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Journal

Circulation Research, 114(3)

ISSN

0009-7330

Authors

Israeli-Rosenberg, Sharon
Manso, Ana Maria
Okada, Hideshi
et al.

Publication Date

2014-01-31

DOI

10.1161/circresaha.114.301275

Peer reviewed

Published in final edited form as:
Circ Res. 2014 January 31; 114(3): 572–586. doi:10.1161/CIRCRESAHA.114.301275.

Integrins and Integrin-Associated Proteins in the Cardiac Myocyte

Sharon Israeli-Rosenberg[#], **Ana Maria Manso**[#], **Hideshi Okada**[#], and **Robert S. Ross**
Department of Medicine, Cardiology, UCSD School of Medicine, La Jolla, CA, USA, and Veterans Administration San Diego Healthcare System, San Diego, CA, USA

[#] These authors contributed equally to this work.

Abstract

Integrins are heterodimeric, transmembrane receptors that are expressed in all cells, including those in the heart. They participate in multiple critical cellular processes including adhesion, extracellular matrix organization, signaling, survival, and proliferation. Particularly relevant for a contracting muscle cell, integrins are mechanotransducers, translating mechanical to biochemical information. While it is likely that cardiovascular clinicians and scientists have highest recognition of integrins in the cardiovascular system from drugs used to inhibit platelet aggregation, the focus of this article will be on the role of integrins specifically in the cardiac myocyte. Following a general introduction to integrin biology, the manuscript will discuss important work on integrin signaling, mechanotransduction, and lessons learned about integrin function from a range of model organisms. Then we will detail work on integrin-related proteins in the myocyte, how integrins may interact with ion channels and mediate viral uptake into cells, and also play a role in stem cell biology. Finally, we will discuss directions for future study.

Keywords

Integrin; mechanotransduction; cell-matrix adhesion; cardiac myocyte; integrin-related proteins

INTRODUCTION

Cellular adhesion, mechanosensing and signaling influence fundamental properties of organogenesis and impact the physiological function of all tissues, including the heart. Integrins are cell surface receptors that incorporate all of these functions as they are instrumental in cell adhesion, extracellular matrix (ECM) organization, signaling, survival, and proliferation[1]. Particularly relevant for a contracting muscle cell, integrins can also translate mechanical to biochemical information. Over the last several decades there has been a large body of data published on the basic biology, function and even therapeutic manipulations, relevant to integrins. Indeed, the cardiovascular clinician and scientist both likely have highest recognition of integrins in the cardiovascular system from drugs now estimated to have been used by over 8 million patients worldwide: antagonists of the αIIbβ3 integrin on the platelet surface that inhibit platelet aggregation[2].

A current *PubMed* search yields over 56,000 entries citing integrin or integrins. Given this gargantuan literature, the focus of this article will be on the role of integrins specifically in

Address correspondence to: Robert S. Ross, MD UCSD School of Medicine and VA Healthcare San Diego Cardiology 111A 3350 La Jolla Village Drive San Diego CA 92161 USA Phone: +1-858-642-1138 Fax: +1-858-642-1199 rross@ucsd.edu.

Disclosures: None

the cardiac myocyte (CM). Figure 1 illustrates the wide variety of tasks integrins can orchestrate in the CM. These include ones that are ubiquitous such as adhesion, formation of ECM-cytoskeletal junctions, signaling and viral uptake. Yet there are integrin functions that are more restricted to the CM and that are therefore generally less well understood. Examples are how they modulate ion channel function, stem cell differentiation, homing and engraftment, modify hypertrophic growth responses, transmit mechanical signals (mechanotransduction) and even protect the CM from ischemic stress. Here we will update the reader on many of these functions of CM integrins and integrin binding proteins, highlighting important new work, and also discuss directions for future study.

General introduction and integrin expression

Historically, integrins came to be recognized when it was hypothesized that a connection must exist between the extra- and intra-cellular environments, specifically between the ECM and intracellular actin cytoskeleton. Ultimately, the long sought after proteins that produced this connection were identified and cloned. The receptor was named "*integrin*" since it had an "integral membrane nature" and also a proposed function in maintaining "integrity" of the cellular ECM-cytoskeletal connection[3]. Interestingly, despite the use of this name in 1986, an antipsychotic drug oxypertine, still used in some areas of the world for treatment of schizophrenia and severe anxiety, is also known as *Integrin*, and is the first listing of this term in common databases, dating back to 1967[4]. For a comprehensive treatise on the discovery of integrin receptors, readers are referred to Richard Hynes' informative essay[5].

In most types of cells, the cellular contact point with the ECM is known as the focal adhesion (FA). In general, FA proteins rapidly turnover. In striated muscle, the "FA equivalent" is the costamere, which serves to bridge, anchor and strengthen the muscle Z-disc and its connection to the sarcolemmal membrane. As shown in Figure 2A and B, transmembrane integrins connect the ECM to proteins in the costamere, a major site of integrin localization. Having integrins in the costamere allows a firm connection between the extracellular environment and the sarcomere, at the Z-line. Integrin heterodimers are also found in intercalated discs (ICDs) (Figure 2A). . The ICDs connect myocytes end-to-end and contain structures essential for both mechanical and electrical coupling including actin-binding adherens junctions, intermediate filament interacting desmosomes, as well as gap junctions, which electrically couple cells. External mechanical forces regulate costamere assembly in the CM; arresting contraction can cause loss of integrin from the costamere and stretch leads to increased integrin levels in the myocyte[6].

Mammals express more than 18 α and 8 β integrin subunits, which heterodimerize to form 24 receptors. The integrin subunits range in size from 80–180KDa molecular weight. They generally consist of a large extracellular domain, a single transmembrane spanning domain, and a short cytoplasmic tail. Though α and β subunits share this general organization, detailed structural domains differ between the two subunits. The extracellular and cytoplasmic domains of both subunits are required for proper heterodimerization needed to form the functional integrin receptor[7].

In the CM, the integrin heterodimers most highly expressed are α1β1, α5β1 and α7β1, which are predominantly collagen (Col), fibronectin (FN) and laminin (LN) binding receptors, respectively. In addition, α6, α9 and α10 are detected in myocytes. β1 is the dominant β integrin subunit but β3 and β5 subunit function have also been studied[8–10]. Though we are concentrating our discussion on CMs here, it must be acknowledged that in heart, as in all tissues, integrin subunit expression can vary temporally, by cell type, and also with disease. Thus there are unique integrin profiles in myocytes vs. fibroblasts or endothelial cells, in fetal vs. neonatal or adult myocytes, and also in normal vs. pathological heart (e.g. normal vs. failing or post myocardial infarction (MI) tissue). For example, while the α5 subunit is

prevalent in fetal and neonatal CMs, α7 replaces α5 at the onset of post-natal development and becomes the main subunit detected in mature adult CMs[11]. However, subunit expression can switch and return to the fetal form with stresses producing myocyte hypertrophy, and various disease states. For instance, α5 and α7 subunits have been shown to be significantly increased by ischemia or post-MI[12] and aortic constriction can increase α1, α5, α7 and β1D subunit expression[13].

In addition to variations in subunits noted during development or with disease, alternative splicing of various subunits adds further complexity to the integrin repertoire. For example, β1 integrin has 4 isoforms. Two of these isoforms are the cytoplasmic domain splice variants β1A and β1D, which are both expressed in myocytes. The A-form is expressed predominantly in the embryo, and the D-form is expressed more highly in the adult myocyte[14, 15]. Illustrating their functional differences, each of these isoforms has varied affinity for FA proteins and the actin cytoskeleton[16]. β1D stabilizes costameres and ECM binding, which intuitively is important for the continuously contracting adult myocyte[17]. Further work is necessary to understand how the detailed structural differences between the A and D isoforms lead to their varied function. In addition, work has shown that β1D is down-regulated post-MI. This process has been suggested to play an important role in the decreased function of the post-MI heart, by reducing the ability of the CM to interact appropriately with the ECM[18]. Like β subunits, α7 has multiple alternatively-spliced variants, with α7B being the dominant one expressed in normal adult CMs[11].

Integrin signaling

Though integrins do not possess their own enzymatic activity, they are potent bidirectional signaling receptors, converting events outside the cell to intracellular signals and vice-versa. More precisely, this means that when ECM ligands (e.g. Col, LN, or FN) bind to the extracellular integrin domains, intracellular signaling occurs through a process commonly termed “outside-in” signaling. Ligand binding produces a wide range of intracellular signals, by aggregating a range of adapter and signaling proteins including integrin-linked kinase (ILK), focal adhesion kinase (FAK), paxillin, vinculin (Vcl), talin (Tln), Kindlin, and Src, among others (Figure 2C). Through assembly of these proteins, integrins are able to propagate signals to a variety of intracellular pathways (e.g. Akt, JNK, ERK, p38 or NFκB) (Figure 2D). Later in this manuscript we will detail the function of these and other important integrin binding proteins.

Experiments in cultured cells have shown that the “liganding” of integrins by ECM results first in integrin clustering, then FA protein complex formation, actin polymerization and finally actin-myosin stress fiber formation, ultimately providing rigidity to the cell and a mechanosensitive link between the extra- and intra-cellular environments[19]. Cytoskeletal proteins are recruited to nascent FA in a specific order, allowing the integrin adhesome to develop from an immature to a mature FA complex[20]. Early FA/ pre-costameric formation in neonatal rat ventricular myocytes (NRVMs) includes β1 integrin and Vcl[6]. Ultimately, changes in cell shape, migratory capability, cell differentiation, growth and survival result from integrin-ECM binding.

In contrast to these extracellular events, those happening within the cell can also trigger direct or indirect binding of the integrin cytoplasmic domain, enabling integrin “activation.” This process is known as “inside-out” signaling since events inside the cell direct integrins to change conformation and alter their ECM binding characteristics (Figure 2C). This process has been extensively studied in platelets and leukocytes where integrins are found normally in an inactive/resting or low affinity state. As an example, in platelets the binding of cleaved thrombin to the protein-activated receptor 1 (PAR1) leads to the recruitment of talin1 to the membrane. This facilitates talin binding to the β3 integrin cytoplasmic tail,

disrupts the interactions between the 2 integrin subunits, and activates αIIbβ3. In turn, the increased affinity of this integrin for fibrinogen results in platelet aggregation and thrombus formation[31]. Comprehensive reviews on integrin structure and function are available for additional information on this subject[7, 24, 25].

In cardiac myocytes the process of inside-out signaling has not been explored in detail. Further studies are necessary to elucidate the specific mechanisms whereby this process might play roles in processes such as cardiac hypertrophy, myocardial infarction or heart failure, in which humoral factors have been shown to be involved.

Entwined with these bidirectional signaling mechanisms of integrins, is a further complexity, since integrins may also cooperatively signal with other receptors. This is exemplified by interactions between integrins, cytokines, growth factors and even adrenergic receptors. These cooperative interactions are critical for functions within the CM, but extended discussion about this topic is beyond the focus of this review. The reader is referred to other pertinent papers on this subject[21–23].

Lessons learned from genetic manipulation of integrins in worms, flies, and fish

As with many proteins, important knowledge about myocyte integrin function arose from work performed in worm, fly and fish: *C. Elegans, D. Melanogaster and D. Rerio*. Studies in worms have contributed information about the general properties and function of the integrin complex in striated muscle, while fly studies have added to this and shed light on the role of integrins in cardiovascular development. As one can imagine, in these less complex organisms there are fewer numbers of integrin heterodimers than are found in mouse or man: two α and one β subunit in *C. Elegans*; five α and two β subunits in *Drosophila*. This facilitates analyses with genetic manipulations vs. mammalian models, since there are fewer routes for compensatory responses of alternative integrin subunits if one particular subunit is mutated.

C. Elegans has a LN-binding integrin receptor designated αIna-1 /βPat-3 and another RGD binding integrin termed αPat-2/βPat-3. (It should be noted that RGD (Arg-Gly-Asp) is a peptide motif necessary for binding to FN.) Mutations in βPat-3 disrupt the worm muscle sarcomere and prevent virtually all muscle contraction[32]. Reduction of Ina-1 expression in epidermal tissue perturbs muscle cell migration[33]. Recent work has shown that inactivation of Pat-2 leads to defective recognition and internalization of apoptotic muscle cells during development[34]. Further, a range of integrin associated proteins (see below) including orthologs of kindlin-2 (UNC-112) and ILK (Pat-4) have been associated with important functions in membrane-sarcomere attachment in the worm[35].

Work in *Drosophila* has highlighted the importance of integrin and integrin associated proteins in early cardiogenesis, their ability to set up a template necessary to form the primitive fly heart tube by instructing cardioblast polarization, heart cell specification and lumen formation, and development of a functional cardiac syncytium[38]. In addition, insights have been made into how mechanical forces can regulate integrin turnover during tissue formation[39].

Studies on integrins and related proteins in *D. Rerio* are comparatively new. Zebrafish have been shown to have four α integrin subunit genes and ten coding for β integrin variants[40]. While manipulation of integrins and related proteins has shown their importance in zebrafish, few studies have studied their relevance in myocytes or heart. One study validated how α5 integrin was essential for preservation of left-right symmetry, in agreement with work in mice. Morpholino knockdown of kindlin-2, important in integrin activation, resulted in hypoplastic, dysmorphic hearts that had contractile abnormalities[42].

Lessons learned about integrins from mouse models

Despite the simplicity of non-mammalian systems, it is difficult to directly extrapolate from them to the more complex vertebrate system. Vertebrates have a much larger repertoire of integrin receptors than lower organisms. Genetic manipulation of integrins in the mouse have proven to be quite informative in deciphering their role in cardiac muscle, and also have allowed for more precise analyses in cardiovascular diseases pertinent to man. Most integrin subunits have been interrogated in the mouse. It is impossible to provide complete details on all these various models. However, some relevant data is provided in Supplemental Table I. There are many variations of these models, including ones in different genetic backgrounds, tissue-specific deletion studies, and studies using these models for disease modeling. We will highlight details of several models important for cardiac muscle biology here.

Global ablation of the α5 integrin gene showed that this integrin was essential for preservation of left-right symmetry in the developing mouse embryo[41], similar to models with loss of fibronectin expression[41]. This same group showed more recently that α5 integrin knockout (KO) mice produced in a C57BL/6J background can survive up to E10.5, that α5 is not needed for specification of the cardiac chambers, but if absent, causes abnormalities in outflow tract formation, likely due to defects in fibronectin-mediated adhesion and Fgf8 signaling[43].

A CM-specific transgenic model was produced that had inducible expression of an α5 “gain-of-function” transgene[44]. These mice developed rapid deterioration of ventricular function, rhythm abnormalities with loss of Cx43 containing gap junctions, and increased expression of calreticulin. The authors suggested that transgene expression in adjacent cells produced abnormalities in cell-cell interactions, leading to the rhythm abnormalities which propagated ventricular dysfunction. This work brings up the intriguing potential that proper integrin function is necessary for electro-mechanical linkage in the myocardium, though further work on this subject is required.

The role of integrins in anoikis, cell death that occurs following disturbed cell-ECM adhesion, was investigated in an aortic stenosis model[45]. First it was shown that myocyte apoptosis develops as the left ventricle transitions from a compensated hypertrophic state, towards failure. Along with this was noted increased β1 integrin expression on the CM cell surface. A followup study using both hemodynamically loaded mouse hearts and cultured myocytes, suggested that the extracellular domain of β1 integrin was shed from the cell under hemodynamic and even pharmacologically-induced stress[46]. It was suggested that this “shed” integrin fragment produced with myocardial stress could modify CM cell-ECM interaction and signaling.

Increased expression of α7 integrin produced via compensatory or exercise induced responses, or via transgenesis, has been shown to have a protective effect on skeletal and cardiac myocytes[47]. Overexpression of α7β1 in dystrophic mice helped to maintain muscle integrity, increased regenerative capacity and hypertrophy, and decreased cardiomyopathy[48]. Interestingly, the protective effect in the myocardium was indirect as the transgene was expressed in skeletal muscle, so the mechanism of this effect requires further exploration. Some of our own recent work found that CM-specific transgenic expression of α7β1D integrin protected the myocardium from ischemia/reperfusion injury, while α5β1D did not[49].

Global ablation of the murine β1 integrin gene caused early embryonic lethality[50]. When a floxed β1 integrin gene was excised in CMs early in cardiogenesis using Nkx2.5-Cre, ventricular compaction was perturbed and CM proliferation was reduced, with progressive

cardiac abnormalities seen towards birth[51]. This Nkx2.5-Cre mouse model was chosen to delete the floxed β1 integrin gene since this Cre is expressed from early developmental timepoints, when cells are first directed towards the cardiac lineage. It is expressed intensely in the developing ventricle and outflow tract by E8.5[52]. Heart development likely continued for several days in these mice after excision of the floxed β1 integrin gene because the β1 integrin protein has a long half-life. When the β1 integrin gene was excised specifically in ventricular myocytes later in development using a constitutively active Cre-recombinase driven by the endogenous myosin light chain (MLC) -2 ventricular (v) promoter, we found reduction of β1 integrin protein in the CMs soon after birth[53]. Progressive myocardial fibrosis and development of a dilated cardiomyopathy (DCM) occurred. These mice were intolerant of hemodynamic loading and had increased damage following challenge with ischemia-reperfusion[49, 53]. Induced CM–specific excision of the β1 integrin gene in the adult CM lead to a blunted hypertrophic response and defective hypertrophic-stress signaling in the intact heart[54]. Moreover, adrenergic-mediated signaling was abnormal in the isolated β1 integrin–deficient myocyte.

Lessons about myocardial integrins from large animal models and man

Work in larger animal models, and a small amount of data in man, has been performed to further elucidate the function of integrins. Most of this work has focused on the intact myocardium as opposed to studies in CMs. Canine models of MI, mitral valve regurgitation and ventricular pressure overload, have been used to evaluate the role of myocardial integrin-related signaling. For example, mitral regurgitation produced by chordal rupture decreased FA protein signaling, though left ventricular stress kinase signaling was increased[55]. It was hypothesized that loss of ECM synthesis modulated integrin expression in this volume-overload model. In another study, ventricular hypertrophy with accompanying diastolic dysfunction was produced by prolonged complete atrioventricular (AV) block[56]. Integrins were suggested to be essential for this remodeling process as β1D integrin protein expression was increased. Though not a focus here, it is worth noting that nuclear imaging using an αvβ3 integrin antibody has been suggested to be useful as an imaging tool in ischemic heart disease[57].

Several studies have evaluated tissue from cardiomyopathy patients treated with left ventricular assist devices (LVAD)[58–60]. Comparisons were made between samples obtained from the time of implantation and explantation of the LVAD, or from native heart removal at transplantation. Some studies suggested the importance of integrins in remodeling, as α5 and β5 integrin expression increased slightly, while α7, β1 and β6 decreased. Another study showed that transcript expression of α1, α6 and α10 were significantly different between pre and post-LVAD samples obtained from DCM patients, and that α5, α6 and β6 varied in ischemic cardiomyopathy samples. No differences in these integrins were detectable by immunohistochemistry, likely a less sensitive technique. Western blotting was not performed. Another group compared failing with non-failing tissue samples using Western blotting, immunomicroscopy and PCR and showed that β1D integrin protein was decreased by 36% in ischemic cardiomyopathy samples vs. controls, though transcript levels were not changed[61]. β3 integrin levels were not changed. Interestingly, no changes were documented in DCM samples. The importance of this observational work in man requires additional study.

Integrin-related proteins

Integrins do not possess their own enzymatic or actin-binding activity. Therefore various adaptor proteins are required to bind to the cytoplasmic tails of α and β subunits to mediate integrin activation and subsequent ECM binding, so called “inside-out” signaling discussed above. Adapter assembly on the integrin subunit after receptor ligand (ECM) binding also

produces a signaling and structural complex that allows propagation of signaling events into the cytoplasm via “outside-in” signaling. These adaptor proteins can be divided into three groups:

(1) Adaptors that have a structural function (e.g. Tln, filamin, tensin, Vcl, α actinin). They bind integrins to F-actin and therefore to the cytoskeleton.

(2) Adaptors with a scaffolding function, that provide binding sites for additional FA proteins such as PINCH, parvin and paxillin.

(3) Catalytic adaptors, such as FAK, Src and PP2A that facilitate the propagation of signals from cellular adhesion sites, the FA or costamere.

We illustrate these proteins in Figures 2C and D. Here we will discuss some general concepts about integrin binding proteins, and will focus our attention on those integrin binding or related proteins that play an important role in heart development or postnatal pathologies such as cardiac hypertrophy and heart failure.

ILK-PINCH-Parvin, the IPP complex—ILK, Particularly Interesting New Cysteine-Histidine-rich protein (PINCH) and Parvin, form the IPP complex which is one of the essential hubs that links integrins to the actin cytoskeleton and mediates integrin signaling. This complex ensures both the stability of its individual components and targeting to the adhesion site[62]. ILK is a ubiquitously expressed protein that binds the cytoplasmic tail of the β integrin subunit[63]. Although ILK was initially identified as a serine/threonine kinase and was suggested to phosphorylate targets such as Akt and Gsk3β, recent structural and functional studies indicate that ILK lacks enzymatic activity and rather serves as a scaffolding protein. This issue is still under investigation[64].

Though ILK directly interacts with integrin cytoplasmic tails, it appears that the recruitment of ILK to FAs, and likely muscle costameres, depends upon its binding to other proteins such as kindlin-2[65], α-parvin[66] and/or paxillin[67]. Before its positioning in the FA, ILK forms a ternary complex with the two adaptor proteins PINCH and parvin.

In contrast to Drosophila and *C. elegans*, which have only one PINCH protein, 2 PINCH proteins, PINCH1 and PINCH2, have been described in mammals[68]. PINCH1 and 2 are encoded for by different genes and share high sequence homology and structural similarity. PINCH1 is expressed in the blastocyst, whereas PINCH2 expression starts on E14.5. In adulthood, both are expressed ubiquitously, although expression may vary slightly within different cell types of some organs. Furthermore, PINCH1 and PINCH2 appear to play redundant roles in most organs and compensate for one another under certain circumstances[69].

The three mammalian parvin isoforms (α-,β- and γ-parvin) are encoded by three different genes, and are structurally and functionally related to parvins in invertebrates such as *C elegans*[70]. Parvin proteins bind to the kinase-like domain of ILK and can interact directly with F-actin[71] or recruit actin-binding proteins, such as α-actinin or Vcl, an interaction mediated through paxillin. α and β-parvin are ubiquitously expressed but enriched in heart and skeletal muscle. γ-parvin has a more restricted tissue distribution with predominance in lymphoid and hematopoietic tissues[70].

PINCH and parvin proteins, perhaps akin to ILK, do not have catalytic activity but rather function as adapters for other signaling molecules such as Nck-2[70] and Ras-suppressor-1 (RSU-1)[72]. Through these interactions PINCH can regulate c-Jun N-terminal kinase (JNK)[72] and protein phosphatase 1 α (PP1α), a negative regulator of Akt activation.

In cardiac muscle ILK, PINCH and parvin co-localize at the costamere[69, 73]. Studies in adrenergically-stimulated NRVMs demonstrated that the IPP complex functioned in the hypertrophic response in an integrin-dependent manner, and that this complex protected the myocytes from apoptosis[74]. By extension, transgenic overexpression of ILK in mouse CMs was shown to produce cardiac hypertrophy[75]. Subsequent studies have shown that mice and zebrafish that lack ILK, parvin or PINCH function in CMs, also exhibit fibrosis, CM disaggregation, and ultimately develop a lethal DCM[69, 73, 76]. In all of these models destabilization of the IPP complex was associated with reduced Ser473 phosphorylation of Akt.

A mutation in human ILK (Ala262Val) was identified in 1/736 patients with DCM, with no similar mutation noted in 350 control samples[77]. This mutation was found to reduce ILK kinase activity and was unable to rescue an ILK mutant zebrafish that developed blood vessel dilation and rupture with abnormal formation of the fish ventricle. In contrast, WT ILK RNA could rescue both ILK expression and the phenotype. Clearly more work is required to assess whether this ILK mutation is causal in human cardiomyopathy.

Talin-Vinculin complex—The Tln-Vcl complex also links integrins to the cytoskeleton. Tln is a large dimeric cytoskeletal protein (270 kDa) that links integrins to the actin cytoskeleton via its connections to the cytoplasmic domain of the β integrin subunit. It is essential for FA assembly. In addition to its structural role, Tln is essential for integrin activation, thereby modulating the ligand binding activity of integrins. It functions in signal transduction, recruiting proteins like FAK and phosphatidylinositol(4) phosphate 5 kinase type Iγ to FAs[78]. The Tln-Vcl connection reinforces the actin cytoskeleton through Vcl binding to F-actin and α-actinin[78] (see below).

Lower eukaryotes possess only a single *Tln* gene while vertebrates contain two genes that encode closely related isoforms, Tln1 and Tln2[78]. Global *Tln1* KO mice display an embryonic lethal phenotype by E8.5–9.0 due to gastrulation defects[79], indicating that although both *Tln* genes encode very similar proteins (74% identical), Tln2 cannot replace Tln1 function in the entire embryo. In contrast, *Tln2* KO mice only develop a mild skeletal myopathy later in life, due to defects in myotendinous junction integrity, indicating that the two Tln isoforms have unique properties[80].

Our lab showed that during embryogenesis, Tln1 and Tln2 are both highly expressed in mouse CMs[81]. In adult mouse CMs, Tln1 protein expression becomes reduced and Tln2 becomes the main Tln form. With pressure-overload (POL) induced cardiac hypertrophy in mice, Tln1 protein expression increases in CMs where it specifically becomes localized to costameres, suggesting that it is involved in the adaptive mechanisms triggered in the stressed heart. The role of Tln1 in the cardiac stress response was confirmed by the generation and characterization of Tln1 CM-specific KO mice (Tln1cKO). The Tln1cKO showed normal basal cardiac structure and function, but following chronic POL, had blunted hypertrophy, decreased fibrosis and preserved cardiac function, vs. littermate controls. In addition Tln1cKO showed attenuated Akt, ERK1/2 and p38 activation following acute POL. These data show that normal expression of Tln1 is not necessary to maintain basal cardiac structure and function in adult heart, and also demonstrate Tln1 is important in the response of cardiac muscle to mechanical stress.

Interestingly, analysis of human heart samples showed an increase in Tln1 expression and localization to the costameres in DCM compared to non-failing samples. This human expression data was in agreement with that found in mouse and suggests that the role of Tln1 in the mouse cardiac stress response may also be applicable to man. In support of this

is a recent study that linked mutation of the mechanosensory protein CARP to human familial cardiomyopathy[82]. Interestingly, these CARP mutants lost binding to Tln1.

The fact that Tln2 is the most abundant talin isoform in adult CMs suggests that it may have a more important structural role than Tln1, supporting the connection between integrins and the sarcomeres at the cardiac costameres. Ongoing studies are being performed in our lab to elucidate this hypothesis.

Vinculin is a cytosolic 116 kDa actin-binding protein that localizes at FAs and at cadherin-mediated cell–cell junctions. It is necessary for stabilization of FAs and to transfer mechanical forces from the cell membrane to the cytoskeleton through integrins[83]. Vcl has binding sites for numerous proteins including Tln, α-actinin, paxillin, α-catenin, α-catenin, vinexin, ponsin, VASP, Arp2/3, F-actin, and PKCα. Since Vcl is highly-expressed at both costameres and ICDs in CMs, so it is difficult to dissect its unique functions at these two cellular regions. A 68-amino acid insert splice-variant isoform of Vcl, termed metavinculin (MVcl), is expressed in muscle and platelets[84]. Some work indicates that Vcl and MVcl colocalize in CMs but that each isoform may provide for varied actin organization[85].

Homozygous global Vcl KO mice (VclKO) which are null for both Vcl and MVcl, die by E10.5, with neural defects, aberrant forelimb development, and abnormal heart development with reduced myocyte number[86]. Heterozygous VclKO mice are viable and fertile, and do not have any gross abnormalities. Our analysis of these mice showed that they displayed ICD morphological abnormalities by EM and Cx43/cadherin immunofluorescence, and had widened QRS complexes. When challenged with hemodynamic stress, a subset of the mice displayed subacute sudden death, while the remainder showed a predisposition to cardiac failure, suggesting that Vcl is critical component of the ICD and costamere. This was supported by additional work where we created a Vcl cardiac-myocyte specific KO mouse (cVclKO)[88]. Sudden death was found in 49% of cVclKO mice younger than 3 months of age despite preservation of contractile function. cVclKO mice that survived through the vulnerable period of sudden death developed DCM and died before 6 months of age. Prior to the onset of cardiac dysfunction, ultrastructural analysis of cVclKO heart tissue showed abnormal adherens junctions with dissolution of the ICD structure, reduced expression of the junctional proteins cadherin and β1D integrin, and connexin-43 mislocalization to the lateral myocyte border. These data suggest that Vcl has a dual role in the CM: as a critical organizer of the ICD and also as an important linker at costameres. To dissect Vcl's role at these two different locations requires detailed further studies.

Interestingly, multiple mutations of MVcl and more rarely Vcl have been associated with both dilated and hypertrophic forms of human cardiomyopathy[89–91]. These studies demonstrate the importance of Vcl in the heart and established it as a potential genetic cause of human cardiomyopathy. Vcl is now included in panels used to screen for the genetic causes of cardiomyopathy.

Wech—As discussed above, the IPP and the Tln-Vcl complexes are the two essential structures that connect integrins with the cytoskeleton. Until recently there was no evidence of physical connection or interaction between these complexes. A recent study in *Drosophila* identified W*ech*, a highly conserved regulator of integrin mediated adhesion that interacts with the Tln head domain and the kinase domain of ILK. Embryos deficient in wech have similar phenotypes to integrin and Tln null embryos, including muscle detachment from the body wall[92]. The single murine *Wech* orthologue colocalizes with Tln and ILK in muscle tissue. Global or cardiac-specific deletion studies of wech will be necessary to extend its role to mammalian organisms and specifically cardiac muscle.

Kindlins—Kindlins are cytosolic proteins which directly interact with the cytoplasmic tail of β integrin and are required for the correct assembly of FA[93]. The three mammalian kindlins—kindlin-1, -2 and -3, are encoded by unique genes, exhibit high sequence homology and identical domain architecture, and have varied expression patterns. Kindlin-1 and kindlin-2 are widely expressed in murine and human tissues. In contrast, the expression of kindlin-3 is restricted to hematopoietic tissues, where it is the dominant form of kindlin expressed, although relatively low expression levels of kindlin-1 and kindlin-2 has also been detected. In addition to promoting cytoskeletal reorganization, kindlins act as crucial co-activators of integrins. Recently they have also been linked to roles in integrin trafficking[94]. Like Vcl, kindlins are found at both cell-matrix and cell-cell junctions[93]. Kindlins do not contain catalytic domains and therefore their primary function is to mediate protein interactions. To date, 4 kindlin binding proteins have been identified: ILK, migfilin, β1- and β3-integrin[95].

Kindlin-2, the only kindlin expressed in cardiac muscle, is abundantly expressed in CMs and is found enriched at ICDs and costameres. Loss of kindlin-2 in mice results in peri-implantation embryonic lethality revealing a requirement for kindlin-2 during early development[42, 65]. Global knockdown of kindlin-2 in zebrafish using antisense morpholinos caused a severe disruption in cardiac structure and function. In particular, loss of kindlin-2 disrupted the structure of the ICD and affected ventricle morphology, size, and contractility. These results suggest that kindlin-2 is essential for formation of the vertebrate heart. Kindlin-2 associates with migfilin, another protein that may regulate integrin activation[96]. Interestingly, migfilin appears to associate with Nkx2.5, a transcription factor crucial for normal heart development and CM differentiation[97]. While this could be a possible pathway implicating kindlin-2 in cardiogenesis, recent work demonstrated that global migfilin KO does not have a basal phenotype in the mouse, so additional studies are clearly necessary to explain this link. Further studies with CM deletion of kindlin-2 in mice would be helpful to corroborate the zebrafish results. Also, similar to Vcl, additional studies will be necessary to differentiate the role of kindlin-2 at the ICDs from its importance at the costamere.

FAK and Pyk2—FAK is a ubiquitously expressed non-receptor tyrosine kinase that plays a major role in integrin-mediated signal transduction. FAK can be activated by either ECM (integrins) or growth factors, and therefore is considered an “integrator” which can regulate multiple signaling pathway outputs[99]. The C-terminal domain of FAK promotes its colocalization with integrins through its association with the integrin-associated proteins paxillin and Tln.

When FAK is activated, conformational changes occur that exposes Tyr397, leading to its autophosphorylation and production of a high-affinity binding site for Src family tyrosine kinases. The interaction between FAK and Src leads to the phosphorylation of multiples tyrosines in FAK, as well as ones in other signaling molecules such as 130kD adaptor protein Crk-associated substrate (p130Cas), and paxillin. FAK activation also influences the activity of Rho-family GTPases that can affect actin cytoskeleton organization. Other signaling proteins activated by FAK include Ras, ERK1/2 and Akt[99].

FAK activity can be regulated by a truncated, C-terminal domain of FAK, termed FAK-related nonkinase (FRNK). FRNK is expressed endogenously and acts as a dominant interfering mutant for FAK. FRNK transcription results from the utilization of an alternative start site within the FAK gene, and its expression is independently regulated by a distinct promoter embedded within FAK intronic sequences[101].

Like many other integrin binding / adapter proteins, FAK is expressed in CMs where it localizes to the costameres and ICDs. Early *in vivo* and *in vitro* studies demonstrated that

FAK was rapidly activated by mechanical stretch / hemodynamic loading[13, 102, 103] as well as by "hypertrophic" agonists such as endothelin, angiotensin and phenylephrine. With activation, FAK relocates from the perinuclear region to the costameres[103]. Activation has been associated with increases in CM stress markers such atrial natriuretic factor (ANF).

Global deletion of the mouse FAK gene induces early embryonic lethality (E8.5–E10) with mesodermal and cardiovascular defects[106]. A range of studies have used Cre-loxP approaches to cause CM-specific gene excision of FAK. When the FAK gene was deleted early in development using an MLC-2 atrial (a) promoter to drive Cre, the majority of these mice died at approximately E14.5 and showed thinned ventricular walls with reduced myocyte proliferation[107]. In contrast, when FAK was deleted in CMs perinatally using an MLC-2V-Cre, the mice bred and survived normally, showing that FAK is not required for basal postnatal cardiac function[108, 109]. Despite this, the normal hypertrophic responses to both POL and angiotensin stimulation were blunted in these mice. These results were corroborated by a later study where FAK activity was down-regulated by CM-specific overexpression of FRNK[110]. A "floxed" FRNK transgene was activated by Nkx2.5-Cre during early cardiac development and the mice displayed a ventricular non-compaction phenotype, with reduced myocyte proliferation and lethality by E15.5, with evidence of heart failure. Yet, when FRNK was activated in the CM perinatally by using MLC2V Cre, the mice survived, bred normally, but had a blunted response to POL like the FAK deficient mice.

FAK was also implicated in regulation of CM survival following ischemia/reperfusion (I/R) injury. Mice with postnatal FAK deletion in CMs (using the MLC2VCre) have a significant increase in infarct size and CM apoptosis after I/R. Likewise, transgenic mice with enhanced cardiac-myocyte specific FAK activity, showed a smaller infarct size with attenuated myocyte apoptosis after I/R[111]. In both cases, the anti-apoptotic role of FAK appeared to be mediated through NF-κB-dependent survival signaling.

Proline-rich tyrosine kinase-2 (PYK2) is a tyrosine kinase related to FAK that shares a similar domain structure and has common phosphorylation sites. Despite their similarities, Pyk2 and FAK display a number of significant differences. While FAK is ubiquitously expressed, Pyk2 exhibits a more limited tissue distribution with highest expression in cells of hematopoietic lineage. The activation of Pyk2 is also different from that of FAK. FAK is primarily activated following integrin mediated adhesion to ECM. In contrast, although Pyk2 can be activated following integrin mediated adhesion, Pyk2 is primarily activated in response to a variety of stimuli that increase intracellular calcium levels. Intracellular distribution of FAK and Pyk2 also varies. Pyk2 is localized in FAs in certain cell types, but is more commonly found distributed throughout the cell and is often enriched in perinuclear regions[112]. Global Pyk2 KO mice are viable and fertile and do not show a gross phenotype besides defects of B cell and macrophage motility, and an increase in bone formation[113]. In CMs, Pyk2 is detected in both the cytoplasm and at costameres where it co-localizes with paxillin[114]. Studies using both neonatal and adult rat ventricular myocytes suggest roles for Pyk2 in cytoskeletal remodeling and in mediating both cardioprotective and proapoptotic pathways[115–118]. Pyk2 overexpression in NRVMs resulted in the down regulation of sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA)2 mRNA levels, indicating a potential role for Pyk2 signaling in cardiac Ca^{2+} handling[114]. Dominant-negative inhibition of Pyk2 signaling after MI improved survival and LV function, and also modified LV remodeling responses[119]. Thus Pyk2 could promote deleterious effects in the post-MI heart. Finally, a recent paper demonstrated the protective role of Pyk2 in ventricular tachyarrhythmias[120].

Melusin—Melusin is a muscle-specific protein localized at the costameres that was identified for its ability to bind to the β1 integrin cytoplasmic domain. Melusin does not

possess a kinase or phosphatase domain and has been demonstrated to behave as a chaperone[121]. With POL-induced hypertrophy, melusin expression increased during the compensatory phase and returned to basal levels during the transition to heart failure[122].

Global melusin KO mice have normal survival, normal basal cardiac structure and function, but when subjected to POL, they developed abnormal cardiac remodeling that progressed to a DCM phenotype[26]. Mice with cardiac-myocyte specific melusin overexpression developed mild cardiac hypertrophy with no obvious structural or functional alterations but showed beneficial LV remodeling with preserved contractile function following prolonged POL, in contrast to control mice that developed a DCM with CM apoptosis and fibrosis[122]. Melusin overexpression increased activation of pro-survival Akt, GSk3β and ERK pathways following POL, compared to controls. Recent studies have demonstrated that melusin is able to interact with the MAP kinases and is part of a molecular complex that includes FAK, the scaffold protein IQGAP1 and the chaperon protein Hsp90, and that FAK and IQGAP1 regulate melusin-dependent CM hypertrophy and survival through ERK1/2 activation[121].

Mechanotransduction by integrins in the cardiac myocyte

A key facet of outside-in signaling relevant to the CM is the ability of integrins to transduce mechanical information into biochemical signals. This property is termed mechanotransduction and is illustrated in Figure 2D. With this, ECM can influence cell and tissue development, and overall cellular function. Costameres play central roles in mechanotransduction. Force dependent changes in the load-bearing molecules at these adhesion-rich regions alter their biochemical activities, resulting in stress-dependent remodeling of the costameric site and signal transduction. Mechanical forces applied to integrins can be transmitted deep into the cell, even reaching the nucleus, by being channeled over the discrete cytoskeletal network[19]. This integrin-mediated, sarcolemmal-nuclear connection might at one level alter nuclear shape, but could impact chromatin organization, DNA replication, gene transcription, or RNA processing. Wang and Ingber propose that these mechanical responses could produce intracellular effects in a much more rapid manner than typical membrane receptor-mediated chemical changes[19]. As discussed, CMs have unique signaling molecules that are activated by integrin binding, such as melusin, which can mediate responses to hemodynamic load[26]. In this manner, CMs may use integrin-mediated mechanochemical signaling pathways to enhance their sensitivity to mechanical stresses borne by the ECM[27].

Integrin binding to LN can modulates the β-adrenergic response, affecting excitability, action potential morphology, Ca^{2+} metabolism, force development and resting tension in cardiac cells[28]. This represents a unique physical response in which integrins can alter the sensitivity to a soluble mitogen that affects both electrical and mechanical responses. Integrins provide potential mechanoelectrical coupling amongst cardiac cells, as they can directly mediate the effects of mechanical stress on gap junction function. For example, connexin-43 (Cx43) expression increased along with cellular conduction velocity, within 1 hour following application of mechanical strain to CMs[28, 29]. Dabiri and Parker, et.al. suggest that integrin-mediated mechanoelectrical feedback might predispose the heart to arrhythmias and even sudden cardiac death, as integrin expression is modified post-MI[30]. This is an intriguing hypothesis. While the data supporting modulation of integrin expression post-MI is clear, direct evidence linking integrins to sudden cardiac death requires further study.

Integrin signaling related to Ca^{2+} handling and ion channels

Integrin activation can regulate a range of cellular receptors including L-type Ca^{2+} (LTCC) and potassium (K^+) channels[123]. Extracellular integrin ligation changes intracellular $[Ca^{2+}]i$

in a variety of cell types. For instance, in pulmonary arterial smooth muscle cells integrin-ligand binding mobilizes Ca^{2+} from ryanodine receptor-gated stores[124]. Likewise, integrins contribute to Ca^{2+} release and Ca^{2+} influx by modulating inositol triphosphate-evoked Ca^{2+} release from intracellular stores, and Ca^{2+} influx through voltage-gated, LTCC[125]. We recently showed that overexpression of CM α7β1D integrins reduced ischemia-reperfusion injury[126]. In this study we showed that α7β1D preserved mitochondrial membrane potential during hypoxia/reoxygenation injury via inhibition of mitochondrial Ca^{2+} overload without effects on oxidative stress. Therefore, we assessed Ca^{2+} handling proteins in the CM and found that β1D integrin colocalized with ryanodine receptor 2 (RyR2) in CM T-tubules (Supplemental Figure I). β1 integrin interacted with RyR2 and stabilized RyR2 opening in an ECM dependent manner. While additional studies are necessary, it is possible that this unique interaction of CM integrins with RyR2 may modulate intracellular Ca^{2+} to provide protection of the myocyte from ischemic damage.

Crosstalk between integrins and ion channels has been implicated in the immune response, and has been linked to control of motility and migration of fibroblasts, epithelial cells, and neoplastic cells. These linkages also participate in the physiology of smooth muscle and CMs[127]. Integrin-dependent control of ion channel activity has also implicated in studies showing Src modulation of Ca^{2+} channel activity in vascular smooth muscle cells[128]. In addition to mediating influx of extracellular Ca^{2+}, intracellular Ca^{2+} release, via ryanodine receptors, may also be regulated by α5β1 activation, as evidenced by data from pulmonary arterial smooth muscle cells, in agreement with our work.

Calcium influx via the Transient Receptor Potential Vanilloid-4 (TRPV4) channel is also initiated by forces applied to β1 integrins in bovine capillary endothelial cells[129]. Treatment of rat forebrain neurons and cremaster muscle arteriole smooth muscle cells with anti α5β1 antibody-coated beads resulted in increased LTCC current, which was inhibited by FAK and c-Src inhibitors.

In addition to modulating Ca^{2+} currents, integrins may also affect K^+ channel activity. In bovine pulmonary artery endothelial cells, soluble vitronectin induced K^+ currents via αvβ3 integrin activation. αvβ3 activation also regulated Ca^{2+}- dependent K^+ current in bovine capillary endothelial cells. Integrin-mediated ion channel effects can be found in additional cell types, ranging from osteoclasts, where activation of β1 and β3 integrins upregulated cytosolic Ca^{2+}, to monocytes, where K^+ currents are modulated by α4β1 integrins[134].

The importance of integrin subtype specificity on ion channel conductance may vary by cell type. This suggests a robust system of ionic regulation via integrins. Integrin-mediated effects can occur rapidly, a not surprising result since integrin-mediated pathways have been shown to involve the phosphorylation cascades associated with FAK or Src kinases[132].

Integrin control of ion-channel function has also been found important in CM homeostasis. Lipsius et.al. demonstrated that laminin-β1 interactions could inhibit LTCC current in cat atrial myocytes via inhibition of nitric oxide (NO) and that RGD-mediated integrin stimulation increased NO and Ca^{2+} release via RyR2 in neonatal rat CMs[135]. In embryonic-stem (ES) cell derived CMs, absence of β1 integrin caused loss of muscarinic control of LTCCs[136]. β1 could also activate an outwardly rectifying $C1^-$ current in rabbit myocytes, via a FAK-Src mediated pathway[137]. Illustrating the crosstalk between adrenergic signaling and integrins, we also found that overexpression of β1A integrin could modify the β adrenergic regulation of LTCC[138].

Together, these integrin-mediated effects on ion channel activity further suggest a biologically conserved mechanism for integrin-mediated mechanoelectrical coupling.

Integrin mediated viral uptake into cells – relevance to myocarditis

Viral myocarditis is a major cause of ventricular dysfunction and sudden cardiac death in children and young adults. Integrin receptors may be involved in aspects of viral infection. It is well known that several integrins recognize RGD sequences displayed on ECM proteins. This RGD sequence also exists on the exposed loops of viral capsid proteins[140, 141]. Evidence indicates that integrins may play a role in several viral infections by facilitating virus entry[142–144].

Adenovirus was one of the first examples of a virus that was shown to use multiple receptors to infect host cells. After attachment of adenovirus to cells via the coxsackie adenovirus receptor (CAR), adenovirus serotypes can utilize αv integrins and β1 integrins for cell entry[140, 145]. Adenoviruses can interact with αv integrins via a long flexible RGD loop on the surface of its penton base. There are reports of colocalization of human CAR with αvβ3 and αvβ5 in heart specimens from patients diagnosed with end-stage DCM[146].

Coxsackievirus B3 (CVB3) is a small non-enveloped single-stranded RNA enterovirus that has been implicated in 25–40% of acute myocarditis cases, as well as DCM in infants and young adolescents[147]. It is interesting to note that integrin αVβ6 enhances infectivity of CVB1 into human colon cancer cells[148] and therefore potentially could modulate susceptibility towards myocarditis. Still, one report demonstrated that inhibition of β1 and β3 integrin function with RGD peptides had no effects on CVB3 entry and replication, or virus-induced cytopathic effect[149].

The role of ILK in integrin binding and signaling has been discussed above. ILK plays a critical role in CVB3 pathogenesis, by modulating virus replication and virus-induced cellular injury through an Akt-dependent mechanism.

Stem Cells and Integrins

Growth, maintenance of pluripotency, and differentiation fate of stem cells, including ones of human origin, can be greatly modified by the `niche', including its ECM content and the 2- vs. 3-dimensional environment[150, 151]. This includes stem cell derived cardiac-like cells. Guidance of the undifferentiated cell towards a specific lineage is not simply dependent upon presence or absence of ECM, but can be specifically dictated by the presence of one ECM type vs. another. For example, FN can guide some pluripotent cells towards endothelial differentiation, while LN can push them towards a myocyte path[152]. There is great interest in this area as tremendous strides are being made towards identification of options available for cell replacement therapy useful for repairing the damaged heart. The stiffness of substrate to which immature cells adhere can also influence differentiation of the cells towards various fates, including maturation along a cardiac lineage. Further, it is interesting to contemplate whether the process termed anoikis, or cell death produced following loss of cell-ECM adhesion, influences stem cell differentiation, maturation and survival[154].

With these concepts in mind, it is clear that integrins could be involved in modifying both adhesion and potentially stiffness, of varied types of stem cells, thereby influencing their differentiation[155]. For some general details on the potential importance of integrins in stem cells, the reader is guided towards a nice general summary as we will attempt to focus here on CM-related work. Some studies have shown that key myocyte integrin subunits such as α6a and β1 can influence mouse embryonic stem (mES) cell differentiation towards a CM lineage[157, 158]. Interestingly, though there is a developmental switch from the β1A (ubiquitously expressed) to the β1D (muscle-dominant) isoform of β1 integrin, knock-in substitution of β1D for β1A, altered mES adhesion and migration, but had no significant effect on proliferation or differentiation of these cells[159].

van Laake et.al. assayed the expression of integrin heterodimers in human ES (hES) maintained in culture or transplanted into an infarcted mouse heart[160]. Beating CMs derived from human ESCs were found to be embedded in a variety of ECM substrates (Col1, Col IV, Col XVIII, LN1, LN8, LN10 and FN) all of which are normally detected in the mammalian myocardium. When transplanted to SCID mouse myocardium, the ECM became particularly enriched in Col IV and XVIII. Integrin expression both in the cultured and transplanted cells, paralleled the matrix, so that α integrin subunits (α3, α5, α6, α7, α11, αv) and β1 integrin (which can heterodimerize with all the aforementioned α subunits), were all detected. Interestingly, with increased time following engraftment into the mouse myocardium, the hESCs showed increased α3, reduced α5, αv, and α6 integrin expression. Of course the nuances of cell culture (cell origin, days in culture, density, etc.) could greatly affect the reported expression patterns and this must be considered. Additional work has shown that hES-derived CMs migrate in response to fibronectin and both integrin α5 and αv[161].

Less data is available on induced pluripotent stem cell (iPSC) derived CMs. In one study, immunomicroscopy showed that mouse iPSC-derived CMs expressed a repertoire of proteins similar to neonatal CMs, including α7 integrin, and LN-α2[162]. Another study primarily using transcript analysis, illustrated that mouse iPSC-derived CMs expressed collagen type I, α1 and β1 integrin, which all increased expression as the m-iPS myocytes initially assembled into embryoid bodies[163].

It has even been suggested that growth of cardiac stem cell cluster derivatives from human biopsies, termed “cardiospheres”, maintain an appropriate ECM-rich environment that is akin to that present *in vivo*. Therefore this may be beneficial for survival of the cardiospheres and allow them to improve function of the damaged myocardium[164]. Marbán and his group indicate that integrins are critical in the process they term, “active vascular expulsion” which allows the cardiospheres infused intravascularly to traverse the vasculature via endothelial pocketing, and then become resident in the myocardial muscle[165].

Which specific integrins to modify, for how long, and using what means, are all questions essential to allow highest efficiency of homing, engraftment, and even differentiation and survival of the various types of pluripotent cells that could potentially be used for repair of the myocardium[166]. The number of different integrins expressed on any particular type of pluripotent cell used for myocardial therapies may vary by cell type and maturation stage, making these studies quite complicated. One study has suggested that use of a small molecule agonist of α4β1 may be useful to accelerate homing of endothelial progenitor cells and hematopoietic progenitor cells, though these *in vitro* culture studies must be extended to ones *in vivo*[167].

While quite exciting, much work is necessary to elucidate the specific role that integrins, integrin-ECM interactions, and integrin-related signaling cascades have in stem cell biology related to myocardial repair.

Summary and Future Directions

In this review we have illustrated some of the multifaceted roles that integrins and integrin-binding proteins play in CMs, drawing, as needed, upon lessons from other cell types. Despite the enormous amount of progress made on this topic, extensive work is still required. In particular, we still understand little about the role of specific functional mutations of these proteins in myocardial disease states. With the increased data obtained from whole exome or genome sequencing of samples from patients with inherited cardiomyopathies, we will expand our knowledge base here. There is no significant data on

microRNA control of integrins in the myocardium. Manipulation of stem cells by various integrin-ECM combinations, use of varied stiffness substrates and analysis of cells in 2D vs. 3D environments, are all of importance, both for sound investigation in the research lab, and later to allow cells to be used effectively as cardiovascular therapies. Finally, as noted early on in this review, we are all aware about use of integrin therapeutics as anti-platelet agents, yet use of integrin modulators for treatment of myocardial / myocyte pathologies is completely unknown and deserves future investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Funding: Supported by NIH PO1 HL 46345, RO1 HL115933 and RO1 HL103566 and VA Merit I01 BX001704 to RSR.

Nonstandard abbreviations and Acronyms

ANF	atrial natriuretic factor
AV	atrioventricular
CAR	coxsackie-adenovirus receptor
CARP	cardiac ankyrin repeat protein
CM	cardiac myocytes
Col	collagen
CVB1	Coxsackie virus B1
CVB3	coxsackievirus B3
Cx43	connexin-43
DCM	dilated cardiomyopathy
E-C	excitation-contraction
ECM	extracellular matrix
EPC	endothelial progenitor cell
ERK	extracellular signa-regulated kinases
ES	embryonic stem
FA	focal adhesion
FAK	focal adhesion kinase
FN	fibronectin
FRNK	FAK-related non-kinase
hES	human embryonic stem
HPC	hematopoietic progenitor cell
I/R	ischemia/reperfusion
ICD	intercalated disc
ILK	integrin-linked kinase

IPP	ILK-PINCH-Parvin
iPS	induced pluripotent stem
JNK	c-Jun N-terminal kinase
KO	knockout
LN	laminin
LTCC	L-type Ca^{2+} channel
LV	left ventricle
LVAD	left ventricular assist device
mES	mouse embryonic stem
MI	myocardial infarction
MVcl	metavinculin
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NO	nitric oxide
NRVM	neonatal rat ventricular myocytes
p130Cas	protein Crk-associated substrate
Pax	paxillin
PINCH	particularly interesting new cysteine-histidine-rich protein
POL	pressure overload
PP1α	protein phosphatase-1α
Pyk2	proline-rich tyrosine kinase 2
RSU-1	ras-suppressor-1
RyR2	ryanodine receptor 2
SERCA	sarco(endo)plasmic reticulum Ca^{2+} ATPase
SR	sarcoplasmic reticulum
Tln	talin
TRPV4	transient receptor potential vanilloid-4
Vcl	vinculin
WT	wild-type

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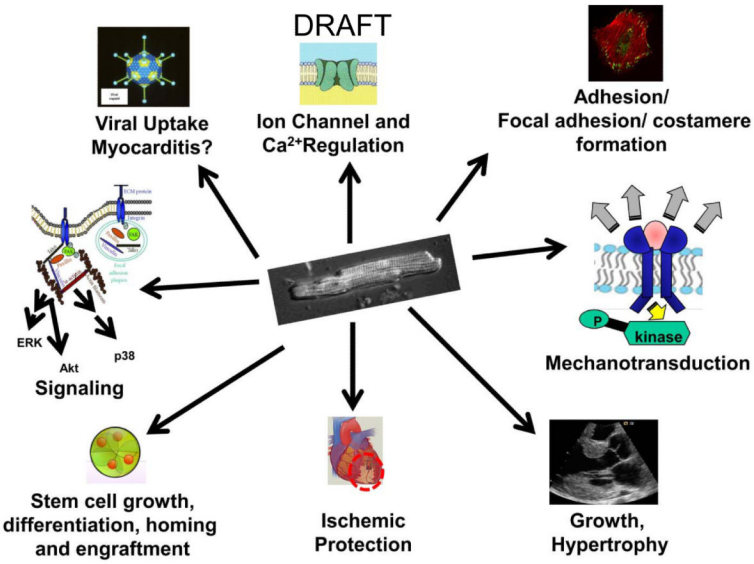

Figure 1. Some of the multiple functions of integrins in the cardiac myocyte

Integrins can have a wide variety of functions. These include ubiquitous ones such as adhesion, formation of ECM-cytoskeletal junctions, signaling or viral uptake. There are also ones that are not as well understood and that are important in the CM, such as modification of ion channel function, or stem cell growth and engraftment; hypertrophic growth, mechanotransduction and ischemic protection.

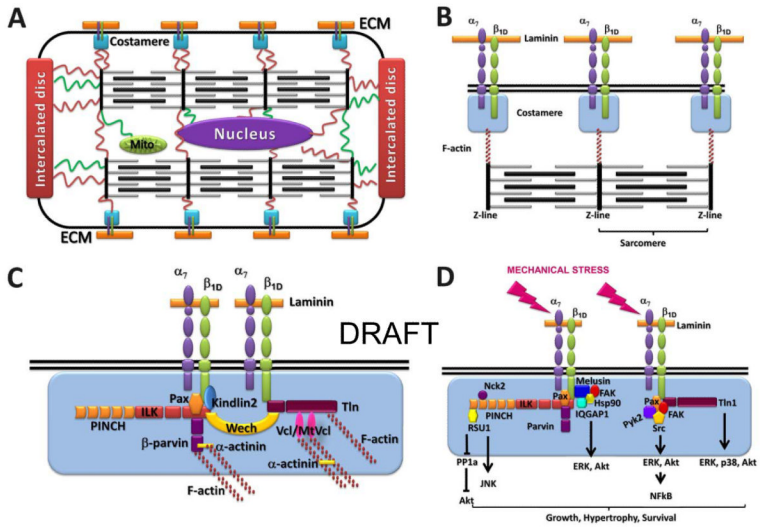

Figure 2. Integrin positioning, complex formation, signaling and mechanotransduction in the myocyte

A) Integrins contact ECM, transverse the sarcolemmal membrane at the costamere where they can interdigitates to the sarcomere as they connect to Z-line structures. Thus they bridge the ECM to sarcomere across the myocyte membrane and may even transmit information to the nucleus. Integrins are also located in the intercalated disc of the CM.

B) Enlarged portion from Panel A showing that integrin α7β1D, a predominant laminin receptor in the mature cardiac myocyte, can bridge the ECM to the sarcomere across the costamere where the integrin forms a complex of structural and signaling proteins.

C) Some important structural connectors which bind to the tails of the integrin receptor in the cardiac myocyte. Integrins connect and aggregate a range of adapter and signaling proteins such as integrin linked kinase (ILK), focal adhesion kinase (FAK), paxillin (Pax), vinculin (Vcl), talin (Tln), Kindlin, PINCH, Parvin, actinin and even actin. This allows both bridging of ECM to the cytoskeleton and perhaps sarcomere, and also allows propagation of signals bidirectionally across the cell membrane. Complex formation between ECM and integrins allows signals from the ECM to be directed into the cell, termed “outside-in” signaling”. Assembly of proteins on the integrin cytoplasmic domain tails can also occur as a result of signals produced from receptors distinct from integrins. With this, the assembled complex can “activate” integrins, change their conformation and allow enhanced interactions of integrins with ECM ligands, a process termed “inside-out” signaling. See text for details.

D) Illustration of some integrin signaling pathways in the cardiac myocyte. When integrins bind ECM ligands (e.g. laminin as illustrated here) they assemble proteins on their intracellular cytoplasmic domains and ultimately orchestrate signals down a variety of pathways such as those produced through Akt, JNK, ERK, p38 or NFκB. In turn, cellular events such as myocyte growth, hypertrophy or cell survival or death can occur. Of note is that mechanical events occuring outside the cardiac myocyte can stimulate the ECM-integrin interaction and also lead to intracellular biochemical changes, a process termed “mechanotransduction.” See text for details.

(ILK=integrin linked kinase; Mito=mitochondria; Pax=paxillin; Tln=talin; Vcl/MtVcl=vinculin and metavinculin, respectively; FAK=focal adhesion kinase)