Cardiac fibrosis occurs after heart injury or inflammation, and during aging. The accumulation of extracellular matrix (ECM) results in stiffening of the heart and decreased cardiac function. Based on its known role in ECM production, the principle cell type implicated in the fibrotic remodeling process is the cardiac fibroblast. Although defining this cell and its behavior is essential for developing approaches to reducing the adverse effects of fibroblast activation, there is still much ambiguity regarding its origin, function, gene expression, and signaling pathways. In this review, we will focus on recent studies that shed light on the nature of these cells and provide evidence that fibroblast origins and gene expression may not be as diverse as previously thought. In addition, we outline new mechanisms for studying these cells. The overall goal is to establish a consensus for identifying and describing resident cardiac fibroblast behaviors in the hopes of discovering signaling pathways for controlling fibroblast activities in pathological situations. The majority of the described studies focus on the cardiac fibroblast population in the mouse, but conservation between human and murine heart biology suggests that findings in the mouse may pertain to human fibrosis and remodeling.

Cardiac Fibroblast Identity

**Definition by Function**

Typically, a cardiac fibroblast is defined as a cell that produces connective tissue. Unlike the connective tissue of bone and tendon, which is organized into regular patterns of collagen, heart ECM is dense, irregular, and composed of collagens, proteoglycans, and glycoproteins. Heart structural components that are produced by fibroblasts include peristin, vimentin, fibronectin, and collagen types I, III, V, and VI (reviewed by Snider et al\(^2\)). Although fibroblasts are considered the predominant manufacturer of these proteins, several other cell types in the heart can also express these ECM components (Table). Basing cell categorization on dynamically and stress-induced genes is a primary difficulty in defining and studying the fibroblast.

Adding to the confusion of understanding the cardiac fibroblast is the use of many different terms, including fibrocyte, telocyte, myofibroblast, protomyofibroblast, mesenchymal cell, and stromal cell. Each of these categories reflects a definition that varies depending on the author and demonstrates a lack of consensus regarding these cells. For the purpose of this review we will refer to fibroblasts in an uninjured heart as “resting” fibroblasts and in an injured heart as “activated” fibroblasts. We use these terms to be inclusive of the various fibroblast populations. Fortunately, recent studies have provided a refined view of the resident cardiac fibroblast and demonstrate that these cells are responsive to injury and are likely the dominant producer of ECM.

**Definition by Origin**

Developmental biologists suggested many years ago that cardiac fibroblasts have a distinct embryonic origin. Specifically, evidence from the avian system demonstrated that the epicardium undergoes the process of epithelial-to-mesenchymal transition (EMT) and contributes to cardiac fibroblasts and vascular smooth muscle cells (VSMC)\(^13-17\) (Figure). With the discovery of epicardium-specific genes, such as WT1, Tcf21\(^19\) and Tbx18,\(^20\) these initial observations have recently been reconfirmed using lineage-tracing methods in the mouse.\(^19-22\) Lineage tracing is a heritable method of tagging cells that permits the later identification of the original cell and its progeny.\(^21\) With the advent of new mouse lines that permit the...
Table. Published Markers for Cardiac Fibroblasts

<table>
<thead>
<tr>
<th>Method of detection</th>
<th>Embryonic stage</th>
<th>Adult</th>
<th>Injury phase</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day E12.5</td>
<td>Day E14.5–18.5</td>
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<tr>
<td>Nuclear</td>
<td></td>
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<td>GT, IHC</td>
<td>Ep, CM</td>
<td>Ep, EC, CM</td>
<td>Ep, EC\textsuperscript{low} Ep, P/V, EC</td>
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<tr>
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<td></td>
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<td>IHC</td>
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<td>Ep, CM</td>
<td>P/V, CM</td>
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<td>F</td>
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<td>Ep</td>
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</tr>
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<td>F\textsuperscript{*}, P/V, IC, EC</td>
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CM, cardiomyocytes; CPC, cardiac progenitor cell; EC, endothelial cells; Ep, epicardium; F, fibroblasts; FC, flow cytometry; GT, gene technology; IC, immune cells; IHC, immunohistochemistry; NE, not expressed; P/V, pericytes/vascular smooth muscle cells. *Marker expressed in a subpopulation of fibroblasts.

Figure. Murine cardiac fibroblast stages and function. Cardiac fibroblasts are derived from epicardial and endocardial progenitors after embryonic day 12.5 birth. It is at this time that fibroblasts begin to deposit and degrade the extracellular matrix (ECM). In the uninjured adult heart, proposed roles for fibroblasts include secretion of trophic factors, ECM surveillance, conduction system insulation, cardiomyocyte electrical coupling, and vascular maintenance. During the injury phase, fibroblasts proliferate, deposit ECM, and recruit inflammatory cells. Recent data have shown that after the proliferative phase of injury, previously activated cardiac fibroblasts can revert to a resting fibroblast gene profile. EMT, epithelial-to-mesenchymal transition.
genetic manipulation of fibroblasts, investigators have elucidated several important findings.

First was the discovery that cardiac fibroblasts develop from 2 origins rather than 1. Two independent groups found that populations of fibroblasts residing in the interventricular septum and right ventricle do not form from the epicardium, but instead have an endothelial origin, constituting roughly 20% of the myocardial resident fibroblasts. Second, in contrast to being a stochastic determined cell population, recent findings demonstrate that differentiation of the cardiac fibroblast requires specific signals. We found that 2 unrelated genes are essential for cardiac fibroblast formation. Disruption of the expression of either Tcf21, a bHLH transcription factor, or PDGFRα, a receptor tyrosine kinase, results in loss of epicardial-derived ventricular fibroblasts. In the absence of either of these genes, not only is there a lack of fibroblasts, but also the expression of ECM components in the left ventricle is disrupted. These findings suggest there are no alternative sources for fibroblasts during developmental stages. It remains to be determined if these same genes affect the developmental program of endocardial-derived fibroblasts. Additionally, these findings demonstrate that the cardiac fibroblast is not a default program of endocardial-derived fibroblasts. Additionally, these studies used lineage tracing to investigate the contribution of other cell lineages to the fibroblast pool after myocardial infarction and catecholamine-induced fibrosis. In sham hearts, fibroblasts genes such as Col1a1, Col1a2, and PDGFRα were expressed at similar levels by the 2 populations of fibroblasts. After injury, ECM and growth factor expression increases were observed when compared with the sham, but there was no significant difference between the 2 fibroblast types. Recent single-cell analyses of fibroblasts have also demonstrated comparable profiles of gene expression (upregulation of Postn, aSMa, Adam12, Lox, Wisp1, and DDR2) after activation.

Markers for Cardiac Fibroblasts

In the past, the markers most often used to identify fibroblasts were CD90 (or Thy1), a receptor tyrosine kinase PDGFRα are required during fibroblast formation and continue to be expressed in adult fibroblasts. Possibly because Tcf21 is a transcription factor, it is often difficult to detect by immunohistochemistry (IHC), PDGFRα expression, on the other hand, is readily detectable by IHC but recognizes rare stem cell populations in the heart. Unfortunately, PDGFRα antibodies designed for flow cytometric applications are not robust for cardiac fibroblasts. Recently, we described a commercially available monoclonal antibody, MEFSK4, which identifies an antigen expressed by PDGFRα+Col1a1+ murine cardiac fibroblasts. One drawback to using this antibody is that it also recognizes surface antigens on pericytes and granulocytes. Periostin, an ECM protein expressed developmentally by cardiac fibroblasts but not adult resting fibroblasts, is highly upregulated after a variety of injuries and appears to be a distinguishing marker for activated fibroblasts.

The use of markers such as collagen, fibronectin, and periostin stem from the functional definition of fibroblasts. As these proteins are secreted, IHC identification of cells expressing these markers can be technically difficult and subjective. Additionally, many ECM proteins are expressed in multiple cell types. For example, collagen can also be expressed by valve interstitial cells, VSMCs, and pericytes.

Cytoskeletal and surface markers, such as vimentin, FSP1, Sca1, CD90 and DDR2, are not secreted and thus can be used to identify fibroblasts directly by IHC or flow cytometry. Unfor - tunately, these markers are not specific and require exclusion of non-fibroblast cell populations (Table). For example, FSP1, originally thought to be fibroblast specific, is found in a limited number of cardiac fibroblasts and is expressed by immune cells. After both pressure overload and myocardial infarction, FSP1 expression broadens and is expressed by VSMCs and endothelial cells.

Some previously used fibroblast markers, such as CD90 and Sca1, have been recently reevaluated with newly developed fibroblast tools, and these proteins appear to be expressed in a

Cardiac Fibroblast Gene Expression

Heterogeneity

Historically, the fibroblast population has been considered heterogeneous based on protein expression, cell size, ability to proliferate after activation, and developmental origin. Until recently, fibroblast studies have been hindered by the lack of means for identification in vivo, which necessitated the use of in vitro culture. The markers initially available to study cardiac fibroblasts (CD90, Sca1, and α-smooth muscle actin [αSMA]) are indeed differentially expressed by fibroblasts, leading to the suggestion that resting fibroblasts are an amalgam of cell populations. These ideas were reinforced by the notion that activated fibroblasts also derive from disparate cell types. The new tools available to study fibroblast biology have demonstrated that the fibroblast population may not be as diverse as previously thought. For example, even though cardiac fibroblasts come from 2 different developmental origins, gene expression analyses observed overlapping genetic profiles when comparing fibroblasts of the 2 origins in either uninjured or pressure overload conditions. In sham hearts, fibroblasts genes such as Col1a1, Col1a2, and PDGFRα were expressed at similar levels by the 2 populations of fibroblasts. After injury, ECM and growth factor expression increases were observed when compared with the sham, but there was no significant difference between the 2 fibroblast types. Recent single-cell analyses of fibroblasts have also demonstrated comparable profiles of gene expression (upregulation of Postn, aSMa, Adam12, Lox, Wisp1, and DDR2) after activation.

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subset of cardiac fibroblasts\textsuperscript{40} (Table). Therefore, previous analyses with these markers may have underestimated the resident fibroblasts. The expression of αSMA, previously the gold standard for identifying activated fibroblasts, has also been reevaluated. Investigators found that αSMA staining identified approximately 15\% of fibroblasts after TAC\textsuperscript{26} and 35\% of fibroblasts after angiotensin II treatment in lesional areas.\textsuperscript{41} Therefore, studies using αSMA expression as the sole readout for activated fibroblasts may have underestimated the activated fibroblast population.

**Genetic Tools**

Mouse lines such as PDGFRα\textsuperscript{ERT2} and Coll1a-GFP are one avenue for reliably observing the resident cardiac fibroblast population (Table).\textsuperscript{33,40} These lines have been used to further characterize and validate fibroblast markers.\textsuperscript{26,33,40} Fibroblast-specific inducible Cre mouse lines such as Tcf21\textsuperscript{mCrem},\textsuperscript{60} Periostin\textsuperscript{Cre},\textsuperscript{33} and Periostin\textsuperscript{CreERT2} provide unique opportunities for tracing and genetically manipulating resident and activated fibroblasts. Given the described heterogeneity of fibroblasts, it is surprising that the PDGFRα\textsuperscript{ERT2}, Coll1a-GFP, and Tcf21\textsuperscript{mCrem} lines were found to label the resident fibroblast population discretely, and this homogeneous cell population uniformly expressed the antigen recognized by the aforementioned MF50K antibody.\textsuperscript{41} The generation of reporter and Cre lines that specifically label both resting\textsuperscript{40} and activated\textsuperscript{33,41} fibroblasts in the heart will enable research to finally examine the role of the fibroblast, and fibroblast-specific genes, during all stages of activation.

**Cardiac Fibroblast Function**

**Development and Resting**

Although the data presented above demonstrate that resident cardiac fibroblasts respond to injury by producing components of the ECM, additional roles of the cardiac fibroblast in uninjured hearts remain a mystery. Without the ability to use genetic tools and well-defined markers, early studies often relied on cell morphology to identify these cells. A common notion was that cardiac fibroblasts comprised a majority of the non-cardiomyocytes of the heart,\textsuperscript{61-63} but we have demonstrated that endothelial cells, not fibroblasts, are the most populous cell type in both the human and murine heart.\textsuperscript{40}

Although not the major constituent, it is likely that cardiac fibroblasts play an important role in normal heart physiology. In fact, many functions have been attributed to fibroblasts, but these proposed cellular activities are often deduced after in vitro culture and need to be verified in vivo (Figure). Matrix degradation, conduction system insulation, cardiomyocyte electrical coupling, vascular maintenance, and stress sensing are all potential aspects of fibroblast cell biology (reviewed in Baudino et al.\textsuperscript{44} Souders et al.\textsuperscript{65} and Snider et al.). Although cardiac fibroblasts are likely to perform these duties, it is unclear if they are the only cells capable of such feats. Certainly, the production of fibrillar collagens during development and disease is an accepted and documented fibroblast activity,\textsuperscript{66} but recent data suggest that pericytes and/or mesenchymal progenitors can also produce ECM components in response to injury.\textsuperscript{67,68}

Another example of a purported fibroblast role is insulation of the conduction system. Although a direct role for fibroblasts has not been proven, the best evidence supporting the idea that the annulus fibrosis buffers the myocardium from the atrioventricular node is the mechanical inhibition of epicardial migration in the avian heart.\textsuperscript{69} An epicardial origin for the cells of the annulus fibrosis has been determined, but other than expression of ECM genes, an insulating role for these cells has not been documented.\textsuperscript{70,71}

Given that in vivo data for the predominant roles of resting fibroblasts is lacking, more effort should be focused on the activities of these cells in non-pathological conditions. A revised understanding of the developing and resting cardiac fibroblast populations will further expand our knowledge of cellular processes assigned to fibroblasts.

**Cardiac Fibroblast Activation (Myofibroblast)**

Because cardiac fibrosis contributes to many forms of heart disease, much attention has focused on the behavior of activated fibroblasts (Figure). The first step in such studies involves the ability to identify the cell of interest. In the field of wound healing and cardiac fibrosis, the terms protomyofibroblast and myofibroblast are often used to indicate the subpopulations of fibroblasts that are responsible for tissue remodeling. The term “myofibroblast” was originally coined to describe a cell that had morphological characteristics of both smooth muscle cells and fibroblasts during skin wound healing.\textsuperscript{72}

The first mention of cardiac myofibroblasts was in the 1970s.\textsuperscript{73,74} These cells could be distinguished from resting cells by their morphology, including serrated nuclei, increased cytoplasm, microfilament bundles, and well-defined endoplasmic reticulum and Golgi complex.\textsuperscript{11,75} Later, skin myofibroblasts were documented to contract collagen in vitro and thus provide a unique and essential role in wound repair by providing tension.\textsuperscript{76} With the advent of an αSMA antibody permitting the identification of these microfilament bundles,\textsuperscript{77} myofibroblasts were found in other injured organs.\textsuperscript{78,79} Expression of the microfilament proteins, αSMA, transgelin, or caldesmon, became the gold standard for identifying myofibroblasts.\textsuperscript{30-32} Subsequent studies suggested that transforming growth factor-β (TGFβ) stimulation induced αSMA\textsuperscript{76} and because TGFβ also induces collagen production, it was suggested that αSMA could be used to identify collagen-producing cells after heart injury. As time passed, these changes in gene expression were considered a process of cell conversion or transdifferentiation into a new cell type.

Given the previous lack of markers and associated difficulty in identifying and studying the fibroblast in vivo, analyses were typically performed in vitro.\textsuperscript{49,83-85} Notably, these in vitro studies may not have appreciated the added mechanical stress caused by substrate stiffness in culture.\textsuperscript{49,86} Researchers observed that fibroblasts in culture fail to acquire quiescent features after stimulation removal, supporting the concept that myofibroblasts were a terminally differentiated cell type.\textsuperscript{86} These findings might be reconsidered in light of the fact that culture on plastic may alter cell physiology. Thus, stating that fibroblast activation is an irreversible differentiation process may not accurately describe the reversible change in gene expression that occurs in vivo.

Recent studies have identified transcription factors that are involved in the functions of activated cardiac fibroblasts. Two of these proteins are scleraxis,\textsuperscript{46,87,88} which is downstream of TGFβ signaling and involved in ECM synthesis, and myocardin-related transcription factors,\textsuperscript{89} which are involved in cytoskeletal changes and upregulation of αSMA expression during fibroblast activation. This information suggests that rather than being a differentiation process, changes in the gene expression of fibroblasts after cardiac injury are more likely to be a response to changes in growth factor signaling and an increase in tissue stiffness (reviewed by van Putten et al\textsuperscript{90}). Given recent findings, we would like to suggest a simplified nomenclature.

\begin{itemize}
  \item Cardiac fibroblasts respond to injury by producing components of the ECM.
  \item Additional roles of the cardiac fibroblast in uninjured hearts remain a mystery.
  \item Genetic tools have been used to characterize and validate fibroblast markers.
  \item Fibroblast-specific inducible Cre mouse lines provide unique opportunities for tracing and genetically manipulating fibroblasts.
  \item Fibroblasts play an important role in normal heart physiology.
  \item Developmental roles of cardiac fibroblasts are often deduced from in vitro studies.
  \item Matrix degradation, conduction system insulation, cardiomyocyte electrical coupling, vascular maintenance, and stress sensing are all potential aspects of fibroblast cell biology.
  \item Cardiac fibroblasts are likely to perform these duties.
  \item Insulation of the conduction system is an accepted and documented fibroblast activity.
  \item Expression of αSMA and microfilament bundles is often used to identify myofibroblasts.
  \item Transforming growth factor-β (TGFβ) stimulation induces αSMA.
  \item Myofibroblasts are terminally differentiated cells.
\end{itemize}
from myofibroblast to activated fibroblast. This would broaden the population of cells to investigate after injury and also to reflect the other dynamic changes in gene expression, such as proliferation, reactive oxygen species production and recruitment of inflammatory cells.20,26,33,41,45,91

**Alternative Cell Sources After Injury**

Contrary to the accepted developmental origin of the resting fibroblast, the origin of the activated fibroblast is historically much less clear and still debated. Because the activated fibroblast was considered a newly differentiated cell type, it was feasible that the cells responding to the injury could come from a variety of sources. Using lineage tracing and the limited tools available to study fibroblast biology, activated fibroblasts were described as differentiating from multiple cell types. Studies suggested that activated fibroblasts differentiated from either endothelial cells via endothelial-to-mesenchymal transition29 or infiltrating immune cells from bone marrow9,27,28. However, those studies relied on lineage tracing using Tie1-Cre and the FSP1-GFP mouse lines.92,93 The recent realization that some populations of fibroblasts derive from an endothelial progenitor could provide an alternative explanation for the presence of endothelial lineages within the fibroblast population. Additionally, FSP1-GFP expression has also been reported in immune and endothelial cells.36,44.

Recently, pericytes, mesenchymal cells associated with the microvasculature, have also been identified as a potential source of injury-induced matrix-producing cells. The ablation of Glia1-expressing pericytes resulted in a pronounced reduction in fibrosis, suggesting a role for pericytes in matrix production.59 Other studies focusing on αv integrin signaling also point to a role for signaling through pericytes in promoting fibrosis after heart injury.60 Although these studies implicate pericytes as an additional contributor to the fibrotic process, the mechanism of these actions remains unclear. For example, another study identified 2 populations of pericytes in the heart, type 1 and type 2. They found that type 1 pericytes expanded after myocardial infarction but did not express collagen type I.57 Intriguingly, the Glia1-expressing pericytes mentioned above comprise only a small portion of the perivascular cell population,67 suggesting that these cells may serve a role in regulating fibrosis rather than directly contributing to the act of ECM deposition.94 These initial studies indicate that more evidence is required before the direct and indirect functions of pericytes during cardiac fibrosis can be elucidated.

**Reversal of Activation**

Generally, it was believed that activated fibroblasts undergo apoptosis and disappear following the completion of tissue repair.55 For example, fibroblast apoptosis occurs via a TNFα-mediated response in skeletal muscle.96 In other organs, however, studies indicate that activated fibroblasts have the capacity to revert to a resting fibroblast as determined by reduction in αSMA expression.97-99 To study the fate of activated cardiac fibroblasts after injury, a reversible model of cardiac fibrosis was investigated. Angiotensin II and phenylephrine (AngII/PE) infusion cause rapid fibroblast activation, but upon drug cessation fibrosis recedes. The activated fibroblast lineage was marked using a mouse line, PeriostinCre, and the cells were followed over time. After 2 weeks the marked fibroblasts were still present, but gene expression had reverted back to a resting fibroblast profile.33 Interestingly, these reverted cells were more susceptible to re-activation, similar to a memory B- or T-cell response. This type of fibroblast reversion has also been observed in liver fibroblasts400,401 and supports the idea that activation is more a change in gene expression than a conversion of the fibroblast into another cell type.

**Cardiac Fibroblast Plasticity**

There is a current concept that fibroblasts are versatile and can interconvert readily into other cell types, but cellular reprogramming efforts have demonstrated that fibroblast reprogramming is often inefficient,102,103 suggesting that these cells may not be as plastic as previously believed. Many past experiments demonstrating fibroblast transdifferentiation were performed in vitro on minimally characterized cell populations.

Nonetheless, recent studies have documented the ability of fibroblasts to convert to other cell types, including adipocytes,104 cardiomyocytes,55,105,106 and endothelial cells.106 One example is the description of the cardiac fibroblast colony-forming unit (cCFU-F). A Scal+, PDGFRα+, CD31+ population of cells from a mouse heart was observed to have long-term culture capabilities and could differentiate into cardiomyocytes, endothelial cells, and smooth muscle cells.105 Although a similar differentiation capacity of heart resident PDGFRα-expressing cells was observed in a recent study, these cells were not identified as fibroblasts and were considered a resident stem cell population.55 Both of those studies relied on in vitro culture with subsequent transplantation to generate nascent cardiomyocytes. Although evidence for spontaneous conversion of fibroblasts to cardiomyocytes in vivo has not been observed, there are several examples of fibroblast to cardiomyocyte conversion with cellular reprogramming after injury.106-109 The efficiency of this conversion was less than 2%106 in the area of reprogramming and would need optimization for any practical application.

Although the ability of fibroblasts to differentiate into adipocytes has been shown in skeletal muscle,106,111 only recently has it been suggested that a cardiac fibroblast progenitor can differentiate into adipocytes.104 It is unclear if fibroblasts themselves can form adipocytes in vivo, but recent data do suggest that a subset of epicardial derivatives contribute to adipocytes that are present in the atrioventricular groove and epicardial fat.112,113 Finally, although the conversion of vascular endothelial cells into fibroblasts appears to be a minor contribution to fibrosis,25,26,33 lineage analysis using a Col1a2CreERT mouse line suggests that some fibroblasts may adopt the properties of endothelial cells after injury.106

**Conclusions**

Recent developments in tools to study fibroblast biology have enabled a more detailed and physiologic understanding of the fibroblast, as most original studies were limited in markers and to in vitro models (Table). Even though cardiac fibroblasts have 2 developmental origins, these populations respond similarly to cardiac injury and are the predominant fibroblast source. The term “myofibroblast” was initially used to distinguish between the fibroblast and a new cell type that arose during the fibrotic response. However, recent advances in fibroblast tools have allowed us to gain a better understanding of fibroblast activation, gene expression, and behavior. These findings suggest that an activated fibroblast arises from a tissue resident fibroblast and can revert back to a resting fibroblast. Although progress is evident in the study of fibroblast biology and fibrosis, there remain key questions to be answered regarding the role of the fibroblast in physiology and disease.

**Disclosures**

None.
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