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Cardiac Fibrosis: Potential Therapeutic Targets

Shuin Park[1,2,3], **Ngoc B. Nguyen**[1,2,3], **Arash Pezhouman**[1,2], and **Reza Ardehali**[1,2,3,4]

[1]Division of Cardiology, Department of Internal Medicine, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA

[2]Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research, University of California, Los Angeles, Los Angeles, CA 90095, USA

[3]Molecular, Cellular and Integrative Physiology Graduate Program, University of California, Los Angeles, Los Angeles, CA 90095, USA

[4]Molecular Biology Institute, University of California, Los Angeles, Los Angeles, CA 90095, USA

Abstract

Cardiovascular disease is a leading cause of mortality in the world and is exacerbated by the presence of cardiac fibrosis, defined by the accumulation of non-contractile extracellular matrix proteins. Cardiac fibrosis is directly linked to cardiac dysfunction and increased risk of arrhythmia. Despite its prevalence, there is a lack of efficacious therapies for inhibiting or reversing cardiac fibrosis, largely due to the complexity of the cell types and signaling pathways involved. Ongoing research has aimed to understand the mechanisms of cardiac fibrosis and develop new therapies for treating scar formation. Major approaches include preventing the formation of scar tissue and replacing fibrous tissue with functional cardiomyocytes. While targeting the renin-angiotensin-aldosterone system is currently used as the standard line of therapy for heart failure, there has been increased interest in inhibiting the transforming growth factor-β signaling pathway due its established role in cardiac fibrosis. Significant advances in cell transplantation therapy and biomaterials engineering have also demonstrated potential in regenerating the myocardium. Novel techniques, such as cellular direct reprogramming, and molecular targets, such as non-coding RNAs and epigenetic modifiers, are uncovering novel therapeutic options targeting fibrosis. This review provides an overview of current approaches and discuss future directions for treating cardiac fibrosis.

Corresponding Author: Reza Ardehali, MD, PhD, MacDonald Medical Research Laboratory Room 3760, 675 Charles E Young Dr S, Los Angeles, CA 90095, RArdehali@mednet.ucla.edu, Phone: 1 (310) 825-0819.

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1. INTRODUCTION

Cardiac fibrosis is a major pathological disorder associated with a multitude of cardiovascular diseases (CVD) and is characterized by excessive extracellular matrix (ECM) protein deposition in the heart[1,2]. Upon ischemic injury or pressure overload, the heart undergoes a dynamic remodeling process that is driven by a multitude of cells including cardiomyocytes, endothelial cells, immune cells, and cardiac fibroblasts[2–4]. Cardiomyocytes rapidly become apoptotic and endothelial cells play a critical role in modulating the inflammatory response[5,6]. In the initial phases of remodeling, immune cells proliferate, infiltrate damaged myocardium to clear dead tissue, and release pro-fibrotic cytokines[3,7]. In response to these cytokines, cardiac fibroblasts become activated and increase production of ECM proteins such as collagens and fibronectin to form scar tissue[1,4,8]. Initially, these responses are critical in removing apoptotic CMs and for stabilizing the chamber walls to prevent rupture and the scar that is formed is deemed as reparative fibrosis. However, the persistent presence of non-contractile collagen-rich tissue leads to the maturation of scar tissue and adverse remodeling, the effects of which include an increased risk of arrhythmias and reduced contractility[9,10]. These effects can have a devastating impact on the clinical outcomes of CVD patients, creating a need to develop strategies to prevent or reverse cardiac fibrosis.

Several obstacles that have limited the development of anti-fibrotic therapies available for CVD patients. First, the regenerative potential of the adult human heart is limited and cardiomyocytes (CMs) are unable to proliferate at a level that can replace damaged myocardium[11]. This restricts therapies that aim to inhibit fibrosis entirely as the endogenous CMs are unable to replace lost muscle tissue, thus increasing risk of cardiac rupture. Second, the molecular mechanisms driving cardiac fibrosis are complex and not fully understood. Although cardiac fibroblasts are the major contributory cells of cardiac fibrosis, further studies are needed to unravel the mechanistic regulation of these cells. There is a need to understand their mechanisms of activation, the temporal nature of their molecular changes, and whether these cells can be “deactivated” or eliminated[12]. Finally, the injured heart, particularly after myocardial infarction (MI), is a volatile microenvironment with dramatic levels of CM apoptosis, immune cell infiltration, and fibroblast proliferation[4,7,13]. This hostile environment may hinder the efficacy of delivering anti-fibrosis therapies. In this review, we aim to describe prominent research areas that are being explored for the treatment of cardiac fibrosis with potential clinical promise.

2. RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

2.1. Overview of the RAAS System

The renin-angiotensin-aldosterone system (RAAS) plays an integral role in the homeostatic control of arterial pressure, tissue perfusion, and extracellular volume[14]. This pathway is

initiated by the secretion of renin from the juxtaglomerular cells of the kidney in response to various stimuli such as decreased renal perfusion, decreased NaCl concentration, or increased sympathetic activity[15,16]. Renin goes on to cleave angiotensinogen, to form a biologically inert peptide, Angiotensin (Ang) I. AngI is then hydrolyzed by angiotensin-converting enzyme (ACE) to form an active AngII, which is a potent vasoconstrictor. AngII is the primary effector of a variety of RAAS-induced physiological and pathophysiological actions. Within the cardiovascular system, these effects include vasoconstriction, increased blood pressure, increased cardiac contractility, and vascular and cardiac hypertrophy[17]. Another important action of AngII include stimulating the production and release of aldosterone from the adrenal cortex. Aldosterone is a major regulator of sodium and potassium balance and thus plays a major role in regulating extracellular volume[18]. Together, the resulting effects of AngII and aldosterone on their target organs serve to maintain blood pressure and restore renal perfusion. Although the RAAS plays an important role in normal circulatory homeostasis, continued or inappropriate activation of this system is thought to contribute to the pathophysiology of diseases such as hypertension and HF.

2.2. Role of RAAS in Cardiac Fibrosis

In vitro experiments using adult rat cardiac fibroblasts have shown that AngII[19–21] and aldosterone[19] stimulate collagen synthesis in a dose-dependent manner. AngII additionally suppresses the activity of matrix metalloproteinase-1 (MMP1), a key enzyme of interstitial collagen degradation[19], that synergistically leads to progressive collagen accumulation within the myocardial interstitium. AngII induces expression of TGFβ1 within cardiac fibroblasts through the Ang type-I receptor (AT_1)[22]. After an MI, increased wall stress resulting from elevated left ventricular end diastolic pressure (LVEDP) stimulates mechanoreceptors that lead to activation of RAAS. The upregulated AngII increases tissue inflammation, and TGFβ, IL-1β, and TNF-α secretion[23–26], leading to enhanced generation of myofibroblasts. Within experimental models of hypertensive heart disease and chronic HF, circulating and local levels of renin-angiotensin-aldosterone promote the development of myocardial fibrosis and diastolic dysfunction[27,28]. Given the significant role of RAAS in the pathogenesis of cardiac fibrosis, therapies have been developed to antagonize or modulate the activity of various components of this system.

2.3. Direct Renin Inhibitors and Renin Receptor Blockers

Direct renin inhibition may be a promising anti-fibrotic therapy since it attenuates the pro-fibrotic effects of renin in addition to that of other effectors of the renin-angiotensin pathway[29]. Renin inhibitors interfere with the initial rate limiting step in the synthesis of AngII by binding directly to renin[30]. Aliskiren is the first orally active renin inhibitor approved by the FDA for the treatment of hypertension in adults[31]. Zhi et al. showed that aliskiren has direct effects on collagen metabolism in cardiac fibroblasts and prevented myocardial collagen deposition in a non-hypertrophic mouse model of myocardial fibrosis[29]. Other groups have shown that aliskiren functions through inhibition of AngII-dependent as well as AngII-independent effects mediated via the (pro)renin receptor (PRR)[32,33]. Cardiac expression of PRR is up-regulated in hypertension and HF and has been shown to be associated with the development of cardiac fibrosis and hypertrophy as well as cardiac

dysfunction[34–39]. Ellmers et al. reported that PRR blockade in a mouse model of MI significantly reduced infarct size and attenuated cardiac fibrosis and adverse remodeling[38].

2.4. ACE Inhibitors and Angiotensin Receptor Blockers (ARBs)

ACE inhibitors such as enalapril, lisinopril, and trandolapril, prevent the conversion of inactive AngI into active AngII and are considered first-line therapy for many cardiovascular and renal diseases. There is a large body of evidence that ACE inhibitors regress myocardial fibrosis and are associated with reduction of ventricular arrhythmias and improvement of myocardial function[40–45]. ARBs are also commonly prescribed clinically and work by preventing the binding of AngII to its receptor (with greater affinity for AT_1 than AT_2). Wu et al. showed that valsartan, an ARB, improved coronary arterial thickening and perivascular fibrosis in a pressure overload mouse model[46]. Similarly, Frimm et al. found that rats treated with losartan had a reduction in cardiac infarct size and collagen content one month after experimental MI[47]. However, despite the efficacy of ACEs and ARBs in a variety of cardiac diseases including heart failure with reduced ejection fraction (HFrEF), recent clinical trials have not shown their benefit in HF patients with preserved ejection fraction (HFpEF)[48–50].

2.5. Aldosterone Antagonists

Aldosterone is a steroid hormone produced by the zona glomerulosa of the adrenal cortex. It plays a key role in regulating blood pressure and plasma sodium levels through its actions on renal tubules to promote sodium retention and extracellular volume expansion. It has also been reported that aldosterone can be produced within the heart[51]. This local aldosterone system responds to short- and long-term physiological stimuli, suggesting that the cardiac-generated aldosterone has possibly autocrine or paracrine actions[52]. Billa et al. demonstrated that chronic administration of aldosterone in the setting of high salt intake causes both interstitial and perivascular fibrosis in the heart[53] and that treatment with an aldosterone antagonist, spironolactone, prevents the increase in total and interstitial collagen in rats[54,55]. Several clinical studies have confirmed survival benefit when aldosterone antagonists are used in HFrEF patients[56–59]. However, the risk of hyperkalemia requires frequent monitoring[60].

Therapies targeting the RAAS have been extensively studied and shown to be effective in preventing collagen deposition and reducing cardiac fibrosis. While RAAS inhibition is the mainstay of clinical care, especially for HFrEF patients, further studies are needed to examine the efficacy and safety of these therapies for patients with HFpEF and other forms of cardiac fibrosis.

3. TGF-β SIGNALING PATHWAY

3.1. Overview of TGF-β Signaling

The Transforming Growth Factor-β (TGFβ) family of peptides is one of the most well-studied regulators of the fibrotic response that plays a central role in the maladaptive remodeling of the heart after injury[61–63]. The expression of TGFβ in myocardial tissue is markedly increased in both animal experimental models of MI and in heart failure (HF) patients[62,64]. The targeting of the TGFβ signaling pathway has long been explored as a

potential therapy to curtail fibrosis[65,66]. One of the challenges of studying the TGFβ family includes the complexity of effects that TGFβ peptides can stimulate across multiple cell types and conditions. TGFβ is known to play key roles in regulating inflammation and ECM deposition, two processes that constitute major phases of the fibrotic response. In inflammation, TGFβ signaling is inhibitory and regulates the synthesis of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNFα)[67,68]. TGFβ1-null mice demonstrate high levels of autoimmunity, supporting the importance of TGFβ in mediating the inflammatory response[69]. On the other hand, TGFβ signaling has been shown to induce fibroblast transition into activated myofibroblasts, the major cellular source for ECM protein deposition that make up the fibrotic area[4]. Due to the multifunctional roles of TGFβ signaling, several studies have revealed that the specificity and timing of targeting this pathway are crucial for effective outcomes[70].

3.2. Inhibitors of TGFβ Receptors I and II

TGFβ signaling is activated by the binding of TGFβ to a tetrameric receptor complex made up of two type I (TGFβR1 or ALK5) and two type II (TGFβR2) receptors[71]. Studies inhibiting either ALK5 or TGFβR2 have shown reduced cardiac fibrosis in mouse models, although adverse effects such as increased mortality and inflammation were observed[72,73]. Furthermore, long-term inhibition has serious side effect such as cardiac toxicities, which limits its clinical application[74]. Despite these limitations, there have been promising reports of novel TGFβ receptor inhibitors on treating cardiac fibrosis. GW788388 was recently identified as a more potent inhibitor of both ALK5 and TGFβR2 with an improved pharmacokinetic profile[75] and minimal toxic effects[76]. Multiple studies have demonstrated that GW788388 reduces myocardial fibrosis in murine heart disease models[77–79]. These studies reveal that GW788388 may be a promising anti-fibrotic agent that requires further exploration.

3.3. Clinical Inhibitors of TGFβ – pirfenidone and tranilast

Pirfenidone and tranilast are two clinically-approved drugs that have a broad range of effects on inflammation and other fibrotic pathways. However, it has additionally been established that these drugs are inhibitory of TGFβ signaling. Both have recently been garnering interest in potentially treating cardiac fibrosis[80]. Pirfenidone is an oral anti-fibrotic drug that was approved by the FDA in 2014 for the treatment of idiopathic pulmonary fibrosis[81]. Pirfenidone has been shown to inhibit the transcription of TGFβ and suppress downstream effects of TGFβ signaling, such as ECM protein upregulation[82]. Several recent studies have additionally demonstrated the anti-fibrotic effects of pirfenidone in cardiac disease. Mirkovic et al. and Nguyen et al. independently showed reduced cardiac scarring after treatment of pirfenidone in hypertensive rats and rats with MI, respectively[83,84]. Similar effects were seen in murine pressure-overload injury; pirfenidone increased survival and attenuated collagen deposition[85,86]. Clinical trials are ongoing to explore the anti-fibrotic effects of pirfenidone in patients with HF and preserved ejection fraction (PIROUETTE).

Tranilast was originally used as an antihistamine to treat bronchial asthma, however, since its conception in the 1980s[87], investigators have found efficacy of tranilast in other medical conditions. One of the main modes of action of tranilast is the suppression of TGFβ

expression and activity[87]. Several studies have reported that tranilast induces downregulation of collagen production in fibroblasts[88–90]. Subsequently, the PRESTO (Prevention of REStenosis with Tranilast and its Outcomes) clinical trial which, despite finding little effects of tranilast on restenosis, noted a reduction in the development of MI in patients treated with tranilast[91]. The effects of tranilast on attenuating myocardial fibrosis have been additionally supported by multiple animal models of cardiomyopathy, including experimental diabetes in rats[92] and viral myocarditis in mice[93]. While the anti-fibrotic effects of tranilast have been attributed to its regulation of TGFβ signaling, Kagitani et al. reported that tranilast treatment is associated with decreased monocyte infiltration, which may also contribute to the reduced fibrosis[94]. Others have reported the anti-inflammatory effect of tranilast to be related to its ability to inhibit prostaglandin E2, thromboxane B2, or interleukin-8. Additionally, the timing of tranilast administration in relation to time of injury is a significant factor to consider. See et al. showed that early tranilast treatment of rats with left anterior descending artery (LAD) ligation (day 0–7 after injury) exacerbated infarct size, implying a potential hazard when used early after injury[95].

Despite the evidences supporting the anti-fibrotic effects of both pirfenidone and tranilast, studies have shown that prolonged dosages of either of these drugs can have hepatic toxicity and may lead to liver failure[66]. Therefore, more research is warranted to explore alternative methods that can safely, but efficaciously, target TGFβ signaling for reduction of cardiac fibrosis.

4. BIOMATERIAL APPLICATIONS

4.1. Overview of Biomaterials

Biomaterials are natural or engineered substances that interacts with biological systems and are used to replace or repair tissues of the body. There has been a vast array of applications of biomaterials for controlling cardiac fibrosis. In addition to providing a platform for controlled release of anti-fibrotic compounds, biomaterials may also provide mechanical support to the infarcted tissue and decrease elevated wall stress, resulting in improved cardiac function[96]. Both naturally-derived biomaterials such as collagen[97–99], fibrin[100–102], and alginate[103–105] in addition to synthetic materials including metals and polymers[106] have been used in cardiac applications. While natural biomaterials tend to offer better compatibility and low immunogenicity, the main benefits of synthetic materials are their strength and durability[107]. When combined with cells or cytokines/growth factors, biomaterials may offer enhanced retention of their payload leading to improved engraftment or biological function[108]. This review will focus on two main classes of biomaterials with cardiovascular applications.

4.2. Injectable Biomaterials

In recent years, injectable biomaterials have seen a significant increase in application towards treating MI[108–110]. Hydrogels based on alginate and chitosan have been shown to decrease cardiac fibrosis, reduce tissue inflammation, and improve vascularization[103,104]. Combined with anti-fibrotic/anti-inflammatory compounds or stem cells, the therapeutic potential of injectable biomaterials can be further expanded. In a rat chronic myocarditis

model, gelatin hydrogel sheets containing hepatocyte growth factor (HGF) were found to improve cardiac function and fibrosis[111]. HGF serves as a favorable candidate as it suppresses fibrosis by inhibiting TGFβ (suppressing collagen synthesis) and activating MMP1 to increase collagen degradation[112,113]. In addition to its anti-fibrotic effects, reports have also indicated their role in angiogenesis[114–116] and tissue regeneration[117]. Other growth factors incorporated with injectable biomaterials include basic fibroblast growth factor[118,119], vascular endothelial growth factor[120–122], and platelet-derived growth factor[123,124]. Collectively, there is significant amount of research on the development of injectable biomaterials with anti-fibrotic compounds or biologics to reduce fibrosis and promote healing.

4.3. Cardiac Patches

Cardiac patches have generally contained cells combined with a natural or synthetic biomaterial although acellular patch therapies and cell sheets have also been investigated. While many *in vivo* studies in small animals have shown an improvement in cardiac function, one limitation with this application is the thickness of the material due to diffusion limitations[125,126]. The use of a collagen scaffold for cardiac patch has been well studied in combination with a variety of cell types[98,127–129]. Fibrin cardiac patches have also contributed to improved cell delivery and cardiac function in large animal models[100,130,131]. Processed decellularized cardiac ECM has also shown promise as an injectable hydrogel[132–134] and patch[135,136]. This is a naturally-derived matrix that provides cells with tissue-specific biochemical cues important for cell migration and differentiation, and tissue regeneration. Pieces of the myocardium (or the entire heart) may be chemically or enzymatically digested to obtain cardiac ECM[132]. The major composition of decellularized cardiac ECM include collagen, elastin, and fibronectin. It should be noted that although fibronectin has been shown to activate cardiac fibroblasts into myofibroblasts[137], it is thought that other factors or cytokines within the cardiac ECM matrix may offset this activation and lead to overall benefit[112]. Other clinical studies on the use of ECM are underway[138–140].

Injectable biomaterials and cardiac patches for the treatment of MI have recently been launched in clinical trials. While many promising studies have been completed in rodent and large animal models, further studies are needed to better understand the mechanisms behind their observed effects as well as utility for clinical applications.

5. CELL TRANSPLANTATION THERAPY

5.1. Overview of Cardiac Cell Therapy

Reduction of blood flow and oxygen to the heart resulting from ischemia can lead to irreversible loss of CMs and replacement with fibrotic scar tissue. Although traditional medical therapies are beneficial, many patients eventually progress to end-stage HF, with cardiac transplantation as the only definitive option. Due to the limited supply of donor hearts and potential complications from chronic immunosuppressive therapy, investigators have turned to therapeutic approaches aimed at improving myocardial function by cell transplantation[141–143]. The inception of the use of stem cells as a form of cardiac therapy

initially emerged in animal studies over 20 years ago[144] and reached clinical trials 10 years thereafter[145]. Despite early promises, there is no evidence to suggest that current approaches for cardiac cell therapy offer any clinical benefit. Although there are many strategies of cell therapy, this review will focus on two main avenues: 1) direct remuscularization of injured heart and 2) targeting endogenous mechanisms of repair.

5.2. Direct Remuscularization of Fibrotic Tissue in Injured Heart

The concept behind this approach is that transplantation of cells into the injured area leads to possible integration with viable cells in the host myocardium thereby improving cardiac contraction and reducing the risk of ventricular rupture or aneurysms. For that reason, many different cell types have been explored as a source for cell transplantation, including autologous skeletal myoblasts[146], bone marrow-derived CD34+ cells (endothelial progenitor cells)[147], C-kit surface antigen-selected cells[148], ESC/iPSC-derived CM precursors[149–153], and ESC/iPSC-derived CM[154–156].

Clinical application of skeletal myoblasts failed due to concerns over arrhythmias generated by the transplanted cells[157]. C-kit surface antigen-selected cardiac progenitor cells initially showed some promise owing to their potential to proliferate and differentiate into the new myocardium, although later studies have challenged the existence of such cells that could generate new cardiomyocytes[158,159]. Since 1998 which human pluripotent stem cells (hPSC) were characterized[160], they have been used to generate CMs *in vitro* and *in vivo.* Nevertheless, injection of hPSC-derived CMs or progenitors in large animal models after an acute MI have raised safety concerns such as ventricular fibrillation/tachycardia[154,161,162]. One possibility is the potential contamination of non-cardiac or pacemaker cells in the hPSC-derived population capable of inducing arrhythmias. Another possibility is failure of transplanted hPSC-derived cardiac cells to physiologically couple with endogenous CMs, leading to disruption of cardiac action potential propagation. Alternative to direct injection of cells into the injured myocardium, others have used bioengineering approaches such as scaffolds or patches for cell therapy (discussed in detail above). Menasch and others developed a sheet of C-derived CMs and applied it onto the surface of the scar and border zone in MI hearts[149]. This single case report study was the first clinical application of hPSC-derived cardiac cell therapy and no safety issues were reported. However, epicardially-administered cells are unlikely to engraft, migrate, and integrate into the host myocardial tissue. Despite the promising advancement in developing contractile cardiac cell products and successfully applying them in animal models[153], further studies are still needed to optimize this strategy to enhance the safety and long-term engraftment of transplanted cells[163].

5.3. Stimulation of Endogenous Cardiovascular Progenitors and/or CMs for Regenerative Therapy

This approach aims to use cells or their by-products to induce endogenous progenitors or CMs to proliferate and replace fibrotic tissue in the injured myocardium via paracrine-mediated effects. However, no study has yet provided unequivocal evidence for the existence of cardiac progenitors in adult human hearts. Studies have shown that certain cells have myogenic differentiation capacity[164] or release by-products, such as exosomes[165], that may

stimulate cardiac regeneration. Other studies have used adult, undifferentiated progenitor cells such as bone marrow aspirated mononuclear cells[166], marrow-derived mesenchymal stem cells (MSCs)[167,168], and resident adult cardiac progenitors (CPCs)[169] to stimulate endogenous pathway for regenerative therapy. A clinical trial involving the use of MSCs is ongoing, despite its uncertain efficacy[170]. Some studies have revealed an improvement in scar size[171], while others have shown no benefit[172]. Pre-clinical studies of CPCs suggested that these cells possess myogenic differentiation capacity, however, further mechanistic studies revealed their anti-inflammatory and antifibrotic properties, as well as stimulation of endogenous cardiac progenitor and CMs[173–176]. Other genetic fate-mapping studies have shown that endogenous CPCs[158,177] and MSCs[173,178,179] produce new CMs, although the percentage of CMs emerging from the CPCs and MSCs was extremely low. Altogether, engraftment of MSCs and CPCs is lower compared to ESC/iPSC-based strategies but leads to significant improvement in left ventricular function and reduction of scar size[153,169,180]. These findings promised a paradigm shift in cardiac biology and new opportunities for future regenerative therapy. However, several years after these findings, a consensus on the biological role of these populations remains obscure.

In summary, although significant advancements have been made in cardiac regenerative medicine and engineering, several critical issues remain. In order to expedite clinical application of cell therapy, a better understanding of the mechanism of action, improvement in cellular delivery and retention, purification of the desired cell types, and functional integration of transplanted cells need to be addressed.

6. DIRECT REPROGRAMMING OF FIBROBLASTS

6.1. Overview of Cardiac Direct Reprogramming

The development of cardiac fibrosis is driven by the proliferation and activation of cardiac fibroblasts, which become the main cellular components of scar tissue[1,4,8]. Considering the abundance of this cell type within the fibrotic region, they may be an ideal target for direct reprogramming to generate CMs to replace the scar and restore cardiac function[181]. In 2010, Ieda et al. demonstrated the ability to reprogram postnatal murine dermal and cardiac fibroblasts into induced CM-like cells by transducing the cells with three factors (Gata4, Mef2c, and Tbx5, hereafter collectively referred to as GMT)[182]. The resulting cells expressed CM-specific markers such as cardiac troponin T (cTnT) and α-myosin heavy chain (αMHC)[182] Wada et al. further demonstrated that transduction with GMT plus additional factors Mesp1 and Myocardin could produce induced CM-like cells from human fibroblasts[183]. This has generated enthusiasm for improving and utilizing direct reprogramming for potential therapeutic purposes.

6.2. *In vivo* Direct Reprogramming by Retroviral Delivery of Transcription Factors

The potential of direct reprogramming to be used for treating ischemic heart disease was first explored in 2012 by several groups that attempted to apply successful *in vitro* results to an *in vivo* setting. Qian et al. and Song et al. independently reported successful reprogramming of resident fibroblasts to induced CMs in murine hearts after LAD ligation by retroviral transduction of GMT and GMT plus Hand2, respectively[184,185]. Both groups

observed increased reprogramming efficiency *in vivo* compared to *in vitro*, suggesting that the cardiac environment may influence the reprogramming process. However, the percentage of cells that were successfully reprogrammed remained low. Inagawa et al. reported an improvement in the reprogramming efficiency with GMT by using a retroviral polycistronic vector[186]. All three studies demonstrated that retroviral delivery of GMT or GMHT into the murine heart after an experimental MI could reduce the extent of cardiac fibrosis, solidifying the therapeutic potential for direct reprogramming.

While numerous groups have reported successful direct reprogramming of cardiac fibroblasts in murine ischemic heart disease models, the clinical translation of this approach has not been fully addressed. Retroviral delivery involves random insertion of DNA into the host cell's genome which make this mechanism of reprogramming potentially pathogenic[187]. Therefore, non-integrative methods of reprogramming that can be safely applied to human patients are warranted. There is an additional need to verify the safety and efficacy of direct reprogramming of cardiac fibroblasts to induced cardiomyocytes-like cells in large animal models as a preclinical prelude to future human studies.

6.3. Potential Clinical Applications of Direct Reprogramming

Non-integrative viral vectors such as Adenovirus (AdV), Adeno-associated viruses (AAV), and Sendai virus (SeV) have recently garnered interest in the reprogramming field. AdVs are a widely used research tool that can transduce a variety of cells with high efficiency. A recent report demonstrated that AdVs encoding GMT were able to induce cardiac reprogramming in a rat infarct model to a similar degree as an integrative viral vector (lentivirus)[188]. However, clinical applications of AdVs have been dampened by their high immunogenicity[189]. AAVs, on the other hand, are a more viable option as they are able to target various cell types similar to AdVs but exhibit significantly reduced immunogenicity[190]. Clinical trials investigating the use of AAVs for gene therapy in various conditions are currently underway[191]. Yoo et al. demonstrated that chimeric-AAVs encoding GMT are able to induce direct cardiac reprogramming and reduce infarct size after LAD ligation in mice[192]. Finally, SeVs are a relatively new tool for gene therapy that is gaining attention due to their lack of integration and high expression of viral genes[193]. Indeed, SeV vectors expressing GMT have been shown to significantly increase the efficiency of cardiac reprogramming in mouse infarct hearts, compared to retroviral vectors, and resulted in lower levels of fibrosis[194,195].

Several studies have also explored the potential to directly reprogram fibroblasts into CMs by non-viral methods. Recent reports have shown the ability to reprogram mouse fibroblasts into CMs by addition of small molecules *in vitro*[196,197]. Additionally, another group has demonstrated the capabilities of miRNA transfection in cardiac reprogramming *in vivo*[198]. These advancements have laid a framework for a future *in vivo* reprogramming without the need for viral transduction.

7. EMERGING NOVEL ANTI-FIBROTIC THERAPEUTIC STRATEGIES

7.1 Non-coding RNAs in Cardiac Fibrosis

There have been several exciting findings for other novel anti-fibrotic therapeutic strategies. Several studies have identified a variety of non-coding RNAs (miRNAs and lncRNA) which may modulate fibrosis[199,200]. miRNA-21[201], miRNA-29[202], and miRNA-34[203] are a few of the identified miRNAs that are being extensively characterized for their role in regulating cardiac fibrosis. Silencing of miRNA-21 and miRNA-34 reduced fibrosis while down-regulation of miRNA-29 exacerbated collagen production. These data suggest that a variety of miRNAs possess both anti-fibrotic and pro-fibrotic roles. Additionally, lncRNAs have gained interest as another family of regulatory non-coding RNAs in cardiac fibrosis. Wisper and MIAT are two recently identified lncRNAs that function to regulate fibrosis-related genes[204,205]. There remain challenges in targeting miRNAs and lncRNAs for therapy due to their broad and non-specific effects. Ongoing efforts to identify the molecular targets of these non-coding RNAs will undoubtedly shed light on this novel therapeutic approach.

7.2 Epigenetic Modifiers in Cardiac Fibrosis

The contributions of epigenetics to the development of cardiac fibrosis is an additional growing field. Evidences have shown that modifications to the epigenetic landscape of various cell types can arise from different stimuli and stresses. These changes can regulate the expression of pro-inflammatory and pro-fibrotic genes in immune cells and cardiac fibroblasts[206]. Therefore, therapies targeting epigenetic modifiers may be promising in reversing pathological symptoms in cardiac fibrosis. Preliminary studies have shown that histone deacetylase inhibitors, such as Mocetinostat, can reverse cardiac fibrosis by targeting cardiac fibroblast activation[207,208]. Additionally, inhibition of the epigenetic reader BRD4 was shown to reduce fibrosis in mice undergoing MI[209]. These findings have been mainly from pre-clinical studies and require further exploration as a promising tool for treating cardiac fibrosis in the future.

8. CONCLUSIONS

In this review, we discussed several potential therapeutic options for preventing or reducing cardiac fibrosis (Figure 1). While the research conducted in these fields have exhibited great promise, there remain challenges for translating these data into clinical practice. Both the RAAS and TGFβ pathway are major signaling cascades that significantly regulate the development of cardiac fibrosis. Inhibitors of components from either of these pathways have shown strong evidences of reducing fibrosis in animal models, although their applications in the clinic require further investigation. The goal of cell transplantation has been to replenish cardiac muscle and replace fibrotic tissue. Questions remain regarding the most suitable cell type for transplantation and how to promote functional integration of transplanted cells into the recipient hearts. The development of engineered biomaterials in the form of hydrogels or cardiac patches have begun to address some of these limiting factors. It is likely that the future success of cell therapy will ultimately involve a combinatorial approach where the ideal cell types are embedded within a scaffold for optimal cell survival, differentiation, and functional integration into the host myocardium

while replacing the scar tissue. Direct reprogramming provides a novel method of replacing pathological fibroblasts with induced CMs. However, the safety of *in vivo* reprogramming still requires validation in large animal models. It is likely that a combination of various therapies will be necessary to address the complex pathology of cardiac fibrosis.

An obstacle not discussed in detail in this review is the significant difficulty in translating results from animal studies to human subjects. The majority of translational research is conducted in rodents (mice or rats), which exhibit significantly different characteristics in cardiac physiology compared with humans (Table 1). These differences have been reflected by poor clinical trial outcomes despite promising pre-clinical data. There has been a movement in recent pre-clinical work to be conducted in larger animal models (pigs and non-human primates), which more closely resemble human physiology. However, there are still species-specific differences that can hinder the development of efficacious therapies. Continued research, considering these factors, on potential anti-fibrosis therapeutic strategies will help to progress these therapies to the clinic.

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Abbreviations:

AAV	adeno-associated viruses
AdV	adenovirus
Ang	angiotensin
CM	cardiomyocyte
CPC	cardiac progenitor cell
cTnT	cardiac troponin T
CVD	cardiovascular diseases
ECM	extracellular matrix
HF	heart failure
HFpEF	heart failure with preserved ejection fraction
HFrEF	heart failure with reduced ejection fraction
HGF	hepatocyte growth factor
hPSC	human pluripotent stem cell
LAD	left anterior descending artery

LVEDP	left ventricular end diastolic pressure
MI	myocardial infarction
MMP1	matrix metalloproteinase-1
MSC	mesenchymal stem cell
PRR	(pro)renin receptor
RAAS	renin-angiotensin-aldosterone system
SeV	sendai virus
TGFβ	transforming growth factor β
TNFα	tumor necrosis factor α
αMHC	α-myosin heavy chain

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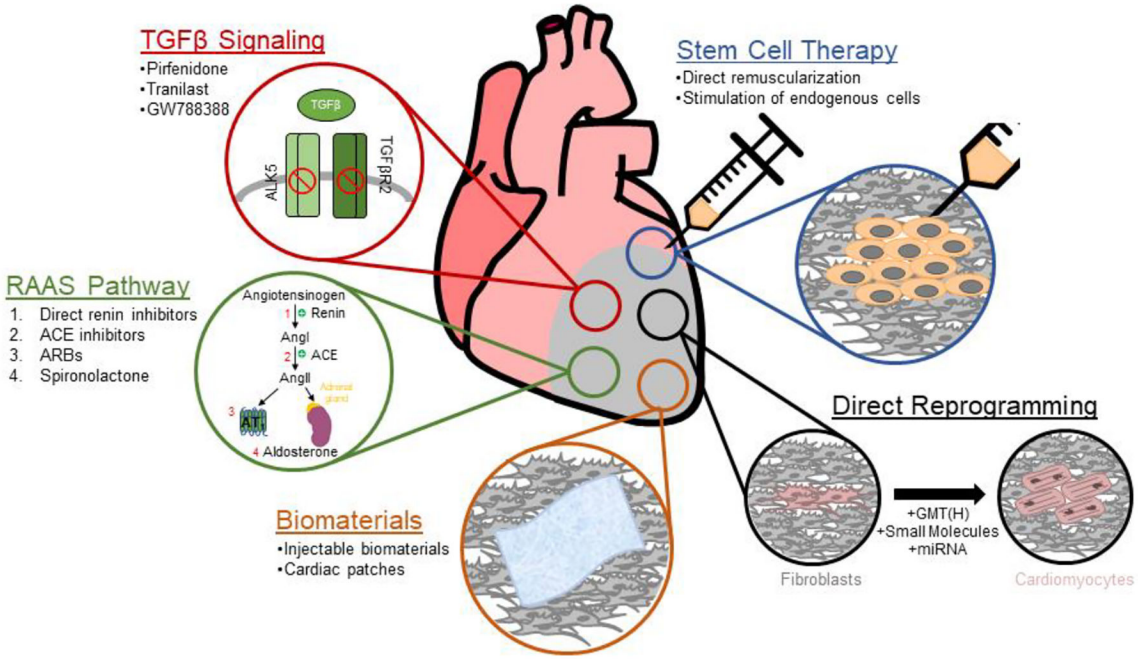

Figure 1:
Schematic diagram depicting potential therapeutic strategies for targeting cardiac fibrosis.

Table 1:

Animal and clinical studies assessing cardiac fibrosis therapies.

Therapeutic Target	Strategy	Year	Model of Fibrosis	Species	Ref
RAAS	Renin inhibition (aliskiren) *	2013	Hyperhomocysteinemia-induced myocardial fibrosis	Mice	29
	Pro-renin receptor blockade	2016	Myocardial infarction	Mice	38
	ACE inhibition (lisinopril) *	1991	Spontaneous hypertension	Rat	40
	ACE inhibition (trandolapril) *	1995	Spontaneous hypertension	Rat	43
	ACE inhibition (captopril) *	1997	Spontaneous hypertension	Rat	44
	ACE inhibition (lisinopril) *	2000	Patients with primary HTN, LV hypertrophy, and LV diastolic dysfunction	Human	42
	ACE inhibition (perindopril) *	2006	HFpEF	Human	48
	ACE inhibition (captopril) *	2017	LPS-induced inflammation	Rat	45
	ARB (losartan) *	1997	Myocardial infarction	Rat	47
	ARB (valsartan) *	2002	Transaortic constriction	Mice	46
	ARB (candesartan) *	2003	HFpEF	Human	49
	ARB (irbesartan) *	2008	HFpEF	Human	50
	Aldosterone antagonist (spironolactone) *	1992	Renovascular hypertension	Rat	54
	Aldosterone antagonist (spironolactone) *	1996	Chronic HF patients	Human	58
	Aldosterone antagonist (spironolactone) *	1999	HFrEF	Human	59
	Aldosterone antagonist (spironolactone) *	2014	HFpEF	Human	57
	Aldosterone antagonist (spironolactone) *	2018	HFpEF	Human	60
TGFβ Signaling	TGFβRII plasmid transfection	2004	Myocardial infarction	Mice	73
	SM16 (inhibitor of ALK5)	2014	Pressure overload	Mice	72
	GW788388 (inhibitor of ALK5 and TGFpRII)	2010	Myocardial infarction	Rats	79
	GW788388 (inhibitor of ALK5 and TGFpRII)	2012	Chagas	Mice	77
	GW788388 (inhibitor of ALK5 and TGFpRII)	2017	Scn5a+/−	Mice	78
	Pirfenidone	2002	DOCA-salt hypertension	Rats	83
	Pirfenidone	2010	Myocardial infarction	Rats	84
	Pirfenidone	2013	Pressure overload	Mice	85
	Pirfenidone	2015	Pressure overload	Mice	86
	Tranilast	2004	DOCA-salt hypertension	Rats	94
	Tranilast	2013	Myocardial infarction	Rats	95
	Tranilast	2016	Viral myocarditis	Mice	93
Biomaterials	Hydrogel (alginate)	2009	Myocardial infarction	Porcine	104

Therapeutic Target	Strategy	Year	Model of Fibrosis	Species	Ref
	Hydrogel (polyester-VEGF)	2011	Myocardial infarction	Rats	121
	Hydrogel (decellularized ECM)	2012	Myocardial infarction	Rats	133
	Hydrogel (alginate-chitosan)	2014	Myocardial infarction	Rats	103
	Hydrogel (gelatin-HGF)	2014	Chronic myocarditis	Rats	111
	Hydrogel (CorMatrix®-ECM)	ongoing	CABG after myocardial infarction	Human	138
	Hydrogel (VentriGel)	ongoing	STEMI undergoing PCI	Human	140
	Patch (alginate-neonatal rat CMs)	2009	Myocardial infarction	Rats	120
	Patch (hiPS-CMs)	2012	Myocardial infarction	Porcine	130
	Patch (decellularized ECM)	2016	Ischemia-reperfusion	Porcine	136
	Glue (fibrin-FGF)	2010	Myocardial infarction	Canine	118
	Microspheres	2006	Myocardial infarction	Canine	119
	Self-assembling peptides (skeletal myoblasts-PDGF)	2008	Myocardial infarction	Rats	123
	Self-assembling peptides (PDGF-FGF)	2011	Myocardial infarction	Rats	124
	Scaffold (fibrin-ECs-SMCs)	2011	Myocardial infarction	Porcine	100
Cell Transplantation	Direct Remuscularization skeletal (myoblasts)	1993		Mice	146
	Direct Remuscularization (BM-MSCs)	2004	Myocardial infarction	Human	145
	Direct Remuscularization	2005	Myocardial infarction	Human	141
	Direct Remuscularization (hematopoietic BM stem cell)	2006	Myocardial infarction	Human	142
	Direct Remuscularization (BM-derived progenitor cells)	2006	Myocardial infarction	Human	143
	Direct Remuscularization (myoblast)	2008	Ischemic Cardiomyopathy	Human	157
	Direct Remuscularization (cardiac stem cell)	2012	Ischemic Cardiomyopathy	Human	210
	Direct Remuscularization (hESC-CMs)	2014	Myocardial infarction	Monkey	154
	Direct Remuscularization (hESC-CMs)	2014	Myocardial infarction	Pig	156
	Direct Remuscularization (hESC-CMs and hESC-CVPs)	2015	Myocardial infarction	Rat	153
	Direct Remuscularization (hESC-CVPs)	2015	Severe heart failure	Human	149
	Direct Remuscularization (hESC-CMs)	2015	Myocardial infarction	Rat	155
	Direct Remuscularization (CD34+ Cell)	2016	Refractory Angina	Human	147
	Direct Remuscularization (myoblast)	2016	Myocardial infarction	Monkey	161
	Stimulation of Endogenous CVPs	2001	Myocardial infarction	Mice	166
	Stimulation of Endogenous CVPs (BM-MSCs)	2008	Ischemic heart disease	Human	168
	Stimulation of Endogenous CVPs (BM-MSCs)	2009	Myocardial infarction	Pig	178
	Stimulation of Endogenous CVPs (BM-MSCs)	2010	Myocardial infarction	Pig	173

Therapeutic Target	Strategy	Year	Model of Fibrosis	Species	Ref
	Stimulation of Endogenous CVPs (cardiac stem cell c-kit+)	2011	Myocardial infarction	Mice	174
	Stimulation of Endogenous CVPs (cardiac stem cell c-kit+)	2012	Heart failure due to Ischemia	Human	169
	Stimulation of Endogenous CVPs (BM-MSCs)	2013	Myocardial infarction & Ischemic heart disease	Human	171
	Stimulation of Endogenous CVPs (cardiosphere-derived cells)	2013	Myocardial infarction	Mice	175
	Stimulation of Endogenous CVPs (MSCs and cardiac progenitor cells)	2015	Myocardial infarction	Pig	176
	Stimulation of Endogenous CVPs	2017	Myocardial infarction	Pig	165
Direct Reprogramming	GMT (retrovirus/lentivirus)	2010	N/A	*In vitro* -> in vivo (Mice)	182
	GMT/GMTMM (retrovirus/lentivirus)	2013	N/A	*in vitro* (Human)	183
	Small molecule cocktail + Oct4	2014	N/A	*In vitro* (Mouse)	196
	Chemical cocktail	2015	N/A	*In vitro* (Mouse)	197
	GMT (retrovirus)	2012	Myocardial infarction	Mice	184
	GMHT (retrovirus)	2012	Myocardial infarction	Mice	185
	GMHT (retrovirus, polycistronic)	2012	Myocardial infarction	Mice	186
	miRNAs (1, 133, 208, and 499)	2015	Myocardial infarction	Mice	198
	GMT (adenovirus)	2017	Myocardial infarction	Rats	188
	GMT (chimeric AAV)	2018	Myocardial infarction	Mice	192
	GMT (sendai virus)	2018	Myocardial infarction	Mice	194

* currently used clinical therapy