Postinfarction gene therapy with adenoviral vector expressing decorin mitigates cardiac remodeling and dysfunction

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1Division of Cardiology, Gifu University Graduate School of Medicine, Gifu; 2Department of Gene Therapy and Regenerative Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima; 3Division of Gene Therapy and Regenerative Medicine, Cognitive and Molecular Research Institute of Brain Diseases, Kurume University, Kurume; and 4Department of Food Science, Kyoto Women’s University, Kyoto, Japan

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Postinfarction gene therapy with adenoviral vector expressing decorin mitigates cardiac remodeling and dysfunction. Am J Physiol Heart Circ Physiol 297: H1504–H1513, 2009. First published August 14, 2009; doi:10.1152/ajpheart.00194.2009.—The small leucine-rich proteoglycan decorin is a natural inhibitor of transforming growth factor-β (TGF-β) and exerts antifibrotic effects in heart and to stimulate skeletal muscle regeneration. We investigated decorin’s chronic effects on postinfarction cardiac remodeling and dysfunction. Myocardial infarction (MI) was induced in mice by left coronary artery ligation. An adenoviral vector encoding human decorin (Ad.CAG-decorin) was then injected into the hindlimbs on day 3 post-MI (control, Ad.CAG-LacZ). Four weeks post-MI, the decorin-treated mice showed significant mitigation of the left ventricular dilatation and dysfunction seen in control mice. Although infarct size did not differ between the two groups, the infarcted wall thickness was greater and the segmental length of the infarct was smaller in decorin-treated mice. In addition, cellular components, including myofibroblasts and blood vessels, were more abundant within the infarcted area in decorin-treated mice, and fibrosis was significantly reduced in both the infarcted and noninfarcted areas of the left ventricular wall. Ten days post-MI, there was greater cell proliferation and less apoptosis among granulation tissue cells in the infarcted areas of decorin-treated mice. The treatment, however, did not affect proliferation and apoptosis of salvaged cardiomyocytes. Although decorin gene therapy did not affect TGF-β1 expression in the infarcted heart, it inhibited Smad2/3 activation (downstream mediators of TGF-β signaling). In summary, postinfarction decorin gene therapy mitigated cardiac remodeling and dysfunction by altering infarct tissue noncardiomyocyte dynamics and preventing cardiac fibrosis, accompanying inhibition of Smad2/3 activation.

heart failure; myocardial infarction; transforming growth factor-β

CHRONIC HEART FAILURE has emerged as a leading cause of mortality and morbidity worldwide. At present, patients with chronic heart failure have a poor prognosis (26) and a high likelihood that they will have to be readmitted to hospital, despite treatment (16, 26). The most common cause of heart failure is myocardial infarction (MI)-induced remodeling of the left ventricle (LV), which is characterized by LV dilatation and diminished cardiac performance (3, 13, 14, 39). Therefore, to improve clinical outcomes among patients with MI, it will be essential to develop therapies that effectively inhibit the resultant LV remodeling.

The most critical determinant of subsequent LV remodeling and eventual heart failure is the magnitude of the acute MI, which can be determined within several hours of an attack (41). The process of cardiac remodeling is complicated, however, and many other factors, including late death or hypertrophy of cardiomyocytes, fibrosis, and the expression of various cytokines, are associated with the continued disease progression during the chronic stage (5, 32, 47, 53). Several lines of evidence point to the critical role played by transforming growth factor-β (TGF-β) during the progression of myocardial fibrosis, suggesting that TGF-β plays a critical role during the healing process following MI and thus affects cardiac remodeling and function during the chronic stage (10, 17, 29). Soluble TGF-β type II receptor (sTβRII) inhibits the action of TGF-β, most likely by adsorbing TGF-β or by acting as a dominant-negative receptor (20). Our laboratory previously reported that the postinfarction gene therapy with adenoviral vector encoding sTβRII mitigated cardiac remodeling and dysfunction at the chronic stage of MI by affecting cardiac fibrosis and infarct tissue dynamics (37). It was thus suggested that a therapy aimed at suppressing TGF-β signaling might represent a new approach to the treatment of post-MI heart failure, applicable during the subacute stage.

Decorin is a small chondroitin-dermatan sulfate proteoglycan, consisting of a core protein and a single glycosaminoglycan chain (25, 43). Importantly, decorin negatively regulates TGF-β by binding it and neutralizing its biological activity, i.e., a natural inhibitor of TGF-β (54). Levels of decorin are reportedly increased in myocardial tissue from patients who have undergone implantation of an LV assist device, which induces regression of fibrosis (21). The most recent study suggests that in vivo transfer of decorin gene promotes skeletal muscle regeneration after injury (28). In the present study, therefore, we hypothesized that postinfarction treatment with decorin may mitigate chronic heart failure by affecting the LV remodeling process. Decorin protein, when intravenously administered, rapidly disappears from the circulation, and ~70% of the dose is trapped by the liver within 10 min (31). Thus a continuous protein supply is necessary to maintain the plasma level of decorin sufficient to display the effect on the target organ, and a gene therapy is appropriate for that purpose.
However, a virus-mediated gene delivery directly into the systemic circulation is potentially harmful, occasionally lethal, through viremia or immune reaction (24, 30). Thus a local gene delivery is more preferable; at the site, decorin is continuously produced and released to the systemic circulation, reaching the heart. Direct injection of therapeutic genes into the heart may be more effective, but we selected less invasive and more feasible method (injection into hindlimb muscles) for gene delivery in the present study. Adenoviruses and adenovirus-associated viruses are currently the most effective vectors for delivering therapeutic genes in the cardiovascular system (42). In the present study, we initiated adenovirus-mediated transduction of the decorin gene into mouse hindlimb muscles on day 3 of MI, times at which the therapy does not affect acute ischemic death of cardiomyocytes, and examined its effects on post-MI heart failure at the chronic stage.

MATERIALS AND METHODS

Recombinant adenoviral vectors. The adenoviral vector plasmid pHAd-decorin, which harbors the cytomegalovirus immediate-early enhancer, a modified chicken β-actin promoter and human decorin cDNA (Ad.CAG-decorin), was constructed using the in vitro ligation method, as previously described (33). Control adenovirus harboring the LacZ gene (Ad.CAG-LacZ) was prepared as previously described (6).

Evaluation of decorin expression in vitro. HeLa cells were transfectected with pHM5-CAG-decorin plasmid using Lipofectamine 2000 (Invitrogen) and cultured for 48 h at 37°C. Levels of human decorin in culture supernatants and in cell lysates were evaluated by Western blotting. In addition, levels of decorin in cultured cells were assessed immunohistochemically using anti-human decorin (R&D Systems) as the primary antibody and Alexa Fluor 488 anti-goat IgG (Invitrogen) as the secondary antibody. Nuclei were stained with 4′,6-diamidino-2-phenylindole. The cells were observed under a laser scanning confocal microscope (LSM510, Zeiss).

Animal experimental protocols. This study was approved by our institutional animal research committee and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (National Institutes of Health publication no. 85-23, revised 1996). MI was induced in 10-wk-old male C57BL/6J mice (Japan SLC, Shizuoka, Japan) by ligating the left coronary artery, as previously described (36, 38). Ad.CAG-decorin (1 × 1011 particles per mouse) was then injected into the hindlimb muscles of the mice. As a control, Ad.CAG-LacZ was injected in the same manner. In sham-operated mice, the suture was passed but not tied. MI was induced in 50 mice. Of those, 34 remained alive on post-MI and were entered into the study, randomly assigned to the decorin (n = 16) or LacZ (n = 18) treatment group, and followed up for 4 wk. Sham-operated mice (n = 8) were injected with the same volume of saline in a similar manner and examined 4 wk later. In another experiment, 14 mice were divided into decorin and LacZ treatment groups (n = 7 each) on day 3 post-MI, and the survivors (n = 5 in the decorin group and n = 4 in the LacZ group) were examined on day 10 post-MI.

Physiological studies. Echocardiography and cardiac catheterization were performed before death, as previously described (36, 38). Animals were anesthetized with halothane (induction, 2%; maintenance, 0.5%) in a mixture of N2O and O2 (0.5 l/min each) via a nasal mask. Echocardiograms were recorded 4 wk post-MI using an echocardiographic system (Vevo770, Visualsonics) equipped with a 45-MHz imaging transducer. The right carotid artery was then cannulated with a micromanometer-tipped catheter (SPR 671, Millar Instrument) that was advanced into the LV via the aorta to record pressures and change in pressure over time.
an ApopTag kit (Chemicon) according to the supplier’s instructions. Mouse mammary tissue served as a positive control. For double immunofluorescent labeling, tissue sections were first stained with Fluorescein-FragEL (Oncogene) and then labeled with anti-α-SMA or anti-vWF, followed by Alexa Fluor 568. In addition, to evaluate cell proliferative activity and apoptosis of the salvaged cardiomyocytes, we performed double immunofluorescence for myoglobin (1:1,000), combined with Ki-67 (1:25) or TUNEL. Tissue sections were first stained with anti-Ki-67 followed by Alexa 488 or Fluorescein-FragEL and then labeled with anti-myoglobin antibody (DAKO) followed by Alexa 568. Nuclei were stained with Hoechst 33342. Immunofluorescence preparations were observed under a confocal microscope (LSM510, Zeiss).

Western blotting. Proteins extracted from cultured cells, whole ventricles of hearts, or hindlimb muscles were subjected to 14% polyacrylamide gel electrophoresis and then transferred to polyvinylidene difluoride membranes. The membranes were then probed with a primary antibody against human decorin, mouse decorin (both from R&D Systems), TGF-β, Smad2, the phosphorylated form of Smad2 (p-Smad2), Smad3, p-Smad3 (all from Cell Signaling), or plasminogen activator inhibitor type 1 (PAI-1; Santa Cruz). Three to five hearts or hindlimb muscles from each group were subjected to the blotting. The blots were visualized using enhanced chemiluminescence (Amersham), and the signals were quantified by densitometry. α-Tubulin (Santa Cruz) served as the loading control.

Electron microscopy. After the hearts were excised, cardiac tissue was quickly cut into 1-mm cubes, immersion fixed in 2.5% glutaraldehyde in 0.1 mol/l phosphate buffer (pH 7.4) overnight at 4°C, and postfixed in 1% buffered osmium tetroxide. The specimens were then dehydrated through a graded ethanol series and embedded in epoxy resin. Ultrathin sections (90 nm), double-stained with uranyl acetate and lead citrate, were examined under an electron microscope (H-800, Hitachi).

Fig. 2. Left ventricular (LV) geometry and function at the chronic stage (4 wk post-MI) in mice receiving decorin or LacZ gene on day 3 after MI. A: echocardiographic data. B: cardiac catheterization data. LVd, LV end-diastolic diameter; LVWT, LV end-diastolic wall thickness at diastole; LVSP, LV peak systolic pressure; dP/dt, change in pressure over time; NS, nonsignificant; bpm, beats/min. Values are means ± SE. *P < 0.05 vs. sham-operated mice; #P < 0.05 vs. LacZ-treated MI mice.

Statistical analysis. Values are shown as means ± SE. Survival was analyzed using the Kaplan-Meier method with the log-rank Cox-Mantel method. The significance of differences was evaluated using t-tests or one-way ANOVA followed by the Newman-Keul’s multiple-comparison test. Values of P < 0.05 were considered significant.

RESULTS

Expression of human decorin in vitro and in vivo. Human decorin was strongly expressed on Hela cells transfected with the human decorin gene (Fig. 1, A and B). In MI mice administered the gene via intramuscular injection into hindlimbs, human decorin was expressed not only in hindlimb muscles, but also in hearts 1 wk after administration (Fig. 1C), indicating that the gene product reached the hearts. However, we failed in immunohistochemical detection of human decorin in the heart (photographs not shown), suggesting that the amount of decorin fixed in the cardiac tissue was too small to be detected by the immunohistochemical assay, and that the decorin protein detected by Western blots was mostly the circulating one within the heart. Four weeks later, the expression was markedly reduced but still detectable in both the hindlimb muscles and hearts. No human decorin was detected in the LacZ-treated (control) mice at any time.

Effect of decorin gene treatment on MI at the chronic stage. Within 10 days after induction of MI, six (34%) of the controls and five (31%) of the decorin-treated mice had died. The remaining mice all survived to the end of the observation period (4 wk post-MI). In total, the survival rate was 66% in
the control group and 69% in the decorin-treated group 4 wk post-MI ($P = $ nonsignificant).

Echocardiography and cardiac catheterization carried out 4 wk post-MI revealed that, compared with the sham-operated mice, control MI mice had marked enlargement of the LV cavity and reduced cardiac function, as indicated by increased LV end-diastolic diameter, increased diastolic thickness of both noninfarct and infarct walls, reduced LV percent fractional shortening, increased LV peak systolic pressure, and increased change in pressure over time (Fig. 2). All of these structural and functional parameters were attenuated in decorin-treated mice, suggesting decorin in some way mitigates post-MI remodeling and cardiac dysfunction. However, the systolic thickening of infarct wall was not increased by the decorin treatment (Fig. 2).

Hearts from control mice showed marked LV dilatation with a thin infarcted segment, while those from decorin-treated mice showed substantially smaller LV cavities and thicker infarcted segments, with shorter circumferential lengths (Fig. 3A and Table 1). On the other hand, both the absolute area of the infarct and the percentage of the LV taken up by the infarct were comparable between the two groups (Table 1).

By 4 wk post-MI, the infarct area had been replaced by fibrous scar tissue in the control mice (Fig. 3B). In the decorin-treated mice, by contrast, not only collagen fibers but also abundant cellular components were present. As a result, the population of noncardiomyocyte interstitial cells within the infarct area was significantly greater, and the percentage of fibrotic tissue was significantly smaller in decorin-treated mice (Table 1). The number of vWF-positive blood vessels within the infarct area and the percent area of extravascular $\alpha$-SMA-positive cells were also greater in decorin-treated mice than control mice (Fig. 3, C and D, and Table 1). On the other hand, there was no significant difference in the populations of CD45-
Table 1. Effects of treatment with the indicated gene on the morphometry and histology of hearts bearing 4-wk-old MI

<table>
<thead>
<tr>
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<th>LacZ</th>
<th>Decorin</th>
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<tr>
<td>Infarcted area</td>
<td></td>
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<tr>
<td>MI wall thickness, μm</td>
<td>2.9±0.4</td>
<td>4.7±0.4*</td>
</tr>
<tr>
<td>MI segment length, mm</td>
<td>14.2±1.1</td>
<td>10.5±0.7*</td>
</tr>
<tr>
<td>%MI segment in LV</td>
<td>50.9±3.9</td>
<td>33.6±2.1*</td>
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<tr>
<td>%MI area in LV</td>
<td>29±8.4</td>
<td>26±6.1</td>
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<tr>
<td>Cell population, no./HPF</td>
<td>1,102±105</td>
<td>1,521±133*</td>
</tr>
<tr>
<td>vWF* vessels, no./HPF</td>
<td>4.8±1.4</td>
<td>9.8±1.3*</td>
</tr>
<tr>
<td>α-SMA* area, %</td>
<td>2.7±0.4</td>
<td>5.1±0.7*</td>
</tr>
<tr>
<td>CD45* leukocytes, no./HPF</td>
<td>2.3±0.3</td>
<td>2.4±0.5</td>
</tr>
<tr>
<td>%Fibrosis</td>
<td>62±1.3</td>
<td>51±2.9*</td>
</tr>
<tr>
<td>Noninfarcted area</td>
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<tr>
<td>Myocyte size, μm</td>
<td>17.6±0.8</td>
<td>15.0±0.7*</td>
</tr>
<tr>
<td>%Fibrosis</td>
<td>7.1±0.5</td>
<td>4.4±0.5*</td>
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Values are means ± SE; n, no. of mice. MI, myocardial infarction; LV, left ventricular; HPF, high-power field; α-SMA, α-smooth muscle actin; vWF, von Willebrand factor. *P < 0.05 vs. LacZ-treated MI mice.

Fig. 4. Cell proliferation within granulation tissue 10 days post-MI. A: photomicrographs showing Ki-67-positive cells (left) and a graph comparing the incidences of proliferating cells in each group (right). B and C, left: confocal photomicrographs of tissue sections from a decorin-treated heart immunolabeled with antibodies against Ki-67 (green fluorescence) plus α-SMA (B) or vWF (C) (red fluorescence). Scale bars, 20 μm. Right: graphs showing the incidences of Ki-67 positivity separately evaluated in myofibroblasts and endothelial cells. HPF, high-powered field. Values in graphs are means ± SE. #P < 0.05 vs. LacZ-treated MI mice.
gene (Fig. 5B). Further analysis using double immunofluorescent labeling revealed that decorin treatment significantly reduced the incidence of TUNEL positivity among myofibroblasts, but not endothelial cells (Fig. 5, C and D). Next, we investigated necrosis by immunohistochemistry for C9, which is a part of the membrane attacking complex C5b-9 (11). No C9-immunopositive necrotic cell was found in granulation tissue cells of any groups (photographs not shown). This finding is consistent with previous electron microscopic studies showing that the dying mode of postinfarct granulation tissue cells is not necrosis, but apoptosis (9, 37, 51).

To check whether the treatment promoted viable cardiomyocytes to replace the function of the infarcted area, we examined cell proliferation activity and apoptosis in the salvaged myocardium. Double immunofluorescence for myoglobin and Ki-67 revealed no proliferating cardiomyocyte in viable myocardium 10 days post-MI (Fig. 6A). We noted TUNEL-positive nuclei of cardiomyocytes in viable myocardium. However, they were extremely rare in each group, and there was no significant difference in the prevalence between the groups (10 days post-MI: sham operated group, $0.012 \pm 0.007\%$; LacZ-treated MI group, $0.010 \pm 0.008\%$; decorin-treated MI group, $0.013 \pm 0.011\%$). These findings do not support the possibility that the present treatments increased regeneration or reduced cell loss due to apoptosis of viable cardiomyocytes to replace the infarcted area.

Western blot analysis showed that expression of TGF-β was significantly upregulated in heart tissues collected on day 10 post-MI (Fig. 7). Moreover, we observed marked activation (phosphorylation) of Smad2 and Smad3, two downstream mediators of TGF-β. Although decorin treatment did not affect TGF-β or endogenous (mouse) decorin expression, it significantly suppressed Smad2 and Smad3 activation (Fig. 7), which implies that inhibition of signaling in the TGF-β/Smad2/Smad3 pathway contributes substantially to the beneficial effects exerted by decorin against post-MI cardiac remodeling and heart failure. PAI-1 is one of the other important team players in fibrosis (56). Myocardial PAI-1 expression was unchanged in the heart 10 days post-MI, which was not influenced by the decorin gene therapy either (Fig. 7).
DISCUSSION

Our findings provide the first evidence that postinfarction decorin gene therapy, started on day 3 post-MI, mitigates the adverse effects on LV geometry and function during the chronic stage.

Pathophysiological mechanisms for the beneficial effects of decorin gene therapy on postinfarction heart failure. One remarkable finding of the present study is that decorin alters the geometry of the infarct scar without affecting its absolute area, i.e., the infarcted segment was thicker and had a smaller circumferential length in decorin-treated hearts during the chronic stage than in control hearts. This is noteworthy because wall stress is directly proportional to cavity diameter and inversely proportional to wall thickness (Laplace’s law) (55) and because wall stress and LV remodeling (dilatation) have a vicious relationship and exacerbate one another. It is thus conceivable that the observed change in infarct geometry would greatly improve the hemodynamic state of the heart.

Our findings also suggest that infarct scar tissue is qualitatively altered by treatment with decorin gene. We observed greater numbers of cells, including abundant myofibroblasts and vascular cells, within the infarct scar in decorin-treated hearts. These cells are normally destined to disappear via apoptosis during the natural course of healing after MI (9, 51), but we found that apoptosis was significantly inhibited in decorin-treated hearts during the subacute stage (10 days post-MI). Moreover, decorin gene therapy also significantly increased cardiac proliferation of both myofibroblasts and vascular endothelial cells. These findings have two important implications. First, both diminished apoptosis and enhanced proliferation among granulation tissue cells during the subacute stage appear to contribute to the observed increase in the cell population within the scar tissue during the chronic stage, which likely preserves the infarct wall thickness. Second, myofibroblasts, which are known to play an important role in wound contraction during the healing process (12), could mediate contraction-induced reduction in the length of the infarct segment, thereby increasing infarct wall thickness. That, in turn, would alter the infarct tissue geometry, reducing wall stress and mitigating LV dilatation and dysfunction.

Vascular endothelial cells also proliferated during the granulation tissue phase in hearts treated with decorin, suggesting an angiogenic effect of decorin. Decorin suppresses malignant tumor cell-mediated angiogenesis (15), whereas it promotes angiogenesis in normal tissue during the healing stage or when ectopically expressed (45, 46). Our finding was well consistent with the latter reports on the role of decorin in angiogenesis. The function of proliferated vascular endothelial cells during the chronic stage of MI remains unclear, although it has been shown that, by supplying blood, newly formed vessels help sustain the cellular components within the infarct area (50). Recently, we reported that blood flow into the infarct area by late reperfusion promotes proliferation and inhibits apoptosis among granulation tissue cells (35). On the other hand, leukocytes continued to die. We suggest that leukocytes may have a higher sensitivity to apoptotic stimuli than other preserved cells, because inflammatory cells generally show very active

Fig. 6. Cell proliferation and TUNEL positivity of the salvaged cardiomyocytes 10 days post-MI. A: confocal photomicrographs of tissue sections from a decorin-treated heart subjected to double immunofluorescence for Ki-67 (green fluorescence) and myoglobin (red fluorescence). There was no Ki-67-positive cardiomyocyte on the preparation where some Ki-67-positive granulation tissue cells or interstitial cells were noted. B: confocal photomicrographs of tissue sections from a LacZ-treated heart subjected to double immunofluorescence for TUNEL (green fluorescence) and myoglobin (red fluorescence). TUNEL-positive nuclei of cardiomyocytes were noted, although very rarely. Graph shows the incidences of TUNEL-positive nuclei in cardiomyocytes. Scale bars, 20 μm. Values in graphs are means ± SE.
proapoptotic interactions through death ligands and receptors (34).

We also observed that decorin gene therapy significantly reduces cardiac fibrosis, confirming the previously reported antifibrotic effect of decorin in hearts (21). Because myocardial fibrosis contributes to both systolic and diastolic dysfunction (5, 22), its reduction is likely another important way in which decorin may mitigate LV remodeling and heart failure.

In contrast to granulation tissue cells, TUNEL positivity in salvaged cardiomyocytes was not affected by the decorin gene therapy, suggesting no contribution of cardiomyocyte death via apoptosis to the beneficial effects of the therapy. The prevalence of TUNEL-positive cardiomyocyte nuclei was very rare (<0.01%). Although the value is consistent with the previous reports, including ours (38, 44), the discrepancy of the values reported is surprisingly great, ranging from 0.02 to 12% among the studies using mouse model of subacute to chronic stage MI: maximally 600-fold difference (38, 44, 49). It may be problematic that such a critical discrepancy in the TUNEL-positive cardiomyocyte rates remains not yet reconciled, the reason of which should be elucidated in the future.

Ki-67-positive nuclei were not immunohistochemically detected in cardiomyocytes under the present staining conditions. However, immunohistochemical negativity does not always deny the slight expression of an antigen, because the sensitivity depends on the staining conditions. On the other hand, too much sensitivity may violate specificity. Beltrami et al. (2) previously reported cardiomyocyte proliferation by immunohistochemistry in human hearts with MI. It is possible that our immunostaining method for Ki-67 is relatively less sensitive compared with that of the previous report. Notwithstanding, we did detect Ki-67 expression in the granulation tissue cells on the same immunohistochemical preparations, suggesting that our immunohistochemistry was not too insensitive. Difference in immunohistochemical sensitivity and difference in species might have yielded the discrepancy between the studies. The infarct wall did not show a significant systolic thickening, even in the decorin-treated groups, unlikely supporting a possible increase of cardiomyocyte population through increased regeneration and/or reduced apoptosis by the treatment.

Molecular mechanisms involved in the beneficial effects of decorin gene therapy. TGF-β signaling controls a diverse set of cellular processes, including cell apoptosis, differentiation, and proliferation (19, 23, 48). It has also been suggested that TGF-β signaling has both proapoptotic and profibrotic effects on the heart (29, 37). SMAD proteins are important downstream mediators of TGF-β signaling (8, 18), and their absence reportedly impairs local inflammatory responses and accelerates wound healing (1). The most recent findings indicate that loss of Smad3 (in Smad3-null mice) significantly increases myofibroblast density in healing infarcts and prevents myocardial fibrosis (4). In the present study, we observed that cardiac expression of TGF-β, p-Smad2, and p-Smad3 was strongly upregulated in control mice during the granulation tissue phase, 10 days post-MI. Notably, mice receiving the decorin gene expressed high levels of human decorin in the heart 10 days post-MI. Although decorin treatment had no effect on TGF-β expression, it largely blocked the activation of Smad2
and Smad3. This finding, together with those of the aforementioned studies by others, suggests that inhibition of the TGF-β/Smad2/Smad3 pathway contributes substantially to the reduction in cardiac fibrosis, as well as the reduced apoptosis and increased proliferation seen among granulation tissue cells in decorin-treated mice. Collectively, these effects would be expected to alter infarct tissue geometry to reduce wall stress and suppress myocardial fibrosis, thereby mitigating post-MI cardiac remodeling and dysfunction.

Hao et al. (17) previously reported increased protein expression levels of Smad2 and 3 in the rat MI model, which may appear to be inconsistent with our results that revealed activation of Smad2 and Smad3, but not upregulation of them. The most important difference may be the timing of examination, as well as difference in species (rat vs. mouse), which caused apparent conflict between the studies; Hao et al. examined MI at 8 wk after the onset, while we used 10-day-old MI. Hao et al. actually found that endogenous decorin expression was stronger in the hearts with older infarction.

It was not unexpected that the decorin gene therapy showed beneficial effects on postinfarction hearts in a strikingly similar manner as the previously reported stTβRII gene therapy, because decorin is a natural inhibitor of TGF-β, while stTβRII competitively inhibits binding of TGF-β with the TGF-β receptor (37). Pathophysiological mechanisms were very similar between these two gene therapies, as discussed above. In the present study, however, we found that decorin could increase cell proliferation in postinfarction granulation tissue and elucidated the inhibitory effect of decorin on TGF-β downstream signaling activation.

Possible clinical implications and limitations. Rapid recanalization of the occluded coronary artery, which salvages ischemic myocardial cells, is the best clinical approach at present to treating acute MI. Unfortunately, most patients actually lose their chance for coronary reperfusion therapy because it is only effective if performed within hours after the onset of infarction (41). The present findings suggest a novel therapeutic strategy, applicable during the subacute stage of MI, that may mitigate chronic progressive heart failure in MI patients who missed their chance for coronary reperfusion during the acute stage. In addition, treatment with decorin may be more promising than the use of stTβRII, which simply inhibits TGF-β signaling, when considering its additive beneficial effects, such as anti-tumor metastasis actions (15, 40, 52).

However, safety of anti-decorin strategies has not been confirmed in humans. In addition, ethical consensus has not been established at all in the safety of a virus-mediated gene therapy. These issues should be resolved before clinical application of the anti-decorin gene therapy.

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REFERENCES


