Cardiac-specific ablation of the STAT3 gene in the subacute phase of myocardial infarction exacerbated cardiac remodeling

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Enomoto D, Obana M, Miyawaki A, Maeda M, Nakayama H, Fujio Y. Cardiac-specific ablation of the STAT3 gene in the subacute phase of myocardial infarction exacerbated cardiac remodeling. Am J Physiol Heart Circ Physiol 309: H471–H480, 2015. First published June 8, 2015; doi:10.1152/ajpheart.00730.2014.—STAT3 is a cardioprotective molecule against acute myocardial injury; however, recent studies have suggested that chronic STAT3 activation in genetically modified mice was detrimental after myocardial infarction (MI). In the present study, we assessed the biological significance of STAT3 activity in subacute MI using tamoxifen (TM)-inducible cardiac-specific STAT3 knockout (STAT3 iCKO) mice. After coronary ligation, STAT3 was rapidly activated in hearts, and its activation was sustained to the subacute phase. To make clear the pathophysiological roles of STAT3 activation specifically in subacute MI, MI was generated in STAT3 iCKO mice followed by TM treatment for 14 consecutive days beginning from day 11 after MI, which ablated the STAT3 gene in the subacute phase. Intriguingly, mortality was increased by TM treatment in STAT3 iCKO mice, accompanied by an increased heart weight-to-body weight ratio. Masson’s trichrome staining demonstrated that cardiac fibrosis was dramatically exacerbated in STAT3 iCKO mice 24 days after MI (fibrotic circumference: 58.3 ± 6.7% in iCKO mice and 40.8 ± 9.3% in control mice), concomitant with increased expressions of fibrosis-related gene transcripts, including matrix metalloproteinase 9, procollagen 1, and procollagen 3.

Echocardiography clarified that cardiac function was deteriorated after MI, which ablated the STAT3 gene in the subacute phase. In conclusion, the intrinsic activity of STAT3 in the myocardium confers the resistance to cardiac remodeling in subacute MI.

signal transduction; cardiac remodeling; signal transducer and activator of transcription 3

NEW & NOTEWORTHY

We examined, for the first time, the cardioprotective role of STAT3 in the subacute phase after myocardial infarction in a time-specific manner. The basal activity of STAT3 in the postinfarct myocardium confers the resistance to cardiac remodeling in subacute myocardial infarction, preventing the progression of heart failure.

ACCUMULATING EVIDENCE has revealed that various kinds of cytokines and growth factors are produced from the myocardium in response to cardiac damages (8, 11, 25) and that their downstream signal transducers are rapidly activated (15, 16, 21) and protect cardiomyocytes from death (1, 14, 26, 30), preventing the onset of heart failure (9, 12). Therefore, these cardioprotective signaling pathways are believed to be therapeutic targets against cardiovascular diseases (5). However, unexpectedly, recent studies have proposed that signaling molecules may exhibit differential functions depending on their kinetics. For example, transient activation of Akt prevents cardiac cell death (6), whereas continuous activation of Akt exhibits detrimental effects, leading to heart failure (17). Thus, the significance of signaling pathways should be evaluated in a time-specific manner during a pathological process.

STAT3 plays protective roles against acute myocardial injury, such as ischemia and ischemia-reperfusion (19, 20). In contrast, a recent study (10) has shown that continuous activation of STAT3 mediated by glycoprotein (gp)130 promotes adverse outcome in subacute myocardial infarction (MI) in constitutively active gp130 mutant mice. Similarly, cardiomyocyte-specific ablation of suppressor of cytokine signaling (SOCS)3, a negative regulator of the gp130 system, results in increased mortality in a gp130-dependent manner, accompanied by contractile dysfunction (29). These results suggest that continuous and forced activation of the gp130/STAT3 pathway is detrimental to the maintenance of cardiac homeostasis; however, it remains to be elucidated whether an intrinsic level of STAT3 activity in subacute MI is required for the prevention of cardiac remodeling.

In the present study, to estimate the effects of STAT3 on cardiac remodeling in the subacute phase of MI, we took advantage of inducible cardiac-specific conditional STAT3-deficient (STAT3 iCKO) mice by generating α-myosin heavy chain (α-MHC)-MerCreMer mice on a STAT3<sup>floxed/floxed</sup> background. Using these mice, time- and tissue-specific ablation of the STAT3 gene was achieved by tamoxifen (TM) treatment. Here, we revealed that an intrinsic level of STAT3 activity functions as an inherent cardioprotective signal in subacute MI.

MATERIALS AND METHODS

Animal care. The care of all animals was approved by the Institutional Animal Care and Use Committee of the Graduate School of Pharmaceutical Science, Osaka University. The investigation conformed with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996).

Coronary artery ligation. MI was generated by ligation of the left anterior descending coronary artery according to a previous report (19) with minor modifications. In brief, mice were anesthetized and
ventilated with 100% O₂ containing 1.5% isoflurane. After a left side thoracotomy, the left coronary artery was ligated with 7-0 silk sutures. The chest and skin were closed with 5-0 silk sutures. Mice were fasted overnight after the operation and thereafter were allowed ad libitum access to standard chow and drinking water. Sham-operated mice were subjected to similar surgery without ligation.

Cardiomyocyte-specific modifications (20). Briefly, STAT3 flox mice were bred with iCKO mice were generated as previously described with minor modifications (20). Briefly, STAT3 flox mice were bred with transgenic mice expressing TM-inducible Cre recombinase fused to mutated estrogen receptor (MerCreMer) under the control of the cardiomyocyte-specific α-MHC promoter to produce α-MHC-MerCreMer/STAT3<sup>fl/fl</sup> mutant mice. α-MHC-MerCreMer/STAT3<sup>fl/fl</sup> mice were used as control mice. At the age of 8–12 wk, mutant mice were subjected to MI as described above. To induce Cre-mediated recombination, mice were intraperitoneally injected with 8 mg/kg TM (Sigma) dissolved in corn oil (Sigma) once a day for 14 consecutive days beginning at day 11 after MI (see Fig. 2A).

Immunoblot analyses. The left ventricle (LV) from the ligation point to the apex was harvested and homogenated in buffer containing 150 mmol/l NaCl, 50 mmol/l Tris·HCl (pH 6.8), 1% Triton X-100, and 0.1% SDS. Proteins were separated by SDS-PAGE and transferred onto a polyvinylidene difluoride membrane (Millipore). The membrane was immunoblotted with anti-phosphorylated (p)-STAT3 (Cell Signaling Technology). The membrane was reprobed with anti-STAT3 (Cell Signaling Technology) or anti-GAPDH (Chemicon) antibody to show equal amount loading. An ECL system was used for detection. The protein expression level was quantified by densitometry.

Immunofluorescent microscopic analyses. Frozen sections (5 μm thick) from hearts were prepared from the portion ~300 μm distal to the ligation point at 4 or 24 days after MI followed by staining with anti-p-STAT3 (Abcam) and anti-troponin I (Santa Cruz Biotechnology) antibodies. Alexa fluor488-conjugated donkey anti-goat IgG (Molecular Probes) and Alexa fluor546-conjugated goat anti-rabbit IgG (Molecular Probes) were used as secondary antibodies. Nuclei were also stained with Hoechst 33258.

Transcription factor ELISA. Hearts homogenates were prepared, and the DNA-binding activity of STAT3 was evaluated using a TransAM STAT3 Transcription Factor Assay Kit (Active Motif) according to the manufacturer’s protocol.

Estimation of the ratio of heart weight to body weight. On day 24 after MI, mice were weighed. After euthanasia by inhalation of isoflurane, the hearts were collected and subsequently washed with cold PBS. The ratio of heart weight to body weight was estimated.

Histological analysis of cardiac fibrosis and cardiac hypertrophy. Hearts were harvested 24 days after MI. Frozen sections (5 μm thick), prepared as described above, were stained with Masson’s trichrome or hematoxylin and eosin (H&E). As for Masson’s trichrome-stained sections, photomicrographs were taken, and fibrotic circumference and infarct wall thickness were measured with the use of ImageJ (NIH) by a researcher who was blinded to the experimental condition. The ratio of the fibrotic circumference to LV circumference was estimated. Similarly, scar wall thickness was measured perpendicular to the scar wall in three separate regions and averaged. Using H&E-stained sections, the cross-sectional area of cardiomyocytes was measured at the border zone.

Quantitative RT-PCR. RT-PCR was performed according to the manufacturer’s protocol. Total RNA was prepared from hearts on day 24 after MI using QIAzol reagent (QIAGEN). First-strand cDNA was synthesized from 1 μg total RNA with oligo (dT) primers and then used to detect expression of each mRNA. Matrix metalloproteinase (MMP)-9, procollagen 1, procollagen 3, IL-6, TNF-α, IL-1β, VEGF, and thrombospondin (Tsp)1 mRNA expressions in the LV from the ligation point to the apex were quantified by real-time PCR with the SYBR green system (Applied Biosystems). As an internal control, GAPDH was used. The primers used in this study were as follows:

- **MMMP:** forward 5′-AGCAACAGCAGTCGACTGAGAAG-3′ and reverse 5′-GCCTTCGGACGGTGAAATGCATCAA-3′; procollagen 1, forward 5′-GCGAGTGGCCTGTTGTTCT-3′ and reverse 5′-GCCCAACAGCTCTTCACCACT-3′; procollagen 3, forward 5′-GCCCAACAGCTCTTCACCACT-3′ and reverse 5′-CGGGGTCACCATTTCCTC-3′; IL-6, forward 5′-AGAGCAGCTTCATCATGACCTGCC-3′ and reverse 5′-ATTACCCAGGAGAGACCTGGAGATAG-3′; TNF-α, forward 5′-CCTGCTGATCTGCTGTTGCTA-3′ and reverse 5′-CTCTGGAAGAGAAACTGTTG-3′; IL-1β, forward 5′-GCAGCTAGTGGAACCTGCTTCT-3′ and reverse 5′-ATGATGTTGACTGCTTTCCG-3′; VEGF, forward 5′-AGATCATCGGCGATTAAACCT-3′ and GAPDH, forward 5′-CATCACCATTCCAGAGGACGC-3′ and reverse 5′-GAGGGCCATCCACAGTCTC-3′.

Gelatin zymography. Hearts were homogenated in buffer containing 0.36 mmol/l NaCl, 1.2 mmol/l Tris-HCl (pH 8.0), and 1% Triton X-100. Total protein concentration was determined with a BCA Protein Assay Kit (Pierce). Using homogenate samples (18 μg protein), MMP-9 enzymatic activity was evaluated with a Gelatin-Zymography Kit (Cosmo Bio) according to the manufacturer’s protocol.

Analysis of cardiac function by echocardiography. M-mode trans-thoracic echocardiography was performed using an IE33 (Philips). Twenty-four days after MI, fractional shortening, ejection fraction, heart rate, LV internal dimension at diastole, and LV internal dimension at systole of mice were estimated under 1.5% isoflurane anesthesia. The investigator was blinded to the identity of the mice for analysis.

Diidroethidium fluorescence analysis. Diidroethidium (DHE) fluorescence analysis was performed to examine the generation of superoxide according to a previous report (20). Frozen sections (5 μm thick) were prepared and stained with 10 μM DHE in Krebs-HEPES buffer composed of (in mmol/l) 99.0 NaCl, 4.69 KCl, 1.87 CaCl<sub>2</sub>, 1.20 MgSO<sub>4</sub>, 1.03 K<sub>2</sub>HPO<sub>4</sub>, 25.0 NaHCO<sub>3</sub>, and 20.0 Na-HEPES, and 11.1 glucose (pH 7.4) at 37°C for 30 min in the dark. The intensities of fluorescence were quantitatively analyzed with ImageJ by a researcher who was blinded to the assay conditions.

Immunohistochemical analyses of capillary density. Frozen sections, prepared as described above, were fixed with ice-cold acetone. Capillaries were stained using a Vectastain ABC kit (Vector Laboratories) with anti-CD31 antibody (BD Bioscience). To estimate capillary density, photomicrographs at the border area, defined as a region <1 mm from the end of the infarct area, were taken, and CD31-positive cells were counted by a researcher who was blinded to the assay condition.

Statistical analysis. Data are presented as means ± SD. Survival rate was estimated by the Kaplan-Meier method and analyzed by a log-rank test. Comparisons between two groups were performed with an unpaired t-test. One-way ANOVA followed by a Tukey-Kramer or Bonferroni test was used for multiple comparisons. Differences were considered to be statistically significant when the calculated (two-tailed) P value was <0.05.

RESULTS

MI-induced STAT3 activation was sustained in the heart from the acute phase to the subacute phase. We investigated the time course of STAT3 activation in the whole heart from the acute phase to the subacute phase after coronary ligation. C57Bl/6 mice were exposed to MI, and STAT3 activation was evaluated at various time points after MI (Fig. 1A). Immunoblot analyses revealed that STAT3 was...
rapidly activated by MI insult and that this activation was sustained from the acute phase to the subacute phase (day 10).

Additionally, the DNA-binding activity of STAT3 was significantly increased after MI, as assessed by transcription factor ELISA (Fig. 1B). To accurately evaluate the activation of cardiac STAT3, immunofluorescent analyses with anti-p-STAT3 antibody were performed. As a result, cardiac p-STAT3 was localized in the nucleus after MI (Fig. 1C).

Ablation of the STAT3 gene in the subacute phase after MI reduced the survival rate. To examine the pathophysiological roles of STAT3 activity in subacute MI using time-specific cardiac STAT3 knockout mice, we generated the α-MHC-MerCreMer mice on the genetic background of STAT3<sup>fl<sup>ox</sup>/fl<sup>ox</sup></sup> mice (STAT3 iCKO mice) and those on STAT3<sup>wild/wild</sup> as a control. At 8–12 wk of age, STAT3 iCKO and control mice were subjected to MI followed by TM treatment (Fig. 2A). Immunofluorescent analyses demonstrated that the expression of p-STAT3 in cardiomyocytes was successfully suppressed after TM treatment (Fig. 2B).

Kaplan-Meier analyses revealed that the survival rate was significantly lower in STAT3 iCKO mice than control mice after TM treatment (Fig. 2C).

Subacute STAT3 activation in the postinfarct myocardium was required for the resistance to cardiac remodeling. We examined whether STAT3 gene ablation results in an enhanced susceptibility to cardiac remodeling. Consistent with the increased mortality of STAT3 iCKO mice subjected to MI, the

![Fig. 1. Cardiac STAT3 was activated in the subacute phase after myocardial infarction (MI). C57Bl/6 mice were exposed to MI operation, and hearts were harvested at the indicated time points. A: lysates were prepared from hearts from the ligation point to the apex and immunoblotted with anti-phosphorylated (p-)STAT3 antibody. Blots were reprobed with anti-STAT3 antibody and anti-GAPDH antibody. **P < 0.01 vs. non-MI mice by one-way ANOVA followed by a Bonferroni test. B: transcription factor ELISA was performed with heart homogenates at the indicated time points. Data are shown as means ± SD; n = 3–4 mice. *P < 0.05 and **P < 0.01 vs. non-MI mice by one-way ANOVA followed by a Bonferroni test. C: heart sections were prepared on day 4 after MI, and immunofluorescent microscopic analyses were performed with anti-p-STAT3 antibody and anti-troponin I antibody. Hoechst 33258 staining was also performed to identify nuclei. Representative data are shown. Bar = 50 μm.](http://ajpheart.physiology.org/)

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heart weight-to-body weight ratio was significantly increased in STAT3 iCKO mice with MI compared with control mice with MI (Fig. 3A).

Histological analyses by Masson’s trichrome staining revealed that cardiac-specific STAT3 ablation in subacute MI considerably exaggerated cardiac fibrosis (fibrotic circumference: 58.3 ± 6.7% in iCKO mice and 40.8 ± 9.3% in control mice, P < 0.01), whereas there were no histological differences between control and STAT3 iCKO mice in sham-operated groups (Fig. 3B). Marked interstitial fibrosis was not observed at the remote area in both groups (data not shown). Moreover, scar wall thickness was significantly reduced in STAT3 iCKO mice compared with control mice. Quantitative RT-PCR analyses showed that mRNA expressions of MMP-9, procollagen 1, and procollagen 3, profibrotic genes, were significantly increased in STAT3 iCKO mice compared with control mice after MI (Fig. 3C). Moreover, zymographic analyses revealed that MMP-9 was enhanced in STAT3 iCKO mice.

We estimated cardiac fibrosis before STAT3 ablation and found that there was no significant difference between iCKO and control mice on day 10 after MI (fibrotic circumference: 39.4 ± 2.3% in iCKO mice and 38.6 ± 5.9% in control mice), indicating that the acute infarct size was almost same between the two groups.

To assess the effects of STAT3 deletion in the subacute phase on cardiac function, we performed echocardiography on day 24 after MI (Table 1). Consistent with cardiac fibrosis, STAT3
gene ablation accelerated the MI-induced fractional shortening decline. These findings indicate that intrinsic STAT3 activity in the postinfarct myocardium plays a cardioprotective role in the subacute phase after MI.

Inflammatory reaction was not altered by cardiac STAT3 deletion in the subacute phase after MI. It has been reported that inflammatory reaction is a critical feature in the postinfarct myocardium (3). Consequently, we performed quantitative RT-PCR to estimate mRNA expressions of proinflammatory cytokines (Fig. 4). It is widely accepted that MI evokes inflammatory reactions with increased expression of IL-6, TNF-α, and IL-1β mRNA in the acute phase (3); however, 24 days after MI, the expression levels of these cytokines were reduced to the levels before MI, and there were no significant differences in the expression of these cytokines between STAT3 iCKO and control mice.
Ablation of cardiac STAT3 evoked the production of ROS. ROS production is closely related to cardiac remodeling after MI. Additionally, we have previously demonstrated that cardiac STAT3 activation inhibited ROS-induced myocardial injury in an ischemia-reperfusion model (20, 23). Therefore, we examined the effects of cardiac STAT3 ablation in subacute MI on ROS generation during cardiac remodeling (Fig. 5). DHE fluorescence staining revealed that MI-induced ROS generation was augmented in STAT3 iCKO mice at 24 days after MI. Cardiac STAT3 deletion resulted in the detrimental cardiomyocyte hypertrophy accompanied by the reduction in capillary density.

The imbalance between cardiomyocytes and the vasculature causes cardiac dysfunction. Since cardiac STAT3 regulates vascular function in the myocardium (24), we analyzed capillary density by immunohistochemical analyses with anti-CD31 antibody (Fig. 6, A and B). Although there was no difference between sham-operated control and iCKO mice, capillary density was significantly reduced in STAT3 iCKO mice relative to control mice in the border zone (iCKO mice: 1,724.2 ± 119.4 capillaries/mm² and control mice: 2,110.5 ± 77.7 capillaries/mm², *P < 0.01).

In a previous study (9), it was reported that the expression of Tsp1, an antiangiogenic factor (13, 27), was upregulated, but not that of VEGF, an angiogenic growth factor (2, 7), in

Table 1. Echocardiographic analyses on cardiac function

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<th>Sham Operation</th>
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<tr>
<td></td>
<td>Control mice</td>
<td>iCKO mice</td>
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<tr>
<td>Fractional shortening, %</td>
<td>45.4 ± 8.7</td>
<td>41.1 ± 8.6</td>
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<tr>
<td>Ejection fraction, %</td>
<td>82.5 ± 9.7</td>
<td>78.6 ± 9.0</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>523.0 ± 53.4</td>
<td>547.5 ± 53.9</td>
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<td>Left ventricular internal dimension at diastole, mm</td>
<td>3.9 ± 0.5</td>
<td>4.2 ± 0.6</td>
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<td>Left ventricular internal dimension at systole, mm</td>
<td>2.1 ± 0.3</td>
<td>2.5 ± 0.7</td>
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Data are shown as means ± SD; n = 7 for control mice with sham operation; 8 for cardiac-specific STAT3 knockout (iCKO) mice with sham operation, 10 for control mice with myocardial infarction (MI), and 11 for iCKO mice with MI. *P < 0.05 vs. control mice with MI by Student’s t-test.

Fig. 4. The expression of proinflammatory cytokines was not influenced by cardiac STAT3 deletion in the subacute phase after MI. At 24 days after MI, total RNA was prepared from hearts from the ligation point to the apex, and quantitative RT-PCR was performed for IL-6 (A), TNF-α (B), and IL-1β (C). The expression of each cytokine was normalized with that of GAPDH. Data are shown as means ± SD; n = 5 for control mice with sham operation, 5 for iCKO mice with sham operation, 7 for control mice with MI, and 5 for iCKO mice with MI. n.s., not significant by one-way ANOVA followed by a Tukey-Kramer test.

Fig. 5. ROS production was enhanced in the STAT3-null myocardium after MI. On day 24 after MI, heart sections were stained with dihydroethidium, as described in MATERIALS AND METHODS. A: representative fluorescent images. Bar = 50 μm. B: fluorescence intensity at the border zone was measured. Data are shown as means ± SD (15 fields from 3 mice for control mice with sham operation, 25 fields from 5 mice for control mice with MI, 20 fields from 4 mice for iCKO mice with MI). *P < 0.01 by one-way ANOVA followed by a Tukey-Kramer test.
conventional cardiac-specific STAT3-null mice. Therefore, the expressions of these genes were analyzed by quantitative RT-PCR (Fig. 6C). Consistently, the expression of Tsp1 was increased in STAT3 iCKO mice relative to control mice, whereas that of VEGF was not altered. However, immunoblot analyses demonstrated that there were no significant differences in Tsp1 protein expression between control and STAT3 iCKO mice (data not shown), suggesting that capillary density was reduced, at least partially, in a Tsp1-independent manner.

Thus, we investigated whether STAT3 deletion exacerbated cardiac hypertrophy, which might relatively reduce capillary density. Intriguingly, H&E staining certified that the cross-sectional area of cardiomyocytes at the border zone was remarkably increased in STAT3 iCKO mice compared with control mice at 24 days after MI (Fig. 6, D and E). As a result, cardiomyocytes/visual field of microscopy were decreased in number (Fig. 6F). Importantly, no significant difference was observed in the capillarite-to-cardiomyocyte ratio between con-
trol and STAT3 iCKO mice (Fig. 6G), resulting in a reduction in capillary density.

**DISCUSSION**

In the present study, using STAT3 iCKO mice, we estimated the pathophysiological roles of STAT3 in the subacute phase of postinfarct cardiac remodeling in a time-specific manner. STAT3 was rapidly phosphorylated in acute MI, and this phosphorylation was sustained through the subacute phase. The ablation of the STAT3 gene in the subacute phase of MI led to an increase of mortality accompanied by an increase in the heart weight-to-body weight ratio. Cardiac fibrosis was exaggerated in STAT3-deficient hearts, concomitant with cardiac dysfunction. STAT3 deletion in subacute MI resulted in severe myocardial injury with increased ROS production and cardiomyocyte hypertrophy. In STAT3 iCKO mice, capillary density was reduced compared with control mice after MI, although the ratio of capillary vessels to cardiomyocytes was not altered, suggesting that cardiac-specific ablation of the STAT3 gene leads to severe hypertrophy without a coordination with capillary growth. Collectively, intrinsic activity of STAT3 is required for the prevention of cardiac remodeling in the subacute phase of MI.

Although a number of studies have demonstrated that activation of STAT3 contributes to the cardioprotection against acute myocardial injury (1, 18, 19, 20, 30), the biological significance of STAT3 activity in subacute or chronic phases after MI is still controversial. Previously, it has been demonstrated that postinfarct cardiac fibrosis is extended in noninducible cardiac-specific STAT3-deficient mice, proposing the antifibrotic effects of STAT3 during cardiac remodeling (9); however, we could not draw the conclusion that STAT3 exhibited the beneficial effects in subacute or chronic remodeling after MI, because the STAT3 gene was ablated before MI in the noninducible model and because cardiac remodeling is influenced by the severity of acute myocardial damage. Interestingly, recent studies have reported that activation of the gp130/STAT3 axis, by expressing the active mutant of gp130 or by ablating the SOCS3 gene, is detrimental to the maintenance of cardiac homeostasis (10, 29). To address the biological functions of STAT3 in the subacute phase after MI, we used inducible STAT3-deficient mice in the present study and found that intrinsic activity of STAT3 is essential for the prevention of cardiac fibrosis. Combined with our previous findings that cardiac activation of STAT3 in the acute stage after MI exhibits resistant to ischemic insults by suppressing cardiomyocyte death, it could be concluded that STAT3 plays critical roles in protection against cardiac remodeling in the subacute phase after MI as well as acute ischemic injury.

Consistent with our results, the continuous activation of gp130/STAT3 by leukemia inhibitory factor does not cause adverse cardiac remodeling but improves heart function (30). Similarly, we have demonstrated that IL-11 administration for 5 consecutive days after MI exhibited cardioprotective effects (19). Taken together, it is likely that the stimulation of the gp130/STAT3 signaling pathway with IL-6 family cytokines, physiological ligands, is cardioprotective (1, 14, 26, 30), whereas the unregulated activation of gp130 might result in heart failure (10). Thus, the detrimental effects of gp130/STAT3 activation in mutant gp130 mice (10) or in SOCS3-deficient mice (29) may be explained by the artificial kinetics of STAT3 activation in genetically modified mice and/or by unbalanced activation of the downstream effectors of gp130, including STAT3, Akt, and ERKs.

A recent study (10) proposed that increased serum IL-6 mediates detrimental signals through STAT3 in failing hearts. In contrast, expression of proinflammatory cytokines, including IL-6, was not upregulated in STAT3 iCKO hearts in the subacute phase, indicating that there is no correlation between the cardiac production of IL-6 and the severity of cardiac dysfunction after MI. Therefore, it is likely that IL-6 is not produced from the failing hearts in subacute or chronic phases after MI, although inflammatory cytokines are transiently induced in the myocardium in the acute phase after MI (22). This observation is consistent with clinical findings that plasma IL-6 is spiked over from the peripheral circulation (28). Moreover, since STAT3 gene ablation exacerbated cardiac remodeling after MI, it is likely that the pathophysiological level of IL-6 after MI does not exhibit detrimental effects through STAT3. Similarly, it has been reported that IL-6 gene ablation does not affect cardiac remodeling (4).

Compared with control mice, capillary density was reduced in STAT3 iCKO hearts after MI. It has been revealed that forced activation of STAT3 induces VEGF expression, contributing to vascular formation in the myocardium (24). In this study, VEGF mRNA expression was not altered by STAT3 deletion in the subacute phase, possibly because VEGF expression was regulated through alternative signaling pathways. Indeed, we have reported that leukemia inhibitory factor-mediated induction of VEGF is inhibited by adenoviral vector expressing dominant negative STAT3, whereas dominant negative STAT3 does not affect the basal expression of VEGF in cardiomyocytes (7). In contrast, the expression of Tsp1 was increased in STAT3 iCKO mice compared with control mice, consistent with the previous observation that capillary density was reduced in noninducible STAT3-null hearts, accompanied by the upregulation of Tsp1 expression (9). Since Tsp1 has antiangiogenic properties (13, 27), it could be proposed that the impaired vasculature in STAT3 iCKO hearts might be derived, at least partially, through the elevated expression of Tsp1. However, it remains to be elucidated whether STAT3 directly suppressed the expression of Tsp1. Moreover, immunoblot analyses could not show remarkable increases in Tsp1 protein in STAT3 iCKO mice after MI (data not shown), proposing the possibility that capillary density was reduced partly in a Tsp1-independent manner. Interestingly, severe cardiomyocyte hypertrophy was observed in the border zone in STAT3 iCKO mice compared with control mice. Importantly, the ratio of capillary vessels to cardiomyocytes was not altered in STAT3 iCKO mice. Thus, pathological stress, including increased ROS generation, might be enhanced in the absence of STAT3, leading to severe hypertrophy and fibrotic enlargement.

It should be noted that STAT3 deficiency is obviously detrimental in subacute MI hearts but not in non-MI hearts. These findings propose that inhibition of the STAT3 signaling pathway increased the susceptibility of the myocardium to chronic injury, although STAT3 inactivation itself induced myocardial damage only to a trivial extent, if any, under stress-free conditions. Our data presented here might provide clinical insights into the cardiotoxicity of molecularly targeted therapies. For example, JAK kinase inhibitors are clinically used for...
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the treatment of patients with autoimmune diseases or cancers. Since JAK kinases are major tyrosine kinases that activate STAT3 downstream of gp130 (15, 16), careful observation would be required to confirm their safety in patients with chronic cardiac stresses, such as old MI, despite their safety in patients without cardiovascular diseases.

In summary, we examined the cardioprotective function of STAT3 in subacute MI in a time-specific manner for the first time. We demonstrated that basal activity of STAT3 confers resistance to cardiac remodeling in the subacute phase after MI. The maintenance of intrinsic STAT3 activity in the subacute phase after MI could be therapeutically beneficial for the prevention from heart failure.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: D.E., M.O., M.M., H.N., and Y.F. drafted manuscript; D.E., M.O., M.M., interpreted results of experiments; D.E., M.O., and A.M. prepared figures; H.N., and Y.F. analyzed data; D.E., M.O., and A.M. performed experiments; D.E., M.M., designed research; D.E., M.O., and A.M. wrote the paper.

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