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Mechanisms of Fibroblast Activation in the Remodeling Myocardium

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Abstract

Purpose of Review—Activated fibroblasts are critically implicated in repair and remodeling of the injured heart. This manuscript discusses recent progress in the cell biology of fibroblasts in the infarcted and remodeling myocardium, highlighting advances in understanding the origin, function and mechanisms of activation of these cells.

Recent Findings—Following myocardial injury, fibroblasts undergo activation and myofibroblast transdifferentiation. Recently published studies have suggested that most activated myofibroblasts in the infarcted and pressure-overloaded hearts are derived from resident fibroblast populations. In the healing infarct, fibroblasts undergo dynamic phenotypic alterations in response to changes in the cytokine milieu and in the composition of the extracellular matrix. Fibroblasts do not simply serve as matrix-producing cells, but may also regulate inflammation, modulate cardiomyocyte survival and function, mediate angiogenesis, and contribute to phagocytosis of dead cells.

Summary—In the injured myocardium, fibroblasts are derived predominantly from resident populations and serve a wide range of functions.

Keywords

fibroblast; myofibroblast; myocardial infarction; cardiac remodeling; cytokine

Introduction

Heart failure is a major cause of morbidity and mortality in western societies [1]. Despite extensive research in the field, prognosis for patients with heart failure remains poor, reflecting our limited understanding of the pathophysiology of the disease and the challenges in development and implementation of new therapeutic strategies [2]. Cardiac fibrosis is one of the major pathophysiologic underpinnings of heart failure [3]. Expansion

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Compliance with Ethics Guidelines

Conflict of Interest

Arti Shinde and Nikolaos Frangogiannis declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent

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of the cardiac interstitium and deposition of extracellular matrix proteins are consistently noted in experimental models of heart failure and in human patients with cardiomyopathic conditions, regardless of etiology. In the injured heart, fibrosis is an important part of the reparative response. Cardiomyocytes have very limited regenerative capacity; as a result, sudden loss of significant amounts of cardiac muscle in myocardial infarction activates a fibrotic response that preserves the structural integrity of the heart, preventing catastrophic events, such as cardiac rupture. The reparative function of cardiac fibrosis is dependent on timely activation and suppression of signals that mediate matrix deposition. Excessive or prolonged fibrogenic activation following myocardial injury increases chamber stiffness causing diastolic dysfunction. Moreover, perturbations of the myocardial architecture in fibrotic hearts can also trigger systolic dysfunction [4].

Fibroblasts are the central cellular effectors of fibrosis. Following myocardial injury, fibroblasts undergo dramatic phenotypic changes in response to microenvironmental alterations in the cytokine milieu and in the composition of the extracellular matrix [5],[6],[7],[8]. Traditional concepts paint a unidimensional picture of cardiac fibroblasts, as the main cellular source of extracellular matrix proteins in the injured myocardium [9]. However, a growing body of evidence suggests that fibroblasts are functionally and phenotypically heterogeneous, and may play diverse roles in cardiac homeostasis and disease [10],[11],[12],[13]. The current review manuscript discusses recent advances in our understanding of the biology of fibroblasts in cardiac remodeling. We will focus on the cellular origin and function of activated fibroblasts in infarcted and remodeling hearts, and we will discuss key molecular signals implicated in fibroblast activation.

Fibroblasts in normal myocardium

Extensive experimental evidence suggests that the adult mammalian heart contains a large population of interstitial cells; many of these cells exhibit fibroblast-like characteristics. Early reports using transmission electron microscopy suggested that fibroblasts may be the most abundant myocardial cells [14]. More recent studies using combinations of markers for cell labeling suggested that in adult mouse hearts less than 20% of non-cardiomyocytes can be identified as fibroblasts [15]. The relative abundance of myocardial fibroblasts reported in different studies varies depending on the species, gender and age of experimental subjects, and on the markers used for cell identification. The absence of specific markers is a major limitation for definitive identification of fibroblast populations in both normal and injured hearts.

The role of fibroblasts in cardiac homeostasis remains poorly understood. In vitro studies have suggested that embryonic cardiac fibroblasts stimulate cardiomyocyte proliferation, whereas adult cells promote hypertrophy [16]. In the absence of injury, resident cardiac fibroblasts may serve to maintain the cardiac extracellular matrix network. Because of their abundance and their close interactions with cardiomyocytes and vascular cells, fibroblasts may also play an important role in regulating baseline cardiac function. However, in vivo experiments testing this intriguing hypothesis have not been performed.

Fibroblasts in the infarcted myocardium

The adult mammalian heart has limited regenerative capacity; as a result, sudden death of a large number of cardiomyocytes following infarction triggers a reparative response, forming a collagen-based scar that preserves the structural integrity of the ventricle [17]. Cardiac repair following myocardial infarction can be divided in three distinct but overlapping phases: the inflammatory phase, the proliferative phase, and the maturation phase [18]. In response to the dramatic changes in the cytokine milieu and to the alterations in composition of the surrounding extracellular matrix following infarction, cardiac fibroblasts exhibit dynamic phenotypic changes during the 3 phases of cardiac repair [5],[19],[20].

The fibroblasts during the inflammatory phase of infarct healing

In the infarcted myocardium, necrosis of cardiomyocytes activates innate immune signaling pathways triggering an intense inflammatory reaction [21],[22], associated with marked upregulation of pro-inflammatory cytokines and chemokines [23]. Upon stimulation with interleukin (IL)-1 or tumor necrosis factor (TNF)-α, cardiac fibroblasts are capable of secreting large amounts of pro-inflammatory mediators and proteases [24],[25],[13]. Considering their relative abundance and their strategic location in close proximity to vessels and cardiomyocytes, fibroblasts may be important cellular effectors of the post-infarction inflammatory response. Although in vivo experiments testing this hypothesis have not been performed, a growing body of evidence suggests that resident cardiac fibroblasts may promote early post-ischemic dysfunction, at least in part, through activation of a pro-inflammatory program [12],[11].

The fibroblasts during the proliferative phase: myofibroblast transdifferentiation

Activation of endogenous pathways that inhibit innate immune signaling and suppress pro-inflammatory activation [26] marks the transition from the inflammatory to the proliferative phase of infarct healing. As the neutrophil infiltrate is cleared by macrophages, fibroblasts expand and undergo myofibroblast transdifferentiation [13], expressing contractile proteins, such as α-smooth muscle actin (αSMA) (Figure 1), and secreting large amounts of extracellular matrix proteins [27],[28]. Activated fibroblasts play a critical role in preservation of the structural integrity of the infarcted ventricle [10]; however excessive or prolonged activation of fibroblast populations may reduce ventricular compliance, promote adverse remodeling and precipitate heart failure [29]. In addition to their established role in matrix synthesis, injury-associated myofibroblasts (or specific subsets of these cells) may serve a wide range of additional roles. In the infarcted myocardium, activated fibroblasts have been implicated in phagocytosis of dead cells [30]. Moreover, activated fibroblasts may modulate cardiomyocyte survival, hypertrophy and function under conditions of stress [31]. Recent evidence has suggested that following injury, myocardial fibroblasts exhibit remarkable phenotypic plasticity and may generate endothelial cells contributing to neovascularization [32].

Although the heart contains abundant resident cardiac fibroblasts that can respond to activating signals, several other potential cellular sources have been proposed to explain the expanding myofibroblast population in the infarcted and remodeling myocardium. Endothelial cells, hematopoietic fibroblast progenitors, pericytes and vascular smooth muscle cells, epicardial epithelial cells have been proposed as important contributors to myocardial fibrotic responses (Figure 1e) [33]. Over the last 10 years several investigative groups have combined bone marrow transplantation experiments, parabiosis and lineage tracing strategies to investigate the cellular origin of fibroblasts in the infarcted and remodeling myocardium [34],[35],[36],[37],[38],[10]. Interpretation of the findings is inherently challenging because of the functional and phenotypic heterogeneity of fibroblast populations and the lack of specific molecular markers to identify fibroblasts [39]. Moreover, it should be emphasized that the relative contributions of various cell types may depend on the type of myocardial injury. In pathophysiologic conditions associated with extensive cardiomyocyte necrosis (such as myocardial infarction), intense upregulation of chemokines may drive recruitment of non-resident populations that may significantly contribute to the activated fibroblast populations. Table 1 provides an overview of recently-published investigations examining the cellular origin of activated fibroblasts in infarcted and pressure-overloaded hearts. Although earlier studies have suggested important contributions of endothelial cells [34],[35] and hematopoietic progenitors [36], recent investigations combining lineage tracing approaches with several distinct Cre drivers suggested that subpopulations of resident cardiac fibroblasts are the main source for activated myofibroblasts in infarcted and remodeling hearts [37],[38],[10].

Signals mediating myofibroblast activation in the remodeling myocardium

Myofibroblast activation in the infarcted and remodeling myocardium requires the co-operation of growth factors and specialized matrix proteins, which signal through cell surface receptors to activate transcription of extracellular matrix proteins. Macrophages, mast cells and lymphocytes infiltrating the remodeling heart play an important role in fibroblast activation by secreting a wide range of bioactive mediators, including cytokines (such as Transforming Growth Factor (TGF)-β and IL-10) and matricellular proteins [40], [41],[42],[43],[44]. Stimulated cardiomyocytes and vascular cells in the area of injury may also activate molecular cascades that modulate fibroblast behavior [45].

Activation of the renin-angiotensin-aldosterone system signaling plays an important role in fibroblast proliferation and activation in the infarcted and remodeling myocardium. Experimental studies have demonstrated that angiotensin type 1 receptor (AT1) and aldosterone signaling activate fibroblasts in healing myocardial infarcts [46],[47]. Clinical studies in human patients with acute infarction support this concept demonstrating that administration of an aldosterone antagonist reduces the levels of circulating markers of collagen synthesis [48]. Moreover in patients with hypertensive heart disease, AT1 blockade significantly reduced indicators associated with myocardial fibrosis [49].

The pleiotropic mediator TGF-β also plays a crucial role in activation of fibroblasts in the remodeling myocardium. TGF-β isoforms are markedly upregulated in the infarcted and remodeling myocardium and are secreted by macrophages, fibroblasts, platelets, vascular

cells and cardiomyocytes in a latent form [50],[51]. Activation of TGF-β in the cardiac interstitium requires protease actions and an interaction with the matricellular protein thrombospondin-1 [52],[53]. Following activation, the TGF-β dimer binds to a heterodimeric complex of TGFβ receptor I and II activating canonical signaling cascades that involve the intracellular effectors Smad2 and Smad3, and triggering Smad-independent pathways. Smad3 signaling appears to play an important role in fibroblast-mediated matrix synthesis and αSMA expression [54],[55]. Although effects of Smad-independent pathways have been documented in hypertrophic remodeling and dysfunction of cardiomyocytes in the pressure-overloaded myocardium [56], the in vivo role of non-Smad signaling in cardiac fibroblast function has not been documented.

Recent studies have revealed that profibrotic mediators, such as angiotensin II or TGF-β act by activating the Transient Receptor Potential (TRP) Channel- calcineurin axis. In fibroblasts, TRPC6 is induced through TGF-β-mediated Smad-independent signaling and is implicated in cardiac myofibroblast transdifferentiation by activating a calcineurin-Nuclear Factor of Activated T cells (NFAT) cascade [57],[58]. Experiments in atrial fibroblasts suggested that TRPM7 is implicated in TGF-βinduced calcium signaling and in myofibroblast transdifferentiation [59] TRPV4 is also involved in cardiac myofibroblast activation by integrating signals from secreted growth factors (such as TGF–β) and mechanosensitive stimuli [60].

Fibroblast de-activation, quiescence and apoptosis in the infarcted and remodeling myocardium

As the healing scar matures, myofibroblasts become quiescent, reducing synthesis of extracellular matrix proteins. Many myofibroblasts in the infarct border zone may undergo apoptosis. Despite their potential importance in protecting the infarcted and remodeling myocardium from overactive fibrosis and dysfunction, the inhibitory signals responsible for myofibroblast de-activation in the healing scar are poorly understood. Our experimental work has suggested that at all stages of repair, fibroblasts are exposed to inhibitory mediators, such as the CXC chemokine Interferon-γ inducible protein (IP)-10/CXCL10 that may serve to prevent excessive fibrosis [61],[62]. However, the role of specific endogenous inhibitory pathways in negative regulation of TGF-β and angiotensin-mediated responses following infarction has not been investigated.

Conclusions and future directions

Cardiac fibroblasts play a crucial role in repair of the infarcted myocardium, but are also implicated in the pathogenesis of adverse remodeling and heart failure following cardiac injury. Despite the recent expansion of our knowledge on the cellular origins of fibroblasts in infarcted and remodeling hearts, our understanding of the molecular signals implicated in fibroblast activation following myocardial injury remains limited. Future research needs to focus on in vivo experiments to identify functionally distinct fibroblast subsets in injured and remodeling hearts, and on studies dissecting the molecular pathways mediating specific fibroblast responses. Moreover, study of endogenous inhibitory signals that inhibit fibroblast

activity is crucial in order to design novel strategies protecting from adverse remodeling and heart failure.

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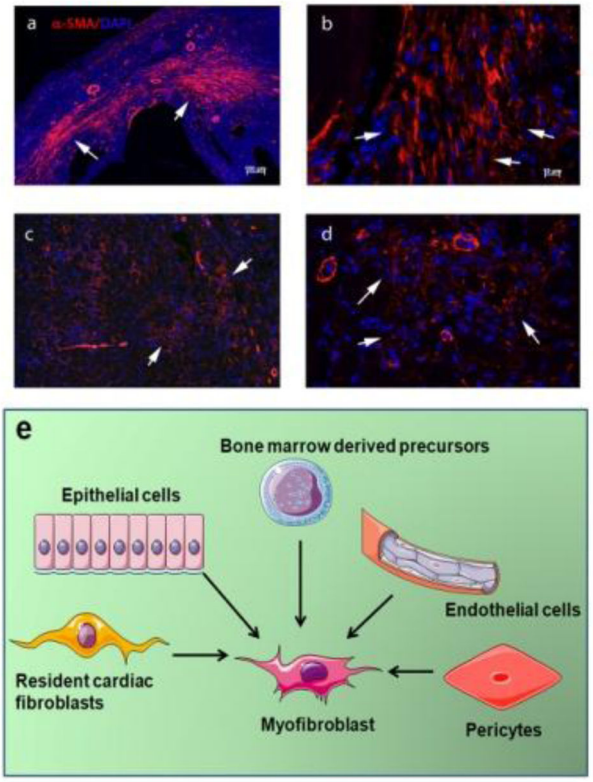

Figure 1. Myofibroblasts in the infarcted and remodeling myocardium

αSMA immunofluorescence identifies abundant myofibroblasts (arrows) in infarcted mouse hearts (after 7 days of coronary occlusion) (a, b) and in pressure overloaded hearts after 7 days of transverse aortic constriction (c, d). (e) Myofibroblasts in injured hearts may originate from a variety of sources, including epicardial epithelial cells, endothelium (through EndMT), vascular pericytes, bone marrow derived precursors, and resident cardiac fibroblasts. Recently published studies in mouse models using lineage tracing approaches suggest that resident cardiac fibroblast populations may be the most important source of activated myofibroblasts in infarcted and pressure-overloaded hearts.

Table 1

Overview of the studies identifying the cellular sources of activated fibroblasts in the infarcted and remodeling myocardium

Reference	Cellular Source(s) of activated fibroblasts	Species	Model of Cardiac Remodeling	Strategies used to identify the cellular origins of fibroblasts	Markers used for fibroblast labeling
Kanisicak et al. [10]	Activated fibroblasts in infarcted and remodeling hearts are derived from Tcf21+ tissue-resident fibroblasts. Endothelial cells, myeloid cells and smooth muscle cells do not significantly contribute to the activated fibroblast population.	Mouse	Myocardial infarction. Pressure overload induced through Transverse aortic constriction (TAC)	Lineage tracing using Periostin^{MCM}, Tcf21^{MCM} (to label resident fibroblasts), LysM-Cre (to label myeloid cells), Cdh5Cre (for endothelial cells) and Myh11Cre^{ERT2} (for smooth muscle cells).	Vimentin, PDGFRα, αSMA
Ruiz- Villalba et al. [63]	Epicardial-derived resident mesenchymal cells (Wt1Cre− eYFP+/ CD31− /CD90+/αSMA low/−) (Major contribution) and bone marrow-derived cells (Lin/cKit+/Sca1+/Flk2+/ CD34) (Minor contribution), mobilized in response to hemotactic stromal cell–derived factor (SDF)-1α gradient	Mouse	Myocardial infarction Pressure overload induced through angiotensin infusion.	Permanent genetic tracing of epicardium- derived cell and bone marrow–derived blood cell lineages using Wt1/IRES/GFP-Cre (Wt1Cre) mice crossed with Rosa26R-eYFP activating permanent reporter enhanced yellow fluorescent protein (eYFP+) expression in the Wt1+ cell lineage (Wt1Cre-YFP+).	Collagen I, FSP1, DDR2, CD90, αSMA
Aisaghboni et al. [34]	A significant proportion of activated fibroblasts (35–40%) is derived from endothelial cells.	Mouse	Myocardial infarction	Cell lineage tracing using TOPGAL reporter transgenic mouse line, which carries the lacZ gene under the control of three tandem β-catenin-responsive consensus TCF/LEF- binding motifs upstream of a minimal fos promoter and double transgenic line carrying the inducible Cre recombinase under the endothelial-specific enhancer of the stem cell leukemia (SCL) gene and the R26RstoplacZ locus	αSMA, Snail, FSP1, Vimentin and Collagen I
Zhou et al. [64]	Epicardium-derived cells differentiated into fibroblasts in the infarcted myocardium.	Mouse	Myocardial infarction	Genetic lineage tracing strategy using tamoxifen induced Cre allele, Wt1CreERT2/+, with epicardium-restricted cardiac activity, crossed with Rosa26mTmG/+ reporter line, which switches from mRFP to mGFP expression following Cre catalysed recombination	FSP1, procollagen I, collagen III, fibronectin, α-SMA
Van Amerongen et al. [65]	Bone marrow-derived cells contributed to the myofibroblast population in the infarcted myocardium (approximately 24% of myofibroblasts were bone marrow-derived).	Mouse	Myocardial infarction	MI induced in C57BL/6 mice reconstituted with BM transgenic for EGFP, as a reporter molecule, or with BM cells that express two reporter genes (luciferase and β-galactosidase) under the control of the promoter and enhancer elements of the collagen I (α2 chain) gene	α-SMA+ cells with spindle shaped morphology
Fujita et al. [66]	Blood-derived cells contributed to the myofibroblast population.	Mouse	Myocardial infarction	Whole Bone marrow or single hematopoietic cell transplantation from GFP-transgenic mice	CD45low/− elongated cells expressing Vimentin and αSMA
Mollmann et al. [36]	A large population of infarct fibroblasts is derived from bone marrow cells (57% on day 7 after infarction, 32% on day 21)	Mouse	Myocardial infarction	Bone marrow transplantation from enhanced green fluorescent protein (eGFP)-transgenic mice	vimentin, αSMA, SMemb
Yano et al. [67]	Circulating bone marrow cells did not contribute to the myofibroblast population.	Rat	Myocardial infarction	Bone marrow transplantation from green fluorescent protein (GFP)+transgenic mice into nude rats	vimentin, αSMA

Reference	Cellular Source(s) of activated fibroblasts	Species	Model of Cardiac Remodeling	Strategies used to identify the cellular origins of fibroblasts	Markers used for fibroblast labeling
Ali et al. [37]	The majority of cardiac fibroblasts in the pressure-overloaded myocardium are derived from epicardial populations, a minority from endothelial cells, and a small fraction from Pax3-expressing cells.	Mouse	Pressure overload through TAC	Fate-mapping models using Pax3Cre/+, Tie2Cre/+, Wt1CreERT2/+, Myh11cre/+-GFP, Vav1Cre/+, Tbx18Cre transgenic mice, Myh6-GFP and R26RmT/mG mice, bone marrow transplantation and parabiosis, global- and fibroblast-specific gene expression analysis	Collagen I, DDR2, PDGFRα, Vimentin, αSMA, CD90 exclusion criteria for hematopoietic cells, macrophages and endothelial cells.
Moore- Morris et al. [38]	Activated fibroblasts in the pressure-overloaded myocardium are derived from 2 resident fibroblast populations, and not from hematopoietic cells, endothelial cells or epithelial cells	Mouse	Pressure overload through TAC	Genetic lineage tracing using transgenic GFP reporter mouse line driven by a collagen1a1 enhancer crossed with Wt1-Cre, Tie2-Cre, Vav-Cre, VE-cadherin-Cre^{ERT2}, Tbx18-Cre, Wt1- Cre^{ERT2}, Nfatc1-Cre and Rosa-tdT-Cre	Vimentin, PDGFRα, Thy1, DDR2
Zeisberg et al. [35]	Activated fibroblasts in the pressure-overloaded myocardium are derived from endothelial cells through endothelial- mesenchymal transition (EndMT) (27–35% of all fibroblasts) either FSP1+ or αSMA+), and from bone marrow-derived cells (13.4% of FSP1+ cells and 21.1% of α-SMA+ cells)	Mouse	Pressure overload through TAC	Lineage tracing using Tie1Cre;R26Rstoplac Z mice, in which cells of endothelial origin are irrevocably marked by lacZ expression, and FSP1-GFP transgenic mice, in which green fluorescent protein (GFP) is expressed under the control of the promoter of fibroblast-specific protein 1 (FSP1), bone marrow transplantation of WT mice with Tie1Cre;R26Rstoplac Z bone marrow	FSP-1, αSMA, DDR2, type I collagen α1
Krammann et al. [68]	Gli-1+ pericytes contribute to the myofibroblast population in the remodeling pressure-overloaded myocardium (approximately 60% of activated fibroblasts are derived from Gli1+ cells)	Mouse	Pressure overload induced through angiotensin infusion or ascending aortic constriction	Lineage tracing using Gli1Cre^{ERT2} mice.	Collagen I, PDGFRα, αSMA