Cardiac fibroblasts: friend or foe?

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THE VERTEBRATE MYOCARDIUM is composed of cardiac myocytes, nonmyocytes, and the surrounding ECM. The organization and relative proportions of these myocardial components play critical roles in heart development and function. In addition, it is clear that interactions between the diverse components of the myocardium impact cardiac development, physiology, and pathology. Most investigations in myocardial development and disease have focused on the cardiac myocytes, and much has been learned regarding the molecular, biochemical, and cellular levels of regulation of this important cell type (40, 127). However, nonmyocyte cell types in the myocardium, including fibroblasts, endothelial cells, mast cells, and others, have received far less attention. Although cardiac myocytes make up the bulk of the myocardial volume, the cardiac fibroblasts are the most numerous cell type in the heart and, as discussed below, play essential roles in myocardial function. The emphasis on the myocyte has relegated the role of fibroblasts to below, play essential roles in myocardial function. The emphasis on the myocyte has relegated the role of fibroblasts to

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More recent studies now document that fibroblasts have much broader functions in the myocardium. These studies have suggested a “sentinel” role for fibroblasts that is intimately associated with the global myocardial response to mechanical, electrical, and chemical signals. Cardiac function, whether developmental, normal growth, or in response to pathophysiological signals, involves the integration of these diverse signals (23, 61, 119). A variety of studies, including those with transgenic models, indicates that there is structural and functional interdependency among the various cell types where a defect in one cell type (altered transgene expression, for instance) has consequences for the other cell types of the heart. Because of their potential role in the regulation of global myocardial function, fibroblasts represent an attractive therapeutic target in heart disease (25). In this review we will discuss the classical role of fibroblasts in the synthesis and remodeling of the ECM, as well as more novel roles in orchestrating the myocardial response to changes in mechanical, chemical, and electrical signals.

DEFINING THE CARDIAC FIBROBLAST

Fibroblasts are widely distributed in vertebrate organisms and are found associated with various forms of connective tissue. They are traditionally defined as cells of mesenchymal origin that produce interstitial collagen (51, 85). However, collagen synthesis and deposition are not usually addressed (i.e., in situ hybridization or immunocytochemical localization) in the context of fibroblast identification. This means that the definition of the fibroblast is based on morphological characteristics that can widely vary with location and metabolic activity within the individual cell, within the organism, as well as the overall activity level of the organism itself.

Fibroblasts tend to lack a basement membrane and display multiple processes or sheet-like extensions. These cells contain an oval nucleus, extensive rough endoplasmic reticulum, a prominent Golgi apparatus, and abundant cytoplasmic granular material. More recently, it has been demonstrated that the discoidin domain receptor 2 (DDR2) is expressed specifically by fibroblasts within the heart (30, 62). DDR1 and DDR2 are cell surface receptors that mediate a variety of cell functions, including growth, migration, morphological changes, and differentiation. DDR2 is a collagen receptor that is expressed in fibroblasts but not endothelial cells, smooth muscle cells, or cardiac myocytes (30). Identification of specific molecular markers of fibroblasts should assist in continued efforts to understand this dynamic myocardial cell.

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Fibroblasts have classically been viewed as a uniform cell type with equivalent functions regardless of the tissue of origin. This view has been challenged by data illustrating extensive phenotypic heterogeneity among fibroblasts from different tissues and from a given tissue under different physiological conditions. This relative phenotypic plasticity has presented a hurdle in attempts to define the cardiac fibroblast. Under appropriate stimulation, relatively quiescent fibroblasts can acquire an active synthetic, contractile phenotype and express several smooth muscle cell markers not typical of fibroblasts (152). However, other smooth muscle markers are not expressed in these differentiated myofibroblasts (i.e., smooth muscle myosin heavy chains). These cells express contractile proteins, are more mobile than undifferentiated fibroblasts, can effectively contract collagen gels, and are believed to be important for wound closure and structural integrity of healing scars (148). Moreover, myofibroblasts are associated with hypertrophic fibrotic scars in various injury models, and differentiation to the myofibroblast is strongly promoted by transforming growth factor-β (TGF-β). Apoptosis of the myofibroblast has been shown to be associated with the progression of granulomatous tissue to a mature scar. On the other hand, failure of apoptosis has been suggested to drive the progression to fibrosis. With the exception of heart valve leaflets, myofibroblasts are not normally found in healthy cardiac tissue. However, upon injury, myofibroblasts appear in the myocardium and are believed to arise from resident interstitial and adventitial fibroblasts. They may also originate from progenitor stem cells in the heart or from the circulation. Whatever their origin, recent data suggest that the production of growth factors, cytokines, ECM proteins, and proteases by myofibroblasts is critical for tissue repair, fibrosis, and organogenesis (24).

ORIGIN OF FIBROBLASTS

Most references (112) cite that the heart is composed of ~70% nonmyocytes and 30% myocytes. These studies have largely depended on histological examination of heart tissue and not on specific cell markers. This results in fibroblasts being defined as cells of mesenchymal origin that produce collagen. This observation has never been confirmed by cell sorting or other modern techniques of quantification of cell number. Nonetheless, fibroblasts account for a large proportion of myocardial cells; however, their origin has been relatively obscure.

Fibroblasts in the heart are thought to arise from various sources at different stages of development. In the embryonic heart, fibroblasts are thought to arise from the differentiation of cells from the proepicardial organ and via epithelial-mesenchymal transformation of endocardial cushion cells (16). In fetal animals, fibroblasts also arise from mesangioblasts. Cossu and Bianco (41) have defined the mesangioblasts as a progenitor of mesodermal tissues. These multipotent progenitors have the ability to differentiate into a variety of vascular and mesodermal tissues (41). The term mesangioblasts means that these bone marrow-derived cells have the ability to be a common progenitor of either the endothelial cells or mesodermal cells (fibroblasts). The origin of these progenitors in the bone marrow is the hematopoietic stem cell. The understanding of lineage is further complicated by the lack of specific lineage markers, which results in a wide variety of phenotypic descriptions.

Several studies (12, 137) have illustrated that increased fibroblast density correlates to myocardial fibrosis during development and disease. It is not clear whether increased fibroblast density is due to proliferation of existing populations of cells or to recruitment of progenitor cells. Investigations using H3 to mark dividing cell populations indicated that only some fibroblasts were labeled (11). Most data indicated that the label was highest near the vessels. These data have been interpreted to mean that only some myocardial fibroblasts have the ability to divide. A variety of studies in the adult suggests that progenitor cells, such as pericytes, adventitial cells associated with the vasculature, and mesenchymal stem cells of the bone marrow, can contribute to the interstitial fibroblast population (130, 149). However, few studies have examined the actual question of fibroblast lineage, proliferation, and turnover.

In studies on collagen deposition in tumors, the collagen-producing cells appeared to arise from the pericytes associated with the vasculature. In response to systemic signals from PDGF, pericytes migrated away from the vessel wall and into the interstitial space whereupon they began producing collagen (149). Recent studies (130, 149) have indicated that cells associated with the intima of vessels, as well as pericytes, have the ability to form fibroblasts or smooth muscle cells. In addition, it has been shown that mononuclear cells derived from the bone marrow have the ability to contribute this intimal population (109). These data suggest that fibroblasts can arise from a stem cell population of the bone marrow.

Organization of cellular components of hearts. The heart is organized as a complex arrangement of both cellular and acellular components. The myocytes are arranged in layers as originally described by Streeter (146) and more recently as laminae (164). These laminae are organized into layers of myocytes two to five cells thick and surrounded by an endomysial collagen network. Fibroblasts form an interconnected network of cells that lies within the endomysial collagen network that surrounds the groups (lamellae) of myocytes (18, 30, 62, 164). This arrangement of fibroblasts in vivo, with interconnected cellular processes forming a network of cells within the collagen network, is analogous to the organization of fibroblasts in a collagen gel. This in vivo arrangement of fibroblasts within the collagen network can allow the fibroblasts to contract the endomysial collagen, exerting force on the myocytes. Changes in fibroblast contractility have been suggested to impact myocardial relaxation. Mast cells also lie in the endomysium but appear to be near the vasculature; however, it is not known whether they contact fibroblasts or other cell types (77). The distribution of endothelial cells and smooth muscle cells is confined to the vasculature (Fig. 1).

Importantly, the intercellular connections of fibroblasts appear to be via at least two different types of cell-cell molecules: connexins and cadherins. In mice and rats, the connexins that connect fibroblasts to myocytes are connexin43 (Cx43) and, to other fibroblasts, Cx45, although the distribution of these connexins may vary in different species (30–32). Cadherin-11 appears to be a specific fibroblast cadherin and has been associated with VEGF-D signaling (114, 156).
FIBROBLASTS AND THE EXTRACELLULAR MATRIX

The acellular components of the heart, referred to collectively as the ECM, include interstitial collagens, proteoglycans, glycoproteins, cytokines, growth factors, and proteases (40, 73). These components form an elaborate three-dimensional (3-D) network (Figs. 1 and 2) that is intimately associated with cardiac myocyte structure and function (2). Collectively, the ECM 1) forms an organizational network that surrounds and interconnects cellular structures, 2) provides a scaffold for the myocyte and nonmyocyte populations of cells, 3) distributes mechanical forces throughout the myocardium, 4) transmits mechanical signals to individual cells via cell surface ECM receptors (mechanotransduction), and 5) participates in fluid movement in the extracellular environment (73). The role of individual components of the ECM is complex and in many cases has been difficult to ascertain. The proteoglycans and glycoproteins appear to be important in various functions of the ECM, including signaling and turnover of the ECM itself. Growth factors and proteases are often found bound as latent factors to the proteoglycans and glycoproteins. Cytokines and growth factors are essentially short-range chemical signals that are critical for response to 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 remodeling. The action of these proteases serves to also produce small peptides termed “matrikines” that are important in signaling biological processes (102, 108, 136).

The organization, composition, and density of the ECM are dynamic, and all impact myocardial development and function. The ECM is qualitatively similar but quantitatively different within various regions of the heart (12). For example, the arrangement of collagen is different in the atria compared with the ventricles, yet in both locations it consists of interstitial collagen types I and III. Similar differences in the distribution of noncollagenous ECM components are also likely. The 3-D organization of the ECM has a significant role in cardiac function, including myocyte alignment, blood flow during systolic contraction, compliance, and diastolic stiffness (17).

The density of the ECM is also an important factor that is intimately tied to cardiac function (100, 161). Collagen expression and accumulation are increased in a number of cardiovascular diseases (38, 160). The density of the ECM affects...
are involved in more than ECM deposition and remodeling. In addition, recent studies (79, 101) have documented that density of the collagen affects the availability of cell receptors and substrates. Density of the ECM also affects the retention of other ECM components, such as proteoglycans. These charged molecules can bind factors that contribute to the concentration of inflammatory components forming chronic inflammation.

Fluid movement in the ECM is important to dilute secreted factors, to facilitate movement of metabolites to the lymphatic system, and to provide lubrication between layers during contraction. Fluid movement affects the microcirculation and is related to ECM composition, interstitial pressure, and tissue oncotic pressure, all of which are important in the regulation of edema (70). In addition, fibroblasts may exert a mechanical force by contraction of the ECM that could affect fluid movement.

Remodeling is generally defined as changes in the geometry of the myocardium and is an essential process that allows the heart to adapt to changes in mechanical, chemical, and electrical signals (23, 37, 145). This is a complex process involving all of the components of the heart, cellular and acellular. Remodeling is a normal process associated with heart growth, especially during the maturation of the heart from the neonatal period to adulthood. When the heart undergoes hypertrophy or dilation, remodeling must again occur. If the modulating signals become pathological, then the process of remodeling becomes detrimental to cardiac function (104, 122, 150). Typically, myocardial remodeling involves changes in the amount and organization of ECM components. These changes in the ECM are correlated with altered physiological parameters of cardiac function. Although much is known concerning the end result of remodeling, comparatively little is known concerning the events that trigger this process. Most studies on remodeling have focused on the myocyte with little emphasis on the other cell types. Few studies have documented the dynamic interaction of ECM, myocytes, and nonmyocyte populations.

Increases in the ECM or fibrosis may be reparative, replacing areas of myocyte loss with a structural scar, or reactive, involving increases in ECM deposition at sites distinct from the focal injury. Fibrosis has important functional consequences for the heart. First, increases in ECM deposition results in mechanical stiffness and contributes to diastolic dysfunction. Progressive increases in fibrosis can cause systolic dysfunction and left ventricular hypertrophy. Additionally, increased collagen levels disrupt electronic communication between myocytes. Moreover, perivascular fibrosis around intracoronary arterioles impairs myocyte oxygen availability and exacerbates myocyte ischemia. Heart failure is characterized by substantial differences in levels of disease severity and progression, even within comparable heart failure etiologies. This most likely reflects polygenic and environmental influences in the heart disease phenotypes in a patient-to-patient manner. Thus it may be that cardiac fibroblasts and their fibrotic remodeling act as disease modifiers and can be used as predictive risk factors in heart failure.

**SENTINEL FUNCTION OF CARDIAC FIBROBLASTS**

Recent investigations (25, 47) have shown that fibroblasts are involved in more than ECM deposition and remodeling. These studies (142, 144) have termed fibroblasts “sentinel cells” and have proposed that these cells play important roles in the myocardial response to chemical and mechanical signals. This role for fibroblasts is based on several lines of evidence. Fibroblasts are intimately associated with the myocardial endomysial network. Thus mechanical forces are rapidly transmitted to the fibroblasts via the ECM. Fibroblasts themselves form a highly organized network within the myocardium (Fig. 2). This is in contrast to classical views of fibroblasts residing in relative isolation within the ECM of connective tissues. In this network, fibroblasts are interconnected with each other and with other cell types, notably cardiomyocytes, via a variety of cell-cell contacts. Cell junctions, such as cadherins and connexins, are important in cellular communications in other systems and are likely to play similar roles in physical communication between fibroblasts and other cells within the myocardium (5). Ion channels also represent a particularly intriguing and important mechanism of signaling because abnormalities in these channels can have very negative effects on cardiac function (139). Recent studies (39) have documented that K+ channels are activated by contact between fibroblasts and myocytes. The activation of the K+ channel has also been associated with ANG II sensitivity (45). In other studies, electrical coupling of myocytes and fibroblasts have been demonstrated both in vivo and in vitro (48). This type of signaling has been shown to have profound implications on repair and remodeling (117).

Several features of the fibroblasts have been described that suggest an important role in the detection and response of the myocardium to changes in the mechanochemical environment: 1) their close association with the ECM, which allows for rapid detection of mechanical, electrical, and chemical events; 2) their organization in an interconnected, 3-D network that is associated with myocytes and other cell types; and 3) their expression and secretion of proinflammatory cytokines in response to myocardial stress (26, 47, 55). These features of the cardiac fibroblasts allow these cells to detect changes in the mechanical, electrical, or biochemical environment and to transmit these changes throughout the myocardium via physical interactions with other cells or via secretion of cytokines.

Despite these intriguing observations, few studies have been performed to test the functional role of fibroblasts as sentinel cells. This is partly due to the fact that in vivo analysis of cellular function in the heart is often compromised by the complexity of this system; therefore, reductionist cell culture models have been used. However, recent studies in divergent fields have shown that normal, planar (2-dimensional) culture systems are also phenotypically and functionally problematic. Culture systems (3-D), such as collagen gels, have demonstrated that these models are more physiologically relevant of the in vivo condition and may be useful in characterizing the dynamic interaction of a variety of factors (44, 60, 65). Cultures (3-D) of myocytes on aligned collagen show an in vivo-like phenotype and are suitable for biochemical experimentation (115, 143). Investigators have also used microfluidic models to pattern the dynamic interaction of myocytes and fibroblasts (34, 129) where mechanical-electrical interactions have been analyzed (34, 60, 150). These approaches are believed to be more physiologically relevant and should provide important insight into the novel functions of fibroblasts.
MECHANICAL SIGNALING

The detection and response to mechanical signals are critical to overall cardiac function (73, 150). Although much is known concerning mechanical signaling of myocytes, comparatively little is understood about fibroblasts or other cell types. Several studies (163) have demonstrated that mechanical stimulation of fibroblasts results in upregulation of ECM components, their specific receptors, as well as the expression of cytokines and growth factors. Interestingly, mechanical deformation of fibroblasts can cause membrane depolarization leading to a concept of mechano-electrical transduction (50, 81, 163).

As discussed above, the ECM plays an essential role in cardiovascular function, and changes in the ECM accompany and contribute to myocardial dysfunction. A number of biochemical factors, including ANG-II, TGF-β, IGFs, and others, regulate ECM production by cardiac fibroblasts (22, 52, 63). Mechanical forces also modulate ECM expression in cardiac fibroblasts (29, 35, 74, 98). Increased cardiovascular load in vivo leads to a rapid increase in collagen mRNA expression (36, 38). Studies (27) with left ventricular assist devices, which generate a chronic reduction in cardiovascular load, illustrate a significant reduction in myocardial collagen content. These studies suggest that mechanical load is important in the regulation of cardiac fibroblast gene expression; however, human and whole animal studies cannot be performed in the isolation of changes in the biochemical milieu. The response of cardiac fibroblasts to mechanical stimulation in vitro appears to be dependent on the presence of growth factor stimulation (8, 13, 29); however, few studies have examined the interactions between these diverse extrinsic factors.

Mechanical stretch of cardiomyocytes induces the release of ANG II, which stimulates the release of growth factors and cytokines from cardiac fibroblasts. These factors exert their effects on both fibroblasts and other cell types, including cardiomyocytes. Although the paracrine factors released by cardiac fibroblasts are required for induction of the cardiomyocyte hypertrophic response, the paracrine activities of cardiomyocytes are also important for regulating the function of cardiac fibroblasts. One such example was demonstrated using chimeric mice that expressed both AT1a receptor-positive and AT1a receptor-null cells (125). Infusion of ANG II caused cardiac hypertrophy and fibrosis in the chimeric mice. Most importantly, the majority of the proliferating fibroblasts were found to be surrounding cardiomyocytes carrying the wild-type AT1a gene. Fibroblasts adjacent to AT1a-null myocytes displayed a lower proliferative index. Consistent with these data, chimeric mice carrying cells overexpressing Gsa protein and wild-type cells displayed clustered fibrosis and hypertrophy in areas containing myocytes overexpressing Gsa (157). Taken together, these data demonstrate the importance of local communication between fibroblasts and myocytes, presumably via a paracrine mechanism.

Mechanical stimulation initiates a complex series of events, including activation of signal transduction pathways, reprogramming of gene expression, and alterations in cellular processes such as proliferation and apoptosis. Strong evidence has emerged identifying a critical role for integrins in initiating the response of cells to mechanical stretch (53, 75, 87, 103). Intense investigations are currently underway to elucidate the molecular mechanisms whereby physical signals transmitted via integrins from the extracellular environment are transduced into biochemical signals within the cytoplasm. Mechanical stimulation activates several signaling networks, including mitogen-activated protein (MAP) kinases, phosphatidylinositol 3-kinase, JAK/STAT, and other pathways. Because integrins themselves do not possess enzymatic activity, their transduction of mechanical signals must depend on interactions with other proteins. It has been postulated that mechanical signals can be transmitted through the physical linkage between the ECM, integrins, and cytoskeletal components (7, 75, 76). Alternatively, a number of enzymatic signaling proteins have been associated with integrins, including focal adhesion kinase (FAK) and Rho family small GTPases. Integrin activation by mechanical stimulation initiates a cascade of phosphorylation events of these associated proteins that intersects with the above-mentioned “classical” pathways.

Focal adhesion kinase. FAK is a nonreceptor tyrosine kinase that was originally identified by its rapid adhesion-induced phosphorylation (96). Autophosphorylation of FAK is a critical early event in the promotion of integrin-induced proliferation and cell motility (134). FAK autophosphorylation promotes the interaction with Src homology 2 homology proteins Src and Fyn and subsequent formation of a signaling complex that initiates changes in gene expression and cell function (132, 133). Recent studies (10, 54, 49) have shown that the expression and activation of FAK and proline-rich tyrosine kinase 2 (PYK2) are enhanced in response to increased myocardial load in vivo. FAK is an important mediator in the transduction of mechanical signals in a number of cell types (110, 140, 158). For instance, load-induced MAP kinase activation and hypertrophy of cardiomyocytes are dependent on FAK activation and downstream Src signaling (4, 153). More recently, FAK and PYK2 have been implicated as downstream targets associated with ANG II-stimulated cell migration and proliferation (111, 131). The fact that FAK is activated both by integrin-mediated mechanical stimulation and growth factor receptor activation suggests that this protein may provide a key integration site between diverse signaling networks (121).

Rho family GTPases. To date, at least 20 proteins have been identified that contain the Rho small GTPase domain characteristic of this protein family. There are several major groups of Rho GTPases, including the Rho-like, Rac-like, and Cdc42-like subfamilies. These proteins have long been known to regulate actin polymerization/dem polymerization necessary for cell-shape changes and motility (58, 68, 107). It is now clear that the functional roles of the Rho GTPases extend to many cellular activities, including proliferation, gene expression, and differentiation.

The Rho GTPases cycle between GDP-bound inactive and GTP-bound active forms. The activation of Rho GTPases is regulated by several associated proteins, including guanine nucleotide exchange factors, GTPase-activating proteins, and guanine dissociation inhibitors. Guanine nucleotide exchange factors catalyze the exchange of GDP for GTP, resulting in GTPase activation. GTPase-activating proteins accelerate Rho GTPase hydrolysis of its GTP-bound state, returning it to its GDP-bound inactive state. Guanine dissociation inhibitors bind GDP-bound GTPase to modulate targeting of Rho-GDP to the cell membrane. The specificity between different Rho GTPases can be regulated at multiple steps, including the activation process and downstream signaling targets.
The Rho family small GTPases play a central role in mediating ANG II- and stretch-induced cardiomyocyte hypertrophy (3, 6, 159). The role of these proteins in cardiac fibroblast signaling has not been extensively studied. Recent data illustrate the expression and function of Rho A and Rac 1 in rat cardiac fibroblasts (56, 65, 66). With the use of a 3-D collagen gel model, these studies showed that the activation of specific Rho GTPases is dependent on the mechanical and biochemical stimuli applied to cardiac fibroblasts. These studies and others suggest that, similar to FAK, the Rho GTPases occupy critical integration sites between various signaling networks in cells.

CHEMICAL SIGNALING

A number of extrinsic factors, including growth factors, cytokines, hormones, and mechanical stretch, affect gene expression and behavior (proliferation, migration, etc.) of fibroblasts and may be promoting load-induced fibrosis. In many cases, there is wide discrepancy in the literature regarding the profibrotic or antifibrotic effects of specific factors. It is possible that some of these discrepancies can be attributed to differences in experimental approaches (doses of factors used, etc.). It is equally likely that the developmental, physiological, or pathological background of fibroblasts modulates the responsiveness to specific factors (28, 48). Recent studies (105, 106) have illustrated that proinflammatory cytokines promote an adaptive response initiated by myocardial stress. After prolonged exposure to these cytokines, this response becomes maladaptive. These studies highlight the dynamic nature of myocardial remodeling in general and fibroblast regulation in particular. They also illustrate the necessity for careful temporal examination of alterations in fibroblast behavior and gene expression during remodeling processes.

Differentiation into cardiac fibroblasts is regulated by various growth factors, including basic (bFGF) and PDGF (162). In addition, phenotypic diversity among fibroblasts is demonstrated from studies examining cellular responses to experimental stimuli in vitro. For example, one study used fibroblasts isolated from skin, lung, liver, and heart and compared their cellular responses to various cytokines, such as IL-1, TNF-α, IFN-γ, and IL-6 (25, 26). The endpoints they examined were fibroblast proliferation, chemotaxis, and ECM metabolism. There were marked changes in responses to cytokines among fibroblasts from different tissues and organs. IL-1 appears to be the most robust and multipotent agonist in cardiac fibroblasts compared with other tissues. Furthermore, studies (20, 78, 113) using kidney, lung, and skin suggest that differences can be identified among fibroblasts even within a single tissue. These data suggest that fibroblasts exist with specialized functions toward specific endpoints, such as proliferation, migration, and/or ECM metabolism.

Fibroblasts are highly sensitive to circulating hormones, which affect their proliferative response to pathological stimuli (21, 64). Studies (14) using histone H3, a marker of cell proliferation, demonstrated that only a small fraction of fibroblasts located near blood vessels contains the marker. These data suggest that tissue fibrosis may involve only a small percentage of fibroblasts that are rapidly proliferating. It is possible that the perivascular fibroblast population in the heart stems from circulating progenitors (2). Indeed, it has been shown that mesoangioblasts can act as progenitors to mesodermal tissues. These multipotent cells have the ability to differentiate into both vascular and mesodermal tissues, including endothelial cells and fibroblasts (41). Another progenitor population may lie in the intima of vascular walls. These progenitor cells have the ability to become fibroblasts, smooth muscle cells, and endothelial cells (130, 149). Therefore, the origin of proliferating cardiac fibroblasts in pathological situations is still under intense investigation.

Fibroblasts are involved in the maintenance of myocardial structure, including ECM homeostasis and production of growth factors, cytokines, and matrix metalloproteinases (MMPs) involved in maintaining a balance between synthesis and degradation of connective tissue components. In cardiovascular disease, fibroblasts play a critical role in myocardial remodeling, which includes cardiomyocyte hypertrophy, migration and proliferation of fibroblasts, and alterations in the deposition and composition of the ECM. Excessive fibroblast proliferation and increases in ECM protein result in fibrosis and subsequent myocardial stiffening leading to cardiac dysfunction (160, 165). Fibrotic tissue remodeling is associated with increased expression of MMPs and factors such as TGF-β, ANG-II, endothelin-1, and TNF-α. By degrading interstitial fibrillar collagen, MMPs play a key role in the ECM remodeling process after myocardial infarction (46, 47, 151).

In the early stages of myocardial repair, MMPs disrupt the collagen ECM network, enhancing inflammatory cell infiltration. This leads to increased production of cytokines and subsequent fibroblast migration, proliferation, and differentiation, followed by deposition of ECM and scar formation. Increased expression and activation of MMPs lead to excessive ECM degradation and impaired healing. MMPs have also been shown to be involved in the cell growth, migration, cell survival, and angiogenesis (42).

Hormones and growth factors. ANG-II, TGF-β, and TNF-α have been shown to be involved in autocrine and paracrine regulation of myocyte hypertrophy, fibroblast proliferation, and ECM protein turnover and degradation. Myocardial remodeling may also be promoted by chronic adrenergic stimulation, which is also an important feature of heart failure. This has been shown to affect myocyte hypertrophy and cell death, as well as leading to increased proliferation of cultured cardiac fibroblasts via secretion of autocrine factors. Surprisingly, statins have been shown to directly inhibit fibroblast proliferation, an effect that may contribute to the prevention of adverse cardiac remodeling. Thus fibroblast activity can play critical and unexpected roles in the structure and function of the heart. Furthermore, given their susceptibility to a wide array of humoral factors, they are a novel therapeutic target for pharmacological interventions.

Factors that affect the phenotype and function of cardiac fibroblasts include ANG II, bFGF, TGF-β, and IGF-1 (15, 19). ANG-II appears to be the most important factor regulating cardiac fibrosis and remodeling, although other factors are clearly important. Indeed, ANG II may activate different signaling cascades in cardiac fibroblasts than in myocytes. ANG II can be activated the ERK pathway, including the Gβγ subunit of G1 and Ras and Raf, whereas G1 and PKC were shown to be important in cardiomyocytes (19). It is possible that these differences in signal activation may lead to the differential activation of genes in these two cell types. In vitro studies using cardiac fibroblasts have shown that ANG II
directly stimulates fibroblast proliferation, collagen production, and the expression of other ECM proteins, such as fibronectin and laminin via AT$_1$ receptors. ANG II also regulates cardiac fibroblast function indirectly through induction of TGF-$\beta$, endothelin-1, and IL-6, all of which function in autocrine and paracrine manners (19, 57, 89, 128). Indeed, recent studies suggest that this indirect effect may be the major mechanism through which ANG II regulates cardiac fibroblasts.

TGF-$\beta$ participates in the regulation of cell proliferation, migration, differentiation, apoptosis, and ECM production. Moreover, it plays a central role in the development of fibrosis. It can stimulate fibroblast chemotaxis and the production of collagen while inhibiting collagen degradation (16, 43). It can also induce expression of $\alpha$-smooth muscle actin in fibroblasts and is thus considered one of the factors responsible for myofibroblast transformation (152). In the heart, TGF-$\beta$ is induced by pressure overload and infarct, as well as by infusion of ANG II. It has been demonstrated that cardiac-specific overexpression of TGF-$\beta$ results in cardiac hypertrophy and fibrosis (120). Furthermore, neutralization of TGF-$\beta$ by antibodies resulted in reduced fibrosis in the pressure of overloaded rats. TGF-$\beta$ activates multiple pathways, but the Smad pathway is believed to be the predominant one. Smads can either activate or repress target genes depending on the binding partners with which they are associated (97). Alterations in Smad expression have been associated with heart disease.

Injury to the myocardium activates a large number of transcription factors. These factors, particularly early factors, are activated in many cell types and induce the expression of genes generally required for stress responses. Microarray analysis has identified numerous transcriptional changes associated with cardiac hypertrophy, myocardial infarction, cardiomyopathy, and heart failure (104). Yet further studies, such as those examining promoter structures and protein interactions, as well as expression profiling, would provide valuable data with which to further analyze the network of signaling and transcriptional regulation.

Activator protein-1 (AP-1) is involved in numerous cellular functions. It generally functions to control cell proliferation and apoptosis (138). AP-1 proteins include homodimers and heterodimers composed of basic region leucine zipper proteins, which include c-jun, c-fos, and jun dimerization partners and activating transcription factors. In cardiac fibroblasts, mechanical stretch and ANG II have been shown to activate AP-1. ANG II directly induces c-fos, c-jun, and JunB expression in cardiac fibroblasts. Furthermore, AP-1 binding sites have been identified in the promoter regions of numerous genes, including transcription factors, ECM proteins, MMPs, cell adhesion molecules, growth factors, cytokines, and cyclins (9).

Early growth response-1 (Egr-1) is a zinc-finger transcription factor that has been shown to be rapidly and transiently activated by growth factors and injurious stimuli (88). In cardiac fibroblasts, Egr-1 is induced by ANG II, EGF, and IGF-1. It has been shown to control genes important for cell growth, as well as those involved in inflammatory responses. In cardiac fibroblasts, Egr-1 has been shown to regulate the expression of PDGF-A, and in other cells, it is involved in the regulation of PDGF-B, bFGF, TNF-$\beta$, tissue factor, plasminogen activator, ICAM-1, and NF-$\kappa$B (88, 141). NF-$\kappa$B is a redox-sensitive transcription factor that controls genes involved in inflammation and apoptosis. It can mediate cell survival or apoptosis depending on the cell type and environment. NF-$\kappa$B target genes include proinflammatory cytokines, MMPs, and nitric oxide synthase, as well as antiapoptotic genes (124, 155).

JAK and STAT proteins are generally found coupled with cytokine receptors. However, recent studies have demonstrated that JAK and STAT proteins are also involved with the PDGF receptor and AT$_1$ receptor. Upon ligand binding, STAT proteins are phosphorylated by JAKs, resulting in translocation to the nucleus where they function as transcription factors (135). The role of STAT3 in heart disease was clearly demonstrated in studies using transgenic and knockout mice. Continuous activation of gp130 signaling (resulting in STAT3 activation) resulted in cardiac hypertrophy, whereas cardiac-specific gp130 loss caused massive apoptosis of cardiomyocytes and dilated cardiomyopathy. Moreover, STAT3 can activate transcription of ANG II, and it has been further demonstrated that ANG II can induce expression of IL-6 in cardiac fibroblasts, which in turn can activate the JAK/STAT pathway in adjacent cardiomyocytes (71, 154). Indeed, we have seen increases in IL-6 expression upon coculturing of cardiac fibroblasts and myocytes (data not shown).

**ELECTROPHYSIOLOGICAL SIGNALING**

Many of the essential functional roles of fibroblasts involve their ability to exhibit excitation-secretion coupling and, therefore, must involve their fundamental cellular electrophysiological properties. However, relatively little is known about the ionic basis of J membrane potential or 2) myocyte-fibroblast or fibroblast-fibroblast electrotonic interactions. The original conventional intracellular microelectrode recordings of membrane potential in atrial cardiac fibroblasts in situ reported resting membrane potentials of $-22 \pm 1.9$ mV (80 – 82). This “resting” membrane potential in mammalian atrial tissue was found to hyperpolarize during atrial relaxation and to depolarize during contraction (83, 84, 91, 92, 95). No regenerative action potentials were identified. These oscillations in membrane potential, termed “mechanically induced potentials,” could be blocked by a 40 $\mu$M gadolinium (80 – 84, 95). Moreover, they appeared to be associated with oscillations in intracellular calcium (91). Subsequent work (81) has suggested a link between these electrotocnic changes and actin and tubulin polymerization.

More recent quantitative studies (82) using patch-clamp methods applied to freshly dissociated atrial fibroblasts provided evidence for a gadolinium-sensitive, nonselective cation conductance that was enhanced by cell compression and inhibited by stretch. The zero current or resting potentials averaged $-37 \pm 3$ mV in these atrial fibroblasts. In this preparation, no expression of voltage-gated Ca$^{2+}$ or K$^+$ currents was identified (82), and no gap junctions were detected by electron microscopy either between fibroblast pairs or between fibroblasts and myocytes. Moreover, injection of fluorescent markers ( Lucifer yellow) into fibroblasts showed no dye intercellular movement from the cell injected. These findings suggest that fibroblasts in the rat atrium do not communicate by intercellular connections. In contrast, recent findings (31, 93) in rabbit sinoatrial node have convincingly demonstrated punctate expression of Cx40 and Cx45. These findings suggest that fibroblasts are coupled...
to myocytes by Cx45 (93, 94). In these recent experiments, dye coupling was also demonstrated both among fibroblasts and between fibroblasts and myocytes in rabbit sinoatrial node (31) and among fibroblasts in rabbit ventricle (32).

Rook et al. (118) have studied the electrophysiological properties of primary cocultures of fibroblasts and myocytes isolated from the hearts of neonatal rats. These fibroblasts, after being seeded and maintained in culture, were not excitable (i.e., applied stimuli failed to initiate action potentials). Their current-voltage relationship showed outward rectification. When these fibroblasts were in physical contact with myocytes within a coculture experiment, their membrane potential hyperpolarized, approximating that of the attached myocytes (−60 to −80 mV). In these fibroblasts, action potential-like responses were negative to those of the myocyte, but with much slower kinetics, could be recorded. In contrast, in isolated pairs of fibroblasts, membrane potential was −20 to −40 mV, and no action potentials were recorded. Detailed biophysical analysis of intercellular coupling revealed a voltage-insensitive conductance, measuring ~21.3 pS between fibroblast pairs and a 32-pS conductance between coupled fibroblasts and myocytes. Staining for Cx43 yielded negative results for both fibroblast-fibroblast and fibroblast-myocyte pairs. In contrast, the more recent work of Gaudesius et al. (60) has reported consistent, punctuate staining for both Cx43 and Cx45 among fibroblasts and between fibroblasts and myocytes in cocultures of neonatal rat heart cells. This study made use of micropatterning methods combined with cell culture, so that groups of cultured myocytes were separated by “inserts” of cardiac fibroblasts. Under these conditions, functional electrical (electrotonic) coupling was observed (60). However, action potential conduction failed when the fibroblast “inserts” exceeded ~400 microns in length. There was no evidence for intrinsic electrical excitability in these cultured neonatal ventricular fibroblasts (60). Neither of these studies attempted to determine the ionic basis for membrane potential or for the electrical properties observed in cultured ventricular fibroblasts.

The ion channels have been described for myofibroblasts and for other organs, e.g., hepatic perisinusoidal stellate cells (123).

Recent studies (39) suggest that modulation of extracellular [K+] results in changes in myofibroblast function. Previous studies (126) have shown that intermediate conductance Ca2+-sensitive K+ channel (IKCa), mRNA, and protein expression increased in rat hearts after myocardial infarction. The increased expression of IKCa appeared localized to myofibroblasts that infiltrated the infarcted area and was correlated with vascular remodeling and perivascular fibrosis (126). In fibroblasts from other tissues, IKCa activity has also been shown to modulate fibroblast phenotype, tending to stabilize them in a proliferative state and to prevent phenotypic switching to a contractile phenotype (116).

The ability of changes in the membrane potential to alter contractile activity of liver myofibroblasts (activated hepatic stellate cell) has also been studied previously. A similarity to ventricular myofibroblasts was that inwardly rectifying K+ currents were recorded from hepatic stellate cells (86). Resting membrane potential in hepatic stellate cells was −81 ± 5 mV (n = 7), suggesting that KIR strongly modulated resting membrane potential (86). Transient outward K+ currents were also recorded in hepatic stellate cells (86).

CONCLUSIONS AND SIGNIFICANCE

Cardiac function is determined by the collective and dynamic interaction of various cell types and the extracellular matrix that composes the heart. As a consequence of this interaction being dynamic, they will likely have significant differences that are dependent on differences in the stage of development and the degree and duration of mechanical, chemical, and electrical signals between the various cell types and the ECM. Understanding how these complex signals interact at the molecular, cellular, and organ levels remains to be a challenge to investigators. Clearly, the cardiac fibroblast plays an important role in the signaling by its ability to respond to a wide variety of chemical signals that are involved in the paracrine and autocrine regulation of cardiac function.

The ECM, which is made primarily by the fibroblasts, is also a key component of understanding the dynamic nature of cardiac function. Its role in mechanical signaling is well established, but how the ECM may function in chemical signaling is poorly understood. The degradation and turnover of both the ECM and fibroblasts within the ECM are important in normal homeostasis as well as in response to pathophysiological signals.

Future studies will require the use of more quantitative approaches to the interaction of fibroblasts and myocytes and the interaction of these cells with the ECM. Both in vitro and in vivo studies will have to recognize the dynamic nature of the mechanical, chemical, and electrical signaling that is critical to cardiac function.

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