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COMPENDIUM ON THE INTERFACE BETWEEN CARDIOIMMUNOLOGY, MYOCARDIAL FUNCTION AND DISEASE

Cardiac Fibroblastic Niches in Homeostasis and Inflammation

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ABSTRACT: Fibroblasts are essential for building and maintaining the structural integrity of all organs. Moreover, fibroblasts can acquire an inflammatory phenotype to accommodate immune cells in specific niches and to provide migration, differentiation, and growth factors. In the heart, balancing of fibroblast activity is critical for cardiac homeostasis and optimal organ function during inflammation. Fibroblasts sustain cardiac homeostasis by generating local niche environments that support housekeeping functions and by actively engaging in intercellular cross talk. During inflammatory perturbations, cardiac fibroblasts rapidly switch to an inflammatory state and actively communicate with infiltrating immune cells to orchestrate immune cell migration and activity. Here, we summarize the current knowledge on the molecular landscape of cardiac fibroblasts, focusing on their dual role in promoting tissue homeostasis and modulating immune cell–cardiomyocyte interaction. In addition, we discuss potential future avenues for manipulating cardiac fibroblast activity during myocardial inflammation.

Key Words: cardiovascular diseases ■ endothelial cells ■ fibroblasts ■ homeostasis ■ inflammation ■ myocarditis

Cardiac function is highly dependent on the well-orchestrated interplay between different cardiac cells within their specific tissue microenvironment. Cardiac fibroblasts represent ≈10% to 20% of all cardiac cells and together with endothelial cells form the cardiac tissue stroma,[1] which is essential for maintaining cardiac function and structural integrity. Fibroblasts are distributed throughout all compartments of the heart, including distinct populations in the septum, the ventricles, the atria, the valves, and the annulus fibrosus.[2] Recent studies in mouse models using genetic targeting of specific fibroblast subsets and single-cell approaches have revealed the remarkable phenotypic and functional diversity of cardiac fibroblasts.[3–5] These studies have revealed a wide range of functions of cardiac fibroblasts in physiological processes, inflammation, and adverse remodeling of the heart. Advances in single-cell mRNA sequencing, including spatial transcriptomics, have further facilitated studying fibroblasts in the context of their topographical cellular niches.[6] Dedicated and versatile fibroblastic niches exist in all tissues throughout the body, and recent advances highlight their heterogeneity and adaptability to meet the specific and current needs of individual tissues.[7–10] Although fibroblasts are adapted to the local requirements and microarchitecture of each specific niche within a particular tissue, they functionally converge to a certain extent through the provision of specific molecular cues that are adapted to either homeostatic or perturbed conditions.[8,11,12] In this review, we summarize the current knowledge on cardiac fibroblastic niches during homeostasis, highlighting changes in fibroblast functions and cellular interactions, and summarize fibroblast reprogramming in response to myocardial inflammation.

ANATOMY OF CARDIAC FIBROBLASTIC NICHES

Origins of Cardiac Fibroblasts

Cardiac fibroblasts are derived from 3 embryonic developmental sources namely epithelium, endothelium, and neural crest with the majority of cardiac fibroblasts in

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Nonstandard Abbreviations and Acronyms

ACTA2	alpha smooth muscle actin 2
Ang II	angiotensin II
BMP4	bone morphogenic protein-4
BMPR1A	bone morphogenic protein receptor 1A
BMPR2	bone morphogenic protein receptor 2
CCL2	C-C motif ligand 2
CCR2	C-C chemokine receptor type 2
CSF-1	colony-stimulating factor 1
CX3CR1	C-X3-C motif chemokine receptor 1
DLL4	delta-like canonical Notch ligand 4
ECM	extracellular matrix
ET-1	endothelin-1
FAPα	fibroblast activation protein alpha
FGF	fibroblast-derived growth factor
FN1	fibronectin-1
Foxp3	forkhead box P3
HIMF	hypoxia-induced mitogenic factor
ICAM-1	intercellular adhesion molecule 1
IFN-γ	interferon-γ
IGF-1	insulin-like growth factor 1
IL	interleukin
LYVE1	lymphatic vessel endothelial hyaluronan receptor 1
MerTK	MER proto-oncogene tyrosine kinase
MHC	major histocompatibility complex
MIF	migration inhibitory factor
MMP	matrix metalloproteinase
MYH	myosin heavy chain
NCAM	neural cell adhesion molecule
NOTCH1	neurogenic locus notch homolog protein 1
POSTN	perostin
SCA-1	stem cell antigen 1
TGF-β	transforming growth factor beta
Thy-1	Thy-1 surface antigen
TIMP	tissue inhibitor of metalloproteinase
TLR	toll-like receptor
TNF	tumor necrosis factor
YAP	yes-associated protein

the adult heart originating from epicardial epithelial cells through epithelial-to-mesenchymal transition during development.[13] The developmental trajectory of epithelium-derived cardiac fibroblasts has been first delineated by using retroviral labeling of putative progenitor cells in the developing mouse embryo.[14] Progeny derived from the epicardium migrate to the underlying tissues to embed in the newly forming myocardium.[15–18] This particular developmental trajectory of cardiac fibroblasts generates the majority of vascular smooth muscle cells and interstitial cardiac fibroblasts,[19] thereby accounting for ≈85% of cardiac fibroblasts in the adult mouse heart.[20] Epithelial-derived murine fibroblasts are characterized by the expression of the transcription factors *Wt1*, *Tbx18*, and *Tcf21*,[13,21,22] which is indicative for specific lineage decisions during development.

A second subset of cardiac fibroblasts is primarily located in the ventricle and has been shown to originate from either vascular endothelial or endocardial endothelial cells, which undergo endothelial-to-mesenchymal transition. In contrast to epicardium-derived fibroblasts, this particular population retains the expression of the angiopoietin-1 receptor encoded by the TEK receptor tyrosine kinase (commonly referred to as TIE2 [tyrosine kinase with immunoglobin and EGF homology domains]) that is primarily expressed on endothelial cells.[20] Finally, a third and rather small subset of fibroblasts that are located primarily in the outflow tract of the adult heart can be lineage traced using *Pax3*-promoter-driven expression of the Cre recombinase in the neural crest.[23]

Although the developmental origins of cardiac fibroblast subsets have been well established, differences in the identity and behavior of fibroblasts in the various cellular niches of the heart have not been fully elucidated. Interestingly, a comparison of gene expression profiles between epithelial-derived and endothelial-derived cardiac fibroblast subsets before and after pressure overload in mice revealed no significant differences between the 2 lineages,[20] a finding that supports the notion of functional convergence of cardiac fibroblasts. It is likely that factors beyond developmental lineage imprinting, such as microenvironmental cues and cellular interactions within specific cardiac niches, play a critical role in determining and shaping the identity and function of cardiac fibroblasts.

Location of Cardiac Fibroblastic Niches

Fibroblasts sense and respond to local tissue signals as they interact with neighboring cells. Such microanatomical regions are characterized by distinct chemical, biomechanical, and cellular factors that shape local fibroblast identity and govern specific response patterns.[11,24] For example, fibroblasts from different regions of the body display unique gene expression profiles and maintain their positional identity even after in vitro culture.[25,26] Moreover, fibroblasts underpin discrete compartments and maintain diverse functional properties as shown for the synovium of joints, where 2 fibroblast populations exist in different compartments: FAPα$^+$ (fibroblast activation protein alpha), Thy-1$^+$ (Thy-1 surface antigen) double-positive fibroblasts located in the synovial sublining layer and FAPα$^+$ Thy-1$^-$ single-positive fibroblasts with destructive potential that are restricted to the synovial lining layer.[27,28] It remains to be determined to what extent such regional differences influence the function

of cardiac fibroblasts. Nevertheless, it is likely that the combination of niche-specific factors present in different microanatomical regions of the heart contributes to the establishment of specific fibroblast identities.

Cardiac fibroblasts can be assigned to 3 major anatomic sites: perivascular, interstitial, and subepicardial (Figure 1). Cardiac perivascular stromal cells are the major fibroblastic stromal cell population in the heart due to the extensive vascularization of the myocardium[29,30] (Figure 1A). Cardiac perivascular stromal cells include different populations of mural cells such as contractile ACTA2 (alpha smooth muscle actin 2)-expressing vascular smooth muscle cells and microvasculature-associated pericytes (PDGFRß+ [platelet-derived growth factor receptor beta-positive], RGS5+ [regulator of G-protein signaling 5-positive], ACTA2+), and adventitial fibroblasts (PDGFRα$^+$, CD34$^+$ [cluster of differentiation 34], SCA-1$^+$ [stem cell antigen-1 positive]; Table). The composition of cardiac mural cells reflects their functional heterogeneity along the vascular tree, which depends on vessel size, contractility, and pressure on the vessel wall.[31] In general, smaller capillaries of the microvasculature are associated with pericytes, while larger blood vessels are lined with multiple layers of vascular smooth muscle cells in the tunica media, which facilitate active vasoconstriction and blood pressure regulation (Figure 1A). The outermost layer, known as the adventitia or tunica externa, serves as a physical anchor for the blood vasculature within the tissue and thus plays an essential role for vascular integrity and function.[32] Adventitial fibroblasts interact with a variety of tissue-resident immune cells including resident cardiac macrophages, dendritic cells, tissue-resident lymphocytes, or innate lymphocytes (Figure 1A).[33] Notably, mural cells and adventitial fibroblasts are among the first cells encountered by incoming immune cells, effectively serving as gatekeepers that control further access to the tissue parenchyma. Perivascular stromal cells respond with proliferation and increased collagen deposition to increased left ventricular wall stress in animal models of hypertension or vascular inflammation.[34–36] However, despite their integral role in vascular development and the maintenance of vascular wall integrity and stability of the heart,[37] relatively little is known about their function and baseline gene expression in the absence of tissue perturbation.

The interstitial space represents the second, distinct cardiac fibroblast niche that responds to changes in mechanical stress, for example, with increased immune cell recruitment and ECM (extracellular matrix) deposition[38] (Figure 1B). Interstitial fibroblasts express common fibroblast markers such as PDGFRα and CD34 and form the interstitial connective tissue that ensheaths cardiomyocytes (Table). Individual cardiomyocytes are surrounded by a layer of connective tissue called the endomysium, whereas bundles of cardiomyocytes that form a functional muscle group are surrounded by the perimysium (Figure 1B). Fibroblasts of the endomysium and perimysium provide structural support for individual muscle cells, transmit tensile forces, and protect the muscle from excessive stretching. The dynamic range of physical stretching of the cardiac interstitium is determined by the composition of ECM fibers such as elastin, collagen I, and collagen III, which allow the interstitium to adapt to rhythmic changes in cardiac volume and pressure.[39–41] Cardiac interstitial fibroblasts respond to altered biomechanical forces or cardiomyocyte death caused by ischemic and nonischemic injury by increasing endomysial and perimysial collagen deposition. This leads to the development of patchy accumulations of interstitial microscars, known as myocardial interstitial fibrosis (Figure 1B). Myocardial interstitial fibrosis is a histological hallmark of several cardiac diseases, including inflammatory cardiomyopathy and hypertension. Interstitial cardiac fibrosis can be classified into 2 types based on the cause and effect on the viability of cardiomyocytes. The first type is reactive interstitial fibrosis, which is typically observed in cases of altered mechanical load or pressure and has minimal effect on cardiac function. The second type is reactive fibrosis, which occurs following cardiomyocyte death and is associated with reduced systolic and diastolic function.[38]

The third fibroblastic niche in the myocardium is located in the subepicardial space (Figure 1C). The epicardium is an evolutionarily conserved structure that forms the outermost mesothelial layer of all vertebrate hearts and acts as a physical barrier between the underlying myocardium and the pericardial cavity.[42] The fetal epicardium serves as a progenitor pool for the majority of fibroblastic stromal cells in the adult heart.[19] Many of the transcription factors expressed in epicardial cells during embryogenesis are reactivated after cardiac injury, leading to the generation of new epicardium-derived cells.[43,44] Pericardial inflammation, induced, for example, through the administration of IL (interleukin)-33, leads to an increase of cells in the subepicardial space[45] (Figure 1C). The subepicardial fibroblastic niche expands in response to myocardial injury shown by the differentiation of epicardium-derived cells into fibroblasts expressing ACTA2, FN1 (fibronectin-1), and collagen III.[46,47] However, the pathways of fibroblast differentiation in the adult subepicardium in response to injury are not yet fully understood. It is possible that subepicardial fibroblasts in the adult heart are derived from mesothelial cells.[21,48] Alternatively, it is conceivable that fibroblasts forming the basement membrane underlying the mesothelium harbor fibroblast progenitors with the potential to respond locally to injury and inflammation (Table). It will be important to investigate in future studies which cells contribute to the pool of fibroblasts in the subepicardial layer and whether there is a dedicated progenitor population under homeostatic conditions that responds to inflammatory perturbations.

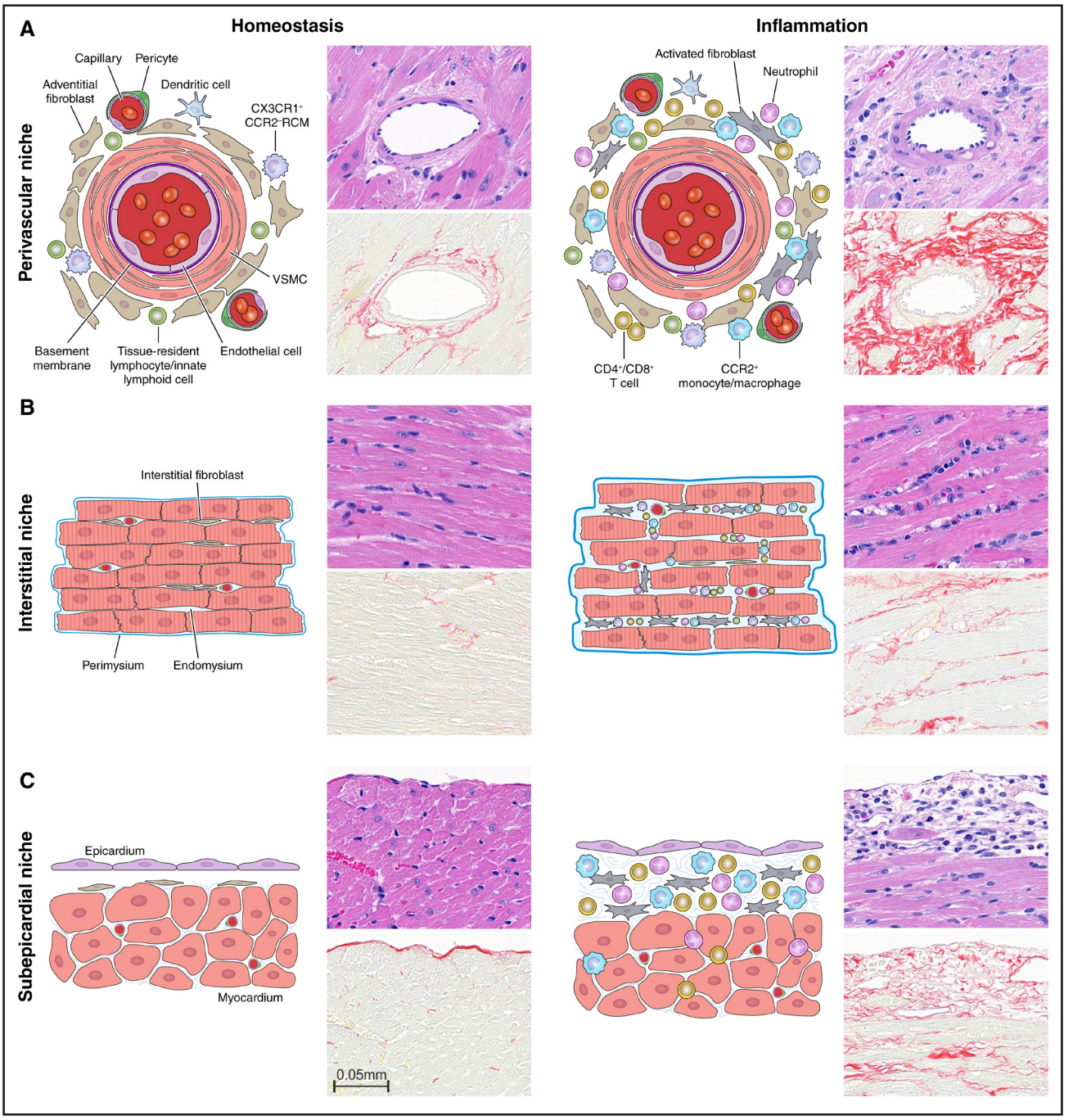

Figure 1. Cardiac fibroblasts underpin different anatomic niches in the heart.
Fibroblasts occupy specific anatomic niches within the heart, where their interactions with neighboring cells contribute to the maintenance of physiological homeostatic organ structure and function (**left**). **A**, Fibroblasts in the perivascular niche localize in concentric layers around blood vessels, providing critical support for vascular integrity and facilitating immune cell immigration. **B**, Cardiac fibroblasts in the interstitial space primarily produce and maintain the cardiac ECM (extracellular matrix) components, allowing both cell anchorage and communication with other cardiac cells. **C**, The subepicardial space is located between the epicardium and the myocardium. Cardiac inflammation alters intercellular communication in the different niche environments (**right**). Inflammation-induced fibroblast activation triggers increased fibroblast-to-immune-cell communication and ECM deposition (**A**–**C**, **right**). Histological images represent hematoxylin and eosin (**top**) or picrosirius red stained sections (**bottom**; scale bars=50 µm) of homeostatic (8-week-old BALB/c mice) or acutely inflamed hearts (8-week-old TCRM mice, a T-cell transgenic mouse model of autoimmune myocarditis). CCR2 indicates C-C chemokine receptor type 2; CX3CR1, C-X3-C motif chemokine receptor 1; RCM, resident cardiac macrophage; and VSMC, vascular smooth muscle cell. Illustration credit: Sceyence Studios.

Table. Fibroblastic Stromal Cell Subsets and Marker Genes in Murine and Human Hearts as Derived From Single-Cell Transcriptomics Analyses

Subset	Location	Genetic markers*		References
		Human	Mouse	
Pan-cardiac fibroblasts	Myocardium	*DCN*, *GSN*, *PDGFRA*, *COL1A2*	*Dcn*, *Gsn*, *Col1a1*, *Pdgfra*, *Pdpn*	4–6,30,49,68,102,110,112,130
Interstitial fibroblasts	Interstitium	Unknown	*Cd34*	5
Subepicardial fibroblasts	Subepicardial space	Unknown	Unknown	
Adventitial fibroblasts	Larger blood vessels, tunica externa	Unknown	*Cd34*, *Ly6a*	5,31
Pericytes	Smaller blood vessels and capillaries	*RGS5*, *ABCC9*, *KCNJ8*, *PDGFRB*	*Rgs5*, *Abcc9*, *Kcnj8*	4–6,49,68,102,112
Vascular smooth muscle cells	Larger blood vessels, tunica media	*MYH11*, *TAGLN*, *ACTA2*, *MYLK*	*Myh11*, *Tagln*, *Acta2*	6,30,49,102,112

*Human and murine gene assignment has been used according to Human Genome Organization (HUGO) Gene Nomenclature Committee and Mouse Genome Informatics (MGI) nomenclature guidelines, respectively.

FIBROBLASTIC NICHES IN HOMEOSTASIS

The phenotype and functions of cardiac fibroblasts change profoundly during myocardial injury or stress.[5,6,30,49] However, the general biology of fibroblasts within the normal heart is less well understood. The high abundance of fibroblasts in the uninjured heart and their specialized functions in different niche environments (Figure 1) are indicative for their critical role in cardiac physiology and homeostasis.

Fibroblasts Are the Source of ECM Components

The cardiac ECM represents an intricate network of filamentous and sheet-forming proteins that are deposited in particular spatial patterns and modified according to the requirements within the niche environment. Recent studies based on transcriptomics analyses of heart cells indicated that—as expected—cardiac fibroblasts are the major cellular source of ECM molecules in healthy murine hearts.[50,51] The ECM is a dynamic entity and affects basic cell behavior including cell growth, migration, differentiation, and survival in the healthy heart[52] and in numerous cardiovascular diseases.[51] Cardiac ECM molecules include type I and III collagens, glycoproteins (eg, collagens, elastins, and fibronectins), glycosaminoglycans (eg, hyaluronan), and proteoglycans (eg, lumican, fibromodulin, and decorin).[53,54] Physical properties of the cardiac tissue are to a large extent determined by the abundance and cross-linking of collagens with increased ventricular and vascular stiffness being frequently observed in various cardiovascular diseases.[55,56] The interstitial ECM is located around cardiomyocytes and muscle bundles. It serves as a mechanical scaffold and an essential element in facilitating the synchronous transmission of contractile forces throughout the myocardium.[57] Biomechanically, these functions necessitate a high adaptability and balancing of ECM properties offering both flexibility to facilitate a dynamic range of muscle contraction, and rigidity to prevent overstretching of the muscle fibers. Type III collagen provides elasticity by forming fine fibrils, whereas collagen type I provides tensile strength by forming thicker and more robust fibers. Consequently, alterations or disruptions in the cardiac collagen network profoundly affect myocardial systolic performance.[56,58]

Cardiac fibroblasts continuously monitor mechanical and chemical cues indicating regional differences in physical stress induced by pathophysiological stimuli. Mechanical tension, vascular hemodynamics, and stress signals are thus important regulators of the extracellular matrix. Fibroblasts are capable of translating mechanical input into biochemical signals that lead to adjustment of ECM composition, cross-linking, and organization (Figure 2). Cellular adhesion molecules (eg, integrins, CD44) that are directly linked to the extracellular matrix, as well as stretch-activated ion channels (eg, transient receptor potential cation channel C6), provide real-time feedback of tension and stress levels in the immediate environment of cardiac fibroblasts.[59] In addition, soluble factors, such as Ang II (angiotensin II) or TGF-β (transforming growth factor beta) are released by cardiac cells upon stress or injury.[60–62] Both Ang II and TGF-β foster fibrotic activity of fibroblasts promoting increased expression of collagens and other matrix molecules, while decreasing MMP (matrix-metalloproteinase) activity through increased TIMP (tissue inhibitor of metalloproteinase) expression.[63] Furthermore, the cardiac ECM provides the substrate for the presentation of various growth factors, cytokines, and chemokines[64–66] indicating that cardiac ECM is critical for supporting intercellular communication.

Homeostatic Cellular Cross Talk in the Heart

Under optimal physiological conditions, the heart beats regularly and adapts dynamically, supplying all heart cells with oxygen and nutrients. The homeostatic state of the system is supported by tunable molecular and cellular interactions that ensure normal organ function. Fibroblasts

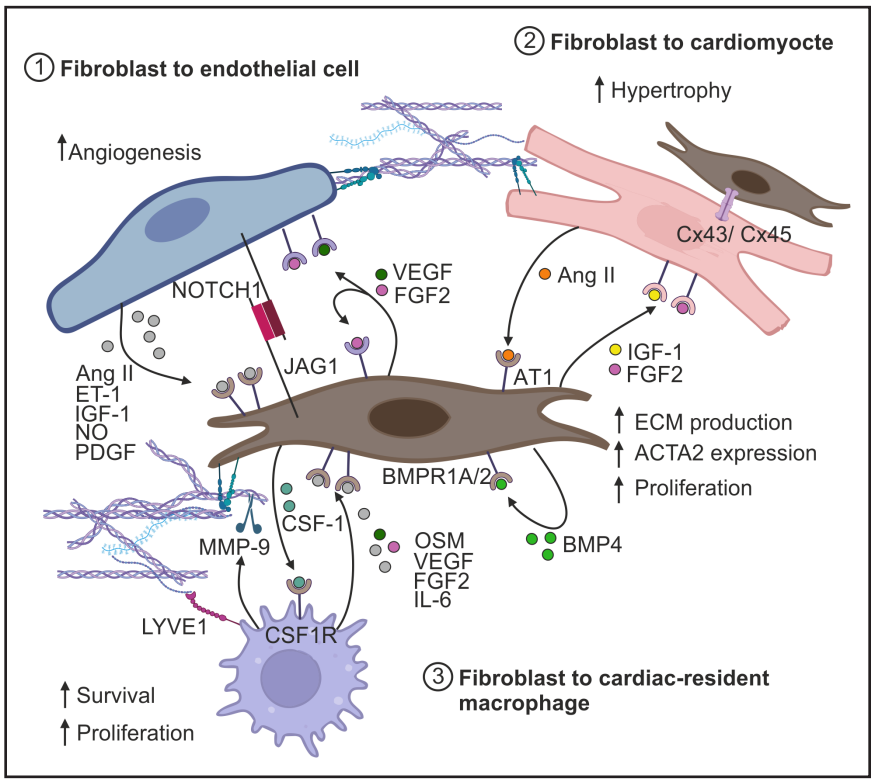

Figure 2. Homeostatic intercellular communication in the heart.
Depending on the specific niche environment, cardiac fibroblasts engage in active cross talk with different cell types, including endothelial cells, cardiomyocytes, and tissue-resident immune cells. In the perivascular niche, fibroblasts communicate with endothelial cells via direct cell-to-cell contacts (NOTCH [neurogenic locus notch homolog protein 1]-JAG [jagged]), soluble signaling molecules, such as Ang II (angiotensin II) or ET-1 (endothelin), or indirectly via the ECM (extracellular matrix) (1). Interstitial fibroblasts are intermingled in the myocardium and support homeostatic cardiomyocyte function and survival via the ECM, growth factor secretion (eg, IGF-1 [insulin-like growth factor 1], or FGF2 [fibroblast growth factor-2]) and through direct cell coupling mediated by Cx (connexin) 43 or 45 (2). Fibroblasts support cardiomyocyte fitness by providing a niche for resident cardiac macrophages (resident cardiac macrophages [RCMs]), which in turn regulate ECM stiffness through the secretion of ECM modulators such as MMP (matrix metalloproteinase) (3). ACTA2 indicates alpha smooth muscle actin 2; AT1, type 1 angiotensin receptor; BMP4, bone morphogenic protein-4; BMPR1A/2, bone morphogenic protein receptor 1A and 2; CSF-1, colony-stimulating factor 1; CSF1R, colony-stimulating factor-1 receptor; LYVE1, lymphatic vessel endothelial hyaluronan receptor 1; OSM, oncostatin M; PDGF, platelet-derived growth factors; and VEGF, vascular endothelial growth factor. Created using BioRender.com.

actively communicate with other cells in their respective niches.[6,67] Depending on the cellular composition of the niche, fibroblasts interact with different cardiac cell populations through various routes of communication including indirect interaction via the extracellular matrix, direct cell-cell contact, or paracrine and autocrine signals (Figure 2). Homeostatic control of cardiac fibroblast function is partially regulated via the BMP4 (bone morphogenic protein 4), BMPR1A (bone morphogenic protein receptor 1A), and BMPR2 (bone morphogenic protein receptor 2) axis[5] (Figure 2). It appears that BMP4, a tissue cytokine of the TGF-β superfamily, acts in an autocrine fashion on cardiac fibroblasts during myocardial inflammation leading to transcriptional downregulation of *Bmp4* expression and restriction of BMP4 protein availability. Consequently, restoration of BMP4 concentration through antibody-mediated sequestration of the BMP4 inhibitors gremlin 1 and 2 was sufficient to reduce cardiac inflammation and preserve cardiac function in a mouse model of autoimmune myocarditis.[5] These findings suggest that the activity of cardiac fibroblasts can be tuned to support their homeostatic cell functions thereby counteracting detrimental cardiac inflammation and resulting fibrotic tissue remodeling.

In the homeostatic perivascular niche, fibroblasts closely interact with endothelial cells and tissue-resident immune cells. Recent in silico predictions of cellular interactions based on single-cell transcriptomics analyses showed that endothelial cells receive incoming signals from cardiac fibroblasts in healthy and aged hearts.[67] Direct cell-cell communication between cardiac fibroblasts and endothelial cells includes NOTCH1 (neurogenic locus notch homolog protein 1) receptor-ligand interactions (eg, NOTCH1-Jagged1, NOTCH2-DLL4 [delta-like canonical Notch ligand 4]) with MYH (myosin heavy chain) 11-expressing vascular smooth muscle cells[68] (Figure 2). Other soluble factors produced by fibroblasts in the perivascular niche that act on endothelial cells include FGF (fibroblast-derived growth factor) and VEGF (vascular endothelial growth factor). These factors bind to their cognate receptors, FGFR1/2 and

VEGFR, respectively, and induce endothelial growth and angiogenesis[69] (Figure 2). The basement membrane, located on the basolateral side of endothelial cells, provides an additional, indirect route of communication between the 2 cell populations. During homeostasis, the intact basement membrane stimulates endothelial cell survival via integrin anchorage and intercellular adhesion through tight junctions.[51] Endothelial cells continuously produce an array of factors that control vascular tension and regulate blood flow in the heart according to the current oxygen demand. These factors include Ang II, ET-1 (endothelin-1), IGF-1 (insulin-like growth factor 1), and TGF-β (Figure 2). In addition, these soluble mediators regulate mural cell and adventitial fibroblast proliferation and expression of contractile elements such as ACTA2.[70] Tight control of intercellular communication is, therefore, key to allowing tissue adaptation while preventing adverse remodeling in the cardiac microenvironment.

The interaction between cardiac fibroblasts and lymphatic endothelial cells represents another crucial yet relatively underexplored aspect of cardiovascular biology. The lymphatic system in the heart is involved in maintaining fluid balance, immune surveillance, and tissue homeostasis.[71] Emerging evidence suggests that interactions between cardiac fibroblasts and lymphatic endothelial cells may influence cardiac remodeling, inflammation, and repair processes.[72] However, the precise mechanisms underlying these interactions remain incompletely understood. Future research is, therefore, needed to understand the interplay between cardiac fibroblasts and lymphatic endothelial cells in shaping physiological cardiovascular functions as well as their functional and structural adaptations in response to tissue perturbations.

Cardiac fibroblasts are in close contact with cardiomyocytes through a continuous network of direct cell-cell junctions that facilitate electrical coupling, for example, through the gap junction proteins Cx (connexin) 43 and 45[73] (Figure 2). In addition to direct contact, fibroblast-cardiomyocyte communication is mediated through the ECM and bidirectional paracrine signals (Figure 2). Integrins on the cell surface provide connections to the ECM for physical anchorage and transmission of mechanical forces.[74] Altered physical forces in the fibroblast-cardiomyocyte interaction lead to increased FGF2 expression, which fosters fibroblast proliferation and collagen production (Figure 2). The partial overlap of these intercellular signaling pathways underscores that intercellular communication in the heart regulates the functional state of specific niches rather than that of individual cells.

Perivascular fibroblastic niches are populated by different tissue-resident immune cells including LYVE1$^+$ (lymphatic vessel endothelial hyaluronan receptor 1), CX3CR1$^+$ (C-X3-C motif chemokine receptor 1 positive), CCR2$^-$ (C-C chemokine receptor type 2 negative) resident cardiac macrophages and tissue-resident T cells (Figure 1A).[75] Resident cardiac macrophages account for ≈7% of noncardiomyocytes in the heart[1] and have been shown to play an important role in cardiac development and vasculature formation.[76] High-resolution transcriptomics analyses, together with genetic approaches to track macrophages in vivo revealed that resident cardiac macrophages are a heterogeneous population with distinct developmental origins, homeostatic cardiometabolic functions, electrical conduction, and other housekeeping tasks during homeostasis.[77–80] As the mammalian heart, unlike the zebra fish, loses its regenerative capacity shortly after birth,[81] cardiomyocyte integrity and functionality in the adult heart must be maintained through recycling of cellular contents rather than regeneration.[82,83] Consequently, cardiac resident macrophages take up cardiomyocyte cellular waste, including defective mitochondria, in a MerTK (MER proto-oncogene, tyrosine kinase)-dependent manner, thereby ensuring cardiomyocyte metabolic fitness and survival.[77] Fibroblasts create niches for macrophages in the tissue, where they intimately interact to support each other.[29,84,85] For example, cardiac fibroblasts express CSF-1 (colony-stimulating factor 1), which promotes macrophage survival, differentiation, and proliferation[85] (Figure 2). In perivascular niches, such as the mouse aorta, LYVE1-expressing resident cardiac macrophages interact with vascular smooth muscle cells. This interaction involves the local release of MMP-9, which enables regulation of perivascular collagen deposition and vascular tone in the steady state by LYVE1$^+$ resident cardiac macrophages[86] (Figure 2). Recent in silico predictions based on single-cell transcriptomics analyses of human myocardial tissue indicated the potential significance of the CD74-macrophage MIF (migration inhibitory factor) axis as an interaction circuit between cardiac fibroblasts and resident cardiac macrophages.[68] However, the full array of molecular pathways that steers this interaction remains largely unexplored in the context of cardiac homeostasis.

REPROGRAMMING OF FIBROBLASTIC NICHES

Cardiac homeostasis is disrupted by numerous perturbations including ischemia, hypertension, or inflammation. Myocardial infarction leads to immediate alterations in the cardiac tissue due to a local lack of oxygenation. In contrast, viral infections, for example, by influenza A virus, coxsackievirus B3, or SARS-CoV2 are associated with both local and diffuse changes in the cardiac microenvironment.[87] Inflammation-associated reprogramming of cardiac fibroblasts during acute autoimmune myocarditis is associated with loss of cardiomyocytes and functional impairment of the heart.[5] Chronic cardiac inflammation precipitates left ventricular remodeling, cardiac dysfunction, and ultimately heart failure.[88] Therefore, it is

important to characterize physical, chemical, microbial, and immunologic triggers of cardiac fibroblast activation and identify critical molecular pathways regulating cardiac fibroblast activity.

Mechanisms of Cardiac Fibroblast Activation

Fibroblasts are ubiquitously distributed throughout all tissues and are equipped with a diverse array of receptors that continuously monitor external stimuli.[89,90] In the cardiac milieu, fibroblasts locally detect and respond to environmental changes or pathological stimuli from multiple sources, including the extracellular matrix, surrounding cells, and soluble mediators from the blood stream.[63,91] Changes in the stiffness of the ECM trigger the activation of the transcription factors YAP (yes-associated protein) and TAZ (transcriptional coactivator with PDZ-binding motif) in fibroblasts, which fosters both fibroblast contractility and further modification of the ECM.[92] Increased tensile or compressive stress causing deformation of the fibroblast membrane activates mechanosensitive ion channels such as TRPV4 (transient receptor potential cation channel subfamily V member 4)[93] or piezo1,[94] triggering fibroblast activation, IL-1β release,[95] increased ECM production, and substrate stiffness (Figure 3A). In addition, fibroblast deformation stimulates mechanosensing transmembrane receptors (ie, integrins) that tether the fibroblast to the ECM.[59]

The cardiac ECM binds and retains various chemokines, cytokines, and growth factors that can be released during inflammation-induced ECM reorganization (Figure 3A). Notably, cardiac injury induces the rapid release of latent TGF-β in a spatially restricted manner, culminating in robust fibroblast activation. Subsequently, TGF-β–activated fibroblasts upregulate extracellular protein synthesis and modulate ECM turnover.[62,96] To meet the elevated energy demands associated with increased protein synthesis, cardiac fibroblasts undergo metabolic adaptations. This amplified energy expenditure in cardiac fibroblasts is primarily fueled by a shift in metabolic preference characterized by increased glycolysis and reduced fatty acid oxidative phosphorylation, allowing for rapid production of energy to support cellular functions under stress.[97] Activation of cardiac fibroblasts is further regulated by the local release of stress-induced soluble factors produced by cardiomyocytes and endothelial cells such as HIMF (hypoxia-induced mitogenic factor; also known as FIZZ1 [found in inflammatory zone 1] or RELMα [resistin-like molecule alpha]) or Ang II[61,98] (Figure 3A).

Cardiac fibroblasts act in concert with resident cardiac macrophages and respond to inflammatory mediators such as IL-6, IL-1β, TNF (tumor necrosis factor), and IFN-γ (interferon-γ) produced by immune cells[99] (Figure 3A). Moreover, fibroblasts can directly sense the presence of microbial agents via pattern recognition receptors such as TLRs (toll-like receptors), which are germline-encoded receptors that recognize a variety of pathogen-associated structures such as lipopolysaccharides or viral nucleic acids. Engagement of pattern–recognition receptors triggers signaling cascades that activate nuclear factor-κB, activator protein 1, IFN regulatory factor transcription factors, or inflammasome assembly[99] and leads to the expression of proinflammatory cytokines and chemokines that further drive inflammatory processes (Figure 3B).[100] In sum, a broad range of inflammatory triggers lead to the modulation of cardiac fibroblast activity covering a wide spectrum of differentiation states.

Inflammation-Driven Fibroblast Reprogramming

Activated fibroblasts are frequently referred to as myofibroblasts reflecting the early observation of increased expression of contractile elements such as ACTA2 in response to injury.[101] However, increased expression of ACTA2 appears to mark only a subset or transitional state of activated fibroblasts in different tissues.[29,50,102] Activation patterns of fibroblasts in different organs can be broadly characterized as either immune interacting (or inflammatory) or ECM producing (or tissue remodeling).[28] For example, in the inflamed synovium of patients with rheumatoid arthritis, sublining layer fibroblasts drive inflammation by promoting lymphocyte recruitment and interaction through the production of proinflammatory and cell-interaction molecules, whereas lining layer fibroblasts drive bone erosion and cartilage damage through ECM remodeling.[27] Likewise, fibroblasts in the heart respond to inflammation with distinct activation patterns including fibroblast subsets that are interacting with immune cells, whereas other cardiac fibroblasts switch to increased production of ECM.[3,5,103] For example, activated cardiac fibroblasts show increased expression of the matricellular protein POSTN (perostin) after hypoxia-induced or hypertensive cardiac damage[49,104] (Figure 3B). During increased influx of activated immune cells caused, for example, by the activation of cardiac myosin-specific T cells, cardiac fibroblasts downregulate homeostatic signaling such as autocrine BMP4 signaling.[5] Local transition of cardiac fibroblasts to an inflammatory state is associated with the swift upregulation of immunomodulatory cytokines, chemokines, and growth factors[5] (Figure 3B). In vitro and in vivo analysis of the fibroblast transcriptome after activation suggested that cardiac fibroblasts directly influence immune cell recruitment and activation in the inflamed myocardium through upregulation of IL-6, CCL2 (C-C motif ligand 2), CSF-1, ICAM-1 (intercellular adhesion molecule 1), and NCAM (neural cell adhesion molecule). Notably, during autoimmune T-cell–driven inflammatory processes, BMP4 downregulation and cardiac fibroblast transition to an inflammatory state seem to be restricted to specific areas.[5] Novel high-dimensional imaging approaches,

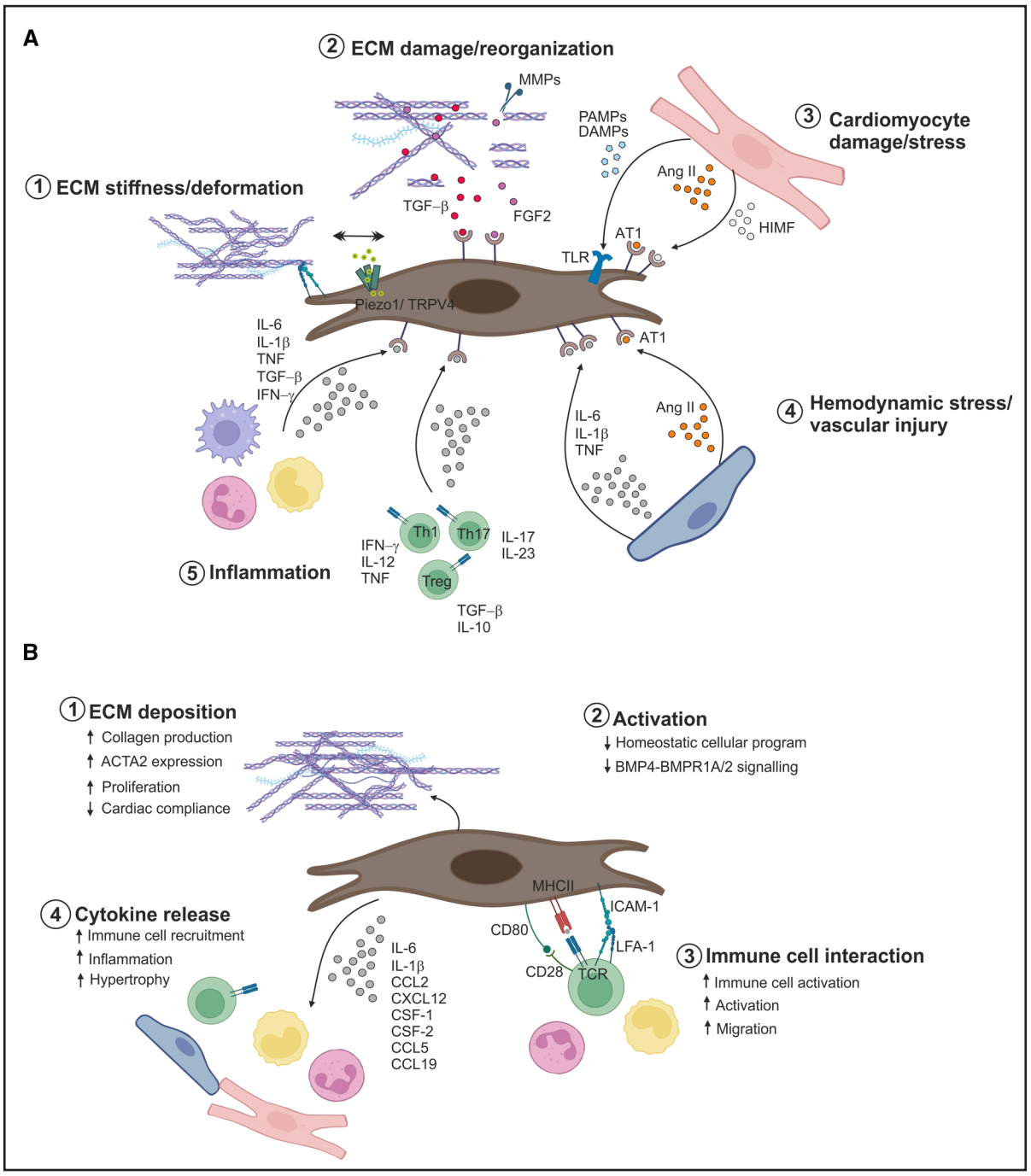

Figure 3. Inflammation leads to reprogramming of cardiac fibroblasts.
A, Cardiac fibroblasts monitor the environment for signs of inflammation, damage, and cellular stress. Fibroblasts are able to integrate information on ECM (extracellular matrix) physical properties (1), ECM quality (2), cardiomyocyte fitness (3), vascular integrity (4), and inflammation (5) derived from multiple cellular and structural sources. Changes in ECM properties and tissue injury trigger mechanosensitive ion channels (eg, piezo1) and the release of latent growth factors such as TGF-β (transforming growth factor beta). Stressed or dying cardiac cells and infiltrating immune cells further release signaling molecules, including Ang II (angiotensin II), interleukins (IL), and IFN-γ (interferon-γ), which foster local inflammation and trigger fibroblast activation. **B**, Following stimulation cardiac fibroblasts transition from a homeostatic to a proinflammatory state. This process is characterized by increased ECM deposition (1), sustained activation (2), enhanced communication with immune cells (3), and localized cytokine release (4). ACTA2 indicates alpha smooth muscle actin 2; AT1, type 1 angiotensin receptor; BMP4, bone-morphogenic protein-4; BMPR1A/2, bone morphogenic protein receptor 1A and 2; CCL, C-C motif ligand; CSF-1, colony-stimulating factor 1; CD, cluster of differentiation; CXCL, C-X-C motif ligand; DAMP, danger-associated molecular patterns; FGF2, fibroblast growth factor-2; HIMF, hypoxia-induced mitogenic factor; ICAM-1, intercellular adhesion molecule 1; LFA-1, lymphocyte function-associated antigen 1; MHC II, major histocompatibility complex class II; MMP, matrix metalloproteinase; PAMP, pathogen-associated molecular patterns; TCR, T-cell receptor; Th, T helper cell; TLR, toll-like receptor; TNF, tumor necrosis factor; and Treg, regulatory T cell. Created using BioRender.com.

such as spatial transcriptomics or multiplex immunohistochemistry, will need to be used in future studies to resolve the spatial context and kinetics of critical signaling pathways and cellular interactions that regulate the local activity of cardiac fibroblasts.

Altered Cellular Interactions During Cardiac Inflammation

Cardiac inflammation is accompanied by substantial accumulation of inflammatory cells including neutrophils, monocytes, and antigen-specific T cells. The relative composition of immune cells in the myocardium varies depending on the cause of the inflammation and its temporal kinetics.[88] For example, hypoxia-induced necrotic cell death during in ischemic injury initiates an inflammatory response to remove cellular debris and to secure short-term adaptation to increased cardiomyocyte stress.[105] The acute inflammatory phase is characterized by increased production of proinflammatory cytokines and chemokines, leading to a rapid influx of neutrophils and monocytes from the circulation. The subsequent resolution phase is characterized by further macrophage-mediated phagocytic clearance of cellular debris and influx of cardiac antigen-specific T cells that support myocardial healing.[105–107] In contrast, during T-cell–driven autoimmune myocarditis, cardiac myosin-specific CD4$^+$ Th (T helper cells) rapidly infiltrate the myocardium and generate proinflammatory cytokines and chemokines that initiate local inflammatory foci, attract and activate inflammatory macrophages and neutrophils, and hence cause the loss of cardiomyocytes.[108,109] Consequently, cardiac fibroblast subsets need to be equipped and be adaptable to interact with immune cells in different disease settings and stages.

The perivascular niche is particularly important for the initial phase of inflammatory responses in the heart due to the mutual stimulation and active communication between perivascular fibroblasts and endothelial cells. Transcriptomics analyses of endothelial cells 1 day after experimental myocardial infarction indicate their contribution to local inflammatory responses by upregulating genes for inflammatory cytokines such as *Il1b*, *Il6*, and *Tnf*.[110] Likewise, during myocardial infarction, fibroblasts upregulate similar proinflammatory and leukocyte-recruiting gene signatures (Figure 3B), which are maintained and modulated at later time points post-infarction with upregulation of key stimulatory pathway components such as *Stat5a/b*, *Mapk7*, and surface receptors such as *Il4ra*, *Fgfr1/2/3*, or *Tgfbr1*.[111] Intriguingly, endothelial cell activation following myocardial infarction appears to be short-lived[111] underscoring the key role of tightly regulated fibroblast activity in the postinjury heart.

Inflammatory fibroblasts in the heart actively support the activity of immune cells, which is most likely directed toward the removal of cardiac cell debris. However, excessive accumulation of neutrophils and inflammatory macrophages can further amplify cardiac fibroblast activity and thereby generate a potentially self-sustaining feedback loop. Indeed, monocyte-derived macrophages expressing triggering receptors expressed on myeloid cells 2 (*TREM2*$^+$) and osteopontin (*SPP1*$^+$) have been identified as major contributors to this process leading to increased *IL6*, *IL1B*, *TNF*, *TGF-β*, and *AREG* expression in human cardiac fibroblasts[30] (Figure 3B). Spatial transcriptomics and in situ hybridization analyses of post-myocardial infarction tissue from human hearts further revealed that CCR2$^+$ monocyte-derived macrophages localize adjacent to activated POSTN-expressing fibroblasts.[112] Notably, CCR2-negative resident macrophages are also necessary for adaptive cardiac tissue remodeling. This is demonstrated by the fact that physical depletion of cardiac resident macrophages in a mouse model of dilated cardiomyopathy results in a reduction in LV systolic function, attenuated LV dilation, and exaggerated myocardial fibrosis.[113] Thus, future experimental and translational studies will be important to elucidate the mechanisms of balanced cardiac myeloid cell–fibroblast interaction.

T lymphocytes, particularly CD4$^+$ helper T cells, modulate both inflammation and repair in several cardiac diseases.[109,114–116] Although CD4$^+$ T cells are not equipped to directly damage cardiomyocytes, they are critical immune effector cells because they orchestrate the migration and activity of other immune cells. The detrimental effects of excessive CD4$^+$ T-cell activity in the heart are demonstrated by the severe disease in transgenic mice harboring MYH6-specific CD4$^+$ T cells with initial acute myocarditis and subsequent inflammatory cardiomyopathy.[108,109] Such heart-specific CD4$^+$ T cells are initially activated in the mediastinal lymph nodes and are guided by fibroblast-derived chemokines CCL19, CCL5, and CX3CL1 into the heart[5,111] (Figure 3B). Within the myocardium, heart-specific CD4$^+$ T cells release potent inflammatory cytokines including IL-17, IL-23, IFN-γ, TNF, and IL-12 and thereby trigger an inflammatory cascade involving recruitment and activation of inflammatory macrophages and local reprogramming of cardiac fibroblasts toward the inflammatory phenotype[5,117,118] (Figure 3A). IL-17 release by Th17 cells induces a proinflammatory state in fibroblasts, which in turn upregulate CCL2, CXCL1, IL-6, and CSF-2, leading to a monocyte influx and further amplification of the inflammatory cascade.[108] Interestingly, *IL17a*$^{-/-}$ deficient mice exhibited primary myocardial inflammation similar to wild-type control mice but were protected from progression to inflammatory cardiomyopathy in models of autoimmune myocarditis,[109,119] further highlighting the role of IL-17 in propagating myocardial inflammation.

In contrast to Th17 cells, other Th cell subsets can contribute to the resolution of myocardial inflammation. Depending on the tissue context, IFN-γ–producing

Th1 cells can foster cardiac inflammation[109,120] or exert disease-attenuating effects.[121] Clearly, in the long term, release of IFN-γ, and associated Th1 cytokines such as IL-12, drives fibroblast activation and cardiac fibrosis.[122] Th1 cells in nonischemic heart failure physically interact with cardiac fibroblasts through integrin α4 (CD49d), inducing TGF-β expression and fibroblast activation in an IFN-γ–dependent manner.[123] Ngwenyama et al[3] recently identified an alternative communication pathway between IFN-γ–producing Th1 cells and cardiac fibroblasts involving the IFN-γ–induced expression of MHC (major histocompatibility complex) class II molecules in cardiac fibroblasts, indicating that cardiac fibroblasts may function locally as antigen-presenting cells and promote antigen-dependent T-cell responses (Figure 3B). It is possible that myosin-specific Foxp3 (forkhead box P3)-expressing regulatory T cells in the heart interact with fibroblasts, macrophages, and other cardiac cells expressing MHC class II molecules to attenuate inflammation and promote wound healing or fibrosis trough the production of TGF-β or IL-10[106,107,124] (Figure 3A). In conclusion, fibroblasts in the cardiac microenvironment closely interact with T lymphocytes during cardiac inflammation.

Chronic Cardiac Inflammation Precipitates Persistent Fibroblast Activation

Tissue responses to inflammatory perturbations can either promote healing, that is, be tissue-protective, or increase the loss of function, that is, be tissue-destructive.[125] Cardiac fibroblasts most likely contribute to the transition from inflammatory to repair programs involving the attenuation of immune cell activity.[105] Conversely, chronic and uncontrolled fibroblast activation is probably one of the major drivers of pathological remodeling of the cardiac tissue with deleterious fibrosis, a hallmark of tissue damage, for example, in inflammatory cardiomyopathy and heart failure.[87] The dual role of cardiac fibroblasts in chronic diseases of the heart can be illustrated by periostin-expressing fibroblasts, which are essential for scar stabilization and prevention of cardiac rupture after hypoxia-induced cardiac injury.[104,126] However, the finding that physical ablation of periostin mRNA-expressing fibroblasts in experimental models of myocardial infarction or chronic Ang II exposure results in reduced fibrosis without compromising scar stability[127] indicates that yet unknown factors such as type of myocardial injury and molecular cues provided by the local environment strongly affect fibroblast response patterns. Identifying factors that control the balance between cardiac homeostasis and inflammation, that is, those cellular and molecular mechanisms that are either cardio-protective or cardio-destructive, will be important in future translational studies.

CONCLUSIONS

Fibroblasts are ubiquitous cells in all organs that are essential for maintaining organ structure and function. In the heart, fibroblasts underpin distinct cellular niches in the perivascular, interstitial, and subepicardial regions of the myocardium. Under unperturbed conditions, cardiac fibroblasts support cardiac function and maintain tissue homeostasis. One of the key functions of cardiac fibroblasts is the control of ECM composition and distribution both in homeostasis and during inflammatory perturbation. Within their specific niches, cardiac fibroblasts constantly interrogate their environment and adapt their phenotypic properties to optimally cater to endothelial cells, mesothelial cells, cardiomyocytes, and resident or immigrating immune cells. Intercellular communication between cardiac fibroblasts and cells in their niche environment integrates information provided by direct mechanical signals, cell-cell contact, and soluble factors such as tissue cytokines, immune cell–derived chemokines and cytokines, and modulators of cellular metabolism.[128] Following activation during tissue injury or inflammation, cardiac fibroblasts undergo substantial changes to support both inflammatory processes and contribute to tissue repair. The outcome of the interaction of cardiac fibroblasts with other cells in their microenvironmental niches is highly dependent on contextual cues. Thus, future studies dissecting the cross talk of cardiac fibroblasts with other cardiac cell populations need to take into account (1) the nature of the particular perturbation, (2) the spatial context, and (3) the kinetics of cellular interactions and decay. Mammalian cardiomyocytes lose their regenerative capacity during the neonatal period.[81] Therefore, approaches to preserve cardiomyocyte function and integrity must be a major goal for future research.

Novel technical approaches such as single-cell RNA sequencing and single-nucleus RNA sequencing have provided unprecedented molecular insights into the complex biology of cardiac fibroblasts. However, several knowledge gaps remain including the loss of spatial context within the tissue. Therefore, it is essential to combine single-cell transcriptomics with topographical analyses in the heart. Topographical resolution can be obtained using spatial transcriptomics or multiplexed error-robust fluorescence in situ hybridization[129] that allow for unbiased transcriptomics analysis of cardiac cells without the need for prior tissue digestion. These techniques will provide valuable information on the identity, function, and interaction of cardiac fibroblastic stromal cells within cellular neighborhoods or niches. Further in-depth analysis of the role and function of cardiac fibroblasts during the transition from homeostasis to inflammatory conditions may lead to the development of novel treatment options such as immunotherapeutical antibodies. The identification of relevant and targetable

molecular circuits in cardiac fibroblasts will be critical to control the balance of regenerative and maladaptive inflammation in the heart.

The niche concept outlined here emphasizes that cardiac fibroblasts are at the nexus of cardiac homeostasis, inflammation-induced cardiac dysfunction, and adverse cardiac remodeling. Modulation of cardiac fibroblast activity to restore homeostasis is a promising avenue for future research and development.

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Disclosures

C. Perez-Shibayama, C. Gil-Cruz, and B. Ludewig are founders and C. Perez-Shibayama, C. Gil-Cruz, and B. Ludewig are shareholders of Stromal Therapeutics AG, Basel, Switzerland. B. Ludewig is a member of the board of Stromal Therapeutics AG, Basel, Switzerland. C. Perez-Shibayama, C. Gil-Cruz, and B. Ludewig are listed as inventors on patent WO 2022/084400 A1. The other author reports no conflicts.

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