Upregulation of Heat Shock Transcription Factor 1 Plays a Critical Role in Adaptive Cardiac Hypertrophy


Abstract—Exercise-induced cardiac hypertrophy has been reported to have better prognosis than pressure overload-induced cardiac hypertrophy. Cardiac hypertrophy induced by exercise was associated with less cardiac fibrosis and better systolic function, suggesting that the adaptive mechanisms may exist in exercise-induced hypertrophy. Here, we showed a critical role of heat shock transcription factor 1 (HSF1), an important transcription factor for heat shock proteins, in the adaptive mechanism of cardiac hypertrophy. We examined expression of 8800 genes in the heart of exercise-induced hypertrophy model using DNA chip technique and compared with pressure overload–induced hypertrophy. Expression of HSF1 and its target molecule heat shock proteins was significantly upregulated in the heart by exercise but not by chronic pressure overload. Constitutive activation of HSF1 in the heart significantly ameliorated death of cardiomyocytes and cardiac fibrosis and thereby prevented cardiac dysfunction as well as hypertrophy induced by chronic pressure overload. Conversely, decreased activity of HSF1 in the heart promoted cardiac dysfunction in response to exercise, a load that normally leads to adaptive hypertrophy with preserved systolic function. Likewise, cardiac function was significantly impaired from the early phase of pressure overload, when HSF1 activation was inhibited. These results suggest that HSF1 plays a critical role in the transition between adaptive and maladaptive hypertrophy. (Circ Res. 2006;99:1411-1418.)

Key Words: pressure overload ■ exercise ■ heart failure

Cardiac hypertrophy is an adaptive response to increased wall stress. At the beginning, cardiac hypertrophy has beneficial effects to maintain cardiac output by reducing wall stress; however, long-term stresses induce systolic dysfunction, leading to heart failure.1 Clinical studies have demonstrated that cardiac hypertrophy is not only a cause of congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for congestive heart failure but also an independent risk factor for heart failure.6 Although there have been reports demonstrating different expression patterns of some genes between these 2 forms of cardiac hypertrophy,7,8 the precise mechanism of different prognosis remains unclear.

In the present study, we examined expression of 8800 genes in the hearts of 2 hypertrophy rat models using DNA chip. In the DNA chip analysis, we found that the heat shock protein (Hsp) genes such as Hsp70 and Hsp27 were markedly upregulated in the heart of the exercise model but not in that of the pressure-overload model. Heat shock transcription factor 1 (HSF1), which regulates Hsps gene expression,9–11 was activated only in the heart of the exercise model. Overexpression of activated HSF1 in the heart prevented cardiomyocyte death and cardiac fibrosis in response to sustained pressure overload and thereby preserved cardiac function.
function. Conversely, decreased expression of HSF1 in the heart impaired the adaptive response to exercise or acute pressure overload and thus caused cardiac dysfunction, suggesting a protective role of HSF1 in cardiac physiology.

Materials and Methods

Rat Models

All protocols were approved by the Institutional Animal Care and Use Committee of Chiba University. Male Wistar rats obtained from Nippon Bio-Supply Center (Tokyo, Japan) were divided into 3 groups: sham-operated model, exercise-induced hypertrophy model, and pressure overload-induced hypertrophy model (n=8, each group). Five-week-old male rats were individually housed and voluntary exercised in a specially manufactured cage equipped with a controlled running wheel and a distance counter as described previously. In this cage, there were 2 rooms (a room for wheel running and another for rest), and therefore rats could run or rest ad libitum. Pressure overload was produced by constriction of abdominal aorta as described previously. Briefly, 8-week-old male rats were anesthetized with an intraperitoneal injection of a cocktail of ketamine HCl (100 mg/kg) and xylazine (5 mg/kg). The abdominal aorta was constricted above the right renal artery by 4-0 silk suture tied around both the aorta and a blunted 22-gauge needle, which was then pulled out.

DNA Chip Analysis

Total RNA (5 μg) was extracted from LV of each model by the lithium/urea method and was used to synthesize biotin-labeled cRNA, which was then hybridized to high-density oligonucleotide array (GeneChip U34A array; Affymetrix, Santa Clara, Calif) according to the previously published protocol. Arrays contain probe sets for approximately 8800 genes and expressed-sequence tags, which were selected from Build no. 34 of the UniGene Database (created from GenBank 107/dbEST, November 18, 1998). The GeneChip 3.3 software (Affymetrix) was used to calculate the average difference for each probe on the array, which was shown as intensity value of gene expression defined by Affymetrix using their algorithm. The average difference has been shown to quantitatively reflect the abundance of particular mRNA molecule in a population.

HSF1 Transgenic Mice

Construction of active form of human HSF1 transgene and generation of the hHSF1 transgenic mice have been previously described. Eight-week-old male transgenic and their wild-type littermates were used. The transgenic mice were apparently healthy, and there were no significant differences in the body weight (BW), heart weight to BW ratio (HW/BW), or hemodynamic parameters, such as blood pressure (BP) and heart rate (HR), between the transgenic mice and wild-type littermates. Strong pressure overload was imposed on the heart for 5 weeks by surgical constriction of transverse aorta (TAC) as described previously.

HSF1-Deficient Mice

The generation of HFS1−/− deficient mice has been described previously. The HFS1−/− deficient heterozygote mice were apparently healthy. There were no significant differences in echocardiogram parameters such as posterior wall thickness (PWTd) and fractional shortening between the mutant mice and wild-type littermates. The mice underwent 4 weeks of exercise (wheel running) or 1 week of TAC as described above.

Histological Analysis

For histological analysis, hearts were fixed by perfusion with 10% formalin. Fixed hearts were embedded in paraffin and sectioned at 4-μm thickness. The myocyte cross-sectional diameter was measured in the sections stained with hematoxylin and eosin, and suitable cross-sections were defined as having nearly circular capillaries and nuclei (n=100, each group). To determine the degree of collagen fiber accumulation, we selected 5 fields at random and calculated the ratio of Masson-stained fibrosis area to total myocardium area with the software “NIH Image” (Bethesda, Md) for image analysis as described previously. Cardiomyocyte death was detected in situ by terminal deoxyribonucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) method using Cardio TACS (Trevigen Inc, Gaithersburg, Md), in paraffin-embedded heart tissue sections.

Ribbonuclease Protection Assay and Northern and Western Blot Analysis

Total RNA (20 μg) was separated on a 1.0% agarose/formaldehyde gel and was hybridized with the cDNA fragments of Hsp70, Hsp27, and HSF1 genes. Ribonuclease protection assay (RiboQuant, Pharmingen, Franklin Lakes, NJ) was performed according to the instructions of the manufacturer. Western blotting was performed as previously described. Antibody for inducible Hsp70 was purchased from Stressgen Biotechnologies (Victoria, Canada) and antibody for HSF1 was from Santa Cruz Biotechnology (Santa Cruz, Calif).

Gel Mobility-Shift Assay

DNA-binding activity of HSF1 was examined using a self-complementary oligonucleotide containing the heat shock element (5′–CTAGAAGCTTCTAGAAGCTTCTAG–3′) as a probe.

ECG Analysis

Transtracheal echocardiography was performed with the HP Sonos 4500 (Hewlett-Packard Co, Palo Alto, CA) with a 10-MHz imaging transducer, and the progress of cardiac hypertrophy was evaluated as described previously.

Hemodynamic Measurements In Vivo

To measure hemodynamic effects of aortic constriction and exercise, the right carotid artery was cannulated with a polyethylene catheter (MILLAR, Houston, Tex). The transducer (model MP 5100, Baxter, Deerfield, Ill) was connected to Mac Laboratory system (model MacLab/4s, AD Instruments, Castle Hill, Australia), and BP, HR, and LV end-diastolic pressure were measured.

Statistical Analysis

Data were shown as mean±SEM. Multiple group comparison was performed by 1-way ANOVA, followed by the Bonferroni procedure for comparison of means. Comparison between 2 groups were analyzed by the 2-tailed Student’s t test or 2-way ANOVA. Values of P<0.05 were considered statistically significant.

Results

Physiological Analysis

After 8 weeks of voluntary exercise or 5 weeks of pressure overload, physiological analyses were performed in 13-week-old rats. Echocardiogram revealed that PWTd and LV end-diastolic dimension were larger in both groups compared with those in the sham-operated group (Table I in the online data supplement, available at http://circres.ahajournals.org). Sustained pressure overload developed cardiac hypertrophy more profoundly than exercise (supplemental Table I). Fractional shortening was decreased in the pressure-overload group but not in the exercise group (supplemental Table I). In hemodynamic parameters, BP measured in the right carotid artery and LV end-diastolic pressure were much higher in the pressure-overload group than the sham-operated group (supplemental Table II). On the other hand, BP was significantly lower and HR was less in the exercise model compared with the pressure-overload model and the sham-operated model (sup-
Expression Level of Hsp70 Protein and Activity of HSF1 Were Elevated in Exercise-Induced Hypertrophy

To determine the molecular difference between exercise- and chronic pressure overload–induced hypertrophy, we performed DNA chip analysis. Expression levels of ≈100 genes were differentially elevated in each model compared with the sham-operated model. mRNA levels of fetal genes such as atrial natriuretic peptide, brain natriuretic peptide, and skeletal α-actin were elevated 2- to 3-fold in the pressure-overload model but not in the exercise model (Figure 1E through 1H). These results indicate that chronic pressure overload but not exercise induces cardiac fibrosis, thereby promoting maladaptive cardiac hypertrophy.

Protective Role of HSF1 in Sustained Pressure Overload–Induced Hypertrophy

To elucidate the role of HSF1 in cardiac hypertrophy, we established the cardiac hypertrophy model by TAC in transgenic mice that express constitutively active HSF1 and analyzed them 5 weeks after the operation. We also performed the same operation in their wild-type littermates as controls. Expression of activated HSF1 was detected in the heart of transgenic mice compared with wild-type mice (Figure 3A). Inducible Hsp70 expression was markedly increased in the heart of transgenic mice but not in wild-type mice (Figure 3A). Pressure studies revealed that there was no difference in BP and HR between transgenic and wild-type mice before the operation (data not shown). After the operation, BP was significantly increased in both transgenic mice and wild-type mice to the same extent (Table 2). LV end-diastolic pressure was significantly elevated in wild-type mice, which was significantly ameliorated in transgenic mice (Table 2). HW/BW was significantly increased in wild-type mice, but the increases in HW/BW were significantly less in transgenic mice than in wild-type mice (Table 2).

Echocardiographic analysis demonstrated that an increase in PWTd...
by TAC was significantly attenuated in transgenic mice compared with that in wild-type mice (Figure 3B). More importantly, cardiac function was significantly better in transgenic mice than in wild-type mice (Figure 3B), suggesting a protective role of HSF1 in cardiac hypertrophy.

Histological analysis showed that TAC induced marked cardiomyocyte hypertrophy in wild-type mice after 5 weeks, whereas transgenic mice developed less cardiomyocyte hypertrophy in response to sustained pressure overload than wild-type mice (Figure 4A). Moreover, cardiac fibrosis was significantly less in transgenic mice compared with wild-type mice (Figure 4B). Because TAC has been reported to induce death of cardiomyocytes,21 we examined death of cardiomyocytes in the heart by TUNEL. TUNEL-positive cells were increased in the heart of wild-type mice compared with sham mice (Figure 4C). The number of TUNEL-positive cells was significantly less in the heart of transgenic mice compared with that of wild-type mice (Figure 4C). Expression of transforming growth factor-β, a cytokine that is crucial for tissue fibrosis,22 was markedly increased in the heart after pressure overload (Figure 4D). This induction was significantly prevented by overexpression of HSF1. It has been reported that HSF1 and Hsps suppress activation of transcription factors such as activator protein-1 and nuclear factor-κB,23–25 both of which are known to upregulate proinflammatory cytokines including transforming growth factor-β. Thus, HSF1 may prevent cardiac fibrosis as well as cardiomyocyte death by antagonizing these proinflammatory pathways, thereby preventing the transition from adaptive to maladaptive hypertrophy.

Cardiac hypertrophy (PWTd) and systolic function (percentage of fractional shortening [%FS]) were examined 5 weeks after TAC by echocardiogram. *P<0.05 vs sham, †P<0.05 vs WT-TAC (n=5).
HSF1 Deficiency Impairs the Adaptive Response to Exercise or Pressure Overload

We next determined whether HSF1 has a protective role in exercise-induced hypertrophy. We produced the exercise-induced cardiac hypertrophy model in HSF1-deficient heterozygote (HSF1−/+), and comparing them with their wild-type littermates. Western blot analysis demonstrated that expression of HSF1 was less in the heart of HSF1−/+ mice than wild-type mice (Figure 5A). Expression of inducible Hsp70 was increased in the heart of wild-type mice by exercise, but this increase was attenuated in the heart of HSF1−/+ mice (Figure 5B). There were no differences in HW/BW and wall thickness (PWTd) between wild-type mice and HSF1−/+ mice before exercise (Figure 5C and 5D). After 4 weeks of exercise, both mice developed cardiac hypertrophy to a similar extent (Figure 5C and 5D). In contrast to the preserved function of wild-type mice, cardiac function of HSF1−/+ mice was significantly reduced after exercise (Figure 5D), suggesting a critical role of HSF1 in the adaptive mechanism of cardiac hypertrophy induced by exercise.

We noted that pressure overload by TAC induced adaptive hypertrophy until 1 to 2 weeks after operation to preserve cardiac function; however, this adaptive mechanism could not protect the hypertrophied heart against sustained pressure overload, resulting in systolic dysfunction at 4 to 5 weeks (Figure 3B). To further test the role of HSF1 in adaptive hypertrophy, we produced the TAC model in HSF1−/+ mice and their wild-type littermates and analyzed cardiac function 1 week later. In this adaptive phase, TAC increased expression of inducible Hsp70 in the heart of wild-type mice (Figure 6A). This induction was markedly attenuated in the heart of HSF1−/+ mice (Figure 6A). Consistent with the results of our rat model, expression of inducible Hsp70 was downregulated in the maladaptive phase (supplemental Figure). Both HSF1−/+ mice and wild-type mice developed cardiac hypertrophy to the degree similar to that of 4 weeks of exercise (Figures 5C, 5D, 6B, and 6C). Cardiac function was preserved in wild-type mice, but it was significantly impaired in HSF1−/+ mice (Figure 6C), further supporting a notion that HSF1 has an important role in adaptive hypertrophy.

Discussion

In the present study, we performed DNA chip analysis to elucidate the molecular difference between exercise-induced and sustained pressure overload–induced cardiac hypertrophy. We found that ~100 genes were differentially expressed in each hypertrophy model compared with the sham-operated model. Among them, expression levels of Hsp70 and Hsp27 were elevated only in the heart of the exercise model, which was confirmed by Northern and Western blot analysis. Hsps such as Hsp70 and Hsp27 are ubiquitously expressed and its expression is enhanced by various stresses such as heat shock, reactive oxygen species, heavy metals, and inflammation. It has been reported that stimuli that result in injury. Constitutive expression of Hsps in transgenic mice efficiently recovers cardiac function after ischemia/reperfusion injury. Constitutive expression of Hsps in transgenic mice efficiently recovers cardiac function after ischemia and reperfusion, whereas absence of Hsps leads to cardiac dysfunction and impairs stress response. Several studies have reported that expression of Hsps is rapidly induced in the heart in response to acute pressure overload, as well as volume overload. However, there has been no report concerning expression of Hsps in the chronic stage of cardiac hypertrophy.
hypertrophy. In this study, Hsp70 and Hsp27 were upregulated in the heart after exercise but not in the heart after long-term pressure overload. These results suggest that exercise persistently upregulates expression of Hsps, thereby inhibiting cardiac dysfunction associated with hypertrophy, whereas pressure overload transiently induces expression of Hsps and thus fails to prevent impaired function in the chronic phase.

The transcription of Hsps genes is mainly controlled by HSF1.9–11 Impaired activation of HSF1 in the aged heart results in diminished induction of Hsps by stressful stimuli such as heat shock or ischemia. This impairment is associated with reduced protective effects of mild heat shock or ischemia against subsequent severe ischemic stresses in the aged heart.26 HSF1 deficiency has been reported to decrease cardiac expression of Hsps,37 further supporting a critical role of HSF1 in the regulation of Hsps expression. Although pressure overload and ischemia/reperfusion injury have been reported to rapidly induce HSF1 activation and Hsps expression in the heart,30,38 we found that HSF1 was activated in only the heart of the exercise-induced hypertrophy model but not in pressure overload–induced hypertrophy model at the chronic stage, suggesting that exercise promotes sustained activation of HSF1, which results in constitutive expression of Hsps in the heart. It remains unclear how HSF1 is downregulated under sustained pressure overload. Extracellular signal-regulated protein kinase (ERK) is known to increase HSF1 activity.39 It is also reported that ERK is activated by pressure overload and that its activation is involved in the adaptive mechanism of hypertrophy.40 Moreover, we observed that ERK was activated in exercise-induced hypertrophy but not in the chronic phase of pressure overload–induced hypertrophy (M. Sakamoto and I. Komuro, unpublished data, 2005), suggesting that the ERK signaling pathway may participate in the activation of HSF1 in exercise-induced hypertrophy and that its downregulation may cause the maladaptive response to chronic pressure overload by inactivating HSF1.

Constitutive activation of HSF1 prevented cardiac dysfunction as well as hypertrophy under chronic pressure overload. Decreased activity of HSF1 did not exacerbate hypertrophy but impaired systolic function in response to exercise or acute pressure overload, a load that usually results in adaptive hypertrophy. Thus, HSF1 activation may play an essential role in inhibiting the transition from adaptive to maladaptive hypertrophy rather than in regulating the size of cardiomyocytes. The different manifestations of hypertrophy between the exercise model and the long-term pressure-overload model might be attributable to the differences in the continuity and degree of stimulus. In a recent report, Perrino et al.41 showed that constitutive activation of HSF1 prevented cardiac dysfunction and hypertrophy in response to chronic pressure overload, whereas decreased activity of HSF1 impaired systolic function in response to acute pressure overload. These results suggest that HSF1 activation may play a critical role in inhibiting the transition from adaptive to maladaptive hypertrophy, thereby preventing the development of cardiac dysfunction.
et al. applied intermittent pressure overload to the hearts of mice and tested the roles of duration and nature of the stress on the development of cardiac failure. Despite a mild hypertrophic response, hearts exposed to intermittent pressure overload displayed various pathological features including diastolic dysfunction. Thus, the nature of the stress on the heart, rather than its duration, is the key determinant of the maladaptive phenotype. To test the role of the degree of stimulus, we compared the effect of 1 week of TAC on cardiac function with that of 4 weeks of exercise for the following reasons. First, pressure overload by TAC induces adaptive hypertrophy until 1 to 2 weeks after operation to preserve cardiac function. Second, 4 weeks of exercise did not induce cardiac dysfunction in wild-type mice. Third, 4 weeks of exercise induces cardiac hypertrophy to the degree similar to that of 1 week of TAC. Finally, expression of inducible Hsp 70 is upregulated in the heart of wild-type mice in both conditions. We produced these 2 different models for adaptive hypertrophy in HSF1+/− mice and their wild-type littermates. Cardiac function of HSF1−/− mice was significantly impaired in both models compared with their wild-type littermates. Moreover, expression of inducible Hsp 70 was markedly reduced in the heart of HSF1−/− mice. Although the nature of 2 different stimuli may lead to the differential molecular response, these results suggest a critical role of HSF1 in adaptive hypertrophy. Downregulation of HSF1 activity in the chronic phase may be a key factor to promote the transition from adaptive to maladaptive hypertrophy. Alternatively, the different manifestations of hypertrophy (adaptive or maladaptive) may be partly attributed to the difference in HSF1 activity between the exercise model and the chronic pressure-overload model because reducing its activity caused maladaptive hypertrophy in both conditions.

Sustained pressure overload induced cardiomyocyte death and fibrosis in the heart, both of which have been reported to cause heart failure. In the heart of HSF1 transgenic mice, the number of TUNEL-positive cardiomyocytes and the extent of fibrosis were significantly less than those of wild-type mice. These protective effects may be attributable to the functions of Hsps in protein folding and degradation. In addition to such well-known functions, accumulating evidence indicates that different Hsps directly act on the cell death machinery and inhibit the signaling pathway for cell death at various points. For example, Hsp27 binds to cytochrome c and prevent it binding to Apaf-1, whereas Hsp70 prevents Apaf-1 from recruiting pro-caspase-9, thereby inhibiting apoptotic cell death. Consequently, induction of HSF1 activity rather than individual Hsps may be more effective to protect the heart from severe stresses and will be an attractive therapeutic target in cardiovascular pathophysiology.

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Disclosures

None.

References

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**Supplemental Table 1**

Echocardiographic analysis

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<th>Sham</th>
<th>PO</th>
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<tr>
<td>PWTd (mm)</td>
<td>1.54±0.06</td>
<td>2.19±0.13**†</td>
<td>1.76±0.02*</td>
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<tr>
<td>LVDd (mm)</td>
<td>6.6±0.1</td>
<td>7.61±0.16*</td>
<td>7.14±0.02*</td>
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<tr>
<td>FS (%)</td>
<td>49.43±1.10</td>
<td>41.20±2.33**†</td>
<td>53.42±0.94</td>
</tr>
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</table>

Sham, sham-operated rat; PO, pressure-overloaded rat; EX, exercised rat; PWTd, posterior wall thickness at diastole; LVDd, left ventricular dimension at diastole; FS, fractional shortening. *p<0.05, **p<0.01 vs. Sham; †p<0.01 vs. EX (n=8).
## Supplemental Table 2

Hemodynamic parameters and heart weight

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<th></th>
<th>Sham</th>
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<tr>
<td>BP (mmHg)</td>
<td>99.3±7.2</td>
<td>144.6±9.0**†</td>
<td>84.4±4.3*</td>
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<tr>
<td>HR (bpm)</td>
<td>318±19</td>
<td>323±15 †</td>
<td>250±11**</td>
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<tr>
<td>LVEDP (mmHg)</td>
<td>0.85±0.05</td>
<td>4.77±0.18*</td>
<td>1.12±0.10</td>
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<tr>
<td>LVW (mg)/BW(g)</td>
<td>1.83±0.05</td>
<td>2.77±0.19**†</td>
<td>2.17±0.10*</td>
</tr>
</tbody>
</table>

Sham, sham-operated rat; PO, pressure-overloaded rats; EX, exercised rats; BP, blood pressure; HR, heart rate; LVEDP, left ventricular end-diastolic pressure; LVW, left ventricular weight; BW, body weight. *p<0.05, **p<0.01 vs. Sham; †p<0.05 vs. EX (n=8).
Supplemental Figure

Expression of inducible Hsp70 after pressure overload.

Pressure overload was imposed on wild-type mice. Whole cell lysates were extracted from the hearts at the indicated time points and examined for expression of inducible Hsp70 by Western blot analysis.
Supplemental Figure

![Supplementary figure showing Hsp70 and Actin protein expression over 28 days of TAC treatment.](image-url)