

Integrins, Focal Adhesions, and Cardiac Fibroblasts

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Abstract: How the myocardium undergoes geometric, structural, and molecular alterations that result in an end phenotype as might be seen in patients with dilated cardiomyopathy or after myocardial infarction is still poorly understood. Structural modification of the left ventricle, which occurs during these pathological states, results from long-term changes in loading conditions and is commonly referred to as “remodeling.” Remodeling may occur from increased wall stress in the face of hypertensive heart disease, valvular disease, or, perhaps most dramatically, after permanent coronary occlusion. A fundamental derangement of myocyte function is the most common perception for the basis of remodeling, but the role of cells in the heart other than the muscle cell must, of course, be considered. Although studies of the myocyte have been extensive, cardiac fibroblasts have been studied less than myocytes. The fibroblast has a broad range of functions in the myocardium ranging from elaboration and remodeling of the extracellular matrix to communication of a range of signals within the heart, including electrical, chemical, and mechanical ones. Integrins are cell surface receptors that are instrumental in mediating cell-matrix interactions in all cells of the organism, including all types within the myocardium. This review will focus on the role of integrins and related proteins in the remodeling process, with a particular emphasis on the cardiac fibroblast. We will illustrate this function by drawing on 2 unique mouse models with perturbation of proteins linked to integrin function.

Key Words: integrin function, cardiac fibroblasts

(*J Investig Med* 2009;57: 856–860)

How the myocardium undergoes geometric, structural, and molecular alterations that results in an end phenotype as might be seen in patients with dilated cardiomyopathy or after myocardial infarction (MI) is still poorly understood. Structural modification of the left ventricle, which occurs during these pathological states, results from long-term changes in loading conditions and is commonly referred to as “remodeling.” Remodeling may occur from increased wall stress in the face of hypertensive heart disease, valvular disease, or, perhaps most dramatically, after permanent coronary occlusion.¹ A fundamental derangement of myocyte function is the most common perception for the basis of remodeling, but the role of cells in the heart other than the muscle cell must, of course, be considered. Although studies of the myocyte have been extensive, cardiac fibroblasts (CFs) have been studied less than myocytes. The

fibroblast has a broad range of functions in the myocardium ranging from elaboration and remodeling of the extracellular matrix (ECM) to communication of a range of signals within the heart, including electrical, chemical, and mechanical ones.² Integrins are cell surface receptors that are instrumental in mediating cell-matrix interactions in all cells of the organism, including all types within the myocardium. This review will focus on the role of integrins and related proteins in the remodeling process, with a particular emphasis on the CF. We will illustrate this function by drawing on 2 unique mouse models with perturbation of proteins linked to integrin function.

Integrin Function: Generalities and Emphasis on the CF

Integrins are heterodimeric transmembrane receptors composed of α and β subunits.^{3,4} The general arrangement of integrin subunits is that of a large extracellular domain (700–1100 amino acids), a single transmembrane segment and a short cytoplasmic tail, ranging from 20 to 60 amino acids.⁵ Ligand binding occurs to the extracellular domain of the integrin heterodimer, a process that is modified by range of amino acids spread throughout both the extracellular and the transmembrane domains.⁶ The cytoplasmic domain of many of the β subunits is highly homologous, whereas that of the α subunit sequences is significantly more diverse. It is through the cytoplasmic tail that the integrins bind both a range of cytoskeletal linker molecules and also signal. Importantly, it has been recognized that binding of talin to integrin tails produces a change in integrin affinity for ligand, a process termed integrin activation.^{7,8} Integrin activation may not only be solely restricted to talin binding but also involve binding of other proteins to the integrin tail, such as kindlin-3.⁹ For additional data on detailed integrin structure, the reader is referred to the excellent reviews of Humphries,⁵ Takada et al.,⁶ Green et al.,¹⁰ and Hynes.¹¹

Initial studies determined that integrins were essential for adhesion of cells to the ECM.^{12–14} Therefore, integrins are fundamental components in the interaction between the ECM and the cardiac myocytes or fibroblasts. Still, as shown in Figure 1, it is now clear that integrins are multifunctional receptors with purposes that, in addition to adhesion, include the regulation of cellular phenotype in the developing and postnatal myocardium, modulating processes such as cell survival, cell migration, as well as remodeling of the cellular cytoskeleton and focal adhesion (FA). A most intriguing role of integrins in the heart is their ability to serve as mechanotransducers during normal development and in response to physiological and pathophysiological signals.^{15–19}

Most importantly, it is well accepted that integrins are important signal transducers.^{11,20,21} The integrins function in a bidirectional manner across cell membranes. Ligand binding to the integrins results in intracellular signaling events, a cascade termed “outside-in” signaling. In this role, the integrins can influence a wide range of activities of the type mentioned previously, including alterations in cell morphology, migration, proliferation, differentiation, survival, gene expression, suppression of tumorigenicity, changes in intracellular pH, or concentration of cytosolic Ca^{2+} . Because the integrins themselves do

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Received September 9, 2009.

Accepted for publication September 25, 2009.

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Supported by grants from the Veterans Administration (VA Merit) and the National Institutes of Health (P01 HL066941 and RO1 HL088390) to R.S.R., and the symposium was supported in part by a grant from the National Center for Research Resources (R13 RR023236).

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ISSN: 1081-5589

DOI: 10.2310/JIM.0b013e3181c5e61f

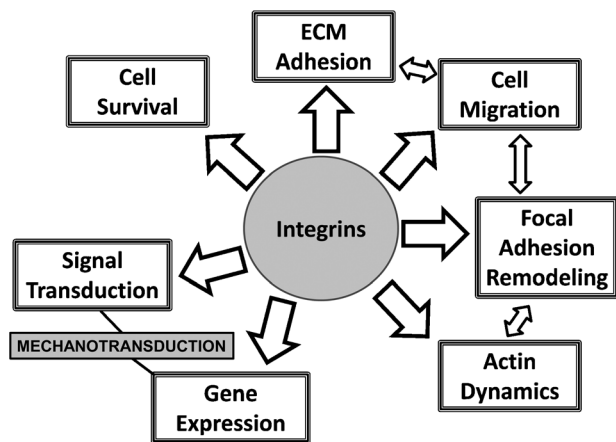


FIGURE 1. Integrins are multifunctional cell surface receptors.

not possess enzymatic activity, they signal through the activation of downstream molecules such as kinases pp125 FA kinase (FAK) or integrin-linked kinase, small GTPases such as Rho or Rac, and regulation of cytoskeletal components such as talin, paxillin, or p130 (CAS), several of which may bind directly to the cytoplasmic domain of the integrin heterodimer. Ultimately, signaling from the integrins may influence pathways through which other cellular effectors (such as growth factors) may also signal, including those requiring Akt, Raf, phosphoinositide 3-kinase, or mitogen-activated protein kinases/extracellularly regulated kinases.

Integrin function can also be modified by agonists that bind to nonintegrin cellular receptors and in turn modify integrin activation, a process termed “inside-out” signaling. Here, both increased binding of integrin to ligand and clustering of multiple integrins in close spacing within the cell membrane occur. It is clear that integrin clustering in both myocytes and fibroblasts in the myocardium is associated with chemical and mechanical signaling.²²

The integrin family is large, with more than 18 α and 8 β subunits identified in mammals. From these various subunits, more than 24 pairs of integrins receptors can arise. Although it is clear that all combinations of $\alpha\beta$ heterodimers can form, how the preferential pairing of subunits occurs in a specific cell

is not clear. The complex nature of this protein family is increased to an even greater extent by the numerous splice variant isoforms of individual subunits, including some expressed in the heart.^{23–25} The integrins expressed on a particular cell type can be unique and can vary depending on developmental stage or pathological state (Table 1). Further complexity is evident because a single integrin receptor can bind to one or several ligands and, in addition, a single ligand can be bound by several integrin heterodimers. For example, CFs express $\alpha_5\beta_1$ (a fibronectin [FN]/osteopontin [OPN] binding integrin) as well as $\alpha_v\beta_1$, $\alpha_v\beta_3$, and $\alpha_v\beta_5$ (which bind FN, OPN, as well as vitronectin).^{26–29} In addition, components of the ECM can bind to several different integrins, allowing potentially for even further functional redundancy and complexity.

Cardiac fibroblasts can play critical roles in cardiac remodeling and may do so via an ECM-integrin interaction. The fibroblasts seem to play diverse functions. For example, Gullberg et al.³⁰ noted that platelet-derived growth factor-stimulated collagen gel contraction by CFs could be partly inhibited by β_1 integrin antibodies. With a stretch of cultured myocytes, release of angiotensin II (AngII) occurred.³¹ Once produced, AngII stimulation of CFs modulated integrin localization and increased expression of both α_8 and β_1 integrins.^{32,33} Furthermore, AngII increased the adhesion of fibroblasts to collagen I via protein kinase C ϵ .³⁴ Fourteen days of AngII infusion in rats caused increased α_8 expression in myofibroblasts.³⁵ Carver et al.³⁶ have shown that $\alpha_8\beta_1$ integrins play a role in collagen gel contraction by rodent CFs. Several studies have focused on various integrins, cytokines, and signaling in the function of CFs. For example, a stretch of rat CFs resulted in both extracellular-signal regulated kinase 2 and JNK1 activation and activation varied dependent on the integrin subunits (eg, α_4 vs α_5) bound by ECM.³⁷ Integrin overexpression can increase the expression and activation of transforming growth factor β (TGF- β), an important cytokine in the fibrotic response of the heart.^{38,39} Conversely, TGF- β stimulation of fibroblasts was found to increase the expression of $\alpha_5\beta_1$ integrins.⁴⁰

Only a few studies on integrins and fibroblasts are available related to the intact heart. Burgess et al.⁴¹ studied rats subjected to treadmill exercise or hypertension. β_1 Integrin was elevated with both hypertrophic stimuli; α_1 and α_2 levels both decreased, but α_5 increased in the exercise group and decreased in the hypertensive one. Sun et al.⁴² studied the role of β integrins after MI using rat and mouse as model systems. Both

TABLE 1. Integrin Expression in CFs in the Basal and Diseased Myocardium

| α Subunit | Subunit(s) Partner | Ligand(s) | Basal Adult | Heart Failure (Relative to Basal Adult) | Hypertension (Relative to Basal Adult) |
|------------------|--------------------------------------|----------------|-------------|---|--|
| α_1 | β_{1A} , β_3 | LN, Col | ++ | = | Decreased |
| α_2 | β_{1A} | LN, Col | + | = | Decreased |
| α_3 | β_{1A} | LN, Col, FN | + | Not known | Not known |
| α_4 | β_{1A} | FN, OPN | +++ | Not known | Not known |
| α_5 | β_{1A} | FN, OPN | +++ | | Decreased |
| α_6 | β_{1A} | LN | + | Not known | Not known |
| α_8 | β_{1A} | FN, VN, OPN | + | Not known | Not known |
| α_v | β_{1A} , β_3 , β_5 | FN, VN, OPN | + | = | Not known |
| β_{1A} | Not applicable | Not applicable | +++ | Decreased | Increased |
| β_3 | Not applicable | Not applicable | ++ | Not known | Not known |
| β_5 | Not applicable | Not applicable | + | Not known | Not known |

Col indicates collagen; FN, fibronectin; LN, laminin; VN, vitronectin; OPN, osteopontin.

β_1 and β_3 integrins were low basally but increased at the infarct zone by day 3 after MI, peaking at day 7 after MI, then returned toward baseline. In line with the cell type-specific expression of integrins, β_{1A} was found primarily on fibroblasts and inflammatory cells, whereas β_{1D} was expressed on myocytes and β_3 was detected principally on endothelial and smooth muscle cells in the peri-infarct vessels. Thus, although several studies of integrin function in the CF have been performed, a full understanding of the role of integrins in these cells, particularly in the face of cardiac pathologies, is far from complete. For the remainder of this review, we would like to use 2 examples to further illustrate the function of integrins and related proteins in the myocardium and CF.

OPN Is Involved in Hypertrophic and Fibrotic Responses of the Heart

Osteopontin is a multifunction glycoprotein that is a member of the small integrin binding ligand N-linked glycoproteins family.⁴³ It is a soluble cytokine, a phosphoprotein that is secreted by many cells including cardiac myocytes and fibroblasts. As a matricellular molecule, it does not serve a structural role in the ECM but rather facilitates ECM responses. It can bind to numerous integrins including $\alpha_v\beta_3$, $\alpha_v\beta_1$, $\alpha_v\beta_5$, $\alpha_4\beta_3$, $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_8\beta_1$, $\alpha_9\beta_1$, and $\alpha_4\beta_7$, as well as the hyaluronic acid receptor CD44. Functioning through a series of downstream signaling pathways, it can direct cellular adhesion, migration, and survival. Despite its multiple functions, OPN homozygous knockout (KO) mice survive⁴⁴ and were therefore used in our prior work by Collins et al.⁴⁵ to study the function of OPN in the myocardium.

Continuous infusion of AngII via osmotic minipump was performed in wild-type (WT) mice at levels that evoked a 50-mm Hg rise in systolic blood pressure. With this, increases in myocardial OPN transcript expression were detected by 4 days into the infusion, with subsequent normalization to baseline levels by 3 weeks. These results suggested that OPN is a component of the myocardial stress response invoked by AngII. Given this, the importance of OPN was investigated by use of the OPN KO mice mentioned previously. With this infusion, hypertension, and related stress, the WT mice underwent the expected increases in heart weight/body weight ratios, indicative of ventricular hypertrophy, but the OPN KO mice showed a blunted morphometric response, despite similar increases in molecular markers of hypertrophy. With this, the amount of AngII-induced fibrosis was assessed in the myocardium, and it was seen that the absence of OPN leads to a reduced fibrotic response, although ECM production (fibronectin, collagen I), A1 integrin, and TGF- α transcripts, all increased similarly in the WT and KO mice. Given this, the effect of OPN on fibroblast function was evaluated using CFs isolated from WT and OPN KO mice. The KO fibroblasts were seen to have reduced adhesion and proliferation, properties that could be partially restored toward normal by the addition of exogenous OPN protein to the cultured KO cells. In support of these results were studies from Trueblood et al.⁴⁶ and Xie et al.,⁴⁷ which showed that OPN KO hearts had a reduced hypertrophic response after aortic constriction and also showed blunted collagen production and fibrosis after MI. Finally, recent data from Lenga et al.⁴⁸ have linked OPN to the formation of mature FAs and transformation of fibroblasts to myofibroblasts, a process important for the formation of scars during processes such as the post-MI state. Thus, it seems that OPN, as a protein produced by both cardiac myocytes and fibroblasts, is critically linked to both hypertrophic and fibrotic response pathways in the myocardium. It provides an important example of a substance involved in cell-

matrix adhesion and modulation of integrin function, which could be potentially modulated in amount or function, to avoid adverse cardiac remodeling.

FAK Is Linked to CF Proliferation, Migration, and Transformation Toward a Myofibroblast Phenotype

A second example of how integrin/FA function is involved in the cardiac stress response comes from our recent work on FAK.⁴⁹ As discussed previously, integrins do not possess their own kinase activity, and therefore, to transduce signals, they use a host of downstream kinases. Principal among them is FAK. We sought to assess the function of FAK in adult CFs by culturing CF from homozygous FAK “floxed” mice originally produced by Beggs et al.⁵⁰ With these cells in hand, we deleted FAK expression by infecting the cells with a recombinant adenovirus producing Cre recombinase. We thus produced cells devoid of FAK. First, we assessed how these cells displayed FAs and expressed FA proteins. We found that the FAs were morphologically preserved and that the FA proteins vinculin, paxillin, and talin were expressed equally in KO and control cells but that in the FAK-deficient CFs, the expression of Pyk2, a cytoplasmic tyrosine kinase that shares approximately a 45% amino acid sequence identity with FAK, was upregulated. The FAK KO CF had normal adhesion, increased proliferation, and reduced migration, when stimulated by platelet-derived growth factor BB. Using FAK-related nonkinase (FRNK) as an inhibitor of FAK function, we found that Pyk2 was not increased in the FRNK-expressing WT CF. The FRNK caused reduction of CF migrations but did not alter cell proliferation, suggesting that Pyk2 was at least partly responsible for the proliferation defects in the FAK KO CF but that it was not involved in the migratory defect seen in these cells.

The interesting work conducted by Clemente et al.⁵¹ has begun to study the function FAK in vivo and showed that FAK was present in both myocytes and fibroblasts of the murine heart but increased dominantly in nonmyocytes after aortic constriction. When mice were treated via single jugular vein injection with small interfering RNA directed toward FAK, FAK expression was reduced more than 70%, and the mice were seen to have reduced fibrosis and collagen production after aortic constriction. Importantly, the work by Swaney et al.⁵² has linked FAK, like OPN, to the transformation of fibroblasts to myofibroblasts. Thus, FAK as an important mediator of integrin signaling seems also linked to CF proliferation, migration, and conversion of CFs to myofibroblasts.

SUMMARY

In this review, we have begun to illustrate how integrins and their related ECM/matricellular and FA proteins, such as OPN and FAK, seem critically involved in the proliferative and fibrotic responses of the stressed or injured myocardium. Study into proteins of these types has just begun, and a great deal of work is necessary to more fully understand their importance in cardiac disease processes. In this regard, caveats must be appreciated as we seek additional information about the function of these proteins in fibroblast function including the organ- and species-specific nuances of fibroblasts, that is, “not all fibroblasts are created equal”; that studies of cells from one developmental stage may yield unique results compared with another (ie, embryonic, neonatal, and adult fibroblasts form the heart may have distinct properties); that culture studies may not always port-over to ones produced in the intact animal; and similarly, that study in 2-dimensional culture may be different

than that performed in 3-dimensional culture. Much work is still necessary, but our progress to date surely illustrates how modulation of FA proteins may be an interesting future therapeutic target for cardiovascular diseases.

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