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Phosphatase PTEN is Critically Involved in Post-Myocardial Infarction Remodeling through the Akt/Interleukin-10 Signaling Pathway

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Abstract

The inflammatory cytokines interleukin (IL)-10 and tumor necrosis factor (TNF)- α play an important role in left ventricular (LV) remodeling after myocardial infarction (MI). Phosphatase and tensin homologue deleted on chromosome ten (PTEN) inactivates protein kinase Akt and promotes cell death in the heart. However, it is not known whether PTEN promotes post-MI remodeling by regulating IL-10 and TNF- α . MI was induced in wildtype (WT) mice and *Pten* heterozygous mutant (HET) mice. *Pten* adenoviruses (*adPten*) or empty vectors (*adNull*) were injected into the peri-infarct area of WT mice. LV dilation was attenuated and fractional shortening was increased in HET mice compared with WT mice. Survival rate and fractional shortening were decreased in *adPten* mice compared with *adNull* mice. Leukocyte infiltration into the peri-infarct area was attenuated in HET mice and worsened in *adPten* mice. PTEN expression was upregulated in the infarcted heart of WT mice. Partial inactivation of PTEN increased production of IL-10 and decreased expression of TNF- α and matrix metalloproteinase (MMP)-2 and -9 after MI in HET mice. PTEN overexpression caused opposite effects in the infarcted heart. Moreover, in the infarcted heart of HET mice, Akt inhibition decreased Stat3 phosphorylation and IL-10 expression, and blockade of the IL-10 receptor increased TNF- α and MMP-2 expression. Both Akt inhibition and IL-10 receptor blockade abolished the attenuation of post-MI remodeling in HET mice. In conclusion, PTEN is critically involved in post-MI remodeling through the Akt/IL-10 signaling pathway. Therefore, targeting PTEN may be an effective approach to post-MI remodeling.

Keywords

Interleukin-10; tumor necrosis factor- α ; metalloproteinase; myocardial infarction; ventricular remodeling

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Conflict of interest None

Introduction

Myocardial infarction (MI) is a major cause of death in developed countries. Although early diagnosis and intervention has improved its survival rate, approximately one fifth of the patients will develop heart failure within 5 years [1]. Following MI, mechanical stress, hypoxia, and endogenous ligands from damaged tissues stimulate release of cytokines in the infarcted myocardium [9,14,25]. These cytokines initiate inflammation, induce expression of matrix metalloproteinase (MMP), and affect cell survival [13,45]. Severe inflammation, increased degradation of extracellular matrix, and massive loss of cardiomyocytes are related to adverse cardiac remodeling, which changes left ventricular geometry and impairs cardiac contraction, leading to heart failure [26,46,47].

Tumor necrosis factor- α (TNF- α) plays an important role in post-MI remodeling [25,44,46]. Two types of TNF- α receptors have been identified in the heart, TNF- α receptor type 1 (TNFR1) and TNF- α receptor type 2 (TNFR2). TNFR1 is critically involved in inflammation and cardiac remodeling after MI while TNFR2 is associated with cardioprotection [11,15,40]. Through TNFR1, TNF- α promotes myocyte apoptosis and inhibits cardiac function [13,25,51]. Loss of TNF- α decreases leukocyte infiltration into infarcted myocardium, inhibits the activities of MMP-2 and MMP-9, and attenuates collagen degradation and left ventricular rupture in mice [46]. TNF- α production can be inhibited in the infarcted heart by interleukin (IL)-10 [13,26].

IL-10 is a potent anti-inflammatory cytokine. Treatment with exogenous IL-10 inhibits apoptosis, production of inflammatory cytokines, and expression of MMP-9 in the infarcted heart [26]. The IL-10 receptor consists of at least two subunits R1 and R2. The R1 subunit binds to IL-10 with high affinity, and anti-IL-10R1 monoclonal antibodies can block all known IL-10 activities [30]. IL-10R1 has been identified in many types of cells including fibroblasts, whereas IL-10R2 is ubiquitously expressed [30]. IL-10 expression is upregulated in the heart after ischemia-reperfusion [14]. Emerging evidence suggests that IL-10 promotes cardiac repair after MI [4,26,48,53,54]. However, the molecular mechanisms that regulate IL-10 expression in the infarcted heart are poorly understood.

Phosphatase and tensin homologue deleted on chromosome ten (PTEN) antagonizes the actions of phosphatidylinositol (PI)-3-kinase (PI3K) by dephosphorylating PI-3,4,5-trisphosphate (PIP3) to PI-4,5-bisphosphate (PIP2) and Pi [42]. It promotes cell death and increases myocardial contractility in the heart [8,29,37,41]. Pharmacological inhibition of PTEN has been shown to enhance PI3K activity and attenuate cardiac injury after ischemia-reperfusion [24]. Moreover, cardiac-specific PTEN inactivation protects the heart from functional failure and fibrosis in a mouse model of pressure overload [31], and constitutive PI3K (p110 α) and chronic Akt activation attenuates post-MI remodeling [19,23]. PTEN has been shown to promote inflammatory responses in bacterial pneumonia but suppress them in bronchial asthma [22,27,39]. It is not clear whether PTEN is involved in post-MI remodeling.

In the present study, we have investigated the hypothesis that PTEN promotes post-MI remodeling by regulating IL-10 production. We manipulated PTEN activity by haploinsufficiency or overexpression of the PTEN gene in mice. We found that partial inactivation of PTEN increases Akt phosphorylation and IL-10 expression and decreases TNF- α , MMP-2, and MMP-9 expression in the infarcted heart of *Pten* heterozygous mutant (HET) mice, with attenuated inflammatory responses and myocardial remodeling. Conversely, we found that PTEN overexpression causes the opposite effects in mice. Moreover, in HET mice, we found that Akt inhibition decreased IL-10 expression, and that IL-10 receptor blockade increased TNF- α and MMP-2 expression and promoted post-MI

remodeling. Therefore, we conclude that PTEN regulates post-MI remodeling through the Akt/IL-10 signaling pathway.

Methods

Animals

All experiments were performed with age-matched male mice. At the time of the experiments, mice were 8-12 weeks old. HET mice, generated by Dr. Parsons's group, have been backcrossed to C57BL6 mice for at least 10 generations [35]. Littermate wildtype (WT) mice were used as controls. The generation of myocyte-specific *Pten* knockout (PKO) mice (*Pten^{loxP/loxP};ckm-Cre^{+/-}*) and control (CON) mice (*Pten^{loxP/loxP};ckm-Cre^{-/-}*) and their phenotype have been described previously [56]. PTEN overexpression by adenoviral gene transfer was also performed with WT mice. All procedures were approved by the Johns Hopkins University Institutional Animal Care and Use Committee (Protocol No. MO10M166) and conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Mouse MI model

MI was induced in mice as described previously [5,32]. Briefly, the chest was opened via a left thoracotomy. The left coronary artery was identified visually using a stereo microscope, and a 7-0 suture was placed around the artery 1-2 mm below the left auricle. The electrocardiogram (ECG) was monitored continuously. Permanent occlusion of the left coronary artery resulted from its ligation with the suture. Myocardial ischemia was confirmed by pallor in heart color and ST-segment elevation. No ligation was applied to sham-treated animals. The chest was closed with a 6-0 silk suture. Once spontaneous respiration resumed, the endotracheal tube was removed. The animals were monitored until fully conscious. After they were returned to their cages, standard chow and water were provided. HET mice were randomly divided into three groups. Akt Inhibitor (AI) [2.5 mg/kg], anti-IL-10R1 antibody (RA) (5µg in 200 µl saline) (BioLegend, San Diego, CA, USA), or normal mouse IgG was separately given to one group of mice via intraperitoneal injection, once a day, for 5 days, starting before the mice were returned to the animal facility. Antibody can enter the blood through lymphatic drainage after intraperitoneal injection [2]. In a separate experiment, WT and HET mice were observed for 4 weeks after surgery.

Measurement of myocardial infarct size and changes in LV geometry

Myocardial infarct size was measured in the heart 24 h or 5 days after MI as we described previously [5]. Since no living tissue was detected in the infarcted area, Evans blue was not used. After freezing, hearts were transected into five pieces, followed by incubation with 1.5% triphenyltetrazolium chloride (TTC) for 15 min. Cardiac sections were photographed and weighed. The infarcted area and noninfarcted areas of each section were measured by computerized planimetry (Imagine J; National Institutes of Health, Bethesda, MD, USA). Infarct size was calculated as a percentage of LV = total weight of white tissue to weight of LV. To determine changes in LV geometry after 5-day MI, the ratio of the thickness of interventricular septum (Se) to the diameter of LV section was measured. Because of the distribution of the septum in the heart, only the middle three sections were assessed. Their averages are presented as a percentage of Se/LV. Myocardial infarct size in the heart 4 weeks after MI was measured as previously described with modifications [34]. Hearts from WT and HET mice were collected. After incubation with 1.5% TTC for 15 min, scars stained white. The infarcted LV wall was identified as thin white tissue. To consider the variation induced by sectioning, we measured the length of infarcted LV wall and the entire

endocardial circumference of each section. Infarct size was calculated as a percentage of total infarcted LV wall length to the sum of circumferences.

PTEN overexpression by adenoviral gene transfer

Adenoviral gene transfer into the peri-infarct area was performed as described previously [57]. After the left coronary artery was permanently occluded, a dose of 10^8 plaque-forming units (p.f.u)/30 μ l of *Pten* adenoviruses (*adPten*) containing human *Pten* expressing sequence or empty vectors (*adNull*) (Vector Biolabs, Philadelphia, PA, USA) was injected into the left ventricular wall at three equidistant points by a 30G syringe. Five days later, hearts were isolated for protein analysis and measurement of mononuclear cell infiltration.

Echocardiography

In vivo cardiac function was assessed by transthoracic echocardiography (Acuson Sequoia C256, 13MHz transducer; Siemens) in conscious mice, as described previously [50]. The mouse heart was imaged in the two-dimensional mode in the parasternal short axis at a papillary level. From this view, LV chamber dimensions were measured. Fractional shortening (FS) was calculated from left ventricular (LV) end-diastolic diameter (EDD) and end-systolic diameter (ESD). M-mode EDD and ESD were averaged from 3-5 beats. Studies and analyses were performed by investigators blinded to genotype or treatments.

Histological studies

Hearts were fixed with 10% buffered formalin, embedded in paraffin, and sectioned at 6 μ m, as described previously [56]. Three or more sections per heart were stained with hematoxylin and eosin (H&E) or Masson's trichrome. Mononuclear cell infiltration was assessed in cardiac sections by H&E staining. Mononuclear cells were counted in eight randomly selected fields (400x) from the peri-infarct area under a microscope. The numbers of mononuclear cells were averaged from two peri-infarct areas of each cardiac section. Each group comprised at least three hearts, and 48 fields were counted in each group. Fibrosis was assessed in the peri-infarct area by Masson's trichrome staining. Fibrotic blue area and whole peri-infarct area were measured using computerized planimetry (Image J). The fibrotic area was presented as a percentage of fibrotic area to the peri-infarct area. A total of 18 fields (100x) were counted in each group. The observer was blinded to the origin of the cardiac sections.

Immunohistochemistry

CD45⁺ cells were detected in paraffin-embedded cardiac sections as described previously [56]. After heating at 61°C, cardiac sections were deparaffinized in xylene and rehydrated. The endogenous peroxidase activity was quenched with 3% (v/v) H₂O₂ in methanol. Sections were incubated with primary anti-CD45 antibody (1:200) (BioLegend, San Diego, CA, USA), and then with a peroxidase-conjugated secondary antibody. Nuclei were counterstained with Mayer's hematoxylin. CD45⁺ cells were counted in the same way as mononuclear cells.

Preparation of isolated perfused hearts

Isolated hearts were prepared as described previously [5]. Briefly, the chest was opened, the heart was excised, and the ascending aorta was cannulated with a blunt needle. The heart was perfused at a constant pressure of 100 cm H₂O with modified Krebs-Henseleit (KH) buffer (in mmol/L: glucose 17, NaCl 120, NaHCO₃ 25, CaCl₂ 2.5, KCl 5.9, MgSO₄ 1.2, and EDTA 0.5), which was maintained at 37°C and bubbled continuously with a mixture of 95% O₂ and 5% CO₂. After 15 min of perfusion, hearts were homogenized in the lysis buffer for protein analysis.

Western blot analysis

At the end of experiments, hearts were collected and stored at -80°C . Before protein analysis, frozen hearts were crosscut into four sections. The peri-infarct myocardium was isolated from the lower middle section under a stereo microscope. Isolated myocardium was homogenized in lysate buffer (in mmol/L: Tris 20, pH 7.5, NaCl 150, EDTA 1, EGTA 1, PMSF 1, Na_3VO_4 1, 1% Triton). Proteins were separated on a precast NuPAGE Bis-Tris gel (Invitrogen) and transferred to a nitrocellulose membrane. Proteins were detected by using primary antibodies, followed by horseradish peroxidase-conjugated secondary antibody and enhanced chemiluminescence. Antibodies against PTEN, Akt, p-Akt, Erk1/2, p-Erk1/2, p-c-Jun, c-Jun, p-Stat3, Stat3, $\text{I}\kappa\text{B}\alpha$, MMP-2, and MMP-9 were purchased from Cell Signaling Technology (Danvers, MA, USA). Antibodies against TNF- α and IL-10 were from BD Biosciences (San Jose, CA, USA).

Statistical analysis

Data are presented as mean $\pm$ standard error of the mean. The difference among groups was analyzed by using Student's t-test or two-way ANOVA with the Tukey's post hoc test. Survival curves were analyzed by the log-rank test. Differences were considered significant if $p < 0.05$.

Results

Partial inactivation of PTEN attenuates LV dilation and dysfunction in HET mice after MI

We measured PTEN protein levels in WT and HET mouse hearts under basal conditions. PTEN protein levels were decreased by approximately 50% in HET mice compared with WT mice (Fig. 1a). There was no significant difference in heart size and weight (Fig. 1a and Table S1). To determine whether partial inactivation of PTEN attenuates cardiac remodeling following MI, we induced MI in WT and HET mice, and measured myocardial infarct size in these mice 24 h later. No significant difference was found between these two groups (Fig. 1a). Echocardiography was performed at 2 and 4 weeks post-MI. LV dilation became deteriorated in WT mice over time, with an increase in LVESD and LVEDD (Table S1). Compared with WT mice, HET mice had decreased LV dilation and heart weight (Table S1). FS was improved in HET mice compared with WT mice at each time point post-MI (Fig. 1b). There was no significant decrease in FS from 2 to 4 weeks in HET mice; however, the decrease from 2 to 4 WS was significant in WT mice (Fig. 1b). By the end of the experiments, three WT mice died [WT vs. HET, 3/15 (20%) vs. 1/17 (6%)]. To assess the degree of post-MI remodeling, we measured infarct size in the heart. Infarct size was significantly decreased in HET mice compared with WT mice at 4 weeks post-MI (Fig. 1c). We also measured fibrotic area in the peri-infarct area at 4 weeks post-MI. Fibrotic area was significantly decreased in HET mice compared with WT mice (Fig. 1d). Taken together, these results suggest that post-MI remodeling is attenuated in HET mice after MI.

PTEN overexpression exacerbates cardiac dysfunction and increases mortality in *adPten* mice

To determine whether PTEN overexpression increases cardiac injury, we injected *Pten* adenoviruses or empty vectors into the peri-infarct area. Survival rate was decreased in mice overexpressing PTEN at 5 days post-MI [Fig. 2a]. Autopsy indicated that the mice died of LV rupture (Fig. 2b). At 5 days post-MI, echocardiography was performed on the remaining mice. FS was deteriorated in *adPten* mice compared with *adNull* mice (Fig. 2c). Although LVEDD and LVESD were not different between these two groups (Table S2), infarct size was increased in *adPten* mice (Fig. 2d). These results suggest that PTEN overexpression promotes cardiac dysfunction and cell death and increases mortality.

Leukocyte infiltration into the peri-infarct area is attenuated by partial inactivation of PTEN and worsened by PTEN overexpression

Since the degree of inflammation is related to left ventricular rupture and cardiac remodeling, we wondered whether PTEN regulates inflammatory responses. We measured infiltration of leukocytes in cardiac sections at day 5 post-MI by using H&E staining and CD45 immunohistochemistry. Mononuclear cells in the peri-infarct area were decreased in HET mice compared with WT mice (Fig. 3a). PTEN overexpression increased the number of mononuclear cells in the peri-infarct area (Fig. 3a). Similarly, CD45⁺ cells were decreased in HET mice (Fig. 3b), increased in *adPten* mice (Fig. 3b).

PTEN suppresses Akt phosphorylation and IL-10 expression and promotes TNF- α expression in the infarcted heart

IL-10 and TNF- α are important inflammatory cytokines. We first determined whether PTEN regulates IL-10 and TNF- α expression in the heart independent of inflammation. Hearts from PKO and CON mice were isolated and perfused with KH buffer to wash out leukocytes. Levels of PTEN, p-Akt, Akt, IL-10, and TNF- α proteins were measured in heart lysates. Myocyte-specific *Pten* deficiency in mice decreased PTEN protein levels and increased Akt phosphorylation in the heart, suggesting inactivation of PTEN in cardiac muscle (Fig. 4a). Furthermore, IL-10 protein levels were increased and TNF- α protein levels were decreased in PKO hearts (Fig. 4b). These results suggest that PTEN inactivation in cardiomyocytes promotes IL-10 production and inhibits TNF- α expression in the heart.

To determine the effects of partial inactivation of PTEN on Akt phosphorylation and IL-10 and TNF- α expression in sham-treated and infarcted mice, we analyzed PTEN, p-Akt, Akt, IL-10, and TNF- α protein levels in the hearts of sham-treated WT (WTs) and HET (HETs) mice, and infarcted WT and HET mice at 5 days after surgery. PTEN protein levels were upregulated in WT mice after MI, but not in HET mice (Fig. 5a). Consistent with PTEN protein levels, Akt phosphorylation was higher after MI in HET mice than in WT mice (Fig. 5a). AI and RA blocked Akt phosphorylation (Fig. 5a). Total Akt levels were not different among these groups. IL-10 protein levels were increased in HETs mice compared with WTs mice (Fig. 5b). The difference in IL-10 protein levels was further increased between HET and WT mice after MI. AI inhibited IL-10 protein expression in HET mice. Interestingly, RA also blocked IL-10 production in HET mice (Fig. 5b). TNF- α protein levels were decreased in HETs mice (Fig. 5b). After MI, the differences in TNF- α protein levels were further increased between HET and WT mice. Both AI and RA upregulated TNF- α protein expression (Fig. 5b). These results suggest that PTEN protein expression is upregulated in the infarcted myocardium of WT mice, and that the *Pten* heterozygous mutant mice are resistant to the upregulation of PTEN, leading to an increase in Akt phosphorylation and IL-10 production and a decrease in TNF- α expression in the heart after MI. Moreover, these results indicate that Akt signaling is required for IL-10 production in HET mice, and that its blockade leads to increased expression of TNF- α . Interestingly, these results also suggest that activation of IL-10 receptors is required for Akt phosphorylation, and that blocking IL-10 receptors could impair IL-10 protein expression and increase TNF- α production in HET mice.

To determine whether PTEN overexpression causes an opposite effect on Akt phosphorylation and IL-10/TNF- α expression, we analyzed levels of these proteins in the peri-infarct area of WT mice at 5 days after injecting *Pten* adenoviruses or empty vectors. PTEN expression was increased in *adPten* mice (Fig. 5c). PTEN overexpression decreased Akt phosphorylation and IL-10 expression, and increased TNF- α expression (Fig. 5c and 5d). These results support the notion that PTEN inhibits Akt phosphorylation and IL-10 expression and stimulates TNF- α expression in the infarcted heart.

PTEN negatively regulates Stat3 phosphorylation in the infarcted heart

Stat3 has been implicated in the regulation of IL-10 protein expression [3,46,47]. We analyzed p-Stat3 and total Stat3 in the infarcted heart at 5 days post-MI. Stat3 phosphorylation was increased in HET mice compared with WT mice (Fig. 6a). Stat3 phosphorylation in HET mice was inhibited by AI and RA (Fig. 6a). In HETs and WT mice, there was no difference in Stat3 phosphorylation. PTEN overexpression decreased Stat3 phosphorylation in *adPten* mice (Fig. 6b). These results suggest that PTEN activity is negatively related to Stat3 phosphorylation in the infarcted heart, and that both Akt signaling and IL-10 signaling are required for Stat3 activation in HET mice.

Nuclear factor κ B (NF- κ B) plays a central role in TNF- α expression in the heart after MI [13,25]. We measured I κ B α , p-p65, and p65 protein levels in the infarcted heart at 5 days post-MI, with GAPDH as a control protein. I κ B α protein levels were increased in HET mice (Fig. S1a). AI and RA decreased I κ B α protein levels (Fig. S1a). Consistent with the changes in I κ B α protein levels, p65 phosphorylation was decreased in HET mice (Fig. S1a). AI and RA increased p65 phosphorylation in HET mice (Fig. S1a). Moreover, in *adPten* mice, I κ B α protein levels were decreased (Fig. S1b) and p65 phosphorylation was increased (Fig. S1b). These results suggest that PTEN promotes NF- κ B activation in the infarcted heart.

It has been reported that activator protein (AP)-1 and extracellular signal regulated kinases (ERK) 1/2 are involved in the regulation of IL-10 and TNF- α expression [16,38]. We measured p-c-Jun, c-Jun, p-Erk1/2, and Erk1/2 in the infarcted heart at 5 days post-MI. c-Jun phosphorylation was decreased in HET mice after MI (Fig. S2a). AI and RA increased c-Jun phosphorylation in HET mice (Fig. S2a). PTEN overexpression also increased c-Jun phosphorylation in *adPten* mice (Fig. S2b). Similarly, p-Erk1/2 was decreased in HET mice after MI (Fig. S3a). AI and RA increased Erk1/2 phosphorylation in HET mice (Fig. S3a). PTEN overexpression increased Erk1/2 phosphorylation in *adPten* mice (Fig. S3b). These results suggest that PTEN has stimulatory effects on AP-1 and Erk1/2 activity in the infarcted heart.

PTEN regulates MMP-2 expression through the IL-10 signaling pathway

MMP-9 and MMP-2 play important roles in regulating degradation of extracellular matrix and cardiac remodeling [45]. Thus we examined whether PTEN regulates MMP-9 and MMP-2 expression in the infarcted heart through the Akt/IL-10 signaling pathway. MMP-9 protein levels were decreased in the peri-infarct area in HET mice compared with WT mice (Fig. 7a). AI increased MMP-9 protein levels in HET mice, but RA did not significantly increase MMP-9 protein levels in HET mice (Fig. 7a). In contrast, MMP-2 protein levels were decreased in HET mice (Fig. 7a). AI increased MMP-2 protein levels in HET mice, and RA had a similar effect on MMP-2 protein levels in HET mice (Fig. 7a). PTEN overexpression increased MMP-9 and MMP-2 protein levels in the peri-infarct area in *adPten* mice compared with *adNull* mice (Fig. 7b). These results suggest that PTEN promotes MMP-2 and MMP-9 expression in the infarcted heart through the Akt signaling pathway, and that partial inactivation of PTEN inhibits MMP-2 protein expression through the IL-10 signaling pathway.

Partial inactivation of PTEN attenuates post-MI remodeling through the Akt/IL-10 signaling pathway

AI and RA increase TNF- α production and MMP expression in HET mice. To determine whether Akt inhibition and blockade of IL-10 receptors affect post-MI remodeling in HET mice, we performed echocardiography on the mice at 5 days post-MI. After MI, LV contraction was impaired in all mice (Fig. 8a). Compared with WT mice, HET mice had decreased LVESD (Fig. 8a and 8b). AI and RA abolished this effect (Fig 8a and 8b).

Consistent with these changes, FS and EF were increased in HET mice (Fig. 8c). These effects were completely blocked by AI or RA (Fig. 8c). Nonetheless, infarct size was not significantly decreased in HET mice (Fig. 8d). Mortality was not different among these groups (one mouse from HET+RA group died). Since cardiomyocytes continue to be lost in the heart after MI, resulting in LV dilation [45], we determined the changes in LV geometry by measuring the ratio of the thickness of interventricular septum to the diameter of LV section. The ratio was significantly increased in HET mice (Fig. 8d). AI and RA blocked this effect (Fig. 8d). These results were in agreement with the findings from echocardiography, suggesting that the attenuation of post-MI remodeling in HET mice is mediated through the Akt/IL-10 signaling pathway.

Discussion

In the present study, we report that PTEN regulates post-MI remodeling through the Akt/IL-10 signaling pathway. First, we demonstrate that post-MI remodeling is negatively related to PTEN activity in the infarcted heart. Second, myocyte-specific PTEN inactivation increases IL-10 and decreases TNF- α expression in the heart independent of inflammation. Third, PTEN expression is increased in the infarcted heart, and it negatively regulates IL-10 protein levels under basal conditions and post-MI through the Akt signaling pathway. Finally, endogenous IL-10 has inhibitory effects on TNF- α and MMP-2 protein expression and post-MI remodeling. Taken together, our findings suggest that PTEN plays an important role in post-MI remodeling by regulating the Akt/IL-10 signaling pathway (Fig. 8e).

PTEN is critically involved in post-MI remodeling

Cardiac-specific PTEN deletion or pharmacological inhibition of PTEN has been shown to inhibit apoptosis and limit infarct size after ischemia-reperfusion [24,37]. Post-MI remodeling is largely dependent on infarct size [13,36,45]. However, partial inactivation of PTEN did not protect the heart from ischemia-reperfusion injury in mice with *Pten* haploinsufficiency, while Akt phosphorylation was increased [42]. In the present study, we used the same strain of mice and did not find a decrease in infarct size at 24 h and 5 days post-MI, suggesting that the attenuation of post-MI remodeling did not result from decreased infarct size in HET mice. However, decreased PTEN activity was associated with subsequent reduced extracellular matrix remodeling and improved LV function. The regulatory role of PTEN in post-MI remodeling was further supported by our findings in mice overexpressing PTEN, which exhibited increased cardiac dysfunction and mortality. Heart failure and LV rupture might result from massive cell death and degradation of extracellular matrix [25,49]. Moreover, it has been reported that PI3K/Akt signaling is required for angiogenesis and cardiac repair after MI, and that this signaling pathway was impaired in the infarcted heart (28,43). We demonstrated that PTEN expression is upregulated in WT mice after MI, suggesting that increased PTEN activity may inhibit the activation of the PI3K/Akt signaling pathway in the infarcted heart. In sharp contrast with the changes in WT mice, PTEN expression was not increased in HET mice after MI, leading to sustained activation of Akt and attenuation of post-MI remodeling. Furthermore, in the present study, we demonstrated that PTEN promotes post-MI remodeling by regulating inflammation, cytokine production, and MMP expression.

PTEN promotes post-MI inflammation

PTEN has been shown to regulate inflammation in many pathological conditions [22,27,39], but its role in post-MI inflammation has not been studied previously. In the present study, we showed that PTEN activity is negatively related to infiltration of leukocytes, as measured by morphology and expression of CD45⁺. Myeloid PTEN inactivation decreases neutrophil infiltration and increases phagocytosis of macrophages in a mouse model of bacterial

pneumonia, leading to attenuated pulmonary inflammation [39]. A similar protective mechanism may play a role in post-MI remodeling of HET mice since the neutrophils and macrophages have *Pten* haploinsufficiency. Moreover, increased basal IL-10 in the infarcted myocardium may inhibit inflammation by decreasing production of reactive oxygen species (ROS) [10,13]. Increased leukocyte infiltration in mice overexpressing PTEN may result from decreased IL-10 and increased ROS levels. Previously, we reported that PTEN promotes ROS production in the heart [57]. Although PTEN inactivation attenuates inflammation in the infarcted heart, it is not clear whether PTEN inactivation affects its initiation and/or resolution. Further studies will be needed to determine the effect of PTEN inactivation on the time-course of inflammation.

PTEN is a key regulator of IL-10 and TNF- α expression in the infarcted heart

After MI, the ischemic myocardium releases inflammatory factors to recruit leukocytes into the infarcted heart [9,13,20,52]. TNF- α is one of the early cytokines released by the infarcted heart. TNF- α expression is induced in the infarcted heart following ROS production and activation of Toll-like receptors [13,25]. In the present study, we have demonstrated that PTEN inactivation increases IL-10 production and decreases TNF- α expression. Since this regulatory mechanism is present in isolated perfused hearts and sham-treated HET mice, it appears that IL-10 and TNF- α expression are dependent on PTEN activity in the myocardium, not on inflammatory cells. TNF- α can be released by cardiac cells, but the relevant source of IL-10 in the heart is less clear [25]. Fibroblasts and endothelial cells potentially produce IL-10 [13,30]. What types of cells produce IL-10 in HET mice and in myocyte-specific PTEN knockout mice requires further investigation. Moreover, we showed that PTEN overexpression decreases IL-10 production and increases TNF- α expression in the infarcted heart, providing additional evidence that PTEN is an upstream regulator of both inflammatory cytokines. Since blood levels of IL-10 and TNF- α have been indicated as independent prognostic determinants in ischemic heart disease [18,55], this regulatory mechanism of IL-10 and TNF- α may have important therapeutic implications.

In the present study, we delineate a signaling cascade, PTEN/Akt/IL-10/TNF- α , in the infarcted heart. It has been reported that IL-10 expression is regulated through the Akt signaling pathway in leukocytes [33]. Constitutive activation of Akt or PTEN inactivation promoted lipopolysaccharide-induced IL-10 production in macrophages, and PI3K inhibition decreased Akt phosphorylation and IL-10 expression and increased TNF- α production [17,33,39]. We demonstrated that the Akt/IL-10/TNF- α signaling cascade was regulated in myocyte-specific PTEN knockout mice, HET mice, and adPten mice in a PTEN-dependent manner. Moreover, Akt inhibition decreased IL-10 expression and increased TNF- α production in the infarcted heart of HET mice. Similarly, IL-10 receptor blockade abolished the inhibitory effect of endogenous IL-10 on production of TNF- α . Therefore, the signaling cascade PTEN/Akt/IL-10/TNF- α is active in the infarcted heart. IL-10 production may be subjected to positive feedback regulation or autoinduction in the infarcted heart, as blockade of the IL-10 receptor inhibited Akt phosphorylation and IL-10 expression in HET mice. This observation is consistent with a previous report, in which IL-10 induced its own expression in macrophages [46]. Moreover, we demonstrated that IL-10 expression is associated with Stat3 phosphorylation in the infarcted heart, but not under basal conditions, suggesting that IL-10 expression may be regulated by Stat3-dependent and independent mechanisms [30]. Further studies are needed to determine the specific transcription factor that induces expression of IL-10 in the infarcted heart. Conversely, IL-10 can mediate protective effects by activating Akt and Stat3 in the infarcted heart [26,54]. Surprisingly, IL-10 receptor blockade completely inhibited Akt and Stat3 phosphorylation, suggesting that IL-10 is an essential survival factor in the infarcted heart.

Thus, increased IL-10 may promote cardiac repair in HET mice. It has been reported that IL-10 represses the cytokine mRNA-stabilizing protein HuR in the infarcted heart, leading to decreased TNF- α expression [26]. Here, we demonstrated that IL-10 may also inhibit TNF- α expression at a transcriptional level because IL-10 receptor blockade increased the phosphorylation of p65, a major subunit of NF- κ B, and decreased I- κ B α protein levels. Activation of NF- κ B has been reported to promote TNF- α expression and post-MI remodeling [7,13,21]. Therefore, IL-10 may tightly control TNF- α expression in the infarcted heart through transcriptional and post-transcriptional mechanisms. We also demonstrated that Erk1/2 phosphorylation and c-Jun phosphorylation were associated with TNF- α expression in the infarcted heart. Since Erk1/2 and AP-1 have been shown to promote cell survival and IL-10 expression [6,12,38], further studies are required to determine their roles in regulating TNF- α expression and cell death.

PTEN regulates MMP-2 expression in the infarcted heart through the IL-10 signaling pathway

Matrix metalloproteinases degrade extracellular proteins and promote post-MI remodeling [45]. We demonstrated that IL-10 protein levels are negatively related to MMP-2 and MMP-9 protein levels in the infarcted heart of HET mice and *adPten* mice. It has been reported that TNF- α deficiency causes decreased expression of MMP-2 and MMP-9 in the infarcted heart [49]. Thus, partial inactivation of PTEN may attenuate degradation of extracellular matrix by regulating the IL-10/TNF- α /MMP pathways. Moreover, we demonstrated that the blockade of IL-10 receptors increased MMP-2 expression in HET mice, suggesting that IL-10 signaling is required for the inhibition of MMP-2. In contrast to our results, treatment with exogenous IL-10 has been shown to inhibit MMP-9 gene expression and enzymatic activity in the infarcted heart [26]. Despite the difference in MMP isoforms, both studies suggest that IL-10 may attenuate post-MI remodeling by decreasing MMP. In the infarcted heart, an IL-10-independent pathway may suppress MMP-9 protein expression in HET mice.

Conclusions

We demonstrate that partial inactivation of PTEN in HET mice increases IL-10 production and decreases TNF- α and MMP-2 expression in the infarcted heart with attenuated inflammation and LV remodeling. We also show that PTEN overexpression causes the opposite effects on IL-10/TNF- α and MMP-2 protein expression and increases leukocyte infiltration and mortality in *adPten* mice. Our further studies suggest that partial inactivation of PTEN increases IL-10 expression by activating the Akt signaling pathway in the infarcted heart, and that IL-10 inhibits TNF- α and MMP-2 production. Therefore, PTEN regulates the Akt/IL-10/TNF- α and MMP-2 signaling cascades and promotes post-MI remodeling in the infarcted heart. Targeting PTEN may be an effective approach to enhance cardiac repair following MI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AI	Akt Inhibitor
AP-1	activator protein-1
EF	ejection fraction
Erk1/2	extracellular signal regulated kinases ½
FS	fractional shortening
IL-10	interleukin 10
LVEDD	left ventricular end-diastolic diameter
MI	myocardial infarction
MMP	matrix metalloproteinase
NF-κB	nuclear factor kappa B
PI3K	phosphatidylinositol-3-kinase
PTEN	phosphatase and tensin homologue deleted on chromosome ten
RA	anti-IL-10 receptor antibody
TNF-α	tumor necrosis factor-α
TTC	triphenyltetrazolium chloride

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Fig. 1.

Partial inactivation of PTEN attenuates post-MI remodeling a PTEN protein levels (upper panel) and infarct size (lower and right panels) in the heart from WT and HET mice 24 h after MI. Each Western blot and heart is representative of at least four mice. The five sections from one infarcted heart were presented. b Fractional shortening (FS) was increased in HET mice at each time point post-MI. *: $p < 0.01$ vs. WT. c Infarct size was decreased in HET mice at 4 weeks after MI. Representative cardiac sections from at least 12 WT and HET mice are shown. *: $p < 0.05$ vs. WT. d Fibrotic area was decreased in the peri-infarct area of HET mice at 4 week after MI. *: $p < 0.01$ vs. WT. N=3.

Fig. 2.

PTEN overexpression increases mortality and decreases cardiac function a survival curves of *adPten* mice and *adNull* mice. *Pten* adenoviruses (*adPten*) or empty vectors (*adNull*) were injected into the peri-infarct area after MI. Survival was decreased in *adPten* mice (6/23) compared with *adNull* mice (8/11). *: $p < 0.05$ vs. *adNull*. b Representative hearts are shown from 11 *adNull* mice and 23 *adPten* mice at 5 days post-MI. LV rupture (yellow arrow) was

located in the apex (upper panel). PTEN protein levels were increased in *Pten* adenovirus-treated hearts (lower panel). Scale: 1 mm. c FS was decreased in *adPten* mice. *: $p < 0.01$ vs. *adNull*. d Infarct size was increased in *adPten* mice. *: $p < 0.05$ vs. *adNull*. N=6-8.

Fig. 3. PTEN regulates leukocyte infiltration in the infarcted heart. Hearts were collected and sectioned at 5 days post-MI. Mononuclear cells (a) and CD45⁺ cells (b) were counted in the peri-infarct area. Mononuclear cells and CD45⁺ cells (red arrows) were decreased in HET mice and increased in *adPten* mice. Cardiac muscle area was increased in HET mice and decreased in *adPten* mice. Each image is representative from at least three hearts per group. *: p<0.01 vs. WT. †: p<0.05 vs. *adNull*.

Fig. 4.

PTEN inactivation in cardiac myocytes increases IL-10 and decreases TNF- α protein levels in the heart. Hearts isolated from myocyte-specific *Pten* knockout (PKO) mice and control (CON) mice were perfused with KH buffer for 15 min. a Akt phosphorylation was increased in PKO mice. b IL-10 production was increased and TNF- α expression was decreased in PKO mice. *: $p < 0.01$ vs. CON. N=3.

Fig. 5.

PTEN regulates IL-10 and TNF- α protein levels in the infarcted heart. Hearts were collected from sham-treated mice (WTs and HETs) and MI mice at 5 days after surgery. Cardiac tissue from the peri-infarct area and corresponding area in sham mice was analyzed for protein levels. a PTEN protein expression and Akt phosphorylation in the infarcted heart. *: $p < 0.01$ vs. WT; †: $p > 0.01$ vs. WT, ‡: $p < 0.05$ vs. HET. N=3-5. b Protein expression of IL-10 and TNF- α in the infarcted heart. Akt Inhibitor (AI) and IL-10 receptor antibody (RA) blocked IL-10 expression and increased TNF- α production in HET mice. *: $p < 0.05$ vs. WTs. †: $p < 0.01$ vs. WT. ‡: $p < 0.01$ vs. HET. #: $p < 0.05$ vs. HET. N=3-5. c PTEN expression and Akt phosphorylation in *adPten* and *adNull* mice. *: $p < 0.01$ vs. *adNull*. N=3-4. d Protein expression of IL-10 and TNF- α in *adPten* mice and *adNull* mice. *: $p < 0.01$ vs. *adNull*. N=3-4.

Fig. 6. PTEN inhibits Stat3 phosphorylation in the infarcted heart a Stat3 phosphorylation was increased after MI in HET mice. AI and RA abolished this effect. *: $p < 0.01$ vs. WT, †: $p < 0.01$ vs. HET, ‡: $p < 0.01$ vs. HET. N=3. b Stat3 phosphorylation was decreased in the infarcted heart of *adPten* mice. #: $p < 0.01$ vs. *adNull*. N=3.

Fig. 7.

PTEN increases MMP-2 and MMP-9 expression in the infarcted heart. Hearts were isolated from sham-treated and MI animals at 5 days after surgery. MMP-2 and MMP-9 protein levels were analyzed in the peri-infarct area. a MMP-2 and MMP-9 protein levels were decreased in HET mice. AI increased MMP-2 and MMP-9 protein levels in HET mice. RA increased MMP-2 but not MMP-9 protein levels in HET mice. *: $p < 0.01$ vs. WT. †: $p < 0.01$ vs. HET. ‡: $p > 0.05$ vs. HET. #: $p < 0.01$ vs. HET. N=3-5. b MMP-2 and MMP-9 protein levels were increased in *adPten* mice. *: $p < 0.01$ vs. *adNull*. N=3-4.

Fig. 8.

Akt inhibition and IL-10 receptor blockade impair cardiac repair in HET mice a Representative images of echocardiography from at least six mice within each group. Arrows indicate LV end-diastolic diameter (EDD). b LVEDD and end-systolic diameter (ESD). ESD was decreased in HET mice compared with WT mice. AI and RA increased ESD in HET mice. *: $p < 0.05$ vs. WT. †: $p < 0.01$ vs. HET. ‡: $p < 0.01$ vs. HET. N=6-7. c LV fractional shortening (FS) and ejection fraction (EF) were increased in HET mice compared with WT mice. AI and RA inhibited the protective effects in HET mice. *: $p < 0.001$ vs. WT. †: $p < 0.001$ vs. HET. ‡: $p < 0.001$ vs. HET. N=6-7. d LV infarct size and geometry after MI. Infarct size was not significantly different between groups (left panel). The ratio of the

thickness of interventricular septum (Se) and the diameter of LV section was increased in HET mice (right panel). AI and RA blocked this effect. *: $p < 0.01$ vs. WT or AI or RA. e A scheme suggesting how PTEN promotes post-MI remodeling. PTEN is induced in the infarcted heart. Increased PTEN suppresses elevation of PIP3 and Akt activity. Akt inactivation decreases induction of IL-10. Decreased activation of IL-10 receptors leads to increased expression of TNF- α and MMP-2 and loss of cardiomyocytes, resulting in adverse LV remodeling. AI, Akt Inhibitor; RA, anti-IL-10 receptor antibody.