Gene silencing of myofibrillogenesis regulator-1 by adenovirus-delivered small interfering RNA suppresses cardiac hypertrophy induced by angiotensin II in mice

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Dai W, He W, Shang G, Jiang J, Wang Y, Kong W. Gene silencing of myofibrillogenesis regulator-1 by adenovirus-delivered small interfering RNA suppresses cardiac hypertrophy induced by angiotensin II in mice. Am J Physiol Heart Circ Physiol 299:H1468–H1475, 2010. First published August 27, 2010; doi:10.1152/ajpheart.00582.2009.—Our previous studies proved that myofibrillogenesis regulator (MR)-1 has a close relationship with cardiac hypertrophy induced by ANG II. In the present study, we developed a recombinant adenoviral vector (AdSiR-MR-1) driving small interfering (si)RNA against MR-1 to evaluate its effect on cardiac hypertrophy in vivo. Cardiac hypertrophy was induced by chronic ANG II infusion in mice; AdSiR-MR-1 was administered via the jugular vein through one bolus injection. Thirteen days after the injection, viral DNA was still detectable in the heart, validating the efficiency of gene transfer. Expression levels of MR-1 mRNA and protein were increased by 2.5-fold in the heart after ANG II infusion; AdSiR-control, which contained a scrambled siRNA sequence, had no effect on them. AdSiR-MR-1 treatment abolished the upregulation of MR-1 induced by ANG II. The silencing effect of AdSiR-MR-1 was observed in many other tissues, such as the liver, lung, and kidney, except skeletal muscle. ANG II-induced cardiac hypertrophy was suppressed in mice treated with AdSiR-MR-1, as determined by echocardiography. Morphological and immunohistochemical examinations revealed that interstitial cardiac fibrosis as well as infiltrating inflammatory cells were increased after ANG II infusion; AdSiR-MR-1 greatly ameliorated these disorders. In ANG II-infused mice, MR-1 silencing also blocked the upregulation of other genes related to cardiac hypertrophy or metabolism of the extracellular matrix. In summary, our results demonstrate the feasibility of MR-1 silencing in vivo and suggest that MR-1 could be a potential new target to treat cardiac hypertrophy induced by ANG II.

RNA interference

CARDIAC HYPERTROPHY, characterized by the enlargement of cardiomyocytes, may develop in response to a variety of pathophysiological stimuli, such as pressure and/or volume overload as well as neurohumoral activations (2, 27). Although it is believed that cardiac hypertrophy initially represents an adaptive process, sustained and decompensated hypertrophy can ultimately progress to heart failure, arrhythmia, and sudden death (20, 25). In patients, cardiac hypertrophy is a powerful and independent predictor of subsequent mortality (8).

The causes for cardiac hypertrophy are extremely complex, and some hypertrophy-related genes play important roles (15). Previously, a novel human myofibrillogenesis regulator (MR)-1 gene was identified from a human skeletal muscle cDNA library in our laboratory (21, 23, 24). The MR-1 gene (GenBank Accession No. AF417001) is located on human chromosome 2q35 (GenBank Accession No. AC021016) and encodes a 142-amino acid protein with a hydrophobic transmembrane structure between residue 75 and 92. Orthologs of MR-1 were found in mammals but not in invertebrates or microbial genomes. The expression level of the MR-1 gene in human tissues is especially high in the skeletal muscle and myocardium (23). Yeast two-hybrid screening and an in vitro glutathione-S-transferase pull-down assay revealed that MR-1 interacts with three proteins involved in muscle contraction: myosin regulatory light chain, myomesin, and β-enolase. The interaction of MR-1 with sarcomeric proteins and its presence in the human myocardium indicate that it participates in the regulation of muscle contraction and has a close relationship with cardiac hypertrophy. Indeed, we found that the expression level of MR-1 was significantly elevated in ANG II-treated cardiomyocytes as well as in heart tissues from abdominal aortic stenosis hypertensive rats (24). Furthermore, overexpression of MR-1 aggravates cardiac hypertrophy induced by ANG II in mice via the activation of the NF-κB signaling pathway (21).

Our previous work also proved that knockdown of MR-1 expression with small interfering (si)RNA prevents ANG II-induced hypertrophy in cultured cardiomyocytes (24). To determine whether our in vitro findings have physiological relevance, we investigate the effects of MR-1 silencing in ANG II-mediated cardiac hypertrophy in vivo. In this study, a recombinant adenoviral vector carrying MR-1 siRNA (AdSiR-MR-1) was constructed and injected into mice with cardiac hypertrophy induced by ANG II. We demonstrated that adenoviral vector-delivered siRNA effectively silences MR-1 expression in the heart and that MR-1 silencing protects cardiac hypertrophy induced by ANG II. We suggest that MR-1 could be a potential new target for the treatment of cardiac hypertrophy in the future.

MATERIALS AND METHODS

Preparation of the recombinant adenoviral vectors. The oligonucleotide containing the siRNA target sequence for mouse MR-1 (5′-AACAGGGCTTTCTACAAACG-3′) and the appropriate restriction sites was synthesized and inserted into pSilencer 3.0-H1 (Ambion, Austin, TX) as previously described (24). A fragment containing the H1 promoter and the inserted sequence (H1 promoter-MR-1) was then excised by EcoRI/HindIII digestion and ligated into the pShuttle-Basic vector (SinoGenoMax Research Center, Beijing, China). After digestion with I-CeuI/I-SceI, the H1 promoter-MR-1 fragment was inserted into pAdxsi (SinoGenoMax Research Center),...
generating the recombinant adenoviral vector AdSiR-MR-1. AdSiR-control was constructed through the same procedure using a scrambled sequence (5'–GCAGACAAACGACGTCAAAGT-3'), which has a nucleotide composition close to that of the MR-1 target sequence. It was used as a control for the exclusion of nonspecific interference.

For the production of adenoviruses, the packaging cell line [human embryonic kidney (HEK)-293 cells] was maintained in DMEM supplemented with 10% FBS and the appropriate antibiotics. One day before transfection, cells were seeded into six-well plates with 5 × 10⁶ cells/well and incubated in an atmosphere of 5% CO₂ at 37°C. AdSiR-MR-1 and AdSiR-control were linearized with PstI and transfected into the cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the protocol. Six hours after transfection, fresh growth medium was added to the cells; cells were incubated and observed for pathological changes. Viruses were harvested and amplified as previously reported (12).

Animal experiments. Male C57BL/6J mice (weighing ~20 ± 2 g) were purchased from the Institute of Laboratory Animal Sciences (Beijing, China) and fed with regular rodent chow. Animals were housed in an air-conditioned room with 6 mice/cage and with a 12:12-h light-dark cycle. All of the animal experiments followed the instructions of the Laboratory Animal Management Statute of Beijing Municipality. Protocols were approved by the Ethics Committee of the Institute of Medicinal Biotechnology. Animals were cared for according to the institutional guidelines of Chinese Academy of Medical Sciences.

After 1 wk of accommodation, mice were randomly divided into the following three groups: the noninfused group, PBS-infused group, and ANG II-infused group, respectively. The noninfused group of animals was left unoperated. Other mice were anesthetized through an intraperitoneal injection of 2.5% avertin (Sigma Chemical, St. Louis, MO) at a dose of 14 µl/g body wt. Alzet mini-osmotic pumps (model 2002, Alza, Mountain View, CA) were then implanted subcutaneously in the flank. In the ANG II-infused group, ANG II (Sigma Chemical) was dissolved in sterile PBS containing 0.01 mol/l acetic acid and infused through the mini-osmotic pumps at a rate of 2.5 µg·kg⁻¹·min⁻¹. PBS was infused at the same rate. The infusions lasted for 2 wk.

Each of the three groups of animals was divided into three subgroups: untreated, treated with AdSiR-control, or treated with AdSiR-MR-1. The initial concentration of AdSiR-MR-1 and AdSiR-control was 5 × 10¹¹ plaque-forming units (pfu/ml). They were diluted in sterile PBS and administered to the mice through a single bolus rapid injection via the jugular vein on the second day of infusion. Animals were anesthetized with avertin during the siRNA injection.

Echocardiography. Two weeks after infusion, echocardiography was performed using an SSD-5500 ultrasonographic system (Aloka) as previously reported (32). After being anesthetized with 2.5% avertin, mice were placed in a supine position. A 12-MHz transducer was applied to the left hemithorax. Two-dimensional targeted M-mode imaging was obtained from the short-axis view at the level of the left ventricular free wall (LVFS), ventricular septum thickness in diastole (IVSd), and LV fractional shortening (LVFS). Tissue Doppler imaging was performed using an SSD-5500 ultrasonographic system (Aloka). Longitudinal 12-µm sections were cut transversely and stained with hematoxylin and eosin or Masson's trichrome. Cross-sectional areas of cardiomyocytes and the percentage of the fibrotic area in the LV were measured using NIH Image 1.61 software (National Institutes of Health, Bethesda, MD). Five fields were randomly selected for analysis on one section. Immunohistochemical determination of MR-1 protein expression and quantification were performed as previously described (24).

To detect infiltrating inflammatory cells, heart sections were incubated with neutrophil antibody 7/4 (AbD Serotec, Oxford, UK) or an anti-Mac-1 antibody (BD Biosciences, San Jose, CA) in the immunohistochemical examinations as described in our previous study (21). Positively stained cells were counted in 5 randomly selected fields/animal.

DNA isolation and Southern blot analysis. For viral DNA isolation, heart tissues were triturated and suspended in Hanks solution. After centrifugation at 3,000 rpm for 15 min, the supernatants were harvested; viral DNA was isolated using the Virus DNA/RNA Isolation Spin-Kit (AppliChem). After being electrophoresed on 0.7% agarose gel and transferred to a nitrocellulose membrane (Amer sham Pharmacia Biotech, Uppsala, Sweden), viral DNA was detected by a 33P-labeled probe specific for the adenoviral vector pAdxsi. Blots were scanned; signal intensities were determined with the Kodak 1D Image Analysis Software (Eastman Kodak, Rochester, NY).

Quantitative real-time RT-PCR. Total RNA was isolated from tissues using TRIzol reagent (Invitrogen, Carlsbad, CA). For reverse transcription, 1 µg of total RNA from each sample was used as a template in a 20-µl reaction system containing random primers and avian Myeloblastosis virus reverse transcriptase (Promega, Madison, WI). Reverse transcription reactions were conducted at 42°C for 20 min and then inactivated at 95°C for 5 min.

Quantitative real-time PCR was performed using the iQ5 Multicolor Real-Time PCR Detection System (Bio-Rad, Hercules, CA). The reaction volume was 25 µl, which included cDNA and Power SYBR green PCR Master Mix (Applied Biosystems, Foster City, CA) as well as 0.5 µmol/l of mouse gene-specific sense and antisense primers (Table 1). Reactions were performed at 95°C for 10 min followed by 30 cycles at 95°C for 15 s and 60°C for 1 min. Each experiment was repeated three times. β-Actin was used as an internal control for the relative quantification of target genes using the comparative threshold cycle method. Normalized mRNA expression levels were plotted as the fold difference from noninfused or PBS-infused untreated mice, which was designated as 1.

<table>
<thead>
<tr>
<th>Table 1. Primers used in real-time PCR</th>
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<tr>
<td>Target</td>
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</tr>
<tr>
<td>MR-1</td>
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<tr>
<td>Collagen type I</td>
</tr>
<tr>
<td>Collagen type III</td>
</tr>
<tr>
<td>Matrix metalloproteinase-2</td>
</tr>
<tr>
<td>Tissue inhibitor of metalloproteinase-2</td>
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<tr>
<td>β-Actin</td>
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MR-1, myofibrillogenesis regulator-1.
Western blot analysis. Proteins were extracted from tissues as previously described (24). Protein samples were subjected to SDS-PAGE, transferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Uppsala, Sweden), and probed with monoclonal antibodies against MR-1 (prepared by Jingmei Biotech, Beijing, China), atrial natriuretic factor (ANF; Santa Cruz Biotechnology, Santa Cruz, CA), β-myosin heavy chain (Santa Cruz Biotechnology), or β-actin (Santa Cruz Biotechnology). After an incubation with a peroxidase-conjugated secondary antibody, signals were visualized by a chemiluminescence kit (Amersham Pharmacia Biotech). Bands were scanned and quantified using Kodak 1D Image Analysis software. MR-1, ANF, and β-myosin protein levels were normalized to that of β-actin and plotted as indicated.

Statistical analysis. After validation of the test for homogeneity of variance, differences among study groups were examined by one-way ANOVA followed by the Newman-Keuls test for multiple comparisons. *P* values of <0.05 were considered as statistically significant.

RESULTS

MR-1 expression is significantly elevated in ANG II-infused mice. Cardiac hypertrophy in mice was induced by a chronic infusion of ANG II through implanted pumps. After 2 wk of infusion, echocardiography was performed. As shown in Fig. 1A, LVPWT in ANG II-infused mice significantly increased by ~31.8% compared with noninfused or PBS-infused mice (*P* < 0.01), indicating the development of LV hypertrophy. Mice were then killed, and their hearts were dissected and photographed. Hearts of the ANG II-infused mice were significantly enlarged compared with noninfused and PBS-infused mice (Fig. 1B). Heart weight-to-body weight ratios of the mice were determined. As shown in Fig. 1C, the mean heart weight-to-body weight ratio of ANG II-infused mice increased by 37.5% compared with noninfused or PBS-infused mice (*P* < 0.01), which was in agreement with echocardiographic data. Heart tissues from the LV were used for histological examination. As expected, the cross-sectional area of cardiomyocytes increased markedly in ANG II-infused mice compared with noninfused mice (608 ± 67 vs. 352 ± 45 μm², *P* < 0.01). Using Masson’s trichrome staining, we found that cardiac fibrosis significantly increased about threefold after ANG II infusion (*P* < 0.01), as indicated by the increase of the blue-stained area (Fig. 1D).

MR-1 expression levels were analyzed in all mice. The cardiac MR-1 mRNA level was increased by ~2.5-fold after ANG II infusion (*P* < 0.01), as determined by real-time RT-PCR (Fig. 2A). Accordingly, the MR-1 protein expression level increased to a similar extent in ANG II-infused mice (*P* < 0.01), as determined by immunohistochemical analysis of cardiac sections and quantification (Fig. 2B). Taken together, these results proved that MR-1 expression was greatly elevated in ANG II-induced cardiac hypertrophy in mice.

AdSiR-MR-1 silences MR-1 expression in vivo. AdSiR-MR-1 was administered to mice on the second day of ANG II infusion by one bolus injection, and mice were killed 13 days later. To determine if the recombinant adenoviral vector still existed in the mice at that moment, possible viral DNA was isolated from the heart and detected by Southern blot analysis. As shown in Fig. 3, 13 days after the jugular vein injection, adenoviral DNA was still visible in the heart of recombinant vector-treated mice, as seen in the AdSiR-MR-1 and AdSiR-control groups. In contrast, viral DNA was not detected in untreated mice.

We then treated the ANG II-infused mice with different doses of AdSiR-MR-1 to find its effective silencing dose; 1 × 10⁹, 2 × 10⁹, or 3 × 10⁹ pfu AdSiR-MR-1 were diluted in 100 or 300 μl PBS and administered to the mice via the jugular vein. Thirteen days later, mice were killed; cardiac MR-1 protein expression was determined by Western blot analysis. We found that significant silencing was observed with 2 × 10⁹ pfu AdSiR-MR-1 (data not shown). Therefore, we used this dose in subsequent experiments.

AdSiR-MR-1 or AdSiR-control at a dose of 2 × 10⁹ pfu was used to treat all three groups of mice. MR-1 mRNA expression levels in the heart were analyzed by real-time RT-PCR. Because the data of noninfused mice were the same as those of PBS-infused mice, we used the latter as a control. As shown in Fig. 4A, in the PBS-infused group, intrajugular delivery of AdSiR-MR-1 silenced MR-1 mRNA basal expression by...
nearly 50% compared with untreated or AdSiR-control treated mice \( (P < 0.01) \). ANG II infusion elevated the MR-1 mRNA level by ~2.5-fold in the heart, and AdSiR-control had no effect on MR-1 expression. However, one bolus injection of AdSiR-MR-1 almost completely abolished the overexpression of MR-1 induced by ANG II. The expression level of MR-1 mRNA returned to near baseline after AdSiR-MR-1 treatment \( (P < 0.01 \text{ vs. untreated or AdSiR-control-treated mice in the ANG II-infused group}) \).

Because these siRNAs were administered systematically, we also analyzed the expression of MR-1 in other tissues, such as the liver, lung, kidney, and skeletal muscle. Results (Fig. 4B) showed that after AdSiR-MR-1 treatment, reduction of MR-1 protein expression was observed in the heart, liver, lung, and kidney of ANG II-infused mice \( (P < 0.01 \text{ vs. AdSiR-control}) \). We found that the effect of AdSiR-MR-1 was not significant in the skeletal muscle; this is perhaps due to the inefficient binding of the adenovirus to skeletal muscle cells (34).

**AdSiR-MR-1 suppresses cardiac hypertrophy, fibrosis, and inflammation in ANG II-infused mice.** Echocardiography was performed in the mice before death to determine if AdSiR-MR-1 could reduce cardiac hypertrophy. Immediately after death, hearts were dissected, heart weight-to-body weight ratios were calculated, and tissues from the LV were subject to morphological analysis. The results are shown in Table 2. AdSiR-control had no effect on the increased heart weight-to-body weight ratio induced by ANG II infusion. Jugular vein injection of \( 2 \times 10^9 \text{ pfu AdSiR-MR-1} \) on the second day of infusion prevented the increase of heart weight and kept the heart weight-to-body weight ratio at baseline level \( (P < 0.01 \text{ vs. untreated or AdSiR-control-treated mice infused with ANG II}) \).

Echocardiography showed that LVEDD, LVESD, LVPWT, and IVSd were greatly dilated, whereas LVFS was reduced, after ANG II infusion, indicating the development of cardiac hypertrophy and decline of heart function (Table 2). AdSiR-MR-1 had no effect on these parameters in PBS-infused mice. However, in ANG II-infused mice, AdSiR-MR-1 reduced cardiac hypertrophy and improved heart function significantly, as indicated by the reduction of LVEDD, LVESD, LVPWT, and IVSd as well as the increase of LVFS \( (P < 0.05 \text{ vs. untreated or AdSiR-control-treated mice}) \).

To further determine the influence of MR-1 silencing, the expression levels of hypertrophy-related genes in the heart were analyzed. As shown in Fig. 5, protein expression levels of ANF and \( \beta \)-myosin heavy chain increased significantly after ANG II infusion \( (P < 0.01 \text{ vs. PBS-infused mice}) \). Whereas AdSiR-control did not affect their expression, \( 2 \times 10^9 \text{ pfu AdSiR-MR-1} \) inhibited the upregulation of ANF and \( \beta \)-myosin heavy chain \( (P < 0.01 \text{ vs. untreated or AdSiR-control-treated mice}) \).

Heart tissues of the mice were submitted to morphological analysis. Our results revealed that the increased cross-sectional area (Table 2) of cardiomyocytes in ANG II-infused mice was significantly reduced after AdSiR-MR-1 injection \( (P < 0.01) \). Interstitial cardiac fibrosis was examined by the Masson’s trichrome staining and quantified. As shown in Table 2, the increased LV interstitial fibrosis induced by ANG II decreased dramatically after AdSiR-MR-1 treatment \( (P < 0.01) \). The reduction of cardiac fibrosis was accompanied with a decrease of collagen contents. Figure 6A shows that LV collagen type I
and collagen type III mRNA levels were greatly elevated after ANG II infusion; AdSiR-MR-1 treatment abolished this effect.

We also analyzed the mRNA expression levels of matrix metalloproteinase (MMP)-2 and tissue inhibitor of metalloproteinase (TIMP)-2, which participate in the turnover of the extracellular matrix (ECM) and cardiac remodeling (6, 13). Figure 6B shows that ANG II infusion dramatically elevated the MMP-2 mRNA level by sixfold. The expression level of TIMP-2 mRNA was also upregulated by ANG II. AdSiR-MR-1 largely blocked the activity of ANG II on MMP-2 and totally inhibited the upregulation of TIMP-2 (P < 0.01 vs. untreated or AdSiR-control-treated mice).

Because adenoviral gene delivery has been reported to stimulate immune responses and cause inflammation in the host (14), we examined whether this happened in our experiments. Mac-1 (specific for monocytes/macrophages) and 7/4 (specific for neutrophils) antibodies (28) were used to stain the heart sections; positively stained cells were counted. As shown in Table 2, we found that in PBS-infused mice, administration of the recombinant adenoviral vectors did not cause an increase

### Table 2. Echocardiographic and quantitative histological results in PBS- and ANG II-infused C57BL/6J mice treated with AdSiR-control or AdSiR-MR-1

<table>
<thead>
<tr>
<th></th>
<th>PBS-Infused Mice</th>
<th>Ang II-Infused Mice</th>
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<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>AdSiR-control</td>
<td>AdSiR-MR-1</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Heart weight/body weight, mg/g</td>
<td>2.8 ± 0.2</td>
<td>3.1 ± 0.8</td>
<td>2.9 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>LV end-diastolic diameter, mm</td>
<td>3.7 ± 0.36</td>
<td>3.74 ± 0.32</td>
<td>3.59 ± 0.29</td>
<td>5.36 ± 0.52*</td>
</tr>
<tr>
<td>LV end-systolic diameter, mm</td>
<td>2.38 ± 0.26</td>
<td>2.41 ± 0.21</td>
<td>2.31 ± 0.22</td>
<td>3.61 ± 0.37*</td>
</tr>
<tr>
<td>LV posterior wall thickness, mm</td>
<td>0.72 ± 0.06</td>
<td>0.72 ± 0.1</td>
<td>0.74 ± 0.07</td>
<td>1.12 ± 0.11*</td>
</tr>
<tr>
<td>Intraventricular septal thickness at diastole, mm</td>
<td>0.55 ± 0.06</td>
<td>0.56 ± 0.07</td>
<td>0.55 ± 0.05</td>
<td>0.78 ± 0.09*</td>
</tr>
<tr>
<td>LV fractional shortening, %</td>
<td>56.2 ± 5.6</td>
<td>55.6 ± 6.3</td>
<td>54.5 ± 5.5</td>
<td>41.8 ± 4.1*</td>
</tr>
<tr>
<td>Cross-sectional area of myocytes, μm²</td>
<td>343 ± 39</td>
<td>337 ± 36</td>
<td>355 ± 37</td>
<td>615 ± 60*</td>
</tr>
<tr>
<td>Fibrosis, %</td>
<td>4.4 ± 0.47</td>
<td>4.61 ± 0.46</td>
<td>4.66 ± 0.46</td>
<td>15.2 ± 1.6*</td>
</tr>
<tr>
<td>Antibody 7/4-positive cells, no. of cells/mm²</td>
<td>12.4 ± 1.5</td>
<td>11.7 ± 1.4</td>
<td>14.4 ± 1.6</td>
<td>68.9 ± 7.7†</td>
</tr>
<tr>
<td>Antibody Mac-1-positive cells, no. of cells/mm²</td>
<td>85.1 ± 7.6</td>
<td>89.1 ± 9.8</td>
<td>88.2 ± 8.5</td>
<td>253 ± 32.5*</td>
</tr>
</tbody>
</table>

Values are mean ± SE; n = 6 mice/subgroup. LV, left ventricular. *P < 0.01 and †P < 0.001 vs. PBS-infused mice; ‡P < 0.05 and §P < 0.01 vs. untreated or AdSiR-control-treated mice in the ANG II-infused group.
of infiltrating inflammatory cells. However, after ANG II infusion, the number of Mac-1- and 7/4-positive cells significantly increased. AdSiR-control had no effect on it; AdSiR-MR-1 treatment significantly reduced the number of infiltrating inflammatory cells (P < 0.01 vs. untreated or AdSiR-control-treated mice).

**DISCUSSION**

The prevention and treatment of cardiac hypertrophy as well as related heart failure have been great challenges for decades. In the present study, we demonstrate, for the first time, that MR-1 could be a potential new target to suppress cardiac hypertrophy induced by ANG II.

As a member of the renin-angiotensin-aldosterone system, ANG II plays a key role in the development of cardiac hypertrophy and remodeling (18). Here, we successfully used ANG II to induce cardiac hypertrophy in mice. In (11, 15) previous reports, 0.2–3 g·kg⁻¹·min⁻¹ ANG II were used to induce cardiac hypertrophy and fibrosis in mice. The dose of ANG II we used here was within that dose range. In agree with other studies (7, 35, 37) have demonstrated the ability of adenovirus to transduce foreign genes into various tissues in animals, including the heart. Indeed, our results showed that viral DNA still existed in the heart nearly 2 wk after systemic delivery, confirming the existence of the transgene. AdSiR-MR-1 effectively reduced the basal expression as well as ANG II-stimulated overexpression of MR-1. Because the siRNA expressed by AdSiR-MR-1 was specific for the mouse MR-1 gene, it did not influence the basal expression of other genes in our experiments. As for the blocking effect of AdSiR-MR-1 on the ANG II-induced upregulation of genes such as ANF, β-myosin, and collagens, we think that it should be explained by mechanisms other than direct silencing (see below for a detailed discussion).

MR-1 expression in the skeletal muscle was not obviously silenced in our experiment. The Adxsi system that we used here was a vector based on E1/E3-deleted adenovirus serotype 5, whereas adenovirus serotype 5-mediated transduction was dependent on binding to the coxsackie adenovirus receptor (33). The lesser silencing efficiency of AdSiR-MR-1 in the skeletal muscle was in correlation with the lesser expression of coxsackie adenovirus receptors in skeletal muscle cells (34).

The most significant finding of this study is that silencing MR-1 can effectively prevent cardiac hypertrophy induced by ANG II. ANG II treatment leads to obvious cardiac hypertrophy and remodeling (18). Here, we successfully used ANG II plays a key role in the development of cardiac hypertrophy induced by ANG II.

MR-1 silencing attenuates the upregulation of extracellular matrix-related genes. Animals were grouped and treated as described in Fig. 4A. Mice were killed 2 wk after infusion, and total RNA was extracted. A and B: real-time RT-PCR was used to determine collagen type I/III (A) and matrix metalloproteinase (MMP)-2/tissue inhibitor of metalloproteinase (TIMP)-2 (B) mRNA levels. Normalized mRNA expression levels were plotted as fold differences compared with the PBS-infused untreated group, which was designated as 1. Values are means ± SEM of 6 mice/subgroup. **P < 0.01 and ***P < 0.001 vs. PBS-infused untreated mice; ###P < 0.01 vs. untreated or AdSiR-control-treated mice in the ANG II-infused group.
of heart weight-to-body weight ratios, echocardiographic parameters, and histological examinations. A single bolus injection of AdSiR-MR-1 successfully abolished the deteriorating effect of ANG II on heart size and function, validating the feasibility of using MR-1 as a potential target to reduce or prevent cardiac hypertrophy. In addition, beneficial effects of MR-1 silencing were observed in the morphological analysis, in which AdSiR-MR-1 greatly relieved the cardiac fibrosis and inflammation induced by ANG II.

ANG II increases cardiac fibrosis through multiple mechanisms, which include the stimulation of fibroblast proliferation and collagen synthesis (3, 16, 17). Our results confirmed the upregulation of collagen type I/III by ANG II, and this is in agree with previous reports (16, 17). However, the detailed mechanisms used by AdSiR-MR-1 to reduce cardiac fibrosis, collagen expression, and inflammation are still not clear and need further investigation. The MR-1 gene is expressed in fibroblasts (22), and the fibroblasts could be infected by the adenoviruses (31), but MR-1 silencing is not likely to be the direct mechanism responsible for the reduction of cardiac fibrosis induced by ANG II. Instead, it is possible that certain cell signaling pathways, such as the NF-κB pathway, could be involved. NF-κB plays an important role in the pathophysiological processes of the heart, such as fibrosis, remodeling, and inflammation; inhibition of NF-κB improves these disorders (10, 29). Our previous study (21) indicated that overexpression of MR-1 enhances the activation of NF-κB in cultured cardiomyocytes. The in vivo effect of MR-1 silencing on NF-κB signaling and its relationship with cardiac fibrosis/inflammation are currently under investigation in our laboratory. Furthermore, our results also proved that ANG II infusion upregulates the expression of MMP-2 and TIMP-2 in the LV. In agree with another report (36), the ANG II-induced elevation of MMP-2 is more potent than that of TIMP-2 (6- vs. 2-fold in our experiment). This could destroy the dynamic balance of MMP to TIMP and cause cardiac remodeling (6, 13). AdSiR-MR-1 treatment partially restored the MMP-2:TIMP-2 ratio, suggesting that it could have a beneficial influence on cardiac remodeling.

In recent years, with the in-depth understanding of the molecular mechanisms and signaling pathways involved in cardiac hypertrophy, novel therapeutic strategies have been explored. These include gene therapies and small-molecular-weight compounds that can modulate hypertrophic pathways (4, 19, 26, 30). For gene therapy, efforts have been made to target key factors or receptors mediating cardiac hypertrophy, such as angiotensinogen, the ANG II type I receptor, activator protein 1 transcription factor, and focal adhesion kinase. The antisense technique, dominant negative mutants, and RNA interference have been used to suppress these molecules in rat or mouse models and proven to be effective in reducing cardiac hypertrophy (4, 19, 30). Despite these efforts, new targets in the treatment of cardiac hypertrophy are still in great demand because of the complexity and recurrence of this disease (26).

In conclusion, our results suggest that MR-1 could be a potential new target to prevent or suppress cardiac hypertrophy induced by ANG II. Further studies are required to investigate the relationship between MR-1 and cardiac hypertrophy in clinic pathology. Also, the cross-talks between MR-1 and other factors in the signaling network of cardiac hypertrophy are of great significance.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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