Heart failure, the clinical manifestation of numerous forms of cardiovascular disease, is a devastating disorder characterized by interstitial fibrosis, chamber remodeling, and reduced ventricular compliance. Heart disease remains the predominant cause of mortality in the United States, accounting for nearly 800,000 deaths per year. Furthermore, it presents a considerable economic burden, with estimated direct and indirect costs in 2011 of ≈$320 billion and predictions suggesting that costs will rise to ≈$918 billion by 2030. Despite substantial improvements in therapeutic strategies, cardiovascular disease remains the leading cause of death worldwide indicating an urgent need for innovative treatment strategies.

Nearly all etiologies of heart disease involve pathological myocardial remodeling characterized by excessive deposition of extracellular matrix (ECM) proteins by cardiac fibroblasts (CFs), which reduces tissue compliance and accelerates the progression to heart failure. The CF is an essential cell type, predominantly of embryonic epicardial and endothelial origins, which resides within the myocardial interstitium, epicardial and perivascular regions. Physiologically, CFs are responsible for homeostasis of the ECM, which provides a structural scaffold for cardiomyocytes, distributes mechanical forces through the cardiac tissue, and mediates electric conduction. Previously, identification of these cells was largely based on phenotypic observations; morphologically, fibroblasts are flat, spindle-shaped cells with multiple processes when propagated on tissue culture plastic. The cardiac cellular milieu can vary greatly depending on the species being examined and between healthy and injured myocardium. Although previous studies suggested that CFs accounted for the majority of cells within the adult rodent and human myocardium, recent reports with more accurate delineation of the CF population have proposed that CFs may comprise <20% of the total cell population in the adult murine heart, substantially less than previously suggested. Although it is now appreciated that a majority of resident fibroblasts originate from the embryonic epicardium, the contribution of various resident and infiltrating cells to the population of activated cardiac myofibroblasts remains under active investigation, including further refinement of specific molecular markers for CFs.

Unlike other organs, the heart has limited regenerative capacity after injury, and instead, repair processes involve the removal of necrotic cardiomyocytes followed by fibrotic scar tissue replacement that acts to preserve myocardial structural and functional integrity. To perform these functions, CFs within the connective tissue convert to their activated form, often known as myofibroblasts, which secrete elevated levels of ECM proteins to promote a profibrotic environment. Cardiac fibrosis provokes pathological changes that culminate in chamber dilatation, cardiomyocyte hypertrophy, and apoptosis, and ultimately lead to the development of congestive heart failure. Although the sources of these activated fibroblasts remain under intense investigation and debate, the refinement of molecular markers and the development of new techniques for lineage tracing are helping to enhance our understanding of their origins.

**Abstract:** Myocardial fibrosis is a significant global health problem associated with nearly all forms of heart disease. Cardiac fibroblasts comprise an essential cell type in the heart that is responsible for the homeostasis of the extracellular matrix; however, upon injury, these cells transform to a myofibroblast phenotype and contribute to cardiac fibrosis. This remodeling involves pathological changes that include chamber dilation, cardiomyocyte hypertrophy and apoptosis, and ultimately leads to the progression to heart failure. Despite the critical importance of fibrosis in cardiovascular disease, our limited understanding of the cardiac fibroblast impedes the development of potential therapies that effectively target this cell type and its pathological contribution to disease progression. This review summarizes current knowledge regarding the origins and roles of fibroblasts, mediators and signaling pathways known to influence fibroblast function after myocardial injury, as well as novel therapeutic strategies under investigation to attenuate cardiac fibrosis.

**Key Words:** disease progression | extracellular matrix | fibroblasts | fibrosis | heart failure | therapeutics
Role of CFs in Injury

The CF is now recognized for its fundamental contributions to the heart’s response to various forms of injury (Figure 1). In general, the critical phases of this response consist of inflammation, proliferation of nonmyocytes, and scar maturation, with the CF intimately involved in all of these processes. After an acute myocardial injury, the expression of various proinflammatory cytokines and profibrotic factors is upregulated in CFs, leading to increased proliferation of these cells and ultimately, the transition to the myofibroblast phenotype. During this maturation phase, myofibroblasts begin to secrete elevated levels of collagens and other ECM proteins. The purpose of this adaptive fibrosis is to maintain the structural integrity and pressure-generating capacity of the heart because a loss of integrity in the mechanical strength of the ventricle may lead to myocardial dysfunction or rupture. In the advanced phases of fibrotic scar formation, the tensile strength of collagen increases within the site of injury. In this setting, a subset of activated myofibroblasts acquire new phenotypic characteristics, including the expression of the contractile protein α-smooth muscle actin (α-SMA), and contribute to pathological cardiac remodeling. Although initially adaptive, these processes eventually lead to the development of adverse changes in ventricular structure and compliance, and a concurrent progression into overt heart failure.

Pathological cardiac remodeling is characterized by fibroblast accumulation and excessive deposition of ECM proteins, which leads to distorted organ architecture and has significant consequences on cardiac function. Fibrogenesis contributes to impaired cardiac function as fibrotic ECM increases ventricular stiffness and can lead to contractile dysfunction. In addition, excess ECM and fibroblasts impair mechano-electric coupling of cardiomyocytes, thus reducing cardiac contraction and increasing the risk of arrhythmogenesis and mortality. This pathological process can also include hypertrophy and dysfunction of cardiomyocytes via paracrine mechanisms, which further contribute to impaired cardiac function.

Furthermore, inflammation and fibrosis within perivascular regions may decrease tissue availability to oxygen and nutrients and increase the pathological remodeling response. After the initial wound healing process in many tissues, a majority of collagen-secreting fibroblasts undergo apoptosis and leave a mature scar composed of cross-linked collagen and other matrix components. However, when this process persists in the heart, myofibroblasts within the cardiac scar tissue continue to release maladaptive proinflammatory and prohypertrophic signals, resulting in cardiomyocyte hypertrophy and necrosis followed by replacement fibrosis.

Importantly, after an acute injury such as myocardial infarction (MI), activated fibroblasts not only increase the synthesis of ECM proteins at the site of injury but also within the healthy tissue remote from the immediate infarct. Commonly referred to as reactive fibrosis, this potentiates the pathophysiological response after acute myocardial injury by reducing chamber compliance and increasing stiffness of the ventricles. A portion of myofibroblasts remain embedded in mature cardiac scars long after the initial myocardial injury, probably because of a resistance to apoptosis, which perpetuates these pathological processes.

Cardiac fibrosis can be categorized into 2 forms, namely reactive interstitial fibrosis or replacement fibrosis, each of which can be recapitulated by several models of heart failure. Transverse aortic constriction is an animal model of left ventricular pressure overload (similar to aortic stenosis) characterized initially by reactive interstitial fibrosis, an adaptive response to preserve cardiac structure and function, and followed by replacement fibrosis in areas of cardiomyocyte necrosis. In animal models of acute ischemic injury, such as MI or ischemia/reperfusion injuries, the initial injury is characterized by an immediate, robust inflammatory response, and extensive cardiomyocyte death; replacement fibrosis restores this region devoid of viable cardiomyocytes to prevent cardiac rupture. Cardiac fibrosis also occurs in the context of right ventricular volume overload that can result from congenital malformations, such as repaired tetralogy of Fallot or...
pulmonary hypertension. In a mouse model of right ventricular volume overload, fibrosis is evident first in the subendocardial right ventricle, but relatively little is known of the cellular origins or molecular mechanisms of fibrosis in this context.40 These models represent critical yet differing recapitulations of human disease, and as such, show distinct functions of the fibroblast response to injury.39

CF Markers and Limitations

One major area of study is an attempt to quantify relative contributions of the various cardiac cell lineages to the CF population after injury. Such studies rely on molecular biomarkers, but experimental interpretation is difficult because this cell population can include fibroblasts, endothelial cells, pericytes, and immune cells. Further complicating this effort, although lineage-specific markers have long been established for several cell types within this group, robust markers of both quiescent CFs and activated cardiac myofibroblasts remain elusive. With numerous molecular markers now being recognized for their ability to identify particular populations of CFs, the controversy surrounding this cell population remains unresolved. In the heart, as with other organs, there is an increasing interest in the investigation of novel CF and myofibroblast markers, and transcriptomics is proving an effective method for the uncovering of new markers.41 Aside from the immediate benefits for cell identification, biomarkers in use for other organs, for example the kidney, can be used as tools to diagnose the progression of disease and also as novel therapeutic targets. Here, we present several commonly used molecular markers to identify quiescent and activated CFs, concerns about their use, and how the refinement of these markers may potentially provide for the identification of a purer fibroblast population (Table 1).

Among the first markers used to identify CFs was discoidin domain receptor 2, which acts as a receptor for several ECM proteins.48 Although expression is limited in other cell types, including endothelial cells, smooth muscle cells, and myocytes, it does not seem that all CFs are positive for discoidin domain receptor 2.9 Fibroblast-specific protein 1 (FSP1 and S100A4), one of the initial markers identified, was believed to be a reliable fibroblast indicator.49 FSP1 was commonly used to designate quiescent and activated CFs in myocardial injury, including pressure overload and infarction-induced fibrosis models. However, recent in vivo work using FSP1–green fluorescent protein (GFP) and FSP1 immunostaining has demonstrated that it is not fibroblast-specific, based on positive expression in several cell types, including a subset of immune and endothelial cells.68-70 Another marker used to identify CFs is thymus-cell antigen 1 (Thy1, CD90), a membrane glycoprotein expressed on the surface of CFs.44 Although this receptor does seem to be expressed on all CFs, other cell types are known to express thymus-cell antigen 1, including immune cells, lymphatic endothelial cells, and pericytes.68-70 Vimentin is an intermediate filament protein that has
Table 1. Fibroblast Markers and Mouse Models for Gene Targeting

<table>
<thead>
<tr>
<th>Marker/Mouse Line</th>
<th>Function</th>
<th>Expression in CFs</th>
<th>Expression in Other Cell Types Found in the Heart</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Smooth muscle actin</td>
<td>Contractile intermediate filament–associated protein</td>
<td>Expression limited to subset of activated CFs in fibrotic regions</td>
<td>Epicardium, smooth muscle cells, pericytes, and cardiac muscle cells</td>
<td>Moore-Morris et al,4 Porter et al,4 Zeisberg et al,42 and Skalli et al43</td>
</tr>
<tr>
<td>Collagen 1α1</td>
<td>Extracellular matrix protein</td>
<td>Majority of CFs</td>
<td>Epicardium, adventitia of large vessels, and valve interstitial cells</td>
<td>Moore-Morris et al,1 Acharya et al,44 Yata et al,46 Zheng et al,46 and Bochmann et al47</td>
</tr>
<tr>
<td>Discoidin domain receptor 2</td>
<td>Tyrosine kinase receptor for several ECM proteins</td>
<td>Limited expression in resident CFs</td>
<td>Limited expression in epicardium</td>
<td>Banerjee et al48 and Morales et al49</td>
</tr>
<tr>
<td>Fibroblast-specific protein 1</td>
<td>Calcium-binding protein and intermediate filament–associated protein</td>
<td>Activated fibroblasts in ischemic and nonischemic heart failure models (typically perivascular)</td>
<td>Endothelial, smooth muscle, and immune cells</td>
<td>Moore-Morris et al,4 Strutz et al,46 Kong et al,50 Österreicher et al,51 and Zeisberg et al42</td>
</tr>
<tr>
<td>Periostin</td>
<td>Matricellular protein secreted by activated CFs</td>
<td>CFs in development and re-expressed in activated CFs after injury</td>
<td>Epicardium, vascular smooth muscle cells, and valve interstitial cells</td>
<td>Lajiness and Conway,77 Kong et al,10 Katsuragi et al,52 Kudo,73 Stanton et al,54 Visconti et al,55 Kruzynecka-Frejtag et al,56 Lindsley et al,57 Takeda et al,58 Snider et al,59 Conway et al,60 and Litvin et al61</td>
</tr>
<tr>
<td>Platelet-derived growth factor receptor-α</td>
<td>Mitogenic tyrosine kinase receptor</td>
<td>CFs during development and after injury</td>
<td>Epicardium</td>
<td>Acharya et al,44 Smith et al,62 Chong et al,63 and Miwa and Era64</td>
</tr>
<tr>
<td>The transcription factor 21</td>
<td>Regulates mesenchymal cell transitions</td>
<td>Resident CFs in development and after myocardial injury</td>
<td>Epicardium</td>
<td>Acharya et al,44 Braitsch et al,53 Acharya et al,65 and Song al66</td>
</tr>
<tr>
<td>Thymus cell antigen 1 Membrane glycoprotein for cell adhesion</td>
<td>Limited expression in quiescent resident CFs</td>
<td>Endothelial cells, pericytes, and immune cells</td>
<td>Pinto et al,71 Acharya et al,44 Crisan et al,68 Jurisic et al,69 and Raff70</td>
<td></td>
</tr>
<tr>
<td>Vimentin</td>
<td>Intermediate filament protein</td>
<td>CFs in healthy and injured heart</td>
<td>Endothelium</td>
<td>Ali et al,72 Franke et al,73 Lane et al,72 and Mark et al73</td>
</tr>
</tbody>
</table>

This table lists a selection of molecular markers that have been used for the identification of quiescent cardiac fibroblasts and activated myofibroblasts; these markers have been translated into numerous mouse lines for lineage tracing and gene targeting studies. Limited expression in all cardiac fibroblasts or positive expression in alternate cardiac cells types are among the problems associated with the use of these markers. Multiple mouse lines exist for many of the markers listed with varying levels of specificity, and complications associated with some lines include Cre expression in the absence of induction, germ line expression, and low recombination efficiencies. CF indicates cardiac fibroblast; and ECM, extracellular matrix.

been used extensively both in vitro and in vivo for the identification of fibroblasts because it is expressed in the majority of fibroblasts both in the healthy and in the injured myocardium. Although CFs are positive for vimentin, concerns with its expression in other cell types, including the endothelium, restrict its efficiency and specificity. In general, the major complications associated with many of the markers currently in use involve either expression in other cell types or limited expression in all forms of CFs.

The transcription factor TCF21 has been used successfully to trace the development of resident CFs from their epicardial precursors. In addition to its extensive expression during development, TCF21 is also broadly expressed in CFs both within interstitial and perivascular fibrotic regions in response to heart failure models of both pressure overload and ischemic etiologies. Importantly, TCF21 is not expressed in infiltrating immune cells identified by positive CD45 expression. In addition, the TCF21 transcription factor appears to be required for CF cell fate determination in development. The inducible TCF21αERCreERT2 transgenic mouse line represents one of the more powerful inducible lines for the study of CFs in development and disease. In development, TCF21 is essential for the formation of CFs in utero, and the TCF21 signal remains active in adult mice, which enables this line to provide significant insight into the contribution of resident fibroblasts to the development of fibrosis in the injured heart.

Platelet-derived growth factor receptor α (PDGFRα) has emerged as a reasonable marker of CFs both during development and in healthy and injured adult tissues. It is also expressed in multiple other organs, including lung fibroblast lineages, oligodendrocyte progenitors, and bladder interstitial cells. In the heart, PDGFRα is robustly expressed in fibroblasts, with relatively limited expression in other cell types, including smooth muscle cells and possible expression in cardiac progenitor cells; it has been used for the development of an inducible Cre system, the PDGFRα-GFPCreERT2 knockin mouse line. This will likely prove a useful tool in characterizing CFs and their role in fibrosis. PDGFRβ has also been used as a marker of fibroblasts. However, expression of PDGFRβ has been observed in numerous other cell types including smooth muscle cells, pericytes, neurons, kidney mesangium, myoblasts, and muscle lineages, thus restricting its specificity and efficiency.

As CFs are likely the principal cells responsible for the secretion of ECM proteins, a new line of thinking involves the identification of CFs by the expression of markers of ECM production. As the predominant protein of the myocardial ECM, Collα1 promoter sequences linked to GFP has been used to create a reporter mouse for identification of CFs.
An inducible form of the collagen1α2 Cre driver has also been developed, allowing for both genetic lineage tracing of collagen1α2 Cre-positive cells and conditional gene deletion for functional studies. However, the expression of collagen1α2 Cre-positive cells and conditional gene deletion for functional studies. An inducible form of the collagen1α2 Cre driver has also been developed, allowing for both genetic lineage tracing of collagen1α2 Cre-positive cells and conditional gene deletion for functional studies. 46 However, the expression of collagen1α2 Cre-positive cells and conditional gene deletion for functional studies. Positive collagen1α1-GFP expression has been reported within cells of both the epicardium and the adventitia of large vessels, suggesting caution in the interpretation of fibroblast-centric conclusions from studies using these lines.

In addition to the molecular markers discussed above, several are expressed after the transition to the activated myofibroblast. Identification of myofibroblasts in many tissues has almost universally relied on the expression of the contractile protein αSMA. However, recent work using a pressure-overload model of heart disease showed that this marker is restricted to a subset of activated fibroblasts and is not expressed in all fibroblasts associated with fibrosis. Periostin is an ECM protein expressed in development, which is also robustly upregulated by activated CFs after injury. A periostin-Cre transgenic mouse line has been developed that expresses Cre under the control of the endogenous periostin promoter that is expressed in resident CFs and epicardial cells in the developing heart, making it well suited to developmental investigations. As periostin-Cre is robustly expressed within activated CFs/myofibroblasts and myocardial infarct sites after injury, it presents one of the more promising tools for lineage tracing and genetically manipulating CFs and myofibroblasts. The development of an inducible knockin system in periostin-expressing cells will likely be an enormously effective tool for the study of activated fibroblasts in disease. Of note, interstitial cells of the valves also express some of the ECM proteins that mark fibroblasts, such as collagen 1α1 and periostin. Overall, advancements in the identification and reliability of CF molecular biomarkers remain essential areas of research, and will eventually allow for a greater understanding of this critical cell type.

Sources of CFs in Disease
The source(s) of activated CFs that accumulate in response to various pathological insults remains somewhat unclear. In other organs, including the lung, kidney and liver in particular, the cellular and molecular mechanisms of fibrosis, and the origins and characteristics of myofibroblasts have also been extensively studied, yet some ambiguity remains. Lineage tracing studies have recently been used in cardiac fibrosis studies, and numerous precursors to the fibroblast population in the injured heart have been proposed, including resident fibroblasts, cells of vascular origin, hematopoietic cells, and pericytes (Figure 2).

Resident Fibroblasts
It is traditionally recognized that activated fibroblasts in the fibrotic heart derive from the proliferation and activation of resident fibroblasts because these cells are remarkably sensitive to circulating pathological stimuli. This theory coincides with numerous studies in the kidney, which suggest that after renal injury a majority of myofibroblasts originate from nephrogenic progenitors that give rise to resident fibroblasts as well as pericytes, and also discount the contribution of other cell types. Similarly, it is hypothesized that hepatic fibrosis is based on the activation of hepatic stellate cells and their transformation into myofibroblasts that express a broad spectrum of matrix components. The majority of resident CFs seem to arise from the embryonic epicardium, and recent lineage tracing studies suggest that these resident epicardial-derived CFs account for the majority of fibroblasts responsible for fibrotic response. Studies have also revealed that cells of the endocardium, a specialized cardiac endothelial lining, that undergo endothelial–mesenchymal transition during development to form the cardiac valves also contribute to resident fibroblasts of the interventricular septum. Proliferation of these endocardial-derived CFs has also been shown to contribute to the fibrotic response after pressure overload. These studies also discount the contribution of cells other than resident fibroblasts to the development of fibrosis in a pressure-overload model of heart failure, as well as in a model of parabiosis. Although these data contradict some previous studies, they indicate that myofibroblast accumulation after injury predominantly results from the proliferation of resident fibroblasts. In addition, resident fibroblasts have been suggested as a feasible source of matrix-producing cells during fibrosis, which could identify them as an important potential target for antifibrotic therapies. Several studies have evaluated the contribution of alternative sources to the activated fibroblast population in pathological cardiac remodeling as individually outlined below.

Vascular Endothelium
Endothelial cells of the coronary vasculature have been proposed as contributors to the myofibroblast population after injury through endothelial–mesenchymal transition. Lineage tracing studies using the constitutive endothelial/hematopoietic restricted Tie1Cre mouse line have suggested that FSP1-positive cells contribute substantially to the fibroblast population in a pressure-overload model of heart failure. These endothelial cells are thought to acquire a fibroblast-like phenotype and respond to proinflammatory stimuli in a manner similar to resident CFs. This phenotypic conversion involves the migration of these cells into the interstitium, where they exhibit typical myofibroblast markers and are believed to contribute to the fibrotic response. However, recent studies have demonstrated that, in addition to fibroblasts, FSP1 also marks a substantial number of immune cells. Furthermore, Tie1Cre is not specific to the endothelium, with fibroblasts being labeled in addition to immune cells and other cell types. In an attempt to resolve this controversy, several studies have since been performed using refined fibroblast markers, including tamoxifen-inducible Cre recombinase under the control of the vascular endothelial cadherin (VECad/Cdh5) promoter (VECad-Cre-ERT2), in concert with collagen-1α and PDGFRα as fibroblast markers. This was complemented by a study using the Tie2Cre mouse line to label and identify cells with endothelial origins. These studies concluded that, at least in the pressure-overload model of heart failure, the endothelium does not significantly contribute to the myofibroblast population.
Epicardium
Resident fibroblasts of the cardiac interstitium are now generally recognized to derive predominantly from cells of the embryonic epicardium. During development, these cells undergo epithelial–mesenchymal transition (EMT) under the influence of several growth factors; subsequently, a portion of these mesenchymal cells invade the myocardium to become the resident CFs. Signals regulating the transition of epicardial cells to fibroblasts are tightly regulated and factors known to influence this transition can include fibroblast growth factors and members of the transforming growth factor β (TGF-β) superfamily. Once these cells have undergone EMT, PDGF and TGF-β are thought to promote their transition into the CF phenotype.

Perivascular Cells
Pericytes, which lie in the perivascular space of cardiac vessels, can differentiate into collagen-producing cells in models of dermal scarring and may contribute to the fibroblast population after cardiac injury. This cell type has been defined in other tissues, including the retina and kidneys, where they have demonstrated phenotypic and functional overlap with fibroblasts; however, they are more thoroughly characterized in the central nervous system. Pericytes surround the neurovasculature and interact with other cell types, including astrocytes and endothelial cells. Here, they maintain the blood–brain barrier and regulate angiogenesis, immune responses, and scar formation, which are affected by factors such as PDGF and TGF-β. The integrity of retinal microvessels is particularly dependent on pericytes, as loss of retinal pericytes contributes to diabetic retinopathy through the formation of microaneurysms. Recent work has revealed the presence of Gli1+ mesenchymal stem cell-like cells within cardiac perivascular regions. Lineage tracing studies demonstrated that the proliferation of these resident cells after kidney, lung, liver, or cardiac injury generates myofibroblasts that contribute to organ fibrosis. Furthermore, ablation of these cells ameliorates cardiac fibrosis and preserves overall cardiac function in a pressure-overload model of heart failure. These cells may present an interesting source of activated CFs, but more work is needed to characterize the particular functional contribution(s) of this cell type in vivo.

Figure 2. Proposed sources of cardiac myofibroblasts. The source(s) of activated cardiac fibroblasts that accumulate in response to various pathological insults remains under active investigation. Mounting evidence suggests that activated myofibroblasts in the fibrotic heart derive from the proliferation and activation of resident fibroblasts, as these cells are remarkably sensitive to pathological insult and represent a feasible source of matrix-producing cells during cardiac fibrosis. Numerous additional precursors to the fibroblast population in the injured heart have been proposed; these include endothelial and epicardial cells, through epithelial–mesenchymal transition and endothelial–mesenchymal transition, respectively, hematopoietic bone marrow–derived cells, perivascular cells, and fibrocytes. Although supporting evidence exists for cellular sources of activated fibroblasts denoted by question marks, controversy remains about the functional contribution of these cell types to the cardiac myofibroblast population. Biomarkers with greater specificity will be required to fully characterize the pathophysiological relevance of these cell types in the cardiac fibrotic response, which will enable potential targeting for antifibrotic therapies.
also positive for several other markers including PDGFRα, these studies do indicate that perivascular cells seem to be an important source of cardiac myofibroblasts and may represent a potential therapeutic target for ameliorating cardiac and general organ fibrosis.107

**Hematopoietic Bone Marrow–Derived Progenitor Cells**

Bone marrow–derived progenitor cells are considered a potential source of fibroblasts in the fibrotic heart. This assertion is based on studies of GFP-labeled bone marrow transplants, which identified GFP-expressing cells within fibrotic regions after both pressure overload and ischemic myocardial injuries.42,106,107 It has been suggested that bone marrow–derived cells may account for up to 60% of all fibroblasts within the site of cardiac injury. However, this cell population is significantly reduced after the initial reparatory process and, therefore, is unlikely to contribute to a persistent fibrotic response.109 CD45-positive monocytes have been identified as a potential source of fibroblasts because they seem to co-express myofibroblast markers110 and inhibition of monocyte recruitment diminished the CF population and myocardial remodeling after MI.111

Several studies have proposed that infiltrating cells are instead likely to consist of a specific phenotype of inflammatory cells, that may not contribute to the cardiac fibroblast population. Both a lineage tracing study using the hematopoietic-specific Vav-Cre line and a study performing genetically labeled bone marrow transplants incorporating more specific markers of fibroblasts have supported this idea, demonstrating that the contribution of circulating hematopoietic cells to the CF population is minimal.54 Future studies will determine whether these data from a pressure-overload heart failure model will extend to models of ischemic heart failure etiology.

**Fibrocytes**

The contribution of cells of hematopoietic origin to the fibroblast population is also under intense investigation after the identification of fibrocytes in the circulation.115 In fact, subsequent studies have reported the contribution of these fibrocytes to the CF population, as well as to the development of cardiac fibrosis in several injury models.42,109,110,113,114 Their pathophysiological relevance to cardiac fibrosis has been suggested in studies linking the inhibition of fibrocyte recruitment to reduced fibrosis and remodeling.110 Fibrocytes represent a unique fibroblast progenitor population that coexpress fibroblast markers, such as procollagen I and vimentin, along with typical hematopoietic markers.115 These circulating fibrocytes seem to originate from hematopoietic stem cells in the bone marrow; however, their pathophysiological relevance in cardiac disease and fibrosis has yet to be fully characterized.

**Alternate Growth Substrates**

Mechanical stretching of the CF, which potentially occurs secondary to cardiac dilatation or changes in the hemodynamic burden of the heart, may induce expression of profibrotic cytokines and expression of ECM proteins and receptors on CFs.116 Mechanical stress could play a contributory or pre-eminent role for the spontaneous activation observed when CFs are cultured on the stiff surface of standard tissue culture plastic. The elasticity (Young’s Modulus) of most cell culture plastic is >1 GPa, and for glass, this measure exceeds 70 GPa. In contrast, the Young’s Modulus of the normal heart is estimated to be <10 kPa. Thus, the natural growth environment for CFs is many orders of magnitude more elastic than standard cell culture conditions. Attempts to circumvent the issue of spontaneous activation in standard cell culture conditions have focused predominantly on the modulation of growth substrate tensile modulus. Polyethylene glycol-based hydrogels with a physiologically relevant elasticity (~7 kPa) have shown promise in preserving the quiescent fibroblast phenotype of valvular interstitial cells when compared with standard tissue culture polystyrene. Cells grown on this substrate exhibited reduced levels of typical myofibroblast markers, including αSMA and connective tissue growth factor (CTGF).117,118 The PI3K/AKT pathway is upregulated when these cells are mechanically activated by stiff substrates, and activation of this pathway was significantly attenuated in cells grown on the more compliant substrate.118 Using tissue culture substrates with a physiologically relevant tensile modulus will aid in the characterization of fibroblast biology in vitro.

**Therapeutic Targets in Cardiac Fibrosis**

Numerous signaling pathways have been implicated in the early activation of CFs as well as in the pathological remodeling that persists long after the initial injury. Modulation of these signals is of intense scientific interest because they represent potentially novel therapeutic targets or strategies. Here, we explore mediators and signaling pathways known to influence fibroblast function after myocardial injury as well as developing therapeutic strategies to combat pathogenic cardiac fibrosis (Table 2; Figure 3).

**Transforming Growth Factor β**

The TGF-β family of growth factors is perhaps the most extensively studied mediator of fibroblast activation, of which TGF-β1 is likely to play the greatest role in pathological fibrosis.166 TGF-β is known to play a major role in regulating fibrotic processes in many organs, and in fact, serum levels are used as diagnostic tools and also correlate with the severity of chronic liver and kidney diseases.83 TGF-β1 is initially secreted in a complex with latent TGF-β–binding proteins that restrict its activity; this complex is proteolytically cleaved and can be activated in an integrin-mediated process.167,168 The type I TGF-β receptor, which is also known as activin receptor-like kinase (ALK) 5, represents the subtype thought to be predominantly responsible for the fibrotic activities of TGF-β1. The canonical pathway of TGF-β1 signaling involves the phosphorylation of Smad2/3, which subsequently bind Smad4 and translocate to the nucleus. The complex then acts as a transcription factor, inducing the activation of numerous profibrotic genes.169,170 The ability of TGF-β to induce the production of ECM proteins in vitro is thought to depend on Smad3,169,171 and furthermore, fibroblasts isolated from Smad3-deficient mice seem resistant to TGF-β1–induced expression of ECM proteins.172,173 Perhaps equally detrimental to overall cardiac function as the initial infarct, the canonical TGF-β pathway is also activated in the infarct border and remote zones where it mediates the formation of pathological
reactive fibrosis. Finally, the persistence of activated fibroblasts is thought to be the result of perpetual TGF-β signaling, which may prevent CF apoptosis after the initial injury through Smad-mediated pathways. In addition to the Smad-mediated pathways, TGF-β can also induce noncanonical signaling that involves several mitogen-activated protein kinases, including c-Jun N-terminal kinase, and p38. This pathway involves the activation of TGF-β–activated kinase (TAK) 1, which is thought to contribute to pathological cardiac remodeling because cardiac overexpression of constitutively active TAK1 induces cardiac hypertrophy and heart failure. Although the myofibroblast-activating properties of TGF-β have previously been attributed to the canonical signaling pathway, growing evidence suggests the noncanonical pathway may actually be the dominant driving force. Thus, attempts to prevent this noncanonical signaling may prove efficacious for the treatment of fibrosis and heart failure. The TGF-β noncanonical signaling pathway is thought to propagate primarily through the type II TGF-β receptor, given that cardiomyocyte-specific deletion of the type II TGF-β receptor has resulted in reduced fibrosis and remodeling in the transverse aortic constriction model of heart failure. As the main effector of the TGF-β noncanonical pathway, TAK1 also represents a viable therapeutic target, as inhibition of TAK1 reduced TGF-β–induced fibroblast ECM protein production. Further downstream, inhibition of p38 is being actively investigated for its antifibrotic potential. Inhibitors of p38 signaling can attenuate TGF-β–induced myofibroblast activation and reduce the expression of collagen, fibronectin, and αSMA in mouse embryonic fibroblasts; moreover, overexpression of p38 can induce the myofibroblast transition. In vivo, inhibition of p38 reduces fibrosis and the expression of αSMA after MI. Several clinical trials evaluating the efficacy of p38 inhibitors of the TGF-β receptor ALK5 are currently under investigation as potential antifibrotic mediators. ALK5 inhibitors can decrease TGF-β signaling, rescuing cardiac dysfunction and ameliorating the remodeling that occurs post-MI. These inhibitors can also attenuate the development of fibrosis and expression of collagen after pressure overload by transverse aortic constriction, and reduce the expression of collagen in response to TGF-β stimulation in vitro. Several groups are also evaluating TGF-β neutralizing antibodies in experimental myocardial fibrosis models. Although fibroblast activation and collagen transcript levels were reduced, there were no substantial improvements in overall cardiac function, and there is evidence that this strategy may actually increase mortality. Although disparate, these results may have been the result of the disruption of the initial reparatory response leading to a reduction in cardiac function and increased mortality. Similar contradictory results have been observed in studies investigating TGF-β inhibition in animal models of chronic kidney disease. Although there is a significant body of evidence in preclinical trials showing antifibrotic effects of TGF-β inhibition, the clinical translation of these studies has been limited. Furthermore, recent data show that TGF-β plays a role in renal autophagy as a cytoprotective mechanism, which suggests that therapies inhibiting TGF-β activity should be approached with caution. Although inhibition of the canonical TGF-β signaling pathway remains appealing, this approach may require further investigation and refinement before it will have significant clinical impact.

In addition to the Smad-mediated pathways, TGF-β can also induce noncanonical signaling that involves several mitogen-activated protein kinases, including c-Jun N-terminal kinase, and p38. This pathway involves the activation of TGF-β–activated kinase (TAK) 1, which is thought to contribute to pathological cardiac remodeling because cardiac overexpression of constitutively active TAK1 induces cardiac hypertrophy and heart failure. Although the myofibroblast-activating properties of TGF-β have previously been attributed to the canonical signaling pathway, growing evidence suggests the noncanonical pathway may actually be the dominant driving force. Thus, attempts to prevent this noncanonical signaling may prove efficacious for the treatment of fibrosis and heart failure. The TGF-β noncanonical signaling pathway is thought to propagate primarily through the type II TGF-β receptor, given that cardiomyocyte-specific deletion of the type II TGF-β receptor has resulted in reduced fibrosis and remodeling in the transverse aortic constriction model of heart failure. As the main effector of the TGF-β noncanonical pathway, TAK1 also represents a viable therapeutic target, as inhibition of TAK1 reduced TGF-β–induced fibroblast ECM protein production. Further downstream, inhibition of p38 is being actively investigated for its antifibrotic potential. Inhibitors of p38 signaling can attenuate TGF-β–induced myofibroblast activation and reduce the expression of collagen, fibronectin, and αSMA in mouse embryonic fibroblasts; moreover, overexpression of p38 can induce the myofibroblast transition. In vivo, inhibition of p38 reduces fibrosis and the expression of αSMA after MI. Several clinical trials evaluating the efficacy of p38 inhibitors of the TGF-β receptor ALK5 are currently under investigation as potential antifibrotic mediators. ALK5 inhibitors can decrease TGF-β signaling, rescuing cardiac dysfunction and ameliorating the remodeling that occurs post-MI. These inhibitors can also attenuate the development of fibrosis and expression of collagen after pressure overload by transverse aortic constriction, and reduce the expression of collagen in response to TGF-β stimulation in vitro. Several groups are also evaluating TGF-β neutralizing antibodies in experimental myocardial fibrosis models. Although fibroblast activation and collagen transcript levels were reduced, there were no substantial improvements in overall cardiac function, and there is evidence that this strategy may actually increase mortality. Although disparate, these results may have been the result of the disruption of the initial reparatory response leading to a reduction in cardiac function and increased mortality. Similar contradictory results have been observed in studies investigating TGF-β inhibition in animal models of chronic kidney disease. Although there is a significant body of evidence in preclinical trials showing antifibrotic effects of TGF-β inhibition, the clinical translation of these studies has been limited. Furthermore, recent data show that TGF-β plays a role in renal autophagy as a cytoprotective mechanism, which suggests that therapies inhibiting TGF-β activity should be approached with caution. Although inhibition of the canonical TGF-β signaling pathway remains appealing, this approach may require further investigation and refinement before it will have significant clinical impact.

In addition to the Smad-mediated pathways, TGF-β can also induce noncanonical signaling that involves several mitogen-activated protein kinases, including c-Jun N-terminal kinase, and p38. This pathway involves the activation of TGF-β–activated kinase (TAK) 1, which is thought to contribute to pathological cardiac remodeling because cardiac overexpression of constitutively active TAK1 induces cardiac hypertrophy and heart failure. Although the myofibroblast-activating properties of TGF-β have previously been attributed to the canonical signaling pathway, growing evidence suggests the noncanonical pathway may actually be the dominant driving force. Thus, attempts to prevent this noncanonical signaling may prove efficacious for the treatment of fibrosis and heart failure. The TGF-β noncanonical signaling pathway is thought to propagate primarily through the type II TGF-β receptor, given that cardiomyocyte-specific deletion of the type II TGF-β receptor has resulted in reduced fibrosis and remodeling in the transverse aortic constriction model of heart failure. As the main effector of the TGF-β noncanonical pathway, TAK1 also represents a viable therapeutic target, as inhibition of TAK1 reduced TGF-β–induced fibroblast ECM protein production. Further downstream, inhibition of p38 is being actively investigated for its antifibrotic potential. Inhibitors of p38 signaling can attenuate TGF-β–induced myofibroblast activation and reduce the expression of collagen, fibronectin, and αSMA in mouse embryonic fibroblasts; moreover, overexpression of p38 can induce the myofibroblast transition. In vivo, inhibition of p38 reduces fibrosis and the expression of αSMA after MI. Several clinical trials evaluating the efficacy of p38 inhibitors of the TGF-β receptor ALK5 are currently under investigation as potential antifibrotic mediators. ALK5 inhibitors can decrease TGF-β signaling, rescuing cardiac dysfunction and ameliorating the remodeling that occurs post-MI. These inhibitors can also attenuate the development of fibrosis and expression of collagen after pressure overload by transverse aortic constriction, and reduce the expression of collagen in response to TGF-β stimulation in vitro. Several groups are also evaluating TGF-β neutralizing antibodies in experimental myocardial fibrosis models. Although fibroblast activation and collagen transcript levels were reduced, there were no substantial improvements in overall cardiac function, and there is evidence that this strategy may actually increase mortality. Although disparate, these results may have been the result of the disruption of the initial reparatory response leading to a reduction in cardiac function and increased mortality. Similar contradictory results have been observed in studies investigating TGF-β inhibition in animal models of chronic kidney disease. Although there is a significant body of evidence in preclinical trials showing antifibrotic effects of TGF-β inhibition, the clinical translation of these studies has been limited. Furthermore, recent data show that TGF-β plays a role in renal autophagy as a cytoprotective mechanism, which suggests that therapies inhibiting TGF-β activity should be approached with caution. Although inhibition of the canonical TGF-β signaling pathway remains appealing, this approach may require further investigation and refinement before it will have significant clinical impact.

In addition to the Smad-mediated pathways, TGF-β can also induce noncanonical signaling that involves several mitogen-activated protein kinases, including c-Jun N-terminal kinase, and p38. This pathway involves the activation of TGF-β–activated kinase (TAK) 1, which is thought to contribute to pathological cardiac remodeling because cardiac overexpression of constitutively active TAK1 induces cardiac hypertrophy and heart failure. Although the myofibroblast-activating properties of TGF-β have previously been attributed to the canonical signaling pathway, growing evidence suggests the noncanonical pathway may actually be the dominant driving force. Thus, attempts to prevent this noncanonical signaling may prove efficacious for the treatment of fibrosis and heart failure. The TGF-β noncanonical signaling pathway is thought to propagate primarily through the type II TGF-β receptor, given that cardiomyocyte-specific deletion of the type II TGF-β receptor has resulted in reduced fibrosis and remodeling in the transverse aortic constriction model of heart failure. As the main effector of the TGF-β noncanonical pathway, TAK1 also represents a viable therapeutic target, as inhibition of TAK1 reduced TGF-β–induced fibroblast ECM protein production. Further downstream, inhibition of p38 is being actively investigated for its antifibrotic potential. Inhibitors of p38 signaling can attenuate TGF-β–induced myofibroblast activation and reduce the expression of collagen, fibronectin, and αSMA in mouse embryonic fibroblasts; moreover, overexpression of p38 can induce the myofibroblast transition. In vivo, inhibition of p38 reduces fibrosis and the expression of αSMA after MI. Several clinical trials evaluating the efficacy of p38 inhibitors of the TGF-β receptor ALK5 are currently under investigation as potential antifibrotic mediators. ALK5 inhibitors can decrease TGF-β signaling, rescuing cardiac dysfunction and ameliorating the remodeling that occurs post-MI. These inhibitors can also attenuate the development of fibrosis and expression of collagen after pressure overload by transverse aortic constriction, and reduce the expression of collagen in response to TGF-β stimulation in vitro. Several groups are also evaluating TGF-β neutralizing antibodies in experimental myocardial fibrosis models. Although fibroblast activation and collagen transcript levels were reduced, there were no substantial improvements in overall cardiac function, and there is evidence that this strategy may actually increase mortality. Although disparate, these results may have been the result of the disruption of the initial reparatory response leading to a reduction in cardiac function and increased mortality. Similar contradictory results have been observed in studies investigating TGF-β inhibition in animal models of chronic kidney disease. Although there is a significant body of evidence in preclinical trials showing antifibrotic effects of TGF-β inhibition, the clinical translation of these studies has been limited. Furthermore, recent data show that TGF-β plays a role in renal autophagy as a cytoprotective mechanism, which suggests that therapies inhibiting TGF-β activity should be approached with caution. Although inhibition of the canonical TGF-β signaling pathway remains appealing, this approach may require further investigation and refinement before it will have significant clinical impact.
inhibition have begun in patients after acute MI. In the study of Losmapimod Treatment on Inflammation and Infarctsize (SOLSTICE) phase II trial, the p38 inhibitor losmapimod was well tolerated in non-ST-segment–elevation MI patients with no major liver toxicities observed. Although infarct size was nonsignificantly reduced, patients receiving losmapimod experienced fewer cardiac events.181 These findings provide strong evidence for the therapeutic potential of targeting the TGF-β noncanonical signaling pathway, specifically through modulation of TAK1 and p38, in the treatment of cardiac fibrosis.

Renin–Angiotensin System

The renin–angiotensin–aldosterone system, of which angiotensin II (Ang II) seems to be the predominant effector, promotes many physiological and pathological functions, including the development of cardiac fibrosis.182,183 The Ang II type 1 receptor (AT1R) mediates many of the effects of Ang II in fibroblasts, including cell proliferation, migration, and the induction of ECM protein synthesis.184,185 Cardiac Ang II levels are quickly elevated after injury, and stimulation of CFs by Ang II induces proliferation and the expression of collagen.186,187 Renal renin–angiotensin–aldosterone system activity, which is reflected by urinary angiotensinogen levels, directly correlates with the extent of renal fibrosis and deterioration of renal function in patients with chronic kidney disease.188 Ang II is also intimately involved with the inflammatory response because it is expressed and activated by macrophages and myofibroblasts.189 It is thought that Ang II is also involved with TGF-β signaling, both in cardiomyocytes and in CFs. Specifically, Ang II activation of ATR1 induces the expression TGF-β1, and it is thought that TGF-β is required for Ang II to induce both cardiac hypertrophy and fibrosis.190,191 Furthermore, Ang II–induced expression of collagen in CFs requires TGF-β/Smad and mitogen-activated protein kinase signaling.186,189

Hemodynamic burden, chronic fibrotic remodeling, and tissue stiffening after a myocardial injury can stimulate the release of endogenous Ang II from cardiomyocyte stores; however, it is now recognized that this mechanical stress can contribute directly to the activation of the AT1R.127 Although HEK293 and COS7 cells do not natively respond to mechanical stretch, expression of the AT1R confers this ability, as seen by an increase in the activation of ERK, suggesting that the AT1R is itself a mechanical sensor. Mechanical stretching of cardiomyocytes isolated from ATG null mice, which do not express endogenous Ang II, also resulted in ERK activation.
In addition, cardiac hypertrophy in ATG−/− mice was induced by pressure overload, indicating that mechanical stress can induce hypertrophy even in the absence of Ang II.127 Of note, these mechanical sensing abilities seem to be specific to the AT1R because other G-protein–coupled receptors (GPCRs) do not respond to mechanical stretch.127 A similar phenomenon occurs in CFs because these cells are highly susceptible to variations in their mechanical microenvironment.21 This stretch-induced activation of the AT1R can be inhibited by the Ang II receptor blocker candesartan,127 revealing a potential antihypertrophic and antifibrotic role for this class of drugs. Because candesartan will reduce blood pressure and thus cardiac load, the mechanism by which it attenuates fibrosis remains unclear and may not necessarily be due to direct inhibition of pathologically activated CFs.

Although not approved for the treatment of cardiac fibrosis, inhibitors of angiotensin signaling, including angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, have already demonstrated significant efficacy in the treatment of heart failure, frequently reducing cardiac fibrosis both in humans and in animal models.128–130 One such angiotensin receptor inhibitor, losartan, is thought to reduce cardiac fibrosis through the inhibition of endothelial–mesenchymal transition, as was demonstrated in mitral valve endothelial cells, by acting to block the Ang II–elicited and TGF-β–induced phosphorylation of ERK1/2.131 Losartan reduced collagen I synthesis and fibroblast activation in response to stimulation by Ang II.132 Stimulation of the AT1R promotes a proinflammatory environment, and losartan can decrease the expression of inflammatory markers, including tumor necrosis factor α, interleukin-1β (IL-1β), and IL-6.133–135 Clinically, losartan is thought to attenuate the progression of myocardial hypertrophy and fibrosis in patients with hypertrophic cardiomyopathy.136 These data present a compelling argument that Ang II is a potent fibrotic mediator, and that the protective effects of Ang II antagonists may partly be due to their ability to reduce cardiac fibrosis. Further studies will be needed to mechanistically establish the effects of Ang II inhibition specifically in CFs, which may lead to refinement of antagonists inverting enzyme/Ang II receptor inhibitors and allow for more direct targeting of the pathway’s pathological activation of this cell population.

Endothelin

The endothelin family of peptides is typically recognized for its vasoconstrictive properties and is now beginning to be appreciated for its potential role in promoting tissue fibrosis. Although originally thought to be secreted predominantly by endothelial cells, endothelin-1 (ET-1) is now understood as a significant profibrotic peptide released by both inflammatory cells and fibroblasts in the lung.192 The endothelin system also plays a major role in kidney disease because ET-1 production is stimulated in disease downstream to both the sympathetic nervous system and the renin–angiotensin–aldosterone system.193 There exist two known receptors for ET-1 in the heart, the ETA and ETB receptors, which have been shown to play differing and sometimes opposing roles. These receptors are primarily expressed by endothelial cells; however, new data define expression on multiple cell types including cardiomyocytes and CFs, along with some immune cells, such as macrophages.194,195 Importantly, ET-1 activation of the ETA receptor is known to increase collagen production in isolated human CFs.196 Furthermore, myofibroblasts isolated from scar tissue after experimental MI possess elevated levels of ET-1, suggesting an important function for endothelin within these cells.197 Although known to have fibroblast-activating properties of its own, ET-1 also acts as a downstream mediator of both Ang II and TGFβ3 to promote myofibroblast persistence in pulmonary fibrosis.137 Further ET-1 signaling is thought to interact with Ang II as well. For example, the development of cardiac fibrosis and hypertrophy in response to Ang II stimulation is impaired in mice in which ET-1 is ablated in endothelial cells. It is thought that these factors are mechanistically linked through the activation of ERK1/2 and the myocardial-related transcription factor (MRTF) A.199–202

Endothelin antagonists are currently approved for the treatment of pulmonary hypertension, and many think they will be additionally efficacious in the treatment of pathological fibrosis in the heart.137 Endothelin receptor blockade has been incorporated extensively both experimentally and in the clinic for its salutary role in the treatment of hypertension. Bosentan, a nonselective endothelin receptor antagonist, although used clinically for the treatment of pulmonary hypertension, can improve cardiac function and reduce infarct size after myocardial ischemia/reperfusion injury in rats.138 Preliminary trials in humans have demonstrated beneficial hemodynamic and cardiac effects in patients with end-stage heart failure.139 However, subsequent clinical trials evaluating endothelin receptor antagonists on coronary artery disease and heart failure have not met primary end points, perhaps because of inefficient selection of potential responders before the initiation of major clinical trials.140–143 Although the reasons for these discrepancies remain unclear, manipulation of ET-1 signaling seems promising. Next generation ETR subtype-specific antagonists, such as ambrisentan and darusentan that are proving to be highly beneficial in the treatment of pulmonary hypertension, may prove more efficacious and help define receptor specificity that may be required for salutary effects. Further studies will be necessary to determine if ET-1 and its receptors will become clinically viable antifibrotic therapeutic targets.

RhoA–MRTF–SRF Signaling Pathway

Serum response factor (SRF), a member of the MADS box-containing family of transcription factors, is critically involved in the regulation of myofibroblast activation, given that numerous SRF-binding sites are located within the promoter regions of genes responsible for fibroblast activation.203,204 Experiments in bleomycin-injured lung tissue have demonstrated upregulation of SRF expression in isolated myofibroblasts,205 and overexpression of SRF can induce the transformation of both lung and CFs.206–208 SRF interacts with cofactors such as members of the MRTF family of transcription factors, which translocate to the nucleus and bind SRF to regulate its transcriptional efficacy.209 In CFs, MRTF-A overexpression alone can induce the myofibroblast transition and the expression of αSMA; conversely, MRTF-A ablation in vivo reduces the fibrotic response after MI.209 SRF activation
These findings have revealed TRPC6-mediated Ca²⁺ entry and phospholipase C signaling pathways to Ca²⁺ entry along with its canonical and noncanonical signaling pathways. Perhaps the inhibition of RhoA, using compounds such as fasudil, presents a potential antifibrotic strategy that complements those described above.

**Transient Receptor Potential Channels**

Receptor-activated Ca²⁺ entry is induced after the activation of GPCRs and receptor tyrosine kinases, including the AT1R or TGF-β receptor, leading to the activation of calcineurin and nuclear translocation of the nuclear factor of activated T cells family of transcription factors. The transient receptor potential proteins are the channels responsible for this receptor-mediated Ca²⁺ entry; the canonical transient receptor potential channel (TRPC) specifically couple receptor-phospholipase C signaling pathways to Ca²⁺ entry and are upregulated in mice subjected to myocardial pressure overload. Cardiomyocyte-specific overexpression of TRPC6 resulted in cardiomyopathy and promoted nuclear factor of activated T cells–dependent promoter activity. Furthermore, siRNA knockdown of TRPC6 reduced hypertrophic signaling in response to ET-1, suggesting a role of TRPC6 in the GPCR-dependent activation of the calcineurin/NFAT pathway.

Recently, the TRPC6 channel was identified in an unbiased genome-wide screen as an important regulator of myofibroblast activation. In primary fibroblasts, TRPC6 overexpression induced the expression of myofibroblast marker genes, and genetic ablation conferred resistance to TGF-β and Ang II–dependent myofibroblast transformation. Furthermore, overexpression of a constitutively active calcineurin in CFs induced the myofibroblast transition, whereas inhibition of calcineurin blocked TRPC6-mediated transformation, indicating a functionally relevant interaction of these proteins in CFs. These findings have revealed TRPC6-mediated Ca²⁺ entry and activation of the calcineurin/NFAT signaling pathway as novel regulatory mediators in the activation of fibroblasts, and suggest that their inhibition may represent an important antifibrotic therapeutic strategy.

**Connective Tissue Growth Factor**

CTGF belongs to the CCN family (the acronym comes from the first 3 members of the family that were found: CYR61 (cysteine-rich angiogenic inducer 61 or CCN1), CTGF (connective tissue growth factor or CCN2), and NOV (nephroblastoma overexpressed or CCN3) of matricellular proteins, which are dynamically expressed nonstructural proteins of the ECM. This family of proteins is thought to play a significant regulatory role in the ECM to modulate cell surface receptors and their responses to cytokines, growth factors, and proteins of the ECM. CTGF is a well-characterized mediator of TGF-β activity during the fibrotic response, and its expression is induced in fibroblasts stimulated by TGF-β.

Furthermore, CTGF is strongly upregulated both in human heart failure and in animal models associated with myocardial fibrosis. Interestingly, induction of CTGF after injury has been observed before the upregulation of TGF-β or deposition of ECM proteins, suggesting an important role in these processes. CTGF also seems to play a significant role directly in isolated CFs, as evidenced by a reduction of various chemokines, matrix metalloproteinases, ECM proteins, and cell adhesion proteins in CFs in which CTGF is ablated using siRNA. Although studies targeting CTGF in animal models of heart failure are relatively limited, preliminary studies in a pressure-overload model have suggested efficacy of CTGF neutralizing antibodies in reducing cardiac remodeling and dysfunction, concomitant with attenuated collagen deposition. These data indicate that the pursuit of therapies targeting CTGF may be of clinical interest for the treatment of cardiac fibrosis.

These data are in stark contrast to a recent study investigating the mechanism of CTGF and definitively characterizing its role in cardiac injury. Several transgenic mouse lines were developed that included heart-specific CTGF knockouts and overexpressors in combination with constitutively active TGF-β and fibroblast-specific CTGF knockout mice. Surprisingly, neither gain nor loss of CTGF affected overall cardiac function or the fibrotic response in multiple models of cardiac injury. However, modulation of CTGF did seem to slightly affect the response to TGF-β in a pressure-overload model, suggesting a potential interaction between these factors. As described above, CTGF is thought to play an important functional role with TGF-β to regulate the fibrotic response, but recent data suggest that CTGF is of limited importance as a TGF-β effector and thus may no longer represent an important therapeutic target for the treatment of cardiac fibrosis.

**Platelet-Derived Growth Factor**

The PDGF family of growth factors is a group of proteins originally recognized for their ability to regulate cell proliferation in smooth muscle cells. Expression of the PDGF family is significantly increased in endothelial cells, macrophages, and myofibroblasts after MI. Enhanced expression of both the ligands and the receptors occur concomitantly with inflammatory and fibrogenic responses after MI, identifying a potential role in the regulation of cardiac repair. Stimulation by PDGF is known to enhance CF proliferation, and also enhances TGF-β expression in vitro. Similarly, cardiac-specific overexpression of PDGF significantly elevates TGF-β transcription and promotes the development of cardiac fibrosis.

The disruption of PDGF signaling is currently under active investigation for the treatment of myocardial fibrosis. The tyrosine kinase inhibitor imatinib, typically recognized for its antitumorigenic properties, can also suppress PDGF signaling by acting as an antagonist against the PDGF receptor. In an MI model of heart failure, imatinib reduced the expression of fibrogenic mediators, including TGF-β and collagen I, significantly reduced fibrotic scar formation and mildly rescued cardiac dysfunction. A neutralizing PDGF receptor antibody has also been shown to attenuate atrial fibrosis in a pressure-overload model of heart failure. Collectively, these data implicate PDGF in the development of cardiac fibrosis,
probably through the induction of TGF-β, and additionally present PDGF inhibition as a potential approach for antifibrotic therapy.

**Integrins**

Integrins are transmembrane proteins comprised of α and β subunits that mediate interactions between the extracellular environment and the actin cytoskeleton. CFs express a wide variety of integrin subtypes that mediate multiple cellular functions, including proliferation, migration, adhesion, differentiation, and apoptosis. Furthermore, it has been reported in cultured myofibroblasts that integrins are required for the activation of latent TGF-β. The integrin-mediated transformation of myofibroblasts is thought to occur through activation of mitogen-activated protein kinase signaling cascades, including ERK1/2 and p38. This regulation of TGF-β activation may be one potential mechanism causing the persistence of myofibroblasts within fibrotic scars, and may offer a target for ameliorating adverse myocardial remodeling after injury. Strategies aimed at blocking the function of integrins have shown preliminary success in limiting maladaptive remodeling. For example, small molecule targeting of the αβ integrin, which is highly expressed in activated fibroblasts, attenuated pulmonary and liver fibrosis through a mechanism involving the inhibition of TGF-β activation. It is possible that therapeutic approaches targeting various integrin subtypes could be therapeutically useful for the treatment of numerous diseases characterized by excessive tissue fibrosis.

**Inflammation/Interferon Receptors**

Inflammation is an essential mediator of the reparative response to an acute cardiac insult. Numerous inflammatory cells, including neutrophils and macrophages, infiltrate the site of injury where various proinflammatory cytokines are released, such as tumor necrosis factor α, IL-1β, and IL-6, and play an important role in the initial induction of resident fibroblast proliferation and myofibroblast activation. In addition to their direct release of ECM components, activated CFs also express numerous cytokines and growth factors that affect wound healing via autocrine and paracrine mechanisms. Cytokine expression by CFs is markedly upregulated after acute myocardial injury, including the expression of the proinflammatory cytokines IL-1β and IL-6. IL-1β promotes fibroblast migration through increasing the expression of proteins involved in ECM turnover, including matrix metalloproteinases, and IL-6 is known to increase fibroblast proliferation and myocardial fibrosis.

Interferons (IFNs) are a family of cytokines that cause a myriad of biological responses and, in addition to immune cells, they can be secreted by other cell types such as CFs. However, the role of IFNs in fibrosis, specifically IFN-γ, remains somewhat controversial. In IFN-γ knockout mice challenged with Ang II, there was a reduction in the myofibroblast marker αSMA. Similarly, mice null for the IFN-γ receptor exhibit a decrease in cardiac hypertrophy and fibrosis, as well as a reduction in the infiltration of macrophages and T cells. Because extensive data have demonstrated a critical role of inflammation in the fibrotic response, inhibiting these inflammatory mediators may prove efficacious in the treatment of cardiac fibrosis.

**GPCR/Adrenergic Signaling**

The adrenergic system plays a fundamental role in the physiological regulation of the myocardium, but chronic overstimulation can induce both cardiac hypertrophy and fibrosis. Although several subtypes of the β-adrenergic receptor (β-AR) are expressed in the heart, the β2-AR seems to be the form that is predominantly expressed by CFs. Chronic stimulation of this receptor can induce cell proliferation, collagen secretion, migration, and transformation to the myofibroblast phenotype. It is well established that acute stimulation of the β-AR increases the levels of cAMP, which can modulate proliferation as well as inhibit the synthesis and secretion of various forms of collagen. In addition, elevated cAMP levels can inhibit the transformation of CFs to myofibroblasts induced by stimulation with TGF-β. Recent studies indicated that failing human CFs isolated from patients with heart failure had higher baseline collagen synthesis that was not inhibited by β-agonist stimulation. Furthermore, β-AR signaling was markedly uncoupled and associated with increased expression and activity of GPCR kinase 2 (GRK2). Similarly, knockdown or inhibition of GRK2 restored β-agonist-stimulated inhibition of collagen synthesis and decreased collagen synthesis in response to TGF-β stimulation, indicating a significant role for GRK2 in the regulation of collagen synthesis and maladaptive ventricular remodeling. GRK2 is also recognized for its role as a RhoA-activated scaffold protein for the ERK mitogen-activated protein kinase cascade, which may have implications in the development of hypertension, and also suggests potential interactions with profibrotic mediators, including TGF-β, Ang II, and ET-1. GRK2 is typically recognized for its role in the downregulation of β-ARs, predominantly through the recruitment of β-arrestin, which is also significantly upregulated in adult human CFs isolated from failing left ventricles. Enhanced β-arrestin signaling in CFs seems to be deleterious in that it promotes a profibrotic phenotype via the uncoupling of β-ARs and potentiates ERK and Smad signaling downstream of TGF-β. Targeting GRK2, RhoA, and β-arrestin may represent plausible therapeutic strategies for the prevention of myocardial fibrosis.

Several approaches for specifically targeting GRK2 have been developed, all of which have shown some promise in reducing the fibrotic response concomitant with protection against overall cardiac dysfunction. The selective serotonin reuptake inhibitor paroxetine was recently identified as an inhibitor of GRK2. Paroxetine is known to reverse left ventricular dysfunction after MI, and reduce both immediate infarct and remote region fibrosis, albeit at a dose substantially in excess of that used in humans. These data suggest that targeting GRK2 signaling may be a potent antifibrotic approach. The idea of inhibiting the interaction between GRK2 and G-protein βγ subunits has been investigated in several models of heart failure, using either small molecules or a truncated form of GRK2 known as βARKct. The βARKct peptide has shown promise in preventing myocardial dysfunction in both small and large animal models of heart failure.
The small molecule Gβγ-GRK2 inhibitor gallein has demonstrated the ability to improve cardiac function and reduce the fibrotic response in several murine models of heart failure.162–165 Of further interest, the introduction of gallein attenuated pathological renal abnormalities, including renal dysfunction, tissue damage, and fibrosis, which occur secondary to myocardial pressure-overload injury (Blaxall Lab, unpublished data, 2016). Although the mechanism of GRK2 and Gβγ-GRK2 inhibition within CFs has yet to be fully delineated, GRK2 represents an exceptionally important target for therapeutic interventions directed against myocardial fibrosis.

Fibroblasts for Cardiomyocyte Regeneration

With the advent of stem cell–based therapies for the treatment of cardiovascular disease, there is now a growing interest in the development of reprogramming techniques to induce the transformation of fibroblasts into functional cardiomyocytes. Although still in its infancy, this technique relies on the creation of induced pluripotent stem cells, which can be differentiated in vitro to potentially generate patient-specific cardiomyocytes for subsequent introduction into the failing or infarcted heart.152 Newer methods are beginning to allow for the direct reprogramming of fibroblasts into cardiomyocytes in vivo through the administration of a specific combination of transcription factors.67,152,153,241 Unfortunately, with these in vivo approaches along with the identification of novel biomarkers will allow for greater investigation of this important cell population. Furthermore, unraveling the intricate mechanisms underlying this fibrotic signaling is of utmost importance for the development of effective therapies for the treatment of cardiac fibrosis. Therapeutic interventions targeting CFs and the pathological fibrosis they promote will inevitably lead to significant advancements in the treatment of heart failure.

Conclusions

It is abundantly clear that a deeper understanding of CFs will be critical to achieving exciting advancements in the treatment of cardiac fibrosis and disease. A limited knowledge of the role of this essential and dynamic cell type continues to hinder progress in the design and application of meaningful new therapies. The development of more specific in vivo approaches along with the identification of novel biomarkers will allow for greater investigation of this important cell population. Furthermore, unraveling the intricate mechanisms underlying this fibrotic signaling is of utmost importance for the development of effective therapies for the treatment of cardiac fibrosis. Therapeutic interventions targeting CFs and the pathological fibrosis they promote will inevitably lead to significant advancements in the treatment of heart failure.

Acknowledgments

We wish to acknowledge Jeffery Molkentin for his astute suggestions during the development of this review.

Sources of Funding

This work was supported by National Institutes of Health grants R01 HL091475, R01 HL129772, R01 HL132551, R01 GM097347, U54 HL119810 (B.C. Blaxall), and P01 HL069779 (B.C. Blaxall, J. Robbins, and K.E. Yutzey), an American Heart Association Postdoctoral Fellowship (F.A. Kamal), and a Predoctoral Fellowship from the Pharmaceutical Research and Manufacturers of America Foundation (J.G. Travers).

Disclosures

None.

References


endocardial cushion lineage-restricted periostin enhancer. Identification and characterization of a novel Schwann and outflow tract 1 (Thy1, CD90) is expressed by lymphatic vessels and mediates cell 

Kado A. Periostin in fibrillogenesis for tissue regeneration: periostin actions inside and outside the cell. 


Conway SJ. Periostin (an osteoblast-specific factor) is expressed inside and outside the cell. 

Kado A, Harada M, McSweeney S, et al. PDGFRα demarcates the cardiogenic clonogenic Sca1+ stem/progenitor cell in adult murine myocardium. 

Noseda M, Harada M, McSweeney S, et al. PDGFRα demarcates the cardiogenic clonogenic Sca1+ stem/progenitor cell in adult murine myocardium. 

Travers et al New Frontiers in Cardiac Fibrosis 1035

Downloaded from http://circres.ahajournals.org/ by guest on December 27, 2017


251. Gaudeuis O, Miragoli M, Thomas SP, Rohr S. Coupling of cardiac electrical activity over extended distances by fibroblasts of cardiac origin. Circ Res. 2003;93:421–428. doi: 10.1161/01.RES.0000089258.40661.0C.

252. Kohl P. Heterogeneous cell coupling in the heart: an electrophysiological role for fibroblasts. Circ Res. 2003;93:381–383. doi: 10.1161/01.RES.0000091364.90121.0C.


Cardiac Fibrosis: The Fibroblast Awakens
Joshua G. Travers, Fadia A. Kamal, Jeffrey Robbins, Katherine E. Yutzey and Burns C. Blaxall

Circ Res. 2016;118:1021-1040
doi: 10.1161/CIRCRESAHA.115.306565

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/118/6/1021

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/