



Cardiac Fibrosis: Cellular Effectors, Molecular Pathways, and Exosomal Roles

Wenyang Jiang^{1†}, Yuyan Xiong^{1†}, Xiaosong Li¹ and Yuejin Yang^{2*}

¹ State Key Laboratory of Cardiovascular Disease, National Center for Cardiovascular Diseases, Fuwai Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China, ² State Key Laboratory of Cardiovascular Disease, Department of Cardiology, National Center for Cardiovascular Diseases, Fuwai Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China

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*Correspondence:

Yuejin Yang
yangyjfw@126.com

[†]These authors have contributed
equally to this work

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Cardiac fibrosis, a common pathophysiologic process in most heart diseases, refers to an excess of extracellular matrix (ECM) deposition by cardiac fibroblasts (CFs), which can lead to cardiac dysfunction and heart failure subsequently. Not only CFs but also several other cell types including macrophages and endothelial cells participate in the process of cardiac fibrosis *via* different molecular pathways. Exosomes, ranging in 30–150 nm of size, have been confirmed to play an essential role in cellular communications by their bioactive contents, which are currently a hot area to explore pathobiology and therapeutic strategy in multiple pathophysiologic processes including cardiac fibrosis. Cardioprotective factors such as RNAs and proteins packaged in exosomes make them an excellent cell-free system to improve cardiac function without significant immune response. Emerging evidence indicates that targeting selective molecules in cell-derived exosomes could be appealing therapeutic treatments in cardiac fibrosis. In this review, we summarize the current understandings of cellular effectors, molecular pathways, and exosomal roles in cardiac fibrosis.

Keywords: cardiac fibrosis, cellular effectors, mechanisms, exosome, treatment

INTRODUCTION

Cardiac fibrosis, marked by an excess of extracellular matrix (ECM) deposition by cardiac fibroblasts (CFs), is a common pathophysiologic process in most heart diseases such as myocardial infarction (MI), hypertensive heart disease, and different types of cardiomyopathies (1, 2) and impair the heart physically and electrically. Taking acute MI (AMI) as an example, sudden massive loss of cardiomyocytes triggers an intense inflammation and causes the dead myocardium to be replaced with a collagen-based scar (3), which is critical to prevent cardiac rupture. However, prolonged or excessive fibrotic responses could remarkably lead to excessive ECM deposition, which results in hardening of myocardium, poor tissue compliance, and worsening of cardiac dysfunction. According to the location of cardiac scars and underlying cause (4, 5), cardiac fibrosis can be classified into various forms, among which reactive interstitial fibrosis and replacement fibrosis are the most relevant type of the ischemic adult heart. Being the major cell type in the adult myocardium, CFs perform a critical role in maintaining ECM protein homeostasis. The activation of CFs can lead to the transition into myofibroblasts, which is a critical step in the development of cardiac fibrosis. Besides CFs, there are various types of cells involved in the process of cardiac fibrosis *via* different pathways. It is widely known that cardiac fibrosis can provoke

chamber dilation, cardiomyocyte hypertrophy, and apoptosis and finally result in congestive heart failure (6–8). Therefore, it is essential to discover potential diagnostic or therapeutic targets for cardiac fibrosis.

Exosomes, ranging in 30–150 nm of size, play an essential role in cellular communications by their bioactive contents (9, 10). As a cell-free system, exosomes could lead to improvement in cardiac function without triggering an immune response by including cardioprotective components such as miRNAs and proteins, emerging as an appropriate candidate for cardiac fibrosis treatment. Recent research studies show that inhibiting exosome secretion or targeting specific molecules in CF-derived exosomes could be a promising therapeutic strategy in ischemic heart disease (11, 12). In this review, we demonstrate the current understandings of the cellular effectors, molecular pathways, and exosomal roles in cardiac fibrosis.

CELLULAR EFFECTORS OF CARDIAC FIBROSIS

After a myocardial injury, CFs convert to their activated form (termed as myofibroblasts) by upregulating expression of pro-inflammatory cytokines, which is defined as the key cellular event in cardiac fibrosis. Though activated myofibroblasts have been the primary effector cells in the fibrotic heart by producing ECM proteins directly, macrophages/monocytes, mast cells (MCs), lymphocytes, cardiomyocytes, and vascular cells (Figure 1) can also play vital roles in the fibrotic response *via* secretion of a variety of fibrogenic mediators such as matricellular proteins and growth factors.

Abbreviations: ECM, extracellular matrix; CF, cardiac fibroblast; AMI, acute myocardial infarction; EMT, epithelial–mesenchymal transition; MMP, matrix metalloproteinases; DDR2, discoidin domain receptor 2; FSP1, fibroblast-specific protein 1; S100a4, S100 calcium-binding protein A4; Thy1, thymus cell antigen 1; TCF21, the transcription factor 21; PDGFR α , platelet-derived growth factor receptor α ; α -SMA, α -smooth muscle actin; ED-A, extradomain A; ROS, reactive oxygen species; MC, mast cells; DAMPs, damage-associated molecular patterns; TNF, tumor necrosis factor; IL, interleukin; MCG, mast cell granule; VEGF, vascular endothelial growth factor; MSC, mesenchymal stem cell; ET, endothelin; EndMT, endothelial to mesenchymal transition; HIF, hypoxia-inducible factor; IP, interferon- γ -inducible protein; T β RII, TGF- β receptor II; IGF, insulin-like growth factor; AT2, angiotensin II type 2 receptor; PAI, plasminogen activator inhibitor; PDGF, platelet-derived growth factor; RAAS, renin–angiotensin–aldosterone system; Ang II, angiotensin II; ACE, angiotensin-converting enzyme; AT 1, angiotensin type 1; AR, adrenergic receptor; CCN2, cellular communication network factor 2; GRK2, G protein-coupled receptor kinase 2; MRTF, myocardin-related transcription factor; TSP-1, thrombospondin 1; STAT, signal transducer and activator of transcription; JAK, Janus kinase; mIL-6R, membrane-bound IL-6R; sIL-6R, soluble IL-6R; RAS, renin–angiotensin system; HIME, hypoxia-induced mitogenic factor; TN-C, Tenascin-C; EE, early endosome; LE, late endosome; ILV, intraluminal vesicle; MVB, multivesicular body; TSG101, tumor susceptibility gene 101; MHC, major histocompatibility complex; PDCD6IP, programmed cell death 6-interacting protein; HSP, heat shock protein; SORBS2, orbin and SH3 domain-containing protein 2; PDLIM5, PDZ and LIM domain; Nrf2, nuclear factor erythroid 2-related factor 2; FoxO, forkhead transcription factors of the O class; YBX-1, Y box binding protein 1; Smurf1, SMAD-specific E3 ubiquitin protein ligase 1; HDAC4, histone deacetylase 4; MKK6, mitogen-activated protein kinase kinase 6; Dyrk2, dual-specificity tyrosine phosphorylation-regulated kinase 2; PTEN, phosphatase and tensin homolog; Mecp2, methyl CpG binding protein 2; BMP2, bone morphogenetic protein 2; EGR1, early growth response factor 1; HuR, human antigen R; Ang, angiotensin.

Fibroblasts and Myofibroblasts

It is recognized that the transdifferentiation from CFs to myofibroblasts is the core cellular event in cardiac fibrosis. In order to clarify the role of CFs and myofibroblasts in cardiac fibrosis, several markers have been found to identify and distinguish CFs and myofibroblasts (13–37) (Table 1). It has been clear that most CFs derived from the epicardium, a protective epithelial layer that entirely covers the four cardiac chambers, undergoing epithelial–mesenchymal transition (EMT) (38, 39). Smaller populations are derived from the endocardium (40, 41) and cardiac neural crest (20) and are mostly found in the interventricular septum and right atrium, respectively (Figure 2). However, the origin of the myofibroblasts forming fibrotic lesions in failing hearts has been a source of debate. Most investigations in the last 10 years have revealed that activated myofibroblasts in remodeling and the infarcted hearts are primarily derived from resident CFs (20), and it is well-established that the transformation of CFs to myofibroblasts is a core cellular event involved in fibrotic response under cardiac injury. Cardiac myofibroblasts, a contractile and secretory cell type, not only contribute to the structure of ECM proteins in fibrotic hearts but also play an important role in matrix remodeling regulation through the production of proteases including the matrix metalloproteinases (MMPs) as well as their inhibitors.

So far, few factors that can independently induce CF activation have been identified. Evidence has revealed that, following mechanical stress, fibroblasts change to proto-myofibroblasts (an intermediate cell) (42). Proto-myofibroblasts contain unique cell markers including a splice variant of fibronectin called the fibronectin extradomain A (ED-A) and stress fibers (43). The cytokine TGF- β further stimulates proto-myofibroblasts, causing them to develop into the myofibroblast cell phenotype; thus, it, in turn, leads to heart failure associated with cardiac remodeling. Inflammation, MI, changes in mechanical tension, reactive oxygen species (ROS), age, and other factors can all alter the activation of CFs (Figure 3). We will discuss the specific molecular pathways contributing to CF activation in the remodeling heart in section Fibrogenic growth factors.

The Monocytes/Macrophages

According to increasing evidence, macrophages and monocytes have both been confirmed to play vital roles in the regulation of cardiac fibrosis. Macrophages and monocytes in the injured heart appear to be increasingly heterogeneous depending on their different subpopulation (44), and their phenotypic and functional flexibility allow them to perform diverse functions in fibrotic responses, such as serving as a primary source of fibrogenic growth factors and cytokines, producing matricellular proteins, and secreting matrix remodeling proteases (45). Moreover, circulating fibroblast progenitors may be implicated in the progression of cardiac fibrosis as suggested by numerous studies using bone marrow transplantation techniques (46). These hematopoietic progenitors could be monocyte subsets that can differentiate fibroblasts, comparable with the CD14+ “fibrocytes” discovered in humans (47), which imply that macrophages and monocytes in fibrotic hearts could be sources of myofibroblasts.

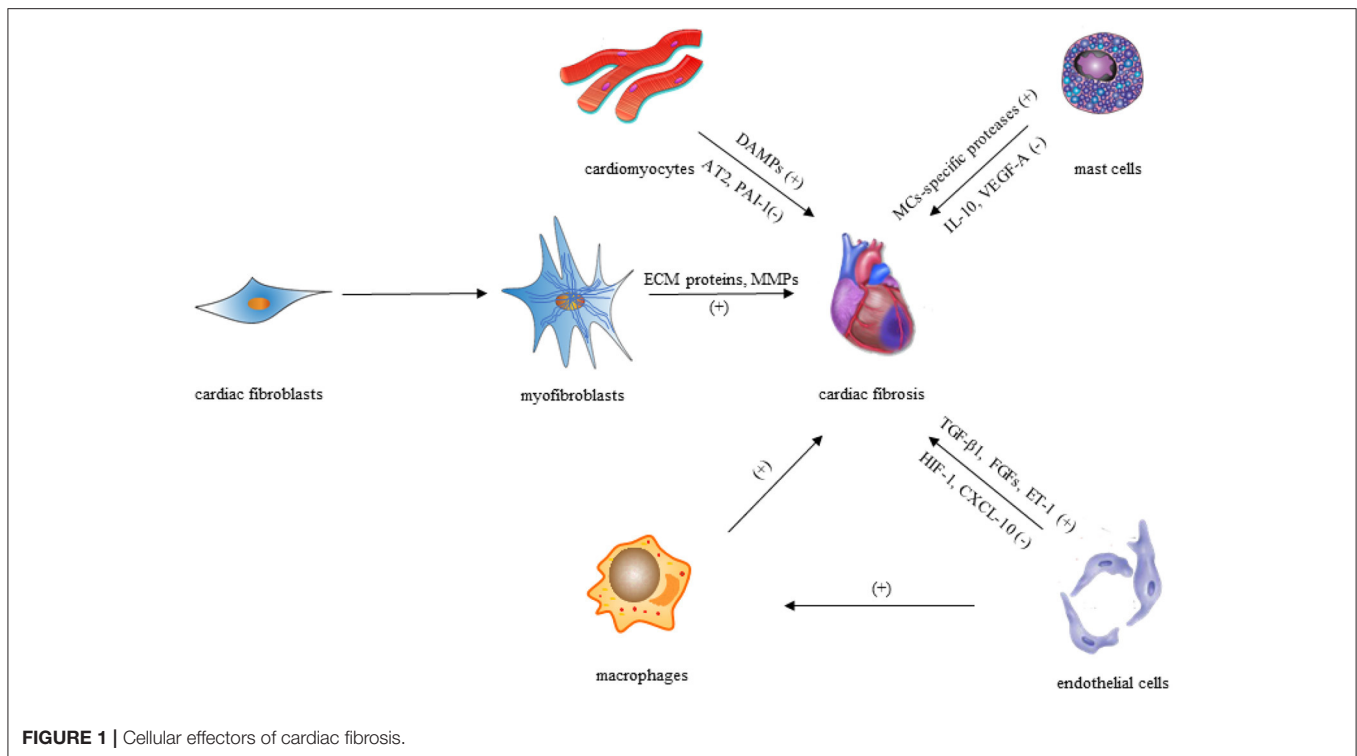


FIGURE 1 | Cellular effectors of cardiac fibrosis.

The Mast Cells

MCs are innate immune cells found almost everywhere of the body, including the heart. Resident MCs in the heart can respond to damage-associated molecular patterns (DAMPs) after injury, thus influencing the development of cardiac remodeling. However, the precise function of MCs in cardiac fibrosis is debatable, as the secretory proteins produced by MCs can be both anti- and profibrotic in nature. MC-specific proteases such as chymase and tryptase released by degranulation could induce TGF-β1 production (48–50), which plays a role in cardiac fibrosis through collagen synthesis, myofibroblast differentiation, and fibroblast stimulation (1, 51). In addition, cytokines like tumor necrosis factor (TNF) (52) and interleukin (IL)-1β (53) stored in MC granule (MCG) can also promote cardiac fibrosis through cardiomyocyte apoptosis during degranulation (54).

MCs, on the other hand, secrete anti-inflammatory mediators including IL-10 (55), which has been shown to inhibit excessive cardiac remodeling by activating STAT3 and suppressing NF-κB (56–58). Besides, MCs can produce vascular endothelial growth factor (VEGF)-A (52, 53), as one of the important anti-fibrotic mediators, which can increase capillary density in damaged tissues and promote proper repair in cardiac fibrosis (59–61). Over the past few years, various studies have been carried out for investigating the functions of MCG in fibrosis. MCG therapy of mesenchymal stem cells (MSCs) *in vitro* reduced TGF-β1-mediated transition of MSCs to myofibroblasts, while *in vivo* delivery of MCGs from rats to the myocardium during AMI lowered fibrosis and enhanced capillary density (62). These findings suggest that MCs have anti-fibrotic properties and could be used as therapeutic targets in cardiac remodeling.

The Endothelial Cells

The prevalence of perivascular fibrosis in the injured heart may indicate that endothelial cells are involved in cardiac fibrosis (63). Under pathophysiologic conditions, endothelial cells may enhance fibrotic responses *via* a variety of mechanisms after the myocardial injury. First, several profibrotic mediators produced by endothelial cells, such as FGFs, TGF-1, and endothelin (ET)-1, may play key roles in the development of cardiac fibrosis (64, 65). Second, endothelial cells may produce pro-inflammatory cytokines and chemokines, contributing to recruitment of lymphocytes and macrophages with fibrogenic actions (66). Third, although low numbers of endothelial-derived fibroblasts were detected in the remodeling myocardium, endothelial cells may undergo endothelial to mesenchymal transition (EndMT), increasing the number of fibroblasts (34).

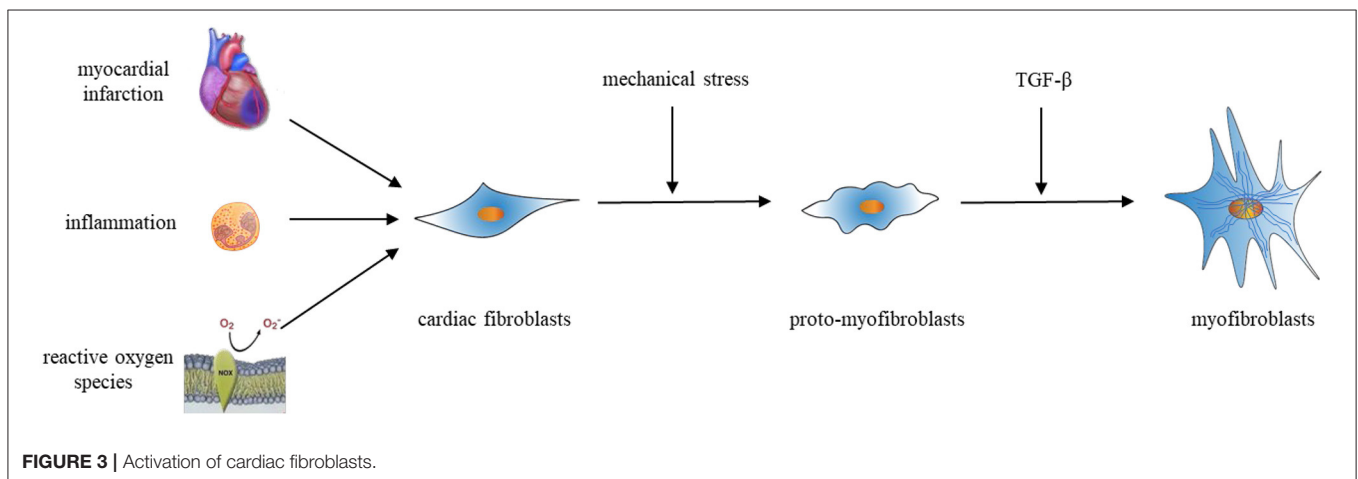
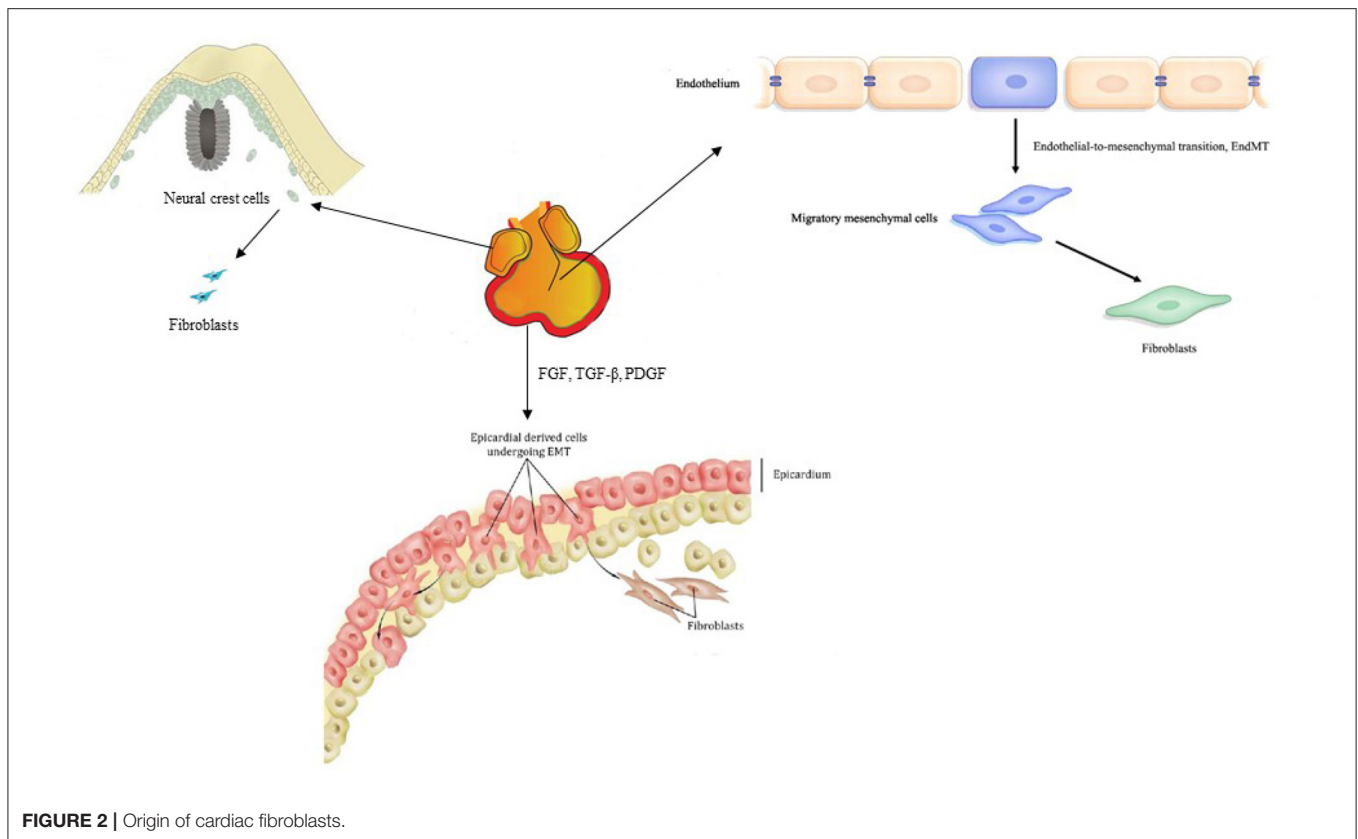
On the contrary, anti-fibrotic mediators could also be produced by endothelial cells. Endothelial cells have been found to express hypoxia-inducible factor (HIF)-1 for protecting the pressure-overloaded myocardium from fibrosis *via* suppression of TGF-β signaling partially (67). Furthermore, endothelial cells exert inhibitory actions on cardiac fibrosis by producing and secreting interferon-γ-inducible protein (IP)-10/CXCL10, a CXC chemokine that prevents the migration of CFs in the infarcted heart (68).

The Cardiomyocytes

The roles of cardiomyocytes in the process of cardiac fibrosis are two sides of the coin. For one thing, cardiomyocytes may promote interstitial fibrosis through neurohumoral and growth factor-mediated pathways, such as cardiomyocyte-specific

TABLE 1 | Summary of molecular markers used for the identification of cardiac fibroblasts and myofibroblasts.

Biomarker	Location	Function	Expression in cardiac fibroblast	Expression in cardiac myofibroblast	Expression in other cell types	References
Discoidin domain receptor 2 (DDR2)	Cell surface	Collagen-specific receptor tyrosine kinase mediating cell growth, migration, and differentiation	Yes	Yes	Epicardium	(13–18)
Vimentin	Cytoskeletal	Intermediate filaments for motility and cell shape	Yes	Yes	Endothelial cells, macrophages	(19–22)
Fibroblast-specific protein 1 (FSP1)/S100 calcium-binding protein A4 (S100a4)	Cytosolic	Calcium-binding protein for motility and tubulin polymerization	Yes	Unknown	Immune cells	(23, 24)
Thymus cell antigen 1 (Thy1, CD90)	Cell surface	Membrane glycoprotein for cell adhesion	Yes	No	Immune cells, lymphatic endothelial cells and pericytes	(25–27)
The transcription factor 21 (TCF21)	Nucleus	Regulates mesenchymal cell transitions	Yes	Yes	Epicardium	(25, 28, 29)
Platelet-derived growth factor receptor α (PDGFR α)	Cell surface	Tyrosine kinase receptor	Yes	Unknown	Platelets, epicardium	(30)
Collagen 1 α 1-GFP	Transgene	Targeting collagen I protein-producing cells	Yes	Unknown	Endothelial and vascular smooth muscle cells	(22, 32)
α -Smooth muscle actin (α -SMA)	Cytoskeletal	Intermediate filament-associated protein for cell contraction	No	Yes	Epicardium, smooth muscle cells, pericytes, and cardiomyocytes	(33, 34)
Periostin	Extracellular matrix (ECM)	Cardiac development, remodeling and ECM organization	No	Yes	Epicardium, vascular smooth muscle cells, and valve interstitial cells	(35–37)



mineralocorticoid receptor signaling (69), TGF- β receptor II (T β RII) signaling (70), and insulin-like growth factor (IGF)-1 signaling (71). Moreover, necrotic cardiomyocytes trigger an inflammatory response that finally leads to activation of fibroblasts *via* release of DAMPs, which means cardiac fibrosis may occur due to cardiomyocyte death, instead of cardiomyocyte-derived fibrogenic signals (51). For another, cardiomyocyte-specific overexpression of angiotensin II (Ang II) type 2 (AT2) receptor or the plasminogen activator inhibitor (PAI)-1 exerts anti-fibrotic actions *via* the kinin/NO system activation or inhibition of TGF- β synthesis, respectively (72, 73).

MOLECULAR PATHWAYS IN CARDIAC FIBROSIS

The complexity of interconnections and the extensive range of molecular pathways involved in the fibrotic response have restricted our understanding of the mechanism of cardiac fibrosis. High-throughput transcriptomic and genomic techniques have recently been employed to find new molecular signals and pathways linked to the fibrotic response's initiation, regression, and progression (74); and in the development of cardiac fibrosis, various molecular routes have been identified.

Most fibrotic heart diseases, regardless of cause, appear to include the aldosterone/angiotensin axis and fibrogenic growth factors such as platelet-derived growth factor (PDGF) and TGF- β . Moreover, several inflammatory signals (3, 75) such as TNF- α and IL-6 may regulate reparative and ischemic fibrosis by transducing the cascades of intracellular signaling that result in the transcription of ECM genes and translation of matrix remodeling-related proteins. Here, we demonstrate signaling pathways and mediators known to influence process of cardiac fibrosis after myocardial injury, hoping to find novel therapeutic targets or strategies.

Neurohumoral Pathways

The Renin–Angiotensin–Aldosterone System

During the progression of cardiac fibrosis, the renin–angiotensin–aldosterone system (RAAS), of which Ang II appears to be the primary effector, is persistently engaged. In fibrotic hearts, the oligopeptide Ang II, which induces vasoconstriction and high blood pressure, is raised. Angiotensin-converting enzyme (ACE) and renin, which are required for the production of Ang II, are produced by fibroblasts and macrophages invading the damaged heart (76, 77). Both *in vivo* and *in vitro* investigations suggest that Ang II is involved in TGF signaling. TGF-1 expression is induced by Ang II in fibroblasts and cardiomyocytes *via* the Ang II type 1 (AT 1) receptor, which plays a crucial role in profibrotic signaling (78–80); and *in vivo*, TGF- β is necessary for Ang II to induce both cardiac fibrosis and hypertrophy (81, 82). Besides, Ang II is also intimately involved with the inflammatory response, and in CFs, Ang II enhances their collagen-synthetic activity through extracellular signal-regulated kinase by an IL-6-dependent mechanism indeed. Another mechanism underlying the fibrotic capability of Ang II could involve miR-29b. *In vitro*, miR-29b suppression promotes Ang II-induced collagen type I and α -SMA expression, but overexpression of miR-29b inhibits it. It is indeed possible that miR-29b targets a sequence within the TGF- β 1 coding area, which explains this observation. On the contrary, AT2 signaling may inhibit AT1-mediated actions, suppressing CF proliferation and matrix synthesis, serving as a negative regulator of Ang II-mediated profibrotic responses.

Aldosterone is also capable of inducing fibrotic responses in the myocardium after cardiac injury, suggested by patients with adrenal adenomas and experimental animal studies. Several potential mechanisms have been involved in the profibrotic activities of aldosterone in the heart. First, aldosterone may have pro-inflammatory effects on vascular cells by increasing the production of cytokines like TNF- α *via* NF- κ B activation. Second, aldosterone may induce a fibrogenic phenotype in macrophages *via* the mineralocorticoid receptor. Third, aldosterone may activate cardiomyocyte-derived fibrotic signals, involving regulation of MMP-2/9 activity and the TGF- β -connective tissue growth factor profibrotic pathway. Fourth, aldosterone may exert a direct effect on CFs, stimulating proliferation and increasing collagen synthesis.

GPCR/Adrenergic Signaling

It has been reported that activation of adrenergic signaling *via* β -adrenergic receptor (AR) can induce cardiomyocyte death

and subsequent reparative fibrosis, thus leading to cardiac remodeling. Although there are several subtypes of β -AR expressed in the heart, the predominant form of β 2-AR seems to be expressed on CFs. Collagen secretion, cell proliferation, migration, and transformation to the myofibroblast phenotype can all be induced by direct activation of β 2-AR on CFs, mediated through p38 MAPK signaling partially. In addition, β -AR signaling can also regulate cytokine expression by macrophages and induce growth factor synthesis by cardiomyocytes, which plays an important role in promoting cardiac fibrosis. However, not all types of β -ARs are involved in the profibrotic responses. On the contrary, several studies have proved that, in a model of pressure overload-induced cardiac fibrosis, β 3-AR signaling in cardiomyocytes may protect the heart, due to downregulation of the matricellular protein CCN2 by cardiomyocytes.

Adrenergic stimulation causes structural changes in G protein $\beta\gamma$ subunits in the damaged myocardium, culminating in activation of G protein-coupled receptor kinase 2 (GRK2). In an experimental model of MI, GRK2 activation in CFs has been shown to have substantial fibrotic effects. Although the specific fibrotic signals activated by GRK2 remain unclear, GRK2 represents a critical target for therapeutic interventions against cardiac fibrosis.

Endothelin-1

The endothelin family of peptides was mostly known for its vasoconstriction capabilities; however, it is now being recognized for its potential role in tissue fibrosis. ET-1, one of the significant endothelin isoforms in humans, is thought to be secreted predominantly by endothelial cells but also can be produced by other cells including fibroblasts, cardiomyocytes, and macrophages. The ETA and ETB receptors, which have been found to perform opposite roles, are two recognized ET-1 receptors in the heart. At first, it was thought that these two receptors were only expressed on endothelial cells; however, the latest evidence suggests (83, 84) that they can also be expressed in other types of cells such as macrophages, cardiomyocytes, and CFs.

Both *in vitro* and *in vivo* studies suggest that ET-1 appears to be a potent fibrogenic mediator. *In vitro*, ET-1 enhances proliferation and collagen production in isolated human CFs *via* ETA receptor; *in vivo*, overexpression of ET-1 in the heart induces myocardial fibrosis associated with biventricular systolic and diastolic dysfunction. In addition to fibroblast-activating characteristics of its own, ET-1 can also act as a downstream of cytokines and neurohumoral mediators such as TGF- β and Ang II, serving as a link between fibrosis and inflammation. For example, the development of cardiac fibrosis in response to Ang II is impaired in mice with vascular endothelial cell-specific ET-1 deficiency, regulated by the myocardium-related transcription factor (MRTF)-A.

Moreover, endothelin antagonists are now approved to treat pulmonary hypertension, and many believe they will also be beneficial in the treatment of heart pathological fibrosis. Bosentan, a non-selective endothelin receptor antagonist routinely used to treat pulmonary hypertension, has also been shown to reduce fibrotic myocardium remodeling in hypertensive and reparative cardiac fibrosis animal models (85).

Despite the failure of several randomized controlled studies exploring the impact of endothelin antagonists in heart failure and coronary artery disease, manipulating ET-1 signaling appears to be promising. More researches are needed to explore whether ET-1 and its receptors may be appropriate clinically viable anti-fibrotic treatment targets.

Fibrogenic Growth Factors

TGF- β

The TGF- β family is a group of pleiotropic and multifunctional peptides activated in experimental models of cardiac fibrosis and fibrotic human hearts markedly (1, 3). TGF- β is found in three isoforms (TGF- β 1, TGF- β 2, and TGF- β 3) in mammals (86), among which TGF- β 1 acts as the predominant isoform in the cardiovascular system and expresses ubiquitously. In the injured heart, TGF- β 1, which is present in the normal heart as a latent complex, is transformed from the latent form to the active form *via* a variety of mediators. Proteases, including MMP-2, MMP-9, and plasmin, are widely acknowledged to participate in the activation of TGF- β as well as the matricellular protein thrombospondin 1 (TSP-1) (1), which plays an important role in cardiac remodeling. Upon activation, a group of studies have revealed that TGF- β was involved in the pathogenesis of cardiac fibrosis through Smad-mediated pathways where TGF- β binds to the constitutively active T β RII on the cell surface, transphosphorylates the cytoplasmic domain of the type I receptor (T β RI), and then gets connection with the Smads; or through Smad-independent pathways, in which TGF- β /TAK-1 signaling may exert profibrotic actions (1). Meanwhile, negative regulation of TGF- β signaling may be crucial in preventing cardiac fibrosis. A study conducted in a mouse model of pressure overload-induced heart failure has suggested that cleavage and release of a soluble endoglin may inhibit fibrogenic actions of TGF- β (87).

In addition, TGF- β is a critical fibrogenic mediator that may have the potential to affect all cell types involved in cardiac fibrotic response. MCGs are known to contain TGF- β in a large amount, while TGF- β -induced Smad-dependent pathways are activated by MC chymase, which results in fibrogenic effects (88). Besides, profibrotic growth factors including TGF- β can be produced and secreted in significant quantities by macrophages and monocytes. In return, TGF- β -mediated actions of these cell types may also play a paracrine role in fibrotic response. Moreover, endothelial cells may promote fibrotic cardiac remodeling through the expression of profibrotic mediators, such as TGF- β 1, FGFs, or ET-1.

What is more, TGF- β -stimulated myofibroblast transdifferentiation is induced by activation of the Smad3 signaling cascade, which promotes α -SMA transcription in fibroblasts (89) and enhances ECM protein synthesis. Furthermore, cardiomyocyte-specific T β RII knockdown significantly reduced fibrosis in the pressure-overloaded heart (70), implying that cardiomyocyte-specific TGF- β signaling is essential in the pathogenesis of fibrotic remodeling.

Platelet-Derived Growth Factor

The PDGF family includes homo- or hetero-dimeric growth factors (such as PDGF-AA, PDGF-BB, PDGF-AB, PDGF-CC,

and PDGF-DD) that signal *via* two distinct receptors: PDGFR- α and PDGFR- β (1). *In vivo*, PDGF-A and PDGF-C bind to PDGFR α , while PDGF-B and PDGF-D bind to PDGFR β in general (90). PDGF-B and PDGF-D are expressed by endothelial cells, whereas PDGFR β is expressed by vascular mural cells (pericytes and smooth muscle cells). Myocardial cells express both PDGF-A and PDGF-C, while PDGFR α -positive interstitial cells have been found in the myocardium, epicardium, and endocardium (90). With pleiotropic effects of PDGF signaling, all PDGFs have been reported to play a certain role in the development of cardiac fibrosis. Overexpression of PDGF-C (91) and PDGF-D (92) from the α -myosin heavy chain promoter (α -MHC), as well as PDGF-A (both splice variants) and PDGF-B, has been reported to generate cardiac fibrosis and hypertrophy in transgenic mice, though the degree and location of fibrosis vary between the different ligands (90). Besides, a group of studies suggested that PDGF stimulates fibroblast proliferation and differentiation to myofibroblasts *in vitro*, whereas PDGF blockade reduces interstitial fibrosis of the infarcted hearts in rats and suppresses atrial-selective canine fibroblast activation, removing the distinctive atrial-ventricular fibroblast activation differences (93). Moreover, a study implied that PDGF may also act to promote fibrosis by elevating TGF levels, for it can significantly upregulate profibrotic TGF-1 mRNA and accelerate cardiac fibrosis and arteriosclerosis when three of the isoforms, PDGF-A, PDGF-C, or PDGF-D, was introduced into the heart using adenovirus-mediated delivery (94). Similarly, PDGFR α appears to be a strong CF marker, possibly implicated in the production of CFs from epicardium, while PDGFR- β regulates the development of vascular smooth muscle cells from epicardium-derived cells (22, 24). Injection of a neutralizing PDGF receptor-specific antibody was also shown to reduce atrial fibrosis in several studies (95). These findings strongly imply that PDGF and PDGFR could be useful targets for anti-fibrotic treatment in the heart.

Inflammatory Cytokines

Tumor Necrosis Factor- α

TNF- α is a powerful pro-inflammatory cytokine that exerts pleiotropic effects on a variety of cell types and is reported to be crucial in the process of cardiac fibrosis. Transmembrane TNF- α , a precursor of the soluble TNF- α , is expressed on activated lymphocytes and macrophages as well as other cell types, exerting its biological actions by binding to type 1 and 2 TNF receptors (TNF-R1 and TNF-R2) (96), which can play different roles. Studies have shown that TNF- α in deficient mice after non-reperfused MI exacerbates cardiac remodeling, hypertrophy, NF- κ B activity, and inflammation as well as border zone fibrosis through TNF-R1, whereas it ameliorates these events through TNF-R2 (97). In addition, an increasing number of studies suggest that cardiac fibrosis is promoted by TNF- α *via* a range of mediators and the interaction with other cell types. Heart failure was accelerated in transgenic mice by cardiac-specific overexpression of TNF- α , which was associated with increased collagen synthesis, deposition, and denaturation, and dramatically elevated MMP-2 and MMP-9 activities (98). Studies have also shown that fibrotic remodeling in the TNF- α overexpressing heart is associated with increased expression

of TGF- β s and the interactions between CFs and MCs (98). Complementally, in models of heart pressure overload induced by Ang II infusion or aortic banding, it is demonstrated that global genetic deletion of TNF- α reduced interstitial and perivascular fibrosis (99).

Interleukin-6

IL-6 is a pleiotropic cytokine that has a wide range of biological functions in hematopoiesis, immunological regulation, inflammation, and cardiac fibrosis. It was first identified as a B-cell differentiation factor (100). Secreted by various types of cells, IL-6 influences a group of cell types and exerts its multiple biological activities through two different signaling pathways: classic signaling and trans-signaling. Both intracellular signaling pathways involve the signal transducer and activator of transcription (STAT) pathway and Janus kinase (JAK) pathway, though they are activated following interaction of signal transducing membrane-bound IL-6R (mIL-6R), soluble IL-6R (sIL-6R), or glycoprotein (gp130) (100, 101). Emerging evidence suggests that IL-6, as a multifunctional cytokine, has a role in cardiac fibrosis. A study using the animal model suggested that elevated production of IL-6 induced by aldosterone could further promote collagen production and cardiac hypertrophy *via* the IL-6 trans-signaling pathway (102). Similarly, increased IL-6 levels and ROS generation in rats could activate the renin-angiotensin system (RAS) and JAK1/2-STAT1/3 signaling pathways, thus ultimately leading to activation of TGF- β /Smad3 fibrotic pathway (103). Moreover, a study of neonatal rats under hypoxic conditions showed that overexpression of IL-6 was sufficient for inducing myofibroblastic proliferation, differentiation, and fibrosis, probably through improved TGF- β 1-mediated MMP-2/MMP-3 signaling (102). Furthermore, IL-6 is a downstream signal of hypoxia-induced mitogenic factor (HIMF), and it plays a key role in cardiomyocyte hypertrophy and cardiac fibrosis *via* the MAPK and CaMKII-STAT3 pathways (104). Directly, by activating CFs to secrete Tenascin-C (TN-C), ET-1, and collagen, IL-6 produced by macrophages can also cause cardiac fibrosis (105). However, different studies on the role of IL-6 in cardiac fibrosis can be conflicting. In models of left ventricular pressure overload, genetic loss of IL-6 reduced cardiac dysfunction and fibrosis, whereas another study utilizing a model of pressure overload caused by transverse aortic constriction found no effect of germline IL-6 loss on ECM protein deposition and cardiac fibrosis (98). Therefore, we conclude that IL-6 and IL-6Rs may act as therapeutic targets of cardiovascular disease in the near future (Figure 4).

ROLE OF EXOSOMES IN CARDIAC FIBROSIS

Exosome-mediated intercellular signaling, which can transfer various functional modulators including proteins, lipids, and RNA, plays an increasingly important role in cardiovascular diseases. CFs are major components of the heart, ischemia/hypertrophy activates these fibroblasts, and they are involved in cardiac fibrosis and remodeling (106). Post-cardiac

injury, fibroblast-derived miR-21-enriched exosomes can lead to cardiac myocyte hypertrophy and remodeling (11). In addition, miR-155 enriched in macrophage-derived exosomes led to enhanced proliferation and differentiation of resident fibroblasts and further exacerbated inflammation (12). Furthermore, exosomes *via* use of a targeting cardiac homing peptide or encapsulated in functional peptide hydrogels exhibit better ability in improving cardiac function and reducing fibrosis (107, 108). Besides, changes of miRNAs or proteins in exosomes derived from plasma or peripheral blood are considered as novel biomarkers for cardiac fibrosis or cardiac remodeling. Therefore, exosomes could be potential therapeutic treatments in cardiac fibrosis. Thorough knowledge of exosomes and exosome-mediated intercellular communication in cardiac fibrosis will provide better understanding to develop novel strategies for cardiac fibrosis treatments.

Exosomes: Biogenesis, Isolation, and Uptake

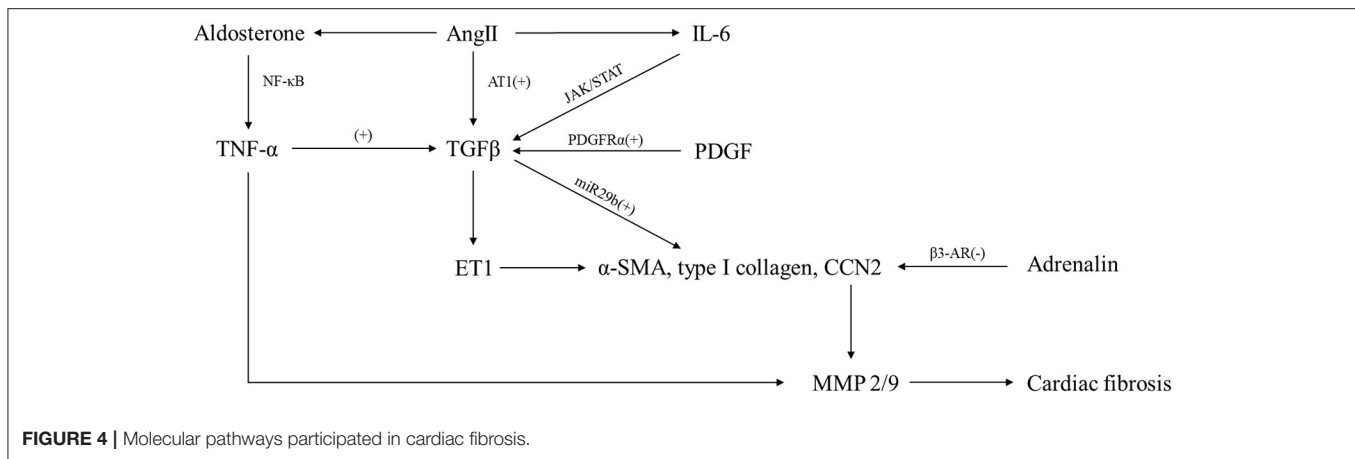
In response to different physiological states, exosomes are secreted by various cell types, such as MCs (109), macrophages, CFs, and exogenous MSCs, whose size range from 30 to 150 nm. Initially, transmembrane proteins are endocytosed and trafficked to early endosomes (EEs). EEs then mature into late endosomes (LEs) and generate intraluminal vesicles (ILVs) in the lumen of the organelles. Multivesicular bodies (MVBs), namely, LEs containing these ILVs, can fuse with plasma membrane and release exosomes into the extracellular space or fuse with lysosomes and degrade exosomes (110, 111). Different cell type- and microenvironment-derived exosomes transport distinct proteins, lipids, and nucleic acid cargoes (112, 113). Generally, exosomes are formed with tetraspanin family (CD9, CD63, and CD81) transmembrane proteins, tumor susceptibility gene 101 (TSG101), major histocompatibility complex (MHC) class II molecules, programmed cell death 6-interacting proteins (PDCD6IPs), heat shock proteins (HSPs) (HSP60, HSP70, and HSP90), cytoskeletal proteins (actin and tubulin), annexins (regulate cytoskeletal changes in membranes and membrane fusion), and membrane transport proteins (114).

Different techniques including microfiltration, gel filtration, ultracentrifugation, and commercial exosomes isolation kits are used to isolate exosomes from body fluids, plasma, or cell culture medium (115). Among these, ultracentrifugation is regarded as the gold standard for exosomes isolation and is also the most common method. Exosomes can enter recipient cells *via* distinct mechanisms including lipid membrane fusion, internalization by receptor-mediated endocytosis, receptor-mediated binding, and activation of downstream signaling (116). Total understanding of the biogenesis, isolation, and uptake of exosomes may contribute to find novel strategies for the treatment of cardiac fibrosis.

Exosome Contents for the Treatment of Cardiac Fibrosis

MicroRNAs

MiRNAs, small endogenous oligonucleotides of 21–25 nucleotides, are critical in regulating post-transcriptional



gene. Additionally, exosomes, containing different numerous miRNAs, could contribute to or alleviate a variety of pathologies including cardiac fibrosis. Exosomes, derived from distinct cell types including fibroblasts and exogenous MSCs, with upregulation or downregulation of certain miRNAs, can exhibit better ability in attenuating cardiac fibrosis and improving cardiac function (Table 2).

It has been confirmed that miR-21 played an essential role in fibroblast biology and that the levels were selectively increased in the failing heart, which makes it a target in heart failure (146). Bang et al. (11) revealed that miR-21 was enriched in fibroblast-derived exosomes, and the transfer of miR-21 to cardiomyocytes led to cellular hypertrophy. Additionally, Kang et al. demonstrated that miR-21-loaded human peripheral blood derived-exosomes enhanced fibrosis, making it a novel therapeutic target for cardiac fibrosis (137). Another research indicated that miR-27a-, miR-28a-, miR-34a-enriched fibroblast-derived exosomes could regulate cardiomyocyte antioxidant enzymes, thus contributing to cardiac hypertrophy (117). Therefore, exosomes derived from fibroblasts, especially those changing miRNAs contents, are a promising target for cardiac fibrosis.

Furthermore, exosomes derived from cardiomyocytes also exert therapeutic effects in cardiac fibrosis. Exosomes that contain high levels of miR-29b and miR-455 can downregulate MMP-9, thus reducing matrix degradation and mitigating fibrosis and myocyte uncoupling (122). MiR-378 secreted by cardiomyocytes mediated cardiac fibrosis *via* targeting the p38 MAPK-Smad2/3 signaling pathway and then regulating collagen and MMP expression in CFs (123). However, cardiomyocyte-derived miR-217- and miR-208-containing exosomes resulted in cardiac dysfunction and worsened cardiac fibrosis *via* targeting phosphatase and tensin homolog (PTEN) and dual-specificity tyrosine phosphorylation-regulated kinase 2 (Dyrk2) separately (124, 147). Evidence indicated that miR-142-3p-enriched exosomes derived from activated CD4⁺ T cells contributed to the activation of WNT signaling pathway and CF activation, making it a promising target for treating cardiac fibrosis post-MI (138).

Cell therapy, including different types of stem cells, has been widely considered as a therapeutic approach for the treatment of cardiac fibrosis. Placenta-derived MSCs decreased the expression of TGF-β and reduced fibrosis in cardiac muscles *via* transferring exosomal miR-29c (130). MiR-92a from CDC-derived exosomes can be enriched *via* the activation of β-catenin and contribute to attenuation of cardiac fibrosis and improved cardiac function (131).

Proteins

Functional proteins, as the vital contents of exosomes, also exhibit an ability in regulating cardiac remodeling and cardiac fibrosis. It is generally considered that heat shock response is a cellular intrinsic defense mechanism (148) and that the increased expression of HSPs is beneficial for cells or tissues to fight against stress stimuli and pathological conditions (149). The overexpression of HSP20 in cardiomyocytes contributes to the secretion of exosomes *via* interaction with TSG101 and leads to the elevation of HSP20 in exosomes, which remarkably improved cardiac function and attenuated adverse remodeling (139). However, myocyte-derived HSP90 exerted a profibrotic role through orchestrating the synthesis of IL-6 and activating STAT-3 in fibroblasts, leading to excess collagen secretion and deposition, thus exaggerating cardiac hypertrophy and fibrosis (140). Emerging evidence indicated that proteins of WNT family are involved in the activation of cardiac fibrotic pathologies (150–152). Działo et al. confirmed that WNT3a-rich exosomes could specifically activate WNT/β-catenin signaling pathway and promoted fibrogenesis in post-infarcted hearts, whereas WNT5a-rich exosomes only activated non-canonical WNT pathways and induced production of profibrotic IL-6 (145). Summarizing, exosomes containing WNT proteins can regulate cardiac fibrosis *via* canonical and non-canonical WNT pathways and provide a novel strategy to treat cardiac fibrosis. The upregulated decorin and downregulated periostin in cardiomyocyte-derived exosomes had been confirmed to regulate cardiac fibrosis through targeting Ang II. Additionally, upregulated human antigen R (HuR) in macrophages significantly increased inflammatory and profibrogenic responses in fibroblast and

TABLE 2 | MiRNAs and proteins involved in exosomes for the treatment of cardiac fibrosis.

Name	Level	Derivation	Disease	Target gene/pathway	Effects	References
MiRNAs						
MiR-21-3p	Downregulation	Cardiac fibroblasts	Heart failure (HF)	Orbin and SH3 domain-containing protein 2 (SORBS2) PDZ and LIM domain 5 (PDLIM5)	Cardiac hypertrophy↓	(11)
MiR-27a, MiR-28-3p, MiR-34a	Upregulation	Cardiac fibroblasts	HF	Nuclear factor erythroid 2-related factor 2 (Nrf2)	Oxidative stress↑ Cardiac remodeling↑	(117)
MiR-155	Upregulation	Macrophages	Uremic cardiomyopathy	Forkhead transcription factors of the O class (FoxO3a)	Cardiomyocyte pyroptosis↑ Cardiac hypertrophy and fibrosis↑	(118)
MiR-19a-3p	Upregulation	Endothelial cells	MI	MiR-19a-3p/Thrombospondin 1	Vascularization↑ Myocardial fibrosis↓ Left ventricular ejection fraction↑	(119)
MiR-133	Upregulation	Endothelial cells	Myocardial fibrosis	Y box binding protein 1 (YBX-1)	Angiogenesis↑ mesenchymal-endothelial transition of cardiac fibroblast↑	(120)
MiR-10b-5p	Upregulation	Endothelial cells	MI	SMAD-specific E3 ubiquitin protein ligase 1 (Smurf1) Histone deacetylase 4 (HDAC4)	Cardiac fibroblast activation↓	(121)
MiR-29b, MiR-455	Upregulation	Cardiomyocytes	Diabetes	Matrix metalloproteinase 9 (MMP-9)	Fibrosis and myocyte uncoupling↓	(122)
MiR-378	Upregulation	Cardiomyocytes	Myocardial fibrosis	Mitogen-activated protein kinase kinase 6 (MKK6)/P38 MAPK pathway	Fibrosis↓	(123)
MiR-208a	Upregulation	Cardiomyocytes	Cardiac fibrosis	Dual-specificity tyrosine phosphorylation-regulated kinase 2 (Dyrk2)	Cardiac fibroblast↑ Myofibroblast differentiation↑ Cardiac fibrosis↑	(124)
MiR-19a	Upregulation	Mesenchymal stem cells (MSCs)	MI	Phosphatase and tensin homolog (PTEN)/Akt pathway	Infarct size↓ Fibrosis↓ Cardiac function↑	(125)
MiR-210	Upregulation	MSCs	MI	MiR-210/hypoxia-inducible factor-1 α (HIF-1 α)	Fibrosis↓ Angiogenesis↑ Apoptosis↓	(126)
MiR-22	Upregulation	MSCs	MI	Methyl CpG binding protein 2 (MeCP2)	Cardiac fibrosis↓ Anti-apoptosis↑	(127)
MiR-24	Upregulation	Human umbilical MSCs	MI	MiR-24/Bim pathway	Cardiac fibrosis↓ Cardiac function↑	(128)
MiR-26a	Upregulation	Satellite cells	Uremic cardiomyopathy	FBXO32/atrogin-1 TRIM63/MuRF1	Cardiac fibrosis lesions↓	(129)
MiR-29c	Upregulation	Placenta-derived MSCs	Duchenne muscular dystrophy	TGF- β	Fibrosis in the diaphragm and cardiac muscles↓ Inflammation↓ Utrophin↑	(130)
MiR-92a	Upregulation	Cardiosphere-derived cells (CDCs)	MI	Bone morphogenetic protein 2 (BMP2)	Contractility↑ Fibrosis↓	(131)

(Continued)

TABLE 2 | Continued

Name	Level	Derivation	Disease	Target gene/pathway	Effects	References
MiR-126	Upregulation	Adipose-derived stem cells (ADSCs)	MI	–	Cardiac fibrosis↓ Inflammation↓ Apoptosis↓ Angiogenesis↑	(132)
MiR-133a	Upregulation	Cardiac progenitor cells (CPCs)	MI	Bim Bmf bFgf Vegf	Apoptosis↓ Fibrosis↓ Hypertrophy↓	(133)
MiR-146a-5p	Upregulation	CPCs	Doxorubicin/ trastuzumab- induced cardiac toxicity	Traf6 Smad4 Irak1 Nox4 Mpo	Myocardial fibrosis↓ CD68+ inflammatory cell infiltrates↓ Inducible nitric oxide synthase expression↓ Left ventricular dysfunction↓	(134)
MiR-146a	Upregulation	ADSCs	MI	Early growth response factor 1 (EGR1)/TLR4/NFκB	Apoptosis↓ Inflammatory response↓ Fibrosis↓	(135)
MiR-425, MiR-744	Downregulation	Plasma	HF	TGF-β1	Collagen formation↑ Fibrogenesis↑	(136)
MiR-21	Upregulation	Human peripheral blood	MI	Smad7 PTEN MMP-2	Fibrosis↑	(137)
MiR-142-3p	Upregulation	CD4+ T cells	MI	WNT pathway	Cardiac fibrosis↑ Dysfunction↑	(138)
Proteins						
HSP20	Upregulation	Cardiomyocytes	Diabetic cardiomyopathy	Phosphorylated Akt Survivin SOD1	Cell death↓ Cardiac adverse remodeling↓	(139)
HSP90	Downregulation	Cardiomyocytes	Cardiac hypertrophy	STAT3	Collagen synthesis↓	(140)
Decorin Periostin	Upregulation Downregulation	Cardiomyocytes	Cardiac fibrosis	Ang II	Transformation into myofibroblast↓ Fibroblast migration↓	(141)
HSP70	Upregulation	Serum	Aging-related cardiac fibrosis	–	Fibroblast proliferation↓ Myofibroblast differentiation↓	(142)
Lamp2b Ischemic myocardium- targeting peptide CSTSMLKAC (IMTP)	Upregulation	MSCs	MI	–	Inflammation↓ Apoptosis↓ Fibrosis↓ Vasculogenesis↑ Cardiac function↑	(143)
Human antigen R (HuR)	Upregulation	Macrophages	Cardiac fibrosis	Ang II	Inflammatory and profibrogenic responses↑ Cardiac fibrosis↑	(144)
WNT3a WNT5a	Overexpression	Cardiac fibroblasts	Cardiac fibrosis	WNT pathways	Cardiac fibroblast activated↑ Cardiac fibrosis↑	(145)

↑ means the corresponding miRNA or protein has a positive effect on the process, or could increase the number/area of the subject; ↓ means the corresponding miRNA or protein has a negative effect on the process, or could decrease the number/area of the subject.

cardiac fibrosis, suggesting that HuR might be targeted to alleviate macrophage dysfunction and pathological fibrosis (144).

Exosomes Act as Biomarkers in Cardiac Fibrosis

Recently, researches have been devoted to using miRNAs or other molecules in serum or plasma as diagnostic or prognostic biomarkers in cardiovascular diseases. Exosomes, as the carrier of those molecular constituents, are highly associated with concurrent physiological or pathological condition. It has been shown that the level of plasma exosomal miR-425 and miR-744 was decreased while the level of miR-21 was increased during the development of heart failure, which makes them novel biomarkers for heart failure and represent the conditions of the CF (136). In addition, surface HSP70 expression in serum exosomes was obviously decreased during senescence in the model of cardiac fibrosis, while HSP70 overexpression attenuated these effects, making it a new biomarker in aging-related cardiac fibrosis (142). Therefore, exosomes may act as a promising diagnostic biomarker in cardiac fibrosis.

CONCLUSION

Cardiac fibrosis, a common pathophysiologic event in most heart disease, can lead to poor tissue compliance, hardening of myocardium, and worsening of cardiac dysfunction. CFs, a major cell type of adult myocardium, play a vital role in the process of cardiac fibrosis. MCs, macrophages/monocytes, endothelial

cells, and cardiomyocytes, in addition to CFs, also have a role in the fibrotic response through fibrogenic growth factors, the aldosterone/angiotensin axis, or inflammatory signals. Thus, cardiac fibrosis is a complex process involving multiple cells and regulated by multiple molecular pathways. Based on this, exosomes derived from various cell types are rich in a variety of miRNAs and proteins and could participate in intercellular communication to mediate cardiac fibrosis process, thus providing a novel strategy for the prediction and treatment of cardiac fibrosis.

AUTHOR CONTRIBUTIONS

WJ and YX determined the topic, wrote the initial draft, and revised the manuscript according to the reviewers' comments. XL searched the related literatures and supplemented the content of the manuscript. YY supervised the planning and execution of the research activity. All authors have given approval to the final version of the manuscript, responsible for the accuracy, and authenticity of the article.

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