Cardiac fibrosis: Myofibroblast-mediated pathological regulation and drug delivery strategies

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Abstract

Cardiac fibrosis remains an unresolved problem in heart diseases. After initial injury, cardiac fibroblasts (CFs) are activated and subsequently differentiate into myofibroblasts (myoFbs) that are major mediator cells in the pathological remodeling. MyoFbs exhibit proliferative and secretive characteristics, and contribute to extracellular matrix (ECM) turnover, collagen deposition. The persistent functions of myoFbs lead to fibrotic scars and cardiac dysfunction. The anti-fibrotic treatment is hindered by the elusive mechanism of fibrosis and lack of specific targets on myoFbs. In this review, we will outline the progress of cardiac fibrosis and its contributions to the heart failure. We will also shed light on the role of myoFbs in the regulation of adverse remodeling. The communication between myoFbs and other cells that are involved in the heart injury and repair respectively will be reviewed in detail. Then, recently developed therapeutic strategies to treat fibrosis will be summarized such as i) chimeric antigen receptor T cell (CAR-T) therapy with an optimal target on myoFbs, ii) direct reprogramming from stem cells to quiescent CFs, iii) "off-target" small molecular drugs. The application of nano/micro technology will be discussed as well, which is involved in the construction of cell-based biomimic platforms and "pleiotropic" drug delivery systems.

Keywords

Cardiac fibrosis; Cardiac fibroblast; Myofibroblasts; Myocardial remodeling; Reprogramming; Drug delivery systems

1. Introduction

Cardiovascular diseases (CVDs) cause approximately 31% of all deaths worldwide [1], and cardiac fibrosis contributes to end-stage extracellular matrix (ECM) remodeling and heart failure [2]. Cardiac fibroblasts (CFs) are not only the source of ECM in healthy heart, they are also critical mediator cells in response to cardiac pathological changes. During injury,

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functional CFs differentiate into myofibroblasts (myoFbs) that are typical cells secreting contractile proteins [3]. In response to disease stimuli, myoFbs exhibit proliferative and invasive properties, and initiate the reparative would healing response. MyoFbs also remodel the interstitium by secreting ECM-degrading metalloproteinases (MMPs) and increasing collagen turnover. However, myoFbs show a persistent proliferation feature, which augments pro-inflammatory responses and causes collagen net formation [4].

The limited understanding of fibrosis is the cause of slow progress in anti-fibrosis therapies. Specifically, it is complicated to clarify the fibrotic mechanism since fibrosis occurs in various types of cardiac disorders. MyoFbs, the most specialized cells in fibrosis, not only come from resident CFs and endothelial cells, they also differentiate from circulating cells that migrate into the injured heart. The diversity of myoFb origination is a barrier for optimizing specific markers on this type of cell. Recently, stem cells have been propelled to the forefront of cardiac regeneration, presenting unexpected anti-fibrosis capabilities. Prescribed therapeutic agents for CVDs treatment such as Angiotensin II (Ang II) inhibitors have shown "off-target" effects on fibrosis alleviation by inhibiting the activation and proliferation of myoFbs [3]. However, new drug screening is difficult due to lack of cell models to mimic complex cellular interactions *in vivo*. Nano/micro-technology has been extensively used to deliver drugs, cells and genes via negative or positive targeting. Nano/micro-scale delivery systems such as polymeric nanoparticles and liposomes show multifunctional characteristics including bio-mimic, biocompatible and degradable natures [5–7]. Not confined to the heart, activated fibroblasts participate in fibrosis and adverse remodeling in many tissues like liver, lung and tumor, which allows for the development of fibroblast-targeted therapeutics [8–10]. For instance, nanoscale drug delivery systems have been developed to target tumor-associated fibroblasts for cancer therapy [11,12]. In addition, compelling evidence has shown enhanced targeting and retention efficacies of stem cells in cardiac tissues by nano/micro-technologies [13,14].

In this review, we will give a detailed insight into the progress of fibrosis, myoFb origination and differentiation, and myoFb-mediated fibrotic remodeling in CVDs. The communication between myoFbs and other cell types will also be discussed, elucidating potential approaches to fibrotic inhibition. The investigation of myoFb's role in responding to disorder stimuli serves to highlight myoFb's potential as a therapeutic target. Additionally, we will summarize emerging strategies for direct or indirect attenuation of fibrosis. For instance, a specific marker on myoFbs has been discovered, and the engineered chimeric antigen receptor T cell (CAR-T) has be used to target myoFbs and eliminate fibrosis [15]. Unlike previous cell reprogramming of CFs into cardiomyocytes, single cell ribonucleic acid (RNA) sequencing has been used to indicate a new method that induces stem cells into quiescent CFs for fibrosis treatment [16]. Notably, nano/micro-technology-mediated bio-mimic cell delivery systems will also be briefly discussed.

2. Cardiac fibrosis

2.1. Cardiac fibrosis characteristics

Cardiac fibrosis, a scar event in the cardiac muscle, occurs in almost all types of heart diseases including myocardial infarction (MI), hypertrophic cardiomyopathy, dilated

cardiomyopathy, diabetic cardiomyopathy and aortic stenosis [17,18]. Turnover of ECM components plays a prominent role in fibrosis that is pathologically characterized by increased deposition of collagens (mainly type I and III) [19,20]. During the process of fibrosis, CF, the key cell type, becomes activated and differentiate into the myoFb [21]. This pathological remodeling results in increased matrix stiffness and abnormal cardiac function, leading to heart failure with reduced ejection fraction [22]. Cardiac fibrosis also causes heart failure with preserved ejection fraction since fibrosis causes myocardial stiffness, impacting filling capacity of the heart and compromising distensibility of ventricles [23].

Myocardial fibrosis presents as either perivascular, focal, replacement, or interstitial depending on the disease. Interstitial fibrosis can be further divided into reactive and infiltrative fibrosis [24]. Reactive interstitial fibrosis can be induced by increased ECM deposition without a significant loss of cardiomyocytes. Therefore, this type of fibrosis normally leads to pressure overload and cardiomyopathies. In comparison, infiltrative interstitial fibrosis occurs in patients with Fabry disease, a rare genetic disease characterized by dysfunctional catabolism of sphingolipids [21,25]. Replacement fibrosis occurs in cardiac injury like MI where cardiomyocytes are damaged and replaced by activated fibroblasts with formation of predominant scar containing type I collagen. In human fibrotic hearts, histological staining of type I collagen has indicated four types of texture: compact, interstitial, patchy and diffuse [26]. Compact fibrosis is deposition of dense and large collagens, where cardiomyocytes are totally devoid. By contrast with compact fibrosis, collagens in interstitial fibrosis deposit in between cells. Patchy fibrosis is characterized by long collagen fiber strands, while diffuse fibrosis exhibits short stretches of fibrosis [26].

Collagen deposition is a critical feature of cardiac fibrosis, specifically the expression of type I and III collagens. For instance, an upregulation of type I collagen was observed in the fibrosis of MI model [27,28], while the expression of type III collagen was increased in patients with ischemic cardiomyopathy [27,29]. Type I and III collagen significantly increase CF proliferation but do not affect myoFb differentiation [30]. The activation of extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), a major mitogenic signal in CFs, has been reported to be involved in the mechanism of type I collagen-induced CF proliferation. However, type III and VI collagen had little or no effect on ERK1/2 phosphorylation [30]. Type VI collagen has been reported to facilitate myoFb differentiation, though the mechanism remains unclear [27]. Specifically, *in vitro* incubation of type VI collagen with myoFb induced differentiation and had negligible effects on CF proliferation [30]. Bryant *et al.* found that type VI collagen was significantly elevated in both non-infarcted (1.48 ± 0.13 fold) and infarcted (2.27 ± 0.13 fold) regions when compared to sham operated control 7 days after MI. As for the possible mechanism, they further found that the type VI collagen interacted with α-integrin receptor in CFs, and the blockade of α-integrin receptor-attenuated type VI collagen induced myoFb differentiation [31]. Interestingly, the deletion of type VI collagen in the knockout MI model played a protective role by limiting infarct size and fibrosis [32]. Another feature of cardiac fibrosis is the collagen maturation and fibrotic scar formation with crosslinking density, which leads to an increase in tensile strength of those scars [1,33]. These scar tissues impact on the extent of cardiac relaxation and contractility, which limits cardiac function [1]. Recently, fibrillar type V collagen has been reported to limit scar size after ischemic cardiac injury. Lacking type V collagen

enabled scars to initiate integrin-dependent mechanosensitive feedback on CFs, leading to the augment of CF activation, ECM secretion and scar size increase [34]. In detail, the *Col5a1* gene in CFs has been shown to regulate type V collagen secretion in CFs. A *Col5a1* knockout led to the deficiency of type V collagen, which caused scar size increase and grossly abnormal scar architecture. The altered mechanical features of scar further increased the expression of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, which acted as feedback cues to drive myoFb differentiation. To develop novel fibrosis therapies, further studies are needed to explore the biology of collagen formation and the crosstalk between collagens and CFs.

2.2. Biological pathogenesis in cardiac fibrosis

The pathogenesis of cardiac fibrosis remains unclear. It is well-established that the inflammation response plays a prominent role in cardiac fibrosis where the nucleotide-binding domain and leucine-rich repeat containing the PYD-3 (NLRP3) inflammasome are critical determinants [35]. Activation of the NLRP3 inflammasome can promote TGF-β signaling and drive fibrosis by inducing maturation of interleukin (IL)-1β (IL-1β) and IL-18 in CFs instead of in cardiomyocytes [36,37]. A recent report revealed that yes-associated protein (YAP) was a regulator of macrophage-mediated pro-inflammatory response after MI and cardiac fibrosis [38].

Recently, there is growing evidence to indicate the central role of mitochondrial dysfunction in fibrosis [39]. Mitochondrial dysfunction is mainly characterized by mitochondrial structure and gene damage, as well as changes in cellular oxidative protein activities. Mitochondria serves as the main source of reactive oxygen species (ROS) that is a byproduct of oxygen metabolism. The imbalance between biogenesis and scavenging of ROS results in oxidative damage to mitochondrial proteins, genes and lipids [40,41]. In cardiac fibrosis, ROS may directly regulate interstitial ECM production by modulating the expression and metabolism of matrix proteins [42]. In a recent study, peroxisome proliferator activated receptor gamma coactivator 1-alpha (PGC-1α), a master metabolic regulator, was upregulated in cardiac diseases and altered mitochondrial biogenesis, causing myocardial fibrosis [43]. Ca2$^+$/calmodulin-dependent protein kinase II (CaMKII) functions as a cellular ROS sensor, which may be activated in the presence of ROS [44]. CaMKII is also activated in cardiac diseases with inflammatory environments such as MI and ischemia/reperfusion injury. It regulates pro-inflammatory signaling nuclear factor kappa-B (NF-κB), thus contributing to pathological cardiac remodeling [45]. Recently, mitochondria-targeted strategies have been proposed. For example, antioxidant enzyme CAT alleviated mitochondrial oxidative damage [46], while mitoquinone also inhibited fibrosis in pressure-overloaded hearts [47]. Notably, the dysfunction of metabolic-associated enzymes and the oxidative respiratory chain also serves as a critical factor for cardiac fibrosis. The ablation of nuclear-encoded mitochondrial inorganic pyrophosphatase (PPA2) has been reported to contribute to fibrosis, via the mechanism of activity reduction of the respiratory chain complex I and IV [48].

Further, endoplasmic reticulum (ER) stress and activation of the unfolded protein response also act as pro-fibrotic stimuli. Studies on the fibrotic remodeling via ER stress contain the activation of pro-apoptotic pathways, epithelial-to-mesenchymal transition, and induction of

pro-inflammatory responses [49]. Luo *et al.* demonstrated that the administration of 4-phenylbutyric acid (4-PBA), an ER stress attenuation agent, could effectively alleviate post-MI complications [50]. Additionally, obesity is also a trigger for cardiac fibrosis, which is highly associated with metabolic dysfunction and the inflammatory response [51]. The excessive synthesis of aldosterone is a remarkable characteristic of obesity. Adipocyte-derived hormone leptin has been reported to be a direct regulator in aldosterone secretion, which promotes endothelial dysfunction followed by cardiac fibrosis [52]. Both type I and II diabetes are related with cardiac fibrosis as well. Hyperglycemia directly activates a fibrogenic program, resulting in accumulation of advanced glycation end-products (AGEs) and activation of AGE receptor-mediated pathways. Diabetes-associated fibrosis is mainly characterized by CF activation. Generated AGEs activate resident CFs and induce a matrix-synthetic phenotype [53]. Growing evidence has shown that peroxisome proliferator-activated receptor α (PPARα), a ligand-activated transcription factor, regulates fibrosis by modulating endothelial nitric oxide synthase and anti-inflammatory signaling pathways. Currently, novel PPARα drugs are used to alleviate fibrosis [54].

3. The role of myofibroblasts in fibrosis and cardiac diseases

3.1. The origin of pro-fibrotic myofibroblasts

MyoFbs are rarely observed in healthy cardiac tissues, while these cells replace damaged non-regenerated cardiomyocytes in injured tissues. Except for resident CFs, epithelial-derived cells (EPDCs) and endothelial-derived cells potentially adopt the myoFb phenotype via the process of epithelial-to-mesenchymal transition and endothelial-to-mesenchymal transition, respectively [55,56]. *In vivo* differentiation of these epithelial and endothelial-derived cells can be achieved by undergoing a defined sequence of events that allows them to present the fibrotic phenotype. These processes are strictly regulated by a sequence of coordinated expression of numerous growth factors such as fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) [55,57]. It needs to be mentioned that during embryonic development, EPDCs can alternate between spindle-shaped CFs and α-smooth muscle actin (α-SMA)[+] phenotypes, which can then be activated to myoFbs in adult life during injury-induced pathological remodeling [58]. Specifically, endothelial-to-mesenchymal transition of resident endothelial cells was reported to generate 70% of the myoFbs in the heart with overload pressure [56,59]. Pericytes, which acts as contractile cells with mesenchymal origin and wraps around the microvasculature, were also proposed to adopt newly generated α-SMA[+] myoFbs [60]. Kramann *et al.* found that resident Gli1^{+} mesenchymal stem cell (MSC)-like cells in the perivascular niche expressed typical MSC markers and presented trilineage differentiation capacity, contributing to cardiac fibrosis. Genetic lineage tracing analysis revealed that rather than circulating Gli1^{+} cells, it was the tissues-resident Gli1^{+} cells that proliferated to generate myoFbs after heart injury [60]. Furthermore, bone marrow-derived stem cells (BMSCs) have a fibroblast-myoFb-like phenotype by the name of fibrocytes, which has been suggested to be recruited into the injured heart. These circulating fibrocytes express characteristics of fibroblasts and play a major role in scar formation during wound healing [61–64]. Using gene labeling, Amerongen *et al.* confirmed that 21% of BMSCs in the infarct area were myoFbs with peak numbers on day 7 post-MI. These bone marrow-derived myoFbs presented in the infarct area

and actively secreted collagen I [62]. The fibroblast lineage and signals that triggers the differentiation into myoFb are not well understood.

More methods have been used to investigate myoFb origins. Using a single-cell engraftment mice model, Norris *et al.* have demonstrated two extracardiac sources of CFs: the embryonic proepicardial organ and the recruitment of circulating BMSCs of hematopoietic stem cell (HSC) origin. Interestingly, periostin, a profibrogenic matricellular protein, was highly expressed in pathological remodeling and heart failure, and induces the differentiation of nonmyocyte progenitor cells into myoFbs [58]. Kanisicak *et al.* developed postn (periostin) gene-targeted mice containing a tamoxifen-inducible Cre for cellular lineage-tracing analysis. Their research revealed the postn-expressing myoFbs in the heart mainly derived from tissue-resident fibroblasts of transcription factor 21 (Tcf21) lineage, but not from endothelial, immune, or smooth muscle cells [65]. Taken together, more detailed studies on the myoFb origin and triggers are needed, and the deep exploration on differentiated myoFb activities are essential for developing cardiac repair therapies.

3.2. The differentiation from cardiac fibroblasts to myofibroblasts

CFs are the most prevalent cell types in the heart and play an essential role in regulating normal structure and function. One of important stimuli for the phenotype transition of CFs into myoFbs is a change of mechanical microenvironment. In intact healthy tissues, CFs are protected from stress by the crosslinked ECM framework. However, in an injury microenvironment, there is loss of architectural integrity allowing mechanical stress to reach fibroblasts and induce differentiation into proto-myoFbs [66]. Proto-myoFbs are identified by stress fibers, including cytoplasmic β-actin and γ-actin. When exposed to transforming growth factor-β (TGF-β, which is produced by CFs), those proto-myoFbs are induced to fully differentiate into myoFbs with the aid of the ED-A splice variant of fibronectin [67,68]. The assessment of RNA expression between CFs and myoFbs revealed a large difference between them, specifically the overexpression of actin alpha 2 and Postn with reduced expression of Tcf21 (a significant marker for quiescent fibroblasts) in myoFbs [69]. Different expression of said markers provide a pathway for assessment of the phenotype of active or quiescent fibroblasts. Nowadays, there are few unique markers of myoFbs, one of the reasons being that many cell types exist in the differentiation process (eg. CF-myoFb transition phenotype). Another reason is the fact that myoFb markers are also expressed on other cell types such as endothelial cells, mesenchymal cells and smooth muscle cells (SMCs), which are all sources of myoFbs. It is widely accepted that overexpressed α-SMA and other SMCs differentiation markers are characteristic markers for fully differentiated myoFbs (Fig. 1). Another differentiation marker is the cytoskeletal protein smoothelin, which was reported for contractile SMCs and can be used to distinguish SMCs from myoFbs [70]. However, smoothelin was also observed in lung myoFbs after the treatment of TGF-β1, making it an unreliable target [71]. Similarly, TGF-β1-treated myoFbs expressed of 4Ig paladin which is also expressed in SMCs [72,73]. The lack of specific myoFb markers still hinders the advancement of targeted therapies. Interestingly, fibroblast activation protein (FAP) is specifically expressed on the surface of differentiated myoFbs, and is currently being studied as a potential marker [15]. Moreover, a recent report shows that cardiac ECM in the failing heart could activate CFs via hyperactivated YAP signaling [74]. The discovery

and optimization of unique identifiers of myoFbs can facilitate the development of targeted drug delivery systems. MyoFbs can further induce the activation of CFs via autocrine signaling, which provides positive feedback to increase fibroblast activation [75], making delivery of myoFbs even more beneficial.

3.3. The role of myofibroblasts and their crosstalk with other cell types

It has been established that the phenotypically transformed myoFbs are found at infarct sites where synthesis and deposition of collagens cause scar formation and fibrosis. Chronic or repeated injury enables fibrous tissue deposition to be persistent. MyoFbs can persist at infarct scars and have been observed at six months post-MI in a rat model [76]. Generally, myoFbs express type I collagen, TGF-β, α-SMA, and other factors, which in turn stimulates other cells to become myoFbs and promote fibrotic effects. Ang II, which can be secreted by myoFbs, enhances the synthesis of collagens in infarct sites especially via the activation of type 1 angiotensin receptor (AT1R) [3]. It is also reported that Ang II stimulates TGF-β expression, revealing the downstream role of TGF-β [77]. Smad proteins are part of the classical signaling cascade after the stimulation of TGF-β receptor, which can also be regulated by Ang II [78]. In this case, the Smad signaling pathway may be directly inhibited for deactivating myoFbs and reducing fibrosis. Moreover, myoFbs contribute to ECM synthesis and deposition, secreting collagens, MMP and other proteins [79]. These responses can also be induced by inflammation related factors in injured cardiac tissues. To date, myoFbs have been highly investigated, indicating the role of myoFb as a mediator for adverse remodeling. The most obvious role is the crosstalk between myoFbs and cardiomyocytes. Injury to the heart modifies the microenvironment by increasing expression of ECM proteins, cytokines, and exosomes, which leads to a fibrotic heart. Under this injured condition, cardiomyocytes correspondingly alter paracrine signaling, which augments activation and *trans*-differentiation of myoFbs [80].

Chronic activation of the myocardial renin angiotensin system increases local Ang II expression, leading to cardiac hypertrophy. In detail, Ang II has been reported to induce myoFbs to secrete exosomes containing miR-21 and miR-423, which resulted in overexpression of AT1R and Ang II receptor type 2 (AT2R) in cardiomyocytes via the activation of mitogen-activated protein kinases (MAPKs) [81]. In cardiac fibrosis, cardiomyocytes can even secrete miR-208a-containing exosomes into CFs, leading to myoFb differentiation followed by fibrosis development [82]. More miRNAs and cytokines have been identified as paracrine signaling factors between cardiomyocytes and CFs in cardiac diseases. For instance, miR-133 expression was decreased during hyperglycemia from diabetic cardiomyopathy, and overexpressed miR-133a prevented ERK1/2 and Smad2 phosphorylation [83]. MiR-378 has been reported to be secreted from cardiomyocytes during mechanical stress, which inhibited the excessive cardiac fibrosis through the suppression of p38 MAPK phosphorylation [84]. Interestingly, CF-specific p38 MAPK in turn induced cardiomyocyte hypertrophy via secreting paracrine factors including IL 6. The IL 6 signaling between fibroblasts and cardiomyocytes further caused cardiac dysfunction such as cardiac hypertrophy [85]. A current report indicated that in transverse aortic constriction (TAC) induced pressure overload, cardiomyocytes activated CFs followed with fibrosis via Wnt5a and Wnt11 signaling. Overexpression of cardiomyocyte-specific LRP6

interacted with the protease cathepsin D and facilitated the degradation of Wnt5a and Wnt11 [86]. Macrophage-secreted exosomes have also been reported to affect myoFbs and fibrosis [87]. For example, macrophages transferred miR-155 abundant exosomes into myoFbs to decrease collagen production [88]. In addition, intra-cardiac injection of stem cells has been shown to induce regional accumulation of macrophages. These accumulated macrophages downregulated CF activity, reduced ECM deposition, and enhanced cardiac function [89]. Together, these data highlight the critical role of cellular paracrine communication in cardiac fibrosis and pathology (Fig. 2)

4.   Therapeutic strategies targeting myofibroblasts

4.1.   Direct reprogramming

The regeneration of adult mammalian hearts is highly limited due to the low regenerative capacity of cardiomyocytes. Endogenous CFs, which accounts for a significant portion of the mammalian heart, are a potential source of cardiomyocytes for regeneration therapy. A promising method to reprogram fibroblasts into induced cardiac-like myocytes (iCLMs) has been developed. Those iCLMs are expected to act as cardiomyocytes to improve cardiac function. To achieve this goal, different combinations of cardiac-specific factors are being explored, mainly transcription factors, fibroblast activation inhibitors, small molecules, and microRNAs (miRNAs). Viral and non-viral vectors, especially nano/micro delivery systems, have been developed to deliver this cargo into CFs to initiate reprogramming.

4.1.1.   Direct reprogramming of murine fibroblasts—In 2010, Srivastava *et al.* found that three transcription factors: Gata4, Mef2c and Tbx5(GMT) enabled postnatal cardiac and dermal fibroblasts to be reprogrammed into iCLMs *in vitro* [90]. They further demonstrated successful reprograming of murine CFs into iCLMs via retroviral delivery of GMT, leading to reduced infarct size and fibrosis [91]. GMT were the core components for direct programming [90], and Tbx5 was further reported to promote the differentiation of transfected cells into beating cardiomyocytes [92]. Following studies from Olson *et al.* demonstrated enhanced reprogramming capacity of CFs both *in vitro* and *in vivo* after adding Hand2 in combination with GMT, which increases cardiac troponin T (cTnT) and tropomyosin expression [93]. These findings showed the feasibility of gene therapy for heart regeneration. Recently, various methods have been explored to increase GMT reprogramming efficacy. Qian *et al.* developed a complex set of polycistronic constructs containing GMT with identical 2A sequences in a single messenger RNA (mRNA). Adjusted ratio expression of Gata4: Mef2c:Tbx5 led to distinct protein expression and altered differentiation efficacy [94]. Inhibitors are alternative adjuvants for the induced expression of cardiac transcription factors. For instance, SB431542, an inhibitor of TGF-β pathway, increased the conversion rate of embryonic fibroblasts into iCLMs. The reprogramming efficacy of SB431542 was up to 5-fold higher than that of GMT, Hand2 and Nkx2.5 combined as measured by *Ryr2* gene expression [95]. When TGF-β and Rho-associated kinase pathways are inhibited, a high percentage of embryonic fibroblasts were converted into iCLMs, and about 60% expressed cTnT [96]. In addition, iCLMs could also be inducted and matured using various cytokines such as FGF2, FGF10, and vascular endothelial growth factor (VEGF) [97]. The conversion into iCLMs is a complex process

since there are several barriers in epigenetic modulation like *Bmi1*. Reduced expression of *Bmi1* changes chromatin modification at cardiogenic loci by increasing the active histone marker H3K4me3 and decreasing repressive H2AK119ub [98]. This means that cardiogenic gene expression is derepressed in the conversion process of iCLMs. These results reveal that, in addition to transcription factors, many other factors influence fibroblast reprogramming, and inhibitors with sequential addition are promising adjuvants for optimizing the induction efficiency of functional iCLMs.

MiRNAs are alternative families with potential for reprogramming since they can enhance the expression of transcription factors such as GMT and Nkx2.5. MiRNAs participate in every aspect of cardiac development, making them promising therapeutics for functional iCLM reprogramming. The representative miRNA is miR-1, a cardiac muscle-specific molecule, that accounts for about 40% of total miRNAs in the mammalian heart [99]. The primary role of miR-1 is to promote cardiomyocyte proliferation and inhibit apoptosis [100]. MiR-133 also plays an essential role in promoting cardiomyocyte proliferation via miR-133-mediated *Snai1* repression [101]. Recently, similar to the application of transcription factors, combination therapy of miRNAs has been explored. For instance, a "miRNA combo" (miR-1, miR-133, miR-208, miR-499) was assembled to convert CFs into functional iCLMs both *in vitro* and *in vivo* [102,103]. Furthermore, in a three-dimensional environment, such as hydrogel, the "miRNA combo" enhanced neonatal CF reprogramming [104]. In terms of mechanism, the combination of these miRNAs contributed to the altered expression of H3K27 methyltransferase and demethylase [105]. What is more, associated virus (AAV) vectors act as excellent vehicles for miRNAs, making them more accessible, and the small size of miRNAs potentially enables the convenient loading and delivery of a "miRNA combo" using one AAV vector [98].

Nano and micro delivery systems are alternative non-viral based systems for gene loading and delivery. Although viral vectors have been widely used for miRNA delivery, side effects have been reported, such as genetic aberration and alternative gene expression. Notably, cancer has been confirmed as an induced side effect [106]. In comparison, non-viral vehicles exhibit reduced immunogenic response and high safety properties. For instance, lipofectamine, an excellent lipid nano/micro-reagent, has been commercially approved and presents with low toxicity. The application of nanoparticles was firstly developed by Monica *et al.* to directly target and reprogram CFs. Dual small molecules were loaded into dextran-functionalized nanoparticles and delivered into CFs, leading to efficient CF reprogramming to iCLMs [107]. Notably, Muniyandi *et al.* loaded poly(lactide-*co*-glycolide) (PLGA) microparticles with two miRNAs (miR-1 and miR-133a) for direct CF reprogramming. In detail, polyetherimide, a most commonly used cationic vector for gene transfection, was used to compress these two miRNAs and form a core. The core was then encapsulated by outer biodegradable PLGA nanospheres. Mature late-stage markers, including troponin T and α-actinin, were both screened and had significant enhancement, revealing successful reprogramming into iCLMs [108]. Typical methods to reprogram murine fibroblasts are summarized in Table 1.

4.1.2. Direct reprogramming of human fibroblasts—Reprogramming therapy can only be translated into clinics once it succeeds on human fibroblasts. Compared with murine

fibroblasts, the process of human fibroblast reprogramming is difficult and time-consuming. In 2013, Nam *et al.* discovered the partial conversion from fibroblasts into iCLMs using the combination of Gata4, Hand2, Tbx5, Myocd (myocardin), miR-1 and miR-133. The milestone discovery was the expression of active cardiac markers [109]. Meanwhile, Wade *et al.* reported that cell morphology changed from a spindle shape to a rod-like shape when they reprogrammed fibroblasts with the "transcription factor combo" of Gata4, Mef2c, Tbx5, and Myocd (referred to Gmtm) [110]. Despite the reprogramming success on murine fibroblasts, Srivastava and colleagues found that it was insufficient to reprogram human fibroblasts using only GMT. The addition of ESRRG and MESP1 to GMT enabled induced iCLMs to express cardiac-specific genes and form sarcomeres. Interestingly, when adding Myocd and Zep-m2 to GMT, more cardiomyocytes-like features were achieved, such as phenotypic shift to the cardiac state and global cardiac gene expression [111].

Further advancement has appeared with the application of small molecules. Inspired by the reprogramming ability of small molecules [112–114], Ding and colleagues discovered a combination of nine small molecules which programmed human somatic cells into iCLMs in 2016. The molecules contained CHIR99021, A83–01, BIX01294, AS8351, SC1, Y27632, OAC2, SU16F, and JNJ10198409 [115]. After their induction, these iCLMs exhibited naïve human cardiomyocytes properties in terms of their transcriptome, epigenetics and electrophysiology. On this basis, the researchers confirmed the successful conversion of human fibroblasts into iCLMs, showing enhanced cardiac function in the infarcted heart [115]. It is noteworthy that these findings demonstrate great potential for clinical application, considering that low conversion rates can be further overcome in future studies. We summarized representative methods for human fibroblast reprogramming in Table 1.

4.2. Chimeric antigen receptor T cell (CAR-T) based immunotherapies

Fibroblast-targeting therapies remain limited due to the difficulty of identifying fibroblasts. The obvious obstacle is the lack of specific markers for activated myoFbs only when compared to other types of cells. On this end, engineered T cells have caused breakthroughs in cancer immunotherapy [15]. After being modified with a specific T cell receptor or a chimeric antigen receptor, cytotoxic T cells are redirected *in vivo* to recognize specific antigens on cancer cells and ablate them [129,130]. Through expression analysis of gene signatures, Aghajanian *et al.* recently discovered FAP, an endogenous protein marker, that was overexpressed on myoFbs when compared with quiescent CFs. They engineered CAR-T against FAP *in vivo*, which resulted in dramatical reduction of cardiac fibrosis at injured mice (Fig. 3) [15]. These results reveal a proof-of-concept which makes the use of CAR-T to target cardiac fibrosis, though more studies are needed to optimize therapeutic efficacy [131]. On one hand, continued search for alternative, unique antigens that are expressed solely on myoFbs may yield more targets or target combinations for fibrosis ablation. On the other, even when targeting the same antigen, specific CAR-T was able to more significantly affect targeting affinities and anti-fibrotic efficacies [132–134].

5. Other therapeutic strategies against fibrosis

5.1. Stem cell-based therapies

The application of stem cells with or without biomaterial scaffolds is another direction for the treatment of cardiac fibrosis and heart failure. Cells that have been explored in cardiac regeneration mainly include cardiac-derived stem/stromal cells (CSCs), BMSCs (eg. HSCs, MSCs, endothelial precursor cells (EPCs)), and induced pluripotent stem cells (iPSCs) [135,136], which can be delivered into heart tissue via intramyocardial, intrapericardial or intravenous injection [137–139]. Clinical trials of stem cell therapy continue, especially in this decade [140]. Unfortunately, no significant benefits have been observed, probably due to the variation between trials or exceptional end points, such as death [22]. One reason for modest clinical success is poor survival and engraftment of injected stem cells [136]. Another reason is negligible differentiation capabilities of stem cells towards fully functional cardiomyocytes [22] especially HSCs [141]. Additional studies suggest that stem cells achieve cardiac repair by secreting paracrine factors or interacting with other cells, such as macrophages [89,136,142]. For instance, Ronald J. *et al.* recently found that transplanted stem cells initiated an acute immune response and wound healing process via facilitating regional accumulation of CCR2$^+$ and CX3CR1$^+$ macrophages. Macrophages with these alternated phenotypes further reduced border zone ECM contents and alleviated fibrosis [89]. Notably, Wu *et al.* found specific transcriptome profiles using single-cell RNA-sequencing, which allowed for successful induction of human iPSCs to quiescent CFs for anti-fibrosis therapy (Fig. 4) [16]. This developed protocol helps deepen studies on the signaling pathway involved in cardiac fibrosis and also sheds light on the potential role of IPSCs as a platform to screen anti-fibrotic drugs in the future.

After being intravenously injected, stem cells are likely to aggregate in blood circulation with a high lung homing property [143]. Without shielding, which is the protection of cells by the use of an external vehicle, the shape, size and location of stem cells in heart tissues are difficult to control. These challenges lead to suboptimal cardiac repair. Recently, biomaterial-based scaffolds have been developed to load and deliver stem cells for cardiac regeneration. Using biomaterials, especially biodegradable polymers, it is easy to construct multifunctional nano/micro-scale delivery systems with high cell loading efficacies. As local delivery systems, patches [144] and hydrogels [145] present good niches for CSCs. Microneedles, which are potential *in situ* or transdermal therapeutic systems [146–148], were also used to form CSC-loaded patches for cardiac repair [149]. Moreover, hydrogels can not only act as vehicles for epicardial placement of stem cells [150], but also could be injectable delivery systems for cardiac healing [151,152]. Recently, platelets have been used for cardiac homing for stem cells. Myocardial ischemia induces endothelial denudation and inflammation, recruiting circulating platelets to injured heart sites such as vessel walls [153] and endothelial cells [154]. Using this cardiac homing property, platelet membranes have been conjugated onto CSC surfaces to boost the injured heart targeting efficacy of CSCs [154]. In addition, antibody systems have been designed to recognize platelets and stem cells simultaneously as a novel cell therapy strategy. For instance, circulating stem cells can be delivered to the injured heart via pre-targeting polymer modified CD34 antibodies which recognize circulating EPCs, and CD41 antibodies which bind circulating platelets [155].

Further, inhaled bispecific antibodies (BsAbs) were designed to redirect stem cells from the lungs to the heart [143]. In detail, the lung has been reported to be a reservoir for HSCs and platelets [156]. Based on this, Liu *et al.* constructed CD34-CD42b BsAbs to catch HSCs and platelets simultaneously in the lungs, leveraging the lung's biogenesis to alleviate cardiac fibrosis [143]. Another advancement are scaffold-free cell sheets, which were developed by culturing cells in a dish grafted with thermo-sensitive polymers like poly(N-isopropylacrylamide), and provide the advantage of solely delivering cells [157]. Representative biomaterial-based cell therapies are summarized in Table 2.

5.2. Therapeutic strategies based on stem cell secreted factors

Besides stem cells for therapy, using stem cell-secreted factors is a cost-effective alternative that has been used to treat cardiac injury. These therapies consist mainly of stem cell-derived extracellular vesicles (EVs), secretomes, exosomes, proteins and cytokines [168]. Effective exosomes or secretomes come from stem cells (MSCs, CSCs, ESCs, etc.), or from cells exposed to pathological environments. These exosomes or secretomes contain growth factors and miRNAs that exert anti-apoptosic, anti-fibrotic, and proangiogenic effects [169]. This therapeutic cargo can be loaded into bio-mimicking nano/micro-particles for cardiac repair [170]. One such example is that Tang *et al.* constructed a regenerative patch by spraying platelet-rich plasma. The cardiac patch could release growth factors, such as VEGF, at the MI site, leading to reduced scar fibrosis and attenuated left ventricular remodeling [162]. Another acellular patch made of a porcine extracellular-matrix scaffold was developed to repair myocardial tissues, which can be notably maintained at the targeted site for up to 28 days [163]. In addition, cell membrane-coated nano/microparticles have emerged as novel targeting platforms, exhibiting homing capabilities to cardiac injury [166]. For instance, cell-mimicking microparticles were fabricated by coating MSC and CSC membranes onto the surface of degradable PLGA microparticles and loading MSC and CSC secretomes [164,165]. Platelet membrane-coated nanoparticles conjugated with PEG2 exhibited desirable injured cardiomyocytes (PEG2 receptors) targeted efficiencies [166]. Additionally, monocyte-mimicking systems have been designed as novel EV delivery vehicles for heart repair [167]. Representative biomaterials-based cell-free therapies are summarized in Table 2.

5.3. Therapeutic strategies developed from "off-target" drugs

Compelling evidence shows the reversion abilities of the myoFb phenotype into quiescent fibroblasts [65,171,172]. Therefore, a potential strategy is to target signaling pathways associated with fibroblast-to-myoFb phenotype differentiation. Interestingly, therapeutic agents against CVDs have "off-target" effects on CFs, which may underlie their benefits on other cells or their unknown effects on myoFbs. These unexpected anti-fibrotic effects are considered as "pleiotropic". Small molecules presenting "pleiotropic" effects on myoFbs mainly contain anti-hypertensive agents such as angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs) (especially Ang II blocker) and lipid-lowering drugs, and pharmacological agents, such as thiazolidinediones (TZDs), with the aim to lower insulin resistance in diabetic patients) [3].

The major task of ACE inhibitors and ARBs is to antagonize Ang II effects. Normally, Ang II binds and activates AT1R and AT2R followed with the activation of downstream pathways. Stimulated by growth factors and cytokines like IL-1 and tumor necrosis factor-α (TNF-α), myoFbs exhibit increased expression of AT1R and AT2R in heart failure when compared to normal CFs in healthy cardiac tissue [173]. MyoFbs also express the required components to the Ang II synthesis [174]. ACE inhibitors specifically inhibit ACE-mediated cleavage of Ang I and thereby block Ang II synthesis, whereas ARBs are used to inhibit AT1R [175]. TGF-β-induced myoFb differentiation also increases ACE expression, which makes TGF-β inhibition an alternated anti-fibrosis mechanism of ACE inhibitors [176]. Besides differentiation, the pro-fibrotic ability of Ang II is also characterized by an increase in ECM protein synthesis, reducing MMP activity expression of tissue inhibitor of metalloproteinase (TIMP). Imidaprilat, an ACE inhibitor, was shown to reduce the expression of IL-1 and MMP-2 in human CFs [177]. Curcumin can attenuate myocardial fibrosis by modulating AT1R and AT2R in rats [178]. Interestingly, another ACE inhibitor lisinopril can reduce TGF-β-induced collagen synthesis in rat CFs [176].

Moreover, statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-coa) and are involved in the established treatment of hypercholesterolaemia, where they exhibit anti-fibrotic effects. For instance, simvastatin inhibits TNF-α induced MMP-9 secretion in human myoFbs [179], and pravastatin exhibits MMP-3 and MMP-9 inhibition abilities in Ang II-overexpressed murine CFs [180]. Pravastatin also had anti-proliferation effects on myoFbs by inhibiting TGF-β signaling [181]. In addition, TZDs, agonists of PPARγ, reduced insulin resistance but increased vascular benefits in patients with type-2 diabetes mellitus [182]. Another candidate is the first-in-class thienodiazepine small molecule JQ1, an inhibitor of bromodomain-containing protein 4 (BRD4) [183]. In heart failure, JQ1 not only treated cardiac hypertrophy [69], but also reduced myoFb activation [184] via inhibition of BRD4 signaling. Moreover, as inflammation is a fibrosis pathogenesis factor, colchicine acts as an anti-inflammatory agent and has been reported to exhibit fibrosis alleviation properties [185].

6. Clinical trials for the alleviation of cardiac fibrosis

Recently, new mediators such as miRNAs and cytokines have emerged to alleviate cardiac fibrosis. However, most of them have not been clinically translated. In current clinical trials, anti-fibrosis results trials are disappointing, with promising data resulting mainly from treatments with renin-angiotensin-aldosterone system (RAAS) inhibitors [186]. In general, the first family of antifibrotic drugs against cardiac fibrosis is that of angiotensin II inhibitors. For instance, ACE inhibitors have been reported to exhibited anti-fibrotic benefits not only in animals (as mentioned in 5.3), but also in human patients [186]. In addition, drugs like inflammatory modulators and relaxin also exhibited fibrosis attenuation effects in clinical studies. Some currently representative clinical trials against cardiac fibrosis are shown in Table 3. The effective drugs in clinical studies, such as RAAS, only show modest regression of cardiac fibrosis [186]. Patients with heart failure still encounter persistent cardiac fibrosis even when receiving official standard of care [187]. The failure of anti-fibrotic drugs clinical trials suggests that translating research data from animal models to

humans requires further development due to the vast genetic differences between animals and humans.

7. Conclusion

The pathological remodeling of cardiac disorders normally accompanies cardiac fibrosis. Anti-fibrotic therapies are still limited due to the fact that various factors are involved in the pathogenesis of fibrosis, such as inflammation and mitochondrial dysfunction. Different cell types also contributed to the fibrotic progress, with comprehensive crosslinks, especially interactions between myoFbs and cardiomyocytes. In the latest literature, single-cell RNA-sequencing offers a promising platform for identifying novel cellular and molecular protagonists that possibly initiate cardiac fibrosis [201]. For instance, activated CFs in MI mice were found to have high expression of collagen triple helix repeat containing 1 (Cthrc1), which played an crucial role in the process of cardiac fibrosis [202]. These results suggest a potential application of single-cell RNA-sequencing for further investigation on fibrosis and the development of anti-fibrotic strategies.

MyoFbs are dominating cells in cardiac fibrosis which regulate cytokine secretion, collagen synthesis, and non-functional scar formation [203–205]. The secretion of myoFbs can further activate CFs. In myoFb-based therapeutic strategies, the latest decades have seen anti-fibrosis advances with reprogramming and cell engineering. However, further studies are needed since myoFb function and its role in fibrosis is still not entirely understood. Specifically, studies can be conducted on the biology of myoFb activation and signaling pathways, which facilitates the design of myoFb deactivation strategies. Secondly, compared with quiescent fibroblasts, discovery of more myoFb-specific markers would faccilitate the design of myoFb-targeted therapeutics. Lastly, studies can be carried on to investigate the crosstalk between myoFbs and other cell types like macrophages, cardiomyocytes and inflammatory cells. Notably, exosomes exert pleiotropic repair effects using miRNAs, so exploration on specific miRNA for fibrosis inhibition would be beneficial. Specifically, precise anti-fibrotic therapy can be achieved by delivering specific miRNA-loaded nanoparticles to myoFbs. Finally, given the "off-target" anti-fibrotic effects of drugs, an impeccable platform is needed to design drug screening. In addition to therapy, there is also a demand for developing diagnostic tools which enable early and reliable fibrosis detection.

Acknowledgements

Abbreviations:

CFs	cardiac fibroblasts
myoFbs	myofibroblasts
ECM	extracellular matrix
CAR-T	chimeric antigen receptor T cell

CVDs	cardiovascular diseases
MMPs	metalloproteinases
Ang II	Angiotensin II (Ang II)
RNA	single cell ribonucleic acid
MI	myocardial infarction
IL	interleukin
ERK1/2	extracellular signal-regulated protein kinases 1 and 2
YEP	yes-associated protein
ROS	reactive oxygen species
PGC-1α	peroxisome proliferator activated receptor gamma coactivator 1-alpha
CaMKII	Ca2+/calmodulin-dependent protein kinase II
NF-κB	nuclear factor kappa-B
ER	endoplasmic reticulum
4-PBA	4-phenylbutyric acid
AGEs	advanced glycation end-products
PPARα	peroxisome proliferator-activated receptor α
EPDCs	epithelial-derived cells
FGF	fibroblast growth factor (FGF)
PDGF	platelet-derived growth factor
α-SMA	α-smooth muscle actin
MSC	mesenchymal stem cell
BMSCs	bone-marrow derived stem cells (BMSCs)
HSC	hematopoietic stem cell
Tcf21	transcription factor 21
TGF-β	transforming growth factor-β
SMCs	smooth muscle cells
FAP	fibroblast activation protein
ATR	angiotensin II receptor
AT1R	type 1 angiotensin receptor

AT2R	Ang II receptor type 2
MAPKs	mitogen-activated protein kinases
iCLMs	induced cardiac-like myocytes
GMT	Gata4, Mef2c and Tbx5
cTnT	cardiac troponin T
mRNA	messenger RNA
miRNAs	microRNAs
VEGF	vascular endothelial growth factor
AAV	ssociated virus
PLGA	poly(lactide-co-glycolide)
CSCs	cardiac-derived stem/stromal cells
EPCs	endothelial precursor cells
iPSCs	induced pluripotent stem cells
DEG	differentially expressed genes
BsAbs	bispecific antibodies
EVs	extracellular vesicles
ACE	angiotensin converting enzyme
ARBs	angiotensin receptor blockers
TZDs	thiazolidinediones
TNF-α	tumor necrosis factor-α
RRAS	renin-angiotensin-aldosterone system
Cthrc1	collagen triple helix repeat containing 1

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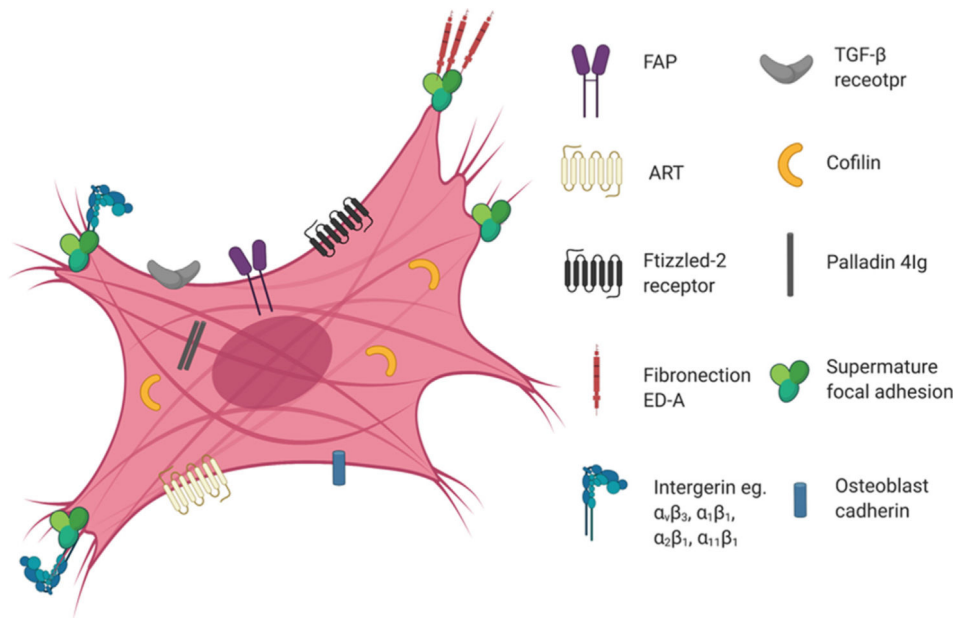
Fig. 1. Surface markers on differentiated myoFbs.
Due to their extracellular nature, FAP, Frizzled-2 receptor, ATR and TGF-β receptor are reasonable target candidates for the design of myoFb-targeted systems. Interestingly, $\alpha_v\beta_3$ integrins expressed in supermature focal adhesions and their combination are also potential targets. However, because many of these markers are also expressed on other cell types, the specificity needs to be optimized with further studies. Abbreviations: myoFbs, myofibroblasts; FAP, fibroblast activation protein; ATR, angiotensin II receptor; TGF-β, transforming growth factor-β.
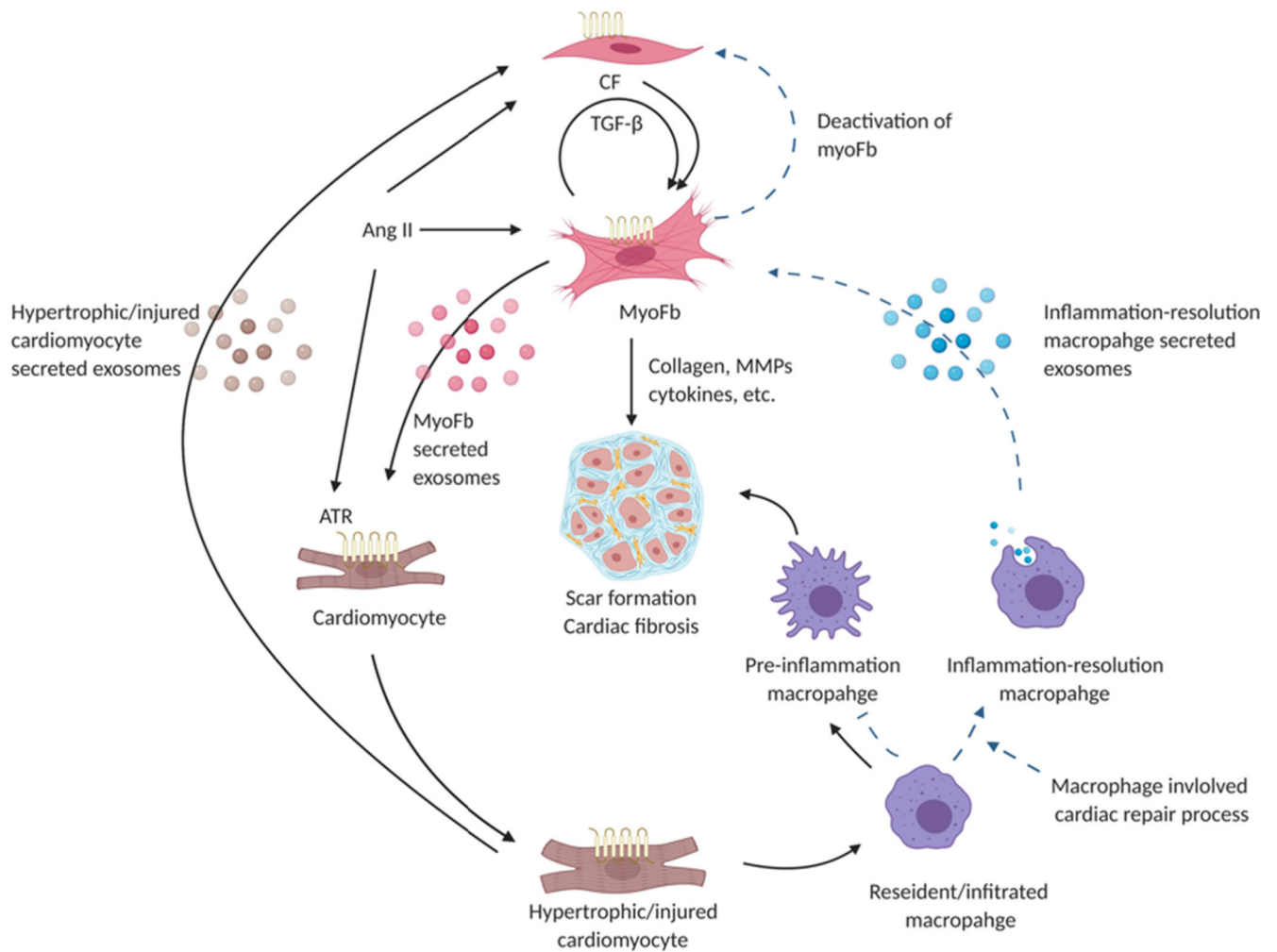
Fig. 2. Representative crosstalk between myoFbs and other cells in cardiac fibrosis.
During cardiac injury, CFs differentiate into myoFbs, leading to ECM deposition and cardiac interstitium expansion. Many molecules are involved in the fibrosis process, including proteins, exosomes and cytokines. For instance, highly expressed Ang II binds ATR in cardiomyocytes and worsens their hypertrophy. Hypertrophic or injured cardiomyocytes alter paracrine signaling which facilitates myoFbs differentiation, while myoFbs in turn also aggravate cardiomyocyte injury. Pro-inflammatory macrophages aggravate fibrosis, while pro-resolution subtypes block myoFb differentiation via paracrine signaling. Abbreviations: CFs, cardiac fibroblasts; myoFbs, myofibroblasts; ECM, extracellular matrix; Ang II, Angiotensin II; ATR, Ang II receptor.
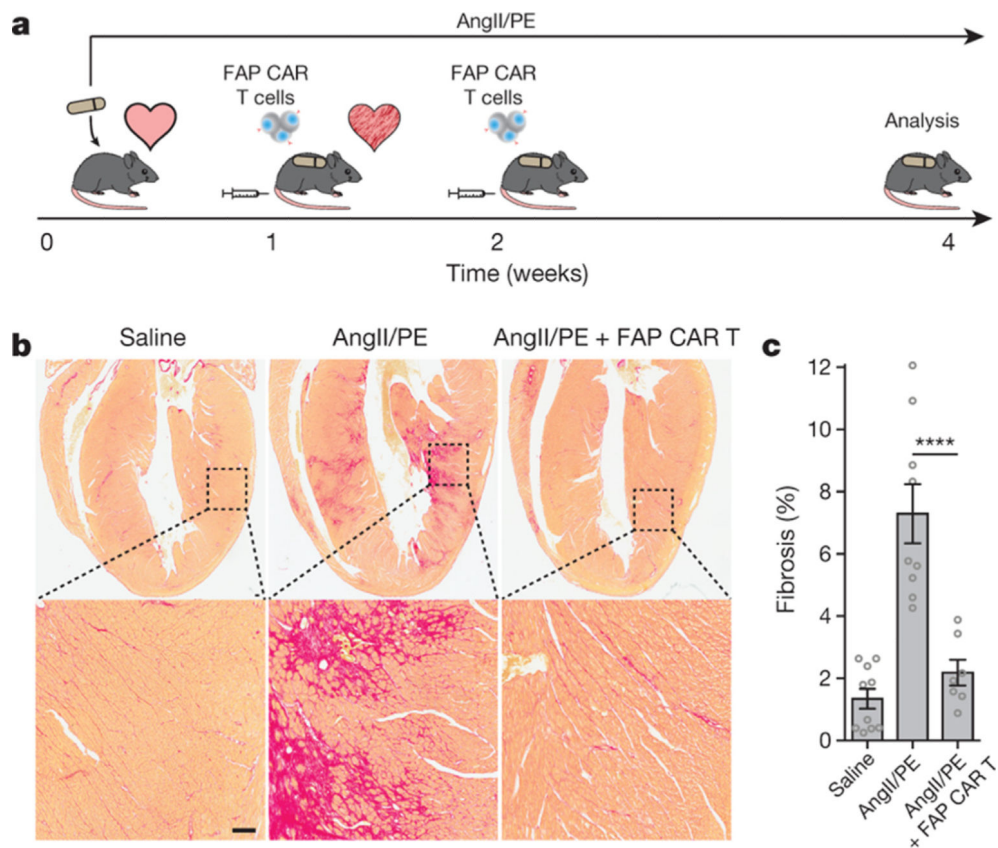
Fig. 3. FAP CAR-T targets cardiac fibrosis.

a, Scheme of experiments for CAR-T that targets FAP expressing cells. b, Top, Picro-sirium red staining of heart coronal sections in mice to evaluate fibrosis (red) with treatments of saline (left), Ang II/PE (center) or FAP CAR-T cells with Ang II/PE (right). Bottom, magnification of top results to evaluate left ventricular fibrosis. Scale bar, 100 μm. c, Quantitative results of cardiac fibrosis. ****$P < 0.0001$; one-way ANOVA between groups, $P < 0.0001$; post hoc multiple comparisons, Tukey's test; n = 10, 9, and 7 biologically independent mice, from left to right. Abbreviations: Ang II, angiotensin II; PE, phenylephrine [15]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
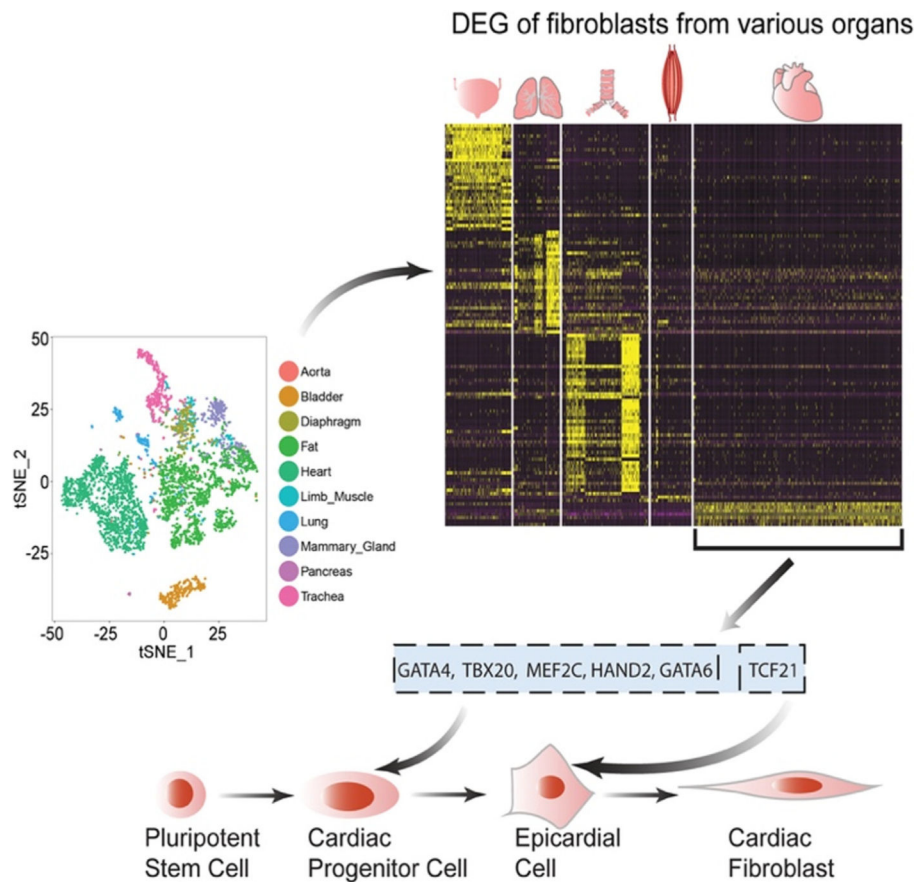
Fig. 4. Flow diagram illustrating the process and protocol of inducing human iPSCs into quiescent CFs.

Using mouse single-cell transcriptomic data, tissue-specific marker gene expressing in fibroblast subpopulations are revealed and used to differentiate human iPSCs-CFs. DEG analysis highlights the critical role of tissue-specific transcription factors in regulating the developmental trajectories of fibroblast subpopulations. Finally, human iPSCs-CFs were generated by sequentially differentiation of intermediate cell types including cardiac progenitor and epicardial cells. Abbreviations: iPSC, induced pluripotent stem cell; CF, cardiac fibroblast; DEG, differentially expressed genes [16].

Table 1

Representative factors and results in murine and human cardiac fibroblast reprogramming.

Combination of factors	Original cell types	Markers and efficiencies	AP	Beating
GMT [90]	MCF, TTDF	cTnT+: ~30% of α-MHC cells	+	+
GMT [91]	MCF	α-MHC-EYFP+: ~40% at border zone	+	+
GHMT [116]	MEF	Sarcomere+: ~32%	+	+
GHMT, MyoD domain [117]	MHF, MLBF, MTTF	cTnT+: ~4.9%	ND	+
GHMT, myoD domain[118]	MHF	cTnT+: ~19%	ND	+
GHMT, SB431542 [119] 19	MCF	Activity of Troponin T-GCaMP5+: 9.27%	ND	–
GMT, Mesp1, Myocd, miR-133 [101]	MEF, MCF	α-MHC-GFP & cTnT+: 8.1%; α-actinin+: 19.9%	ND	–
GMT mRNA, C_lipo [120]17	MCF	α-MHC-GFP+: 0.5% of total MCF	ND	–
GHMT, miR-1, miR-133, Y-27632, A83–01 [121]	MEF, MAF	cTnT+: ~60% (with A83–01); α-actinin+: ~60% (with A83–01)	+	+
OSKM, PEG hydrogel [122]	MEF; MTTF	α-actinin+: 1.72 fold to control	ND	+
Hand2, Nkx2.5, Gata4, Mef2c, Tbx5 [123]	MEF, MCF	Activity of Troponin T-GCaMP5+: 1.6%	ND	+
miR-1, miR-133, miR-208, miR-499 [124]	MCF	tdTomato+ Troponin T+: ~12%	+	+
CHIR99021, RepSox, Forskolin, VPA [125]	MEF, MTTF	α-actinin+: 14.5%; α-MHC+: 9%	+	+
OSKM, Ascorbic acid [126]	MEF	Gata4+: ~40%; MHC+: ~24%	+	+
GMT, MESP1, Myocd [127]	HCF	cTnT+: 5.9%; α-actinin+: 5.5%;	+	+
GMT, Mesp1, Myocd, Esrrg, Zfpm2 [111]	HESC	α-MHC-mCherry+: 15.8%	+	ND
GMT, Mesp1, Myocd, miR-133 [101]	HCF	cTnT+: 27.8%; α-actinin+: 8%	ND	+
Gata4, Hand2, Tbx5, Myocd, miR-1, miR-133 [128]	HFF	cTnT+: 34.1%	ND	+

Different combinations of diverse factors and original cells for fibroblast reprogramming. Results are assessed by the expression of cardiac markers, electrophysiological characteristics and beating abilities.

GMT, Gata4, Mef2c, Tbx5; GHMT, Gata4, Hand2, Mef2c, Tbx5; MCF, murine cardiac fibroblast; HCF, human cardiac fibroblast; MTTDF, murine tail-tip dermal fibroblast; MTTF, murine tail tip fibroblast; cTnT, cardiac troponin T; MHF, murine head fibroblast; LBF, murine low body fibroblast; MEF, mouse embryonic fibroblast; MAF, murine adult fibroblast; HESC, human embryonic stem cell; HFF, human foreskin fibroblast; ND, not detected.

Table 2

Representative cell and derivates loaded nano/micro delivery systems for cardiac fibrosis ablation.

Vesicle	Biomaterials	Cell type/factors	Delivery route	Efficiencies Potency against fibrosis
Vascularized cardiac Patch [144]	Biomimetic micro-vessels (BMVs), which are encapsulated in a fibrin gel spiked with human CSCs	CSCs	*In situ* delivery	NM
Cardiac patch [158]	Biomimetic micro-vessels (BMVs)	CSCs	*In situ* delivery	Reduced scar size
Microneedle patch [149]	PVA Microneedle	CSCs	*In situ* delivery	Reduced scar fibrosis
Hydrogels [150]	Poly(2-alkyl-2-oxazoline) (POx) derivative	MSCs	*In situ* delivery	Reduced interstitial fibrosis
Nanogel [145]	Thermosensitive poly(N-isopropylacrylamine-co-acrylic acid) or P(NIPAM-AA) nanogel	CSCs	*In situ* gelation	Reduced fibrosis
Hydrogels [151]	Chitosan/dextran/beta-glycerophosphate based hydrogels	MSCs	Intravenous injection	Reduced fibrosis area
Platelet membrane coated CSCs [154]	Decorating platelet nanovesicles onto the surface of CSCs	CSCs	Intravenous injection	Reduced fibrosis
Biorthogonal conjugation system [155]	CD41attached DBCO polymer; azide modified CD34 antibody	Catching bone marrow derived EPCs in blood circulation	Intravenous injection	Attenuated interstitial and perivascular fibrosis compared with control-injected hearts
CD34 antibody attached platelet [159]	DSPE-PEG-CD34 antibodies, which were bonded to the surface of platelets	Catching bone marrow derived MSCs in blood circulation	Intravenous injection	NM
Bispecific antibodies (CD34-CMHC) [160]	Anti-CD34-F(ab')₂, which were bonded to anti-CMHC- F(ab')₂	Redirecting BMSCs to injured myocardial cells	Intravenous injection	NM
Bispecific antibodies (CD42b-CD34) [143]	Biorthogonal reaction between TCO modified CD34 antibodies and TZ modified CD42b antibodies	Combining HSCs and platelets in the lung & Redirecting HSCs to the heart injury	Inhalation	Reduced scar fibrosis; reduced α-SMA protein expression
Cell sheets [161]	NM	Adipocyte cells	*In situ* transplantation	Inhibit fibrosis
Platelet fibrin Patch [162]	Double-lumen syringe including platelet-rich plasma and calcium-containing media solution	platelet-rich plasma	Local syringe	Reduced scar fibrosis
Artificial patch [163]	Myocardial extracellular matrix scaffold	CSC secreted factors		Reduced amount of scarring in rat MI model; less myocardial fibrosis in pig MI model.
Synthetic MSCs [164]	PLGA microparticles loading MSC secreted factors, which are coated with MSC membrane	MSC secreted factors	Intramyocardial injection	Reduced scar fibrosis
Synthetic CSCs [165]	PLGA microparticles loading CSC secreted factors, which are coated with CSC membrane	CSC-conditioned media with grow factors	Intramyocardial injection	Reduced scar size
Platelet membrane coating nanoparticles [166]	CSC secretome loaded nanoparticles, coating with PEG2 conjugated platelet membrane	CSC secretome	Intravenous injection	Reduced scar size
Monocyte mimic [167]	Monocyte membrane	MSC derived EVs	Intravenous injection	Alleviated fibrosis remodeling

Abbreviation: CSC, Cardiac stem/stromal cell; MSC, mesenchymal stem cell; PVA, poly(vinyl alcohol); PLGA, poly(lactic-co-glycolic acid); EPC, endothelial progenitor cell; DBCO, dibenzocyclooctyne; DSPE-PEG, 1, 2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-Poly(ethylene glycol); BMSC, bone marrow-derived stem cell; CMHC, cardiac myosin heavy chain; TCO, *trans*-cyclooctene; TZ, tetrazine; HSC, hematopoietic stem cell; α-SMA, α-smooth muscle actin; EV, extracellular vesicle; NM: not mentioned.

Table 3

Representative anti-fibrotic therapies against cardiac fibrosis in clinical trials.

Study	Agent	Length of treatment	Patient included (n)	Discoveries
RAAS inhibitor [188]	Candesartan	24 months	153	PIINP in patients with atrial fibrillation were reduced.
RAAS inhibitor [189]	Spironolactone	6 months	80	PICP and PIINP in patients with metabolic syndrome were reduced.
RAAS inhibitor [190]	Eplerenone	6 months	44	PIINP and PICP in patients with heart failure (ejection fraction was preserved) were reduced.
RAAS inhibitor [191]	Losartan	12 months	20	The progression of cardiac fibrosis was attenuated in patients with nonobstructive hypertrophic cardiomyopathy.
RAAS inhibitor [192]	Eplerenone	6 months	113	PICP and PIINP was reduced while myocardial deformation was improved in patients with obesity and mild LV diastolic dysfunction.
Inflammation modulator [193]	Atorvastatin	6 months	56	PIINP was decreased in patients with heart failure.
Endothelin inhibitor [194]	Enrasentan	6 months	72	LVEDVI was improved in asymptomatic patients with LV dysfunction after the treatment of enrasentan compared to that with enalapril treatment.
Loop diuretic [195]	Torsemide	8 months	36	PICP and CVF were both reduced in hypertensive patients with symptomatic heart failure after the treatment of Torsemide.
Loop diuretic [196]	Torsemide	8 months	22	PCP was decreased in patients with chronic heart failure.
Loop diuretic [197]	Torsemide	8 months	24	Collagen crosslinking was enhanced accompanied with normalization of LV chamber stiffness in patients with heart failure.
Cyclic GMP-specific phosphodiesterase type-5A inhibitor [198]	Sildenafil	3 months	59	LV contraction parameters were improved while TGF-β and MCP-1 were reduced in patients with diabetic cardiomyopathy.
Cyclic GMP-specific phosphodiesterase type-5A inhibitor [199]	Sildenafil	24 weeks	216	No obvious improvement in cardiac activities in patients with heart failure (ejection fraction was preserved).
Pre-relaxin-AHF [200]	Relaxin	48 h	234	Cardiovascular deaths were lowered in patients with acute heart failure.

Abbreviations: PIINP, the amino-terminal peptide of type III procollagen; PICP, the carboxy-terminal peptide of procollagen type I; PINP, the amino-terminal peptide of type I procollagen; LV, left ventricular; LVEDVI, LV end diastolic volume index; CVF, collagen volume fraction; PCP, procollagen type I carboxy-terminal proteinase; TGF-β, transforming growth factor-β.