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Role of the Transcription Factor Erythroblastosis Virus E26 Oncogen Homolog-1 (ETS-1) as Mediator of the Renal Proinflammatory and Profibrotic Effects of Angiotensin II

Wenguang Feng, **Phillip Chumley**, **Ping Hua**, **Gabriel Rezonzew**, **David Jaimes**, **Madison W. Duckworth**, **Dongqi Xing**, and **Edgar A. Jaimes**
Divisions of Nephrology (W.F., P.C., P.H., G.R., D.J., M.W.D., E.A.J) and Cardiovascular Diseases (D.X.), University of Alabama at Birmingham, Veterans Affairs Medical Center, Birmingham, AL

Abstract

Angiotensin II (Ang II) plays a major role in the pathogenesis of end-organ injury in hypertension via its diverse hemodynamic and nonhemodynamic effects. Erythroblastosis virus E26 oncogen homolog-1 (ETS-1) is an important transcription factor recently recognized as an important mediator of cell proliferation, inflammation, and fibrosis. In the present studies, we tested the hypothesis that ETS-1 is a common mediator of the renal proinflammatory and profibrotic effects of Ang II. C57BL6 mice (n=6 per group) were infused with vehicle (control), Ang II (1.4 mg/kg per day), Ang II and an ETS-1 dominant-negative peptide (10 mg/kg per day), or Ang II and an ETS-1 mutant peptide (10 mg/kg per day) via osmotic minipump for 2 or 4 weeks. The infusion of Ang II resulted in significant increases in blood pressure and left ventricular hypertrophy, which were not modified by ETS-1 blockade. The administration of ETS-1 dominant-negative peptide significantly attenuated Ang II-induced renal injury as assessed by urinary protein excretion, mesangial matrix expansion, and cell proliferation. Furthermore, ETS-1 dominant-negative peptide but not ETS-1 mutant peptide significantly reduced Ang II-mediated upregulation of transforming growth factor-β, connective tissue growth factor, and α-smooth muscle actin. In addition, ETS-1 blockade reduced several proinflammatory effects of Ang II, including macrophage infiltration, nitrotyrosine expression, and NOX4 mRNA expression. Our studies suggest that ETS-1 is a common mediator of the proinflammatory and profibrotic effects of Ang II-induced hypertensive renal damage and may result in the development of novel strategies in the treatment and prevention of end-organ injury in hypertension.

Correspondence to Dr Edgar A. Jaimes, Division of Nephrology, University of Alabama at Birmingham, Ziegler Research Building 637, 1530 3rd Ave South, Birmingham, AL 35294, ejaimes@uab.edu.

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Keywords

angiotensin II; hypertension (kidney); ETS-1; extracellular matrix; physiology/pathophysiology; growth factors and cytokines; oxidative stress (kidney)

Maladaptive activation of the renin-angiotensin system plays a critical role in the pathogenesis of glomerulosclerosis and chronic kidney disease of different causes, including hypertension[1] and diabetes mellitus.[2] Angiotensin II (Ang II) is a potent systemic vasoconstrictor and modulator of renal microcirculation.[3] In addition to these hemodynamic actions, Ang II activates downstream signaling cascades that trigger the increased production of several growth factors,[4,5] cytokines,[6] chemokines,[7] and other mediators that stimulate mesangial cell hypertrophy and proliferation,[8] extracellular matrix deposition,[9] and inflammation.[10,11] It is not clear, however, whether these effects of Ang II are mediated via the independent activation of multiple pathways or whether alternatively common mediators induce these diverse profibrotic and proinflammatory pathways in response to Ang II.

The ETS factors are a family of transcription factors that participate in the regulation of a wide variety of biological processes, including normal development and differentiation.[12] Erythroblastosis virus E26 oncogen homolog-1 (ETS-1) is a member of the ETS transcription factor family involved in the expression of a variety of genes, including growth factors, chemokines, and adhesion molecules.[13] ETS-1 is also a well-known proto-oncogene in the pathogenesis of several different types of cancer.[14,15] Several studies have also supported the role of ETS-1 as a mediator of vascular inflammation,[16] recruitment of inflammatory cells to the vessel wall, and proliferation and migration of vascular smooth muscle cells.[17] In recent studies, we demonstrated that ETS-1 mediates the expression of proinflammatory cytokines and adhesion molecules that participate in the formation of neointima after balloon injury.[18] In previous studies, we also demonstrated that ETS-1 in large part mediates the production of fibronectin in response to Ang II in cultured mesangial cells.[19]

Herein, we performed a series of studies to determine the role of ETS-1 in the proinflammatory and profibrotic effects of Ang II in the kidney in vivo, including cell proliferation, macrophage infiltration, mesangial expansion, oxidative stress, and fibrosis. We took advantage of the availability of an ETS-1 dominant-negative (ETS-1 DN) peptide, which competes with ETS-1 for binding to the target genes.[20]

Methods

Animals and Treatments

Eight-week-old male C57BL6 mice (Jackson Labs, Bar Harbor, ME) were divided into the following groups (n=6 per group): group 1, control mice infused with vehicle; group 2, mice treated with Ang II (1.4 mg/kg per day) for 28 days via osmotic minipump; group 3, mice treated with Ang II and ETS-1 DN (10 mg/kg per day) for 28 days via osmotic minipump; and group 4, mice treated with Ang II and ETS-1 mutant peptide (ETS-1 MU, 10 mg/kg per day) for 28 days via osmotic minipump. Additional groups of mice were also studied after 2

weeks of infusion of Ang II only or Ang II plus the ETS-1 DN or ETS-1 MU peptide. Details on the peptide sequences and synthesis are available in the online-only Data Supplement. A urine sample was collected before euthanasia and saved for protein and creatinine measurements. The mice were euthanized by exsanguinations and kidneys and hearts collected for subsequent analysis. The animal protocols were approved by the institutional animal care and use committee at the University of Alabama at Birmingham and were consistent with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Blood Pressure Measurements

We used a radio telemetry system (DSI, St Paul, MN) to monitor blood pressure in conscious mice. Blood pressure was measured at baseline and then weekly for 6 hours continuously until euthanasia. See the online-only Data Supplement for expanded methods.

Real-Time Quantitative Reverse Transcriptase Polymerase Chain Reaction Analysis of mRNA Expression

Total RNA was extracted from renal cortices with TRIzol (Invitrogen, Carlsbad, CA), treated with DNAase I and then purified with an RNA purification kit (Invitrogen). The protein- and DNA-free RNA was reverse-transcribed to cDNA (Invitrogen), amplified by PCR with specific primers (Table S1 in the online-only Data Supplement), and quantified using SYBR Green and a 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA), as previously described.[21] Levels of specific mRNAs were normalized using GAPDH as an internal control.

Immunofluroscence

Five-micrometer sections of kidney cortex were prepared from paraffin-embedded tissues. Sections were incubated with a rabbit antibody to ETS-1 (sc-350, Santa Cruz Biotechnology) or a rabbit antibody to nitrotyrosine (06–284, Upstate) at 4°C overnight. See the online-only Data Supplement for expanded methods.

Immunohistochemistry

The avidin-biotin-peroxidase immunohistochemical technique (ABC kit; Vector) was used to detect microphage infiltration, cell proliferation, and α-smooth muscle actin expression, using primary antibodies against macrophages (F4/80, Ab6640; Abcam), Ki67 (m7249; Dako), and α-smooth muscle actin (A2547; Sigma). See the online-only Data Supplement for expanded methods.

Morphometric Analysis

Light microscopy of periodic acid Schiff–stained kidney sections from the 4 experimental groups was used for morphometric analysis. See the online-only Data Supplement for expanded methods.

Western Blot

Western blot analysis was performed as described before.[22] The blots were incubated with the primary antibodies against ETS-1 (sc-350; Santa Cruz Biotechnology), transforming growth factor-β (TGF-β, MAB1835; R&D), or connective tissue growth factor (CTGF, sc-14940; Santa Cruz Biotechnology) at 4°C for 24 or 48 hours. The blots were washed and incubated with secondary antibodies and the signal detected by luminol chemiluminescence. See the online-only Data Supplement for expanded methods.

Statistical Analysis

Results are expressed as mean±SEM. The data were evaluated by 1-way or 2-way ANOVA. When the overall *F* test result of ANOVA was significant, a multiple-comparison Dunnett test was applied. Student *t* test was used in 2-mean comparisons. Differences were reported as significant when P was <0.05.

Results

Blood Pressure and Left Ventricle

Blood pressure as measured by radio telemetry was increased by Ang II and not modified by the administration of either ETS-DN (Figure S1 in the online-only Data Supplement) or ETS-1 MU, an inactive peptide used as control. The administration of Ang II resulted in significant increases in left ventricular weight, which were not significantly changed by the administration of either ETS-1 DN or ETS-1 MU peptide (Table S2 in the online-only Data Supplement).

Ang II Increases Cortical ETS-1 Expression

To determine whether ETS-1 expression is increased in the renal cortex of mice infused with Ang II, we measured ETS-1 mRNA expression by real-time quantitative reverse transcriptase PCR and protein expression by Western blot. As shown in Figure 1, 12 weeks of Ang II infusion resulted in a 3-fold increase in ETS-1 mRNA expression and a 4-fold increase in ETS-1 protein expression compared with control mice. By immunofluorescence we determined that the increase in cortical ETS-1 expression was mostly glomerular (Figure 1). After 4 weeks of Ang II, the levels of ETS-1 mRNA and protein returned to baseline (Figure 1). The administration of either ETS-DN or ETS-1 MU peptide had no effect on ETS-1 expression.

ETS-1 Blockade Reduces Proteinuria and Matrix Expansion in Mice Infused With Ang II

As previously shown by others,[23] the infusion of Ang II resulted in modest albeit significant increases in urinary protein excretion, which were reduced by ETS-1 DN but not by ETS-1 MU (Figure S2). As also previously shown by others,[23] the administration of Ang II for 4 weeks resulted in significant increases in glomerular matrix expansion as assessed by morphometric analysis of periodic acid Schiff–stained kidney sections, which was significantly reduced by the administration of ETS-1 DN but not ETS-1 MU (Figure 2). By immunohistochemistry, we determined that the cortical staining of α-smooth muscle actin, a

well-validated marker of renal fibrosis,[24] was significantly increased by the infusion of Ang II and reduced by the administration of ETS-1 DN but not ETS-1 MU (Figure 3).

ETS-1 Blockade Inhibited Profibrotic Gene Expression in Mice Infused With Ang II

To determine whether these changes were linked to changes in known mediators of mesangial matrix expansion, we measured the expression of TGF-β and CTGF,[25] 2 profibrotic cytokines with promoter sequences that indicate the existence of several ETS-1 binding sites. As shown in Figures 4 and 5, the administration of Ang II resulted in a significant increase in the mRNA expression of TGF-β and CTGF after 2 and 4 weeks of Ang II, which were significantly reduced by the administration of ETS-1 DN but not ETS-1 MU. These changes in mRNA expression were accompanied by similar directional changes in the protein expression of TGF-β and CTGF after 4 weeks of Ang II as assessed by Western blot (Figures 4 and 5).

ETS-1 Mediates Proinflammatory Effects of Ang II

To evaluate the role of ETS-1 on the proinflammatory effects of Ang II, we measured macrophage infiltration as assessed by the number of F4/80-positive cells in kidney sections. In the control group, there were scattered low numbers of F4/80-positive cells, which increased significantly after 2 and 4 weeks of infusion with Ang II. The number of F4/80-positive cells was higher at 2 weeks compared with 4 weeks of Ang II infusion and was reduced by treatment with ETS-1 DN peptide but not by ETS-1 MU peptide. F4/80-positive cells were present in the tubulointerstitium, as well as in glomerular spaces (Figure 6).

Cell Proliferation

The number of Ki-67 cells, a marker of cell proliferation, was assessed by immunohistochemistry. The infusion of Ang II for either 2 or 4 weeks resulted in significant increases in cell proliferation (Figure 7), which were ameliorated by the administration of ETS-1 DN peptide but not ETS-1 MU peptide. Ki-67–positive cells were found in the glomerular and tubulointerstitial areas.

Nitrotyrosine Formation

Nitrotyrosine, a marker of oxidative stress, was detected by immunofluorescence. Kidneys from mice infused with Ang II for 4 weeks had increased nitrotyrosine immunofluorescence, both interstitial and glomerular (Figure 8). Treatment with ETS-1 DN but not with ETS-1 MU resulted in significant reductions in the intensity of immunofluorescence. These changes in nitrotyrosine formation were accompanied by concomitant increases in NOX4 mRNA expression, which were reduced by treatment with ETS-1 DN but not ETS-1 MU peptide after 2 and 4 weeks of Ang II (Figure 8). The infusion of Ang II for 2 weeks resulted in significant increases in NOX2 mRNA that were not modified by ETS-1 DN: vehicle, 1.05 ± 0.21; Ang II, 1.86 ± 0.23; Ang II+ETS-DN, 2.24 ± 0.47 (mRNA relative expression, n=6; $P<0.05$ versus vehicle). After 4 weeks of Ang II, the mRNA levels of NOX2 were no different from the control: vehicle 1.1 ± 0.14 versus Ang II 1.17 ± 0.2 (mRNA relative expression, n=6; *P* value was not significant). The mRNA expression for NOX1 was

detected only after 35 cycles of PCR amplification in both vehicle and Ang II–infused mice, indicating low expression of this NOX isoform.

Discussion

ETS-1 is a member of the ETS family of transcription factors that share a highly conserved DNA-binding domain (ETS domain).[26] The ETS originates from the sequence described in the E26 avian erythroblastosis virus (E26 transformation-specific sequence). ETS-1 is involved in the regulation of normal development and differentiation[12,27] and as a proto-oncogene[28] is implicated in the pathogenesis of different types of cancer.[14] Several studies have shown that ETS-1 is required for the normal development of the mammalian kidney and for the maintenance of glomerular integrity.[29,30] Indeed, ETS-1 knockout animals have fewer glomeruli, and among the existing glomeruli, a higher percentage are immature.[31] The renal expression of ETS-1 is increased in several models of acute kidney injury, including ischemia-reperfusion and cisplatin toxicity,[32] and in models of glomerular injury, including the anti-Thy model of glomerulonephritis[33] and antiglomerular basement-induced glomerulonephritis.[34] In previous studies, we also demonstrated that Ang II increases the cortical expression of ETS-1 in rats and that knockdown of ETS-1 reduces Ang II–stimulated fibronectin production in rat mesangial cells.[19] In the current studies, we observed that the expressions of ETS-1 mRNA and protein were increased after 2 weeks but returned to baseline at week 4. Similarly studies by others have demonstrated that some of the proinflammatory effects of Ang II are more pronounced during the first 2 weeks of infusion with Ang II and then reduced at later time points.[23] We hypothesize that ETS-1 functions as an initiator of proinflammatory and profibrotic pathways, which at the same time trigger feedback mechanisms that modulate these responses.

In our studies, ETS-1 blockade in mice infused with Ang II did not modify blood pressure or left ventricular hypertrophy, but the severity of kidney damage was significantly reduced compared with Ang II alone, suggesting that these effects were independent of the hemodynamic effects of Ang II. As we and others have demonstrated, Ang II has important proinflammatory effects by promoting the expression of several proinflammatory mediators, including monocyte chemotactic protein 1, tumor necrosis factor-α, interleukin 1, reactive oxygen species (ROS), and cyclooxygenase 2 among others.[22,35,36] In addition, Ang II has profibrotic effects that are mediated via increases in the expression of growth factors such as TGF-β[37] and CTGF.[38] In agreement with previous reports by others, the infusion of pressor doses of Ang II for 4 weeks resulted in significant increases in proteinuria, mesangial expansion,[23] and α-smooth muscle actin expression,[24] an important marker of renal fibrosis. These renal effects of Ang II were significantly reduced by the administration of an ETS-1 DN peptide but not by an ETS-1 MU peptide, suggesting that ETS-1 regulates and initiates the activation of pathways involved in the development of fibrosis in response to Ang II. TGF-β and CTGF are 2 important growth factors that mediate the profibrotic effects of Ang II.[25,38,39] Analysis of the TGF-β and CTGF promoter sequences reveals the existence of several ETS-1 binding sites. Based on our results, we hypothesize that ETS-1 may be directly regulating the expression of these growth factors.

The infusion of Ang II resulted in significant increases in the number of macrophages in the renal cortex, which were also reduced by ETS-1 blockade. Most of the macrophage infiltration was found in the tubulointerstitial space and to a lesser degree in the glomeruli. In the kidney, Ang II induces inflammation by promoting the production of ROS and proinflammatory cytokines and chemokines, leading to increased chemotaxis and macrophage infiltration.[35,36] Macrophages subsequently secrete a variety of mediators that participate in the pathogenesis of renal damage in response to Ang II.[23] Our results indicate that ETS-1 is an important mediator of the proinflammatory effects of Ang II in the kidney cortex, likely by regulating the expression of chemokines and cytokines involved in macrophage infiltration.[13,18]

The administration of Ang II also resulted in significant increases in renal cell proliferation, which was predominantly tubulointerstitial and significantly reduced by ETS-1 blockade. As we and others have shown, ROS are important mediators of cell proliferation in response to Ang II.[40,41] In our studies, we observed significant increases in nitrotyrosine expression, a well-validated marker of oxidative stress.[42] The expression of nitrotyrosine was accompanied by concomitant increases in the mRNA expression of NOX4, the most abundant NOX isoform and source of ROS in response to Ang II in the kidney.[43] Blockade of ETS-1 resulted in significant reductions in nitrotyrosine and NOX4 mRNA expression, suggesting that ETS-1 may be directly regulating NOX4 expression or alternatively regulating the expression of proinflammatory mediators that stimulate the production of ROS in response to Ang II. In contrast, although the administration of Ang II increased NOX2 mRNA expression, treatment with ETS-DN did not modify its expression, suggesting that ETS-1 does not regulate this NOX isoform.

Perspectives

In these studies, we have unveiled the role of the transcription factor ETS-1 as a common mediator in the simultaneous activation of profibrotic and proinflammatory pathways in the presence of increased activation of the renin-angiotensin system. Given its a role as a common mediator of multiple pathways, we postulate that ETS-1 may be a target for novel strategies in the prevention and treatment of end-organ injury in hypertension, as well as other conditions characterized by increased activation of profibrotic and proinflammatory pathways, including chronic kidney disease of different causes, glomerulonephritis, acute kidney injury, and diabetic nephropathy, among others.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Significance

What Is New?

- These studies establish the role of the transcription factor ETS-1 as a mediator of the proinflammatory and profibrotic effects of angiotensin II.

What Is Relevant?

- In these studies, we have identified ETS-1 as a potential new target for the treatment and prevention of renal injury in hypertension.

Summary

In conclusion, we have characterized the transcription factor ETS-1 as a mediator of several effects of angiotensin II involved in the pathogenesis of renal injury in hypertension. These studies may result in the development of novel strategies in the treatment and prevention of end-organ injury in hypertension.

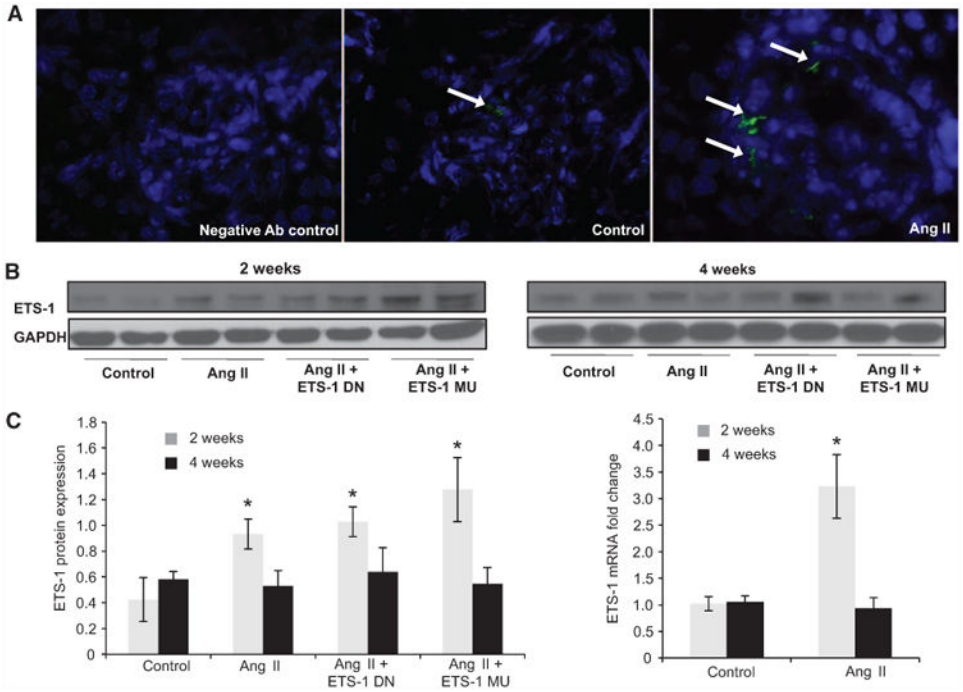

Figure 1.

Angiotensin II (Ang I)I increases cortical ETS-1 expression. **A**, Representative confocal photomicrographs showing low basal expression of ETS-1 (**green**) in control kidney cortex, which is predominantly glomerular and increased by Ang II. **B**, The renal cortical ETS-1 protein expression increases after 2 weeks of Ang II as assessed by Western blot (n=6, **P*<0.05 vs control) and returns to baseline after 4 weeks of Ang II. The expression of ETS-1 was not significantly modified by treatment with either ETS-1 dominant-negative (ETS-1 DN) or ETS-1 mutant (ETS-1 MU) peptide. **C**, Densitometric analysis for ETS-1 showing significant increases in ETS-1 protein expression after 2 but not after 4 weeks of Ang II. Neither ETS-1 DN nor ETS-1 MU modified ETS-1 protein expression. Data expressed as mean±SEM are normalized to GAPDH (**P*<0.05 vs control; #*P*<0.05 vs Ang II group; n=6). **D**, The infusion of Ang II for 2 weeks resulted in increases in cortical ETS-1 mRNA expression as assessed by real-time reverse transcriptase polymerase chain reaction (n=6; **P*<0.05 vs control) and returns to baseline levels after 4 weeks of Ang II.

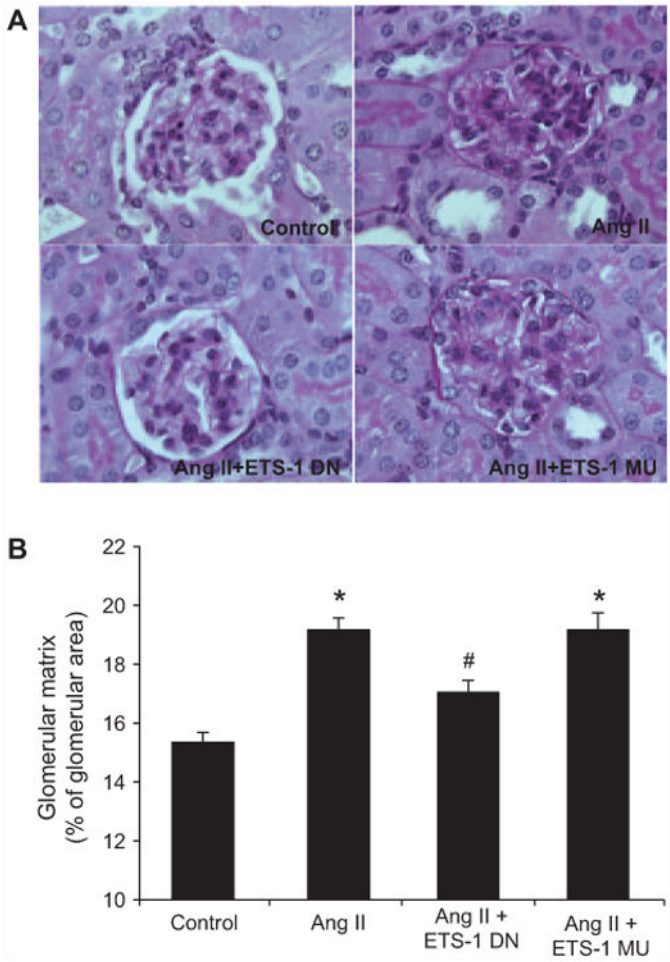

Figure 2.

ETS-1 blockade reduces matrix expansion in angiotensin II (Ang II)–infused mice. **A**, Representative photomicrographs of sections stained with periodic acid Schiff to assess extracellular matrix (**pink stain**). **B**, Quantitative analysis of glomerular matrix expansion from mice infused with Ang II for 4 weeks with and without concomitant infusion of ETS-1 dominant-negative (ETS-1 DN) or ETS-1 mutant (ETS-1-MU) peptide. ETS-1 DN inhibited the Ang II–induced mesangial expansion (n=6 per group; **P*<0.05 vs control; #*P*<0.05 vs Ang II).

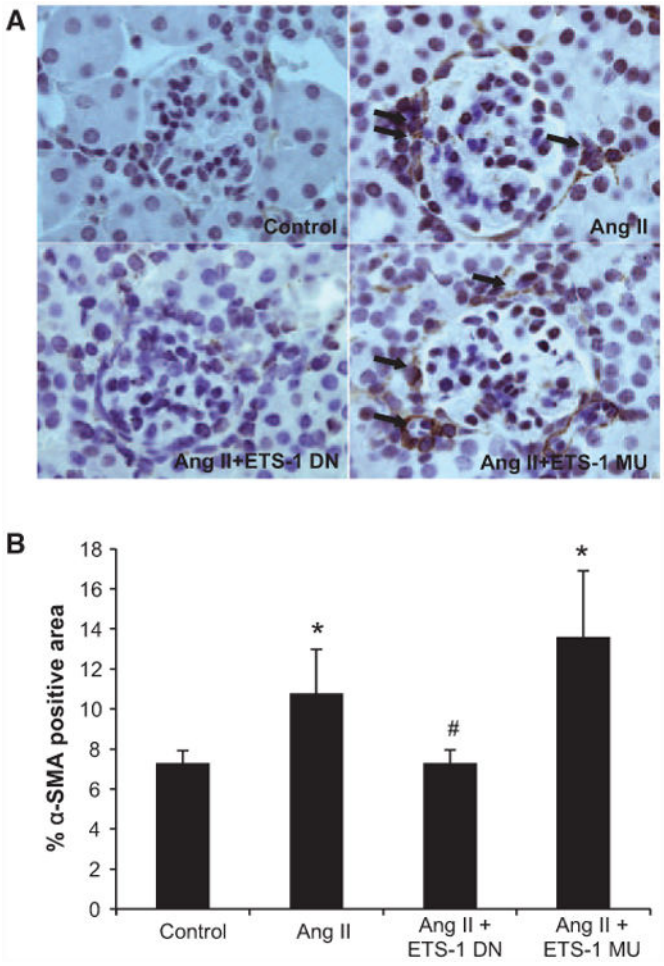

Figure 3.

ETS-1 blockade reduces cortical α-smooth muscle actin (α-SMA) expression in angiotensin II (Ang II)–infused mice. **A**, Representative photomicrographs of immunohistochemistry sections for α-SMA; **Brown staining** is indicative of positive staining that was evident in glomerular and interstitial areas. **B**, Quantitative analysis of glomerular α-SMA immunohistochemistry from mice infused with Ang II for 4 weeks with and without concomitant infusion of ETS-1 dominant-negative (ETS-1 DN) or ETS-1 mutant (ETS-1-MU) peptide. ETS-1 DN inhibited the expression of α-SMA induced by Ang II (n=6 per group; **P*<0.05 vs control; #*P*<0.05 vs Ang II).

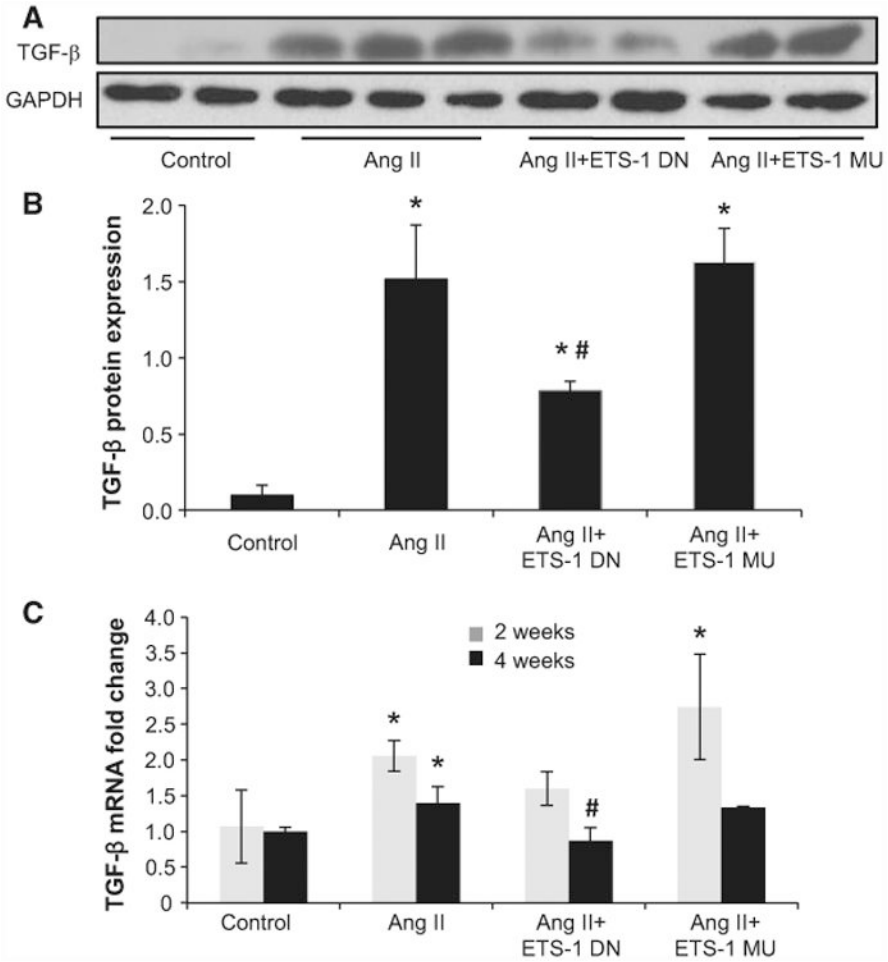

Figure 4.

ETS-1 blockade reduced angiotensin II (Ang II)–induced transforming growth factor-β (TGF-β) upregulation. **A**, Representative Western blots for TGF-β. **B**, Densitometry data analysis for TGF-β protein expression. ETS-1 dominant-negative (ETS-1 DN) peptide inhibited the Ang II–induced TGF-β overexpression in kidney cortex. Data expressed as mean±SEM are normalized to GAPDH (**P*<0.05 vs control; #*P*<0.05 vs Ang II group; n=6). **C**, TGF-β mRNA expression as assessed by real-time polymerase chain reaction after 2 and 4 weeks of Ang II infusion. ETS-1 DN inhibited Ang II–induced TGF-β mRNA expression in kidney cortex. Data are expressed as mean±SEM and normalized to GAPDH (**P*<0.05 vs control; #*P*<0.05 vs Ang II group; n=6). ETS-1 MU indicates ETS-1 mutant.

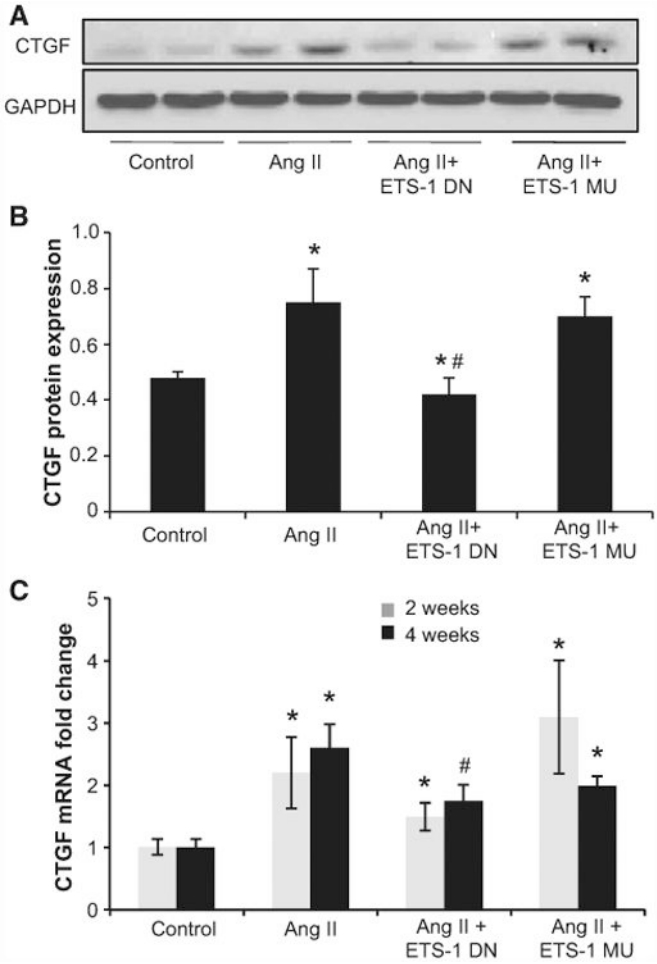

Figure 5.

ETS-1 blockade reduced angiotensin II (Ang II)–induced connective tissue growth factor (CTGF) upregulation. **A**, Representative Western blots for CTGF. **B**, Densitometry data analysis for CTGF protein expression. ETS-1 dominant-negative (ETS-1 DN) peptide inhibited the Ang II–induced CTGF overexpression in kidney cortex. Data are expressed as mean±SEM and are normalized by GAPDH (**P*<0.05 vs control; #*P*<0.05 vs Ang II group; n=6). **C**, CTGF mRNA expression as assessed by real-time polymerase chain reaction after 2 and 4 weeks of Ang II infusion. ETS-1 DN inhibited the Ang II–induced CTGF mRNA expression in mice kidney. Data are expressed as means±SEM and normalized to GAPDH (**P*<0.05 vs control; #*P*<0.05 vs Ang II group; n=6). ETS-1 MU indicates ETS-1 mutant.

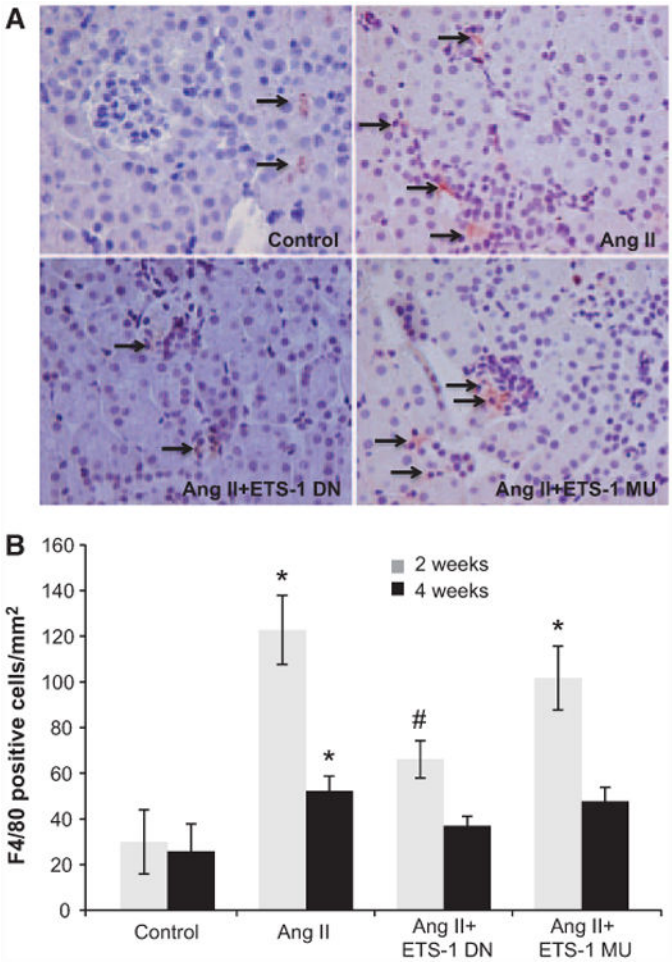

Figure 6.

ETS-1 blockade reduces angiotensin II (Ang II)–induced macrophage infiltration. **A**, Representative photomicrographs of immunohistochemical staining for F4/80-positive cells (macrophages). **Reddish brown color** in the cytoplasm indicates positive stain. Positive staining for macrophages was found in tubulointerstitial and glomerular areas (**arrows**). **B**, Quantitative analysis of immunohistochemical staining for F4/80-positive cells. In mice infused with Ang II, the number of positive cells increased significantly (**P*<0.01 vs control). The administration of ETS-1 dominant-negative (ETS-1 DN) peptide decreased macrophage infiltration in response to Ang II, whereas the ETS-1 mutant (ETS-1 MU) peptide had no effect (#*P*<0.05 vs Ang II; n=6 per group).

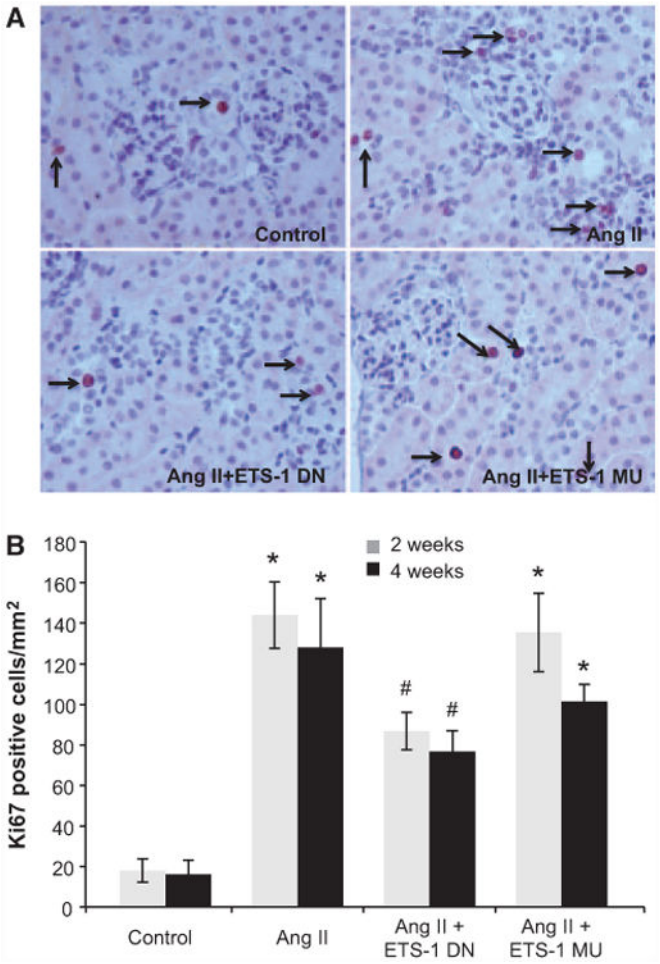

Figure 7.

ETS-1 blockade reduces angiotensin II (Ang II)–induced cell proliferation. **A**, Representative photomicrographs of immunohistochemical staining for Ki-67–positive cells as a marker of cell proliferation. Nuclear **reddish brown color** is considered positive. Positive cells were found in tubulointerstitial area and glomerular areas (**arrows**). **B**, Quantitative analysis of immunohistochemical staining for Ki-67 cells. In mice infused with Ang II, the number of Ki-67–positive cells increased significantly (**P*<0.01, control vs Ang II). The administration of ETS-1 dominant-negative (ETS-1 DN) peptide decreased cell proliferation in response to Ang II, whereas the ETS-1 mutant (ETS-1 MU) peptide had no effect (#*P*<0.05 vs Ang II; n=6 per group).

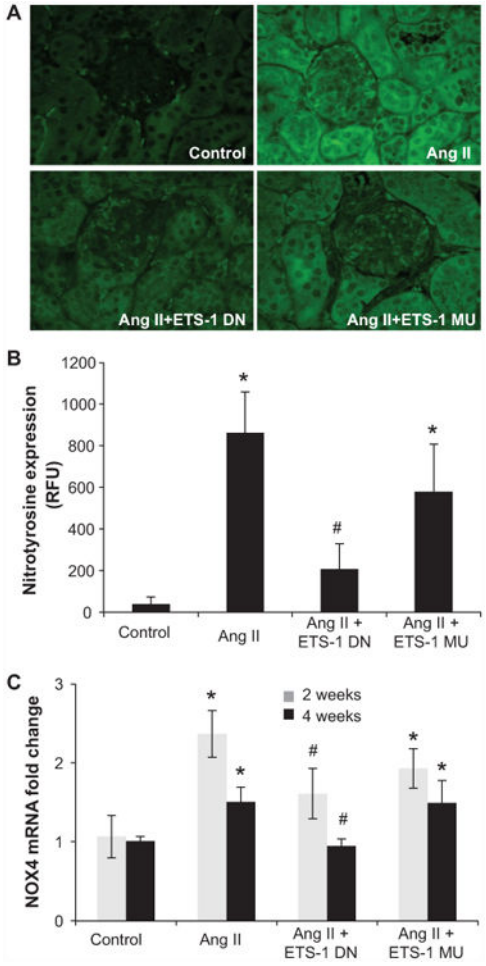

Figure 8.

ETS-1 blockade reduces angiotensin II (Ang II)–induced nitrotyrosine formation and NOX4 expression. **A**, Representative photomicrographs of immunofluorescence for nitrotyrosine. **B**, Quantitative analysis of the intensity of cortical nitrotyrosine immunofluoroscence from mice infused with Ang II for 4 weeks with and without concomitant infusion of ETS-1 dominant-negative (ETS-1 DN) or ETS-1 mutant (ETS-1-MU) peptide showing significant increase in nitrotyrosine in response to Ang II, which is significantly reduced by ETS-1 DN but not by ETS-1 MU (n=6 per group; **P*<0.05 vs control; #*P*<0.05 vs Ang II). **C**, NOX4 mRNA expression as assessed by real-time polymerase chain reaction after 2 and 4 weeks of Ang II, which is increased by Ang II and reduced by ETS-1 DN but not by ETS-MU (**P*<0.01 vs control; #*P*<0.01 vs Ang II and Ang II+ETS-1 MU).