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Role of phosphoinositide 3-kinases in regulating cardiac function

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1. ABSTRACT

Phosphoinositide 3-kinases (PI3Ks) are important signaling proteins in the heart. Class I_A PI3Ks (p110α, β) are critical regulators of physiological heart growth and cell survival, and are generally considered to be beneficial for heart function. In contrast, activation of class I_B PI3K(p110γ) is detrimental for heart function, reducing cardiac contractility. This may have implications for the treatment of heart disease and failure. *In vitro*, *ex vivo* and *in vivo* studies have contributed to our understanding of PI3K signaling in the heart. This review summarizes class I PI3K signaling in the regulation of cardiac function, with a particular focus on the role of different PI3K isoforms in settings of heart disease.

2. INTRODUCTION

Understanding the role of key signaling proteins on cardiac function is of importance as it aids our understanding of the pathophysiological mechanisms involved in heart failure, a condition in which cardiac function is compromised. Heart failure has reached near-epidemic proportions in much of the Western world, affecting approximately 1-2% of the population (1-3). Phosphoinositide 3-kinases (PI3Ks) have been identified as important regulators of cardiomyocyte growth, survival, and contractility. This review will summarize the roles of different PI3K isoforms in regulating cardiac function, both in the healthy heart and in settings of disease.

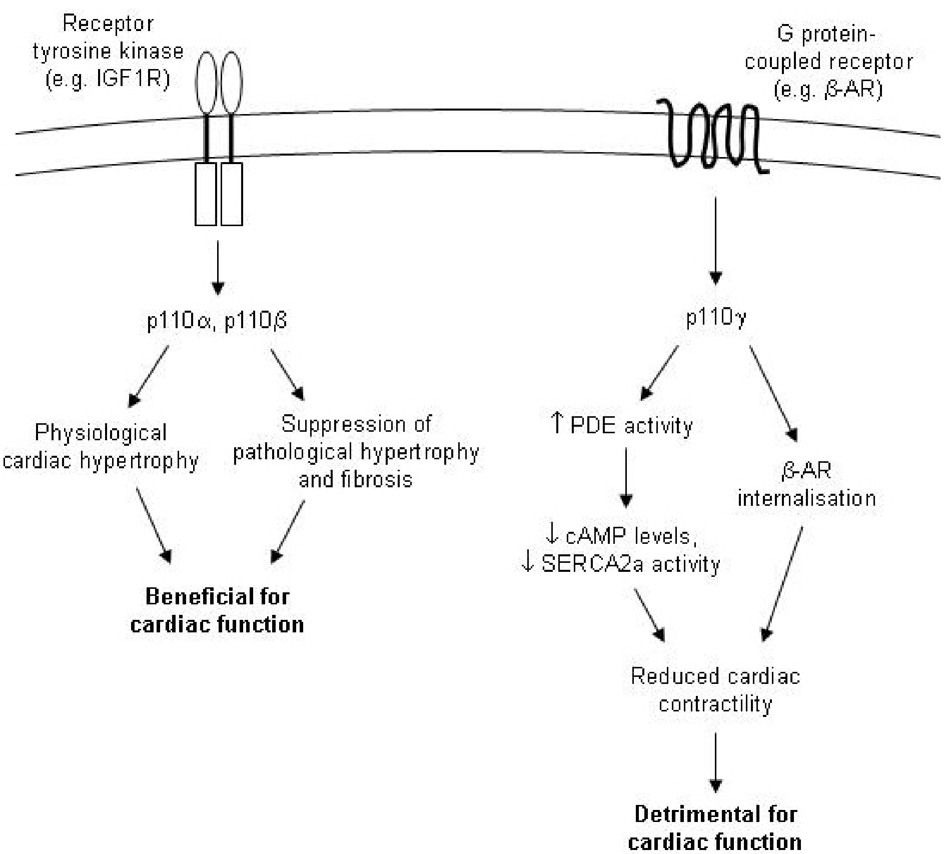

Figure 1. Differential role of class I PI3Ks in regulating cardiac function.

3. PHOSPHOINOSITIDE 3-KINASE (PI3K)

PI3Ks are important signaling proteins in numerous cell types. PI3Ks catalyze the phosphorylation of lipids in the cell membrane, leading to the generation of second messengers such as phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3). There are three major classes of PI3Ks (classes I-III). These are determined based on amino acid sequence, homology of the lipid-kinase domains, and specificity for substrate binding (4, 5). Class I PI3Ks consist of a 110kDa catalytic subunit (p110) complexed with a regulatory subunit, and can be divided into two subclasses: I_A and I_B. Class I_A PI3Ks (p110α, p110β and p110δ) associate with the regulatory proteins p85α, p85β and p55γ (as well as spliced variants of p85α), while p110γ (the only class I_B PI3K identified to date) is regulated by p101 (6). p110α, β and γ are expressed in the heart (7) and vasculature (8-10), while p110δ is found predominantly in leukocytes (see (6)) and will not be addressed in this review. p85α is the most abundant isoform of the class I_A regulatory subunits expressed in the heart (see (11)).

Class I_A PI3Ks are activated by receptor tyrosine kinases, such as platelet-derived growth factor (PDGF) receptor, epidermal growth factor (EGF) receptor, and insulin-like growth factor 1 (IGF1) receptor (see (12)) (Figure 1). Binding of growth factors to these receptors results in autophosphorylation of specific tyrosine residues in the intracellular domains, providing a docking site for p85 (13). Subsequent activation of the p110 catalytic subunit results in phosphorylation of lipid substrates in the cell membrane, namely phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2). The product of this reaction, PtdIns(3,4,5)P_3, acts as a second messenger, recruiting proteins to the cell surface where they can be modified by other enzymes (12). Class I_B PI3Ks are activated by binding to the Gβγ subunits of heterotrimeric G proteins following stimulation of G protein-coupled receptors (14, 15) (Figure 1).

4. ROLE OF PI3K SIGNALING IN THE HEART: EVIDENCE FROM *IN VITRO* AND *EX VIVO* STUDIES

In vitro and *ex vivo* studies using PI3K inhibitors (e.g. Wortmannin, LY294002) have implicated PI3K in a diverse range of cellular processes. For example, administration of IGF1 reduced myocardial injury and improved cardiac function in rat hearts subjected to ischemia-reperfusion injury (16). Wortmannin reduced the beneficial effects of IGF1 in this model, suggesting that PI3K plays an important role in the regulation of contractile function. In another study, ischemia-reperfusion induced activation of Akt (a downstream target of PI3K), and this was blocked by Wortmannin, implicating PI3K in stress responses (17). Inhibition of PI3K by LY294002 significantly increased cell contractility and transient calcium levels in isolated rat cardiomyocytes following stimulation by a β_2AR agonist (18). Another study demonstrated that IGF1 protected rat fibroblasts from apoptosis induced by UV radiation (19). This protection was completely inhibited by the addition of Wortmannin, implicating PI3K in cell survival (19).

In vitro and *ex vivo* studies are limited, however, as the PI3K inhibitors available are not isoform-specific. Additionally, LY294002 has been shown to specifically inhibit slowly inactivating K^+ currents leading to increased Ca^{2+} release and myocyte contractility (20), consequently limiting the usefulness of this inhibitor to examine the role of PI3K in regulating cardiac function. Thus, the specific functions of different PI3K isoforms are still being elucidated. Of particular interest is the role of class I PI3Ks in the heart, as components of class I PI3K signaling pathways are emerging as potential therapeutic targets for the treatment of cardiovascular disease and heart failure (21).

5. *IN VIVO* STUDIES INVESTIGATING PI3K SIGNALING IN THE HEALTHY HEART

Transgenic and knockout mouse models have provided a powerful approach for understanding the specific roles of different PI3K isoforms in the heart. In the following section, we have focused on PI3K(p110α) and PI3K(p110γ), as these isoforms have been identified as important regulators of heart structure and function.

5.1. PI3K(p110α) mediates physiological cardiomyocyte hypertrophy

Studies in cardiac-specific PI3K transgenic mice have demonstrated that the p110α isoform of PI3K is critical for developmental, IGF1-induced and exercise-induced heart growth (physiological cardiac hypertrophy) (22-24). Unlike pathological cardiac hypertrophy (heart growth due to disease), physiological hypertrophy is characterized by normal cardiac structure and function, and does not lead to decompensation. Mice expressing a cardiac-specific constitutively active (ca) form of PI3K(p110α) displayed a 6.5 fold increase in PI3K(p110α) activity, which was associated with a 20% increase in heart size compared with control mice (non-transgenics) (24). Mice expressing a dominant negative (dn) PI3K mutant displayed a 77% decrease in PI3K activity, and had 20% smaller hearts compared with non-transgenics (23, 24). Importantly, caPI3K and dnPI3K mice showed no signs of cardiomyopathy (such as fibrosis) and had normal cardiac function and lifespan under basal conditions (23, 24). dnPI3K mice showed a blunted response to exercise (a stimulus that induces physiological heart growth), but not to pressure overload (a pathological stimulus that leads to maladaptive heart growth, cell death and fibrosis), suggesting that PI3K(p110α) is critical for physiological, but not pathological, induced cardiac growth (23). These studies were later confirmed using a knockout approach. Deletion of class I_A PI3Ks from cardiac myocytes in mice led to a reduction in heart size that was similar in magnitude to that observed in dnPI3K mice (25). Knockout mice also showed a blunted cardiac hypertrophic response to exercise training (25).

IGF1 is an upstream agonist for class I_A PI3K (i.e. p110α, β) activation in the heart (22, 26). Transgenic mice overexpressing IGF1 receptors in the heart exhibited a 40% increase in heart size and no signs of histopathology, as well as enhanced left ventricular systolic function (approximately 15% increase) at three months of age (22). Heart growth due to the overexpression of IGF1 receptors was completely blocked by crossing IGF1 receptor transgenic mice with dnPI3K transgenic mice, suggesting that IGF1-induced hypertrophy is PI3K(p110α)-dependent (22) (Figure 1).

PI3K(p110α) signaling appears to promote cardiomyocyte hypertrophy via activation of Akt (also known as protein kinase B). Akt is a known downstream effector of PI3K, and is activated by phosphoinositide-dependent kinase 1 (PDK1) upon recruitment to the cell surface by PtdIns(3,4,5)P_3 (27). Akt phosphorylation was elevated in hearts of caPI3K mice and decreased in hearts of dnPI3K mice, consistent with the differences in cardiac PI3K activity in these mice (24). Akt1 has been identified as an essential mediator of physiological cardiac hypertrophy, as Akt1 knockout mice were resistant to increases in heart size induced by chronic exercise training (28).

5.2. PI3K(p110γ) negatively regulates cardiac contractility

The p110γ isoform of PI3K appears to be a negative regulator of cardiac contractility, as knockout mice lacking PI3K(p110γ) displayed enhanced contractile function (7) (Figure 1). Loss of PI3K(p110γ) in these mice had no effect on heart size under basal conditions (7), however PI3K(p110γ) appears to contribute to cardiac hypertrophy in settings of pathological stress (15, 29) (see section 6.4).

PI3K(p110γ) mediates cardiac contractility by regulating the activity of phosphodiesterases (PDEs; enzymes that degrade cyclic AMP, cAMP), as loss of PI3K(p110γ) in mouse myocardium eliminated PDE4 activity with beneficial consequences for cardiomyocyte contractility (30). Production of cAMP following β-adrenergic receptor stimulation results in activation of cAMP-dependent protein kinase (PKA), an enzyme that regulates intracellular Ca^{2+} levels by phosphorylating proteins involved in excitation-contraction coupling (such as L-type Ca^{2+} channels, ryanodine receptors, and phospholamban (PLN), a protein that regulates sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a) activity) (31). Binding of PI3K(p110γ) to PDEs results in degradation of cAMP, reducing contractility.

PI3K(p110γ) also regulates heart rate via negative modulation of the spontaneous firing rate of sinoatrial node myocytes (32), possibly by affecting sarcoplasmic Ca^{2+} cycling rates (33, 34).

6. PI3K SIGNALING IN THE FAILING HEART

Heart failure occurs when the heart loses its ability to provide sufficient perfusion to meet the metabolic demands of the body, and can result from a variety of conditions that impair and overload the heart (such as hypertension, cardiomyopathy, and myocardial infarction) (35, 36). Numerous structural events contribute to the depressed function of the failing heart. These include pathological cardiac hypertrophy, which is associated with distinct changes in gene expression; cell death (via apoptosis and necrosis), which reduces the number of cardiomyocytes available for contraction; and fibrosis, which leads to mechanical stiffness. The following section will outline the role of PI3K in preventing or mediating these events.

6.1. PI3K(p110α) attenuates pathological cardiac hypertrophy and associated changes in gene expression

Pathological cardiac hypertrophy is a key event in the progression from a cardiac insult to heart failure. Pathological hypertrophy is initially considered an adaptive response to pressure or volume overload on the heart, however prolonged exposure to a pathological stimulus can cause the heart to decompensate (37). Pathological hypertrophy is associated with distinct changes in gene expression, increased interstitial fibrosis, cell death, and depressed cardiac function (21, 38-40).

Constitutive activation of PI3K(p110α) in transgenic mice (i.e. caPI3K) attenuated pathological cardiac hypertrophy induced by pressure overload (aortic-banding), a pathological stimulus that mimics hypertension in the ventricle (41). In contrast, mice expressing a dominant negative PI3K mutant (i.e. dnPI3K) exhibited a massive (approximately 80%) increase in heart weight, increased interstitial fibrosis, and severe lung congestion in response to pressure overload (41). Thus, in response to a physiological stimulus a decrease in PI3K(p110α) activity blunts hypertrophic growth (see section 5.1); whereas in response to a pathological stimulus a decrease in PI3K(p110α) activity exaggerates pathological heart growth. Hearts of aortic-banded dnPI3K transgenic mice also showed significantly more dilation and decompensated more rapidly than non-transgenics. These data suggest that PI3K(p110α) is critical for maintaining cardiac structure and function in a setting of pressure overload, via the suppression of pathological cardiac hypertrophy, ventricular dilation, and the regulation of fibrosis (Figure 1). Of note, disruption of other receptor tyrosine kinases (e.g. ErbB2 receptors) also impairs growth and promotes cardiac dilation (42, 43).

Differential regulation of gene expression during pathological hypertrophy is a likely contributory factor for depressed cardiac function (44, 45). Aortic-banding studies have shown that pathological hypertrophy is associated with re-expression of fetal genes, including atrial and B-type natriuretic peptides (ANP and BNP respectively), β-myosin heavy chain (β-MHC) and α-skeletal actin (23, 46, 47). Severe/advanced pathological hypertrophy is accompanied by a decrease in SERCA2a expression (48-50). SERCA2a plays an important role in excitation-contraction coupling, promoting relaxation by pumping Ca^{2+} ions into the sarcoplasmic reticulum. Downregulation of SERCA2a is detrimental for heart function (45, 51), and transgenic mice with reduced SERCA2a activity developed heart failure more rapidly than non-transgenics in response to pressure overload (52).

PI3K(p110α) may protect the heart in a setting of pressure overload by attenuating changes in gene expression associated with pathological cardiac hypertrophy. As noted earlier, PI3K(p110α) is critical for exercise-induced heart growth. Swimming training reversed molecular abnormalities associated with pathological cardiac hypertrophy in hypertensive rats (53), and increased PI3K(p110α) activity in caPI3K mice attenuated the drop in SERCA2a expression due to pressure overload (41). IGF1-PI3K signaling might also improve cardiac structure and function by upregulating genes involved in cardiomyocyte contraction, such as α-skeletal actin, tropomyosin 3γ, and actin capping protein α (22). Overexpression of α-skeletal actin or tropomyosin 3γ in the mouse heart enhanced contractility (54, 55), while actin capping proteins are important for filament length regulation, as well as sarcomere organization and muscle function (56-58). As previously mentioned, α-skeletal actin is re-expressed during pathological cardiac hypertrophy; this may be an attempt to preserve function in a setting of cardiac dysfunction.

6.2. Class I_A PI3Ks promote cell survival

Cell death (i.e. apoptosis and necrosis) is a key feature of heart failure (59-61), contributing to the depressed function of the failing heart by reducing the number of cardiomyocytes available for contraction. Numerous studies have implicated PI3K-Akt signaling in cell survival [e.g. (62, 63)], however mechanisms in the heart are still being elucidated. Administration or transgenic overexpression of IGF1 reduced the death of cardiomyocytes in settings of disease (64, 65), suggesting that the class I_A PI3Ks (which lie downstream of IGF1 receptors) are the isoforms responsible for PI3K-dependent cell survival in the heart.

In non-cardiac cells, Akt promotes cell survival via inhibition of pro-apoptotic factors such as procaspase-9 and Bad (66, 67). *In vitro* studies have demonstrated that PI3K-dependent activation of Akt in neonatal cardiomyocytes resulted in Bad phosphorylation and prolonged cell survival (68, 69). However, expression of Bad is significantly downregulated during the neonatal period and is very low in the adult heart (70), suggesting that inhibition of Bad via phosphorylation by Akt is not a major contributor to cell survival in the adult heart. Caspase-9 activity was elevated in neonatal cardiomyocytes subjected to hypoxia, but decreased when the cells were pre-treated with epoxyeicosatrienoic acid, a fatty acid that induces activation of the PI3K-Akt pathway (69). Activation of PI3K-Akt was also associated with an increase in X-linked inhibitor of apoptosis protein (XIAP) expression (69). XIAP is an anti-apoptotic protein that prevents cell death by binding to caspases (71). Thus, in the neonatal heart, PI3K-Akt signaling appears to promote cell survival via activation of XIAP and inhibition of Bad and caspase-9. Further investigation is required to identify the downstream substrates responsible for the anti-apoptotic effects of PI3K signaling in the adult heart.

6.3. PI3K(p110α) reduces cardiac fibrosis

A fine network of collagen fibres surrounds cardiac myocytes under normal conditions. This collagen matrix is important for structural support and myofibrillar alignment when the heart contracts. However, a build-up of collagen in the interstitial space between cardiomyocytes (i.e. increased interstitial fibrosis) is detrimental for heart function, disrupting the cardiac conduction system (72, 73) and causing mechanical stiffness (74).

PI3K(p110α) appears to be a negative regulator of fibrosis, as hearts of dnPI3K mice contained greater interstitial fibrosis compared with non-transgenics in response to pressure overload, while hearts of caPI3K mice contained less fibrosis (41). Microarray analysis suggests that PI3K-dependent regulation of fibrosis during pressure overload occurs at the level of transcription, as dnPI3K mice expressed higher levels of fibrotic genes (such as procollagen, fibronectin and fibrillin), while caPI3K mice expressed lower levels compared with non-transgenics (41).

6.4. The role of PI3K(p110γ) in settings of pathological stress

In general, PI3K(p110γ) activation appears to have a detrimental effect in the heart. PI3K(p110γ) knockout mice were protected from heart failure induced by chronic activation of β-adrenergic receptors (β-ARs), displaying less fibrosis, a significantly smaller hypertrophic response and better heart function than controls (29). PI3K(p110γ) might contribute to cardiac dysfunction via its effects on β-AR internalization (Figure 1). Downregulation and desensitization of β-ARs is a key feature of end-stage heart failure, and is detrimental for heart function (75, 76). Downregulation of β-AR on the plasma membrane is achieved via sequestration of β-ARs in endosomes. This process is dependent on the binding of p110γ to β-AR kinase 1 (β-ARK1) (77). Expression of a catalytically inactive p110γ mutant or disruption of the interaction between β-ARK1 and p110γ restored β-AR signaling and contractile function in transgenic mice subjected to chronic β-AR stimulation (78, 79). The beneficial effects of loss of PI3K(p110γ) may also be related to the maintenance of Ca^{2+} cycling at the level of the sarcoplasmic reticulum (SR) via altered PDE activity. Loss of p110γ causes a decrease in PDE activity resulting in increased intracellular cAMP levels, enhanced phosphorylation of PLN and SERCA2a activity. This leads to an increased SR Ca^{2+} load and enhanced calcium release from the SR (80). Consequently, inhibition of PI3K(p110γ) may be a therapeutic strategy in the treatment of heart failure. Of note, however, the role PI3K(p110γ) in the diseased heart is complex and appears to vary depending on the nature of the pathological stress. PI3K(p110γ) knockout mice displayed an accelerated progression to dilated cardiomyopathy in response to pressure-overload (80, 81).

7. CONCLUSION

PI3K(p110α) and PI3K(p110γ) play very different roles in the heart. PI3K(p110α) is critical for physiological cardiac hypertrophy, and activation of PI3K(p110α) is important for maintaining cardiac structure and function in pathological settings. In contrast, activation of PI3K(p110γ) is generally detrimental for heart function, reducing heart contractility via the internalization of β-ARs and inhibition of SERCA2a activity (Figure 1). Further investigation of the signaling pathways mediated by these isoforms of PI3K might lead to the development of new treatments for cardiovascular disease and failure.

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