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REVIEW ARTICLE

Leveraging Extracellular Non-coding RNAs to Diagnose and Treat Heart Diseases

Zhenyi Zhao[1,2] · Ningning Guo[2,3] · Weixin Chen[4] · Zhihua Wang[2,3]

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Abstract

Extracellular vesicles (EVs), including exosomes and microvesicles, emerge to be crucial mediators of cell-to-cell communication in multiple organs. Non-coding RNAs loaded inside EVs contribute as one major mechanism for remote information transfer among different cell types or organs. Increasing evidence suggests that EV-associated non-coding RNAs derived from cardiovascular or non-cardiac cells regulate cardiovascular pathophysiology in heart development and diseases. The functional relevance of the EV-associated ncRNAs in heart diseases provides an avenue to develop novel diagnostic tools and therapies for heart diseases. In this review, we summarize the recent advancement of EV-associated ncRNAs in different cardiovascular diseases, including myocardial infarction, arrhythmias, cardiac hypertrophy, and heart failure, with an emphasis on the underlying molecular mechanisms.

Keywords Extracellular vesicles · Non-coding RNAs · Cardiovascular diseases · Cell-to-cell communication

Abbreviations

EVs	Extracellular vesicles
MVs	Microvesicles
ncRNA	Non-coding RNA
lncRNA	Long-non-coding RNA
cicrRNA	Circular RNA
micRNA	MicroRNA
AMI	Acute myocardial infarction
AF	Atrial fibrillation
HF	Heart failure
CAD	Coronary artery disease
DCM	Diabetic cardiomyopathy
ECs	Epithelial cells
CPCs	Cardiac progenitor cells
HIF1	Hypoxia-inducible factor-1
CM	CPC-conditioned medium
HUVECs	Human umbilical vein endothelial cells
RISC	RNA-induced silencing complexes
RasGap	Ras GTPase-activating protein
TLR	Toll-like receptor
MIAT	Myocardial infarction-associated transcript
SORB2	H3 domain-containing 2
PTEN	Phosphatase and tension homolog
BM-MSCs	Bone marrow mesenchymal stem cells
PPARδ	Proliferator-activated receptor δ
GAS5	Growth-arrest specific transcript 5
PUNISHER	Agap2-antisense RNA1[AS1]
hnRNPK	Heterogeneous nuclear ribonucleoprotein K
PTP1	Protein tyrosine phosphatase 1
TIMP4	TIMP metallopeptidase inhibitor 4
MMP9	Matrix metallopeptidase 9
TGFB1	Transforming growth factor-beta
COL1A1	Collagen type I alpha 1 chain
CACNA1C	Calcium voltage-gated channel subunit alpha1 C

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Zhenyi Zhao and Ningning Guo contributed equally to this work.

✉ Weixin Chen
cwx526@126.com

✉ Zhihua Wang
wangzhihua@fuwaihospital.org

[1] School of Pharmacy, Health Science Center, Shenzhen University, Shenzhen 518060, China

[2] Shenzhen Key Laboratory of Cardiovascular Disease, Fuwai Hospital Chinese Academy of Medical Sciences, Shenzhen, Shenzhen 518057, China

[3] State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Disease, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100037, China

[4] Department of Cardiology, Fuwai Hospital Chinese Academy of Medical Sciences, Shenzhen, Shenzhen 518057, China

SORB2	SH3 domain-containing 2
PDLIM5	PDZ and LIM domain 5
PPCM	Peripartum cardiomyopathy
IGF-1	Insulin-like growth factor 1
HSP20	Heat-shock protein 20
ATG4a	Autophagy-related 4A cysteine peptidase
MALAT1	Metastasis-associated lung adenocarcinoma transcript 1
VEGFA	Vascular endothelial growth factor A
hnRNPK	Heterogeneous nuclear ribonucleoprotein K
MFACR	Mitochondrial fission and apoptosis-related

Introduction

Extracellular vesicles (EVs) are membrane-bound vesicles released by both normal and transformed cells, carrying bioactive molecules including proteins, lipids, and RNAs. EVs can be detected in all major bodily fluids, like blood, urine and saliva, among which the circulating EVs in the blood have been mostly studied [1]. Cumulative evidence highlights the important role of EVs in cell-to-cell communication by releasing their cargoes into the target cell [2]. This type of cell-to-cell communication is engaged in numerous pathophysiological processes, such as inflammation, immune modulation, neurological diseases, cancer, and cardiovascular diseases [3–7].

It has been well recognized that majority of the human transcriptome are non-coding RNAs (ncRNAs) with only 2% of them corresponding to protein-coding genes [8]. These ncRNAs are grouped into transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), small nucleolar RNAs (snoRNAs), microRNAs (miRNAs), piwi-interacting RNAs (piRNAs), circular RNAs (circRNAs), and long non-coding RNAs (lncRNAs) [9]. The functionality of these ncRNAs in maintaining normal cell physiology has been repetitively verified by mounting studies [3–7]. Interestingly, ncRNAs also appear in plasma, where they are mostly packaged inside EVs rather than as free molecules, so that they are well protected from degradation by endogenous RNases [10, 11]. These circulating ncRNAs can then travel to neighboring or distant cells to transmit complex massages, particularly during the pathogenesis of diseases [12]. Thus, the EV-associated ncRNAs contribute as novel candidates for disease diagnostics or even therapeutics.

As the leading cause of death worldwide, heart diseases represent an important scenario whereby the EV-associated ncRNAs participate in the cross talk among different cell types. Here we summarize a recent finding about functional EV-associated ncRNAs in heart diseases, with an emphasis on the molecular mechanisms. We also discuss the potential application of EV-associated ncRNAs in diagnostics and therapeutics.

Function Mechanisms of EV-Associated ncRNAs

EVs are extracellular structures that are enclosed by a lipid bilayer and secreted by cells into their environment [13]. The two major classes of EVs are exosomes and microvesicles (MVs) [14]. Exosomes (30 ~ 150 nm diameter) are small vesicles released from cells when multivesicular bodies fuse with the plasma membrane. Micovesicles (MVs) are membranous vesicles with diverse sizes (100 ~ 1000 nm) compared to exosomes, and are released from the cell through blebbing of the plasma membrane. MVs are released into the extracellular space from plasma membrane instead of internal membranes by a calcium-dependent mechanism [14]. EV size is determined by its biosynthesis, associating with EV composition correlated with the cell line of origin [15].

Proteins, DNA, lipids, and RNAs are common EV cargoes that elicit diverse cellular responses in recipient cells [16]. Exosomes released from a certain tissue usually possesses a tissue-specific protein signature [17, 18]. Nevertheless, exosomes commonly carry different RNA species compared to their parental cells [19]. Accumulating evidence demonstrates that EVs actually offer a "protective shell" to protect the associated ncRNAs from degradation and maintain their functional integrity during circulation [20]. The EV content is affected by microenvironments, including aging, inflammatory process, oxygen content, and some other factors.

The identification of RNAs in EVs has progressed immensely due to highly sensitive methods of RNA detection (RNA-Seq) and reverse transcription and quantitative polymerase chain reaction (RT-qPCR) analysis in recent years. These RNA populations include various protein-coding transcript mRNAs and many types of non-coding RNAs, including miRNAs, lncRNAs, circRNAs, snoRNAs, snRNAs, tRNAs, rRNAs, and piRNAs [19]. These RNAs can be transferred from parent cells to recipient cells, where they can regulate or serve as templates for protein production [21, 22]. Here we focus on microRNAs, lncRNAs, and circRNAs identified in EVs, which could shed light on the source of diversity in the outcome of the EV-associated ncRNA mechanistic studies. The mechanisms of different RNAs entering the receptor cells are functionally summarized in the following (Fig. 1).

miRNAs

miRNAs participate in multi-biological processes via the subtle and precise regulation of gene expression; miRNAs not only target the 3′ UTR of the mRNA but also interact

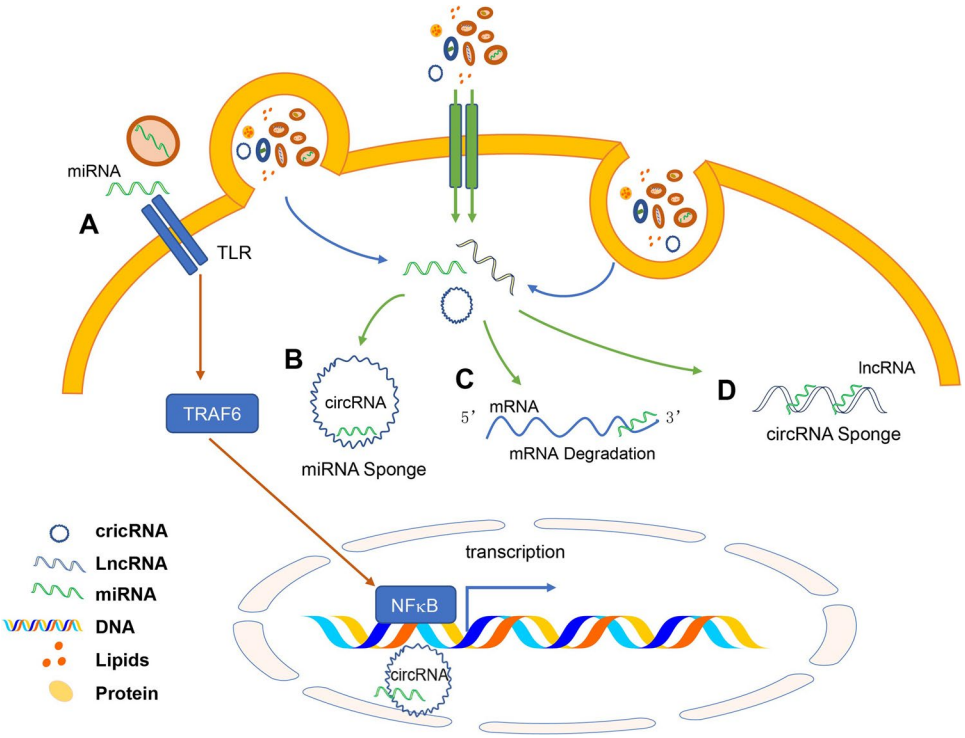

Fig. 1 Regulatory mechanisms of EV-associated ncRNAs. (A) miRNAs participate in intercellular signaling, for example (nuclear factor-kappaB) NF-kB in TLR signaling pathways. (B) circRNAs act as microRNA sponges that regulate target gene expression. (C) miRNAs target mRNAs for degradation. (D) lncRNAs as decoy molecules regulating mRNA molecules or sponging miRNA molecules to block the inhibitory effect of miRNAs on mRNA molecules

with other parts of the mRNA, such as the 5′ UTR or coding sequence, and even the gene promoter [23]. Additionally, microRNAs can also participate in intercellular signaling. A report demonstrates that EV miRNAs can act as a Toll-like receptor (TLR) ligand and induce an immune response or inhibit the activation of macrophages by inhibiting TLR signals [24–28] (Fig. 1). Based on these complicated mechanisms, single miRNAs could impact on a series of pathways or axes to regulate one target gene expression.

circRNAs

CircRNAs mostly comprise protein-coding exons; some circRNAs contain both exons and introns, and others are derived from untranslated regions (5′ or 3′ UTRs) or introns of mRNAs or ncRNAs [29, 30]. One prominent function of circRNAs is to act as ceRNA (miRNA sponges), but they can also modulate RNA transcription, splicing, turnover, and translation, and may even have protein-coding potential [31]. CircRNAs have been shown to sponge some miRNAs and thereby control their function as regulators of mRNA stability and/or translation (Fig. 1). For instance, ciRS-7 or CRD1as is generated from the CDR1 gene, and there are over 70 conserved binding sites for miRNA-7 [32]. Moreover, CRD1as RNA binds to the protein Argonaut 2 (AGO2) and miRNA, forming an RNA-induced silencing complex in the cell [33]. Another study shows that iRS-7 sponges and regulates the function of miR-7. Interestingly, EIciEIF3J interacts with snRNP U1 and the promoter EIF3J to enhance EIF3J transcription [33]. It is important to note that not all circRNAs are enriched for miRNA-binding sites and repress miRNA [34].

lncRNAs

lncRNAs are operationally defined as transcripts of greater than 200 nucleotides that function by means other than coding for proteins. Numerous lncRNAs have been found in EVs that regulate gene transcription through transcriptional interference. For example, a finding indicates that lncRNAs, pseudogenes, and mRNAs cross talk by competing for a common pool of miRNAs and build a complex regulatory network [35] (Fig. 1). The lncRNA growth arrest-specific 5 (GAS5) was generally regarded as a tumor suppressor, acting as a miR-21 sponge, that could inhibit the proliferation and promote the apoptosis of various cancer cells [36]. Interestingly, the study showed that GAS5 contained a binding site for miR-23a and acted as a sponge of miR-23a. An upregulated GAS5 expression inhibited cardiomyocyte hypertrophy through negatively regulating miR-23a and its target forkhead box O3 (Foxo3a) [37, 38].

EV-Associated ncRNAs in Heart Diseases

In the heart, EVs are secreted from multiple cell types, such as cardiomyocytes, cardiac progenitor cells, endothelial cells, epithelial cells, macrophages, and fibroblasts [39, 40]

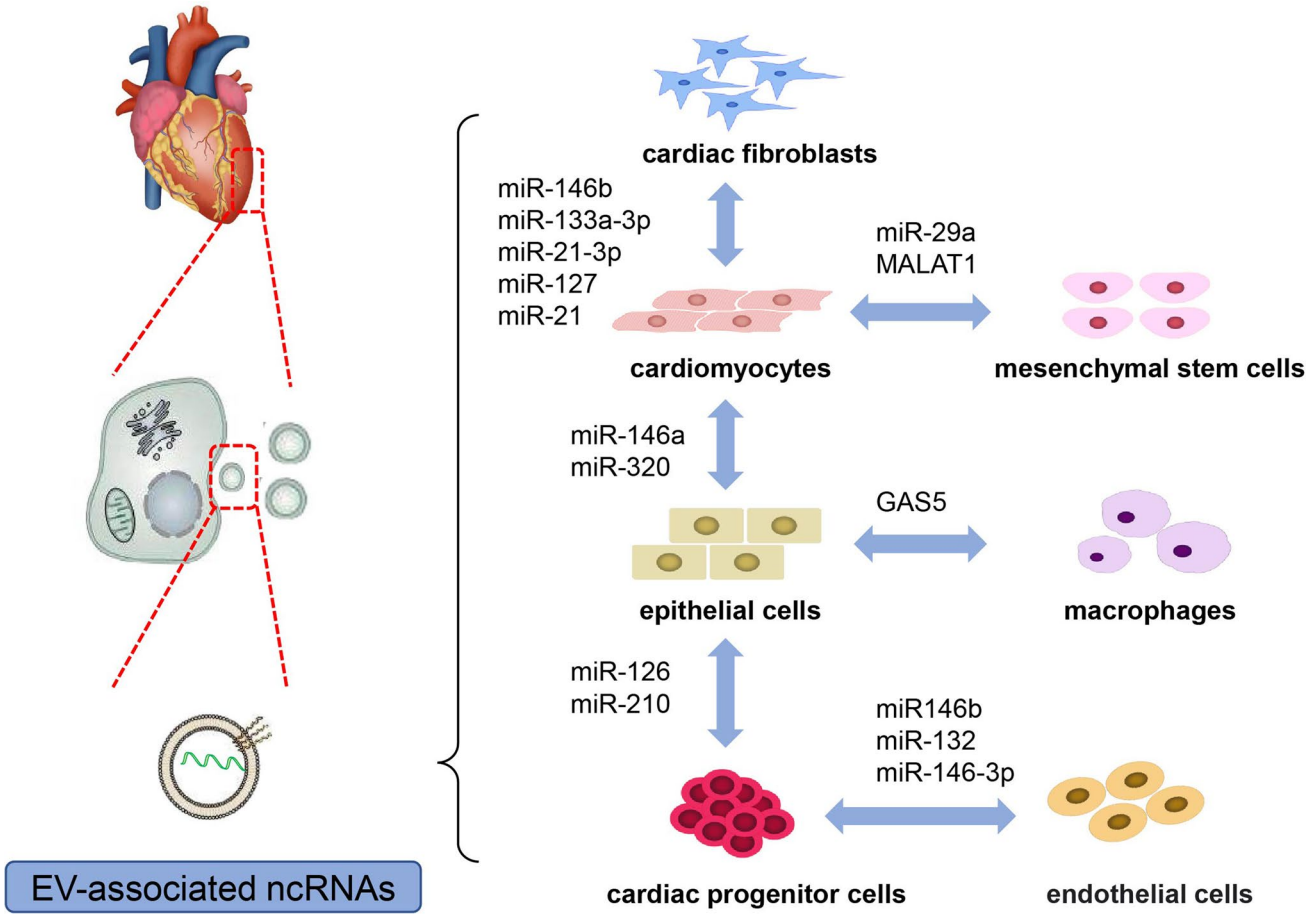

Fig. 2 Schematic of communications among various cell types from the heart via EV-derived ncRNAs

(Fig. 2). A growing body of studies demonstrates that cardiac EVs, particularly the associated ncRNAs, play a crucial role in intercellular communication during the pathogenesis of heart diseases (Table 1).

Myocardial Infarction

Acute myocardial infarction with high mortality and morbidity rate is a life-threatening condition that occurs when blood flowing to the heart muscle is abruptly cut off, causing tissue damage [64].

Ong et al. [65] found that the exosome-associated miR126 and miR-210 mediated the cross talk between epithelial cells (ECs) and cardiac progenitor cells (CPCs). Hypoxia-inducible factor-1 (HIF1) is a transcription factor that mediates adaptive responses to ischemia [66]. Exosomes from ECs overexpressing HIF1 have higher contents of miR-126 and miR-210. These miRNAs regulate pro-survival kinases and induce glycolysis in CPCs, which eventually protect the CPCs in hypoxic stress conditions and improve the survival of CPCs, while depletion of miR-126 and miR-210 exosomes from the ECs abrogates the protective effects [65] (Fig. 2).

Given their cardiac developmental origins, endogenous CPCs have been proposed as candidates for heart repair. CPC-conditioned medium (CM) protects HL-1 cardiomyocytes in mice and promotes tube formation in human umbilical vein endothelial cells (HUVECs) [67]. Surprisingly, CPC-derived exosomes are enriched with several cardioprotective and pro-angiogenic miRNAs, such as miR-210, miR-132, and miR-146a-3p (Fig. 2), compared with cardiac fibroblasts. Particularly, the effect of miR-210 is associated with a functional downregulation of ephrin A3 and protein tyrosine phosphatase 1 (PTP1) [68]. miR-132 promotes tube formation in HUVECs, which is associated with a functional downregulation of the known miR-132 target, Ras GTPase-activating protein (RasGap-p120) [69]. Dosage- and time-dependent assays show an increase in the intracellular concentrations of miR-210 and miR-132 after exposure of the HL-1 cardiomyocyte line to EVs secreted by CPCs [70]. Functional miRNAs associated with CPC-derived exosomes appear to be a plausible mechanism in the recovery of cardiac function during myocardial infarction.

Increasing evidence shows that circulating miRNAs can be used as potential diagnostic biomarkers for myocardial

Table 1 Functional EV-associated ncRNAs in heart diseases

EV-associated ncRNAs	Targets	Effects	References
Arrhythmia			
miR-23a	Vegf	Prediction coronary lesions	[41]
miR-26a	Kcnj2	Protective role for atrial fibrillation	[41]
miR-483-5p	Igf2	Predict postoperative atrial fibrillation	[42]
miR-208a	Myh6	Regulate stress-dependent cardiac growth	[43]
Myocardial infarction			
miR-1	GJA1	Diagnosis of AMI	[44]
miR-138	HIF1-α	Regulating mitochondrial apoptosis	[45]
miR-328	cTnI	Diagnosis of AMI	[46]
miR-29b-3p	TGF-β1	Protection from post-MI heart failure	[47]
miR-21	Smad7	Protection from MI	[48]
miR-423-5p	Nt-BNP	Predict acute STEMI	[49]
miR-499	CK-MBm	Prediction from AMI	[11]
Cardiac hypertrophy			
circRNA_000203	Col1a2; CTGF	Regulate developing diabetic cardiomyopathy	[50]
miR-133a	(IGF)-1; PI-3 K; Akt,	Regulate cardiac hypertrophy	[51]
Bvht	Rnase	Prediction from HF	[52]
circSlc8a1	Srf; Ctgf; Adrb1; Adcy6	Regulate hypertrophy	[53]
miR-21	SORBS2; PDLIM5	Prevented the development of cardiac hypertrophy	[54]
miR-155	Socs1	Regulate hypertrophy progression	[55]
miR-200a	TSC1;mTOR activation	Regulate cardiac remodeling	[56]
Heart failure			
miR-150	Collagen-I levels	Diagnose atrial fibrillation	[57]
miR-212	the 3′ UTR of Kir2.1 (KCNJ2)	Prediction of heart failure	[58]
miR-125a/b-5p	31UTR of prepro-endothelin-1	Modulates the expression of endothelin in endothelial cells	[59]
miR-126		Prediction of heart failure	[60]
Other heart diseases			
PUNISHER	VEGFA	Regulate endothelial cell function in CAD	[61]
GAS5		Regulate apoptosis of vascular endothelial cells in atherosclerosis	[62]
miR-17	Proangiogenic genes	Promotes angiogenesis	[63]

infarction, including miR-133, miR-208b, and miR-499, which play important roles in cell differentiation and function [71]. This hypothesis is strengthened by a report that miRNAs derived from the heart release information into the circulation system upon myocardial injury. The absence of miR-133a expression leads to the ectopic expression of smooth cardiomyopathy and heart failure [72]. One research studying on 312 patients showed that the miR-133a level was higher in AMI patients compared with non-AMI patients [73]. miR-208a can be found in extracellular environments including blood, saliva, and urine [74]. miR-208a is reported as a gold standard marker of myocardial injury from increasing concentrations in myocardial injury mice [75], while in plasma, miR-499 shows a rapid increase in AMI patients, as well as unstable angina and non-ST elevation within 3 h of symptom onset. This provides evidence of miR-499 as a potentially novel biomarker to accelerate the diagnosis of MI or acute coronary syndrome patients [76, 77].

Cardiac Arrhythmias

Cardiac arrhythmias are associated with inflammation, metabolic disorder, and structural and physiological irregularity [78]. Cardiac electrophysiology dysfunction is one of the features in cardiac arrhythmias. It might be affected by ventricular tachycardia and atrial fibrillation (AF) [79]. Atrial fibrillation is a chronic and the most common form of arrhythmia. Variations of miRNA and its targeted genes are related to the initiation and progression of atrial fibrillation [80].

A recent study investigated the role of epicardial fat (eFat)-derived EVs in the pathogenesis of AF [81]. Several miRNAs, including miR-146b, miR-133a, and miR-29a (Fig. 2), released by EVs transfer the messages from cardiomyocytes or mesenchymal stem cells (MSCs) to cardiac fibroblasts to stimulate a cardiac fibroblast that forms the arrhythmogenic substrate for AF. eFat-EVs from patients

with AF carried more profibrotic miRNAs (e.g., mmiR-146b) than patients without AF.

Another example from Ye et al. [82] suggests that exosomal miRNAs target mRNAs and lead to its deregulation. They find that miR-146-5p is released from CMs and represses the expression of the target protein TIMP metallopeptidase inhibitor 4 (TIMP4) in cardiac fibroblasts. Inhibiting miR-146b-5p in CMs of MI heart rescues TIMP4 expression, and consequently reduces fibrotic markers matrix metallopeptidase 9 (MMP9), transforming growth factor-beta (TGFB1), and collagen type I alpha 1 chain (COL1A1).

Yao et al. [83] find that miR-133a-3p is significantly reduced in atrium tissues of rats with AF induction. This provides a perspective that miRNA appears to be loaded selectively into exosomes and interacts with lncRNAs representing new shuttles of cell-to-cell communication. They found that miR-133a-3p from cardiomyocytes is identified as the target gene of lncRNA myocardial infarction-associated transcript (MIAT). Inhibition of MIAT reduces the mRNA expression of fibrosis-related gene TGF-β1, collagen I, and collagen III, while anti-miR-133a-3p administration significantly reversed the alleviation by knocking down MIAT. Thus, upregulating miR-133a-3p can decrease collagen content and inhibit atrial remodeling.

Similarly, miR-29a is transferred to cardiac fibroblasts to suppress collagen synthesis [81]. miR-29a-3p predominantly exists in mesenchymal stem cell (MSC)-derived EVs [84], serving as a mediator for long-distance cell-to-cell communications. A study validates calcium voltage-gated channel subunit alpha1 C (CACNA1C) to be the direct target gene of miR-29a-3p [85]. miR-29a-3p transfection in cardiomyocytes reduces the density of L-type calcium induced by electrical remodeling, which represents a novel approach to prevent atrial fibroblasts.

Cardiac Hypertrophy

Cardiac hypertrophy appears as an abnormal enlargement or thickening of the heart muscle, accompanied by increasing cardiomyocyte death and fibrotic remodeling, while the following reduction in systolic and diastolic function irreversibly develops into heart failure [86].

A recent report analyzed the potential paracrine miRNA cross talk between cardiac fibroblasts and cardiomyocytes in a mouse model of TAC [54]. It shows that miR-21-3p is abundant in fibroblast-derived exosomes. Once taken up by cardiomyocytes, miR-21-3p silences its target genes SH3 domain containing 2 (SORB2) and PDZ and LIM domain 5 (PDLIM5), and subsequently induces cardiomyocyte hypertrophy. Interestingly, administration of an antagonist of miR-21 in mice improves cardiac function in Ang II-induced cardiac hypertrophy.

Another two studies by Nie et al. [87] and Li et al. [88] also reveal interactions between cardiac fibroblasts and cardiomyocytes in the progression of cardiac remodeling. They show that miR-127 is highly expressed in the hearts of CHF patients and pressure overload–induced hypertrophic mice [87, 88]. Their results show that miR-217-enriched exosomes derived from cardiomyocytes induce cardiac hypertrophy, whereas the miR217-TUD-mediated downregulation of miR-217 reversed these effects. miR-217-containing exosomes targeting phosphatase and tension homolog (PTEN) enhances the proliferation of fibroblasts in vivo.

Exosomes are thought to play important roles in MSC-related cardioprotective effects [89, 90]. A study demonstrated the cross talk between bone marrow mesenchymal stem cells (BM-MSCs) and cardiomyocytes [91] (Fig. 2). They select the most relevant miRNA to cardiac hypertrophy from BM-MSC exosomes, and find miR-29 to be a key regulatory cargo contributing to the cardiac protective effects during pressure overload. It has been reported that the circulating miR-29a is upregulated in patients with hypertrophic cardiomyopathy (HCM) [92], representing a potential biomarker for cardiac remodeling assessment in HCM. In addition, miR-29a also plays a cardioprotective role in cardiac hypertrophy events by targeting proliferator-activated receptor δ (PPARδ) and downregulating ANF, which ameliorates the isoproterenol hydrochloride-induced cardiac hypertrophy response [93]. However, the mechanism of miR-29a on BM-MSC exosomes improving the function of the hypertrophic heart needs further investigation.

Heart Failure

Heart failure (HF) is a complex and progressive disease which may be caused by multi-pathological conditions including high blood pressure, coronary artery disease, faulty heart valves, and cardiomyopathy [94].

miR-21 is upregulated in the myocardium of HF murine models as well as in failing human myocardium [95]. It has been studied that miR-21 reveals a common way of communication between fibroblasts and cardiomyocytes (Fig. 2). Exosomes derived from cardiac fibroblasts selectively packaging miR-21 are taken up by cardiomyocytes to enhance cellular hypertrophy through repressing target genes SORB2 and PDLIM5 [96, 97]. miR-21 silencing attenuates the impairment of cardiac function and regression of cardiac hypertrophy fibrosis, which can prevent and even cure the structural and functional defeats in a mouse model of heart failure [98]. However, other research indicates that miR-21 inhibited by intravenous delivery of locked nucleic acid-modified (LNA) antimir oligonucleotides has no effect for remodeling response or preventing cardiac dysfunction in different mouse models of HF [99]. The results remain controversial since some microenvironment factors still need

to be elucidated including individual microRNAs in vivo (Table 1).

Exosome-derived miR-92b-5p is found significantly increased in patients with acute HF caused by dilated cardiomyopathy [100]. Circulating miR-192 is a prognostic marker in ischemic heart failure [101]. It is characterized by accumulation of p53 causing the apoptosis of cardiomyocytes and resulting in the upregulation of miR-192 [102]. However, the exosome-derived miR-192 b-5p communication pathway in cardiac cells needs to be further clarified, since miR-192 molecular pathways still need to be further investigated (Fig. 2).

Other Heart Diseases

Peripartum cardiomyopathy (PPCM) is a life-threatening pregnancy-associated cardiomyopathy in previously healthy women. A finding suggests that miR-146a appears as a major mediator in the development of PPCM via a cross talk between endothelial cells and cardiomyocytes. The 16-kDa N-terminal prolactin fragment (16 K PRL) stimulates the release of miR-146a-loaded exosomes from ECs [103]. The miR-146a-containing exosomes lead to a subsequent decrease in metabolic activity and decrease in the expression of specific miR-146a-target mRNAs. In contrast, the pharmacological blockade of miR-146a or blockade of PRL with bromocriptine in postpartum CKO mice can attenuate miR-146a–target mRNA decrease and improve cardiac function.

Diabetic cardiomyopathy (DCM) is characterized by changes in myocardial structure and function, which increases the incidence of heart failure even after controlling for coronary artery disease and hypertension [104]. A report shows that exosomes derived from diabetic cardiomyocytes contain higher levels of miR-320. miR-320 is secreted by cardiomyocytes into exosomes which can be transferred to endothelial cells, and eventually downregulate the expression of its specific target genes such as insulin-like growth factor 1 (IGF-1), heat-shock protein 20 (HSP20), and transcription factor ETS2. The study reveals that cardiomyocytes could exert an anti-angiogenic effect through the release of miR-320-enriched exosomes in DCM.

Damages of cardiomyocytes and oxidative stress are stated as the main causes of Dox-induced cardiomyopathy [105]. A novel treatment of the human adipose–derived mesenchymal stem cells (MSCs) with hypoxia induced hypoxia-inducible factor lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) accumulation in the secreted exosomes [106]. The exosome-mediated transfer of MALAT1 represses the expression of miR-92a-3p, which results in the destruction of cardiac homeostasis, for example inhibiting cardiomyocyte metabolism and autophagy via targeting ATG4a [107]. MALAT1 acts as a competing endogenous RNA (ceRNA) binding to miR-92a-3p, resulting in the activation of autophagy-related 4A cysteine peptidase (ATG4a), and improving mitochondrial metabolism. The MALAT1/miR-92a-3p/ATG4a axis partially mediates hypoxia in Dox-induced cardiac damage, which shows a new regulatory mechanism underlying the cross talk among lncRNAs, miRNAs, and mRNAs in response to stress stimulation.

Atherosclerosis is a pattern of the disease arteriosclerosis in which the wall of the artery develops abnormalities [108]. It is demonstrated that lncRNA growth-arrest specific transcript 5 (GAS5) plays a critical role in the progression of atherosclerosis [109]. THP-1 cells, when differentiating to macrophage-like cells, after taking up the exosomes derived from GAS5-overexpressing THP-1 cells, promote cell apoptosis [62]. However, exosomes shed by THP-1 cells with GAS5 knockdown inhibit the apoptosis of endothelial cells. This activation of exosomes provides an inspiration of an exosome mechanism for macrophage and endothelial cell communication mediated by lncRNA GAS5 in atherosclerosis [62] (Table 1).

Coronary artery disease (CAD) is caused by the buildup of the oxygen-rich blood plaque in the arteries [110]. A recent study identified that EVs incorporated with circulating lncRNA agap2-ANTISENSE RNA1[AS1] (PUNISHER) significantly increased in CAD [61]. In this case, EVs appear to be vehicles which convey information from circulating lncRNAs to ECs. EVs carrying PUNISHER reduce the expression vascular endothelial growth factor A (VEGFA) in recipient ECs through interacting with heterogeneous nuclear ribonucleoprotein K (hnRNPK). The inhibition of PUNISHER expression is accompanied by an impairment of the angiogenic response and a decrease in cell proliferation. This study reveals that EV-incorporated PUNISHER regulates the transcription and stability of VEGFA, thereby controlling the angiogenic function of ECs and promoting an angiogenic response (Table 1).

Translational Studies of EV-Associated ncRNAs

Cardiovascular disorders are still a major cause of morbidity and mortality worldwide, although much progress has been made in basic research [111]. Substantial studies are devoted to understanding the biology of EVs and their potential application in cardiovascular diseases. Advances in EV research provide a new avenue to develop EV-based therapeutic strategies. Below we summarized recent progresses in translational medicine leveraging EV-associated ncRNAs for diagnostics and personalized therapies of heart diseases.

Native EVs for Cardiovascular Biomarkers and Therapies

Specific miRNAs carried by EVs have been proposed as specific biomarkers for the early monitoring of CVD and other diseases [1]. For example, circRNA mitochondrial fission and apoptosis-related (MFACR) represents a valuable biomarker to predict cardiac cell death [112]. Increasing miR-126 or miR-199a expression in circulating EVs predicts the occurrence risk of CV events in stable CAD patients [113]. Exosomes from patients with MI show higher levels of miR-133a and miR-1 [114].

The therapeutic role of EVs in recipient cells is mostly studied in the delivery of ncRNAs, particularly miRNAs [115]. Some of the miRNAs are considered cell-based therapeutic targets, such as miRNA-34, miRNA-15, miRNA-24, and miRNA-208, which are relevant to cardiovascular repairation [116]. In addition, EV-associated ncRNAs trigger various cardioprotective effects, like improving cell survival in cardiomyocytes and endothelial cells [7, 117, 118] and activating pro-survival signaling pathways, including AKT, ERK, and Toll-like receptors [119]. For example, cardiosphere-derived EVs with miRNA-146 improve heart function after MI by increasing cardiomyocyte proliferation and angiogenesis [11]. Extracellular matrix-derived EVs carrying miRNA-199a-3p rescue electrical function in bioengineered atria by regulating the acetylation of transcription factor GATA4 [120]. EV-derived miRNA-486 plays a cardioprotective role via targeting PTEN and activating AKT signaling [121].

However, usage of EVs as diagnostic biomarkers or therapeutic media remains challenging due to the lack of standardization about sample collection, isolation, and quantification [122], which need to be further established in future. EVs are affected by the time of day when the sample is collected and the amount of physical activity will also influence for collection [122]. Moreover, physiological fluids contain EVs secreted from both diseased and non-diseased cells, which complicates the recovery of disease-specific EVs as biomarkers or therapeutic media [123].

Exosome Engineering

Beyond diagnostic and prognostic applications, many studies have focused on the use of engineered EVs in drug delivery to the injured heart. In one approach, the EV-secreting cell is genetically modified to express targeting peptides on the membrane of the secreted EVs to increase EV homing [124–126]. Wang et al. [124] modified exosomes with enriched membrane protein (Lamp2b) fused with ischemic myocardium-targeting peptide CST-SMLKAC (IMTP). Although no absolute quantification was provided for the accumulation of EVs in the heart, EVs isolated from engineered cardiosphere-derived cells (CDCs) expressed the cardiomyocyte-specific peptide (CMP) on their surface and retained their native physical properties. Peptide-modified EVs resulted in greater accumulation in fluorescence imaging and increased uptake by cardiomyocytes when compared to the EVs without the surface modification [124, 125].

In another approach reported by Gee et al. [127], an extracellular nanovesicle–based ribonucleoprotein delivery system named NanoMEDIC is developed by combining two distinct homing mechanisms to realize gene editing. Chemical-induced dimerization recruits Cas9 protein into extracellular nanovesicles, and then a viral RNA packaging signal and two self-cleaving riboswitches tether and release sgRNA into nanovesicles. This method successfully achieves exon skipping efficiencies in skeletal muscle cells derived from Duchenne muscular dystrophy (DMD) patient iPS cells. Similarly, Campbell et al. [128] develop a specialized extracellular vesicle termed "gesicle" using vesicular stomatitis virus glycoprotein to efficiently deliver Cas9 in a ribonucleoprotein form targeting the HIV long terminal repeat (LTR). This method bypasses the need for transgene delivery, and allows finer control of Cas9 expression.

In addition, EVs have been combined with virus-based gene therapies. A number of researches decipher how EVs influence viral and bacterial pathogenesis by spreading pathogen-derived factor [129]. Meliani et al. [130] utilize exosome-associated AAV (adeno-associated virus) to improve liver gene transfer and protect from pre-existing humoral immunity to the capsid in mice with hemophilia. Ju et al. [131] find that the endosomal sorting complex required for transport (ESCRT) system helps viruses to produce enveloped virions which avoid immune surveillance. These efforts might further accelerate the application of EV-associated ncRNAs in cardiovascular systems.

Perspectives

Before establishing EVs as an effective therapeutic tool, however, several challenges need to be resolved, including identification of specific EV-producing cells, replication of optimal vesicle production conditions, and manipulation of vesicle cargo contents. Further progress is also required to enhance the therapeutic effect of EVs by increasing their stability and targeting the EVs to a specific location, enriching their therapeutic contents, improving their internalization and intracellular trafficking, and controlling their spatial and temporal release from biomaterials. Above all, the molecular mechanisms how EV-associated ncRNAs impact cardiovascular pathophysiology need to be further explored.

Taken together, EV-associated ncRNAs provide a promising therapeutic strategy for CVD based on their advantageous properties as important factors involved in many physiological and pathological cardiovascular processes [132, 133], an essential component of the paracrine effect of stem cell-based therapies [134], low immunogenicity, low toxicity, and high capability to carry bioactive molecules to target cells. Future efforts to resolve the challenges in isolating, purifying, and manipulating EVs would help optimize the translation of EVs in a clinical setting.

Declarations

Conflict of Interest The authors declare no competing interests.

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