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Focal Adhesion Signaling in Heart Failure

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Summary

In this brief review, recent evidence is presented to indicate a role for specific components of the cardiomyocyte costamere (and its related structure the focal adhesion complex of cultured cardiomyocytes) in initiating and sustaining the aberrant signal transduction that contributes to myocardial remodeling and the progression to heart failure (HF). Special attention is devoted to the focal adhesion kinase family of nonreceptor protein tyrosine kinases in bi-directional signal transduction during cardiac remodeling and HF progression. Finally, some speculations and directions for future study are provided for this rapidly developing field of research.

Keywords

costameres; focal adhesion kinase; PYK2; talin; vinculin

Introduction

Heart failure (HF) results from the inability of the heart to pump blood forward at a sufficient rate to meet the metabolic demands of the body, or the ability to do so only at the expense of abnormally high filling pressures. Even before the onset of overt HF, reduced cardiac performance leads to profound mechanical and neurohormonal stresses on the remaining functional myocardium. These stresses trigger signal transduction pathways that ultimately result in additional structural changes in the cardiomyocytes and nonmuscle cells of the heart (so called “myocardial remodeling”) which contribute to further functional deterioration of the already diseased myocardium [7]. The downward spiral in cardiac performance during cardiac remodeling may be initiated by specific signaling molecules that function to integrate both neurohormonal and mechanical signals in order to elicit compensatory responses in an attempt to counteract the functional deterioration. However, some of these compensatory responses ultimately prove detrimental. In this brief review, I will focus on recent evidence to indicate a role for specific components of the costamere (and its related structure the focal adhesion complex) in initiating and sustaining the aberrant signal transduction that contributes to myocardial remodeling and the progression to HF.

Cardiomyocyte costameres are sites of attachment to the extracellular matrix (ECM)

Cardiomyocyte costameres (and their focal adhesion counterparts in cultured cells), are critical cytoskeletal elements involved in bi-directional mechanotransduction [66]. The term “costamere” was first used by Craig and colleagues [58,57] to describe vinculin-containing, rib-like bands that encircle the cardiomyocyte perpendicular to its long axis. In addition to vinculin, costameres contain many other structural elements that coalesce beneath the sarcolemmal membrane, and resemble the metal ribs of a wooden barrel. They flank the Z-disc and overlying I-bands of sarcomeres that run just beneath the cardiomyocyte plasma membrane (FIGURE 1). Immunoelectron and fluorescent microscopic analyses have revealed that cardiomyocyte costameres also form discrete physical attachments between the underlying, outer Z-discs of the muscle cell and their surrounding, 3-dimensional, stress-tolerant ECM. Attachment is mediated in part by integrins, which are cell-surface receptors for a variety of ECM proteins, and the dystrophin-glycoprotein complex, which mediates attachment to the ECM protein laminin. It is through these attachments that costameres directly transmit contractile forces generated within the cardiomyocyte to the surrounding ECM, and where longitudinal displacement of the ECM by adjacent muscle cells is transmitted directly to the internal contractile machinery. Thus, both externally applied and intrinsically generated mechanical loads are transmitted bi-directionally through costameres.

Focal adhesion complexes in cultured cardiomyocytes

Costameres share many of the structural features of cell-to-matrix adherens junctions, and thus may be considered striated muscle-specific elaborations of focal adhesions found in cultured nonmuscle cells [17]. Immunolocalization studies have confirmed that many, if not all of the same proteins that comprise the costamere *in vivo* eventually reassemble within focal adhesions as isolated cardiomyocytes attach and spread in culture. Furthermore, cultured cardiomyocytes that retain contractile activity, or are stimulated to contract in culture, re-assemble their costameric proteins along the cell-substratum interface in register with the overlying Z-discs of their remodeled sarcomeres. These basal, costameric attachment sites, as well as the remodeled cell-to-cell adherens junctions derived from the remaining components of the intercalated disc, provide the major cell adhesion sites of both neonatal and adult cardiomyocytes in culture.

A detailed structural analysis of the various focal adhesion components of cardiomyocyte costameres and focal adhesions has not yet been obtained. However, using photoactivatable fusion proteins of several focal adhesion proteins and interferometric photoactivated localization microscopy, Kanchanawong et al. [34] have described the 3-dimensional organization of focal adhesion complexes in human osteosarcoma and mouse embryonic fibroblast cells (FIGURE 2). Their images reveal a highly organized, vertically layered structure consisting of at least 3 strata: a membrane-apposed integrin signaling layer containing integrin cytoplasmic tails, focal adhesion kinase (FAK) and the adaptor protein paxillin; an intermediate force-transduction layer containing talin and vinculin; and an uppermost actin-regulatory layer containing zyxin, vasodilator-stimulated phosphoprotein (VASP) and α -actinin overlying and attached to the actin cytoskeleton. The integrin cytoplasmic domains and the subcortical actin layer were separated by a distance of

approximately 40nm, indicating an important role for talin, vinculin and other intermediary proteins above the plasma membrane in bi-directional force transmission.

These images provide enormous detail in describing the molecular architecture of focal adhesions, but do not reveal the dynamic nature of focal adhesion formation and dissolution. However, the rapid turnover of focal adhesion components plays a crucial role in cellular differentiation and migration during cardiac development [18,89,30,23,24], and may also be an important regulatory factor during new sarcomere addition in response to hypertrophic stimuli [47,10]. Using fluorescence recovery after photobleaching (FRAP) and mathematical modeling, Ingber and colleagues [42] showed that various components of the focal adhesion complex display residence times that vary from as little as 1 sec for vinculin, and up to 111 sec for talin. Sanger and co-workers [86] had previously observed a similar dynamic range of exchange between costamere/Z-disc proteins and the cytoplasm of spreading skeletal muscle myotubes. Using FRAP, we subsequently demonstrated that the phosphorylation of FAK, a critical component of the signaling layer of cardiomyocyte focal adhesions, regulates the stability of paxillin within cardiomyocyte focal adhesions, and ultimately controls the rate of cell spreading and myofibrillar organization of cultured cardiomyocytes in response to both static stretch, and the hypertrophic agonist endothelin-1 [10]. Thus, the dynamic nature of cytoskeletal assembly and disassembly within focal adhesion complexes appears critical during the response of cultured cardiomyocytes to neurohormonal and mechanical stimuli.

Focal adhesion complexes assemble in response to mechanical overload *in vivo*

In pressure-overloaded feline myocardium, Kuppaswamy and co-workers [39,41] first demonstrated the cytoskeletal assembly of c-Src and other signaling proteins, which was partially mimicked *in vitro* using adult feline cardiomyocytes embedded within a three-dimensional collagen matrix and stimulated with an integrin-binding Arg-Gly-Asp (RGD) peptide [41]. In subsequent elegant studies, Franchini and co-workers [20,15,85], analyzed the rapid assembly of focal adhesion complexes in response to pressure-loading of the isolated perfused rat heart. The assembly of focal adhesion complexes was also an early response of cultured cardiomyocytes to a variety of neurohormonal and mechanical stimuli that ultimately lead to cardiomyocyte hypertrophy [76,16,63,82,84].

Clustering of β_1 -integrins at the sarcolemmal membrane and their attachment to ECM proteins were critical factors in the regulation of focal adhesion assembly in these settings. Ross and colleagues [75] further demonstrated the importance of β -integrins in costamere assembly. They used Cre recombinase driven by the myosin light chain-2 ventricular (*MLC-2v*) promoter to induce β_1 -integrin gene excision exclusively in ventricular cardiomyocytes. They found that β_1 -integrin knockout mice had significantly depressed contractile performance with extensive cardiac fibrosis, and were intolerant to pressure-overload produced by transverse aortic coarctation (TAC). Surviving mice developed spontaneous heart failure by 6 months of age, with abnormal myocyte membrane integrity as determined by Evan's blue dye staining. This group subsequently described the structural and functional consequences of cardiomyocyte-specific excision of the β_1 -integrin gene in adult cardiomyocytes [43]. Using a tamoxifen-inducible promoter to drive expression of

Cre recombinase, even partial excision of the β_1 -integrin gene resulted in multiple defects in mechanotransduction signaling when hearts were stressed by TAC. Adaptive hypertrophy was blunted, and the response to adrenergic stimuli was also markedly impaired. Thus, these results demonstrated a critical function of β_1 -integrins in the postnatal myocardium, and first linked defects in β_1 -integrin-dependent costamere assembly to the development of cardiomyopathy.

In the majority of aforementioned studies, a hemodynamic stress in the form of an increase in ventricular afterload of the intact heart [39,41,20,85,75,43] was used to stimulate focal adhesion assembly. However, Domingos et al. [15] showed that increasing ventricular preload (by raising diastolic pressure from 0 to 15 mmHg in the beating, isolated perfused rat heart) also rapidly increased FAK tyrosine phosphorylation and binding of c-Src and Grb2 to FAK. This was paralleled by activation and binding of ERK1/2 to the cardiomyocyte cytoskeleton, indicating enhanced focal adhesion signaling in response to increased diastolic stress. Balloon inflation to raise intraventricular pressure in hearts perfused with cardioplegic solution also activated FAK and ERK1/2. In contrast, early induction of volume overload 4 weeks after surgically induced mitral regurgitation in the dog decreased focal adhesion signaling [65]. Similarly, rats with surgically induced aortocaval fistulae demonstrated increased ECM degradation, decreased focal adhesion signaling, and increased apoptosis [74]. Downregulation of focal adhesion signaling was mediated by the activation of PTEN, a dual lipid and protein phosphatase. Beta adrenergic blockade prevented the PTEN activation, the downregulation of focal adhesion signaling, and the increased rate of apoptosis, but had little effect on ECM degradation. These data suggest that volume overload *per se* is a relatively weak stimulator of focal adhesion signaling as compared to pressure overload of the intact heart. Attempts to model the differential effects of pressure vs. volume loading by subjecting cardiomyocytes to transverse vs. linear stretch support these observations [78,21,73].

β_1 -integrin cytoplasmic domains form cytoskeletal attachments through talin dimers

Unlike growth factor receptors, the cytoplasmic tails of integrins do not possess intrinsic catalytic activity. However, they interact with other cytoskeletal proteins to transmit extrinsically applied and internally generated mechanical force. A major binding partner of the β_1 -integrin cytoplasmic domain is the cytoskeletal protein talin, which is a rod-shaped, multi-domain protein involved in bi-directional activation of integrins [70]. Cell-surface integrins can exist in either low- or high-affinity states, and cellular modulation of integrin affinity is in part accomplished by reversible binding of the talin N-terminal, globular head domain to the C-terminal, β_1 -integrin cytoplasmic tail [8]. Cardiomyocytes predominantly express the muscle-specific β_{1D} -integrin isoform, which has the highest binding affinity for talin of the various β -integrin cytoplasmic domains. This observation suggests that integrin engagement to the cardiac ECM favors a mechanically strong, activated integrin binding conformation [2].

The mammalian genome contains two genes for talin encoding two structurally similar proteins (talin-1 and talin-2) that share 74% sequence identity [92]. The specific function of each talin isoform is not known, but it appears that the talin-2 isoform plays a unique role in

muscle development and disease. Localization of talin-2 is restricted to costameres and intercalated discs in striated muscle [72], but talin-2 appears dispensable in the presence of talin-1 for normal costamere assembly. However, deletion of both genes produced a severe defect in sarcomere assembly in striated muscle, with profound defects in the assembly of focal adhesion complexes and sarcomeres by cultured myoblasts isolated from double knockout embryos [11]. The failure of normal sarcomere development in these immature muscle cells also highlights the potential importance of talin (and perhaps other cytoskeletal linker proteins) in adaptation to hypertrophic stimuli.

Ross and colleagues [46] recently evaluated talin-1 and talin-2 expression in the normal embryonic and adult mouse heart, as well as in control and failing human adult myocardium. Using isoform-specific antibodies and nonfailing human cardiac tissue, they showed that talin-1 was only weakly detected in the cardiomyocyte plasma membrane, and rarely co-localized with dystrophin, a muscle-specific costameric protein (FIGURE 3). Talin-1 was also more readily detected in nonmuscle cells of the cardiac interstitium. In contrast, talin-2 was readily detected in a costameric pattern in cardiomyocytes as demonstrated by strong co-localization with dystrophin. Talin-1 function was then tested in the basal and mechanically stressed myocardium after cardiomyocyte-specific excision of the talin-1 gene. They found that during embryogenesis, both talin isoforms are highly expressed, but talin-2 is the main isoform expressed in adult mammalian cardiomyocytes, where it was predominantly localized to costameres. However, talin-1 expression was up-regulated during cardiac hypertrophy, suggesting that it plays an important role in the compensatory response of the heart to stress. In human failing heart, cardiomyocyte talin-1 was also increased as compared to control samples from normal functioning myocardium. Talin-1 knockout mice showed normal basal cardiac structure and function, but when subjected to pressure overload, these animals showed blunted hypertrophy, less fibrosis, but improved cardiac function versus controls. Overall, these data suggested that reduction of cardiomyocyte talin-1 expression might lead to improved cardiac remodeling following pressure overload.

Exactly how talin isoforms mediate integrin-dependent signal transduction in the heart remains unknown. Adhesion to ECM proteins via β_1 -integrins causes the recruitment of talin dimers to the cytoplasmic face of the sarcolemmal membrane, leading to integrin transition to its high affinity state. This process is an important, early step in “outside-in” signaling during cell attachment of cultured cardiomyocytes, and may play an important functional role in costamere assembly during the addition of new sarcomeres *in vivo*. Conversely, talin activation by a number of intracellular signaling pathways causes the physical displacement of α -integrin subunits, thereby allowing for high-affinity ECM engagement during “inside-out” signaling [80,2]. Intracellular talin binding alone is sufficient to alter the conformation of integrin extracellular domains and promote their attachment to ECM proteins [90]. The recruitment of talin involves the Src-dependent tyrosine phosphorylation of the β -integrin tail, and its recognition by the talin head region [1]. Thus, intracellular stimuli that cause Src-dependent integrin phosphorylation promote the activation of integrins via talin recruitment to costameres, and stimulate costamere formation during inside-out signaling. Subsequent recruitment of additional cytoplasmic

linker proteins (such as paxillin and vinculin) to the costamere may also be required for Z-disc assembly and premyofibril formation during myofibrillogenesis [13,79].

FAK and cardiomyocyte mechanotransduction

Talin localization during integrin engagement and clustering may initiate outside-in signaling, but talin, like β_1 -integrin cytoplasmic domains, has no intrinsic catalytic activity capable of relaying biochemical signals into the cell interior. However, secondary recruitment and activation of protein tyrosine and serine/threonine kinases to the cytoplasmic adhesion plaque may accomplish this function. FAK is clearly one candidate enzyme that is responsible for integrin-mediated mechanotransduction within cardiomyocyte focal adhesions and costameres. As indicated above, FAK is a nonreceptor protein tyrosine kinase that functions as an “activatable scaffold” [69] in integrin-dependent signal transduction. FAK contains a central kinase domain flanked by long N- and C-terminal extensions (FIGURE 4). An autoinhibitory FERM domain, located within the N-terminal region of FAK, associates with the plasma membrane via its interaction with several different growth factor receptors. The C-terminal region of FAK comprises the focal adhesion targeting (FAT) domain. The FAK FAT domain binds directly to paxillin and talin, which in turn bind to the cytoplasmic tail of β_1 -integrins at sites of integrin clustering. Once localized, FAK phosphorylates itself at a single tyrosine residue (Y₃₉₇). This autophosphorylation site serves as a high-affinity binding site for the SH2 domain of Src-family protein tyrosine kinases [68]. Once bound to FAK, active Src then phosphorylates FAK at residues Y₅₇₆ and Y₅₇₇ within its catalytic domain (which augments FAK kinase activity toward exogenous substrates), and at Y₈₆₁ and Y₉₂₅ near its C-terminus [19]. Phosphorylation of the Y₈₆₁ site promotes the binding of p130^{Cas} to FAK [45]. The Y₉₂₅ phosphorylation site promotes the binding of Grb2 to FAK, and other adapter proteins and kinases containing SH2 domains. The FAK-Src complex can also phosphorylate paxillin, p130^{Cas}, and other cytoskeletal proteins involved in costamere and myofibrillar assembly.

FAK serine phosphorylation and sarcomere assembly

In addition to multiple tyrosine phosphorylation sites, FAK contains several serine residues (S₇₂₂, S₈₄₃, S₈₄₆, and S₉₁₀) that undergo reversible phosphorylation in response to hypertrophic stimuli. These serine residues are in close proximity to critical protein-protein interaction sites within the C-terminal region of FAK, such as the binding site for p130^{Cas}, Grb2, and the adjacent FAT domain. The functional role of FAK serine phosphorylation in cardiomyocytes is largely unknown, but one report indicates that serine (and tyrosine) phosphorylation of FAK increases dramatically in hypertensive rats, with different sites of phosphorylation appearing to regulate FAK subcellular localization [91]. Our group [10] recently demonstrated that endothelin-1 and other hypertrophic factors induced a time- and dose-dependent increase in FAK-S910 phosphorylation. Endothelin-induced FAK-S910 phosphorylation required endothelin Type A receptor-dependent activation of Protein Kinase C δ (PKC δ) and Src via parallel Raf-1 $\rightarrow$ MEK1/2 $\rightarrow$ ERK1/2 and MEK5 $\rightarrow$ ERK5 signaling pathways. Replication-deficient adenoviruses expressing wild-type FAK and a non-phosphorylatable, S910A-FAK mutant were then used to examine the functional significance of FAK-S910 phosphorylation. Unlike wildtype FAK, S910A-FAK increased the half-life of GFP-tagged paxillin within costameres (as determined by total internal

reflection fluorescence microscopy and FRAP) and increased the steady-state FAK-paxillin interaction (as determined by co-immunoprecipitation and Western blotting). These alterations resulted in reduced neonatal rat ventricular myocyte (NRVM) sarcomere reorganization and cell spreading. Finally, we found that FAK was serine-phosphorylated at multiple sites in non-failing, human left ventricular tissue, and both FAK-S910 phosphorylation and ERK5 expression were dramatically reduced in patients undergoing heart transplantation for end-stage dilated cardiomyopathy. These results suggest that reduced FAK-S910 phosphorylation may contribute to sarcomere disorganization that is frequently observed in end-stage heart failure patients [10].

The ERK-dependent, regulated exit of FAK from cardiomyocyte costameres [10] is reminiscent of the cyclic FAK tyrosine and serine phosphorylation that occurs during migration of nonmuscle cells [52]. In this scenario, FAK/Src tyrosine phosphorylation at Y₃₉₇ and Y₉₂₅ promotes the assembly of new costameres in response to hypertrophic agonists or mechanical strain. Subsequent ERK1/2/5 activation then leads to the serine phosphorylation of FAK at S₉₁₀, which promotes FAK exit from newly formed costameres and its replacement by vinculin during premicrofibril formation [67]. The dynamic nature of FAK entry and exit from focal adhesions and costameres is consistent with the time required for new sarcomere addition in response to longitudinal strain, and its dependence on FAK localization and activation [47].

FAK knockout mice and the development of HF

Studies of global and cardiomyocyte-specific FAK knockout mice lend further support for an important role for FAK in cardiomyocyte mechanotransduction and myofibrillar assembly. Global FAK deletion led to lethality at embryonic day 8.5, and the mutant embryos displayed a profound defect in development of all mesodermal structures, including the heart and vasculature [31,32]. Interestingly, the developmental defects found in FAK^{-/-} embryos were phenotypically very similar in timing and phenotype to the morphological defects observed in fibronectin-null mice, suggesting an important relationship between fibronectin- and FAK-dependent signaling, especially with respect to development of the cardiovascular system [69]. In both cases, the developmental defects were attributed to the inability of mesodermal cells to migrate normally. Indeed, fibroblasts isolated from FAK^{-/-} embryos displayed markedly reduced mobility and abnormally large focal adhesions, indicating a defect in focal adhesion turnover. However, cardiomyocyte-restricted FAK knockout mice have a variable cardiac phenotype depending on precisely when during development FAK is deleted. Embryonic deletion of FAK in cardiomyocytes caused perinatal lethality due to the presence of large ventricular septal defects and abnormalities in outflow tract alignment, indicating again that FAK predominantly regulates mammalian cardiomyocyte proliferation and migration during early cardiac development [24,61]. However, cardiomyocyte FAK deletion during later prenatal development was not associated with any congenital heart defects, but led to spontaneous dilated cardiomyopathy in aged animals [60], and a blunted hypertrophic response to angiotensin II infusion [60] or TAC [14] in the adult heart. The inability to respond normally to hypertrophic stimuli supported earlier cell culture studies that demonstrated impaired hypertrophic responses of cultured cardiomyocytes overexpressing FAK-related nonkinase (FRNK, a naturally

occurring inhibitor of FAK), Y397F-FAK (a FAK autophosphorylation mutant), FAK antisense RNA, or just the FAK-FAT domain [63,16,27,38,84,47,53]. FAK also plays an important role in limiting stress-induced cardiomyocyte apoptosis, and improving cardiomyocyte survival after ischemia-reperfusion injury [25,87]. Furthermore, Vander Heide and co-workers recently showed that the tissue-specific, inducible knockout of FAK in adult cardiomyocytes substantially reduced the preconditioning response during ischemia-reperfusion injury [62]. Thus, there are substantial data indicating an important role for FAK and FAK-dependent signal transduction in preserving cardiac function, and preventing disease progression and HF during adaptation to hemodynamic, neurohormonal, and ischemic stress.

PYK2, the other member of the FAK family of protein tyrosine kinases

The FAK family of protein tyrosine kinases actually consists of 2 members [51], both of which are expressed in cardiomyocytes. Proline-rich Tyrosine Kinase 2 (PYK2; also known as FAK2, cell adhesion kinase- β (CAK- β); or Cell Adhesion Tyrosine Kinase (CADTK)), is the other member of the family, and shares ~45% protein sequence homology with FAK. PYK2 has a similar domain structure as FAK (FIGURE 4), and both kinases are believed to be derived from a common, ancient ancestral gene that diverged during early vertebrate evolution [12]. Whereas FAK is expressed at high levels in virtually all cells and tissues, PYK2 expression is much more restricted, and is preferentially expressed in cells of the endothelium, central nervous system and haematopoietic lineages.

Activation of PYK2 occurs in response to integrin engagement and clustering in some cell types, but its cellular distribution is predominantly cytoplasmic. This is particularly true in cardiomyocytes, where PYK2 is found throughout the cytoplasm of both neonatal and adult cells [3] with only a small portion localized to focal adhesions and costameres [28]. There are other important structural differences between PYK2 and FAK that reflect their distinct roles in cardiomyocyte signal transduction. Perhaps most important is the fact that unlike FAK, PYK2 activation is sensitive to intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) signals, and undergoes dimerization in response to Ca^{2+} -calmodulin binding to the FERM F2 subdomain within the N-terminus of the molecule [36]. The calcium dependence of PYK2 activation may be related to the dissociation of the PYK2 FERM domain from the central catalytic domain as occurs during FAK activation [44], resulting in dimerization and intermolecular trans-autophosphorylation. Indeed, overexpression of the PYK2 FERM domain (in the absence of calmodulin overexpression) inhibited the activation of full-length PYK2 by forming a complex with the inactive enzyme [Riggs, 2011 #7050]. These data indicate that the PYK2 FERM domain is also involved in the regulation of PYK2 activity by regulating the formation of PYK2 oligomers that are critical for its activity. Regardless of its role in oligomerization, Ca^{2+} -calmodulin binding to the PYK2 FERM domain provided an answer to the question of how PYK2 activity was regulated by $[\text{Ca}^{2+}]_i$, and this may be an important issue with regard to its regulation during cardiac hypertrophy and HF.

As a Ca^{2+} -calmodulin-dependent protein tyrosine kinase, PYK2 undergoes bimolecular transphosphorylation [59] in response to integrin engagement, increased $[\text{Ca}^{2+}]_i$, and activation of PKCs in many cell types, including cardiomyocytes [3,50,35,4,49,29,28,22,26].

Like FAK, PYK2 serves as an activatable scaffolding protein, and transduces signals from G-protein coupled receptors to the mitogen-activated protein kinases (MAPK) and the phosphoinositol-3-kinase (PI-(3)K)-PDK1-Akt signaling pathway depending upon which adaptor proteins bind to the phosphorylated enzyme [6,56,81,22]. Indeed, many of the same binding partners for FAK also bind PYK2, making it somewhat difficult to assign a specific role for PYK2-dependent signaling in cells expressing both PYK2 and FAK. Furthermore, PYK2 can functionally compensate for the loss of FAK in some cell types [88]. The adaptive capacity of cells to switch to PYK2-dependent signaling after deletion or kinase inhibition of FAK is evidence for some degree of signaling redundancy between the two protein tyrosine kinases. In fact, global deletion of the *PTK2b* gene that encodes PYK2 produces viable offspring without obvious defects in cardiovascular development [54], indicating that FAK may also compensate for the loss of PYK2 in most cell types.

Despite these caveats, PYK2 appears to serve a limited number of specific functions in cardiomyocytes, especially with respect to the recognition of, and response to a variety of stressful stimuli. Unlike FAK, PYK2 undergoes tyrosine phosphorylation during Ca^{2+} overload, UV irradiation, and H_2O_2 and TNF- treatment [83]. Hirotani et al. [29] demonstrated that PYK2 is an essential signaling component in endothelin- and phenylephrine-induced cardiomyocyte hypertrophy, perhaps acting via the Ca^{2+} - and/or PKC-dependent activation of Rac1. Furthermore, recent studies have confirmed that PYK2 is an important upstream regulator of the stress activated protein kinases (p38^{MAPK} and JNK1/2) in cardiomyocytes [49,28,22]. Thus, PYK2 activation has been implicated in hypertrophic gene expression changes during pathological cardiomyocyte hypertrophy [28,26] and in the induction of apoptosis [49].

Regulated PYK2 expression in cardiomyocytes

Unlike FAK, which appears to be constitutively expressed in most cell types, PYK2 expression also appears to vary in response to intracellular and extracellular stimuli. Although PYK2 is highly expressed in the neonatal cardiomyocyte, PYK2 expression in the adult heart is substantially reduced, but undergoes up-regulation in response to cardiomyocyte stress. The mechanisms underlying the regulated expression of PYK2 in cardiomyocytes and other cells are not known. As indicated above, embryonic fibroblasts isolated from FAK-knockout mice demonstrate a 2–3 fold increase in PYK2 expression levels, suggesting a compensatory role for PYK2 in maintaining integrin-dependent signaling events in FAK-deficient cells [77]. A similar increase in PYK2 expression and phosphorylation was observed in mice with conditional deletion of FAK in endothelial cells [88]. However, PYK2 levels did not increase with cardiomyocyte-restricted FAK deletion [14,61,24]. Nevertheless, our group was the first to demonstrate that PYK2 expression and phosphorylation were both significantly increased in cultured NRVM and adult rat ventricular myocytes (ARVM) by increases in $[\text{Ca}^{2+}]_i$ and contractile activity [3], and in ARVM *in vivo* in response to acute LV pressure overload [5]. The increase in PYK2 levels was at least partly mediated by an increase in PYK2 mRNA [64]. Similarly, Melendez et al. [50] showed that PYK2 expression and PYK2 activity were substantially increased in a mouse model of dilated cardiomyopathy due to tropomodulin overexpression, and we have recently demonstrated that PYK2 is substantially up-regulated in the PKC ϵ -overexpressing

mouse during LV remodeling and HF [37]. Nevertheless, very little is known about the factors that regulate PYK2 expression in cardiomyocytes and other cell types.

PYK2-dependent signal transduction in pathological LV remodeling

The up-regulation and activation of PYK2 during cardiac hypertrophy and its transition to HF suggests that this focal adhesion kinase plays an important role in the pathogenesis of contractile dysfunction during cardiomyocyte stress. In general, PYK2 is considered a pro-apoptotic signaling molecule, in contradistinction to FAK, which has been shown to be pro-survival in many cell types. Indeed, our group has recently shown that adenovirally-mediated overexpression of CRNK, the C-terminal domain of PYK2, inhibits PYK2 activation in cardiomyocytes, improves LV contractile function in normal hearts, and improves LV fractional shortening, pathological changes in gene expression, and survival during post-MI ventricular remodeling [26]. These results are in stark contrast to those obtained by conditional deletion of FAK in adult cardiomyocytes, in which FAK depletion alone led to progressive LV dilatation and fibrosis [60] or the impairment of compensatory LVH in response to pressure overload [14]. Thus, FAK and PYK2 appear to have very different, *opposing* functions in cardiomyocytes.

Termination of FAK- and PYK2-dependent signaling in the heart

Activation of both FAK and PYK2 during mechanotransduction signaling is dependent on phosphorylation of specific tyrosine and serine residues, which either augment kinase activity, or induce conformational alterations that promote adaptor protein binding and release. Kinase activation, however, is transient, which suggests a role for cellular phosphatases in attenuating or terminating focal adhesion signaling. Two phosphatases, Shp2 and PTEN, have been implicated in this process.

PTEN is a dual specificity, lipid and protein phosphatase that is highly expressed in cardiomyocytes, and whose expression increases in response to hypertrophic stimuli [71]. PTEN is known to inhibit cardiomyocyte growth, in part via the dephosphorylation of FAK (and PYK2), and inhibition of downstream signaling to the PI(3)K-AKT signaling pathway. PTEN was shown by Seqqat et al. [74] to be activated in response to β_1 -adrenergic stimulation following acute volume overload *in vivo*, and by prolonged isoproterenol treatment in cultured cardiomyocytes. PTEN activation was accompanied by FAK and PYK2 dephosphorylation and the induction of apoptosis. In mice, muscle-specific knockout of PTEN resulted in basal hypertrophy and mild reduction in LV systolic function [55]. But in contrast to control mice, TAC in PTEN-knockout mice resulted in reduced pathological hypertrophy, less interstitial fibrosis, and reduced apoptosis with a marked preservation of LV function, indicating that loss of PTEN prevents the development of maladaptive ventricular remodeling in response to pathological biomechanical stress.

Shp2 is another FAK/PYK2 phosphatase, whose activity is reduced in response to biomechanical stress. Depletion of Shp2 by small interfering RNAs increased FAK phosphorylation and downstream focal adhesion signaling in cultured cardiomyocytes [48]. These findings demonstrate that basal Shp2 tyrosine phosphatase activity controls the size of

cardiomyocytes by downregulating a pathway that involves FAK/Src and the PI(3)K-AKT-mTOR signaling pathways.

Speculation and future directions

The application of inducible gene excision technology to the study of focal adhesion signaling has already provided insight into the role of FAK and other components of the costamere in cardiomyocyte signal transduction. This approach, along with high-resolution fluorescence microscopy, should continue to provide important structure-function relationships between individual components of the cardiomyocyte costamere in health and disease. In contrast, the roles of PYK2 and PYK2-dependent signaling in HF progression remain largely unexplored, despite the fact that this kinase is up-regulated during pressure- and volume overload prior to the development of overt HF. Indeed, there is some controversy as to whether the kinase is cardioprotective in this setting, where it may protect the ventricular myocardium against tachyarrhythmias during vagal stimulation [40]. However, this cardioprotective, electrophysiological effect was examined in global PYK2 knockout mice, where at least some of the deleterious effects of PYK2 deletion may have resulted from PYK2 ablation in noncardiac cell types, including neuronal, inflammatory and vascular cells which all express abundant amounts of PYK2. Inducible gene targeting in cardiomyocytes should clarify this issue. FAK has already been considered a novel drug target for the development of small molecule inhibitors in the treatment of a variety of malignancies in which FAK overexpression is a common finding [33]. However, it remains to be determined whether FAK inhibition will result in significant cardiotoxicity in patients treated with these agents, as has been observed in patients treated with other tyrosine kinase inhibitors [9]. FAK inhibitors currently under evaluation also show significant inhibitory activity against PYK2, which will undoubtedly complicate the interpretation of toxicity studies. Conversely, specific small molecule inhibitors of PYK2 are not currently available, but might have utility in the treatment of HF if indeed PYK2 has deleterious effects during HF progression. Nevertheless, therapeutics aimed at focal adhesion signaling to prevent cardiac hypertrophy and HF may ultimately prove useful in reducing morbidity and mortality during LV remodeling.

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Figure 1. Cellular localization of costameres in striated muscle

(A) A schematic diagram is depicted, illustrating costameres as circumferential elements that physically couple peripheral myofibrils to the sarcolemma in periodic register with the Z-disk. The protein composition of costameres includes integrins, a variety of cytoskeletal and adaptor proteins, and signaling kinases. (B) An inside-out sarcolemma that was mechanically peeled from a single myofiber and stained with antibodies to γ -actin to reveal the costameric cytoskeleton. Bar = 10 μ m. The figure was reproduced from [17] by copyright permission of The American Society for Biochemistry and Molecular Biology.

Figure 2. Nanoscale architecture of focal adhesions

Schematic model of focal adhesion molecular architecture, depicting experimentally determined protein positions. Note that the model does not depict protein stoichiometry. The figure was reproduced from [33] by copyright permission of the Nature Publishing Group.

Figure 3. Expression of talin-1 (Tln1) and talin-2 (Tln2) in adult human cardiac tissue

Adult human cardiac tissue was evaluated for expression of Tln1 and Tln2. Tln1 was weakly detected in the cardiomyocyte membrane, as shown by co-localization with Dystrophin (Dys), a muscle-specific membrane marker. It was also detected in non-myocyte cells (*). Tln2 was detected in a costameric pattern in cardiomyocytes as demonstrated by strong co-localization with Dys (arrows). DAPI staining (blue) shows the position of cell nuclei. Scale bar = 20 μm . The figure was reproduced from [45] by copyright permission of The American Society for Biochemistry and Molecular Biology.

Figure 4. Structural domains of focal adhesion kinase (FAK) and proline-rich tyrosine kinase-2 (PYK2)

Proline-rich tyrosine kinase-2 (PYK2) shares a similar domain arrangement with focal adhesion kinase (FAK), with 60% sequence identity in the central kinase domain, conservation of proline-rich regions (PRRs), and identical positions of four tyrosine phosphorylation sites. PYK2 tyrosines 402, 579, 580 and 881 correspond to FAK tyrosines 397, 576, 577 and 925, respectively. FAK and PYK2 both contain a C-terminal focal adhesion targeting (FAT) domain that binds to paxillin. However, PYK2 shows a perinuclear distribution and is not strongly localized to focal contacts in many cells. The figure was reproduced from [49] by copyright permission of the Nature Publishing Group.