Decreased metalloprotease 9 induction, cardiac fibrosis, and higher autophagy after pressure overload in mice lacking the transcriptional regulator p8

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associates with the MMP9 promoter and is necessary for MMP9 transcription.

We have recently unveiled a role for p8 in controlling autophagy (25). Thus p8 RNA interference (RNAi) increases basal autophagy in cells and decreases cellular viability by regulating the levels of Bnip3 protein, a known pro-autophagic target. Notably, we have shown that p8-deficient mouse hearts have higher basal autophagy and Bnip3 levels. These mice develop LV wall thinning and chamber dilation, with consequent impaired basal cardiac function.

Here we further investigated the in vivo role of p8 in cardiac remodeling induced by transverse aortic constriction (TAC). We found that unstressed p8-deficient mouse hearts have altered patterns of expression of genes associated with fibrosis, p8−/− hearts also manifest higher autophagy and apoptosis. We also show that p8 expression is strongly induced in the LV of p8/+ mice after TAC, and that disruption of p8, despite causing a reduction in MMP9 induction, triggers a striking compensatory upregulation of MMP2 and MMP3 as well as a down modulation of TIMP2, consistent with the observed reduction in fibrosis. By contrast, TAC-induced autophagy is significantly higher in the LV of p8-deficient mice. Our results suggest an unexpectedly complex role for p8 in heart failure, promoting both pathological (increased fibrosis) and protective (autophagy and apoptosis inhibition) processes.

MATERIALS AND METHODS

qPCR and RT-PCR. Total RNA was isolated and PCRs were performed as described in Ref. 12. Quantitative real-time PCR (qPCR) of mouse RNA was performed using Quantitect SYBR Green Kit (Qiagen) according to the manufacturer’s instructions. qPCR was performed using an Eppendorf 96-well thermocycler. The amount of each product was determined from the relative standard curves constructed with serial dilutions of the control cDNA. End-point RT-PCR was performed as by Gorruppi et al. (14), and β-actin or gapdh were co-amplified as controls. The sequences of the oligonucleotide primers used will be provided upon request.

Apoptotic cell assay. The terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL assay) was performed as described by Patten et al. (43). The number of TUNEL-positive cardiomyocytes, identified by α-sarcinomic actin staining, was expressed as percentage of total cells.

Soluble collagen analysis. Sircol soluble collagen assay was performed as indicated by the manufacturer (Biocolor). Briefly, fresh heart samples were weighted and digested overnight at 4°C in 0.5 M acetic acid containing 0.1 mg/ml pepsin (Sigma-Aldrich). One milliliter of Sircol Dye reagent (Sirius red) was added to each sample (100 μl) and incubated for 30 min at room temperature on a rotating platform. Sircol dye binding does not discriminate between collagen types and is reduced if collagens are denaturated. The Eppendorf tubes containing the collagen-dye insoluble complex were centrifuged for 10 min at 12,000 rpm and washed once with 750 μl of cold acid/salt wash reagent, and the pellets were dried after carefully removing all the liquid. Each pellet was then solubilized with 250 μl of alkali reagent and the absorbance read at 550 nm. Serial dilutions of a known concentration of rat collagen (provided by the manufacturer) in water were processed in parallel and used to create the standard curve.

Protein isolation and Western blotting. The Western blots were performed as described by Kong et al. (25). Total proteins were extracted in ice-cold RIPA buffer containing protease inhibitors and quantitated using Bradford solution (Bio-Rad). Expression levels were normalized to loading controls by using ImageJ software (http://rsb.info.nih.gov/ij/). For Western blots, primary antibodies used include anti-LC3B, ATG12–5, poly (ADP-ribose) polymerase (PARP), P-eff2a and were from Cell Signaling Technologies, and antibodies against MMP9, MMP13, atrial natriuretic factor (ANF), CHOP, GRP78, Bax, Sqstm1, Bnip3, tubulin, and actin were from Santa Cruz Biotechnologies. Appropriate peroxidase-conjugated secondary antibodies were from Santa Cruz Biotechnologies or Jackson, whereas ECL reagents were from Boston Bioproducts.

Animal experiments. p8−/− mice and wild-type (p8+/+) litterate controls in C57BL/6j background have been previously described (11). Ten- to twelve-week-old, age-matched, p8−/− (n = 10) and p8+/+ mice (n = 10) underwent TAC or sham operation (n = 5 each), and 4 wk later hearts were harvested while mice were under deep anesthesia for analysis. Both male and female were used in the study. For echocardiography mice were imaged in the left lateral decubitus position using a Phillips-Sonos 7500 echocardiography system equipped with 11 MHz sector array and 10–15 MHz linear array transducers. Digital echocardiographic images were analyzed with Phillips-based analysis software (qLAB) on XP-based platform coupled with the echocardiography system. LV end-diastolic diameter (LVEDD) and end-systolic diameter (LVESD) were measured, and fractional shortening (FS) was calculated as (LVEDD – LVESD/ LVEDD) × 100%. Studies and analysis were done by investigators blinded to the mice genotype. All procedures were performed according to the animal protocols approved by Tufts University Animal Care and Use Committee.

Statistical analysis. Independent experiments were pooled when the coefficient of variance could be assumed to be equal. Statistical significance was evaluated with Prism 5.0 software by using non-paired, two-tailed Student’s t-test (n = number of experiments or animals analyzed). P values below 0.05 were considered significant and if lower as indicated.

RESULTS

To determine the role of p8 in cardiac remodeling, we analyzed the effects of p8 genetic deletion in a pressure-overload model of heart failure. To this end, age-matched p8−/− and wild-type (p8+/+) mice underwent TAC or sham operation, and their hearts were analyzed 4 wk later.

Altered expression of fibrosis markers in p8−/− mice. p8 is a stress-inducible transcriptional regulator, and its genetic deletion is associated with LV dilation, wall thinning, and impaired baseline cardiac function (25). First, to determine the physiological relevance of p8, we analyzed the regulation of its gene expression in the LV of sham- and TAC-operated mice. A low level of basal p8 mRNA was present in the LV of unstressed p8+/+ mice and was significantly increased after TAC, as quantified by end-point RT-PCR (3.2 ± 0.7-fold) or qPCR (4.2 ± 1.3-fold) (Fig. 1, A and B, respectively). We then analyzed in LV samples the expression of genes linked to cardiac hypertrophy and fibrosis. Interestingly, unstressed p8−/− mice exhibited increased basal expression of collagen I (col1a2) and collagen III (col3a1) (Fig. 1C). After TAC, p8+/+ mice displayed a lower induction of col1a2 and col3a1 and a modest upregulation of tnfa (Fig. 1C) compared with p8+/+ mice. In p8+/+ or p8−/− LV samples, no significant differences in the expression of the hypertrophic marker anf or of the cytokines csgf and vegf were observed either at baseline or after TAC (Fig. 1C). We also analyzed the expression of TGFβ and of the plasminogen activator inhibitor-1 (PAI-1), a TGF-β signaling target, and found a similar regulation in p8+/+ and p8−/−/
Our data suggested that p8 might act in vivo to prevent ing occurring in myocardial infarction and heart failure (49). The changes in cardiac collagen deposition described above suggested to us that p8 might act to limit excessive collagen deposition described above suggested to us that p8 might act to limit excessive collagen deposition. We therefore investigated whether p8 deletion similarly interferes with MMP9 upregulation in vivo following TAC. MMP9 levels were similarly low in LV of sham-operated p8−/− and p8+/+ mice. Compared with p8+/+ controls, p8−/− mice exhibited significantly less MMP9 protein induction in their LV after TAC (Fig. 2D).

We then analyzed qPCR mmp9 expression in the LV of sham- and TAC-operated p8+/+ and p8−/− mice. In agreement with the Western blot results and with our previous findings in primary rat cardiac fibroblasts (14), mice lacking p8 had a significantly lower induction of mmp9 following TAC compared with p8+/+ mice (Fig. 2E). These results suggested a conundrum: p8 appears necessary for cardiac collagen deposition, yet it is also important for the expression of a collagen-degrading enzyme-MMP9. Might p8 affect the expression of other MMPs or MMP regulatory mechanisms that affect collagen breakdown?

MMPs have both overlapping substrate specificities and peculiar functions, which are finely tuned through the expression of tissue inhibitors of metalloproteinases (TIMPs). Accordingly, the loss of mmp9 activities has been shown be partially compensated for by the upregulation of MMP2, MMP13, and complemented by upregulation of TIMP1 (8, 50). Hence, we investigated whether p8 deletion might modify the LV expression of mmp2, mmp3, mmp13, timp1, and timp2 following TAC. Their expression levels were normalized to actin and compared with mmp9 expression (Fig. 3). The levels of mmp2, mmp3, mmp13, timp1, and timp2 were similar in LV of sham-operated p8−/− and p8+/+ mice. Compared with p8+/+ controls, p8−/− mice had a significantly higher mmp2 and mmp3 expression along with a lower timp2 induction after TAC. As was shown in Fig. 2, B and C; upregulation of mmp9 was lower in the p8−/− mice. Both mmp13 and timp1 levels were similarly upregulated in the p8−/− and p8+/+ mice after TAC. These data suggest that in p8−/− mice, the lack of MMP-9 upregulation after TAC is associated with a compensatory change in the expression of other mmps and specific timps. This overall increase in enzymes that degrade collagen...
Bax protein and higher levels of cleaved nuclear PARP (Fig. 4) resulted in an increase in apoptosis to near equal levels for p8+/+ and p8−− mice subjected either to sham operation or TAC. A: hematoxylin/eosin (top) and Masson’s trichrome stainings (bottom) of histological sections from LV of p8+/+ and p8−− mice. Here we investigated the apoptosis levels in p8−− mice consequent to TAC.

Increased baseline apoptosis in p8-deficient mice. Cardiomyocyte apoptosis is a key triggering event in the remodeling process that contributes to heart failure. Cardiomyocyte death is accompanied by fibrosis to replace lost cardiomyocytes. We have previously shown that p8 silencing in cells triggers apoptosis (25). Here we investigated the apoptosis levels in vivo in sham- and TAC-operated p8−− and p8+/+ mice. Our findings suggest that p8−− mice have elevated baseline apoptosis; however, TAC triggers an increase in apoptosis to near equal levels for p8+/+ vs p8−− mice.

Thus, at baseline, p8-deficient mice expressed higher levels of Bax protein and higher levels of cleaved nuclear PARP (Fig. 4A). In agreement with previous reports (10, 22, 40), these apoptotic markers were upregulated after TAC, and we found a similar increase in both p8−− and p8+/+ mice. Accordingly, the number of TUNEL-positive cardiomyocytes, identified by α-sarcomeric actin staining, was significantly higher at baseline in the p8−− mice compared with p8+/+ animals and was increased by pressure overload to a similar extent in both genotypes (Fig. 4B).

A large body of evidence supports a role for apoptotic signaling initiated by the ER in the development of heart failure (37). In cancer cells, p8 has been shown to mediate cannabinoid-activated ER-stress signaling and apoptosis (4, 47). We thus investigated ER-stress signaling markers were upregulated after TAC, and we found a similar increase in both p8−− and p8+/+ mice. Equal amounts of total proteins were separated by SDS-PAGE and analyzed with antibodies against MMP9, MMP13, ANF, and actin (ACT). B: area of fibrosis after sham or after TAC is expressed as percentage and represented as means ± SE (n = 4 per group). C: soluble collagen measurement in LV samples from sham- or TAC-operated p8−− and p8+/+ mice. The concentration of soluble collagen is expressed as µg/mg of LV myocardium sample (n = 4 for each group, P < 0.05). D: Western blot analysis of LV samples from sham- or TAC-operated p8−− and p8+/+ mice. The values were normalized to gapdh levels and represent the means ± SE (n = 4 per group, P < 0.05).
Thus the higher level of baseline apoptosis in \( p8 \)-deficient mouse hearts is not associated with increased ER-stress activation. Both \( p8^{+/+} \) and \( p8^{-/-} \) mice develop similar levels of apoptosis and ER-stress signaling upregulation following TAC.

\( p8 \)-deficient mice have higher baseline autophagy, which is enhanced after TAC. Autophagy in the heart is a homeostatic mechanism for the maintenance of normal cardiac function and morphology. Several pathophysiological conditions, including heart failure, have been associated with an increase in autophagy (6, 15, 29, 45). We have previously shown that either \( p8 \) silencing or deletion causes an increase in proautophagic gene expression and autophagic markers, coinciding in vivo with decreased baseline cardiac functionality (25). We thus investigated the effects of \( p8 \) deletion on autophagy levels in sham-operated mice and after TAC by analyzing the protein levels of LC3-II, ATG12–5, and SQSTM1 in Western blots. Consistent with our previous findings, disruption of \( p8 \) led to increased apoptosis in the \( p8 \)-deficient mice; effects after TAC. A: Western blot analysis in LV from sham- or TAC-operated \( p8^{-/-} \) and \( p8^{+/+} \) mice. Equal amounts of total proteins were analyzed with antibodies against BAX, cleaved PARP (clvd PARP), and actin (ACT). The bands were quantified by densitometry and normalized to actin levels (BAX and PARP, \( *P < 0.05 \), \( n = 4 \) each). B: quantification of apoptosis after terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining of the hearts of \( p8^{+/+} \) and \( p8^{-/-} \) mice (\( n = 4 \) each). TUNEL-positive cardiomyocytes, identified by \( \alpha \)-sarcomeric actin staining, were quantified by examination of 8 random fields per heart. Values are means ± SE (\( *P < 0.05 \)). C: endothelial reticulum (ER) stress signaling at baseline and in failing hearts from \( p8^{-/-} \) and \( p8^{+/+} \) mice. Western blot analysis was carried out using anti-P-eIF2\( \alpha \), CHOP, GRP78, and ACT antibodies. Representative membranes are shown; no significant differences between \( p8^{-/-} \) and \( p8^{+/+} \) mice after TAC were found (\( n = 6 \) for each genotype, \( P > 0.05 \)).
significantly elevated levels of LC3-II and ATG12-5 in sham-operated p8−/− animals compared with p8+/+ mice, confirming a greater basal autophagy (Fig. 5A). Autophagy is increased in the pressure-overload model, and accordingly we detected upregulation of LC3-II and ATG12-5 levels after TAC in both p8-deficient and p8+/+ mice. LC3-II levels were significantly higher in p8−/− mice indicating a higher level of TAC-induced autophagy (Fig. 5A) (27). Consistent with this, SQSTM1 protein levels were significantly lower in the hearts of p8-deficient mice compared with p8+/+ controls, indicative of an increase in autophagy levels (39). We have previously reported that both mRNA and protein expression of proautophagic protein Bnip3 are higher in p8−/− mice manifesting a greater basal autophagy (Fig. 5A) (27). Consistent with this, Bnip3 levels were significantly lower in the hearts of sham-operated p8−/− mice developed similar p8+/+ and p8−/− mice with respect to mortality or cardiac enlargement after TAC, as assessed by heart weight-to-body ratio (n = 15 for each genotype). No significant differences were noted in total body, lung, and kidney weights (Fig. 6C). Neither hematoxylin/eosin nor trichrome staining of histocytic sections

*p8 loss is associated with cardiac dysfunction: effects after TAC. We have previously shown that p8 is induced in failing hearts of humans, as well as by stimuli associated with cardiac remodeling (14). Moreover, our previous data indicated that p8 genetic deletion is associated with a decrease in baseline cardiac function (25). We thus analyzed the possible functional consequences of p8 loss in vivo during heart failure induced by pressure overload. We performed echocardiographic recordings of p8+/+ and p8−/− mice and measured their LV wall dimensions and ventricle performance at baseline before operation and after 4 wk, the day before the animal was euthanized.

Echocardiographic analysis indicated that at baseline p8−/− mice manifest a modest, but significant, LV dilation, wall thinning, and lower fractional shortening compared with p8+/+ mice, a result consistent with Kong et al. (25). Four weeks after TAC, p8+/+ and p8−/− mice developed similar LV dilation and decreased LV function, as measured by end-systolic and end-diastolic dimensions and increased heart wall thickness. The decrease in fractional shortening after TAC was similar in p8-deficient and p8+/+ mice (Fig. 6, A and B, and Table 1). No significant differences were noted among p8+/+ and p8−/− mice with respect to mortality or cardiac enlargement after TAC, as assessed by heart weight-to-body ratio (n = 15 per group). No significant differences were noted in total body, lung, and kidney weights (Fig. 6C). Neither hematoxylin/eosin nor trichrome staining of histocytic sections
of liver and kidney revealed significant morphological differences or fibrotic lesions at baseline or following TAC between sham-operated and TAC mice (not shown). These results suggest either that 1) p8 function in the heart might be compensated by other signaling mechanisms in the process of hypertrophy and failure induced by pressure overload, 2) the TAC procedure produces a sufficiently large degree of cardiac stress as to squelch any differences between p8+/+ vs. −/− mice, or, most likely, 3) p8 performs multiple and complex functions in the heart, some of which are attenuated by TAC.

Table 1. Echocardiographic assessment of p8+/+ and p8−/− mice at baseline and 4 wk after TAC

<table>
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<tr>
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<th>Baseline</th>
<th>Sham</th>
<th>TAC</th>
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<tr>
<td></td>
<td>p8+/+</td>
<td>p8−/−</td>
<td>p8+/+</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>3.41 ± 0.6</td>
<td>3.67 ± 0.09</td>
<td>3.33 ± 0.1*</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>2.05 ± 0.07</td>
<td>2.47 ± 0.09</td>
<td>1.87 ± 0.13*</td>
</tr>
<tr>
<td>AWT, mm</td>
<td>0.90 ± 0.03</td>
<td>0.87 ± 0.02</td>
<td>0.85 ± 0.03</td>
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<tr>
<td>PWT, mm</td>
<td>0.86 ± 0.01</td>
<td>0.78 ± 0.02</td>
<td>0.83 ± 0.01</td>
</tr>
<tr>
<td>FS, %</td>
<td>40.19 ± 1.40</td>
<td>32.76 ± 1.42</td>
<td>44.3 ± 2.71†</td>
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LVEDD and LVESD, LV end-diastolic and end-systolic, respectively; AWT, anterior wall thickness; PWT, posterior wall thickness; FS, fractional shortening; TAC, transverse aortic constriction. *P < 0.05 for both LVEDD and LVESD; †P < 0.01 for FS. Baseline animals; n = 16 for p8+/+; n = 17 for p8−/−. Operated animals; n = 6 for sham and n = 10 for TAC, for each genotype.
of which essentially compensate for or cancel out the effects of others.

**DISCUSSION**

Cellular stress response pathways are controlled by a number of signaling molecules and transcriptional regulators, which by sensing and transmitting the information allow an adaptive response (28, 29). The basic helix-loop-helix protein p8 is a transcriptional regulator involved in multiple stress-activated cellular responses (12). Our previous work, using cultured cells, has suggested a function for p8 in key signaling events associated with heart failure (5, 12). The present study investigates p8 role in vivo in LV remodeling induced by pressure overload. Our results suggest a surprisingly complex, often contradictory function for p8 in cardiac physiology and pathology that could not have been predicted from the cell culture models we used previously.

**p8 deletion alters fibrotic gene expression.** The cardiac adaptive response to pathophysiological conditions is associated with changes in gene expression and reemployment of the developmental program. p8 mRNA levels are increased in p8+/+ failing hearts consequent to TAC, which is consistent with our previous studies of p8 regulation in primary rat cardiomyocytes by GPCR agonists and with its induction in failing hearts of humans (14).

Increased cardiac fibrosis compromises the contractile properties of the heart and is a hallmark of pathological cardiac hypertrophy and heart failure (34, 41, 49). Unstressed p8 knockout mice hearts have higher collagen gene expression and fibrotic areas, both of which likely contribute to the reported loss of function (25). Still, the effects we found of p8 disruption on collagen deposition and mmp expression are complex. Upon TAC, p8 absence impairs collagen deposition, raising the possibility that p8 might negatively regulate collagen degradation. Contrary to this, p8 disruption reduces MMP9 levels, which is consistent with our earlier findings from cultured cardiac fibroblasts. However, p8 disruption also increases TAC-induced mmp2, mmp3, and timp2 levels, suggesting that p8 indeed negatively regulates mmp expression and providing a potential explanation for the decreased levels of collagen in p8−/− hearts after TAC. In support of this idea, because of their multiplicity and substantial substrate overlap, MMPs and TIMPs are thought to have redundant functions (46).

Nevertheless, the role of p8 in inducing mmp9 expression may still be important to the pathogenesis of cardiac remodeling. The structural changes in myocardial ECM during LV remodeling requires proteolysis (49). The lower induction of mmp9 may explain why ECM degradation and, on the other hand, collagen deposition are attenuated after TAC in the absence of p8. Indeed, our results are also consistent with previous studies demonstrating a prominent role of MMP-9 in myocardial ECM remodeling in cardiac rupture, infarction, and LV remodeling after pressure overload (8, 18, 38). The enhanced expression of mmp2, mmp3, and the lower timp2 levels in the p8−/− mice after TAC are likely responsible for the apparent conundrum observed here, where p8 mice have a decreased mmp9 induction but less fibrosis after TAC (Fig. 7). The exact mechanism for this compensatory effect is unknown; it might be a direct promoter regulation by p8 or, more likely, an MMP9 substrate might regulate other mmps. In fact, in vivo compensatory upregulation of MMPs has been reported for mmp7 knockout mice and for mmp9 knockout mice both in an infarction and in a vein graft animal models (8, 50). Therefore, considering the lower mmp9 induction and the compensatory enhanced expression of other MMPs that leads to lower fibrosis after TAC, we might consider p8 absence as beneficial, at least with regard to ECM regulation.

**p8-deficient mice have increased autophagy and apoptosis at baseline.** Activation of autophagy plays a causative role in cardiac pathologies. Autophagic cell death as a cause of cellular degeneration has been demonstrated in patients with hypertrophied failing myocardium after ischemia or in dilated cardiomyopathy (6). Incomplete autophagy in lysosome-associated protein 2-deficient mice (Lamp2) causes autophagosome accumulation and cardiomyopathy, whereas atg5 deletion in adult mice leads to hypertrophy and contractile dysfunction (6, 39). Our previous work has shown that unstressed p8-deficient hearts have higher levels of autophagy, which is associated with pro-autophagic beclin 3 gene upregulation (25). In the current study we observed higher Bax protein in unstressed p8−/− hearts. Furthermore, PARP was cleaved, indicating caspase 3 activation, and the number of TUNEL-positive cardiomyocytes was higher in p8−/− mouse hearts compared with p8+/+ controls. All these results support a higher basal apoptosis in p8-deficient mice. Accordingly, we have shown that in vitro, p8 RNAi induces atg5-dependent autophagy and beclin3-dependent apoptosis (25). In vivo, the increase of basal apoptosis might be in part responsible for the wall thinning and the decreased heart function that we have reported and ultimately for the increased fibrosis at baseline. Alternatively, the increase in baseline LVEDD may be due to cardiomyocyte slippage due to the loss of interstitial collagen.

Autophagy represents a maladaptive response to hemodynamic stress (45, 54). TAC-induced upregulation might help eliminate misfolded proteins and damaged mitochondria, thus protecting from reactive oxygen species and apoptosis. However, the point at which autophagic activity becomes autophagic cell death has not yet been defined (29, 33, 45). In
p8−/− mice pressure overload-induced autophagy levels were superimposed on the basal levels. Accordingly, increases of LC3-II and Atg5–12 conjugation indicated autophagy activation after TAC and were higher in p8-deficient mice. The adapter sequestosome (SQSTM1) targets proteins for autophagosomal degradation and accumulates in cardiomyocytes when autophagy is suppressed (39). After TAC, p8-deficient mice had lower SQSTM1 levels, suggesting a higher autophagic turnover. Interestingly, we also found an increase in LC3-I levels after the TAC in both p8-deficient and p8+/+ mice. This might reflect either the increased activity of pro-autophagic transcription factors as FoxO3 or a protein accumulation due to a general delay in the autophagic flux after TAC (23, 53).

In the heart, accumulation of misfolded proteins after pressure overload induces ER stress (42), and p8 was shown to mediate ER stress-induced apoptosis by cannabinoid in cancer (4, 47). When comparing p8-deficient and p8+/+ mice, we found a similar activation of ER-stress markers in either unstressed hearts or after TAC. This suggests that, at least in the heart, p8 absence does not activate nor significantly blocks this cell-damaging signaling.

What is the role for p8 in the failing hearts? p8 deficiency causes upregulation of autophagy, but the role of this process in the heart is still controversial. In an ischemia-reperfusion (I/R) model, upregulation of autophagy can protect from cell death because either pharmacological inhibition or interference of atg5 functions will worsen the consequences. Conversely, the inability to upregulate autophagy during sustained pressure overload causes LV dilatation in mice lacking atg5 in their heart (39), while pressure overload enhances cardiac autophagy and remodeling in Beclin-overexpressing mice (9). Interestingly, much of the I/R damage seems mediated by Bnip3 upregulation, as its overexpression causes myocytes autophagy and cell death, while its silencing rescues both (16, 17).

The reduction in collagen accumulation and mmp induction have been proven to be beneficial (18); however, despite a significant decrease in mmp9 induction and a lower fibrosis, p8−/− mice develop decompensated heart failure and higher myocardial remodeling in their heart as depicted by a general delay in the autophagic flux after TAC. On the other hand, p8 expression might be regulated over the time in the failing heart and the consequences of p8 loss difficult to determine in a total body knockout animal. On this line, p8 protein levels are downregulated in the failing human hearts after therapeutic implant of a LV assisted device (14). All these considerations are in line with the multifaceted and controversial characteristics reported for this molecule so far (3, 12). Our findings support an in vivo role for p8 in ECM remodeling during the onset of heart failure, provide insights on the involvement of autophagy in the heart, and further our understanding of the molecular aspects of cardiovascular diseases.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


