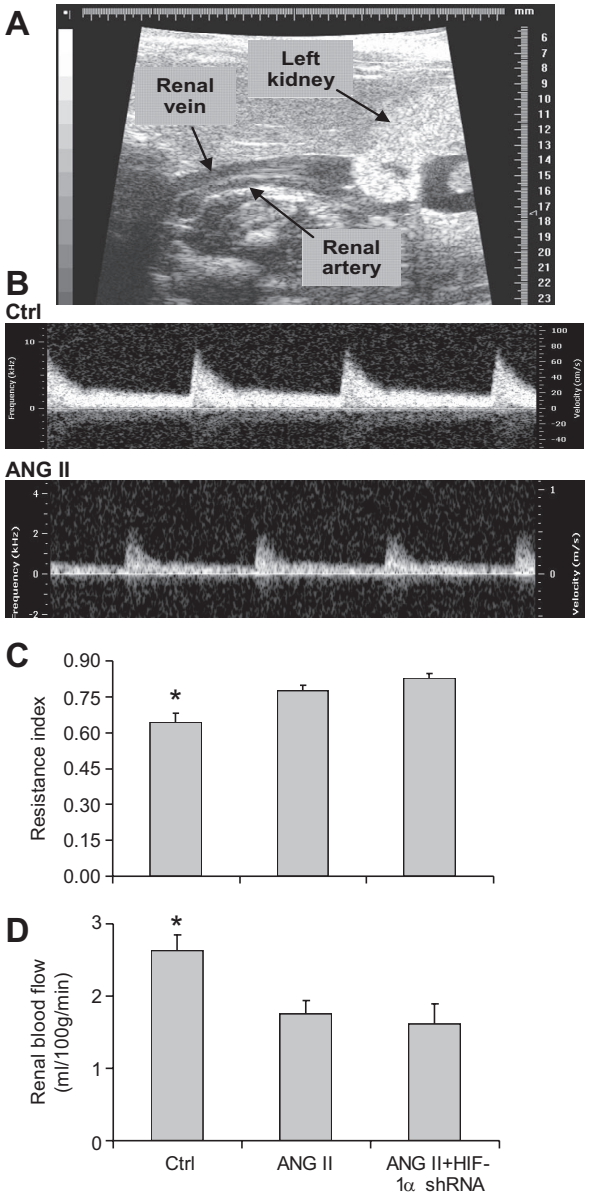

Figure 7. Effect of hypoxia-inducible factor (HIF)-1α small hairpin RNA (shRNA) on angiotensin II (Ang II)–induced decreases in renal blood flow. **A**, Representative image showing renal artery. **B**, Representative images showing renal blood flow velocity Doppler waveform. **C**, Calculated renal vascular resistance index (RI). RI=(Vs−Vd)/Vs, Vs=peak systolic velocity, Vd=end-diastolic velocity. **D**, Summarized renal blood flow velocity. Velocity time integral (VTI) is the area under the curve of Doppler waveform. Multiplying the VTI by cross-sectional area of renal artery ($\pi \times r^2$) and heart rate (HR) provides an estimate of the average velocity of blood flow (in milliliters) during 1 minute. *$P<0.05$ vs other 2 groups (n=4 to 6).

It is suggested that overactivation of HIF-1α in the kidney is a crucial mediator in Ang II–induced chronic renal injury.

Ang II is an important pathogenic factor involved in many different chronic kidney diseases.[1,13,14,16,17] The mechanisms by which Ang II produces renal injuries are not fully understood. Inhibition of urinary albumin excretion and glomerulosclerosis by HIF-1α shRNA in the present study demonstrated that HIF-1α–mediated gene activation participated in Ang II–induced glomerular damage. The effects of

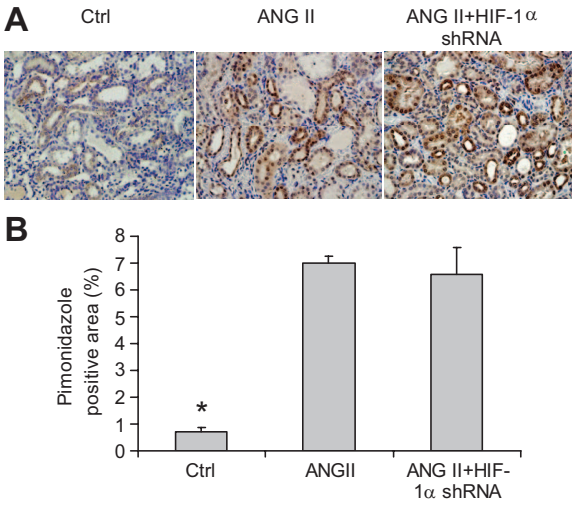

Figure 8. Effect of hypoxia-inducible factor (HIF)-1α small hairpin RNA (shRNA) on angiotensin II (Ang II)–induced hypoxia in the kidneys. **A**, Representative photomicrographs showing the staining of hypoxia probe pimonidazole in outer medulla (brown color). **B**, Calculated percentage of positively stained area. *$P<0.05$ vs other 2 groups (n=5 to 6).

HIF-1α shRNA on HIF-1α levels were different in cortex and medulla. These differences may be attributable to regional differences in vascular architecture/distribution between the renal cortex and medulla, which bring 90% of the blood supply to the renal cortex and 10% to the renal medulla.[40,41] As a result, tissue blood perfusion in the medulla is ≈30% of that in the cortex.[41] Therefore, the injected DNA via renal artery would be delivered into the cortex more than the medulla and consequently knocked down HIF-1α more in the cortex than in the medulla.

Many target genes of HIF-1α have been implicated in chronic renal injury, such as plasminogen activator inhibitor and tissue inhibitor of metalloproteinase.[6–9] VEGF is one of the HIF-1α target genes and Ang II–stimulated podocyte-derived VEGF, as a glomerular permeability factor, has been suggested to be a major cause for the development of proteinuria in diabetic nephropathy.[42–46] We, therefore, detected whether HIF-1α–mediated alteration of VEGF was involved in Ang II–induced glomerular damages. Our results showed that improvement of Ang II–induced glomerular damages was associated with inhibition of VEGF by HIF-1α shRNA, further suggesting that HIF-1α–mediated gene activation participates in Ang II–induced glomerular damage.

In addition, Ang II–induced renal tubulointerstitial damage contributes to the progression of chronic renal injury.[12,47–50] The present study also demonstrated that Ang II–induced tubulointerstitial damage was mediated by HIF-1α, as indicated by the inhibition of collagen I/III and α-SMA accumulation in HIF-1α shRNA–treated animals. An interesting finding in the present study was that HIF-1α shRNA blocked Ang II–induced α-SMA. α-SMA is a well-known marker of epithelial-mesenchymal transition (EMT). Ang II–induced EMT has been indicated as an important mechanism for the progression of chronic kidney diseases.[12,51] Although both HIF-1α[3,9,52] and Ang II[49,53] have been shown to promote EMT, the interaction between HIF-1α and Ang II in the process of EMT remains unclear. We showed recently that HIF-1α mediated Ang II–induced cell transdifferentiation in cultured renal cells.[20] The present in vivo data further suggest that HIF-1α may participate in Ang II–induced EMT in the kidneys. Overall, results from the present study suggest that HIF-1α–mediated gene activation may represent a novel mechanistic pathway in Ang II–induced renal injury.

It has been demonstrated that hypertensive renal injury is largely dependent on renal perfusion pressure.[38,54,55] The present study showed that HIF-1α shRNA remarkably attenuated renal injury without altering Ang II–induced hypertension, indicating that HIF-1α may also be involved in kidney damage caused by increased renal perfusion pressure. Whether increased renal perfusion pressure stimulates HIF-1α and its detailed role in renal injury need to be clarified in future investigations.

Because ischemia/hypoxia is involved in Ang II–induced renal injury,[56,57] we then assessed whether possible changes in the status of hypoxia/oxygenation contributed to the beneficial effects of HIF-1α shRNA. Our results showed that the attenuation of renal injury by HIF-1α shRNA was not through the improvement in renal blood perfusion/oxygenation. Therefore, the beneficial effect of HIF-1α shRNA would probably be through inhibition of HIF-1α–mediated gene activations associated with renal injuries.

The present study demonstrated that accumulation of HIF-1α by Ang II is a critical mediator in Ang II–induced kidney damage. How Ang II activates HIF-1α requires further clarification. In this regard, a direct stimulating effect by Ang II and Ang II–induced ischemia/hypoxia may have contributed to HIF-1α activation in the present study. Consistent with our results, previous reports have also demonstrated that Ang II reduces renal blood perfusion[56,57] and produces hypoxia in the kidneys.[56] Meanwhile, Ang II has been shown to stimulate HIF-1α under normoxia as well.[18–20] Therefore, activation of HIF-1α may be through both direct effect and ischemic effect of Ang II. One possible pathway mediating Ang II–induced HIF-1α activation would be HIF-prolyl hydroxylases, the enzymes that promote the degradation of HIF-1α.[58,59] The activity of HIF-prolyl hydroxylases is inhibited by low oxygen tension[58,59] and is also regulated by mechanisms independent of oxygen levels,[60–63] such as redox signals.[20,60,61] These oxygen-dependent and -independent mechanisms may be involved in Ang II–induced HIF-1α activation in the kidneys. Ang II may inhibit HIF-prolyl hydroxylase to consequently activate HIF-1α, which needs to be elucidated in future.

It should be noted that there are controversial reports regarding the role of HIF-1α in chronic renal injury. It was reported previously that genetic ablation of renal epithelial HIF-1α inhibited the development of renal tubulointerstitial fibrosis in unilateral ureteral obstruction rats.[9] Conversely, it was shown that induction of HIF-1α by $CoCl_2$ ameliorated the renal injuries in progressive Thy1 nephritis rat model[64] and in an obese, hypertensive type 2 diabetes mellitus rat model.[65] More puzzling evidence on this topic came from reports that used the same animal model and obtained conflicting results regarding the role of HIF-1α in chronic renal injury. One study demonstrated that stable expression of

HIF-1α in tubular epithelial cells promoted interstitial fibrosis in 5/6 nephrectomy mice,[15] whereas 2 other reports showed that upregulation of HIF-1α by either $CoCl_2$ or dimethyloxalylglycine protected tubulointerstitium in 5/6 nephrectomy rats.[66,67] These discrepancies might be attributed to the differences in disease models, disease stages, and approaches to manipulate HIF-1α.

By comparing the above controversial studies, those that used genetic approaches to manipulate HIF-1α levels, including upregulation and downregulation, showed that HIF-1α was an injurious mediator, whereas all of the studies using pharmacological approaches to increase HIF-1α levels showed that HIF-1α was a protective factor. The difference between gene manipulation and pharmacological intervention may be the specificity of targeting HIF-1α. Nonspecific actions in addition to stimulating HIF-1α by pharmacological interventions cannot be totally ruled out. In this regard, it has been shown that the reagents used to upregulate HIF-1α levels execute some other actions independent of HIF-1α induction.[68,69] Commonly used hypoxia mimetic agents, such as cobalt, nickel, and desferrioxamine, interfere with iron metabolism and may interrupt many other iron-dependent enzymes.[68,69] Dimethyloxalylglycine is an analog of 2-oxoglutarate and may act as an inhibitor of many other oxoglutarate-dependent enzymes in addition to HIF prolyl-hydroxylases.[69,70] Therefore, the protective role of HIF-1α in those studies using pharmacological reagents may not be firmly concluded until it could be shown that blockade of HIF-1α upregulation eliminates the renal protective effect by those pharmacological reagents. Apparently, more detailed investigations are required regarding the role of the HIF-1α pathway in chronic kidney diseases under different situations. There may be possibilities that activation of HIF-1α is injurious under certain conditions and protective in some other situations, which needs to be clarified in future studies. Nonetheless, our results provided strong evidence that long-term overactivation of HIF-1α mediated Ang II–induced renal damages in the model used in the present study.

Perspectives

The present study demonstrated that blockade of HIF-1α accumulation attenuated Ang II–induced renal injury. It is suggested that overactivation of HIF-1α–mediated gene regulation in the kidney may constitute a new pathogenic pathway mediating renal injury under various pathological conditions associated with excessive Ang II and that normalization of overactivated HIF-1α may be a useful strategy in the treatment of chronic renal injury with elevated levels of Ang II.

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Disclosures

None.

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