ESC HEART FAILURE
ESC Heart Failure 2021; **8**: 3634–3642
Published online 7 July 2021 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.13497

REVIEW

Integrins in cardiac hypertrophy: lessons learned from culture systems

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Abstract

Heart growth and pathological changes are accompanied by extracellular matrix-dependent alterations in integrins and integrin-associated proteins, suggesting their role in heart development and disease. Most of our knowledge on the involvement of integrins in heart pathology is provided by the *in vivo* experiments, including cardiac hypertrophy models. However, *in vivo* studies are limited by the complex organization of heart tissue and fail to discern cell types and particular integrins implicated in hypertrophic signalling. This problem is being addressed by isolated cardiomyocyte primary cultures, which have been successfully used in different *in vitro* disease models. This review aimed to analyse the general approaches to studying integrins and integrin-associated signalling pathways in cardiac hypertrophy focusing on the *in vitro* systems. The lessons learned from culture experiments on the models of hypertrophy induced by stretch, stimulating factors, and/or extracellular matrix components are summarized, demonstrating the major involvement of integrin-mediated signalling in cardiac hypertrophic response and its apparent crosstalk with signal pathways induced by stretch or hypertrophy stimulating factors. The benefits and perspectives of using cardiomyocyte primary culture as a hypertrophy model are discussed.

Keywords Cardiac hypertrophy; Cardiomyocyte culture; Integrins; Integrin signalling

Received: 5 November 2020; Revised: 16 May 2021; Accepted: 16 June 2021
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Introduction

Cardiac tissue is composed of different cell types and the extracellular matrix (ECM), which is a cell-produced organized network of macromolecular proteins. Cardiomyocytes (CMs) are functional heart cells responsible for contractility, whereas cardiac fibroblasts are the main producers of ECM. Myocardial ECM consists of collagens and glycoproteins including fibronectin and laminin, as well as proteoglycans and elastins,[1] with type I collagen being the most abundant structural component.[2] A detailed description of ECM in heart tissue may be found in recent reviews.[3,4]

Cardiac ECM is known to be a highly dynamic system. Its composition and stiffness are altered during physiological as well as pathological changes in the heart,[5] including hypertrophic growth.[6] While it had previously been considered that ECM functions to provide the integrity and mechanical stiffness of the heart, now it seems to be a major regulator of intracellular processes within the cardiac cells leading to changes in their function and phenotype.[7–9] Because the interactions between cells and ECM are provided by transmembrane integrin receptors,[10] there is growing interest over recent years in understanding the role of integrins in heart diseases. This review aimed to analyse the general approaches to studying integrins and integrin-associated signalling pathways in cardiac hypertrophy and focuses on the *in vitro* models.

Integrins in cardiac muscle cells

Integrin receptors are composed of α–β heterodimeric units and are expressed in all cell types. There were identified more than 18 α and 8 β subunits, which may combine to form at least 24 different receptors. In cardiac muscle cells, the most abundant integrins are $\alpha_1\beta_1$, $\alpha_5\beta_1$, and $\alpha_7\beta_1$ being mainly collagen, fibronectin, and laminin receptors, respectively. Some integrins including β_1 and α_7 have additional alternative splicing variants named isoforms,[11,12] which were shown to be developmentally regulated. In cardiogenesis,

β_1D isoform is known to replace the embryonic β_1A variant.[13] Expression of α-chains was also shown to be altered during heart development. Integrin subunit α_5 is mainly expressed in foetal and neonatal CMs, but in postnatal development, it is replaced by α_7 integrin, which remains the major α subunit in adult CMs.[13] Furthermore, foetal and neonatal rat ventricular myocytes were shown to express α_1 and α_3 subunits, whereas in freshly isolated cells of adult hearts, the lack of α_1 chain was demonstrated.[14]

Integrins were initially considered to function as a physical connection between cell cytoskeleton and ECM. However, there is accumulated evidence to indicate that integrins are involved in signal transduction from the extracellular space into cells. This process is known as mechanotransduction, which is the converting of mechanical forces, in particular, ECM tension, into biochemical signals.[15] Integrins do not possess enzymatic activity, so they use downstream molecules to transmit their signals within the cell.[16] Integrin activation is followed by their clustering and the attraction of non-receptor kinases to cytoplasmic domains with the activation of relevant signalling pathways that modulate transcriptional activity and direct particular cellular activities.[4,17] Among non-receptor kinases, focal adhesion kinase (FAK) is believed to be a key player in further proceeding the intracellular signals after integrin activation.[4,18] *In vivo* studies revealed increased FAK activity in the heart following pressure overload,[19,20] and mouse models with the heart-specific knockout of FAK demonstrated its role in regulation of cardiac hypertrophy.[21,22]

Focal adhesion kinase is known to stimulate extracellular signal-regulated kinases (ERK1/2) and small GTPase RhoA, a major regulator of the actin cytoskeleton.[15] Moreover, in different cell types, FAK is involved in the regulation of PI3K/Akt signalling implicated in cell proliferation and survival.[23] In cardiac muscle cells, the interaction of cytoplasmic domains of integrins with FAK was shown to mediate the phosphorylation of mitogen-activated protein (MAP) kinases, such as ERK, p38, and c-Jun N-terminal kinases (JNKs).[24] Despite some studies arguing against a major role for these MAP kinases in hypertrophy,[25,26] ERK1/2, p38, and JNKs were shown to be activated in hypertrophic myocardium[27,28] and have been implicated in the development of pathological cardiac hypertrophy.[29–33]

The genetic ablation of several integrin subunits has clearly demonstrated their essential role in normal development and function.[34] Integrin expression was shown to be altered during heart pathological changes. *In vivo* data demonstrated that cardiac hypertrophy is accompanied by the up-regulation of β_1, α_3, and α_7 integrins as well as redistribution of β_3 integrin along with the re-expression of α_1 and α_5 subunits, which are known to be expressed during cardiogenesis.[34–37] This knowledge generated a renewed interest in studying the involvement of integrins in cardiac hypertrophic response. Most of what we know about the role of integrins in heart diseases is based on *in vivo* integrin modulation studies.[38] In particular, β_1 integrin deficiency was shown to cause hypertrophic changes with the reduced basal contractility and relaxation[39] and to induce the increased myocardial

Table 1 *In vivo* models with modulation of gene expression demonstrating the role of integrins in heart pathology

Integrin subunit	*In vivo* model	Effect on the heart as compared with wild-type animals	Reference
Integrin β_1	Mice with β_1 integrin knockout exposed to myocardial infarction	Higher levels of cardiomyocyte apoptosis and poorer left ventricular function	Krishnamurthy *et al*.[40]
	Mice with β_1 integrin knockout exposed to heart failure	Less hypertrophic growth with reduced heart weight/body weight ratio and myocyte cross-sectional area; higher levels of apoptosis	Krishnamurthy *et al*.[42]
	Mice with cardiomyocyte-specific β_1 integrin knockout exposed to pressure overload	Intolerance to haemodynamic loading with high mortality; blunted hypertrophic response with reduced increases in wall thickness and left ventricular mass	Shai *et al*. and Li *et al*.[41,43]
	Mice with cardiomyocyte-specific β_1 integrin knockout exposed to ischaemia/reperfusion injury	Significant increase of the percentage myocardial infarction area/area at risk	Okada *et al*.[44]
Integrin β_3	Mice with β_3 integrin knockout exposed to pressure overload	Inhibited hypertrophic response with reduced increases in left ventricular mass and wall thickness; increased cell death; reduced cardiac output with increased mortality	Johnston *et al*.[6]
	Mice with β_3 integrin knockout exposed to pressure overload	Enrichment of μ-calpain and programmed cell death	Suryakumar *et al*.[45]
Integrin $\alpha_7\beta_1$D	Mice with cardiomyocyte-specific $\alpha_7\beta_1$D integrin overexpression exposed to ischaemia/reperfusion injury	Substantial reduction of the infarct size	Okada *et al*.[44]
Integrin $\alpha_5\beta_1$D	Mice with cardiomyocyte-specific $\alpha_5\beta_1$D integrin overexpression exposed to ischaemia/reperfusion injury	No effect on the infarct size	Okada *et al*.[44]

DOI: 10.1002/ehf2.13497

dysfunction after myocardial infarction.[40] The excision of the β_1 integrin gene in mice induced their intolerance to pressure overload,[41] while β_3 integrin knockout was shown to inhibit pressure overload-induced hypertrophic growth and result in reduced cardiac output.[6]

The lessons learned from integrin modulation studies in the context of *in vivo* disease models are summarized in *Table 1*. However, these data only indirectly link integrins with heart pathological changes, because knockout models do not exclude the influence of countless signalling pathways within the organism. Moreover, even in heart-specific knockout models, it is hard to distinguish the impact of CM integrin signalling from the engagement of non-muscle cells. In particular, most of the heart pathologies, including hypertrophy, are accompanied by fibroblast-driven fibrosis,[46] which was shown to be controlled through integrin-mediated feedback from the ECM.[47–49] The involvement of certain fibroblast-specific integrins in the onset of the fibrotic programme was confirmed on lung fibrosis models. For example, $\alpha_v\beta_3$ integrin was shown to be crucial in driving progressive pulmonary fibrosis in humans,[50] whereas α_6 integrin conferred an invasive phenotype to human fibroblasts and mediated experimental lung fibrosis in mice.[51] Moreover, fibroblast-specific knockout of β_3 integrin substantially reduced fibrosis in the mouse model of pressure overload hypertrophy.[52] The engagement of integrin signalling associated with cardiac fibroblasts into the general picture of heart pathology interferes with the estimation of CM-specific response accounting for intracellular changes.

To address this problem, hypertrophic models using isolated CM primary cultures may be used. Even though *in vitro* systems are just approximating natural conditions, they allow revealing the involvement of particular components of integrin signalling in CM during their pathological changes.

Culture systems for studying cell–matrix interactions in cardiac muscle cells

Integrin-mediated mechanotransduction has been extensively studied *in vitro* for different non-muscle as well as smooth muscle cells,[53,54] whereas data obtained on cardiac muscle cells are much less. This may be because CM cultures are difficult to prepare and manipulate as compared with the majority of non-muscle cells. However, several *in vitro* systems for CMs culturing have been well established and approved for studying cell–matrix interactions. In these *in vitro* systems, primary cultures of neonatal and adult ventricular CMs isolated from rats and mice are commonly used.

Two-dimensional (2D) culture systems including ECM proteins applied onto the dish surface are the traditional models for CMs culturing. Such models allowed isolating the effect of specific ECM components on cell morphology and function. For example, our previous results show that the organization of contractile apparatus in rat neonatal CMs differs depending on the type of ECM substrate.[7] A further benefit of 2D systems is the opportunity to specify matrix geometry. In one study, CMs cultured on micropatterned islands were shown to develop unique myofibrillar patterns corresponding to ECM geometric cues.[55]

Another approach is a three-dimensional (3D) format using one or a combination of ECM components. 3D culture systems are considered as approaching natural conditions with the cells surrounded by ECM rather than plated onto it. Therefore, 3D systems allow recapitulating cell–matrix interactions observed in heart tissue. To support that opinion, culturing of rat neonatal CMs in 3D collagen gels vs. 2D ECM substrates prevented the rearrangement of their myofibrillar apparatus according to our previous data.[56] Moreover, 3D cardiac ECM scaffold was shown to enhance the maturation of CMs derived from induced pluripotent stem cells as compared with 2D cultures.[57] Importantly, 3D cultures of CMs were shown to differ significantly from traditional 2D models in the formation of focal adhesion complexes and the integrin involvement therein.[58,59]

A particular advantage of 3D culture systems is the ability to finely tune matrix stiffness, which was shown to influence significantly cell behaviour.[60] However, these systems have their apparent drawbacks associated with sample analysing methods. For example, it is much more difficult to recover cells from 3D vs. 2D systems for some experiments, including protein assays.[61] Furthermore, the abundant amount of ECM proteins in 3D cultures makes it difficult to analyse samples of lysed cells using SDS-PAGE protocols as compared with 2D cultures.[61]

In general, both 2D and 3D primary cultures of cardiac muscle cells have their advantages and disadvantages and should complement each other in studying ECM-mediated mechanotransduction.

In vitro models of cardiac hypertrophy

Cardiac hypertrophy

The described 2D and 3D culture systems have been successfully used to generate cardiac disease models, including hypertrophic models.

Cardiac hypertrophy occurs in response to cardiac stress, including an increased mechanical load due to pressure or volume overload. In contrast to physiological hypertrophy, hypertrophic response induced by pathological stimuli generally progresses to heart failure, myocardial infarction, arrhythmias, and death.[62] The differences between

physiological and pathological hypertrophy are governed by distinct cellular signalling pathways dependent on the nature of upstream stimuli rather than the duration of cardiac stress.[63,64] The characteristic feature of pathological hypertrophy is that it is accompanied by the induction of foetal gene programme similar to the developmental pattern, including the expression of myosin heavy chain β-isoform (MYH7), skeletal α-actin, and atrial (ANF) as well as brain natriuretic factor (BNF). Moreover, pathological hypertrophy is defined by the increase in protein synthesis, CM size, and cytoskeletal remodelling, which are not observed in physiological hypertrophy.[64]

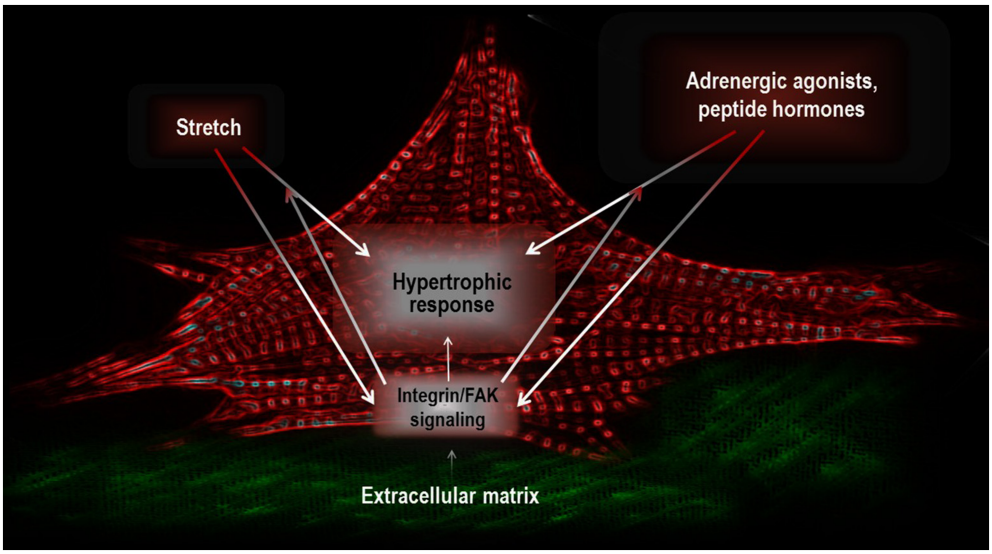
In general, the ANF is considered to be one of the most conserved and well-characterized markers of cardiac hypertrophy.[65] Its induction along with the increased cell size and protein synthesis is commonly used to confirm the hypertrophic response in culture models.

Stretch-induced hypertrophy models

Mechanical stretch is considered to be an initial factor for cardiac hypertrophy induced by haemodynamic overload. This knowledge gave rise to the idea of using mechanically stretched CM cultures as a relevant model of pressure overload-induced hypertrophy. Mechanical forces were shown to regulate integrin dynamics in CMs cultured on ECM proteins.[66] Therefore, stretch-induced hypertrophy models have been used to study the involvement of integrins and integrin-associated proteins in hypertrophic response in cardiac muscle cells.

The role of integrin β_1 in hypertrophic signalling was confirmed on the neonatal rat ventricular myocyte culture exposed to persistent centrifugal force stretch, where anti-integrin β_1-blocking antibodies were shown to partially inhibit stretch-induced hypertrophic response in these cells.[67] The involvement of integrin-associated proteins in stretch-induced hypertrophy was demonstrated on the primary culture of neonatal rat ventricular myocytes plated on type I collagen and exposed to cyclic stretch. The results have shown that hypertrophic response, including ANF gene activation, was accompanied by an increase in FAK phosphorylation and its redistribution from perinuclear regions to aggregates distributed along the myofilaments.[68] Disruption of endogenous FAK/Src signalling using a dominant-negative FAK mutant or an Src kinase pharmacological inhibitor markedly attenuated stretch-induced FAK activation and inhibited stretch-induced ANF expression. These results suggest that FAK signalling is an important component of the early hypertrophic response induced by stretch[68] (*Figure 1*). Another study using neonatal rat ventricular myocytes plated on deformable membranes coated with collagen IV and exposed to equiaxial static stretch revealed the apparent crosstalk between β_1 integrin and angiotensin II receptor signalling in mediating FAK-dependent regulation of ERK1/2 in response to mechanical stretch. Furthermore, β_1 integrin was shown to be required for FAK-independent activation of ERK1/2, p38, and JNK MAP kinases.[24] In neonatal rat ventricular myocytes grown on collagen I-coated stretch plates, mechanotransduction of the stretch signal was associated with a small increase in JNK activity but did not cause p38 MAP kinase activity.[69] However, another study revealed elevated activity of p38 in high-density cultures of CMs plated on collagen I

Figure 1 The interplay between hypertrophic signalling induced by extracellular matrix, stretch, and stimulating factors in cardiac myocytes.



DOI: 10.1002/ehf2.13497

and exposed to stretch, where p38 was shown to induce BNF expression through activation of the transcription factor NF-κB.[70] These data suggest that the engagement of p38 MAP kinase in hypertrophic response may depend on culture density.

The increased phosphorylation of FAK upon cyclic stretching of neonatal rat ventricular myocytes plated on type I collagen in the model of stretch-induced hypertrophy was accompanied by the increase in the amount and DNA-binding activity of transcriptional factor NF-κB in cell nuclear extracts. Treatment with FAK/Src pharmacological inhibitor attenuated NF-κB redistribution and DNA-binding activity induced by cyclic stretch, indicating the involvement of NF-κB in hypertrophic response and suggesting that FAK signalling may regulate NF-κB activation in pressure overloaded cardiac myocytes.[71]

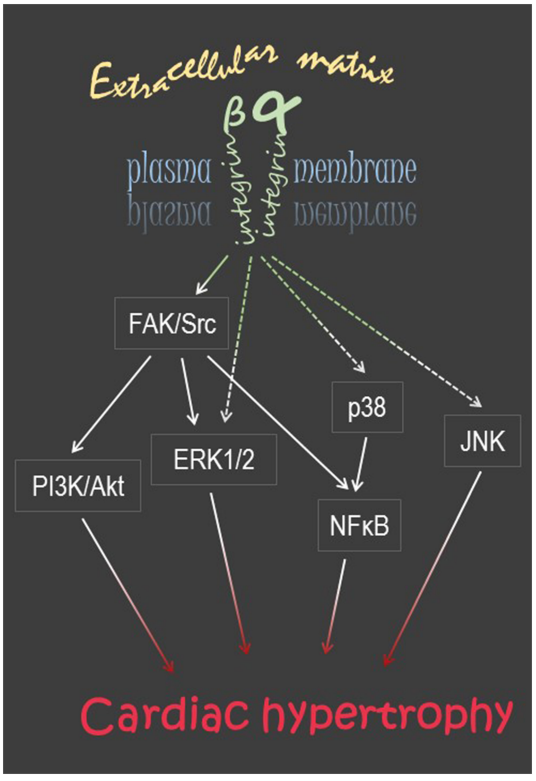
Stimulating factor-induced hypertrophy models

Along with mechanical stretch, several factors have been identified to induce hypertrophic changes in CMs, including adrenergic agonists and peptide hormones.[65,72] These factors have been demonstrated to induce hypertrophy *in vitro*, including ANF expression and an increase in cell size.[73]

Using these models allowed revealing the crosstalk between ECM-mediated transduction and stimulating factor-mediated signalling during the hypertrophic response in CMs (*Figure 1*). For example, the adrenergic signal pathways leading to CM hypertrophy were shown to be strongly dependent on integrin-mediated signalling.[74] Stimulating rat neonatal CMs, cultured on laminin or fibronectin, with phenylephrine, which is known to be a pharmacological agonist of the α_1-adrenergic receptor,[75] induced hypertrophic response, including increased cell size and expression of ANF. In contrast, CMs plated on the non-adhesive substrate gelatin exhibited a reduced capacity to undergo these phenylephrine-stimulated hypertrophic changes. Moreover, in CMs cultured on ECM proteins, phenylephrine stimulated a rapid increase in tyrosine phosphorylation of focal adhesion proteins including FAK, whereas the mutant form of FAK attenuated phenylephrine-stimulated hypertrophic response, indicating the role of ECM-mediated mechanotransduction in phenylephrine-induced hypertrophy.[74] A large increase in the expression of integrins α_1 and α_5 was observed in rat neonatal ventricular myocytes cultured on collagen-coated dishes during phenylephrine-induced hypertrophic growth with the redistribution of these integrins from a diffuse pattern on the cell surface to a sarcomeric banding pattern. Interestingly, phosphorylation of integrin β_1 significantly inhibited phenylephrine-induced hypertrophy, suggesting that integrin β_1 phosphorylation may be regulated during hypertrophic growth of cardiac myocytes.[76]

Phenylephrine was also shown to strongly up-regulate the expression of β_1D integrin and its subcellular redistribution as well as the rapid and sustained increase in FAK phosphorylation in neonatal rat ventricular myocytes cultured on collagen I.[73] In turn, the overexpression of β_1 integrin could augment phenylephrine-induced hypertrophic response, whereas inhibition of β_1 reduced the adrenergic hypertrophy. Likewise, phenylephrine-stimulated hypertrophic response was apparently down-regulated by inhibiting FAK signalling, despite the overexpression of FAK did not enhance hypertrophy.[73] Interestingly, β_1D integrin overexpression *per se* was also shown to cause elevation of endogenous ANF, induction of hypertrophic reporter genes, and increases in cell size similar to those caused by adrenergic stimulation. Likewise, overexpression of FAK induced ANF up-regulation.[73] These data strongly suggest integrin-mediated FAK signalling as an important component of hypertrophic response in cardiac muscle cells and support its engagement with adrenergic hypertrophic signalling.[73,77] Laminin binding to β_1 integrin receptors was also shown to down-regulate the adrenergic hypertrophic signalling via FAK/PI3K/Akt pathway in cat atrial myocytes treated with a β_1-adrenoreceptor stimulator and a selective β_2-adrenoreceptor antagonist.[78]

Figure 2 Schematic of the integrin-mediated regulation of hypertrophic response in cardiomyocyte culture models.



DOI: 10.1002/ehf2.13497

Endothelin-1 and angiotensin II are two peptide hormones that were shown to induce cardiac hypertrophy by an autocrine mechanism.[75] Endothelin stimulation of rat neonatal ventricular myocytes plated on collagen-coated dishes resulted in time-dependent FAK activation,[79] and stimulation of cells by different hypertrophic agonists, including phenylephrine, endothelin-1, and angiotensin II, demonstrated nuclear translocation of NF-κB and stimulation of its transcriptional activity. Importantly, the inhibition of NF-κB activity suppressed hypertrophic agonist-induced expression of ANF and increase in cell size. Conversely, overexpression of NF-κB induced the expression of ANF as well as an increase in cell size, suggesting this transcription factor to be an important participant in cardiac hypertrophic growth.[80]

The key components of integrin signalling involved in hypertrophic response as confirmed by the data of *in vitro* experimentations are summarized in *Figure 2*.

Extracellular matrix-induced hypertrophy models

Besides mechanical stretch and stimulating factors, ECM components *per se* have been also used to induce a hypertrophic response in cardiac myocytes. The hypertrophic effect of fibronectin was demonstrated by the increased cell size and protein synthesis as well as secretion of ANF and BNF in cardiac muscle cells cultured on fibronectin-coated dishes in contrast to the cells grown on non-coated plates. Fibronectin was also shown to induce reorganization of actin structures, co-localization of β_1 integrin with vinculin, formation of focal adhesion complexes, and FAK phosphorylation.[81] Importantly, blocking antibodies against β_1 and β_3 integrin significantly inhibited fibronectin-induced secretion of ANF and BNF. The described effects were inhibited in a dose-dependent manner by GRGDSP, which is a competitive antagonist of the Arg–Gly–Asp (RGD) domains found in fibronectin, vitronectin, and laminin.[81] These data suggest that fibronectin stimulation of cardiac hypertrophy is RGD dependent[82] and justify the use of RGD-stimulating models in hypertrophic studies.

Three-dimensional ECM-based systems including fibronectin/vitronectin or their RGD motifs have been mainly used to recapitulate the *in vivo* formation of focal adhesion complexes in hypertrophic myocardium. For example, adult feline CMs embedded in 3D collagen I matrix with the addition of a low concentration of fibronectin and vitronectin were shown to form FAK-containing β_3 integrin-mediated focal adhesion complexes, characteristic of hypertrophic myocardium *in vivo*.[36,83] The benefit of 3D vs. 2D ECM-based hypertrophy models was demonstrated by differences in the formation of focal adhesion complexes between stimulated with synthetic RGD peptide adult feline CMs that were cultured on laminin support or within a type I collagen matrix. The results demonstrated that only collagen-based 3D model provided for cytoskeletal assembly of FAK, Nck, and Shc as well as c-Src and ERK1/2 activation, as observed in hypertrophic myocardium.[59]

Our previous data show that CMs in a long-term 2D culture system lacking ECM proteins undergo substantial rearrangements, including the significant increase in cell size, reorganization of contractile apparatus, and re-expression of foetal genes,[84,85] which changes are reminiscent of those observed in hypertrophic CMs. Importantly, such alterations were accompanied by deposition and remodelling of ECM by CMs themselves[84,86] with the correlated dynamics in integrins and integrin-linked kinase.[87] Our preliminary data also show the redistribution of FAK during such rearrangements, presumably corresponding to the formation of focal adhesion complexes.[88] These findings offer a new perspective for using monolayer CM culture, considering it *per se* as a hypertrophic model devoid of additional factors, suitable for the investigation of ECM-mediated integrin mechanotransduction in cardiac muscle cells.

Conclusions

For the last decades, cardiac ECM is emerging as an important regulator of cell morphology and function in heart development and disease. As matrix-derived cues are known to be transmitted via transmembrane integrin receptors, the involvement of integrins and integrin-associated proteins in heart pathological changes is under active consideration. *In vivo* studies including knockout models demonstrated the essential role of integrins in cardiac hypertrophy. However, complex organization of heart tissue interferes with studying the engagement of particular integrins within the cardiac muscle cells and the relationships between integrin-mediated signalling and other hypertrophic signalling pathways. In this context, the *in vitro* models are becoming a highly significant component of cardiac hypertrophy research. Despite the limitations of different culture systems, some of them have been demonstrated to sufficiently recapitulate events found inside the hypertrophic heart and have proven themselves as relevant models to study hypertrophy-induced integrin signalling. In general, lessons learned from culture experiments demonstrate the major involvement of ECM-mediated signalling in cardiac hypertrophic response and reveal the apparent crosstalk between integrins and hypertrophic signalling induced by stretch or stimulating factors (*Figure 1*).

Conflict of interest

None declared.

Funding

This work was supported by the Ministry of Science and Higher Education of the Russian Federation (theme No. FMFU-2021-0008) and the Institute of Cytology (INC RAS) Director's Fund.

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