The extracellular matrix (ECM) provides structural support and organization for cardiomyocytes and the vasculature of the heart. Under normal conditions, the ECM scaffold facilitates efficient force transduction for mechanical work, while mediating intercellular communication and metabolic exchange within the myocardial microenvironment. In the setting of increased wall stress, injury, or disease, the ECM can undergo a series of dynamic changes that lead to favorable chamber remodeling and functional adaptation. Over time, sustained matrix remodeling can impair diastolic and systolic function caused by excess deposition of interstitial fibrous tissue. These pathological alterations in ECM structure/function are considered central to the evolution of adverse cardiac remodeling and the development of heart failure. This review discusses the complex dynamics of the cardiac ECM in the setting of myocardial infarction, pressure overload, and volume overload. We also summarize the current status of ECM biomarkers that may have clinical value in prognosticating cardiac disease progression in patients. Finally, we discuss the most current status of drugs under evaluation for use in cardiac fibrosis. (Circ Res. 2014;114:916-927.)

Key Words: biological marker ■ collagen ■ extracellular matrix

The extracellular matrix (ECM) provides structural support and organization for cardiomyocytes and the vasculature of the heart. Under normal conditions, the ECM scaffold facilitates efficient force transduction for mechanical work, while mediating intercellular communication and metabolic exchange. This follows the unique 3-dimensional disposition of myocyte bundles and fibrillar collagens within the myocardium (Figure 1). With stress, injury, or disease, the ECM undergoes changes that potentiate inflammatory processes, myocardial protein turnover, tissue repair, and regeneration, which leads to organ remodeling and functional adaptation. However, sustained ECM remodeling can compromise proper diastolic and contractile functions as seen with fibrosis.

This review will discuss the complex dynamics of the ECM in the setting of cardiovascular conditions of myocardial infarction (MI), pressure overload (PO), and volume overload (VO) as both participant and contributor to the disease pathophysiology. Subsequent sections will focus on the translational aspects of identifying biomarkers that can prognosticate or identify disease progression in patients and the current status of ECM-focused antifibrotic therapies.
Myocardial Infarction

Based on experimental models, the myocardial ECM response to ischemic injury can be arbitrarily divided into 3 phases: (1) early injury response, (2) proliferation, and (3) late maturation. In rodents, the early injury response phase lasts from 0 to 48 hours after coronary occlusion, the proliferative phase between 2 and 5 days, and the maturation phase for ≈1 month. Timing is extended in large mammals, with early injury stage lasting several days, proliferation ≈2 weeks, and maturation 1 to 2 months or longer. Dominant features pertaining to ECM dynamics and associated factors for each phase of the injury response are discussed.

Early Injury Response Phase

Immediately after ischemic injury, endogenous ligands released by damaged cells can trigger host innate immunity, leading to the release of cytokines and chemokines thereby promoting the trafficking of inflammatory cells to site of injury. Local production of reactive oxygen species (ROS) and cytokines activates matrix metalloproteinases (MMPs), extracellular enzymes that degrade the ECM scaffold. MMPs such as interstitial collagenases MMP-1 and MMP-13 can cleave intact fibrillar collagen and proteoglycans. The resulting 75-kDa and 25-kDa fragments of collagen are further degraded by gelatinases (MMP-2 and -9) that are produced by infiltrating leukocytes and local cells. Degradation products of matrix proteins can also modulate inflammation and repair. For example, the degradation of collagen IV by MMP-9 gives rise to tumstatin, and degradation of collagen XVIII leads to increases in endostatin. Both tumstatin and endostatin are potent angiogenesis inhibitors. Similarly, cleavage of fibronectin liberates the extracellular A, which is proinflammatory and modulates transforming growth factor (TGF)-β-mediated myofibroblast transdifferentiation. MMPs can be inactivated by autodigestion or by a family of tissue inhibitors of metalloproteinases (TIMPs), such as TIMP-1, -2, -3, and -4.

Proliferative Phase

The initial injury response sets the stage for myofibroblast proliferation in which TGF-β and thrombospondin-1 (TSP-1) play pivotal roles. The activation of TGF-β is dependent on integrins which can release TGF-β from its binding site to interact with other proteins and their receptors. TGF-β is activated by additional protein interactions including TSP-1, which is an antiangiogenic glycoprotein secreted by macrophages. Transdifferentiation of myofibroblasts from multiple sources such as bone marrow fibrocytes, endothelial cells, epithelial cell, smooth muscle cells, and fibroblasts is considered central to repair. A combination of signals from TGF-β, fibronectin extracellular domain A, and mechanical stretch serves to transform fibroblasts into myofibroblasts. Myofibroblasts in turn secrete fibrillar collagens I and III during ECM remodeling.

MMPs also play key roles during the proliferation phases post-MI. Although they are important in myocardial repair, excessive activation can weaken the extracellular support, is proinflammatory, and ultimately detrimental. For example, membrane type 1 metalloproteinase increases significantly early after MI, yet its deletion in the mouse preserves type I collagen and improves survival. Other matricellular proteins that play important roles include TSP-1, which is also expressed early after MI. Consequently, its deletion induces inflammation and worsens remodeling. Osteopontin is an extracellular glycoprotein that promotes ECM maturation and is maximally upregulated during the proliferative phase. Deletion of osteopontin leads to blunted fibroblast responses to TGF-β, less type I collagen deposition, and worse ventricular remodeling. Other ECM glycoproteins whose expression is modulated after MI are tenascin-C and fibronectin. Tenascin-C is upregulated 3 to 5 days after MI, and levels diminish quickly to the point of being undetectable by day 14. By contrast, fibronectin levels peak during the late proliferative phase. Binding of fibronectin to integrins can also regulate cell growth and differentiation.
Maturation Phase

Continued collagen deposition, along with its maturation via cross-linking by lysyl oxidases, is essential for effective scar formation and healing. The scar’s tensile strength gradually increases, and myofibroblasts and excess vascular cells are then cleared by apoptosis. However, appropriate ECM responses are critical for preservation and restoration of myocardial function.

Defective ECM maturation is associated with compromised mechanical integrity and abnormal geometric expansion resulting in increased risk of cardiac rupture. Microcellular proteins such as secreted protein acidic and rich in cysteine/osteonectin, TSP-1, and periostin (discussed below) are also essential for this maturation-remodeling process. Excessive fibrosis, especially in the border or remote zone, can lead to diastolic stiffness, dyskinetic contraction, and arrhythmias. Their downregulation and deficiency on the contrary can adversely affect anatomic continuity, mechanical function, and ultimately survival.

Periostin is an ECM glycoprotein that is maximally upregulated after day 7 after MI. Periostin activates multiple integrins on cardiomyocytes and regulates their growth. Periostin deficiency can increase the rate of cardiac rupture secondary to lessened myofibroblast activation. Connective tissue growth factor (CTGF) is a matricellular protein of the heparin-binding family, which is stimulated by TGF-β and contributes to wound healing. CTGF also reaches its maximal protein expression around day 7 after MI. CTGF overexpression decreases infarct size with concomitant phosphorylation of the protein kinase B/p70S6 kinase/glycogen synthase kinase-3β salvage kinase pathway. Inhibition of protein kinase B abolishes cardioprotection. Syndecans are a group of cell-surface heparan sulfate proteoglycans that regulate inflammation and cell–matrix interactions during healing. After MI, border zone expression of syndecan-1 increases by day 7. Overexpression of syndecan-1 reduces cardiac dilation and dysfunction after MI. Syndecan-4 expression was shown to be much longer in rodents and patients, with maximal expression occurring late in maturation. Transgenic mice of syndecan-4 was protective after MI and was associated with increased infarct neovascularization, myofibroblast infiltration, decreased inflammation, lower cardiac rupture, and improved survival. Conversely, syndecan-4 knockout mice revealed an increased rate of cardiac rupture and mortality. Biglycan is another ECM protein belonging to the leucine-rich repeat proteoglycan family and is upregulated within the first week after MI. In the mouse, biglycan deficiency was associated with overexpression of MMP-2, -8, -9, and -13, and TIMP-1 and -4. Biglycan knockout mice exhibited a higher mortality because of rupture.

Pressure Overload

Chronic PO imposes excess mechanical load on the myocardium and triggers changes in ECM structure as a means to preserve function. Animal models of PO can be generated with vasopressors, transaortic constriction, or systemic hypertension. They have all shed light on the different dynamic changes that occur in ECM structure and protein composition.

Aortic constriction in rats induces marked increases of myocardial collagen I, III, fibronectin, and laminin, which can be partially reversed by the angiotensin-converting enzyme (ACE) inhibitor ramipril. Similarly, the β-blocker metoprolol attenuates upregulation of collagens I, III, and fibronectin in fibroblasts isolated from rat hearts subjected to aortic constriction. In a murine aortic constriction study, elevated collagen VIII levels were negatively correlated to left ventricular (LV) end-systolic and end-diastolic dimensions and tracked with the transition from LV hypertrophy to heart failure. Interstitial and perivascular fibrosis develops in the myocardium of spontaneously hypertensive rats. These changes are accompanied by increased stiffness and LV hypertrophy. Treatment with the ACE inhibitor lisinopril ameliorates peri-vascular and interstitial fibrosis while normalizing blood pressure and allowing for LV hypertrophy regression.

Matricellular proteins also contribute to ECM dynamics following PO. Microarray analysis of myocardium from spontaneous hypertensive rats with decompensated heart failure
revealed upregulation of TSP-4. TSP-1 and TSP-4 mRNA levels increase with angiotensin infusion and correlate with altered diastolic and systolic function as well as natriuretic peptide release. Osteopontin is also increased with PO and participates in cardiomyocyte hypertrophy. With excessive and prolonged PO stress, a vicious cycle of hypertrophy, fibrosis, and impairment of microvascular blood flow ensues. With continued buildup of contractile mass in parallel, the increase in wall tension leads to further injury and death of the myocyte, increased inflammation and MMP activity, progressive ventricular dilatation, and ultimately heart failure.

**Volume Overload**

VO imposes stress on the myocardial ECM and results in characteristic anatomic changes including ventricular dilatation. Using models of VO, such as aortocaval fistula in the rat, alterations in myocardial ECM structure can be arbitrarily classified into 3 stages: acute (hours to 2 weeks), compensatory remodeling (2–10 weeks), and decompensated failure (>15 weeks). The acute stage is characterized by rapid degradation of ECM and changes in collagen turnover. In the compensatory phase, there is a higher MMP/TIMP ratio, collagen isoform changes from type I to type III, and increased fibronectin and elastin. Mast cell infiltration with degranulation and the activation of the renin–angiotensin system also stimulates fibrosis. In the decompensated stage, there is further chamber dilatation, high ECM turnover, activation of MMP-1, -2, -9, and -13, and downregulation of TIMP-1 and -3. These trends are observed in animal models and in human cardiomyopathic hearts. There is also downregulation of elastin proteins, fibrillin-1 and fibulin-1, as well as decorin, which regulates collagen fiber assembly. Both tenascin-C and periostin levels are also increased, facilitating slippage between the cardiomyocytes and ECM. This is accompanied by the upregulation of inflammatory signals and downregulation of profibrotic cytokines such as TGF-β and CTGF.

**Differences in ECM in PO Versus VO**

Pathological remodeling with PO and VO have distinct characteristics. PO is characterized by progressive concentric hypertrophy of the ventricles, whereas VO is associated with eccentric dilatation (Figure 2). Collagen fibril content and connective tissue density are also much more prominent in pressure overload. Pressure load induces continuous deposition of collagen I and III, fibronectin, and laminin, whereas VO escalates turnover rate of ECM and leads to upregulation of MMPs, TIMPs, and procollagens. In the decompensatory failing stage of VO, increased matrix deposition occurs at later stages of the pathology. In terms of matricellular proteins, TGF-β has been shown to be upregulated in PO but suppressed in VO. Similarly, profibrotic CTGF increases in PO but is downregulated in VO. Moreover, matricellular proteins TSP-4, TIMP-1, and osteopontin are increased in PO.

The differences in the remodeling phenotype are likely the results of differences in relative microvascular blood flow and subsequent rates of matricellular protein turnover. In PO, the increase in hypertrophy without parallel increases in microvascular blood flow leads to increase in cell death, collagen deposition, and decreased matrix removal. The end result is excessive fibrosis with concomitant increases in myocardial stiffness. By contrast, with VO, there is initially preserved microvascular perfusion with increased MMP activity and matrix turnover. This leads to progressive ventricular remodeling without early fibrosis, thus maintaining ventricular compliance. It is only in later stages when wall tension and, thus, oxygen demand are high that it can trigger cell death and increased net collagen deposition, leading ultimately to heart failure.

**Biomarker Protein Profiling of the ECM**

Pathological changes in myocardial ECM structure that occur with adverse remodeling (eg, post-MI, PO, or VO) can be assessed directly via myocardial biopsy techniques and probed noninvasively by imaging modalities. Indirect assessments of ECM dynamics can also be evaluated in the periphery via monitoring of circulating biomolecules of ECM composition (ECM proteins and associated metabolites) as well as proteins involved in ECM remodeling activity. Efforts to discover circulating biomolecules or biomarkers that reflect these dynamic changes in ECM composition, structure, and function in the setting of heart disease have been the focus of

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**Ventricular remodeling with pressure and volume overload**

<table>
<thead>
<tr>
<th>Pathologic Alterations</th>
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<td>TGF-β</td>
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<td>Renin-Angiotensin system activation</td>
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**Figure 2. Changes in the cardiac extracellular matrix (ECM) as reported during ventricular remodeling with pressure (PO) or volume overload (VO).** Select pathological alterations are noted for structural members or recognized regulators of the cardiac ECM. CCN2/CTGF indicates connective tissue growth factor; MMP, matrix metalloproteinase; TGF-β, transforming growth factor-β; TIMP, tissue inhibitors of metalloproteinase.
intense investigation. Several candidate biomarkers that relate to myocardial ECM are reviewed.

**Collagen Metabolites**

It is known that increases in fibrillar collagen type I and III are associated with the evolution of hypertensive and ischemic heart disease. Type I collagen is mainly responsible for increased fibrosis, which leads to decreased compliance and impaired diastolic and systolic function. During post-MI healing, type III collagen is deposited early followed by type I collagen. In the circulation, markers of collagen metabolism have been shown to increase with cardiovascular disease and myocardial fibrosis. Detection of amino- and carboxy-terminal propeptides of procollagen type I and III in serum coincides with increases in tissue collagen synthesis. Biomarker studies have shown that serum concentrations of the carboxy-terminal propeptide of procollagen type I positively correlated with collagen content detected in myocardial biopsies of patients with heart failure. Importantly, serum propeptide of procollagen type I was assayed directly from the coronary sinus of these patients indicating a direct correlation with myocardial fibrosis. The amino-terminal propeptide of procollagen type III has been reported to rise with insulin resistance in normotensive, nondiabetic obese subjects and may be predictive of early LV dysfunction. Support for this concept comes from a recent report by Lopez-Andrés et al., which showed a positive correlation between serum propeptide of procollagen type III and adverse cardiovascular outcomes in subjects with heart failure from the Cardiac Resynchronization in Heart Failure (CARE-HF) trial. Thus, circulating propeptide of procollagen type III might have diagnostic and predictive value as a biomarker of heart failure disease status.

The reciprocal process of myocardial collagen degradation can be detected in the circulation via assessment of the carboxy-terminal telopeptide of type I collagen. Detection of serum/plasma telopeptide of type I collagen has been correlated with pathological remodeling in the context of hypertension as well as heart failure. In patients with dilated cardiomyopathy, serum telopeptide of type I collagen levels >7.6 μg/L were positively associated with increased mortality. These studies are among a long list of positive, as well as some conflicting, demonstrations of these metabolites as biomarkers of cardiovascular disease. Overall, the predictive and diagnostic potential of collagen metabolites in heart failure seems encouraging. Further characterization will undoubtedly provide added insights into their utility as circulating biomarkers.

**Galectin-3**

Recent data suggest involvement of galectin-3 in the evolution of heart failure via myocardial inflammation, cardiac remodeling, and fibrosis. Galectin-3 (also termed Mac-2) is secreted by macrophages and has been shown to bind to a wide array of ECM proteins including laminin, fibronectin, and tenascin. Galectin-3 has carbohydrate recognition and collagen-like domains that enable interactions with a wide variety of matrix molecules. Circulating levels of galectin-3 have been reported to positively associate with increased risk for new onset of heart failure in ostensibly healthy subjects. Among patients with established heart failure, galectin-3 correlates with the severity of disease as well as adverse outcome. Moreover, patients with LV systolic dysfunction who had an adverse cardiovascular event present higher circulating galectin-3 levels than nonevent patients. In the same study, galectin-3 concentrations <20 ng/mL were associated with an overall lower rate of cardiovascular events for the study cohort. Interest in galectin-3 goes beyond that of a surrogate disease biomarker as evidence suggests that it contributes to heart failure pathophysiology. Preclinical studies indicate that continuous infusion of low-dose galectin-3 into the pericardial sac of healthy rats leads to LV dysfunction. At this point in time, the function of galectin-3 as a biomarker versus a mediator of disease is still an area of active research.

**Advanced Glycation End Products**

Nonenzymatic reactions between glucose and collagens can lead to the formation of stable advanced glycated end products (AGEs). Over time, collagen-associated AGEs accumulate within the ECM attributable to their slow turnover rate and exert detrimental effects on cardiovascular function. Accumulation of AGEs within the myocardium diminishes compliance leading to diastolic LV stiffness. Specific cross-linking of AGEs with matrix proteins has been detected on type I collagen and elastin. AGEs monitored in the circulation positively correlate with heart failure severity and progression of disease. These circulating AGEs, although not glycated collagen metabolites per se, were positively associated with increased stiffness of myocardium in diabetic patients with early coronary artery disease. Clinical studies describing the use of AGE cross-link breakers are discussed in a subsequent section.

**Matrix Remodeling Enzymes**

As discussed in previous sections of this review, the degradation of ECM and collagen is mediated in large part by MMPs whose actions can be antagonized by proteins such as TIMPs. The use of MMPs, TIMPs, or their ratios as biomarkers of cardiovascular disease progression such as for hypertension is supported by clinical studies demonstrating relationships between disease status and MMP blood concentration. In a study of 53 patients with MI, circulating MMP-9 was found to rise significantly as early as day 1 after MI. Early elevation of MMP-9 was associated with LV dilation risk. Temporal changes in other MMPs such as MMP-8 and MMP-2 were also described. Circulating TIMP-1 levels rose acutely and TIMP-4 decreased to baseline levels 1 week after MI and remained depressed. These findings are encouraging and suggest the use of MMPs as prognostic markers of cardiovascular disease evolution. However, as to which marker is best suited to reflect changes in specific cardiovascular disease phenotypes is currently unclear. Regardless, their potential application as biomarkers remains promising.

**Antifibrotic Therapeutics**

The development of tissue fibrosis can severely impair organ function. This is true for most if not all tissues and organs including the lungs, kidneys, liver, and peritoneum. Proper heart function relies on having a sufficient number of myocytes to...
provide contractile function and optimal mechanical tissue properties to allow for efficient blood filling. Long-standing impaired contractile function is a hallmark of systolic heart failure. By contrast, chronic impairment in ventricular relaxation can lead to heart failure with preserved ejection fraction. In the setting of congestive heart failure, the myocardium typically suffers both from myocyte death and excess fibrotic tissue deposition where it can comprise >30% of the cardiac mass (versus 2%–3% in normal tissue).66

The development of heart failure and adverse cardiac remodeling is driven by increased activation of various hormonal and local humoral systems. As such, these processes have become the logical target of antifibrotic drug intervention strategies. Antifibrotic therapies have been validated extensively in preclinical studies and to a limited extent in the clinic. The fundamental processes that drive cardiac fibrosis are common to most tissues and organs. For example, chronic inflammation and excess ROS generation play significant roles in the development of fibrosis.62 Similarly, proinflammatory cytokines such as TGF-β also drive fibrosis.62,63 A targeted approach to develop antifibrotic therapies aimed at these pathways has been an area of active research. However, success has been limited due to the prolonged nature of cardiac fibrosis pathology and the difficulty in early stage diagnosis. In addition, fibrosis tends to occur in the setting of complex pathologies and entails a terminal process whose reversibility may be limited.

There are particularities about the heart that warrant special attention. One issue relates to the timing of treatment after MI.64 It is generally accepted that drugs whose effects attenuate collagen production should be avoided early after MI because they may impair the formation of a mature scar. In this category are anti-inflammatory agents such as indomethacin or prednisone. Use of these agents has led to ventricular rupture and is essentially avoided as post-MI therapies.65 The best therapeutic strategy for the treatment of fibrosis is prevention. This implies early risk detection and diagnosis. This may be practical after a major event such as MI but less so for other pathologies that can remain undetected and asymptomatic for years. The following sections cover humoral systems recognized to be altered and thus targeted for treatment in the prevention and therapeutic treatment of diseases that compromising cardiac structure and function. RAAS exists both as a circulating (ie, humoral) and as a local system, which is found within particular cells and tissues such as the myocardium and brain.67 Central to its profibrotic actions are the well-characterized effects of angiotensin II and aldosterone, which are byproducts of the RAAS. The levels of both hormones rise in the setting of various cardiovascular and metabolic diseases.68,69 Their profibrotic effects can be triggered by their actions on macrophages, fibroblasts, and smooth muscle cells. The effects of angiotensin II are mediated by their actions on angiotensin II receptor type I (AT1) receptors. AT1 receptors are present on cardiac myocytes, fibroblasts, myofibroblasts, macrophages, endothelial, and smooth muscle cells.70–73 These receptors can directly couple to pathways that promote fibrous tissue production and deposition in capable cells (eg, myofibroblasts) or indirectly by stimulating the production of autocrine/paracrine factors that can exert profibrotic effects such as TGF-β, osteopontin, or endothelin.74,75 The pharmacodynamic effects of aldosterone are mediated through the mineralocorticoid receptor.76 However, the mechanisms by which aldosterone exerts its profibrotic effects remain unclear.77

Multiple studies using cells in culture, animal models, and clinical trials have documented the antifibrotic potential of ACE inhibitors or AT1 blockers. Cardiac fibroblasts can produce angiotensin II and as such are susceptible to the effects of ACE inhibitors.78 Because fibroblasts also express AT1 receptors that couple to signaling pathways that stimulate collagen production, their blockade also translates into the suppression of fibrosis.71,79 Furthermore, as noted above, the binding of angiotensin II to the AT1 receptor is known to stimulate the production of other profibrotic factors (TGF-β), which further reinforces such responses.80 Crucial to the validation of these agents as antifibrotic compounds are studies using chronic animal models of cardiac remodeling and hypertension. In a study using aged spontaneously hypertensive rats with severe cardiac remodeling and fibrosis, treatment with lisinopril was effective in reducing chamber hypertrophy, fibrosis, and ventricular stiffness.81 In an animal model of cardiac remodeling and fibrosis secondary to a Duchenne-like muscular dystrophy, the use of losartan for 2 years led to an impressive reduction in cardiac fibrosis.82 A limited number of clinical studies have provided further support for the use of angiotensin II–targeted drugs as suppressors of cardiac fibrosis. Brilla et al83 demonstrated that in hypertensive patients treated with lisinopril for 6 months, significant reductions in myocardial fibrosis occurred accompanied by improved diastolic function. In particular, the use of hydrochlorothiazide in a different cohort of patients did not reduce fibrosis or improve diastolic function. An added benefit of ACE inhibitor treatment is the increase in myocardial bradykinin, which is known to suppress collagen production by fibroblasts via a NO-cGMP–dependent pathway.84 Although the aforementioned evidence argues for direct effects of ACE inhibitors or AT1 blockers on fibrosis relevant end points, it is also possible that such effects may follow other actions of such drugs. For example, reports indicate that these classes of drugs can ameliorate cardiac myocyte death.85

Evidence obtained from animal and clinical studies indicates that exposure to high aldosterone levels or activation of the mineralocorticoid receptor can induce tissue damage and inflammation leading to fibrosis.87 Such changes can...
be prevented by adrenalectomy or administration of receptor antagonists.77,87 Two agents currently exist in the market as receptor antagonists. One is spironolactone, a nonspecific blocker of the mineralocorticoid receptor. The second is eplerenone, a more selective mineralocorticoid antagonist. Studies have tested the effects of these drugs in patients with heart failure with preserved ejection fraction at doses that do not lower blood pressure. In one study, patients were randomized to conventional treatment with or without eplerenone and LV function was assessed by echocardiography at 6 and 12 months. In treated patients, deceleration time (ie, LV relaxation) decreased more than those on conventional treatment. At 12 months, improvement in diastolic function was associated with a significantly slower increase in plasma procollagen metabolites.88 Edwards et al89 reported similar data in patients with heart failure with preserved ejection fraction where the effects of spironolactone on LV function and circulating markers of collagen turnover were compared with placebo. After 40 weeks of treatment, spironolactone improved markers of LV relaxation and significantly attenuated increases in type III procollagen peptides.90 Altogether, these results suggest that targeting angiotensin II and aldosterone with readily available drugs can be used to limit the development of fibrosis and potentially mediate partial reversal of profibrotic pathology.

β-Blockers
β-Receptor blockers have been incorporated as standard treatment for limiting cardiac remodeling associated with MI and heart failure.90 The β1 receptor subtype is recognized as the most relevant in driving detrimental changes in remodeling of myocardium.91 Treatment of patients with heart failure with the nonselective blocker carvedilol or the β1-selective blockers metoprolol or bisoprolol has demonstrated reduced mortality.91 However, no studies have reported on reduction of fibrosis, thus bringing into question the use of β-blockers for such purposes. The only agent that has demonstrated a capacity to reduce fibrosis in animal studies is carvedilol. Antifibrotic effects have been observed using animal models of infarction and heart failure.92,93 However, the benefits of carvedilol which has pleiotropic properties may be derived from indirect actions on reducing cytokine levels, via antioxidation (a recognized feature of this drug) or anti-inflammatory effects.93

Endothelin Receptors Blockers
Endothelin-1 is a potent vasoconstrictor that binds to 2 types of receptors, ETα and ETβ.94 As with smooth muscle cells, cardiac fibroblasts express both receptors and their stimulation leads to increased production of collagens.95 Endothelin production is stimulated by humoral factors such as angiotensin II, thus serving to reinforce their profibrotic actions.96 Attenuating circulating endothelin levels with ACE inhibition has been suggested as an effective approach to reduce myocardial fibrosis. However, nonselective endothelin receptor blockers (bosentan) and ETα selective blockers (ambrisentan) are also available in the market and used to treat pulmonary hypertension and fibrosis.96 Because endothelin levels were recognized to be elevated in patients with heart failure, it became an obvious target for blockade. Extensive preclinical studies supported this concept, and associated reports documented reductions in myocardial fibrosis and improved diastolic function.97,98 Clinical trials were implemented using both types of blockers in patients with heart failure (Effects of the Endothelin Receptor Antagonist Bosentan on the Morbidity and Mortality in Patients With Chronic Heart Failure [ENABLE] and Endothelin Receptor Antagonist Trial in Heart Failure [EARTH]). Both trials failed to meet their primary efficacy end points of morbidity, mortality, or chamber size and thus prevented further development for such indications.99 Thus, their use as antifibrotic agents currently remains unclear.

Natriuretic Peptides
An interesting group of molecules recognized as potent antifibrotic are natriuretic peptides and, in particular, brain natriuretic peptide (BNP). Preclinical studies in transgenic animals in which BNP expression was ablated revealed extensive cardiac fibrosis.100 Similarly, mice lacking the natriuretic receptor-A also developed cardiac hypertrophy and fibrosis.101 Subsequent studies demonstrated that cardiac fibroblasts can produce BNP and that treatment of fibroblasts with exogenous BNP exerts antifibrotic effects.102 Such actions seem to be mediated by increases in cGMP levels. Currently, only one drug of this category (nesiritide, a recombiant form of BNP) is approved for acute decompensated heart failure and is only available in intravenously-administered formulation.103 However, efforts are in place to develop more potent engineered forms of peptides that may extend half-life and pharmacological effects. C-terminal tail of dendroasps natriuretic peptide (CD-NP) is a novel peptide designed to activate both guanylyl cyclase A and B. In a proof-of-concept study in patients with chronic heart failure, CD-NP activated cGMP, suppressed aldosterone, and preserved renal function without reducing blood pressure.104 Thus, this class of novel engineered peptides may hold promise as agents that can potently suppress the production of fibrillar collagens in the heart.

Cytokine Modulators
As noted above, there are multiple cytokines that are recognized as potent stimulators of fibrotic responses. One noted example is TGF-β, which has been shown to exert profibrotic effects.63 Over the years, TGF-β has been the focus of therapeutic strategies aimed at suppressing its levels or blocking its receptor binding activity.105 However, as with most other cytokines, their actions are pleiotropic and often compensated by other ligands. In the case of TGF-β, it has strong immunomodulatory effects that make its targeting inherently risky. Pirfenidone is a drug developed for the treatment of idiopathic pulmonary fibrosis and has gained approval for use in Europe, Japan, and Canada.106 Pirfenidone has well-established antifibrotic and anti-inflammatory properties in various in vitro systems and animal models of fibrosis. Several studies have shown that pirfenidone reduces the secretion of TGF-β and of other inflammatory mediators such as tumor necrosis factor-α and interleukin-1β in both cultured cells and isolated human peripheral blood mononuclear cells.107,108 Animal studies of hypertension and diabetes mellitus have demonstrated its capacity to suppress cardiac fibrosis and diastolic dysfunction.109,110 Currently, it has not yet gained approval for use in the United States for the treatment of pulmonary fibrosis, and future trials would need to be implemented to explore its cardiovascular application potential.
Modulators of Inflammation, Oxidative Stress, and MMPs

As discussed above, the development of tissue fibrosis is seen as a terminal process that ensues in the context of a chronic inflammatory response. The development of a profibrotic phenotype in fibroblasts is closely aligned to the presence of inflammatory cells such as macrophages.111 Macrophages have the capacity to secrete multiple humoral factors such as cytokines that can prime fibroblasts to acquire a profibrotic phenotype in addition to releasing a variety of proinflammatory molecules (eg, ROS, proteases) that can further enhance the inflammatory/profibrotic milieu. It follows that strategies aimed at mitigating inflammation could serve to ameliorate tissue fibrosis. Corticosteroids offer the best example of such a strategy because they can effectively ameliorate tissue fibrosis by directly suppressing inflammation and the production of proinflammatory cytokines. Animal studies have validated their effectiveness in reducing cardiac fibrosis.64 However, because their short- or long-term use is fraught with serious risks, this approach has never gained footing. The use of anti-inflammatory agents as antiﬁbrotics for cardiovascular applications is still an area of active investigation, and further studies are needed.

Excess generation of ROS has been recognized as a strong stimulator of myocardial damage and pathological collagen production. ROS-mediated tissue damage caused by oxygen radicals has been shown to active proteases that can degrade the ECM. Studies in animal models have supported the use of antioxidant strategies to lessen tissue damage and reduce fibrosis.112 However, no clinical studies have validated this strategy to ameliorate myocardial fibrosis. For example, the use of vitamin E supplementation in high-risk patients failed to reduce cardiovascular events.113 As a counterpart, compounds such as carvedilol or enalapril may partly owe their pharmacology to attenuation of tissue oxidative stress.90,114

The normal turnover of fibrillar collagen is considered essential for maintaining the structural integrity of the ECM. However, in circumstances in which excess turnover occurs (eg, pathological cardiac remodeling), the sustained activation of MMPs can facilitate pathological remodeling and fibrosis.115 MMPs were recognized as central mediators of fibrosis.116 MMP inhibitor available for clinical use and is marketed under the name Periostat (used to treat periodontal disease). Doxycycline broadly inhibits MMP activity, scavenges ROS, and suppresses cytokine production.116 In animal models of cardiac injury, treatment with doxycycline has been shown to improve cardiac function and attenuate adverse cardiac remodeling.116 Moreover, use of doxycycline in animal models of bleomycin-induced lung injury reduced fibrosis development.117 The Early Short-term Doxycycline Therapy in Patients with Acute Myocardial Infarction and Left Ventricular Dysfunction to Prevent the Ominous Progression to Adverse Remodeling (TIPTOP) clinical trial evaluating the efficacy of submicrobial doses of doxycycline (100 mg for 7 days BID started immediately after percutaneous intervention, n=110) on post-MI remodeling was recently completed and evidenced significant decreases in LV end-diastolic volumes, infarct size, and severity.118 Larger studies will be needed to validate the use of these compounds as a means to limit cardiac remodeling and myocardial fibrosis.

Cross-Link Breakers

The chemical maturation of fibrillar collagens involves the formation of AGE cross-links between adjacent molecules.119 Cross-linking stabilizes the molecule and increases the half-life of fibrillar collagen (which is 3 months), which makes the fibrils more resistant to degradation.120 Cross-linking is known to increase in diseases such as hypertension and diabetes mellitus and with aging.119 Several compounds have been described that can chemically break AGE cross-link including aminoguanidine and alagebrum. For example, in 6-month-old rats, long-term treatment (18 months) with aminoguanidine prevented AGE accumulation in aorta and myocardium and preserved vascular function.121 Alagebrum, also known as ALT-711, was administered to aged dogs and reduced LV stiffness and improved stroke volume.122 This compound was used in multiple phase I and II cardiovascular clinical trials with initial promising outcomes.123 However, the most recent trial in patients with heart failure (n=102) failed to improve exercise tolerance, systolic function, and other end points of cardiovascular function.124 Despite these findings, future studies on AGE-modified collagens may still yield important insights for cardiovascular drug development.

Relaxin

Currently, one of the most promising approaches to treat cardiac fibrosis is treatment with relaxin. Relaxin is a peptide hormone that belongs to the insulin superfamily and binds to leucine-rich repeat-containing G-protein-coupled receptor 7 (relaxin family peptide receptor 1) and leucine-rich-repeat-containing G-protein-coupled receptor 8 (relaxin family peptide receptor 2) receptors.125 Relaxin was initially characterized in the context of female reproductive biology in which it mediates the softening of cervical tissue and the pubic ligament.125 In females, it is produced by the ovary and in males by the prostate, but it is also produced locally by many cells, allowing it to act in an autocrine/paracrine manner.125 Crucial to its understanding as a mediator of matrix degradation was the observation that relaxin knockout mice develop fibrosis at multiple sites (including the heart) with aging and that the accumulation of excess collagen could be reversed by restoring relaxin levels.126 In cultured cardiac fibroblasts, relaxin suppresses multiple profibrotic markers.127 In animal models of MI, relaxin treatment...
reduces cardiac hypertrophy and collagen deposition. In transgenic mice that develop severe fibrosis, the expression of relaxin by adenoviral delivery notably suppresses cardiac collagen.\textsuperscript{1,2} Using spontaneously hypertensive rats, a recent report documented the beneficial effects of relaxin on atrial fibrillation by reversing fibrosis.\textsuperscript{3,4} There have been a limited number of relaxin clinical studies for cardiovascular indications. A recent trial (Relaxin in Acute Heart Failure [RELAX-AHF]) in patients with acute heart failure using recombinant relaxin (Serealxin) was completed and reported beneficial effects on dyspnea and 180-day mortality.\textsuperscript{13} However, its effects on fibrosis are less clear as demonstrated by the lack of efficacy reported in patients with scleroderma.\textsuperscript{12} Regardless, several major drug companies continue to pursue the development of relaxin-based programs using engineered forms of the peptide.

Conclusions

The cardiac ECM is a dynamic entity that is intrinsically regulated by multiple systems whose homeostasis is perturbed in the setting of disease. The need for therapies to treat patients with pathological myocardial fibrosis is becoming increasingly evident. At present, patients with ventricular dysfunction associated with cardiac fibrosis have few therapeutic options available to them. As the incidence of heart failure with preserved ejection fraction continues to rise, the need for targeted approaches that address the underlying pathologies of cardiac fibrosis and prevent disease evolution is critical. One approach is to identify the problem at an early stage and treat proactive-ly using drugs recognized as useful, such as those targeting the RAAS. The other approach is to validate those that appear promising in well-designed clinical studies. In addition, effective appraisal of patient risk and response to treatment using diagnostic and prognostic biomarkers will also enhance the clinical impact of targeted therapeutic interventions. We think that these approaches accompanied by new developments in the field (ie, preclinical model development, novel imaging modalities, diagnostic methods) will improve our understanding of myocardial ECM disease and enable targeted drug development strategies in this important area.

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References


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