This Review is part of a thematic series on **Cardiac Fibroblasts**, which includes the following articles:
Origin of Cardiac Fibroblasts and the Role of Periostin [2009;105:934–947]

Cardiac Fibroblast: The Renaissance Cell

Intramyocardial Fibroblast–Myocyte Communication
Cytokines Controlling Fibroblast Activation
Fate Mapping Cardiac Fibroblasts

Jeff Molkentin, Guest Editor

Cardiac Fibroblast
The Renaissance Cell

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***Abstract*—**The permanent cellular constituents of the heart include cardiac fibroblasts, myocytes, endothelial cells, and vascular smooth muscle cells. Previous studies have demonstrated that there are undulating changes in cardiac cell populations during embryonic development, through neonatal development and into the adult. Transient cell populations include lymphocytes, mast cells, and macrophages, which can interact with these permanent cell types to affect cardiac function. It has also been observed that there are marked differences in the makeup of the cardiac cell populations depending on the species, which may be important when examining myocardial remodeling. Current dogma states that the fibroblast makes up the largest cell population of the heart; however, this appears to vary for different species, especially mice. Cardiac fibroblasts play a critical role in maintaining normal cardiac function, as well as in cardiac remodeling during pathological conditions such as myocardial infarct and hypertension. These cells have numerous functions, including synthesis and deposition of extracellular matrix, cell–cell communication with myocytes, cell–cell signaling with other fibroblasts, as well as with endothelial cells. These contacts affect the electrophysiological properties, secretion of growth factors and cytokines, as well as potentiating blood vessel formation. Although a plethora of information is known about several of these processes, relatively little is understood about fibroblasts and their role in angiogenesis during development or cardiac remodeling. In this review, we provide insight into the various properties of cardiac fibroblasts that helps illustrate their importance in maintaining proper cardiac function, as well as their critical role in the remodeling heart. **(*Circ Res*. 2009;105:1164-1176.)**

Key Words: cardiac fibroblast ■ cardiac cell populations ■ cardiac remodeling ■ extracellular matrix ■ cytokines

Studies in the 1970s and 1980s from Zak and Nag defined and quantified the cellular populations of the adult rat left ventricle based on morphological characteristics using transmission electron microscopy of rat left ventricular sections, as well as gradient centrifugation.[1,2] These seminal studies on specific cardiac regions were further extended to the whole rat heart and suggested that the heart consists of approximately 70% nonmyocytes and 30% cardiac myocytes.[1–5] In addition, various studies using cell-specific markers have demonstrated that the myocardium exhibits distinct regional differences that are influenced by the specific physiological nature of the region.[3–9] These data defining the cardiac cell populations in the rat have effectively been applied to all species. However, over the past decade, the mouse model has become the standard organism to study the heart through the use of various transgenic, knockout, and surgical models. There are specific physiological differences between the rat and mouse, such as heart rate and total collagen.[10,11] Until recently, the cellular populations extrapolated from the early rat experiments had yet to be qualified in the mouse or any other species. During normal cardiac function, cellular components of the heart interact in a dynamic fashion to respond

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Non-standard Abbreviations and Acronyms	
Ang	angiotensin
Cx	connexin
DDR	discoidin domain receptor
ECM	extracellular matrix
EMT	epithelial–mesenchymal transformation
IL	interleukin
JAK-STAT	Janus kinases–signal transducers and activators of transcription
MMP	matrix metalloproteinases
RAS	renin–angiotensin system
TGF	transforming growth factor
TIMP	tissue inhibitor of metalloproteinases
TNF	tumor necrosis factor
VEGF	vascular endothelial growth factor

to changes in developmental, homeostatic, and pathological stimuli. The main cellular constituents of the heart include cardiac fibroblasts, myocytes, endothelial cells, and vascular smooth muscle cells, with the majority of cells consisting of fibroblasts and myocytes.[3–5] These cell types maintain the electric, chemical, and biomechanical responsive nature of the organ. Moreover, these cells help preserve the 3D structure via autocrine and paracrine action of secreted factors, as well as via direct cell–cell interactions.[3–7,9,12] Alterations in these signals or biomechanical input can cause deleterious, adaptive, and/or compensatory changes in the heart.

Gap junctions are essential in maintaining a normal heartbeat, and direct interactions between myocytes and cardiac fibroblasts occur via gap junctional connexins (Cx40, Cx43, and Cx45) to function in electrical conduction in the heart.[3,4,6,7,12] Indeed, Cx43 expression has been linked to arrhythmia and myocardial infarction size.[5,8,12–17] Moreover, abrogation of these interactions via remodeling can cause interference in this system leading to pathological conditions.[3,7,12,18] Additionally, connexins have been shown to play an important role in endothelial cell interactions and may also be important for cardiac fibroblast-endothelial cell interactions, as discussed later in this review.[19–21] In addition to connexins, it has also been demonstrated that cadherins play a critical role in cardiac development and function. Previous studies have shown that N-cadherin is important for cell–cell interactions, as well as myofibril organization in the heart.[22,23] It has also been demonstrated that cadherin-13, also known as T-cadherin, is important for vascular remodeling and may play a critical role in this process following myocardial infarct. Other studies have demonstrated that cytokines, such as interleukin (IL)-6 are critical in myocardial function and cardioprotection.[24–27] In vivo studies have shown that continuous activation of the gp130 receptor leads to myocardial hypertrophy.[25,28] Additionally, studies in rats have demonstrated that constant IL-6 receptor α stimulation can reduce infarct size and protect against apoptosis.[29] Better understanding of these signaling pathways in cardiac fibroblasts may provide insight into potential therapeutic targets for the treatment of the failing heart. In this review, we summarize current studies involving the cardiac fibroblast and its importance in physiological and pathological cardiac remodeling.

The Cardiac Fibroblast

What is a fibroblast? Fibroblasts are widely distributed connective tissue cells that are found in all vertebrate organisms. They are usually defined as cells of mesenchymal origin that produce a variety of extracellular matrix (ECM) components, including multiple collagens, as well as fibronectin.[30,31] In spite of this, synthesis and deposition of collagen are not often addressed in the identification of fibroblasts. This means that the definition of the fibroblast is based solely on morphological characteristics that can vary with location within the organism, as well as the overall activity level of the organism itself. Morphologically, fibroblasts are flat, spindle-shaped cells with multiple processes emanating from the main cell body. One characteristic of the fibroblast is that they lack a basement membrane, and it is one of the defining features that separate it from the other permanent cell types of the heart, all of which do contain a basement membrane.

Fibroblasts have typically been viewed as a uniform cell type with comparable functions regardless of whether they originate from the heart, skin, or other tissue. This reductionist view has been challenged by data demonstrating extensive phenotypic heterogeneity among fibroblasts from different tissues and from a particular tissue under different physiological conditions. Indeed, lung fibroblasts have been shown to be heterogeneous in cell surface marker expression, as well as in their levels of collagen production.[32] Moreover, periodontal fibroblasts also show heterogeneity based on morphology, glycogen pools, and collagen production.[32,33] Furthermore, recent studies comparing the gene expression patterns of 50 fibroblast cultures from multiple sites showed that their gene expression patterns were highly diverse.[40] This phenotypic plasticity has created a unique challenge in attempts to better define the cardiac fibroblast. Further investigation and characterization of these different fibroblast subpopulations could lead to strategies to inhibit or reverse fibrosis.

As mentioned above, fibroblasts lack a basement membrane and tend to display a prominent Golgi apparatus and extensive rough endoplasmic reticulum, especially when active. Although much research has been done examining the fibroblast, no truly definitive cell-specific marker has yet been defined. However, it has been recently shown that discoidin domain receptor (DDR)2 is expressed specifically by cardiac fibroblasts.[4,34] DDR2 is a cell surface receptor that mediates a wide range of cellular functions, including growth, migration, and differentiation. DDR2 is a collagen receptor that is expressed in cardiac fibroblasts but not myocytes, endothelial cells, or vascular smooth muscle cells.[4] DDR2 is also expressed on specific bone marrow–derived cells, termed fibrocytes, which are discussed in further detail below.[35] Another marker that has been proposed to be a fibroblast-specific marker is fibroblast-specific protein-1[36,37]; however, other studies in the literature have shown that fibroblast-specific protein-1 is also expressed in a variety of

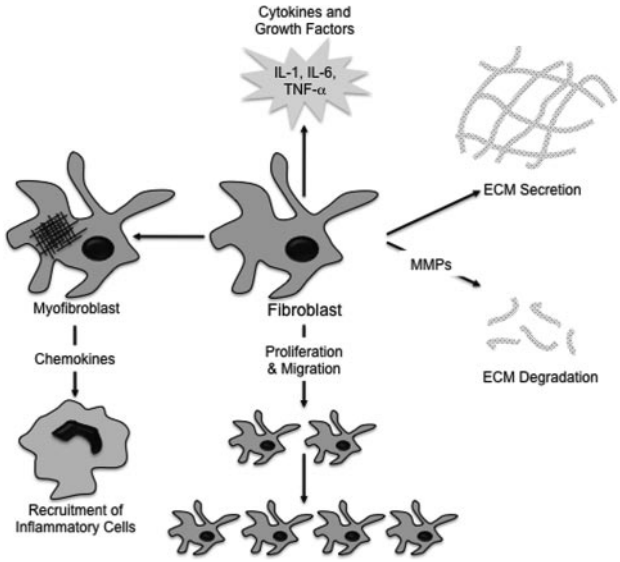

Figure 1. Roles of the cardiac fibroblast. The cardiac fibroblast responds to stimuli in various ways, including secretion of cytokines and growth factors, differentiation into a myofibroblast, proliferation and migration, and altering matrix generation and degradation.

other cell types, including leukocytes and a multitude of cancer cells.[38] It has also been shown that fibroblast-activation protein, a serine protease, is highly expressed on activated fibroblasts.[39,40] Recent studies from our laboratory and others have shown that cadherin-11 is localized to fibroblasts.[41,42] Moreover, work from Orlandini and Oliviero demonstrated that cell–cell interactions mediated by cadherin-11 lead to increased expression of vascular endothelial growth factor (VEGF)-D by fibroblasts.[43] Taken together, these studies suggest that cadherin-11 may be important for vascular remodeling following cardiac injury, such as myocardial infarction. In addition to these fibroblast markers, there are other genes that are highly expressed by cardiac fibroblasts including vimentin,[44,45] β1-integrin,[46,47] fibronectin,[48–50] connexins,[3,4,6,7,51] and the fasciclin gene periostin.[52–54] Further identification of definitive fibroblast-specific markers should assist in continued efforts to understand this dynamic cell type.

Cardiac fibroblasts play numerous roles in cardiac development and remodeling. They also play a prominent role in defining cardiac structure and function. Below, we discuss the numerous roles played by the cardiac fibroblast that help support and maintain proper cardiac function, as well as their role during cardiac pathology. Cardiac fibroblasts are both sources and targets of different stimuli, helping to coordinate chemical, mechanical, and electrical signals between the cellular and acellular components of the heart. The global roles of cardiac fibroblast function, such as proliferation, migration, myofibroblast differentiation, matrix generation and degradation and secretion of cytokines and growth factors are illustrated in Figure 1.

The Myofibroblast

Research over the past 20 years has demonstrated that there is phenotypic heterogeneity among fibroblasts and that some express features of smooth muscle differentiation. These smooth muscle–like cells were originally termed myofibroblasts by Gabbiani, who demonstrated that these cells take part in the growth, development, and repair of various tissues.[55,56] Under appropriate conditions, resting or quiescent fibroblasts can acquire an active, synthetic, contractile phenotype and express several smooth muscle cell markers that are not typically expressed in fibroblasts.[57] However, other smooth muscle cell markers, such as myosin heavy chain, are not expressed in these differentiated myofibroblasts. Additionally, myofibroblasts can be derived from bone marrow–derived cells or epithelial cells via epithelial–mesenchymal transformation (EMT). These cells express contractile proteins, are more mobile than "normal" fibroblasts, can contract collagen gels, and are thought to be important for wound closure and structural integrity of healing scars.[58] Indeed, research has demonstrated that myofibroblasts play a key role in reparative fibrosis in the infarcted heart.[59] In addition, myofibroblasts have been shown to be intimately associated with hypertrophic fibrotic scars in various injury models, and differentiation from fibroblast to myofibroblast is promoted by transforming growth factor (TGF)-β, cytokines, the ECM, and other growth factors.[60–63] Moreover, apoptosis of myofibroblasts has been linked in the progression of granulomatous tissue to a mature scar.[64] On the other hand, loss of apoptosis has been suggested to drive the progression toward fibrosis. With the exception of heart valve leaflets, myofibroblasts are not usually found in normal, healthy cardiac tissue. However, on injury, myofibroblasts appear in the myocardium and seem to arise from interstitial and adventitial fibroblasts. It is also possible that myofibroblasts originate from resident progenitor stem cells in the heart or hematopoietic stem cells from the circulation. Whatever their origin, recent studies suggest that the expression and secretion of growth factors, cytokines, ECM, and proteases by myofibroblasts is critical for tissue repair, fibrosis, and organogenesis.[65] When myofibroblasts are not properly regulated, destructive tissue remodeling can occur.[66,67] Other studies have demonstrated that fibroblasts cultured in vitro at low density will differentiate into myofibroblasts; however, it appears that these cells can be a transient phenotype because they can be reverted back to normal fibroblasts.[68] Indeed, it has been demonstrated that amniotic membrane stromal extract can reverse differentiated myofibroblasts back to fibroblasts in vitro.[69] Furthermore, it has also been observed that fibroblast growth factor can block or reverse the myofibroblast phenotype.[70] As we discuss below, myofibroblasts play a critical role in cardiac pathology and remodeling.

Origin of Cardiac Fibroblasts

Considerable heterogeneity in both morphology and function occur in fibroblasts during development. For these reasons, it is difficult to precisely define a fibroblast. To understand organogenesis, it is essential to know how, when, and where the cell comes from, as well as how the cells migrate to their location in the tissue. Depending on the stage of development, fibroblasts in the heart can arise from various sources (Figure 2).

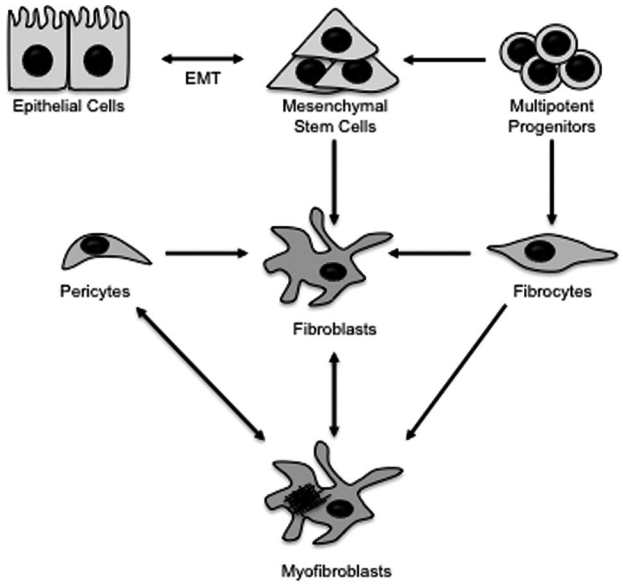

Figure 2. Sources of fibroblasts and myofibroblasts. The pathways leading to fibrosis involve considerable cell plasticity, with the profibrotic cells being derived from several sources. Epithelial cells can be transformed into mesenchymal cells and vice versa. Mesenchymal stem cells can differentiate into cardiac fibroblasts. Fibrocytes can also differentiate into fibroblasts or myofibroblasts. Finally, pericytes surrounding the vasculature can be differentiated into myofibroblasts.

During embryonic development, fibroblasts are mesenchymal in origin and appear to be closely involved in the formation of the heart. Fibroblasts are thought to arise from the differentiation of cells from the proepicardial organ. These epicardial-derived cells are considered to be one of the major sources of cardiac fibroblasts.[71,72] Additional studies have also demonstrated that fibroblasts can arise from mesoangioblasts. These multipotent progenitors have the ability to differentiate into either vascular (endothelial cells) or mesodermal (fibroblasts) tissues.[73] The origins of these progenitors in the bone marrow are hematopoietic stem cells. These cells express many lineage markers of vascular cells, such as CD34 and Flk-1 (VEGF-R2). These studies suggest that there is a progression from the mesoangioblast to endothelial cells and pericytes, which have recently been shown to have the potential to differentiate into myofibroblasts.[74,75] Indeed, numerous studies in the adult animal suggest that cells, such as pericytes and mesenchymal stem cells of the bone marrow, can contribute to the fibroblast population.[76,77]

In the neonatal and adult heart, fibroblasts arise from endogenous cell populations, via EMT and from bone marrow–derived cells. Rapid expansion of the heart occurs during fetal development and neonatal growth, with cardiac fibroblasts contributing ECM to several specific structures of the heart, including the valves, the atrioventricular node, the cardiac skeleton, and the endomysial network. Fibroblastic cells derived through EMT form the cardiac valves.[78] This complex transformation process results in fibroblastic cells that contribute to the formation of cardiac cushions and ultimately formation of the collagenous valve leaflets, including the valve interstitium. Originally, these valve fibroblasts were believed to be long-lived cells; however, recent evidence has demonstrated that interstitial fibroblasts can be replaced by bone marrow–derived cells.[79] It has also been shown that myofibroblast precursors are present in peripheral blood.[80] In the adult heart, cardiac fibroblast turnover is low, with the sources of replacement being endogenous fibroblast populations and those derived through EMT. However, under pathophysiological conditions, such as cardiac hypertrophy or myocardial infarction, the fibroblasts can arise from bone marrow–derived cells. These latter cells are sometimes termed fibrocytes.[81–83]

Fibrocytes are typically defined by their growth characteristics and cell surface phenotype. These cells have many similarities to fibroblasts, in that they constitutively express collagen, but they also express cell surface markers indicative of leukocytes and hematopoietic progenitor cells.[84–89] These cells also express other defining cell surface markers, including chemokine receptors and adhesion molecules.[77,90] Fibrocytes have been shown to deposit ECM during wound healing and fibrosis, as well as functioning in the immune response.[81–83] Taken together, these findings support the fact that hematopoietic stem cells and epicardial-derived cells are major sources of new fibroblasts in the adult cardiac valves, as well as other regions of the heart.

Organization of Cardiac Fibroblasts in the Heart

It has been stated that the adult heart consists of 30% myocytes and 70% nonmyocytes.[1,2] However, recent studies using flow cytometry have demonstrated that the adult murine heart consists of approximately 45% nonmyocytes and 55% myocytes.[91] In addition, these studies confirmed that the adult rat heart consists of 30% myocytes and 70% nonmyocytes as demonstrated by Zak and Nag.[91] These data suggest that the exact cellular makeup of the heart can vary dramatically from species to species. These differences in cardiac cell populations can lead to differences in ECM content as well.

The 3D collagen network of the heart starts to form during fetal development and is primarily laid down during neonatal development. Within this connective tissue network, lie the cardiac fibroblasts. During the formation of this 3D network, myocyte–collagen attachments are made involving integrins.[92] Fibroblasts within the endomysial collagen network then surround the groups of myocytes in a lamellar function (Figure 3).[4,34,93] The cardiac fibroblasts contain interconnected cellular processes that form a network of cells within the collagen network. This arrangement of fibroblasts within the network allows the fibroblasts to contract the endomysial collagen, exerting mechanical force on the myocytes. In addition, this organization allows for the fibroblasts to help maintain structural integrity of the heart through cell–cell and cell–ECM interactions, as well as through proliferation and ECM degradation and synthesis.[4,64,94,95] In doing so, the fibroblasts are able to respond to a number of stimuli including chemical, mechanical and electrical signals. It is through these dynamic interactions that fibroblasts are able to maintain proper form and function of the heart.[96–99] Changes in any of these stimuli by the fibroblasts can also affect the

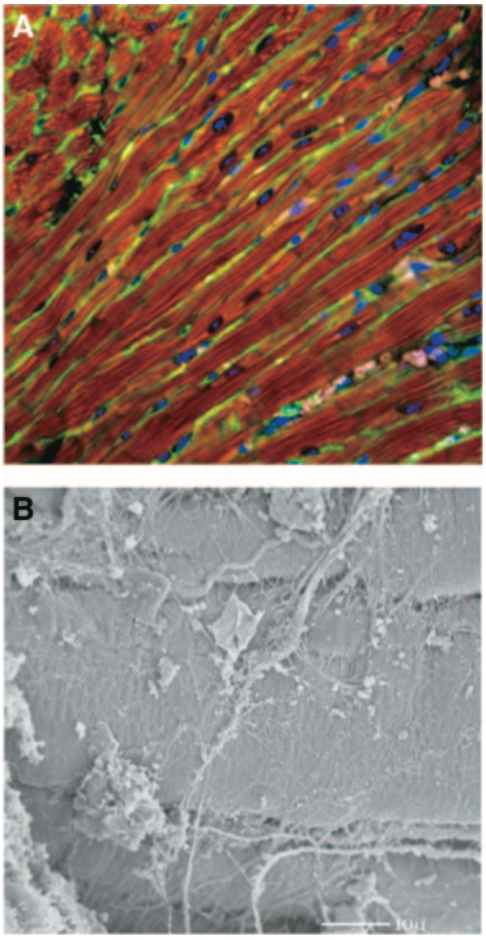

Figure 3. Organization and interactions of fibroblasts and myocytes in the heart. A, Confocal micrograph of adult rat heart stained with phalloidin (red), DAPI (blue), and wheat germ agglutinin (green). The fibroblasts lie within the endomysial collagen in the extracellular space as previously shown. B, Transmission electron micrograph showing cardiac myocytes in contact with a cardiac fibroblast. The cardiac fibroblasts are in contact with collagen, other fibroblasts, and myocytes forming a mechanosensitive 3D arrangement.

function of other cardiac cell types, such as myocytes and endothelial cells.

Cardiac fibroblasts interact with the extracellular matrix through integrins and DDR2, whereas intercellular connections appear to be through 2 families of cell surface proteins, connexins and cadherins. Cx40, Cx43, and Cx45 have all been shown to be involved in these interactions and to provide electrical contact.[96–99] The connexin that connects fibroblasts to myocytes (heterotypic) is Cx43, and the connexin that connects fibroblasts to other fibroblasts (homotypic), Cx45, although the distribution of these connexins can vary depending on species.[4,6,100] Besides connexins, other cell surface molecules that appear to play a role in forming cell–cell contacts are members of the cadherin family. Previous studies have demonstrated that cadherin-11 is highly expressed in fibroblasts and is associated with VEGF-D expression.[41,43] In addition, Western blot analyses have shown that cadherin-11 appears to be specifically expressed in cardiac fibroblasts in the mouse left ventricle (data not shown). Moreover, cadherin-13 has been shown to play a role in vascular remodeling, and may be important following myocardial infarction.[101] Furthermore, it has been demonstrated in vitro that N-cadherin is involved in myocyte-fibroblast and fibroblast–fibroblast interactions.[96] From these studies, it is clear that the fibroblast plays an essential role in chemical, mechanical, and electrical signaling in the heart, and disruption of these signaling pathways can lead to cardiac dysfunction.

Cardiac Fibroblasts and the ECM

Cardiac fibroblasts have been termed "sentinel" cells, because they can sense changes in chemical, mechanical, and electrical signals in the heart and mount the appropriate response. However, one of the primary functions of the cardiac fibroblast is the synthesis and degradation of the ECM to provide a 3D network for myocytes and other cells of the heart to ensure proper cardiac form and function. The ECM, which consists of the acellular components of the heart, includes interstitial collagens, proteoglycans, glycoproteins, cytokines, growth factors, matrikines, and proteases.[102–105] The extracellular matrix serves multiple purposes, because it forms an organizational network that surrounds and interconnects cells and provides a scaffold for cardiac cell populations. In addition, the ECM helps to distribute mechanical forces throughout the myocardium, convey mechanical signals to individual cells via cell surface ECM receptors, and participate in fluid movement in the extracellular environment.[102,103] The role of individual components present within the ECM is highly complicated and, in many cases, has been difficult to define. Proteoglycans and glycoproteins appear to play important roles in various functions of the ECM, including signaling and turnover of the ECM itself. Various growth factors and proteases are often bound as latent factors to the proteoglycans and glycoproteins.[106–108] Cytokines and growth factors are essentially short-range chemical signals that are critical for response to pathological stimuli. Extracellular proteases are part of a biochemical cascade within the ECM that is essential for turnover of ECM components, activation of latent factors and cardiac remodeling and are discussed in detail below.

Cardiac Fibroblasts and Remodeling

Remodeling is broadly defined as changes in the organization of the myocardium and is a critical process that allows the heart to adapt to changes in mechanical, chemical and electrical signals.[109–111] Cardiac fibroblasts are key components of this process because of their ability to secrete and breakdown the ECM. Degradation of collagen requires the presence of matrix metalloproteinases (MMPs).[112,113] In the normal heart, MMP expression and function are tightly regulated; however, in pathological states, MMP expression and activity are increased, leading to excessive ECM degradation, which can have profound effects on cardiac function. Following cardiac injury, fibroblast function can be influenced by chemical signals (ie, cytokines, matrikines and growth factors) in a paracrine or autocrine manner. These factors can cause changes in fibroblast gene expression, as well as cell migration to the injured region to promote wound healing and scar formation.

Depending on the stage of heart failure, there can be considerable myocyte hypertrophy and cell death. Dilatation can also be observed in later stages; however, present at every stage are changes in the ECM, which are regulated by cardiac fibroblasts. There is also activation and differentiation of cardiac fibroblasts into myofibroblasts.[64,94,114] After maturation to myofibroblasts, an increase in the synthesis and secretion of fibronectin is observed.[115] As the heart undergoes remodeling associated with heart failure, an increase in cytokine and growth factor secretion is observed. In response to these various factors, myofibroblasts begin to proliferate, migrate and remodel the cardiac interstitium through increased secretion of MMPs and collagen.[64,94,116,117] To further stimulate the remodeling process, cardiac fibroblasts secrete increased amounts of growth factors and cytokines, specifically IL-1β, IL-6, and tumor necrosis factor (TNF)-α, which, in turn, activate MMPs, leading to further cardiac remodeling.[64,104,118] Initially, all of these changes are critical to the reparative wound healing response; however, over time, these changes become maladaptive leading to fibrosis and reduced cardiac function.

Although not present in normal myocardium, myofibroblasts are highly localized to sites of injury where synthesis and deposition of collagen promotes scar formation and fibrosis.[119] In addition, these cells are also located near or associated with blood vessels. Because myofibroblasts express contractile proteins, such as smooth muscle actin, they are able to provide mechanical tension to the remodeling matrix, helping to close the wound and reduce scarring.[58,65,115] As the scar matures, cells in the scar undergo apoptosis, leaving a scar that consists mainly of collagen and ECM proteins, but myofibroblasts are still present.[120] Indeed, myofibroblasts have been observed in mature scars in a rat model of myocardial infarct, as well as in scarred human tissue.[119,121] Why myofibroblasts persist is unknown, but they are highly involved in regulating cardiac remodeling, cardiac dysfunction, and ultimately cardiac failure.

It has been observed that valve fibroblasts can have an hematopoietic stem cell origin.[79] It has also been demonstrated that fibrocytes can enter wounds, be detected in scar tissue, and possibly participate with local fibroblasts in wound repair and pathological fibrosis. These studies help provide the foundation that different progenitor populations can possibly be harnessed and used as therapeutic agents in the treatment of cardiovascular disease.

In the normal heart, collagen and other ECM components help maintain heart structure and function. ECM is synthesized and degraded by cardiac fibroblasts in a coordinated fashion; however, during heart failure there is disruption of these regulatory pathways, leading to an imbalance of ECM synthesis and degradation that determines the level of cardiac remodeling. Increases in the extracellular matrix or fibrosis may be reparative, replacing areas of myocyte loss with a structural scar, or reactive, involving increases in ECM deposition at sites other than those of the primary injury. Fibrosis has significant consequences for cardiac function, because increases in ECM synthesis and deposition results in increased mechanical stiffness and contributes to diastolic dysfunction. Progressive increases in fibrosis can lead to systolic dysfunction and left ventricular hypertrophy. Moreover, increased levels of collagen can disrupt electrophysiological communication between myocytes. Furthermore, perivascular fibrosis around intracoronary vessels impairs oxygen and nutrient availability and intensifies myocyte ischemia. Heart failure is characterized by considerable differences in levels of disease severity and progression. This most likely reflects polygenic and environmental influences on the level and severity of heart disease in a patient-to-patient manner. Therefore, it is possible that cardiac fibroblasts and their role in cardiac remodeling act as disease modifiers and can potentially be used as predictive risk factors in heart failure.

Cardiac Fibroblasts and Chemical and Mechanical Signaling

Cardiac fibroblasts respond to a wide range of different stimuli during cardiac development and disease, including hypoxia, as well as changes in chemical and mechanical signals. Many of these stimuli function as activators of cardiac fibroblasts; however, chronic stimuli can lead to pathological remodeling and reduced cardiac output. During normal cardiac function, fibroblasts are constantly subject to mechanical stretch. Proper regulation of these mechanical signals is essential to maintaining normal cardiac function.[103,122] Various studies have shown that mechanical stimulation of fibroblasts results in a marked upregulation of ECM components, ECM-specific receptors, as well as increased expression of various cytokines and growth factors.[123–128] In addition, mechanical stretch can induce MMP expression in cardiac fibroblasts, leading to ECM degradation. Moreover, cytokine and growth factor expression has been shown to be upregulated in cardiac fibroblasts in response to mechanical stretch.[129–131] Furthermore, angiotensin II (Ang II), TGF-β, and endothelin can regulate ECM synthesis and deposition by cardiac fibroblasts.[94,132–134] These studies imply that mechanical load plays a critical role in the regulation of cardiac fibroblast gene expression. However, the response of cardiac fibroblasts to mechanical stimulation in tissue culture appears to be dependent on growth factor stimulation.[124,135,136] These observed differences between in vitro and in vivo studies may be attributable to improper cell–cell and/or cell–ECM contacts in vitro. Better in vitro models, as well as future in vivo studies, should help to define the interplay between mechanical and chemical signaling in cardiac fibroblasts.

A major function of cardiac fibroblasts is to produce and secrete growth factors, cytokines, and other signaling molecules. These signaling factors can have effects on all cardiac cell types. The specific signaling factors that are secreted by the cardiac fibroblasts largely depends on the stimuli. These stimuli may be chemical factors, such as pro- or antiinflammatory cytokines and growth factors, electrical signals, hypoxia, or mechanical stretch. In patients with heart failure, high levels of circulating proinflammatory (IL-1β, IL-6, and TNF-α) and profibrotic (TGF-β) cytokines are observed.[137] Because of the different expression levels and the complexity of the various factors involved, it is difficult to delineate the exact effects of individual factors on heart failure.

As mentioned above, cardiac fibroblasts are the main source of the proinflammatory cytokine IL-1β in the heart following injury.[138] Expression of IL-1β can be induced via several different pathways, including hypoxia and TNF-α, whereas its expression can be inhibited by estrogen.[138,139] IL-1β, acting through its receptor (IL-1R) inhibits cardiac fibroblast proliferation, while promoting cell migration.[140–144] Moreover, it also promotes turnover of the ECM through reduced collagen type I and III synthesis and increased secretion of MMPs.[145,146] Furthermore, IL-1β can also induce IL-6 expression, as well as angiotensin type 1 receptor expression in neonatal rat cardiac fibroblasts.[147]

Previous studies have demonstrated a tight connection between plasma TNF-α levels and the progression of left ventricular remodeling and heart failure.[148] Additional studies in animals demonstrated that infusion of TNF-α resulted in cardiac dysfunction and heart failure.[149] Moreover, it has been shown that TNF-α increases expression of IL-1β and IL-6 in cardiac fibroblasts in vitro.[150] These data further demonstrate the multiple levels of gene regulation that occur in the cardiac fibroblast and how this regulation can affect cardiac function.

IL-6 is a pleiotropic cytokine that is responsible for numerous processes, such as regulation of cell growth, apoptosis, differentiation, and survival in various cell types and organs, including the heart. Recent studies have indicated that IL-6 is a critical component in cell–cell interactions that occur between myocytes and cardiac fibroblasts.[151] The biological activity of IL-6 is regulated by binding to the IL-6 receptor α/gp130 signal transduction complex and subsequent activation of several signal transduction pathways.[152,153] Data from several studies suggests that IL-6 and the gp130-JAK-STAT signaling pathway are important in cardiac function and cardioprotection.[24–27] Indeed, cardiac hypertrophy results in a dramatic increase in the expression of IL-6.[154–156] Other studies have shown that continuous activation of gp130 leads to myocardial hypertrophy.[28] Furthermore, constant IL-6/soluble IL-6 receptor α stimulation can reduce infarct size and protect against apoptosis.[29] One mechanism through which IL-6 acts is by inducing proliferation of cardiac fibroblasts and altering ECM turnover.[145] IL-6 can be produced by both cardiac fibroblasts and myocytes[157]; however, the majority of IL-6 appears to be secreted by cardiac fibroblasts, but both cell types can respond to IL-6 stimulation.[151] As mentioned above, IL-1β can induce IL-6 expression, but it can also be regulated by other factors including TNF-α and Ang II.[139,158]

Ang II is the effector molecule of the renin–angiotensin system (RAS) that is important for regulating blood pressure and volume. In the circulatory RAS, renin cleaves angiotensinogen into Ang I, which is further processed into Ang II by angiotensin-converting enzyme. Recently, it has been demonstrated that a local RAS exists in the heart and plays an key role in cardiac function.[159,160] Cardiac fibroblasts can express angiotensinogen, renin and angiotensin-converting enzyme, allowing them to effectively produce Ang II.[161] Moreover, it has been shown that Ang II can increase collagen production and secretion from cardiac fibroblasts, which occurs through the angiotensin type 1 receptor.[162,163] These studies suggest that the intracellular RAS is a critical component of the heart and normal cardiac function and open up alternative avenues for therapeutic intervention.

Another cytokine that is typically upregulated during heart failure is TGF-β, which acts through interactions with its cell surface receptors, with cardiac fibroblasts being the primary producers.[164] TGF-β is involved in cell proliferation, differentiation, migration, apoptosis and ECM production. Stimulation of cardiac fibroblasts by TGF-β results in increased synthesis of fibrillar collagen, fibronectin, proteoglycans, and expression of contractile genes, leading to differentiation into myofibroblasts.[165–169] Additionally, there are conflicting reports of whether TGF-β acts in pro- or antiproliferative manner, suggesting multiple roles for TGF-β in the heart.[68,170–172] Better understanding of cytokine and growth factor regulation and signaling in the future may provide potential therapeutic targets in heart failure patients.

Cardiac Fibroblasts and Electrical Signaling

Recent studies have demonstrated that fibroblasts have other critical functions other than ECM synthesis, deposition, and remodeling.[173] Cardiac fibroblasts have a high membrane resistance, which makes them good conductors. Cell junctions, such as through connexins, are important for cellular communications in other organ systems and likely play similar roles in physical communication between fibroblasts and other cells within the myocardium.[174] Indeed, it has been demonstrated in vitro, through Cx43, that electrical coupling of myocytes and cardiac fibroblasts can occur.[8,96–98] In addition, it has also been shown that fibroblast-myocyte coupling can occur, via Cx45, in the sinoatrial node.[96–98] Studies from Louault et al also demonstrated function coupling of cardiac fibroblasts through Cx40 and Cx43.[99] These studies suggest that cardiac fibroblasts could act as bridges that connect different regions of myocytes that would normally be electrically isolated by connective tissue. Moreover, in vitro cell–cell interaction assays have demonstrated that cardiac fibroblasts and myocytes communicate through the formation of tight cell–cell junctions (Figure 4).[175] Additionally, ion channels also play an intriguing and important method of signaling because abnormalities in these channels can lead to cardiac dysfunction.[176] It has been demonstrated that K^+ channels are activated by contact between cardiac fibroblasts and myocytes.[177] Activation of K^+ channels has also been linked to Ang II sensitivity.[178] Furthermore, recent studies have shown that cardiac fibroblasts express several different voltage-gated K^+ channels.[179] Both of these types of signals can have profound implications on cardiac repair and remodeling.

Cardiac Fibroblasts and Angiogenesis

Formation of blood vessels depends on environmental cues that modulate endothelial cell function.[180,181] During wound healing, various angiogenic factors cooperate to assemble and stabilize endothelial cells into blood vessels. It has been demonstrated by several laboratories that fibroblasts play a critical role in the wound healing process.[182–184] In addition, we have recently observed a tight association between cardiac fibroblasts and endothelial cells both in vitro and in vivo,

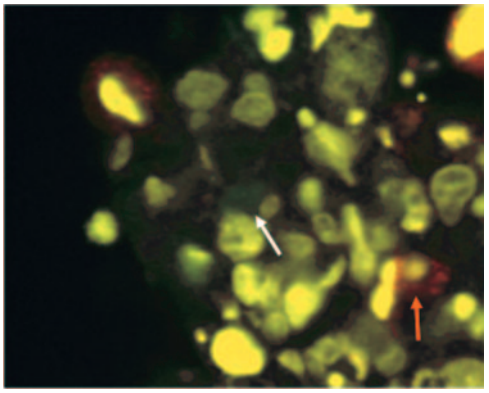

Figure 4. Cell communication between cardiac fibroblasts and myocytes. Z-section of a cell aggregate containing cardiac fibroblasts that were dual loaded with Lucifer yellow and chloromethyl rhodamine-acetylated and myocytes that were unloaded. Cardiac fibroblasts appear as yellow or orange cells. The orange cells are fibroblasts that have transferred their green dye to an adjacent cell (orange arrow). Note the green cells, which are myocytes that have received green dye from an adjacent fibroblast (white arrow).

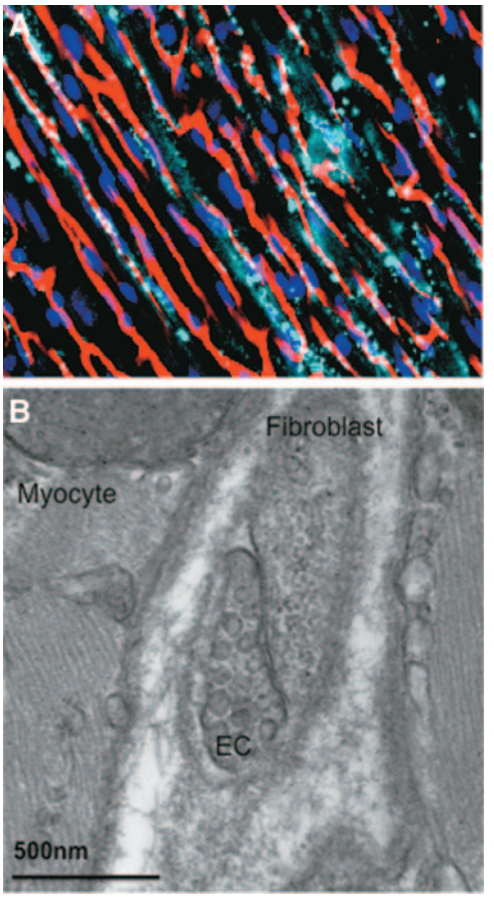

Figure 5. Cardiac fibroblasts and endothelial cells are intimately associated with one another. A, Confocal micrograph demonstrating tight association of fibroblasts and endothelial cells (ECs) in the coronary vasculature. Mice were perfused with fluorescent microspheres (red), hearts collected, and stained with DAPI (blue) to visualize nuclei and DDR2 to visualize fibroblasts (teal). Note the tight association between the fibroblasts and the vasculature. White spots are costaining of microspheres and DDR2. B, Transmission electron microscopy imaging of adult mouse hearts illustrates the close relationship between fibroblasts and ECs. Note the pinocytotic vesicles present in the ECs. Cells in such close proximity have several avenues by which they communicate, including gap junctions and the ECM.

suggesting that fibroblasts are important for blood vessel formation during development and potentially disease (Figure 5). Therefore, understanding the interactions between endothelial cells and fibroblasts is essential for understanding how angiogenesis is regulated.

As mentioned, fibroblasts play a role in the development, growth, and remodeling of tissues. They do so through synthesis and deposition of ECM, cytokines, and growth factors, which allow them to modulate their environment through autocrine and paracrine signaling. Fibroblast involvement in blood vessel formation was first reported several years ago[185,186]; however, their exact role in the angiogenic process remains unresolved. Fibroblast growth factors are potent inducers of angiogenesis. In addition to the cytokines listed above, fibroblasts can also secrete fibroblast growth factor and VEGF.[187] Both fibroblast growth factor and VEGF act on vascular endothelial cells and are important for stimulating angiogenesis and coronary collateral formation for restoring the blood supply to injured myocardium. It is possible that chemokines regulate the fibrotic process through recruitment and activation of fibroblast progenitors, fibrocytes, by exerting direct effects on resident cardiac fibroblasts and regulating angiogenesis. In addition, it has been demonstrated that pigment epithelium-derived growth factor can be expressed by both cardiac fibroblasts and myocytes. Pigment epithelium-derived growth factor was shown to inhibit VEGF-induced endothelial cultures in vitro.[188] Taken together, these studies indicate that cardiac fibroblasts can express both pro- and antiangiogenic factors and that proper regulation of these factors is critical to vascular development and remodeling. To date, few studies have directly investigated how fibroblasts contribute to blood vessel formation.

Other factors that have been shown to play a role in angiogenesis are also produced by fibroblasts, such as MMPs and tissue inhibitors of metalloproteinases (TIMPs).[66] MMPs can regulate endothelial cell proliferation, adhesion, and survival leading to activation or inhibition of angiogenesis. MMPs act by degrading the ECM, which can promote endothelial cell migration and sprouting, or MMPs can cleave and release antiangiogenic factors that inhibit these events.[189,190] On the other hand, TIMPs have been shown to inhibit angiogenesis,[191,192] as well as promote vessel formation.[193,194] Thus, like MMPs, it appears that the function of TIMPs is context-dependent. Indeed, recent studies from the Lilly group demonstrated that TIMP-1 is secreted by fibroblasts and increases vessel formation.[195] In these studies, they observed that the activities of TIMP-1 were MMP-dependent. In addition, they observed that direct interaction between fibroblasts and endothelial cells is necessary for optimal vessel formation.[195] These studies further underline the significance of fibroblast-derived factors and cell–cell interactions in regulating angiogenesis. Future studies should be aimed at defining the cell surface molecules that play a role in these cell–cell interactions.

Conclusions

Recent studies have helped identify, quantify, and map the various cell lineages that are involved in cardiac development, maintenance, and disease. Dynamic interactions between the various cell types, the ECM and the biochemical factors that are present in the heart are essential for proper form and function of the heart. Despite recent progress, our present understanding of the how and where cardiac fibroblasts arise from is still relatively unclear. Identification of better cellular markers with absolute specificity for fibroblasts will help in understanding these lingering lineage issues. In addition, identification of cell-specific markers defining cardiac fibroblast progenitor cells (ie, mesenchymal stem cells, fibrocytes, pericytes) would prove invaluable for the isolation and further characterization of these cells for potential therapeutic applications.

The ECM is also a crucial component in trying to understand the dynamic nature of the heart. The role of the extracellular matrix in mechanical signaling is well known, but how the ECM functions in chemical signaling is relatively unknown. Future studies will involve more quantitative approaches to examine interactions between the fibroblasts and other cardiac cell types (myocytes and endothelial cells), as well as the interactions of these cells with the ECM.

The unique features of cardiac fibroblasts make it an appealing target for reducing pathological remodeling. Recent studies demonstrating the importance of the fibroblast to blood vessel formation opens up additional avenues for cardiac therapies. Moreover, studies have demonstrated that the heart does have some intrinsic reparative responses ranging from recruitment of progenitor cells from the bone marrow to the injured myocardium, as well as fibroblast and pericyte differentiation into myofibroblasts at the site of injury. A better understanding of the underlying mechanisms of these processes holds the key to successful therapy of the injured heart.

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Disclosures

None.

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