Annu. Rev. Pharmacol. Toxicol. 2005. 45:657–87
doi: 10.1146/annurev.pharmtox.45.120403.095802

THE CARDIAC FIBROBLAST: Therapeutic Target in Myocardial Remodeling and Failure

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Key Words fibrosis, myocardial infarction, heart failure, risk factors, clinical trials

■ **Abstract** Cardiac fibroblasts play a central role in the maintenance of extracellular matrix in the normal heart and as mediators of inflammatory and fibrotic myocardial remodeling in the injured and failing heart. In this review, we evaluate the cardiac fibroblast as a therapeutic target in heart disease. Unique features of cardiac fibroblast cell biology are discussed in relation to normal and pathophysiological cardiac function. The contribution of cardiac fibrosis as an independent risk factor in the outcome of heart failure is considered. Candidate drug therapies that derive benefit from actions on cardiac fibroblasts are summarized, including inhibitors of angiotensin-aldosterone systems, endothelin receptor antagonists, statins, anticytokine therapies, matrix metalloproteinase inhibitors, and novel antifibrotic/anti-inflammatory agents. These findings point the way to future challenges in cardiac fibroblast biology and pharmacotherapy.

INTRODUCTION

As global human populations develop economically and technologically, human physiological function is faced with challenges outside evolutionary experience. The consequence is a paradigm shift in the causes of human mortality away from extrinsic factors, such as infectious disease or nutritional insufficiency, and toward failures of intrinsic physical function owing to longer life span or inherent genetic abnormalities. These circumstances have resulted in a pandemic of heart disease. In the United States alone, heart failure accounts for 400,000–700,000 deaths per year, $20–$40 billion in yearly healthcare costs, and is the leading hospital discharge diagnosis (1). These considerations provide the impetus for an ongoing search for novel approaches to therapy.

Heart failure reflects the end result of a variety of primary or secondary causes, including the hereditary and idiopathic cardiomyopathies, and the sequelae of

hypertension, coronary artery disease, infectious myocarditis, and alcohol abuse or other toxic insults (2). Irrespective of the underlying cause, heart failure is defined functionally as an insufficient pumping capacity to meet the metabolic needs of the tissues. This operational definition reflects the historical development of cardiology, which until recent years has focused almost exclusively on the muscular compartment of the heart, and at the cell and molecular level upon the biology of the cardiac myocyte.

However, the nonmyocyte cell populations of the heart are increasingly appreciated to contribute to the performance of the normal and failing heart. In particular, cardiac fibroblasts have been recognized to constitute the major nonmyocyte cell type of the heart numerically and contribute importantly to multiple aspects of myocardial function and pathophysiology.

In this review, we evaluate the cardiac fibroblast as a therapeutic target in heart disease. We explore the biology of the cardiac fibroblast as a unique cell type, distinct from fibroblasts in other organs and tissues. The role of the cardiac fibroblast in the function of the heart is discussed in the context of the etiology and progression of heart failure. Established and emerging therapeutic agents that derive benefit through actions on cardiac fibroblasts are summarized. Key unanswered questions in cardiac fibroblast biology are identified as they relate to novel therapeutic targets in cardiac fibrosis and heart failure.

BIOLOGY OF THE CARDIAC FIBROBLAST

Fibroblast Function in Normal, Injured, and Failing Myocardium

Cardiac fibroblasts are recognized as the cell type primarily responsible for homeostatic maintenance of extracellular matrix (ECM) in the normal heart. Cardiac ECM is a highly differentiated structure (3; Figure 1). Myocytes are surrounded by a basement membrane whose principal structural component is nonfilamentous type IV collagen. Collagen fibrils composed primarily of collagen I with smaller amounts of collagen III are arranged in successive layers of organization. The endomysium consists of a loose weave of fibrils wrapped around individual myocytes, plus collagen struts that interconnect adjoining myocytes. Bundles of myocytes are surrounded by a collagen sheath termed the perimysium. Perimysial bundles are encased in a collagenous fascia, the epimysium. In addition, the intracoronary arterioles, which possess their own connective tissue tunica adventitia around the outer vessel lamina, are integrated into the cardiac ECM by additional collagen fibers.

In keeping with the structural and mechanical role of cardiac ECM, the major constituents are the fibrillar collagens I ($\sim$80%) and III ($\sim$10%), with smaller amounts of collagens IV, V, VI, elastin, laminin, proteoglycans, glycosaminoglycans, and others (4, 5). In addition, the ECM is decorated with a diverse assortment of growth factors, proteases, and other molecules. These entities, many of which

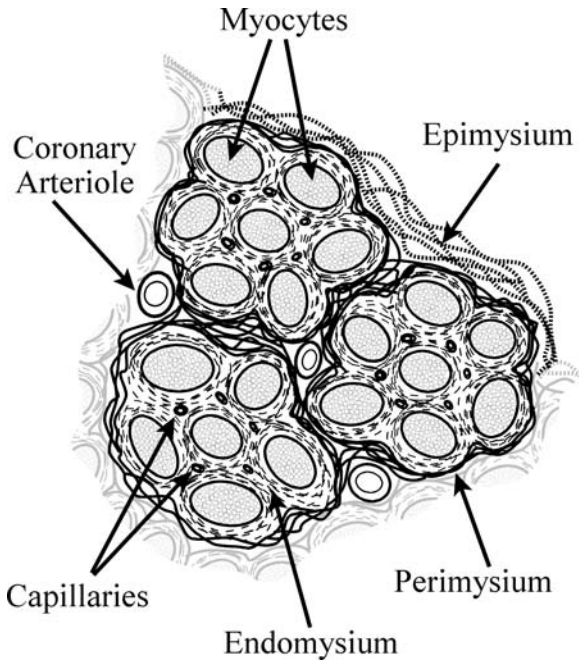

Figure 1 Structural organization of cardiac ECM. Schematic arrangement of fibrillar collagen in relation to cardiac myocytes and the coronary vasculature is shown. Collagen weave surrounding individual myocytes and collagen struts tethering adjacent myocytes comprise the endomysium. Groups of myocytes are bundled within the perimysium. The epimysium encloses groups of perimysial bundles. Capillaries and coronary microvessels have free diffusion access to cardiac myocytes throughout the ECM. Adapted from References 3 and 49.

are sequestered in inactive forms, serve important roles in regulating cell function upon disruption of the ECM (6).

Extracellular matrix homeostasis involves ongoing cycles of synthesis and degradation. Both synthetic and degradative aspects of collagen metabolism are tightly regulated (5, 7). Fibrillar collagen is synthesized as a precursor polypeptide, exported from the cell, and proteolytically processed by removal of amino- and carboxy-terminal propeptides before insertion into nascent fibrils. Collagen monomers are then cross-linked through hydroxyproline and hydroxylysine residues to produce the mature structure. Mature fibrillar collagen is highly stable, with a turnover half-life of around 100 days in normal myocardium. Collagen biosynthesis is regulated transcriptionally by fibrogenic growth factors, particularly TGFβ, and posttranscriptionally by the rate-limiting enzyme prolyl-4-hydroxylase (5, 7). Collagen degradation is accomplished in stepwise fashion by members of the matrix metalloproteinase (MMP) superfamily, which are

themselves under multiple levels of regulatory control. MMP production is regulated by three mechanisms: transcriptionally; posttranscriptionally by the activation of the proenzyme to the active form; and posttranslationally by endogenous pseudosubstrate antagonists, the tissue inhibitors of metalloproteinases (TIMPs).

Of diagnostic significance, collagen metabolism has been monitored in vivo by immunochemical measurement of key metabolic products and enzymes in serum (Figure 2). Synthesis of collagens I or III produce stoichiometric equivalents of the procollagen amino-terminal peptides (PINP, PIIINP) and procollagen carboxy-terminal peptides (PICP and PIIICP), respectively, which are cleared from the circulation by the liver. Degradation of collagens I and III releases the corresponding carboxy telopeptides, ICTP or IIICTP, which are excreted by the kidney (5, 7, 8). Serum concentrations of specific MMPs and TIMPs reflect the release of these

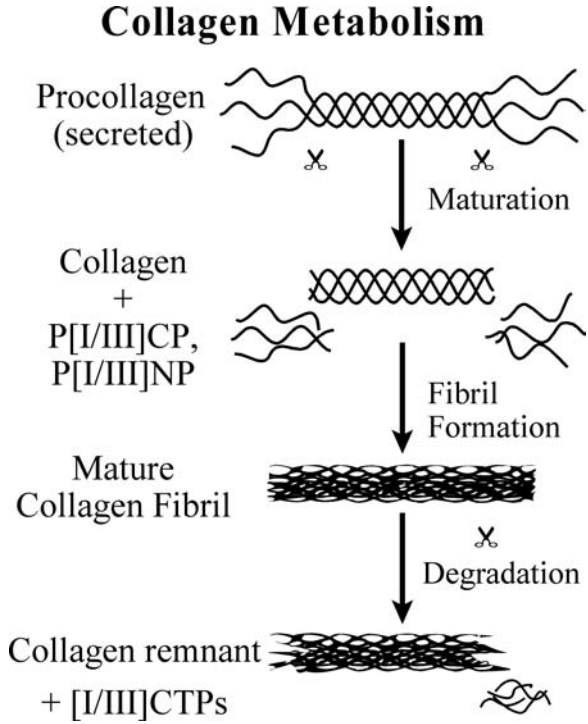

Figure 2 Extracellular metabolism of fibrillar collagen. Monomers of collagens I and III are exported from the cell as propeptides and assembled. Amino- and carboxy-terminal propeptides (P[I/III]NP and P[I/III]CP, respectively) are released into the interstitial space during assembly. Mature collagen fibrils are further processed by cross-linking through hydroxylated lysine and proline residues. Degradation of collagen fibrils by collagenase (MMP-1) releases stable carboxy-terminal telopeptides ([I/III]CTP).

molecules into the interstitial space and are measured by ELISA techniques. These methods have been validated to monitor altered ECM metabolism in the context of heart disease (9).

Cardiac fibroblasts are activated in response to myocardial infarction (MI) or injury and participate as key cells in the wound healing response. In common with injury responses in other tissues, myocardial injury sets in motion a complex sequence of events involving coordinated interactions among multiple cell types in the wound environment (10). The sequential steps in response to injury include hemostasis, infiltration of immune and inflammatory cells, degradation and phagocytosis of necrotic myocytes and cellular debris, repopulation of cardiac fibroblasts within the zone of injury by chemotaxis and increased proliferation, reconstruction of a granulomatous scar, and subsequent ECM remodeling to produce a mature scar. Thus, net ECM degradation, resulting from increased MMP expression, dominates the initial phase of the injury response, whereas net ECM deposition, arising from enhanced collagen synthesis, dominates the later phase of healing.

Transitions of fibroblast phenotype and functional capabilities are regulated by cytokines and growth factors released from other cell types and by the fibroblasts themselves. Cardiac fibroblasts serve important roles as intermediate sensors and amplifiers of signals from immune cells and myocytes, through production of autocrine and paracrine mediators such as cytokines, growth factors, prostaglandins, and nitric oxide (NO) (reviewed in 10, 17). These agents are presumed to regulate the functional responses of cardiac fibroblasts through intracellular signaling networks, which converge at the level of transcription of coordinated gene programs.

In the heart, as in other systems, termination of injury responses appears to occur by apoptosis of activated cells (11). Chronic or repeated injury in the heart ultimately overcomes the compensatory reactions of the myocardium. The ensuing progression to heart failure is accompanied by persistent inflammation and fibrosis. The mechanisms that govern the resolution of acute injury responses, versus the transition to chronic activation of cardiac fibroblasts, are not well understood.

Myofibroblasts have been described as a specialized phenotype of activated fibroblasts (12). These cells express contractile proteins, including smooth muscle α-actin, vimentin, and desmin; effectively contract collagen gels in vitro; and are postulated to be important for wound closure and structural integrity of healing scars. In addition to normal wound healing, myofibroblasts are associated with hypertrophic fibrotic scars in injury models from multiple organ systems, and differentiation to the myofibroblast phenotype is strongly promoted by the reference fibrogenic growth factor TGFβ. Myofibroblast apoptosis has been associated with progression of granulomatous tissue to a mature scar, whereas failure of myocyte apoptosis has been suggested to drive the progression to fibrosis (13). Cardiac myofibroblasts were shown to persist in mature infarct scars (14).

The Cardiac Fibroblast is a Unique Cell Type

Fibroblasts traditionally have been viewed as a uniform (and rather boring!) cell type with equivalent functional capacity regardless of the tissue of origin. More recently, this view has been challenged by experimental evidence suggesting that intrinsic phenotypic heterogeneity exists among fibroblasts from different tissues.

First, cardiac fibroblasts originate from a specific spatiotemporal locus in the developing embryo and undergo a specific developmental sequence to acquire their differentiated phenotype (15, 16). Following formation of the primitive heart tube, extracardiac cells from the same coelomic splanchnopleura that produced the heart tube migrate onto the external surface of the developing heart and give rise to the proepicardium. These cells undergo an epithelial to mesenchymal transition (EMT) and migrate into the developing heart to form the coronary vasculature and the cardiac fibroblasts. Differentiation to cardiac fibroblasts is regulated by programmed sequences of growth factors, including FGF and PDGF (16, 16a).

Additional evidence for phenotypic diversity among fibroblastic cells comes from comparative studies on cellular responses to experimental stimuli in vitro. In this regard, we reviewed the published literature on fibroblasts from skin, joint synovium, lung, liver, and heart to compare responses to the proinflammatory cytokines IL-1, TNFα, IFNγ, and IL-6. We examined the functional endpoints of fibroblast proliferation, chemotaxis, and extracellular matrix metabolism (17). Superimposed on common themes of fibroblast function, we found significant variations in responses to specific cytokines among individual tissues.

Work from our laboratory using cultured neonatal rat cardiac fibroblasts has demonstrated unique features of cytokine responses in these cells. We find that IL-1 strongly inhibits cardiac fibroblast proliferation (18) and enhances chemotaxis, in concert with increased expression of cell adhesion molecules (19) and MMPs, but diminished collagen biosynthesis (R.D. Brown, G.M. Jones, M. Atz, K. Spicka & C.S. Long, unpublished data). In addition, IL-1 activates coordinate expression of mRNAs for pro- and antiinflammatory cytokines and mediators, including TGFβ1 and inducible nitric oxide synthase (20–22). IL-1 is by far the most robust and multipotent agonist in these cells. TNFα and IFNγ are less effective by themselves but potentiate antiproliferative and pro-migratory responses of IL-1. The dominant actions of IL-1, and its striking antiproliferative and pro-migratory effects contrast with phenotypic responses in other fibroblast types and are likely to reflect specific properties of the cardiac fibroblast.

Moreover, reports from kidney (23), lung (24), and skin (25) suggest that heterogeneities can be identified among fibroblasts even within a single tissue. These observations argue that fibroblasts exist with specialized functional portfolios toward endpoint responses such as proliferation, migration, or ECM metabolism.

Further diversity has recently been recognized among fibroblasts regarding their mobilization in response to injury. The conventional view holds that quiescent fibroblasts present in normal tissue undergo a phenotypic transition in response

to injurious stimuli. However, emerging data from other systems suggest three possible sources: (*a*) epithelial-to-mesenchymal transition from epithelial cells (26, 27); (*b*) recruitment of circulating, collagen-secreting, bone marrow–derived hematopoietic precursor cells described as fibrocytes (28, 29); and (*c*) activation of resident fibroblasts (28, 30). More than one route of recruitment may occur within a single tissue (26, 28). In irradiation-induced pulmonary fibrosis, Hashimoto et al. observed that myofibroblasts arose from resident fibroblasts, whereas the principal collagen-producing fibroblasts actually derived from bone marrow recruitment (28). By contrast, peritoneal myofibroblasts were reported to derive from circulating precursor cells (31). These observations provide exciting new insights into the biology of wound healing.

CLINICAL AND PATHOPHYSIOLOGICAL CONSEQUENCES OF CARDIAC FIBROSIS

Changes in myocardial structure and function in response to injury, collectively referred to as myocardial remodeling (32), may initially augment cardiac performance, but over the longer term may progress to a maladaptive response and heart failure. In terms of the cardiac myocyte, these alterations include myocyte hypertrophy; disarray of myocyte organization; and increased wall thickness, which in a majority of cases is followed by wall thinning and chamber dilation, with accompanying myocyte apoptosis or necrosis. Concomitant changes in cardiac fibroblasts include increased fibroblast proliferation as well as accelerated and aberrant remodeling of extracellular matrix and net accumulation of ECM, resulting in cardiac fibrosis (7, 33). This fibrosis may be reparative, replacing areas of myocyte loss with structural scar, or reactive, involving diffuse increases in ECM deposition at sites unrelated to focal injury. Perivascular fibrosis surrounding coronary arterioles is also noted. Differences in the characteristics of fibrosis are observed depending on the heart disease etiology.

Fibrosis has important functional consequences for the heart. First, increased ECM content results in exaggerated mechanical stiffness and contributes to diastolic dysfunction. Progressive increases in fibrosis can cause systolic dysfunction and left ventricular hypertrophy (LVH). Second, increased collagen content disrupts electrotonic connectivity between cardiac myocytes and provides an electrical substrate for reentrant arrhythmogenesis. Third, perivascular fibrosis surrounding intracoronary arterioles impairs myocyte oxygen availability, reduces coronary reserve, and exacerbates myocyte ischemia.

Within this framework, heart failure is characterized by substantial heterogeneity of disease severity and progression even in cases of comparable heart failure etiologies, presumably reflecting polygenic and environmental influences in the disease phenotypes of individual patients. In this regard, we may consider the hypothesis that properties of cardiac fibroblasts, and consequently fibrotic remodeling, act as disease modifiers, and more specifically, as independent predictive risk factors in heart failure.

Hypertrophic Cardiomyopathy

Hypertrophic cardiomyopathy (HCM; defined as increased septum and LV wall thickness inappropriate for hemodynamic load) in affected individuals may be asymptomatic or lead to alternate outcomes, including atrial fibrillation, symptomatic cardiac dysfunction progressing to heart failure, or sudden cardiac death (SCD) from ventricular tachycardia (34). The latter result is encountered in young competition athletes, where sudden death may be the first symptom of HCM. At the cellular level, HCM is most commonly associated with myocyte hypertrophy and myocyte disarray, accompanied by replacement fibrosis at foci of myocyte death.

HCM in most cases has been shown to arise from hereditary or spontaneous mutations in myocyte sarcomeric proteins responsible for force generation (35). This observation provides proof of the principle that a single gene defect in the cardiac myocyte is sufficient to drive the full syndrome of cardiac hypertrophy and failure. However, the relationship between mutational genotype and disease phenotype is highly variable and not well understood (36). These findings clearly suggest the presence of additional modifiers of disease.

The association of cardiac fibrosis with diastolic dysfunction and SCD has been studied in HCM. Both myocyte disarray and fibrosis are associated with diastolic dysfunction and electrical instability in HCM patients (37, 38). Varnava et al. (39), in examination of autopsy specimens from HCM-induced sudden death, concluded that myocyte disarray correlated most strongly with SCD in young patients, whereas fibrosis was associated with SCD in older patients. By contrast, a second autopsy study by Shirani et al. (40) found morphologic abnormalities and increased amounts of ECM in children and young-adult SCD victims, arguing that expanded ECM is involved early in the disease process. These data clearly suggest an association between cardiac fibrosis and HCM severity, although the link to SCD needs clarification.

Dilated and Ischemic Cardiomyopathies

Dilated heart failure is the most commonly encountered end-stage of heart failure progression, accounting for approximately 80% of cases, and may result from primary and secondary causes, including ischemic heart disease, hypertensive heart disease, or the idiopathic dilated cardiomyopathies (reviewed in 41). Up to 30% of dilated heart failure cases appear to be due to a diverse array of mutations in proteins for force generation, force transmission, energy metabolism, and nuclear structure. The variable penetrance of familial dilated and ischemic cardiomyopathies (DCM) suggests the presence of disease modifier genes. Chamber dilation and wall thinning are associated with mild to moderate myocyte hypertrophy, but less myocyte disarray than accompanies HCM. Diffuse interstitial and perivascular fibrosis are often evident but vary substantially (42).

Significant associations of increased collagen deposition and elevated collagen I:collagen III composition have been observed in autopsy specimens (43) or failing explanted hearts (44). Increased ECM remodeling on endomyocardial biopsy

was associated with deterioration of LV performance on echocardiography (45). Importantly, a prospective study on serum markers of collagen metabolism in idiopathic or ischemic DCM showed that patients at the upper strata of values for serum collagen markers were at increased risk for advanced clinical stage of heart failure, poor hemodynamic condition, transplantation, or death during follow-up (46). These results suggest a definite association of DCM with fibrosis; more severe fibrotic reactions may act as an independent factor for risk stratification. It should be noted that the effects of somatic mutations in noncontractile proteins described in DCM have not been evaluated in cardiac fibroblasts.

Hypertensive Heart Failure

Hypertension exerts characteristic adverse effects on the heart, including LV hypertrophy, thickening of intracoronary arterioles, and cardiac fibrosis (47). The causes of cardiac fibrosis in hypertensive heart disease have been attributed to a combination of hemodynamic (pressure overload) and humoral factors (AngII, ET-1, TGFβ; 48).

Using formalin fixation combined with alkaline digestion of cellular constituents to selectively visualize the cardiac extracellular matrix, Rossi (49) obtained striking microscopic images showing changes in the ultrastructural organization of collagen fibers relative to increasing degrees of LVH in autopsy heart specimens with hypertensive heart disease. In mild hypertension, there was diffuse reactive fibrosis with net increased collagen accumulation in endomysium and perimysium and evidence of myocyte hypertrophy, but the overall structure of the ECM was preserved. With increasing LVH, and particularly in the severely affected group, greatly expanded and disorganized ECM deposition was observed, reflecting areas of myocyte death and replacement fibrosis interspersed with extreme myocyte hypertrophy.

Previous studies have shown a correlation between fibrosis and diastolic dysfunction in hypertensive heart disease (50). These findings were extended by Querejeta et al. (9) to show a predictive relationship between serum concentrations of PICP and endomyocardial fibrosis in hypertensive patients. In a second study, these authors showed that hypertensives with LVH and renal fibrosis had higher serum PICP than uncomplicated hypertensives or control subjects. Further, six months of treatment with the angiotensin receptor blocker losartan revealed a nonresponder subpopulation of these highly fibrotic patients whose serum markers were not improved by therapy, despite normalization of blood pressure. These data indicate that fibrotic responses may contribute to hypertensive disease and therapeutic outcomes independent of hemodynamic factors (33).

Cardiac Fibrosis and Arrhythmia

As alluded to above, fibrosis exerts adverse impacts on cardiac electrical properties in addition to effects on the mechanical properties of the myocardium. The specific relationship between fibrosis and arrhythmogenesis depends on the cardiomyopathy and the structural details of myocardial remodeling (reviewed in 51). Myocyte

loss owing to apoptotic or necrotic mechanisms is followed by replacement fibrosis, resulting in electrical isolation of myocytes, and introduction of alternate conductance pathways for reentrant arrhythmias. Arrhythmogenesis owing to reentrant pathways commonly arise as a consequence of coronary artery disease resulting in ischemic heart failure (52), MI (53), congestive (pressure-overload) heart failure (54), or atrial fibrillation in elderly patients (55). These conductance disturbances may be exacerbated by perivascular or reactive fibrosis, which impairs myocyte oxygen availability. By contrast, arrhythmias may arise from focal mechanisms not associated with sites of fibrosis, presumably arising from primary myocyte dysfunction and the resultant myocyte disarray, in idiopathic cardiomyopathy (56), primary atrial fibrillation (57), or hypertrophic cardiomyopathy in young patients (39). However, even in these cases fibrosis may provide adjunct sites for conduction delay or block (56).

Arrhythmogenic right ventricular dysplasia (ARVD) is an extreme example of a fibrotic cardiomyopathy and its consequences (41, 58). ARVD is at least partly hereditary, of variable severity, characterized by fibrofatty replacement of myocytes particularly in the right ventricle, and associated with a high frequency of sudden cardiac death before middle age. Initial genetic mapping studies have identified mutations in the ryanodine receptor, which is involved in intracellular Ca release, and in desmoplakin, a constituent of myocyte desmosomes consistent with a primary myocyte dysfunction leading to cell death. Arrhythmogenesis in ARVD commonly appears to be associated with reentrant pathways arising from replacement fibrosis at sites of myocyte loss.

In summary, cardiac fibrosis is clearly associated with altered myocardial mechanical performance and arrhythmogenesis. In some studies this association has been extended to a statistical correlation of fibrosis severity with myocardial function. However, there are relatively few data that quantitate cardiac fibrosis as an independent and predictive risk factor for heart disease outcome or therapeutic effect.

THERAPIES DIRECTED AT THE CARDIAC FIBROBLAST

The importance of fibrosis as a determinant of myocardial performance and disease outcome is increasingly appreciated. Nevertheless, efforts to develop novel therapies that specifically target the cardiac fibroblast are at a relatively early stage, in common with approaches to fibrotic disease in other organ systems. This section discusses established and emerging therapies directed at cardiac fibroblasts in heart disease. A summary of this discussion appears in Table 1.

Renin-Angiotensin System: ACE Inhibitors and Angiotensin Receptor Antagonists

The renin-angiotensin system (RAS), through the production and actions of angiotensin II (AngII) at its receptors, plays a key role in the compensatory

TABLE 1 Antifibrotic actions of current drug therapies

Agent class	Mechanism of action, if known	Therapeutic status and actions on cardiac fibrosis	References
Renin-angiotensin-aldosterone inhibitors			
• ACE inhibitors	Enzymatic inhibitors of angiotensin converting enzyme, reduce Ang II production	Approved for heart failure; probable beneficial actions on cardiac fibrosis	(60, 61, 63–65)
• Angiotensin receptor blockers	AT_1 receptor antagonists	Approved for heart failure; probable beneficial actions on cardiac fibrosis	(66–69)
• Aldosterone antagonists	Mineralocorticoid receptor antagonists	Approved for heart failure; probable beneficial actions on cardiac fibrosis	(71–73)
Endothelin receptor antagonists	ET_A-ET_B endothelin receptor antagonists	Failed Phase III trial for heart failure	(77)
Statins	HMG-CoA reductase inhibitors	Approved lipid lowering drugs; investigational for heart failure; effects on fibrosis unknown	(81–83)
Cytokine therapies			
• Anti-TNFα	Inhibit TNFα availability	Approved for rheumatoid arthritis; failed Phase III for heart failure	(84)
• IFNγ	Inhibit myofibroblasts, collagen synthesis	Investigational, Phase III in progress for pulmonary fibrosis	(85)
• Anti-TGFβ	Inhibit TGFβ availability or action	Investigational, Phase III in progress for post-opthalmologic surgery	(89)
MMP inhibitors	Enzyme inhibitors of MMPs, prevent ECM remodeling	Failed Phase III trials for different applications; approved for periodontal disease	(103, 123, 124)
Pirfenidone	Unknown; inhibits TGFβ1, anti-inflammatory	Investigational for pulmonary and renal fibrosis	(109, 110)
Tranilast	Unknown; antifibrotic and antiinflammatory	Failed Phase III for antiatherosclerosis	(111)
Nuclear receptor agonists	Activate nuclear PPARα or PPARγ, respectively		
• PPARα agonists		Approved lipid lowering drugs	(114, 115)
• PPARγ agonists		Approved for Type II diabetes	(118)

neurohumoral response to myocardial injury. The myocardium of animals and humans contains an endogenous RAS independent of the renovascular RAS (reviewed in 7, 59). Stimulation with AngII results in cardiac fibroblast proliferation and net accumulation of fibrillar collagen in vitro and cardiac fibrosis in vivo. These responses are transduced by AT1 receptors, and their expression in cardiac fibroblasts far exceeds that in myocytes (20). An important aspect of AngII function occurs through upregulation of additional fibrogenic growth factors, which mediate or augment the direct effects of AngII, including endothelin-1 (ET-1), TGFβ1, and, as discussed below, aldosterone. In this context, Weber et al. have reported upregulation of angiotensin production, angiotensin AT1 receptors, and increased collagen mRNA in myofibroblasts associated with healing infarct scars (14).

Large clinical trials have shown that angiotensin-converting enzyme inhibitors (ACE-I) reduce morbidity and mortality, slow the progression of established heart failure (60), and reduce cardiovascular events in patients at risk but without symptomatic heart failure (61). A significant component of the therapeutic benefit has been interpreted to be independent of blood pressure–lowering effects, suggesting actions on cardiac remodeling (but see 62).

Benefits of ACE-I therapy on cardiac fibrosis and myocardial performance have been shown in limited populations of hypertensive patients with symptomatic heart disease. In a prospective study, Brilla et al. found that six months of treatment with the ACE-I lisinopril in patients with hypertensive heart disease reduced cardiac fibrosis and improved left ventricular diastolic function, whereas the diuretic hydrochlorothiazide had comparable blood pressure–lowering effects but did not improve fibrosis or diastolic function (63). Schwartzkopff et al. reported that treatment of patients with hypertensive heart disease with the ACE-I perindopril for 12 months caused a significant regression of periarteriolar fibrosis and a marked improvement in coronary blood flow reserve (64).

In addition to benefits in the treatment of chronic heart failure, ACE-I have consistently been shown to improve survival when administered within the first seven days following acute MI (65). Studies in experimental animals suggest that ACE-I regulate both synthetic and collagenolytic aspects of ECM metabolism in the early response to MI. However, AngII regulation of ECM metabolism in the setting of acute MI is not understood. This will be an important area for continuing research.

Advances in the molecular pharmacology of the AT1 receptor have led to the development of angiotensin AT1 receptor blockers (ARB) as an alternate therapeutic approach to the ACE inhibitors. ARBs appear to offer clinical benefits similar to ACE-I in heart failure therapy (66, 67). Antifibrotic actions of the ARB losartan have also been studied in two small prospective studies. In a series of 37 patients with hypertensive heart disease, 12 months of treatment with losartan reduced cardiac fibrosis and serum collagen markers, whereas the calcium channel blocker amlodipine had no effect despite similar hemodynamic normalization (68). In a second series of patients with hypertensive heart disease, losartan treatment had selective benefit to reduce collagen deposition and LV stiffness in more severely

fibrotic patients than in patients with nonsevere fibrosis. These results suggest heterogeneity of fibrosis responses to therapy (69).

In summary, inhibitors of the RAS clearly appear to derive a significant portion of their therapeutic benefit from actions on cardiac fibroblasts and fibrotic remodeling of the heart. Despite a tremendous amount of research, however, the specific mechanisms of these actions, and the underlying role of the RAS in myocardial remodeling and homeostasis remain enigmatic. Identifying the primary mechanisms should provide further opportunities for therapeutic development.

Aldosterone Antagonists: Spironolactone, and Eplerenone

The mineralocorticoid aldosterone has been strongly implicated in the fibrogenic response of the myocardium upon stimulation with AngII or through AngII-independent mechanisms (70). The myocardium expresses enzymes for biosynthesis and metabolism of aldosterone as well as mineralocorticoid receptors (70). Aldosterone infusion in animal models subjected to sodium overload resulted in diffuse fibrosis of both RV and LV, combined with focal replacement fibrosis, which appeared attributable to hyperkalemic myocyte loss. Aldosterone-induced fibrosis was independent of blood pressure elevation but reversed by the receptor antagonist spironolactone (7, 70).

This research came to fruition with the RALES clinical trial, which demonstrated that treatment with spironolactone in heart failure patients receiving an ACE inhibitor and standard therapy produced marked reductions in all-cause cardiovascular mortality and improved NYHA classification (71). These findings were confirmed and extended in a large-scale trial (EPHESUS) of the selective mineralocorticoid antagonist eplerenone in post-MI patients (72). A substudy arising from the RALES trial demonstrated that the serum collagen III metabolic marker PIIINP predicts cardiovascular risk, and spironolactone therapy normalizes ECM metabolism and the progression of LV dilatation (73). However, only patients whose baseline PIIINP levels were above the median responded to spironolactone therapy with improvement in event-free survival. In light of the linkage between myocardial fibrosis and arrhythmogenesis, it is noteworthy that both RALES and EPHESUS found reductions in sudden cardiac death from aldosterone antagonist therapy. Additional trials are ongoing to compare the effectiveness of aldosterone antagonists in combination with ARBs versus ACE inhibitors.

Despite the successful outcomes of these trials, fundamental questions remain regarding the actions of aldosterone on cardiac fibroblasts. Attempts to identify mineralocorticoid receptors and to elicit aldosterone-stimulated collagen synthesis in cardiac fibroblasts in vitro have been problematic (7, 70). Induction of fibrosis by aldosterone in experimental animal models exhibits slow onset (four weeks) and is attenuated by treatment with endothelin antagonists or angiotensin receptor blockers, suggesting indirect rather than direct interaction with cardiac fibroblasts (7, 70). Regardless, mineralocorticoid antagonists represent an important new

therapy that demonstrates the value of targeting cardiac fibrosis to improve cardiac performance and prognosis in appropriately selected patients.

Endothelin Receptor Antagonists

Endothelin-1 (ET-1) regulates activities of cardiac fibroblasts in vitro and in vivo. Cardiac fibroblasts express a mixed population of ET_A and ET_B receptor subtypes. ET-1 increases cell proliferation, MMP activity, and net collagen synthesis in cultured cardiac fibroblasts (74). AngII-elicited increases in ECM metabolism in cultured fibroblasts were blunted by an ET_A receptor antagonist (75), and complex interactions among ET-1, AngII, and TGFβ occur in the myocardium as well. ET-1 is upregulated in failing LV of human heart failure patients, and elevations in serum endothelin concentrations correlate with heart failure severity (76). Despite these promising indications, ET-1 antagonists failed to show benefit following acute MI and in chronic heart failure (reviewed in 77).

Statins

The proven clinical benefit of statins as cholesterol-lowering drugs in atherosclerotic disease was more recently complemented by the unexpected finding that these agents exert pleiotropic effects on a variety of cell signaling pathways through inhibition of protein prenylation (78, 78a). These observations have fueled interest in the potential utility of statins in heart failure. Studies in experimental animal models provide support for beneficial actions of statins directed toward cardiac fibroblasts. Treatment with statins reduces myocardial remodeling, fibrosis, and collagen synthesis in models of myocardial injury including surgical infarction (79), transgenic models of hypertrophic cardiomyopathy (79a) or NaCl-induced pressure overload (80). In many of these studies, statins exert concordant antifibrotic and antiinflammatory actions. These results underscore that inflammation and fibrosis represent aspects of a continuum of responses of cardiac fibroblasts to myocardial injury.

In theory, statin therapy might confer either positive or negative impacts in the setting of congestive heart failure (discussed in 81). Statins may act on cardiac fibroblasts to attenuate inflammatory signaling through reduced prenylation of small GTPases. In this regard, elevated serum concentrations of the inflammatory marker C-reactive protein were shown to predict the likelihood of nonfatal MI or fatal coronary events following an initial infarct. Treatment with pravastatin normalized serum concentrations of CRP and reduced the risks of cardiac events equivalent to normal subjects (82). Moreover, amelioration of coronary artery disease and ischemic events would relieve proapoptotic stress on cardiac myocytes and indirectly diminish replacement fibrosis. The large-scale prospective CORONA trial is underway to explicitly test therapy with rosuvastatin in heart failure (83). This trial does not include measurement of fibrotic or inflammatory markers as a primary endpoint, but such a substudy would be of considerable interest.

Anticytokine Therapies: TNFα, IFNγ, and TGFβ

Proinflammatory and profibrotic cytokines play central roles in coordinating the activities of multiple cell types in the injured and failing myocardium. In light of the importance of cardiac fibroblasts as cytokine effectors, these agents offer a promising direction for therapeutic development. However, the complexity and redundancy of cytokine signaling networks have posed a challenge for drug development. Initial efforts with anticytokine therapy in heart failure focused on TNFα (84). Strong experimental evidence indicates an important role for this cytokine in the initial activation of MMPs requisite to myocardial remodeling following acute MI and in the progression to chronic heart failure (17, 84). However, sequestration of TNFα with a receptor:antibody chimera (sTNFR1:Fc) was ineffective to ameliorate or regress symptomatic heart failure in a large-scale clinical trial (17, 84).

Promising results have been obtained with IFNγ in therapy of idiopathic pulmonary fibrosis. This cytokine exerts important actions to prevent the phenotypic activation of myofibroblasts and to induce myofibroblast apoptosis in opposition to the profibrotic actions of TGFβ. A randomized, double-blind trial in patients with idiopathic pulmonary fibrosis demonstrated that IFNγ improved pulmonary function, oxygenation, and symptoms, and decreased fibrogenic markers in lung biopsies (85). A larger multicenter trial is near completion. This cytokine should be investigated further in the context of myocardial fibrosis.

Considering the importance of TGFβ as a ubiquitous controller of fibrosis, efforts have been undertaken to intervene directly on the production and activation of this cytokine. Experimental animal studies have offered promising results. Inhibition of TGFβ with neutralizing antibodies; soluble TGFβ receptor:antibody chimeras (sTGFβRII:IgG Fc); or adenoviral-mediated gene transfer of decorin, a TGFβ binding protein, reduce fibrosis in rodent models of pressure overload cardiac hypertrophy, bleomycin-induced pulmonary fibrosis, or experimental glomerulonephritis (86, 87). Inhibition of TGFβ with neutralizing antibodies or antisense oligonucleotides reduces injury and scarring in ocular surgery in animals (88). Preliminary studies in patients undergoing glaucoma filtration surgery indicate that a humanized neutralizing monoclonal antibody to TGFβ2 reduces postoperative scarring (89). In this procedure, a single administration of TGFβ2 antibody at the time of surgery is sufficient to produce a favorable healing response. A Phase III trial is in progress.

Systemic inhibition of TGFβ might be expected to lead to adverse side effects owing to the pleiotropic actions of this growth factor. Additional strategies to inhibit TGFβ have targeted its interaction with binding partners in the ECM, including latent TGFβ binding protein (90) or latency activated peptide (91), in order to restrict therapeutic modulation of TGFβ to a specific tissue or physiological context. Further, the fibrogenic actions of TGFβ in many cases are mediated through expression of connective tissue growth factor. Research is in progress to target this effector molecule (92). Although these approaches are in a preliminary stage, modulation of TGFβ production and actions is likely to remain a focus of research in antifibrotic therapy.

Inhibitors of ECM Metabolism: Collagen Synthesis, Matrix Metalloproteinases, and Plasmin Systems

Drugs that act on the enzymatic steps of ECM metabolism present clear-cut therapeutic opportunities to modulate myocardial remodeling. These approaches intersect key functions of the cardiac fibroblast (17). Few studies have explored inhibitors of collagen biosynthesis to limit cardiac fibrosis (5, 93), whereas extensive research focuses on MMP inhibitors (MMPI) in the context of pathologic remodeling.

The rationale to develop inhibitors of MMPs to modulate ECM metabolism is based on the demonstrated involvement of MMPs in at least three major aspects of myocardial injury and failure (94, 95). First, activation of MMPs underlies myocyte slippage, ventricular wall thinning, and chamber dilation following acute MI. Second, chronic MMP activation contributes importantly to the aberrant remodeling of ECM during the progression to chronic heart failure. Third, excess MMP activation is a key contributor to instability and rupture of atherosclerotic plaques. In this context, it is important to recognize that systemic inhibition of MMPs might exert contravening effects on atherosclerotic plaque stability versus cardiac fibrosis.

Studies in animals have investigated the effects of MMP inhibition with pharmacological agents or by gene deletion in transgenic animals in models of cardiac injury and failure. Rohde et al. showed that a nonselective pharmacological MMP inhibitor (CP-471 474) reduced LV dilatation four days following surgical infarction in mice (96). However, follow-up at later time intervals (15 days) post-MI in MMP-9-deficient mice showed defective wound healing with diminished collagen accumulation in the infarct zone and decreased infiltration of macrophages compared to wild type (97). These results emphasize the relationship between degradation and repair of ECM.

Activation of MMPs by the plasmin system has been demonstrated in elegant studies by Heymans et al. (98). Using surgical infarctions in transgenic mouse strains, the authors showed that mice deficient in urokinase plasminogen activator (uPA) or MMP-9 were protected from acute post-MI cardiac rupture, but subsequently exhibited reduced inflammatory infiltrates and defective healing. Plasminogen-deficient mice failed to activate MMP-2 and -9 and exhibited similar defects in infarct healing (94). Transient overexpression of plasminogen activator inhibitor-1 (PAI-1), the principal physiological antagonist of plasmin, by adenoviral gene transfer following infarction in wild-type mice prevents cardiac rupture but permits infarct healing to normalize as the expression of the adenoviral construct wanes (98). These findings have important clinical implications for the use of lytic agents on ECM remodeling versus thrombosis and for potential drug interactions between lytic agents and MMP inhibitors in acute myocardial infarction. In this context, PAI-1 is expressed by cardiac fibroblasts, upregulated by AngII, and promotes cardiac fibrosis (98, 99). Additionally, therapy with thiazolidinedione ligands of nuclear PPARγ receptors is associated with reduced serum levels of PAI-1 (reviewed in 99).

The effects of MMP inhibitors in the progression to heart failure have also been examined. Pacing-induced supraventricular tachycardia of three-weeks duration in pigs produces congestive heart failure characterized by LV dilation; increased activities of MMP-1, -2, and -3; and decreased collagen content. Pathological remodeling was attenuated and cardiac function was preserved by treatment with the nonselective MMPI, PD-166 793 (100). Similar results were obtained more recently with a newer generation MMPI, PGE 7113313, designed to spare inhibition of MMP-1, which is downregulated in chronic human heart failure (101). It should be noted that inhibitors were administered prior to and throughout the duration of pathological stimulus rather than after the establishment of congestive heart failure.

Preliminary studies in humans lend further support for MMPs as targets in heart disease (reviewed in 94). Gene polymorphisms have been identified in the promoters of MMP-1, -3, -9, and -12, which influence MMP expression. Polymorphisms in the promoters for MMP-3, -9, and -12 were shown to confer susceptibility to coronary artery disease and abdominal aortic aneurysm. Concentration ratios of serum MMP/TIMP correlate with functional values of LV volume and ejection fraction and predict clinical outcome of myocardial infarction. A Phase II trial was recently completed to test the effect of MMP inhibitor PG 116800 to prevent adverse cardiac remodeling following a first myocardial infarction (102). The tetracycline derivative Periostat (doxycycline) is the only MMP inhibitor currently approved for clinical use, but its application is limited to periodontal disease. Treatment of coronary heart disease patients with Periostat reduced serum inflammatory markers (C-Reactive Protein, IL-1, and IL-6) as well as circulating concentrations of MMP-9 (103). Considering its safety, efficacy, and cost, clinical trials of doxycycline in myocardial remodeling also appear worth pursuing.

Novel Antifibrotic/Antiinflammatory Agents: Pirfenidone, Tranilast, and Nuclear Receptor Ligands

Pirfenidone (PD), 5-methyl-1-phenyl-2(1H) pyridone, has been shown to exert protective actions in animal models of tissue injury and fibrosis. The mechanism of PD action is not fully understood but appears to involve inhibition of fibroblast proliferation and collagen synthesis, potentially through disruption of TGFβ1 expression (106). PD regressed LVH and fibrosis in DOCA-salt hypertension (104) and protected against doxorubicin-induced myocardial and renal oxidative injury and resulting fibrosis (105). PD also reduced the fibrotic and inflammatory responses of bleomycin-induced pulmonary fibrosis (106) and LPS-induced toxic shock in rodents (107). These results suggesting combined antifibrotic and antiinflammatory actions of PD were extended with in vitro studies, where PD demonstrated antifibrotic activities against smooth muscle leiomyoma cells and rat renal myofibroblasts (108) and antiinflammatory actions in the RAW 246.7 macrophage cell line (107). PD is under active investigation (Phase II) in idiopathic pulmonary fibrosis (109) and in renal tubulointerstitial fibrosis (110). Based on its spectrum

of activities, continued investigations of mechanisms of PD actions in cardiac fibroblasts are warranted.

Tranilast [N(3,4-dimethoxy cinnamoyl)-anthranilic acid] is a second agent that showed very promising antifibrotic and antiinflammatory characteristics in experimental studies and early phase clinical trials. Based on its abilities to inhibit smooth muscle cell migration and proliferation, this agent was targeted for therapy to prevent coronary vascular restenosis following angioplasty. However, a large-scale clinical trial failed to show benefit (111). Nonetheless, recent studies in the DOCA-salt hypertensive rat model show that tranilast blocks myocardial fibrosis and suppresses inflammatory cell infiltrates (112). It should be emphasized that tranilast, like PD, antagonizes production and activity of TGFβ (113).

Recent attention has focused on ligands of the peroxisome proliferator-activated receptors, PPARα and PPARγ, for their actions on the myocardium (reviewed in 114, 115). PPARs are nuclear receptors that regulate lipid storage and metabolism. Activators of PPARα and PPARγ are used clinically in dyslipidemias and in diabetes, respectively. The PPAR receptors are expressed by multiple cell types in the cardiovascular system, including cardiac myocytes and fibroblasts. PPAR receptors share common properties to suppress production of inflammatory cytokines, cellular adhesion proteins, and chemotactic peptides by inhibiting the transcription factor NF-κB. PPARα and PPARγ ligands are cardioprotective in experimental infarction (116), and they prevent interstitial fibrosis, preserve diastolic function, and inhibit inflammatory activation in pressure overload cardiac hypertrophy (117). These studies do not distinguish primary actions on cardiac fibroblasts from secondary actions via other cell types. Further, adverse effects of PPARγ ligands in congestive heart failure have been reported (118). Better understanding of PPAR mechanisms in cardiac fibroblasts is important to complement ongoing research in other cardiac cell types.

FUTURE CHALLENGES FOR THERAPY

The concepts that fibrosis and inflammation contribute to the myocardial response to injury are generally accepted, as is the importance of the cardiac fibroblast as a key cell type mediating these processes. From this perspective, the rationale for development of therapies that target the cardiac fibroblast is clear. Indeed, we have seen that established therapies that target the renin-angiotensin-aldosterone system actually may derive a significant part of their benefit from actions on cardiac fibroblasts. However, fundamental unresolved issues stand between these generalizations and the development of effective new therapies. These may be broadly divided between issues of fibroblast biology and issues of pharmacotherapy.

Unanswered Questions in Cardiac Fibroblast Biology

The ability to develop rational therapies targeted to the cardiac fibroblast ultimately depends on a sound knowledge of the biology and the physiological role of this cell

type in myocardial remodeling. Questions remain that bear on the therapeutic issues discussed above and that may provide novel opportunities for drug development.

First, what are the fibroblast phenotypes of normal, injured, and failing myocardium? It will be important to resolve whether a single activated fibroblast phenotype is capable of multiple functional responses, such as proliferation, migration, ECM metabolism, and production of autocrine/paracrine mediators, or whether different subsets of fibroblasts subserve distinct endpoint responses. To address this question, it will be necessary to define the cellular precursors of activated fibroblasts; for example, whether activated fibroblasts derive from homogeneous or polyclonal populations of quiescent resident fibroblasts, from epithelial-mesenchymal transitions of undifferentiated resident precursor cells, or from recruitment of circulating precursor cells. These same issues need to be addressed for cardiac myofibroblasts.

These ideas lead to related thoughts about the mechanisms that underlie the termination of the myocardial injury response in normal wound healing compared to the transition to maladaptive responses in fibrotic heart failure. One may speculate that heart failure progression results from unresolved cardiac myocyte dysfunction. Within this environment of chronic injury, cardiac fibrosis could reflect either a failure to terminate a normal injury phenotype, or alternatively, the de novo appearance of a novel failure phenotype of cardiac fibroblasts. The role of fibroblast apoptosis as a termination mechanism in adaptive myocardial healing versus the role of hyperplasia as a mechanism for fibroblast recruitment in fibrotic progression should be a specific focus for investigation. Identification of populations of (myo)fibroblasts that are involved in distinct functional aspects or disease stages of myocardial remodeling would offer obvious opportunities for therapeutic intervention.

Continued research on the signaling mechanisms that regulate cardiac fibroblast phenotype in response to inflammatory and fibrotic stimuli represents a second major area of emphasis. The unique properties of the cardiac fibroblast relative to other fibroblastic cells, the ability of cardiac fibroblasts to integrate many stimuli through receptor-specific signaling pathways, and the diversity of endpoint responses all point to a more complex regulatory organization than has been appreciated (Figure 3). The functional response of regulated gene expression reflects combinatorial inputs in the evolving wound environment. These regulatory interactions underlie the spatiotemporal sequencing of events that occurs in wound healing and provide the basis for determining the intervals of opportunity for defined therapeutic targets. This model further suggests that genomic or proteomic pattern recognition analyses will help to identify groups of genes that are expressed in concert, in turn generating hypotheses for conserved regulatory motifs within the gene promoters and associated signaling pathways. Additional levels of complexity come from the discovery that signaling molecules as well as transcriptional regulators are spatially organized within the cell (119, 119a). In theory, these elements offer a wealth of targets for therapy to intersect the inflammatory-fibrotic cascade, but the dissection of key mechanisms will require acquisition of detailed molecular information. Genomic analyses of myocardial injury models, and particularly of

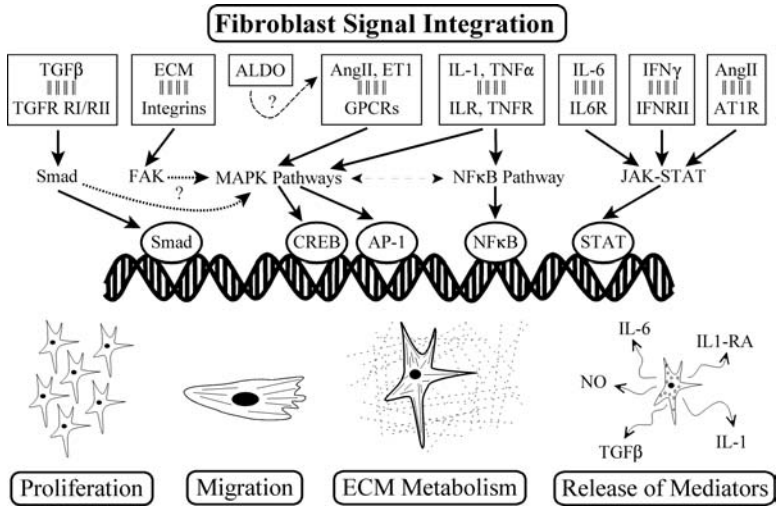

Figure 3 Signaling and transcriptional regulation of fibroblast function. Cardiac fibroblasts respond to diverse humoral and mechanical inputs through cell surface receptors. Environmental stimuli are integrated through receptor-mediated signaling pathways leading to activation of nuclear transcription factors and altered gene expression. Integration of nuclear and cytoplasmic signaling networks controls fibroblast phenotype. Abbreviations: ALDO, aldosterone; R, receptor; GPCR, G protein–coupled receptor; FAK, focal adhesion kinase; IL-1 RA, IL-1 receptor antagonist; MAPK, mitogen activated protein kinase.

cardiac fibroblasts, are at an early stage, but a torrent of data is surely coming. We are unaware of clinical trials utilizing agents that focus on cardiac fibroblast intracellular signaling pathways. However, a number of agents are in preclinical or early phase clinical trials for other applications, and it is likely that coming years will see their evaluation in myocardial remodeling (120).

A third area for research will be to explore further the mechanisms of intercellular communication in the normal and failing heart. Homeostatic maintenance and remodeling of the heart requires communication among cardiac myocytes, fibroblasts, and immune cells, as well as interactions with the coronary vasculature (Figure 4). Mechanotransduction via integrins and the extracellular matrix, direct cell-cell communication via gap junctions, or humoral transmission by diffusible chemokines and cytokines all may contribute to this process. Therapies aimed at intramyocardial signaling by AngII and aldosterone demonstrate the value of this approach.

Conversely, cardiovascular exercise training has been shown to promote physiologically adaptive cardiac hypertrophy and nonfibrotic ECM remodeling, which are associated with improved cardiac performance, and protect against the risk of adverse cardiac events (121, 121a). These results raise questions of how the

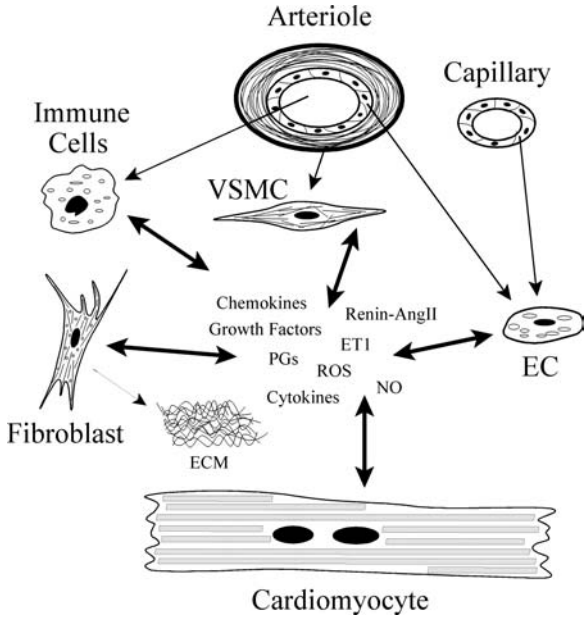

Figure 4 Intercellular communication in the myocardium. Cell types within the myocardium respond to humoral and mechanical stimuli and, reciprocally, release autocrine-paracrine mediators. Therapeutic modulation of intercellular signaling can interrupt negatively reinforcing cycles of inflammation and fibrosis in failing myocardium (e.g., antagonism of RAS). Abbreviations: EC, endothelial cell; VSMC, vascular smooth muscle cell; PG, prostaglandin; ROS, reactive oxygen species.

myocardium distinguishes the healthy stress of exercise from the pathologic stress of injury. It is intriguing to speculate that exercise activates survival pathways in cardiac myocytes, resulting in intercellular signals to cardiac fibroblasts, which are distinct from signals of injury (121b). Knowledge of cardiac fibroblast responses to exercise training may provide additional insight into approaches to oppose the progression of fibrosis. An observational study is ongoing to evaluate cardiac fibrosis by serum collagen markers and MRI in relation to exercise tolerance in hypertrophic cardiomyopathy (122).

Unresolved Issues for Pharmacotherapy

Despite strong physiologic rationales and abundant evidence from in vitro and in vivo basic research, spectacular and costly failures have occurred in clinical trials for novel agents in heart failure (see, for examples, the discussions above on anti-TNFα therapy, ET-1 receptor antagonists, and tranilast). A further cautionary example comes from attempts to develop MMP inhibitors as therapeutic agents for pathophysiologic ECM remodeling. Staggering investments of time and money by

basic and pharmaceutic research sectors so far have yielded a single approved agent, doxycycline (Periostat), for periodontal disease. Lessons from these experiences have been thoughtfully reviewed and are relevant to cardiac fibroblasts (123, 124).

Cardiac fibrosis is not a primary cause, but rather a disease modifier, that affects the progress, severity, and outcome of heart disease. Furthermore, fibrotic remodeling of the heart represents a spectrum of responses that may vary depending on the specific etiology of myocardial injury; the stage of disease progression; and differences in age, gender, ethnicity, and genetic polymorphisms between individual patients. It therefore is essential to develop algorithms for stratification of risk in large patient populations. Preliminary results discussed above suggest that more extensive fibrosis may confer measurable risk and that individuals differ in their responses to therapy. However, these studies are in their initial stages compared to the quantitative databases that have accumulated for prognostic indicators, such as LV hypertrophy, serum cholesterol, or inflammatory status. Analysis of serum collagen metabolites in archival samples from large-scale clinical trials in relation to clinical outcome could offer a cost-effective starting point to obtain this type of information.

Development and standardization of surrogate markers of fibroblast activation or fibrosis are prerequisite to risk stratification and to evaluation of the efficacy of pharmaceutical agents. The utility and economy of serum collagen metabolites have been validated in this regard (5, 7–9). However, these measurements are limited because of their lack of specificity for cardiac versus extracardiac ECM remodeling, and the lack of sensitivity to detect key mechanistic steps in the remodeling process. Analysis of coronary sinus blood may provide a means to identify specific markers of cardiac ECM metabolism compared to general ECM markers in the systemic circulation.

Selection of appropriate clinical endpoints is critical to assess therapeutic benefit and to evaluate the relationship between target efficacy and clinical outcome. Reduction in mortality may be the preferred endpoint in younger patients, whereas reversal of disease progression or adverse events may assume greater importance in the elderly.

Antifibrotic agents will likely be useful adjuncts for combination therapy in selected patient populations, as is currently recommended for aldosterone antagonists. This approach offers the appeal of targeting complementary therapies for cardiac myocytes and cardiac fibroblasts. The development of agents that combine antifibrotic and antiinflammatory actions offers promise. However, issues of cost and safety of polypharmacy, especially in older patients, must be considered.

Identification of the windows of opportunity for antifibrotic therapy is likely to be crucial for effective intervention. Myocardial remodeling is progressive and cumulative as the heart passes from the initial response to injury through the transition to fibrosis and failure. It will be necessary to apply therapy at intervals that are appropriate to the molecular target. Furthermore, it may be more feasible to prevent fibrotic remodeling than to reverse it once it occurs. This is most likely to be the case for replacement fibrosis following myocyte loss. On the other hand,

reversal of reactive fibrosis may be more feasible as has been seen with regression of LVH and fibrosis in hypertension.

Finally, choice of physiologically appropriate experimental models is important to extrapolate preclinical findings to positive results in human trials. Studies with cultured fibroblasts in vitro typically examine responses to acute stimuli, and animal studies examine the initial onset of myocardial remodeling and failure in younger animals. By contrast, human heart failure is more commonly a progressive disease of the elderly, and clinical trials are aimed at therapy of established disease. Transgenic mouse technology has provided powerful insights into the roles of specific gene products in cardiovascular physiology, but the consequences of transgene expression throughout the life span of the animal may not accurately reflect the sequential and coordinated activation of myocardial remodeling in response to a pathological insult. Newer methodologies allowing conditional transgenic expression will help address this disparity.

In conclusion, the available evidence provides a strong rationale for therapies directed toward cardiac fibroblasts to improve outcomes in heart disease. Continued basic and translational research, and perseverance through the inevitable setbacks, will be needed to bring this promise to fruition.

ACKNOWLEDGMENT

Work in the authors' laboratories was supported by NIH (HL59428).

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Volume 45, 2005

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