MiniReview

Integrin-Linked Kinase at the Heart of Cardiac Contractility, Repair, and Disease

Gregory E. Hannigan, John G. Coles, Shoukat Dedhar

Abstract—Recent advances in cardiac physiology identify the integrin-linked kinase (ILK) as an essential molecule regulating cardiac growth, contractility, and repair. A key transducer of biochemical signals initiated at the plasma membrane by cell–matrix interactions, ILK now emerges as a crucial player in mechanotransduction by integrins. Animal models have been particularly instructive in dissecting the cardiac functions of ILK and its associated proteins, such as parvins and PINCH, and have clearly established ILK as a major contributor to cardiac health. ILK gene knockouts in mice, flies, and worms result in early embryonic lethality because of cell adhesion defects and cytoskeletal disorganization. Although widely distributed in mammalian tissues, ILK expression is highest in the heart, and cardiac-specific ablation of ILK causes cardiomyopathy and sudden death in mice. ILK protein complexes are found in the sarcomere, which is the basic contractile unit of myocytes. A natural inactivating mutation in the kinase domain of ILK disrupts ILK protein interactions in the sarcomere, causing a contractile defect in the zebrafish heart. The relatively subtle phenotype of mutant ILK hearts, compared with ILK-ablated hearts, suggests multiple cardiac ILK functions. Cardiac-specific expression of ILK in transgenic mice induces a hypertrophic program, pointing to ILK as a proximal regulator of multiple hypertrophic signal transduction pathways. ILK protein interactions may also be important in mediating postinfarct cell migration and myocardial repair. (Circ Res. 2007;100:1408-1414.)

Key Words: integrin–linked kinase  ■ cardiac hypertrophy  ■ cardiomyopathy

Integrin-linked kinase (ILK) is a widely expressed serine/threonine protein kinase that binds to the cytoplasmic tail of β integrins, linking cell–matrix interactions to signals regulating cytoskeletal remodeling and cellular processes such as growth, proliferation, survival, and differentiation. Genetic ablation in Caenorhabditis elegans and Drosophila confirms that ILK provides an essential link mediating recruitment of actin filaments to the plasma membrane at muscle attachment points, and deletion of ILK in mouse embryos lethally impairs actin dynamics at integrin attachment points in the epiblast. ILK functions as a molecular scaffold, assembling multiprotein complexes that maintain the integrity of integrin adhesion points. ILK also plays a central role in transducing biochemical signals initiated by cell–matrix interaction.

ILK is most abundant in the heart, suggesting that ILK transduces β integrin–dependent biomechanical stresses in contractile cells. Cardiomyocyte-targeted ablation of β integrin causes dilated cardiomyopathy (DCM) in response to pressure overload, further suggesting a role of β integrin in maintaining myocardial structure and function. The cardio-protective function of β integrin indicates that it initiates essential biochemical signals for cardiomyocyte viability. β integrin localizes to costameres, junctional complexes linking the extracellular matrix (eg, collagen IV, laminin) to actin and myosin-containing sarcomeres. Costameres are thus analogous to focal adhesions in other cell types, such as endothelium. Costameres contain proteins, including talin, vinculin, melusin, and α-actinin, which provide structural linkage to sarcomeric actin filaments. ILK localizes to costameres, and genetic experiments indicate it is a key component of the sarcomeric contractile apparatus in vertebrate hearts.

ILK Protein Complexes With PINCH and Parvin Play Critical Roles in the Heart

Originally discovered through its physical interaction with β integrin, ILK transduces signals from growth factors, cytokines and chemokines, in addition to those triggered by cell-matrix interactions. At sites of integrin attachment, ILK is found in ternary complexes with 2 adaptor proteins, parvin and PINCH. PINCH is found in complexes containing ILK and the actin sequestering protein thymosin β4 (Tβ4), which mediates cardiomyocyte migration on extracellular matrix substrata. The 3 members of the parvin family also bind to...
ILK and link it to the actin cytoskeleton. α-, β-, and γ-parvin are involved in regulating cell adhesion and ILK signaling, and an essential role for ILK/β-parvin complexes in cardiac contractile function has recently come to light.8

ILK protein complexes are essential components of the cardiac mechanical stretch sensor in zebrafish.8 The zebrafish main squeeze (msq) mutant causes lethal heart failure attributable to a point mutation (L308P) in the kinase domain of zILK, which disrupts kinase activity and ILK interaction with β-parvin (ParvB).8 ParvB protein levels and colocalization with zILK in costameres are normal in ilkL308P animals, indicating that the ILK–ParvB complex is critical in regulating cardiac contractility. Moreover, the msq phenotype is mimicked by RNA interference–mediated ParvB knockdown and is not rescued by ILKE350K, a partially kinase defective mutant that also impairs ParvB interaction.11 Expression of the stretch-responsive genes atrial natriuretic factor (anf) and vascular endothelial growth factor (vegf) is markedly reduced in ilkL308P mutant hearts. Protein kinase B (PKB) (also known as Akt) is a prosurvival kinase that has been identified as an important target of ILK (reviewed elsewhere).5 The phosphorylation state of PKB at Ser473 was equivalent in whole embryo lysates of both wild-type and msq zebrafish, although cardiac-specific Ser473 phosphorylation was not analyzed. Expression of wild type human ILK or activated PKB (but not catalytically inactive ILKE211K) rescued the msq phenotype, suggesting a conserved ILK–PKB signaling requirement in cardiac contractility.8

ILK regulation of PKB is likely complex. Genetic studies in invertebrates have identified a noncatalytic adaptor function of ILK in linking integrins to the actin cytoskeleton,3,4 and because the ILK sequence lacks residues thought to be critical for catalytic function, this has led to some questions regarding the kinase activity of ILK.12 However, in-gel kinase assays using PKB and myosin light chain as substrates provide rigorous biochemical evidence supporting ILK kinase activity.13,14 ILK-mediated PKB Ser473 phosphorylation is commonly seen in many cancer cells (reviewed elsewhere) and is likely cell context–dependent. Ser473 phosphorylation is not impaired in ilkL308P mouse fibroblasts5 or embryonic neurons,15 but is inhibited in ilkL308P hearts16; thus it is critical to establish whether this results from direct phosphorylation, or the noncatalytic function of ILK.

In addition to Ser473, phosphorylation at Thr308 is thought to be required for full PKB activation and signaling. Mammalian target of rapamycin (mTOR) is a prohypertrophic serine/threonine kinase also implicated in Ser473 phosphorylation.17 In noncardiac cells, Thr308 phosphorylation is sufficient for signaling downstream targets such as glycogen synthase kinase (GSK)3β and p70S6K, but not other PKB targets,18,19 suggesting the lack of Ser473 phosphorylation in ilkL308P hearts could selectively affect survival signaling by targets such as Foxo1/3a.18,19 An outstanding question is that of potential molecular interactions among ILK, PKB, and mTOR during cardiac hypertrophy, and Ser473 phosphorylation in mTOR-deleted hearts needs to be examined.

Conditional ILK deletion in the mouse heart causes spontaneous DCM and sudden death at 6 to 12 weeks of age (Figure 1).16 Cre expression driven by the muscle creatine kinase promoter also resulted in efficient deletion of ILK from skeletal muscle. Surprisingly, these mice showed no obvious muscle defects and grew normally, although early death from DCM would preclude development of later defects in ilkL308P skeletal muscle. Melusin is a muscle-specific, β1 integrin–binding protein that localizes in costameres.20 Melusin-null mice have normal cardiac development and basal function; however, in response to pressure overload, melL−/− hearts rapidly progress to DCM and heart failure.21 The phenotype in ilkL308P hearts is similar to mice with cardiac-specific ablation of β1 integrin,7 FAK,22 or melusin. However, loss of ILK causes early spontaneous onset of DCM, whereas β1 integrin, FAK, or melusin ablation causes DCM in response to an applied cardiac stress, suggesting ILK is an effector of other critical signaling pathways. Notably, ILK ablation resulted in reduced β1 integrin protein and abrogated FAK Tyr576 (ie, activating) phosphorylation, demonstrating that loss of ILK in the heart disrupts β1 integrin–FAK signaling. PKB Ser473 phosphorylation was suppressed in ilkL−/− and mel−/− hearts,21 suggesting ILK and melusin may interact to protect against DCM. Interestingly, ilkL−/− mouse hearts exhibit disrupted myocyte architecture and extensive fibrosis, whereas in ilkL308P zebrafish, cardiomyocytes are structurally intact, suggesting distinct ILK catalytic and scaffolding functions in vertebrate cardiomyocytes.

Tβ4 is highly expressed in migrating cardiomyocytes and is also secreted into the extracellular space. PINCH was identified as a Tβ4 binding protein, and Tβ4–PINCH–ILK complexes were demonstrated in cardiac cell lysates.19 Treatment of explant-derived cardiomyocytes with Tβ4 stimulated migration on collagen matrices, concurrent with PI3K–PKB phosphorylation, decreased infarct scar volume and postinfarct apoptosis, and improved ventricular function, all suggesting a role for ILK in post-myocardial infarction cardiac repair. Tβ4 did not increase the number of infarct-resident c-kit+ or Sca-1+ cardiomyocytes, suggesting Tβ4–PINCH–ILK complexes mediate cardioprotection early after infarction, rather than stimulating stem cell recruitment to the damaged tissue. Together with the zebrafish and mouse cardiac knockout data, these results have highlighted critical roles for ILK–PINCH–parvin complexes in cardiac physiology and demonstrate the catastrophic outcome accompanying loss of ILK in the heart.

**Protein Kinase Signaling in Cardiac Hypertrophy**

Cardiac hypertrophy is thought to be an acute compensatory or adaptive response to pressure or volume overload, thus preserving cardiac function. There is a strong association of prolonged hypertrophy with heart failure, suggesting distinct hypertrophic phenotypes.23,24 Clinically, physiological hypertrophy is defined as an increase in cardiac mass in response to normal postnatal maturation, exercise, and hemodynamic loading and is characterized by preserved myocardial architecture, function, and lifespan. Pathological hypertrophy refers to detrimental effects on cardiac structure and function caused by stressors such as hypertension, excess mechanical loading caused by structural lesions, and mutations in myo-
The general view is that hypertrophy associated with heart disease is invariably maladaptive, eventually leading to dilated heart failure. Induction of a “fetal” gene program is also associated with pathological hypertrophy, further suggesting distinct molecular profiles of physiologic and pathologic hypertrophy. It is commonly thought that the initial hypertrophic response is adaptive, whereas chronic hypertrophy is associated with progression to heart failure. However, this idea does not explain why the hearts of high-performance athletes, a classic instance of physiologic hypertrophy, do not routinely deteriorate to dilatation and failure. Thus, additional factors likely superimpose on, or synergize with, hypertrophy to instigate decompensation.

Work over the last decade has identified protein kinase signal transduction pathways that are capable of stimulating cardiac hypertrophy. These studies have used gene transfer to primary cardiomyocyte cultures, and, more importantly, a number of transgenic mouse hypertrophy models have been developed that extend the in vitro findings to a more physiologic setting. Typically, the cardiac-restricted α-myosin heavy chain (MHC) promoter is used to drive expression of signaling molecules, facilitating cross-study comparison of their effects on cardiomyocyte growth. Experimental models indicate that cardiac cell growth is regulated by many of the same protein kinase cascades that regulate noncardiac cell growth and proliferation. Interestingly, cardiomyocytes generally do not proliferate in response to these same signals, even when overexpressed to levels that cause oncogenic transformation of noncardiac cells. This is typically attributed to the terminally differentiated state of cardiomyocytes; however, the role of growth regulatory pathways in resident cardiac stem/progenitor cell populations warrants increased attention. Here we summarize recent work identifying ILK as a proximal regulator of cardiac hypertrophic pathways.

Recent reviews of prohypertrophic cardiac signaling pathways propose that signals can be adaptive or maladaptive and, consistent with this notion, calcineurin (Cn) and Ras activities have been selectively linked to pathologic hypertrophy. However, prohypertrophic molecules may be involved in pathologic signaling, and biochemical analysis of hypertrophied and failing human hearts suggests partial overlap in the pathways underlying these 2 conditions. Cn-deficient mice have smaller than normal hearts and cannot mount a hypertrophic response to pressure overload. There appears to be progressively robust activation of Cn signaling.
ILK Is a Membrane-Proximal Activator of Hypertrophic Signal Cascades

Transgenic models identify a number of molecules, including β1 integrin and ILK, as mediators of cardiomyocyte hypertrophy. Hypertrophy in rat ventricular myocytes requires cell–matrix interaction, and adenosine virus expression of β1 integrin potentiates hypertrophy,37 indicating that β1 integrin transduces signals regulating myocyte size. Integrin-mediated adhesion of cardiomyocytes to fibronectin stimulates PINCH-dependent recruitment of ILK to costameres, and deletion of ILK induces marked myocyte apoptosis, indicating a role for integrin–ILK signaling in regulation of cardiac cell growth.38 In noncardiac cells, ILK activation initiates a cascade of protein kinase phosphorylations, including PKB, GSK3β, p38 mitogen-activated protein kinase (p38MAPK), extracellular signal-regulated kinases (ERKs), and mTOR, all implicated in cardiac cell growth.2

Phosphoinositide-3′-OH kinases (PI3K) are key regulators of fundamental cell functions such as growth, proliferation, differentiation, and survival.39 ILK signaling by cell–matrix or growth factor activation is dependent on PI3K activity,2 and purified recombinant ILK is activated in vitro by the phosphatidylinositol-3,4,5-trisphosphate product of PI3K activity,40 suggesting this lipid binds to the pleckstrin homology (PH)-like domain of ILK. A point mutation in this domain (ILK R211A) renders ILK catalytically inactive, further supporting a potential mechanism for PI3K-dependent activation. Cardiac-specific overexpression of constitutively active PI3K catalytic subunit (p110α) in mice results in enlarged hearts and myocytes, whereas expression of a dominant negative mutant PI3K regulatory subunit (p85α) mutant leads to smaller than normal hearts.41,42 Cardiac expression of the insulin-like growth factor-1 receptor induces physiologic hypertrophy in transgenic mice. A dominant negative p110α subunit blocks insulin-like growth factor-1 receptor–induced hypertrophy, indicating that PI3K signaling mediates physiologic hypertrophy in vivo.43,44

Consistent with PI3K-dependent activation, ILK signaling is inhibited by PTEN, a phosphoinositide lipid phosphatase antagonist of PI3K signaling.47,48 Cardiac-specific knockout of PTEN is sufficient to induce hypertrophy,49 likely by upregulating ILK and PKB (Figure 2). Cardiac overexpression of PKB induces hypertrophy in transgenic mice,50 whereas a catalytic mutant of PKB inhibits PI3K-induced hypertrophy.51 PKB also phosphorylates and inactivates GSK3β, which may lead to nuclear translocation of β-catenin and activation of β-catenin/T-cell factor/lymphocyte enhancer factor (Tcf/Lef)-dependent gene transcription implicated in pathological hypertrophy.52 Some reports have implicated β-catenin and PKB in physiological growth of the heart.53 Inhibition of GSK3β activity promotes cardiac hypertrophy,54 and, conversely, expression of a constitutively active GSK3β S9A mutant (not phosphorylated at Ser9) suppresses both isoproterenol- and pressure-induced hypertrophy in vivo,55 supporting the idea that inhibition of GSK3β activity promotes diverse types of hypertrophy.

Cardiac-specific expression of ILK, but not a dominant negative ILK R211A mutant, induces cardiomyocyte hypertrophy in transgenic mice.56 Biochemically, ILK-induced hypertrophy is characterized by activation and increased phosphorylation of a number of prohypertrophic cytoplasmic protein kinases. ILK-induced hypertrophy does not impair basal cardiac function, and there is no evidence of fibrosis, suggesting the PI3K–ILK–PKB pathway mediates physiologic hypertrophy. Conversely, loss of ILK in the heart causes an initial hypertrophy, marked by disorganized cardiomyocyte architecture with deposition of interstitial fibrotic tissue, and ultimately impaired contractility.16

Protein synthesis is required to drive increased cardiac mass in response to hypertrophic stimuli.24 mTOR is a serine/threonine protein kinase that plays a key role in controlling protein synthesis levels in response to changes in the microenvironment of the cell, including nutrient and growth factor levels. S6 kinases promote protein translation by phosphorylating the S6 ribosomal subunit protein, and p70S6K is phosphorylated by mTOR in an ILK- and PKB-dependent manner.57 Inhibition of mTOR in vivo by rapamycin suppresses p70S6K phosphorylation and attenuates overload-induced hypertrophy by 70%.58 Cardiac expression of activated PKB in transgenic mice stimulates mTOR-dependent induction of VEGF,59 and blockade of VEGF signaling promotes transition from compensatory hypertrophy to heart failure.60 In endothelial cells, VEGF induces ILK activity, which in turn stimulates VEGF production,61 indicating a potential positive feedback loop of VEGF–ILK
signaling in physiologic cardiac hypertrophy (Figure 2). ILK-induced hypertrophy in transgenic mice stimulates phosphorylation of p70S6K, whereas cardiac expression of the dominant negative ILK<sup>R211A</sup> mutant does not induce hypertension or p70S6K phosphorylation. These results highlight the potentially critical role that PI3K–ILK–PKB–mTOR signaling plays in the hypertrophic response to myocardial pressure overload.

The 3 classes of MAPKs, ERK1/2, c-Jun N-terminal kinase (JNKs), and p38<sup>MAPK</sup>, have all been shown to modulate ventricular hypertrophy. In noncardiomyocytes, ILK mediates phosphorylation of all 3 classes of MAPKs, suggesting that ILK–MAPK signaling is also involved in cardiac hypertrophy. Cardiac expression of MEK1, an upstream activator of ERKs, induced compensated hypertrophy. Mice did not develop cardiomyopathy up to 12 months of age, indicating that activation of MEK1/ERK signaling is sufficient to induce physiologic hypertrophy. Cardiac MEK1 expression induces phosphorylation of ERK1/2, but not JNK or p38<sup>MAPK</sup>, whereas ILK induces ERK1/2 and p38<sup>MAPK</sup> phosphorylation, consistent with ILK being a proximal activator of MAPK signaling pathways.

Members of the small GTPase (Smg) superfamily regulate remodeling of the actin cytoskeleton by transducing signals initiated by activated growth factor, cytokine, and G protein–coupled receptors and by cell–matrix interactions. Many targets of Smg-dependent signaling have been identified, accounting for their widespread involvement in regulating cell processes, such as migration, gene expression, and vesicle trafficking. Ras and Rho/Rac/Cdc42 subfamily members have also been studied for their role in myocardial hypertrophy and ventricular remodeling. ILK overexpression activates Rac1 and Cdc42 in fibroblasts, and RNA interference–mediated ILK knockdown suppresses extracellular matrix–stimulated Rac/Cdc42 activation in epithelial cells. Cardiac expression of ILK, but not ILK<sup>R211A</sup>, in mice induces selective activation of Rac1 and Cdc42, thus identifying an ILK-Rac1 hypertrophic pathway that is also acutely activated in cardiomyocytes by infection with adenoviruses expressing ILK. In vivo and in vitro, activation of Rac1/Cdc42 is blunted either by infecting with a dominant negative ILK<sup>R211A</sup> adenovirus or by a small molecule inhibitor of ILK, suggesting that ILK activity is required for this induction. ILK activation of Rac1 in epithelial cells occurs via β-parvin binding to α-PIX, a Rac/Cdc42 guanine nucleotide exchange factor. Although this association has not been tested in cardiac cells, it seems likely that ILK/β-parvin/α-PIX complexes are responsible for the observed activation of Rac1, and it may also be possible that contractile functions of β-parvin in the myocardium involve α-PIX and Rac activation.

**ILK Is Upregulated in Hypertrophied Human Hearts**

It is important to know whether prohypertrophic protein kinases are active in human cardiac hypertrophy. Studies addressing protein kinase signaling in human hypertrophy indicate the relevance of the in vivo models. Haq et al<sup>35</sup> reported differential activation of PKB/Akt and MAPK pathways between hypertrophic and failing hearts. ERK1/2, JNK, and p38 were all phosphorylated (activated) in failing hearts, but not in the cases of stable hypertrophy. We reported elevated levels of ILK protein in human hypertrophy caused by congenital or acquired outflow tract obstruction. We saw marked activation of ERK1/2, p70S6K, as well as Rac1 and Cdc42, raising the possibility that pressure overload-induced ILK upregulation activates these signaling events.

Prohypertrophic signaling via ERK1/2, p70S6K, p38<sup>MAPK</sup>, Rac1, and Cdc42 is activated in cardiac ILK transgenic mice and adeno-ILK–infected cardiomyocytes, suggesting that ILK upregulation in response to pressure overload activates these events in human hypertrophy. With respect to ILK signaling, some differences between published studies of mouse and human hypertrophic signaling bear discussion. ERK1/2 and p38 MAPKs are activated in hypertrophic Tg ILK mouse hearts, whereas, in failing human hearts, there is activation of these kinases as well as JNK and PKB, suggesting hyperactivation of a compensatory stress response. These differences also reflect single-agent induction of hypertrophy in the absence of pressure overload in the ILK transgenic mouse, although clinical cases of compensated hypertrophy result from more complex inputs including pressure overload. The activation status of ILK in the failing human heart is unknown.

**Conclusions and Therapeutic Implications**

Upregulation of ILK activity induces compensatory hypertrophy in mouse models, whereas inactivating mutations or loss of expression may contribute to DCM syndromes in humans. If this turns out to be the case, then gene and stem cell therapies could be considered, and the mckCRE ILK<sup>56</sup> and α-MHC ILK<sup>56</sup> mice may prove to be a very useful models to evaluate the efficacies of such therapies. Similarly, ILK activation can promote cardiac repair after myocardial infarction, and new ways to stimulate ILK activity or expression may provide novel approaches for therapeutic interventions post–myocardial infarction. The extent and kinetics of ILK signaling by potential therapeutic agents, such as Tβ4, will need to be established in preclinical trials using these models to obtain maximum efficacy and minimal toxicity. Given the central role of ILK in heart physiology, future studies in this area may prove to be important for a healthy heart.

**Sources of Funding**

Supported by the Canadian Institutes of Health Research, the Heart and Stroke Foundation of Canada (G.E.H. and J.G.C.), the National Cancer Institute of Canada (S.D.), and the Canadian Breast Cancer Research Alliance (G.E.H.).

**Disclosures**

None.

**References**


Integrin-Linked Kinase at the Heart of Cardiac Contractility, Repair, and Disease
Gregory E. Hannigan, John G. Coles and Shoukat Dedhar

Circ Res. 2007;100:1408-1414
doi: 10.1161/01.RES.0000265233.40455.62
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/100/10/1408

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/