

Review

Molecular Targets and Emerging Therapeutics in Cardiac Fibrosis

Neethu Mohan ^{1,*} and Sruthi Radhakrishnan ²

¹ Division of Cellular and Molecular Cardiology, Department of Pathology, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum 695011, Kerala, India

² Division of Pulmonary, Critical Care, Sleep, and Occupational Medicine, Department of Medicine, Indiana University, Indianapolis, IN 46202, USA; srradha@iu.edu (S.R.)

* Corresponding author. E-mail: neethum@sctimst.ac.in (N.M.)

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ABSTRACT: Cardiac fibrosis represents a global health crisis, observed in nearly all forms of heart disease, and contributes significantly to the progression of heart failure. Driven by diverse etiologies such as chronic hypertension, myocardial infarction, and metabolic disorders, cardiac fibrosis is characterized by the excessive deposition of extracellular matrix proteins. At the cellular level, the activation of cardiac fibroblasts into myofibroblasts serves as the primary mechanism for this structural remodelling. Excessive collagen deposition, crosslinking, and pathological scarring lead to increased ventricular stiffness, electrical arrhythmias, and a profound decline in cardiac function, affecting the quality of life for millions of patients worldwide. The review discusses the existing well-known profibrotic signals and molecular signalling pathways leading to cardiac fibroblast activation, collagen synthesis, and crosslinking. Mechanosensitive pathways, signalling mechanisms involved in collagen crosslinking, and epigenetic factors of cardiac fibrosis are also discussed along with their potential antifibrotic targets and therapeutic drugs. Further, small-molecule inhibitors, peptide-based therapies, natural compounds, and repurposed drugs for fibrosis are also discussed. This review concludes with recent approaches of chimeric antigen receptor (CAR)-T cell therapy for cardiac fibrosis.

Keywords: Cardiac fibrosis; Therapeutic targets; Collagen crosslinking; Mechanosensing signalling pathways of fibrosis; Epigenetic targets of fibrosis; Small molecule inhibitors; Peptide-based therapies

1. Introduction

Cardiac fibrosis is a maladaptive structural consequence of various cardiovascular pathologies, including myocardial infarction, hypertension, cardiomyopathies, and valvular heart disease. It is characterised by pathological remodelling of extracellular matrix (ECM), particularly excessive deposition of collagen, within the interstitium, which progressively compromises myocardial elasticity. The resulting architectural distortion leads to arrhythmias and heart failure. Cardiac fibrosis can generally be classified as replacement fibrosis, interstitial fibrosis, and perivascular fibrosis. Replacement fibrosis is a reparative response to cardiomyocyte death and post-infarct scarring to maintain structural integrity. Replacement fibrosis leads to the accumulation of profibrotic proteins, collagen types I and III, and fibronectin. Further, increased crosslinking of the cardiac



wall results in enhanced myocardial stiffness. This leads to impaired diastolic filling, disrupted electrical conduction by attenuating action potential propagation, life-threatening arrhythmias, and eventual systolic dysfunction. Interstitial fibrosis or reactive fibrosis is a diffuse expansion of the endomysium and perimysium, typically seen in pressure overload, metabolic syndrome, and dilated cardiomyopathy. In genetic cardiomyopathies, both replacement and interstitial fibrosis are observed. Perivascular fibrosis is characterised by an increased amount of connective tissue, particularly collagen, around the vessels. Perivascular and fine interstitial fibrosis are observed in fibrosis of heart failure with preserved ejection fraction and are often associated with systemic inflammation. Different forms of fibrosis (replacement, interstitial, and perivascular) observed in heart failure are schematically represented in Figure 1.

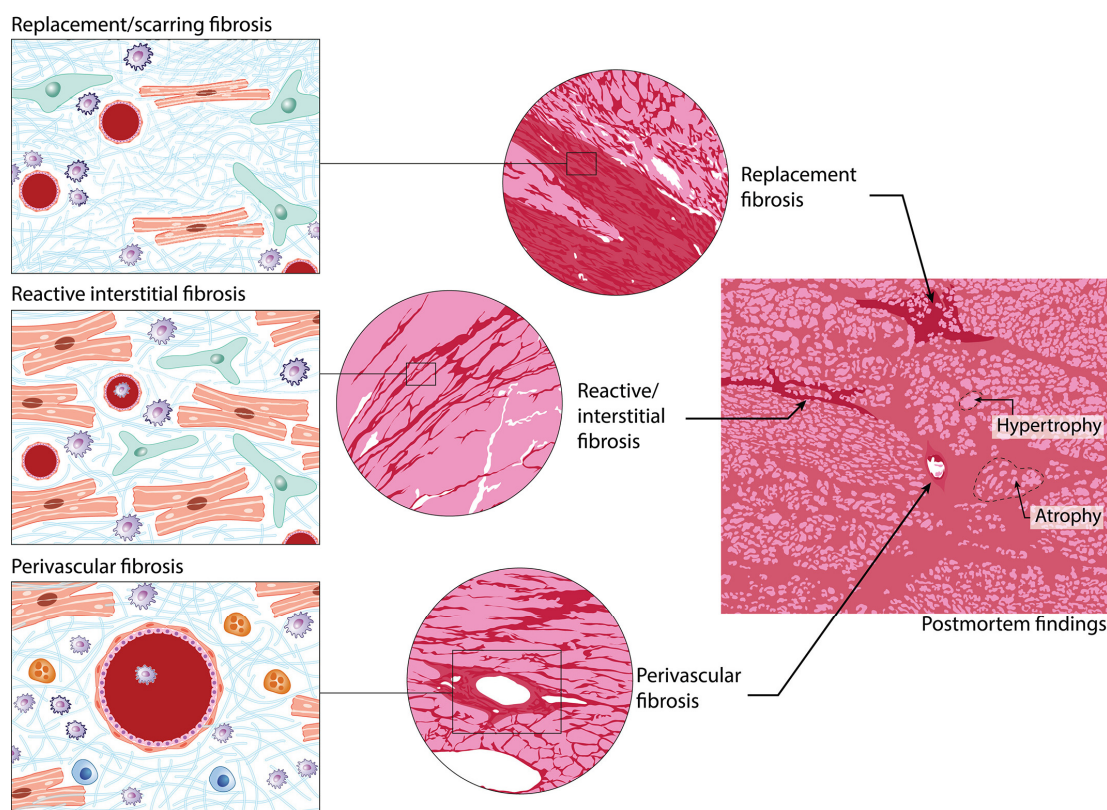


Figure 1. Schematic representation of different forms of fibrosis observed in heart failure. The major cell types: fibroblasts (green), inflammatory cells (blue and grey), and myocytes (orange-red), and their distribution within collagen fibrils are shown on the left. The right panel shows that all forms of fibrosis are observed in a failing heart. Adapted from Boer et al., 2019 [1], an open-access article distributed under the terms of the Creative Commons CC BY-NC 4 license.

A road map for defining, quantifying, and treating fibrosis in heart failure was defined by the Committee of Translational Research of the Heart Failure Association of the European Society of Cardiology [1]. Some of the therapeutic approaches for heart failure have direct or indirect anti-fibrotic activities. Current clinical management relies on repurposing agents that inhibit the renin-angiotensin-aldosterone system (RAAS), such as angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), and mineralocorticoid receptor antagonists (MRAs), which exhibit protective effects to hypertension and heart failure. Newer therapies, like sodium-glucose cotransporter 2 (SGLT2) inhibitors and angiotensin receptor-neprilysin inhibitor (ARNI) (sacubitril/valsartan), have shown secondary anti-fibrotic effects. These therapies manage symptoms or slow down the progression of heart failure, but they often fail to stop the underlying scarring process. Direct inhibition of transforming growth factor- β 1 (TGF- β 1) signalling has largely failed in clinical trials due to its pleiotropic, context-dependent roles in tissue homeostasis and repair, combined with pathway redundancy and a lack of cell-specific targeting, resulting

in limited efficacy and unacceptable on-target toxicity [2]. Clinical trials using inhibitors of the main driver of fibrosis, the pro-fibrotic cytokines, TGF- β 1, have also not shown completely satisfactory results, emphasising a critical need to identify drug targets and specific drugs to prevent the progression of fibrosis. In this review, we revisit classical signalling pathways and profibrotic signals that promote fibroblast activation and collagen production. Additionally, we discuss current strategies for identifying more precise molecular signalling mechanisms and the newer targets, and discuss mechanosensitive and epigenetic mechanisms in cardiac fibrosis. Small-molecule inhibitors of these drug targets, peptide-based therapies for cardiac fibrosis, natural compounds used as antifibrotic agents, and important drugs repurposed for cardiac fibrosis are also reviewed. Further, chimeric antigen receptor (CAR) T-cell therapy, designed to selectively deplete activated myofibroblasts, is also briefly reviewed.

Myocardial Remodelling Post-Cardiac Injury and Progression to Fibrosis

Prolonged ischemia generated during myocardial infarction results in cardiomyocyte death. The loss of functional tissue triggers an inherent wound-healing response aimed at maintaining the structural integrity of the heart. The wound-healing process post-myocardial injury can be categorized into three distinct, but overlapping phases: the inflammatory phase, the proliferative or reparative phase, and the maturation/remodelling phase. The inflammatory phase is a tightly regulated response that is essential for clearing necrotic debris before initiating the ECM remodelling. This process is triggered by the release of damage-associated molecular patterns (DAMPs) from dying cardiomyocytes and fragments of degraded ECM components. These signals activate resident macrophages, which secrete cytokines such as tumor necrosis factor α (TNF- α), interleukin- 1β (IL- 1β), and interleukin- 1α (IL- 1α), which in turn attract circulating monocytes and neutrophils via Toll-like receptors (TLRs), and other resident cells, including endothelial cells and fibroblasts. Monocytes and neutrophils clear cell debris by phagocytosis. During the resolution phase, the anti-inflammatory cytokines are secreted, promoting M1-M2 macrophage polarisation and programmed neutrophil apoptosis. Cardiac fibroblasts play an important role in the ECM remodelling. During the pro-inflammatory phase, the quiescent cardiac fibroblasts that reside within the interstitial space and perivascular space are activated by DAMPs, ROS, and cytokines. Activated fibroblasts proliferate, migrate, transdifferentiate into myofibroblasts, and secrete ECM to remodel the matrix. Myofibroblasts are migratory, secretory, and contractile cells, expressing α -smooth muscle actin (α -SMA) stress fibres, matricellular protein periostin, and predominantly secrete collagen type 1. While the resident fibroblasts are the major source of myofibroblasts, studies indicate that heterogeneous cell types, including endothelial, epicardial-derived cells, smooth muscle cells, and circulating bone marrow-derived fibrocytes, transdifferentiate to myofibroblasts. The remodelled ECM, particularly collagen type I, protects the cardiac wall from rupture. However, the sustained activation of various signaling pathways, such as the RAAS, the overproduction of TGF- β , dysregulated immune response, elevated proinflammatory cytokines, and imbalance in regulators of ECM remodeling, all lead to persistent activation of myofibroblasts and excessive deposition of collagen type I and its crosslinking, resulting in pathological remodeling. The signalling pathways that drive cellular responses and the progression to fibrosis are discussed in detail in Section 2.

2. Signalling Pathways Regulating Wound Healing and Cardiac Fibroblast Response

2.1. Inflammatory Signalling Pathways

The early inflammatory phase is characterized by the recruitment of innate immune cells to the injured myocardium. Neutrophils are among the first responders, clearing necrotic debris and releasing proteolytic enzymes and reactive oxygen species [3]. These cells, along with subsequently recruited immune cells, secrete pro-inflammatory cytokines such as IL-1 and TNF- α , which amplify inflammation and promote

leukocyte recruitment [4]. IL-1 signalling plays a key role in linking the inflammation to fibroblast activation, as IL-1 α activates multiple intracellular pathways in cardiac fibroblasts, including extracellular signal-regulated kinase (ERK1/2), p38 mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinase (JNK), phosphoinositide 3-kinase (PI3K)/AKT serine/threonine kinase 1 (Akt), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), thereby regulating fibroblast function and matrix remodelling [5]. IL-1 β further contributes to fibrotic remodelling by upregulating angiotensin II type 1 (AT1) receptor expression and enhancing cardiac fibroblast migration, while also inducing matrix metalloproteinases (MMP) expression in cardiac fibroblasts [6]. Following neutrophil infiltration, circulating monocytes are recruited to the injured myocardium and differentiate into macrophages, which orchestrate both inflammatory and reparative processes [7]. In addition to their role as inflammatory mediators, monocytic fibroblast precursors have been shown to directly contribute to fibrotic remodelling, as demonstrated in angiotensin II-induced cardiac hypertrophy, where these cells promote ECM deposition and fibrosis [8].

IL-6 family cytokines, acting primarily through the Janus kinase/signal transducer and activator of transcription (JAK/STAT3) signalling pathway, are key mediators linking inflammation to cardiac fibroblast activation and fibrosis [9]. In cultured cardiac fibroblasts, IL-6 enhances STAT3 phosphorylation, whereas STAT3 inhibition attenuates IL-6-induced collagen synthesis and reverses cardiac hypertrophy [10]. Moreover, genetic deletion of IL-6 has reduced myocardial STAT3 phosphorylation and has improved adverse remodelling in a mouse model of coxsackievirus B3-induced dilated cardiomyopathy [11]. Clinically, elevated circulating levels of IL-6, together with TNF- α , have been shown to predict the future development of heart failure in asymptomatic elderly individuals, highlighting the translational relevance of IL-6-STAT3 signalling in cardiac disease [12].

TNF- α is a key pro-inflammatory cytokine that signals through the TNF receptors (TNFR1 and TNFR2), with soluble TNF- α preferentially activating TNFR1 and membrane-bound TNF- α signalling through TNFR2 [13]. TNF- α exerts context-dependent and sometimes opposing effects during cardiac remodelling. TNF- α has promoted pro-fibrotic responses by driving endothelial-to-mesenchymal transition [14] and increasing lysyl oxidase expression through TGF- β and PI3K signalling [15]. However, transmembrane TNF- α signalling via TNFR2 has exerted protective effects by attenuating pressure overload-induced cardiac hypertrophy [16] and limiting myocarditis and cardiac death through suppression of activated heart-reactive CD4⁺ T cell expansion [17].

Innate immune receptors, particularly TLRs, play a critical role in sensing tissue damage and initiating inflammatory responses in cardiac fibroblasts [3]. Cardiac fibroblasts have been identified as significant TLR9-responsive cells with important functional roles in myocardial inflammatory signalling [18]. Cell-autonomous TLR4 signalling has been shown to modulate TGF- β -induced activation of human cardiac fibroblasts [19]. Recent studies have further highlighted the contribution of inflammasome signalling to fibroblast activation, demonstrating that inflammasome activation in cardiac fibroblasts is essential for myocardial ischemia-reperfusion injury [20], and establishing immune-fibroblast cross-talk as an important amplification loop in chronic cardiac inflammation.

2.2. Transforming Growth Factor- β Signalling

TGF- β is the most extensively studied and central mediator of cardiac fibroblast activation and pathological cardiac fibrosis [21]. In mammals, the TGF- β subfamily comprises three isoforms: TGF- β 1, TGF- β 2, and TGF- β 3. Among these, TGF- β 1 is the predominant and most intensively investigated isoform [22]. In the healthy myocardium, TGF- β is stored in a latent complex bound to latency-associated peptide and latent TGF- β -binding proteins within the ECM. Following cardiac injury, latent TGF- β is rapidly activated through proteolytic cleavage by MMPs, MMP-2, MMP-9, plasmin, integrins, and matricellular proteins such as thrombospondin-1 [23].

Multiple cardiac cell types contribute to the TGF- β pool in the injured heart, including macrophages, monocytes, endothelial cells, cardiomyocytes, platelets, pericytes, and resident fibroblasts [24]. Macrophage-derived TGF- β plays an important paracrine role in shaping the fibrotic niche [7], while endothelial cell-specific activation of TGF- β promotes microvascular fibrosis [25]. Cardiomyocyte-specific TGF- β signalling has also emerged as a contributor to fibrotic remodelling, as cardiomyocyte-specific deletion of TGF- β RII has reduced fibrosis in pressure-overload models [26]. Nevertheless, accumulating genetic and lineage-tracing evidence indicates that fibroblast-intrinsic TGF- β signalling represents the dominant driver of pathological ECM deposition and scar formation [27].

TGF- β exerts its profibrotic effects through both canonical intracellular signalling proteins that act as key transducers for TGF- β , suppressor of mother against decapentaplegic (SMAD)-dependent, and non-canonical SMAD-independent signalling pathways. Canonical signalling is initiated when TGF- β binds to the type II TGF- β receptor (TGF- β RII), leading to recruitment and activation of the type I receptor, anaplastic lymphoma kinase-5 (ALK5) (TGF- β RI), a serine/threonine kinase present on the cell membrane. Activated ALK5 phosphorylates receptor-regulated SMADs, SMAD2 and SMAD3, which subsequently form complexes with the common mediator SMAD4 and translocate into the nucleus to regulate transcription of profibrotic target genes [22]. Although both SMAD2 and SMAD3 are involved in canonical TGF- β signalling, *in vivo* evidence demonstrates that SMAD3 plays a critical and indispensable role in cardiac fibrosis, whereas SMAD2 appears largely dispensable [28]. SMAD3 null mice have shown attenuated α -SMA expression, reduced cardiac fibroblast migration, and decreased synthesis of ECM proteins including collagens I, III, and fibronectin [29].

In parallel, TGF- β activates multiple non-canonical signalling cascades, including p38 MAPK, ERK1/2, JNK, transforming growth factor- β -activated kinase 1 (TAK1), and Rho family guanosine triphosphatases (GTPases) [30,31]. These SMAD-independent routes regulate fibroblast activation and proliferation, cytoskeletal dynamics, and epithelial to mesenchymal transition (EMT), thereby reinforcing the activated myofibroblast phenotype. Although these kinases integrate signals from numerous cytokines and mechanical stimuli, experimental inhibition of TAK1 or p38 α MAPK has attenuated TGF- β -induced ECM production and myofibroblast differentiation, underscoring their functional relevance downstream of TGF- β [32,33]. Together, genetic, pharmacological, and single-cell transcriptomic evidence indicate that TGF- β signalling, particularly fibroblast-intrinsic SMAD3-dependent pathways, acts as a central molecular determinant of myofibroblast activation and cardiac fibrotic remodelling [34–36].

Studies have shown that cardiac fibroblast-specific deletion of SMAD2/3 or TGF- β receptors (TGF- β RI/2) from cardiac fibroblasts inhibited the profibrotic genes and ECM remodelling [35]. Several studies have reported the potential of Pirfenidone (5-methyl-1-phenyl-2-[1H]-pyridone), a synthetic small-molecule drug, to inhibit cardiac fibrosis by targeting the TGF- β 1/SMAD pathway or by reducing both collagen expression and vascular permeability [37,38]. Further, bioinformatics analysis and preclinical analysis have shown that Pirfenidone targeted p38 γ -MAPK12, the TGF- β 1-SMAD2/3 pathway, and other effector proteins such as MMP2, MMP14, PDGF- α / β , and IGF-1 in the post-MI LV remodelling [39].

2.3. The Renin–Angiotensin–Aldosterone Signalling

The RAAS signalling is a complex hormonal network that plays a crucial role in cardiovascular homeostasis. Although it is classically known for controlling blood pressure, blood volume, and sodium balance, RAAS activity also influences inflammation, ECM turnover, and cardiac remodelling [40–42]. Renin, an aspartyl protease, cleaves angiotensinogen, synthesized primarily in the liver and expressed in cardiac tissue, into angiotensin I. Angiotensin I is subsequently converted by angiotensin-converting enzyme (ACE) into angiotensin II (Ang II).

In the setting of myocardial injury, cardiomyocytes, immune cells, and fibroblasts locally generate renin and ACE, leading to increased interstitial Ang II levels [43]. Ang II acts via autocrine, paracrine, and

endocrine mechanisms, triggering intracellular signalling pathways involving MAPK, ERK, PKC, and Smad proteins. These pathways promote cardiac fibroblast proliferation, migration, resistance to apoptosis, differentiation into myofibroblasts, and excessive synthesis of collagen and fibronectin, ultimately contributing to cardiac hypertrophy, fibrosis, and adverse ventricular remodelling [44–46]. In addition, Ang II further stimulates myocardial aldosterone production, and aldosterone further contributes to cardiac fibrosis by directly promoting fibroblast activation and matrix synthesis, as well as by modulating cardiomyocyte, immune, and vascular cell function [43].

The profibrotic actions of the classical RAAS are counterbalanced by protective mechanisms, including signalling through the AT2 receptor and the alternative ACE2/angiotensin-(1–7)/Mas pathway. ACE2 converts Ang II into angiotensin-(1–7), which exerts vasodilatory, anti-inflammatory, and antifibrotic effects through Mas receptor activation and downstream pathways involving nitric oxide, cyclic AMP-dependent protein kinase, and reduced MAPK activity [47]. Experimental studies have shown that ACE2 deficiency has worsened cardiac remodelling [48], whereas ACE2 upregulation has attenuated fibrosis [49]. The complexity of RAAS signalling is further increased by additional angiotensin peptides, including Ang III, Ang IV, Ang A, and alamandine, which act through multiple receptors in a tissue and dose-dependent manner [50]. Collectively, these mechanisms explain the central role of RAAS in cardiac remodelling and fibrosis and provide the biological basis for therapeutic strategies aimed at restoring balance between the detrimental classical RAAS axis and its protective counter-regulatory pathways.

2.4. The Wnt/ β -Catenin Signalling

Canonical Wnt signalling is initiated when Wnt ligands bind Frizzled family transmembrane receptors and the co-receptors LRP5/6, leading to dissociation of the β -catenin destruction complex. This process results in stabilization and cytoplasmic accumulation of β -catenin, followed by nuclear translocation and interaction with T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) transcription factors and co-activators to regulate gene expression [51]. Wnt ligands may also activate non-canonical β -catenin-independent pathways, highlighting the complexity of Wnt signalling responses in the injured heart.

In the uninjured myocardium, Wnt signalling is tightly regulated at multiple levels by endogenous inhibitors and intracellular mediators. Members of the Dickkopf (Dkk) family inhibit Wnt signalling by preventing the formation of the Frizzled–LRP receptor complex, while soluble frizzled-related proteins (sFRPs) act extracellularly by binding Wnt ligands and limiting their availability for receptor activation, thereby reducing β -catenin-dependent transcription [52,53]. In addition to these extracellular regulators, Disheveled functions as a key intracellular signalling intermediate that integrates both canonical and non-canonical Wnt pathways [54].

Wnt signalling is reactivated following myocardial injury. In mouse models of myocardial infarction, myocardial injury has induced transcriptional upregulation of multiple Wnt ligands and antagonists, including Wnt2, Wnt4, Wnt10b, Wnt11, Dkk1, and Dkk2, within the injured myocardium [55]. Concomitantly, acute ischaemic cardiac injury has triggered organ-wide Wnt-dependent epicardial activation that has promoted epithelial to mesenchymal transition and expansion of cardiac fibroblast populations, driving profibrotic remodelling [56]. Consistent with these experimental findings, nuclear accumulation of β -catenin and its transcriptional partner, T cell factor (TCF-4), has been detected in cardiac fibroblasts of failed paediatric heart allografts [57] and conditional deletion of β -catenin in cardiac fibroblasts has attenuated interstitial fibrosis and preserved cardiac function [58].

Extensive crosstalk between Wnt/ β -catenin and TGF- β signalling pathways coordinates cardiac fibroblast activation and fibrotic remodelling [59]. At the molecular level, β -catenin cooperates with Smad 2 downstream of TGF- β receptors to co-regulate the expression of α -SMA, thereby reinforcing the myofibroblast phenotype [60]. Disruption of either pathway attenuates fibroblast activation, underscoring

the functional interdependence of Wnt and TGF- β signalling in driving persistent fibroblast activation and pathological cardiac fibrosis.

2.5. MAPK Signalling (ERK, p38, JNK)

Among the intracellular signalling cascades that govern fibroblast activation, MAPK pathways, including ERK, p38 MAPK, and JNK, play critical and functionally distinct roles [61]. ERK1/2 signalling is predominantly activated downstream of extracellular cues sensed by three major classes of membrane receptors: integrins, G-protein-coupled receptors (GPCRs), and receptor tyrosine kinases. Following cardiac injury, growth factors and neurohormones such as platelet-derived growth factor (PDGF), endothelin 1, and Ang II are released and activate receptor tyrosine kinases and GPCRs, leading to ERK activation. In parallel, integrin-mediated mechanotransduction provides an additional route for ERK1/2 activation [62]. Beyond the canonical Smad-dependent pathway, TGF- β further amplifies fibroblast activation through non-canonical signalling cascades, including ERK, JNK, and p38 MAPK pathways, thereby reinforcing profibrotic responses and positioning ERK as a central signalling hub [63]. Sustained or aberrant activation of ERK and JNK MAPK signalling promotes excessive cardiac fibroblast proliferation and matrix-remodelling, thereby driving pathological myocardial fibrosis and contributing to the progression of heart failure [64,65]. In cardiac fibroblasts, p38 MAPK, particularly the p38 α isoform, plays a central role in the reactive oxygen species and cytokine-dependent induction of IL-6 and MMP-3, highlighting the involvement of MAPK pathways in inflammatory signalling [66,67].

JNK signalling represents a stress-activated MAPK pathway that contributes to cardiac fibroblast activation and broader pathological remodelling in the heart. Beyond canonical activation, post-translational modulation of JNK, including S-nitrosylation of c-Jun N-terminal kinase, has been implicated in pressure overload-induced cardiac dysfunction and fibrosis [68], highlighting the importance of context-specific regulation of this pathway. In human atrial fibroblasts, Ang II-induced differentiation has been mediated through p21-activated kinase 1-dependent activation of the JNK/c-Jun axis [69], further linking JNK signalling to fibroblast phenotypic conversion. Similarly, resistin has promoted cardiac fibroblast differentiation through coordinated activation of JAK/STAT3 and JNK/c-Jun pathways [70], and Wnt4-dependent cardiac repair has been shown to involve phospho-JNK/JNK signalling [71]. Collectively, these findings establish MAPK pathways as key signalling hubs that integrate mechanical stress, inflammatory mediators, and neurohormonal stimuli to sustain persistent fibroblast activation and pathological cardiac fibrosis.

TGF-Wnt β -catenin and MAPK pathways intersect to drive cardiac fibroblast activation, differentiation into myofibroblasts, and collagen production, promoting fibrosis. TGF-triggers Smad-dependent profibrotic responses, while canonical Wnt β -catenin signalling synergises with TGF- β to enhance IL-11 production, increasing ECM deposition. Additionally, non-coding microRNAs that regulate proteins in the above pathways are also targets for fibrosis. The important miRNAs miR-101a, miR-145, miR-675, miR-10a, miR-15, MiR-323a-3p, miR-202-3p, miR-433, miR-29b, miR-503 targeting TGF- β and Wnt pathways are reviewed in detail [72].

A schematic of pathways, targeting cardiac fibroblast activation, transdifferentiation to myofibroblasts, collagen synthesis, ECM remodelling, and crosslinking, is represented in Figure 2. Targeting general TGF- β , inflammatory, and Wnt pathways for cardiac fibrosis is challenging due to their pleiotropic nature, as they play essential roles in normal tissue homeostasis, immune regulation, and repair, leading to significant off-target effects and toxicity when blocked systematically. Targeting specific proteins or effectors downstream of these signalling pathways would be a more appropriate approach for cardiac fibrosis.

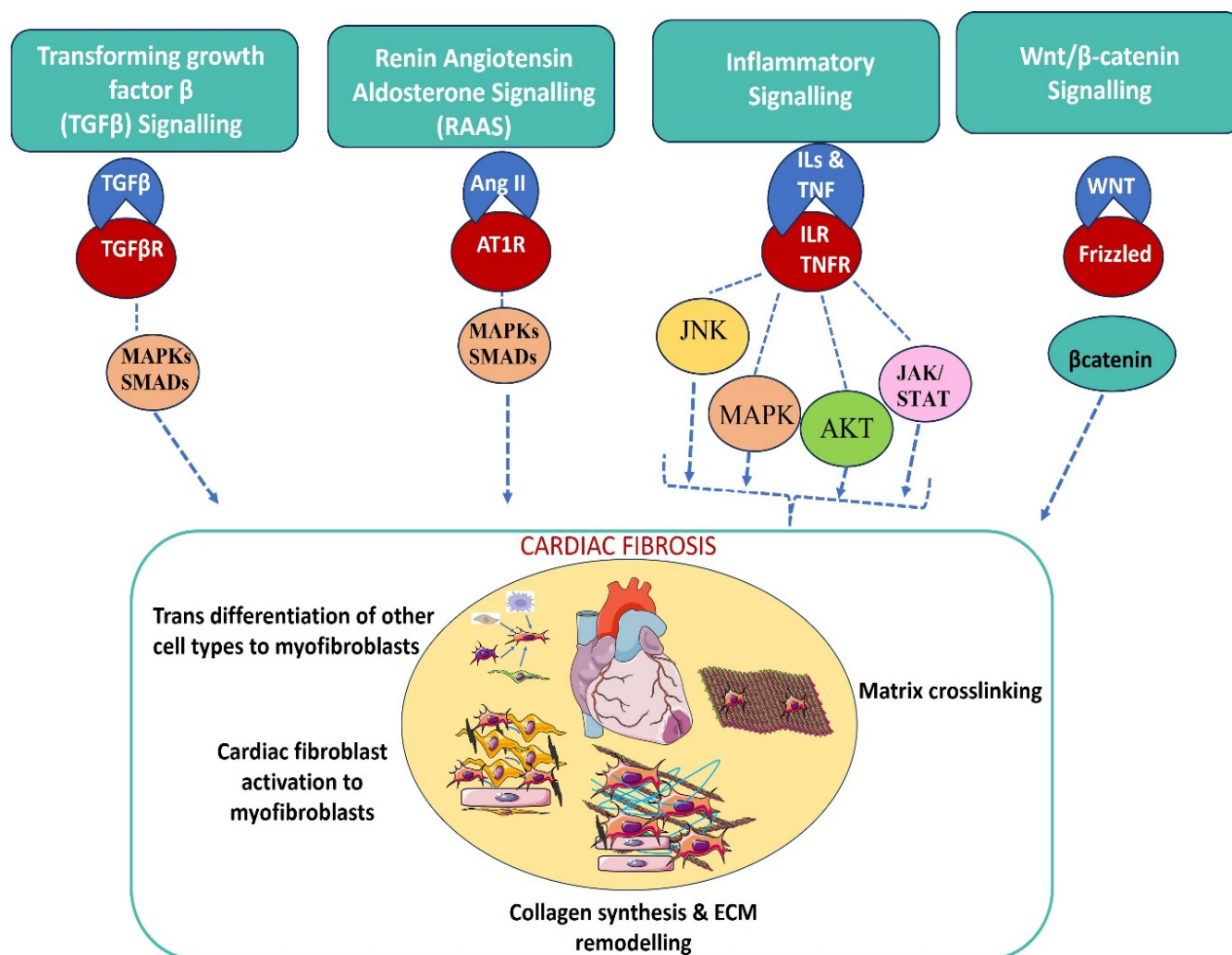


Figure 2. Schematic of signalling pathways involved in cardiac fibrosis. Profibrotic stimuli, receptors, and a few proteins in signalling pathways, and their regulators are all targets for cardiac fibrosis. The pathways regulate the transdifferentiation of cardiac fibroblasts and other cell types to myofibroblasts, matrix remodelling, collagen synthesis and crosslinking.

3. Proteins and Signalling Pathways That Regulate Collagen Crosslinking and Myocardial Stiffness, and Their Drug Targets

The relative abundance of collagen types I and III, and their extent of cross-linking, dictate the passive mechanical properties of the heart [73]. Collagen cross-linking in the cardiac ECM is a fundamental post-translational modification that dictates myocardial stiffness and structural integrity. The structural stability of the cardiac wall is maintained by a delicate equilibrium involving MMPs and tissue inhibitors of metalloproteinases (TIMPs). The process is primarily driven by the enzyme lysyl oxidase (LOX), secreted by cardiac fibroblasts. LOX catalyses the oxidative deamination of lysine and hydroxylysine residues in collagen. This process leads to the formation of aldehyde groups, which then crosslink adjacent collagen fibrils, thereby increasing tensile strength. We have investigated the role of periostin, a matricellular protein, in the regulation of LOX in activated cardiac fibroblasts in response to Ang II and TGF β 1. Our results indicate that periostin regulates LOX via ERK1/2 MAPK signalling and serum response factor (SRF) in myofibroblasts *in vitro*. The expression of periostin is also correlated with upregulated expression of collagen and LOX in the infarcted myocardium in a rat model of MI [64]. Transglutaminase 2 (TG2) functions as a calcium-dependent acyltransferase that catalyses the formation of stable crosslink ϵ (γ -glutamyl)lysine isopeptide bonds, providing an alternative enzymatic pathway for collagen cross-linking independent of the LOX-mediated oxidative deamination. TG2 is involved in early ECM remodelling,

while LOX contributes to crosslinking at later stages. The degree of cross-linking determines the solubility, stiffness, and resistance to degradation of the resulting fibrils. Enhanced matrix stiffness in turn leads to myofibroblast activation, creating a loop for fibrosis. The imbalance in the equilibrium of collagen synthesis, MMPs, TIMP production, and upregulation of LOX, often observed in the progression of hypertensive heart disease and heart failure, results in a stiff, non-compliant ventricle. The different phases of cardiac ECM remodelling post myocardial infarction, the role of Lysyl oxidase and TG2 in collagen crosslinking, and the role of periostin in the regulation of Lysyl oxidase are schematically represented in Figure 3.

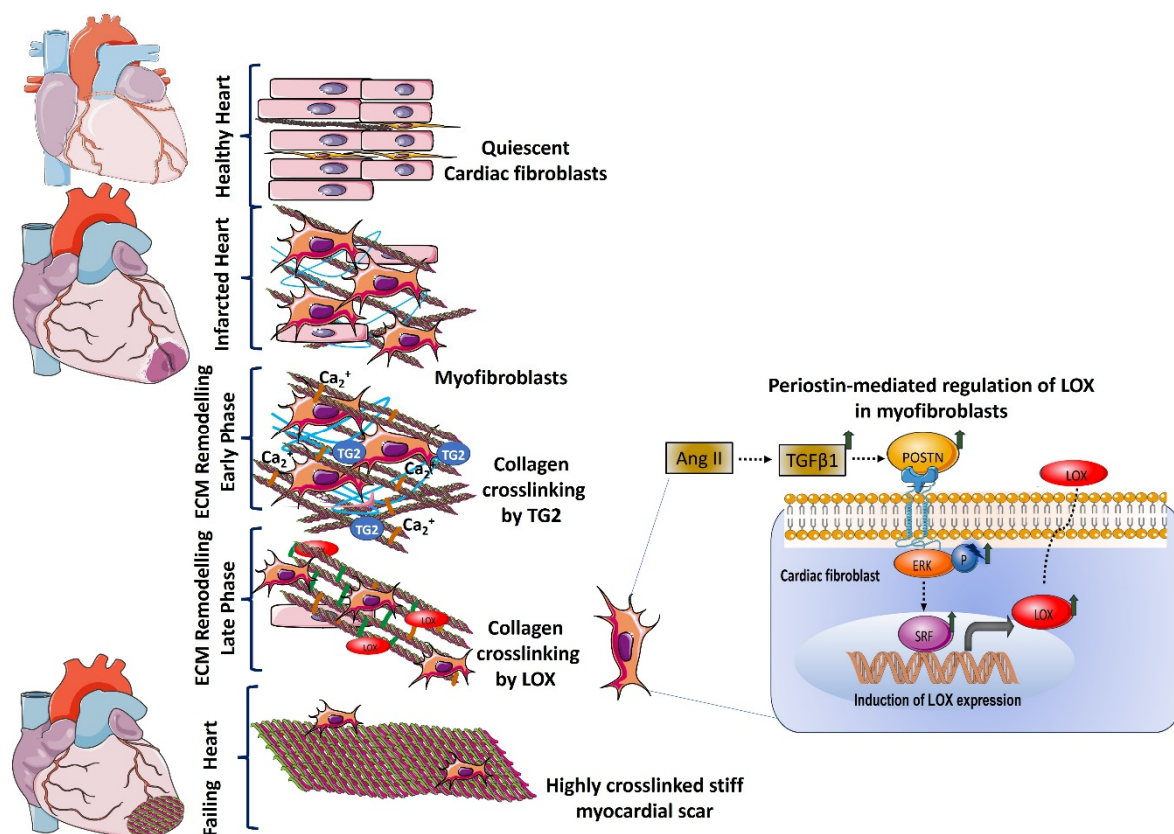


Figure 3. Schematic representation of different phases of ECM remodelling in an infarcted heart, the role of LOX and TG2 in collagen crosslinking, and the role of periostin in the regulation of LOX. Periostin regulates LOX via ERK signalling through the involvement of serum-responsive factor (SRF) in myofibroblasts in response to profibrotic stimuli such as Angiotensin II and TGFβ1.

While collagen synthesis can be targeted by regulating RAAS and other signalling pathways, collagen crosslinking requires targeting of specific proteins like LOX and transglutaminase. LOX was inhibited using β -aminopropionitrile (BAPN) in a volume overload model in rats, which attenuated collagens, MMPs, tissue inhibitor expression, and ventricular wall stress, partially attenuated ventricular hypertrophy, and decreased the decline in cardiac function. BAPN had a direct effect on collagen crosslinking; however, its effect on MMPs and TIMP could be secondary [74]. In an MI model, SNT-5382, a potent, selective lysyl oxidase-like 2 (LOXL2), exhibited reduced collagen volume fraction at the border zone of the infarct region. SNT-5382 reduced mature collagen crosslinks, while the total collagen content was unaffected. Further, a randomised, double-blind, placebo-controlled Phase 1 clinical trial (ACTRN12617001564347) conducted in healthy subjects showed that the LOXL2 inhibitor was very well tolerated, with no serious adverse events [75]. Since periostin regulates LOX, periostin is also a potent target for collagen crosslinking. A highly selective TG2 small-molecule inhibitor, 1–155, reduced both TG2 activity and its export into the ECM by blocking its cell-surface interaction with its binding partner, syndecan, thereby reducing secretion. The

antifibrotic ability of this inhibitor was tested in two *in vivo* models, such as an interstitial cardiac fibrosis model of pressure overload using angiotensin II infusion and an acute myocardial infarction model induced by ligation of the left anterior descending coronary artery. In both models, there was a 50% significant reduction in infarct size, Sirius red staining for collagen deposition, and in levels of the TG2-mediated protein crosslink $\epsilon(\gamma\text{-glutamyl})\text{lysine}$ [76]. In another study, the tissue transglutaminase (tTG) inhibitor ERW1041E, an irreversible pharmacological inhibitor of enzymatically active tTG, attenuated cardiomyocyte hypertrophy and interstitial fibrosis by reducing collagen protein levels and collagen crosslinking in a mouse model of pressure-overload; however, it did not affect ejection fraction. *In vitro*, the inhibitor did not affect collagen transcription but induced MMP3 and TIMP1 synthesis in cardiac fibroblasts through non-enzymatic action [77]. Complete inhibition of collagen crosslinking is lethal, as it may lead to cardiac wall rupture and other serious complications, such as aortic aneurysm. Hence, the timing of targeting collagen crosslinking is crucial for reducing myocardial stiffness while maintaining cardiac wall integrity.

Matricellular Proteins as Therapeutic Targets for Cardiac Fibrosis

Matricellular proteins are a class of non-structural ECM proteins that function as dynamic signalling adapters and regulate cell-matrix interactions by binding to cell-surface receptors, growth factors, and other structural proteins. They act as molecular “switches” that are rapidly upregulated following heart injury to orchestrate the transformation of quiescent fibroblasts into active, collagen-secreting myofibroblasts. They also play an important role in collagen chain alignment and crosslinking. Cellular communication network (CCN) family proteins, CCN1, CCN2, CCN5, and periostin are key matricellular proteins involved in cardiac fibrosis. Endogenous CCN1 was essential for scar formation and had a role in the arrangement of collagen fibrils in the maturing scar. CCN1 knockout animals exhibited reduced alignment and heightened tortuosity of collagen fibres, and reduced organizational coherency, packing, and size of collagen fibrils [78]. Cardiac remodelling and fibrosis are also observed in chronic kidney disorders (CKD); CCN1 expression was markedly enhanced in the serum and heart, accompanied by an increase in COL1, TGF- β , heavy-chain cardiac myosin, ANP, disordered myocardial arrangement, cardiac fibrosis, hypertrophy, and decreased cardiac systolic function in a mouse model of CKD. Knockdown of CCN1 reversed these effects, reduced MAPK signalling and fibrosis [79]. Connective tissue growth factor (CTGF, CCN2) is an autocrine regulator of cardiac fibrosis. TGF- β , Ang II, and ET-1 induce CCN2, which is a downstream mediator of these proteins. CCN2 was upregulated in cardiac fibroblasts in response to Ang II, and CCN2 deletion from activated fibroblasts inhibited the fibrotic remodelling [80]. CCN2 silencing inhibits the activation of the MAPK signalling pathway and alleviates myocardial fibrosis and left ventricular hypertrophy [81]. Both CCN1 and CCN2 deletions led to decreased fibrosis. CCN5 is a secreted matricellular protein that mediates anti-fibrotic activity by inhibiting cardiac fibroblast-to-myofibroblast transition and TGF- β_1 signalling pathway. Serum CCN5 level decreased in hypertensive patients, but significantly increased in hypertensive patients on an ACE inhibitor, indicating a negative association between CCN5 and Ang II-induced hypertension, in heart failure patients [82]. A therapeutic target stimulating endogenous CCN5 production could reduce cardiac fibrosis.

Periostin is another matricellular protein that is not present in the healthy adult heart; it is expressed after myocardial infarction and plays a role in the progression of fibrosis. Studies from our lab indicate that, *in vitro*, periostin is upregulated in cardiac fibroblasts in response to Ang II and TGF- β_1 treatment, and that periostin is expressed in the infarct scar post-myocardial injury. Further, we have shown that knockdown of periostin decreases collagen crosslinking enzyme LOX, and that periostin regulates LOX via ERK-MAPK, involving SRF [64]. Single-cell RNA sequencing revealed that Postn-positive fibroblasts played a significant role in the progression of myocardial fibrosis, and knocking down Postn decreased cardiac fibroblast activity, inhibited their migration, and induced apoptosis [83]. Targeted ablation of periostin-

expressing cardiac fibroblasts in chronic AngII-exposed mice and in the MI model resulted in reduced fibrosis without compromising scar stability and cardiac function [84]. A recent study showed that periostin is a pivotal target of microRNA-150-5p in primary adult human cardiac fibroblast activation and chronic MI mouse model [85].

4. Mechanosensitive Pathways in Cardiac Fibroblasts and the Drug Targets

The stiffness of the heart is contributed to by the adult cardiomyocyte and the cardiac ECM. Cardiac fibroblasts function as sophisticated mechanosensors that respond to physical cues such as stretch, compression, and stiffness. At the cellular level, force is transmitted from the ECM or neighbouring cells to cardiac fibroblasts through specific transmembrane proteins, focal adhesion molecules, GPCRs, stretch-activated ion channels, and cytoskeletal proteins, which sense the mechanical signals (stretch or ECM stiffness) into specific signal transduction pathways. In cardiac fibroblasts ($\alpha1\beta1$, $\alpha2\beta1$, and $\alpha11\beta1$), integrins that bind to collagen, and $\beta3$ integrins that bind to fibronectin, are the sensors for changes in extracellular matrix stiffness [86]. Integrins and focal adhesion complexes (paxillin and vinculin) transmit mechanical signals across the membrane to the cytoskeleton. Cytoskeletal actin polymerization phosphorylates Rho kinases and Lin-11, Isl-1, and Mec-3 kinases (LIM kinases), further promoting nuclear translocation of transcription factors, myocardin-related transcription factors (MRTF), a cofactor for transcription factor SRF, that binds the promoter region of α -SMA and SM22. Actin polymerisation during cytoskeletal dynamics modulates the Hippo signalling pathway, regulating nuclear translocation of the Yes-Associated Protein (YAP). Cyclic strain and ECM stiffness directly promote cardiac fibroblast activation and proliferation through YAP activation [86]. Studies have shown that YAP promotes myofibroblast differentiation and associated ECM gene expression through engagement of TEA domain transcription factor 1 and subsequent de novo expression of MRTF-A. Cardiac fibroblast YAP is a promising therapeutic target to prevent fibrotic remodelling and heart failure [87]. YAP inhibitor, Verteporfin, reduced profibrotic activation of cardiac stromal cells induced by mechanical cues, even in the presence of TGF β . Further, verteporfin significantly reduced fibrosis and remodelling in an ischemic *in vivo* model; however, it did not improve cardiac functions [88]. Z11, a small-molecule inhibitor of nucleosome assembly protein 1-like 1 (NAP1L1), is found to prevent the interaction between NAP1L1 and YAP1, promote ubiquitination degradation of YAP1, and alleviate cardiac fibrosis by inhibiting the AKT/mTOR signalling, cardiac fibroblasts activation, and collagen deposition. Z11 decreased the expression of α -SMA, NAP1L1, and YAP1, collagen I, and collagen deposition in a mouse MI model [89].

Increased ECM Stretch can mechanically open the latent complex, activate TGF- β , and release active TGF- β from the ECM. DAMPs are released into the extracellular space during cellular stress, and ECM fragments of hyaluronic acid, and ECM-localized small leucine-rich proteoglycans (SLRPs) are released during tissue damage. This further activates TLRs and transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B), which in turn leads to enhanced fibrosis. Mechanical stress can also activate TLRs. Syndecan-4 also has a role in mechanotransduction in the myocardium. Syndecan binds to GAG chains to extracellular molecules and to the cytoplasmic domain to the cytoskeleton. Syndecan-4 signalling via nuclear factor of activated T-cells (NFAT) regulates ECM production and cardiac myofibroblast differentiation in response to mechanical stress. The mechanotransduction pathways in cardiac fibroblasts are reviewed in detail by Herum et al. [90].

In cardiac fibroblasts, the intracellular transmission of mechanical cues can be elicited through mechanically responsive ion channels. During mechanosensing, deformation of the cell membrane induces changes in ion influx, particularly Ca²⁺ ions. Though not electrically excitable, cardiac fibroblasts are sensitive to membrane potential oscillations that happen following myocardial infarction and ECM remodelling. Transient receptor potential canonical type 6 (TRPC6), a nonselective receptor-operated cation channel, and Piezo 1 are the most studied mechanosensitive ion channels in cardiac fibroblasts.

TRPC6 is sensitive to stretch, stress, and osmotic pressure and is highly permeable to Ca^{2+} during cardiac pathology, and is a mediator of fibrosis [91]. *In vitro*, cardiac fibroblasts showed upregulation of TRPC6 in response to TGF- β 1 and Angiotensin II, via p38 MAPK activation and by serum response factor transcriptional regulation of the TRPC6 promoter. This, in turn, increased Ca^{2+} permeability in cultured cardiac fibroblasts, which induced myofibroblast transformation via calcineurin–NFAT signalling. Overexpression of TRPC6 induced spontaneous differentiation of fibroblasts to myofibroblasts, while knockdown attenuated myofibroblast differentiation induced by TGF- β 1, confirming its role in myofibroblast activation [92]. Further, orally bioavailable TRPC6 antagonist BI 749327, selectively inhibited TRPC6 expression of profibrotic genes and interstitial fibrosis in mice subjected to sustained pressure overload and improved cardiac function [93]. Piezo 1 is a mechanosensitive cation channel that plays an important role in cardiac fibroblast activation and progression of fibrosis. Cardiac fibroblasts express mechanically activated Piezo1 channels, which stimulate paracrine IL-6 secretion via the p38 mitogen-activated protein kinase downstream of Ca^{2+} entry [94]. Emig et al., have shown that Piezo1-overexpression in human atrial fibroblasts induced cytoskeletal reorganisation by affecting the architecture of actin stress fibres. The study showed that in human atrial fibroblasts seeded on soft and stiff hydrogels, Piezo1 was required to adapt the cell stiffness to the substrate stiffness. Further, Piezo-induced cell stiffening is mediated by an autocrine mechanism via IL-6 secretion, and can transmit cell stiffening to neighbouring cells in a paracrine manner mediated by IL-6 [95], as depicted in Figure 4. Braidotti et al. reviewed Piezo1-induced autocrine, paracrine, and paratensile effectors in activated fibroblasts, highlighting the role of Piezo1 in mechanosensing in cardiac fibroblasts and suggesting Piezo1 as a potential target for cardiac fibrotic remodelling [96].

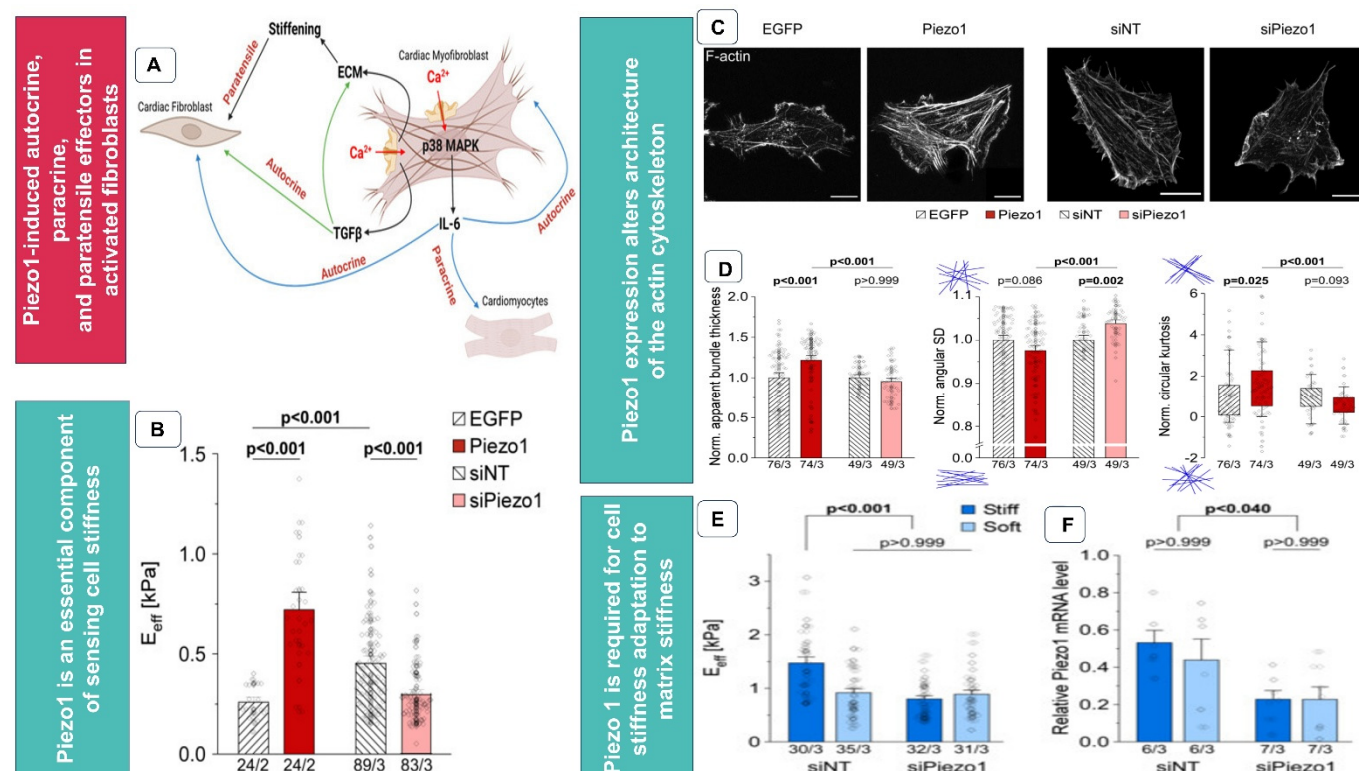


Figure 4. (A) Schematic representation of Piezo1-induced autocrine, paracrine, and paratensile effectors in activated fibroblasts. (Adapted from Braidotti et al., [96] an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license). (B) Stiffness of human atrial fibroblast line, 3 days after overexpression of EGFP, Piezo1, non-targeting siRNA (siNT), and siRNA mediated knock-down of Piezo1 (siPiezo1), (C) Phalloidin staining of actin cytoskeleton in human atrial fibroblasts 3 days post-transfection (D) apparent thickness, angular standard deviation (SD), and circular kurtosis of actin bundles, (E,F) average stiffness and mRNA levels of Piezo 1 of cells after 4 days of culture on stiff (~6 kPa) or soft (~3

kPa) hydrogels respectively. (B–F) Adapted from Emig et al., [95] an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

5. Targeting Epigenetic Factors

Epigenetic modifications are changes in DNA, 3D chromatin architecture, mRNA, and post-translational modifications of histone (acetylation, methylation, ubiquitination, and phosphorylation). The epigenetic modifiers (DNA modifiers, histone protein modifiers, and mRNA modifiers) are broadly classified as writers, readers, and erasers. Writers are enzymes that catalyse the addition of chemical groups to DNA or histone tails, leading to gene silencing or activation. DNA methyl transferases (DNMTs), histone acetyltransferases (HATs), RNA methylases, acetylases, and protein arginine methyltransferase (PRMTs) are examples of “Writer” enzymes. Readers are proteins that recognise, bind, and interpret the covalent modifications laid down by writers, leading to transcription or chromatin remodelling (e.g., Bromodomains). Erasers are enzymes that remove the chemical tags deposited by writers and modulate gene expression (e.g., histone deacetylases (HDACs) and demethylases). Histone acetylation increases gene expression while histone deacetylation suppresses gene transcription. Histone acetylation mainly occurs at the ϵ -amino groups of lysine residues in the N-terminal tails of histones H3 and H4 and is associated with transcriptional activation by promoting chromatin relaxation. HMTs or histone methyl transferases induce methylation, which, depending on the type of methylation, is associated with both gene repression and active transcription of genes. Perez et al. recently reviewed the epigenetic modifications in cardiac fibrosis in detail and how chromatin-modifying enzymes orchestrate the transcriptional changes in cardiac fibroblasts [97].

A study in a hypertensive model showed that H3K9 acetylation in myocardial tissue correlated with increased α -SMA expression. Inhibition of HAT p300 using the small-molecule inhibitor L002 reduced myofibroblast differentiation and fibrosis in a mouse model of hypertension [98]. Curcumin, a natural compound, inhibited p300 and reduced fibrosis, BNP expression, perivascular fibrosis, and downregulated acetylation levels of GATA4 and pro-hypertrophic gene expression in a chronic heart failure Dahl salt-sensitive rat model [99]. However, a clinical trial (UMIN000014232) showed curcumin intake in patients with initial signs of hypertensive heart disease with left ventricular ejection fraction $\geq 60\%$, decreased plasma BNP levels without altering ventricular diastolic function [100]. DNMTs also has a role in regulating α -SMA activation. DNMT3a promotes CFs activation and fibrosis through the ERK1/2 pathway [101]. DNMT3a inhibition using low-intensity pulsed ultrasound (LIPUS) prevents hypoxia-induced cardiac fibroblast activation and cardiac fibrosis [102].

Recent studies indicate that epigenetic modifications affecting RNA are also involved in cardiac fibroblast activation and profibrotic gene expression. N⁶-methyladenosine (m⁶A) is the most common modification observed in mRNA. Methyltransferase-like protein 3 (METTL3) is the primary catalytic “writer” enzyme that makes m⁶A modifications on mRNA. METTL3 overexpression upregulated cardiac fibroblast activation to myofibroblast and collagen deposition, and its downregulation attenuated these changes. Transcriptome and m⁶A profiling analyses revealed that the expression and m⁶A levels of collagen-related genes were altered [103]. A recent study showed that inhibition of METTL3-METTL14 using controlled delivery of its inhibitor S-adenosyl-l-homocysteine from microneedles reduced mitochondrial fragmentation, differentiation to myofibroblasts, and expression of collagen I and collagen III [104]. FTO (Fat mass and obesity-associated) protein is an m⁶A-demethylase of mRNA. Ang II is found to suppress FTO expression, and leonurine, a natural compound with a cardioprotective role, reverses these effects [105]. Overexpression of protein arginine methyltransferase 5 (PRMT5) induces cardiac fibroblast differentiation to myofibroblasts, and its inhibition reverses the effects [106]. Further, PRMT5 inhibition downregulates Col1A1 and Acta2, and its interaction with Smad3. EPZ015666, a selective inhibitor of PRMT5, improved fibrosis and left ventricular dysfunction in an *in vivo* mouse model [107].

Bromodomain-containing protein 4 (BRD4), an epigenetic reader, has been identified as a central regulator of the pro-fibrotic cardiac fibroblast phenotype induced in response to TGF- β stimulation. Chromatin targeting of BRD4 is controlled via a p38-dependent signalling circuit for epigenetic reprogramming [108]. JQ1, a specific BET (bromodomain and extraterminal) bromodomain inhibitor, decreased cardiac fibrosis in a pressure-overload or MI model. JQ1, blocked the expression of periostin, a marker of myofibroblasts [109]. HDACs induce chromatin condensation and suppress gene transcription. HDAC8 has a role in cardiac fibrosis. HDAC8 interacts with signal transducer and activator of transcription 3 (STAT3), facilitating its binding to the MMP12 promoter. An HDAC inhibitor, YAK577, inhibited HDAC8, thereby reducing Col1A1 and fibronectin gene expression and MMP12 mRNA levels in an isoproterenol-induced heart failure model [110]. A small-molecule activator of SIRT3 (a class3 HDAC), (2-APQC), alleviates isoproterenol-induced cardiac hypertrophy and myocardial fibrosis *in vitro* and *in vivo* rat models. It inhibited the activation of AKT, mTOR, (mTOR)-p70 ribosomal protein S6 kinase (p70S6K), c-jun N-terminal kinase (JNK), TGF- β , and Smad3 pathways to improve isoproterenol-induced cardiac hypertrophy and myocardial fibrosis [111]. Although several *in vitro* and preclinical studies in animals have identified epigenetic enzymes as potential therapeutic targets for cardiac fibrosis, clinical studies are limited.

6. Small Molecule Inhibitors and Peptide-Based Therapies for Cardiac Fibrosis

Small-molecule inhibitors are low-molecular-weight organic compounds for pharmacological approaches targeting various signalling pathways. Unlike larger biologics, these low-molecular-weight compounds easily penetrate cell membranes to interact with specific intracellular targets. Their increased bioavailability, reduced immune response, and ability to precisely target proteins and pathways make them a good candidate to develop as a drug. Several small-molecule inhibitors have been explored in targeting collagen crosslinking, mechanosensing pathways, and epigenetic factors regulating cardiac fibrosis. Some of the key small-molecule inhibitors targeting signalling pathways discussed in Sections 3–5 are summarised in Table 1. While these molecules show promising results in preclinical trials, the toxicity of a few inhibitors has to be carefully addressed. Another disadvantage of small molecules in treating cardiac fibrosis is that while they specifically target one pathway, they do not target other pathways that promote fibrosis.

Peptide-based therapies represent an emerging class of precision medicine for cardiac fibrosis, utilising short chains of amino acids to modulate cellular signalling with higher specificity than small molecules. They disrupt specific protein-protein interactions and exhibit more specificity than small-molecule inhibitors. Glucagon-like peptide-1 receptor (GLP-1R) agonists, a class of glucose-lowering drugs, exhibit cardioprotective effects. Liraglutide, a glucagon-like peptide-1 receptor agonist, decreased interstitial cardiac fibrosis and expression of inflammatory and oxidative stress markers in Ang II-induced hypertension and age-induced cardiac fibrosis mice model [112]. Results from STEP-HFpEF (NCT04788511) and STEP-HFpEF-DM (NCT04916470) trials indicate that Semaglutide, a GLP-1R agonist, targets the underlying inflammatory processes and contributes to better overall cardiovascular outcomes in patients with HFpEF [113]. Obesity-driven systemic inflammation can cause coronary microvascular dysfunction, leading to myocardial fibrosis, stiffness, and diastolic dysfunction. A meta-analysis also indicates that GLPRAs exhibit protective effects in obese patients with chronic heart failure [114]. Natriuretic peptides, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP), cardiac-derived hormones, regulate the renin–angiotensin–aldosterone–vasopressin and sympathetic nervous systems and exhibit pleiotropic effects on myocardial and vascular remodelling, and immune responses. ANP and BNP are synthesised by myocytes in response to myocardial stretch, volume overload, and during heart failure, while CNP is predominantly produced by endothelial cells in response to shear stress and inflammation. They exhibit cardioprotective effects by inhibiting RAAS and have anti-fibrotic agents in the heart by increasing cyclic guanosine monophosphate (cGMP) in cardiac

fibroblasts, which inhibit collagen synthesis. The decrease in the level of CNP, secreted by endothelial cells, is positively correlated to increased cardiac fibrosis in mice [115]. Another study showed that Angiotensin-receptor-neprilysin inhibitors increase the bioavailability of ANP. This decreased myocardial fibrosis by increasing protein kinase G; by inhibiting SMAD signalling in cultured cardiac fibroblasts and cardiac fibrosis in Type 2 diabetic rats [116]. Cenderitide is a designer CNP, fused to the C-terminus of Dendroaspis natriuretic peptide (DNP). Cenderitide was engineered to co-activate the two NP receptors, particulate guanylyl cyclase (pGC)-A and pGC-B, to enhance the anti-fibrotic properties of dual-receptor activation. Cenderitide significantly attenuated the development of cardiac fibrosis and preserved diastolic function by enhancing pGC-A-mediated suppression of the potent pro-fibrotic factor aldosterone and by inhibiting fibroblast proliferation and collagen synthesis linked to pGC-B activation [117,118]. Relaxin, a heterodimeric peptide and insulin-like hormone, was found to decrease cardiac fibroblast to myofibroblast transition by increasing Wnt ligands and Wnt signalling, and decreasing fibrosis, in a rat model of ageing [119]. Based on these findings, recombinant human relaxin or longer-acting analogues of relaxin [120] are currently being evaluated in various clinical trials as treatments for HFpEF [121]. Another study reported that the recombinant human relaxin reduced chronic NLRP3 inflammasome activation which promotes fibrosis through IL-1 β . Relaxin reduced myofibroblast NLRP3 inflammasome through the involvement of relaxin family peptide receptor-1(RXFP1), angiotensin II type 2 receptor (AT₂R), ATP receptor-P2X₇R, and by the inhibition of TLR-4, ROS, and caspase-1 [122]. A recent update on a clinical trial (NCT05592275) using volenrelaxin showed improvement in left atrial function at a low dose; however, the treatment with this long-acting form of human relaxin was associated with worsening congestion in patients with recently decompensated HFpEF [123].

Humanin, an endogenous mitochondria-derived peptide, has been used to reverse cardiac fibrosis and apoptosis in the ageing heart in a mouse model. Humanin reduced oxidative stress, cardiac fibroblast proliferation, decreased TGF- β 1, collagen deposition, and apoptosis of cells [124]. Cathelicidin-related antimicrobial peptide (CRAMP) is another peptide that reduced hyperglycemia-induced endothelial-to-mesenchymal transition (EndMT) in mouse heart endothelial cells and reduced fibrosis in diabetic mice by attenuating TGF- β /Smad signalling and AMP-activated protein kinase (AMPK α 1) and mechanistic Target of Rapamycin (mTOR) signalling [125]. Peptides that exhibited therapeutic benefits for cardiac fibrosis are summarized in Table 1.

Table 1. (A) Small molecule inhibitors targeting collagen crosslinking, mechanosensing pathway proteins, and epigenetic modifiers of cardiac fibrosis. Their mechanism of action and effects on cardiac fibroblasts and cardiac fibrosis are detailed based on *in vitro* and preclinical *in vivo* models of fibrosis. (B) Peptides targeting signalling pathways that activate cardiac fibroblasts and fibrosis.

SI No.	Inhibitors That Target Cardiac Fibrosis	Target	Mechanism	<i>In Vitro/In Vivo</i> Effects on Cardiac Fibrosis	Refs.
(A). Small molecule inhibitors					
1	1–155	transglutaminase 2 (TG2)	Inhibits TG2 activity and the export of TG2 to the ECM by blocking cell surface interaction with its binding partner, syndecan, and reducing its secretion, thereby decreasing collagen crosslinking by TG2	Significant reduction in infarct size, collagen deposition and in the levels of the TG2-mediated protein crosslinks in the interstitial fibrosis model and in the MI model	[76]
2	ERW1041E	tissue transglutaminase (tTG)	Inhibits enzymatically active tTG and reduces collagen crosslinking	<i>In vitro</i> , the inhibitor did not affect collagen transcription but induced MMP3 and TIMP1 synthesis in cardiac fibroblasts through non-enzymatic action. It attenuated interstitial fibrosis by reducing collagen protein levels and collagen crosslinking in a pressure-overloaded model of mice; however, it did not affect ejection fraction.	[77]
3	β -aminopropionitrile (BAPN)	lysyl oxidase (LOX)	BAPN inhibits collagen crosslinking and has secondary effects by inhibiting MMPs and TIMP	In a volume overload model in rats, BAPN attenuated collagen, MMPs, TIMP, and ventricular wall stress. It partially attenuated ventricular hypertrophy and decreased the decline in cardiac function.	[74]
4	SNT-5382	lysyl oxidase-like 2	Reduce mature collagen crosslinks	Reduced collagen volume fraction at the border zone of the infarct region in an MI model.	[75]
5	Verteporfin	Yes-associated protein 1 (YAP)	Decrease profibrotic activation of cardiac stromal cells induced by mechanical cues	Significantly reduced fibrosis and remodelling in an ischemic <i>in vivo</i> model; however, it did not improve cardiac functions.	[88]
6	Z1149421873	nucleosome assembly protein 1-like 1 (NAP1L1)	Prevents the interaction between NAP1L1 and YAP1, promotes ubiquitination and degradation of YAP1 and inhibits the AKT/mTOR signalling	Inhibited cardiac fibroblast activation and collagen deposition. Decreased the expression of α -SMA, NAP1L1, and YAP1, collagen I and collagen deposition in a mouse MI model.	[89]
7	BI 749327	transient receptor potential canonical 6 (TRPC6) antagonist	Prevents Ca ²⁺ overload, has 85- and 42-fold selectivity over related channels, TRPC3 and TRPC7 and prevent activation of nuclear factor of activated T cells (NFAT)	Inhibited TRPC6 expression of profibrotic genes and interstitial fibrosis in mice subjected to sustained pressure overload, and improved cardiac function.	[93]
8	L002 and C646	histone acetyltransferase p300 (HAT p300)	Binds to the acetyl-CoA binding pocket of the p300 catalytic domain (specifically targeting the Factor Acetyltransferase or FAT domain)	Reduced myofibroblast differentiation and fibrosis in a mouse model of hypertension.	[98]

9	S-Adenosyl-l-homocysteine (SAH)	methyltransferase-like 3 (METTL3)-methyltransferase-like 14 (METTL14)	Inhibits m6A methylation levels	Reduced cardiac fibroblast proliferation, α -SMA expression, differentiation to myofibroblasts, and expression of FGF2, Fn1, Col3a1, and Col6a3, as well as myofibroblast migration. There was a lower degree of myocardial fibrosis in SAH-treated rats compared to MI rats	[104]
10	EPZ015666	protein arginine methyltransferase 5 (PRMT5)	PRMT5 forms a protein complex with transcription factors, and its binding to promoter sites	Suppressed α -SMA expression induced by TGF- β in cultured cardiac fibroblasts. Improved fibrosis and left ventricular dysfunction in an <i>in vivo</i> mouse model of pressure overload.	[107]
11	JQ1	bromodomain-containing protein 4	Inhibits transactivation of a common pathologic gene regulatory program for NF κ B and TGF- β signalling networks regulating inflammatory and profibrotic proteins	Blocked the expression of periostin, a marker of myofibroblasts, decreased cardiac fibrosis in a pressure-overload or MI model.	[109]
12	YAK577	histone deacetylase 8 (HDAC8)	Reduce <i>HDAC8</i> mRNA levels	Reduced Col1A1 and fibronectin gene expression and MMP12 mRNA levels in an isoproterenol-induced heart failure model.	[110]
(B). Peptides therapies for cardiac fibrosis					
13	Liraglutide (Glucagon-like peptide-1 receptor agonist)	glucagon-like peptide-1 receptor	Attenuates NF κ B activity and translocation	Decreased interstitial cardiac fibrosis and expression of inflammatory and oxidative stress markers in Ang II-induced hypertension and age-induced cardiac fibrosis mouse model.	[112]
14	Cenderitide	natriuretic peptide receptors	Enhance particulate guanylyl cyclase(pGC-A)-mediated suppression of aldosterone and pGC-B-mediated collagen synthesis	Attenuated the development of cardiac fibrosis and preserved diastolic function.	[117,118]
15	Relaxin	relaxin family peptide receptor-1(RXFP1), angiotensin II type 2 receptor ATP receptor (P2X7R), Wnt signalling	Increase Wnt ligands and Wnt signalling, reduced myofibroblast NLRP3 inflammasome through RXFP1, P2X7R	Decrease cardiac fibroblast to myofibroblast transition, decreased fibrosis in a rat model of ageing, inhibit ROS and caspase-1.	[122]
16	Humanin, an endogenous mitochondria-derived peptide	Akt and glycogen synthase kinase 3 β	Attenuates fibrosis by activating the Akt/glycogen synthase kinase 3 β pathway	Reduced oxidative stress, cardiac fibroblast proliferation, TGF- β 1, collagen deposition and apoptosis of cells, reversed cardiac fibrosis and apoptosis in the ageing heart in a mouse model.	[124]
17	CRAMP	TGF β /Smad, AMPKa1 and mTOR	Attenuates TGF- β /Smad signalling, AMPKa1 and mTOR signalling	Reduced hyperglycemia-induced endothelial-to-mesenchymal transition and reduced fibrosis in diabetic mice.	[125]

7. Repurposing of Drugs for Cardiac Fibrosis

Many promising anti-fibrotic candidates that were successful in preclinical trials fail in human trials due to their toxicity, off-target effects, or other reasons. By leveraging existing pharmacokinetic and safety profiles, drug repurposing significantly truncates the bench-to-bedside timeline, offering an accelerated pathway to address the profound unmet need of treating cardiac fibrosis in heart failure. Hence, several FDA-approved drugs developed for liver, lung, and pulmonary fibrosis are also being researched for their effectiveness in cardiac fibrosis. Metformin, an FDA-approved anti-diabetic drug derived from *Galega officinalis*, has been researched for cardioprotective effects. *In vitro* studies have shown that metformin decreased the proliferation and migration of cardiac fibroblasts treated under high-glucose conditions. Metformin decreased p-Stat3 expression and upregulated mitochondrial complex I protein Grim-19 and Sirt1 expression to inhibit proliferation and migration [126]. *In vitro* and *in vivo* studies in animals have shown that metformin, via activation of the AMPK pathway, inhibits mitochondrial complex I, increases AMP concentration, and modulates energy metabolism and cardiac contraction. Metformin also inhibited proinflammatory cytokines, TGF- β , reduced cardiac fibrosis, and improved ventricular remodelling. Additionally, metformin modulated eNOS-mediated vascular relaxation [127]. Another study in diabetic db/db mice showed that a combination of exercise and metformin inhibits the TGF- β 1/Smad pathway, attenuating myocardial fibrosis. Metformin reduced NF- κ B-mediated inflammatory response to enhance the inhibition of the positive feedback loop in the TGF- β 1/Smad pathway, which in turn attenuated myofibroblast activation and ECM deposition in the diabetic mice model [128]. Based on the promising cardiovascular outcomes in preclinical studies, a Met-HeFT trial (NCT03514108) (Metformin in Patients with Chronic Heart Failure), a Danish, randomised, double-blind, placebo-controlled study (DANHEART), is examining the efficacy and safety of metformin in patients with heart failure (HFrEF) and diabetes, insulin resistance, or obesity [129]. A meta-analysis showed that metformin improved cardiovascular outcomes, including reduced all-cause mortality, lower rates of myocardial infarctions, and improved heart failure outcomes [130].

Mesalazine (5-aminosalicylic acid) is structurally similar to aspirin and is also repurposed for cardiac fibrosis. It is used to treat inflammatory bowel disease and ulcerative colitis. Mesalazine reduced proliferation, myofibroblast differentiation, and collagen deposition in the TGF- β -treated human cardiac fibroblast line. Further, it decreased cell stiffness, stress relaxation, and cell adhesion. The molecular mechanisms of antifibrotic effects of mesalazine have been identified as dual inhibition of SMAD2/3 and ERK1/2 phosphorylation [131]. Pirfenidone, an FDA-approved antifibrotic medicine for idiopathic pulmonary fibrosis, is repurposed for cardiac fibrosis. In the mouse model of pressure overload, pirfenidone reduced fibrosis via repressing TGF- β 1/Smad3 signalling. In the PIROUETTE trial, a randomised phase 2 clinical trial (NCT02932566), treatment with pirfenidone over 52 weeks in patients with heart failure with preserved ejection fraction led to a reduction in myocardial extracellular volume, a marker for cardiac fibrosis [132]. However, larger trials are required to prove the clinical benefits. Nintedanib is an FDA-approved tyrosine kinase inhibitor used for idiopathic pulmonary fibrosis and chronic fibrotic interstitial lung diseases. *In vitro*, it reduced the expression of profibrotic genes in cardiac fibroblasts and modulated hypertrophy in cardiomyocytes [133]. Nintedanib-treated mice subjected to transverse aortic constriction did not show cardiac functional decline at 4 weeks. Further, it decreased the expression of collagen Type 1A and collagen Type 3A genes at 8 weeks. Additionally, it reduced myocardial and systemic inflammation by inhibiting pro-inflammatory subsets and promoting regulatory T cells.

8. Natural Compounds

Resveratrol, a non-flavonoid polyphenol isolated from grapes, giant knotweed, peanuts, and other plants, is the most widely explored plant compound that has anti-fibrotic effects. Studies indicate it acts by

targeting multiple proteins and signalling pathways. Several *in vitro* studies in cardiac fibroblasts and *in vivo* studies in small animal models have shown that resveratrol has improved myocardial fibrosis and pulmonary fibrosis induced by isoproterenol, streptozotocin-induced diabetes, doxorubicin, and viral myocarditis [134]. Following these reports, a clinical trial (ChiCTR1800016122) investigated whether supplementation of resveratrol improved remodelling in patients with hypertension. The group treated with resveratrol had lower biomarkers indicating cardiac fibrosis, evidenced by decreased procollagen type I C-peptide and galectin-3 at 6 months. The study suggested that resveratrol could be used as an adjunct to conventional therapies for hypertensive heart disease [135].

Several products derived from natural compounds used in traditional Chinese medicine have been investigated for their antifibrotic effects. Evodiamine, a quinolone alkaloid isolated from *Evodia rutaecarpa*, a traditional Chinese medicine, attenuated TGF- β 1-induced activation of primary rat cardiac fibroblasts into myofibroblasts and activation of Smad2, Smad3, ERK1/2, and Akt, and the nuclear translocation of Smad4. The drug also prevented endothelial to mesenchymal transition *in vitro*, highlighting its ability to target other cell types that contribute to fibrosis [136]. A cardiac glycoside bufalin derived from Chinese toad venom (*ChanSu* or *Bufo gargarizans*) with known anti-cancer and anti-inflammatory properties, is reported to inhibit the proliferation of primary human cardiac fibroblasts, decrease collagen type I, α 1 protein levels *in vitro*, with significant reductions in interstitial fibrosis, perivascular fibrosis, and diastolic dysfunction in an Ang II infusion model in mice. The mechanism of action of this glycoside was shown by regulating miRNA miR-671-5p, which targets selenoprotein P1 and modulates myocardial fibrosis and stiffness [137]. Qifu yixin prescription (QYP), a traditional Chinese medicine formula used in the treatment of heart failure, was tested for its antifibrotic effects *in vitro* and *in vivo*. QYP effectively suppresses cardiac fibroblast activation by stimulating soluble guanylate cyclase activation and inhibition of ERK phosphorylation. In a mouse model of heart failure by thoracic aortic constriction, QYP reduced interstitial and perivascular fibrosis [138]. Salvianolic acid B, an active component of the Chinese drug *Salvia miltiorrhiza*, is used to treat angina pectoris, hyperlipidemia, and coronary heart disease is reported to decrease myocardial fibrosis in diabetic cardiomyopathy by decreasing collagen and regulating TGF- β 1/Smad7 expression. *In vitro* the drug inhibited cardiac fibroblast proliferation, migration, phenotypic transformation, and collagen secretion, in a high glucose-induced diabetic fibrosis model [139]. Tongxinluo, a traditional Chinese drug made from 12 distinct herbs, is found to inhibit myocardial fibrosis by inhibiting endothelial-mesenchymal transition through activating the NRG-1/ErbB- PI3K/AKT signalling cascade, in a rat reperfusion injury model [140]. The oral administration of ethanolic extract of *Boerhavia diffusa*, a leafy vegetable, which has quercetin, boeravinone B, kaempferol, and caffeic acid as bioactive components, decreased myocardial oxidative stress, increased endogenous antioxidant enzymes, by regulating the metabolism of glutathione, and decreased myocardial collagen content in an Ang II-induced MI rats [141]. Forskolin, derived from the roots of *Coleus forskohlii*, decreased the expression of fibronectin, collagen I, TGF- β , and α -SMA, reduced myocardial fibrosis, and improved cardiac diastolic function through its antioxidative stress ability [142]. Plumbagin, derived from *Plumbagin zeylanica*, partially inhibited TGF- β 1-induced myocardial fibroblast fibrosis by promoting autophagy activation and by suppressing the AKT/mTOR pathway both *in vitro* and *in vivo* in an animal MI model [143].

These *in vitro* and *in vivo* studies in small animals clearly indicate that many of these phyto compounds exhibit cardioprotective effects, including antifibrotic effects by regulating multiple pathways, molecular mechanisms, and multiple cell types of the heart, such as fibroblasts, immune cells, endothelial cells, and by autophagy. However, more in-depth investigations, toxicity analysis, and efficacy studies in large animals are required before exploration in clinical trials.

9. Chimeric Antigen Receptor (CAR)-T Cell Therapy

Chimeric antigen receptor (CAR)-T cell therapy is used to target activated cardiac fibroblasts and treat cardiac fibrosis. CAR-T cells were designed to recognize and kill cells expressing fibroblast activation protein (FAP) alpha. This approach led to a significant reduction in cardiac fibrosis and restored cardiac function after injury in mice [144]. The clinical use of CAR T cells, based on viral-based gene therapy, is limited by safety concerns, cytokine release syndrome, cardiotoxicity, and sustained transgene expression. Since FAP is required for wound healing to prevent cardiac wall rupture, sustained transgene expression of FAP in CAR-T cells might interfere with normal wound healing [145]. Later studies demonstrated that injection of lipid nanoparticles (LNPs) encoding mRNA for CAR against FAP produced functional, transient antifibrotic CAR-T cells *in vivo*, which reduced fibrosis and restored cardiac function after injury [146]. A recent study used a CD3-targeted LNP system enabling efficient mRNA delivery into T cells for *in vivo* CAR-T generation. This study has shown that FAP-CAR-T cells selectively target FAP-positive cells with high FAP expression without harming normal cardiomyocytes. *In vivo*-generated FAP-CAR-T cells significantly alleviated myocardial fibrosis and effectively improved cardiac function [147].

Another study explored the use of FAP-targeted CAR-T cells and tested their efficacy in autoimmune (EAM) and viral (CVB3) myocarditis models. Human cardiac organoids treated with interleukin-17A (IL-17A) were used to model inflammatory fibrosis. Cardiac organoids treated with FAP-CAR-T cells showed 55% less fibrosis. In EAM and CVB3 models, FAP-CAR-T cells reduced fibrosis by 65% and 55%, respectively, and restored ejection fraction [148]. In yet another study, a reactive oxygen species (ROS)-responsive hydrogel was designed for intrapericardial delivery of FAP-specific CAR-T cells to treat post-myocardial infarction fibrosis. The hydrogel selectively degrades in the ROS-enriched microenvironment, enabling the controlled release of encapsulated FAP-CAR-T cells. In murine models of MI-induced fibrosis, the hydrogel-based delivery enhanced FAP-CAR-T cell infiltration, persistence, and effector function, resulting in significant depletion of activated fibroblasts, attenuating fibrotic remodelling, and preserving cardiac structure and left ventricular function [149].

10. Conclusions

Targeting cardiac fibrosis is a critical clinical necessity, an unmet medical need to prevent progression into heart failure. However, cardiac fibrosis is a complex molecular mechanism with multi-layered crosstalk between various cell types and signalling pathways. This makes it nearly impossible for a single-target drug to prevent the progression of tissue scarring. Current research is focused on identifying specific molecular “brakes” that can be applied at three distinct stages of the scarring process: myofibroblast transformation, collagen synthesis, and collagen crosslinking. The window for targeting cardiac fibrosis is very crucial and depends on the progression of fibrosis. Too early intervention following myocardial infarction may result in cardiac wall rupture, and too late intervention often results in the formation of a mature crosslinked ECM scar that is difficult to degrade. The small-molecule inhibitors of specific protein targets and signalling pathways have shown better selectivity, minimise off-target toxicity, and improve efficiency in reversing fibrosis. Small-molecule inhibitors of epigenetic regulators can restore normal, quiescent fibroblast function rather than permanently deleting a gene or protein, providing a safer, tunable therapeutic approach. Epigenetic inhibitors act as master switches that simultaneously suppress a wide network of profibrotic and proinflammatory genes. However, the challenge with epigenetic modifiers as drug targets is the lack of selectivity for specific genes, leading to unintended activation or silencing of genes throughout the genome. Safety concerns about the long-term consequences of using these drugs, which fundamentally alter gene regulation, also need to be addressed. Drug repurposing for cardiac fibrosis is a more favourable strategy, which accelerates clinical translation by bypassing early-stage safety trials, reducing clinical trial costs, and utilising medications with already-proven pharmacological profiles. The use of advanced platforms enables

high-throughput screening for toxicity, with high-content imaging and functional analysis, allowing for the simultaneous measurement of multiple, highly relevant phenotypes. Beyond pharmacological and repurposed approaches, the FAP-targeted CAR-T cell therapy offers a strategy for selectively identifying and eliminating activated myofibroblasts and reversing cardiac fibrosis.

Statement of the Use of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this manuscript, the author(s) used Grammarly to improve grammar correction and clarity of writing. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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N.M. and S.R. performed the design concept, bibliographic search and manuscript drafting. N.M. prepared the figures and revised the draft critically for its intellectual content. Both authors have read and agreed to the final version of the manuscript.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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