Mechanobiology of myofibroblast adhesion in fibrotic cardiac disease

Alison K. Schroer and W. David Merryman

ABSTRACT
Fibrotic cardiac disease, a leading cause of death worldwide, manifests as substantial loss of function following maladaptive tissue remodeling. Fibrosis can affect both the heart valves and the myocardium and is characterized by the activation of fibroblasts and accumulation of extracellular matrix. Valvular interstitial cells and cardiac fibroblasts, the cell types responsible for maintenance of cardiac extracellular matrix, are sensitive to changing mechanical environments, and their ability to sense and respond to mechanical forces determines both normal development and the progression of disease. Recent studies have uncovered specific adhesion proteins and mechano-sensitive signaling pathways that contribute to the progression of fibrosis. Integrins form adhesions with the extracellular matrix, and respond to changes in substrate stiffness and extracellular matrix composition. Cadherins mechanically link neighboring cells and are likely to contribute to fibrotic disease propagation. Finally, transition to the active myofibroblast phenotype leads to maladaptive tissue remodeling and enhanced mechanotransductive signaling, forming a positive feedback loop that contributes to heart failure. This Commentary summarizes recent findings on the role of mechanotransduction through integrins and cadherins to perpetuate mechanically induced differentiation and fibrosis in the context of cardiac disease.

KEY WORDS: Cadherin, Integrin, Myofibroblast

Introduction
The heart is a highly dynamic mechanical environment that stretches and contracts over three billion times during the course of the average human life. Proper functioning of cardiac structures is dependent on their active and passive mechanical properties, and alteration of these properties is a hallmark of fibrotic cardiac disease. Given the high societal cost of heart disease, the mechanisms regulating fibrotic disease progression in heart valves and the myocardium have been extensively studied. In both tissues, inflammation and fibrosis drive extensive tissue remodeling that significantly impairs cardiac function (Fig. 1A). Inflammation triggers extracellular matrix (ECM) degradation and the release of profibrotic factors, such as angiotensin II (AGT; hereafter referred to as AngII), transforming growth factor β1 (TGF-β1), and fibroblast growth factor (FGF), that promote accumulation of ECM and fibrosis of the valves and myocardium (Chen and Frangogiannis, 2013; Mahler and Butcher, 2011). However, drugs targeting these pathways have had limited success, which has been attributed to their failure to address the concurrent mechanical signals that play a crucial role in the initiation and progression of disease (Frangogiannis, 2014). Conditions that favor increased local tissue strains and stresses increase the risk of developing fibrotic disease in affected structures by altering the behavior and phenotype of cardiac cells, which contributes to maladaptive tissue remodeling (Cuniberti et al., 2006; Merryman et al., 2006). Therefore, determining how various cardiac cells respond to changing mechanical environments will aid our understanding of the development of heart disease and potentially uncover new targets for future therapy (Fig. 1A).

Valvular interstitial cells (VICs) and cardiac fibroblasts (CFs) are primarily responsible for maintaining the ECM, and are sensitive to mechanical forces in addition to chemical cues. Mechanically induced signaling promotes myofibroblast (MyoFB) differentiation of VICs and CFs, resulting in cells that exhibit increased contractility and increased secretion of growth factors and ECM proteins (Fig. 1B) (Tomasek et al., 2002). MyoFBs interact with the local ECM and sense tissue forces through transmembrane ECM receptors called integrins. Upon adhesion to the ECM, integrins recruit intracellular proteins to form a focal adhesion (FA), which links integrins to the actin cytoskeleton and initiates force-dependent signaling. In addition to force transduction from the ECM to the cells, integrins also regulate force transmission from intracellular stress fibers to the local microenvironment (Baker and Zaman, 2010). MyoFBs are identified by their expression of α-smooth muscle actin (α-SMA), a specialized cytoskeletal protein that allows for enhanced generation of cellular force (Hinz et al., 2004). These forces are, in turn, transmitted to the local microenvironment, altering ECM structure, and substantially affecting tissue mechanics (Petersen et al., 2012).

Intracellular forces are also transmitted to neighboring cells through adhesions junctions (AJs), which contain Ca2+-dependent adhesion proteins called cadherins. Classical cadherins form homotypic bonds with cadherins on neighboring cells and mechanically link the cytoskeletal elements of both cells. Different cadherins are found in various cell types, and major shifts in cell phenotype are often accompanied by a corresponding shift in cadherin expression. Neuronal (N)-cadherin is typically expressed in cardiomyocytes (CMs) and quiescent fibroblasts, whereas osteoblast (OB)-cadherin (also known as cadherin-11) is highly expressed in MyoFBs (Aisagbonhi et al., 2011; Chalpe et al., 2010; Heuberger and Birchmeier, 2010; Hinz et al., 2004; Li et al., 2012). OB-cadherin forms significantly stronger bonds than N-cadherin and is, thus, able to transmit increased levels of intercellular tension generated by α-SMA in MyoFBs (Hinz et al., 2004; Hutcheson et al., 2013). Recent studies have revealed new signaling roles for cadherins in addition to their structural function (Leckband and de Rooij, 2014). Signaling effectors downstream of cadherins, including β-catenin, undergo a substantial crosstalk with cytokine and integrin signaling in the regulation of MyoFB differentiation and function (Caraci et al., 2008; Charbonney et al., 2011; Xu et al., 2012).

This Commentary focuses on mechanotransduction in VICs and CFs during cardiac fibrotic disease, and its effects on the regulation of MyoFB differentiation. Mechanotransduction in endothelial cells is certainly relevant to cardiovascular disease and has been described in an excellent recent review (Conway and Schwartz, 2015).
2013), so this aspect will not be discussed in detail. Likewise, mechanotransduction in CMs is also important to disease progression and has been the subject of another recent review (McCain and Parker, 2011). The adhesion molecules of particular interest for this Commentary are integrins and cadherins, which are both relevant to mechanically regulated signaling and force transmission in the heart. Other important mechanotransducers, including mechano-sensitive Ca\(^{2+}\)-channels and syndecans are either not involved in adhesion (Teng et al., 2014), or have already been extensively reviewed elsewhere (Frangogiannis, 2010). This Commentary will describe the interactions between mechanical forces and biochemical cues from tissue level down to intracellular signaling, focusing on the particular integrins and cadherins that perpetuate these signals and contribute to the progression of fibrotic cardiac disease.

**Etiology of fibrotic cardiac disease**

Fibrotic disease affects both the valves and the myocardium, and is characterized by significant changes in tissue composition and biochemical signaling that, in turn, affect cell behavior and accelerate progression of disease (Fig. 1A). Several cardiac conditions with large clinical impacts are perpetuated by active remodeling of the ECM by VICs in valves and by CFs in the myocardium.

**Heart valve disease**

Valve disease affects over six million Americans and is associated with changes in mechanical properties of the leaflets that impair normal blood flow through the heart (Go, 2013). The mitral and aortic valves on the left side of the heart are most susceptible to disease, which manifests most often as regurgitation or stenosis (Go et al., 2013). Mitral regurgitation is the most common type of heart valve disorder (1.7% of adults), but aortic valve disorders are associated with higher mortality, especially when calcification and stenosis of the valve is evident (Go et al., 2013). The initial causes of these conditions are often linked to altered mechanical loading (e.g. hypertension, ventricular remodeling, bicuspid aortic valve mutation) and, in all cases, tissue remodeling is perpetuated by inflammation and fibrosis, which trigger ECM degradation and accumulation, respectively (de Marchena et al., 2011; Hutcheson et al., 2014). The mitral valve is particularly susceptible to tissue weakening in a process known as myxomatous remodeling, whereas the aortic valve is susceptible to
tissue stiffening in response to fibrosis and sclerotic remodeling (Geirsson et al., 2012; Merryman and Schoen, 2013). Myxomatous remodeling is characterized by increased expression of TGF-β1 (TGFβ1), matrix metalloproteinases (MMPs) and several ECM components, and results in valve billowing and regurgitating blood flow (Aupperle and Disatian, 2012; Geirsson et al., 2012; Hagler et al., 2013). Calcific aortic valve disease (CAVD), a stenotic disease that often necessitates valve replacement, is characterized by increased expression of TGF-β1 and collagen – especially collagen-1 – and the development of calcified nodules, resulting in a stiff, partially occluded, stenotic valve (Le Polain de Waroux et al., 2007; Mohler et al., 2001). These pathologic alterations to the mechanical properties of the tissue are primarily mediated by VICs, a heterogeneous population of fibroblast-like cells that differentiate into active MyoFBs in response to fibrotic signaling factors and mechanical cues (Fig. 1) (Liu et al., 2007).

Cardiac fibrosis

Cardiac fibrosis is a hallmark of heart failure and results in an increased passive stiffness of the heart wall, diastolic dysfunction and poor long-term prognosis (Azevedo et al., 2010; Burlew and Weber, 2002; Dusenbery et al., 2014). Many chronic cardiovascular conditions, including valve disease and hypertension, can cause pressure overload in the ventricles that subsequently develops into hypertrophy and fibrosis (Azevedo et al., 2010; Dusenbery et al., 2014; Huang et al., 2010). Another common initiator of cardiac fibrosis is scar formation after myocardial infarction, which affects over one million Americans annually (Go, 2013). In both chronic conditions and myocardial infarction, inflammatory cytokines induce tissue remodeling and degradation of ECM in the myocardium (Gullestad et al., 2012). The loss of ECM can result in a temporary decrease in the passive wall stiffness and increase in diastolic strains in the infarct region, which increase the chance of myocardial wall rupture (Banerjee et al., 2006; Gao et al., 2005; Ma et al., 2013). Deposition of de novo ECM is necessary to maintain structural integrity and requires the switch from inflammatory to profibrotic signaling factors (Frangogiannis, 2014). In both chronic and acute myocardial remodeling, AngII inhibits the degradation of collagen-1 and promotes the expression of FGF-2 and TGF-β1 that, in turn, promote cell growth and collagen production in the myocardium (Frangogiannis, 2014; Huang et al., 2010; Porter and Turner, 2009; Schuleri et al., 2012; Virag et al., 2007). TGF-β1 inhibits inflammation and promotes the differentiation of fibroblasts into MyoFBs, and the accumulation of dense ECM in the

**Fig. 2. Mechano-sensitve mechanisms of MyoFB differentiation.** (A–C) Myofibroblasts (MyoFBs) play a central role in the progression of fibrotic disease in the heart because of their roles in the generation and transmission of cellular force, intercellular signaling and ECM remodeling. One important mechanism yielding MyoFBs is EndMT (A), by which endothelial cells (ECs) lose their endothelial markers (including VE-cadherin) and become migratory and contractile. Valvular interstitial cells (VICs) and cardiac fibroblasts (CFs) can also differentiate into MyoFBs in response to high mechanical strain (B) – which is often experienced during inflammation – with the degradation of initial ECM and a corresponding decrease in tissue stiffness. Quiescent VICs and CFs can also differentiate into MyoFBs in response to high mechanical stress (C), caused by both increased tissue stiffness and increased tissue forces. MyoFBs increase the overall stress in the environment by producing excess ECM and contracting existing ECM through increased cellular contractility. MyoFBs also release profibrotic signaling factors, including TGF-β1 and Wnt, that promote further MyoFB differentiation and tissue stiffening. This forms a positive feedback loop leading to progressively worsening fibrosis. Tissue stiffening also often leads to compensatory increases in ventricular pressure, which increases the applied tissue forces and reinforces this positive feedback loop.
myocardial interstitium (Ikeuchi et al., 2004). This increases stress on the remaining contractile myocardium, resulting in further adverse remodeling and fibrosis largely mediated by CFs that have differentiated into active MyoFBs (Fig. 1A) (Ersbøll et al., 2014).

Cardiac cell responses to mechanical stress

In healthy valves and healthy myocardium, quiescent fibroblasts maintain tissue homeostasis by a controlled and balanced release of ECM proteins and proteases (Davis and Molkentin, 2014). However, these fibroblasts transition to an active MyoFB phenotype during injury or disease in response to the synergistic contributions of growth factor signaling (primarily TGF-β1) and mechanical cues (Fig. 2) (Merryman et al., 2007; Walker et al., 2004). Decreasing substrate stiffness and treatment of MyoFBs with FGF-2 have been shown to reverse MyoFB differentiation and promote the quiescent fibroblast phenotype in vitro (Greenberg et al., 2006; Kloxin et al., 2010). However, the increased mechanical stimulation during fibrotic disease may prevent such a de-differentiation and could be responsible for the long-term persistence of MyoFBs that are observed in disease. The three main mechano-sensitive mechanisms of cellular differentiation that give rise to MyoFBs in the heart during disease initiation are described below (Fig. 2).

Endothelial cells directly contribute to tissue remodeling by differentiating into fibroblast-like cells in response to chemical and mechanical signals through a process known as endothelial-to-mesenchymal transition (EndMT) (Fig. 2A). These cells lose their endothelial adhesions, including vascular-endothelial (VE)-cadherin, and express migratory, mesenchymal cell markers, including N-cadherin, matrix metalloproteinase 2 (MMP2) and α-SMA (Wylie-Sears et al., 2014). A similar mechanism is responsible for the origin of both VICs and CFs during development (see Box 1) and is enhanced by active contraction of the myocardium and surrounding matrix (Sewell-Loftin et al., 2014). Inflammation and TGF-β1 signaling both promote EndMT in valve endothelial cells during the initiation of valve disease (Farrar and Butcher, 2013; von Gise and Pu, 2012; Wylie-Sears et al., 2014). EndMT also accounts for ~25% of the α-SMA-positive, MyoFB-like cells that are found in the myocardium after infarction; this transition is dependent on Wnt, a signaling factor that also promotes fibrosis in concert with TGF-β1 (Aisagbonhi et al., 2011; Akhmetshina et al., 2012). MyoFB-like cells participate in ECM remodeling during the transition from inflammation to fibrosis, but future work is needed to completely characterize this cell population and how it contributes to disease manifestation.

Another well-established mechanism for MyoFB differentiation that is relevant to progressing cardiac disease is the differentiation of quiescent VICs and CFs in response to high strains (Fig. 2B). Pressure overload increases strains in the valves and myocardium, and initiation of inflammation causes a breakdown of the ECM that can further increase local strains. Ex vivo aortic valves that were exposed to pathologic strains (15–20% of original length) expressed more matrix metalloproteinases — other than MMP2 (i.e. MMP1 and MMP9) — and collagen-1 than valves that are exposed to physiologic valve strains (10%) (Balachandran et al., 2009). MMPs and other proteases are also expressed by MyoFBs in infarct regions to break down any damaged ECM, and allow for increased fibroblast migration into the infarct region (Aisagbonhi et al., 2011; Davis and Molkentin, 2014; Schuleri et al., 2012). CFs proliferate and express increased levels of α-SMA and MMP2 in vitro after exposure to ~ approximately physiologic — cyclic strains between 5% and 15% (Dalla Costa et al., 2010). In addition, expression of MMPs and α-SMA increases the ability of CFs to migrate through and contract 3D substrates (Wang et al., 2014b). High local strains can also signal transition from inflammation to fibrosis. In response to increased cyclic strain, aortic VICs (AVICs) express reduced levels of inflammatory markers and increased levels of profibrotic factors including TGF-β1 (Smith et al., 2010). Strain and TGF-β1 together enhance MyoFB differentiation and cell contractility, and increase the formation of calcified nodules by AVICs in dynamic culture (Fisher et al., 2013; Merryman et al., 2007; Santiago et al., 2010). This increase in the formation of calcified nodules depends on the establishment and intercellular transmission of cellular tension through α-SMA and OB-cadherin, respectively (Fisher et al., 2013; Hutcheson et al., 2013).

A third mechanical trigger for MyoFB differentiation is increased mechanical stress (Fig. 2C). In vitro, increased substrate stiffness promotes MyoFB differentiation in AVICs, leading to increased formation of calcified nodules, α-SMA expression, and expression of TGF-β receptor type 1 (Kloxin et al., 2010; Yip et al., 2009). On stiff 2D substrates, CFs exhibit an increase in the expression of α-SMA, TGF-β1 and collagen-1 (Galie et al., 2011). MyoFBs generate increased internal cellular tension to balance the increase in extracellular tension they experience on stiffer substrates and, in turn use this tension to remodel their local microenvironment. After cells have increased their intracellular tension, the surrounding ECM becomes more taut, which can promote the differentiation of nearby fibroblasts and perpetuate disease progression (Petersen et al., 2012).

Besides their active role in ECM modification after differentiation into MyoFBs, CFs can alter myocardial structure by affecting the behavior and function of CMs. Direct intracellular contacts between CFs and CMs in vitro decrease the contraction velocity of CMs, and increase the expression of inflammatory cytokines therein (Banerjee et al., 2006). CF-induced changes in ECM
stiffness and composition can also promote CM hypertrophy (Ieda et al., 2009). Finally, the release of profibrotic factors, such as TGF-β1, AngII and FGF by CFs have all been shown to promote CM hypertrophy (Rohr, 2011), leading to thickening of the muscular walls that are then able to generate stronger contractions and enhance mechanotransductive signaling throughout the heart. These tissue-level forces are transmitted to the different cardiac cells through the cardiac ECM and through intercellular adhesions between them. In the following sections, we highlight recent research that provided new insight to the molecular mechanisms by which mechanical signals are transduced from the cellular microenvironment in order to elicit these tissue-level changes.

Mechanosensors of stress

Mechano-sensitive adhesion proteins, including integrins and cadherins, transduce mechanical signals between cells and their microenvironment and can stimulate cellular responses including cell growth and differentiation. Both integrins and cadherins are large proteins families, and expression of specific isoforms within these families is associated with changes in cellular phenotype and progression of disease (Tables 1 and 2). The following sections summarize those integrin and cadherin isoforms that are upregulated in fibrotic disease in the heart and discuss the mechanosensitive signaling pathways they initiate.

Integrins sense mechanical signals from the ECM

Integrins are a diverse class of ECM receptors comprising heterodimers of α and β subunits that determine ECM binding specificity and intracellular signal transduction. Upon ECM engagement, integrins recruit FA proteins that mechanically link the cytoskeleton to the ECM, mediating a force balance between stress fibers and ECM fibrils, and initiating downstream signaling pathways (Baker and Zaman, 2010). This signaling is sensitive to ECM composition, stiffness, and applied strains, and regulates the phenotype of the cell, which in turn affects ECM synthesis and integrin expression throughout the progression of disease.

The β1 subunit is part of most collagen-binding integrins in the heart and can induce force-dependent cellular responses, including cell growth and MyoFB differentiation through activation of PTK1 (also known as and hereafter referred to as FAK), MAPK3 and MAPK1 (also known as ERK1 and ERK2, respectively; hereafter referred to as ERKs), MAPK14 (also known as and hereafter referred to as p38), and other mitogen activated kinases (MAPKs) and their downstream signaling cascades. The exact functional effects depend on the cell type and the local microenvironment. For example, CM-specific deletion of β1-mediated adhesion results in cardiac fibrosis and heart failure in response to pressure overload by disrupting CM membrane integrity and contractile function (Shai et al., 2002). In addition, α7β1 integrin, a laminin receptor, has also been shown to have a protective effect in CMs that are exposed to ischemic stress (Okada et al., 2013). Removal of the β1-integrin-associated mechanosensitive protein melusin (ITGB1BP2), alters signaling through ERK1/2 and glycogen synthase kinase 3β (GSK-3β), and leads to dilated cardiomyopathy and fibrosis in response to pressure overload in the heart (Branccacio et al., 2003; Penna et al., 2014). These studies highlight that β1 integrin helps to protect CMs from adverse effects of the mechanical strains they experience.

Another β1-containing integrin that protects cardiac cells against fibrosis is α2β1 integrin, the primary receptor for collagen-1, which

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Integron type</th>
<th>ECM-binding partner</th>
<th>Normal tissue</th>
<th>Fibrotic disease</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valves</td>
<td>α2β1</td>
<td>Collagen-1</td>
<td>Arranged circumferentially, primarily in the fibrous layer</td>
<td>Increased, disorganized expression throughout valve</td>
<td>(Stephens et al., 2010)</td>
</tr>
<tr>
<td></td>
<td>α1β1</td>
<td>Collagens IV and VI</td>
<td>Basal lamina of endothelium and fibroa</td>
<td>Increased expression throughout valve</td>
<td>(Aupperle and Disatian, 2012)</td>
</tr>
<tr>
<td></td>
<td>α3β1</td>
<td>Collagens IV and VI</td>
<td>Basal lamina of endothelium and fibroa</td>
<td>Increased expression throughout valve</td>
<td>(Apek et al., 1999)</td>
</tr>
<tr>
<td></td>
<td>α5β1</td>
<td>Fibronectin</td>
<td>Limited expression in basal lamina</td>
<td>Increased expression throughout valve</td>
<td>(Gu and Masters, 2010)</td>
</tr>
<tr>
<td></td>
<td>ανβ3</td>
<td>RGD</td>
<td>Minimal expression and exposure</td>
<td>Primarily exposed and expressed in areas of MyoFB differentiation</td>
<td>(Benton et al., 2009)</td>
</tr>
<tr>
<td>Myocardium</td>
<td>α2β1</td>
<td>Collagen-1</td>
<td>Organized network surrounding CMs and CF</td>
<td>Main component of scar after myocardial infarction and general cardiac fibrosis</td>
<td>(Burgess et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>α1β1</td>
<td>Collagen-1</td>
<td>Organized network surrounding CMs and CF</td>
<td>Main component of scar after myocardial infarction and general cardiac fibrosis</td>
<td>(Burgess et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>α5β1</td>
<td>Fibronectin</td>
<td>Limited expression in basal lamina</td>
<td>Increased throughout the myocardium</td>
<td>(Burgess et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>α7β1</td>
<td>Laminin</td>
<td>Expresed throughout the myocardium</td>
<td>Increased throughout the myocardium</td>
<td>(Burgess et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>β1</td>
<td>Fibronectin or/and collagen</td>
<td>Organized network surrounding CMs and CF</td>
<td>Increased throughout the myocardium</td>
<td>(Okada et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>α8β1</td>
<td>RGD</td>
<td>Organized network surrounding CMs and CF</td>
<td>Increased collagen and fibronectin throughout myocardium</td>
<td>(Bouzeghrane et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>ανβ3</td>
<td>RGD</td>
<td>Organized network surrounding CMs and CF</td>
<td>Increased collagen and fibronectin throughout myocardium</td>
<td>(Balasubramanian et al., 2012; Luo et al., 2014)</td>
</tr>
</tbody>
</table>

*Each valve leaflet is composed of three layers of tissue, atrialis, fibrosa and spongiosa.*
forms the largest fraction of healthy ECM in both valves and the myocardium (Chen and Simmons, 2011; Gershak and Black, 2015). Fibrillar collagen-1 networks are maintained in a tightly regulated homeostasis that has been shown to be dependent on α2β1-integrin-mediated adhesion in fibroblasts (Fujimura et al., 2007). Consequently, blocking α2β1 adhesion causes a build-up of ECM in the skin and in collagen gel lattices, which is prevented by α2β1-integrin-induced release of the collagen-1 protease MMP-1 (Fujimura et al., 2007; Liu and Leask, 2013; Riikonen et al., 1995; Zhang et al., 2014b).

However, whereas α2β1 integrin inhibits fibrosis in healthy tissues, other β1 integrins promote MyoFB differentiation and fibrosis. For instance, α1β1 has been shown to promote both inflammation and MyoFB differentiation in adult connective tissue (Kriegstein et al., 2002; Rodriguez et al., 2009). In addition, α3β1 integrin promotes EndMT and MyoFB differentiation in fibrillar lungs and mediates crosstalk between factors involved in TGF-β1-and Wnt-associated signaling (Kim et al., 2009). These integrins are expressed in the heart and bind to non-fibrillar collagens IV and VI that are upregulated during valve disease and myocardial fibrosis (Arango et al., 2008; Aupperle and Disatian, 2012; Naugle et al., 2006). Another β1 integrin that has been linked to cardiac fibrotic disease is α5β1 integrin, the classic fibroconnectin receptor. Expression of α5β1 is increased in fibrotic myocardium and signaling downstream of α5β1 promotes the expression of additional fibroconnectin in an example of positive feedback (Sarrazyn et al., 2014; Wang et al., 2010). Secretion of fibroconnectin contributes to further MyoFB differentiation by initiating signaling through FAK that facilitates the formation of new integrin adhesions and increases matrix stiffness (Fig. 2C). Fibroconnectin-induced signaling promotes MyoFB differentiation, and increased MMP15 expression by and increased activity of MMP9 in fibroblasts in 3D in vitro systems, but these effects are reduced or reversed by the addition of collagen-1 to the matrix (Cushing et al., 2005; Zhang et al., 2014a). Another fibroconnectin-binding integrin, α8β1, is specifically enhanced in MyoFBs within fibrotic hearts (Bouzeghrane et al., 2004). Overall, β1 integrin expression is increased in fibrotic and hypertrophic hearts and, moreover, fibroblast-specific deletion of β1 integrin causes insufficient wound healing and reduced MyoFB differentiation in a dermal model (Burgess et al., 2002; Liu and Leask, 2013; Liu et al., 2010). Taken together, these data suggest that, despite their protective effect on CMs, β1 integrins exert a pro-fibrotic effect in heart fibroblasts during disease.

Another important integrin type that has been directly linked to mechanotransduction and MyoFB differentiation are the β3 integrins (Roca-Cusachs et al., 2009). β3 integrins recognize the RGD peptide sequence that is found in fibroconnectin, collagen and vitronectin, and are highly expressed during development and disease (Luo et al., 2014). In accordance with this, AVICs cultured on RGD-coated substrates express increased levels of MyoFB markers and of αvβ3 integrins and are more prone to calcification (Benton et al., 2009; Gu and Masters, 2010). Furthermore, β3 integrin expression in the heart is significantly increased after myocardial infarction, and expression of β3 integrins in CFs is necessary for the accumulation of collagen and fibroconnectin in response to pressure overload (Balasubramanian et al., 2012; Luo et al., 2014). This effect is likely to be mediated by the FA component talin, which links integrins to the cytoskeleton and is required for αvβ3-integrin-mediated mechanotransduction (Roca-Cusachs et al., 2009). The talin1 isoform is expressed in the heart during development and disease, and its deletion prevents pressure-induced hypertrophy and fibrosis in the myocardium by altering signaling through p38, ERK1/2, protein kinase B (Akt) and GSK-3β (Mano et al., 2013). All of these kinases are involved in TGF-β1-induced MyoFB differentiation and promote the expression of α-SMA and increased intracellular tension. Furthermore, β3 integrin engagement with ECM proteins enhances TGF-β1 signaling through Src and p38 to further promote the expression of α-SMA and of β3 integrins, thereby forming another positive feedback loop (Pechkovsky et al., 2008).

The convergence on TGF-β1 signaling is one example of the significant crosstalk between integrin- and growth-factor signaling that is involved in the regulation of MyoFB differentiation in the heart (Fig. 3) (Friedland et al., 2009; Schroer, 2014). Non-canonical TGF-β1 signaling through Src and p38 promotes the production of α-SMA in AVICs by the transcription factors myocardin related transcription factor (MRTF) and serum response factor (SRF) (Elberg et al., 2008; Hutcheson et al., 2012; Watanabe et al., 2009). FGF-2 signaling through FAK and ERK1/2 has been shown to prevent MyoFB differentiation in MEFs and to reverse TGF-β1-mediated expression of α-SMA (Greenberg et al., 2006; Kawai-Kowase et al., 2004; Schroer, 2014). Src and FAK are directly activated by β3 and β1 integrins, respectively, and the effects of the adhesions they mediate on MyoFB differentiation is mirrored in this crosstalk (Fig. 3). Specifically, Src promotes α-SMA expression, whereas FAK can both inhibit and promote the expression of MyoFB markers. Compounding this crosstalk, integrin signaling also regulates the expression and activation of growth factors. For example, β1 integrins regulate the expression of the angiotensin gene (AGT) in CFs through p38 signaling in response to mechanical stretch. This effect is mediated by activation of Rac1 and inhibition of RhoA, intracellular kinases involved in cytoskeletal organization and contraction (Verma et al., 2011).

In addition to such ‘outside-in’ signaling, integrins can also participate in ‘inside-out’ signaling, by which FA proteins can modify the intracellular domain of integrin subunits to activate them, resulting in stronger adhesions to the ECM (Baker and Zaman, 2010; Katsumi et al., 2004; Michael et al., 2009). Vinculin is a force-sensitive FA protein that is recruited to FAs in a tension-dependent manner, and has been shown to strengthen and stabilize the FA when held under high tension, allowing for greater transmission of force (Carisey et al., 2013; Grashoff et al., 2010; Humphries et al., 2007).

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### Table 2. Expression of cadherin isoforms in normal tissue and during progression of fibrotic disease

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normal tissue</th>
<th>Fibrotic disease</th>
<th>Cell type</th>
<th>Mechanotransductive effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valves</td>
<td>N-cadherin</td>
<td>OB-cadherin</td>
<td>AVIC</td>
<td>Increased cell tension, formation of calcified nodules</td>
<td>(Hutcheson et al., 2013)</td>
</tr>
<tr>
<td>VE-cadherin</td>
<td>N-cadherin</td>
<td>N-cadherin</td>
<td>VEC</td>
<td>EndMT, migratory, α-SMA-positive cells</td>
<td>(Meadows et al., 2009)</td>
</tr>
<tr>
<td>Myocardium</td>
<td>N-cadherin</td>
<td>OB-cadherin</td>
<td>CF</td>
<td>Unknown</td>
<td>(Thompson et al., 2014)</td>
</tr>
<tr>
<td>N-cadherin</td>
<td>N-cadherin</td>
<td>CM</td>
<td>Reduced cell contraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VE-cadherin</td>
<td>N-cadherin</td>
<td>EC</td>
<td>EndMT, migratory, α-SMA-positive cells</td>
<td>(Aisagbonhi et al., 2011)</td>
<td></td>
</tr>
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Cadherins mediate mechanically induced signaling between cells through AJs, which link cadherins to the cytoskeleton as described in a recent review (Leckband and de Rooij, 2014). Differential signaling or mechanical perturbations that disrupt cadherin–cadherin interactions between cells can be shown to release β-catenin into the cytoplasm where it acts in concert with growth factor signaling to promote a mesenchymal cell phenotype (Heuberger and Birchmeier, 2010). For example, exposure to oscillatory fluid flow triggers an N-cadherin-mediated release of β-catenin and a subsequent osteogenic differentiation in stem cells (Arnsdorf et al., 2009). Typically, free cytoplasmic β-catenin is rapidly marked for degradation by GSK-3β; however, inhibition of GSK-3β by TGF-β1 or Wnt allows for an accumulation of β-catenin in the cytoplasm and its subsequent translocation to the nucleus where it activates its target genes (Caraci et al., 2008). In addition to signaling through β-catenin, several recent studies have characterized a force-dependent interaction between vinculin and α-catenin, another protein linking AJs to the cytoskeleton (Huveneers et al., 2012; le Duc et al., 2010; Yao et al., 2014; Yonemura et al., 2010). Vinculin is recruited to AJs in a force-dependent manner by α-catenin, which results in strengthening of the adhesion and increased cell contractility (Barry et al., 2014; Yao et al., 2014). This result indicates that integrins and cadherins might share a common mechanosensitive mechanism, in which vinculin-induced stabilization of either FAs or AJs affects downstream signaling pathways. p120-catenin is also involved in stabilizing AJs and increases activation of Rac1 when it is bound to either N- or OB-cadherin, leading to increased expression of mesenchymal cadherins (Yanagisawa and Anastasiadis, 2006).

N-cadherin is the classic cell–cell adhesion protein that is expressed by quiescent fibroblasts and CMs in the heart. N-cadherin expression and localization at cell–cell contacts is associated with increased stability of β-catenin and decreased expression of α-SMA (Xu et al., 2012). CFs form N-cadherin-mediated interactions with CMs, which influences CM structure and contractility during development, normal function and disease (Chopra et al., 2011; Kudo-Sakamoto et al., 2014; Vreeker et al., 2014). In a recent study, N-cadherin bonds between CFs and CMs were shown to dynamically deform CM membranes in response to MyoFb contraction and induce a measurable slowing of CM conduction velocity (Thompson et al., 2014). This study demonstrates a mechanical signal from MyoFbs that affects the electrophysiology of CMs and may contribute to risk of arrhythmia and other cardiovascular conditions that are associated with low conduction velocity (King et al., 2013).

Whereas quiescent AVICs and CFs express N-cadherins, during injury and disease these cells differentiate into MyoFbs; this process is characterized by expression of OB-cadherin, which has recently garnered much interest as a mechanosensitive regulator of inflammation and fibrotic disease (see Box 2). During differentiation of MyoFbs, TGF-β1 signaling induces an increase in OB-cadherin concurrently with a decrease in the expression of N-cadherin (Hinz et al., 2004). Within cell–cell adhesions, OB-cadherin is able to withstand significantly higher forces than N-cadherin, which allows for stronger matrix contraction and the transmission of higher intracellular tension (Hinz et al., 2004; Hutcheson et al., 2013; Pittet et al., 2008). OB-cadherin is highly expressed in diseased heart valves in both VECs and VCs, and has been implicated in the development of calcified nodules in the aortic valve in CAVD through increased transmission of intracellular force (Hutcheson et al., 2013; Wang et al., 2014a; Zhou et al., 2013). It is also expressed in CFs, but the functional significance of this expression in the context of cardiac fibrosis and wound healing has not been studied. Given the known roles of OB-cadherin in inflammation and fibrosis in joint connective tissue and lungs, OB-cadherin is likely to have an important role in myocardial remodeling (Chang et al., 2011; Schneider et al., 2012).

In addition to functional mechanical roles, OB-cadherin can also potentiate downstream signals to control cell behavior. Although the downstream signaling of OB-cadherin is still relatively uncharacterized, recent studies have shown significant crosstalk with MyoFb regulatory signals. For instance, it was found that OB-cadherin engagement promotes differentiation of smooth muscle.
Box 2. OB-cadherin – 20 years of insight
OB-cadherin (also known as cadherin 11) was first described in 1994 in the context of osteogenesis and bone development, but recent work has shown its importance in a variety of tissues (Okazaki, 1994). Over 100 studies have been published in the past few years that examine the role of OB-cadherin in cancer and fibroblast-mediated disease. In the context of cancer, OB-cadherin expression is associated with increased migration and metastasis, especially metastasis to bone (Deng et al., 2013). As cell differentiation and migration in cancer and inflammatory disease show some similarities, OB-cadherin has been proposed as a common, possibly therapeutic, target for both types of disease (Assefnia et al., 2014). OB-cadherin plays an important role in inflammation in rheumatoid arthritis; it stimulates synovial fibroblasts to release proinflammatory cytokines upon cadherin engagement (Chang et al., 2011; Ding et al., 2014). Celecoxib, a pharmacological inhibitor of Cox-2 that inhibits inflammation during rheumatoid arthritis, has been shown to bind to OB-cadherin (Assefnia et al., 2014). Inflammation often leads to fibrosis, which is mediated by activated MyoFBs. In this context, OB-cadherin has been implicated in the progression of dermal and pulmonary fibrosis, and suggested to promote MyoFB differentiation through interaction with β-catenin (Kim et al., 2009; Schneider et al., 2012; Wu et al., 2014). In accordance with this, injection of a function-blocking antibody against OB-cadherin improves bleomycin-induced dermal and pulmonary fibrosis, but its effect has not yet been investigated in other organ systems (Schneider et al., 2012; Wu et al., 2014). Taken together, these studies indicate that OB-cadherin is a promising target for further research in the field of fibrotic disease.

Conclusions and future challenges
There are mechanistic similarities between progression of fibrotic disease in the heart wall and valves that can inform future studies of both diseases. Namely, both myocardial and valve tissue show increased numbers of active MyoFBs that remodel ECM, and alter cardiac mechanics and function. These cells are sensitive to mechanical signals that are largely transduced through integrins and cadherins. Integrins react to the composition and mechanics of the microenvironment of a cell, and can promote cell differentiation in a context-dependent manner. MyoFB differentiation potentiated by integrin and cadherin signaling can contribute to further ECM remodeling and tissue stiffening, which – in turn – enhances mechanotransductive signaling in nearby cells (Fig. 2). Such a system of positive feedback loops can then perpetuate the progression of fibrotic disease. Prolonged inflammatory responses further compound the problem by initiating and propagating ECM remodeling. Both integrin- and OB-cadherin-mediated signaling have been implicated in inflammation, and strategies aimed at blocking the functions of these adhesion molecules have shown preliminary success in limiting inflammation-triggered maladaptive remodeling (Chang et al., 2011; Kriegstein et al., 2002; Lee et al., 2007; Schneider et al., 2012). The overlapping and integrated networks of chemical and mechanical signals that regulate the progression of fibrotic heart disease will continue to challenge the development of promising therapies. Nevertheless, the crucial role of cadherins and integrins in both chemical and mechanical signaling makes them excellent potential targets for therapy and future study (Agarwal, 2014).

Competing interests
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