Mission Impossible: IGF-1 and PTEN Specifically “Akt”ing on Cardiac L-Type Ca\(^{2+}\) Channels

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The complex functional properties of cardiomyocytes are precisely regulated, oftentimes with millisecond precision. There are a multitude of signaling cascades and associated molecules that participate in this regulation. Insulin-like growth factor-1 (IGF-1), IGF receptors, phosphoinositides, phosphatidylinositol 3-kinase (PI3K), Akt or phosphokinase B (PKB), L-type Ca\(^{2+}\) channels (LTCC), and intracellular Ca\(^{2+}\) include some of the most studied and essential molecules governing many aspects of cardiac biology. In fact, it is difficult to name a cellular process in cardiomyocytes that is not in some way regulated by one or more of these molecules. This raises a fundamental question—is it possible for this group of diverse proteins and second messengers to work together, and yet provide the needed specificity in the cellular responses? The article by Sun et al in this issue of Circulation Research provides powerful evidence for a critical interface between cardiomyocyte Ca\(^{2+}\) signaling and PI3K-regulated pathways. Perhaps more importantly, the work begins to unravel some of the mystery behind the specificity of these seemingly diffuse pathways.

Rapid progress in recent years has provided strong evidence for the crucial role of a family of evolutionary conserved lipid kinases, PI3Ks, in mediating a wide range of cellular processes in response to stimulation by growth factors and hormones. This mediation sets in motion a coordinated series of events leading to cell growth, cell cycle entry, cell migration, and cell survival. Various signaling proteins, including protein serine-threonine kinases, protein tyrosine kinases, and exchange factors that regulate heterotrimeric guanosine triphosphate (GTP)-binding proteins (G proteins), have domains that specifically bind to phosphorylated phosphoinositides. These proteins are located in the cytosol of unstimulated cells but, in response to lipid phosphorylation, accumulate at the plasma membrane. These proteins become activated at the membrane and initiate a wide array of local responses, including assembly of signaling complexes, and priming of protein kinase cascades.

Multiple forms of PI3Ks exist in higher eukaryotes. Class I PI3K can be activated by either receptor tyrosine kinase (RTK)/cytokine receptor activation (class I\(_{\alpha}\), PI3K\(_{\alpha}\), \(\beta\), and \(\delta\)) or G protein–coupled receptors (GPCR) (class I\(_{\beta}\), PI3K\(_{\gamma}\)). Class I enzymes are heterodimers of catalytic subunits (the ubiquitous p110\(\alpha\) or more tissue-restricted p110\(\beta\) or p110\(\delta\)) and the regulatory adapter subunit (p85\(\alpha\), p85\(\beta\), p55). The regulatory subunit maintains the p110 catalytic subunit in a low-activity state in quiescent cells and mediates its activation by direct interaction with phosphorytrosine residues of activated growth factor receptors or adaptor proteins. The activated PI3K converts the plasma membrane lipid phosphatidylinositol-4,5-bisphosphate [PI(4,5)P\(_2\)] to phosphatidylinositol-3,4,5-trisphosphate [PI(3,4,5)P\(_3\)], leading to the recruitment of protein kinase Akt and its activator, 3-phosphoinositide-dependent protein kinase-1 (PDK1), to the cell membrane via interactions between kinase pleckstrin homology domains and the 3'-phosphorylated lipid. The resultant colocalization of Akt and PDK1 causes the latter to phosphorylate and activate the former. The final results are the activation of a range of downstream targets.

Class I\(_{\alpha}\) and class I\(_{\beta}\) PI3Ks mediate distinct phenotypes in the heart and are under the negative control by the 3'-lipid phosphatase, phosphatase and tensin homolog on chromosome ten (PTEN), which dephosphorylates PI(3,4,5)P\(_3\) into PI(4,5)P\(_2\). A large body of evidence has shown that PI3K\(_{\alpha}\), \(\gamma\), and PTEN are expressed in cardiomyocytes, fibroblasts, endothelial cells, and vascular smooth muscle cells, where they modulate cell survival/apoptosis, hypertrophy, and contractility. Indeed, several transgenic and knockout models that have been generated in recent years support a fundamental role of PI3K/PTEN signaling in the regulation of myocardial contractility and hypertrophy.

Genetic ablation of PTEN caused both hypertrophy and impaired contractility in the knockout mice. Interestingly, the hypertrophy effect of PTEN knockout in the heart was specifically attributable to the PI3K\(_{\alpha}\)-mediated pathways, whereas the decrease in contractility was directed by PI3K\(_{\gamma}\). This earlier work demonstrated a critical dichotomy in PI3K signaling in the heart based on the distinct PI3K isoforms, yet the downstream mediators ultimately responsible for the different phenotypic responses have not been fully defined. Given the central roles of cardiac LTCCs in both cellular hypertrophy and in contractility, these channels seemed a logical target to investigate for differential regulation by the PI3K\(_{\alpha}\) and PI3K\(_{\gamma}\).

In this issue of Circulation Research, Sun and colleagues, using combinations of genetically altered mice, provide new evidence linking the regulation of cardiac LTCCs by IGF-1...
and PTEN to PI3Kα-mediated hypertrophy response, but not the PI3Kγ-mediated impairment in contractility. Sun et al demonstrate that IGF-1 or inactivation of PTEN enhances L-type Ca\(^{2+}\) current (\(I_{\text{Ca,L}}\)) via PI3Kα-dependent Akt activation (see the Figure). This is consistent with the previous notion that IGF-1 receptor tyrosine kinase stimulation activates Akt via PI3Kα, as well as the previous findings that constitutively active overexpressed Akt in the myocardium increases \(I_{\text{Ca,L}}\) in ventricular myocytes. On the other hand, specific regulation of LTCCs by PI3K isoforms have previously been shown in portal vein myocytes in which platelet-derived growth factor (PDGF) increases \(I_{\text{Ca,L}}\) via the class I\(_1\) isoform, p110β, whereas angiotensin II–mediated increases in \(I_{\text{Ca,L}}\) are dependent on the class I\(_2\) isoform, p110γ. Adding to the complexity is one previous study showing the negative modulation of the LTCCs by activated G\(_{\text{aiq}}\) in cardiac myocytes and its possible mediation via PI3Kα.

Sun and colleagues further documented that basal Akt activity was insufficient to upregulate cardiac \(I_{\text{Ca,L}}\) when PTEN phosphatase activity was normal. Indeed, when PTEN activity is abolished, the PI3Kα activity was sufficient to activate Akt and increase \(I_{\text{Ca,L}}\). In contrast, knockout of PI3Kγ in PTEN-null mice did not impact basal or IGF-1–stimulated \(I_{\text{Ca,L}}\).

The study raises many mechanistic questions. An obvious one is how does Akt regulate the channel. The authors suggest it could be by direct phosphorylation of the channel based on consensus Akt sites present in the pore-forming Ca\(_{1,2}\) subunit; however, no biochemical data or other evidence are presented to support such direct phosphorylation of Ca\(_{1,2}\). Perhaps direct phosphorylation of the channel occurs, but it could equally well involve any of the subunits of the channel including the various Ca,β subunit isoforms present in the heart. Alternatively, it is equally plausible that other intermediate proteins may be the actual substrate for Akt. For example, elegant studies of IGF-1 stimulation of neuronal Ca\(_{1,2}\) channels previously demonstrated an essential role for Akt signaling, but ultimately these studies revealed that activation of c-Src and tyrosine phosphorylation of Y2122 in the C terminus of Ca\(_{1,2}\) was the final pathway for regulation of the channel. Although this neuronal isoform of Ca\(_{1,2}\) was initially suggested not to be expressed in cardiac muscle, it now seems possible that some level of expression occurs in the heart. Thus, defining the final steps linking Akt to the LTCCs in cardiomyocytes represents an important area of future investigation.

Perhaps one of the most important and intriguing questions is whether the basis for the differential effects of PI3Kα and PI3Kγ in the heart are largely attributable to differences in compartmentalization of these isoforms. In other words, who are the different neighbors for each PI3K isoform, where is the neighborhood in the cell, and what sort of traffic is routed through the neighborhood? A role for PI3K in the compartmentalization of the β\(_1\)-adrenergic/cAMP/PKA regulation of cardiac contractile responses has previously been identified. In the case of LTCCs, there is growing evidence of differentially localized subpopulations of channels in cardiomyocytes that are partly manifest by differences in auxiliary Ca,β subunit composition. In addition, colocalization of LTCCs in caveolae with proteins of the cAMP/PKA signaling cascade is essential for the regulation of LTCCs by β\(_2\)-adrenergic signaling. Does PI3Kα show a comparable colocalization with Ca\(_{1,2}\) channels in caveolae? Given that caveolae have typically not been defined as rich in phosphoinositols, potentially other subdomains of the sarcolemma that are enriched in inositol phospholipids may be critical sites of compartmentalization. Adding to the challenge of defining this critical neighborhood is the dynamic nature of PI3K localization in the cell as the kinase is activated.

Although the focus of Sun et al and our discussion has been on the PI3Kα-mediated stimulation of \(I_{\text{Ca,L}}\) via Akt, one should not jump to the conclusion that PI3Kγ cannot play an important role in regulation of \(I_{\text{Ca,L}}\). Under basal conditions, there is no clear evidence for any impact of PI3Kγ on \(I_{\text{Ca,L}}\); however, in the presence of β\(_1\)-adrenergic stimulation, Xiao and colleagues demonstrated that PI3K activity blunts the stimulation of \(I_{\text{Ca,L}}\). Putting together previous studies showing that PI3Kγ activity reduces cellular cAMP levels and the work of Xiao et al, it seems possible that PI3Kγ may be the isoform specifically responsible for the blunted β\(_1\)-adrenergic stimulation of \(I_{\text{Ca,L}}\). However, this hypothesis remains to be tested.

Ultimately, defining these complex and interrelated signaling pathways is essential for advancing our understanding of normal physiology and pathological alterations such as hypertrophy and heart failure. The importance of precise cellular signaling using this PI3Kα-Akt and Ca\(_{1,2}\) pathway is
demonstrated by the essential role of PI3Kα signaling in physiological hypertrophy. In contrast, signaling via PI3Kγ has been implicated in pathological hypertrophy. IGF-1 via Akt can stimulate LTCCs and induce a positive inotropic response in the failing human heart, but whether this response is altered in heart failure or contributes to the pathogenesis is unknown. The involvement of multiple signaling pathways represents a challenge for specificity; on the other hand, it is likely the precise integration of these signaling pathways in defined cellular compartments that enables the appropriate cellular responses. It will take more time and research before we fully learn how cardiomyocytes can accomplish the impossible mission of IGF-1 and PTEN, specifically “Akt”ing on LTCCs, and before we understand where the mission goes wrong in disease.

Acknowledgments
This work was supported by the Department of Veteran Affairs Merit Review Grant and the National Institutes of Health Grants (HL 75274 to N.C.).

References
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Circ Res. 2006;98:1349-1351
doi: 10.1161/01.RES.0000228347.79970.24

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