Loss of PTEN attenuates the development of pathological hypertrophy and heart failure in response to biomechanical stress

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Aims The maladaptive response to biomechanical stress is a fundamental response in heart disease. Loss of the 3'-lipid phosphatase, phosphatase and tensin homolog deleted on chromosome ten (PTEN), is associated with increased phosphorylation of Akt/protein kinase B and glycogen synthase kinase-β. We hypothesize that these key changes will halt the development of pathological hypertrophy and the progression to heart failure in response to pressure overload.

Methods and results In mice, muscle-specific knockout of PTEN, mckCRE-PTENlox/lox (PTEN KO), resulted in basal hypertrophy and mild reduction in left ventricular (LV) systolic function. Male mice were subjected to aortic banding (AB) or sham operation. In contrast to mckCRE-PTEN+/+ control mice, pressure overload in PTEN KO mice resulted in reduced pathological hypertrophy, less interstitial fibrosis, and reduced apoptosis with a marked preservation of LV function. Western blot analysis of mitogen-activated protein kinase (MAPK) signalling showed equivalent phosphorylation of extracellular signal-regulated kinase (ERK)1 and ERK2 with markedly reduced phosphorylation of jun N-terminal kinase (JNK)1 and JNK2, and p38 in PTEN KO mice subjected to AB. Loss of PTEN was associated with increased expression of the proangiogenic factors, vascular endothelial growth factor-A and angiopoietin-2, with preservation of the myocardial capillary density in response to pressure overload. Moreover, banded PTEN KO mice maintained the expression of several key metabolic genes that are known to be dysregulated in heart failure. In contrast, a subpressor dose of the G protein-coupled receptor (GPCR) agonist angiotensin II (Ang II) leads to increased pathological hypertrophy and MAPK activation in PTEN KO mice.

Conclusion Loss of PTEN prevents the development of maladaptive ventricular remodelling with preservation of angiogenesis and metabolic gene expression in response to pressure overload but not in response to the GPCR agonist, Ang II. Inhibition of PTEN signalling in the heart may represent a novel approach to slow the progression of heart failure in response to pathological biomechanical stress.

KEYWORDS Heart failure; Hypertrophy; Angiogenesis; Angiotensin II; Signalling

1. Introduction

The phosphatidylinositol-3 kinase (PI3K) system has a fundamental role in cell signalling and is involved in cell survival and growth.1,2 In the heart, both class IA and IB PI3Ks are expressed, with class IA isoforms playing a key role in mediating physiological hypertrophy and basal heart size3,4, while the class IB isoform PI3Kγ controls G protein-coupled receptor (GPCR) signalling and myocardial contractility.2,3,5,6 The phosphatase and tensin homolog deleted on chromosome ten (PTEN) is a membrane-bound lipid phosphatase and functions essentially as a negative regulator of PI3K signalling in multiple systems.2,7 In the cardiovascular system, PTEN is a negative regulator of PI3Kα and PI3Kγ isoforms in cardiomyocytes and endothelial cells.2,3,8 In the heart, loss of PTEN leads to increased phosphorylation of Akt/protein kinase B (PKB), glycogen synthase kinase-3β (GSK3β) and 70-kDa S6 kinase resulting in physiological-like hypertrophy,3 ischaemic preconditioning9, and stimulation of L-type Ca2+ channel current.10 PTEN also

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plays a critical role in normal cardiovascular morphogenesis and post-natal angiogenesis. Diabetes and isoproterenol infusion increased myocardial PTEN protein levels, while PTEN expression is reduced in a murine model of human hypertrophic cardiomyopathy, suggesting that PTEN may play a key role in heart disease.

Mechanotransduction and the ability to respond to biomechanical stress plays a fundamental role in cardiac (and vascular) function and involves interaction between extracellular matrix and intracellular cytoskeletal proteins via cell adhesion complexes, which are modulated by both class IA and IB PI3Ks. Muscle cells respond to mechanical stretch stimuli by triggering downstream signals for cardiomyocyte growth and survival, and defects in the cardiomyocyte stretch sensor machinery can lead to dilated cardiomyopathy and heart failure in humans. While PI3K and lipid phosphatases can modulate cytoskeletal interactions, mechanical stretch increases Akt/PKB and GSK3β activity in both cardiomyocytes and Langendorff-perfused hearts. Enhanced Akt/PKB signalling has been shown to mediate physiological hypertrophy while antagonizing pathological stress and to prevent heart failure. In this study, we investigated the role of PTEN in the cardiac response to biomechanical stress using the aortic banding (AB) model. Our results provide a crucial link between PTEN and the cellular responses to biomechanical stress in which the loss of PTEN was associated with a wide variety of adaptive responses necessary to resist the progression of maladaptive ventricular remodelling and progression to heart failure.

2. Methods

2.1 Experimental animals

The muscle-specific conditional PTEN knockout (PTEN KO) (mckCRE-PTEN/foxfox) mouse has been previously described. Only male littermate mice of 10–11 weeks of age were used as controls which were mckCRE-PTEN/fox (CONT). All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), Institutional Guidelines and the Canadian Council on Animal Care.

2.2 Aortic banding and angiotensin II infusion

The AB protocol was used as a means of pressure overload and biomechanical stress as previously described. Briefly, the descending aortic arch was accessed via a left thoracotomy and the descending thoracic aorta was surgically constricted to generate a transtentorial pressure gradient of 50–60 mmHg. Male CONT or PTEN KO mice at 10–11 weeks of age underwent sham operation (SHAM) or AB and were followed for 9–12 weeks. In a separate group of animals, subpressor dose of angiotensin II (ang II) (0.15 mg/kg/day) or saline (vehicle) was infused using subcutaneously implanted micro-osmotic pumps (model 2004; Durect, Cupertino, CA) into male CONT and PTEN KO mice at 10–11 weeks of age for 2 weeks as previously described.

2.3 Histomorphometry and TUNEL assay

For heart morphometry, hearts were arrested with KCl, fixed with 10% buffered formalin, and embedded in paraffin. Trichrome and picro-sirius red (PSR) staining and visualization were carried out as previously described. Trichrome-stained sections were used for measurement of cardiomyocyte cross-sectional area. Myocardial interstitial fibrosis is shown as collagen volume fraction obtained from PSR-stained sections using two-photon confocal microscopy (Zeiss LSM 510 META NLO) and quantified using the Image Pro Plus (Media Cybernetics, Crofton, MA) computer image analysis software. Myocardial tissue was also stained using a rat anti-mouse monoclonal antibody (Pharmingen) against CD31 (also known as platelet-endothelial cell adhesion molecule-1, PECAM-1) and capillary counts were obtained from 5 sections from each heart. In situ DNA fragmentation was labeled using the terminal deoxynucleotidyl transferase biotin-dUTP nick end labelling (TUNEL) assay (ApopTag Plus kit) (Oncor, Gaithersburg, MD) and positive TUNEL cells were obtained from five sections from each heart.

2.4 Echocardiography and hemodynamic measurements

Echocardiographic assessments and invasive hemodynamic measurements were carried out as previously described in mice anesthetized with 0.75% isoflurane/oxygen. Briefly, trans-thoracic echocardiography was carried out using an Acuson Sequoia C256 equipped with a 15-MHz linear transducer (Version 4.0, Acuson Corporation, Mountain View, California) and hemodynamic measurements were made using a 1.4 French Millar catheter (Millar Inc., Houston) which was advanced into the proximal aorta via the right carotid artery and then through the aortic valve into the left ventricle.

2.5 Real-time TaqMan polymerase chain reaction and Western blot analyses

RNA levels for the indicated genes were quantified by real-time TaqMan reverse transcription–polymerase chain reaction (RT–PCR) with 18S rRNA used as an endogenous control as previously described (see Table 1 for primers and probes). Western blot analysis of total and phosphorylated ERK1 (44 kDa), ERK2 (42 kDa), jun N-terminal kinase 1 (JNK1) (46 kDa), JNK2 (54 kDa), and p38MAPK (38 kDa) was carried out using commercial antibodies from Cell Signaling Inc., as previously described.

2.6 Statistical analysis

The effects of genotype and AB were evaluated using ANOVA followed by the Student Neuman–Keuls test for multiple comparison testing. Comparison testing in Figure 1 was made using the Mann–Whitney U non-parametric test. All statistical analyses were performed with the SPSS software (version 10.1). Data are presented as mean ± SD; n refers to the sample size.

3. Results

3.1 Loss of PTEN prevents the development of heart failure in response to pressure overload

Given that PTEN is a negative regulator of PI3K system and a loss of PTEN is associated with chronic Akt/PKB activation in the heart, we hypothesized that loss of PTEN will result in a protection from biomechanical stress. Consistent with our previous results, transthoracic echocardiographic and hemodynamic assessment confirmed a mild reduction in systolic function in sham-operated PTEN KO mice (Table 2). The CONT mice showed a predictable and marked ventricular dilation (Figure 1A) and reduction in systolic function following 9 and 12 weeks of AB (Table 2). In contrast, in PTEN KO mice, there was a minimal ventricular dilation and loss of heart function in response to 9 and 12 weeks of AB (Figure 1A and Table 2). Given the baseline changes in myocardial contractility, we next analysed the relative changes in left ventricular (LV) size and function. Banded CONT mice showed a drastic increase in LV end diastolic...
### Table 1: Real-time polymerase chain reaction TaqMan primers, probes, and TaqMan assays

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Probe</th>
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<tr>
<td>ANF</td>
<td>5'-GGA GGA GAA GAT GCC GGT AGA fnb</td>
<td>5'-GCT TCA TCT TCA GTC TCA CTC A-3'</td>
<td>5'-FAM-TGA GGT CAT GCC CCC GCA GG-TAMRA-3'</td>
</tr>
<tr>
<td>BNP</td>
<td>5'-CTG CTG GAG GTC ATA AGA GA-3'</td>
<td>5'-TGC CCA AAG CAG CTT GAG AT-3'</td>
<td>5'-FAM-CTC AAG GCA CCC TCC GGG-TAMRA-3'</td>
</tr>
<tr>
<td>β-MHC</td>
<td>5'-GTG CCA AGG GCC TGA ATG A-3'</td>
<td>5'-GCA AAG GCT CCA GCT GTG A-3'</td>
<td>5'-FAM-ATC TTT TAC CCA GCT CTA A-TAMRA3'</td>
</tr>
<tr>
<td>VEGF-A</td>
<td>5'-GAG CGG AGA AAG CAT TGG TTT G-3'</td>
<td>5'-GCA ACG GCA GTG TCT GTG-3'</td>
<td>5'-FAM-CTT GCA ACG CGA TCT GTG-TAMRA-3'</td>
</tr>
<tr>
<td>TGFβ1</td>
<td>5'-CCT GCA AGG ATC TGC ACA GAC-3'</td>
<td>5'-ACA GGA TGT GGC CAC GAC TAC-3'</td>
<td>5'-FAM-CTG GTG AAA CGG AAG CGC ATC GAA-TAMRA-3'</td>
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**Gene Descriptions:**
- ANF: atrial natriuretic factor
- BNP: B-type natriuretic peptide
- β-MHC: beta-myosin heavy chain
- VEGF-A: vascular endothelial growth factor-A
- TGFβ1: transforming growth factor beta1

**Assay ID:**
- Ang-2: Assay ID: Mm00545823_m1
- IGFBP-5: Assay ID: Mm01253846_g1
- PPARα: Assay ID: Mm01301752_m1
- MCAD: Assay ID: Mm00431615_g1
- LCAD: Assay ID: Mm01256456_m1

**Notes:**
- Assay ID refers to the commercial assay available from Applied Biosystems.

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**Figure 1:** Loss of PTEN slows ventricular dilation and the deterioration in left ventricular function in response to nine weeks of pressure overload. (A) Representative cross-sectional views of hearts from sham-operated and aortic banded CONT and PTEN KO mice; scale bar represents 5 mm. (B–E) Preservation of LV dimension and function based on changes in LVEDD (B), FS (C), +dP/dt (D) and −dP/dt (E). LVEDD, left ventricular end diastolic dimension; FS, fractional shortening; +dP/dt, maximum; −dP/dt, minimum change in the first derivative of the LV pressure. Open bars = CONT, grey bars = PTEN KO. n = 8 for each group; *P < 0.01 compared with the CONT group. CONT, mckCRE-PTEN+/+ mice; PTEN KO, mckCRE-PTENflx/flx mice.
dimension (Figure 1B) and reductions in fractional shortening (Figure 1C) and hemodynamic measures of myocardial contractility, +dP/dt (Figure 1D) and −dP/dt (Figure 1E), indicative of heart failure. In contrast, banded PTEN KO mice showed minimal ventricular dilation and loss of myocardial contractility (Table 2 and Figure 1). These data show that loss of myocardial PTEN results in marked and persistent protection against pathological biomechanical stress.

3.2 Reduced pathological hypertrophy and maladaptive ventricular remodelling in banded PTEN KO mice

A hallmark response to pressure overload is the development of pathological hypertrophy. Examination of the cardiomyocyte cross-sectional area (Figure 2A and B) and LV weight (Figure 3A) confirmed that PTEN KO mice have a basal hypertrophy as previously reported.5 While the littermate

<table>
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<th>Table 2</th>
<th>Echocardiographic and hemodynamic parameters following aortic banding</th>
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<tr>
<td>CONT + SHAM</td>
<td>PTEN KO + SHAM</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>561 ± 8</td>
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<tr>
<td>PWT (mm)</td>
<td>0.71 ± 0.06</td>
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<tr>
<td>LVEDD (mm)</td>
<td>3.81 ± 0.11</td>
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<tr>
<td>LVESD (mm)</td>
<td>1.71 ± 0.09</td>
</tr>
<tr>
<td>FS (%)</td>
<td>55.1 ± 2.1</td>
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<tr>
<td>VCF, (circ/s)</td>
<td>11.2 ± 0.32</td>
</tr>
<tr>
<td>+dP/dtmax (mmHg/s)</td>
<td>0.10 ± 0.05</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>1.71 ± 0.09</td>
</tr>
<tr>
<td>LVMD (g)</td>
<td>1.71 ± 0.09</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>66.1 ± 10.2</td>
</tr>
<tr>
<td>EF (%)</td>
<td>66.1 ± 10.2</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>5.13 ± 1.84</td>
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<tr>
<td>LWV/BW (mg/g)</td>
<td>3.75 ± 0.13</td>
</tr>
<tr>
<td>HW/BW (mg/g)</td>
<td>4.91 ± 0.12</td>
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*P < 0.05 compared with CONT + SHAM group; **P < 0.01 compared with CONT + SHAM group; ***P < 0.001 compared with CONT + SHAM group.

Figure 2 Reduced myocardial hypertrophy, interstitial fibrosis and apoptosis in PTEN KO mice in response to nine weeks of pressure overload. (A and B) Representative trichrome stained sections (A) showing less interstitial fibrosis (blue staining) and reduced cardiomyocyte cross-sectional area (MCSA) in banded PTEN KO mice. MCSA is quantified and shown in (B). (C and D) Representative Picrosirius Red (PSR) stained sections imaged using confocal microscopy (C) showing markedly reduced interstitial fibrosis in banded PTEN KO mice. Collagen volume fraction (CVF) is quantified and shown in (D). (E and F) Representative sections stained for TUNEL positive cells (E) showing reduced apoptosis in banded PTEN KO mice which is quantified and shown in (F). Scale bar represents 50 μM, n = 4 for each group; *P < 0.05 compared with the CONT + SHAM group; **P < 0.01 compared with all other groups. CONT, mckCRE-PTENflox/flox mice; PTEN KO, mckCRE-PTENflox/flox mice.
CONT mice developed marked hypertrophy following 9 weeks of AB, banded PTEN KO mice developed significantly less hypertrophy based on the cardiomyocyte cross-sectional area (Figure 2A and B) and the morphometric assessment of LV hypertrophy, LV weight corrected by tibial length (Figure 3A). Consistent with the differential hypertrophic response, myocardial interstitial fibrosis (Figure 2C and D) and degree of apoptosis as determined using the TUNEL assay were significantly attenuated in banded PTEN KO mice (Figure 2E and F). The molecular profiling of the hypertrophic response confirmed a drastic reduction in the increased expression of atrial natriuretic factor (ANF) (Figure 3B) and B-type natriuretic peptide (BNP) (Figure 3C) without a differential response in beta-myosin heavy chain (βMHC) expression in banded PTEN KO mice (Figure 3D).

The development of pathological hypertrophy coupled with interstitial fibrosis and increased apoptosis is closely linked to the phosphorylation and activation of the mitogen-activated protein kinase (MAPK) pathways. As such, we examined the phosphorylation status of the different common MAPK pathways using Western blot analysis. Consistent with less adverse ventricular remodelling in banded PTEN KO mice, phosphorylation of the adaptive ERK1 and 2 pathways were equivalent (Figure 4A–C), while there was a drastically lowered phosphorylation of the maladaptive JNK1 and 2 (Figure 4D–F) and p38 MAPK pathways (Figure 4G and H) compared with banded CONT mice. These data show that PTEN-deficient hearts are resistant to maladaptive ventricular remodelling characterized by less pathological hypertrophy, interstitial fibrosis, and apoptosis in association with reduced activation (phosphorylation) of JNK1 and 2 and p38 MAPK pathways.

### 3.3 Preservation of angiogenesis and metabolic gene expression in pressure overload PTEN-deficient heart

Given the critical role of Akt/PKB and PTEN in coronary angiogenesis and cardiac metabolism, we...
hypothesized that loss of PTEN may enhance the early angiogenesis response and prevent the detrimental changes in expression of cardiac metabolic genes. Coronary angiogenesis was assessed by staining for CD31 (PECAM-1), a marker for coronary endothelial cells. While AB in CONT mice lead to a marked decrease in myocardial capillary density characteristic of pathological hypertrophy (Figure 5A and B), coronary angiogenesis was maintained in banded PTEN KO mice despite the increase in ventricular mass (Figure 5A and B). The ability of early activation of Akt/PKB to prevent heart failure has been linked to up-regulation of expression of the pro-angiogenic factors, vascular endothelial growth factor-A (VEGF-A) and angiopoietin-2 (Ang-2). Indeed, in PTEN KO mice the basal expression of VEGF-A (Figure 5C) and Ang-2 (Figure 5D) were both increased without changes in the expression of TGF\(\beta\)1 (Figure 5E) and VEGFR1 (Flt1) (data not shown) which is consistent with recent data showing that early activation of Akt/PKB is critical in mediating coronary angiogenesis in cardiac hypertrophy. While the banded CONT mice showed a predictable increase in VEGF-A (Figure 5C), Ang-2 (Figure 5D), and TGF\(\beta\)1 (Figure 5E), lack of an increased expression of TGF\(\beta\)1 in banded PTEN KO mice is consistent with the absence of increased interstitial fibrosis (see Figure 2). These results further implicate vascular rarefaction as a key determinant of the functional cardiac response to biomechanical stress.

Pathological ventricular hypertrophy and heart failure is associated with alteration in expression of genes controlling glucose and fatty acid metabolism. Activation of Akt/PKB changes the expression of metabolic genes which may enhance the ability of the heart to cope with pathological stimuli. We therefore assess the expression of several key cardiac metabolic genes. Insulin-like growth factor binding protein-5 (IGFBP-5), an important target of insulin signalling via PI3K\(\alpha\), is increased at baseline and in response to banding in PTEN KO mice (Figure 5F). Decreased expression of the mitochondrial genes, medium-chain acyl CoA dehydrogenase (MCAD) and long-chain acyl CoA dehydrogenase (LCAD) and the mitochondrial transcriptional activator, peroxisome proliferator-activated receptor alpha (PPAR\(\alpha\)), are reliable markers of advanced heart failure. Indeed, pressure overload in CONT mice lead to a predictable decreased expression of PPAR\(\alpha\) (Figure 5G) and MCAD (Figure 5H). In contrast, in PTEN KO mice the expression of PPAR\(\alpha\) (Figure 5G), MCAD (Figure 5H), and LCAD (Figure 5I) was up-regulated at baseline and in response to pressure overload which may minimize the mitochondrial dysfunction and altered fatty acid metabolism seen in heart disease. We conclude that in the setting of pathological hypertrophy, loss of PTEN preserves coronary angiogenesis and metabolic gene expression thereby preventing the development of heart failure.

3.4 Enhanced pathological hypertrophy in response to subpressor dose of angiotensin II in PTEN KO mice

The protective effects seen in the PTEN KO mice in response to pressure overload may represent a unique response to biomechanical stress rather than to GPCR signalling. Indeed, PTEN is a master negative regulator of GPCR signalling (and tyrosine receptor kinase signalling) by controlling the intracellular PI3K-mediated effects. As such, the
pathological effects of GPCR signalling may be exacerbated in the absence of PTEN. In response to 2 weeks of subpressor dose of Ang II (0.15 mg/kg/day), PTEN KO mice showed a greater hypertrophic response compared with CONT mice based on morphometric assessment of hypertrophy, LV weight corrected for tibial length (Figure 6A), and analysis of hypertrophy markers, ANF (Figure 6B) and BNP (Figure 6C). We next examined the phosphorylation status of the different MAPK pathways using Western blot analysis. Consistent with enhanced pathological hypertrophy in PTEN KO mice treated with Ang II, phosphorylation of the adaptive ERK1 and 2 pathways were equivalent (Figure 6D–F), while there was a drastic increase in phosphorylation of the maladaptive JNK1 and 2 (Figure 6G–I) and p38 MAPK pathways (Figure 6J and K) in PTEN KO compared with CONT mice. These results suggest that in contrast to biomechanical stress, loss of PTEN facilitates GPCR-mediated pathological hypertrophy in association with greater activation of the maladaptive MAPK pathways, JNK1/2 and p38.

4. Discussion

The response to biomechanical stress is a fundamental characteristic response in heart disease. Our data provide definitive evidence that loss of PTEN plays a key role in the suppression of the development of pathological hypertrophy with preservation of myocardial function in response to biomechanical stress. Changes in PTEN expression in experimental and human models of heart disease clearly supports a key role of PTEN in heart disease.11–13 In the cardiovascular system, PTEN is a negative regulator of PI3Kα and PI3Kγ isoforms in cardiomyocytes, vascular smooth muscle, and endothelial cells.2 Loss of PTEN is associated with increased phosphorylation and activation of Akt/PKB by various extracellular stimuli depending on PI3K activity and regulates multiple aspects of cellular functions including survival, growth, angiogenesis, and metabolism.2,22 Tyrosine receptor kinase agonists, such as insulin and insulin-like growth factor-1 (IGF-1) activate Akt/PKB via PI3Kα leading to physiological hypertrophy2, while loss of PTEN leads to the development of physiological hypertrophy in the setting of activated Akt/PKB and its downstream pathways.2 Interestingly, lower serum levels of IGF-1 have been linked to increased incidence of heart failure in humans.35 Importantly, we have shown that muscle-specific loss of PTEN results in reduced basal myocardial contractility which is associated with a relative resistance to pressure overload-induced heart failure. Consistent with a critical
role for Akt/PKB in cell survival, loss or gain of PTEN activity leads to reduced or enhanced apoptosis, respectively. Increased expression of PTEN increases apoptosis in neonatal cardiomyocytes. The increased basal phosphorylation of Akt/PKB and GSK coupled with increased expression of IGFBP-5 are likely to have contributed to the decreased apoptosis seen in banded PTEN KO mice. The importance of the MAPK proteins in mediating the activation and maintenance of cardiac hypertrophy has been demonstrated in animal models and these pathways play a central role in the progression to failure in the human heart. The reduced activation of the JNK1 and 2, and p38 MAPK pathways with decreased expression of TGFβ1 mitigated against the development of pathological hypertrophy and interstitial fibrosis in PTEN KO mice. Increased basal Ang-2 and VEGF levels in the setting of basal ‘physiological’ hypertrophy in PTEN KO mice allowed for normal angiogenesis to maintain coronary capillary density, an important component of physiological hypertrophy. Under conditions of increased cardiac growth, a
prompt increase in Ang-2 and VEGF-A levels are required to stimulate coronary angiogenesis in order to maintain the balance between myocardial mass and coronary blood flow, thereby preserving cardiac function. The ability of Akt/PKB to maintain coronary angiogenesis and vascular homeostasis in the setting of cardiac hypertrophy is dependent on the mTOR pathway. Enhanced expression of VEGF-A and Ang-2 in PTEN KO mice was clearly associated with a preservation of coronary angiogenesis in response to hypertrophic stress. The differential expression of pro-angiogenic factors in cardiomyocyte vs. endothelial-specific knockout of PTEN suggest that cardiomyocyte Akt/PKB signalling is the primary determinant of VEGF-A and Ang-2 expression.

The activation of Akt/PKB pathway is known to mediate important metabolic effects which may exert beneficial effects in the setting of pathological hypertrophy. Both PPARα, the principal transcriptional regulator of cardiac fatty acid beta-oxidation, and MCAD are down-regulated in pathological hypertrophy leading to reduced fatty acid utilization and cardiac dysfunction. Loss of PTEN prevented the down-regulation of both PPARα and MCAD which may have minimize the metabolic dysfunction following pressure overload-induced heart disease. Given the similarity of PTEN signalling in heart and skeletal muscle, increase insulin-stimulated glucose uptake in the heart as shown in the skeletal muscle of PTEN KO mice may also mediate protective effects in certain forms of heart disease.

In contrast to pressure overload-induced hypertrophy, we showed that loss of PTEN enhanced the hypertrophic response to the GPCR agonist, Ang II. These results were predictable based on the ability of PTEN to function as a negative regulator of PI3K signalling. Indeed, Ang II activates PI3Kγ via its GPCR, AT1 receptor, and PI3Kα via a transactivation mechanism involving the release of growth factors. Our results are consistent with changes seen in cultured cardiomyocytes whereby Ang II-induced hypertrophy was blocked by PTEN overexpression and PTEN can directly inhibit Ang II-mediated synthesis of inflammatory mediators. We conclude that the reduced pathological hypertrophy seen in banded PTEN KO mice is a unique response to biomechanical stress and that the pathways mediating pressure overload-induced hypertrophy can be dissociated from those mediating hypertrophy in response to GPCR agonists. Our results are consistent with recent findings in other murine models such as the PI3Kγ Ko mice which showed reduced GPCR-induced hypertrophy but not in response to pressure overload, while the melusin KO mice developed enhanced eccentric remodelling and hypertrophy in response to biomechanical stress without a differential response to GPCR stimulation.

Despite the reduction in basal myocardial contractility, PTEN KO mice were resistant to pressure overload suggesting that basal myocardial contractility and the adaptive response to biomechanical stress can be uncoupled. Similarly, PI3Kγ and phospholamban KO mice which have increased basal myocardial contractility were clearly more susceptible to biomechanical stress compared to normal wild-type mice. The uncoupling between basal myocardial contractility and the ability to tolerate pathological biomechanical stress strongly suggest that there are unique determinants of the cardiac response to pressure overload (see Figure 7). Indeed, given the important role of lipid phosphatases including PTEN as key regulators of cell-matrix adhesion and migration, we cannot rule out an important effect of cell adhesion on the observed phenotype in the pressure overload PTEN-deficient hearts. PTEN regulates several distinct pathways involved in remodelling of cell adhesion complexes and cytoskeletal organization (see Figure 7). For example, PTEN directly dephosphorylates Shc and focal adhesion kinase which in turn modulates cell adhesion complexes and the intracellular actin cytoskeleton. Independent of Akt/PKB activity.

Our results suggest that inhibition of PTEN may help to prevent adverse myocardial remodelling in the setting of pressure overload such as hypertension and aortic valvular stenosis. Similarly, inhibition of calcineurin, a calcium-calmodulin-regulated serine-threonine phosphatase, and mTOR are associated with reduced pathological hypertrophy in response to pressure overload. Collectively, these results support the notion that certain key signalling pathways can be successfully targeted to prevent the progression of adverse ventricular remodelling and heart disease. More definitive studies will be needed to establish whether loss of PTEN can be beneficial in already hypertrophied and diseased hearts.

Acknowledgements

G.Y.O. is a recipient of a Postdoctoral Fellowship from the Canadian Institute for Health Research, Heart and Stroke Foundation of Canada and from the TACTICS program.

Conflict of interest: none declared.

Funding

Canadian Institute for Health Research (Grant MOP84279 to Z.K.) and the EuGeneHeart (EU 6th Framework programs), the Austrian National Bank and IMBA (J.M.P).

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