Integrins in cardiac hypertrophy: lessons learned from culture systems

Natalya Bildyug*

Institute of Cytology, Russian Academy of Sciences, Saint Petersburg, 194064, Russia

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
*Correspondence to: Natalya Bildyug, Institute of Cytology, Russian Academy of Sciences, Saint Petersburg 194064, Russia. Email: nbildyug@gmail.com

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, with type I collagen being the most abundant structural component. A detailed description of ECM in heart tissue may be found in recent reviews. 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, including hypertrophic growth. 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. Because the interactions between cells and ECM are provided by transmembrane integrin receptors, 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 α1β1, α5β1, and α7β1 being mainly collagen, fibronectin, and laminin receptors, respectively. Some integrins including β1 and α7 have additional alternative splicing variants named isoforms, which were shown to be developmentally regulated. In cardiogenesis,
β1D isoform is known to replace the embryonic β1A variant. Expression of α-chains was also shown to be altered during heart development. Integrin subunit α5 is mainly expressed in foetal and neonatal CMs, but in postnatal development, it is replaced by α7 integrin, which remains the major α subunit in adult CMs. Furthermore, foetal and neonatal rat ventricular myocytes were shown to express α1 and α2 subunits, whereas in freshly isolated cells of adult hearts, the lack of α1 chain was demonstrated.

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. Integrins do not possess enzymatic activity, so they use downstream molecules to transmit their signals within the cell. 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. Among non-receptor kinases, focal adhesion kinase (FAK) is believed to be a key player in further proceeding the intracellular signals after integrin activation. In vivo studies revealed increased FAK activity in the heart following pressure overload, and mouse models with the heart-specific knockout of FAK demonstrated its role in regulation of cardiac hypertrophy.

Focal adhesion kinase is known to stimulate extracellular signal-regulated kinases (ERK1/2) and small GTPase RhôA, a major regulator of the actin cytoskeleton. Moreover, in different cell types, FAK is involved in the regulation of PI3K/Akt signalling implicated in cell proliferation and survival. 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). Despite some studies arguing against a major role for these MAP kinases in hypertrophy, ERK1/2, p38, and JNKs were shown to be activated in hypertrophic myocardium and have been implicated in the development of pathological cardiac hypertrophy.

The genetic ablation of several integrin subunits has clearly demonstrated their essential role in normal development and function. 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 β1, α3, and α7 integrins as well as redistribution of β3 integrin along with the re-expression of α1 and α6 subunits, which are known to be expressed during cardiogenesis. 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. In particular, β1 integrin deficiency was shown to cause hypertrophic changes with the reduced basal contractility and relaxation and to induce the increased myocardial

| Integrin subunit | Mice model | Effect on the heart as compared with wild-type animals | Reference |
|-----------------|------------|-------------------------------------------------------|-----------|
| Integrin β1D    | Mice with β1 integrin knockout exposed to myocardial infarction | Higher levels of cardiomyocyte apoptosis and poorer left ventricular function | Krishnamurthy et al. |
|                 | Mice with β1 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. |
|                 | Mice with cardiomyocyte-specific β1 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. |
| Integrin β3     | Mice with β2 integrin knockout exposed to ischaemia/reperfusion injury | Significant increase of the percentage myocardial infarction area at risk | Okada et al. |
| Integrin α5β1D  | Mice with cardiomyocyte-specific α5β1D integrin overexpression exposed to ischaemia/reperfusion injury | 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. |
| Integrin α6β1D  | Mice with cardiomyocyte-specific α6β1D integrin overexpression exposed to ischaemia/reperfusion injury | Enrichment of μ-calpain and programmed cell death | Suryakumar et al. |

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

ESC Heart Failure 2021; 8: 3634–3642
DOI: 10.1002/ehf2.13497
dysfunction after myocardial infarction. The excision of the β3 integrin gene in mice induced their intolerance to pressure overload, while β3 integrin knockout was shown to inhibit pressure overload-induced hypertrophic growth and result in reduced cardiac output.

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, whereas data obtained on cardiac fibroblasts and mediated experimental lung fibrosis in mice. Moreover, fibroblast-specific knockout of β3 integrin substantially reduced fibrosis in the mouse model of pressure overload hypertrophy. 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, 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. 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.

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. Moreover, 3D cardiac ECM scaffold was shown to enhance the maturation of CMs derived from induced pluripotent stem cells as compared with 2D cultures. 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.

A particular advantage of 3D culture systems is the ability to finely tune matrix stiffness, which was shown to influence significantly cell behaviour. 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. 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.

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. 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 β1 in hypertrophic signalling was confirmed on the neonatal rat ventricular myocyte culture exposed to persistent centrifugal force stretch, where anti-integrin β1-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 β1 integrin and angiotensin II receptor signalling in mediating FAK-dependent regulation of ERK1/2 in response to mechanical stretch. Furthermore, β1 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
and exposed to stretch, where p38 was shown to induce BNF expression through activation of the transcription factor NF-κB. 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.

**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. These factors have been demonstrated to induce hypertrophy in vitro, including ANF expression and an increase in cell size.

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. Stimulating rat neonatal CMs, cultured on laminin or fibronectin, with phenylephrine, which is known to be a pharmacological agonist of the α1-adrenergic receptor, 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. A large increase in the expression of integrins α1 and α5 was observed in rat neonatal ventricular myocytes cultured on collagen-coated dishes under 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 β1 significantly inhibited phenylephrine-induced hypertrophy, suggesting that integrin β1 phosphorylation may be regulated during hypertrophic growth of cardiac myocytes.
Endothelin-1 and angiotensin II are two peptide hormones that were shown to induce cardiac hypertrophy by an autocrine mechanism. Endothelin stimulation of rat neonatal ventricular myocytes plated on collagen-coated dishes resulted in time-dependent FAK activation, 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.

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 β1 integrin with vinculin, formation of focal adhesion complexes, and FAK phosphorylation. Importantly, blocking antibodies against β1 and β3 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. These data suggest that fibronectin stimulation of cardiac hypertrophy is RGD dependent 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 β3 integrin-mediated focal adhesion complexes, characteristic of hypertrophic myocardium in vivo. 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.

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, which changes are reminiscent of those observed in hypertrophic CMs. Importantly, such alterations were accompanied by deposition and remodelling of ECM by CMs themselves with the correlated dynamics in integrins and integrin-linked kinase. Our preliminary data also show the redistribution of FAK during such rearrangements, presumably corresponding to the formation of focal adhesion complexes. These findings offer a new perspective for using monolayer 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.

References

1. Municie JM, Weaver VM. The physical and biochemical properties of the extra-cellular matrix regulate cell fate. Curr Top Dev Biol 2018; 120: 1–37.
2. Bowers SL, Baudino TA. Cardiac myocyte–fibroblast interactions and the coronary vasculature. J Cardiovasc Transl Res 2012; 5: 783–793.
3. Pinkert MA, Hortensius RA, Ogle BM, Elcicer KW. Imaging the cardiac extracellular matrix. Adv Exp Med Biol 2018; 1098: 21–44.
4. Valiente-Alandi I, Schafer AE, Blaxall BC. Extracellular matrix-mediated cellular communication in the heart. J Mol Cell Cardiol 2016; 91: 228–237.
5. Takawale A, Sakamuri SS, Kassiri Z. Extracellular matrix communication and turnover in cardiac physiology and pathology. Compr Physiol 2015; 5: 687–719.
6. Johnston RK, Balasubramanian S, Kasigianesan H, Baicu CF, Zile MR, Kuppuswamy D. β1 integrin-mediated ubiquitination activates survival signaling during myocardial hypertrophy. FASEB J 2009; 23: 2759–2771.
7. Bildyg NB, Pinaev GP. Extracellular matrix dependence of organization of the cardiomyocyte contractile apparatus. Cell Tissue Biol 2014; 8: 38–49.
8. Bildyg N. Extracellular matrix in regulation of contractile system in cardiomyocytes. Int J Mol Sci 2019; 20: 5054.
9. Ward M, Iskratsch T. Mix and (mis-)match—the mechanosensing machinery in the changing environment of the developing, healthy adult and diseased heart. Biochim Biophys Acta Mol Cell Res 1867; 2020: 118436.
10. Bachmann M, Kukurainen S, Hytönen VP, Wehrle-Haller B. Cell adhesion by integrins. Physiol Rev 2019; 99: 1655–1699.
11. de Melker AA, Schafer AE, Blaxall BC. Extracellular matrix-mediated cellular communication in the heart. J Mol Cell Cardiol 2016; 91: 228–237.
12. de Melker AA, Schafer AE, Blaxall BC. Extracellular matrix-mediated cellular communication in the heart. J Mol Cell Cardiol 2016; 91: 228–237.
13. Brancaccio M, Cabodi S, Belkin AM, Collo G, Koteliansky VE, Tomatis D, Allruda F, Silengo L, Tarone G. Differential onset of expression of α7 and β1d integrins during mouse heart and skeletal muscle development. Cell Adhes Commun 1998; 5: 193–205.
14. Terracio L, Rubin K, Gulberg D, Balog E, Carver W, Jyring R, Borg TK. Expression of collagen binding integrins during cardiac development and hypertrophy. Circ Res 1991; 68: 734–744.
15. Sun Z, Guo SS, Fassler R. Integrin-mediated mechanotransduction. J Cell Biol 2016; 215: 445–456.
16. Liu S, Calderwood DA, Ginsberg MH. Integrin cytoplasmic domain-binding proteins. J Cell Sci 2000; 113: 3563–3571.
17. Green HJ, Brown NH. Integrin intracellular machinery in action. Exp Cell Res 2019; 378: 226–231.
18. Graham ZA, Gallagher PM, Cardozo CP. Focal adhesion kinase and its role in skeletal muscle. J Muscle Res Cell Motil 2015; 36: 305–315.
19. Franchini KG, Torsoni AS, Soares PH, Saad MJ. Early activation of the multi-component signaling complex associated with focal adhesion kinase induced by pressure overload in the rat heart. Circ Res 2000; 87: 558–565.
20. Tucci AR, Oliveira FOR Jr, Lechuga GC, Oliveira GM, Eleuterio AC, Mesquita CB, Farani PSG, Britto C, Moreira OC, Pereira MCS. Role of FAK signaling in chagasic cardiac hypertrophy. Braz J Infect Dis 2020; 24: 386–397.
21. DiMichele IA, Doherty JT, Rojas M, Escalora DI, Kirouac DI, Taylor JM. Myocyte-restricted focal adhesion kinase deletion attenuates pressure overload-induced hypertrophy. Circ Res 2006; 99: 636–645.
22. Peng X, Kraus MS, Wei H, Shen TL, Pariurut R, Alcaraz A, Ji G, Cheng L, Yang Q, Kodloff MI, Chen J, Chien K, Gu H, Guam JL. Inactivation of focal adhesion kinase in cardiomyocytes promotes eccentric cardiac hypertrophy and fibrosis in mice. J Clin Invest 2006; 116: 217–227.
23. Hao J, Zhang Y, Ye R, Zheng Y, Zhao Z, Guo SS, Fassler R. Integrin-β1-integrin and organized actin filamentation during myocardial hypertrophy. Mol Cell Cardiol 2007; 43: 137–147.
24. Nemoto S, Sheng Z, Lin A. Opposing effects of Jun kinase and p38 mitogen-activated protein kinases on cardiomycocyte hypertrophy. Mol Cell Biol 1998; 18: 3518–3526.
25. Keat I, Molkentin JD. Extracellular signal-regulated kinases 1/2 (ERK1/2) signaling in cardiac hypertrophy. Ann N Y Acad Sci 2010; 1188: 96–102.
26. Rossetto G, Prasad SV, Aracauillo A, Mao J, Koch WJ, Rockman HA. Cardiac overexpression of a G12 inhibitor blocks induction of extracellular signal-regulated kinase and e-Jun NH2-terminal kinase activity in vivo pressure overload. Circulation 2001; 103: 1453–1458.
27. Kojonazarov B, Novoyatleva T, Boehm M, Happe C, Sibinska Z, Tian X, Sajjad A, Luitel H, Kriefling P, Posern G, Evans SM, Grimminer F, Ghofrani HA, Weissmann N, Bogaard HJ, Seeger W, Schermuly RT. p38 MAPK inhibition improves heart function in pressure-loaded right ventricular hypertrophy. Am J Respir Cell Mol Biol 2017; 57: 603–614.
28. Yang C, Li B, Wang G, Xing Y. The attenuation of myocardial hypertrophy by atorvastatin via the intracellular calcium signal and the p38 MAPK pathway. Int J Clin Exp Pathol 2012; 5: 798–807.
29. Muslin AJ. MAPK signalling in cardiovascular health and disease: molecular mechanisms and therapeutic targets. Clin Sci (Lond) 2008; 115: 203–218.
30. Lorenz K, Schmitt JP, Vidal M, Lohse MJ. Cardiac hypertrophy: targeting Raf/MEK/ERK1/2-signaling. Int J Biochem Cell Biol 2009; 41: 2351–2355.
31. Ji Q, Tan Y, Fan D, Pan W, Yu J, Xu W, Wu J, Zhang M. Songling Xuemaikang Capsule inhibits isoproterenol-induced cardiac hypertrophy via CaMKII and ERK1/2 pathways. J Ethnopharmacol 2020; 253: 112660.
32. Souza DS, De Oliveira Barreto T, Menezes-Filho JER, Heimfarth L, Rhana P, Rabelo TK, Santana MNS, Durço AO, Conceição MRL, Quintans-Júnior LJ, Guimaraes AG, Cruz JS, Vasconcelos CM. Myocardial hypertrophy is prevented by farnesol through oxidative stress and ERK1/2 signaling pathways. Eur J Pharmacol 2020; 873: 173583.
33. Ross RS, Borg TK. Integrins and the myocardium. Circ Res 2001; 88: 1112–1119.
34. Wei L, Wang L, Carson JA, Agan JE, Imanaka-Yoshikai K, Schwartz RJ. β1 integrin and organized actin filament...
facilitate cardiomyocyte-specific RhoA-dependent activation of the skeletal α-actin promoter. FASEB J 2001; 15: 785–796.

36. Kuppuswamy D, Kerr C, Narishige T, Kasi VS, Menick DR, Cooper G. Association of tyrosine-phosphorylated c-Src with the cytoskeleton of hypertrophying myocardium. J Biol Chem 1997; 272: 4500–4508.

37. Babbitt CJ, Shai SY, Harpf AE, Pham CG, Ross RS. Modulation of integrins and integrin signaling molecules in the pressure-loaded murine ventricle. Histochem Cell Biol 2002; 118: 431–439.

38. Israeli-Rosenberg S, Manso AM, Okada H, Ross RS. Integrins and integrin-associated proteins in the cardiac myocyte. Circ Res 2014; 114: 572–586.

39. Keller RS, Shai SY, Babbitt CJ, Pham CG, Okada H, Ross RS. Disruption of integrin function in the murine myocardium leads to perinatal lethality, fibrosis, and abnormal cardiac performance. Am J Pathol 2001; 158: 1079–1090.

40. Krishnamurthy P, Subramanian V, Singh M, Singh K. Deficiency of β1 integrins results in increased myocardial dysfunction after myocardial infarction. Heart 2006; 92: 1309–1315.

41. Shai SY, Harpf AE, Babbitt CJ, Jordan MC, Fischlein MC, Chen J, Omura M, Leil Ki TA, Becker KD, Jiang M, Smith DJ, Cherry SR, Loftus JC, Ross RS. Cardiac myocyte-specific excision of the β1 integrin gene results in myocardial fibrosis and cardiac failure. Circ Res 2002; 90: 458–464.

42. Krishnamurthy P, Subramanian V, Singh M, Singh K. β1 integrins modulate β-adrenergic receptor-stimulated cardiac myocyte apoptosis and myocardial remodeling. Hypertension 2007; 49: 865–872.

43. Li R, Wu Y, Manso AM, Gu Y, Liao P, Harpf AE, Sajima T, Nguyen Y, Huang MS, Dalton ND, Peterson KL, Ross RS. β1 integrin gene excision in the adult murine cardiac myocyte causes defective mechanical and signaling responses. Am J Pathol 2012; 180: 952–962.

44. Okada H, Lai NC, Kawaraguchi Y, Liao P, Copps J, Sugano Y, Okada-Maeda S, Banerjee I, Schilling JM, Gingras AR, Asfaw EK. Integrins protect cardiomyocytes from ischemia/reperfusion injury. J Clin Invest 2013; 123: 4294–4308.

45. Suryakumar G, Kasi gasesan H, Balasubramanian S, Kuppuswamy D. Lack of β3 integrin signaling contributes to calpain-mediated myocardial cell loss in pressure-overloaded myocardium. J Cardiovasc Pharmacol 2010; 55: 556–567.

46. Porter KE, Turner NA. Cardiac fibroblasts: at the heart of myocardial remodeling. Pharmaco ther 2009; 123: 255–278.

47. Lindahl GE, Chambers RC, Papakrivopoulos J, Dawson SJ, Jacobsen MC, Bishop JE, Laurent GJ. Activation of fibroblast procollegen α1(I) transcription by mechanical strain is transforming growth factor-β-dependent and involves increased binding of CCAAT-binding factor (CBF/NF-Y) at the proximal promoter. J Biol Chem 2002; 277: 6153–6161.

3641

48. Wang J, Chen H, Seth A, McCulloch CA. Mechanical force regulation of myofibrillar differentiation in cardiac fibroblasts. Am J Physiol Heart Circ Physiol 2003; 285: H1871–H1881.

49. Hinz B. The myofibrillar: paradigm for a mechanically active cell. J Biomech 2010; 43: 146–155.

50. Fiore VF, Wong SS, Tran C, Tan C, Xu W, Sulchek T, White ES, Haggood JS, Barker TH. avβ3 Integrin drives fibroblast contraction and strain stiffening of soft pro-visional matrix during progressive fibrosis. JCI Insight 2018; 3: e97597.

51. Chen H, Qu J, Huang X, Kurundkar A, Zhu L, Yang N, Venado A, Ding Q, Liu G, Antosy VB, Thannickal VJ, Zhou Y. Mechanosensing by the αvβ3 integrin confers an invasive fibroblast phenotype and mediates lung fibrosis. Nat Commun 2016; 7: 12564.

52. Balasubramanian S, Quiones L, Kasiganesan H, Zhang Y, Pleasant DL, Sundaramurthy KP, Zile MZ, Bradshaw AD, Kuppuswamy D. β3 Integrin in cardiac fibroblast is critical for extracellular matrix accumulation during pressure overload hypertrophy in mouse. PLOS ONE 2012; 7: e45076.

53. Li Z, Lee H, Zhu C. Molecular mechanisms of mechanotransduction in integrin-mediated cell-matrix adhesion. Exp Cell Res 2016; 349: 85–94.

54. Tyson J, Bundy K, Roach C, Douglas H, Banerjee I, Schilling JM, Gingras AR, Tyson J, Bundy K, Roach C, Douglas H, Botvinick EL, George SC, Hughes CCW. Contractile apparatus organization of three-dimensional adult cardiac extracellular matrix promotes maturation of cardiomyocytes from ischemia/reperfusion injury. J Cell Biol 2013; 192: 872–882.

55. Geisse NA, Sheehy SP, Parker KK. Control of myocyte remodeling in vitro with engineered substrates. In Vitro Cell Dev Biol Anim 2009; 45: 343–350.

56. Bil'diug NB, Iudintseva NM, Pinaev GP. Contractile apparatus organization of cardiomyocytes upon their cultivation in collagen gels. Tsitologiya 2014; 56: 822–827.

57. Fong AH, Romero-López M, Heylman CM, Keating M, Tran D, Sobrino A, Tran AQ, Pham HH, Fimbres C, Gershon PD, Botvinick EL, George SC, Hughes CCW. Three-dimensional adult cardiac extracellular matrix promotes maturation of human induced pluripotent stem cell-derived cardiomyocytes. Tissue Eng Part A 2016; 22: 1016–1025.

58. Cuikerman E, Pankov R, Stevens DR, Yamada KM. Taking cell-matrix adhesions to the third dimension. Science 2001; 294: 1708–1712.

59. Wildey CD, Balasubramanian S, Rosas MCR, Ross RS, Kuppuswamy D. Focal complex formation in adult cardiomyocytes is accompanied by the activation of β3 integrin and c-Src. J Mol Cell Cardiol 2003; 35: 671–683.

60. Bhana B, Iyer RK, Chen WJ, Zhao R, Sider KL, Likhitpanichkul M, Simmons CA, Radisic M. Influence of substrate stiffness on the phenotype of heart cells. Biotechnol Bioeng 2010; 105: 1146–1160.

61. Keely PJ, Conklin MW, Gehler S, Ponik SM, Provenzano PP. Investigating integrin regulation and signaling events in three-dimensional systems. Methods Enzymol 2007; 426: 27–45.

62. Burke MA, Cook SA, Seidman JD, Seidman CE. Clinical and mechanistic insights into the genetics of cardiomyopathy. J Am Coll Cardiol 2016; 68: 2871–2886.

63. Shimizu I, Minamino T. Physiological and pathological cardiac hypertrophy. J Mol Cell Cardiol 2016; 97: 245–262.

64. Nakamura N, Sadoshima J. Mechanisms of physiological and pathological cardiac hypertrophy. Nat Rev Cardiol 2018; 15: 387–407.

65. Silberbach M, Gorenc T, Hershberger RE, Stork PJ, Stegger PS, Roberts CT Jr. Extracellular matrix signal-regulated protein kinase activation is required for the anti-hypertrophic effect of atrial natriuretic factor in neonatal rat ventricular myocytes. J Biol Chem 1999; 274: 24858–24864.

66. Sharp WW, Simpson DG, Bong TK, Samarel AM, Terracio L. Mechanical forces regulate focal adhesion and costamere assembly in cardiac myocytes. Am J Physiol 1997; 273: 546–556.

67. Yutao X, Geru W, Xiaojun B, Tao G, AiQun M. Mechanical stretch-induced hypertrophy of neonatal rat ventricular myocytes is mediated by β3-integrin/microtubule signaling pathways. Eur J Heart Fail 2006; 8: 16–22.

68. Torsoni AS, Constancio SS, Nadruz W, Hanks SK, Franchini KG. Focal adhesion kinase is activated and mediates the early hypertrophic response to stretch in cardiac myocytes. Circ Res 2003; 93: 140–147.

69. Ruwolof C, Egas JM, van Wamel AET, van der Laarse A. Signal transduction mechanisms of mechanosensitive channels in adult cardiac myocytes. Tissue Eng Part A 2016; 22: 1016–1025.

70. Cuikerman E, Pankov R, Stevens DR, Yamada KM. Taking cell-matrix adhesions to the third dimension. Science 2001; 294: 1708–1712.

71. Wildey CD, Balasubramanian S, Rosas MCR, Ross RS, Kuppuswamy D. Focal complex formation in adult cardiomyocytes is accompanied by the activation of β3 integrin and c-Src. J Mol Cell Cardiol 2003; 35: 671–683.

72. Bhana B, Iyer RK, Chen WJ, Zhao R, Sider KL, Likhitpanichkul M, Simmons CA, Radisic M. Influence of substrate stiffness on the phenotype of heart cells. Biotechnol Bioeng 2010; 105: 1146–1160.
72. Gray MO, Long CS, Kalinyak JE, Li HT, Karliner JS. Angiotensin II stimulates cardiac myocyte hypertrophy via paracrine release of TGF-β1 and endothelin-1 from fibroblasts. *Cardiovasc Res* 1998; 40: 352–363.

73. Pham CG, Harpf AE, Keller RS, Vu HT, Shai SY, Loftus JC, Ross RS. Striated muscle-specific β1-integrin and FAK are involved in cardiac myocyte hypertrophic response pathway. *Am J Physiol Heart Circ Physiol* 2000; 279: H2916–H2926.

74. Taylor JM, Rovin JD, Parsons JT. A role for focal adhesion kinase in phenylephrine-induced hypertrophy of rat ventricular cardiomyocytes. *J Biol Chem* 2000; 275: 19250–19257.

75. Chien KR, Knowlton KU, Zhu H, Chien S. Regulation of cardiac gene expression during myocardial growth and hypertrophy: molecular studies of an adaptive physiologic response. *FASEB J* 1991; 5: 3037–3046.

76. Kim DJ, Park SH, Lim CS, Chun JS, Kim JK, Song WK. Cellular localization of integrin isoforms in phenylephrine-induced hypertrophic cardiac myocytes. *Cell Biochem Funct* 2003; 21: 41–48.

77. Ross RS, Pham C, Shai SY, Goldhaver JI, Fenczik C, Glembotski CC, Ginsberg MH, Loftus JC. β1 integrins participate in the hypertrophic response of rat ventricular myocytes. *Circ Res* 1998; 82: 1160–1172.

78. Wang YG, Ji X, Pabbidi M, Samarel AM, Lipsius SL. Laminin acts via focal adhesion kinase/phosphatidylinositol-3-kinase/protein kinase B to down-regulate β1-adrenergic receptor signalling in cat atrial myocytes. *J Physiol* 2009; 587: 541–550.

79. Heidkamp MC, Bayer AL, Scully BT, Eble DM, Samarel AM. Activation of focal adhesion kinase by protein kinase Cε in neonatal rat ventricular myocytes. *Am J Physiol Heart Circ Physiol* 2003; 285: H1684–H1696.

80. Purcell NH, Tang G, Yu C, Mercurio F, DiDonato JA, Lin A. Activation of NF-κB is required for hypertrophic growth of primary rat neonatal ventricular cardiomyocytes. *Proc Natl Acad Sci U S A* 2001; 98: 6668–6673.

81. Heidkamp MC, Bayer AL, Scully BT, Eble DM, Samarel AM. Activation of focal adhesion kinase by protein kinase Cε in neonatal rat ventricular myocytes. *Cell Biochem Funct* 2018; 36: 372–383.

82. Ogawa E, Saito Y, Harada M, Kamitani S, Kuwahara K, Miyamoto Y, Ishikawa M, Hamanaka I, Kajiya M, Takahashi N, Nakagawa O, Masuda I, Kishimoto I, Nakao K. Outside-in signalling of fibronectin stimulates cardiomyocyte hypertrophy in cultured neonatal rat ventricular myocytes. *J Mol Cell Cardiol* 2000; 32: 765–776.

83. Nagai T, Laser M, Baicu CF, Zile MR, Cooper G IV, Kuppuswamy D. Beta3-integrin-mediated focal adhesion complex formation: adult cardiocytes embedded in three-dimensional polymer matrices. *Am J Cardiol* 1999; 83: 38H–43H.

84. Bildyug N, Bozhokina E, Khaitлина S. Contribution of α-smooth muscle actin and extracellular matrix to the in vitro reorganization of cardiomyocyte contractile system. *Cell Biol Int* 2016; 40: 472–477.

85. Bildyug N, Khaitлина SY. Redistribution of sarcomeric myosin and α-actinin in cardiomyocytes in culture upon the rearrangement of their contractile apparatus. *Cell Tissue Biol* 2019; 13: 360–365.

86. Bildyug N, Voronkina IV, Smagina LV, Yudintseva NM, Pinaev GP. Matrix metalloproteinases in primary culture of cardiomyocytes. *Biochemistry (Mosk)* 2015; 80: 1318–1326.

87. Bildyug N. Dynamics of integrin-linked kinase during the rearrangement of contractile apparatus in rat neonatal cardiomyocytes. *Febs Open Bio* 2019; 9: 197.

88. Bildyug N. Redistribution of focal adhesion kinase in rat neonatal cardiomyocytes in culture. *J Muscle Res Cell Motil* (Special Issue: The European Muscle Conference 2019) 2019; 40: 244. 

*ESC Heart Failure* 2021; B: 3634–3642
DOI: 10.1002/ehf2.13497