Overexpression of Myofibrillogenesis Regulator-1 Aggravates Cardiac Hypertrophy Induced by Angiotensin II in Mice

Hong-Liang Li, Zhi-Gang She, Tian-Bo Li, Ai-Bing Wang, Qinglin Yang, Yu-Sheng Wei, Yi-Guang Wang, De-Pei Liu

Abstract—Myofibrillogenesis regulator-1 (MR-1) augments cardiomyocytes hypertrophy induced by angiotensin II (Ang II) in vitro. However, its roles in cardiac hypertrophy in vivo remain unknown. Here, we investigate whether MR-1 can promote cardiac hypertrophy induced by Ang II in vivo and elucidate the molecular mechanisms of MR-1 on cardiac hypertrophy. We used a model of Ang II–induced cardiac hypertrophy by infusion of Ang II in female mice. In wild-type mice subjected to the Ang II infusion, cardiac hypertrophy developed after 2 weeks. In mice overexpressing human MR-1 (transgenic), however, cardiac hypertrophy was significantly greater than in wild-type mice as estimated by heart weight:body weight ratio, cardiomyocyte area, and echocardiographic measurements, as well as cardiac atrial natriuretic peptide and B-type natriuretic peptide mRNA and protein levels. Our further results showed that cardiac inflammation and fibrosis observed in wild-type Ang II mice were augmented in transgenic Ang II mice. Importantly, increased nuclear factor κB activation was significantly increased higher in transgenic mice compared with wild-type mice after 2 weeks of Ang II infusion. In vitro experiments also revealed that overexpression of MR-1 enhanced Ang II–induced nuclear factor κB activation, whereas downregulation of MR-1 blocked it in cardiac myocytes. In conclusion, our results suggest that MR-1 plays an aggravative role in the development of cardiac hypertrophy via activation of the nuclear factor κB signaling pathway. (Hypertension. 2007;49:1399-1408.)

Key Words: hypertrophy ■ myocardium ■ NF-κB ■ myofibrillogenesis regulator-1 ■ fibrosis ■ angiotensin II

The heart undergoes adaptive hypertrophic growth to augment cardiac output in response to a variety of pathological stimulations, including hypertension, myocardial infarction, pressure overload, and abnormalities in contractility and structure because of inherited gene mutations.1,2 Although initial cardiac hypertrophy acts like an adaptive mechanism, prolonged and severe hypertrophy is a risk factor for arrhythmias, sudden death, and heart failure. Because cardiac hypertrophy is a highly complex disorder that results from an interaction of genetic, physiological, and environmental factors, many genes and their products participated in its pathogenesis through different pathways.1,2 Therefore, the discovery and functional clarification of cardiac hypertrophy-related novel human genes are important for understanding the molecular mechanisms underlying cardiac hypertrophy. Myofibrillogenesis regulator-1 (MR-1) was identified from a human skeletal muscle cDNA library, which encodes a protein of 142 amino acids with a hydrophobic transmembrane structure from 75 to 92 amino acids.3 MR-1 protein is mainly located on the nucleic membrane and, to a lesser extent, in the cytoplasm. Northern blot and serial analysis of gene expression revealed that the transcription level of MR-1 in human tissues is especially high in myocardium and skeletal muscle.3 Yeast 2-hybrid screening and in vitro glutathione S-transferase pull-down assay revealed that MR-1 interacts with 3 proteins involved in muscle contraction (myosin regulatory light chain, myomesin, and β-enolase) and several cell signal transduction–related proteins.3,4 The interaction of MR-1 with sarcomeric structural proteins involved in muscle contraction and its presence in human myocardial myofibrils indicate that MR-1 is involved in the regulation of contractile proteins in the myocardium and might be associated with cardiac hypertrophy. Indeed, our previous study showed that MR-1 expression was in-
duced in angiotensin II (Ang II)–treated cardiomyocytes and hypertrophied myocardium, whereas knockdown the expression of MR-1 prevented Ang II–induced hypertrophy. These findings suggest that MR-1 plays an important role in Ang II–induced cardiac hypertrophy. However, to date, the mechanism of MR-1 on cardiac hypertrophy remains unclear. To determine whether our in vitro findings have physiological relevance, we investigated the effects of MR-1 in the Ang II–mediated model of cardiac hypertrophy in vivo using transgenic mice. Therefore, the purpose of this study was to determine whether MR-1 can promote cardiac hypertrophy induced by Ang II in vivo and to elucidate the molecular mechanisms of MR-1 on cardiac hypertrophy in vitro and in vivo.

**Methods**

**Animals and Chronic Ang II Infusion**

All of the protocols were approved by institutional guidelines. All of the surgeries and subsequent analyses were performed in a blinded fashion for genotype. The details for transgenic mice production and Ang II infusion are given in the online data supplement available at http://hyper.ahajournals.org.

**Blood Pressure and Echocardiography**

Heart rate and systolic blood pressure were measured by tail-cuff plethysmography (BP-2000 system; Visitech Systems). The details are given in the online data supplement.

**Cardiac Morphology and Histomorphometric Analysis**

After blood samples were taken, hearts were cleared by perfusion with PBS at 70 mm Hg, arrested in diastole with 60 mmol/L of KCl, weighted, fixed by perfusion with 4% paraformaldehyde, embedded in paraffin, and sectioned at 5 μm or snap frozen in liquid nitrogen for RNA and protein analysis. Sections were stained with hematoxylin/eosin and Masson’s trichrome for histopathologic analysis. The details are given in the online data supplement.

**Measurement of Myeloperoxidase Activity**

Myeloperoxidase (MPO), an enzyme that is specific for neutrophils, was determined in cardiac tissue by the method described previously. One unit of MPO was defined as the quantity of enzyme that hydrolyzed 1 mmol of peroxide per minute at 25°C. The assays were performed without knowledge of the group from which each sample originated.

**Neurohormonal Factors**

Plasma atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), Ang II, endothelin-1, and renin activity levels were determined with commercially available kits (Phoenix Pharmaceuticals, Inc.).

### TABLE 1. Echocardiographic Data in Sham- and Angiotensin II–Treated Mice

<table>
<thead>
<tr>
<th>Variable</th>
<th>Basal</th>
<th>Sham</th>
<th>Ang II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>TG</td>
<td>WT</td>
</tr>
<tr>
<td>Lung weight, mg</td>
<td>181.0±7.1</td>
<td>177.0±5.6</td>
<td>169.0±7.9</td>
</tr>
<tr>
<td>Liver weight, mg</td>
<td>1415±211</td>
<td>1334±178</td>
<td>1411±201</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>113.0±4.8</td>
<td>116.0±6.7</td>
<td>119.0±6.2</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>424±11</td>
<td>429±8</td>
<td>418±3</td>
</tr>
<tr>
<td>HW/BW, mg/g</td>
<td>2.6±0.9</td>
<td>2.8±2.2</td>
<td>3.0±0.2</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>2.5±0.4</td>
<td>2.1±0.7</td>
<td>2.4±0.2</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>4.2±0.3</td>
<td>4.4±0.3</td>
<td>4.4±0.3</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>2.6±0.5</td>
<td>2.4±0.6</td>
<td>2.5±0.3</td>
</tr>
<tr>
<td>Ns, mm</td>
<td>0.63±0.03</td>
<td>0.64±0.05</td>
<td>0.65±0.05</td>
</tr>
<tr>
<td>LVPPd, mm</td>
<td>0.68±0.05</td>
<td>0.70±0.05</td>
<td>0.69±0.03</td>
</tr>
<tr>
<td>FS, %</td>
<td>55.0±7.3</td>
<td>56.0±3.3</td>
<td>52.0±9.0</td>
</tr>
<tr>
<td>Fibrosis, %</td>
<td>6±1</td>
<td>6±3</td>
<td>7±2</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; HR, heart rate; BW, body weight; HW, heart weight; PWT, posterior wall thickness; LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter; Nsd, left ventricular septum, diastolic; LVPPd, left ventricular posterior wall, diastolic; FS, fractional shortening. All of the values are mean±SEM (n=10 to 12).

*P<0.05 vs WT sham group.
†P<0.05 vs WT Ang II.
**Northern Blot and Western Blot Analysis**

Whole RNA was extracted from cardiac tissue with TRIzol reagent according to the manufacturer’s instructions (Invitrogen). RNA (30 μg) was run on a 1% agarose/formaldehyde gel at 120 V for 2 hours. The RNA was transferred to a nylon membrane by vacuum for 1.5 hours and cross-linked by UV wave. Probes of MR-1 (human and mouse), ANP, and BNP cDNA were generated by reverse transcription of heart mRNA and amplification of the resulting cDNA by the PCR, as described previously.5,8 Protein extracts from different groups of myocardium (50 μg) were fractionated on a 10% polyacrylamide gel under reducing conditions, transferred to nitrocellulose membranes, and probed with various antibodies. After incubation with a secondary peroxidase-conjugated antibody, signals were visualized by Chemiluminescence kit (Amersham).

**Electrophoretic Mobility Shift Assay and Inhibitor of Nuclear Factor κB Kinase Assay**

Nuclear proteins were isolated using the method of Haudek et al.9 Protein concentrations were measured by BCA protein assay reagents (Pierce) using BSA as a standard. Electrophoretic mobility-shift assays were performed according to the manufacturer’s instructions (Gel Shift Assay System E3300, Promega). Inhibitor of nuclear factor κB kinase (IKK) assay was performed by a method described previously.10,11

**Recombinant Adenoviral Vectors and Cell Culture**

We generated recombinant adenoviral vectors encoding MR-1 and MR-1 silencing RNA using the small interference (si)RNA sequence

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**Table 2. Plasma ANP, BNP, Angiotensin II, Renin Activity, and LV Tissue Angiotensin-Converting Enzyme Activity**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham</th>
<th>Ang II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>TG</td>
</tr>
<tr>
<td>Plasma BNP, pg/mL</td>
<td>16.7±3.1</td>
<td>21.3±3.1</td>
</tr>
<tr>
<td>Plasma ANP, pg/mL</td>
<td>23.2±4.4</td>
<td>28.9±3.4</td>
</tr>
<tr>
<td>Plasma Ang II, pg/mL</td>
<td>23.5±4.2</td>
<td>30.1±4.8</td>
</tr>
<tr>
<td>Plasma renin activity, ng/mL per h</td>
<td>1.4±0.3</td>
<td>1.8±0.3</td>
</tr>
<tr>
<td>LV tissue ACE activity, nmol mg wet per weight per hour</td>
<td>0.18±0.01</td>
<td>0.21±0.03</td>
</tr>
</tbody>
</table>

Plasma ANP, BNP, and renin activity were measured from blood samples taken while animals were conscious. Data are expressed as mean±SEM (n=10 to 12).

*P<0.05 vs WT sham group.
†P<0.05 vs WT Ang II group.
described previously\(^4\) and expanded them in 293 cells. Control adeno-
viral GFP was produced as described previously.\(^12\) We infected cardio-
myocytes with adeno viral vectors encoding MR-1 and adeno viral GFP, as well as MR-1 silencing RNA and AdSiR-control at a multiplicity of in-
fection of 100, resulting in 95% to 100% of cells expressing the trans-
genomes without toxicity. Primary cultures of cardiac myocytes were pre-
pared as described.\(^10,11\) The details are given in the online data sup-
plement.

**[^H]Leucine Incorporation**

[^H]Leucine incorporation was measured as described previously.\(^10\) Briefly, cardiac myocytes were infected with different adeno virals (AdIκBα [S32A/S36A] was bought from Vector BioLabs) for 24 hours and subsequently stimulated with Ang II and coinfected with [^H]leucine (1 μCi/mL, Moravek Biochemicals Inc) for 48 hours. At the end of the experiment, the cells were washed with Hanks’ solution, scraped off the well, and then treated with 10% trichloro-
acetic acid at 4°C for 60 minutes. The precipitates were then dissolved in NaOH (0.4 N). Aliquots were counted with a scintilla-
tion counter. The details are given in the online data supplement.

**Statistical Analysis**

All of the values are expressed as mean±SEM. The differences in the data simply between 2 groups were determined by Student’s t test. Comparisons between groups on Western blotting data were assessed by one-way ANOVA followed by Bonferroni’s posthoc test. A value of \(P<0.05\) was considered statistically significant.

**Results**

**Characterization of Human MR-1 Transgenic Mouse**

To assess the effect of constitutive human MR-1 expression, we generated transgenic mice with full-length human MR-1 cDNA. Four lines of transgenic (TG) mice were confirmed by PCR (data not shown) and Western blot (Figure 1A). These lines were viable and fertile, and there were no detectable differences in cardiac size and structure between TG and wild-type (WT) mice either macroscopically or microscopically. We analyzed MR-1 protein levels in various tissues of TG mice by Western blot analysis using an anti-human MR-1 antibody prepared by our groups. We found a robust expression of human MR-1 protein in the heart and skeletal muscle, and a lower MR-1 expression was detected in the lung, brain, liver, and kidney (Figure 1B). Among 4 established lines of TG mice, 1 line that expressed the highest level of the human MR-1 protein in the heart was used for further experiments. Northern blot showed that the endogenous expression level of the mouse MR-1 mRNA was not modified or downregulated by expression of the human MR-1 gene (Figure 1C).

**Cardiac Performance After Ang II Infusion in MR-1 Transgenic Mice and Control**

We next assessed cardiac function after 2 weeks of Ang II infusion in WT and TG mice by echocardiography. The cardiac functional parameters evaluated by echocardiography after 2 weeks of Ang II infusion are shown in Table 1. There was no significant difference in heart rate among the 4 groups of mice. In WT Ang II mice, decreased fractional shortening and ejection fraction with dilated left ventricular end systolic dimension and left ventricular end diastolic dimension were seen, corresponding with cardiac hypertrophy. In TG Ang II mice, significantly higher left ventricular end systolic dimension and left ventricular end diastolic dimension and lower fractional shortening and ejection fraction were observed compared with WT Ang II mice, indicating that TG mice with Ang II infusion have a greater level of systolic dysfunction compared with WT mice with Ang II infusion. Sham-operated animals had no evidence of myocardial dysfunction as assessed by echocardiography. Importantly, although Ang II significantly increased systolic blood pressure in WT and TG mice, there is not a marked difference in both groups responding to Ang II.

**MR-1 Expression Facilitates Left Ventricular Remodeling in Response to Ang II**

Cross-sectional area of cardiac myocytes, an index of cellular hypertrophy, increased in the left ventricle (LV) from WT Ang II and was markedly increased in TG Ang II (Figure 2A, 2B, and S1). We next examined whether MR-1 overexpres-
sion affects cardiac expression of hypertrophy-related genes, in which we used ANP and BNP as markers. In WT mice, the expression level of ANP and BNP mRNA and protein increased significantly at 2 weeks of Ang II infusion com-
pared with that in sham-operated mice (Figure 2C and 2D), indicating that Ang II induced gene expression along with the development of cardiac hypertrophy. In TG Ang II mice, expression levels were significantly higher than those in WT
Ang II mice, suggesting an additive effect of MR-1 on ANP and BNP mRNA and protein expression. Plasma ANP, BNP, Ang II, endothelin-1, and renin activity are also established markers of cardiac hypertrophic response. Therefore, we also measured them from blood samples taken while animals were conscious. As shown in Table 2, plasma ANP, BNP, endothelin-1, and Ang II concentrations, as well as renin activity, were increased in the WT Ang II group, and such increase was markedly fortified in the TG Ang II group. Consistent with the above-mentioned results, heart weight:body weight ratio was also significantly increased in the WT Ang II group compared with the WT sham group. Importantly, the heart weight:body weight ratio of TG Ang II mice was significantly higher than WT Ang II mice (Table 1). Lung and liver weights were found to be unaltered among all of the groups investigated (Table 1). These results indicate that MR-1 efficiently facilitates cardiac remodeling by modulating the expression and concentration of hypertrophy-related genes in Ang II–infused hearts.

**MR-1 Expression Facilitates the Activation of NF-κB Mediated by Ang II In Vitro and In Vivo**

As shown in Figure 3A, 2 weeks of Ang II infusion increased activation of NF-κB in the myocardium in WT mice, whereas it was markedly increase in TG mice. The specificity of NF-κB DNA binding activity was confirmed by supershift assays (data not shown). To determine the molecular mechanisms that MR-1 used to enhance NF-κB activation in vivo, we first analyzed IkBα phosphorylation and IKK activation processing after 2 weeks of Ang II infusion. Heart lysates were prepared and Western blot analysis was performed using appropriate antibodies. We detected IkBα phosphorylation and IkBα degradation clearly after 2 weeks of Ang II infusion in WT mice. Interestingly, IkBα phosphorylation and IkBα degradation were evidently enhanced in TG Ang II mice (Figure 3B). Because phosphorylation of IkBα is mediated through IKKβ, these results suggested that MR-1 maybe promote IKKβ activation mediated by Ang II. Indeed, as shown in Figure 3C, in immune-complex kinase assays, Ang II infusion activated IKKβ in WT mice, and MR-1 overexpression markedly enhanced the activation. Under these conditions, MR-1 had no effect on IKKα and IKKβ protein levels. The translocation of p65 to the nucleus is important to NF-κB activation. Therefore, we also tested the effect of MR-1 on Ang II–induced phosphorylation of p65. As shown in Figure 3D, Ang II induced the phosphorylation of p65 and nuclear translocation determined by Western blot analysis after 2 weeks of Ang II infusion in WT mice. More importantly, p65 phosphorylation and nuclear translocation were elevated in TG Ang II mice (Figure 3D). Lamin B1 was used as a loading control for nuclear extracts. Consistent with our in vivo results, further in vitro results showed that overexpression of MR-1 by infection of adenoviral vectors encoding MR-1 increased Ang II–induced NF-κB activation and transcriptional activity by promoting IKKβ and p65 activation, whereas downregulation of MR-1 by infection MR-1 silencing RNA expression blocked Ang II–induced NF-κB activation (Figure 3E). To further investigate the molecular mechanisms of MR-1 cardiac hypertrophy, we examined the effects of NF-κB activation on cardiac hypertrophy. Our data showed that blocking NF-κB activation by infection of AdIkBα (S32A/S36A) attenuated MR-1 and Ang II–mediated cardiac hypertrophy, whereas activation of NF-κB by infection of AdIKKβ augmented cardiac hypertrophy as demonstrated by [3H]leucine incorporation and ANP promoter activity assays (Figure 3F).

**MR-1 Expression Enhanced Proinflammatory Response Induced by Ang II Infusion**

To determine whether expression of MR-1 promotes the inflammatory responses in the hearts, cellular infiltrates were characterized by immunohistochemical analyses. Sections from the LV of TG and WT mice were immunostained with 7/4, Mac-1 and Mac-3 antibodies. 7/4 is a marker that labels mainly neutrophils, and Mac-1 is mainly expressed on monocytes/macrophages, whereas Mac-3 is mainly expressed on activated macrophages. The numbers of 7/4–, Mac-1– and Mac-3–positive cells were increased in the hearts of WT mice after 2 weeks of Ang II infusion compared with the age-matched WT-sham mice (Figure 4A through 4C). Interestingly, the number of 7/4–, Mac-1– and Mac-3–positive cells was greatly increased in the hearts of TG Ang II mice (Figure 4A through 4C). These results showed that MR-1 overexpression enhanced the increase of the number of neutrophils and macrophages mediated by Ang II. To further quantify the extent of inflammatory cells infiltration, we measured the activity of cardiac MPO, an enzyme specific to activated neutrophils. When myocardial MPO activity was measured in cardiac tissues after 2 weeks of Ang II infusion, we found that the activity significantly increased within the heart of both TG and WT mice but that it was significantly higher in the TG Ang II (Figure 4D). Our results further showed that TG mice have significantly higher TNF-α, IL-6, and MCP-1 protein expression after 2 weeks of Ang II infusion compared with WT Ang II infusion mice (Figure 4E). To further examine the effects of MR-1 on inflammation, we investigated the effects of MR-1 on TNF-α, IL-6, and MCP-1 promoter activities. Our data showed that overexpression of MR-1 by infection of adenoviral vectors encoding MR-1 increased TNF-α, IL-6, and MCP-1 promoter activities, whereas downregulation of MR-1 by infection MR-1 silencing RNA expression blocked TNF-α, IL-6, and MCP-1 promoter activities mediated by Ang II and MR-1 (Figure 4F).

**MR-1 Expression Augments Ang II–Induced Fibrosis in Heart**

Cardiac hypertrophy induced by Ang II is associated with increased fibrosis in the myocardium, which is characterized by overproduction of extracellular matrix proteins. Fibrosis detected by Masson’s trichrome staining and picrosirius red staining (data not shown) for collagen was negligible in hearts from sham-operated TG and WT mice, whereas fibrosis significantly increased in WT and TG mice after Ang II infusion (Table 1 and Figure 5A). Compared with WT mice, this increased fibrosis was significantly higher in TG mice at 2 weeks after Ang II infusion. We also focused on the synthetic processes involved in collagen turnover by exam-
ining the expression of protein encoding transforming growth factor (TGF)-β1, TGF-β3, and collagen I (Figure 5B), which are known to be involved in cardiac fibroblast proliferation and the biosynthesis of extracellular matrix proteins. We found that their expression was significantly higher in TG mice than in WT mice after Ang II treatment.

**Discussion**

The present study provides the first direct evidence that overexpression of MR-1 in mice exacerbates cardiac hypertrophy, inflammation, and fibrosis caused by Ang II infusion, leading to diminished cardiac function by promoting activation of the NF-κB signal pathway.

Ang II plays a critical role in cardiac remodeling in hypertension, a process resulting from cardiomyocyte hypertrophy, inflammation, and fibrosis, resulting in decreased compliance and increased risk of heart failure. Ang II infusion causes increased expression of TGF-β1, TGF-β3, and collagen I. These proteins are involved in cardiac fibroblast proliferation and extracellular matrix protein biosynthesis. In our study, we observed that the expression of these proteins was significantly higher in TG mice compared to WT mice after Ang II treatment. This suggests that MR-1 overexpression exacerbates cardiac remodeling in response to Ang II.

As estimated by the heart weight:body weight ratio, cardiomyocyte area, echocardiographic measurements, and LV ANP and BNP mRNA and protein levels, cardiac function was impaired in TG mice compared to WT mice after Ang II treatment. These findings support the role of MR-1 in mediating the adverse effects of Ang II on cardiac structure and function.

**Figure 4.** MR-1 overexpression facilitates proinflammatory response induced by Ang II. A to C, Quantitative showing the number of 7/4-, Mac-1-, and Mac–positive cells in the hearts of WT and TG mice (n=4). D, Myocardial MPO activity in cardiac tissue samples from TG and WT mice (n=6). MPO activity is expressed as units per 100 mg of wet tissue weight. E, Western blot analysis of TNF-α, IL-6, and MCP-1 protein expression in myocardium obtained from TG and WT mice (n=6). Top, representative blots; bottom, quantitative results. Values are mean±SEM. *P<0.01 for difference from WT sham values. §P<0.01 for difference from WT Ang II values. F, Effects of MR-1 on Ang II-induced TNF-α, MCP-1, and IL-6 promoter activities in cardiac myocytes.
by high blood pressure. Therefore, further studies are needed to elucidate this point.

The findings of the present study are also suggestive of the mechanism by which MR-1 exerts its prohypertrophic effects. Compelling evidence has accumulated to show that NF-κB plays an important role in the induction of cardiac hypertrophy in response to both biomechanical strain and neurohormonal stimuli, such as phenylephrine and Ang II.\textsuperscript{10,11,18} Therefore, the deteriorative mechanisms of MR-1 on cardiac hypertrophy were examined for its effects on the IKKβ/NF-κB pathway. The phosphorylation of IKKβ is a key regulatory step that dictates NF-κB activation.\textsuperscript{19} In nearly all instances, IKK directly phosphorylates 2 serine residues at the N terminus of IKKα (Ser-32 and Ser-36) and IKKβ (Ser-19 and Ser-23). This phosphorylation event triggers the polyubiquitination of IKKα and the subsequent degradation by the 26S proteasome. Among the parameters involved in IKKβ/NF-κB activation, the IKK activity, IKKα/β degradation, and NF-κB–specific DNA protein complex formation were examined. The results showed that MR-1 had significant promotions on these parameters. The phosphorylation of the p65 subunit on serine 536 by IkB kinases has been shown to increase transcriptional activity. To gain further insight into the mechanism of MR-1 on increase of the Ang II–induced activation of NF-κB, we investigated the phosphorylation and translocation of p65. Data show that p65 does phosphorylate and translocate to the nucleus in WT mice after Ang II infusion, and such effect was markedly increased in TG mice, further suggesting a role for MR-1 in transcriptional activation of NF-κB. Our in vitro data further confirm that overexpression of MR-1 promotes NF-κB activation, whereas down-regulation of MR-1 expression attenuates NF-κB pathway activation mediated by Ang II. These results suggest that MR-1 enhances NF-κB activation by augmenting Ang II–induced phosphorylation and nuclear translocation of p65, as well as activation of IKK, which led to increased IKK phosphorylation, ubiquitination, and subsequent degradation. Importantly, blocking NF-κB activation abrogated cardiac hypertrophy mediated by MR-1 and Ang II, whereas activation of NF-κB promoted cardiac hypertrophy, indicating that MR-1 augmented Ang II–mediated cardiac hypertrophy by enhancing NF-κB activation.

It has also been shown that phosphorylation at serine 536 on the p65 subunit is induced in response to a variety of proinflammatory stimuli, including Ang II.\textsuperscript{19–23} The increased phosphorylation of p65 at serine 536 in the TG mice after Ang II infusion suggests that the upregulation of NF-κB maybe involved in the progression and regulation of genes controlling cardiac inflammation. During the inflammatory phase, infiltration by inflammatory cells, particularly neutrophils and macrophages, is increased after Ang II infusion. We observed remarkable neutrophil and macrophage infiltration of the heart at 2 weeks after Ang II infusion, which composed the acute inflammatory response. Importantly, the increased infiltration by inflammatory cells was augmented by MR-1 expression. Ang II has also been shown to induce inflammatory response in the mammalian heart.\textsuperscript{24–26} Myocardial expression of proinflammatory cytokines may aggravate hypertrophy mediated by chronic infusion of Ang II. In this investigation, we found that TNF-α, MCP-1, and IL-6 protein levels observably increased in TG Ang II mice compared with WT Ang II mice and MR-1 overexpression enhanced TNF-α, MCP-1, and IL-6 promoter activities, further indicating that MR-1 overexpression promotes inflammatory response mediated by Ang II. One possible mechanism for
such a promotive effect is that MR-1 expression would directly activate NF-κB activation, resulting in potentiation of the inflammatory response and subsequent myocardial hypertrophy as the expression of many cytokines, including TNF-α, MCP-1, and IL-6, regulated by NF-κB activation. Each molecule has a κB binding domain in its promoter site.

Because Ang II-induced cardiac hypertrophy was also accompanied by cardiac fibrosis, we next investigated whether the prohypertrophic role of MR-1 on cardiac hypertrophy relates on its effect on fibrosis. In WT mice, Ang II induced fibrosis, and such fibrosis was significantly enhanced in TG mice. The fibrogenic effect of Ang II involves several mechanisms, namely, a direct trophic effect on myocytes and a proliferative effect on fibroblasts (through the activation of TGF-β1), which may lead to differentiation to myofibroblasts. It has been well documented that TGF-β is a key cytokine that promotes accumulation of collagen and other major components of the extracellular matrix in many fibrotic disorders, including cardiac and pulmonary fibrosis, glomerulonephritis, and vascular restenosis.27,28 In the present study, we found that, after Ang II infusion, TG mice hearts had considerable collagen deposition associated with markedly increased TGF-β1 and TGF-β3, as well as collagen deposition, compared with that in WT mice hearts, suggesting that the profibrotic effect of MR-1 may be at least partially because of its effect on TGF-β1 and TGF-β3 and then increased fibroblast proliferation and collagen synthesis by regulating NF-κB signaling.

In conclusion, our present work provided in vitro and in vivo evidence that cardiac MR-1 exacerbated cardiac hypertrophy by Ang II via regulating NF-κB signaling. MR-1 is a critical signaling molecule responsible for Ang II–induced cardiac hypertrophy, inflammation, and fibroblast. We propose that attenuation MR-1 expression seems to be the promising therapeutic target for cardiac hypertrophy.

**Perspectives**

In this study, we demonstrated that MR-1, a new myofibrologenesis regulator, enhances Ang II–mediated cardiac hypertrophy, inflammation, and fibrosis. MR-1 is a key signaling molecule responsible for Ang II–induced cardiac hypertrophy, inflammation, and fibrosis. We propose that attenuation MR-1 expression seems to be the promising therapeutic target for cardiac hypertrophy.

**Sources of Funding**

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**Disclosures**

None.

**References**


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In the Hypertension article by Li et al (Li H-L, She Z-G, Li T-B, Wang A-B, Yang W, Wei Y-S, Wang Y-G, Liu D-P. Overexpression of myofibrillogenesis regulator-1 aggravates cardiac hypertrophy induced by angiotensin II in mice. Hypertension. 2007;49:1399–1408), the axis labels on Figure 4A–D were changed after the article was posted on the Hypertension Web site on April 9, 2007. The revised Figure 4 is shown here and has been updated in the final printed and online versions of the article.

![Figure 4](image)

**Figure 4.** MR-1 overexpression facilitates proinflammatory response induced by Ang II. A–C, Quantitative showing the number of 7/4-, Mac-1-, and Mac-positive cells in the hearts of WT and TG mice (n=4). D, Myocardial MPO activity in cardiac tissue samples from TG and WT mice (n=6). MPO activity is expressed as units per 100 mg of wet tissue weight. E, Western blot analysis of TNF-α, IL-6, and MCP-1 protein expression in myocardium obtained from TG and WT mice (n=6). Top, representative blots; bottom, quantitative results. Values are mean±SEM. *P<0.01 for difference from WT sham values. §P<0.01 for difference from WT Ang II values. F, Effects of MR-1 on Ang II-induced TNF-α, MCP-1, and IL-6 promoter activities in cardiac myocytes.

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