Cardiac regulation by phosphoinositide 3-kinases and PTEN

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The diverse effects mediated by PI3K/PTEN (phosphoinositide 3-kinase/phosphatase and tensin homologue deleted on chromosome 10) signalling in the heart clearly support an important biological and pathophysiological role for this signalling cascade. PI3Ks are a family of evolutionarily conserved lipid kinases that mediate many cellular responses to physiological and pathophysiological stimuli. Class I PI3K can be activated by either receptor tyrosine kinase/cytokine receptor activation (class IA) or G-protein-coupled receptors (class IB), leading to the generation of phosphatidyl inositol (3,4,5)P3 and recruitment and activation of Akt/protein kinase B, 3'-phosphoinositide-dependent kinase-1 (PDK1), or monomeric G-proteins, and phosphorylation of a wide range of downstream targets including glycosylation synthase kinase 3β (GSK3β), mTOR (mammalian target of rapamycin), p70 S6 kinase, endothelial nitric oxide synthase, and several anti-apoptotic effectors. Class IA PI3Ks mediate distinct phenotypes in the heart under negative control by the 3'-lipid phosphatase PTEN, which dephosphorylates PtdIns(3,4,5)P3 to generate PtdIns(4,5)P2. PI3Ks are expressed in cardiomyocytes, fibroblasts, endothelial cells, and vascular smooth muscle cells, where they modulate cell survival, hypertrophy, contractility, metabolism, and mechanotransduction. The PI3K/PTEN signalling pathways are involved in a wide variety of diseases including myocardial hypertrophy and contractility, heart failure, and preconditioning. In this review, we discuss the signalling pathways mediated by PI3K class I isoforms and PTEN and their roles in cardiac structure and function.

1. Structure and function of phosphoinositide 3-kinases

Phosphatidylinositols (PtdIns) are phospholipids that comprise a phosphoglyceride esterified to the hydroxyl group of an inositol ring which can be phosphorylated and dephosphorylated at various positions by lipid kinases and phosphatases, respectively.1-4 Phosphatidylinositol-4,5-phosphate [PtdIns(4,5)P2] is the main precursor for PtdIns(3,4,5)P3 biosynthesis by class I PI3K enzymes which are activated by a range of agonists.1,3,5,6 PtdIns(3,4,5)P3 is degraded by the lipid phosphatases, PTEN (phosphatase and tensin homologue deleted on chromosome 10) and SHIP2 (and SHIP1), functioning effectively as 3'- and 5'-phosphatases to generate PtdIns(4,5)P2 and PtdIns(3,4)P2, respectively.3,4,7 Phosphoinositide 3-kinases (PI3Ks) and PTEN are the primary regulators of PtdIns(3,4,5)P3 and PtdIns(3,4)P2 [and PtdIns(4,5)P2] levels, which in turn mediate selective targeting and activation of many downstream effectors by binding to selective domains such as the pleckstrin homology (PH) domain.3,4,8 The PH domain has a unique three-dimensional structure with an affinity for PtdIns(3,4,5)P3 that is two orders of magnitude greater than for other phosphorylated PtdIns.3,8 PI3Ks are classified on the basis of their substrate specificity, mode of activation, and molecular structure.1,3,5,6 Class IA PI3Ks are heterodimeric enzymes composed of a regulatory subunit (accessory) subunit coupled to a catalytic subunit.3,5,6 PI3K catalytic subunits include p110α, p110β, and p110δ, whereas the class IB catalytic subunit is p110γ,3,6; however, more recent data suggest that p110β may also effectively couple to G-protein-coupled receptors (GPCRs) and therefore could function as a class IB catalytic subunit.9,10 The class IA PI3K adapter (accessory) subunits are encoded by three genes which generate highly homologous products, p85α (85 kDa) (pik3r1), p85β (85 kDa) (pik3r2), and p55γ (55 kDa) (pik3r3), with p85α being the predominant isoform in the heart.1,3,6,11,12 The class IB PI3K adapter (accessory) subunit (p101) is tightly associated with the catalytic subunit p110γ.3,6,13

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Class I PI3Ks phosphorylate Ptdlin, Ptdlin(4)P and Ptdlin(4,5)P2, to form Ptdlin(3)P, Ptdlin(3,4)P2, and Ptdlin(3,4,5)P3 in vitro, respectively, whereas phosphorylation of Ptdlin(4,5)P2 to form Ptdlin(3,4,5)P3 is the predominant in vivo response to agonist stimulation. All catalytic subunits of class I PI3Ks contain four conserved homology domains (HDs) (from N- to C-terminus): Ras-binding domain (RBD or HR4), C2 domain (HR3), PIK (helical) domain (HR2), and the catalytic kinase domain (HR1). The RBD binds the monomeric G-protein, Ras, whereas the C2 and PIK (helical) domains are involved in phospholipid membrane binding and protein/protein interaction, respectively. The p110γ subunit also contains two Gβγ interaction sites, located in the N-terminal p101-binding and C-terminal catalytic domains which are critical for GPCR-mediated signalling. The p110α and β isoforms are expressed in the heart and vasculature, whereas p110γ is expressed in cardiomyocytes, cardiac fibroblasts, vascular smooth muscle cells (VSMCs), and endothelial cells.

Adaptor (accessory) proteins have an expression pattern which mirrors their corresponding catalytic subunits and bind their catalytic subunits via p110-binding regions. Class Iα adaptor p110-binding domains are flanked by two Src-homology 2 (SH2) domains which upon receptor stimulation bind to phosphotyrosines in the activated receptor allowing the class Iα PI3K heterodimer to phosphorylate lipid substrate(s) in membranes. Phosphotyrosine docking sites are created by autophosphorylation in response to receptor tyrosine kinase (RTK) or Janus-activated kinase activation. In contrast to class Iα PI3Ks, p110γ binds Gβγ subunits following GPCR stimulation, leading to translocation from the cytosol to the cell membrane and kinase activation. This activation of p110γ by GPCR stimulation is amplified by simultaneous binding of Gβγ subunits to the p101 adaptor subunit. Catalytic activities of both class Iα and Iγ PI3Ks are further increased by conformational changes and membrane reorientation of the catalytic domains induced by interaction between the RBD and guanosine triphosphate-bound monomeric G-protein Ras. In addition to lipid kinase activity, class I PI3K catalytic subunits have intrinsic protein kinase activity in vitro, which can lead to autophosphorylation of both catalytic and adaptor subunits as well as activation of MAPK.

2. Structure and function of PTEN: a 3′ lipid phosphatase and a negative regulator of phosphoinositide 3-kinases

PTEN is a 3′-lipid phosphatase and is known as ‘mutated in multiple advanced cancers’ (MMAC1) and TGFβ-regulated and epithelial cell-enriched phosphatase (TEP1). Mammanian PTEN has a molecular weight of 40–50 kDa and comprises an N-terminal PIP2-binding polybasic tail, an N-terminal phosphate domain, a C2 domain, and a C-terminal tail region that contains the PSD-95/Dlg/ZO-1 (PDZ) HD as well as multiple phosphorylation sites. Dephosphorylation of focal adhesion kinase and the adaptor protein Shc by PTEN leads to inhibition of cell migration, suggesting that PTEN may exhibit some protein phosphatase activity. However, PTEN functions predominantly as a 3′-lipid phosphatase in vivo owing to its unique catalytic site allowing accommodation of the bulky acidic Ptdlin(3,4,5)P3 substrate. The main physiological substrate of PTEN is membrane-bound Ptdlin(3,4,5)P3, and activation of PTEN involves recruitment to the plasma membrane in part due to its high anionic lipid content. The polybasic N-terminal region, catalytic and C2 domains of PTEN are involved in electrostatic binding to Ptdlin(4,5)P2 and spatial orientation of PTEN. However, phosphorylation of C-terminal cationic residues maintains PTEN in an inactive state until dephosphorylation of PTEN triggers targeting to the plasma membrane via electrostatic interactions between the dephosphorylated C-terminal residues and Ptdlin, followed by recruitment into a protein-associated complex involving its PDZ-binding domain. Allosteric activation of PTEN by its substrate, Ptdlin(4,5)P2, provides an additional mechanism for regulating PTEN activity independent of membrane binding. In addition, PTEN can be found in the nucleus, where it has a distinct role from cytoplasmic PTEN.

PTEN is also sensitive to the cellular redox status by the modification of cysteine residues in the active site, whereas certain agonists induce changes in the expression of PTEN by both transcriptional and translational mechanisms. Antagonistic actions of PTEN on PI3K signalling is evolutionary-conserved and occurs in various mammalian tissues including the heart. Overexpression of catalytically active PTEN decreases basal and insulin-stimulated Ptdlin(3,4,5)P3 levels, whereas overexpression of wild-type PTEN into PTEN-null cell lines causes a decrease in intracellular levels of Ptdlin(3,4,5)P3 [and Ptdlin(3,4)P2]. In contrast, in embryonic stem cells lacking PTEN, Ptdlin(3,4,5)P3 levels are increased with greater insulin-like growth factor-1 (IGF-1)-induced accumulation of Ptdlin(3,4,5)P3, whereas expression of a catalytically inactive PTEN causes increased cellular Ptdlin(3,4,5)P3 levels. PTEN is widely expressed in many cells including cardiomyocytes, VSMCs, and endothelial cells.

3. Molecular targets of phosphoinositide 3-kinase and PTEN signalling

3.1 Phosphoinositide-dependent kinase-1

Phosphoinositide-dependent kinase-1 (PKD1) is a ubiquitously expressed 67 kDa kinase containing an N-terminal catalytic domain and a C-terminal PH domain. PDK1 phosphorylates and activates a number of kinases including Akt/PKB, protein kinase C, and other phosphotyrosine phosphatases. Cardiac-specific PDK1 ablation leads to reduced cardiomyocyte size and cardiomyopathy (Table 1).

3.2 Akt/protein kinase B

Akt/protein kinase B (PKB) is a serine/threonine protein kinase family comprising three closely related members: Akt1 (PKBα), Akt2 (PKBβ), and Akt3 (PKBγ). Akt/PKB contains an N-terminal PH domain, a central catalytic domain, and a C-terminal regulatory domain. The activity of Akt/PKB is primarily controlled by PI3K and PTEN via the modulation of Ptdlin(3,4,5)P3 levels.
PH domain, followed by phosphorylation of a threonine residue (threonine-308)\(^4,34\) in the catalytic domain by PKD1 and a serine residue (serine-473) in the C-terminal regulatory domain by the rictor–mTOR complex (mTOR complex 2).\(^37\) Akt/PKB is directly inactivated following dephosphorylation of the two regulatory sites by Mg\(^2+\)-ATP-dependent protein phosphatase 2A (PP2A).\(^38\) Akt/PKB controls a variety of regulatory responses in mammalian cells, including inhibition of apoptosis and regulation of cellular proliferation, metabolism, and hypertrophy\(^4,36,39\) and is activated by both RTKs/cytokines\(^6,36,39\) and GPCRs.\(^6,12,35\) Activated Akt/PKB phosphorylates several downstream targets including glycogen synthase, protein phosphatase 2A (PP2A), and GSK3\(\beta\) isoforms being the most abundant in the myocardium, with the Akt1/PKB\(\beta\) and Akt2/PKB\(\beta\) isoforms being the most abundant (Table 1).\(^12,35,41,42\) Following the recruitment and activation of Akt/PKB at the plasma membrane, activated Akt/PKB exerts multi-compartmental effects in the nuclear, cytoplasmic, and mitochondrial compartments, where they can mediate distinct physiological effects.\(^40,51,52\) In response to RTK/GPCR activation, phosphorylation of serine-9 residue in the N-terminal region of GSK3\(\beta\) by Akt/PKB inhibits GSK3\(\beta\), thereby leading to diverse effects including improved cell survival and hypertrophy\(^40,51,53\) possibly resulting from the up-regulation of calcineurin/NFAT and c-Jun NH\(_2\)-terminal kinases (JNKs).\(^51,53\) In contrast, GSK3\(\alpha\) is phosphorylated at the serine-21 residue by Akt/PKB, which plays a compensatory role in the setting of pathological hypertrophy possibly by the inhibition of ERK1/2 signalling.\(^52\)

### Table 1 Gene-targeted and transgenic models with altered phosphoinositide 3-kinase and PTEN signalling in the heart

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac-specific DN p85(\alpha)</td>
<td>Reduced heart and cardiomyocyte size; normal myocardial contractility</td>
<td>12,64,66</td>
</tr>
<tr>
<td>Cardiac-specific CA p110(\alpha)</td>
<td>Increased heart size; increased cardiomyocyte size</td>
<td>64</td>
</tr>
<tr>
<td>Cardiac-specific and temporally controlled p110(\alpha) overexpression</td>
<td>Increased myocardial contractility; no hypertrophy</td>
<td>79</td>
</tr>
<tr>
<td>Whole-body p110(\alpha) KO(\ast)</td>
<td>Increased myocardial contractility; no hypertrophy</td>
<td>12</td>
</tr>
<tr>
<td>Whole-body p110(\alpha) KO(\ast)</td>
<td>Increased myocardial contractility; no hypertrophy</td>
<td>71</td>
</tr>
<tr>
<td>Whole-body p110(\alpha) KO(\ast)</td>
<td>Increased myocardial contractility; no hypertrophy</td>
<td>67</td>
</tr>
<tr>
<td>Whole-body p110(\alpha) kinase dead mutant</td>
<td>Normal myocardial contractility; no hypertrophy</td>
<td>64</td>
</tr>
<tr>
<td>Cardiac-specific p110(\alpha) (\text{inact})</td>
<td>Normal myocardial contractility; no hypertrophy</td>
<td>45</td>
</tr>
<tr>
<td>Conditional PTEN KO</td>
<td>Increased heart and cardiomyocyte size; reduced myocardial contractility</td>
<td>12,23</td>
</tr>
<tr>
<td>Whole-body Akt1/PKB(\alpha) KO</td>
<td>Reduced somatic growth; normal glucose tolerance; enhanced pathological hypertrophy</td>
<td>42,70</td>
</tr>
<tr>
<td>Cardiac-specific CA Akt(\text{T308D/S473D})</td>
<td>Hypertrophy; reduced myocardial contractility</td>
<td>45</td>
</tr>
<tr>
<td>Cardiac-specific CA Akt(\text{T498D}) (myr-Akt)</td>
<td>Hypertrophy; normal myocardial contractility</td>
<td>47,48</td>
</tr>
<tr>
<td>Cardiac-specific CA Akt(\text{E40K})</td>
<td>Hypertrophy; enhanced myocardial contractility</td>
<td>46,49</td>
</tr>
<tr>
<td>Cardiac-specific KD Akt(\text{K179M})</td>
<td>Reduced heart and cardiomyocyte size; normal myocardial contractility</td>
<td>45</td>
</tr>
<tr>
<td>Conditional PDK1 KO</td>
<td>Cardiomyopathy; reduced cardiomyocyte size; increased sensitivity to hypoxia</td>
<td>34</td>
</tr>
<tr>
<td>Cardiac-specific CA GSK3(\beta)</td>
<td>Impaired hypertrophic response</td>
<td>53</td>
</tr>
<tr>
<td>Cardiac-specific inducible GSK-3(\beta)</td>
<td>Reversal of pre-existing hypertrophy</td>
<td>54</td>
</tr>
<tr>
<td>Whole-body knockin of GSK3(\beta) S9A and GSK3(\alpha) S21A</td>
<td>GSK3(\beta) serine-9 and GSK3(\alpha) serine-21 phosphorylation mediates and suppresses pathological hypertrophy, respectively</td>
<td>52</td>
</tr>
<tr>
<td>Whole-body p70(\text{S6K}) KO</td>
<td>Reduced cell and body size; no effect on cardiac hypertrophy</td>
<td>59,60</td>
</tr>
</tbody>
</table>

\(\ast\)Different strains.

\(\alpha\)Different strain, expression level, and/or type of Akt.

3.3 Glycogen synthase kinase-3

GSK3 is a serine/threonine kinase which was originally identified to phosphorylate and inactivate glycogen synthase.\(^40\) GSK3 has two mammalian isoforms: GSK3\(\alpha\) and GSK3\(\beta\), which are both expressed in the heart.\(^12,40,50,52\) GSK3\(\beta\) is constitutively active in unstimulated cells, where it phosphorylates several targets in addition to glycogen synthase, including cyclin D, c-Jun, NFAT, and the canonical Wnt/\(\beta\)-catenin, leading to their inactivation and/or degradation.\(^40\) Cardiac-specific overexpression of GSK3\(\beta\) is associated with reduced agonist and pressure-overload-induced hypertrophy, confirming that GSK3\(\beta\) functions as a negative regulator of hypertrophy in vivo (Table 1).\(^31,33,34\) GSK3\(\alpha\) and GSK3\(\beta\) are both present in the cytoplasmic and nuclear compartments, where they can mediate distinct physiological effects.\(^40,52\) For example, cytoplasmic GSK3\(\beta\) phosphorylation of the canonical Wnt/\(\beta\)-catenin leading to its degrada
tion may modulate N-cadherin/\(\beta\)-catenin cell adhesion complexes, whereas the nuclear effects of GSK3\(\beta\) phosphorylation of NFAT may relate to its anti-hypertrophic effects.\(^40,51,52\) In response to RTK/GPCR activation, phosphorylation of serine-9 residue in the N-terminal region of GSK3\(\beta\) by Akt/PKB inhibits GSK3\(\beta\), thereby leading to diverse effects including improved cell survival and hypertrophy\(^40,51,53\) possibly resulting from the up-regulation of calcineurin/NFAT and c-Jun NH\(_2\)-terminal kinases (JNKs).\(^51,53\) In contrast, GSK3\(\alpha\) is phosphorylated at the serine-21 residue by Akt/PKB, which plays a compensatory role in the setting of pathological hypertrophy possibly by the inhibition of ERK1/2 signalling.\(^52\)

3.4 Mammalian target of rapamycin and p70 S6 kinase

mTOR, also termed FRAP (FKBP-12 rapamycin-associated protein), is a 289 kDa evolutionary-conserved serine/threonine kinase that is inhibited by the drug rapamycin.\(^4,55,56\) RTK and Akt/PKB activation as well as elevated amino
acids activate mTOR via both translational and phosphorylation-dependent mechanisms leading to altered metabolism and increased growth mediated by changes in gene transcription and translation.\textsuperscript{4,55} For example, mTOR phosphorylates and thereby inactivates the eukaryotic translation initiation factor called 4E-binding protein 1 (4E-BP1) leading to increased protein translation.\textsuperscript{5,56} mTOR also phosphorylates and activates p70S6K, which is a short isoform of the ribosomal S6 kinase (S6K1).\textsuperscript{51} Ribosomal S6 kinases (S6K1 and S6K2) are key regulators of cell growth through the control of protein translation particularly for mRNA transcripts containing polyuridylic acid sequences at the transcriptional start site by phosphorylating the S6 protein of the 40S ribosomal subunit.\textsuperscript{57} Both mTOR and p70S6K are expressed in the heart and VSMCs.\textsuperscript{12,58} Whereas the whole-body knockout of p70S6K is associated with reduced somatic growth,\textsuperscript{59} loss of S6K1 and/or S6K2 has no impact on the development of physiological, patho-
logical, or PI3K-mediated cardiac hypertrophy, suggesting that S6K is a redundant hypertrophy pathway (Table 1).\textsuperscript{60}

4. Cardiac effects of phosphoinositide 3-kinase and PTEN signalling

4.1 Cell survival

Activation of PI3K enhances cell survival and antagonizes apoptosis via Akt/PKB activity in many cell types including cardiomyocytes, cardiac fibroblast, VSMCs, and endothelial cells.\textsuperscript{4} The anti-apoptotic action of Akt/PKB involves both cytoplasmic and nuclear compartments via modulation of Bad, caspase-3, forkhead transcription factors, and IκB kinase.\textsuperscript{4,41} Growth factors that reduce apoptosis such as insulin, IGF-1, erythropoietin, and cytokines rely almost exclusively on the PI3K/Akt pathway, whereas GPCR-induced PI3K/Akt activation and cardioprotection occur in response to several peptide agonists including urocortin, ghrelin, and adrenomedullin as well as β2-AR stimulation.\textsuperscript{4,43,61,62} Consistent with the biochemical effects of Akt/PKB, overexpression of Akt/PKB in the heart causes resistance to apoptosis,\textsuperscript{4} whereas knock out of Akt2/PKBβ enhances apoptosis in response to myocardial ischaemia.\textsuperscript{41} Consistent with a critical role for Akt/PKB in cell survival, loss or gain of PTEN activity leads to reduced or enhanced apoptosis, respectively,\textsuperscript{63} whereas increased expression of PTEN induces an expected increase of apoptosis in neonatal cardiomyocytes.\textsuperscript{31}

4.2 Myocardial hypertrophy

PI3K activity is essential for both basal cell growth and adaptive (physiological) and maladaptive (pathological) hypertrophy.\textsuperscript{4,12,15,64,67} PI3K inhibitors attenuate basal rates of protein synthesis and abolish increases in protein synthesis induced by insulin in neonatal cardiomyocytes.\textsuperscript{68} Cardiac-specific overexpression of constitutively active PI3Kα (CA-PI3Kα) in mice results in enlarged hearts owing to increased cardiomyocyte size, whereas overexpression of dominant-negative PI3Kα (DN-PI3Kα) produces smaller hearts with reduced cardiomyocyte size without affecting cell number.\textsuperscript{12,64,66} These changes in heart size are associated with corresponding alterations in Akt/PKB and p70S6K phosphorylation and activities, thereby providing evidence that PI3Kα activation regulates adaptive (physiological) growth and hypertrophy.\textsuperscript{4,12,64,66,69} Indeed, PI3Kα plays a critical role in adaptive growth in response to various physiological stimuli including exercise and IGF-1 via increased phospho-Akt/PKB and p70S6K levels and activities.\textsuperscript{62,64,66} Activation of cardiac PI3Kα during chronic exercise training is important for maintaining heart structure and function in various pathological settings and may explain the beneficial effects of exercise in patients with heart failure.\textsuperscript{69}

Moreover, cardiac-specific overexpression of IGF-1 receptor in mice leads to a similar phenotype as the CA-PI3Kα transgenic mice\textsuperscript{44,66} Consistent with a central role of Akt/PKB in mediating hypertrophy, overexpression of CA-Akt or DN-Akt results in mice with larger or smaller hearts, respectively.\textsuperscript{4,45} Enhanced Akt/PKB activity was associated with increased p70S6K activity and increased phospho-GSK3β,\textsuperscript{45} whereas reduced Akt/PKB activity decreases p70S6K activity.\textsuperscript{45} The hypertrophy driven by enhanced PI3Kα (and Akt/PKB) activity results in a unique foetal gene expression profile without altering the relative dimensions of cardiomyocytes unlike the changes seen in concentric (reduced long/short axis) and eccentric (increased long/short axis) pathological hypertrophy.\textsuperscript{12,64} Consistent with the biochemical effects of Akt/PKB, overexpression of Akt/PKB in the heart causes hypertrophy and resistance to apoptosis,\textsuperscript{45,66} whereas knockout of Akt1/PKBα attenuates normal physiological growth while promoting pathological myocardial hypertrophy (Table 1).\textsuperscript{42,70} The Akt1/PKBα-mediated suppression of pathological hypertrophy may occur owing to Akt1/PKBα antagonism of signalling via ERK1/2 and JNK1/2 pathways while also preventing the antihypertrophic effects of GSK3β.\textsuperscript{61,42} Inactivation of PTEN increases cell size and growth by amplifying PI3K signalling, whereas overexpression of PTEN inhibits PI3K signalling, resulting in decreased cell size.\textsuperscript{4,63} Expression of DN-PTEN in neonatal cardiomyocytes activates Akt/PKB, increases protein synthesis and cell size, although overexpression of wild-type PTEN impaired these responses.\textsuperscript{31} Conditional knockout of cardiac PTEN increases myocardial levels of phospho-Akt/PKB (serine-473), phospho-GSK3β (serine-9), and phospho-p70S6K (threonine-412), resulting in increased cardiomyocyte and heart size.\textsuperscript{12}

Although PI3Kα/Akt appears to control physiological growth during development, PI3Kγ/Akt activation following GPCR stimulation typically leads to pathological (maladap-
tive) hypertrophy,\textsuperscript{4,15,65,67,71,72} which may explain the increased PI3K/Akt activity observed in various models of cardiac pathological hypertrophy induced by pressure-overload, Fas-ligand, and chronic β-adrenergic stimulation.\textsuperscript{15,65,71} Indeed, pressure-overload selectively activates p110γ without affecting p110α in the setting of pathological hypertrophy.\textsuperscript{65} Various GPCR agonists (Gαq) such as Ang-II, endothelin-1 and α-AR and β-AR agonists lead to PI3K/Akt activation and hypertrophy,\textsuperscript{4} whereas loss of PI3Kγ prevents activation of Akt/PKB and ERK1/2 pathways in response to β-AR stimulation, resulting in a marked reduction in hypertrophy.\textsuperscript{15} Several downstream signalling pathways from Akt/PKB appear to mediate this pathological (maladaptive) hypertrophy including GSK3β\textsuperscript{53,54} and mTOR.\textsuperscript{58} Consistent with the key role of PTEN as a negative regulator of the PI3K system, loss of myocardial PTEN enhances the pathological hypertrophy and activation of MAPK in response to the GPCR agonist Ang-II.\textsuperscript{23}
4.3 Myocardial contractility: cAMP and β-adrenergic signalling

Under basal conditions and following β-AR stimulation, cAMP levels in cardiomyocytes are regulated in a complex manner by interaction between adenylate cyclase-induced synthesis and phosphodiesterase-mediated degradation in well-defined microdomains. Protein kinase A (PKA) is the main effector of cAMP in cardiomyocytes and mediates positive chronotropic, inotropic, and lusitropic effects via phosphorylation of many target proteins involved in excitation–contraction coupling including phospholamban (PLN). Loss of PI3Kγ increases basal myocardial cAMP levels without affecting the maximal forskolin-induced cAMP levels and myocardial expression of adenylyl cyclase. Loss of PI3Kγ appears to reduce PDE4 activity in subcellular compartments containing the SR Ca<sup>2+</sup>-ATPase but not RyR2 or L-type Ca<sup>2+</sup> channels (LTCCs), leading to increased phospho-PLN (serine-16) at baseline, which is consistent with the ability of PDEs to spatially restrict the actions of cAMP in the heart (Figure 1B). Consistent with these biochemical changes, loss of PI3Kγ is associated with sustained increases in contractility and relaxation both in vivo and in single cardiomyocyte without pathological hypertrophy or increased interstitial fibrosis (Table 1). In contrast, loss of PTEN leads to reduced cAMP levels in cardiomyocytes, which is rescued by the pharmacological inhibition of PI3K and is associated with reduced myocardial contractility and relaxation. PI3Kγ also modulates β<sub>2</sub>-AR signalling by confining and reducing β<sub>2</sub>-AR/G<sub>α<sub>1</sub></sub>mediated elevation in cAMP levels and PKA activation but not affecting β<sub>1</sub>-AR stimulation-induced cAMP generation. Indeed, PI3Kγ ablation selectively increases cAMP levels, PLN-phosphorylation on serine-16, as well as positive inotrope and lusitropic effects mediated by β<sub>2</sub>-AR stimulation without affecting β<sub>1</sub>-AR effects possibly owing to interactions between β<sub>2</sub>-AR/G<sub>α<sub>1</sub></sub> and PI3Kγ, resulting in the modulation of cAMP generation. Interaction between PI3Kγ with other GPCRs may also regulate myocardial contractility such as activation of purinergic receptor (P2 receptors) diminishing Ca<sup>2+</sup> oscillations via PI3Kγ in neonatal cardiomyocytes, whereas the negative inotropic effects of platelet activating factor, a GPCR agonist released during ischaemia, is completely dependent on PI3Kγ. PI3Kγ might also regulate myocardial contractility by mediating downregulation of β-ARs in response to agonist stimulation. In response to agonist stimulation, βARK1 (GRK2) mediates PI3K translocation to β-AR, thereby triggering recruitment of phosphoinositide-binding endocytic proteins such as β-arrestin and AP-2, ultimately leading to β-AR internalization. Loss of PI3Kγ does not prevent downregulation of β<sub>1</sub>-AR and β<sub>2</sub>-AR in response to chronic β-AR stimulation in vivo. Rather, cardiac-specific overexpression of inactive p110 catalytic subunit (PI3K<sub>inactive</sub>) prevented β-AR desensitization following chronic β-AR stimulation, suggesting that both PI3Kα and PI3Kγ are involved in β-AR desensitization. Despite in vitro evidence showing that PTEN inhibition facilitates β-AR downregulation, neither basal β-AR density and agonist affinity nor βARK1 activity is altered in the hearts of PTEN knockout mice. Although PI3Kγ regulates myocardial contractility, PI3K<sub>α</sub> (and Akt/PKB) can also influence myocardial contractile strength. Cardiac-specific and temporally controlled overexpression of PI3Kγ results in increased myocardial contractility, owing to increased expression of multiple Ca<sup>2+</sup>-regulating proteins without resulting in cardiac hypertrophy (Table 1). Cardiac-specific expression of IGF-1 receptor and knockout of insulin receptor result in enhanced and reduced myocardial contractility, respectively, whereas IGF-1 induces a positive inotropic effect in cardiomyocytes. Cardiac-specific overexpression of CA-Akt induces variable effects on myocardial contractility, including enhanced (E40K-Akt mutant) and reduced (myr-Akt mutant) contractility, which may be related to the differential subcellular targeting of CA-Akt and/or differences in transgene expression (Table 1).

4.4 Electrophysiology

PI3Ks can modulate electrical (and contractile) function in the heart by regulating several ion channels/exchanger. IGF-1, a potent activator of PI3K and Akt/PKB, induces positive inotropic effects in human ventricular myocytes, which is linked to increased I<sub>K<sub>Ca</sub></sub>. Although the mechanism for the increase in I<sub>K<sub>Ca</sub></sub> is unclear, cardiac I<sub>K<sub>Ca</sub></sub> is increased in transgenic mice with cardiac-specific overexpression of Akt/PKB, suggesting that Akt/PKB might directly phosphorylate LTCCs as do protein kinase A and protein kinase C. Indeed, loss of PTEN results in increased cardiac I<sub>K<sub>Ca</sub></sub> owing to Akt/PKB-mediated upregulation of LTCC activity via the PI3K<sub>γ</sub> pathway. Alternatively, Akt/PKB phosphorylation and extranuclear localization of AHNAK (at serine 5535) into the T-tubular/intercalated disk region regulate F-actin interaction with the β<sub>2</sub> subunit of LTCC, which may affect I<sub>K<sub>Ca</sub></sub>. The Akt/PI3K system is also involved in the rapid recovery and rebound activation of I<sub>K<sub>Ca</sub></sub> following acetylcholine-mediated cholinergic stimulation.

The inward rectifier family of cardiac K<sup>+</sup> channels also appears to be regulated by the PI3K system. PtdIns(4,5)P<sub>2</sub> interaction with Kir2.1 (Kir) inward rectifier K<sup>+</sup> channels promotes subunit assembly and enhances ion channel opening, whereas activation of cardiac phospholipase C and PI3K can lead to local depletion of PtdIns(4,5)P<sub>2</sub>, thereby reducing channel activity. These effects may explain the loss-of-function phenotypes for several mutations associated with Andersen’s syndrome (LQT7) involving altered interaction between PtdIns(4,5)P<sub>2</sub> and cardiac Kir2.1. Cardiac Kir3.1 (I<sub>K<sub>ACH</sub></sub>) channels are activated by M<sub>2</sub> receptors (G<sub>α</sub>q) which mediate acetylcholine-negative chronotropic effects and are strongly modulated by PtdIns(3,4,5)P<sub>3</sub>, suggesting a role for PI3Kγ via M<sub>2</sub> receptor-mediated activation of G<sub>α</sub>q. Akt/PI3K increases HERG (human ether-a-go-go) current, which encodes for the rapid component of delayed rectifier K<sup>+</sup> current (I<sub>K<sub>H</sub></sub>), suggesting that voltage-gated K<sup>+</sup> channels can also be regulated by the Akt/PI3K system.

4.5 Metabolism and mechanotransduction

PI3K plays an important role in myocardial metabolism and mechanotransduction. Activation of RTK (e.g. insulin and epidermal growth factor) or GPCR (e.g. α- and β-AR agonists) promotes glucose uptake via translocation of insulin-responsive glucose transporters...
(GLUT4) to the plasma membrane in an Akt/Pi3K-dependent manner. Enhanced glucose uptake following Pi3K activation as a consequence of increased myocardial contraction may increase cardiac glycogen synthesis owing to Akt/PKB-mediated inhibition of GSK3β as shown in skeletal muscle. Akt/PKB activation also decreases α-AMP-activated protein kinase (α-AMPK) activity, a key determinant of cardiac energy substrate utilization and preference especially in response to insulin while stimulating mTOR activity which senses changes in amino acid levels and regulates protein synthesis. Although Akt2/PKBβ negatively regulates fatty acid uptake and metabolism, activation of Pi3Kα is sufficient to increase myocardial fatty acid oxidation, a characteristic response seen in physiological hypertrophy, independent of the Akt/PKB pathway.

Mechanotransduction 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 Iα and Iγ Pi3Ks. Phosphorylated Ptdlns bind and activate several cell adhesion proteins, including gelsolin and profilin, via basic residues, thereby fostering connections between the sarcolemmas and the intracellular actin cytoskeleton.

and loss of myocardial PTEN attenuates the development of heart failure in response to biomechanical stress.

4.6 Coronary angiogenesis

Coronary neoangiogenesis occurs during physiological hypertrophy in response to angioptin-2 and VEGF-A in order to maintain a balance between myocardial mass and coronary blood flow, whereas vascular rarefaction in the setting of pathological hypertrophy may cause tissue hypoxia and lead to contractile dysfunction. The Akt/PKB-induced expression of angiogenic growth factors such as VEGF maintains coronary angiogenesis and vascular homeostasis in the setting of cardiac hypertrophy and is dependent on the mTOR pathway. Endothelial progenitor cells (EPCs) which are recruited to ischaemic regions leading to neovascularization is under control by PI3Kγ. Increased basal angioptin-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. The differential expression of pro-angiogenic factors in cardiomyocyte vs. endothelial-specific knockout of PTEN indicates that cardiomyocyte Akt/PKB signalling is the primary determinant of increased VEGF-A and Ang-II expression.

5. Isoform-specific signalling by class I phosphoinositide 3-kinases

Cell signalling involves spatial and temporal integration of multiple signalling pathways including Pi3K/Pten signalling,
6. Role of phosphoinositide 3-kinase and PTEN in myocardial preconditioning and ischaemia–reperfusion injury

Ischaemic preconditioning (IPC) refers to a phenomenon where brief periods of mild ischaemia can protect the heart from subsequent ischaemia. Acute IPC lasts ~2 h, and a second window of protection (i.e. delayed IPC) develops 24 h later and lasts ~3 days. Preconditioning involves the release of several autacoids that trigger protection by activating various receptors, cell signalling cascades, and effectors. PTEN/PI3K/Akt and several downstream pathways are activated in myocardial ischaemia–reperfusion. Gene expression analysis of human ventricular myocardium in response to reversible myocardial ischaemia–reperfusion revealed an immediate genomic response characterized by the activation of multiple signalling pathways regulated by the PI3K system. In acute IPC, there is increased phosphorylation of Akt/PKB, whereas PI3K inhibitors, wortmannin and LY294002, block reductions in infarct size, improvement in post-ischaemic function, and increases in phospho-Akt/PKB. PTEN inhibition also blocked reductions in infarct size and the associated increase in phosphorylation of PDK1 and Akt/PKB observed with delayed IPC. The precise connection between Akt/PKB activation and protection induced by IPC is unclear but may be related to enhanced cell survival and shifts in metabolism induced by Akt/PKB stimulation and/or inhibition of GSK3β in response to phosphorylation. PTEN is also regulated by PtdIns-mediated phosphorylation of Akt/PKB in the mitochondria may also trigger additional protective mechanisms such as opening of mitochondrial KATP channels. Interestingly, cardiac-specific knockout of PDK-1 is associated with enhanced sensitivity to hypoxia, whereas rapamycin, an inhibitor of mTOR, blocks delayed IPC.

Animal models with increased class IA PI3K and/or Akt/PKB signalling have enhanced cell survival and smaller infarct size in ischaemia–reperfusion. Both RTK (e.g. insulin, IGF-1) and GPCR (e.g. adenosine, acetylcholine, opioid, and bradykinin) agonists can induce cardioprotective effects in IPC consistent with the possible role of Akt/PKB and other downstream signalling pathways. In response to ischaemia–reperfusion, loss of PI3Kγ results in reduced phosphorylation of Akt/PKB and GSK3β, impaired functional recovery, and increased cell death, in addition to mitigating the protective effects of adenosine. In contrast, PDK-1 knockout hearts responded in a ‘preconditioned’ manner owing to increased signalling via the other PI3K isoforms such as PI3Kδ, resulting in enhanced phosphorylation of Akt/PKB and GSK3β, better functional recovery, and reduced cell death. These results provide strong evidence for a pivotal role of GPCR-mediated activation of the PI3K signalling in IPC. Similarly, ischaemic postconditioning mediates cardioprotection in the remodelled rat myocardium primarily via the activation of the PI3K-PKB/Akt reperfusion injury salvage kinase pathway, whereas erythropoietin-mediated reduction in myocardial ischaemia–reperfusion injury is mediated in part by activation of the Akt/PI3K system. Loss of PTEN activity due to reactive oxygen species-mediated degradation of PTEN leads to early Akt/PKB activation and induction of IPC, whereas delayed feedback mechanisms restore PTEN levels, leading to the decay of IPC. Consistent with the role of PTEN as a negative regulator of PI3K signalling, PTEN haploinsufficiency is sufficient to reduce the threshold of protection induced by IPC in association with increased phospho-Akt/PKB levels. Collectively, these observations highlight the involvement of PI3K isoforms in different aspects of IPC and the potential use of selective modulation of PI3K signalling in optimizing protection from myocardial ischaemia/reperfusion.
7. Role of phosphoinositide 3-kinase and PTEN in heart failure

PI3K/Akt and several downstream pathways are activated in heart disease including diabetic cardiomyopathy,62 adriamycin-induced cardiomyopathy,62,112 chronic β-AR stimulation,15,71 pressure-overload-induced hypertrophy,65,67,71 or in advanced human heart failure.4,92 Class Ia and Ib PI3K isoforms appear to have distinct roles in the pathogenesis of heart disease. Activation of PI3Kα in the heart during chronic exercise training is important for maintaining cardiac structure and function in the setting of pathological hypertrophy and may underlie the beneficial effects of exercise in patients with heart failure.69 Furthermore, mesenchymal stem cells overexpressing Akt/PKB prolonged cardiomyocyte survival, prevented remodelling, and improved cardiac performance in infarcted hearts.113 Cardiac-specific overexpression of DN-PI3Kα, knockout of the insulin receptor, and Akt1/PKBα accelerate the progression to dilated cardiomyopathy in response to GPCR stimulation and/or pressure-overload, which may be related to reduce phospho-Akt/PKB and cell survival, and/or altered mechanotransduction.4,42,66,90 In addition, enhanced nuclear phospho-Akt/PKB is associated with delayed cellular ageing and death in cardiomyocytes, and phospho-Akt/PKB is higher in cardiomyocytes from adult premenopausal women compared with men or postmenopausal women, which may explain some of the age-related and gender-dependent differences in susceptibility to cardiovascular diseases.4,43,113

PI3Kγ activation has been linked with left ventricle enlargement and decomposition in pressure-overload-induced heart disease, suggesting that impaired PI3Kγ signalling may have therapeutic benefits in experimental models of heart failure.4,65,67 Loss of PI3Kγ prevents isoproterenol-induced increases in cardiac Akt/PKB activity and ERK1/2 phosphorylation, resulting in reduced hypertrophy and preserved heart function.4,15 Increased PTEN expression associated with sustained β-adrenergic stimulation may serve to negate β-adrenergic receptor-mediated increases in PI3K activity.15,31 On the other hand, β-AR and angiotensin II-mediated Akt/PI3Kγ and MAPK activation in cardiac fibroblasts may result in remodelling of the myocardial extracellular matrix as commonly seen in heart failure.4,15,114 On the basis of the ability of PI3Kγ in mediating negative inotropic effects of Gαi signalling and excitation–contraction coupling, inhibiting PI3Kγ may provide an important means to negate the increased Gαi and/or to restore impaired Ca2+ cycling in heart failure.4,12,72,74,76,77 Moreover, since several downstream pathways of PI3Kγ including GSK3β5,53 and mTOR58 are also altered in heart disease, targeting these pathways may also provide clinical benefit in the treatment of pathological hypertrophy and heart failure.

Despite the well-defined role of PI3Kγ in GPCR signalling, excitation–contraction coupling, and pathological hypertrophy, recent evidence using the PI3Kγ knockout mice has produced discordant findings in experimental models of heart failure. The key role of PI3Kγ in minimizing myocardial ischemia–reperfusion injury and in the recruitment of EPCs to ischaemic regions18 suggests that loss of PI3Kγ should result in worsening heart function in response to chronic myocardial ischaemic injury. However, in response to myocardial infarction, adverse ventricular remodelling and ventricular function in PI3Kγ knockout mice did not differ from wildtype mice,115 suggesting that responses controlling acute myocardial ischaemic reperfusion injury are distinct from those mediating long-term remodelling following myocardial infarction. In addition, although PI3Kγ knockout mice are protected from isoproterenol-induced heart failure,15 pressure-overload induced by aortic constriction in PI3Kγ knockout mice leads to a rapid cardiac dilation and dysfunction compared with wild-type controls.67,72 These results highlight a potential role of PI3Kγ in cell adhesion and mechanotransduction, which, if compromised, may result in adverse ventricular remodelling in response to biomechanical stress.4,23 Consistent with these observations, titin isoform transitions are mainly regulated through PI3K/Akt-dependent signalling triggered particularly by 3,5,3′-triiodo-l-thyronine via a rapid action pathway and is a major determinant of the extracellular matrix stiffness.116 Despite baseline reduction in myocardial contractility, loss of PTEN prevents the development of maladaptive ventricular remodelling with preservation of angiogenesis and metabolic gene expression in response to pressure-overload, suggesting that 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 (Figure 1C).23 The pro-angiogenic effects associated with loss of PTEN and activation of PI3K signalling are consistent with a critical role of PI3Kα in endothelial migration and angiogenesis.117 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, such as the cardiomyocyte–fibroblast–ECM adhesion network (Figure 1C).23

8. Future perspectives

The clearest evidence that our understanding of the PI3K system is suboptimal is the emerging trend of the uncoupling between PI3K cellular effects and those seen using in vivo models of heart disease, such as pressure-overload and myocardial infarction in PI3K mutant models, suggesting that PI3K signalling has many unexplored roles such as in cardiac mechanotransduction and metabolism. In view of what we have learnt so far, a major open question to be addressed is the residual role of PI3Kβ in cardiac signalling and heart disease, and the recent availability of the PI3Kβ knockout mice is a major step in the right direction. The availability of isoform-specific blockers of the PI3K system also provides a useful research tool to examine the short-term effects of isoform-specific PI3K signalling. The GSK3 system has also emerged as a major downstream effector of the PI3K system, and the use of genetic knockin and cardiac-specific knockout of GSK3α and GSK3β isoforms will provide useful insight into their role in cardiac structure and function. Given the critical role of PI3K/GSK3 system in the cardiovascular system, the possibility of using PI3K isoform-specific and/or GSK3β inhibitors for a variety of medical conditions must be closely monitored for cardiovascular toxicity in order to avoid the many pitfalls encountered by several recent pharmaceutical therapeutic agents.

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