# Protective Role of Endogenous Erythropoietin System in Nonhematopoietic Cells Against Pressure Overload–Induced Left Ventricular Dysfunction in Mice

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***Background***—Erythropoietin (Epo) receptors (EpoRs) are expressed in the heart. We have recently demonstrated that the endogenous Epo-EpoR system plays an important protective role in myocardial ischemia in mice and humans. In the present study, we tested our hypothesis that the endogenous Epo-EpoR system in nonhematopoietic cells also plays a protective role against pressure overload–induced cardiac dysfunction in vivo.

***Methods and Results***—Transgene-rescued EpoR-null mutant mice ($EpoR^{-/-}{}_{rescued}$) that express EpoR exclusively in the hematopoietic cells were subjected to transverse aortic constriction (TAC). At 1 week after TAC, left ventricular weight and lung weight were significantly increased in $EpoR^{-/-}{}_{rescued}$ mice compared with wild-type mice, although the fibrotic area was comparably increased after TAC in the 2 genotypes. In the $EpoR^{-/-}{}_{rescued}$ mice with TAC, left ventricular end-diastolic diameter was significantly increased, left ventricular fractional shortening was significantly decreased, and survival rate was significantly decreased compared with wild-type mice with TAC. Phosphorylation of STAT3 at 5 hours and 1 week after TAC and that of p38 at 5 hours after TAC were significantly increased in wild-type mice but not in $EpoR^{-/-}{}_{rescued}$ mice. Vascular endothelial growth factor protein expression and capillary density in left ventricular myocardium were significantly decreased in $EpoR^{-/-}{}_{rescued}$ mice with TAC compared with wild-type mice with TAC.

***Conclusions***—These results suggest that the endogenous Epo-EpoR system in the nonhematopoietic cells plays an important protective role against pressure overload–induced cardiac dysfunction in vivo. **(*Circulation*. 2007;115:2022-2032.)**

**Key Words:** angiogenesis ■ erythropoietin ■ heart failure ■ hypertension ■ hypertrophy ■ remodeling



Left ventricular (LV) pressure overload resulting from systemic hypertension or aortic stenosis causes LV hypertrophy, an adaptive response to compensate for the increased LV wall stress.[1] Although heart failure resulting from LV pressure overload has been shown to be associated with upregulations of angiotensin II, endothelin-1, fibroblast growth factor-2, and transforming growth factor-$\beta_1$,[2–5] the precise molecular mechanisms for the development of pressure overload–induced cardiac dysfunction remain to be elucidated. Recently, Shiojima et al[6] and Izumiya et al[7] demonstrated that inhibition of vascular endothelial growth factor (VEGF)–mediated angiogenesis promotes the development of heart failure in mouse models of cardiac hypertrophy. Furthermore, Friehs et al[8] reported that administration of VEGF delays the onset of heart failure in a newborn rabbit model of pressure overload–induced cardiac hypertrophy. These findings highlight the importance of coordinating cardiac hypertrophy and angiogenesis to prevent the development of contractile dysfunction in the hypertrophied heart.

## Clinical Perspective p 2032

Erythropoietin (Epo) is a cytokine that plays a critical role in the proliferation and terminal differentiation of erythroid progenitors and precursors by preventing apoptosis.[9] Recent studies have demonstrated that Epo receptors (EpoRs) are expressed not only in hematopoietic lineage cells but also in nonhematopoietic organs, including the heart.[10,11] We have recently reported that the endogenous Epo-EpoR system contributes to the mobilization of endothelial progenitor cells, their recruitment to the pulmonary artery, and the prevention of hypoxia-

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**The online-only Data Supplement, consisting of Methods, is available with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.106.659037/DC1.**

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**TABLE 1. Baseline Characteristics of Mice**

| | WT (n=10) | $EpoR^{-/-}_{rescued}$ (n=10) | P |
|---|---|---|---|
| Body weight, g | 23.9±0.6 | 23.7±0.3 | 0.74 |
| Tibial length, mm | 1.78±0.01 | 1.77±0.01 | 0.64 |
| Heart weight, mg | 114±3 | 121±3 | 0.092 |
| LV weight, mg | 84±2 | 89±2 | 0.091 |
| Heart weight/tibia length ratio, mg/mm | 6.41±0.16 | 6.83±0.14 | 0.061 |
| LV weight/tibia length ratio, mg/mm | 4.72±0.12 | 5.04±0.11 | 0.062 |
| Systolic blood pressure, mm Hg | 104±3 | 104±5 | 1.00 |
| Heart rate, bpm | 718±7 | 714±9 | 0.89 |
| Serum Epo, pg/mL | 111±9 | 287±3 | <0.001 |

Results are expressed as mean±SEM.

**TABLE 2. Baseline Findings With Echocardiography and Cardiac Catheterization**

| | WT (n=10) | $EpoR^{-/-}_{rescued}$ (n=10) | P |
|---|---|---|---|
| LVDd, mm | 3.67±0.04 | 3.75±0.05 | 0.24 |
| LVDs, mm | 1.86±0.05 | 1.99±0.05 | 0.047 |
| LVFS, % | 49.4±0.5 | 47.0±1.0 | 0.044 |
| IVS thickness, mm | 0.57±0.01 | 0.59±0.01 | 0.13 |
| LV posterior wall thickness, mm | 0.54±0.01 | 0.53±0.01 | 0.71 |
| Heart rate (echocardiography), bpm | 519±10 | 507±14 | 0.45 |
| LV systolic pressure, mm Hg | 84.0±1.0 | 83.6±0.6 | 0.76 |
| LV end-diastolic pressure, mm Hg | 2.8±0.5 | 2.1±0.4 | 0.30 |
| LV dp/dt max, mm Hg/s | 6102±252 | 6025±223 | 0.82 |
| LV −dp/dt min, mm Hg/s | 5810±271 | 5479±153 | 0.30 |
| Heart rate (catheterization), bpm | 469±9 | 461±13 | 0.61 |

IVS indicates interventricular septum. Results are expressed as mean±SEM; LV dp/dt max, maximum first time derivative of LV pressure; and LV −dp/dt min, minimum first time derivative of LV pressure.

induced pulmonary hypertension in mice.[12] We also have demonstrated that endogenous Epo-mediated signaling plays an important role in the reduction in infarct size in patients with acute myocardial infarction[13] and in mice with myocardial ischemia and reperfusion.[14] Furthermore, it has been shown that in patients with heart failure associated with anemia, administration of Epo improves exercise capacity, quality-of-life scores, LV ejection fraction, and number of rehospitalizations, although it remains unknown whether such beneficial effects of Epo can be due simply to the improvement of anemia or to its direct effects on the heart.[15–17] Finally, administration of Epo at 3 weeks after the onset of myocardial infarction in rats has been shown to promote angiogenesis in the noninfarcted viable myocardium and to improve cardiac function.[18]

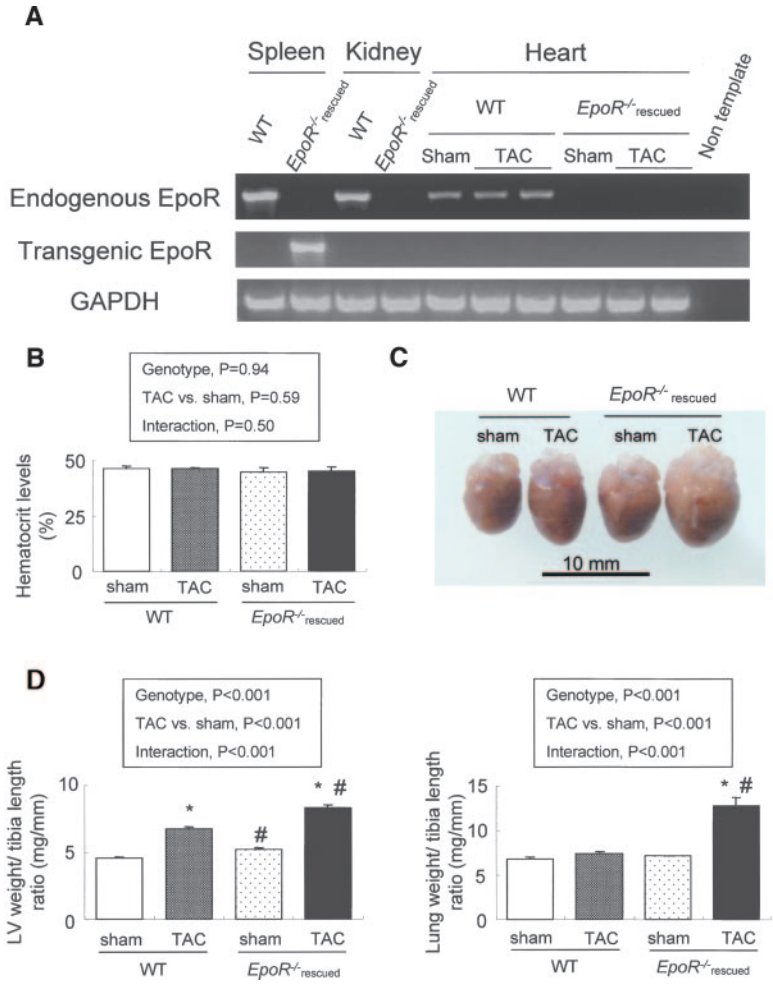


**Figure 1.** EpoR mRNA expression, hematocrit, and heart and lung weights. A, Endogenous EpoR mRNA was expressed in the spleen, kidney, and heart in WT but not $EpoR^{-/-}_{rescued}$ mice. In contrast, transgenic EpoR mRNA was expressed only in the spleen of $EpoR^{-/-}_{rescued}$ mice. B, Hematocrit levels at 1 week after sham operation or TAC in the 2 genotypes (n=5 to 6). C, Representative photographs of the hearts at 1 week after sham operation or TAC. D, Ratios of LV weight to tibia length and lung wet weight to tibia length at 1 week after sham operation or TAC (n=18 to 22). Results are expressed as mean±SEM. *$P$<0.01 vs sham; #$P$<0.01 vs WT mice.

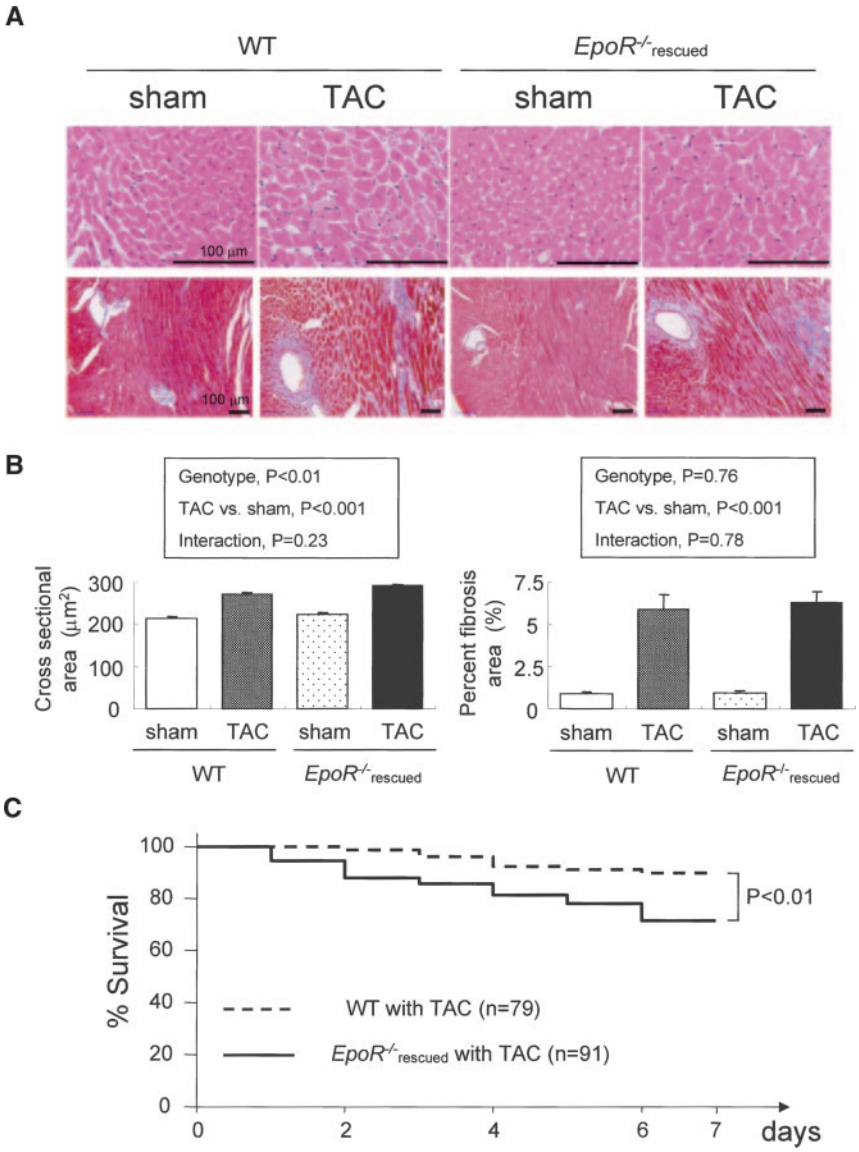


**Figure 2.** Histological data and survival. A, Representative histological micrographs of the LV myocardium stained with hematoxylin-eosin (top) and Masson's trichrome (bottom) at 1 week after sham operation or TAC. B, Quantitative analysis of the cross-sectional area of cardiomyocytes and the fibrosis area of LV myocardium (n=7 to 11). C, Kaplan-Meier survival curves of WT and $EpoR^{-/-}_{rescued}$ mice after TAC. Results are expressed as mean±SEM.



These previous findings raise an important question as to whether the endogenous Epo-EpoR signaling system in nonhematopoietic cells plays an important protective role against pressure overload–induced LV dysfunction.

Thus, in the present study, we examined the effects of LV pressure overload on the extent of LV hypertrophy, LV function, and survival in transgene-rescued EpoR-null mutant mice ($EpoR^{-/-}_{rescued}$),[19] which are characterized by the absence of endogenous Epo-EpoR signaling in the cardiovascular system but have normal hematopoietic function.

## Methods

The institutional animal care and use committee of Tohoku University School of Medicine approved all the protocols and experimental procedures of the present study.

### Animals

Because EpoR-null mice die on embryonic day 13 because of severe anemia,[20] we used $EpoR^{-/-}_{rescued}$ mice with a C57BL6/J background that had been developed by Suzuki et al.[19] These mice possess the transgene that drives EpoR mRNA expression only in the hematopoietic cells with the activity of the hematopoietic regulatory domain of the GATA-1 gene.[19] $EpoR^{-/-}_{rescued}$ mice normally develop and are fertile despite the lack of EpoR expression in nonhematopoietic cells. In the present study, we used $EpoR^{-/-}_{rescued}$ mice that express ≈40% of the normal EpoR level in erythroid cells[19] and age- and gender-matched wild-type (WT) C57BL/6J mice as controls. Echocardiographic and histological data were analyzed in a blinded manner with regard to mice genotype. Male WT and $EpoR^{-/-}_{rescued}$ mice, 10 to 12 weeks of age, were subjected to LV pressure overload by transverse aortic constriction (TAC) for 1 week (see the online Data Supplement).[21]

### Measurements of Blood Pressure, Hematocrit, and Serum Epo Levels

Details for measurements of these parameters are provided in the online Data Supplement.

### Echocardiography and Cardiac Catheterization

Details for echocardiography and cardiac catheterization are provided in the online Data Supplement.

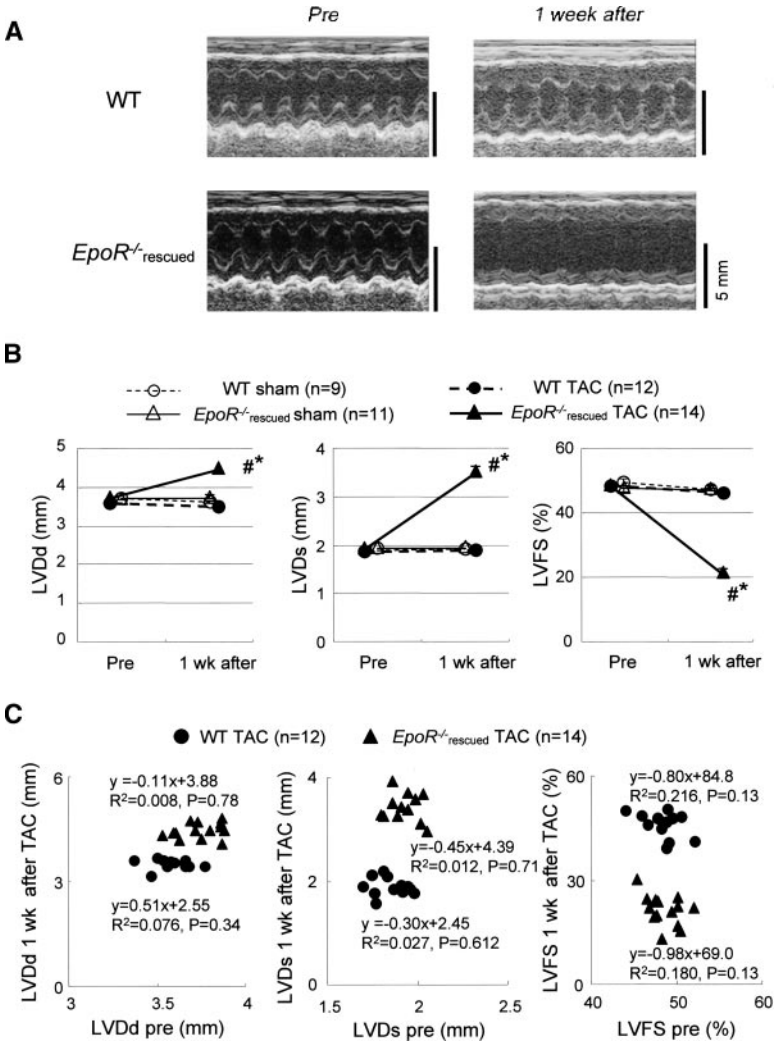


**Figure 3.** Echocardiographic data before and after operations. A, Representative transthoracic M-mode echocardiographic tracings from a WT and an $EpoR^{-/-}{}_{rescued}$ mouse before and at 1 week after TAC. B, Changes in LVDd, LVDs, and LVFS after operations. C, Relationships between the echocardiographic parameters obtained before TAC and those at 1 week after TAC. See text for details. Results are expressed as mean±SEM. *$P<0.05$ vs sham-operated $EpoR^{-/-}{}_{rescued}$ mice; #$P<0.05$ vs WT mice with TAC.

## Reverse-Transcription Polymerase Chain Reaction for EpoR mRNAs

We performed reverse-transcription polymerase chain reaction to detect both endogenous and transgenic EpoR mRNAs in the LV, kidney, and spleen of WT and $EpoR^{-/-}{}_{rescued}$ mice. Details for reverse-transcription polymerase chain reaction for EpoR mRNAs are provided in the online Data Supplement.

## Northern Blot Analysis

For Northern blot analysis, 10 $\mu$g total RNA was hybridized with cDNA probes, which included those for atrial natriuretic factor, collagen 1, collagen 3, and sarcoplasmic reticulum $Ca^{2+}$ ATPase-2 (see the online Data Supplement).

## Western Blot Analysis

The LV tissue was homogenized with lysis buffer, and the total protein homogenate (20 to 50 $\mu$g) was separated by SDS-PAGE and transferred onto polyvinylidene difluoride membranes. Details for Western blot analysis are provided in the online Data Supplement.

## Histological Analysis

Details for histological analysis are provided in the online Data Supplement.

## Statistical Analysis

Results are presented as mean±SEM. Comparisons of data between 2 groups were performed with unpaired Student *t* test (Tables 1 and 2). Two-factor ANOVA, followed by Bonferroni's test, was performed to compare the effect of LV pressure overload on various parameters between WT and $EpoR^{-/-}{}_{rescued}$ mice. The Kaplan-Meier method was used to draw survival curves, and survival was assessed by log-rank test. We performed ANOVA with repeated measures, followed by Bonferroni's test for comparisons of serial echocardiographic data among groups. Simple linear regression analysis was used to assess correlations between echocardiographic parameters obtained before and after TAC. Values of $P<0.05$ were considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

# Results

## Baseline Characteristics of $EpoR^{-/-}{}_{rescued}$ Mice

Baseline characteristics of WT and $EpoR^{-/-}{}_{rescued}$ mice at 10 weeks of age are shown in Table 1. Body weight, systolic blood pressure, and heart rate were comparable between the 2 genotypes under basal conditions. Heart weight and LV weight, when normalized by tibia length, tended to be increased in $EpoR^{-/-}{}_{rescued}$ mice compared with WT mice, but these differences did not reach statistical significance. Serum Epo level was significantly increased in $EpoR^{-/-}{}_{rescued}$ mice compared with WT mice, which was consistent with our

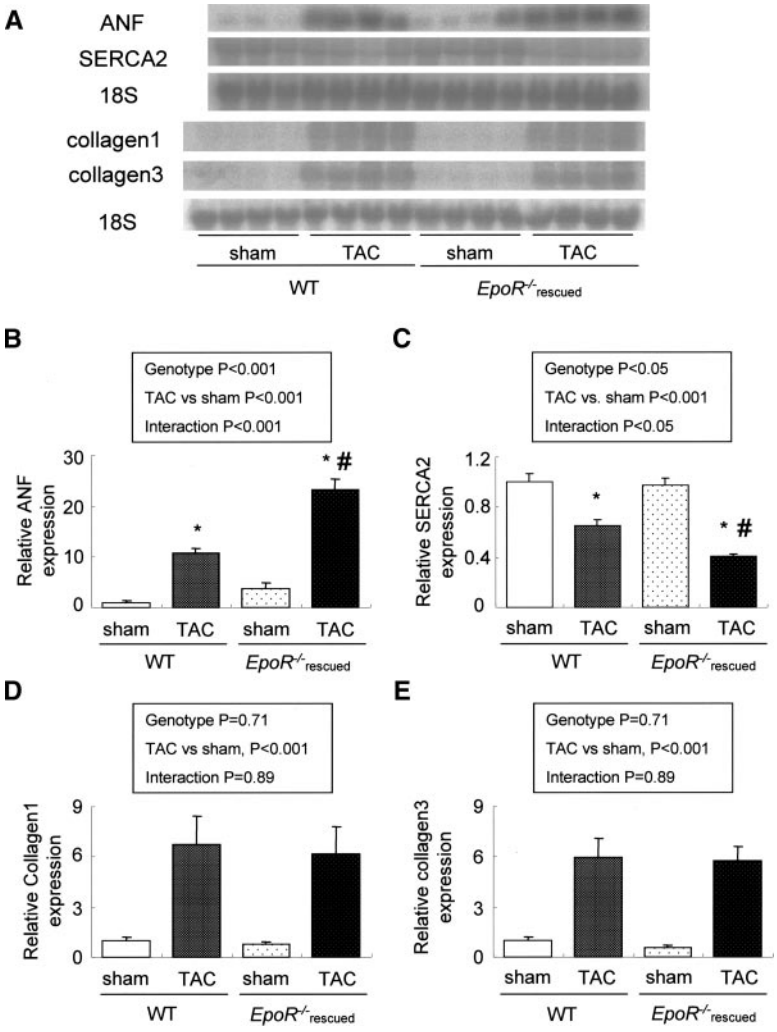


**Figure 4.** A, Representative Northern blots of LV myocardial tissue at 1 week after sham operation or TAC. B through E, Results of quantitative analysis of mRNA expressions in LV myocardial tissue at 1 week after operations. Each mRNA expression level was normalized using that of 18S (n=6 to 10). Results are expressed as mean±SEM. *$P<0.01$ vs sham; #$P<0.01$ vs WT mice.

previous study[14] and that by Suzuki et al.[19] The baseline mRNA expression levels of atrial natriuretic factor, collagen 1, collagen 3, and sarcoplasmic reticulum $Ca^{2+}$ ATPase-2 were comparable between WT and $EpoR^{-/-}_{rescued}$ mice (data not shown).

Echocardiographic studies under basal conditions at 10 weeks of age showed that LV end-systolic diameter (LVDs) was slightly but significantly increased by 7% and that LV fractional shortening (LVFS) was slightly but significantly decreased by 5% in $EpoR^{-/-}_{rescued}$ compared with WT mice(Table 2). LV end-diastolic diameter (LVDd) and septal and posterior wall thicknesses were comparable between the 2 genotypes. Cardiac catheterization revealed that no differences existed between the 2 genotypes in heart rate, LV systolic pressure, LV end-diastolic pressure, or maximum and minimum first time derivative of LV pressure (Table 2).

## Accelerated LV Hypertrophy, LV Dysfunction, and Reduced Survival in $EpoR^{-/-}_{rescued}$ Mice With Pressure Overload

At 1 week after TAC, the difference in mean blood pressure between the right and left carotid arteries was comparable between WT (32±3 mm Hg; n=12) and $EpoR^{-/-}_{rescued}$ mice (32±2 mm Hg; n=12), indicating comparable LV pressure overload in the 2 genotypes. Endogenous EpoR mRNA was expressed in the spleen and kidney in WT but not in $EpoR^{-/-}_{rescued}$ mice under basal conditions. This also was the case in the heart regardless of the types of operation (Figure 1A). Transgenic EpoR mRNA was expressed only in the spleen of $EpoR^{-/-}_{rescued}$ mice. Hematocrit at 1 week after operation was comparable between the 2 genotypes (Figure 1B).

The heart at 1 week after TAC was larger in $EpoR^{-/-}_{rescued}$ compared with WT mice(Figure 1C). This also was the case when heart size was expressed as the ratio of LV weight to tibia length (Figure 1D). Although this ratio was slightly but significantly greater in sham-operated $EpoR^{-/-}_{rescued}$ than in sham-operated WT mice, analysis by 2-factor ANOVA revealed that the increase after TAC was significantly greater in $EpoR^{-/-}_{rescued}$ than in WT mice ($P<0.001$ for the interaction). Lung weight at 1 week after TAC, when expressed as the ratio of lung wet weight to tibia length, was significantly increased only in $EpoR^{-/-}_{rescued}$ mice with TAC (Figure 1D).

Representative histological micrographs of the LV in the 2 genotypes at 1 week after sham operation or TAC are shown in Figure 2A. TAC significantly and comparably increased cross-sectional area of cardiomyocytes in both genotypes (Figure 2B). The extent of TAC-induced interstitial fibrosis

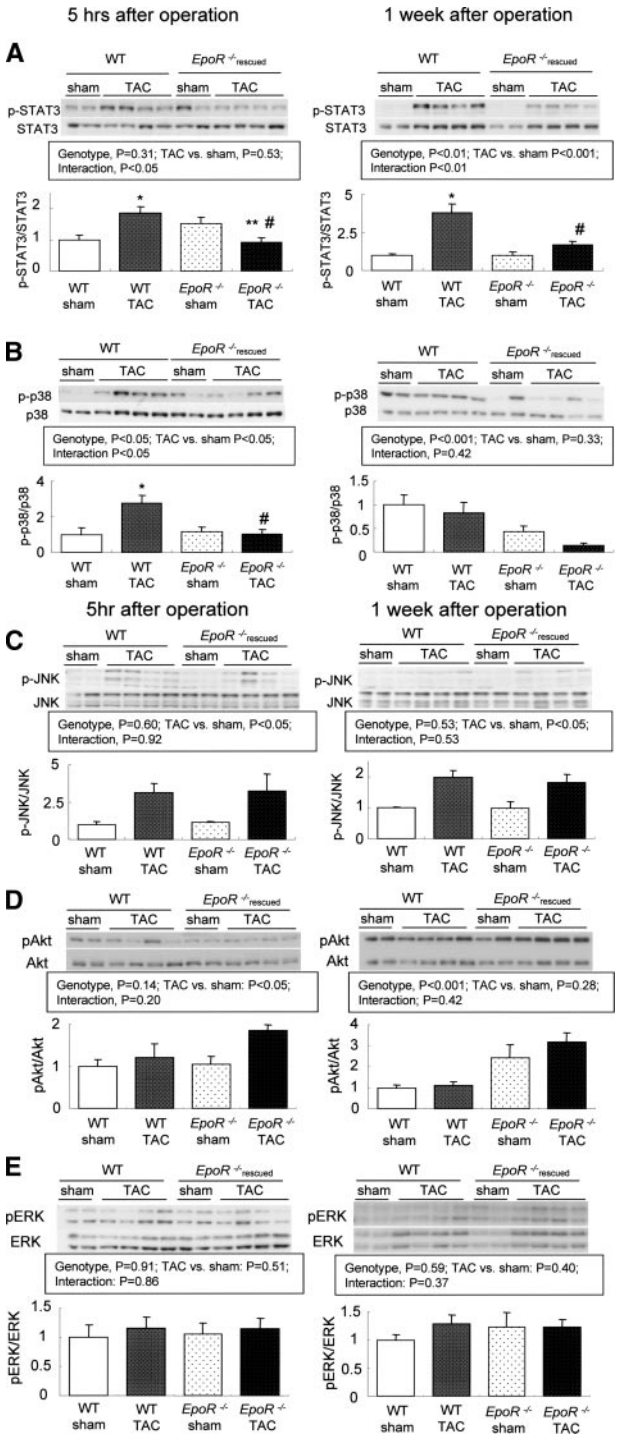


**Figure 5.** Representative Western blots and the results of quantitative analysis of LV myocardial tissue at 5 hours and 1 week after sham operation or TAC (n=5 to 6). Results are expressed as mean±SEM. *$P<0.01$ vs sham; **$P<0.05$ vs sham; #$P<0.01$ vs WT mice.

was comparable between the 2 genotypes (Figure 2B). The survival rate of $EpoR^{-/-}{}_{\text{rescued}}$ mice with TAC (n=79) was significantly reduced compared with that of WT mice with TAC (n=91) (71% versus 89%; $P<0.01$; Figure 2C). Postmortem examination revealed severe pulmonary congestion in most of the mice that died within 1 week after TAC in both genotypes (data not shown).

Representative M-mode echocardiographic tracings of the 2 genotypes at 1 week after operations are shown in Figure 3A. LVDd and LVDs were significantly increased after TAC in $EpoR^{-/-}{}_{\text{rescued}}$ compared with WT mice (Figure 3B), whereas LVFS was significantly deteriorated after TAC in $EpoR^{-/-}{}_{\text{rescued}}$ compared with WT mice (Figure 3B).

To assess the possibility that the clear differences in the echocardiographic parameters at 1 week after TAC between the 2 genotypes can be attributed to the slight but significant differences that had already been found before operation (Table 2), we examined the relationship between those echocardiographic parameters at 1 week after TAC and those obtained from the same animals before operation. As shown in Figure 3C, none of the parameters obtained at the 2 different time points were significantly correlated in WT or $EpoR^{-/-}{}_{\text{rescued}}$ mice. Figure 3C also indicates that the parameters at 1 week after TAC corresponding to any given numerical values before TAC always were larger with regard to LVDd and LVDs or lower with regard to LVFS in $EpoR^{-/-}{}_{\text{rescued}}$ than in WT mice. These results suggest that the clear differences in the echocardiographic parameters at 1 week after TAC between the 2 genotypes cannot be explained by the slight differences before operation.

## Altered Gene Expressions and Impaired Phosphorylations of Signaling Proteins in $EpoR^{-/-}{}_{\text{rescued}}$ Mice With Pressure Overload

Although TAC significantly increased myocardial atrial natriuretic factor expression in both genotypes, the increase was significantly greater in $EpoR^{-/-}{}_{\text{rescued}}$ compared with WT mice (Figure 4A and 4B). Similarly, although TAC significantly decreased myocardial sarcoplasmic reticulum $Ca^{2+}$ ATPase-2 expression in both genotypes, the decrease was greater in $EpoR^{-/-}{}_{\text{rescued}}$ than in WT mice (Figure 4A and 4C). TAC also significantly increased myocardial expression of collagen 1 and 3, but to the same extent, in the 2 genotypes (Figure 4A, 4D, and 4E).

TAC significantly increased phosphorylation of STAT3 at both 5 hours and 1 week only in WT mice, not in $EpoR^{-/-}{}_{\text{rescued}}$ mice (Figure 5A). TAC also significantly increased phosphorylation of p38 in WT mice at 5 hours but not at 1 week, and this increase was not noted in $EpoR^{-/-}{}_{\text{rescued}}$ mice (Figure 5B). In contrast, TAC increased JNK phosphorylation to the same extent in both genotypes at both 5 hours and 1 week after TAC (Figure 5C). Phosphorylation of Akt was significantly increased at 5 hours after TAC (Figure 5D). Although the extent of the increase tended to be greater in $EpoR^{-/-}{}_{\text{rescued}}$ than in WT mice, the difference did not reach statistical significance. Phosphorylation of Akt at 7 days after operation was significantly increased in $EpoR^{-/-}{}_{\text{rescued}}$ compared with WT mice (Figure 5D). Although phosphorylation of extracellular signal–regulated kinase (ERK) in WT mice tended to be increased by 15% at 5 hours and by 29% at 7 days after TAC compared with sham-operated WT mice, the difference did not reach statistical significance (Figure 5E). Furthermore, neither cleaved caspase 3 nor poly (ADP-ribose) polymerase was detected in the LV myocardial tissue of the 2 genotypes with sham operation or TAC (data not shown).

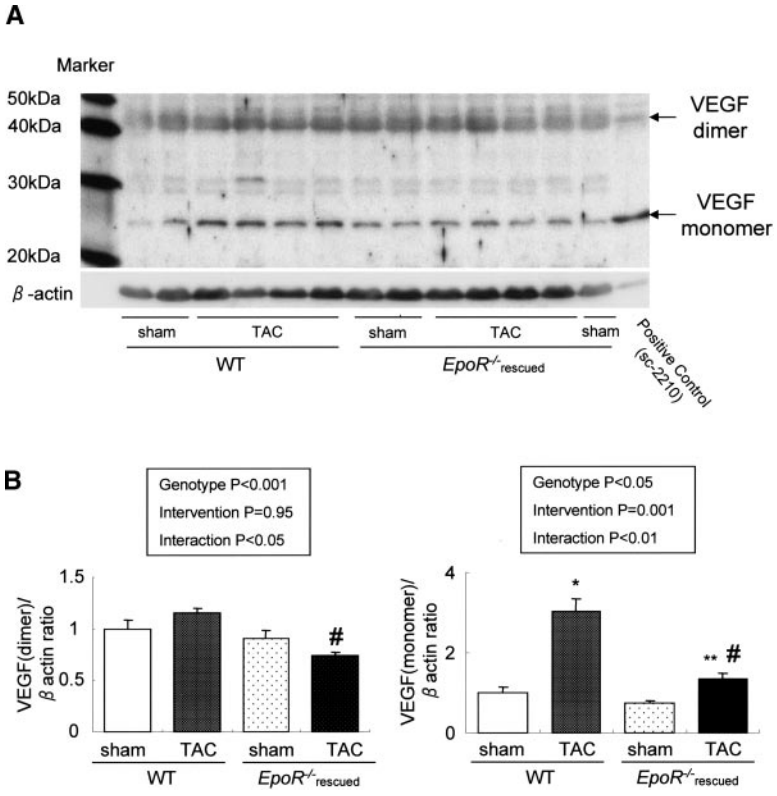


**Figure 6.** Protein expression of VEGF and capillary density in LV myocardium after TAC. A, Representative Western blots for VEGF in LV myocardial tissue at 1 week after operations. B, Quantitative analysis of the protein expression levels of VEGF in LV myocardial tissue (n=5 to 6). Results are expressed as mean±SEM. *$P<0.01$ vs sham; **$P<0.05$ vs sham; #$P<0.01$ vs WT mice.



### Impaired VEGF Upregulation and Angiogenesis in $EpoR^{-/-}{}_{rescued}$ Mice With Pressure Overload

Representative Western blots for VEGF in LV myocardium are shown in Figure 6A. VEGF protein expression in LV myocardium was significantly decreased in $EpoR^{-/-}{}_{rescued}$ mice with TAC compared with WT mice with TAC (Figure 6A and 6B). The number of capillaries normalized by that of cardiomyocytes was significantly increased after TAC in WT but not in $EpoR^{-/-}{}_{rescued}$ mice(Figure 7A and 7B).

## Discussion

The novel finding of the present study was that the deletion of EpoR in nonhematopoietic cells results in enhanced susceptibility to LV dilatation, LV dysfunction, and cardiac death in mice with LV pressure overload. The enhanced susceptibility to LV failure in $EpoR^{-/-}{}_{rescued}$ mice with TAC was associated with impaired phosphorylation of STAT3 and p38, decreased protein expression of VEGF, and impaired capillary growth in the LV myocardium. These results suggest that the endogenous Epo-EpoR system in nonhematopoietic cells plays an important protective role against pressure overload–induced cardiac dysfunction (Figure 8).

### Enhanced Susceptibility to Heart Failure in $EpoR^{-/-}{}_{rescued}$ Mice With Pressure Overload

Although the ratio of LV weight to tibia length was slightly but significantly greater in sham-operated $EpoR^{-/-}{}_{rescued}$ mice than in sham-operated WT mice, the increase after TAC was significantly greater in $EpoR^{-/-}{}_{rescued}$ than in WT mice (analysis by 2-factor ANOVA). Interstitial fibrosis cannot explain the greater increase because percent fibrosis area and mRNA expression levels of collagens 1 and 3 were increased comparably in the 2 genotypes with TAC. Because the cross-sectional area of cardiomyocytes was comparably increased after TAC in $EpoR^{-/-}{}_{rescued}$ and WT mice, the greater increase in LV weight in $EpoR^{-/-}{}_{rescued}$ mice with TAC might possibly be attributable to a greater increase in cardiomyocyte length, although we do not have direct evidence. Echocardiography further revealed different characteristics between the 2 genotypes with TAC. At 1 week after TAC, LV chamber was significantly dilated and LVFS was significantly reduced in $EpoR^{-/-}{}_{rescued}$ compared with WT mice. Finally, the survival rate after TAC was significantly reduced in $EpoR^{-/-}{}_{rescued}$ compared with WT mice. Because the ratio of lung wet weight to tibia length after TAC was significantly increased in $EpoR^{-/-}{}_{rescued}$ mice and postmortem examination revealed severe pulmonary congestion in most of the mice that died within 1 week after TAC, it is highly possible that the predominant cause of death was progression of heart failure.

### Possible Mechanisms for the Protective Role of Epo-EpoR Signaling in Nonhematopoietic Cells

The Epo-EpoR system plays a critical role in the proliferation and differentiation of erythroid progenitors and precursors by preventing apoptosis via JAK-STAT, PI3-Akt, and mitogen-activated protein kinase pathways.[22–24] In neurons and cardiomyocytes, exogenous Epo elicits a protective effect on ischemia and reperfusion injury.[10,11,25] We have recently demonstrated that a higher serum level of endogenous Epo predicted a smaller infarct in patients with acute myocardial infarction who underwent primary percutaneous coronary intervention, suggesting that the endogenous Epo-EpoR system plays an important protective role against myocardial ischemia/reperfusion injury in humans.[13] Furthermore, using

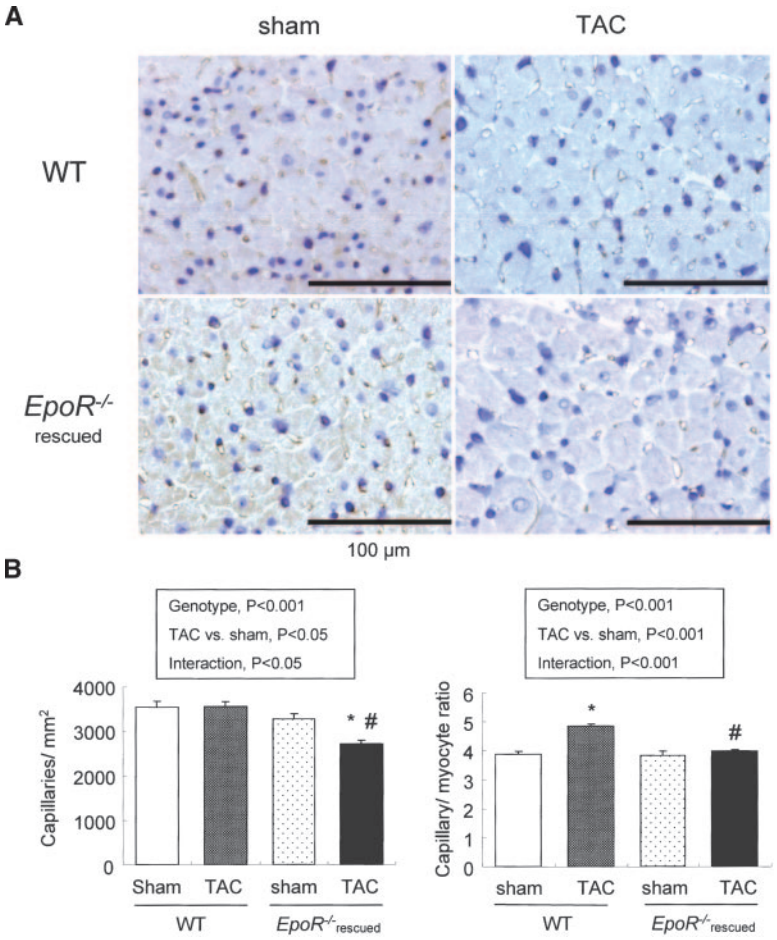


**Figure 7.** A, Representative CD31 immunostaining images of LV myocardium at 1 week after operations. B, Numbers of CD31-positive capillaries in LV myocardium (n=9 to 10). Results are expressed as mean±SEM. *$P<0.01$ vs sham; #$P<0.01$ vs WT mice.



$EpoR^{-/-}{}_{rescued}$ mice, we were able to demonstrate that the endogenous Epo-EpoR system in nonhematopoietic cells plays an important protective role against myocardial ischemia/reperfusion injury, at least in part, by preventing apoptosis.[14] We also have recently reported that the endogenous Epo-EpoR system contributes to mobilization of endothelial progenitor cells, their recruitment to the pulmonary artery, and prevention of the development of hypoxia-induced pulmonary hypertension.[12] In addition to these protective effects on the cardiovascular system, the present study revealed a novel protective role of the endogenous Epo-EpoR system in nonhematopoietic cells against pressure overload–induced cardiac dysfunction.

In the present study, the enhanced susceptibility to heart failure in $EpoR^{-/-}{}_{rescued}$ mice with TAC was associated with the impairments of STAT3 phosphorylation, VEGF protein expression, and capillary growth in LV myocardium. This result may be in line with previous reports by others demonstrating that STAT3 is required for VEGF upregulation and resultant capillary growth in the heart,[26–28] that the disruption of coordinated cardiac hypertrophy and endogenous VEGF-induced angiogenesis accelerates the development of heart failure in hypertrophied hearts,[6,7] and that administration of Epo promotes coronary angiogenesis in animal models of myocardial infarction.[18,29] The deletion of the endogenous Epo-EpoR system in nonhematopoietic cells in mice with LV pressure overload may impair STAT3 activation, VEGF upregulation, and capillary growth, resulting in heart failure. The impaired capillary growth in $EpoR^{-/-}{}_{rescued}$ mice with TAC may result in failure to supply sufficient blood flow to hypertrophied cardiomyocytes. It also is possible that inappropriate angiogenesis in $EpoR^{-/-}{}_{rescued}$ mice with TAC results in insufficient delivery of paracrine factors from vascular endothelial cells that are required for appropriate hypertrophic response in cardiomyocytes.[6,30]

Our finding that the ratio of LV weight to tibia length was significantly increased despite the significant impairment of coronary angiogenesis in $EpoR^{-/-}{}_{rescued}$ mice with TAC compared with WT mice with TAC is not consistent with the finding of Izumiya et al[7] that decoy VEGF receptors impaired both coronary angiogenesis and the development of TAC-induced cardiac hypertrophy. We have several explanations for this discrepancy. First, a substantial difference exists in the extent of the impairment of coronary angiogenesis after TAC between our study and the Izumiya et al study (17% versus 40% decrease in the capillary-to-myocyte ratio, respectively, compared with corresponding control mice with TAC). The insufficient but relatively well-maintained level of myocardial VEGF expression (Figure 6B) and the resultant relatively mild decrease in capillary-to-myocyte ratio (Figure 7B) in $EpoR^{-/-}{}_{rescued}$ mice with TAC may contribute to the greater extent of LV hypertrophy after TAC in $EpoR^{-/-}{}_{rescued}$ mice than in WT mice. The different extent of impaired angiogenesis also may explain the different degree of myo-

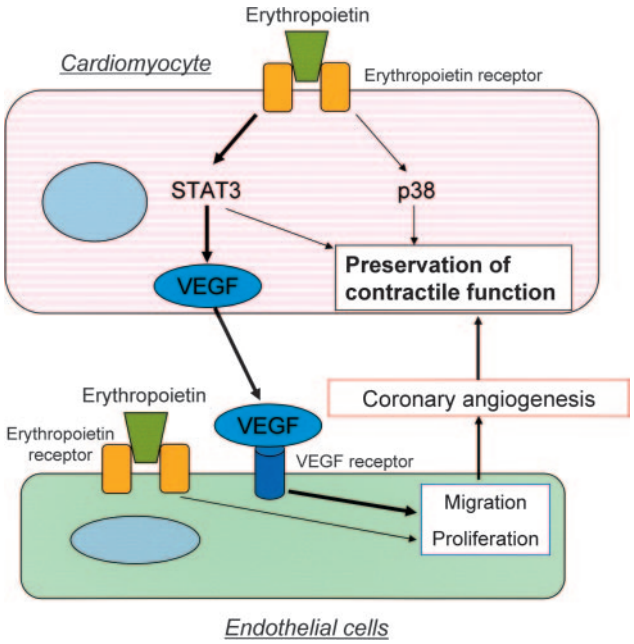


**Figure 8.** Summary of the present study on the possible mechanisms for the protective roles of the endogenous Epo-EpoR system in hypertrophied hearts. The myocardial Epo-EpoR system mediates STAT3 and p38 activation, VEGF production, and capillary growth, resulting in the preservation of contractile function. The endothelial Epo-EpoR system also may play a role in coronary angiogenesis in hypertrophied hearts.

cardial fibrosis after TAC between our study and that of Izumiya et al[7] (8% versus 240% increase in fibrosis area, respectively, compared with corresponding control mice with TAC). Second, in $EpoR^{-/-}{}_{\text{rescued}}$ mice compared with WT mice, phosphorylation of Akt after TAC tended to be increased at 5 hours and was significantly increased at 7 days (Figure 5D), although we do not know the precise mechanisms. The increased LV hypertrophy after TAC in $EpoR^{-/-}{}_{\text{rescued}}$ compared with WT mice also may be attributed to the increased phosphorylation level of Akt.

We also found that p38 phosphorylation in LV myocardium is accelerated in WT mice with TAC but not in $EpoR^{-/-}{}_{\text{rescued}}$ mice with TAC. Nishida et al[21] reported that cardiac-specific p38$\alpha$ knockout mice develop LV dilatation and LV dysfunction in response to TAC and that this abnormal response to pressure overload was associated with massive cardiac fibrosis and accelerated cardiomyocyte apoptosis compared with control mice, although the extent of cardiomyocyte hypertrophy was comparable between the 2 groups. Therefore, the characteristics of cardiac-specific p38$\alpha$ knockout mice with TAC are completely different from those of $EpoR^{-/-}{}_{\text{rescued}}$ mice with TAC in the present study. More recent studies have shown that inhibition of p38 improves cardiac function and cardiomyocyte apoptosis in a rat model of myocardial injury[31] or cardiac function and LV remodeling in a rat model of myocardial infarction.[32] Therefore, future study is required to determine the significance of the impaired p38 phosphorylation in $EpoR^{-/-}{}_{\text{rescued}}$ mice with pressure overload.

Although phosphorylation of ERK in WT mice tended to be increased by 15% at 5 hours and by 29% at 7 days after TAC compared with sham-operated WT mice, the difference did not reach statistical significance. Many investigators reported that phosphorylation of ERK is increased after LV pressure overload, although Babiker et al[33] reported no change in the phosphorylation of ERK after TAC. Because the phosphorylation level of ERK fluctuates after TAC,[34] the extent of the increase may depend on elapsed time after TAC and some experimental conditions. Because neither cleaved caspase 3 nor poly (ADP-ribose) polymerase was detected in the LV myocardial tissue of WT or $EpoR^{-/-}{}_{\text{rescued}}$ mice with TAC in the present study, the different characteristics between the 2 genotypes with TAC cannot be explained by the difference in the extent of cardiomyocyte apoptosis.

## Study Limitations

Several limitations for the present study should be mentioned. First, LVDs was slightly but significantly increased and LVFS was slightly but significantly decreased in $EpoR^{-/-}{}_{\text{rescued}}$ mice before TAC operation. In our previous study,[14] LVDs was increased by 3% and LVFS was decreased by 3% in $EpoR^{-/-}{}_{\text{rescued}}$ mice compared with WT mice under basal conditions; however, the differences were not statistically significant. Different anesthetic conditions between the present study (tribromoethanol) and the previous study (conscious state) might explain the different echocardiographic results. One might argue that the preexisting differences in those echocardiographic parameters between the 2 genotypes can explain the differences at 1 week after TAC. However, we confirmed that the remarkable differences in LVDs and LVDd and performance at 1 week after TAC between the 2 genotypes cannot be explained by the slight differences before TAC operation (Figure 3C). Second, we did not confirm whether the enhanced VEGF production in LV myocardium of WT mice with TAC is attributed to the increased expression of VEGF in cardiomyocytes or vascular endothelial cells. However, recent studies have demonstrated that Epo-EpoR signaling of vascular endothelial cells accelerates angiogenesis through a mechanism independent of VEGF.[35,36] Possible roles of the endogenous Epo-EpoR system of vascular endothelial cells in coronary angiogenesis in hypertrophied hearts remain to be elucidated.

## Conclusions

We were able to demonstrate that the deletion of EpoR in nonhematopoietic cells results in enhanced susceptibility to the development of heart failure in response to TAC, suggesting that the endogenous Epo-EpoR system in the nonhematopoietic cells plays an important protective role against pressure overload–induced cardiac dysfunction in vivo.

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## Disclosures

None.

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## CLINICAL PERSPECTIVE

The results of our present study suggest that the endogenous erythropoietin (Epo)-Epo receptor (EpoR) system in nonhematopoietic cells plays an important protective role against pressure overload–induced cardiac dysfunction in vivo. Patients with end-stage renal disease are frequently associated with severe anemia, which is due to a failure to produce a sufficient amount of Epo in the kidney. Cardiovascular disease mortality in dialysis patients, whose endogenous Epo-EpoR signaling is supposed to be downregulated, is definitely higher than that in the general population, and long-term Epo therapy improves the prognosis primarily through its beneficial effects on cardiovascular mortality and morbidity. However, it remains to be elucidated whether the beneficial effects of treatment with exogenous Epo of an impaired endogenous Epo-EpoR system are due primarily to the improvement in anemia or to its direct effects on the cardiovascular system. From our present and previous findings, we propose a novel mechanism for the protective cardiovascular effects of the endogenous Epo-EpoR system that is mediated by EpoR in nonhematopoietic cells, namely a mechanism not related to erythrocytosis. Finally, patients with preserved left ventricular systolic performance develop heart failure as those with low ejection fraction do. Because our mouse model develops severe systolic dysfunction, our present conclusion may not be extrapolated to heart failure patients or animal models with preserved left ventricular systolic function. A future study using different animal models is required to address this important issue.

# Protective Role of Endogenous Erythropoietin System in Nonhematopoietic Cells Against Pressure Overload–Induced Left Ventricular Dysfunction in Mice

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