

Non-coding RNAs in cardiac fibrosis: Emerging biomarkers and therapeutic targets

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Abstract

Non-coding RNAs (ncRNAs) are a class of RNA molecules that do not encode proteins. ncRNAs are involved in cell proliferation, apoptosis, differentiation, metabolism, and other physiological processes as well as the pathogenesis of diseases. Cardiac fibrosis is increasingly recognized as a common final pathway in advanced heart diseases. Many studies have shown that the occurrence and development of cardiac fibrosis is closely related to the regulation of ncRNAs. This review will highlight recent updates regarding the involvement of ncRNAs in cardiac fibrosis, and their potential as emerging biomarkers and therapeutic targets. (Cardiol J 2018; 25, 6: 732–741)

Key words: cardiac fibrosis, ncRNAs; biomarkers, therapeutic targets

Introduction

Cardiac fibrosis is a histopathologic hallmark of a variety of advanced heart diseases, and is an important event in the cardiac remodeling process, characterized by a disproportionate accumulation of extracellular matrix (ECM) components. Progressive fibrotic changes can be triggered by varied pathophysiological conditions, such as myocardial infarction (MI), pressure overload (e.g. hypertension and aortic stenosis [AS]), hypertrophic cardiomyopathy (HCM), post-viral dilated cardiomyopathy (DCM), toxic insults (e.g. alcohol or anthracyclines) [1], and metabolic disturbances (e.g. diabetes and obesity) [2, 3]. Although it initially serves as an adaptive remodeling, a persistent fibrotic response will eventually result in ventricular dysfunction and heart failure. In addition, cardiac fibrosis is a proposed substrate for sudden cardiac death (SCD) and tachyarrhythmias [4, 5].

Cellular effectors and molecular pathways that are implicated in the pathogenesis of cardiac fibrosis and the transformation of cardiac fibro-

blasts (CFs) to myofibroblasts play a critical role in the development of fibrosis [6]. These molecules include inflammatory cytokines and chemokines, transforming growth factor- β (TGF- β), matricellular proteins, and the renin–angiotensin–aldosterone system. Experimental and clinical evidence suggests that cardiac fibrotic alterations may be reversible [7]. Understanding the mechanisms responsible for the initiation, progression, and resolution of cardiac fibrosis is crucial for enabling the design of anti-fibrotic treatment strategies for patients with heart disease. Although the clinical use of angiotensin converting enzyme inhibitors (ACEI) and angiotensin II receptor antagonists can partially reverse this remodeling, currently, there are no licensed treatments for cardiac fibrosis. The lack of an effective therapy for cardiac fibrosis is critically responsible for its serious adverse effects. Thus, novel anti-fibrotic strategies are urgently needed.

Non-coding RNAs (ncRNAs) are a class of RNA molecules that do not encode proteins, and function directly at the RNA level. Although once thought

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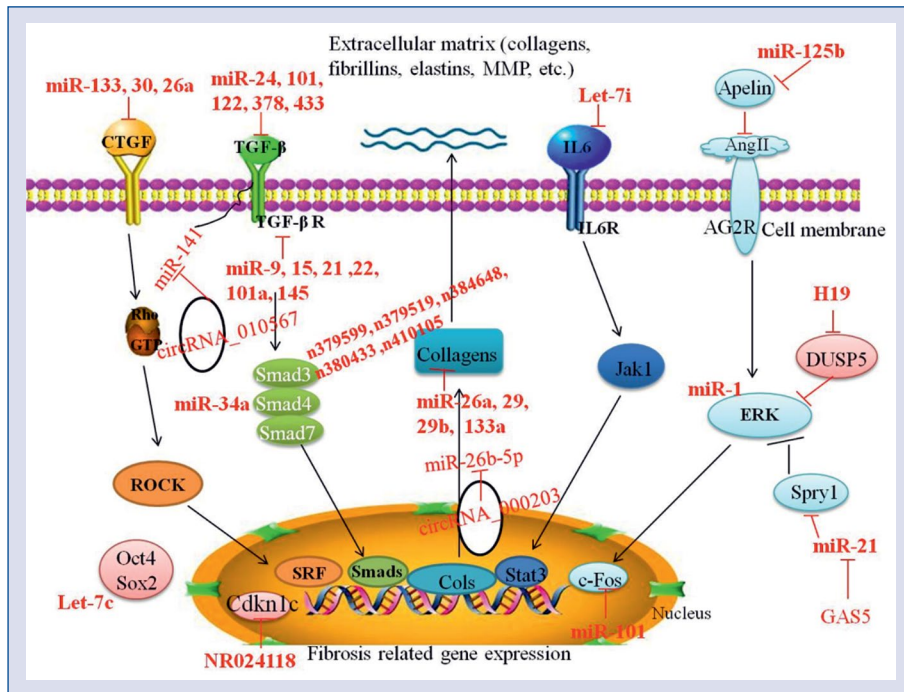


Figure 1. ncRNAs involved in the cardiac fibrosis pathways; ncRNAs regulate cardiac fibrosis processes by targeting the key molecules that mediate transcription of extracellular matrix (ECM) genes and transforming growth factor- β (TGF- β) signaling; CTGF — connective tissue growth factor; Rho-GTP — Rho-GTPase-activating protein; ROCK — Rho associated coiled-coil containing protein kinase; SRF — serum response factor; MMP — matrix metalloproteinases; IL6 — interleukin-6; Jak1 — Janus kinase 1; Stat3 — signal transducer and activator of transcription 3; c-Fos — FBJ murine osteosarcoma viral oncogene homolog; Spry1 — sprouty homolog 1; ERK — extracellular signal-regulated kinases; DUSP5 — dual-specificity phosphatase 5.

to be genomic ‘junk’, ncRNAs are attracting more and more attention in physiological and pathological research fields due to their novel roles in transcription, RNA processing and translation [8]. ncRNAs are divided broadly into two categories based on their length: short or small ncRNAs (< 200 nucleotides, including microRNAs [miRs]), and long ncRNAs (lncRNAs, > 200 nucleotides). Circular RNAs (circRNAs) are another class of ncRNAs, and are known for their closed ring structure. ncRNAs can either work alone, or interact with other ncRNAs in mechanisms such as the competitive endogenous RNA (ceRNA) mechanism (as molecular sponges for miRs) [9, 10]. Recognition of the roles played by ncRNAs in human disease has unveiled new mechanistic understanding, and will lead to novel diagnostic and therapeutic approaches. Recently, research on the role of ncRNAs in cardiovascular disease, including cardiac fibrosis, has developed rapidly. In this review, the latest research progress highlighting the molecular mechanism played by ncRNAs in cardiac fibrosis and also the aim to identify novel diagnostic biomarkers and therapeutic strategies will be summarized.

ncRNAs in the pathogenesis of cardiac fibrosis

In normal heart tissue the ECM components, primarily fibrillar collagen (mainly type I collagen) combined with other molecules (e.g. elastin, glycoproteins and proteoglycans), constitute the laminar scaffolds surrounding cardiomyocytes. This interstitial matrix is solid and flexible, holding the cardiomyocyte layers together, and is important for transmission of the contractile force. CFs play an important role in maintaining the integrity of the matrix network. However, homeostasis between the synthesis and degradation of ECM components is disrupted under pathologic conditions with trans-differentiation and activation of myofibroblasts, which are capable of increased ECM secretory activity [11]. Over-accumulation of ECM leads to reparative or reactive fibrosis. Emerging evidence suggests that the abnormal expression of ncRNAs is linked to the physiological processes of CF differentiation, proliferation, and induction of fibrosis. Herein is summarized the role of ncRNAs in cardiac fibrosis (Fig. 1).

miRs in cardiac fibrosis

miRs are a class of endogenous single-stranded ncRNAs of 18 to 24 nucleotides in length, and are the most widely studied ncRNAs. miRs regulate gene expression at the post-transcriptional level by targeting the 3'-untranslated region of mRNA sequences, thus controlling a variety of cellular processes essential to human health and disease [12]. In the heart, miRs play a crucial role in the mechanism of cardiac remodeling, such as cardiac hypertrophy, fibrosis, and apoptosis. There has been remarkable progress in methods for the modulation of miR expression (e.g. miR-mimics, antagomiRs, and miR sponges) both *in vitro* and *in vivo* [13], and the understanding of pathways and molecules regulating cardiac fibrosis has likewise improved significantly.

Many miR molecules are involved in the regulation of cardiac fibrosis. miR-21 is one of the most studied anti-cardiac fibrosis factors. Early work from Thum et al. [14] demonstrated that miR-21 promotes cardiac fibrosis by targeting extracellular regulated kinase inhibitor sprouty homolog 1 (Spry1) with stimulation of mitogen-activated protein kinase (MAPK) signaling in CFs. In cardiac fibrosis related to angiotensin II, miR-21 is transcriptionally activated and targets phosphatase and tensin homologue (PTEN) resulting in increased fibroblast survival. Osteopontin knockout mice are protected from miR-21 increase and fibrosis development due to impaired activation of transcription factor activator protein 1 (AP-1) and fibroblast [15]. A recent study performed by Gupta et al. [16] revealed that miR-21 together with cardiac fibrosis was increased in cardiac allografts compared with isografts. Conversely, both genetic and pharmacological inhibition of miR-21 successfully reduced fibrosis and fibrocyte accumulation in cardiac allografts. Further mechanistic study found that overexpression of miR-21 in monocyte cell line activated a fibrotic gene programme and promoted monocyte-to-fibrocyte transition together with activation of monocyte chemoattractant protein 1 via the PTEN/AP-1 regulatory axis. Thus, inhibition of miR-21 may be a novel strategy to target fibrosis development in cardiac allografts. Moreover, miR-21 was also reported play a role in the inhibition process of interleukin-10 mediated bone marrow fibroblast progenitor cells homing and transdifferentiation to myofibroblasts in pressure-overloaded myocardium. Verma et al. [17] demonstrated that restoration of miR-21 levels suppressed the interleukin-10 effects on TGF- β -induced fibrotic

signaling in bone marrow fibroblast progenitor cells and thus modulates cardiac fibrosis.

Another prominent miR that control cardiac fibrosis is miR-29. Van Rooij et al. [18] found that the level of miR-29 was significantly reduced under stress, increasing the synthesis of its targets, including collagen, elastin, fibrin, and other extracellular matrix proteins, thereby promoting cardiac fibrosis. Conversely, over-expression of miR-29 can inhibit the synthesis of collagen and further reduce myocardial fibrosis. Additionally, miRs are also involved in age-associated cardiac fibrosis. Boon et al. [19] show that miR-34a is induced in the ageing heart and that *in vivo* silencing or genetic deletion of miR-34a reduces age-associated cardiomyocyte cell death. Moreover, miR-34a inhibition reduces cell death and fibrosis following acute MI and improves recovery of myocardial function. Mechanistically, they identified PNUTS (also known as PPP1R10) as a novel direct miR-34a target, which reduces telomere shortening, DNA damage responses and cardiomyocyte apoptosis, and improves functional recovery after acute MI. Moreover, miR-34a was also involved in the regulation of post-ischemic cardiac fibrosis [20]. miR-34a was upregulated in the MI heart. *In vivo*, inhibition of miR-34a reduces the severity of experimental cardiac fibrosis in mice. TGF- β 1 increased miR-34a expression in CFs. Overexpressing miR-34a levels increased the profibrogenic activity of TGF- β 1 in CF, whereas inhibition miR-34a levels weakened the activity via targeting Smad4. Another study performed by Jazbutyte et al. [21] demonstrated that miR-22 also prominently upregulated during cardiac aging. Functionally, miR-22 overexpression induced cellular senescence and promoted migratory activity of CFs. Small interference RNA-mediated silencing of mimecan in CFs mimicked the miR-22-mediated effects. Rescue experiments revealed that the effects of miR-22 on CFs were only partially mediated by mimecan. However, Hong et al. [22] revealed that miR-22 was a negative regulator of fibrogenesis. In their study, miR-22 was dynamically downregulated following MI induced by permanent ligation of the left anterior descending coronary artery for 7 days, an effect paralleled by significant collagen deposition. Inhibition of miR-22 resulted in increased expression of Col1 α 1, Col3 α 1 and fibrogenesis in cultured CFs. Conversely, overexpression of miR-22 in cultured CFs significantly abrogated angiotensin II-induced collagen formation and fibrogenesis. Furthermore, they found that TGF β RI is a direct target for miR-22, and downregulation of TGF β R may have

mediated the antifibrotic effect of miR-22. Since converse effects existing, more studies are needed to validate the role of miR-22 in cardiac fibrosis.

Recently, Lichan Tao et al. [23] revealed that miR-433 plays a crucial role in the regulation of cardiac fibrosis and is a potential target for ameliorating cardiac fibrosis. According to their investigation, miR-433 levels were increased in the heart tissues of myocardium with fibrosis in DCM or MI, and miR-433 inhibition exhibited a cardioprotective effect by targeting the AZIN1 and JNK1 genes through the TGF- β 1, ERK, and p38 kinase pathways. Interestingly, miRs can also regulate cardiac fibrotic process via preserving lipid raft cholesterol. Nishiga et al. [24] revealed that decreased miR-33, a well-studied miR in atherosclerosis, was associated with worsened cardiac function in patients with DCM. Subsequently, in transverse aortic constriction mice model, cardiac fibrosis was ameliorated in miR-33-deficient hearts compared with wild-type hearts, despite no difference in hypertrophic responses. Moreover, they also found that CFs were mainly responsible for miR-33 expression in the heart. Deficiency of miR-33 impaired CF proliferation, which was considered to be caused by altered lipid raft cholesterol content. As systemic miR-33-deficient mice, CF-specific miR-33-deficient mice also showed decreased cardiac fibrosis induced by transverse aortic constriction. This study provides a novel angle of miR regulation in cardiac fibrosis. Other miRs, such as miR-15, miR-30, miR-1, miR-378, and miR-133, that are involved in the pathogenesis of cardiac fibrosis are summarized in Table 1.

Overall, cardiac fibrosis is a complex process involving the coordinative interaction of numerous miRs. Particularly, as summarized in Table 1, many miRs were involved in the same pathologically fibrotic process, such as Let-7c, miR-21, miR-24, miR-29, miR-34a, miR-101, miR-101a, and miR-433 for post-infarction fibrosis, and miR-15, miR-21, miR-26a, miR-125b, and miR-378 for pressure-overload fibrosis induced by transverse aortic constriction. However, the interactions among these miRs, the effects of altering one of the miRs on the unintended targets and pathological processes of the disease are not well-studied. In addition, not only CFs, other cell types, such as macrophages, lymphocytes, mast cells, vascular endothelial cells, and cardiomyocytes, also participate in the process of fibrogenesis. Fibroblast-enriched miRs (e.g. miR-15, and miR-30), can directly affect the fibrotic process, while non-fibroblast enriched miRs (e.g. miR-1,

miR-378, and miR-133) may regulate it by paracrine mechanism. Thus, the mechanism for the involvement of miRs in the paracrine regulation of different cells during fibrogenesis needs more meticulous research. A specific delivery system or virus vehicle also lacks a design for selective manipulation of miRs in cardiac myocytes and nonmyocytes.

lncRNAs in cardiac fibrosis

lncRNAs, a class of transcripts larger than 200 nucleotides, have a more heterogeneous distribution in the genome, with nested and overlapping, sense and antisense transcripts. They had an mRNA-like structure, as they can also incorporate exons, have a 3' poly-A tail and CpG islands, and display alternate splicing [25]. However, lncRNAs generally do not encode proteins, share less sequence conservation, and show lower expression levels. They participate in diverse biological processes, including histone modification, transcript regulation, mRNA fragmentation, endo-sponge activity, and direct protein interaction, and play important roles in various diseases. Recently, there have been significant advances in the identification of lncRNAs involved in cardiac fibrosis (Table 2).

Tao et al. [26] revealed that overexpression of lncRNA H19, a highly abundant and conserved imprinted gene, increased the proliferation of CFs and the resulting cardiac fibrosis by inhibiting dual-specificity phosphatase 5 (DUSP5), whereas H19 silencing induced the opposite effect. Further mechanistic studies unveiled that the lncRNA H19 promotes CF proliferation and fibrosis partly through the repression of DUSP5 and the activation of p-ERK1/2, a well-known pro-proliferative and pro-fibrotic signaling pathway [26]. The same group also recently investigated the role and function of lncRNA growth arrest-specific 5 (GAS5) in cardiac fibrosis, and demonstrated that GAS5 plays a suppressive role in cardiac fibrosis via negative regulation of miR-21 [27]. Furthermore, they found that the modulation of miR-21 regulated the expression of matrix metalloproteinase-2 (MMP-2) via a phosphatase and tensin homologue (PTEN) pathway in CFs.

Another study demonstrated the potential role of NR024118 in CF showed that angiotensin II dynamically regulated the expression of lncRNA-NR024118 and Cdkn1c in CFs [28]. Huang et al. [29] observed an altered lncRNAs expression in ischemic cardiomyopathy and revealed that mouse CF-enriched lncRNAs n379599, n379519, n384648, n380433 and n410105 regulate the fibrosis related genes expression via targeting TGF- β signaling

Table 1. miRs involved in the regulation of cardiac fibrosis.

miRs name	Modulation	Targets/signaling pathway	Pathological factors/species	Effects	Ref.
Let-7c	Up	Activate Oct4 and Sox2	MI/mouse; NRCFs	Pro-	[34]
Let-7i	Down	Suppress interleukin-6 and collagens	Ang II/mouse; NRCFs	Anti-	[35]
miR-1	Down	Activate Fibullin-2/MAPK	AAB/rat	Anti-	[36]
miR-9	Down	Suppress TGFβ receptor II	High glucose/human CFs	Anti-	[37]
miR-15	Up	Suppress TGFβ receptor I, p38, endoglin, Smad3/7	TAC/mouse	Anti-	[38]
miR-21	Up	Activate sprouty homologue 1/ERK-MAP kinase	TAC/mouse	Pro-	[14]
		Suppress TGFβ receptor III/ /p-Smad3	MI/mouse		[39]
		Activate osteopontin/PTEN and Smad7	Ang II/mouse		[15]
miR-22	Up	Activate PTEN/AP-1	Allografts/mice; monocyte		[16]
		Suppress mimecan (osteoglycin)	Aging/mouse; NRCFs	Pro-	[21]
miR-24	Down	Suppress TGFβ receptor I	Ang II/mouse	Anti-	[22]
		Suppress Furin/TGF-β	MI/mouse	Anti-	[40]
miR-26a	Down	Suppress collagen I and CTGF	Ang II/NRCFs TAC/lkBa tg mouse	Anti-	[41]
miR-29	Down	Activate collagens, fibrillins, and elastin	MI/mouse and human	Anti-	[18]
miR-29b	Down	Suppress multiple collagens, MMP, IGF-1, LIF, and PTX-3	Mouse CFs		[42]
miR-30, 133	Down	Activate CTGF	Renin-2 tg rat	Anti-	[43]
miR-34a	Up	Suppress PNUMS	Aging, MI/mice, human	Pro-	[19]
		Activate Smad4	MI/mouse		[20]
miR-101	Down	Suppress c-Fos/TGF-β1	Ang II, MI/rat	Anti-	[44]
miR-101a	Down	Suppress TGFβ receptor I, p-Smad3	MI, hypoxia/rat	Anti-	[45]
miR-122	Down	Activate TGF-β1	AS/human	Anti-	[46]
miR-125b	Up	Suppress Apelin, p53	TAC, AngII/mouse;	Pro-	[47]
			human CFs		
miR-133a	Down	Suppress Snai1	Mouse embryonic fibroblasts	Anti-	[48]
		Suppress Col1A1	Ang II/rat		[49]
miR-145	Up	Suppress TGFβ receptor II	Smooth muscle cells; Ang II/mouse	Anti-	[50]
miR-208a	Up	Activate endoglin	Aorta-caval shunt/rat	Pro-	[51]
miR-378	Down	Activate RTK, GRB-2/TGFβ	AngII, TAC/mouse; NRCFs	Anti-	[52]
miR-433	Up	Suppress AZIN1 and JNK1/ /TGF-β1, ERK, p38 kinase, and Smad3	MI/mice; NRCFs	Pro-	[23]

NRCFs — neonatal rat cardiac fibroblasts; Ang II — angiotensin II; Col — collagen; ERK — extracellular signal-regulated kinases; MAPK — mitogen-activated protein kinase; LIF — leukemia inhibitory factor; IGF-1 — insulin-like growth factor 1; PTX-3 — pentraxin 3; c-Fos — FBJ murine osteosarcoma viral oncogene homolog; MI — myocardial fibrosis; TAC — transverse aortic constriction; AAB — ascending aortic banding; TGF-β — transforming growth factor-β; MMP — matrix metalloproteinases; CTGF — connective tissue growth factor; PTEN — phosphatase and tensin homologue; AS — aortic valve stenosis; RTK — receptor-tyrosine kinase

pathway. In a mice model of myocardial infarction, Qu et al. [30] have reported an altered lncRNAs expression pattern. Furthermore, using bioin-

formatics analysis, they found lncRNA-NONM-MUT022554 may affect ECM-receptor interactions and the phosphoinositid-3 kinase/protein kinase B

Table 2. lncRNAs and circRNAs involved in the regulation of cardiac fibrosis.

ncRNAs name	Modulation	Targets/signaling pathway	Pathological factors/species	Effects	Ref.
H19	Up	Suppress DUSP5 and activate p-ERK1/2	ISO/rat; TGF- β 1/NRCFs	Pro-	[26]
GAS5	Down	Suppress miR-21 / PTEN/MMP-2	ISO/rat; TGF- β 1/NRCFs	Anti-	[27]
NR024118	Down	Suppress cell cycle inhibitor Cdkn1c	Ang II/adult rat CFs	?	[28]
n379599, n379519, n384648, n380433 and n410105	Up	Activate ECM genes (e.g. Col8A1, Col3A1, and fibronectin)/TGF- β pathway (PAI-1, Snai1, Snai2, and p-Smad2/3)	ICM/human; mouse CFs	Pro-	[29]
NONM-MUT022554	Up	Activate ECM-receptor interactions and PI3K-Akt	MI/mouse	?	[30]
circRNA_010567	Up	Suppress miR-141/TGF- β 1	Diabetic mouse	Pro-	[32]
circRNA_000203	Up	Suppress miR-26b-5p/colla2 and CTGF	AngII/mouse CFs	Pro-	[33]

DUSP5 — dual-specificity phosphatase 5; PTEN — phosphatase and tensin homologue; MMP — matrix metalloproteinases; NRCFs — neonatal rat cardiac fibroblasts; ERK — extracellular signal-regulated kinases; ECM — extracellular matrix; ICM — ischemic cardiomyopathy; ISO — isoproterenol; TGF- β — transforming growth factor β ; MI — myocardial infarction; PI3K-AKT — phosphoinositid-3 kinase/protein kinase B; CTGF — connective tissue growth factor; Ang II — angiotensin II

(PI3K-Akt) signalling pathway and thus regulate cardiac fibrosis. However, most of these studies were performed in isolated cells or animal models and lack in-depth mechanistic research. Since poorly conservation between species, the role of these lncRNAs in the fibrotic pathogenesis of patients warrant further investigation.

circRNA in cardiac fibrosis

Initially, it was thought that circRNAs were produced by RNA splicing errors; however, these ncRNAs have recently become a new hot spot for research. Depending on their genomic structures, circRNAs can be classified into one_exon, annot_exon, intronic, exon_intron, intergenic, and antisense [31]. Each type has distinctive properties, including the potential for circle amplification of RNA, the ability to reorganize the order of genomic information, protection from exonucleases, and constraining RNA folding. With the advent of deep sequencing technology and advanced data analysis methods, the regulatory mechanisms of circRNAs have been identified, including sponging of miRs, regulating adjacent gene expression, and binding to specific proteins to alter their cellular localization.

Zhou et al. [32] showed that circRNA-010567 promotes myocardial fibrosis via suppressing miR-141, by targeting TGF- β 1 in a diabetic mouse model. In another recent study, circRNA_000203 identified from CFs has pro-fibrotic effects as a miR-26-5p sponge, thus blocking the interactions between miR-26-5p and its target fibrosis-

associated genes Col1a2 and connective tissue growth factor (CTGF) [33]. Despite all of this, knowledge of circRNAs is extremely limited and their regulatory role in cardiac fibrosis remains to be fully understood.

ncRNAs as emerging biomarkers for cardiac fibrosis

The evaluation of cardiac fibrosis can serve as key indicators for risk stratification and guide SCD prevention. Histopathological analysis is the gold standard to visualize extracellular compartment of myocardium and quantify myocardial fibrosis. However, the clinical application of this invasive method is largely limited. Cardiac magnetic resonance (CMR) is emerging as a gold standard among the non-invasive imaging modalities to identify these pathological changes. Nonetheless, this methodology is associated with a number of intrinsic limitations. For example, ionizing, extensive imaging time, and this method cannot be used for patients with implanted metal devices. Therefore, there is an ongoing search for novel serum biomarkers of cardiac fibrosis assessment. It has become evident that ncRNAs, especially miRs, are potential novel biomarkers for cardiovascular disease due to their ideal characteristics. ncRNAs can be released into the blood from dying cells or actively secreted from living cells under stimulation. Since combined with lipoprotein or existed in vesicles, miRs show a high degree of stability in the circulation, and can be easily detected [53].

Studies show that miR21 and miR-19b might be useful to estimate intracardiac fibrotic processes in patients with AS. Villar et al. [54] revealed that both the myocardial and plasma levels of miR21 were significantly higher in AS compared with healthy individuals. miR-21 overexpression was confined to interstitial cells and absent in cardiomyocytes. Moreover, circulating levels of miR-21 together with miR-21 target genes predicted the variance of myocardial collagen expression levels. In another study, investigators analyzed the potential associations of 7 myocardial fibrosis-related miRs with the degree of collagen fibril cross-linking (CCL) and the enzyme lysyl oxidase (LOX) responsible for CCL in 28 patients with AS [55]. From the studied miRs only miR-19b presented a direct correlation between myocardial and blood samples. In addition, myocardial and serum miR-19b were inversely correlated with CCL, LOX, and left ventricular stiffness in AS patients. In *in vitro* studies miR-19b inhibition increased connective tissue growth factor protein and LOX protein expression in human fibroblasts. However, their use needs to be verified in a large cohort. Moreover, their ability to characterize the degree of cardiac fibrosis also needs to be further assessed, combined with CMR or myocardial biopsies.

Another cardiac pathology usually associated with high levels of cardiac fibrosis is HCM. Among a set of 21 different miRs detected in the plasma of patients with HCM, only miR29a correlated with CMR detected levels of cardiac fibrosis. In this study, and in contrast to patients with AS, miR21 levels did not correlate with the cardiac fibrosis process (although miR21 levels were still increased in patients with HCM) [56]. Another study attempted to determine the plasma levels of miRs profile in HCM patients with diffuse myocardial fibrosis (defined as T1 \geq 470 ms of CMR) by miR array analysis. After polymerase chain reaction validation, a total of 11 miRs, including miR-21-5p and miR-29a-3p, were significantly inversely correlated with post-contrast T1 values [57]. The involvement of miRs as potential biomarkers for cardiac fibrosis was also demonstrated in patients with DCM. Rubiś et al. [58] investigated relationships between circulating levels of a set of 5 different miRs and ECM fibrosis assessed by endomyocardial biopsy in patients with DCM. Circulating miR-26 and miR-133a were found to be independently associated with fibrosis.

In conclusion, although these reports demonstrated that circulating miRs provide attractive candidates as putative biomarkers for cardiac fibrosis in a variety of cardiovascular diseases, the

results need to be validated in larger cohorts. In addition, its prognostic value for adverse events, such as SCD and ventricular dysfunction, are lacking. Recently, no study has reported if the circulating levels of lncRNAs and circRNAs are sufficient to serve as biomarkers for cardiac fibrosis. This requires urgent attention in future studies.

ncRNAs as potential therapeutic targets for cardiac fibrosis

RNA therapeutics is the use of sophisticated chemically synthesized/modified nucleic acids delivered via 'carrier' molecules (e.g. liposomes and nanoparticle, and/or ligands), or produced *in vivo* by recombinant viral vectors. Viral vectors have the capacity to continuously transcribe a therapeutic RNA sequence, whereas chemical synthesis allows introduction of RNA modifications that cannot be generated biologically [53]. Currently the most advanced and frequently used RNA therapeutics are RNA interference, target gene silencing by small interfering RNAs and related structures.

For example, the RNA interference drug ablating proprotein convertase subtilisin/kexin type 9 (PCSK9), which aims to lower the low density lipoprotein cholesterol level, has an ongoing clinical phase 2 trial [59]. miR therapeutics for ablation or enhancement of miR functions by miR mimics or antagomiRs is another hotspot approach. With recent developments in the affinity and specificity of delivery systems, experimental studies and preclinical or clinical research has made significant progress in cancer therapy [60]. Although there are no ongoing clinical trials for investigating the role of miRs in cardiac fibrotic changes, the technical processes used and research progress achieved in these trials also provides a reference and basis for ncRNA-therapeutic modulation of cardiac fibrosis.

Since high targeting efficacy and specificity is critical for therapeutic success, local delivery, such as catheter-based miR eluting stents and light-induced antimiR activation, is particularly desirable, in order to arrive at the cells of interest. Adeno-associated virus serotype 9 (AAV9) denotes a cardiac-targeting recombinant viral vector, which is highly selective for cardiac myocytes. Ramanujam et al. [61] developed a viral vector-based strategy for gene targeting of nonmyocyte cardiac cells *in vivo* and compared global to cardiac myocyte-specific and nonmyocyte-specific deletion of miR-21 in chronic left ventricular pressure overload. In their study, AAV9 and moloney murine leukemia virus (MMLV) were engineered to encode improved

Cre recombinase for cardiac myocyte-specific deletion and nonmyocyte ablation in miR-21fl/fl mice. Pericardial injection of MMLV-improved Cre recombinase to neonates achieved highly selective genetic ablation of miR-21 in nonmyocyte cardiac cells, identified as CFs and endothelial cells. Upon left ventricular pressure overload, cardiac function was only preserved in mice with miR-21 deficiency in nonmyocyte cardiac cells, but not in mice with global or cardiac myocyte-specific ablation. This study encouraged further development of anti-miR therapy toward cellular tropism. The “off-target” effects of modulating ncRNAs as novel therapeutic targets is also worth-noting, since multiple mRNA genes and signaling pathways can be targeted by one miR.

In light of the current lack of therapeutic options for anti-fibrotic treatment as well as the key players of ncRNAs in cardiac fibrosis, ncRNAs have now fundamentally expanded our spectrum of therapeutic options. In the past decade, using different animal models, scientists could use loss or gain of ncRNA function-strategies to treat, and in certain cases, reverse cardiac fibrosis (as summarized in Tables 1 and 2). However, selective targeting of CFs, key players in the pathology of cardiac fibrosis, in humans is currently not possible. The heterogeneity in the function of ncRNAs, the disease phenotype, and the therapeutic response to ncRNAs, which exists between animal models and humans, has made the transition from rodents to humans challenging. Further studies are urgently needed to fully understand the functions of fibrosis-related ncRNAs, and to translate these fascinating results to clinical applications.

Conclusion and clinical perspectives

Despite its adverse effects, there is currently no efficient therapeutic approach available for cardiac fibrosis. Increasing evidence indicates that ncRNAs play an important role in the pathogenesis of cardiac fibrosis, and they therefore have potential as novel biomarkers and therapeutic targets. Various *in vitro* and animal ncRNA studies have demonstrated fascinating results; however, no ongoing clinical trials investigating the role of ncRNAs in cardiac fibrotic changes are currently underway. Despite obstacles, modulating ncRNAs has promising potential as an attractive therapeutic strategy for treating cardiac fibrosis, particularly with the remarkable progress in the development of ncRNA drug design and delivery.

Conflict of interest: None declared

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