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REGENERATIVE MEDICINE (SM WU, SECTION EDITOR)

Role of Cardiac Fibroblasts in Cardiac Injury and Repair

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Abstract

Purpose of Review The pathological remodeling of cardiac tissue after injury or disease leads to scar formation. Our knowledge of the role of nonmyocytes, especially fibroblasts, in cardiac injury and repair continues to increase with technological advances in both experimental and clinical studies. Here, we aim to elaborate on cardiac fibroblasts by describing their origins, dynamic cellular states after injury, and heterogeneity in order to understand their role in cardiac injury and repair.

Recent Findings With the improvement in genetic lineage tracing technologies and the capability to profile gene expression at the single-cell level, we are beginning to learn that manipulating a specific population of fibroblasts could mitigate severe cardiac fibrosis and promote cardiac repair after injury.

Summary Cardiac fibroblasts play an indispensable role in tissue homeostasis and in repair after injury. Activated fibroblasts or myofibroblasts have time-dependent impacts on cardiac fibrosis. Multiple signaling pathways are involved in modulating fibroblast states, resulting in the alteration of fibrosis. Modulating a specific population of cardiac fibroblasts may provide new opportunities for identifying novel treatment options for cardiac fibrosis.

Keywords Cardiac fibroblasts · Origins of fibroblasts · Fibroblast states · Signaling pathways

Introduction

Heart failure is becoming the leading cause of morbidity and mortality worldwide [1]. In the course of cardiomyocyte death, the cardiac tissue is replaced by a reparative fibrotic scar, which progressively remodels the tissue. A healthy heart consists of cardiomyocytes and nonmyocytes. Cardiomyocytes occupy only 30% of the cell numbers and are responsible for contraction and relaxation [2]. Nonmyocytes consist of epicardial cells, endothelial cells, fibroblasts, pericytes, smooth muscle cells, lymphocytes, macrophages, and some other cell types. Of these, cardiac fibroblasts play an irreplaceable role in regulating the extracellular environment by increasing or decreasing the extracellular matrix (ECM) [3]. Cardiac injuries, including pressure overload-induced heart failure and ischemic injury, usually result in cardiac fibrosis because of the excessive deposition of ECM, which is produced by myofibroblasts differentiated from resident fibroblasts in the injured heart [4, 5]. Given the significant role of cardiac fibroblasts in pathophysiological conditions, it is important to learn more about fibroblasts and myofibroblasts, including their origin, state, regulating genes, heterogeneity, and signaling pathways, in order to develop novel therapeutic targets for treating cardiac fibrosis. Recent advances in technology, such as genetic lineage tracing and single-cell RNA sequencing, have provided knowledge on the role of cardiac fibroblasts in cardiovascular disease [5–7, 8•, 9–11]. This review aims to provide updates on the recent advancements in fibroblast research and on the roles of fibroblasts after cardiac injuries.

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Origins of Cardiac Fibroblasts

As cardiac fibroblasts play a significant role in maintaining the structural integrity of the injured heart by differentiating into myofibroblasts and modulating the ECM, it is necessary to clarify their origin. However, the identification of fibroblasts remains difficult because of a lack of clear markers that can specifically label fibroblasts [12, 13]. According to recent findings based on flow cytometric and histological analyses, fibroblasts account for approximately 13% of cells in the healthy mouse heart [14, 15]. Fibroblasts could also be divided into subpopulations based on gene expression files during tissue homeostasis and after injury. In addition, resident cardiac fibroblasts have distinct developmental origins, which may indicate their unique gene profiles and functions in cardiac diseases.

For understanding the developmental origin, genetic lineage-tracing based on the Cre–loxP recombination system is used to permanently trace labeled cells and follow their fate over time during tissue repair and regeneration [16]. Using the mouse lines that express Cre, including *Tbx18Cre* [17, 18], *Gata5Cre* [19], *Sema3D-Cre* [20], and *Wt1Cre* [21–23], even the inducible CreER, such as $Wt1^{CreERT2}$ [21], $Tcf21^{mCrem}$ [24, 25], and $Tbx18^{CreERT2}$ [26], previous studies have demonstrated that a large population of cardiac fibroblasts is derived from the epicardium through epithelial-to-mesenchymal transition (EMT) [27] (Fig. 1a). In the developing heart, some epicardial progenitor cells delaminate from the epicardial layer and invade the myocardium on embryonic day (E) 12.5. Fibroblasts with distinct molecular markers are formed and distributed throughout the ventricle at later embryonic stages [25, 28–31]. Moreover, single-cell RNA sequencing (scRNA-seq) data have suggested that epicardium-derived fibroblasts express genes associated with cell migration and cell metabolism and account for a major population of cardiac fibroblasts [32].

In addition to the epicardium being a major source of fibroblasts, recent genetic lineage tracing studies have suggested that the embryonic endothelium represents an indispensable developmental source of fibroblasts. By tracing the endothelial lineage with *Tie2Cre*, Ali and Moore-Morris reported that around 10% of fibroblasts in the left ventricle and around 65% of fibroblasts in the interventricular septum are derived from endothelial progenitor cells [5, 33] (Fig. 1b). By exploring the fate map of these endothelium-derived fibroblasts, Moore-Morris clarified that an endothelial-to-mesenchymal transition (EndoMT) process by E9.5 was responsible for this fibroblast lineage [5] (Fig. 1a). Furthermore, according to scRNA-seq data, endothelium-derived fibroblasts express genes associated with valve leaflets and account for a small population of

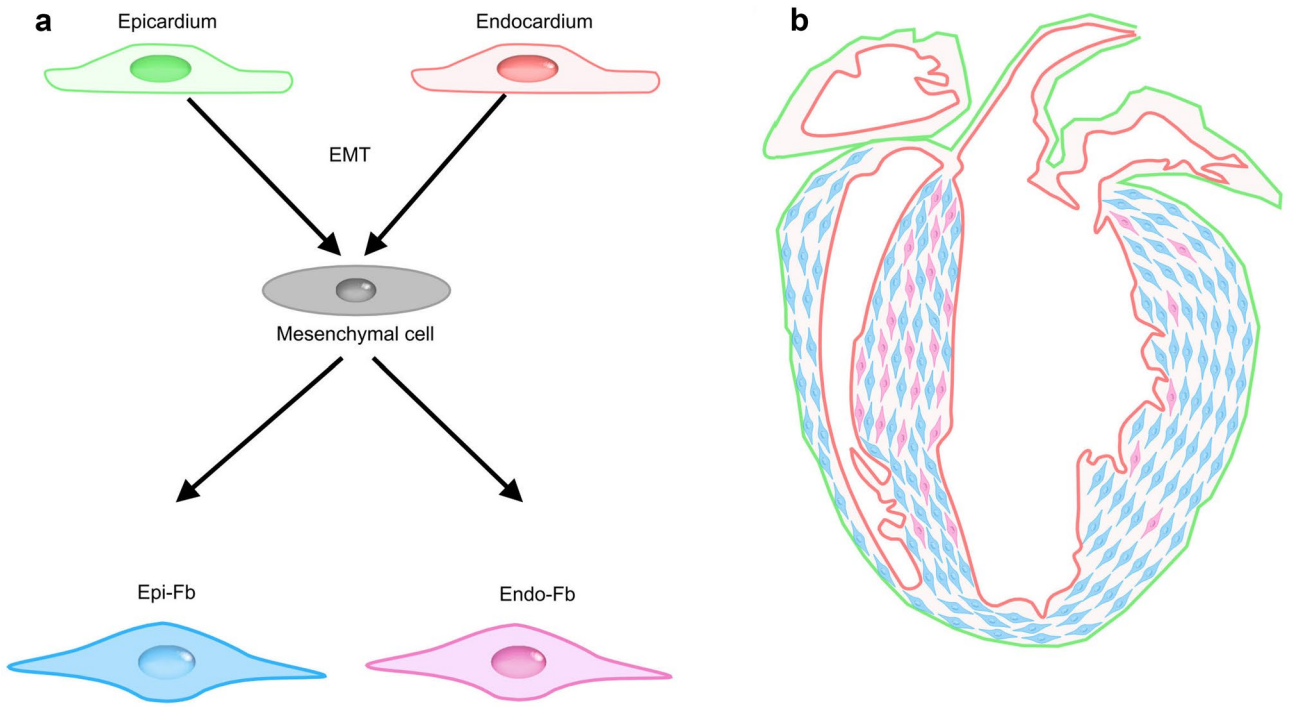

Fig. 1 Origins of fibroblasts. **a**, Epicardium and endocardium to fibroblasts by EMT. **b**, Epi-Fb and Endo-Fb occupy distinct regions of hearts. Epi-Fb, epicardium-derived fibroblasts; Endo-Fb, endocardium-derived fibroblasts

cardiac fibroblasts [32]. With the development of the lineage tracing strategy, dual lineage tracing tools have been used to specifically trace endocardium-derived cells and have revealed that endothelial cells have the potential to produce intermediate mesenchymal cells that can subsequently differentiate into fibroblasts, smooth muscle cells, pericytes, and adipocytes [34]. In addition to epicardium- and endothelium-derived cells, neural crest cells are reported to contribute to a small population of cardiac fibroblasts [35].

Cardiac fibroblasts have multiple developmental origins. Whether these different origins affect their behaviors and functions under pathophysiological conditions remains unknown. The mouse model for myocardial infarction (MI) has recently been used to study the functions of fibroblasts having different origins. After MI, adult epicardial cells are reactivated and generate a thickened epicardial layer consisting of fibroblasts in response to cardiac injury [36, 37]. In previous studies using the thoracic aortic constriction (TAC) model, few fibroblasts originating from the adult epicardium were found [5, 33], indicating that fibroblasts derived from the epicardium are injury type-dependent in adults. To analyze the gene expression profiles in distinct fibroblast populations based on their origin, epicardium-derived fibroblasts from *Tbx18Cre;Rosa26mTmG* mice and endothelium-derived fibroblasts from *Tie2Cre;Rosa26mTmG* mice were sorted by flow cytometry. Little difference was noted between their RNAs after pressure overload-induced heart failure [5, 38, 39]. The proliferative activity of the two fibroblast populations was found to be similar as the proportion of fibroblasts of each origin did not change significantly after pressure overload-induced heart failure in comparison with the sham control [33, 40]. The aforementioned data indicated that the origins of fibroblasts have rare effects on their function and performance in pressure overload-induced cardiac injury. However, the epicardium or endothelium contributes to not only cardiac fibroblasts but also pericytes, adipocytes, and other cell types. In addition, there is preference in terms of the location where the two fibroblast populations reside. Immunostaining for fibroblast markers and lineage tracing markers may obscure the true difference between the two fibroblast populations in response to injury. In the future, more advanced lineage tracing strategies could be developed in order to re-assess whether fibroblasts of different origins have different functions and gene expression profiles during heart injury and repair.

State of Cardiac Fibroblasts After Cardiac Injury

As a plastic cell type, fibroblasts exhibit variable differentiated states to respond to wound healing and to remodel for scar formation. Recently, Fu et al. identified four different states of fibroblasts, namely resident fibroblasts, active fibroblasts, myofibroblasts, and matrifibrocytes, after MI using genetic lineage tracing to monitor the fate of fibroblasts [41••]. They used $Tcf21^{MCM/+};R26^{EGFP}$ mice to trace tissue-resident cardiac fibroblasts and calculated the proliferation rate of fibroblasts through 5-ethynyl-2′-deoxyuridine (EdU) and Ki67 staining of heart sections collected from mice during different periods after MI. They reported that the activation and proliferation of fibroblasts are the highest within 2–4 days after MI [41••]. After the activation of fibroblasts, the activated fibroblasts get converted to myofibroblasts that express smooth muscle α-actin (αSMA), contain extensive endoplasmic reticulum [42], and secrete adequate extracellular matrix proteins to fill the injured area within 3–7 days [41••]. Furthermore, the activated fibroblasts and myofibroblasts reach the exhausted proliferation rate by 7 days after MI; this finding is consistent with that of another study conducted by an independent group [43]. By 7–10 days, the myofibroblasts enter an alternate differentiated state without proliferation ability and αSMA expression, while the scar fully matures with increased extracellular matrix deposition [41••, 44]. The final stage for fibroblasts is matrifibrocytes. They exhibit weak contractile and secretory abilities but express universal and specific extracellular matrix and tendon genes. Specifically, they express *Cilp2* and *Comp*, which are related to bone and fiber remodeling, and may play an important role in maintaining the mature scar [41••] (Fig. 2).

A subset of cardiac fibroblasts undergoes senescence in response to injury and aging. These senescent fibroblasts modulate innate immunity by secreting inflammatory molecules [45]. The p53 signaling pathway is involved in promoting the occurrence of senescent fibroblasts and in modulating the inflammatory domains and ECM deposition [46]. As matrifibrocytes have a poor proliferation rate in the final stage of injury, they are considered to be a fibroblast cell type with some senescent characteristics [47]. Although senescent fibroblasts have been reported to express differential genes in comparison with other fibroblast subtypes, further studies should be conducted to assess the impact of senescent fibroblasts on tissue fibrosis and cardiac remodeling after injury.

Using scRNA-seq, many studies have started to unravel the diversity and heterogeneity of fibroblast populations [48]. Ren et al. analyzed the transcriptomes of 11,492 single cells in a pressure overload-induced cardiac injury model in order to unravel the heterogeneity of fibroblasts by grouping them into six clusters (FB1–FB6) [8•]. Skelly et al. defined a new cluster of fibroblasts that could express both fibroblast and immune cell markers through scRNA-seq of cells isolated from uninjured hearts; however, their functional role in the heart has not yet been studied [32]. Another study reported the emergence of two new fibroblast subpopulations, fibroblast-*Cilp* and fibroblast-*Thbs4*, after tissue stress and accelerated fibrosis [49]. As

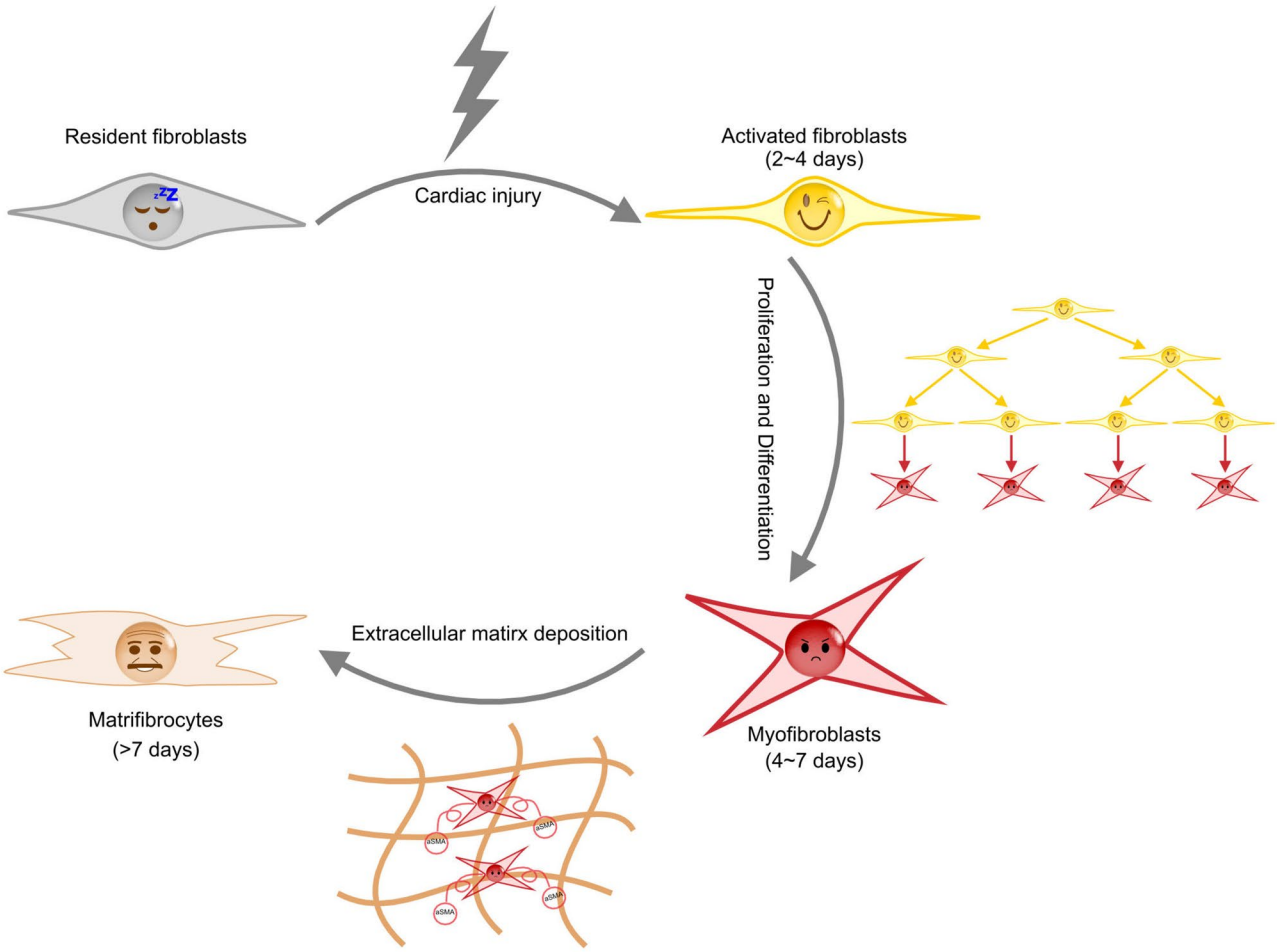

Fig. 2 Different states of fibroblasts respond to wounds healing in a time-dependent way

the transcriptional profile of fibroblasts is variably modified in response to external stimulation, it will be helpful to reveal their indispensable phenotypic plasticity using single-cell transcriptomic technologies.

The aforementioned data demonstrate the different states of fibroblasts in different injury models and generalize the function of each fibroblast state during fibrosis; the correlations between fibroblasts in each state remain unknown. Further research will be needed to reveal their functions and contributions to cardiac injury and repair.

Table 1 Marker genes expression in different states of fibroblasts (summarized from [41••]). *Col1a1,* collagen type I alpha 1; *Col13a1,* collagen type III alpha 1; *PAGFRa*, platelet-derived growth factor receptor alpha; *Tcf21*, transcription factor 21; *Acta2,* actin alpha 2, smooth muscle; *Lox,* lysyl oxidase; *Cilp2,* cartilage intermediate layer protein 2; *Comp,* cartilage oligomeric matrix protein. +, represents marker genes expression, more"+" indicates higher expression.

Table 1 Marker genes expression in different states of fibroblasts

Sub-types of fibroblasts				
Markers	Resident fibroblasts	Active fibroblasts	Myofibroblasts	Matrifibrocytes
Col1a1	+	++	+++	++
Col3a1	+	++	+++	++
PDGFRa	+++	++	+	++
Tcf21	+++	+	+	++
Vimentin	+++	+++	+++	+++
Periostin	+	++	+++	++
Acta2		++	++	
Lox		++	+++	++
Cilp2				++
Comp			++	+++

Pathways Implicated in Cardiac Fibroblasts After Cardiac Injury

Transforming growth factor beta (TGF-β) is one of the most well-studied factors involved in the pathogenesis of cardiac fibrosis, including tissue inflammation, myofibroblast differentiation, ECM synthesis, and gene expression [50–56]. TGF-β1, TGF-β2, and TGF-β3 are the three distinct isoforms of TGF-β [57]. Once TGF-βs bind to the heteromeric serine–threonine kinase receptors TGFBRs on the cell surface, downstream signaling cascades, including the Smad tri-complex (Smad2–Smad3–Smad4) [58–60] or Smad-independent pathways, are activated to execute important functions in tissue fibrosis [59]. TGF-β–Smad2/3 signaling is considered to be the canonical signaling pathway for inducing the differentiation of fibroblasts to myofibroblasts [52]. Accordingly, Khalil et al. provided direct genetic evidence that fibroblast-specific TGF-β–Smad2/3 signaling plays a role in cardiac fibrosis using a tissue-specific knockout model in vivo [61]. Specifically, they used lineage tracing strategies to explore cardiac fibrosis in pressure overload-induced heart failure by using fibroblast-specific and myofibroblast-specific inducible Cre mouse lines simultaneously with the conditional deletion of Tgfbr1/2, Smad2, or Smad3 [61]. They found that the specific deletion of Tgfbr1/2 or Smad3, instead of Smad2, in fibroblasts remarkably reduced the fibrotic response after TAC. The deletion of TGF-β receptor 1/2 in fibroblasts could relieve pressure overload-induced cardiac hypertrophy by adjusting abundant regulatory genes engaged in cardiomyocyte homeostasis and disease compensation [61]. This finding was consistent with that of Li et al., who proved that Smad2 plays a redundant role in ovarian granulosa cells in vivo [62]. Furthermore, mitogen-activated protein kinase (MAPK) signaling pathways, including p38 MAPK[62], ERK1/2[63, 64], PI3K[65, 66], and JNK[67], are considered to be the noncanonical signaling pathways in response to TGF-β stimulation; they form a complicated network of signaling pathways for modulating cardiac fibrosis.

Bone morphogenetic proteins (BMPs) are believed to play an indispensable role in the TGF-β–Smad signaling pathway [68–71]. TGF-β1 and BMPs directly bind to type II receptors, which dimerize with and stimulate type I receptors for signal transduction through Smads [72]. TGF-β1 triggers counterregulatory pathways to reduce its own activity. For instance, ALK5/Smad3 and ALK1/Smad1 stimulated by TGF-β1 counterregulate each other to modulate the TGF-β1 state in cardiac fibrosis [68–71]. BMP7 has been found to reduce cardiac fibrosis by activating Smad1 in endothelial cells [73]. In order to assess the effect of BMPs on cardiac fibroblasts, Kevin et al. subjected $BMP9^{-/-}$ mice to TAC and observed that the deletion of BMP9 aggregated cardiac fibrosis by increasing phosphorylated Smad3 (pSmad3) levels in the left ventricle [72]. Their findings suggest that the manipulation of the BMP signaling pathway could be a potential therapeutic target for reducing cardiac fibrosis and improving cardiac repair.

There is extensive evidence that Wnt/β-catenin signaling is involved in inflammation, immune response, and scar formation in multiple mouse models [74, 75]. The Wnt signaling system consists of 19 lipophilic proteins [76]. Wnts play an indispensable role in the induction of cardiogenesis [77]. Recent research has revealed that Wnt1 could promote the proliferation of cardiac fibroblasts and the expression of specific genes that are associated with the profibrotic process in injured regions of the infarcted heart [74]. Wnt5a expression is increased in regions of immune cell infiltration during cardiac fibrosis caused by autoimmune myocarditis [75]. To assess the role of β-catenin in pressure overload-induced heart failure, the β-catenin gene in resident cardiac fibroblasts was manipulated and cardiac fibroblasts were activated in $Tcf21^{MerCreMer}$ and $Periostin\ (Postn)^{MerCreMer}$ mouse lines, respectively. The loss of function study revealed that the inactivation of β-catenin in cardiac fibroblasts was extremely beneficial in alleviating cardiac hypertrophy by reducing interstitial fibrosis without changing the number of activated fibroblasts in vivo [78]. Notably, cardiomyocyte hypertrophy was found to be reduced with the loss of β-catenin after TAC [78], indicating some non-cell autonomous effect. Additional research should be conducted to study the interaction of the Wnt/β-catenin signaling pathway with other pathways in cardiac fibroblasts in order to provide new insights into cardiac injury and repair.

The Hippo pathway is known for its key role in cardiomyocyte proliferation [79, 80]. Large tumor suppressor kinase1 (Lats1) and Lats2 are two negative regulators of Yap, which is the core transcriptional coactivator in the Hippo pathway [81]. The results of scRNA-seq have revealed that uninjured Lats1/2-mutant cardiac fibroblasts could automatically transition to the myofibroblast state [82]; this is consistent with the finding of another study [83]. Jamie et al. used $Yap^{F/F};Tcf21^{MCM}$ mouse line to knock down Yap specifically in cardiac fibroblasts and found that Yap induced myofibroblast differentiation and gene expression involved in ECM through the activation of the TEAD domain transcription factor 1 and the subsequent de novo expression of myocardin-related transcription factor A [79]. These findings suggest that the manipulation of the Hippo signaling pathway is a prospective therapeutic strategy for improving cardiac remodeling in heart failure. As Wnt/β-catenin and Hippo pathways interact to regulate cardiomyocyte proliferation in the developing heart [84], it would be interesting to understand whether these two pathways converge or interact with each other to regulate fibroblast activation and proliferation in cardiac fibrosis.

Manipulation of Cardiac Fibroblasts for Cardiac Repair

As cardiac fibrosis is an important predictor of sudden cardiac death [85], manipulating the number and activity of cardiac fibroblasts to alleviate severe fibrosis could be a promising therapeutic target for treating cardiac fibrosis. In terms of gene regulation, the selective knockdown of Tgfbr1/2 or Smad3 in cardiac fibroblasts could significantly inhibit the fibrotic response and reduce fibrosis [61, 86]. The deletion of Yap in fibroblasts could prominently attenuate cardiac fibrosis and ameliorate cardiac dysfunction after MI or other injuries [79]. Another study reported that the activation of TGF-β1 could be inhibited by decreasing nuclear Yap levels [87]. In addition, recombinant BMP9 acts as a potential candidate to reduce cardiac fibrosis and improve cardiac function in heart failure patients [72]. The deletion of Mapk14 from fibroblasts can also arrest fibrosis by blocking the process of myofibroblast differentiation [88].

With the exploration of the underlying mechanism, many therapeutic approaches for potentially targeting tissue fibrosis have been reported in patients. Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) have already shown considerable potency in alleviating cardiac fibrosis in human and animal models of heart failure; however, they have not been approved for treating cardiac fibrosis [89]. TβRI, an inhibitor of ALK5, reduces TGF-β activity by restraining collagen synthesis and slowing fibrosis progression; it can alleviate cardiac dysfunction and improve cardiac remodeling in heart injury patients [90, 91]. Evodiamine reduces the expression of α-SMA in rat cardiac fibroblasts to enhance cardiac function after injury [92]. Moreover, tranilast and pirfenidone are two antifibrotic agents that could limit TGF-β activation; however, the underlying mechanisms remain unknown [93]. The profibrotic Wnt/β-catenin signaling pathway has been reported to be controlled by angiotensin II receptor 1 in experimental autoimmune myocarditis [94]. XAV939, an inhibitor of the Wnt/β-catenin pathway, reduces cardiac fibroblast activation induced by TGFβ1 and promotes cardiac repair [78]. With regard to Smad-dependent and Smad-independent pathways, halofuginone and the c-abl kinase inhibitor imatinib are proposed to inhibit fibrosis [95]. Halofuginone resists fibrosis by inhibiting the activity of Smad3 and rapidly increasing the expression of Smad7 [96]. Imatinib alleviates renal [97] and pulmonary [98] fibrosis by affecting the Smad-independent TGF-β signaling pathway. Osthole treatment decreases the expression of α-SMA, pSmad2/3, Smad4, and TβRI and the deposition of collagen I and III but increases the expression of Smad7 [99]. Verteporfin inhibits the YAP–TEAD association and decreases the expression of MRTF-A and α-SMA to attenuate cardiac fibrosis [79]. CCG-203971, the inhibitor of MRTF-A, also reduces collagen lattice contraction [79, 100]. Moreover, cilengitide reduces the scar size in collagen V-deficient mice by inhibiting specific integrins [101]. JQ1, a small-molecule BET bromodomain inhibitor, has been verified to reduce fibrosis in TAC models [102].

Through scRNA-seq analysis, collagen triple helix repeat containing 1 (CTHRC1) has been found to be a novel regulator that plays a crucial role in cardiac fibroblasts to heal scars after MI [103]. Moreover, scRNA-seq and transposase-accessible chromatin sequencing (scATAC-seq) have revealed that Meox1 is highly upregulated in myofibroblasts after TAC; it is considered to be a central mediator of fibroblast activation that could be targeted to regulate cardiac dysfunction [104•]. Recently, chimeric antigen receptor (CAR) T cells have been engineered to recognize and prompt the ablation of myofibroblasts [105]. These advances in the modulation of cardiac fibroblasts provide us with new approaches for treating cardiac fibrosis and promoting tissue repair after injury.

Conclusion

With technological advancements, such as lineage tracing and scRNA-seq, the origins of fibroblasts, the relationships between different fibroblast states, and fibroblast heterogeneity have begun to be unraveled in recent years. The coordination between tremendous complex and diverse signaling pathways that regulate the fate of fibroblasts and modulate their transformation during diseases has also been elucidated in recent studies. While some existing treatments targeting cardiac fibroblasts could alleviate fibrosis after cardiac injury, their specific mechanisms and pharmacokinetics remain unknown. As fibroblasts are a type of nonmyocytes in the heart that play an indispensable role in cardiac injury and repair, efforts will also be needed to understand the interaction of fibroblasts with other cell lineages during the pathological process of cardiac fibrosis. It is necessary to conduct more basic research using state-of-the-art technology to understand fibroblast biology in depth and explore more therapeutic targets for treating cardiac fibrosis.

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Compliance with Ethical Standards

Conflict of Interest The authors confirm that there is no conflict of interest between them.

Human and Animal Rights and Informed Consent The present study did not involve human or animal subjects.

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•• Of major importance

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