Smad3 Mediates Cardiac Inflammation and Fibrosis in Angiotensin II–Induced Hypertensive Cardiac Remodeling

Xiao R. Huang, Arthur C.K. Chung, Fuye Yang, Wensheng Yue, Chuxia Deng, Chu Pak Lau, Hung Fat Tse, Hui Y. Lan

Abstract—Although Smad3 is a key mediator of fibrosis, the functional role of Smad3 in hypertensive cardiovascular disease remains unclear. The present study tested the hypothesis that angiotensin II may activate the transforming growth factor-β/Smad3 pathway to mediate hypertensive cardiac remodeling in Smad3 knockout (KO) and wild-type mice by subcutaneous angiotensin II infusion and in the primary culture of Smad3 KO cardiac fibroblasts. Fourteen days after angiotensin II infusion, both Smad3 KO and wild-type mice developed equal levels of high blood pressure. However, hypertensive cardiac fibrosis and inflammation were developed in Smad3 wild-type but not in Smad3 KO mice. This was demonstrated by the findings that mice lacking Smad3 were protected against a fall in left ventricular ejection fraction (P<0.05), an increase in left ventricular mass (P<0.05), and the development of cardiac fibrosis and inflammation, including upregulation of transforming growth factor-β1, connective tissue growth factor, collagen I/III, α-smooth muscle actin, interleukin 1β, tumor necrosis factor-α, monocyte chemoattractant protein 1, intercellular adhesion molecule 1, and an increase in macrophage and T-cell infiltration in left ventricular tissues (all P<0.01, respectively). Additional studies in vitro also revealed that angiotensin II–induced cardiac fibrosis and inflammation were prevented in Smad3 KO cardiac fibroblasts. Inactivation of both Smad3 and nuclear factor κB/p65 signaling pathways was a key mechanism by which Smad3 KO mice were protected from angiotensin II–mediated hypertensive cardiac remodeling. In conclusion, Smad3 plays an essential role in hypertensive cardiac remodeling. Results from this study suggest that targeting Smad3 may be a novel therapeutic strategy for hypertensive cardiovascular disease. (Hypertension. 2010;55:1165-1171.)

Key Words: hypertension • cardiac remodeling • TGF-β, Smad3 • angiotensin II

Hypertensive cardiac remodeling, characterized by left ventricular (LV) hypertrophy and cardiac inflammation and fibrosis, is a major risk factor for cardiovascular morbidity and mortality and a leading cause of chronic heart failure.1–3 It is generally agreed that activation of the renin-angiotensin system plays an important pathophysiological role in chronic cardiovascular disease. This is supported by the finding that blockade of angiotensin II (Ang II) with either angiotensin-converting enzyme inhibitors or its type 1 receptor blockers significantly improves cardiac function and regresses cardiac remodeling in patients with hypertension and in animal models after myocardial infarction.1,3,4

It is now clear that Ang II may act in both an autocrine and paracrine manner to activate a number of signaling pathways to mediate cardiac hypertrophy, inflammation, and fibrosis. This may involve mitogen-activated protein kinases, reactive oxygen species, receptor or nonreceptor-associated tyrosine kinases, and nuclear factor κB (NF-κB).5 Increasing evidence has also shown that Ang II, via its Ang II type 1 receptor, upregulates transforming growth factor (TGF)-β1 to induce cardiomyocyte hypertrophy, myofibroblast transition, and production of the extracellular matrix.6 This is further confirmed by the finding that blockade of Ang II inhibits cardiac TGF-β expression and cardiac remodeling.7,8 It is now well accepted that TGF-β1 is a key mediator in cardiac remodeling.9 Thus, functional blockade of TGF-β attenuates cardiac remodeling and cardiac dysfunction in a number of animal models.10–12

A recent discovery of TGF-β signaling provides insights into a better understanding of TGF-β1 in cardiac remodeling. It is now clear that TGF-β1, after binding to its receptors, activates the downstream mediators, mainly Smad2 and Smad3, to exert its biological effects, which are negatively regulated by Smad7 via a negative feedback mechanism.13 In the postinfarct heart, although expression of Smad2, 3, and 4 is increased, decreased Smad7 expression is also noted,14,15 suggesting the imbalance of TGF-β signaling during cardiac remodeling. The finding that blockade of Ang II with the Ang II type 1 receptor antagonist attenuates activation of Smads, and cardiac remodeling demonstrates the close link between
the Ang II and TGF-β/Smad signaling pathways. It was further confirmed in recent studies that Ang II is capable of activating the TGF-β/Smad signaling pathway to mediate vascular fibrosis via the extracellular signal-regulated kinase/p38 mitogen-activated protein kinase-Smad cross-talk pathway, in addition to the classic TGF-β-dependent mechanism. Furthermore, we also provided evidence for Smad3, not Smad2, as a key mediator of TGF-β/Smad signaling in Ang II–induced vascular fibrosis. A recent finding that mice lacking Smad3 were resistant to cardiac fibrosis and improved cardiac dysfunction after myocardial infarction delineates an essential role for Smad3 in cardiac remodeling. Because Ang II is able to activate Smad signaling to mediate vascular fibrosis, we, thus, hypothesized that Smad3 may be essential in hypertensive cardiac remodeling. This was examined in a mouse model of hypertension induced by Ang II in Smad3 knockout (KO) mice and in vitro in Smad3 KO cardiac fibroblasts.

**Methods**

**Mouse Model of Ang II–Induced Hypertension**

Smad3 KO and wild-type (WT) mice, congenic to the C57BL/6 strain, were used in this study. Hypertensive cardiac remodeling was induced in genetically identical littermates of Smad3 KO and WT mice by subcutaneous infusion of Ang II at a dose of 1.46 mg/kg per day for 14 days via osmotic minipumps as described in the Methods section of the online Data Supplement (available at http://hyper.ahajournals.org).

**Echocardiography**

Echocardiography was performed in both Smad3 KO and WT mice before and at day 14 after Ang II infusion as described previously and in the Methods section of the online Data Supplement.

**Immunohistochemistry**

Immunohistochemistry was performed in paraffin sections using a microwave-based antigen retrieval method, as described previously. Antibodies and the detailed protocol were presented in the Methods section of the online Data Supplement.

**Real-Time PCR**

The ventricular total RNA was isolated using the RNeasy kit (Qiagen Inc). Expression of collagen I, III, α-smooth muscle actin (α-SMA), TGF-β1, connective tissue growth factor (CTGF), tumor necrosis factor-α (TNF-α), interleukin (IL) 1β, intercellular adhesion molecule 1, monocyte chemoattractant protein (MCP) 1, and GAPDH was detected by real-time PCR using the primers as described previously. The ratio against GAPDH for individual mRNAs was calculated and expressed as the mean ± SEM.

**Western Blot Analysis**

Proteins extracted from cultured cardiac fibroblasts were analyzed by Western blotting, as described previously. The detailed protocol was described in the Methods section of the online Data Supplement.

**In Vitro Study of Smad3 KO Cardiac Fibroblasts**

Cardiac fibroblasts were isolated from both Smad3 WT and KO mice and characterized as described previously. Cardiac fibroblasts at passage 3 were stimulated with Ang II (1 μmol/L) as described in the Methods section of the online Data Supplement.

**Statistical Analyses**

Data obtained from this study are expressed as the mean ± SEM. Statistical analyses were performed using 1-way ANOVA followed by Newman-Keuls multiple comparison test from GraphPad Prism 4.0 (GraphPad Software, Inc).
Results

Smad3 KO Mice Are Protected Against Ang II–Induced Cardiac Remodeling Histologically and Functionally

As shown in Figure S1A, Smad3 WT and KO mice treated with saline had normal systolic blood pressure. However, Ang II infusion resulted in an equal increase in high blood pressure in both Smad3 WT and KO mice, reaching the maximal levels at day 7 after Ang II infusion. Interestingly, cardiac dysfunction, including a significant decrease in LV ejection fraction and an increase in LV mass, was developed in Smad3 WT but not in Smad3 KO mice (Figure S1B). Histologically, although Smad3 WT mice developed focal inflammation and fibrosis within the myocardium and the perivascular area (Figure S1C), Smad3 KO mice exhibited a normal cardiac histology (Figure S1C).

Ang II–Induced Cardiac Fibrosis Is Prevented in Smad3 KO Mice

Ang II–induced cardiac fibrosis in Smad3 WT and KO mice at the mRNA and protein levels was examined by immuno-histochemistry and quantitative real-time PCR. As shown in Figure 1A through 1D, compared with the saline-treated mice, Ang II infusion significantly increased collagen I and III mRNA and protein expression in LV tissues of Smad3 WT mice, particularly in the focal area with severe myocardium damage and in the perivascular area. In contrast, Ang II–induced cardiac fibrosis was inhibited in Smad3 KO mice. Similarly, mice lacking Smad3 also attenuated Ang II–induced upregulation of \( \alpha \)-SMA mRNA expression (Figure 1E) and \( \alpha \)-SMA\(^{+}\) myofibroblast accumulation (data not shown).

Mice Lacking Smad3 Are Protected From Ang II–Induced Cardiac Inflammation

Immunohistochemistry detected that no leukocytic infiltration was evident in the myocardium in both normal and saline-treated Smad3 WT and KO mice (data not shown). However, Ang II infusion resulted in a large number of leukocytes infiltrating the myocardium in Smad3 WT mice, primarily localizing to the damaged areas of cardiac tissues and resulting in a severe perivascular inflammation, as

![Figure 2](https://hyper.ahajournals.org/)

**Figure 2.** Smad3 KO mice are protected against Ang II–induced leukocytic infiltration in cardiac tissues. A, Representative immunostaining pictures for CD45\(^{+}\), F4/80\(^{+}\), and CD3\(^{+}\) cells infiltrating the perivascular region from Smad3 WT (top panel) and KO (bottom panel) mice. B, Semiquantitative analysis of CD45\(^{+}\) leukocytes. C, Semiquantitative analysis of F4/80\(^{+}\) macrophages. D, Semiquantitative analysis of CD3\(^{+}\) T cells. Data represent the mean±SEM for a group of 6 mice. *P<0.05, **P<0.001 vs saline control; ###P<0.001 vs Ang II–infused Smad3 WT mice. Magnifications: ×200.
demonstrated by the accumulation of many CD45<sup>+</sup> total leukocytes, F4/80<sup>+</sup> macrophages, CD3<sup>+</sup> T cells, and a marked upregulation of TNF-α and MCP-1 expression (Figures 2 and 3A and 3B). In contrast, Ang II–induced cardiac inflammation was inhibited in Smad3 KO mice with an absence in leukocytic infiltration (Figure 2) and normal levels of TNF-α and MCP-1 expression (Figure 3A and 3B). By real-time PCR, mice lacking Smad3 were protected against

![Figure 3](image3.png)

**Figure 3.** Deletion of Smad3 prevents Ang II–induced cardiac TNF-α and MCP-1 expression. A, Representative immunostaining pictures for cardiac TNF-α expression. B, Representative immunostaining pictures for cardiac MCP-1 expression. Note that perivascular TNF-α and MCP-1 expressions are markedly upregulated. C, TNF-α mRNA expression detected by real-time PCR. D, MCP-1 mRNA expression detected by real-time PCR. Data represent the mean±SEM for a group of 6 mice. ***P<0.001 vs saline control; ##P<0.01, ###P<0.001 vs Ang II–infused Smad3 WT mice. Magnifications: ×200.

![Figure 4](image4.png)

**Figure 4.** Smad3 KO mice are prevented from upregulation of cardiac TGF-β1 and CTGF in response to Ang II infusion. A, Representative immunostaining pictures for cardiac TGF-β1 expression. B, Representative immunostaining pictures for cardiac CTGF expression. C, TGF-β1 mRNA expression detected by real-time PCR. D, CTGF mRNA expression detected by real-time PCR. Data represent the mean±SEM for a group of 6 mice. **P<0.01 vs saline control; ##P<0.01 vs Ang II–infused Smad3 WT mice. Magnifications: ×200.
Ang II–upregulated mRNA expression of cardiac TNF-α and MCP-1 (Figure 3C and 3D), as well as IL-1β and intercellular adhesion molecule 1 (Figure S2).

Loss of Smad3 Signaling and Inactivation of the NF-κB Signaling Pathway Are Key Mechanisms by Which Smad3 KO Mice Are Protected From Ang II–Induced Hypertensive Remodeling

We then investigated the mechanisms by which Smad3 KO mice were protected against Ang II–mediated cardiac fibrosis and inflammation. As shown in Figures 4 and 5, immunohistochemistry and real-time PCR revealed that Ang II infusion markedly upregulated cardiac TGF-β1 and CTGF expressions in Smad3 WT mice (Figure 4), resulting in a strong activation of Smad2/3, as demonstrated by numerous nucleated phospho-Smad2/3 cells (Figure 5A). These changes were particularly intensified in the damaged cardiac tissues and the perivascular region (Figures 4A, 4B, 5A, and 5C) but were absent in Ang II–infused Smad3 KO mice (Figures 4, 5A, and 5C).

Additional study by immunohistochemistry also delineated that Ang II infusion caused a marked activation of NF-κB signaling, as demonstrated by the increased nuclear localization of the phosphorylated NF-κB/p65 subunit in the damaged cardiac tissues, including the perivascular region of Smad3 WT mice (Figure 5B and 5D). In contrast, Smad3 KO mice showed no significant NF-κB/p65 activation in response to Ang II infusion (Figure 5B and 5D).

In Vitro Deletion of Smad3 in Cardiac Fibroblasts Inhibits Ang II–Induced Cardiac Inflammation and Fibrosis

Cardiac fibroblasts isolated from Smad3 WT and KO mice were stimulated with Ang II. Consistent with the in vivo findings, Western blot and real-time PCR showed that Ang II–induced expression of collagen I and α-SMA mRNA and protein expression was significantly increased in Smad3 WT but not in Smad3 KO cardiac fibroblasts (Figure 6A and 6B). This was associated with marked differentiation of cardiac fibroblasts into α-SMA⁺ myofibroblasts (55%) in Smad3 WT but not in Smad3 KO mice (Figure S3). Furthermore, the addition of Ang II was also able to upregulate TNF-α, IL-1β, and MCP-1 mRNA expression in Smad3 WT but not in Smad3 KO cardiac fibroblasts (Figure 6B). All of the results obtained in vitro again indicated that Smad3 signaling is essential for Ang II–induced cardiac fibrosis and inflammation.

Discussion

A growing body shows that Ang II may act as a growth factor to directly or indirectly (by stimulating TGF-β1) promote cardiac remodeling and dysfunction. In the present study, we identified that Smad3 is essential for hypertensive cardiac remodeling induced by Ang II, because mice null for Smad3 were resistant to Ang II–mediated cardiac fibrosis and inflammation, despite hypertension. Loss of the TGF-β/Smad3 and the NF-κB signaling pathways may be mechanisms by which deletion of Smad3 prevents hypertensive cardiac remodeling in response to Ang II.
Inhibition of Ang II–mediated cardiac fibrosis may be a key mechanism by which Smad3 KO mice were protected against hypertensive cardiac remodeling. This was supported by the findings that Ang II–induced cardiac fibrosis and cardiac dysfunction, such as a fall in LV ejection fraction and an increase in LV mass index, were inhibited in Smad3 KO mice. Furthermore, in vitro studies have also shown that Ang II (0.1 μmol/L) and TGF-β1 can induce cardiac fibroblasts to express α-SMA and differentiate into collagen-producing myofibroblasts to some degree.26,27 In the present study, higher levels of α-SMA expression were induced in cardiac fibroblasts under a higher concentration of Ang II (1 μmol/L), which resulted in >55% of cardiac fibroblasts to differentiate into α-SMA+ myofibroblasts in Smad3 WT but not in Smad3 KO cardiac fibroblasts. This finding also demonstrated a critical role for Smad3 in Ang II–induced myofibroblast differentiation during cardiac remodeling, which is consistent with a known role for Smad3 but not Smad2 in TGF-β and Ang II–induced vascular and renal fibrosis in a dosage-dependent manner in vitro17,18,24 and in vivo in postinfarcted cardiac remodeling.19 Thus, loss of Smad3 signaling may be a central mechanism by which Smad3 KO mice were protected from Ang II–induced cardiac fibrosis.

In the present study, we also found that Smad3 is critical in Ang II–mediated cardiac inflammation. This was evidenced by the findings that Ang II–induced cardiac inflammation, particularly perivascular inflammation, was inhibited in Smad3 KO mice. Indeed, Ang II infusion resulted in a marked increase in perivascular CD45+ leukocytes, F4/80+ macrophages, and CD3+ T cells. This was associated with upregulation of proinflammatory cytokines (IL-1β and TNF-α), chemokine (MCP-1), and intercellular adhesion molecule 1 in cardiac tissues of Smad3 WT mice. In contrast, cardiac inflammation was absent in Smad3 KO mice, despite Ang II–induced hypertension. The resistance of Smad3 KO mice to Ang II–induced cardiac inflammation was consistent with the previous studies that showed that mice null for Smad3 inhibit inflammatory response in the postinfracted heart.19 Although mechanisms whereby loss of Smad3 inhibits inflammation remain largely unclear, they may be associated with the inhibitory effect of TGF-β/Smad3 on Ang II–induced MCP-1 and NF-κB–dependent inflammatory responses.28,29 It is known that Smad3 is critical for TGF-β–induced MCP-1 expression during vascular inflammation30, therefore, activation of cardiac Smad3 in response to Ang II and TGF-β1 may contribute to an increase in macrophage infiltration in the hypertensive heart. This may be a mechanism whereby macrophage infiltration was inhibited in the hypertensive heart of Smad3 KO mice. Moreover, blockade of Ang II–induced NF-κB activation may also be a signaling mechanism of suppressed cardiac inflammation in Smad3 KO mice. It is now well accepted that Ang II acts by stimulating the NF-κB signaling pathway to mediate cardiovascular inflammation.2,3,5,28,29 We have also shown that inactivation
of Smad2/3 by overexpressing Smad7 inhibits NF-κB–dependent inflammatory response by inducing inhibitor of NF-κB expression under high Ang II disease conditions. In contrast, loss of Smad7 promotes Smad2/3 activation and renal inflammation. Taken together, these findings suggest that the interaction between the TGF-β/Smad and NF-κB signaling pathways may be important in Ang II–induced cardiac inflammation.

**Perspectives**

Results from this study clearly demonstrate that Smad3 is essential for hypertensive cardiac remodeling. Smad3 may be a key mediator of downstream TGF-β signaling in chronic cardiovascular disease in response to Ang II and high blood pressure. Activation of Smad3 not only causes extracellular matrix synthesis by cardiovascular cells but also promotes cardiac inflammation, possibly via the Smad3–dependent MCP-1 mechanism. Findings that Smad3 KO mice were protected against Ang II–mediated cardiac fibrosis and inflammation suggest that targeting Smad3 may be a novel therapeutic strategy for hypertensive cardiovascular disease.

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**Disclosures**

None.

**References**


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Smad3 mediates cardiac inflammation and fibrosis in angiotensin II-induced hypertensive cardiac remodeling

Running title: Smad3 mediates hypertensive cardiac remodeling

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Methods

Mouse model of Angiotensin II-Induced Hypertension
Smad3 KO and wild-type (WT) mice, congenic to the C57BL/6 strain [1], were used in this study. Although Smad3 KO mice are viable, but they have lower body weight when compared to the WT littermate (30% reduced) and also have impaired immunity [1]. Hypertensive cardiac remodeling was induced in genetically identical littermates of Smad3 KO and WT mice (both sexes, age 8-10 weeks, n=6-8/group) by subcutaneous infusion of Ang II at a dose of 1.46 mg/kg/day for 14 days via osmotic minipumps (Model 2004; ALZA Corp., Palo Alto, CA). The use of a higher dose of Ang II infusion may allow to examine a critical role for Smad3 in cardiac remodeling on Smad3 KO mice under more stringency conditions, which is similar to other studies such as in ApoE KO mice [2]. Control animals followed the same experimental procedure, but received saline infusion only. In addition, groups of 6 Smad3 KO and WT mice without treatment were used as age-matched controls. Systolic and diastolic blood pressure (BP) and resting heart rates before treatment and at days 7 and 14 after Ang II infusion were measured by tail plethysmography using the BP2000 blood pressure analysis system (Visitech Systems, Inc., Apex, NC) in conscious mice. Mice were euthanized at day 14 post Ang II infusion. The left and right ventricles were collected for histology, immunohistochemistry, and real-time PCR analyses. The experimental procedures were approved by the Committee on the Use of Live Animals for Teaching and Research at the University of Hong Kong.

Echocardiography
Echocardiography was performed in both Smad3 KO and WT mice before and at day 14 after Ang II infusion. Left ventricular (LV) function was assessed by two-dimensional and M-mode echocardiography using a high resolution ultrasound system (Vivid-I, GE Medical Systems, Milwaukee, Wisconsin, USA) with a 10S-RS sector transducer (11.5 MHz). In brief, mice were anesthetized with intraperitoneal administration of ketamine/ xylazine. All the animals were examined in the left lateral position with an ultrasound gel pad positioned in the anterior chest wall. Standard M-mode parameters, including intraventricular septum thickness (IVS), posterior wall thickness (LVPW), LV end-systolic diameter (LVSD) and LV end-diastolic diameter (LVDD) were measured according to the American Society of Echocardiography recommendation [3]. The LV ejection fraction (LVEF=[(LVDD)^3−(LVSD)^3]/LVDD^3), LV fractional shortening (%FS=[(LVDD−LVSD)/LVDD] × 100%), and LV mass (mg) =1.055×[(IVS+LVDD+LVPW)^3−(LVDD)^3] were calculated. The mean value of three different cardiac cycles was used for each individual mouse at each point of recordings.

Immunohistochemistry
Immunohistochemistry was performed in paraffin sections using a microwaved-based antigen retrieval method as described previously [4]. Antibodies used in this study included: rat anti-mouse monoclonal antibody to leukocyte common antigen (CD45+), macrophages (F4/80+) (Serotec, Ltd, Oxford, UK), and rabbit polyclonal antibodies to CD3+ T cells (SP7) (Abcam, Cambridge, UK), rabbit or goat polyclonal antibodies to collagen I and III (Southern Tech, Birmingham, AL), α-SMA (Sigma, St.
Louis, MO), TGF-β1, CTGF, phospho-Smad2/3, TNF-α, MCP-1 (Santa Cruz Biotech Inc., Santa Cruz, CA), and an activated NF-κB p65 (Ser276) subunit (Cell Signaling Tech. Beverly, MA). An irrelevant isotype rabbit or goat IgG was used as a negative control. The stained sections were developed with diaminobenzidine to produce brown products and counterstained with hematoxylin. The number of positive cells for CD45, F4/80, CD3, phospho-Smad2/3, and phospho-p65 was counted in entire LV tissues under high-power fields (x40) by means of a 0.025-mm² graticule fitted in the eyepiece of the microscope and positive cells were expressed as cells/cm².

**Western Blot Analysis**
Proteins extracted from cultured cardiac fibroblasts were analyzed by Western blotting as previously described [5]. After blocking with 5% non-fat milk, the membranes were incubated overnight at 4°C with primary antibodies against collagen I and α-SMA (Southern Biotech) and GAPDH (Chemicon Inc., Temecula, CA) and then were incubated with IRDyeTM800 conjugated secondary antibodies (Rockland Immunochemical Inc., Gilbertsville, PA) in dark for 1 hour at room temperature. Signals were scanned and visualized by Odyssey Infrared Imaging System (LiCor Inc., Lincoln, NE). The ratio of the protein interested was subjected to GAPDH and was densitometrically analyzed by Image J software (NIH, Bethesda, MD).

**In vitro study of Smad3 KO cardiac fibroblasts**
Cardiac fibroblasts were isolated from both Smad3 WT and KO mice and were characterized as described previously [6]. Cardiac fibroblasts at passage 3 were stimulated with Ang II (1 μM) for 6 hours (for mRNA expression) and 24 hours (for protein expression) for collagen I, α-SMA, TNFα, IL-1β, and MCP-1 expression examined by both Western blot and real-time PCR analyses. The time selected for the study was determined by the preliminary time-dependent assays (hours 0, 1, 3, 6, 12, 24) with the findings that Ang II (1μM) induced the peaked mRNA expression at 3-6 hours and protein expression at 24 hours.

**Statistical analyses**
Data obtained from this study are expressed as the mean ± SEM. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Newman-Keuls Multiple Comparison Test from GraphPad Prism 4.0 (GraphPad Software, Inc. San Diego, CA).

**Results**
References


Figure S1. Effect of Smad3 KO mice on the systolic blood pressure, LV ejection fraction, LV mass, and histology in response to Ang II infusion. (A) The systolic blood pressure. (B) Echocardiography at day 14 after Ang II infusion. (C) Histological changes at day 14 after Ang II infusion. Results show that although Ang II infusion increases blood pressure equally in both Smad3 KO and WT mice, Smad3 WT mice develop cardiac dysfunction such as a significant fall in LV ejection fraction (EF) and an increase in LV mass and severe perivascular (see insert picture) and focal myocardial inflammation and fibrosis (*), which is prevented in Smad3 KO mice. Data represent the mean ± SEM for a group of 6 mice. *p<0.05, *** p<0.001 vs the baseline value or saline-treated animals. #p<0.05 as indicated. Magnifications x 200.
Figure S2. Real-time PCR shows that Ang II-induced upregulation of cardiac IL-1β and ICAM-1 mRNA are inhibited in Smad3 KO mice at day 14 after Ang II infusion. (A) IL-1β mRNA expression detected by real-time PCR. (B) ICAM-1 mRNA expression detected by real-time PCR. Data represent the mean ± SEM for a group of 6 mice. **p<0.01 vs saline control; ## p<0.01, ###p<0.001 as compared to Ang II-infused Smad3 WT mice.
Figure S3. Immunohistochemistry detects Ang II-induced myofibroblast transition in Smad3 WT, but not in Smad3 KO cardiac fibroblasts. (A) Normal cardiac fibroblasts from a Smad3 WT mouse. (B) Normal cardiac fibroblasts from a Smad3 KO mouse. (C) Ang II (1 μM)-treated cardiac fibroblasts for 24-hour from a Smad3 WT mouse. (D) Ang II (1 μM)-treated cardiac fibroblasts for 24-hour from a Smad3 KO mouse. (E) Quantitative analysis of α-SMA+ cardiac myofibroblasts. Arrows indicate α-SMA+ cells. Data represent the mean ± SEM for three-independent experiments. ***p<0.01 vs medium control; ###p<0.001 as compared to Ang II-treated Smad3 WT cells. Magnifications x 400.