Involvement of Reductive Stress in the Cardiomyopathy in Transgenic Mice With Cardiac-Specific Overexpression of Heat Shock Protein 27

Xia Zhang, Xiaoyan Min, Chuanfu Li, Ivor J. Benjamin, Bo Qian, Xiaojin Zhang, Zhengnian Ding, Xiang Gao, Yuzhen Yao, Yujie Ma, Yunling Cheng, Li Liu

Abstract—Oxidative stress plays an important role in cardiac diseases, which has been well demonstrated, whereas the role of reductive stress has been poorly investigated. We and others have shown previously that heat shock protein 27 (Hsp27) plays a role as an antioxidant. To investigate whether overexpression of Hsp27 could lead to reductive stress and result in cardiomyopathy, we generated transgenic mice with different expression levels of Hsp27. We observed that transgenic mice with high levels of Hsp27 developed cardiomyopathy. The myopathic hearts were under reductive stress, which was evidenced by an increased ratio of reduced glutathione/oxidized glutathione and a decreased level of reactive oxygen species. In addition, upregulated glutathione peroxidase 1 and decreased iron content were revealed in the myopathic hearts. More importantly, inhibition of glutathione peroxidase 1 significantly attenuated the development of cardiomyopathy. The data indicate that the Hsp27-induced cardiomyopathy could be attributed to, at least in part, upregulation of glutathione peroxidase 1. Our findings suggest that reductive stress plays an important role in the development of cardiomyopathy and that Hsp27 may serve as a potential target for the treatment of patients with cardiomyopathy. (Hypertension. 2010;55:1412-1417.)

Key Words: Hsp27 ■ oxidation ■ redox ■ cardiomyopathy ■ iron ■ cardiac function

Oxido-reduct homeostasis is essential for normal metabolism of cardiomyocytes.1 Oxidative stress has been well demonstrated to play critical roles in the pathophysiology of many cardiac diseases, including hypertrophy,2 cardiac ischemic injury,3 and congestive heart failure.4

“Reductive stress” is the counterpart of oxidative stress, which is defined as an abnormal increase of reducing equivalents, such as the elevated ratio of reduced glutathione (GSH)/oxidized glutathione (GSSG).5 Similar to oxidative stress, reductive stress has also been shown to have a deleterious effect in lower eukaryotes.6,7 For example, in yeast with reductive stress, some proteins showed delayed folding, disordered transport, and failed oxidation and were aggregated.7 In addition, reductive stress enhanced protein aggregation and reduced life span of yeast and Caenorhabditis elegans.6–10 More significantly, Rajasekaran et al15 reported a striking discovery that reductive stress can cause cardiomyopathy by protein aggregation in R120G αB-crystalline transgenic (Tg) mice, which mimic the clinical manifestations, phenotypic heterogeneity, and late onset of clinical signs and symptoms observed in desmin-related myopathy patients. Desmin-related myopathy is a heterogeneous group of muscle disorders morphologically defined by intrasarcoplasmic aggregates of desmin. The authors found that the myopathic hearts were under reductive stress, and the hearts exhibited dramatically increased levels of heat shock protein (Hsp) 25, which were positively correlated with protein aggregation.5 However, it is unclear whether the upregulated Hsp25 will contribute to reductive stress and mediate the development of cardiomyopathy.

Hsp27 is one of the best characterized members of the small Hsp family, with the molecular weight of 27 kD (Hsp27) in humans (25 kD in rodents [Hsp25]).11 Hsp27 has been demonstrated to play important roles in regulating intracellular redox homeostasis and antiapoptosis.12,13 We have shown previously that moderate expression of Hsp27 in cardiomyocytes protects the myocardium against doxorubicin-induced cardiac dysfunction through antioxidative stress11 and attenuates endotoxin-induced myocardium injury.14 Interestingly, numerous studies have shown that expression of exogenous Hsp27 in murine fibroblasts that do not express Hsp27 in normal growth conditions can generate a proreduced state.12,15–17 Arrigo et al18 reported that, in murine fibroblasts, the expression of Hsp27 significantly decreased the

Received November 3, 2009; first decision November 30, 2009; revision accepted April 7, 2010.

From the Departments of Geriatrics (Xia Z., X.M., B.Q., Xiao Z., Y.Y., Y.M., Y.C., L.L.) and Anesthesiology (Z.D.), First Affiliated Hospital With Nanjing Medical University, Nanjing, China; Department of Surgery (C.L.), East Tennessee State University, Johnson City, Tenn; Center of Cardiovascular Translational Biomedicine (I.J.B.), University of Utah School of Medicine, Salt Lake City, Utah; Model Animal Research Center (X.G.), Nanjing Medical University, Nanjing, China; E-mail liulil652003@yahoo.com.cn

© 2010 American Heart Association, Inc.

Hypertension is available at http://hyper.ahajournals.org DOI: 10.1161/HYPERTENSIONAHA.109.147066

1412
basal level of intracellular reactive oxygen species (ROS) and upregulated the total glutathione level. It is possible, therefore, that cardiac overexpression of Hsp27 may lead to reductive stress and subsequently cause cardiomyopathy.

To test this possibility, we generated Tg mice with cardiосpecific expression of Hsp27 (Hsp27 Tg) at different expression levels. We found that reductive stress and cardiomyopathy were exhibited in the hearts of high levels of Hsp27 Tg mice while not in the moderate levels of Hsp27 Tg mice. Our data suggest that high expression of Hsp27 in the myocardium will lead to reductive stress that plays a critical role in cardiomyopathy. Our observation also indicates that Hsp27 could be a potential target of the therapeutic approach for patients with cardiomyopathy.

Materials and Methods

Antibodies and Reagents
Please see the online Data Supplement at http://hyper.ahajournals.org for detailed information.

Animals
Hsp27 Tg mice were generated as described previously. Two-month–old Hsp27 Tg mice were used in the present study. Age- and sex-matched wild-type littermates (WT) served as control. All of the experiments were performed with the guidelines for the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996). Animal care and experimental protocols were approved by the Nanjing University Committee on Animal Care.

Cardiac Hypertrophy and Blood Pressure
The ratios of heart weight (HW)/body weight (BW) and HW/tibia length were measured (n=7 to 12 per group). Blood pressure was measured in mice using a noninvasive tail-clf computerized system (Softron BP_99a; n=10 to 13 per group).

Histology
Hearts were prepared for paraffin section. The stainings of hematoxylin-eosin and fluorescein isothiocyanate–labeled wheat germ agglutinin were performed as described previously. The images were taken under light or confocal microscope (Zeiss Ltd). Myocyte size from each heart was calculated by measurement of all of the myocytes in the field at a magnification of 200 (n=4 per group). Electron microscopy was performed as described previously. (n=3 per group). Immunohistochemistry for Hsp27 was performed on paraffin section as described previously (n=3 per group).

Echocardiography
Echocardiography was performed using a vivo770 cardiac system (Visualsonics), as described previously. Parameters of cardiac function were measured digitally and averaged ≥5 continuous cardiac cycles (n=6 to 10 per group).

Other Measurements
Animal survival was carefully monitored over 88 weeks (n=19 WT; n=11 Tg85; and n=33 Tg10). Western blot was performed as described previously (n=4 to 9 per group). (n=4 to 9 per group). RT-PCR assays for the mRNA levels of atrial natriuretic peptide, brain natriuretic peptide, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were performed as described previously (n=6 per group).

Oxidation Parameters
Intracellular ROS content (n=4 to 5 per group) and protein carbonylation (n=6 to 9 per group) were analyzed as described previously. (n=7 per group).

Figure 1. Hsp27 expression was analyzed by immunoblot in hearts. *P<0.01 vs Tg94, #P<0.01 vs Tg85; n=4 per group.

Malondialdehyde levels (n=9 to 11 per group) and glutathione peroxidase (GPx) activities (n=6 to 10 per group) were examined using assay kits according to the manufacturer’s instructions. Total GSH and GSSG were measured using 5,5'-dithio-bis(2-nitrobenzoic acid)-GSSG reductase recycling assay as described previously (n=7 per group).

Ferritin Assay
The cardiac ferritin level was determined by the Access Ferritin assay system according to the manufacturer’s instructions. Briefly, the protein extract was added to a reaction vessel followed by the addition of the chemiluminescent substrate Lumi-Phos® 530. The reaction was measured with a luminometer (Beckman Coulter Inc; n=6 per group).

Iron Level Measurement
Intracellular iron measurement was performed using the VITROS Fe slides and the VITROS chemistry products calibrator kit 4 on the VITROS Chemistry Systems Instrument (Johnson & Johnson; n=8 per group [heart]; n=6 per group [lung and liver]).

GPx Inhibition
Mercaptosuccinate (MS) is widely used as the inhibitor of GPx1. We initially determined an optimal dose of MS by examining cardiac GPx activity in Tg10 mice 1 hour after injection (IP) with MS at 0, 10, 50, and 100 mg/kg of BW, respectively. We observed that 50 mg/kg of BW will be an optimal dose for the experiment.

We then treated 4-week–old Tg10 mice with 50 mg/kg of MS once a day for 4 weeks. Cardiac function was measured subsequently by echocardiography (n=8 per group). The ratios of HW/BW and HW/tibia length were calculated (n=12 to 15 per group). Age-matched untreated Tg10 mice served as controls.

Iron Supplementation
Tg10 breeding mice started to take drinking water containing 1.4 mg/mL of ferrous fumarate. The mother mice continuously took the ferrous water during lactation. After ablactation, the newborn mice were fed with the same ferrous water to 8 weeks of age. The dosage of iron was selected according to a previous study. Cardiac function was subsequently measured by echocardiography. Age-matched untreated Tg10 mice served as controls (n=6 to 10 per group).
Statistical Analysis

The data were expressed as mean±SD. Comparison data between groups was performed using 1-way ANOVA. The Tukey procedure was performed for multiple-range tests. Survival curves were compared by the log-rank test. \( P<0.05 \) was considered to be significant.

Results

Differential Levels of Hsp27 Expression Among the Hearts of Independent Tg Lines

As shown in Figure S1 (please see the online Data Supplement at http://hyper.ahajournals.org), Hsp27 was expressed only in the hearts of the Tg lines. Figure 1 shows cardiac Hsp27 levels among Tg lines. Based on the protein expression levels, the Hsp27 Tg lines were designated as low (Tg94), moderate (Tg85), and high (Tg10 and Tg21).

High Hsp27 Tg Mice Developed Cardiac Hypertrophy

Low and moderate Tg lines showed comparable heart size with WT mice, whereas high Tg lines exhibited pronounced cardiac hypertrophy. Whole hearts and 4-chamber view sections (Figure 2, top and middle) showed that the hearts were much larger in Tg10 mice than in WT or Tg85 mice. The myocyte size and the ratios of HW/BW and HW/tibia length in Tg10 mice were higher than in WT or Tg85 mice (\( P<0.01 \); Figure 2, bottom; Figure S2A and S2B). The mRNA levels of cardiac atrial natriuretic peptide and brain natriuretic peptide increased significantly in Tg10 mice compared with WT mice (\( P<0.01 \); Figure S2C). The blood pressure was comparable between WT and Tg10 mice (Figure S2D). Based on these data, Tg10 and Tg85 mice were chosen for further studies.

High Hsp27 Tg Mice Exhibited Cardiac Dysfunction

As shown in Figure 3 and Table S1, Tg10 mice exhibited significant enlargement of the left ventricular chamber and increased LV mass compared with WT or Tg85 mice, whereas there were no significant differences between Tg85 and WT mice. Percentages of ejection fraction and fractional...
shortening, which indicate cardiac contractility, and early/late
diastolic filling ratio, which indicates cardiac diastolic func-
tion, significantly decreased in Tg10 compared with WT and
Tg85 mice (P<0.01 or 0.05), respectively.

High Hsp27 Tg Mice Showed Lower Survival Rate
The first mouse in the Tg10 group died at the age of 43 weeks.
When the mice were 85 weeks old, the survival rates were
100.00% in both WT and Tg85 mice, whereas the rates were
only 63.64% in the Tg10 mice. Tg10 mice showed signifi-
cantly lower survival rates within 88 weeks compared with
WT or Tg85 mice (Figure S3; P<0.01 or 0.05).

Histological Examination
Figure S4A and S4B show that the diameter of myocytes was
increased in Tg10 compared with WT and Tg85 mice. Arrows indicate protein aggregation within cardiomyocytes.
There were no obvious widespread losses of myocytes among
all of the groups. Figure S4C demonstrates that there was no
Hsp27 protein in the WT myocardium. Hsp27 protein mainly
appeared around the Z bands in the myocytes of Tg85 mice,
whereas Hsp27 protein was shown predominantly found
along the inner surface of membranes.

Ultrastructure of Cardiomyocytes
WT and Tg85 mice showed normal mitochondria, sarco-
plasmic reticulum, myofilaments, and other ultrastruc-
tures. However, in some areas of heart sections from Tg10
mice, neither myofilaments nor Z bands could be observed.
Additionally, vacuolation of the sarcoplasmic reticulum
and increased density of mitochondria were observed in
Tg10 mice (Figure S5).

Table.  GSH and GSSG Contents

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WT</th>
<th>Tg10</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH, nmol/mg of protein</td>
<td>857.4±88.6</td>
<td>1167.9±76.5*</td>
</tr>
<tr>
<td>GSSG, nmol/mg of protein</td>
<td>21.9±4.1</td>
<td>22.3±4.7</td>
</tr>
<tr>
<td>GSH/GSSG</td>
<td>40.1±6.6</td>
<td>53.7±7.4*</td>
</tr>
</tbody>
</table>

n=7 per group.
*P<0.01 vs WT.

Figure 5. Upregulation of GPx1 in Tg10 hearts. A, Expressions of GPx1 and GAPDH in hearts were analyzed by immunoblot.
*P<0.01; n=5 to 9 per group. B, Cardiac GPx activities of mice treated with or without MS for 1 hour. *P<0.01, n=6 to 10 per
group. C and D, Cardiac function (n=8 per group) and HW (n=12 to 15 per group) of Tg10 mice was improved by 4 weeks of
treatment with MS (50 mg/kg). Tg10 indicates untreated age-matched Tg10 control mice; Tg10+MS, Tg10 mice treated with MS.
*P<0.01; #P<0.05.
High Cardiac Expression of Hsp27 Caused Reductive Stress

The cardiac levels of ROS (Figure 4) and malondialdehyde of Tg10 were lower than WT and Tg85 mice (Figure S6A; P<0.01 or 0.05). Protein carbonyl content of Tg10 was also lower than WT mice (Figure S6B; P<0.01). In contrast, the GSH and the ratio of GSH/GSSG in Tg10 were increased significantly compared with WT mice (Table). These data indicate that cardiac expression of Hsp27 at high levels leads to reductive stress in the heart.

Cardiac Expression of Hsp27 at High Levels Resulted in Upregulation of GPx1

The expression levels of GPx1 in Tg10 mice were higher than in WT and Tg85 mice (P<0.01), whereas G6PDH levels were comparable among all of the groups (Figure 5A). Consistently, GPx activity of Tg10 mice was significantly greater than WT mice (Figure 5B).

We then treated Tg10 mice with 50 mg/kg of MS for 4 weeks. This optimal dose of MS was selected from a series of dose-ranging studies, which indicated that cardiac GPx activity in 50 mg/kg of MS-treated Tg10 mice was comparable with WT (Figure 5B). We observed that, after 4 weeks of MS administration, the percentages of both ejection fraction and fractional shortening were significantly improved compared with age-matched untreated Tg10 control mice (Figure 5C). The ratios of HW/BW and HW/tibia length were also significantly reduced after MS treatment (Figure 5D).

Hearts From High Hsp27 Tg Mice Showed Decreased Iron Content

Figure 6A shows that the levels of iron and ferritin were lower in Tg10 mice than in WT and Tg85 mice (P<0.01). In contrast, the level of transferrin receptor 1 (TFR1) in Tg10 mice was higher than in WT and Tg85 mice (P<0.01; Figure 6B). The iron contents in lungs or livers were comparable between WT and Tg10 mice (Figure S7A). To investigate the role of cardiac iron deficiency in the development of cardiomyopathy, we supplied mice with ferrous fumarate in drinking water. The results revealed that exogenous iron supplement neither improved cardiac function nor changed cardiac iron contents (Figure S7B and S7C), suggesting that cardiac iron deficiency may be caused by the decreased iron uptake into myocytes by Hsp27.

Discussion

The significant findings in the present study are that Tg mice with cardiac-specific expression of high levels of Hsp27 lead to reductive stress and cardiomyopathy. Our findings suggest that reductive stress plays an important role in the development of cardiomyopathy and that Hsp27 could be a potential target of the therapeutic approach for reductive stress-related cardiomyopathy in humans, such as desmin-related myopathy.

It is still unclear why high levels of Hsp27 expression lead to reductive stress. However, our observation was supported by several studies.5,14,23 It is reported that high expression of Hsp25 increased the glutathione pool through upregulation of GPx,15,22 whereas decreased Hsp27 levels were associated with a decrease of GPx.21 GPx1 is an inducible antioxidant enzyme, which catalyzes the disposition of \( \text{H}_2\text{O}_2 \) into \( \text{H}_2\text{O} \) and \( \text{O}_2 \).5 We observed that GPx1 and GSH were significantly increased in the myopathic hearts. Our data suggest that reductive stress induced by Hsp27 could be caused, at least in part, by the upregulation of GPx1, which results in both facilitation of the glutathione-redox cycle and detoxification of existing ROS.

Iron plays a critical role in the generation of superoxide through Haber-Weiss-Fenton reactions.23 Therefore, lowering the intracellular iron content will diminish the ROS generation. Indeed, we found that the contents of both iron and ROS were reduced in the myopathic hearts. Our observation was supported by numerous other studies. For example, expression of Hsp27 in L929 cells dramatically decreased the intracellular iron level and protein carbonyl content.12 In CCL39 cells, Hsp27 overexpression caused less iron uptake, whereas downregulation of Hsp27 increased the iron level.24 We also observed that ferritin levels were significantly decreased, whereas TFR1 levels were increased in myopathic hearts. Ferritin can safely store excess iron,25 and the TFR1-dependent pathway is the main route of cellular iron uptake.26 Low intracellular free iron will downregulate ferritin and upregulate TFR1 by feedback regulation.21 We observed that cardiac-specific expression of Hsp27 at high levels only leads to iron deficiency in hearts, whereas not in lungs and livers. We also observed that exogenous iron supplement did not
increase cardiac iron content, which indicates that cardiac iron deficiency could be caused by the decreased uptake into cardiomyocytes by Hsp27. Although we do not understand why high levels of Hsp27 decrease the uptake of iron, Chen et al. reported recently that Hsp27 downregulates TfR1-mediated iron uptake via stabilization of the cortical actin cytoskeleton in CCL39 cells.

We observed that the hearts in high Hsp27 Tg mice developed cardiomyopathy. Although we do not understand the mechanisms by which high Hsp27 Tg mice developed cardiomyopathy at present, Rajasekaran et al. reported recently that cardiomyopathy could be caused by reductive stress and protein aggregation. A similar phenomenon was found in the present study. We found that inhibition of GPx1 by MS, a widely used GPx1 inhibitor, significantly improved cardiac function and decreased HW in myopathic hearts. Our data suggest that expression of Hsp27 at high levels will induce reductive stress and cardiomyopathy, in part, through upregulation of GPx1 expression.

Perspectives
Oxido-redox homeostasis is essential for normal metabolism of cardiomyocytes. The role of oxidative stress in the pathogenesis of cardiac diseases has been intensively investigated, whereas the role of reductive stress is poorly studied. Our findings provide evidence that high expression of Hsp27 will lead to reductive stress and cardiomyopathy and that Hsp27 could be a potential target for the therapeutic approach of patients with cardiomyopathy. Further intensive studies need to address how reductive stress results in cardiomyopathy and whether the mitochondrial and sarcoplasmic reticulum is involved.

Acknowledgments
We are grateful to Dr Jim Kelley (East Tennessee State University) for critical reading of the article. We are also grateful to Drs Yanzhu Ha (East Tennessee State University) and Liangjun Yan (University of North Texas Health Science Center, Fort Worth, TX) for the helpful discussions.

Sources of Funding
This work was supported by the National Natural Science Foundation of China (grant 30972856) and the Jiangsu Province Natural Science Foundation of China (grants BK2007247 and BK2008467). This work was also supported by the Chinese Medical Association (grant 09010320187).

Disclosures
None.

References
Involvement of Reductive Stress in the Cardiomyopathy in Transgenic Mice With Cardiac-Specific Overexpression of Heat Shock Protein 27
Xia Zhang, Xiaoyan Min, Chuanfu Li, Ivor J. Benjamin, Bo Qian, Xiaojin Zhang, Zhengnian Ding, Xiang Gao, Yuzhen Yao, Yujie Ma, Yunling Cheng and Li Liu

Hypertension. 2010;55:1412-1417; originally published online May 3, 2010;
doi: 10.1161/HYPERTENSIONAHA.109.147066

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/55/6/1412

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2010/04/30/HYPERTENSIONAHA.109.147066.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/
Involvement of Reductive Stress in the Cardiomyopathy in Transgenic Mice with Cardiac-specific Overexpression of Hsp27

ONLINE SUPPLEMENT

Xia Zhang*, Xiaoyan Min*, Chuanfu Li†, Ivor J. Benjamin‡, Bo Qian†, Xiaojin Zhang*, Zhengnian Ding§, Xiang Gao#, Yuzhen Yao*, Yujie Ma*, Yunling Cheng* and Li Liu*

*Departments of Geriatrics and §Departments of Anesthesiology, First Affiliated Hospital with Nanjing Medical University, Nanjing 210029, China; †Departments of Surgery, East Tennessee State University, Johnson City, TN37614; ‡Center of Cardiovascular Translational Biomedicine, University of Utah School of Medicine, Salt Lake City, UT 84132; #Model Animal Research Center, Nanjing University, Nanjing 210061, China

Running Head: Hsp27 induces reductive stress and cardiomyopathy

Corresponding to: Li Liu, MD, PhD. and Yunlin Cheng, MD. Department of Geriatrics, First Affiliated Hospital with Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, China. Tel: 01186-25-83718836-5021; Fax: 01186-25-83724440; Email: liuli652003@yahoo.com.cn
Supplemental Methods

Antibodies and Reagents

Primary antibodies for Hsp27 were obtained from Stressgen (Victoria BC, Canada). FITC labeled lectin wheat germ agglutinin (WGA), primary antibodies for GAPDH and dinitrophenylhydrazine (DNPH), mercaptosuccinate (MS), oxidized glutathione (GSSG), glutathione reductase, 5-sulphosalisilic acid and 2-vinylpyridine were purchased from Sigma-Aldrich (St Louis, MO). Primary antibodies for G6PD, glutathione peroxidase 1(GPx1) and transferrin receptor 1 (TRF1) were purchased from Bioworld (Minneapolis, MN). 2’,7’-Dichlorofluorescein diacetate (DCFH-DA) was obtained from Merck (Darmstadt, Germany). Protease inhibitor cocktail was purchased from Roche (Mannheim, Germany). Trizol reagent was purchased from Invitrogen Life Technologies (Carlsbad, CA). Oligo-dT was purchased from Promega (Madison, WI). Access Ferritin assay kit was obtained from Beckman Coulter (Fullerton, CA). Analytical kits for malondialdehyde (MDA) and glutathione peroxidase activity were purchased from Jiancheng BioTech (Nanjing, China). Ferrous fumarate suspension was from Hayao Medicinal Manufacture (Haerbin, China).

Animals

The strain of the founder of Hsp27 transgenic mice was CBA/BL6. The founders and all subsequent generations were crossed to C57/BL6 mice. All experiments were conducted with F10 and later generations. Total 217 mice (66 of WT, 61 of Tg85, 82 of Tg10, 4 of
Tg94 and 4 of Tg21) were used in the present study.

**Animal anesthesia**

Mice were anesthetized with tribromoethanol/amylene hydrate (2.5% wt/vol, 8 μl/g body weight, I.P injection) for the measurement of echocardiography.
# Table S1. Cardiac Function Measured by Echocardiography

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WT</th>
<th>Tg85</th>
<th>Tg10</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVSd(mm)</td>
<td>0.70±0.08(n=10)</td>
<td>0.65±0.06(n=10)</td>
<td>0.72±0.02(n=10)</td>
</tr>
<tr>
<td>IVSs(mm)</td>
<td>1.07±0.06(n=10)</td>
<td>1.01±0.11(n=10)</td>
<td>1.12±0.07(n=10)</td>
</tr>
<tr>
<td>LVPWd(mm)</td>
<td>0.76±0.13(n=10)</td>
<td>0.69±0.09(n=10)</td>
<td>0.72±0.02(n=10)</td>
</tr>
<tr>
<td>LVPWs(mm)</td>
<td>1.10±0.10(n=10)</td>
<td>1.04±0.10(n=10)</td>
<td>1.06±0.09(n=10)</td>
</tr>
<tr>
<td>LVIDd(mm)</td>
<td>3.29±0.47(n=10)</td>
<td>3.25±0.29(n=10)</td>
<td>3.77±0.38(n=10) *†</td>
</tr>
<tr>
<td>LVIDs(mm)</td>
<td>2.17±0.36(n=10)</td>
<td>2.14±0.32(n=10)</td>
<td>2.87±0.43(n=10) ‡§</td>
</tr>
<tr>
<td>LVVd(ul)</td>
<td>45.10±15.31(n=10)</td>
<td>43.06±9.21(n=10)</td>
<td>61.87±14.60(n=10) *†</td>
</tr>
<tr>
<td>LVVs(ul)</td>
<td>16.37±6.55(n=10)</td>
<td>15.75±5.64(n=10)</td>
<td>32.61±11.25(n=10) ‡§</td>
</tr>
<tr>
<td>EF(%)</td>
<td>64.23±4.67(n=10)</td>
<td>64.28±6.87(n=10)</td>
<td>48.40±8.01(n=10) ‡§</td>
</tr>
<tr>
<td>FS(%)</td>
<td>34.15±3.20(n=10)</td>
<td>34.34±5.13(n=10)</td>
<td>24.12±4.69(n=10) ‡§</td>
</tr>
<tr>
<td>LV Mass(mg)</td>
<td>75.39±13.77(n=10)</td>
<td>66.18±11.87(n=10)</td>
<td>92.87±15.12(n=10) *§</td>
</tr>
<tr>
<td>LV Mass Corrected(mg)</td>
<td>60.32±11.01(n=10)</td>
<td>52.94±9.50(n=10)</td>
<td>74.30±12.10(n=10) *§</td>
</tr>
<tr>
<td>MV E/A</td>
<td>1.68±0.19(n=6)</td>
<td>1.71±0.25(n=9)</td>
<td>1.32±0.24(n=10) *§</td>
</tr>
<tr>
<td>AV Peak V(mm/s)</td>
<td>1202.38±217.30(n=9)</td>
<td>1245.69±291.80(n=9)</td>
<td>1266.13±287.13(n=10)</td>
</tr>
<tr>
<td>AV Peak Gradient</td>
<td>5.97±1.89(n=9)</td>
<td>6.52±3.02(n=9)</td>
<td>6.72±2.96(n=10)</td>
</tr>
<tr>
<td>Ao Root(mm)</td>
<td>1.49±0.10(n=7)</td>
<td>1.55±0.10(n=7)</td>
<td>1.50±0.14(n=10)</td>
</tr>
</tbody>
</table>
IVSd: interventricular septal thickness at diastolic phase; IVSs: interventricular septal thickness at systolic phase; LVPWd: left ventricular posterior wall thickness at diastolic phase; LVPWs: left ventricular posterior wall thickness at systolic phase; LVIDd: left ventricular internal diameter at diastolic phase; LVIDs: left ventricular internal diameter at systolic phase; LVVd: left ventricle end-diastolic volume; LVVs: left ventricle end-systolic volume; %EF: Ejection fraction; %FS: Fractional Shortening; LV Mass: Left ventricular mass; MV E/A: mitral valve E wave / A wave; AV Peak V: aortic valve peak velocity; AV Peak Gradient: aortic valve peak Gradient; Ao Root: aortic Root diameter. 

*P<0.05, vs. WT; †P<0.05, vs. Tg85; ‡P<0.01, vs. WT; §P<0.01, vs. Tg85; n=6~10 each group.
Figure Legends:

**Figure S1.** Hsp27 expression was analyzed by immunoblot in different tissues. Lv: liver; Sp: spleen; Ln: lung; Kd: kidney; Ht: heart; Br: brain; and Sk: skin.

**Figure S2.** High levels of Hsp27 cause cardiac hypertrophy. (A) Ratios of HW/BW and HW/TL. *P*<0.01, n=7~12/group. (B) Myocyte size was determined by FITC-WGA staining. *P*< 0.01, n=4/group. (C) The mRNA levels for ANP and BNP were analyzed by RT-PCR. *P*< 0.01, n=6/group. (D) Blood pressure in Tg10 mice and WT mice. n=10~13/group.

**Figure S3.** Mortality analysis. *P*<0.01 vs. WT mice, #P<0.05 vs. Tg85 mice. n=19/WT group, n=11/Tg85 group, n=33/Tg10 group.

**Figure S4.** Histological examination. (A, B) H&E stained tissues were examined using the light microscope and the confocal microscope. (C) Immunohistochemistry for Hsp27. n=3/group.

**Figure S5.** Representative electron micrographs in cardiac sections. Note that myofilaments were absent (*) and the sarcoplasmic reticulum was vacuolated (→) in some areas of myocytes of Tg10 mice. n=3/group.
**Figure S6.** High levels of Hsp27 caused reductive stress in hearts. (A) Lipid peroxidation was measured as MDA content. *P<0.01, †P<0.05, n=9~11/group. (B) Protein oxidation was measured as carbonyl-containing 2,4-DNPH adducts by Western blot. *P<0.01, n=6~9/group.

**Figure S7.** Iron content and supplement. (A) Basal iron contents of lungs and livers in WT and Tg10 mice. n=6/group. (B, C) Cardiac function and iron content was not significantly changed after Fe supplementation in Tg10 mice. Tg10: untreated age-matched Tg10 control mice, Tg10+Fe: Tg10 mice supplemented with iron. n=6~10/group.
Figure S1
Figure S2

A. Ratios

B. Area of Single Myocyte (μm²)

C. Relative Intensity

D. Blood Pressure

Figure S2
Survival Time (weeks)

Survival Rate (%)

WT (n=19)

Tg85 (n=11)

Tg10 (n=33)

Figure S3
Figure S5
Figure S6
Figure S7

A

Fe Content
(mg/g protein)

WT

Tg10

Tg10+Fe

Lung  Liver

B

Tg10

Tg10+Fe

C

cardiac Fe content
(mg/g Protein)

Tg10  Tg10+Fe