Reduced Cardiac Remodeling and Function in Cardiac-Specific EP₄ Receptor Knockout Mice With Myocardial Infarction

Jian-Yong Qian, Pamela Harding, Yunhe Liu, Ed Shesely, Xiao-Ping Yang, Margot C. LaPointe

Abstract—We have shown previously that cyclooxygenase-2 inhibition reduces cardiac hypertrophy and fibrosis postmyocardial infarction (MI) in a mouse model and that prostaglandin E₂ stimulates cardiomyocyte hypertrophy in vitro through its EP₄ receptor. Because the role of cardiac myocyte EP₄ in cardiac function and hypertrophy in vivo is unknown, we generated mice lacking EP₄ only in cardiomyocytes (CM- EP₄ knockout [KO]). Twelve- to 14-week–old mice were evaluated using echocardiography and histology. There were no differences in ejection fraction, myocyte cross-sectional area, and interstitial collagen fraction between KO mice and littermate controls. To test the hypothesis that EP₄ is involved in cardiac remodeling after MI, we induced MI by ligating the left anterior descending coronary artery. Two weeks later, the mice were subjected to echocardiography, and hearts were removed for histology and Western blot. There was no difference in infarct size between KO mice and controls; however, KO mice showed less myocyte cross-sectional area and interstitial collagen fraction than controls. Also, CM-EP4 KO mice had reduced ejection fraction. Because the transcription factor Stat-3 is involved in hypertrophy and protection from ischemic injury, we tested whether it was activated in control and KO mouse hearts after MI. Western blot indicated that Stat-3 was activated in control hearts after MI but not in KO hearts. Thus, CM-EP4 deletion decreased hypertrophy, fibrosis, and activation of Stat-3. However, cardiac function was unexpectedly worsened in these mice. We conclude that cardiac myocyte EP₄ plays a role in hypertrophy via activation of Stat-3, a process that seems to be cardioprotective.

Key Words: PG receptor ■ hypertrophy ■ EP₄ ■ PGE ■ MI, remodeling

Cardiac hypertrophy is a common pathological lesion induced by various extracellular stimuli, including mechanical stress (eg, systemic hypertension), hormones (eg, angiotensin II and β-adrenergic agents), and cytokines (eg, transforming growth factor-β). During the early stages of various cardiac diseases, hypertrophy is compensatory, but sustained stimulation may lead to excessive cardiac remodeling and, ultimately, heart failure. Understanding the cellular basis of cardiac hypertrophy is important when designing an appropriate therapy to regulate the hypertrophic response.

Prostaglandin (PG) E₂ is a well-known proinflammatory prostanoid. It is synthesized from arachidonic acid by cyclooxygenase (COX) and PGE₂ synthase. There are 2 COX isoforms in mammalian cells. COX-1 is expressed constitutively in almost all tissues, and its PG products mediate physiological responses, such as vascular homeostasis and gastroprotection. COX-2, although often undetectable in resting cells, is readily induced as an immediate early gene in response to cytokines, growth factors, phorbol esters, and bacterial lipopolysaccharides. In addition to inflammatory effects, PGE₂ has also been shown to promote growth. PGE₂ enhanced cell proliferation in murine aortic smooth muscle cells and NIH 3T3 cells. It also increased protein synthesis, cell size, and brain or B-type natriuretic peptide (BNP) expression in cardiac myocytes, all markers of hypertrophic growth. We have also shown that myocardial infarction (MI) increased generation of PGE₂ accompanied by upregulation of COX-2 in the mouse heart, and treatment with a specific COX-2 inhibitor for 2 weeks improved cardiac function and reduced hypertrophy and fibrosis. Given that COX-2 products have both deleterious and protective effects in the heart and vasculature, it is important to understand how PGE₂ affects the prohypertrophic response.

PGE₂ exerts its biological actions through 4 different G protein-coupled receptors, EP₁, EP₂, EP₃, and EP₄, which differ in structure, ligand-binding properties, activation of signal transduction pathways, and tissue distribution. EP mRNAs have been detected in the heart of several species, including humans, and EP₃ is the most abundantly expressed EP subtype. Activation of EP₃ leads to accumulation of intracellular cAMP through interaction with a cholinergic-sensitive Gα protein. EP₃ has also been linked to activation...
of p42/44 mitogen-activated protein kinase, and in cardiac myocytes it is involved in regulation of protein synthesis and epidermal growth factor receptor transactivation. All 4 of the EPs have been knocked out in mice with no cardiovascular phenotypes in unstressed conditions, except that EP4 knockout (KO) mice died shortly after birth from patent ductus arteriosus. Using the global EP4 KO mouse (EP4 knocked out in all cells), the role of EP4 has been examined acutely (≤24 hours) in ischemia/reperfusion models. An EP4 agonist reduced infarct size (IS) when administered before ischemia/reperfusion injury, whereas ischemia/reperfusion injury enhanced IS in EP4 KO mice, suggesting that EP4 is cardioprotective in this model. It is hard to extrapolate these results to predictions of how EP4 contributes to cardiac function and structure during chronic disease processes (eg, cardiac remodeling post-MI). Because the aforementioned study used a global EP4 KO model, the role of EP4 in cardiac myocytes is not clear. Thus, we developed a novel mouse model where EP4 is deleted only in cardiac myocytes and used these mice to study the effect of EP4 on cardiac remodeling after MI.

**Materials and Methods**

**Generation and Genotyping of CM-EP4 KO Mice**

The CM-EP4 KO mouse was created by the Cre-loxP mechanism through interbreeding 2 transgenic mouse lines. The EP4flox/flox mouse was generated by gene targeting and possessed loxP sites flanking exon 2 of the EP4 receptor gene (“EP4 floxed”) (obtained from Dr Matthew Breyer, Vanderbilt University Medical Center). This strain can generate a conditional EP4 “knockout” in that exon 2 will be deleted in cells expressing Cre recombinase. Importantly, the loxP sites do not alter the wild-type (WT) EP4 phenotype in the absence of Cre. The second line has a Cre recombinase introduced as a transgene driven by an α-myosin heavy chain promoter (obtained from Dr Michael Schneider, Baylor College of Medicine). The α-myosin heavy chain is expressed primarily in adult cardiac myocytes, obviating the complications of EP4 deletion early during fetal development. The colony was maintained by breeding mice transgene to mice homozygous for the floxed EP4 allele and lacking the Cre transgene. This scheme generated roughly equal numbers of CM-EP4 KO and “WT floxed” littermates, and the use of littermates as controls assured that the backgrounds matched. The majority of each strain of mice was alive after birth and had similar survival rates.

Total DNA was extracted from heart and other tissues using a DNeasy tissue kit (Qiagen) following the manufacturer’s instructions. A total of 100 ng of each DNA sample was amplified by PCR (40 cycles of 30 seconds at 94°C, 30 seconds at 60°C, and 30 seconds at 72°C). The sense primer for EP4 was 5′-GTG AGA TGG GGG GAG GGG ACA ACT-3′, and the antisense primer was 5′-TCT GTG AAG CGA GTT CTT AGG CT-3′. The primers distinguished the WT and floxed EP4 genes by amplifying a 243-bp fragment in the WT and a 344-bp fragment in EP4flox/flox. The sense primer for Cre was 5′-CCAGCTAAACATGCTTCATCGTCG-3′, and the antisense primer was 5′-ATTCTCCCACCGT CAGTACCGTGA-3′. The Cre primers amplified a 300-bp fragment. We also performed Western blot for Cre (antibody 69050-3 from Novagen) to confirm its expression in CM-EP4 KO mouse hearts.

**Animal Protocols**

All of the animal experiments were approved by the Henry Ford Health System Institutional Animal Care and Use Committee. A total of 141 mice were used for this study (KO=62; littermate controls=79).

Protocol 1 looked at the effect of CM-EP4 KO on cardiac phenotypes in unstressed mice. Twelve- to 14-week-old male CM-EP4 KO and WT littermates were used in these studies. Normal, unstressed mice were subjected to 2D M-mode echocardiography, and then hearts were harvested. There were 2 sets of samples for both KO and WT mice, with the first set used for extraction of DNA, RNA, and protein and the second for histological analysis. Protocol 2 studied the effect of CM-EP4 KO on cardiac remodeling post-MI. To induce MI, 12- to 14-week-old (22- to 25-g) male mice were anesthetized with sodium pentobarbital (50 mg/kg IP), and a left thoractomy was performed. The left anterior descending coronary artery was ligated with an 8-0 silk suture placed near its origin at the edge of the left atrium, as described previously. Ligation was deemed successful when the anterior wall of the left ventricle became pale. The ligature was positioned so as to produce a moderate infarct (20% to 50%). Mice were kept on a heating pad and monitored until awake. Two weeks post-MI, mice were subjected to 2D echocardiography, and then hearts were harvested. In all of the groups of KO and WT mice, half of the hearts were used for extraction of DNA, RNA, and protein, and half for histological analysis. The mortalities after MI (from surgery to sacrifice) were similar between KO and WT mice (42%).

2D transthoracic echocardiography was performed on conscious mice using an Acuson 256 system with a 15-MHz linear transducer, as reported previously. M-mode images from both a short and long axis were used to determine left ventricular end-diastolic/systolic dimension (LVd/LVsDs) and shortening fraction. Ejection fraction (EF) was obtained from the short-axis view and calculated by the following formula: diastolic area−systolic area/diastolic area. Diastolic measurements were made at the maximum left ventricle cavity dimension, whereas systolic parameters were measured during maximum anterior motion of the posterior wall.

**Histological Assessment of Intersitial Collagen Fraction, Myocyte Cross-Sectional Area, IS, and Wall Thickness of the Infarct Area**

Mouse hearts were harvested and sectioned transversely into 4 slices from apex to base. Three sections (sections A, B, and D) were frozen in isopentane and stored at −70°C for determination of intersitial collagen fraction (ICF), myocyte cross-sectional area (MCSA), and IS, as described previously. The wall thickness of the infarct area was measured from the section with the longest infarct zone. The infarct length of the epicardium was evenly divided into 6 portions, and 5 evenly spaced points were selected for measurement. The distance between the epicardium and endocardium of each point was measured and averaged to obtain wall thickness of the infarct area. Measurements were made using MicroSuite Biological Imaging Software.

**RT-PCR Analysis of EP1 to 4 and BNP mRNAs**

The infarcted region of the mouse heart was discarded, and the remaining portion of the left ventricle was homogenized. Total RNA was extracted from tissue homogenates using an RNaseasy Fibrous Tissue mini kit (QIAGEN) following the manufacturer’s instructions. Reverse transcription was performed using the Omniscript RT Kit (Qiagen) with 2 μg of total RNA, 1 μg of random primer (Invitrogen), and 10 μL of RNAasin (Promega) in a 20-μL volume for 1 hour at 37°C. Primers were as follows: EP1 sense: TCC CCA ATA CAT CTG TGG TGC; EP1 antisense: TCC CCA GCG CCG CCG ATC ATC (718 bp); EP2 sense: GTG GCC CTG GCT CCC GAA AGT C; EP2 antisense: GTC AAG GAG CAT ATG GCG AAG GTG (536 bp); EP3 sense: CAT GAT GTT CAC TGG CTT CGT; EP3 antisense: GTC ACC ACC AGA GCC AGC AA (438 bp); EP4 sense: TGC TTT GTC GAA CAT CCC C; EP4 antisense: GTG GTG TCT GTG TGG GTC A (326 bp); BNP sense: TGA AAG TGG TGT CCC AGA TGA; BNP antisense: GTG CTG CCT TGA GAC CA (241 bp); GAPDH sense: ATT CAA CGG CAC AGT CAA GG; and GAPDH antisense: TGG ATG CAG GGA TGA TGT TC (482 bp). PCR conditions were: 94°C for 30 seconds, 58°C to 62°C for 30 seconds, 72°C for 90 seconds, and a final extension at 72°C for 10 minutes.
72°C for 5 minutes. Cycle number was 40 for EP3; 35 for EP1, EP2, and EP4; 30 for BNP; and 25 for GAPDH. The GAPDH signal was used for evaluating input RNA values. The PCR bands were quantified by densitometry and normalized by GAPDH, and ratios between different groups were compared.

Western Blotting
Mouse hearts were homogenized in sample buffer (Tris 47.5 mmol/L, 2% SDS, 10% glycerol [pH 6.8]) or Tris-HCl 25 mmol/L, EDTA 0.5 mmol/L, and EGTA 0.5 mmol/L (pH 7.5) containing a proteinase and phosphatase inhibitor mixture (Roche). A total of 40 μg of protein per sample was electrophoresed and transferred to a polyvinylidene fluoride membrane. Phospho(p)-Stat-3 and total(t)-Stat proteins were detected with the appropriate antibodies (Cell Signaling). The antibody-antigen reaction was detected using a secondary antibody linked to horseradish peroxidase. The protein bands were scanned, quantified, phosphorylated Stat normalized to total Stat-3, and ratios between different groups compared.

Statistics
Data were expressed as mean±SE and analyzed by unpaired 1-tailed Student t test (to compare data from WT littermates with MI versus CM-EP4 KO mice with MI). P<0.05 was considered significant.

Results
Characterization of CM-EP4 KO Mice
Using specific PCR primers and DNA extracted from the heart and kidneys, we tested whether EP4 was knocked out only in the heart. In the top panel of Figure 1, EP4flox/flox is a 344-bp PCR product generated from the EP4flox/flox gene. EP4 WT represents a 243 bp PCR product of the WT EP4 gene generated from tail DNA of a C57Bl6/J mouse. GAPDH is the control gene. The DNA ladder lane shows the position of the 500-bp DNA fragment. The graph is the densitometric quantification of PCR-amplified DNA from the hearts of WT floxed littermates and CM-EP4 KO mice (n=5 to 6).

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Deletion of a receptor may result in a compensatory change in other receptors. To investigate the effect of EP4
deletion on other EPs, we performed RT-PCR to amplify EP₁, EP₂, and EP₃ mRNAs. The mRNA levels of EP₁, EP₂, and EP₃ were similar in the hearts of both CM-EP₄ KO and WT littermates, indicating that other EPs were not changed to compensate for the loss of EP₄ (Figure 4).

We performed 2D-M-mode echocardiography of mice and found no difference in those parameters that measure the pumping function of the heart (eg, EF) and its shape (LVDs and LVDd) between CM-EP₄ KO and WT floxed littermates (Table 1). Thus, EP₄ gene deletion does not change cardiac function in the absence of stress.

Finally, we used histological methods to evaluate the size of myocytes (MCSA) and the degree of fibrosis (ICF) in hearts of CM-EP₄ KO mice and WT littermates. As shown in Table 1, neither MCSA nor ICF was different between KO and littermate controls. In contrast, MI increased chamber dilatation tended to be more severe in CM-EP₄ KO mice, indicating that EP₄ deletion exacerbated MI-induced cardiac dysfunction and LV dilatation (Table 2).

**Effect of EP₄ Deletion on BNP Gene Expression**

Because we have shown previously that PGE₂, acting though EP₄, stimulates BNP gene expression, we used RT-PCR to assess the effect of cardiac-specific EP₄ deletion on this marker gene of hypertrophy. As shown in Figure 5, MI induced BNP gene expression in littermate controls, but this effect was abrogated in KO mice.

**Discussion**

PGE₂ is a proinflammatory prostanoid with proliferative and hypertrophic properties. Recently we and others found that PGE₂, acting through EP₄, is involved in cardiomyocyte hypertrophy in vitro. The availability of an EP₄ KO mouse would represent the best model to test whether EP₄ plays a role in cardiac remodeling in vivo. Global EP₄ gene KO mice have been reported, but 95% of homozygotes for the null allele have patent ductus arteriosus after birth and soon die. Experiments have been done on these mice by separately breeding F2 generation global EP₄ KO survivors and F2 WT mice. However, this results in colonies of different genetic backgrounds and artificially selects for those EP₄ KO mice that have adapted to survive this genetic deletion. Thus, understanding the role of EP₄ in the heart requires a conditional KO approach, as well as a breeding scheme that produces KO and littermate controls of the same overall background.

In this study, we have reported the generation and characterization of mice with reduced EP₄ in the heart. An explanation for the <100% reduction of EP₄ in the heart is that EP₄ should still be present in fibroblasts, endothelial cells, and smooth muscle cells. Also, previous studies using this technology have described some variability in the percentage of
myocytes expressing Cre and thus, not all of the myocytes are assumed to have excised the floxed EP4 gene.18,27

Because there were no cardiac phenotypes, we used the CM-EP4 KO mice to study the effect of EP4 deletion on several parameters of MI-induced cardiac remodeling, including IS and scar thickness, hypertrophy, fibrosis, and function. Cardiac remodeling subsequent to MI involves a number of different processes, including infarct expansion, pressure overload, and volume overload.28 Early remodeling occurs in the first 3 or so days after MI and involves the infarct and peri-infarct zones. Remodeling also occurs in the noninfarcted regions of the left ventricle and involves myocyte hypertrophy, accumulation of extracellular matrix, and changes in the size and geometry of the entire left ventricle. The initial adaptive hypertrophic remodeling of the noninfarcted myocardium is hemodynamically beneficial to reduce wall stress. Late remodeling involves changes in the extracellular matrix and in the size and shape of the heart. When the changes in the left ventricle are no longer compensatory, left ventricular remodeling becomes maladaptive, leading to ventricular dilatation and overt heart failure.29,30

We found that EP4 signaling in cardiac myocytes had no effect on IS in our model. In contrast, Xiao et al12 reported that systemic EP4 KO mice had a larger IS than WT controls in an acute model of ischemia/reperfusion (24 hours of injury), suggesting that EP4 is cardioprotective. It is possible that EP4 has a different function in the very early events resulting from ischemia/reperfusion injury than in the late remodeling that we are studying in our permanent left anterior descending coronary artery ligation model. Another possibility is that, in the global EP4 KO mouse subjected to ischemia/reperfusion injury, the absence of EP4 in other cells, such as inflammatory cells, fibroblasts, vascular smooth muscle cells, and endothelial cells in the heart and other tissues, may have an influence on the extent of ischemia/reperfusion injury. Finally, there is also the possibility that differences in the genetic backgrounds of the global EP4 KO mice and the controls could influence IS differences.

Our data suggest that EP4 signaling is involved in part in cardiac hypertrophy in vivo. The results are supported by publications showing that PGE2, acting through EP4, increased cardiomyocyte hypertrophy and the hypertrophic marker gene BNP in vitro.6,14,21 Our data are also consistent with our previous publication showing that COX-2 inhibition reduced MCSA after MI.7 However the studies

Table 2. Histology and Echocardiography of CM-EP4 KO Mice After MI

<table>
<thead>
<tr>
<th>Parameters Studied</th>
<th>Littermate Controls</th>
<th>CM-EP4 KO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham MI</td>
<td>Sham MI</td>
</tr>
<tr>
<td>IS, mean±SE, %</td>
<td>31.4±2.9</td>
<td>37.3±4.3</td>
</tr>
<tr>
<td>WTIA, mean±SE, μm</td>
<td>447.9±26.9</td>
<td>446.4±38.2</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>MCSA, mean±SE, μm²</td>
<td>160.6±0.6</td>
<td>200.3±4.1</td>
</tr>
<tr>
<td>ICF, mean±SE, %</td>
<td>5.4±0.4</td>
<td>9.3±0.3</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>EF, mean±SE, %</td>
<td>81.42±2.66</td>
<td>80.06±1.09</td>
</tr>
<tr>
<td>SF, mean±SE, %</td>
<td>67.67±1.26</td>
<td>65.05±0.94</td>
</tr>
<tr>
<td>LVDs, mean±SE, mm</td>
<td>1.04±0.06</td>
<td>1.01±0.04</td>
</tr>
<tr>
<td>LVDD, mean±SE, mm</td>
<td>3.19±0.07</td>
<td>3.17±0.11</td>
</tr>
<tr>
<td>n</td>
<td>14</td>
<td>12</td>
</tr>
</tbody>
</table>

SF indicates shortening fraction.

Statistical significance: *P<0.05 and †P<0.005 for littermate control MI vs CM-EP4 KO MI.

**Figure 5.** RT-PCR of BNP. RNA was extracted from mouse hearts. RT-PCR for BNP and GAPDH was performed (bottom panel is a gel of PCR products), and the BNP/GAPDH ratio was determined after quantification by densitometry, as shown in the graph (n=4 to 10).

**Figure 6.** Western blot for Stat-3. Two weeks after MI, hearts were removed, protein extracted, and Western blot performed. The bottom panel is a representative Western blot of phosphorylated (p) Stat-3 and total (t)-Stat-3, and the upper graph is the densitometric quantification of the phosphorylated Stat-3/t-Stat-3 ratio (n=12).
with the COX-2 inhibitor could not identify which COX-2 products and prostanoid receptors were involved in the effect. Thus, our present studies with the CM-EP\textsubscript{4} KO mouse point to EP\textsubscript{4} signaling in cardiac myocytes as part of the hypertrophic growth program.

Regarding fibrosis post-MI, the increase in ICF was less in the KO mouse hearts than in the littermate controls, implying that cardiac myocyte EP\textsubscript{4} is somehow involved in fibrosis. Given that PGE\textsubscript{2} levels increase in the heart post-MI, the PGE\textsubscript{2} may also act on myocytes in an EP\textsubscript{4}-dependent manner to stimulate the synthesis and secretion of growth factors. For example, PGE\textsubscript{2} has been shown to stimulate IL-6 expression in neonatal cardiac myocytes, and IL-6 stimulates fibroblast proliferation. Thus, it will be of interest to use our CM-EP\textsubscript{4} KO mice to study the role of myocyte EP\textsubscript{4} in fibroblast proliferation.

After MI, cardiac function was worsened in CM-EP\textsubscript{4} KO mice compared with littermate controls. This reduction in function occurred despite the reductions in fibrosis and hypertrophy. This result is in contrast with our previous study showing that COX-2 inhibition improved cardiac function in C57BL6/J mice after MI. There may be several reasons for these disparate results. The first is that the species of mice is different in the 2 studies. A second potential reason is that COX-2 inhibition reduces all of the prostanoid generation, including reductions in other deleterious prostanoids, such as PGF\textsubscript{2\alpha} and thromboxane A\textsubscript{2}, but these are likely still present in the CM-EP\textsubscript{4} KO mouse heart. Third, the experimental design of the studies is slightly different. In the COX-2 inhibition study, the COX-2 inhibitor was administered either 2 days or 2 weeks post-MI, whereas in the present study, EP\textsubscript{4} was deleted in the mouse hearts before inducing MI. This timing issue could be important, because cardiac remodeling has both an early and a late component, and early inflammatory responses are necessary for wound healing and infarct repair. Finally, the COX-2 inhibitor, but not cardiac-specific EP\textsubscript{4} deletion, would have systemic anti-inflammatory effects.

In a study in rats, it was shown that treatment with a COX-2 inhibitor during MI reduced fibroblast proliferation, macrophage infiltration, and inflammation in the heart. Thus we hypothesize that the reduction in hypertrophy and fibrosis may not have a beneficial effect if the overall level of inflammation in the heart is not appropriately regulated. This is an area for future studies.

Other studies have described reductions in fibrosis with further impairment of systolic function. For example, Cingolani et al showed that the small peptide Ac-SDKP infused into spontaneously hypertensive rats reduced LV collagen content but not hypertrophy and further reduced systolic performance. The authors suggested that the reduction in systolic function might be caused by the lack of a proper extracellular matrix support (either collagen type or cross-linked collagen) for the hypertrophied myocytes. Additional studies are required to study the role of EP\textsubscript{4} in systolic function.

There are many signaling mechanisms involved in cardiac hypertrophy. Activation of the transcription factor Stat-3 and the p42/44 mitogen-activated protein kinase protein kinase and phosphoinositide 3-kinase-α signaling pathways is involved in compensatory hypertrophy and appears protective. Phosphoinositide 3-kinase, which lies downstream of many receptor tyrosine kinases, activates the kinase Akt, which, in turn, activates Stat-3. Activated Stat-3 promotes cardiomyocyte survival by upregulating antiapoptotic and cytoprotective proteins. Overexpressed EP\textsubscript{4} has been shown to activate phosphoinositide 3-kinase/Akt. Also in vitro, PGE\textsubscript{2} acting via EP\textsubscript{4} induces cardiomyocyte hypertrophy through activation of Stat-3 and p42/44 mitogen-activated protein kinase. Normally, Stat-3 is activated by IL-6 and other cytokines binding to receptors (eg, gp130), which are coupled to a kinase called Janus kinase (JAK). The effect of PGE\textsubscript{2} to activate Stat-3 in myocytes may occur indirectly through stimulation of synthesis and secretion of IL-6 or may result from the effects of other signaling kinases, such as p42/44 mitogen-activated protein kinase protein kinase or phosphoinositide 3-kinase/Akt. Cardiovascular effects of the KO of components of the Stat-3 signaling pathway have been studied. Cardiomyocyte-restricted KO of Stat-3 increased cardiac dysfunction, sensitivity to inflammation, and fibrosis in aged mice. Similarly, cardiomyocyte-specific deletion of the gp130 receptor prevented hypertrophy in response to thoracic aortic constriction. These gp130 KO mice showed a rapid onset of cardiac dysfunction with dilated cardiomyopathy and massive myocyte apoptosis. Thus, results from our present study showing that CM-EP\textsubscript{4} KO mice are defective in their ability to activate Stat-3, have reduced cardiac hypertrophy, and a worsening of systolic function after MI are mostly consistent with the aforementioned studies on KO of gp130 and Stat-3 and suggest a role for both EP\textsubscript{4} and Stat-3 in a growth/survival pathway to protect the heart from pathophysiological stimuli. Additional studies will be required to investigate the role of myocyte EP\textsubscript{4} in the regulation of IL-6, inflammatory mediators, signaling kinases, and apoptosis in the mouse heart after MI.

**Perspectives**

In summary, we are the first to have generated CM-EP\textsubscript{4} KO mice in which the systemic complications of EP\textsubscript{4} deletion are obviated. This is also the first report on cardiac myocyte EP\textsubscript{4}’s effect on cardiac hypertrophy, fibrosis, and function. After MI, EP\textsubscript{4} is involved in cardiac remodeling, activation of Stat-3, and cardiac function. Moreover, we suggest that EP\textsubscript{4} is involved in compensatory hypertrophy, perhaps coupled to Stat-3 activation, and when this is disrupted, cardiac function is impaired. Therefore, an EP\textsubscript{4} agonist may have a therapeutic benefit to enhance compensatory hypertrophy and improve function in patients with MI.

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**Disclosures**

None.

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