Review

Integrin signalling: The tug-of-war in heart hypertrophy

Mara Brancaccio a,c, Emilio Hirsch a,c, Antonella Notte b, Giulio Selvetella b, Giuseppe Lembo b,d, Guido Tarone a,c,*

a Department of Genetics, Biology, and Biochemistry, Turin University, Via Santena, 5bis, 10126 Turin, Italy
b Department of Angiocardiocnecurology, I.R.C.C.S. “Neuromed”, 86077 Pozzilli (IS), Italy
c Experimental Medicine Research Center, San Giovanni Battista Hospital, 10126 Turin, Italy
d Department of Experimental Medicine and Pathology, “La Sapienza” University of Rome, Italy

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Abstract

The mechanical stress imposed by hemodynamic overload on heart walls is a primary event in triggering the cardiac hypertrophic response. Integrins, a class of membrane receptors, are major players in transmitting the mechanical force across the plasma membrane and sensing the mechanical load in cardiomyocytes. In fact, integrins, together with a number of associated cytoskeletal proteins, connect the sarcomeric contractile apparatus to the extracellular matrix across the plasma membrane and trigger intracellular signaling pathways activating the cardiomyocyte hypertrophy program.

In this review, we will discuss the role of the muscle-specific integrin isoform β1D and of associated proteins such as FAK, melusin, vinculin, zyxin, VASP, and migfilin that are the most upstream elements (“initiators”) activated by mechanical strain. These molecules trigger a coordinated downstream signaling cascade involving proteins such as AKT, RAS, and MAPKs that execute the biochemical program leading to cardiomyocyte hypertrophy. Better understanding of the functional role of the initiator elements is of key importance to developing novel strategies to control cardiac hypertrophy and prevent heart failure.

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1. Introduction

Cardiac hypertrophy is the result of a number of different stimuli, such as mechanical stress, neurohumoral activation, growth factors and cytokines (TNF, TGFβ, CNTF, CT-1, LIF). An altered balance between these stimuli triggers distinct patterns of cardiac remodelling and profoundly affects the functional properties of the hypertrophic heart [1,2]. Each stimulus usually starts from a membrane receptor that together with a restricted group of associated proteins (Initiators) triggers a downstream signalling pathway involving the regulation of a cascade of molecules (Executioners) responsible for the execution of the hypertrophic program. The complexity of the signalling pathways is enormous with reciprocal positive and/or negative feedback loops between distinct pathways and consequent generation of a signalling network in which each executioner molecule is regulated with precise timing and kinetics. The detailed characterization of these signalling pathways and networks is, thus, the ultimate goal to define the molecular basis of specific patterns of cardiac remodelling. Accomplishment of this goal unavoidably requires a detailed knowledge of the initiator proteins acting as switches of precise signalling networks in analogy to the orchestra director which coordinates the timely intervention of multiple players resulting in correct execution of the “concerto”. Moreover, initiator molecules represent ideal targets for a strategy of intervention to control the cardiac hypertrophy, since targeting executioner molecules in some case can result in...
“out-of-tune playing”. AKT represents a paradigmatic example of this concept [3]. In fact AKT can be activated via three different upstream lipid kinases, PI3Kα, β or γ. While PI3Kα, and β respond mainly to growth factor stimuli and control a compensatory hypertrophy [4], the PI3Kγ responds to adrenergic stimuli and activates a maladaptive hypertrophy program [5]. Thus activation of AKT can lead to a different pattern of heart remodeling [3], likely to depend on the specific signaling molecules which are concomitantly activated. Therefore, targeting of the most upstream initiator
components of the signaling cascade represents a better strategy to activate a coordinated signaling leading to beneficial heart remodeling.

In this review we focus our discussion on the mechanical stimuli imposed on cardiac walls by hemodynamic overload since they represent a primary event triggering cardiac hypertrophic response. Indeed, application of mechanical forces to isolated cardiomyocytes in culture triggers a hypertrophic response indicating the existence of a cell autonomous mechano-transducer apparatus [6]. A major apparatus transducing mechanical forces is represented by integrins, a class of receptors extending across the plasma membrane and physically connecting the intracellular sarcomeric contractile machinery to the extracellular matrix proteins (Fig. 1). These receptors are concentrated at specific sites at the plasma membrane known as costameres and intercalated discs. Costameres (Fig. 1 boxed area) are located in correspondence of the Z discs and allow lateral connections of myofibrils to the plasma membrane along the major cell axis [7]. Intercalated discs are junctional structures ensuring end-on connection of actin filaments to plasma membrane and providing mechanical coupling between cardiomyocytes during heart beating.

The integrin–actin association is mediated by a complex molecular machinery comprising two classes of molecules: structural and signaling proteins (Fig. 2). The structural proteins (Fig. 2, left panel), include the β1 integrin itself as well as cytoplasmic molecules such as vinculin and talin. The signaling proteins include a number of enzymes and adaptor molecules (Fig. 2, right panel) that initiate a cascade of biochemical events culminating in the activation of downstream molecules such as AKT, RAS and ERK1/2, which execute the hypertrophy program. These downstream signaling molecules acting as executioners of the cardiac hypertrophy program have been discussed in recent excellent reviews [8–11]. Here we focus our attention on the most upstream and membrane proximal molecules representing the initiator components of the mechanotransduction cascade. Moreover, emphasis is placed on those proteins whose role in heart hypertrophy has been demonstrated by in vivo genetic analysis.

We apologize, if for space reason, we are quoting only a limited number of the scientific contributions that have appeared in the literature on this topic.

1.1. Integrin-linked molecular machinery triggering signal transduction

To introduce the complexity of the system, we first describe the major molecular components of the integrin associated molecular machinery initiating the transduction of intracellular signals in response to mechanical stimuli. In the following paragraphs we will discuss the role of specific elements of such molecular machinery in heart hypertrophy.

Integrins are a family of cell-surface receptors, consisting of non-covalently associated α and β subunits, anchoring cells to the extracellular matrix. Both α and β subunits extend across the plasma membrane and are characterized by a large extracellular domain and a short cytoplasmic portion. For integrin structure and regulation we refer the reader to a number of recent reviews [12–15]. Briefly, mammalian integrins comprise 8 β and 18 α subunits, which can associate in different combinations generating more than 24 heterodimers (Fig. 3a). Each integrin heterodimer has its own binding specificity, even though the major adhesive proteins of the matrix, such as fibronectin and laminin, are recognized by multiple integrins. In addition, each integrin heterodimer has a characteristic expression pattern. Adult cardiomyocytes, in particular, express the laminin binding α7β1 heterodimer as the major integrin, while the α5β1 fibronectin receptor and the α6β1 laminin receptor are expressed in cardiomyocytes during embryonic development [16,17]. In addition, different splicing isoforms of the β1 integrin subunit are expressed in embryonic (β1A) and adult cardiomyocytes (β1D) (Fig. 3b), which are characterized by specific amino acid sequences at the cytoplasmic domain and unique property of interaction with cytoskeletal and signaling molecules [18] (see below).

At the cytoplasmic face of the plasma membrane, integrins interact with actin filaments indirectly via a number of proteins that are part of the molecular machinery initiating the signaling response. The proteins include talin, vinculin, α-actinin, ILK, parvin/ILKBP, paxillin, filamin, migfilin, zyxin, VASP, p130CAS, SRC, FAK and melusin (Fig. 2).

Even though the precise role of each of these proteins is not fully understood, some general features of this molecular complex have been outlined. In particular, some of these proteins, such as talin and vinculin, are devoid of enzymatic function and play a structural role providing the physical link between integrin and actin [19]. Talin can dimerize and bind β1 integrin cytoplasmic domain, promoting the formation of integrin clusters at adhesion sites. At the same time, vinculin can bind both talin and actin, thus anchoring the actin filaments to the membrane (Fig. 2).

In addition to talin and vinculin, ILK, a serine/threonine integrin-linked kinase, also plays an important role in integrin–actin interaction (Fig. 2). This protein has both a signaling and a structural role in integrin function. In fact, while ILK can phosphorylate both AKT and GSK-3β regulating cell survival and proliferation [20], its genetic ablation in vivo has disclosed a kinase-independent role of ILK in physically linking integrin to actin [21–23]. ILK, in fact, binds to paxillin and parvin, and this latter interacts with actin [24] (Fig. 2). Recently, evidence of a role of ILK/Parvin complex in cardiomyocyte hypertrophy in response to both integrin and adrenergic receptor signaling has been presented [25].

The talin/vinculin and ILK/Parvin complexes thus represent two apparently independent systems of linking integrin to actin. Both of these complexes are likely to play specific roles in heart since both talin and ILK are highly expressed in cardiomyocytes. Whether the two systems can
sense different types of mechanical stimuli, such as those generated by pressure or volume overload, is unknown at present. Thus, dissecting their role in cardiac hypertrophy and their regulation during the pathophysiological response to hemodynamic overload will be of great interest.

The complexity of this molecular machinery is further increased by the presence of a number of signaling proteins such as the kinases SRC, FAK, PIPK\(\gamma\) or the adaptor proteins p130CAS, melusin, zyxin, migfilin. The physical connection of these proteins to the structural elements of the integrin–actin complex is indeed instrumental for the transduction of mechanical stimuli into biochemical events inside the cell. This concept can be exemplified by examining the mechanism of talin and vinculin regulation. Both proteins can exist in two functionally distinct conformations: in the “closed/inactive” conformation the head and tail domains bind to each other preventing interaction with other molecules (Fig. 4); in the “extended/active” conformation head and tail domains are spread apart and available for intermolecular interactions [19]. The tyrosine kinases FAK and SRC [26,27] and PIPK\(\gamma\), a phosphatidylinositol phosphate kinase type 1 that synthesizes PIP2 (phosphatidylinositol (4,5) bis-phosphate) [28], play an important role in regulating the switch from a “closed” to an “extended” conformation of both talin and vinculin, thus, promoting the organization of actin anchorage to integrins at the sarcolemma. In the current model, integrin signaling triggered by cell matrix interaction and/or by mechanical stretch, causes activation of FAK (see also below) and SRC kinases that phosphorylate and activate PIPK\(\gamma\) (Fig. 4 left panel). Activated PIPK\(\gamma\) binds to talin inducing its extended/activated conformation followed by

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Fig. 3. The integrin family and muscle specific isoforms. a) Mammalian integrins comprise 8 \(\beta\) and 18 \(\alpha\) subunits. Based on their association in heterodimers, the integrin family can be clustered in the \(\beta 1\) and \(\alpha V\) subgroups that are ubiquitously expressed and the \(\beta 2\) subgroup selectively expressed in leukocytes. The bars connecting different \(\alpha\) and \(\beta\) subunits indicate all known heterodimers. Each integrin heterodimer binds to specific extracellular matrix proteins or cell surface counter receptors (ligands for the \(\beta 2\) subgroup are omitted). Adult cardiomyocytes, in particular, express the laminin binding \(\alpha 7\beta 1\) as the major integrin heterodimer. b) Muscle cells express \(\beta 1D\), an isoform of \(\beta 1\) integrin subunit generated by alternative splicing and characterized by specific amino acid sequences (indicated by the one letter amino acid code) at the cytoplasmic domain (slashed area). \(\beta 1D\) is expressed in newborn and adult cardiomyocytes while embryonic cardiomyocytes express mainly the ubiquitous form \(\beta 1A\). The short horizontal gray bar represents the plasma membrane. Coll: Collagen; E-Cad: E-cadherin; Fg: fibrinogen; Fn: fibronectin; Lm: laminin; Tn: tenascin; V-Cam: vascular cell adhesion molecule; Vn: vitronectin.
binding to the integrin cytoplasmic domain (Fig. 4 central panel). This leads to local synthesis of high amounts of PIP2 that induce the extended/active conformation of vinculin and its binding to talin and actin, leading to the assembly and stabilization of the molecular machinery that anchors integrin to actin filaments (Fig. 4 right panel). Such machinery represents a paradigmatic model of a molecular sensor capable of strengthening the extracellular matrix–actin link across the plasma membrane in response to increased mechanical workload.

2. β1D muscle specific integrin isoform: a stiff mechano-sensor?

The role of β1 integrin in cardiomyocyte function has been investigated by genetic and biochemical approaches (Table 1). Since inactivation of the β1 integrin gene leads to very early embryonic lethal phenotype, the role of this molecule in heart hypertrophy has been investigated by tissue restricted inactivation of the β1 gene in cardiomyocytes after birth [29]. Lack of β1 integrin expression in cardiomyocytes leads to myocardial fibrosis and development of dilated cardiomyopathy within six months of age. A more severe phenotype is generated when β1 integrin function is disrupted by high level expression of a dominant-negative β1 mutant in cardiomyocytes under the control of the α1 myosin heavy chain promoter. These transgenic mice die perinatally displaying diffuse fibrotic replacement of the myocardium [30]. Altogether, these findings indicate that the actin–extracellular matrix interaction via β1 integrin is necessary for the organization and maintenance of cardiomyocytes in cardiac tissue.

![Diagram](image-url)

Fig. 4. Schematic model of the actin–membrane link assembly in response to integrin signaling. A sequence of signaling events is represented from left to right. Cell matrix interaction and/or mechanical stretching trigger integrin signaling causing activation of FAK and SRC kinases (left panel), which phosphorylate PIPK1γ. This results in PIPK1γ/talin head domain binding, which induces transition to the talin extended/active conformation and, in turn, binding to the integrin cytoplasmic domain (central panel). PIPK1γ catalytic activity is increased upon talin binding and thus P(4–5)P2 (phosphatidyl inositol 4, 5 bis-phosphate) accumulates at the plasma membrane. P(4–5)P2 can interact with vinculin favoring its binding to talin and actin, thus promoting integrin–actin association (right panel). Mechanical forces acting on integrins stimulate this signaling pathway inducing an increased assembly and stabilization of the integrin–actin association. This molecular machinery can thus respond to excessive strain by increased recruitment of integrin/talin/vinculin complexes at junctional sites on the sarcolemma allowing to withstand the increased mechanical load at cellular level. (FAK and SRC are omitted from the central and right panel for the sake of simplicity). Tal: talin; Vin: vinculin; FAK: focal adhesion kinase; ECM ligand: Extra Cellular Matrix protein; PIPK1: phosphatidyl inositol phosphate kinase type 1.

Table 1
Experimental evidence of a role of integrins and associated cytoskeletal proteins in heart hypertrophy

<table>
<thead>
<tr>
<th>Gene/protein</th>
<th>In vitro evidences</th>
<th>In vivo evidences</th>
<th>In vivo knockout/transgenic</th>
<th>Mutated in cardiomyopathies</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integrin β1D/A</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>[29,30,32,46,51]</td>
</tr>
<tr>
<td>FAK</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>[33,38,42,43,45–50]</td>
</tr>
<tr>
<td>PYK2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>[52,54]</td>
</tr>
<tr>
<td>P130CAS</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>[50]</td>
</tr>
<tr>
<td>Vinculin/metavinculin</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>[66,68–70]</td>
</tr>
<tr>
<td>Melasin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>[57,58]</td>
</tr>
<tr>
<td>VASP</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td>[76]</td>
</tr>
<tr>
<td>MENA</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td>[76]</td>
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</table>

* The role of the protein has been investigated by analyzing cardiomyocyte hypertrophy on isolated cells grown in vitro.

* The role of the protein has been investigated by analyzing signaling pathways activated by hypertrophic stimuli in vivo in the heart.

* The role of the protein on cardiac hypertrophy in vivo has been investigated by inactivation of the corresponding gene and/or forced expression of the natural/mutated protein in genetically modified mice strains.

* Gene mutations in human patients have been linked to pathological phenotype.
As mentioned above, cardiomyocytes as well as skeletal muscle cells express β1D, a specific β1 integrin isoform characterized by a unique amino acid sequence at the cytoplasmic domain (Fig. 3b) which binds the cytoskeletal protein talin (see above) with a higher affinity compared to β1A, the β1 integrin isoform expressed in non-muscle cells [18,31]. Talin binding to the β1D integrin cytoplasmic domain also induces a conformational change that propagates across the plasma membrane to the extracellular domain, switching β1D to a high affinity state for its extracellular matrix ligands [15,14]. Thanks to this unique allosteric regulation, the β1D muscle specific integrin binds with higher affinity both cytoskeletal elements and extracellular ligands [18], thus, providing a stronger link between extracellular matrix and sarcomeric protein as compared to the non-muscle isoform β1A [18].

The functional in vivo relevance of this isoform has been investigated by generating knock-in mice expressing only the non-muscle β1A isoform [32]. Indeed β1A can substitute β1D in cardiomyocytes without causing major structural or functional defects in basal heart function. β1A is localized, as β1D, at intercalated discs and costameres excluding a specific role for β1D in targeting integrins to these sites. Moreover the sarcomeric organization and overall heart tissue structure is not perturbed by forced expression of β1A. The lack of an obvious phenotype in these mutant mice is somehow surprising given the different functional properties of β1A and β1D isoforms discussed above [18]. A possible explanation can be that mice are less susceptible to muscle defects compared to man as indicated by the mild dystrophic phenotype of dystrophin deficient mice. Interestingly, however, β1A expressing cardiomyocytes show increased ANP and β myosin heavy chain mRNA levels, indicating a partial activation of two important hypertrophy genes [32]. Even though echocardiographic analysis was not reported for these mice, biochemical markers indicate that forced β1A expression in cardiomyocytes induces a modest, but significant, degree of heart hypertrophy in basal conditions [32]. These findings suggest the hypothesis that β1A is more sensitive to mechanical stretch and generates hypertrophy signals under conditions of normal work-load. It is, thus, likely that the major functional role of the stiffer link generated by β1D is to increase the threshold level of the response to mechanical stretch. This concept is in line with the fact that the mechanical tension exerted on cardiomyocytes is considerably higher than that acting on β1A-expressing non-muscle cells. It is, then, conceivable that the muscle specific integrin isoform acts as a switch with a higher activation threshold to mechanical stretching. This hypothesis can be tested by evaluating the cardiac hypertrophic response to pressure overload in heart expressing only the non-muscle β1A integrin isoform.

In addition to β1, β3 integrin subunit is thought to play a role in cardiac hypertrophy, as documented in a right ventricle pressure overload model. Upon right ventricle pressure overload β3-integrin, c-SRC and FAK associate in a cytoskeleton-bound complex [33]. This association is seen as early as 4 h after right ventricular pressure overload and is likely to result from specific integrin signaling. In fact, stimulation of β3 integrin with Arg-Gly-Asp peptide in isolated adult cardiomyocytes triggers activation of c-SRC accompanied by binding to p130CAS and phosphorylation of FAK Tyr925 [34]. In addition, activation of p70S6 kinase, via mTOR, MEK/ERK, and phosphatidylinositol 3-kinase is also promoted by Arg–Gly–Asp stimulation of β3 integrin in cardiomyocytes [35]. While these studies clearly indicate an important role of β3 integrin in isolated cardiomyocytes, its involvement in cardiac hypertrophy in vivo remains to be clarified. Indeed analysis of β1 and β3 expression in the infarcted myocardium indicates that, while β1D integrin is present in cardiomyocytes, β3 subunit is mainly expressed in vasculature and stroma [36], suggesting a major function of this integrin in these compartments of cardiac tissue. However, in cardiomyocytes, β3 expression is likely to be modulated, as shown by the finding that Spironolactone [37] increases β3 integrin expression in cultured cardiomyocytes. It is thus, possible that, in the course of cardiac remodelling induced by mechanical, neurohumoral and/or cytokine stimuli, β3 expression is upregulated in cardiomyocytes in parallel with alteration of the extracellular matrix components acting as ligand for such an integrin. An intriguing possibility is that cardiomyocyte β3 integrin comes into play when vitronectin or osteopontin, two well known extracellular matrix ligands for β3, are deposited in the stroma of overloaded failing heart [38–40].

3. FAK, a survival and hypertrophy transducer activated by both mechanical and humoral stimuli

Focal Adhesion Kinase (FAK) is a cytoplasmic tyrosine kinase playing a major role in integrin signaling [41]. FAK localizes to cell–matrix adhesion sites and to costameres in cardiomyocytes through its interaction with talin and paxillin and the integrin β subunit. This protein consists of an N-terminal FERM domain, a central kinase domain, and a C-terminal Focal Adhesion Targeting (FAT) domain, but it lacks SH-2 and SH-3 domains common in most tyrosine kinases [41]. Clustering of integrins leads to the recruitment of FAK to the cell–matrix adhesions and results in activation of FAK via autophosphorylation at Tyr-397. Tyrosine phosphorylation of FAK creates a binding site for the SH2 domain of SRC kinase, which further phosphorylates Tyr-397 sustaining FAK activation. Phosphorylated Tyr-397 also binds to the p85 subunit of PI3K that provides a local source of PIP3 for the initiation of downstream signals that regulate cell survival and proliferation. In addition, an homologous protein, known as FRNK (FAK-Related Non-Kinase), is also produced by the FAK gene by the use of an alternative intronic promoter. FRNK is
composed of the noncatalytic C-terminal region of FAK [41] and is expressed in a variety of embryonic tissues, including the chick embryo heart. Although the function of FRNK has not been firmly established, this protein is believed to serve as an endogenous inhibitor of FAK-dependent signal transduction.

FAK has an important role both in hypertrophic growth and survival of cardiomyocytes (Table 1). FAK tyrosine phosphorylation is increased within 3 min after pressure overload (60-mm Hg) in rat heart [42]. Pressure overload also induces activation of c-SRC [33,42] as well as a number of downstream adapter and signaling proteins such as p130CAS, SHC, NCK [38], GRB2 and the p85 subunit of phosphatidylinositol 3-kinase [42]. ERK1/2 and AKT, two possible downstream effectors of FAK via GRB2 and phosphatidylinositol 3-kinase, are also activated. [42]. Increased contractile activity, induced by rising calcium concentration in the cardiac perfusate, does not activate the FAK signaling complex nor ERK1/2, indicating that these signaling events are triggered selectively by mechanical stretch [43]. FAK tyrosine phosphorylation is rapidly induced in isolated rat cardiomyocytes in culture upon mechanical stretching, further indicating the ability of this pathway to respond to mechanical events [44].

Besides stimulating ERK1/2 (see above), FAK activation protects cultured cardiomyocytes from apoptosis, as shown by enhanced cell death of FRNK expressing cardiomyocytes, a dominant negative mutant interfering with FAK signaling [45]. This FAK protective effect most likely occurs via the phosphatidylinositol 3-kinase/AKT pathway, and can be of key importance in preventing the transition from compensated hypertrophy to heart failure.

FAK signaling plays a key role in cardiomyocyte hypertrophy, not only in response to mechanical stress, but also in response to soluble factors such as the adrenergic agonist phenylephrine [46,47], endothelin and angiotensin [48–50], three well known hypertrophic stimuli acting via G protein coupled receptors (GPCR). Phenylephrine stimulation induces rapid FAK tyrosine phosphorylation in cultured cardiomyocytes and this signaling event is necessary for the adrenergic mediated hypertrophic response. In fact, the dominant negative FAK mutant, FRNK, blunted PE-induced ANF expression [46]. Phenylephrine-mediated ANF expression is also prevented by the expression of a dominant negative form of integrin β1D [51], suggesting that integrins cooperate with GPCR in phenylephrine-mediated FAK activation. Even though the molecular basis of integrin-GPCR cooperation in cardiomyocytes has not been investigated so far, it is likely that the extracellular matrix provides consensus signals allowing full GPCR signaling. The role of integrins in imparting consensus signals necessary for proper biological function of different classes of cell surface receptors aroused from studies of growth factor and cytokine receptors signaling [13]. Such a role fits in with the notion that proper tissue homeostasis requires cells to respond to humoral stimuli only when correctly positioned within the belonging tissue via the appropriate integrin–extracellular matrix interaction.

Whether the mechanical stretch and endothelin/phenylephrine-induced FAK activation result in identical biological response in cardiomyocytes remains to be investigated. A signaling molecule, in fact, can mediate different biological responses when stimulated by different effectors. It can be speculated that integrin-mediated FAK activation mainly regulates cell survival, while GPCR-induced FAK activation mostly impinges on the hypertrophic growth.

The in vivo role of FAK has recently been investigated using cardiac specific inactivation of the gene in the heart of adult mice (DiMichele L. and Taylor JM personal communication). Interestingly, mice lacking FAK expression in heart display normal cardiac structure and function in the absence of stress condition, but upon prolonged pressure overload they are more prone to develop a dilated phenotype as compared to wild type littermates. Moreover, FAK null hearts also expressed reduced levels of the hypertrophy marker ANP upon aortic banding compared to controls. Thus, FAK signaling seems to be dispensable for basal adult cardiac function, but it is required to support a proper compensatory hypertrophy in overloaded hearts.

An apparently opposite function seems to be played by the FAK homologue PYK2 (proline-rich tyrosine kinase 2, also known as RAFTK, CADTK, CAKβ, or FAK2) [41]. PYK2 is expressed in cardiomyocytes and its phosphorylation level is increased in a rat model of pressure overload cardiac hypertrophy [52] as well as in dilated hearts overexpressing tropomodulin [53]. A recent report suggests that PYK2 overexpression reduces SERCA2 mRNA levels in neonatal rat cardiomyocytes in vitro [54] suggesting that this kinase plays a role in the abnormal cardiac Ca2+ handling, a major event leading to heart failure.

4. Melusin, a survival and hypertrophy transducer activated only by mechanical stimuli

Melusin is a muscle specific protein localized at costameres, identified on the basis of its ability to bind to the membrane proximal region of β1 integrin cytoplasmic domain [55] (Fig. 2 right panel and Table 1). Melusin has a unique structure [56] with the N-terminal portion containing tandemly repeated CHORD domains (Cysteine and Histidine Rich Domain) capable of Zn2+ binding and separated by a spacer region. The C terminal portion of the protein, containing the integrin binding site, is characterized by a CS domain found in proteins, such as alpha-crystalline and the co-chaperon p23. A stretch of 44 residues rich in glutamic and aspartic acids and binding Ca2+ with low affinity is present at the extreme C-terminal end of the protein [56].

The role of melusin in heart hypertrophy has been established both by loss and gain of function experiments in two mouse strains: a melusin-null mouse lacking melusin
expression and a melusin transgenic mouse that over-expresses the protein in cardiomyocytes. The phenotype of these mice clearly indicates that melusin is not required for heart development, sarcomere organization or cardiac function in basal conditions [57]. Melusin ablation, however, strongly impairs the left ventricle hypertrophy response to pressure overload, and dramatically accelerates the transition to cardiac dilation. Melusin function is restricted to mechanical stimuli, since melusin null mice still develop unaltered cardiac hypertrophy in response to sub-pressor doses of angiotensin II or phenylephrine, that do not impose mechanical overload to the left ventricle [57].

An opposite phenotype is observed when Melusin is over-expressed in the heart of transgenic mice. The left ventricle of these mice retain concentric compensatory hypertrophy with full contractile function and are protected from dilation when subjected to long standing pressure overload [58]. These functional properties are accompanied by protection from cardiomyocyte apoptosis and lack of stromal tissue deposition, hallmarks of beneficial heart remodeling. Interestingly, endogenous Melusin levels are up-regulated during the initial phase of compensatory hypertrophy in mice subjected to aortic banding, but return to basal levels in hearts that have undergone the transition toward dilation [58]. At biochemical level, Melusin controls the phosphorylation of AKT and GSK3β in response to mechanical load, in fact lack of melusin leads to impaired phosphorylation of these proteins, while melusin over-expression causes their over-phosphorylation in response to mechanical stimuli [57,58]. AKT is known to control phosphorylation of mTor, p70S6 and GSK3β, three serine/threonine kinases responsible for increased protein synthesis and cardiomyocyte hypertrophy [59]. Evidence from different laboratories [4,60–62] indicates that these molecules control increased cardiomyocyte size and concentric hypertrophy and trigger a beneficial compensatory cardiomyocyte hypertrophy [63].

Melusin is thus dispensable in physiological working conditions, but is required to trigger the beneficial hypertrophic response, and prevent left ventricle dilation in condition of exceptional mechanical overload. These properties qualify melusin as a potential modifier gene in human cardiomyopathies [64].

Inactivation of vinculin gene, and of its muscle isoform metavinculin (see below), results in an early embryonic lethal phenotype due to heart and brain development defect [65], indicating that vinculin, as β1 integrin, is required for correct cardiomyocyte organization in heart tissue. Interestingly, decreased vinculin/metavinculin expression in heterozygous Vin+/− mice causes misalignment of α-actinin containing Z-discs and abnormal myocardial ultrastructure with preserved basal cardiac function [66]. Vin+/− heterozygous mice, however, show increased mortality following acute hemodynamic stress imposed by transverse aortic constriction, indicating that these structural changes predispose to stress-induced cardiomyopathy [66].

Vinculin gene also codes for Metavinculin, a vinculin isoform specifically expressed in cardiomyocytes and smooth muscle cells generated by alternative splicing of exon 19 [67]. This isoform is characterized by a 68 amino acid insert in the tail region of the molecule containing the actin binding domain. Mutational analyses of vinculin gene revealed the existence of mutations falling within the metavinculin-specific exon 19 or causing complete absence of this isoform in patients with dilated cardiomyopathy [68–70]. This is accompanied in some cases by a grossly abnormal ultrastructure of the intercalated discs. Metavinculin is, thus, an important player in the adhesive apparatus of contractile force transmission in cardiomyocytes, and alteration of its function can contribute to development of heart hypertrophy and failure either directly or as a “modifier” gene [64].

Zyxin is present at sites of cell-substratum and consists of three tandemly arrayed LIM domains supporting specific interactions with proteins involved in cytoskeletal organization and dynamics [71]. Zyxin-binding proteins include α-actinin, the small GTPases exchange factor VAV and the adaptor p130CAS. Zyxin also displays four proline-rich ActA repeats that can interact directly with members of the ENA/VASP protein family (ENAbled Vasodilator-Stimulated Phosphoprotein) [72]. The latter proteins are involved in modulation of actin assembly and organization and VASP is enriched at intercalated discs of cardiomyocytes. Zyxin plays an important role in localizing members of this family to sites of cell-substratum adhesion, and proper subcellular targeting of ENA/VASP family members is essential to fulfill their roles in vivo. Mice lacking either Zyxin [73], MENA (Mammalian ENA) [74] or VASP [75] are viable and fertile and do not show obvious developmental defects or alteration in cardiac structure and function, indicating that these proteins are either dispensable for heart development and function in basal conditions or that other molecules can replace their function. However, expression of a dominant negative VASP mutant in heart of transgenic mice results in specific displacement of both VASP and MENA from cardiac intercalated discs [76]. These mice, moreover, develop dilated cardiomyopathy with myocyte hypertrophy and bradycardia, which results in early postnatal lethality [76]. Interestingly, however, VASP represents the best

5. Molecules anchoring actin to integrins

As shown in Fig. 2, in addition to FAK and Melusin, a number of proteins are closely associated to the integrin cytoplasmic domains and participate in the link to actin as well as the generation of intracellular signaling in response to mechanical stimuli. Here we focus our discussion on Vinculin, Zyxin, ENA/Wasp and Migfilin, which are of interest in heart hypertrophy, as indicated by either genetic mutations in cardiomyopathies or by the phenotypic analysis of genetically modified mice.
known target of the nitric oxide (NO)-activated cGMP-dependent kinases (PKG). It is also largely accepted that NO, through activation of soluble guanylyl cyclase and cGMP formation, attenuates the hypertrophic response to growth factor stimulation in cardiomyocytes, independently of blood pressure. The role of VASP phosphorylation in this response is not fully understood. Nonetheless it is possible that VASP phosphorylation plays a role downstream of PKG-I to mediate the anti-hypertrophic effect of NO [77]. Despite this hypothesis, the lack of phenotype in ENA-VASP null mice suggests that these genes are unlikely to play a structural role in sarcomere organization or connection to the plasma membrane. On the other hand, the appearance of a pathological cardiac phenotype in the transgenic mice expressing dominant-negative forms of these proteins points to a function as regulators of the sarcomere–plasma membrane connection, a function which requires a dynamic control during continuous stretching and compression in contracting cardiomyocytes. ENA-VASP can thus be interesting candidates as “modifier genes” affecting the response of heart to pressure overload or other stress conditions.

Migfilin is a LIM domain-containing protein binding to filamin and VASP and recruited to cell–matrix contacts in response to cell–matrix adhesion [78,79]. Migfilin translocates to the nucleus in a calcium regulated manner and binds to NKX2-5, a transcription factor essential for heart development, and promotes its transcriptional activity. Interestingly, mutations in NKX2-5 are involved in the pathogenesis of cardiac diseases causing human cardiac malformations and atrioventricular conduction abnormalities [80] and its level is increased in cardiac hypertrophy [81,82]. These findings raise the interesting hypothesis that migfilin can play an important role in the heart hypertrophic response to mechanical stimuli by functionally linking integrin signaling to gene transcription.

6. Summary and perspectives

The results discussed above illustrate the important role of integrins and of the associated cytoskeletal/signaling proteins as initiators of the mechano-transduction signaling in heart hypertrophy. In particular, β1 integrin cytoplasmic domain and vinculin are key structural links required for correct sarcomeric structure organization, cardiac development and function. Subtle alteration of these components results in defective hypertrophy response to mechanical load. FAK, Melusin as well as Zyxin, and ENA-VASP are signaling proteins whose function is not required for cardiomyocyte differentiation and basal cardiac function. These molecules, however, are required for a correct hypertrophy response when the heart is subjected to hemodynamic overload and, thus, represent potential “modifier” genes whose mutations increase the susceptibility to cardiomyopathy [64].

A number of additional proteins of the integrin signaling machinery, such as talin and ILK, are still to be characterized for their role in cardiac hypertrophy in vivo. Analysis of their role is technically demanding, as it requires cardiac-specific gene inactivation, but it can shed light on still unexplored questions.

One important question still poorly investigated is whether pressure and volume overload involve different mechano-transduction and signaling molecules. In pressure overload, generated by aortic stenosis and hypertension, the mechanical stimulus is mainly imposed during the systolic phase. Conversely, in volume overload, documented in myocardial infarction and aortic regurgitation, the mechanical strain occurs mainly during the diastolic phase. The question is of particular relevance since the two stimuli lead to very different cardiac remodelling. In fact, pressure overload results in increased ventricular wall thickness, little or no chamber dilation, and the parallel addition of sarcomeres (concentric hypertrophy), while volume overload is characterized by modest increase in wall thickness, large increase in chamber volume, and serial addition of sarcomeres (eccentric hypertrophy). Using both in vitro [83] and in vivo [84] models, it has been shown that the two types of hemodynamic stimuli activate distinct patterns of signal transduction pathways. In particular, the MAPK ERK1/2, but not p38 and JNK, are differentially activated by the two stimuli. Also AKT, and its downstream targets GSK3β and p70S6 kinase, appear to be regulated by a different kinetic [84]. While these data point to important differences in the regulation of the executioner signaling molecules, it is not known whether different initiator molecules are responsible for sensing these two types of mechanical stimuli.

An additional important issue concerns the role of different signalling pathways in triggering compensatory vs maladaptive cardiac hypertrophy. Cardiac hypertrophy is often seen as a negative event, since in patients affected by cardiovascular pathologies it is commonly associated with deterioration of heart function and the development of heart failure (maladaptive hypertrophy). On the other hand, athletes subjected to intense physical exercise develop the so-called “physiological” or “compensatory” hypertrophy, which greatly improves myocardial function. Indeed, maladaptive hypertrophy is characterized by increased deposition of stromal tissue and by cardiomyocyte apoptotic death with consequent contractile dysfunction. On the other hand, physiological hypertrophy is characterized by increased cardiomyocyte volume with adequate angiogenesis in the absence of fibrosis and apoptosis. Thus, referring to cardiac hypertrophy without defining the cytoarchitectural pattern of heart remodelling can be misleading in terms of the functional outcome. Based on these considerations, reinforcing and/or sustaining hypertrophy of the muscle and vascular components of cardiac tissue should be regarded as positive interventions to support heart function during hemodynamic overload. Mechano-transduction signalling...
in cardiomyocytes is likely to operate in this direction, as suggested by the fact that pro-hypertrophic signalling of both Melusin and FAK does indeed lead to sustained compensatory hypertrophy.

References


