Hepatocyte Growth Factor Reduces Cardiac Fibrosis by Inhibiting Endothelial-Mesenchymal Transition

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

Abstract—The purpose of this study was to investigate the effect of hepatocyte growth factor (HGF) on the pathogenesis of cardiac fibrosis induced by pressure overload in mice. Although cardiac fibrosis is attributed to excess pathological deposition of extracellular matrix components, the mechanism remains unclear. Recent reports revealed that α-smooth muscle actin–expressing myofibroblasts are primarily responsible for fibrosis. It is believed that myofibroblasts are differentiated from resident fibroblasts, whereas the transformation of vascular endothelial cells into myofibroblasts, known as endothelial-mesenchymal transition, has been suggested to be intimately associated with perivascular fibrosis. Thus, we hypothesized that HGF prevents cardiac fibrosis by blocking these pathways. We analyzed the pressure-overloaded HGF-transgenic mouse model made by transverse aortic constriction. Human coronary artery endothelial cells and human cardiac fibroblasts were examined in vitro after being treated with transforming growth factor-β1 or angiotensin II with or without HGF. The amount of cardiac fibrosis significantly decreased in pressure-overloaded HGF-transgenic mice compared with pressure-overloaded nontransgenic controls, particularly in the perivascular region. This was accompanied by a reduction in the expression levels of fibrosis-related genes and by significant preservation of echocardiographic measurements of cardiac function in the HGF-transgenic mice (P<0.05). The survival rate 2 months after transverse aortic constriction was higher by 45% (P<0.05). HGF inhibited the differentiation of human coronary artery endothelial cells into myofibroblasts induced by transforming growth factor-β1 and the phenotypic conversion of human cardiac fibroblasts into myofibroblasts. We conclude that HGF reduced cardiac fibrosis by inhibiting endothelial-mesenchymal transition and the transformation of fibroblasts into myofibroblasts. (Hypertension. 2012;59:958-965.) ● Online Data Supplement

Key Words: HGF ■ fibrosis ■ myofibroblast ■ EndMT ■ pressure overload

Heart disease is currently the leading cause of death; it is responsible for >25% of all deaths, according to the latest report of the Centers for Disease Control and Prevention.1 The number of cardiovascular deaths has been reduced, but in spite of a marked development in recent devices and medicines, cardiovascular disease still impacts the mortality rate in almost all nations.2 Cardiac fibrosis is often present in end-stage heart failure and is caused by various factors, such as ischemia,3 pressure overload,4 and cardiomyopathy,5 so antifibrotic therapy is believed to be beneficial in preventing heart failure. Although fibrosis, which is attributed to an excess deposition of extracellular matrix (ECM) components, is one of the most common pathological changes found in various organs, including the heart, the detailed mechanism remains unclear. It is worth noting that myofibroblasts are characterized by α-smooth muscle actin (α-SMA) expression and appear to play a major role in the pathogenesis of fibrosis by secreting numerous cytokines, growth factors, and ECM proteins.6 Myofibroblasts were originally thought to be differentiated from resident fibroblasts activated by acute or chronic stimuli, such as myocardial infarction and pressure overload. On the other hand, it is also possible for vascular endothelial cells to be transformed into myofibroblasts by a process known as endothelial-mesenchymal transition (EndMT),7 which is mediated by TGF-β1 and associated with perivascular fibrosis. EndMT is one of the forms of the epithelial-mesenchymal transition (EMT), which is considered to be a driving process in cell migration to other locations according to a fate pathway during the organization of the body plan in the embryonic period and a central mechanism for diversifying cells in complex tissues.8,9 Likewise, endocardial cells migrate into the atrioventricular cushion and the primordia of the valvoseptal tissues as mesenchymal cells during EndMT.10

Received September 26, 2011; first decision October 16, 2012; revision accepted February 14, 2012.


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The online-only Data Supplement is available with this article at http://hyper.ahajournals.orglookup/suppl/doi:10.1161/HYPERTENSIONAHA.111.183905/-DC1.

Hypertension is available at http://hyper.ahajournals.org

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DOI: 10.1161/HYPERTENSIONAHA.111.183905
HGF is an active heterodimer composed of a 69-kDa \( \alpha \)-chain and a 34-kDa \( \beta \)-chain, and it is known to have multiple biological functions through its specific tyrosine-kinase receptor, c-Met.\(^{11,12} \) Interestingly, HGF has been reported to show antifibrotic actions in various pathological conditions, like pulmonary fibrosis and renal fibrosis.\(^{13,14} \) We reported previously that the overexpression of the \( HGF \) gene improved cardiac function and fibrosis through microvascular angiogenesis in a porcine ischemic cardiomyopathy model\(^{15} \); however, the mechanism of HGF in cardiac fibrosis is still unclear. On another front, TGF-\( \beta 1 \) and angiotensin II (Ang II) are known to be involved in the hypertensive heart and resulting fibrosis.\(^{16–20} \) Based on these findings, it seems of interest to explore the role of HGF regarding myofibroblasts in the context of cardiac fibrosis induced by pressure overload. In this study, we further focused on the preventing effect of HGF on the pathogenesis of cardiac fibrosis after pressure overload through the inhibition of EndMT and the transformation of fibroblasts into myofibroblasts.

**Materials and Methods**

Detailed materials and methods are given in the online-only Data Supplement. To create cardiac fibrosis, we used the mouse pressure-overload model by TAC. We generated transgenic mice (C57BL6 background) with cardiac-specific (\( \alpha \)-major histocompatibility complex–driven) overexpression of \( HGF \) (\( HGF \)-Tg).\(^{21–22} \) Histological, physiological, and quantitative real-time PCR analyses were conducted by widely accepted methods, as noted in the online-only Data Supplement.

All of the procedures were performed in accordance with the guidelines of the institutional animal care and use committee of Osaka University Graduate School of Medicine. Values are shown as mean±SE. Statistical analysis was performed using ANOVA and \( t \) test (unpaired, 2-tailed), followed by Bonferroni adjustment for multiple comparison for comparing \( \geq 2 \) groups. Values of \( P<0.05 \) were considered to be statistically significant.

**Results**

**Reduction of Cardiac Fibrosis in HGF-Tg Mice**

In pressure-overloaded mice, cardiac hypertrophy occurred 48 hours after surgery and led to fibrosis within 5 days.\(^{23} \) As shown in Figure 1A, the fibrotic area could be detected in the heart of non-Tg control mice. In contrast, the fibrotic area in the heart of HGF-Tg mice was significantly reduced, especially around the perivascular region, as compared with non-Tg mice from 14 days after operation (Figure 1B through 1D; \( P<0.05 \)). Consistent with the histological data, echocardiography revealed poor values for cardiac performance, including fractional shortening, diastolic left ventricular diameter, and left ventricular mass in the TAC-operated group as compared with sham-operated mice at 56 days after operation (Figure 2; \( P<0.05 \)). However, fractional shortening, diastolic left ventricular diameter, and left ventricular mass values were preserved in HGF-Tg mice after TAC operation as compared with non-Tg mice (Figure 2A through 2D; \( P<0.05 \)). Different patterns of left ventricular filling in constricted mice indicated that diastolic function was also maintained in HGF-Tg mice (Figure 2E; \( P<0.05 \)). A marked change in intraventricular septum thickness and posterior left ventricular wall thickness indicated left ventricular hypertrophy attributed to pressure overload after constriction, whereas there was no significant difference in those parameters between constricted Tg mice and littermate controls (Figure S1A and S1B, available in the online-only Data Supplement). Blood pressure was reduced slightly at 8 weeks in all of the post-TAC mice, whereas there was no significant difference between the non-Tg TAC and HGF-Tg TAC groups (Figure 3A; \( P<0.05 \)). Compared with the non-Tg TAC group, heart weight and lung weight normalized by tibial length significantly decreased in the HGF-Tg TAC group (Figure 3B and 3C; \( P<0.05 \)), as was consistent with the echocardiographic findings. Importantly, these changes resulted in a drastic improvement in mortality, and the survival rate at 2 months after TAC operation was significantly higher in the HGF-Tg group as compared with the non-Tg group (Figure 4A;
The heart and serum HGF levels were kept higher in HGF-Tg TAC mice compared with the non-Tg TAC mice at 8 weeks post-TAC operation (Figure 4B and 4C; \( P < 0.05 \)). Therefore, we next explored how HGF prolonged survival against cardiac pressure overload. Initially, we focused on cardiac fibrosis. Along with the progression of fibrosis, the expression of TGF-\( \beta \)1 mRNA was significantly elevated immediately after TAC operation in the hearts of non-Tg mice (Figure 5A; \( P < 0.05 \)). Similarly, mRNA expression of ECM proteins, such as collagen I and collagen III, was also

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\[ LV mass \]

\[ E/A \]

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significantly increased in correlation with fibrotic response (Figure 5B and 5C; \( P < 0.05 \)). However, HGF-Tg mice showed a pronounced reduction in the mRNA expression of these fibrotic markers as compared with non-Tg mice from 1 or 2 weeks after TAC operation (Figure 5A through 5C; \( P < 0.05 \)). In fact, at 4 weeks after TAC operation, the collagenous area was markedly decreased in HGF-Tg mice (Figure 5D and 5E; \( P < 0.05 \)).

Inhibition of TGF-\( \beta \)-Induced EndMT by HGF

Interestingly, the localization of \( \alpha \)-SMA–positive cells almost matched the Masson trichrome–positive stained area, except in the vasculature (Figure 6A). Above these findings, we further explored the mechanisms of HGF-induced reduction in fibrosis from the view of myofibroblasts, one of the key drivers in the fibrotic process. As shown in Figure 6B, \( \alpha \)-SMA–positive myofibroblasts transdifferentiated from vas-

Figure 5. Analysis of fibrosis-related genes in pressure-overloaded mice. Effects of hepatocyte growth factor (HGF) on the mRNA expression of transforming growth factor (TGF)-\( \beta \) (A), collagen I (B), and collagen III (C) in pressure-overloaded mice at 8 weeks post-transverse aortic constriction (TAC). D, Typical Sirius Red staining of the heart in pressure-overloaded mice at 4 weeks post-TAC. Scale bars, 50 \( \mu \)m. E, Collagenous area of the hearts in TAC-operated HGF-transgenic (Tg) and non-Tg mice. \( \circ \) HGF-Tg indicates human hepatocyte growth factor transgenic group; non-Tg \( \bullet \), littermate group.

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**Figure 4.** Survival rate and hepatocyte growth factor (HGF) levels after transverse aortic constriction (TAC). A, Cumulative death according to the Kaplan-Meier method. \( \circ \) HGF-transgenic (Tg) TAC vs non-Tg TAC. B and C, Heart and serum levels of HGF protein at 8 weeks post-TAC. \( \circ \) HGF-Tg sham vs non-Tg sham; \( \Delta \) non-Tg TAC vs non-Tg sham; \# HGF-Tg TAC vs non-Tg TAC; \( ** \) HGF-Tg TAC vs HGF-Tg sham. Numbers in parentheses or bars indicate group size. Non-Tg sham \( \bullet \) indicates sham-operated without TAC littermate group; HGF-Tg sham \( \circ \), sham-operated without TAC HGF-Tg group; non-Tg TAC \( \Delta \), pressure-overloaded littermate group; HGF-Tg TAC \( \circ \), pressure-overloaded HGF-Tg group.
Inhibited EndMT induced by TGF-β1 and Ang II (Figure 8D; P<0.01). HGF significantly inhibited the expression of collagen I and collagen III mRNA, as well (Figure 8E and 8F; P<0.05). The addition of HGF also resulted in a significant decrease in the viability of myofibroblasts induced by TGF-β1 and Ang II stimulation (Figure S2A and S2B; P<0.05).

Discussion

Hypertension is one of the most common diseases in middle-aged and older people, and heart failure attributed to chronic hypertension is a major complication, along with coronary artery disease and stroke.24–26 To date, with the advent of effective drugs to lower blood pressure, concern is now shifting toward how to manage the causal complications typified by hypertensive heart failure with extremely poor prognosis.24–26 Cardiac fibrosis is highly correlated with cardiac dysfunction in patients with a hypertensive heart.27 Therefore, the reduction or improvement of cardiac fibrosis might be a useful treatment for heart failure. Under pressure overload, TGF-β1 was observed in the initial step of myocardial remodeling accompanied by the activation of fibroblasts, leading to the induction of ECM proteins, such as collagen and fibronectin.18–20 In this regard, TGF-β1 seems to be a key modulator causing cardiac fibrosis in the hypertensive heart. Indeed, recent clinical studies demonstrated an increase in myocardial TGF-β1 expression during cardiac hypertrophy and fibrosis.28 In contrast, HGF is a pleiotropic growth factor, which is known to work as an antagonist to decrease TGF-β1 expression and counteract TGF-β1 signaling.14 In fact, it has been reported that HGF prevented renal fibrosis in nephrectomized or spontaneously nephrotic mice.14,29,30 However, the molecular mechanisms by which HGF caused the antifibrotic effects are still unknown. Thus, we hypothesized that administration of HGF might achieve cardioprotective effects against fibrosis and explored the mechanisms of the antifibrotic actions of HGF in cardiac fibrosis using a pressure-overload model. In this study, we have shown that HGF-Tg mice with high levels of heart and serum HGF21,22 were immune to cardiac fibrosis attributed to pressure overload as compared with controls. In HGF-Tg mice, the fibrotic area was significantly smaller than in littermates under the pressure-overloaded condition, which was followed by the pronounced reduction in the expression of TGF-β1, collagen I, and collagen III mRNA. It is noteworthy that the cardiac performance, as well as survival rate, in the HGF-Tg group was markedly improved regardless of the high mortality in the constricted littermate group.

To elucidate the mechanism behind the relationship between HGF and cardioprotection, we focused on myofibroblasts in the fibrotic milieu, because they have been reported to be critical players in a large variety of fibropathological conditions by secreting ECM proteins and profibrotic mediators, represented by TGF-β1. The cellular origins of myofibroblasts are thought to be resident fibroblasts, whereas vascular endothelial cells can be transformed into myofibroblasts markedly reduced in pressure-overloaded HGF-Tg mice (P<0.05). On the other hand, intramyocardial myofibroblasts, which were assumed to be differentiated from resident fibroblasts, were also reduced in the HGF-Tg group (Figure 6C; P<0.05).

To clarify the effect of HGF on these myofibroblasts separately, we performed in vitro experiments. TGF-β1 significantly induced the differentiation of human coronary endothelial cells to the myofibroblast phenotype, as indicated by α-SMA expression, in a dose-dependent manner (Figure 7A through 7D; P<0.05). Similarly, TGF-β1 significantly increased the expression of fibrosis-related genes, including fibroblast-specific protein 1 and collagen I (Figure 7C, 7E, and 7F; P<0.05). In contrast, endothelial cells incubated with HGF showed a significant decrease in the expression of α-SMA, fibroblast specific protein 1, and collagen I mRNA (Figure 7; P<0.05). These data demonstrated that HGF inhibited EndMT induced by TGF-β1.

Inhibition of the Transformation of Fibroblast Into Myofibroblast by HGF

In addition to endothelial cells, fibroblasts were also transformed into myofibroblasts. As shown in Figure 8A, Ang II markedly increased α-SMA expression, which was accompanied by the phenotypic conversion of fibroblasts into myofibroblasts. TGF-β1 and Ang II significantly increased the expression of α-SMA mRNA in human cardiac fibroblasts after a 24- or 48-hour stimulation (Figure 8B and 8C; P<0.05). In contrast, the addition of HGF significantly inhibited the increase in α-SMA expression induced by TGF-β1 and Ang II (Figure 8D; P<0.01). HGF significantly inhibited the expression of collagen I and collagen III mRNA, as well (Figure 8E and 8F; P<0.05). The addition of HGF also resulted in a significant decrease in the viability of myofibroblasts induced by TGF-β1 and Ang II stimulation (Figure S2A and S2B; P<0.05).
Hepatocyte growth factor (HGF) suppresses endothelial-mesenchymal transition (EndMT) induced by transforming growth factor (TGF)-β1 with or without HGF (A) and the ratio of α-smooth muscle actin (SMA)–positive cells (B). α-SMA (red) and vascular endothelial cadherin (green). Nuclei were counterstained with 4’,6-diamidino-2-phenylindole (blue). *P<0.05 vs control, †P<0.05 vs TGF-β1 stimulation without HGF. Numbers in bars indicate group size. C through F, Effects of HGF on EndMT in TGF-β1-stimulated HCAECs evaluated by the mRNA expression of α-SMA (D), fibroblast-specific protein 1 (FSP1; E), and collagen I (F). *P<0.05 vs 10 ng/mL of TGF-β1 stimulation without HGF; †P<0.05 vs 1 ng/mL of TGF-β1 stimulation without HGF. Numbers in bars indicate group size.

Interestingly, after hepatic or renal injury, the expression of the HGF gene was reported to be upregulated not only in the liver and kidney but in other distant organs, such as the spleen and lung. It is believed that an elevation in circulating HGF levels would rescue the damaged organ. Clinically, after myocardial infarction, the expression of HGF, as well as its secretion into the blood circulation, was reported to be upregulated from 3 hours after onset. Similarly, we have confirmed that the heart and serum HGF levels of pressure-overloaded mice were significantly higher than that of sham-operated mice just after operation (data not shown). Such an increase in intrinsic HGF levels might play a role as a protective mechanism against the excess accumulation of matrix proteins during the healing process of cardiac injury under pressure overload. In this sense, HGF is thought to be an inhibitory regulator in cell matrix homeostasis.

**Perspectives**

Overall, our present study demonstrates that an increase in HGF resulted in a significant improvement of cardiac performance and mortality in a pressure-overload model. The reduction of cardiac fibrosis would be caused by the inhibition of multiple pathways, including EndMT and the trans-
formation of fibroblasts into myofibroblasts. Cardiac fibrosis is one of the adaptive responses or repair reactions to pathological injury caused by pressure overload or disruption of coronary blood flow in hypertension or myocardial infarction. On the other hand, myocardial fibrosis leads not only to heart failure through reduced contractility and compliance but also to fatal arrhythmia through disordered conductance of the heart. Pathologically cardiac fibrosis could be defined as excess deposition of ECM components in the heart. The accumulation of ECM causes the increase in myocardial elastance, whereas the reduction of ECM causes ventricular dilation, both of which are the driving forces for heart failure. So the point is how to manage the imbalanced fibropathological conditions. In this study, the inhibition of fibrosis was necessary and sufficient for the preservation of cardiac performance and the improved survival rate; however, further research is needed to control fibrosis. Because fibrotic change is believed to be irreversible at this time, administration and/or stimulation of HGF might offer potential for the preservation of cardiac performance in the failing heart attributed to sustained pressure overload.

Acknowledgment
We thank all of the members of the Department of Clinical Gene Therapy for their expert technical assistance and highly supportive advice.

Sources of Funding
This work was partially supported by Grant-in-Aids from the Organization for Pharmaceutical Safety and Research (Tokyo, Japan); the Ministry of Public Health and Welfare (Tokyo, Japan); the Japan Society for the Promotion of Science (Tokyo, Japan); and through special coordination funds of the Ministry of Education, Culture, Sports, Science, and Technology of the Japanese Government (Tokyo, Japan).

Disclosures
None.

References
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Hypertension. 2012;59:958-965; originally published online March 5, 2012;
doi: 10.1161/HYPERTENSIONAHA.111.183905

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

The online version of this article, along with updated information and services, is located on the
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Hepatocyte Growth Factor Reduces Cardiac Fibrosis
by Inhibiting Endothelial-Mesenchymal Transition

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Running title: HGF reduces cardiac fibrosis by inhibiting EndMT

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Expand materials and methods

Mouse pressure overload model by transverse aortic constriction (TAC)

To create cardiac fibrosis, we employed the mouse pressure overload model by TAC. We generated transgenic mice (C57BL6 background) with cardiac-specific (α-MHC-driven) over-expression of HGF (HGF-Tg) \(^1,^2\). In these mice, there was no pathological change in the heart under normal conditions, as described previously \(^1,^2\). Briefly, mice at 8 weeks of age underwent experimental surgery to induce pressure overload under inhalation anesthesia with 2 % isoflurane, as mentioned previously \(^3\). Placed in a supine position, mice were performed endotracheal section by a midline cervical incision using a blunt 22-gauge needle connected to a volume-cycled rodent ventilator (MicroVent Perinatal Mouse Ventilator Model 848, Harvard Apparatus, Holliston, USA) with a tidal volume of 200 μl and a respiratory rate of 220 /min. After the transverse aorta and a 27-gauge needle were bound together with a 7-0 silk suture, the needle was promptly removed to yield a 65 to 70 % constriction.

All procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Osaka University Graduate School of Medicine.

Physiological analysis

On day 7, 14, 21, 28 and 56 post-TAC, cardiac function was measured under 2.0 % isoflurane anesthesia by transthoracic echocardiography using the Xario (Toshiba, Tokyo, Japan) with a 10-MHz probe, as described previously \(^4\). M-mode tracing was recorded at the level of the papillary muscles, and fractional shortening (FS), diastolic left ventricular diameter (LVDd), intraventricular septum thickness (IVST), posterior left ventricular wall thickness (PWT) and the ratio of peak early diastolic ventricular filling velocity to peak atrial filling velocity (E/A) were evaluated. LV mass values were calculated by using Teicholz formula. Blood pressure was measured by BP-98A computerized tail-cuff system (Softron, Tokyo) in unanesthetized mice. Both tissue and serum levels of HGF protein were determined by Mouse HGF EIA kit (Institute of Immunology, Tokyo).

Histological analysis

After the physiological analysis, mice were sacrificed for sections. Isolated perfused hearts were fixed in 10 % buffered formalin, embedded in paraffin and sliced into horizontal 5-μm sections for Masson’s trichrome (MTC) and Sirius Red staining. These perfused hearts were also embedded in Tissue-Tek O.C.T. compound (Sakura
Finetek USA, Inc., Torrance, USA) and sliced into 5-μm cryosections for α-SMA immunostaining. The fibrotic area was measured using the ImageJ program (NIH, USA). The antibody against α-SMA was purchased from Dako (Carpinteria, USA). The pericardial and intramyocardial fibrosis was analyzed respectively from in-plane MTC staining. We have measured the fibrotic area of the hearts from both Non-Tg TAC and HGF-Tg TAC groups in every 5 randomly chosen views of each sample. The collagenous area was measured in the same way by Sirius Red staining to evaluate the expression levels of collagen protein.

Cell culture and proliferation assays
Cryopreserved human coronary artery endothelial cells (HCAEC) were purchased from Sanko Junyaku (Ibaragi, Japan). HCAEC were cultured in EBM-2 medium (Sanko Junyaku) with 10 % FBS/penicillin-streptomycin in standard dishes at 37°C. At passage 5, they were stimulated with 1 or 10 ng/ml TGF-β1 (Sigma-Aldrich, St. Louis, USA) and incubated with or without the addition of 50 ng/ml recombinant human HGF (PeproTech EC, London, UK) for 48 hours in 5 % CO2 at 37°C. Then, the gene expression of α-SMA and other fibrotic markers, including fibroblast-specific protein-1 (FSP1, also termed S100A4) and collagen I, was quantified by real-time PCR. For immunofluorescence staining, the immunofluorescence antibody against α-SMA was purchased from Sigma, anti-vascular endothelial cadherin (VE-cadherin) antibody was from Cell Signaling Technology (Danvers), and the Alexa Fluor 488 Conjugate (Invitrogen, Carlsbad, USA) was employed as the fluorescent secondary antibody.

Cryopreserved human cardiac fibroblasts (HCF) were purchased from Cell Systems Corporation, (Kirkland, USA). Fibroblasts were cultured in CSC 4Z3-500-S medium (Cell Systems) with 10 % FBS/penicillin-streptomycin in standard dishes at 37°C. At passage 5, they were stimulated with 1.0 ng/ml TGF-β1 or 10⁻⁶ mol/l Ang II with or without 50 ng/ml HGF for 48 hours in 5 % CO2 at 37°C. After 48 hours, the gene expression of α-SMA was quantified by real-time PCR.

For the evaluation of cell viability and proliferation, HCF was seeded at a concentration of 1×10⁴ cells per well in a 96-well plate, and 50 ng/ml HGF was added after a 24-hour stimulation by 1.0 ng/ml TGF-β1 or 10⁻⁶ mol/l Ang II. After another 12- and 24- hour incubation, mitogenic activity was assessed by the 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assay using the Cell Titer 96 Aqueous One Solution Cell Proliferation Assay (Promega, Madison, USA), in which the formazan product reflecting the number of living cells in
culture was quantified by measuring absorbance at 490 nm with a Mithras LB 940 spectrophotometer (Berthold Technologies, Bad Wildbad, Germany). The cell number was counted with a hemocytometer. Briefly, $1 \times 10^6$ HCF was seeded on 100-mm-well plates and incubated, each cell monolayer was resuspended in 2 mL trypsin-EDTA (0.25 % trypsin, 1 mmol/l EDTA-4Na), and cell number was counted with a hemocytometer.

**Quantitative real-time PCR**

For reverse transcription, total RNA from deep frozen hearts was prepared according to standard methods. RNA was directly collected using RNeasy Mini Kit (Qiagen, Hilden, Germany), from *in vitro* cytokine-treated HCAEC or HCF with lysis buffer according to the manufacturer’s instruction. The total RNA was quantified, and integrity was confirmed. The TaKaRa PCR Thermal Cycler Dice Standard (TAKARA BIO Inc., Shiga, Japan) was used to synthesize cDNA, and the Applied Biosystems 7900HT Fast (Life Technologies Corporation, Carlsbad, USA) was used for detection, in accordance with the manufacturer’s instructions. In each experiment, mouse $\beta$-actin, human GAPDH or 18s-rRNA was amplified as a reference standard. Triplicate analysis was employed in all PCR studies. PCR primer details are as follows:

1. **Mouse TGF-$\beta$1**
   - sense, 5'-CAGACATTCGGGAAGCAGTG-3'
   - antisense, 5'-CAGCCACTCAGGCGTATCAG-3'

2. **Mouse collagen I**
   - sense, 5'-TGGATCGACCCCTAACCAAGG-3'
   - antisense, 5'-TCGGTCATGCTCTCTCTCAA-3'

3. **Mouse collagen III**
   - sense, 5'-CATGGGTTTCCCTGGTCTA-3'
   - antisense, 5'-ACCACCGGTACCAGGTATGC-3'

4. **Human $\alpha$-SMA**
   - sense, 5'-GTCTGCTGGCATCCATGAA-3'
   - antisense, 5'-AGAAGCATTTGCGGTGGA-3'

5. **Human collagen I**
   - sense, 5'-CTTGGCTTTGAAGACCCCATGGA-3'
   - antisense, 5'-TTGGCAGTCTGAGAACCCCA-3'

6. **Human collagen III**
   - sense, 5'-AACACGCAAGGGCTGTGACT-3'
   - antisense, 5'-TTTTGTGCTGACTTGCACTG-3'
(7) Human FSP1 (S100A4)
sense, 5’-TCTTTCTTGTTTGATCCTG-3’
antisense, 5’-GCATCAAGCACGTGCTGAA-3’

References
Fig. S1. Effect of HGF on echocardiographic parameters in pressure-overloaded mice. (A & B) Interventricular septum thickness (IVST) and Posterior wall thickness (PWT). *P<0.05 Non-Tg TAC vs. Non-Tg sham. Non-Tg sham = sham-operated without TAC littermate group, HGF-Tg sham = sham-operated without TAC HGF-Tg group, Non-Tg TAC = pressure-overloaded littermate group, HGF-Tg TAC = pressure-overloaded HGF-Tg group.
Fig. S2. Effect of HGF on myofibroblast cell viability.
(A & B) The cell viability of myofibroblasts was inhibited by HGF after 12 to 24 hours of incubation with TGF-β1 and Ang II.

*P<0.05 vs. 1.0 ng/ml TGF-b1 or 10^{-6} mol/l Ang II without HGF for 12 hrs,
†P<0.05 vs. 1.0 ng/ml TGF-b1 or 10^{-6} mol/l Ang II without HGF for 24 hrs. N = 6.