Hepatocyte growth factor prevents tissue fibrosis, remodeling, and dysfunction in cardiomyopathic hamster hearts

Teruya Nakamura,1,2 Kunio Matsumoto,1 Shinya Mizuno,1 Yoshiki Sawa,2 Hikaru Matsuda,2 and Toshikazu Nakamura1

1Division of Molecular Regenerative Medicine, Course of Advanced Medicine, and 2Department of Surgery, Course of Interventional Medicine, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

Submitted 30 December 2003; accepted in final form 5 January 2005

AN ULTIMATE PHENOTYPIC RESPONSE of heart failure is depicted by dilated cardiomyopathy (16). Biochemical and mechanical stress on the myocardium due to genetic abnormalities and/or functional and numerical loss of myocytes drives persistent signals toward eccentric hypertrophy of cardiomyocytes and interstitial fibrosis (11, 16). Such a remodeling results in dilatation of the chamber lumen, increased myocardial stiffness, and contractile dysfunction (38). A large body of evidence suggests that transforming growth factor-β1 (TGF-β1) and angiotensin II (ANG II) have pivotal roles in promoting unfavorable myocardial remodeling (9, 18, 19, 21, 46). ANG II increases expression of TGF-β1 in cardiomyocytes, and TGF-β1 acts on fibroblasts to increase extracellular matrix production and strengthen the cell adhesion (9, 19, 21). TGF-β1 induces hypertrophy and apoptotic cell death in cardiomyocytes (18), and it is an important mediator of the hypertrophic cardiomyocyte growth induced by ANG II (36).

Hepatocyte growth factor (HGF), originally identified and cloned as a mitogen for hepatocytes in primary culture (30, 31), has mitogenic, motogenic, and morphogenic activities in various cell types via e-Met/HGF receptor tyrosine kinase. HGF also has cytoprotective and angiogenic (2, 8, 45) activities and plays roles in organogenesis and tissue regeneration (3, 24). Previous studies revealed that exogenous HGF prevents tissue fibrosis and dysfunction in chronic disease models, including liver cirrhosis (23, 44), lung fibrosis (48), and renal fibrosis (26). It is worth noting that HGF has a biological character that opposes expression and activity of TGF-β1. We recently found that HGF is an endogenous cardioprotective factor, and the possible therapeutic application of HGF for myocardial ischemia-reperfusion injury was considered (29). However, it remained to be determined whether HGF has a role in myocardial remodeling or any beneficial effects on dilated cardiomyopathy.

The cardiomyopathic Syrian hamster is a representative model of idiopathic dilated cardiomyopathy. These animals have a defect in δ-sarcoglycan, one of four sarcoglycans that bind one another at extracellular domains and constitute the dystrophin-associated sarcoglycan subcomplex, rendering cardiomyocytes more susceptible to mechanical stress (35). Using the hamster model, we investigated the following: 1) the relationship between myocardial HGF/TGF-β1 and the amount of collagen in the natural course of dilated cardiomyopathy, and 2) the effect of HGF administration on progression of myocardial remodeling and dysfunction in late-stage cardiomyopathy and modulation of gene expression involved in myocardial remodeling. We obtained evidence that HGF suppresses myocardial fibrosis, hypertrophy, and dysfunction in cardiomyopathic hamsters, even in the case of late-stage treatment.

METHODS

Preparation of recombinant HGF. Human recombinant HGF was purified from culture media of Chinese hamster ovary cells transfected with expression plasmid containing human HGF cDNA (37). The purity of HGF exceeded 98%, as determined by SDS-PAGE followed by protein staining.

Address for reprint requests and other correspondence: T. Nakamura, Course of Advanced Medicine, Osaka Univ. Graduate School of Medicine, 2-2-B7 Yamadaoka, Suita, Osaka 565-0871, Japan (E-mail: nakamura@onbich.med.osaka-u.ac.jp).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Animal model and experimental design. Male cardiomyopathic Syrian hamsters (TO-2; Bio Breeders, Wilmington, MA) and age-matched control hamsters (F1β) were bred with humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Pub No. 85-23, Revised 1996). All cardiomyopathic hamsters manifested clinical signs such as loss of activity, anorexia, and poor body weight gain at 26 wk after birth. After 26 wk of age, some of the animals died suddenly due to cardiac failure, as determined at autopsy (data not shown). Twenty-seven-week-old TO-2 hamsters were given recombinant HGF daily (300 μg·kg⁻¹·day⁻¹ or 1 mg·kg⁻¹·day⁻¹, n = 8 in each group) subcutaneously for the next 3 wk. In the control group (n = 8), only saline was injected. After the administration of HGF, all animals were killed and hearts were histologically examined.

Evaluation of fibrotic and hypertrophic changes and immunohistochemistry. Hearts were transversely sectioned, fixed in 70% ethanol or 10% neutralized formalin, and subsequently embedded in paraffin. To evaluate fibrotic changes of the heart, we stained tissue sections with Masson-trichrome. The fibrotic area of the total area was assessed by computer-aided image analysis of tissue sections. At least 6 fields at 200-fold magnification were captured and assessed in all the samples. For measurement of fiber areas, cross sections of cardiac tissue were stained with 50 μg·ml·wt germ agglutinin (WGA) conjugated with tetramethylrhodamine isothiocyanate (Sigma Chemical, St. Louis, MO) as described previously (7). At least 3 fields at 200-fold magnification were captured and assessed in all samples. For immunohistochemical staining of α-smooth muscle actin (α-SMA), tissue sections were incubated with horseradish peroxidase-conjugated monoclonal anti-human α-SMA mouse IgG antibody (1:2000) (clone 1A4; DAKO, Glostrup, Denmark), and sections were subsequently visualized in chromogenic substrate solution containing 3,3′-diaminobenzidine hydrochloride and 0.01% hydrogen peroxide (26). For detection of c-Met receptor, anti-c-Met antibody (1:400) (SP-260; Santa Cruz Biotechnology, Santa Cruz, CA) was applied to the 70% ethanol-fixed sections, followed by incubation with biotin-labeled goat anti-rabbit IgG (Vector, Burlingame, CA) (27). The antigenic signals were visualized with peroxidase-labeled avidin-biotin complex using a kit (Vectastain Elite ABC; Vector). For double-immunofluorescent staining of c-Met and α-SMA, sections were incubated with rabbit anti-c-Met antibody (SP-260; Santa Cruz Biotechnology) and mouse monoclonal anti-human α-SMA antibody (DAKO), followed by detection of immunofluorescence using an Alexa488-conjugated goat anti-rabbit IgG (Vector, Burlingame, CA) (27). The images were captured and intensities of the signals were quantified with Fluorochrom (Alpha Innotech, San Leandro, CA). Data were obtained as the ratio mRNA/28S ribosomal RNA signals, and values were compared with those of control samples (46).

Statistical analysis. All values are expressed as means ± SE. The differences in the data were determined using unpaired Student’s t-test or one-way ANOVA with multiple comparisons. Data were considered to be significantly different when the two-tailed P value was <0.05.

RESULTS

Histopathological change in cardiomyopathic hamsters. As a baseline for serial observations of growth factor expression in δ-sarcoglycan-mutated cardiomyopathy, we first examined the time course of changes in cardiac structures. Compared with age-matched control F1β hamsters, the heart-to-body weight ratio increased in TO-2 hamsters at 26 and 32 wk after birth (Fig. 1A). In the control, microscopic findings were normal, without fibrosis and hypertrophy (Fig. 1B). In contrast, TO-2 hamsters showed evidence of myocardial destruction, fibrosis, and focal infiltration of inflammatory cells, and myofibroblasts that expressed α-SMA appeared in granulomatous regions (Fig. 1C). Fibrosis was increased in most hearts at an age of 16 wk. Widespread fibrosis and loss of cardiomyocytes were seen uniformly at 26 wk (Fig. 1D), and these changes were even more severe at 32 wk after birth (Fig. 1E). Consistent with...
these histological changes, death from cardiac failure (cardiac enlargement, pleural and peritoneal effusion, liver and lung congestion) occurred after 26 or more weeks of age. Thus decompensated cardiac failure was manifest between 26 and 32 wk of age in this model.

**Changes in myocardial TGF-β1 and HGF expression.** To define the temporal relationships between TGF-β1 or HGF and the pathological phenotype in our model, these growth factors were analyzed during the progression of dilated cardiomyopathy. Among the three isoforms of TGF-β (i.e., TGF-β1, -β2, and -β3), TGF-β1 has been thought to play a predominant role in cardiac hypertrophy (46) and has been shown to be essential for the cardiac hypertrophy induced by ANG II (36). In controls, myocardial TGF-β1 showed no change. In contrast, TGF-β1 levels showed significant increases at 32 wk in TO-2 hamsters (Fig. 1F). TGF-β1 levels showed a positive correlation with the soluble collagen concentration (Fig. 1F). Conversely, cardiac HGF levels in TO-2 hamsters decreased at 32 wk, and there was a negative correlation with the soluble collagen concentration (Fig. 1G). In immunohistochemistry, TGF-β1 was expressed in cardiomyocytes, interstitial cells, and endothelial cells, whereas HGF was predominantly expressed in endothelial cells and interstitial cells (data not shown). Together, these results indicate that the decrease in myocardial HGF levels, as well as the increase in TGF-β1 levels, may play a role(s) in the progression of cardiac fibrosis.

**Localization of c-Met/HGF receptor.** To further define potential involvement of HGF in the cardiomyopathic phenotype, we analyzed localization of the c-Met receptor by using immunohistochemistry. In the heart of 26-wk-old TO-2 hamsters, c-Met receptor was detected in spindle-shaped cells in the interstitial areas with fibrosis (Fig. 2A, I and II), several cardiomyocytes, and blood vessel cells (Fig. 2A, III). In contrast, c-Met was detectable in vascular vessels in the heart of age-matched wild-type hamster (Fig. 2A, IV). On the basis of appearance and accumulation of c-Met-positive spindle-shaped cells in the interstitial areas, the cells were considered to be myofibroblasts. We therefore analyzed localization of the c-Met receptor and α-SMA in interstitial areas with fibrotic change by using double-immunohistochemistry. Most of the α-SMA-positive cells were also positive for the c-Met receptor (Fig. 2B).Appearances of these cells indicate that c-Met receptor was localized in α-SMA-positive interstitial myofibroblasts and blood vessel cells (possibly endothelial and vascular smooth muscle cells). These findings suggest that in addition to cardiomyocytes and blood vessel cells, cardiac myofibroblasts in interstitial areas are a potential target of HGF, similar to the case in myofibroblasts/myofibroblastic cells involved in fibrotic change in distinct organs (4, 17, 27).

**Suppression of ANG II-induced fibrogenic gene expression by HGF in vitro.** On the basis of the findings that α-SMA-positive myofibroblasts/myofibroblastic cells are responsible for overproduction of extracellular matrix (9, 19, 21) and that the c-Met receptor is expressed in α-SMA-positive interstitial myofibroblasts in the heart with cardiomyopathic pathology, we analyzed the effect of HGF on TGF-β1 and type I collagen in α-SMA-positive myofibroblasts. Because cardiac fibroblasts undergo phenotypic change into myofibroblasts and mediate...
hypertrophic effects of ANG II on cardiomyocytes through upregulation of TGF-β1, and because cultivation and characterization of neonatal rat fibroblasts have been established (18, 19), we used cardiac fibroblasts obtained from neonatal rats. Although rat cardiac fibroblasts did not express α-SMA immediately after cultivation, most of the cells expressed α-SMA and underwent morphological changes characterized by multiple cellular processes associated with stress fibers of α-SMA after 4 days, strongly suggesting that cardiac fibroblasts underwent phenotypic change into myofibroblasts (Fig. 3, A and B). TGF-β1 and type I collagen expressions were upregulated by ANG II after 12 h of stimulation. However, simultaneous treatment of HGF decreased TGF-β1 and type I collagen expression, suggesting that HGF may downregulate these fibrogenic gene expressions in myofibroblasts (Fig. 3, C and D).

**Effect of HGF administration on physiological conditions and cardiac function in TO-2 hamsters.** On the basis of the findings that the cardiac level of endogenous HGF is decreased and c-Met receptor is expressed in cardiac myocytes and α-SMA-positive myofibroblasts in late-stage cardiomyopathy, we considered that supplemental administration of recombinant HGF would be particularly significant in later stages of cardiomyopathy. Twenty-seven-week-old TO-2 hamsters were subjected to daily subcutaneous injections of 300 μg/kg or 1 mg/kg recombinant human HGF for 3 wk (n = 8 in each group) (Fig. 4A). When HGF was subcutaneously administered at 1 mg/kg, plasma HGF level reached a peak (21 ng/ml) at 5 h postinjection, and the level decreased below 1 ng/ml at 24 h. HGF in cardiac tissue also reached a peak (135 ng/g tissue) at 5 h but decreased much more slowly than in the plasma. Because HGF as low as 1 ng/ml exerts biological activities, changes in HGF levels in the plasma and cardiac tissues suggest that administered HGF may be physiologically functional. Control animals treated with saline showed decreased water and food intake, and body weight loss was remarkable during the observation period. In contrast, activities of the hamsters administered HGF were conserved, and body weight reverted to the level seen at pretreatment (Fig. 4B).

Cardiac function was assessed weekly by echocardiography. TO-2 hamsters treated with saline showed chamber dilatation, wall thinning, and reduced wall motion compared with observations in F1β hamsters. TO-2 hamsters administered HGF showed improved wall motion (Fig. 4C). The fractional shortening (%FS) of TO-2 hamsters administered saline was also much lower than that of F1β hamsters, and it further decreased over 3 wk, thereby indicating that contractile dysfunction was progressing. In contrast, HGF administration significantly increased the %FS, and, more importantly, the %FS during HGF treatment was higher than in cases of pretreatment (Fig. 4D). These results suggest that cardiac function was compensated by late-stage administration of HGF in TO-2 hamsters.

HGF reduces myocardial fibrosis, myocyte hypertrophy, and apoptosis. Histologically, HGF treatment for 3 wk reduced infiltration of interstitial cells and myocardial fibrosis in the left

---

**Fig. 2. Localization of c-Met/HGF receptor in normal and cardiomyopathic hamster hearts.** A: immunohistochemical detection of c-Met receptor in cardiomyopathic (I, II, and III) and normal (IV) hearts. Distribution of c-Met in the interstitial area (smaller frame in I) and parenchymal area (larger frame in I) are shown at higher magnifications in II and III, respectively. Twenty-six-week-old F1β and TO-2 hamsters were used. In III, some cardiomyocytes and blood vessels positive for c-Met expression are indicated by pound signs and asterisks, respectively. Original magnification, ×160 for I and IV. B: double-immunofluorescent staining for α-SMA (red fluorescence) and c-Met (green fluorescence) in fibrous area in the heart of a 26-wk-old TO-2 hamster. Colocalization of α-SMA and c-Met is shown in the merged image. Original magnification, ×160. Top and bottom insets in merged image, respectively, indicate localization of α-SMA and c-Met in blood vessels and myofibroblasts.
Fig. 3. Suppression of ANG II-induced TGF-β1 and type I collagen (Col) expression by HGF in rat cardiac myofibroblasts. A and B: induction of α-SMA expression during cultivation, as determined by Western blot analysis (A) and immunohistochemistry (B). Bar, 50 μm. C and D: suppression of TGF-β1 and type I collagen mRNA expression by HGF, as determined by Northern blot analysis (C) and quantitated by densitometric analysis (D). 28S ribosomal RNA bands were visualized using ethidium bromide, indicating equal loading of RNA samples. Four independent experiments were evaluated, and each value represents the mean ± SE. #P < 0.05, ##P < 0.01, ###P < 0.001 vs. control. *P < 0.05, **P < 0.01 vs. treatment with ANG II alone.

Fig. 4. Improvement of body weight, heart-to-body weight ratio, and cardiac contractility by HGF administration. A: protocol for recombinant human HGF (rhHGF) administration. W, weeks; s.c., subcutaneous. B: change in body weight (BW) by HGF administration. *P < 0.05, saline vs. HGF (1 mg·kg⁻¹·day⁻¹). C: M-mode tracing images. Note that both septal (s) and posterior wall (p) motions were improved by HGF treatment. HGF (high) represents treatment with 1 mg·kg⁻¹·day⁻¹ HGF. D: change in fractional shortening (%FS). Each value represents the mean ± SE (n = 7–8 in each group). *P < 0.05, **P < 0.01, ***P < 0.001 vs. same time points in the saline group.
ventricle (Fig. 5, A–D). The percentage of fibrotic change in TO-2 hamsters treated with saline was 23.3 ± 2.2%, and the value was fivefold higher compared with that in F1β hamsters (Fig. 5E). In contrast, fibrotic changes in the left ventricles of TO-2 hamsters were respectively decreased to 19.7 ± 1.2 and 16.4 ± 1.1% by 300 μg·kg⁻¹·day⁻¹ HGF; HGF (low), 1 mg·kg⁻¹·day⁻¹ HGF treatment (Fig. 5E). To confirm these results, we checked the expression of TGF-β1 and type I collagen in the left ventricle. Compared with that in F1β hamsters, TGF-β1 and type I collagen mRNA were increased in TO-2 hamsters. HGF treatment, however, reduced these expressions (Fig. 6).

To determine the effect of HGF on hypertrophic change in cardiomyocytes, we quantified the fiber area in cross sections by using WGA staining (7). In TO-2 hamsters, hypertrophic change was manifested and the fiber area was significantly increased compared with that in F1β hamsters. On the other hand, HGF administration reduced the fiber area (Fig. 7, A–D). The decrease in the hypertrophic change was consistent with the decrease in ANP and TGF-β1 expressions (Fig. 6).

Because myocyte hypertrophy is associated with apoptotic cell death of cardiac myocytes in cardiomyopathic hearts (16) and because HGF inhibits apoptosis of cardiac myocytes (29), we analyzed whether HGF would alter apoptosis of cardiomyocytes in cardiac sections by using TUNEL staining. Apoptotic cardiomyocytes were mostly undetectable in the heart of 30-wk-old F1β hamsters (Fig. 7, E and H), whereas TUNEL-positive apoptotic cardiomyocytes were seen in the heart of age-matched, saline-treated TO-2 hamsters (Fig. 7, F and H). HGF decreased the number of TUNEL-positive cardiomyocytes to 25% of the value in saline-treated TO-2 hamsters (P < 0.05) (Fig. 7, G and H), indicating that HGF suppresses apoptosis of cardiac myocytes associated with cardiac hyper-

Fig. 5. Suppression of myocardial fibrosis in TO-2 hamster by HGF treatment. A–D, histological appearance of the left ventricle. Tissue sections were stained with hematoxylin and eosin (H&E) (A and C) or Masson-trichrome dye (B and D). Bar, 100 μm. E, change in a fibrous area in left ventricles after HGF administration. Fibrous area was quantified by image analysis in Masson-trichrome staining. At least 6 areas were evaluated in each section, and each value represents the mean ± SE (n = 5 in each group). *P < 0.05, **P < 0.01 vs. saline (S). HGF (low), 300 μg·kg⁻¹·day⁻¹ HGF; HGF (high), 1 mg·kg⁻¹·day⁻¹ HGF.

Fig. 6. Effect of HGF treatment on TGF-β1, atrial natriuretic peptide (ANP), and type I collagen mRNA expression in the left ventricles. Changes in TGF-β1, ANP, and type I collagen mRNA expressions were analyzed using Northern blotting. Similar results were obtained from 3 independent experiments, and typical results are shown. 28S ribosomal RNA was visualized using ethidium bromide, indicating equal loading of RNA.
trophy and fibrosis. Collectively, these data indicate that administration of HGF reduced cardiac fibrosis, myocyte hypertrophy, and apoptosis, thereby suppressing myocardial remodeling and dysfunction in the late stage of cardiomyopathy.

DISCUSSION

Our findings demonstrate that administration of HGF suppressed myocardial hypertrophy, fibrosis, and dysfunction in cardiomyopathic hamsters. In controls not given HGF, the hamsters showed marked cardiac dysfunction and loss of body weight and activity, and some died of cardiac failure, which means that the physiological status of dilated cardiomyopathy progressed to the decompensated stage. Administration of HGF attenuated cardiac dysfunction and suppressed loss of body weight and activity. We also showed suppression of TGF-β1, type I collagen, and ANP expression by exogenous HGF, which is consistent with the improvement of established myocardial fibrosis and hypertrophy. The biological function of HGF on TGF-β1 and type I collagen was also shown in vitro using cultured myofibroblasts. There are reports on therapeutic approaches for treatment of cardiomyopathic hamsters (28, 33, 47), but there is no previous documentation on amelioration of such a late-stage pathophysiology.

The upregulation of TGF-β1 is closely associated with tissue fibrosis (5). TGF-β1 induces transdifferentiation and fibrogenic gene expression in myofibroblasts, which are responsible for fibrotic change in tissues (9, 19, 21). For cardiac myocytes, TGF-β1 stimulates excess hypertrophy and induces contractile dysfunction (18). In human myocardial samples, cardiac hypertrophy and fibrosis were significantly correlated with upregulation of TGF-β1 and angiotensin-converting enzyme (ACE) (14). Using a genetic approach, Schultz et al. (36) showed that a lack of TGF-β1 blocked the increase in left ventricular mass, the increase in myocyte size, and the deterioration in cardiac contractility induced by ANG II injection, suggesting that regulation of TGF-β1 may improve cardiac hypertrophy and remodeling. Our findings that supplemental HGF conversely downregulated cardiac TGF-β1 levels also explain the possible mechanisms of the decreased susceptibility of cardiac cells to myocardial remodeling and fibrosis. Therefore, it is noteworthy that endogenous cardiac HGF was downregulated in the late stage of TO-2 hamsters and that the

Fig. 7. Suppression of hypertrophic and apoptotic changes in cardiomyocytes by HGF treatment. A–C: appearances of fibers visualized using wheat germ agglutinin staining in F1β hamster (A), TO-2 hamster treated with saline (B), and TO-2 hamster treated with 1 mg·kg⁻¹·day⁻¹ HGF (C). Bar, 100 μm. D: quantification of fiber area. At least 3 areas were evaluated in each section, and each value represents the mean ± SE (n = 5 in each group). *P < 0.05, **P < 0.01 vs. saline. E–G: distribution of apoptotic cardiomyocytes as detected by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining in F1β hamster (E), TO-2 hamster treated with saline (F), and TO-2 hamster treated with 1 mg·kg⁻¹·day⁻¹ HGF (G). H: quantification of cardiomyocyte apoptosis. At least 500 cardiomyocytes were evaluated in each hamster, and each value represents the mean ± SE (n = 5 in each group). *P < 0.05 vs. saline. n.d., Not detectable.
decreased cardiac HGF levels were inversely related to the increase in cardiac soluble collagen levels, in terms of a pathognomonic point of view. Because transcription of HGF is negatively regulated by TGF-β1 in various cell types (13, 25), the decrease in cardiac HGF levels seems attributable to the increase in TGF-β1 levels. HGF also seems to be involved in regenerative and therapeutic mechanisms in tissue fibrosis, such as liver cirrhosis and chronic renal disease, where TGF-β1 pathologically plays an essential role (26, 44). Antifibrotic actions of HGF on hepatic and renal fibrosis have been associated with the suppression of TGF-β1 expression in these tissues (26, 44).

Although the present findings of gene expression involved in myocardial remodeling and fibrosis explain therapeutic effects of HGF, previously noted biological activities of HGF seem to explain additional mechanisms responsible for improvement of dilated cardiomyopathy by HGF. HGF directly activates transcriptional factor ets-1 and upregulates the transcription of a collagenase (matrix metalloproteinase-1) and possibly other types of proteases involved in breakdown of extracellular matrix proteins (2, 27, 32, 40, 43). Another possibility involves nitric oxide (NO) production. NO derived from endothelial cells regulates not only vascular homeostasis but also cardiac contractility (6, 15).

Long-term inhibition of NO synthesis leads to upregulation of tissue ACE and accelerated cardiac fibrosis (39, 42), which could be prevented by neutralization of TGF-β1 (22). On the other hand, previous studies have indicated that HGF enhances NO production in distinct types of cells, including endothelial cells, and that NO mediates biological activities of HGF (10, 22, 34).

It is well-documented that inhibition of ANG II by either an inhibitor for ACE or type I receptor antagonist attenuates not only myocyte hypertrophy but also fibrotic change of the heart in cardiomyopathic hamsters (28, 41, 47), although it is not yet clear how these agents elicit an antifibrogenic effect. Interestingly, antifibrogenic action of ANG II blockade in cardiomyopathic hamsters has been associated with an increased expression of HGF (41). Together with our findings that HGF suppressed myocardial fibrosis, these results indicate that attenuation of the fibrotic change by ANG II blockade can be attributed, to some extent, to the antifibrotic effect of HGF.

It is also notable that in pacing-induced heart failure in the canine model, which otherwise shows significant myocardial dilatation and dysfunction, HGF gene transfection improved cardiac contractility, attenuated wall thinning and left ventricular dilatation, and increased myocardial microvascular perfusion (1). Although we should consider hemodynamic dissimilarity between that model and our present model, a mechanism by which HGF improved cardiac contraction has yet explained. Perhaps it may be attributable, at least in part, to biological activities of HGF, including the improvement of myocardial remodeling and/or hemodynamic overload (49) and the increase in cardiac angiogenesis (2, 40, 43), as well as the suppression of TGF-β1 expression and apoptosis in cardiomyocytes. Although further studies on mechanisms by which HGF improves untoward events in dilated cardiomyopathy are needed, our results may provide a rationale for the potential therapeutic value of HGF for treatment of dilated cardiomyopathy.

ACKNOWLEDGMENTS

We are grateful to M. Ohara (Fukuoka) for language assistance and pertinent comments and to Dr. S. Miyagawa (Osaka University Graduate School of Medicine) for technical support.

GRANTS

This work was supported by the 21st Century Center of Excellence Program and Grant-In-Aid 14207005, both from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, as well as a research grant from the Japan Heart Foundation and an IBM Japan Research Grant.

REFERENCES

18. Kim NN, Villarreal FJ, Printz MP, Lee AA, and Dillmann WH. Trophic effects of angiostatin II on neonatal rat cardiac myocytes are


