microRNAs and long noncoding RNAs in heart failure: Biogenesis, subcellular functions, and clinical applications

Yuyan Tang[1,2], Rong Xie[1,2], Xiang Cheng[3], Xin Joy Wang[4], Sofia-Iris Bibli[5,6,7], Huaping Li[1,2], Chen Chen[1,2]

[1]Department of Cardiology, Tongji Hospital, Tongji Medical College and State Key Laboratory for Diagnosis and Treatment of Severe Zoonotic Infectious Diseases, Huazhong University of Science and Technology, Wuhan, Hubei 430030, China;
[2]Hubei Key Laboratory of Genetics and Molecular Mechanisms of Cardiological Disorders, Wuhan, Hubei 430030, China;
[3]Department of Cardiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430022, China;
[4]Faculty of Biology, Medicine and Health, University of Manchester, Manchester, Greater Manchester M13 9PL, United Kingdom;
[5]European Center for Angioscience, Department of Vascular Dysfunction, Medical Faculty Mannheim, Heidelberg University, Mannheim, Baden-Württemberg 68167, Germany;
[6]German Centre for Cardiovascular Research, Berlin 13347, Germany;
[7]Helmholtz-Institute for Translational AngioCardioScience of the Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Heidelberg University, Heidelberg, Baden-Württemberg 69120, Germany., Baden-Württemberg 69120, Germany.

Abstract

Cardiovascular diseases remain the leading causes of mortality and disability, with heart failure being the end stage of various cardiovascular diseases. The well-established biological pathogenesis of heart failure includes cardiomyocyte hypertrophy, cardiac fibrosis, and endothelial dysfunction. Despite advanced therapies, mortality from heart failure remains high. Therefore, novel targets and therapeutic strategies for heart failure are urgently needed. The discovery of microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) extends understanding of the pathophysiological mechanisms of diseases and provides new perspectives on potential clinical applications. Emerging evidence shows that miRNAs and lncRNAs may be located in different subcellular fractions and possess various regulatory functions; this deepens the understanding of miRNA and lncRNA functions. Furthermore, miRNA- or lncRNA-based therapies may be optimized based on their subcellular functions. In this review, we have summarized the diverse functions of subcellular miRNAs and lncRNAs and the current clinical applications of miRNAs and lncRNAs in the treatment of heart failure.

Keywords: microRNA; Long non-coding RNA; Heart failure; Subcellular compartments; Clinical application

Introduction

Heart failure is a clinical syndrome caused by functional or structural cardiac abnormality, accompanied by elevated natriuretic peptide levels and pulmonary or systemic congestion.[1] Because of the high morbidity and mortality, as well as a significantly impaired quality of life, heart failure remains a major global public health burden.[2–3] Heart failure is a common chronic end-stage resulting from various heart diseases, including ischemic heart disease, cardiomyopathy, and hypertension.[4–5]

Although heart failure has diverse etiologies, it typically shares common pathophysiological changes in cardiomyocytes, fibroblasts, endothelial cells, and the extracellular matrix. Early-stage cardiomyocyte hypertrophy is usually compensatory; however, in later stages, this is accompanied by cardiomyocyte elongation and death, leading to ventricular dilation.[6] During heart failure, proliferation and activation of fibroblasts result in interstitial collagen deposition, reducing ventricular compliance.[7] Endothelial cell dysfunction impairs vasodilation, decreasing cardiac perfusion and increasing afterload.[8]

Despite advances in understanding the pathophysiology and treatment of heart failure, its prevalence continues to rise, and prognosis remains poor.[2] Therefore, it is urgent to identify new biomarkers and novel therapeutic approaches for heart failure.

Although 80% of the human genome is transcribed, protein-coding genes account for only 1%; the majority of the transcriptional products are described as non-coding RNAs (ncRNAs).[9–10] Since their discovery, ncRNAs have been identified as important regulators in cardiovascular

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000004177

Correspondence to: Chen Chen, Department of Cardiology, Tongji Hospital, Tongji Medical College and State Key Laboratory for Diagnosis and Treatment of Severe Zoonotic Infectious Diseases, Huazhong University of Science and Technology, No. 1095 Jiefang Avenue, Wuhan, Hubei 430030, China
E-Mail: chenchen@tjh.tjmu.edu.cn

Chinese Medical Journal 2026;XX(XX)
Received: 28-07-2025; **Online:** 17-07-2026 **Edited by:** Rongman Jia

development and in the processes of cardiovascular diseases.[11] Among them, microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are the most extensively studied.[12]

miRNAs are small, single-stranded ncRNAs with a length of approximately 22 nucleotides (nt).[13] Studies have demonstrated that miRNAs are widely distributed throughout various subcellular fractions.[14–15] The function of miRNAs in the cytoplasm has been widely studied. Generated in the nucleus and then transported into the cytoplasm, miRNAs are commonly loaded into the RNA-induced silencing complex (RISC), where they mediate gene silencing by binding to the 3′ untranslated region (UTR) of target messenger RNA (mRNA) molecules, leading to mRNA degradation or translational repression.[16–17] In addition to the cytoplasmic mechanism, an increasing number of studies have found that miRNAs in other subcellular compartments also play important roles in cells. For example, miRNAs in the nucleus can regulate nuclear transcripts and alternative splicing.[18–19]

Importantly, increasing evidence shows that the subcellular localization of miRNAs is not a simple reflection of their distribution, but a key factor that determines their functions. The same miRNA can have distinct molecular functions depending on the subcellular compartment where it exists. For instance, microRNA-320 (miR-320) acts as a conventional translational repressor in the cytoplasm.[20] However, when it is transported into the nucleus, it switches to a transcriptional activator that directly binds to gene promoters and drives transcription.[21] Similarly, miR-126-5p functions as a canonical post-transcriptional silencer in the cytoplasm.[22] However, in the nucleus of endothelial cells under shear stress, miR-126-5p directly binds to caspase-3 in an aptamer-like manner to inhibit apoptosis, a mechanism entirely independent of mRNA targeting.[23] These examples illustrate that subcellular localization fundamentally reshapes the mode of action of miRNAs, converting them from post-transcriptional regulators into transcriptional modulators or direct protein interactors.

lncRNAs are RNAs longer than 200 nt that lack protein-coding potential.[24–25] The functions of lncRNAs are complex and diverse, and are closely related to their subcellular localization. For instance, in the nucleus, lncRNAs act as molecular scaffolds, while in the cytoplasm, they modify the stabilization of mRNA and regulate translation.[26–27]

In the nucleus, lncRNAs may directly interact with chromatin through triplex or R-loop structures to regulate transcription, recruit chromatin-modifying complexes such as polycomb repressive complex 2 (PRC2) to silence or activate specific gene loci, or modulate alternative splicing by recruiting splicing factors to precursor mRNAs.[28–30] In the cytoplasm, lncRNA shifts to post-transcriptional roles, stabilizing mRNAs by recruiting RNA-binding proteins, acting as miRNA sponges to relieve translational repression, or interfering with ribosome-associated factors to modulate global translation.[31–33] In mitochondria, lncRNAs directly bind to metabolic enzymes and enhance their activity through allosteric mechanisms.[34] This localization-dependent functional flexibility reveals that subcellular localization is not a simple consequence of lncRNA biogenesis, but an active determinant that decides the specific molecular function of lncRNAs.

Studies have shown that both miRNAs and lncRNAs play important roles in the cardiovascular system. For example, conditional knockout of *Dicer*, which encodes the rate-limiting enzyme for miRNA maturation, in cardiomyocytes of 3-week-old mice led to atrial enlargement and early death. When it was knocked out in cardiomyocytes of adult mice, rapid and dramatic biventricular enlargement occurred.[35] Multiple lncRNAs have been demonstrated to be essential for cardiovascular development. For instance, lncRNAs such as *TERMINATOR*, *ALIEN*, and *PUNISHER* were, respectively, expressed in undifferentiated pluripotent stem cells, cardiovascular progenitors, and differentiated endothelial cells, and played key roles during their corresponding developmental stages.[36]

The subcellular localization of ncRNA as a key driver is important for understanding heart failure in the context of clinical translation. First, it reveals previously hidden disease mechanisms. For example, miR-92a-3p and miR-212/132 both can promote cardiac hypertrophy. However, miR-92a-3p functions in the nucleus by driving pathological myosin heavy chain 7 (MYH7) expression by a transcriptional activation mechanism that is invisible to conventional cytoplasm-focused studies and fundamentally different from the post-transcriptional silencing mediated by cytoplasmic miR-212/132 targeting forkhead box O3 (FoxO3). Without the subcellular perspective, these two mechanistically distinct pathways would be indistinguishable.[37–38] Besides, it redefines therapeutic target selection. A miRNA whose pathological effect is primarily in the nucleus requires nuclear-penetrating delivery systems, whereas a cytoplasmic miRNA mechanism can be targeted by conventional antisense oligonucleotides (ASOs). Ignoring subcellular localization may lead to therapeutic failure due to drug delivery to the wrong compartment.

Therefore, in this review, we adopt subcellular localization of ncRNAs as the central organizing framework. Rather than simply cataloguing ncRNAs by disease mechanism or molecular type, we organize the roles of miRNAs and lncRNAs across subcellular compartments within the three key cell types involved in heart failure. Through this framework, we aim to demonstrate how subcellular localization determines ncRNA function in heart failure, identify mechanistic gaps overlooked by conventional perspectives, and discuss how this spatial understanding may guide the optimization of ncRNA-based diagnostics and therapeutics.

Important Roles of Cardiomyocytes, Fibroblasts, and Endothelial Cells in Heart Failure

Cardiomyocytes

Cardiomyocyte hypertrophy is a hallmark of heart failure. To maintain perfusion of organs, the heart and individual cardiomyocytes enlarge in response to increased preload and afterload. Different from reversible physiological hypertrophy occurring during exercise, pathological

hypertrophy induced by chronic hypertension or myocardial infarction is irreversible and characterized by lengthening of individual cardiomyocytes with impaired function. Pathological hypertrophy of cardiomyocytes leads to ventricular dilatation and wall thinning, which finally results in systolic dysfunction and heart failure.[39–40]

In the normal heart, the proportion of dead cardiomyocytes is extremely low, whereas in the failing heart, the number of dying cardiomyocytes increases remarkably.[41] Cardiomyocyte death is the core progression of heart failure. Because of the limited regenerative ability of the adult mammalian cardiomyocytes, damaged and dead cardiomyocytes are replaced by scar rather than proliferating cardiomyocytes, leading to irreversible cardiac dysfunction.[42]

Fibroblasts

Cardiac fibrosis is primarily mediated by activated cardiac fibroblasts and can be divided into three types: replacement fibrosis, interstitial fibrosis, and perivascular fibrosis. When cardiomyocytes are damaged, fibroblasts migrate to the site of injury, proliferate, and are activated into reparative myofibroblasts, which secrete collagen to form scars, resulting in replacement fibrosis.[7,43] Although replacement fibrosis is usually reparative and prevents fatal mechanical complications such as cardiac rupture, the scar tissue lacks the ability of rhythmic contractile function of healthy cardiomyocytes, resulting in contractile dysfunction. Interstitial fibrosis is characterized by diffuse fibrillar collagen accumulation throughout the myocardial interstitium, while perivascular fibrosis is defined by collagen deposition around the adventitia of microvessels. These two types of fibrosis commonly coexist in heart failure associated with pressure overload, such as that seen with hypertension or aortic stenosis, and can reduce left ventricular compliance and impair diastolic function.[44–45]

Endothelial cells

The myocardial microvascular system is crucial for maintaining homeostasis. In heart failure, vasodilation of the coronary and peripheral arteries is both impaired, leading to impaired myocardial contractility and increased cardiac afterload, which in turn exacerbates heart failure.[46–47] In addition, reduced capillary density has been observed in the hearts of patients with heart failure, resulting in myocardial hypoxia and cardiac dysfunction.[48] Both processes are related to endothelial cells. On the one hand, endothelial cell dysfunction reduces vasodilatory response to blood flow and vasodilators; on the other hand, endothelial cell death leads to microvascular rarefaction.[12,49] Therefore, reversing endothelial dysfunction and promoting angiogenesis have protective roles in the treatment of heart failure.

Biogenesis of miRNA

Canonical miRNA biogenesis

The typical miRNA biogenesis initiates in the nucleus with the production of primary miRNAs (pri-miRNAs) by RNA polymerase II (Pol II).[50–51] The classical pri-miRNAs are characterized by a hairpin-shaped secondary structure, with a stem composed of two complementary RNA sequences at a length of approximately 35 base pair (bp), a terminal loop at the top, and unpaired single-strand RNA segments present at both sides of the base of the hairpin.[52–53] Once generated, pri-miRNAs are cleaved by the microprocessor complex, consisting of Drosha and its cofactor DiGeorge syndrome critical region 8 (DGCR8), into a short hairpin-like structure with a 2-nt 3′ overhang, named precursor miRNAs (pre-miRNAs).[54–58] After cleavage, the pre-miRNAs are transported to the cytoplasm from the nucleus by Exportin 5 (Xpo5), a member of Ran-dependent nuclear transport receptor.[59–61] In the cytoplasm, a miRNA duplex is processed from pre-miRNAs by DICER,[62–64] an RNase III enzyme whose stability, processing efficiency, and cleavage site selection are influenced by two double-stranded RNA-binding proteins, trans-activation response RNA-binding protein (TRBP), and protein activator of interferon-induced protein kinase R (PACT).[65–67] The miRNA duplexes are loaded into Argonaute (Ago) proteins with the assistance of heat shock protein 70 (Hsp70) and heat shock protein 90 (Hsp90) chaperones, which keep the conformation of Ago open.[68–69] Two strands of the duplex are then separated into the "guide" and "passenger" strands by two characteristics: base identity and thermodynamic stability.[70–71] The strand with 5′ termini being uracil or adenine, or whose 5′ end is thermodynamically unstable, tends to be selected as the guide strand by Ago.[72] With the passenger strand removed, the remaining guide strand and Ago proteins compose RISC, which is responsible for target gene-silencing.[73]

Non-canonical miRNA biogenesis

Besides the canonical route, miRNAs can be generated bypassing DROSHA, DICER, or utilize alternative pathways. For example, some spliced introns, such as mirtrons, escape immediate degradation and form a hairpin-shaped structure after debranching, bypassing processing by DROSHA.[74–75] Drosha-independent miRNAs can also come from 5′ methylguanosine-capped pre-miRNAs directly transcribed by Pol II.[76] In addition, partial small nucleolar RNAs (snoRNAs), such as H/ACA box snoRNA ACA45[77] and transfer RNAs (tRNAs), such as isoleucine tRNA,[78] can serve as sources of miRNAs bypassing Drosha. There are also DICER-independent cases. For instance, after being processed by DROSHA, pre-miR-451 is directly cleaved by AGO2 and subsequently trimmed by the 3′-to-5′ exoribonuclease PARN rather than being processed by DICER, because its unusually short hairpin cannot be recognized by DICER.[79–80]

The biogenesis pathway determines the initial subcellular destination of each miRNA. Canonical processing delivers mature miRNAs predominantly to the cytoplasm via Xpo5, whereas specific sequence motif or association with nuclear import adaptors can redirect miRNAs to the nucleus.[81–82] Importantly, during heart failure, perturbations in biogenesis components such as altered Xpo5 expression[83] or changes in AGO2[84] may impair nuclear import efficiency and redistribute miRNAs across subcellular compartments,

shifting their functions from post-transcriptional silencing to other non-classical functions. In the following sections, we first describe the distinct functions of miRNAs in each subcellular compartment, and then demonstrate how these compartment-specific functions are co-opted during heart failure progression.

Biogenesis of lncRNA

Similar to the process of generation of mRNA, many lncRNA species are transcribed by Pol II,[85] undergo splicing and folding, with 5′ end m^7G caps and 3′ poly(A) tails.[86] In addition to the classical maturation processes involving 5′ m^7G capping and 3′ polyadenylation, lncRNAs can also be cleaved by RNase P, generating a mature 3′ end.[87–88] They may be capped at both ends or at the 5′ end by snoRNAs,[89–91] or may form circular structures to avoid degradation.[92] For example, the 3′ end of *NEAT1* and *MALAT1* form a triplex structure, processed by RNase P.[93–94] The 5′ ends of 5′ snoRNA-capped and 3′ polyadenylated long non-coding RNA (SPAs) are capped by snoRNA-associated ribonucleoproteins and contain 3′ poly(A) tails.[91] Both the 5′ and 3′ ends of a sno-lncRNA are wrapped by snoRNA structures; the lncRNA sequence lies between the sequences of snoRNA. It lacks the conventional m^7G caps and poly(A) tails.[89]

Although several species of lncRNAs possess an mRNA-like structure, lncRNAs prefer to remain in the nucleus rather than the cytoplasm, for unknown reasons. The phosphorylation state of the C-terminus of Pol II C-terminal domain (CTD) corresponds to different stages of transcription. For example, S5P is associated with early elongation, 5′ capping, and active splicing, while S2P is related to late elongation and 3′ end processing.[95–96] Mammalian native elongating transcript sequencing (mNET-seq) reveals that several lncRNA genes are transcribed by Pol II with abnormal phosphorylation status. Pol II tends to pause more at the transcription start site (TSS) of protein-coding genes with specific phosphorylation signals, while these signals in lncRNA genes are weaker and more evenly distributed. The transcription end site (TES) of protein-coding genes show a strong threonine 4 phosphorylation (T4P) signal, indicating that Pol II terminates at these sites. In contrast, with more scattered termination signals, Pol II is shown to terminate at multiple positions at lncRNA genes. The above findings indicate that the transcription process of lncRNAs is less precise than that of mRNAs. This type of lncRNA exhibits low efficiency during co-transcriptional splicing, and its transcription termination is not dependent on polyadenylation signals, resulting in the transient accumulation of lncRNA on chromatin and subsequent rapid degradation by the RNA exosome.[85]

The splicing efficiency of lncRNAs is lower than that of mRNAs.[97] Their internal splicing signals are weaker, and the distance from the 3′ splice site to the branch point is longer.[98] Studies have shown that splicing can significantly promote nuclear export of mRNAs. The coupling of splicing and nuclear export is widespread and conserved in higher eukaryotes. Therefore, weak splicing is related to strong nuclear retention of lncRNAs.[99]

Some chromatin-located lncRNAs possess many U1-binding motifs, which recruit U1 small nuclear ribonucleoprotein particles (snRNP) to Pol II that is transcriptionally active. Mutation of the U1-binding sequence, depletion of the U1-snRNP component SNRNP70, or inhibition of Pol II transcription all weaken the chromatin association of lncRNAs. This suggests that lncRNAs are attached to chromatin through the binding of U1-snRNP to their U1-binding motifs.[100]

There are also motifs in lncRNA sequences that recruit specific nuclear factors that can assist in nuclear retention, as well as enhance the function of lncRNAs. For instance, an element in 42 nt length, called SINE-derived nuclear RNA localization element (SIRLOIN), including three C-rich segments from the Alu sequence, promotes lncRNA accumulation in the nucleus by binding with heterogeneous nuclear ribonucleoprotein K (hnRNP K) When hnPNP K is knocked down in MCF-7 human breast cancer cells cells, lncRNAs with SIRLOIN tend to export to the cytoplasm.[101]

The biogenesis and processing pathways described earlier are not merely background information but directly determine the subcellular fate of lncRNAs. Unlike mRNAs, lncRNAs exhibit a strong tendency toward nuclear retention due to their inefficient splicing, atypical Pol II phosphorylation patterns, and presence of specific nuclear retention motifs such as SIRLOIN and U1-binding sequences. These features establish biogenesis-driven subcellular localization. lncRNAs with efficient splicing and canonical processing are more likely to be exported to the cytoplasm, where they engage in post-transcriptional regulation. Those with weak splicing signals or nuclear retention elements remain in the nucleus, where they participate in chromatin remodeling, transcriptional regulation, and alternative splicing.[27] Importantly, during heart failure, pathological stimuli may alter the expression or activity of splicing factors, nuclear export machinery, and RNA-binding proteins, potentially redistributing lncRNAs across subcellular compartments, thereby shifting their functional output.

Functions of miRNAs in Different Subcellular Localizations

Classical function of miRNAs in cytoplasm

Classical post-transcriptional gene silencing

The most extensively studied and well-defined function of miRNAs is to promote mRNA degradation or inhibit mRNA translation by binding to the 3′ UTR of target mRNAs through sequence complementarity.

For sequence complementarity, miRNAs usually recognize the target mRNAs by their “seed region” located at the second–seventh nt of miRNAs. In addition, another pairing at the eighth nucleotide can enhance binding. Therefore, the second–eighth region is also called “extended seed”. If the first base of target mRNAs opposite miRNAs is an adenosine, or there is supplementary pairing at 13th–16th nucleotide of miRNAs, the interaction

may be further improved.[102–103] miRNA target sites, also known as microRNA response elements (MREs), are not only situated at the 3′ UTR region of mRNAs, but can also be found in the 5′ UTR region or coding sequence region. Bioinformatics algorithms predict that each mRNA possesses four or five MREs.[103–104]

After recognition, miRNAs may suppress gene expression by two different models. On the one hand, miRNAs may induce mRNA slicing. The Ago proteins possess four key domains: the N-terminal, Piwi/Argonaute/Zwille (PAZ), middle, and P-element-induced wimpy testis (PIWI) domains.[105–107] The PIWI domain has an RNase H-like structure with nuclease activity and can directly cleave target mRNA.[105] Among the four human Ago proteins, only AGO2 possesses strong slicing activity.[108] However, this slicing action is rare in humans because it requires nearly perfect pairing between miRNA and its target mRNA, which rarely occurs in animals. In contrast, this pathway commonly occurs in plants. On the other hand, the more common effects of miRNAs in animals are translational repression.[109–110] Trinucleotide repeat-containing protein 6 (TNRC6) directly binds to Ago proteins and recruits the carbon catabolite repression 4–negative on TATA-less (CCR4–NOT) complex and poly(A)-specific ribonuclease subunit 2–poly(A)-specific ribonuclease subunit 3 (PAN2–PAN3), thereby removing the poly(A) tail at the end of mRNAs and triggering rapid decay.[111–112] Besides, TNRC6 with CCR4–NOT complex not only recruits DEAD-box helicase 6 (DDX6), promoting mRNA decapping, but also recruits 4E–T to compete with eukaryotic translation initiation factor 4G (eIF4G), for binding to the cap-binding protein eIF4E, thereby inhibiting translation initiation.[113] Dissociation of poly(A)-binding protein is also induced by TNRC6, preventing the translation initiation complex eIF4F, which is composed of eIF4E, eIF4G, and eIF4A, from binding to the 5′ cap, thereby blocking translation initiation.[114]

Regulation of non-conventional MREs

miRNAs bind to target mRNAs through seed sequences and the MREs within the 3′ UTR region of mRNAs. However, recent studies have identified non-conventional MREs, characterized by the following features: Pairing is not limited to the seed region or MREs may be located in the coding sequence (CDS) or other non-3′ UTR regions.[115–116] This interaction demands extensive pairing at the 3′ side instead of the seed region and requires only Ago proteins rather than GW182. Ribosome profiling shows that these MREs cause transient ribosome stalling rather than mRNA destabilization.[117]

miRNAs interacting with non-Ago proteins

From the traditional perspective, miRNAs mainly work in RISC by binding to Ago proteins, to interact with mRNAs, and result in translation inhibition or mRNA decay.[118] However, a part of mature miRNAs interacts with non-Ago proteins.[119] Early studies found that miRNAs competitively bind to non-Ago proteins. And certain miRNAs form a secondary structure similar to RNA aptamers, resembling artificially designed nucleic acid aptamers that specifically bind to proteins.[120]

Functions of miRNAs in the Nucleus

Although miRNAs and RISC components are commonly located in the cytoplasm, they are also present in the nucleus. Their nuclear localization can be achieved by two mechanisms. Some miRNAs contain specific motifs that may promote their nuclear localization. For instance, human miR-29b is predominantly located in the nucleus rather than the cytoplasm, mainly due to the hexanucleotide terminal motif AGNGUN at miR-29b.[81] Besides, Ago proteins can be transported into the nucleus by binding to adaptor proteins, which carry a nuclear localization signal.[121] When miRNAs are imported into the nucleus, they activate transcription or inhibit transcription by interacting with promoters or enhancers.

Transcriptional regulation by interaction with promoters

There are three types of interactions between miRNAs and gene promoters.[82]

(1) RNA–RNA model: miRNAs can bind to the non-coding transcripts derived from the promoter region with the help of Ago proteins, altering the modification status of histones, as a scaffold to recruit epigenetic modifiers.

(2) RNA–DNA hybrid model: Guided by Ago proteins, miRNAs can bind to the unwinding single-stranded DNA at the gene promoters, which may affect the assembly of the pre-initiation transcription complex and the binding of transcription factors (TFs). For transcriptional activation, miRNAs directly bind TATA-box motifs, which are thymine–adenine–thymine–adenine-rich core promoter elements, of specific genes, helping the formation of pre-initiation transcription complexes.[122] In contrast, the interaction of miRNAs with DNA-binding motifs of TFs causes transcription regression.[123]

(3) RNA–DNA triplex model: Some purine- or pyrimidine-rich miRNAs can form a triple-helix structure in the major groove of purine-rich double-stranded DNA through Hoogsteen or reverse Hoogsteen pairing. The formation of such structures can influence DNA conformation, promoting or inhibiting the binding of TFs.[124]

miRNAs serve as enhancer triggers

An enhancer is a short non-coding DNA sequence, 100–1000 bp in length, which belongs to *cis*-regulating elements playing a key role in initiating transcription.[125–126] miRNAs also target enhancer regions to regulate the expression of nearby genes. For example, miRNAs regulate histone modifications such as increased histone H3 lysine 27 acetylation (H3K27ac) and decreased H3K9me3. Besides, they promote the binding of p300 and RNAPII to enhancers, both of which are markers of enhancer activation. Furthermore, this process requires the involvement of AGO2 protein.[127]

Nuclear RNA interference

Studies show that in human cells, the RNA interference-related proteins such as AGO2, DICER, and GW182 are not only located in the cytoplasm but also in the nucleus, and are able to assemble delicate protein complexes. However, the process by which duplex small RNAs load onto Ago proteins is absent, and known loading factors are lacking in the nuclear fraction. Therefore, the assembly of RISC and loading of small RNAs happen only in the cytoplasm. Once finished, the complex is transported into the nucleus with the help of the chaperone proteins importin 8, mex-3 RNA binding family member A (MEX3A), and TNRC6A, since AGO2 lacks a nuclear localization signal.[128] Similar to RISC in the cytoplasm, the nuclear RISC targets transcripts, lncRNAs, and pre-miRNAs. For instance, nuclear miR-9-5p targets lncRNA *MALAT1* in myeloid cells.[129] miR-709 is able to regulate pri-miR-15a/16-1 in the nucleus.[130]

Functions of miRNAs in the Mitochondria

Mitochondria have their own circular DNA, which encodes 37 genes: 13 protein-coding genes involved in oxidative phosphorylation, 22 tRNA genes, and two rRNA genes.[131] Although mitochondria themselves do not generate miRNAs, AGO2 protein is present in mitochondria; meanwhile, other RISC components, such as GW182, are absent.[132] Nuclear-encoded miRNAs can enter mitochondria and target mitochondrial-encoded genes,[133–134] either enhancing or inhibiting their expression, thereby playing an important role in various biological processes.[135]

miRNAs in Different Subcellular Localizations during Heart Failure

Having established that miRNAs possess distinct molecular functions in different subcellular compartments, we now examine how these compartment-specific functions are engaged during heart failure. Importantly, the pathological processes of heart failure are compartment-specific: Pathological transcriptional reprogramming occurs in the nucleus, aberrant protein synthesis operates in the cytoplasm, and metabolic dysfunction originates in the mitochondria. By mapping miRNAs to these subcellular compartments within cardiomyocytes, fibroblasts, and endothelial cells, a more precise understanding can be achieved of their roles in heart failure. The following sections are organized by cell type, and within each cell type, by subcellular compartment.

Cytoplasmic miRNAs in cardiomyocytes

As described in the previous sections, cytoplasmic miRNAs primarily function through post-transcriptional gene silencing by RISC, including mRNA degradation and translational repression via 3′ UTR binding. In addition, miRNAs in the cytoplasm interact with non-Ago proteins through aptamer-like secondary structures, directly modulating protein activity independent of mRNA targeting. In the context of cardiomyocyte hypertrophy and dysfunction, both of these cytoplasmic mechanisms are actively engaged, as illustrated by the following examples.

To identify miRNAs directly related to cardiac hypertrophy, a precursor-miRNA library was transfected into neonatal rat cardiomyocytes, and changes in cardiomyocyte size and B-type natriuretic peptide (BNP) secretion were measured at the same time. As a result, 26 miRNAs that promoted cardiac hypertrophy were selected, ten of which also strongly increased BNP secretion. Among them, miR-212 showed the strongest effect and, together with miR-132, belonged to the evolutionarily conserved miR-212/132 family. *In vivo* and *in vitro* studies showed that miR-212/132 promoted cardiac hypertrophy by binding to the 3′ UTR of FoxO3, inhibiting its expression [Figure 1A].[12]

miR-1-3p directly binds to cardiac membrane proteins, especially the inward-rectifier potassium channel Kir2.1. The binding site of miR-1-3p on the Kir2.1 channel was identified to be located at the C-terminus of the protein, mainly through the pore-facing G-loop of Kir2.1; the core sequence AAGAAG of miR-1-3p was beyond the canonical miRNA seed region. This interaction resulted in quick and significant I_{K1} suppression in cardiomyocytes at a biophysical level. And a human single-nucleotide polymorphism of miR-1-3p associated with arrhythmia damaged this biophysical modulation, but retained its RNA interference function [Figure 1B].[142]

Nuclear miRNAs in cardiomyocytes

As outlined earlier, nuclear miRNAs regulate gene expression through multiple mechanisms, including binding to promoter-associated transcripts, direct interaction with promoter DNA, and nuclear RNA interference targeting nuclear transcripts. During pathological cardiac remodeling, these nuclear mechanisms are co-opted to drive abnormal transcriptional programs, particularly the reactivation of fetal gene expression that is central to heart failure progression. The following examples demonstrate how nuclear miRNAs contribute to cardiac hypertrophy through transcriptional regulatory mechanisms that are different from cytoplasmic post-transcriptional silencing.

In transverse aortic constriction (TAC)-operated mouse models, miR-92a-3p bound to ankyrin repeat domain 1 (*Ankrd1*) sense promoter-associated transcripts (PATs), recruiting nuclear AGO2 to the *Ankrd1* promoter DNA, ultimately enhancing *Ankrd1* transcription. In turn, nuclear ankyrin repeat domain 1 (ANKRD1) recaptured key features of cardiac remodeling by activating pathological MYH7 expression [Figure 1C].[37]

miR-665 was enriched in the nucleus of cardiomyocytes; expression of miR-665 was improved both in TAC-operated mouse models and in cultured cardiomyocytes treated with phenylephrine. Nuclear miR-665 aggravated heart failure by inhibiting the phosphatase and tensin homolog (PTEN) expression. Mechanistically, miR-665 may directly suppress *PTEN* transcription by binding to its promoter, resulting in suppression of PTEN expression. Furthermore, when AGO2 protein was knocked down by siRNA, the miR-665-induced reduction in PTEN expression was reversed, indicating that this regulatory effect was dependent on the presence of AGO2 [Figure 2D].[143]

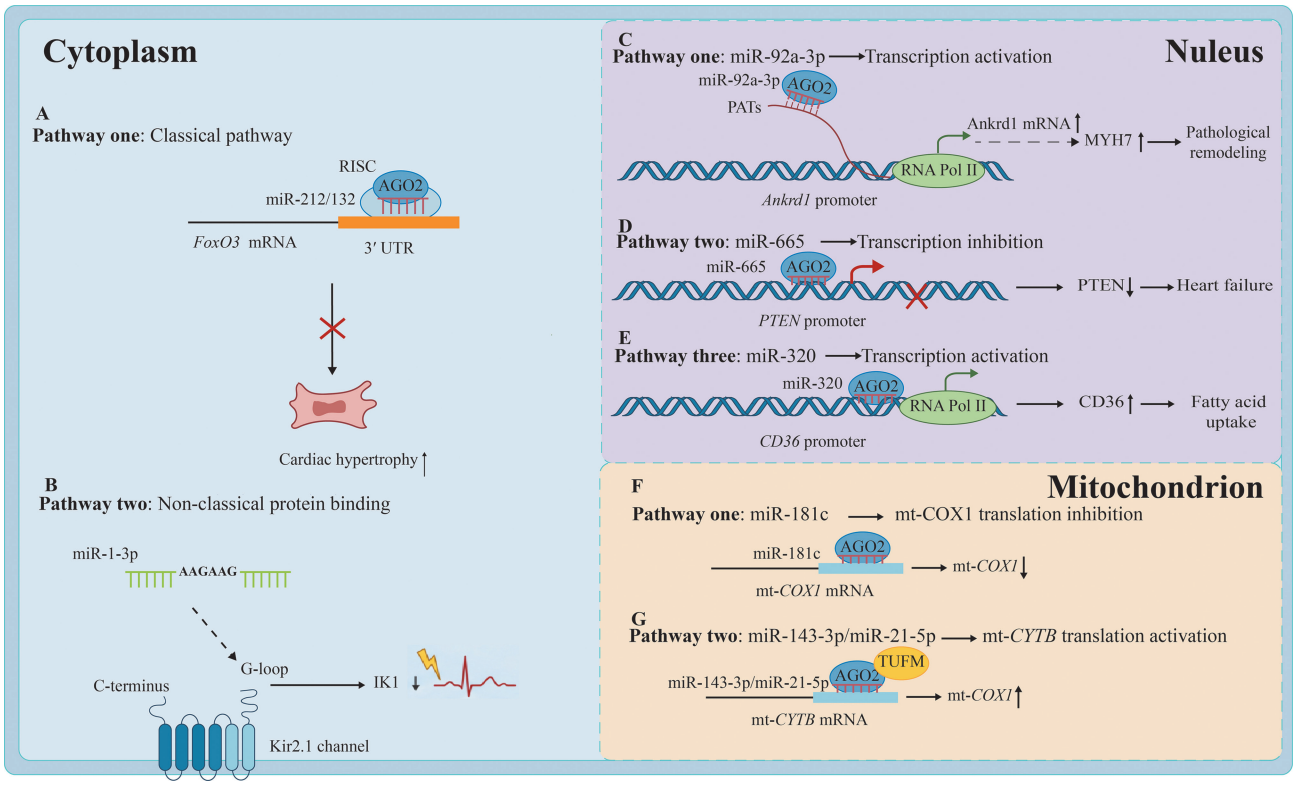

Figure 1: Subcellular compartment-specific miRNA mechanisms in cardiomyocytes during heart failures. (A) Classical cytoplasmic pathway. The miR-212/132 family, loaded onto AGO2 within the RISC complex, binds to the 3′ UTR of FoxO3 mRNA, repressing FoxO3 protein expression and thereby promoting cardiac hypertrophy. (B) Non-classical protein binding in the cytoplasm. miR-1-3p directly interacts with the Kir2.1 inward-rectifier potassium channel protein at its C-terminal G-loop domain via the non-seed core sequence AAGAAG, resulting in suppression of the IK1 current and predisposing to arrhythmia. (C) Nuclear transcriptional activation by miR-92a-3p. In the nucleus, miR-92a-3p binds to *Ankrd1* sense PATs, recruiting AGO2 and RNA Pol II to the *Ankrd1* promoter, thereby enhancing *Ankrd1* transcription. Upregulated nuclear ANKRD1 subsequently activates pathological MYH7 expression, contributing to cardiac remodeling. (D) Nuclear transcriptional inhibition by miR-665. miR-665, enriched in the cardiomyocyte nucleus, binds to the *PTEN* promoter in an AGO2-dependent manner, suppressing *PTEN* transcription and aggravating heart failure. (E) Nuclear transcriptional activation by miR-320-3p. In the diabetic heart, miR-320-3p forms a complex with AGO2 that translocates into the nucleus, functioning as an RNA-induced transcriptional activation (RITA) complex. This complex binds the *CD36* promoter and cooperates with RNA Pol II to activate *CD36* transcription, leading to increased fatty acid uptake. (F) Mitochondrial translational inhibition by miR-181c. Nuclear-encoded miR-181c is transported into the mitochondria, where it binds mt-*COX1* mRNA via AGO2, repressing mt-COX1 protein translation without affecting its mRNA level. (G) Mitochondrial translational activation by miR-143-3p/miR-21-5p. Mitochondrial AGO2, guided by miR-143-3p and miR-21-5p, recruits the translation elongation factor TUFM to enhance translation of mt-*CYTB* from the mitochondrial genome. During diabetic cardiomyopathy, decreased expression of these miRNAs and reduced mitochondrial AGO2 entry leads to mt-*CYTB* suppression, mt-ROS accumulation, and heart failure. Ankrd1: Ankyrin repeat domain 1; AGO: Argonaute; CD36: CD36 molecule; FoxO3: Forkhead box O3; lncRNAs: Long noncoding RNAs; miR: microRNA; miR-143-3p: microRNA-143-3p; miR-51-5p: microRNA-51-5p; miR-181c: microRNA-181c; miRNAs: microRNAs; mt-COX1: Mitochondrially encoded cytochrome c oxidase I; mt-CYTB: Mitochondrially encoded cytochrome b; MYH7: Myosin heavy chain 7; PATs: Promoter-associated transcripts; Pol II: RNA polymerase II; PTEN: Phosphatase and tensin homolog; RISC: RNA-induced silencing complex; TUFM: Mitochondrial translation elongation factor Tu; UTR: Untranslated region.

miR-320-3p was upregulated in both type 2 diabetes mellitus db/db mouse models and patients with heart failure. miR-320-3p was more abundant than other candidates such as miR-340-5p and miR-34a-5p in the heart. Furthermore, in multiple diabetes-related models, including db/db model and high-fat diet (HFD)-induced diabetic mice, miR-320-3p expression increased before cardiac dysfunction. Further experiments showed that overexpression of miR-320-3p exacerbated heart failure, while its inhibition attenuated it in db/db mice. Through *in situ* hybridization, miR-320-3p was found to be located in the nucleus in cardiomyocytes. The cytoplasmic and nuclear fractions were separately examined, revealing that miR-320-3p expression in the nucleus was significantly upregulated, while its level in the cytoplasm remained unchanged. In the diabetic heart, miR-320-3p bound to AGO2. The complex was then activated and translocated into the nucleus, where it served as a RNA-induced transcriptional activation (RITA) complex to bind the promoter of *CD36* and, together with Pol II, activated *CD36* transcription, which was responsible for increased fatty acid uptake [Figure 1E].[144]

Mitochondrial miRNAs in cardiomyocytes

miR-181c is transcribed in the nucleus, assembled in the cytoplasm, and eventually transported to the mitochondria of cardiomyocytes. Immunoprecipitation experiments revealed that AGO2 protein in the mitochondria bound both mitochondrially encoded mitochondrially encoded cytochrome c oxidase I (mt-*COX1*) mRNA and miR-181c. Luciferase reporter assays demonstrated that miR-181c binds to the 3′ UTR of mt-*COX1* mRNA. Overexpression of miR-181c precursor in neonatal rat ventricular myocytes did not change the mRNA levels of mt-*COX1*, but significantly reduced its protein levels, indicating that miR-181c regulated mt-*COX1* primarily by repressing its translation. These results show that miRNAs enter mitochondria and regulate the mitochondrial genome under physiological conditions [Figure 1F].[135]

A previous study demonstrated that AGO2 exists in mitochondria.[132] This study showed that mitochondrial AGO2 in cardiomyocytes enhanced translation of mitochondrially encoded *CYTB* (mt-CYTB) from the mitochondrial genome with the help of miR-143-3p and miR-21-5p to recruit mitochondrial translation elongation factor Tu (TUFM). During diabetic cardiomyopathy, the expression of miR-143-3p and miR-21-5p and the entry of AGO2 into mitochondria were decreased, resulting in suppression of mt-CYTB. The decreased mt-CYTB caused mitochondrial reactive oxygen species (mt-ROS) accumulation and diabetic heart failure [Figure 1G].[84]

Cytoplasmic miRNAs in fibroblasts

In the β1-adrenergic receptor transgenic mouse model of heart failure, miRNA profiling of hearts shows that miR-21 was most prominently upregulated during the progression of heart failure. miR-21 is also found to be markedly upregulated in failing hearts of humans. Using *in situ* hybridization and separate analysis of cardiac cell components, miR-21 was found to be expressed mainly in cardiac fibroblasts, while its expression in cardiomyocytes was extremely low. In fibroblasts, miR-21 inhibitors increased the apoptosis of these cells. In transgenic mice with cardiomyocyte-specific overexpression of miR-21, no significant phenotypic changes were observed, indicating that miR-21 had limited functions in cardiomyocytes. In the TAC mouse model, inhibition of miR-21 with antagomir-21 remarkably improved cardiac fibrosis and cardiac dysfunction. Bioinformatics analysis identified sprouty RTK signaling antagonist 1 (SPRY1) as a downstream target of miR-21. Luciferase reporter assays showed that the luciferase activity of a vector containing the 3′ UTR of SPRY1 3′ UTR decreased significantly when miR-21 was co-transfected, demonstrating direct binding. However, miR-21 did not significantly reduce *SPRY1* mRNA levels, but it markedly inhibited SPRY1 protein expression, indicating that miR-21 acted through translational repression.[145]

Nuclear miRNAs in fibroblasts

Mature miR-9 is detected in the nucleus and is enriched at promoters and intronic regions. Sequence alignment shows that miR-126-5p, miR-9, and miR-29b-3p share a conserved sequence region reported as the nuclear shuttling motif from miR-29b-3p. Analysis of miR-9 levels in cell fractionations of different cell types revealed that miR-9 was enriched in the nuclei of fibroblasts. Mechanistically, miR-9 helped the formation of G-quadruplex (G4) structures at super-enhancers and promoters of transforming growth factor beta 1 (TGF-β1)-responsive genes. Once TGFB1 signaling was stimulated, miR-9 promoted G4 formation, increased Histone H3 lysine 4 trimethylation (H3K4me3) modification, and chromatin looping, bringing super-enhancers and promoters into close physical proximity, thereby increasing gene transcription. Since TGFB1 signaling is a critical pathway in fibrosis, miR-9 plays a role in the fibrosis pathway.[145]

To provide a systematic overview of the subcellular localization and functional roles of miRNAs discussed above, Table 1 summarizes the key miRNAs implicated in heart failure, organized by cell type and subcellular compartment.[23,37,38,84,135,142,145–148,257,258] For each miRNA, the table lists its direct targets, functional consequences, and the experimental models used for validation.

Cytoplasmic miRNAs in endothelial cells

miR-15a and miR-16 are transcribed from the miR-15a/miR-16-1 cluster; they are induced during ischemia. The analysis of miRcode showed that miR-15a and miR-16 can bind to the CDS of Tie2 mRNA. Further experiments revealed that miR-15a and miR-16 overexpression decreased Tie2 protein expression but did not affect Tie2 mRNA levels. In human umbilical vein endothelial cells, Tie2 was an angiogenesis regulator. Therefore, miR-15a and miR-16 inhibited post-ischemic angiogenesis by suppressing Tie2 in a non-classical way [Figure 2A].[147]

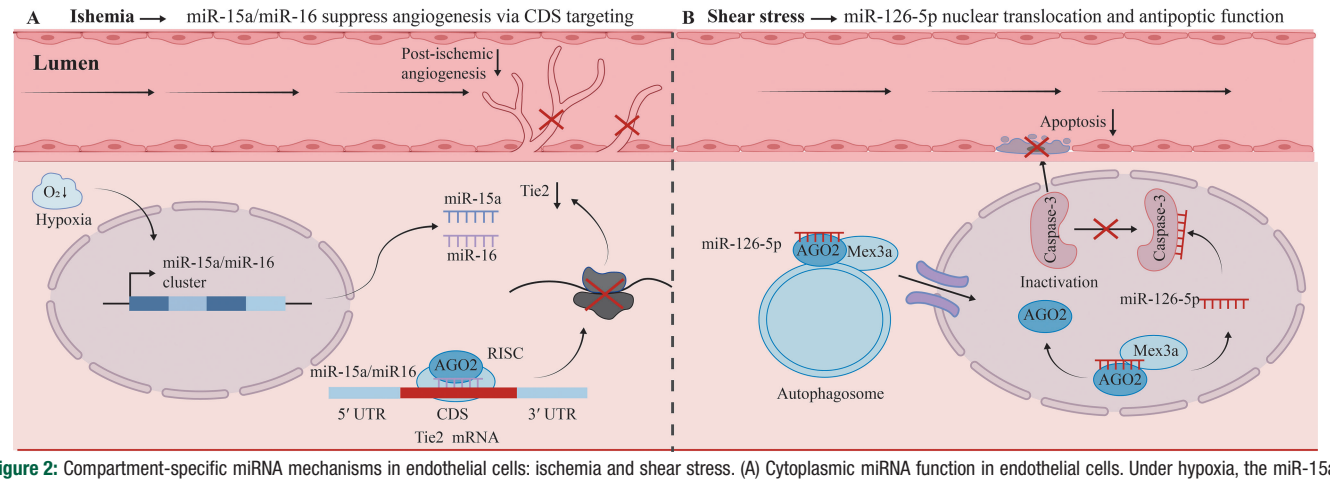

Figure 2: Compartment-specific miRNA mechanisms in endothelial cells: ischemia and shear stress. (A) Cytoplasmic miRNA function in endothelial cells. Under hypoxia, the miR-15a/miR-16-1 cluster is activated. Mature miR-15a and miR-16 are loaded onto AGO2 within RISC and bind to the CDS region of Tie2 mRNA, repressing Tie2 protein expression without affecting its mRNA level, thereby inhibiting post-ischemic angiogenesis. (B) Nuclear miRNA function in endothelial cells. Under shear stress, miR-126-5p binds AGO2 and Mex3a on the surface of autophagosomes. The complex is transported into the nucleus via Mex3a. Once inside, miR-126-5p dissociates from AGO2 and directly binds to caspase-3 in an aptamer-like manner, preventing its dimerization and activation, thereby inhibiting endothelial cell apoptosis. AGO: Argonaute; CDS: Coding sequence; lncRNAs: Long noncoding RNAs; miR: microRNA; miRNAs: MicroRNAs; RISC: RNA-induced silencing complex; UTR: Untranslated region.

Table 1: Subcellular localization and functions of key miRNAs in heart failure.

miRNA name	Cell type	Subcellular localization	Key function in HF	Direct target	Experimental model	Reference
miR-212/miR-132	Cardiomyocytes	Cytoplasm	Promote pathological cardiac hypertrophy; Increase BNP secretion	mRNA: 3′ UTR of FoxO3	*In vitro*: Neonatal rat cardiomyocytes; *in vivo*: mouse models	[38]
miR-1-3p	Cardiomyocytes	Cytoplasm	Direct biophysical suppression of IK1 current via Kir2.1 channel; Associated with arrhythmia risk	Protein: C-terminus of Kir2.1 channel	*In vitro*: cardiomyocytes	[142]
miR-92a-3p	Cardiomyocytes	Nucleus	Enhances *Ankrd1* transcription; Promotes pathological cardiac remodeling and MYH7 expression	Non-coding RNA: *Ankrd1* sense promoter-proximal transcripts; Recruits nuclear AGO2 to *Ankrd1* promoter DNA	*In vivo*: TAC mouse model	[37]
miR-665	Cardiomyocytes	Nucleus	Aggravates heart failure by suppressing PTEN transcription	DNA: *PTEN* gene promoter	*In vivo*: TAC mouse model; *in vitro*: cardiomyocytes treated with phenylephrine	[257]
miR-320	Cardiomyocytes	Nucleus	Forms RITA complex with AGO2; Activates CD36 transcription; Increases fatty acid uptake in diabetic cardiomyopathy	DNA: *CD36* gene promoter	*In vivo*: db/db mice; HFD-induced diabetic mic	[258]
miR-181c	Cardiomyocytes	Mitochondria	Represses translation of mitochondrial-encoded mt-*COX1*; Regulates mitochondrial function under physiological conditions	mRNA: 3′ UTR of mitochondrial mt-*COX1*	*In vitro*: neonatal rat ventricular myocytes	[135]
miR-143-3p/miR-21-5p	Cardiomyocytes	Mitochondria	Enhance translation of mitochondrial mt-*CYTB* by recruiting TUFM via mitochondrial AGO2; Decreased expression leads to mt-ROS accumulation and diabetic heart failure	mRNA: mitochondrial mt-*CYTB*; Recruits TUFM	*In vivo*: diabetic cardiomyopathy models	[84]
miR-21	Fibroblasts	Cytoplasm	Promotes cardiac fibrosis and dysfunction; Inhibits fibroblast apoptosis; Represses translation of *SPRY1*	mRNA: 3′ UTR of *SPRY1*; Translational repression without mRNA degradation	*In vivo*: β1-adrenergic receptor transgenic mice; TAC mouse model; failing human hearts	[146]
miR-9	Fibroblasts	Nucleus	Promotes fibrosis by facilitating G4 structure formation at super-enhancers and promoters of TGFB1-responsive genes; Increases H3K4me3 modification and chromatin looping	DNA/Chromatin: super-enhancers and promoters of TGFB1-responsive genes; Promotes G4 structure formation	*In vitro*: fibroblasts	[145]
miR-15a/miR-16	Endothelial cells	Cytoplasm	Inhibit post-ischemic angiogenesis by suppressing Tie2 protein expression	mRNA: CDS of *Tie2* mRNA; Protein suppression without mRNA level change	*In vitro*: human umbilical vein endothelial cells (ischemia model)	[147]
miR-126-5p	Endothelial cells	Nucleus	Inhibits cell apoptosis under shear stress by preventing caspase-3 dimerization and activation	Protein: Caspase-3; Direct binding in aptamer-like manner using seed sequence; Dissociates from AGO2 in nucleus	*In vitro*: endothelial cells (shear stress conditions); complex formation with AGO2 and Mex3a	[23,148]

AGO: Argonaute; CD36: Cluster of differentiation 36; CDS: Coding sequence; FoxO3: Forkhead box O3; HF: Heart failure; HFD: High-fat diet; IK1: Inward rectifier potassium current 1; lncRNAs: Long noncoding RNAs; miR: MicroRNA; miRNAs: microRNAs; mt-COX1: Mitochondrially encoded cytochrome c oxidase I; mt-CYTB: Mitochondrially encoded cytochrome b; mt-ROS: Mitochondrial reactive oxygen species; MYH7: Myosin heavy chain 7; PTEN: Phosphatase and tensin homolog; RISC: RNA-induced silencing complex; RITA: RNA-induced transcriptional activation; SPRY1: Sprouty RTK signaling antagonist 1; TAC: Transverse aortic constriction; TGFB1: Transforming growth factor beta 1; TUFM: mitochondrial translation elongation factor Tu; UTR: Untranslated region.

Nuclear miRNAs in endothelial cells

Under conditions of shear stress, miR-126-5p binds to AGO2 and interacts with another protein, Mex3a. This complex forms on the surface of autophagic vesicles and is transported into the nucleus with the help of Mex3a. Once translocated, using its own seed sequence in an aptamer-like manner, miR-126-5p dissociates from AGO2 and directly binds to the histone protease, caspase-3, preventing it from dimerization and activation. Thus, cell apoptosis is inhibited [Figure 2B].[23,148]

Functions of lncRNAs in Different Subcellular Compartments

lncRNA function in the nucleus

lncRNA function in *cis*

Many lncRNAs are not randomly located within the genome, but in specific conserved patterns such as being antisense to, or divergent from, protein-coding genes. This suggests the unique biological significance of lncRNA organization wherein lncRNAs may have evolved to

conveniently regulate the expression of neighboring genes, to adapt to different physiological or environmental conditions.[136] Divergent lncRNAs, widespread in mammalian genomes, are a typical example. They share the same promoter region with a protein-coding gene or are located adjacent to the same promoter, forming a "head-to-head" structure. However, the lncRNAs and protein-coding genes are transcribed in opposite directions. This is a common structural basis for *cis*-regulation.[137] *cis*-regulation can occur in three modes: DNA-elements-dependent regulation in *cis*, transcript-dependent regulation in *cis*, and transcription- or splicing-dependent regulation in *cis*.

DNA-elements-dependent regulation in *cis*

Here, the regulation is actually caused by DNA regulatory elements within the lncRNA promoter region or the lncRNA genes themselves and is neither related to the process of transcription nor the presence of RNA. In this case, the deletion of the DNA element rather than the knockdown at the RNA level has an impact.

To confirm this kind of regulation, certain lncRNAs must be deleted at the DNA level, inhibited at the transcriptional level, and suppressed at the RNA level, followed by a careful observation of the changes in expressions of neighboring genes. *Meteor* lncRNA is an example; by analyzing the three-dimensional interactions between embryonic stem cell (ESC)-specific enhancers and mesendoderm-specifying TF loci, an important enhancer region called *Meteor* was identified, where lncRNAs were also transcribed. When the *Meteor* locus was deleted, ESCs differentiated into the neuroectoderm rather than mesendoderm during development, with a global change in gene expression profiles. When transcription at the *Meteor* locus was activated by guide RNAs (gRNAs), expression of both *Meteor* lncRNAs and downstream target genes related to mesendoderm specification was increased. To prematurely terminate *Meteor* lncRNA transcription, a poly(A) signal was inserted downstream of the lncRNA TSS without disturbing the integrity of the DNA enhancer. The results showed that disrupting *Meteor* lncRNA expression did not affect target gene expression. Next, the short interfering RNA (siRNA) was used to specifically decrease the expression of the *Meteor* lncRNA without influencing DNA integrity and transcription activity; it did not affect the expression of target genes. This suggests that the expression of the lncRNA itself does not matter. It is the *Meteor* DNA enhancer region, rather than the lncRNA transcript, which is essential for the regulation of targeted genes related to mesendoderm specification [Figure 3A].[138]

Transcription-dependent regulation in *cis*

The regulatory functions of some lncRNA loci mainly rely on the processes of transcription or splicing themselves, rather than on the mature lncRNA molecules or their sequences. These processes can regulate the expression of

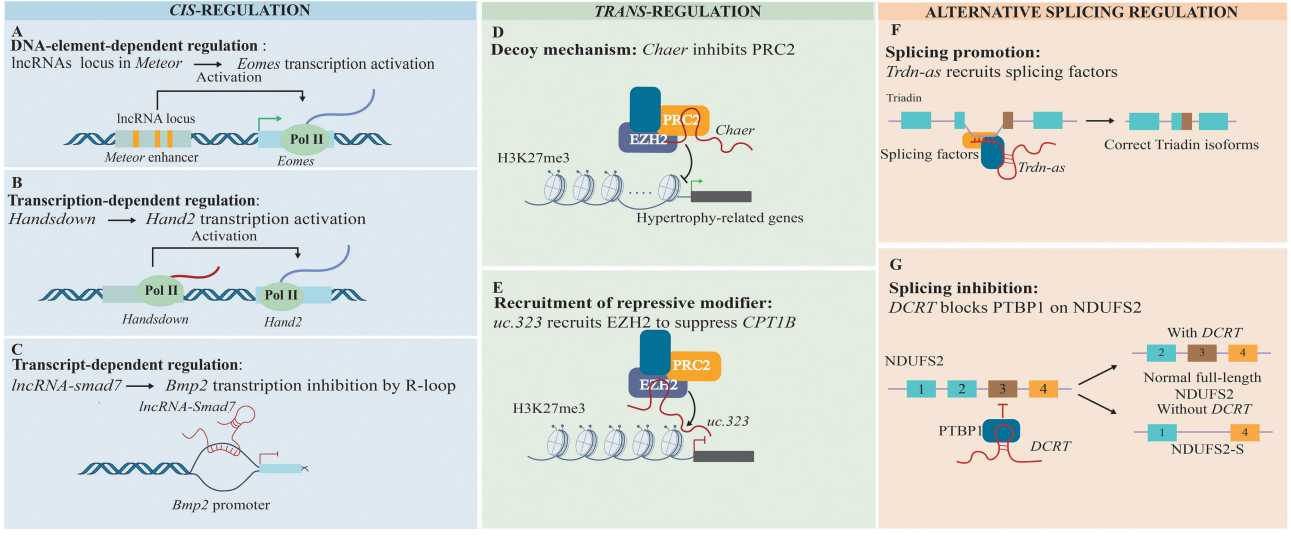

Figure 3: Nuclear lncRNAs regulate gene expression through *cis*-regulation, *trans*-regulation, and alternative splicing modulation during heart failure. (A) DNA-element-dependent *cis*-regulation. The *Meteor* locus contains a DNA enhancer element from which lncRNAs are also transcribed. The enhancer element itself, rather than the lncRNA transcript, drives transcriptional activation of neighboring mesendoderm-specifying genes such as *Eomes*. (B) Transcription-dependent *cis*-regulation. Transcriptional activation at the *Handsdown* (*Hdn*) locus represses the expression of the neighboring *Hand2* gene. This regulation depends on the act of transcription by Pol II at the *Hdn* locus, not on the *Hdn* lncRNA transcript itself. (C) Transcript-dependent *cis*-regulation via R-loop formation. *lncRNA-Smad7* forms an R-loop structure with single-stranded DNA at the *Bmp2* promoter, mediating transcriptional repression of *Bmp2* and promoting cardiomyocyte differentiation. (D) *Trans*-regulation by decoy mechanism. lncRNA *Chaer* directly binds to the catalytic subunit EZH2 of the PRC2 complex, sequestering PRC2 away from hypertrophy-related gene loci. This reduces H3K27me3 repressive marks and facilitates the activation of hypertrophy-related genes. (E) *Trans*-regulation by recruitment of repressive modifiers. lncRNA *uc.323* binds to EZH2 and recruits the PRC2 complex to the *CPT1b* promoter, increasing H3K27me3 modification and suppressing *CPT1b* transcription, thereby protecting against cardiac hypertrophy. (F) Splicing promotion. *Trdn-as* directly binds to splicing factors (SRSF1, SRSF10) and the Triadin precursor mRNA simultaneously, recruiting splicing factors to ensure the production of correct Triadin protein isoforms essential for cardiac calcium homeostasis. (G) Splicing inhibition. lncRNA *DCRT* binds to the RNA-binding protein PTBP1, blocking PTBP1-mediated exon 3 skipping of NDUFS2 pre-mRNA. In the presence of *DCRT*, normal full-length NDUFS2 is produced; without *DCRT*, aberrant exon skipping generates the pathological NDUFS2-S isoform associated with mitochondrial damage in DCM. CPT1: Carnitine palmitoyltransferase 1; DCM: Dilated cardiomyopathy; DCRT: Dilated cardiomyopathy repressive transcript; EZH2: Enhancer of Zeste Homolog 2; lncRNAs: Long noncoding RNAs; miRNAs: microRNAs; NDUFS2: NADH:Ubiquinone Oxidoreductase Core Subunit S2; Pol II: RNA polymerase II; PRC2: Polycomb Repressive Complex 2; PTBP1: Polypyrimidine Tract Binding Protein 1; RISC: RNA-induced silencing complex; TF: Transcription factor; UTR: Untranslated region.

neighboring genes by affecting chromatin state, TF binding, or other mechanisms.

Some enhancer RNAs act in this manner. The difference between transcript-dependent regulation and DNA-element-dependent regulation is that the activation of transcription affects the expression of neighboring genes. *Handsdown* (*Hdn*) is an example in cardiomyocytes. The lncRNA locus *Hdn* in mice was identified close to *Hand2* gene locus and was found to be expressed early in cardiac cells. When the *Hdn* locus was deleted, *Hand2* gene expression increased, indicating that *Hdn* repressed *Hand2* in *cis*. When the *Hdn* transcript was significantly suppressed by GapmeRs, *Hand2* gene expression remained unchanged. However, when the *Hdn* locus was strongly activated by dCas9–VP64 with MS2–p65–HSF1 system, which significantly increased transcription without altering the genomic sequence, the expression of *Hand2* and related genes decreased. Conversely, when $Hdn^{pA\text{-}MAZ/pA\text{-}MAZ}$ was used to terminate *Hdn* transcription, the expression of *Hand2* increased, which was the opposite effect of *Hdn* transcription activation. The key finding was that the transcriptional activation of the *Hdn* locus itself, rather than the function of the lncRNA that it produced, can repress the expression of the neighboring *Hand2* gene [Figure 3B].[139]

Transcript-dependent regulation in *cis*

Chromatin exists in two states: active and repressive. The active chromatin is permissive to transcription, whereas repressive chromatin is less accessible for transcription. These states determine whether a gene can be expressed. lncRNAs can help bring chromatin modifiers such as architectural proteins or histone-modifying enzymes to the regions near neighboring genes, making this part of the DNA either active or silent. Through this mechanism, lncRNAs regulate the expression of nearby genes locally. The typical feature of this mechanism is that the deletion of lncRNA locus and premature transcriptional termination of lncRNA do not influence the expression of neighboring genes, but siRNAs or ASOs do.

lncRNA function in trans

Besides affecting nearby genes, lncRNAs also regulate distant genes. There are two ways through which they can modify the chromatin environment; indirectly, they rely on proteins that are able to bind both RNA and DNA, or directly, they target specific DNA sequences for binding.

Protein–lncRNA interaction on chromatin

Many lncRNAs are directly located in the chromatin and interact with chromatin-associated proteins. Such interactions can either promote or inhibit the binding and function of these proteins at specific DNA regions, thereby regulating gene expression. For instance, lncRNAs recruit histone-modifying enzymes that activate gene expression or factors that cause gene silencing. In addition, some lncRNAs act as "decoys" that sequester proteins that repress gene expression, thereby promoting gene expression.

DNA–lncRNA interaction on chromatin

An important characteristic of lncRNAs is that they can form hybrid structures with DNA, which influence chromatin accessibility. This kind of interaction usually occurs as triplexes or R-loops. The key distinguishing feature between the triplex and R-loop structures is that the R-loop is sensitive to the digestion of RNase H, while the triplex is not.

lncRNA-Smad7 is an example of an identifying feature of the R-loop structure in promoter regions. In mouse embryonic stem cells, knockdown of *lncRNA-Smad7* did not affect stem cell survival or basic characteristics but prevented them from cardiomyocyte differentiation in terms of increased proliferation but poor maturation. This demonstrated that *lncRNA-Smad7* is essential for promoting the maturation of stem cells into mature cardiomyocytes. Electrophoretic mobility shift assay (EMSA) showed that *lncRNA-Smad7* preferred to bind to the $ss(TG)_{17}$ repeat in single-stranded DNA rather than to the $ds(CA{:}TG)_{17}$ in double-stranded DNA form, in the *Bmp2* promoter *in vitro*. In *in vitro* EMSA experiments, the complex formed by *lncRNA-Smad7* and *Bmp2* promoter DNA was recognized by the S9.6 antibody, showing a supershift, and was eliminated by RNase H treatment. This result demonstrated that *lncRNA-Smad7* and *Bmp2* promoter DNA formed an R-loop rather than a triplex due to susceptibility to RNase H digestion. In DNA–RNA immunoprecipitation followed by quantitative PCR (DRIP-qPCR) and DNA–RNA immunoprecipitation sequencing (DRIP-seq) experiments, the R-loop in the *Bmp2* promoter region was dependent on the presence of *lncRNA-Smad7*. The R-loop that *lncRNA-Smad7* formed at the *Bmp2* promoter region mediated transcriptional repression of *Bmp2* [Figure 3C].[28]

Roles in alternative splicing

RNA splicing is a highly-regulated process, where spliceosomes, *cis*-acting elements, and trans-acting splicing factors are all involved and cooperate with each other to remove introns and join exons together to produce a mature mRNA.[140–141]

Recent studies have found that during the progression of heart failure, lncRNAs regulate alternative splicing events. For example, lncRNAs may recruit and stabilize splicing factors to promote the splicing of precursor mRNAs, or compete with splicing factors for binding, thereby preventing these factors from associating with their target pre-mRNAs.

lncRNA function in cytoplasm

Interaction with proteins and RNAs

lncRNAs influence protein function by interacting with RNA-binding motifs or specific structures of proteins and forming lncRNPs, which in turn regulate mRNA degradation and translation. In addition, lncRNAs interact with other RNAs by base pairing, subsequently recruiting protein complexes to promote mRNA degradation, maintain

its stability, or influence translation, ultimately regulating the fate of mRNA.

miRNA sponges

An important post-transcriptional regulatory function of lncRNAs is acting as miRNA sponges. lncRNAs contain multiple binding sites that are complementary to miRNAs, allowing them to sequester miRNAs and reduce the opportunities for miRNAs to bind to their original mRNA targets. As miRNAs normally suppress mRNA expression and promote its degradation, lncRNAs that absorb miRNAs may indirectly increase the expression levels of mRNA targets of miRNAs. Whether lncRNAs effectively serve as miRNA sponges depends on their own abundance and their quantitative relationship with miRNAs. If the expression levels of lncRNAs are much lower than those of miRNAs, their sponge effect is limited. It is when their expression is sufficiently high that they may have a significant influence on gene expression.

lncRNA in mitochondria

Some lncRNAs are localized in mitochondria and encoded either by nuclear genes or by the mitochondrial genome itself.[149–150] These lncRNAs play important roles in regulating mitochondrial energy metabolism, programmed cell death, and communication between the mitochondria and the nucleus.[151] Studies have shown that lncRNAs influence the development of heart failure by regulating mitochondrial activity.

lncRNAs in Different Subcellular Localizations in Heart Failure

Having established the diverse molecular mechanisms that lncRNAs can execute in different subcellular compartments, we now examine how these compartment-specific functions are engaged during heart failure. In addition to being classified based on their genomic location, lncRNAs can also be divided into two categories according to their cellular functions: those that act in *cis*, affecting the expression and/or chromatin state of neighboring genes, and those that work in *trans*, having functions at distant genomic loci or in different subcellular compartments. In the following sections, we organize lncRNAs by cell type and subcellular compartment to illustrate how the same organizational principle demonstrated for miRNAs that subcellular localization fundamentally determines functional output, applies equally to lncRNAs in the context of heart failure.

Nuclear lncRNAs in cardiomyocytes

In contrast to the post-transcriptional mechanisms operating in the cytoplasm, nuclear lncRNAs in cardiomyocytes regulate gene expression at the transcriptional and co-transcriptional levels. As described earlier, these mechanisms include recruiting or sequestering chromatin-modifying complexes, modulating alternative splicing by interacting with splicing factors and pre-mRNAs, and forming direct RNA–DNA interactions at specific genomic loci. During pathological cardiac remodeling, these nuclear mechanisms are co-opted to drive aberrant epigenetic reprogramming and splicing dysregulation that are central to heart failure progression.

During the progression of cardiomyocyte hypertrophy, lncRNA *Chaer* functioned in *trans* in the nucleus, acting as a decoy to prevent inhibitory proteins from approaching hypertrophy-related genes. Among the abnormally expressed lncRNAs detected in the hearts of mice subjected to pressure overload, lncRNA *Chaer* was highly enriched in the heart and displayed significant dynamic changes during disease progression. When *Chaer* gene was deleted by clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9, knockout mice showed no cardiac phenotypes under resting conditions. However, under pressure overload, these mice showed greatly attenuated cardiac hypertrophy, fibrosis, and dysfunction, indicating that *Chaer* was essential for such pressure-induced cardiac pathology. The function of *Chaer* was independent of its neighboring gene *Hopx*, involved in *trans*-acting regulation. *Chaer* directly bound to the catalytic subunit enhancer of zeste homolog 2 (Ezh2) of the PRC2 complex, which is responsible for epigenetic modification. The binding of *Chaer* to PRC2 disturbed the normal localization of PRC2 within the genome. As a result, the histone modification H3K27me3 mediated by PRC2, a signature of gene silencing, was also suppressed. Therefore, the inhibition of methylation by *Chaer* facilitated the activation of hypertrophy-related genes [Figure 3D].[30]

uc.323 is a protective lncRNA in cardiac hypertrophy, which acts in *trans* in the nucleus to recruit repressive chromatin modification enzymes to inhibit the expression of hypertrophy-related genes. Transcribed ultraconserved regions (T-UCRs) are lncRNAs transcribed from ultraconserved regions (UCRs) in the genome. These regions possess completely identical DNA sequences across mammals. Therefore, they may be associated with important and conserved biological functions. Microarray analysis showed that expression of uc.323, a T-UCR, decreased during cardiac hypertrophy in a TAC mouse model. *In vitro* experiments demonstrated that high expression of uc.323 protected cardiomyocytes from hypertrophy, while *uc.323* knockout mice developed cardiac hypertrophy. *uc.323* was mainly localized in the nucleus and directly bound to EZH2, a histone methyltransferase and core component of the PRC2 complex, to increase H3K27me3 of CPT1b, inhibiting its transcription. As a result, cardiac hypertrophy was suppressed [Figure 3E].[152]

Trdn-as is an lncRNA that functions in the nucleus to promote alternative splicing of *Triadin* by recruiting splicing factors to the precursor mRNA of *triadin*. Abnormal calcium homeostasis was associated with arrhythmias and heart failure, and the triadin protein played an important role in maintaining Ca^{2+} homeostasis. Multiple isoforms were generated from *Triadin* through alternative splicing, and the correct ratio of these isoforms was crucial for maintaining cardiac health. *Trdn-as*, an lncRNA specifically expressed in cardiomyocytes, was found to regulate the alternative splicing of *Triadin*. Mice with knockout of *Trdn-as* were more likely to develop calcium metabolism disorders, arrhythmias, and premature death. Mechanistically, *Trdn-as* can directly bind to splicing factors such

as SRSF1, SRSF10, and the precursor mRNA of *triadin* through base pairing, simultaneously. By recruiting splicing factors to the *triadin* precursor mRNA, *Trdn-as* ensured the production of proper triadin protein isoforms, protecting cardiac function [Figure 3F].[153]

Different from *Trdn-as*, lncRNA *DCRT* prevented alternative splicing by blocking the interaction of polypyrimidine tract binding protein 1 (PTBP1) and precursor mRNA of *NDUFS2*, which encodes NADH:ubiquinone oxidoreductase core subunit S2. DCM is characterized by left ventricular enlargement and persistent systolic dysfunction. The lncRNA *DCRT* was found to be highly enriched in normal heart tissue; however, its expression was significantly decreased in the myocardium of patients with DCM. *DCRT* knockout mice were generated by CRISPR-Cas9 technology. They spontaneously developed cardiac dysfunction and heart enlargement, accompanied by mitochondrial damage. Chromatin isolation by RNA purification assays identified that *DCRT* RNA mainly bound to the RNA-binding protein PTBP1, and they colocalized in the nucleus of cardiomyocytes. PTBP1 is an RNA-binding protein that primarily participates in exon skipping during pre-mRNA splicing. Through single-molecule long-read sequencing, RNA immunoprecipitation sequencing (RIP-seq), and quantitative polymerase chain reaction (qPCR), it was found that *DCRT* inhibited PTBP1-induced skipping of the third exon of the *NDUFS2* gene. Aberrant splicing of NDUFS2 produced a short isoform, namely NDUFS2-S. The levels of this abnormal NDUFS2-S isoform were significantly increased in DCM models and hearts of human patients, mediating mitochondrial damage in cardiomyocytes [Figure 3G].[154]

Cytoplasmic lncRNAs in cardiomyocytes

As described in the preceding sections, cytoplasmic lncRNAs regulate gene expression through multiple post-transcriptional mechanisms, including direct interaction with proteins to modulate mRNA stability and translation, base-pairing with mRNAs to recruit regulatory complexes, and sequestering miRNAs as ceRNAs/sponges. In the context of cardiomyocyte hypertrophy and dysfunction, all three of these cytoplasmic mechanisms are actively engaged, as illustrated by the following examples.

The lncRNA *CARDINAL* was found to be specifically expressed in the heart and inhibited whole mRNA translation by interfering with the function of developmentally regulated GTP-binding protein 1 (DRG1), a ribosome-associated protein that promoted mRNA translation. *CARDINAL* bound to DRG1 and prevented DRG1 from forming a complex with its chaperone DFRP1. Formation of the DRG1–DFRP1 complex stabilized DRG1 protein and promoted translation in the whole cell. *CARDINAL* inhibited the formation of this complex, resulting in decreased DRG1 protein levels and thus helped maintain protein translation at normal levels. Under stress conditions such as hypertension, the expressions of both *CARDINAL* and DRG1 were upregulated. Although *CARDINAL* continued to suppress protein translation in cardiomyocytes by binding with DRG1, it could no longer fully counteract the translation-promoting effect of DRG1 due to the greater increase in DRG1 levels. This led to an overall increase in protein translation and protein accumulation in cardiomyocytes, resulting in cardiac hypertrophy [Figure 4A].[31]

lncRNA *CHKB-DT* functioned in the cytoplasm of cardiomyocytes by recruiting proteins to stabilize the mRNA of *ALDH2*, which encodes aldehyde dehydrogenase 2. Expression of lncRNA *CHKB-DT* was significantly decreased both in patients with dilated cardiomyopathy (DCM) and in mice with heart failure induced by TAC. Heterozygous knockout of *CHKB-DT* in cardiomyocytes led to cardiac enlargement and dysfunction and reduced the contractile capacity of primary cardiomyocytes. Mechanistically, *CHKB-DT* physically binds to *ALDH2* mRNA. Through separate truncation experiments on *CHKB-DT* and *ALDH2* mRNA, it was found that the 700–1218 nt sequence of *CHKB-DT* directly binds to the 1–500 nt sequence of *ALDH2* mRNA. By recruiting the fused in sarcoma (FUS) protein via a "GGUG" motif, *CHKB-DT* protected *ALDH2* mRNA from degradation [Figure 4B].[32]

lncRNA *Gm15834* acted as a miRNA sponge in the cytoplasm of cardiomyocytes. It was upregulated *in vivo* in the TAC mouse model and *in vitro* in the angiotensin II (AngII)-induced cardiac hypertrophy model. lncRNA *Gm15834* was mainly localized in the cytoplasm of cardiomyocytes. It contained binding sites for miR-30b-3p, preventing the suppression of the downstream of miR-30b-3p, autophagy protein Unc-51-like kinase 1 (ULK1), which led to enhanced autophagy and aggravated cardiac hypertrophy [Figure 4C].[33]

Mitochondrial lncRNAs in cardiomyocytes

Beyond the nucleus and cytoplasm, a subset of lncRNAs localizes to the mitochondria, where they regulate metabolic enzyme activity through direct protein binding and allosteric modulation. Given that metabolic remodeling is a hallmark of heart failure, mitochondrial lncRNAs represent a mechanistically distinct layer of regulation that cannot be captured by studies that are focused solely on nuclear or cytoplasmic compartments.

In one study, mouse hearts were fractionated into mitochondrial and non-mitochondrial components. A mitochondria-enriched lncRNA was identified by RNA-seq, referred to as *mitolnc*. *mitolnc* was knocked out by two different strategies, either by deleting the *mitolnc* locus or by inserting a poly(A) cassette into its first exon. Neither approach affected the expression of the neighboring gene *PCBP1* nor caused any obvious developmental abnormalities. Mechanistically, *mitolnc* contained a conserved sequence motif in its first exon. However, *mitolnc* directly bound to branched-chain keto acid dehydrogenase E1 subunit alpha (BCKDHA), a key subunit of the branched-chain α-keto acid dehydrogenase (BCKDH) complex, without altering the mitochondrial localization or increasing the inhibitory phosphorylation of BCKDHA. Instead, *mitolnc* enhanced BCKDH function through allosteric activation. In other words, *mitolnc* bound to the enzyme and induced conformational changes that increased enzymatic activity. Inhibition of *mitolnc* led

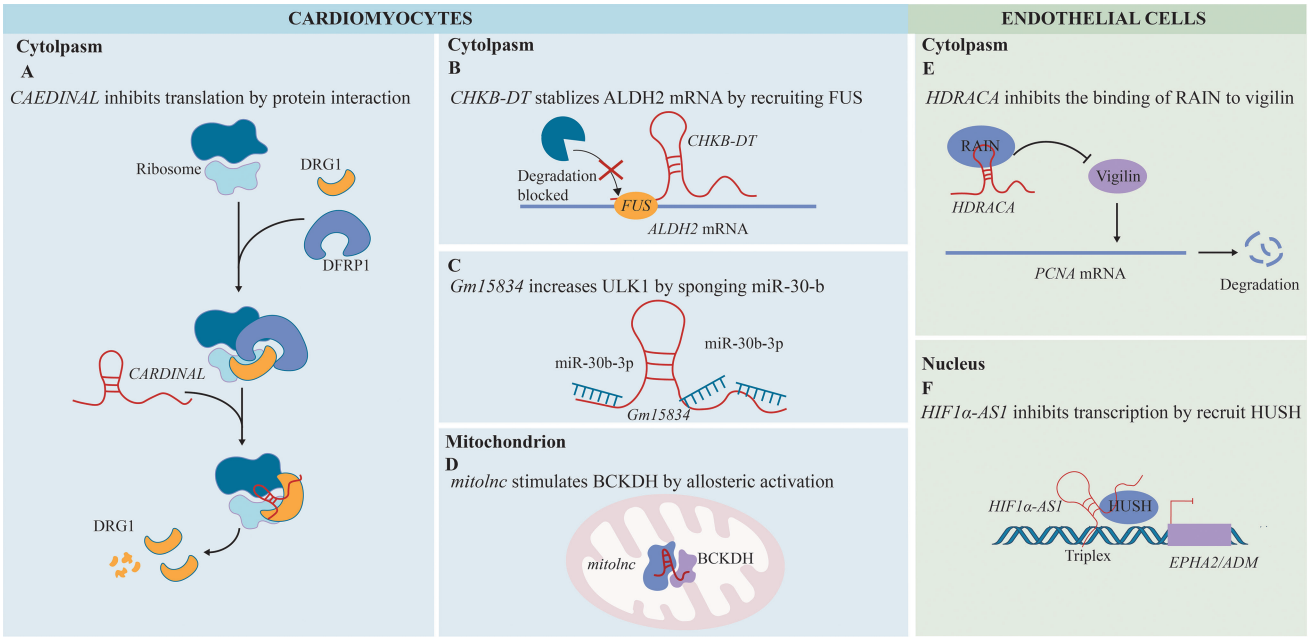

Figure 4: lncRNAs regulate heart failure through distinct mechanisms in different subcellular compartments of cardiomyocytes and endothelial cells. (A) Cytoplasmic lncRNA *CARDINAL* inhibits translation by protein interaction in cardiomyocytes. *CARDINAL* binds to the ribosome-associated protein DRG1, preventing DRG1 from forming a complex with its chaperone DFRP1. This leads to decreased DRG1 protein levels and reduced overall mRNA translation, maintaining normal protein homeostasis in cardiomyocytes. (B) Cytoplasmic lncRNA *CHKB-DT* stabilizes ALDH2 mRNA in cardiomyocytes. *CHKB-DT* physically binds to ALDH2 mRNA and recruits the FUS protein, blocking ALDH2 mRNA degradation and protecting cardiac function. (C) Cytoplasmic lncRNA *Gm15834* acts as a miRNA sponge in cardiomyocytes. Gm15834 contains multiple binding sites for miR-30b-3p, sequestering miR-30b-3p and relieving its suppression on the autophagy protein ULK1, leading to enhanced autophagy and aggravated cardiac hypertrophy. (D) Mitochondrial lncRNA *mitolnc* stimulates BCKDH by allosteric activation in cardiomyocytes. *mitolnc* directly binds to BCKDHA, a key subunit of the BCKDH complex, inducing conformational changes that enhance enzymatic activity and promote branched-chain amino acid catabolism. (E) Cytoplasmic lncRNA *HDRACA* inhibits angiogenesis in endothelial cells. When dysfunctional HDL fails to downregulate *HDRACA*, *HDRACA* binds to RAIN and blocks the interaction between RAIN and vigilin. Free vigilin then binds to PCNA mRNA, promoting its degradation and inhibiting angiogenesis. (F) Nuclear lncRNA *HIF1α-AS1* suppresses gene transcription in endothelial cells via triplex formation. *HIF1α-AS1* forms a triplex structure with double-stranded DNA at target gene loci and recruits the repressive HUSH complex, suppressing the expression of EPHA2 and ADM. ADM: Adrenomedullin; ALDH2: Aldehyde dehydrogenase 2; BCKDH: Branched-chain α-keto acid dehydrogenase; DRG: Developmentally regulated GTP binding protein 1; EPHA2: EPH receptor A2; FUS: Fused in sarcoma; HDL: High-density lipoprotein; HUSH: Human silencing hub PCNA: Proliferating cell nuclear antigen; RAIN: Ras-interacting protein 1; ULK1: Unc-51 Like Autophagy Activating Kinase 1.

to reduced BCKDH activity, resulting in the accumulation of BCAAs and ultimately causing cardiac hypertrophy. Therefore, *mitolnc* plays an important role in maintaining normal cardiac metabolism and physiology [Figure 4D].[34]

Cytoplasmic lncRNAs in endothelial cells

Endothelial dysfunction, characterized by impaired angiogenesis and increased vascular permeability, is a key contributor to heart failure progression. In the cytoplasm of endothelial cells, lncRNAs regulate angiogenic capacity through post-transcriptional mechanisms, particularly by modulating protein–protein interactions that control mRNA stability.

The lncRNA *HDRACA* plays a key role in high-density lipoprotein (HDL)-regulated angiogenesis. *HDRACA* expression was downregulated by the regulation of normal HDL, allowing the protein Ras-interacting protein 1 (RAIN) to bind to the protein vigilin and preventing it from binding to proliferating cell nuclear antigen (*PCNA*) mRNA. As a result, the stability and expression of *PCNA* mRNA were increased, which in turn promoted endothelial cell proliferation and angiogenesis. In contrast, *HDRACA* is not effectively downregulated by the dysfunctional HDL, resulting in binding to RAIN and blockage of the interaction between RAIN and vigilin. Therefore, vigilin was able to bind to *PCNA* mRNA, leading to decreased stability and expression of PCNA mRNA, thereby inhibiting angiogenesis. In summary, lncRNA *HDRACA* regulated the interaction between RAIN and vigilin, affecting the stability of *PCNA* mRNA and its capacity for angiogenesis [Figure 4E].[155]

Nuclear lncRNAs in endothelial cells

In the nucleus of endothelial cells, lncRNAs use distinct mechanisms from their cytoplasmic counterparts. As demonstrated later, nuclear lncRNAs form triplex structures with genomic DNA to recruit transcriptional repressor complexes, directly modulating the chromatin environment at specific gene loci. This mechanism is fundamentally different from the regulation of cytoplasmic mRNA stability described earlier, further illustrating how subcellular localization determines the mode of action of lncRNAs.

HIF1α-AS1 is an lncRNA that forms a triplex structure in endothelial cells, acting in *trans* to recruit inhibitory proteins to suppress gene expression. The Triplex-Seq technique was used to selectively identify lncRNAs involved in the formation of triplex structures. To exclude conventional RNA–DNA hybrids such as R-loops, RNase H was used to screen RNA–DNA complexes resistant to digestion. To further validate these candidate lncRNAs, RIP experiments were performed in human endothelial cells with an anti-double-stranded DNA antibody, with

or without RNase H treatment. After RNase H digestion, *HIF1α-AS1* was found to be the strongest triplex-associated lncRNA. *HIF1α-AS1* is located on the antisense strand of the *HIF1A* gene and is an oxygen-dependent lncRNA whose expression decreases under hypoxic conditions in endothelial cells. Acting as an adapter, *HIF1α-AS1* recruits a repressive complex known as human silencing hub (HUSH) to specific genomic loci, suppressing the expression of genes such as erythropoietin-producing hepatoma (EPH) receptor A2 (*EPHA2*) and Adrenomedullin (*ADM*) [Figure 4F].[29]

To summarize the diverse subcellular functions of lncRNAs in heart failure, Table 2 compiles the key lncRNAs discussed in this review, categorized by cell type and subcellular localization.[28,29,30–34,139,152–155] The table highlights their direct molecular targets, functional roles in heart failure pathogenesis, and the corresponding experimental evidence supporting each mechanism.

Integrative Conceptual Framework: A Compartment-Based Regulatory Model for ncRNAs

The mechanisms described earlier for miRNAs and lncRNAs can be unified into a compartment-based regulatory model [Figure 5]. In the cytoplasm, both miRNAs and lncRNAs converge on post-transcriptional control of mRNA fate. miRNAs act primarily through RISC-mediated translational repression and mRNA decay, and lncRNAs through direct mRNA stabilization/destabilization, protein sequestration, or as miRNA sponges. In the nucleus, miRNAs and lncRNAs both regulate transcription, but through fundamentally different molecular functions: miRNAs, guided by AGO2, interact with promoter-associated RNAs (paRNAs) or DNA to modulate TF access and histone modifications; lncRNAs, by contrast, exploit their greater length and structural complexity to act either in *cis* or in *trans*. Notably, the cis/*trans* distinction is largely specific to lncRNAs, reflecting their genomic positional relationship with target genes, a feature not shared by miRNAs, which are typically encoded at loci distant from their targets. In the mitochondria, miRNAs regulate mitochondrial gene expression by binding with AGO2, while lncRNAs affect metabolic enzyme activity by allosteric regulation.

Intercellular ncRNA Crosstalk Through Extracellular Vesicles in Heart Failure

In the previous sections, the subcellular functions of miRNAs and lncRNAs within individual cardiac cell types during heart failure have been discussed. However, these cell types do not function in isolation. The heart is a highly integrated organ in which cardiomyocytes, fibroblasts, and endothelial cells are in close physical proximity and engage in continuous bidirectional communication to maintain cardiac homeostasis. During heart failure progression, this intercellular crosstalk becomes dysregulated and contributes to pathological remodeling.

A major route for such communication is via extracellular vesicles (EVs), which include primarily exosomes (30–150 nm) and microvesicles (100–1000 nm). EVs transfer bioactive cargo, notably miRNAs and lncRNAs, from donor to recipient cells, enabling cell-type-to-cell-type reprogramming of gene expression and contributing to the pathogenesis of heart failure.[156]

Cardiomyocyte EVs may be enriched in miR-217 under pressure-overload conditions. Once delivered to fibroblasts, miR-217 drives fibroblast proliferation and collagen synthesis.[157] Besides miRNAs, cardiomyocyte-derived exosomal lncRNAs also participate in this process. lncRNA *Neat1*, elevated after myocardial ischemia, functions in recipient fibroblasts, promoting expression of profibrotic genes.[158] Activated cardiac fibroblasts also secrete EVs that directly remodel cardiomyocyte signaling, contributing to hypertrophy and dysfunction. An example is fibroblast-derived exosomal miR-21-3p. In cardiomyocytes, miR-21-3p silences sorbin and SH3 domain containing 2 (SORBS2) and PDZ and LIM domain 5 (PDLIM5), promoting cardiac hypertrophy.[159]

Endothelial cells and cardiomyocytes further engage in reciprocal EV-mediated ncRNA exchange that shapes cardiomyocyte survival and vascular angiogenesis. Endothelial exosomal miR-210 improves hypoxia tolerance by inhibiting ferroptosis.[160] miR-320 derived from cardiomyocytes impairs angiogenesis by suppressing the expression of insulin-like growth factor 1 (*IGF1*), heat shock protein family B (small) member 6 (*HSPB6*) , and ETS proto-oncogene 2 (*Ets2*).[161]

The functional outcome of an ncRNA is determined not only by its subcellular localization within the cell of origin but also by its subcellular destination in recipient cells following intercellular transfer. Several lines of evidence suggest that EV-delivered ncRNAs may function in multiple subcellular compartments of recipient cells, including the nucleus. For example, some miRNAs can translocate to the nucleus through a mechanism involving AGO2 in association with importin 8 and Mex3a. It is therefore possible that exosomal miRNAs, once released into the cytoplasm of recipient cells, may similarly be imported into the nucleus to exert transcriptional regulatory functions. Although direct experimental evidence for this specific hypothesis is currently lacking, the nuclear import machinery for miRNA–AGO2 complexes is well established, and the possibility of nuclear functions for EV-delivered miRNAs represents an exciting frontier. Similarly, exosomal lncRNAs, such as *Neat1*, that are known to function in the nucleus, may potentially be re-imported into the nucleus of recipient cells to exert chromatin- or splicing-regulatory functions. The subcellular sorting mechanisms that determine whether an EV-delivered ncRNA remains in the cytoplasm or is transported to the nucleus and mitochondria of the recipient cell remain largely unknown and represent a critical knowledge gap.

Clinical Implications of miRNAs and lncRNAs

miRNAs and lncRNAs as biomarkers of heart failure

The sensitive expression changes in miRNAs and lncRNAs in pathological tissues make them ideal biomarkers

Table 2: Subcellular localization and functions of key lncRNAs in heart failure.

ncRNA name	Cell type	Subcellular localization	Key function in HF	Direct targets	Experimental models	References
CARDINAL	Cardiomyocytes	Cytoplasm	Inhibits whole mRNA translation by interfering with DRG1; Under stress, cannot fully counteract DRG1's translation-promoting effect, leading to protein accumulation and cardiac hypertrophy	Protein: DRG1; Prevents DRG1-DFRP1 complex formation	*In vivo*: hypertension-induced stress models	[31]
CHKB-DT	Cardiomyocytes	Cytoplasm	Stabilizes *ALDH2* mRNA by recruiting FUS protein; Protects against cardiac enlargement and dysfunction; Maintains cardiomyocyte contractility	mRNA: *ALDH2* mRNA; Protein: FUS	*In vivo*: TAC mouse model; Heterozygous CHKB-DT knockout mice; Clinical: DCM patients	[32]
Gm15834	Cardiomyocytes	Cytoplasm	Acts as miRNA sponge; Enhances autophagy by preventing miR-30b-3p from suppressing ULK1; Aggravates cardiac hypertrophy	miRNA: miR-30b-3p; Indirectly upregulates ULK1	*In vivo*: TAC mouse model; *In vitro*: angiotensin II-induced cardiac hypertrophy model	[33]
Chaer	Cardiomyocytes	Nucleus	Functions in trans as a decoy; Binds PRC2 complex to disrupt its genomic localization; Reduces H3K27me3 modification; Activates hypertrophy-related genes; Essential for pressure-induced cardiac pathology	Protein: Ezh2; Disrupts PRC2-mediated H3K27me3 histone modification	*In vivo*: pressure overload mouse model; CRISPR-Cas9 *Chaer* knockout mice	[30]
uc.323	Cardiomyocytes	Nucleus	Protective T-UCR; Functions in trans; Recruits EZH2 to increase H3K27me3 at CPT1b promoter; Inhibits CPT1b transcription; Suppresses cardiac hypertrophy	Protein: EZH2; DNA/Chromatin: *CPT1b*	*In vivo*: TAC mouse model; *uc.323* knockout mice; *in vitro*: cardiomyocytes	[152]
Trdn-as	Cardiomyocytes	Nucleus	Promotes alternative splicing of Triadin pre-mRNA by recruiting splicing factors; Maintains proper triadin isoform ratios; Essential for Ca^{2+} homeostasis; Prevents arrhythmias and premature death	RNA: Triadin precursor mRNA; Proteins: SRSF1, SRSF10	*In vivo*: *Trdn-as* knockout mice (develop Ca^{2+} metabolism disorders, arrhythmias, premature death)	[153]
DCRT	Cardiomyocytes	Nucleus	Prevents aberrant alternative splicing of NDUFS2 by blocking PTBP1 binding; Inhibits PTBP1-induced exon 3 skipping; Prevents production of abnormal NDUFS2-S isoform; Protects mitochondrial function; Prevents DCM development	Protein: PTBP1; RNA: NDUFS2 precursor mRNA	*In vivo*: CRISPR-Cas9 *DCRT* knockout mice; Clinical: DCM patients	[154]
Handsdown (*Hdn*)	Cardiomyocytes	Nucleus	Transcription-dependent *cis*-regulation; Transcriptional activation of *Hdn* locus represses neighboring *Hand2* gene expression; Regulation depends on transcription process, not mature lncRNA transcript	DNA/Chromatin: *Hand2* gene locus	*In vitro*: cardiac cells; Transcriptional manipulation experiments (dCas9-VP64, GapmeRs, $Hdn^{pA\text{-}MAZ/pA\text{-}MAZ}$)	[139]
lncRNA-Smad7	Mouse embryonic stem cells	Nucleus	Forms R-loop at *Bmp2* promoter; Mediates transcriptional repression of *Bmp2*; Essential for stem cell maturation into cardiomyocytes; Prevents increased proliferation and poor maturation	DNA: Bmp2 promoter	*In vitro*: mouse embryonic stem cells; EMSA, DRIP-qPCR/DRIP-seq experiments	[28]
mitolnc	Cardiomyocytes	Mitochondria	Mitochondria-enriched lncRNA; Contains conserved sequence	Protein: BCKDH	*In vivo*: *mitolnc* knockout mice	[34]
HDRACA	Endothelial cells	Cytoplasm	Regulates HDL-mediated angiogenesis by modulating RAIN-vigilin interaction, affecting PCNA mRNA stability and endothelial cell proliferation	Protein: RAIN Indirect effect on: *PCNA* mRNA stability via vigilin	*In vitro*: endothelial cells with normal HDL *vs*. dysfunctional HDL treatment	[155]
HIF1α-AS1	Endothelial cells	Nucleus	Acts in trans to recruit HUSH repressive complex to suppress gene expression under normoxic conditions; forms triplex structure	DNA: Genomic loci of *EPHA2* and *Adrenomedullin* genes	*In vitro*: human endothelial cells under normoxic *vs*. hypoxic conditions; Techniques: Triplex-Seq, RIP with anti-dsDNA antibody ± RNase H	[29]

ALDH2: Aldehyde dehydrogenase 2; BCKDH: Branched-chain α-keto acid dehydrogenase; bp: Base pair; CARDINAL: Myocardin-adjacent long noncoding RNA; Cas9: CRISPR-associated protein 9; Chaer: Cardiac hypertrophy-associated epigenetic regulator; CHKB-DT: Choline kinase beta divergent transcript; CPT1b: Carnitine palmitoyltransferase 1B; CRISPR: Clustered regularly interspaced short palindromic repeats; dCas9–VP64: Nuclease-deactivated CRISPR-associated protein 9 fused to the VP64 transcriptional activation domain; DCM: Dilated cardiomyopathy; DCRT: Dilated cardiomyopathy repressive transcript; DFRP1: DRG family regulatory protein 1; DRG1: Developmentally regulated GTP-binding protein 1; DRIP-qPCR/DRIP-seq: DNA–RNA immunoprecipitation followed by qPCR/sequencing; dsDNA: Double-stranded DNA; EMSA: Electrophoretic mobility shift assay; EPHA2: EPH receptor A2; EZH2: Enhancer of zeste homolog 2; FUS: FUS RNA-binding protein; H3K27me3: Histone H3 lysine 27 trimethylation; HDL: high-density lipoprotein; $Hdn^{pA\text{-}MAZ/pA\text{-}MAZ}$: *Hdn* pA-MAZ knock-in, carrying a 99-bp pA-MAZ transcriptional stop signal (polyadenylation signal from the MAZ locus); HDRACA: High-density lipoprotein-regulated angiogenesis in coronary artery disease; HF: Heart failure; HIF1α-AS1: HIF1A antisense RNA 1;PCNA: Proliferating cell nuclear antigen; HUSH: Human silencing hub; lncRNAs: Long noncoding RNAs; MAZ: MYC-associated zinc finger protein; miR: microRNA; miRNAs: microRNAs; ncRNAs: Non-coding RNAs; NDUFS2: NADH:ubiquinone oxidoreductase core subunit S2; NDUFS2-S: NDUFS2 short isoform; PRC2: Polycomb repressive complex 2; PTBP1: Polypyrimidine tract-binding protein 1; qPCR: Quantitative polymerase chain reaction; RAIN: Ras-interacting protein 1; RIP: RNA immunoprecipitation; RNase H: Ribonuclease H; SRSF: Serine/arginine-rich splicing factors; TAC: Transverse aortic constriction; TRDN-AS: Triadin antisense long noncoding RNA; T-UCR: Transcribed ultraconserved region; ULK1: Unc-51 like autophagy activating kinase 1.

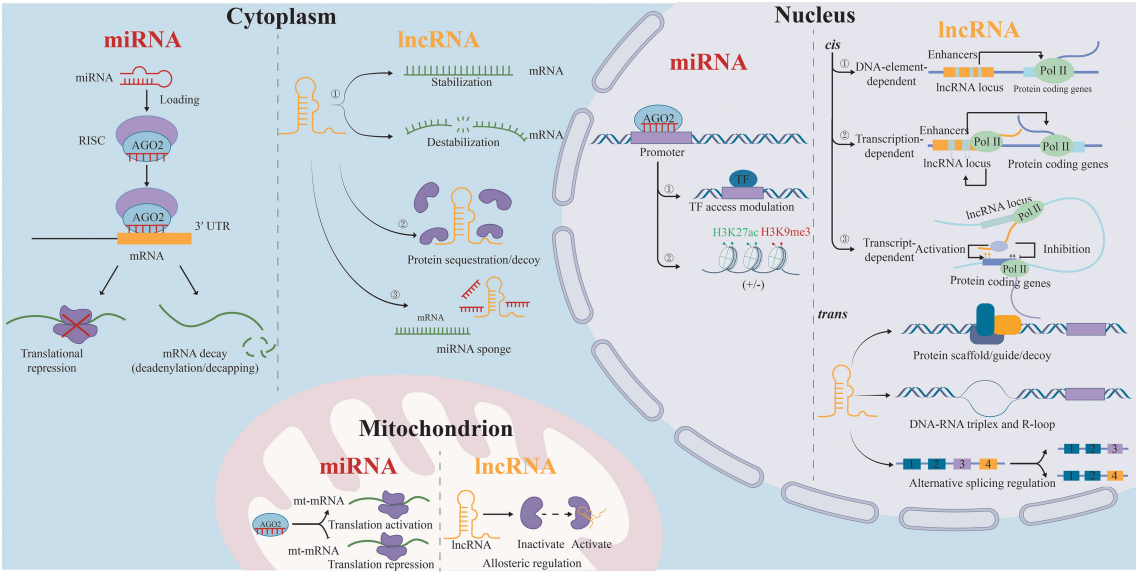

Figure 5: Compartment-based regulatory model for miRNAs and lncRNAs. Schematic summary of how miRNAs and lncRNAs regulate gene expression across subcellular compartments. Cytoplasm: miRNAs are loaded into RISC and bind the 3′ UTR of target mRNAs to induce translational repression and/or mRNA decay. Cytoplasmic lncRNAs modulate mRNA fate by promoting mRNA stabilization or destabilization, sequestering RNA-binding proteins, or acting as miRNA sponges. Nucleus: AGO2-guided miRNAs interact with chromatin to influence transcription by modulating TF access and altering histone marks such as H3K27ac and H3K9me3, resulting in activation or repression. Nuclear lncRNAs regulate gene expression through *cis* or *trans* mechanisms, including DNA-element-, transcription-, or transcript-dependent *cis* regulation, and *trans* actions as scaffolds/guides/decoys, formation of DNA–RNA triplexes or R-loops, and control of alternative splicing. Mitochondria: miRNAs, together with AGO2, regulate mitochondrial transcripts and metabolic output, whereas mitochondrial lncRNAs can directly modulate metabolic enzyme activity through allosteric regulation that shifts enzyme conformation between inactive and active states. AGO: Argonaute; lncRNAs: Long noncoding RNAs; miRNAs: microRNAs; mt-mRNA: mitochondrial messenger RNA; Pol II: RNA polymerase II; RISC: RNA-induced silencing complex; TF: Transcription factor; UTR: Untranslated region.

for disease states.[184] To accurately detect the changes in miRNA and lncRNA levels in different tissues and under various preservation conditions, various techniques such as PCR and next-generation sequencing have been developed, making the quantitative detection of miRNAs and lncRNAs possible. Besides, miRNAs and lncRNAs can be detected in cells, subcellular fractions, and a variety of body fluids such as serum, plasma, saliva, and urine, making them suitable for liquid biopsy applications.[185] It is their sensitive response to disease states, ease of preservation, and the ability to be detected using minimally invasive methods that make them highly promising as biomarkers.

miRNAs as biomarkers

A study enrolled a total of 953 patients with symptomatic chronic heart failure, covering a wide range of clinical characteristics such as age, renal function, heart failure classification, and etiology. Among them, 782 patients completed follow-up testing after 3 months. During the follow-up, baseline levels of miR-132 were found to be negatively correlated with the risk of cardiovascular death and heart failure hospitalization, but not with all-cause mortality. After adjusting for N-terminal pro-B-type natriuretic peptide (NT-proBNP), miR-132 alone remained independently associated with the risk of heart failure hospitalization, especially in patients with ischemic heart failure. Involving miR-132 in a multivariable risk model improved the prediction of heart failure hospitalization risk profoundly, though it provided limited improvement in predicting cardiovascular death. These findings suggest that miR-132 holds potential for enhancing interventions aimed at reducing heart failure rehospitalizations.[186]

A total of 47 myocardial tissue samples were included in a study consisting of 21 patients with DCM at the end-stage of heart failure, 13 patients with ischemic cardiomyopathy at the end-stage of heart failure, eight controls without heart disease, and five fetal heart tissue samples. In addition to tissue samples, peripheral blood samples were also obtained from three cohorts: 13 healthy controls, 51 patients with advanced heart failure, and 14 patients with stable heart failure. Small RNA-sequencing technology was used to analyze the miRNA expression profiles in myocardial tissue and plasma/serum. Cardiac-specific protein biomarkers such as cardiac troponin I (cTnI) and BNP were measured and analyzed with miRNA levels. In the peripheral blood of patients with advanced heart failure, the levels of cardiac/skeletal muscle-specific miRNAs so-called myomirs, including miR-208a, miR-208b, miR-499, miR-1-1, and miR-133b, were significantly increased up to 140-fold, consistent with the elevation of the myocardial injury marker cTnI. This result suggests their potential as biomarkers of myocardial injury, with diagnostic performance comparable to that of cTnI. However, myomirs did not have an advantage in reflecting cardiac function when compared with BNP, indicating that they mainly represent myocardial injury rather than cardiac function in heart failure.[187]

A total of 113 heart transplant recipients were enrolled in another study, including a test cohort of 60 cases composed of 30 patients with biopsy-proven acute rejection and 30 matched controls without rejection, and a validation cohort of 53 cases consisting of 31 patients with rejection and 22 without rejection. In the test cohort, RNA was extracted from paired cardiac transplant biopsy tissue and serum samples, and qPCR was performed to

detect the expression of 14 miRNAs related to allograft rejection, endothelial activation, inflammation, and tissue specificity. Differentially expressed miRNAs were screened by unsupervised clustering and principal component analysis. The discrimination capability of serum miRNAs for rejection was assessed using receiver operating characteristic (ROC) curve analysis. The findings were then validated in the validation cohort. In cardiac transplant biopsy tissue, seven miRNAs, including miR-10a, miR-21, miR-31, miR-92a, miR-142-3p, miR-155, and miR-451, were found to be significantly different between the rejection and control groups. Among the sera samples, four miRNAs, including miR-10a, miR-31, miR-92a, and miR-155, still showed significant differences. miR-31, miR-92a, and miR-155 in serum were elevated in the rejection group, while miR-10a was reduced. These four serum miRNAs demonstrated excellent discrimination ability for rejection. In the validation cohort, these four miRNAs distinguished patients with and without rejection effectively. These results demonstrate their potential as non-invasive biomarkers for cardiac transplant rejection.[188]

To evaluate the diagnostic performance of miRNAs in both heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF), a meta-analysis included 45 relevant studies for qualitative analysis, among which 29 studies were included in the quantitative meta-analysis. The overall study population consisted of 3419 patients with CHF (chronic heart failure) and 2590 controls, including healthy individuals and non-CHF patients. The analysis showed that traditional biomarkers performed better than miRNA testing alone. A combination of miRNAs with traditional biomarkers further improved diagnostic performance. For HFrEF, a panel of eight miRNAs was selected, achieving a diagnostic AUC of 0.91, with a sensitivity of 0.85 and a specificity of 0.88. For HFpEF, seven miRNAs were selected, with a diagnostic AUC of 0.79, sensitivity of 0.82, and specificity of 0.61.[189]

A total of 2763 participants were enrolled in a prospective observational cohort study, and 398 circulating extracellular RNAs were measured in the plasma. During follow-up, left ventricular remodeling was assessed by left ventricular mass, end-diastolic volume, and left atrial diameter using echocardiography, and new-onset heart failure events were recorded. Twelve extracellular RNAs were found to be associated with left ventricular mass and at least one other cardiac structural parameter, such as left atrial diameter or end-diastolic volume. Among them, three miRNAs, miR-17, miR-20a, and miR-106b, were strongly associated with the risk of heart failure. For each twofold increase of miR-17, miR-20a, and miR-106b levels, the risk of new-onset heart failure was reduced by approximately 15%, and this association remained significant after adjustment for multiple risk factors.[190]

lncRNAs as biomarkers

A cross-sectional study enrolled 40 patients after heart transplantation, including 12 cases without rejection (0R), 16 cases with mild rejection (1R), and 12 cases with moderate to severe rejection (≥2R). Whole transcriptome analysis of serum lncRNAs and paired endomyocardial biopsy were performed. The types and expression levels of circulating lncRNAs in serum were detected and compared among different acute cellular rejection (ACR) grades (0R, 1R, ≥2R). Fifty-seven lncRNAs were identified as potential biomarkers for ACR among all 11,062 detected lncRNAs. Six of them, lncRNA *AC008105.3*, *AC006525.1*, *AC011455.8*, *AL359220.1*, *AC025279.1*, and *HAGLR* showed significant differential expression across ACR grades, with expression levels increasing alongside the severity of rejection. Five lncRNAs demonstrated outstanding diagnostic abilities for 1R and ≥2R grades of ACR. In particular, *AL359220.1* and *AC025279.1* were independent predictors for moderate to severe ACR (≥2R) and were expected to become novel biomarkers for rejection after heart transplantation.[191]

Three groups were included in another study, consisting of 95 healthy volunteers, 149 patients with a non-acute myocardial infarction (AMI), defined as patients with similar clinical manifestations but not diagnosed with AMI, and 103 AMI patients with ischemic time ≤12 h. Peripheral whole blood samples were collected, and qPCR was used to detect 15 cardiovascular disease-related lncRNAs. The differential expressions of the 15 lncRNAs among the healthy volunteers, non-AMI, and AMI groups were compared. Among them, only lncRNA *ZFAS1* and *CDR1AS* showed significant differences between patients with AMI and other groups. *ZFAS1* levels in peripheral blood in AMI patients were significantly decreased, which was 25.7% lower than that in the non-AMI group and 39.2% lower than that in healthy volunteers. *CDR1AS* levels were significantly increased, which was 2.2 times higher than that in the non-AMI group and 2.0 times higher than that in healthy volunteers. Besides, *ZFAS1* was negatively correlated with myocardial injury markers such as aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine kinase (CK), while *CDR1AS* was positively correlated. However, neither of them showed significant associations with cardiovascular risk factors such as hypertension, diabetes, or smoking history. In conclusion, both lncRNAs had some sensitivity and specificity for the early diagnosis of AMI. When combined, they may show greater diagnostic value.[192]

In one study, lncRNA *LIPCAR* was identified as an independent predictive factor for cardiovascular death. The study included three independent patient cohorts, adding up to 788 patients. Two hundred and forty-six patients belonged to the post-myocardial infarction left ventricular remodeling cohort. They suffered from AMI and left ventricular (LV) remodeling, which was defined as an increase in LV end-diastolic volume >20% and were assessed after 1-year follow-up. Three hundred and forty-four patients belonged to the chronic systolic heart failure (HF) cohort. A hundred and ninety-eight patients with chronic systolic heart failure belonged to the heart failure prognosis cohort. They were followed up for 3 years and divided into cardiovascular death and surviving groups to validate the ability of lncRNA to predict mortality risk. lncRNA microarray analysis of plasma RNA was conducted on patients with MI with and without LV remodeling, and

15 candidate lncRNAs with high abundance and ≥3-fold change were selected. Among them, seven were detected in all samples. These seven lncRNAs were then validated by real-time quantitative PCR in the 246 patients with MI. uc022bqs.1 (*LIPCAR*) and uc004cos.4 were found to be significantly downregulated, and able to predict ventricular remodeling in the future.

LIPCAR was then measured in 344 patients with chronic heart failure to analyze its association with the risk of cardiovascular mortality. *LIPCAR* was downregulated at the early stages after MI and gradually upregulated with disease progression at the 1st, 3rd, and 12th months. *LIPCAR* increased remarkably, especially in patients who developed LV remodeling and chronic HF. Thus, the expression level of *LIPCAR* can distinguish patients who may develop ventricular remodeling in the future.

In addition to being a predictor of ventricular remodeling, *LIPCAR* has also been found to be an independent predictive factor for cardiovascular death. In 198 patients with heart failure, the association between expression levels of *LIPCAR* and 3-year cardiovascular mortality was assessed. Multivariate analysis showed that the higher the *LIPCAR* levels, the higher the risk of cardiovascular death over the subsequent 3 years was, after adjusting for multiple clinical risk factors. Further analysis showed that *LIPCAR* was a prognostic factor, independent of traditional risk factors such as age, sex, etiology, New York Heart Association (NYHA) classification, left ventricular ejection fraction (LVEF), and BNP.[193]

However, two studies have raised issues about the independent predictive value of *LIPCAR*. One study included 234 patients with hypertensive heart disease, all with a long-term history of hypertension and established chronic heart failure. They were followed up for an average of 4.73 years. Based on estimated glomerular filtration rate (eGFR), the patients were divided into two groups: non-chronic kidney disease (CKD) group (n = 134) and CKD group (n = 100). Analysis of the overall population showed that *LIPCAR* levels were positively correlated with age and negatively correlated with eGFR. Univariate analysis showed that elevated levels of *LIPCAR* were associated with an increased risk of rehospitalization for heart failure and cardiovascular death. However, multivariate analysis revealed that these associations were primarily influenced by eGFR. When eGFR was adjusted, the independent association between *LIPCAR* and outcomes disappeared. This suggests that the independent predictive value of *LIPCAR* was influenced mainly by renal function. Subgroup analysis showed that the group with high *LIPCAR* levels had a higher risk of heart failure for rehospitalization and composite endpoints in non-CKD group; however, only the risk of rehospitalization for heart failure was independently associated with *LIPCAR*. In the CKD group, there was no significant association between high levels of *LIPCAR* and any outcome.[194]

In a study using the Gruppo Italiano per lo Studio della Sopravvivenza nell'Insufficienza Cardiaca–Heart Failure (GISSI-HF) cohort, where 967 patients with symptomatic chronic heart failure were enrolled and followed up for 3.9 years on average, high *LIPCAR* levels were found to be associated with an increased risk of cardiovascular hospitalization, in the univariate analysis. However, this association was no longer significant after multivariate adjustment. In addition, there was no significant relationship between *LIPCAR* levels and endpoints such as all-cause mortality, cardiovascular death, or hospitalization for heart failure. These results suggest that *LIPCAR* may be more suitable as a marker for the health status of patients rather than as an independent prognostic predictor. The study pointed out that the positive findings in the previous study may be attributed to the small sample size.[195]

miRNA-related therapeutic strategies against heart failure

Currently, there are two main therapeutic strategies based on ncRNAs. The first approach is ncRNA antagonism, which is mainly used in conditions where ncRNAs exacerbate diseases. Antisense RNAs are used to inhibit the expression or block the function of target ncRNAs. The second approach is known as ncRNA replacement therapy, which is applied when ncRNAs play a protective role in disease. Small RNAs are introduced to restore the expression or compensate for the function of the target ncRNAs.[196]

According to whether they are double-stranded or single-stranded and their different mechanisms of action, ncRNAs involved in ncRNA therapy can be divided into three categories: ASOs, RNA duplexes, and aptamers.[197]

ASOs are single-stranded oligonucleotides with a length of 17–22 nt, composed of deoxyribonucleotides or ribonucleotides.[198–199] According to the mechanisms of action, ASOs can be divided into gapmer ASOs and steric block ASOs. In addition, if ASOs are designed to bind to miRNAs and inhibit their function, they are referred to as antagomiRs.[200]

Gapmer ASOs typically have a structure in which 8–10 deoxynucleotides, without any modification, are located in the central region and flanked by 2′-modified nt. The central DNA sequence binds to the target mRNA via complementary pairing to form a DNA–RNA hybrid, which recruits RNase H and triggers RNA degradation. The 2′-modified nt increase the affinity of ASOs for the target RNA and prevent them from nuclease degradation, thus prolonging their life span.[201]

Steric block ASOs are fully modified oligonucleotides without the 8–10 deoxynucleotide core. Because RNase H is sensitive to modifications in the deoxynucleotide core, steric block ASOs do not mediate RNA degradation but instead function by sterically blocking key proteins. Their most common application is to regulate alternative splicing by blocking regulatory proteins near splice junctions.[202–203]

Duplex RNA refers to double-stranded RNA molecules that function by RNA interference, mainly including siRNA and miRNA mimics.[204] Unlike ASOs, which rely on RNase H for degradation, duplex RNAs act mainly

via the RISC complex to achieve gene silencing. miRNA mimics are designed to imitate the activity of endogenous miRNAs and are usually partially complementary to their target mRNAs. Similar to endogenous miRNAs, they usually target multiple mRNAs simultaneously, leading to RISC-mediated deadenylation and translational repression of the target mRNAs.[197]

CDR132L is a synthetic locked antisense oligonucleotide (ASO) with a fully phosphorylated backbone that targets miR-132. Previous studies have shown that both miR-212 and miR-132 directly target the TF FoxO3, which has anti-hypertrophic and pro-autophagic effects. Mice deficient in miR-212/132 were resistant to heart failure induced by pressure overload, whereas cardiomyocyte-specific overexpression of the miR-212/132 led to pathological cardiac hypertrophy, heart failure, and death. Injection of antagomir drugs to inhibit miR-132 can reverse cardiac hypertrophy and heart failure in mice.[38]

A Phase Ib clinical trial of CDR132L (NCT04045405) enrolled a total of 28 patients with chronic heart failure. Twenty patients were randomly assigned to the CDR132L group, and eight patients to the placebo group. The 20 patients in the CDR132L group were further divided into four subgroups, each receiving different doses (0.32, 1.00, 3.00, or 10.00 mg/kg) of CDR132L by intravenous injection. Patients were followed up for 4 months to assess safety, tolerability, pharmacokinetics, pharmacodynamics, as well as several biomarkers and clinical parameters related to heart failure. Results showed that CDR132L caused a sustained reduction of plasma miR-132 levels in a dose-dependent manner. Besides, compared with the placebo group, patients in the CDR132L treatment group showed improvement in cardiac function indicators such as decreased NT-proBNP levels and increased LVEF, cardiac fibrosis, and biomarkers related to heart failure. In addition, CDR132L exhibited a favorable safety profile.[205]

A multicenter, randomized, double-blind, placebo-controlled Phase II clinical trial (NCT05350969) of CDR132L is currently ongoing. The trial is planned to enroll 280 patients with left ventricular dysfunction and remodeling after AMI and would receive different doses of CDR132L (5 and 10 mg/kg) intravenously for a total of three injections, with each dose given 28 days apart. The study aims to assess the efficacy and safety of CDR132L on cardiac remodeling and outcomes related to heart failure in addition to standard therapy. The main endpoint is the improvement in left ventricular end-systolic volume index at 6 months, while secondary endpoints include changes in LVEF, NT-proBNP levels, and other heart failure-related indicators, as well as safety.[206]

Challenges and Limitations

Limitations of disease models and translational relevance

It should be noted that current research on the roles of ncRNAs in heart failure is based predominantly on rodent models such as the TAC mouse model and diabetic db/db model, which differ significantly from human heart failure. First, the physiological characteristics of mouse hearts differ substantially from those of humans, which may affect the expression and regulatory patterns of ncRNAs.[162] For example, the mouse heart rate is approximately 600 beats per minute, while that of humans is approximately 70 beats per minute. Second, the mouse disease models may not simulate the pathological process of human diseases entirely. Basic research relies predominantly on single-factor models such as TAC for pressure overload or coronary ligation for ischemia, which are established in young, genetically homogeneous animals free of comorbidities. In contrast, human heart failure clinically presents as a complex syndrome driven by multifactorial etiologies, where patients frequently present with comorbidities such as ageing, metabolic disorders, and mixed pathologies such as co-existing ischemia and hypertension.[163] Lacking in clinical complexity, the robustness of translational findings is limited in standardized animal models. For instance, while miR-21 demonstrates a consistent pro-fibrotic role in controlled TAC mouse models,[146,164] its expression and functional correlation in human heart failure patients exhibit significant variability.[165–166] This variation is likely attributed to the high heterogeneity of clinical etiologies and the influence of confounding factors that are absent in standardized laboratory mouse models.

Inconsistencies in functional studies of ncRNAs

It is noteworthy that functional studies of certain ncRNAs have yielded contradictory conclusions. Taking miR-21 as an example, the study by Thum *et al*[147] demonstrated that miR-21 promotes cardiac fibrosis by targeting SPRY1, whereas Patrick *et al*,[167] using genetic knockout models, found that miR-21 deficiency did not affect the cardiac response to pressure overload. These discrepancies may arise from three reasons. First, there are differences in experimental approaches. Chemical inhibitors such as antagomirs may have off-target effects, while genetic knockout may trigger compensatory mechanisms. Second, the animal backgrounds are different; different mouse strains may respond differently to cardiac stress. Finally, disease models also differ; the role of miRNAs may differ between acute and chronic stress models.

Limitations of research methods for ncRNAs

Limitations in miRNA research methods

Several limitations of current research methods should be considered. First, although luciferase reporter assays are widely used to validate direct binding between miRNAs and target mRNAs, this method is performed in artificial overexpression systems and may not fully reflect regulatory relationships under endogenous conditions, and the mutation of 3′ UTR may create another miRNA binding site.[168] Second, the miRNA overexpression or inhibition strategies employed in many studies may result in non-physiological miRNA concentrations, potentially generating false-positive results or off-target effects.[168] Third, the inconsistent sources of cardiomyocytes used

across different studies, such as neonatal rat cardiomyocytes, and human induced pluripotent stem cell-derived cardiomyocytes, may reduce the comparability of results.[169]

Limitations in lncRNA research methods

Although studies of subcellular localization of lncRNAs are emerging, it is important to know that methodological challenges remain in identifying lncRNA localization and function. The identification of lncRNA–protein interactions often relies on RNA immunoprecipitation (RIP) or crosslinking and immunoprecipitation (CLIP) techniques, which may be affected by non-specific binding and crosslinking artifacts.[170] The conformation of lncRNA–DNA interactions, particularly triplex structures and R-loops, needs precise controls, including RNase H sensitivity assays to distinguish between these two types of structures.[171] Furthermore, functional studies of lncRNAs using knockdown by siRNA or ASO *vs*. knockout via CRISPR-Cas9 approaches may yield different results, as knockdown targets the mature transcript while knockout may affect the DNA regulatory elements or the act of transcription itself. These distinctions are particularly important for lncRNAs that function in *cis*, where the DNA locus, transcription process, and RNA transcript may each contribute independently to gene regulation.[172] Additionally, the function of lncRNAs as miRNA sponges requires certain stoichiometric conditions to be met.[173] The expression levels of lncRNAs must be comparable to those of their target miRNAs.However, some studies have failed to adequately verify this prerequisite.

Technical challenges in subcellular localization studies

Accurate determination of the subcellular localization of ncRNAs is a precondition for understanding their functions; however, existing methods face several technical challenges that require cautious interpretation of relevant data.

Contamination in subcellular fractionation

Nuclear-cytoplasmic fractionation is the most commonly used method for studying subcellular localization of ncRNAs; however, this approach faces challenges of cross-contamination between fractions. During cell lysis, cytoplasmic RNA may remain in the nuclear fraction, leading to false-positive conclusions of nuclear localization.[174] Similarly, nuclear membrane rupture may cause nuclear RNA to leak into the cytoplasmic fraction.[175] Studies show that different lysis buffer components, centrifugation conditions, and operation times may all affect purity.[176] Therefore, reliable nuclear-cytoplasmic fractionation experiments should follow quality control measures using Western blotting to detect nuclear marker proteins, such as Lamin B1, Histone H3, and cytoplasmic marker proteins, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), to assess purity.[177]

Challenges in mitochondrial RNA research

Mitochondrial isolation faces more complex technical challenges. Mitochondrial fractions obtained by traditional differential centrifugation typically contain contamination from endoplasmic reticulum, lysosomes, and peroxisomes.[178] Although density gradient centrifugation may improve purity, it may cause mitochondrial damage and RNA degradation.[179] Furthermore, physical contact sites exist between the outer mitochondrial membrane and endoplasmic reticulum called mitochondria-associated endoplasmic reticulum membranes, making it difficult to separate the two organelles completely.[180] Therefore, studies of mitochondrial ncRNAs should use mitochondrial marker proteins, such as voltage-dependent anion channel (VDAC), cytochrome c oxidase subunit IV (COX IV), or HSP60 and endoplasmic reticulum marker proteins, such as Calnexin, to verify purity, use RNase A to remove mitochondrial membrane-associated RNAs, and combine immunofluorescence co-localization experiments for verification.[181]

Limitations of in situ detection methods

Fluorescence *in situ* hybridization (FISH) and *in situ* hybridization (ISH) can detect RNA localization while maintaining cell integrity, avoiding contamination issues associated with fractionation. However, these methods also have limitations. Probe design specificity directly affects the reliability of results, especially for ncRNAs with similar sequences. Besides, detection sensitivity for low-abundance RNAs is limited. Fixation and permeabilization processes may affect the localization of RNA.[182] In addition, the resolution of confocal microscopy, which is approximately 200 nm, is insufficient to distinguish certain adjacent subcellular structures. Single-molecule fluorescence *in situ* hybridization (smFISH) technology developed in recent years has improved detection sensitivity and specificity[183]; however, this technique has not yet been widely applied in the cardiovascular field.

Challenges of miRNAs and lncRNAs as Biomarkers

Although the results discussed above are promising, several critical challenges remain before miRNAs and lncRNAs can be translated into clinically reliable biomarkers for heart failure.

Specificity

A main concern is the limited disease specificity of many candidate ncRNA biomarkers because most miRNAs and lncRNAs are not specifically expressed in the heart but are broadly expressed across multiple tissues and pathological conditions. For instance, miR-155, identified as a biomarker in cardiac transplant rejection,[188] is also a biomarker of metabolic disorders and malignant diseases.[207–210] Similarly, myomirs such as miR-208b, miR-499, and miR-133b are expressed in both cardiac and skeletal muscle, suggesting that their circulating levels may be elevated not only by myocardial injury but also by exercise-induced muscle damage or rhabdomyolysis.[211–213] These findings highlight the following challenge: Changes in circulating ncRNA levels often represent a mixed signal from multiple organs and disease processes, making it difficult to attribute them specifically to heart failure without careful adjustment for confounders.

Stability

Although circulating miRNAs are generally considered relatively stable due to their encapsulation in EVs, or binding to AGO proteins and lipoproteins, their levels are highly sensitive to the processing methods used before measurements.[214–217] Sample type, such as serum versus whole blood, choice of anticoagulant, and processing time may all introduce essential variability.[218–220] Among them, hemolysis is a particularly important factor, as red blood cells contain high concentrations of certain miRNAs such as miR-451 and miR-16, and even mild hemolysis can dramatically change the circulating miRNA profile.[221] Furthermore, there is no optimal endogenous reference gene for normalization of circulating miRNA levels. Commonly used references, such as U6 snRNA and miR-16, have limitations, and the choice of normalization may significantly influence the results. The levels of serum U6 show large individual variation and changes in some diseases.[222] miR-16 often serves as an endogenous reference but is sensitive to hemolysis, resulting in variability in normalization.[223] For lncRNAs, the situation is even more challenging. Compared with miRNAs, the abundance of lncRNAs is normally lower in circulation, and they are more likely to be degraded by RNase due to their greater length and less association with protective carriers.[224] This lower stability and abundance make their reliable quantification in plasma or serum technically more difficult and more prone to inter-assay variability.

Advantages over existing biomarkers

From a clinical perspective, it is important to note that most studies of ncRNAs have shown that a single type of miRNA or lncRNA is not superior to established biomarkers such as NT-proBNP and BNP. As demonstrated in the meta-analysis discussed earlier, traditional biomarkers still exhibited better diagnostic performance compared with miRNAs alone; the addition of miRNAs to traditional biomarkers provided only modest incremental improvement.[186] Similarly, myomirs showed comparable diagnostic performance to cTnI for myocardial injury but had no advantage over BNP for assessing cardiac function.[187] Given the higher cost and technical complexity of ncRNA-based assays compared with conventional immunoassays for natriuretic peptides and troponins, the value of ncRNAs as biomarkers in routine clinical workflows remains to be developed.[225]

Challenges of miRNA-based therapeutic strategies

Although the results of early clinical ncRNA-based therapies for heart failure are encouraging, several important problems must be addressed before these approaches can be broadly applied in clinical practice.

Tissue-targeted delivery

Efficient and specific delivery of ncRNA therapeutics to cardiomyocytes still remains a significant barrier to clinical translation. Compared with the liver, which naturally accumulates most systemically delivered ncRNA drugs due to its anatomical features and abundant receptor-mediated uptake pathways, the heart lacks such favorable pharmacokinetic properties.[226] Currently, the most widely used delivery systems, including lipid nanoparticles (LNPs) and N-acetylgalactosamine (GalNAc) conjugates, are primarily optimized for hepatic delivery and show limited cardiac targeting.[227] Adeno-associated viruses (AAVs), particularly AAV9, have shown relatively higher cardiac transduction efficiency; however, their clinical application is limited by immune responses, packaging capacity, and serious adverse events.[228] CDR132L, administered via systemic intravenous injection without any cardiac-targeting moiety, has raised questions about what fraction of the administered dose actually reaches the myocardium and how much is taken up by the liver, kidneys, and other organs. Insufficient cardiac accumulation not only reduces therapeutic efficacy but also increases the dosage required, which in turn increases the risk of systemic toxicity. Novel strategies such as cardiac-homing peptide conjugates and antibody-oligonucleotide conjugates targeting cardiac surface markers are under active investigation but remain in early preclinical stages.[229–230]

Off-target effects

The multi-target nature of miRNAs presents a unique challenge for therapeutic specificity. A single miRNA typically targets hundreds of mRNAs across diverse biological pathways, which becomes a disadvantage as a therapeutic strategy.[231] For CDR132L, the therapeutic target miR-132 is not only involved in cardiac hypertrophy and fibrosis but also plays important roles in neuronal development, synaptic plasticity, immune regulation, and angiogenesis.[232–234] Therefore, systemic inhibition of miR-132 may potentially impair normal neurological function, immune responses, or vascular homeostasis. Effects may not be apparent in short-term clinical trials but may emerge with prolonged treatment.

Perspective

The studies summarized in this review demonstrate that miRNAs and lncRNAs are not merely passive byproducts of transcription but are spatially organized, functionally versatile regulators, and their biological activities are fundamentally determined by their subcellular localization. In heart failure, the same ncRNA can exert entirely different—and sometimes opposing—effects depending on whether it resides in the cytoplasm, nucleus, or mitochondria. This principle has profound implications for both basic science and clinical translation; however, several critical challenges and opportunities remain.

Unresolved questions and future directions

Although the roles of miRNAs and lncRNAs in different subcellular compartments during heart failure have been increasingly recognized, several key questions remain unanswered.

The mechanisms of subcellular redistribution of ncRNAs during heart failure progression are poorly understood.

Several studies have documented that the subcellular localization of specific ncRNAs shifts during disease. For instance, miR-320 is upregulated in the nucleus but remains unchanged in the cytoplasm of diabetic cardiomyocytes.[144] miR-126-5p is translocated into the nucleus of endothelial cells under shear stress.[23] However, what upstream signals trigger such redistribution, whether this is a general phenomenon across ncRNAs and different cell types during heart failure, and whether it is reversible upon therapeutic intervention, remain largely unknown. Further systematic profiling of subcellular ncRNA distribution at different stages of heart failure, combined with single-cell resolution, will be an important step.

The transport machinery for ncRNAs entering the mitochondria is not yet clearly elucidated. Although it is established that nuclear-encoded miRNAs can transport into the mitochondrial matrix and regulate mitochondrial gene expression,[132,135] the role of molecular carriers such as hPNPase remains controversial,[235] and recognition signals of this transport process have not been identified. Whether specific RNA-binding proteins or mitochondrial outer membrane channels mediate this import, and whether this pathway is altered in failing hearts, remains to be explored.

The cell-type specificity of ncRNA subcellular localization is another problem that requires consideration. Some of the ncRNAs tend to be enriched in a specific cell type. For example, miR-21 is predominantly expressed in cardiac fibroblasts with minimal expression in cardiomyocytes,[146] and miR-9 is enriched in the nucleus of fibroblasts.[145] However, most studies have examined ncRNA localization in only one cell type at a time. A comprehensive comparison of the subcellular distribution and function of the same ncRNA across cardiomyocytes, fibroblasts, and endothelial cells within the failing heart is lacking.

From a clinical perspective, the subcellular localization of ncRNAs has important implications for therapeutic design that have not been fully considered. Current ncRNA-based therapeutic approaches, such as ASO, miRNA mimics, or inhibitors, are generally designed to target intracellular ncRNAs as a whole, rather than ncRNAs with specific subcellular localization. The same ncRNA may have distinct, and even independent functions, depending on its subcellular localization within the same cell. For example, miR-1 has been reported to be protective in cardiac injury and is therefore considered a potential therapeutic target.[236] However, another study has shown that miR-1 may also localize to the cell membrane and modulate cardiac electrophysiology through direct interactions with ion channel proteins.[142] This suggests that although therapeutic manipulation of miR-1 may produce minimal adverse effects in the short term, long-term intervention may disrupt its membrane-related electrophysiological regulation and increase the risk of arrhythmias. Therefore, developing compartment-specific delivery strategies represents another important challenge in this field.

Addressing these questions requires more precise experiments and technologies such as accurate subcellular fractionation, single-cell and spatial transcriptomics, live-cell imaging of ncRNA trafficking, and compartment-specific gain- and loss-of-function approaches. Such efforts will not only deepen our understanding of ncRNA biology in heart failure but also make a difference in the area of ncRNA-based diagnosis and therapies.

Methodological bottlenecks

The development of subcellular ncRNA biology requires overcoming several technical limitations. Current subcellular fractionation protocols inevitably introduce cross-contamination between compartments, and the yield of ncRNAs from purified organelles is often insufficient for comprehensive profiling.[181] single-molecule fluorescence *in situ* hybridization (smFISH) provides spatial resolution but is limited in throughput and cannot easily distinguish functional ncRNAs from passively diffusing transcripts.[237] Emerging spatial transcriptomics platforms such as multiplexed error-robust fluorescence *in situ* hybridization (MERFISH) hold promise for mapping ncRNA localization at subcellular resolution in intact tissue sections, but their application to short ncRNAs such as miRNAs remains technically challenging due to the limitation of probe design.[238] Furthermore, functional validation of compartment-specific ncRNA activities requires tools that can manipulate ncRNA levels within a defined compartment without affecting other compartments. However, this is not yet reliably achieved by current ASO and CRISPR-based approaches.

Challenge of subcellular compartment-targeted delivery

The function of an ncRNA is inseparable from its subcellular location. Therefore, effective ncRNA-based therapies may require delivery not only to the correct cell type but also to the correct subcellular compartment within that cell. Current delivery platforms, including LNPs, GalNAc conjugates, and AAV vectors, predominantly achieve a cytoplasmic or endosomal release of their cargo. Although this is sufficient for targeting cytoplasmic mRNA degradation pathways, it may be insufficient for therapeutic strategies that require nuclear or mitochondrial ncRNA targets.

For nuclear-targeted delivery, the conjugation of ASO or miRNA mimics/inhibitors with nuclear localization signal peptides has shown proof-of-concept efficacy; however, endosomal entrapment and inefficient nuclear localization remain major hurdles.[239–241] For mitochondrial-targeted delivery, triphenylphosphonium-modified nanocarriers have been explored, leveraging the mitochondrial membrane potential to drive accumulation.[242] The MITO-Porter system with mitochondrial fusogenic lipid envelopes has also been developed, acting through membrane fusion.[243] However, these approaches face challenges, including toxic effects via increased proton leak, and may have limited benefits *in vivo*.[244] Therefore, the development of clinical-grade, compartment-specific delivery platforms represents one of the most important translational bottlenecks in the field.

A subcellular perspective as a driver of clinical translation

Subcellular ncRNA profiling can refine biomarker discovery and diagnostic precision. Current circulating ncRNA biomarker

studies typically measure total plasma or serum levels of candidate miRNAs or lncRNAs without considering their subcellular origin in the diseased tissue. However, the compartment from which an ncRNA is released may carry distinct diagnostic information. For example, the selective enrichment of mitochondrial-derived miRNAs in circulation may serve as a specific indicator of mitochondrial dysfunction and bioenergetic failure in cardiomyocytes, distinguishing mitochondrial-driven heart failure phenotypes from those driven primarily by fibrosis or inflammation. Taking subcellular origin into consideration may therefore improve the specificity of ncRNA-based liquid biopsies, enabling a more precise differentiation of patients and diseases.

In addition, the subcellular perspective may guide more rational therapeutic target selection. The same ncRNA may be protective in one compartment and pathogenic in another. This knowledge is critical for therapeutic design. Globally inhibiting or overexpressing a multifunctional ncRNA without considering its compartment-specific activities can neutralize beneficial functions while targeting deleterious ones, or *vice versa*. By mapping the compartment-specific functional profiles of candidate therapeutic ncRNAs, researchers can identify the precise subcellular localization in which an intervention is needed and design strategies that modulate ncRNA activity in a compartment-selective manner. This approach may substantially reduce off-target effects and improve the therapeutic effects of ncRNA-based drugs.

Collectively, these considerations argue that the subcellular localization of ncRNAs should be integrated into the translational roadmap for ncRNA-based heart failure therapies, from biomarker discovery to therapeutic design.

Translational extensions beyond heart failure

The subcellular ncRNA framework established in this review for heart failure has broad relevance to other diseases in which ncRNA-mediated regulation of signaling pathways, mitochondrial function, and cellular remodeling may play a central pathogenic role. We highlight three important disease contexts where future investigation is particularly warranted.

In pulmonary arterial hypertension (PAH), progressive pulmonary vascular remodeling leads to right ventricular failure. Numerous ncRNAs involved in left ventricular hypertrophy, fibrosis, and endothelial dysfunction are similarly dysregulated in the pulmonary vasculature and right ventricle.[245] miR-132, for example, promotes both cardiomyocyte hypertrophy and pulmonary artery smooth muscle cell proliferation.[246] Nuclear lncRNAs that regulate chromatin remodeling in cardiac fibroblasts may likewise drive epigenetic reprogramming of pulmonary vascular cells. Given that mitochondrial dysfunction and glycolytic shift are hallmarks of PAH,[247] mitochondrial miRNAs controlling oxidative phosphorylation may serve as shared therapeutic targets in left heart failure and PAH-associated right ventricular failure. A systematic mapping of subcellular ncRNAs in pulmonary vascular cells and RV cardiomyocytes is required to define such compartment-specific targets.

Metabolic diseases and diabetic complications: In diabetes mellitus and its cardiovascular complications, subcellular ncRNA biology is tightly linked to disease mechanisms. Diabetic cardiomyopathy is characterized as mitochondrial dysfunction, impaired fatty acid oxidation, ROS overproduction, and cardiomyocyte apoptosis, which are all influenced by mitochondrial and cytoplasmic ncRNAs.[248] For instance, miRNAs that target mitochondrial-encoded oxidative phosphorylation subunits may contribute to the bioenergetic deficit observed in diabetic hearts. In diabetic nephropathy, lncRNAs regulating TGF-β signaling in proximal tubular cells resemble profibrotic lncRNAs in cardiac fibroblasts.[249–250] Furthermore, circulating ncRNAs released from metabolically stressed tissues may serve as systemic mediators of inter-organ crosstalk in diabetes, and their subcellular origin may determine their stability, cargo content, and signaling capacity in recipient cells.[251–252] Integrating subcellular ncRNA biology may therefore yield novel biomarkers and therapeutic strategies for treating metabolic disease.

Male infertility and mitochondrial ncRNA biology: Spermatogenesis is highly energy-demanding and depends on mitochondrial oxidative phosphorylation.[253] Mitochondrial dysfunction, such as excess ROS and reduced ATP, is a recognized driver of poor sperm motility and male infertility.[254] Mature sperm are known to contain diverse ncRNAs, particularly miRNAs, which play important roles in fertilization and early embryonic development.[255] Given that mitochondrial miRNAs have been well-characterized in somatic cells such as cardiomyocytes, where they regulate genes associated with oxidative phosphorylation,[135,256] it is possible that similar mitochondrial ncRNA networks exist in spermatogenic cells. However, the subcellular localization and functional roles of miRNAs specifically within sperm mitochondria remain largely unexplored, representing a significant knowledge gap and translational frontier.

Funding

This work was supported by grants from Noncommunicable Chronic Diseases-National Science and Technology Major Project (Nos. 2025ZD0547100 and 2025ZD0547302), the National Natural Science Foundation of China (Nos. 82525005, U22A20266, 82270363, and 82470289), the Natural Science Foundation of Hubei Province (No. 2025EHA037), and the Basic Research Program of Huazhong University of Science and Technology (No. 2024BRA020).

Conflicts of interest

None.

References

1. Bozkurt B, Coats AJS, Tsutsui H, Abdelhamid CM, Adamopoulos S, Albert N, *et al*. Universal definition and classification of heart failure: A report of the Heart Failure Society of America, Heart Failure Association of the European Society of Cardiology, Japanese Heart Failure Society and Writing Committee of the Universal Definition of Heart Failure: Endorsed by the Canadian Heart Failure Society, Heart Failure Association of India, Cardiac Society of Aus-

tralia and New Zealand, and Chinese Heart Failure Association. Eur J Heart Fail 2021;23:352–380. doi: 10.1002/ejhf.2115.
2. Savarese G, Becher PM, Lund LH, Seferovic P, Rosano GMC, Coats AJS. Global burden of heart failure: A comprehensive and updated review of epidemiology. Cardiovasc Res 2023;118:3272–3287. doi: 10.1093/cvr/cvac013.
3. Balagopalan JP, Abdullakutty J. Heart Failure Registries in Asia–What Have We Learned? Cardiovasc Innov and Appl 2024;9:935. doi: 10.15212/cvia.2024.0026.
4. Ziaeian B, Fonarow GC. Epidemiology and aetiology of heart failure. Nat Rev Cardiol 2016;13:368–378. doi: 10.1038/nrcardio.2016.25.
5. Roger VL. Epidemiology of heart failure: A contemporary perspective. Circ Res 2021;128:1421–1434. doi: 10.1161/CIRCRESAHA.121.318172.
6. Tham YK, Bernardo BC, Ooi JY, Weeks KL, McMullen JR. Pathophysiology of cardiac hypertrophy and heart failure: Signaling pathways and novel therapeutic targets. Arch Toxicol 2015;89:1401–1438. doi: 10.1007/s00204-015-1477-x.
7. Liu M, Lopez de Juan Abad B, Cheng K. Cardiac fibrosis: Myofibroblast-mediated pathological regulation and drug delivery strategies. Adv Drug Deliv Rev 2021;173:504–519. doi: 10.1016/j.addr.2021.03.021.
8. Tanai E, Frantz S. Pathophysiology of heart failure. Compr Physiol 2015;6:187–214. doi: 10.1002/cphy.c140055.
9. Consortium EP. An integrated encyclopedia of DNA elements in the human genome. Nature 2012;489:57–74. doi: 10.1038/nature11247.
10. Pennisi E. Genomics. ENCODE project writes eulogy for junk DNA. Science 2012;337:1159, 1161. doi: 10.1126/science.337.6099.1159.
11. Robinson EL, Baker AH, Brittan M, McCracken I, Condorelli G, Emanueli C, *et al*. Dissecting the transcriptome in cardiovascular disease. Cardiovasc Res 2022;118:1004–1019. doi: 10.1093/cvr/cvab117.
12. Zuchi C, Tritto I, Carluccio E, Mattei C, Cattadori G, Ambrosio G. Role of endothelial dysfunction in heart failure. Heart Fail Rev 2020;25:21–30. doi: 10.1007/s10741-019-09881-3.
13. Lu TX, Rothenberg ME. MicroRNA. J Allergy Clin Immunol 2018;141:1202–1207. doi: 10.1016/j.jaci.2017.08.034.
14. Trabucchi M, Mategot R. Subcellular heterogeneity of the microRNA machinery. Trends Genet 2019;35:15–28. doi: 10.1016/j.tig.2018.10.006.
15. Wei W, Lu H, Dai W, Zheng X, Dong H. Multiplexed organelles portrait barcodes for subcellular microRNA array detection in living cells. ACS Nano 2022;16:20329–20339. doi: 10.1021/acsnano.2c06252.
16. Gebert LFR, MacRae IJ. Regulation of microRNA function in animals. Nat Rev Mol Cell Biol 2019;20:21–37. doi: 10.1038/s41580-018-0045-7.
17. Leung AKL. The whereabouts of microRNA actions: Cytoplasm and beyond. Trends Cell Biol 2015;25:601–610. doi: 10.1016/j.tcb.2015.07.005.
18. Roberts TC. The microRNA biology of the mammalian nucleus. Mol Ther Nucleic Acids 2014;3:e188. doi: 10.1038/mtna.2014.40.
19. Billi M, De Marinis E, Gentile M, Nervi C, Grignani F. Nuclear miRNAs: Gene regulation activities. Int J Mol Sci 2024;25:6606. doi: 10.3390/ijms25116066.
20. Tian ZQ, Jiang H, Lu ZB. MiR-320 regulates cardiomyocyte apoptosis induced by ischemia-reperfusion injury by targeting AKIP1. Cell Mol Biol Lett 2018;23:41. doi: 10.1186/s11658-018-0105-1.
21. Tong M, Sadoshima J. Nuclear miR-320 controls lipotoxicity. Circ Res 2019;125:1121–1123. doi: 10.1161/CIRCRESAHA.119.316199.
22. Schober A, Nazari-Jahantigh M, Wei Y, Bidzhekov K, Gremse F, Grommes J, *et al*. MicroRNA-126-5p promotes endothelial proliferation and limits atherosclerosis by suppressing Dlk1. Nat Med 2014;20:368–376. doi: 10.1038/nm.3487.
23. Santovito D, Egea V, Bidzhekov K, Natarelli L, Mourao A, Blanchet X, *et al*. Noncanonical inhibition of caspase-3 by a nuclear microRNA confers endothelial protection by autophagy in atherosclerosis. Sci Transl Med 2020;12:eaaz2294. doi: 10.1126/scitranslmed.aaz2294.
24. Ulitsky I, Bartel DP. lincRNAs: Genomics, evolution, and mechanisms. Cell 2013;154:26–46. doi: 10.1016/j.cell.2013.06.020.
25. Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. Nat Rev Genet 2016;17:47–62. doi: 10.1038/nrg.2015.10.
26. Bridges MC, Daulagala AC, Kourtidis A. LNCcation: lncRNA localization and function. J Cell Biol 2021;220:e202009045. doi: 10.1083/jcb.202009045.
27. Statello L, Guo CJ, Chen LL, Huarte M. Gene regulation by long non-coding RNAs and its biological functions. Nat Rev Mol Cell Biol 2021;22:96–118. doi: 10.1038/s41580-020-00315-9.
28. Kong X, Yan K, Deng P, Fu H, Sun H, Huang W, *et al*. LncRNA-Smad7 mediates cross-talk between Nodal/TGF-beta and BMP signaling to regulate cell fate determination of pluripotent and multipotent cells. Nucleic Acids Res 2022;50:10526–10543. doi: 10.1093/nar/gkac780.
29. Leisegang MS, Bains JK, Seredinski S, Oo JA, Krause NM, Kuo CC, *et al*. HIF1alpha-AS1 is a DNA:DNA:RNA triplex-forming lncRNA interacting with the HUSH complex. Nat Commun 2022;13:6563. doi: 10.1038/s41467-022-34252-2.
30. Wang Z, Zhang XJ, Ji YX, Zhang P, Deng KQ, Gong J, *et al*. The long noncoding RNA Chaer defines an epigenetic checkpoint in cardiac hypertrophy. Nat Med 2016;22:1131–1139. doi: 10.1038/nm.4179.
31. He X, Yang T, Lu YW, Wu G, Dai G, Ma Q, *et al*. The long noncoding RNA CARDINAL attenuates cardiac hypertrophy by modulating protein translation. J Clin Invest 2024;134:e169112. doi: 10.1172/JCI169112.
32. Nie X, Fan J, Dai B, Wen Z, Li H, Chen C, *et al*. LncRNA CHKB-DT Downregulation Enhances Dilated Cardiomyopathy Through ALDH2. Circ Res 2024;134:425–441. doi: 10.1161/CIRCRESAHA.123.323428.
33. Song C, Qi H, Liu Y, Chen Y, Shi P, Zhang S, *et al*. Inhibition of lncRNA Gm15834 attenuates autophagy-mediated myocardial hypertrophy via the miR-30b-3p/ULK1 axis in mice. Mol Ther 2021;29:1120–1137. doi: 10.1016/j.ymthe.2020.10.024.
34. Weiss M, Hettrich S, Hofmann T, Hachim S, Gunther S, Braun T, *et al*. Mitolnc controls cardiac BCAA metabolism and heart hypertrophy by allosteric activation of BCKDH. Nucleic Acids Res 2024;52:6629–6646. doi: 10.1093/nar/gkae226.
35. da Costa Martins PA, Bourajjaj M, Gladka M, Kortland M, van Oort RJ, Pinto YM, *et al*. Conditional dicer gene deletion in the postnatal myocardium provokes spontaneous cardiac remodeling. Circulation 2008;118:1567–1576. doi: 10.1161/CIRCULATIONAHA.108.769984.
36. Kurian L, Aguirre A, Sancho-Martinez I, Benner C, Hishida T, Nguyen TB, *et al*. Identification of novel long noncoding RNAs underlying vertebrate cardiovascular development. Circulation 2015;131:1278–1290. doi: 10.1161/CIRCULATIONAHA.114.013303.
37. Xie R, Yuan S, Hu G, Zhan J, Jin K, Tang Y, *et al*. Nuclear AGO2 promotes myocardial remodeling by activating ANKRD1 transcription in failing hearts. Mol Ther 2024;32:1578–1594. doi: 10.1016/j.ymthe.2024.03.018.
38. Ucar A, Gupta SK, Fiedler J, Erikci E, Kardasinski M, Batkai S, *et al*. The miRNA-212/132 family regulates both cardiac hypertrophy and cardiomyocyte autophagy. Nat Commun 2012;3:1078. doi: 10.1038/ncomms2090.
39. Shimizu I, Minamino T. Physiological and pathological cardiac hypertrophy. J Mol Cell Cardiol 2016;97:245–262. doi: 10.1016/j.yjmcc.2016.06.001.
40. Nakamura M, Sadoshima J. Mechanisms of physiological and pathological cardiac hypertrophy. Nat Rev Cardiol 2018;15:387–407. doi: 10.1038/s41569-018-0007-y.
41. Olivetti G, Abbi R, Quaini F, Kajstura J, Cheng W, Nitahara JA, *et al*. Apoptosis in the failing human heart. N Engl J Med 1997;336:1131–1141. doi: 10.1056/NEJM199704173361603.
42. Bergmann O, Zdunek S, Felker A, Salehpour M, Alkass K, Bernard S, *et al*. Dynamics of cell generation and turnover in the human heart. Cell 2015;161:1566–1575. doi: 10.1016/j.cell.2015.05.026.
43. Ravassa S, López B, Treibel TA, San José G, Losada-Fuentenebro B, Tapia L, *et al*. Cardiac fibrosis in heart failure: Focus on non-invasive diagnosis and emerging therapeutic strategies. Mol Aspects Med 2023;93:101194. doi: 10.1016/j.mam.2023.101194.
44. Frangogiannis NG. Cardiac fibrosis. Cardiovasc Res 2021;117:1450–1488. doi: 10.1093/cvr/cvaa324.
45. Ghazal R, Wang M, Liu D, Tschumperlin DJ, Pereira NL. Cardiac fibrosis in the multi-omics era: implications for heart failure. Circ Res 2025;136:773–802. doi: 10.1161/CIRCRESAHA.124.325402.
46. Treasure CB, Vita JA, Cox DA, Fish RD, Gordon JB, Mudge GH, *et al*. Endothelium-dependent dilation of the coronary micro-

vasculature is impaired in dilated cardiomyopathy. Circulation 1990;81:772–779. doi: 10.1161/01.cir.81.3.772.
47. Ramsey MW, Goodfellow J, Jones CJ, Luddington LA, Lewis MJ, Henderson AH. Endothelial control of arterial distensibility is impaired in chronic heart failure. Circulation 1995;92:3212–3219. doi: 10.1161/01.cir.92.11.3212.
48. Karch R, Neumann F, Ullrich R, Neumuller J, Podesser BK, Neumann M, *et al*. The spatial pattern of coronary capillaries in patients with dilated, ischemic, or inflammatory cardiomyopathy. Cardiovasc Pathol 2005;14:135–144. doi: 10.1016/j.carpath.2005.03.003.
49. Manning D, Rivera EJ, Santana LF. The life cycle of a capillary: Mechanisms of angiogenesis and rarefaction in microvascular physiology and pathologies. Vascul Pharmacol 2024;156:107393. doi: 10.1016/j.vph.2024.107393.
50. Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, *et al*. MicroRNA genes are transcribed by RNA polymerase II. EMBO J 2004;23:4051–4060. doi: 10.1038/sj.emboj.7600385.
51. Cai X, Hagedorn CH, Cullen BR. Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. RNA 2004;10:1957–1966. doi: 10.1261/rna.7135204.
52. Han J, Lee Y, Yeom KH, Nam JW, Heo I, Rhee JK, *et al*. Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. Cell 2006;125:887–901. doi: 10.1016/j.cell.2006.03.043.
53. Zeng Y, Yi R, Cullen BR. Recognition and cleavage of primary microRNA precursors by the nuclear processing enzyme Drosha. EMBO J 2005;24:138–148. doi: 10.1038/sj.emboj.7600491.
54. Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, *et al*. The nuclear RNase III drosha initiates microRNA processing. Nature 2003;425:415–419. doi: 10.1038/nature01957.
55. Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN. The drosha-DGCR8 complex in primary microRNA processing. Genes Dev 2004;18:3016–3027. doi: 10.1101/gad.1262504.
56. Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, *et al*. The microprocessor complex mediates the genesis of microRNAs. Nature 2004;432:235–240. doi: 10.1038/nature03120.
57. Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ. Processing of primary microRNAs by the Microprocessor complex. Nature 2004;432:231–235. doi: 10.1038/nature03049.
58. Landthaler M, Yalcin A, Tuschl T. The human DiGeorge syndrome critical region gene 8 and its D. melanogaster homolog are required for miRNA biogenesis. Curr Biol 2004;14:2162–2167. doi: 10.1016/j.cub.2004.11.001.
59. Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev 2003;17:3011–3016. doi: 10.1101/gad.1158803.
60. Bohnsack MT, Czaplinski K, Gorlich D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA 2004;10:185–191. doi: 10.1261/rna.5167604.
61. Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. Science 2004;303:95–98. doi: 10.1126/science.1090599.
62. Hutvagner G, McLachlan J, Pasquinelli AE, Balint E, Tuschl T, Zamore PD. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science 2001;293:834–838. doi: 10.1126/science.1062961.
63. Ketting RF, Fischer SE, Bernstein E, Sijen T, Hannon GJ, Plasterk RH. Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in C. elegans. Genes Dev 2001;15:2654–2659. doi: 10.1101/gad.927801.
64. Grishok A, Pasquinelli AE, Conte D, Li N, Parrish S, Ha I, *et al*. Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control C. elegans developmental timing. Cell 2001;106:23–34. doi: 10.1016/s0092-8674(01)00431-7.
65. Chendrimada TP, Gregory RI, Kumaraswamy E, Norman J, Cooch N, Nishikura K, *et al*. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature 2005;436:740–744. doi: 10.1038/nature03868.
66. Haase AD, Jaskiewicz L, Zhang H, Laine S, Sack R, Gatignol A, *et al*. TRBP, a regulator of cellular PKR and HIV-1 virus expression, interacts with Dicer and functions in RNA silencing. EMBO Rep 2005;6:961–967. doi: 10.1038/sj.embor.7400509.
67. Lee Y, Hur I, Park SY, Kim YK, Suh MR, Kim VN. The role of PACT in the RNA silencing pathway. EMBO J 2006;25:522–532. doi: 10.1038/sj.emboj.7600942.
68. Iwasaki S, Kobayashi M, Yoda M, Sakaguchi Y, Katsuma S, Suzuki T, *et al*. Hsc70/Hsp90 chaperone machinery mediates ATP-dependent RISC loading of small RNA duplexes. Mol Cell 2010;39:292–299. doi: 10.1016/j.molcel.2010.05.015.
69. Tsuboyama K, Tadakuma H, Tomari Y. Conformational activation of argonaute by distinct yet coordinated actions of the Hsp70 and Hsp90 chaperone systems.Mol Cell 2018;70:722–729.e4. doi: 10.1016/j.molcel.2018.04.010.
70. Schwarz DS, Hutvagner G, Du T, Xu Z, Aronin N, Zamore PD. Asymmetry in the assembly of the RNAi enzyme complex. Cell 2003;115:199–208. doi: 10.1016/s0092-8674(03)00759-1.
71. Khvorova A, Reynolds A, Jayasena SD. Functional siRNAs and miRNAs exhibit strand bias. Cell 2003;115:209–216. doi: 10.1016/s0092-8674(03)00801-8.
72. Frank F, Sonenberg N, Nagar B. Structural basis for 5′-nucleotide base-specific recognition of guide RNA by human AGO2. Nature 2010;465:818–822. doi: 10.1038/nature09039.
73. Kawamata T, Seitz H, Tomari Y. Structural determinants of miRNAs for RISC loading and slicer-independent unwinding. Nat Struct Mol Biol 2009;16:953–960. doi: 10.1038/nsmb.1630.
74. Ruby JG, Jan CH, Bartel DP. Intronic microRNA precursors that bypass Drosha processing. Nature 2007;448:83–86. doi: 10.1038/nature05983.
75. Okamura K, Hagen JW, Duan H, Tyler DM, Lai EC. The mirtron pathway generates microRNA-class regulatory RNAs in Drosophila. Cell 2007;130:89–100. doi: 10.1016/j.cell.2007.06.028.
76. Xie M, Li M, Vilborg A, Lee N, Shu MD, Yartseva V, *et al*. Mammalian 5′-capped microRNA precursors that generate a single microRNA. Cell 2013;155:1568–1580. doi: 10.1016/j.cell.2013.11.027.
77. Ender C, Krek A, Friedlander MR, Beitzinger M, Weinmann L, Chen W, *et al*. A human snoRNA with microRNA-like functions. Mol Cell 2008;32:519–528. doi: 10.1016/j.molcel.2008.10.017.
78. Babiarz JE, Ruby JG, Wang Y, Bartel DP, Blelloch R. Mouse ES cells express endogenous shRNAs, siRNAs, and other Microprocessor-independent, Dicer-dependent small RNAs. Genes Dev 2008;22:2773–2785. doi: 10.1101/gad.1705308.
79. Cheloufi S, Dos Santos CO, Chong MM, Hannon GJ. A dicer-independent miRNA biogenesis pathway that requires Ago catalysis. Nature 2010;465:584–589. doi: 10.1038/nature09092.
80. Yoda M, Cifuentes D, Izumi N, Sakaguchi Y, Suzuki T, Giraldez AJ, *et al*. Poly(A)-specific ribonuclease mediates 3′-end trimming of Argonaute2-cleaved precursor microRNAs. Cell Rep 2013;5:715–726. doi: 10.1016/j.celrep.2013.09.029.
81. Hwang HW, Wentzel EA, Mendell JT. A hexanucleotide element directs microRNA nuclear import. Science 2007;315:97–100. doi: 10.1126/science.1136235.
82. Liu H, Lei C, He Q, Pan Z, Xiao D, Tao Y. Nuclear functions of mammalian MicroRNAs in gene regulation, immunity and cancer. Mol Cancer 2018;17:64. doi: 10.1186/s12943-018-0765-5.
83. Molina-Navarro MM, Rosello-Lleti E, Tarazon E, Ortega A, Sanchez-Izquierdo D, Lago F, *et al*. Heart failure entails significant changes in human nucleocytoplasmic transport gene expression. Int J Cardiol 2013;168:2837–2843. doi: 10.1016/j.ijcard.2013.03.192.
84. Zhan J, Jin K, Xie R, Fan J, Tang Y, Chen C, *et al*. AGO2 protects against diabetic cardiomyopathy by activating mitochondrial gene translation. Circulation 2024;149:1102–1120. doi: 10.1161/CIRCULATIONAHA.123.065546.
85. Schlackow M, Nojima T, Gomes T, Dhir A, Carmo-Fonseca M, Proudfoot NJ. Distinctive patterns of transcription and RNA processing for human lincRNAs. Mol Cell 2017;65:25–38. doi: 10.1016/j.molcel.2016.11.029.
86. Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, *et al*. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res 2012;22:1775–1789. doi: 10.1101/gr.132159.111.
87. Wilusz JE, Freier SM, Spector DL. 3′ end processing of a long nuclear-retained noncoding RNA yields a tRNA-like cytoplasmic RNA. Cell 2008;135:919–932. doi: 10.1016/j.cell.2008.10.012.
88. Sunwoo H, Dinger ME, Wilusz JE, Amaral PP, Mattick JS, Spector DL. MEN epsilon/beta nuclear-retained non-coding RNAs are up-regulated upon muscle differentiation and are essential components of paraspeckles. Genome Res 2009;19:347–359. doi: 10.1101/gr.087775.108.

89. Yin QF, Yang L, Zhang Y, Xiang JF, Wu YW, Carmichael GG, *et al*. Long noncoding RNAs with snoRNA ends. Mol Cell 2012;48:219–230. doi: 10.1016/j.molcel.2012.07.033.
90. Zhang XO, Yin QF, Wang HB, Zhang Y, Chen T, Zheng P, *et al*. Species-specific alternative splicing leads to unique expression of sno-lncRNAs. BMC Genomics 2014;15:287. doi: 10.1186/1471-2164-15-287.
91. Wu H, Yin QF, Luo Z, Yao RW, Zheng CC, Zhang J, *et al*. Unusual processing generates SPA LncRNAs that sequester multiple RNA binding proteins. Mol Cell 2016;64:534–548. doi: 10.1016/j.molcel.2016.10.007.
92. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, *et al*. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 2013;495:333–338. doi: 10.1038/nature11928.
93. Wilusz JE, JnBaptiste CK, Lu LY, Kuhn CD, Joshua-Tor L, Sharp PA. A triple helix stabilizes the 3′ ends of long noncoding RNAs that lack poly(A) tails. Genes Dev 2012;26:2392–2407. doi: 10.1101/gad.204438.112.
94. Brown JA, Valenstein ML, Yario TA, Tycowski KT, Steitz JA. Formation of triple-helical structures by the 3′-end sequences of MALAT1 and MENbeta noncoding RNAs. Proc Natl Acad Sci U S A 2012;109:19202–19207. doi: 10.1073/pnas.1217338109.
95. Heidemann M, Hintermair C, Voss K, Eick D. Dynamic phosphorylation patterns of RNA polymerase II CTD during transcription. Biochim Biophys Acta 2013;1829:55–62. doi: 10.1016/j.bbagrm.2012.08.013.
96. Hsin JP, Manley JL. The RNA polymerase II CTD coordinates transcription and RNA processing. Genes Dev 2012;26:2119–2137. doi: 10.1101/gad.200303.112.
97. Mele M, Mattioli K, Mallard W, Shechner DM, Gerhardinger C, Rinn JL. Chromatin environment, transcriptional regulation, and splicing distinguish lincRNAs and mRNAs. Genome Res 2017;27:27–37. doi: 10.1101/gr.214205.116.
98. Rosenberg AB, Patwardhan RP, Shendure J, Seelig G. Learning the sequence determinants of alternative splicing from millions of random sequences. Cell 2015;163:698–711. doi: 10.1016/j.cell.2015.09.054.
99. Valencia P, Dias AP, Reed R. Splicing promotes rapid and efficient mRNA export in mammalian cells. Proc Natl Acad Sci U S A 2008;105:3386–3391. doi: 10.1073/pnas.0800250105.
100. Yin Y, Lu JY, Zhang X, Shao W, Xu Y, Li P, *et al*. U1 snRNP regulates chromatin retention of noncoding RNAs. Nature 2020;580:147–150. doi: 10.1038/s41586-020-2105-3.
101. Lubelsky Y, Ulitsky I. Sequences enriched in Alu repeats drive nuclear localization of long RNAs in human cells. Nature 2018;555:107–111. doi: 10.1038/nature25757.
102. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005;120:15–20. doi: 10.1016/j.cell.2004.12.035.
103. Bartel DP. Metazoan MicroRNAs. Cell 2018;173:20–51. doi: 10.1016/j.cell.2018.03.006.
104. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 2009;19:92–105. doi: 10.1101/gr.082701.108.
105. Song JJ, Smith SK, Hannon GJ, Joshua-Tor L. Crystal structure of Argonaute and its implications for RISC slicer activity. Science 2004;305:1434–1437. doi: 10.1126/science.1102514.
106. Schirle NT, MacRae IJ. The crystal structure of human Argonaute2. Science 2012;336:1037–1040. doi: 10.1126/science.1221551.
107. Elkayam E, Kuhn CD, Tocilj A, Haase AD, Greene EM, Hannon GJ, *et al*. The structure of human argonaute-2 in complex with miR-20a. Cell 2012;150:100–110. doi: 10.1016/j.cell.2012.05.017.
108. Meister G, Landthaler M, Patkaniowska A, Dorsett Y, Teng G, Tuschl T. Human argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. Mol Cell 2004;15:185–197. doi: 10.1016/j.molcel.2004.07.007.
109. Wu L, Fan J, Belasco JG. MicroRNAs direct rapid deadenylation of mRNA. Proc Natl Acad Sci U S A 2006;103:4034–4039. doi: 10.1073/pnas.0510928103.
110. Mathonnet G, Fabian MR, Svitkin YV, Parsyan A, Huck L, Murata T, *et al*. MicroRNA inhibition of translation initiation in vitro by targeting the cap-binding complex eIF4F. Science 2007;317:1764–1767. doi: 10.1126/science.1146067.
111. Liu J, Rivas FV, Wohlschlegel J, Yates JR, 3rd, Parker R, Hannon GJ. A role for the P-body component GW182 in microRNA function. Nat Cell Biol 2005;7:1261–1266. doi: 10.1038/ncb1333.
112. Eulalio A, Huntzinger E, Izaurralde E. GW182 interaction with Argonaute is essential for miRNA-mediated translational repression and mRNA decay. Nat Struct Mol Biol 2008;15:346–353. doi: 10.1038/nsmb.1405.
113. Mathys H, Basquin J, Ozgur S, Czarnocki-Cieciura M, Bonneau F, Aartse A, *et al*. Structural and biochemical insights to the role of the CCR4-NOT complex and DDX6 ATPase in microRNA repression. Mol Cell 2014;54:751–765. doi: 10.1016/j.molcel.2014.03.036.
114. Fukaya T, Tomari Y. MicroRNAs mediate gene silencing via multiple different pathways in drosophila. Mol Cell 2012;48:825–836. doi: 10.1016/j.molcel.2012.09.024.
115. Helwak A, Kudla G, Dudnakova T, Tollervey D. Mapping the human miRNA interactome by CLASH reveals frequent noncanonical binding. Cell 2013;153:654–665. doi: 10.1016/j.cell.2013.03.043.
116. Chi SW, Zang JB, Mele A, Darnell RB. Argonaute HITS-CLIP decodes microRNA–mRNA interaction maps. Nature 2009;460:479–486. doi: 10.1038/nature08170.
117. Zhang K, Zhang X, Cai Z, Zhou J, Cao R, Zhao Y, *et al*. A novel class of microRNA-recognition elements that function only within open reading frames. Nat Struct Mol Biol 2018;25:1019–1027. doi: 10.1038/s41594-018-0136-3.
118. Burroughs AM, Ando Y, de Hoon MJ, Tomaru Y, Suzuki H, Hayashizaki Y, *et al*. Deep-sequencing of human Argonaute-associated small RNAs provides insight into miRNA sorting and reveals Argonaute association with RNA fragments of diverse origin. RNA Biol 2011;8:158–177. doi: 10.4161/rna.8.1.14300.
119. Flores O, Kennedy EM, Skalsky RL, Cullen BR. Differential RISC association of endogenous human microRNAs predicts their inhibitory potential. Nucleic Acids Res 2014;42:4629–4639. doi: 10.1093/nar/gkt1393.
120. Belter A, Gudanis D, Rolle K, Piwecka M, Gdaniec Z, Naskret-Barciszewska MZ, *et al*. Mature miRNAs form secondary structure, which suggests their function beyond RISC. PLoS One 2014;9:e113848. doi: 10.1371/journal.pone.0113848.
121. Weinmann L, Hock J, Ivacevic T, Ohrt T, Mutze J, Schwille P, *et al*. Importin 8 is a gene silencing factor that targets argonaute proteins to distinct mRNAs. Cell 2009;136:496–507. doi: 10.1016/j.cell.2008.12.023.
122. Zhang Y, Fan M, Zhang X, Huang F, Wu K, Zhang J, *et al*. Cellular microRNAs up-regulate transcription via interaction with promoter TATA-box motifs. RNA 2014;20:1878–1889. doi: 10.1261/rna.045633.114.
123. Guo H, Pu M, Tai Y, Chen Y, Lu H, Qiao J, *et al*. Nuclear miR-30b-5p suppresses TFEB-mediated lysosomal biogenesis and autophagy. Cell Death Differ 2021;28:320–336. doi: 10.1038/s41418-020-0602-4.
124. Paugh SW, Coss DR, Bao J, Laudermilk LT, Grace CR, Ferreira AM, *et al*. MicroRNAs form triplexes with double stranded DNA at sequence-specific binding sites; a eukaryotic mechanism via which microRNAs could directly alter gene expression. PLoS Comput Biol 2016;12:e1004744. doi: 10.1371/journal.pcbi.1004744.
125. Friedman MJ, Wagner T, Lee H, Rosenfeld MG, Oh S. Enhancer–promoter specificity in gene transcription: molecular mechanisms and disease associations. Exp Mol Med 2024;56:772–787. doi: 10.1038/s12276-024-01233-y.
126. Kawasaki K, Fukaya T. Regulatory landscape of enhancer-mediated transcriptional activation. Trends Cell Biol 2024;34:826–837. doi: 10.1016/j.tcb.2024.01.008.
127. Xiao M, Li J, Li W, Wang Y, Wu F, Xi Y, *et al*. MicroRNAs activate gene transcription epigenetically as an enhancer trigger. RNA Biol 2017;14:1326–1334. doi: 10.1080/15476286.2015.1112487.
128. Gagnon KT, Li L, Chu Y, Janowski BA, Corey DR. RNAi factors are present and active in human cell nuclei. Cell Rep 2014;6:211–221. doi: 10.1016/j.celrep.2013.12.013.
129. Leucci E, Patella F, Waage J, Holmstrom K, Lindow M, Porse B, *et al*. microRNA-9 targets the long non-coding RNA MALAT1 for degradation in the nucleus. Sci Rep 2013;3:2535. doi: 10.1038/srep02535.
130. Tang R, Li L, Zhu D, Hou D, Cao T, Gu H, *et al*. Mouse miRNA-709 directly regulates miRNA-15a/16-1 biogenesis at the posttranscriptional level in the nucleus: Evidence for a microRNA hierarchy system. Cell Res 2012;22:504–515. doi: 10.1038/cr.2011.137.
131. Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, *et al*. Sequence and organization of the human mito-

chondrial genome. Nature 1981;290:457–46. doi: 10.1038/290457a0.
132. Zhang X, Zuo X, Yang B, Li Z, Xue Y, Zhou Y, *et al*. MicroRNA directly enhances mitochondrial translation during muscle differentiation. Cell 2014;158:607–619. doi: 10.1016/j.cell.2014.05.047.
133. Bian Z, Li LM, Tang R, Hou DX, Chen X, Zhang CY, *et al*. Identification of mouse liver mitochondria-associated miRNAs and their potential biological functions. Cell Res 2010;20:1076–1078. doi: 10.1038/cr.2010.119.
134. Kren BT, Wong PY, Sarver A, Zhang X, Zeng Y, Steer CJ. MicroRNAs identified in highly purified liver-derived mitochondria may play a role in apoptosis. RNA Biol 2009;6:65–72. doi: 10.4161/rna.6.1.7534.
135. Das S, Ferlito M, Kent OA, Fox-Talbot K, Wang R, Liu D, *et al*. Nuclear miRNA regulates the mitochondrial genome in the heart. Circ Res 2012;110:1596–1603. doi: 10.1161/CIRCRESAHA.112.267732.
136. Seila AC, Calabrese JM, Levine SS, Yeo GW, Rahl PB, Flynn RA, *et al*. Divergent transcription from active promoters. Science 2008;322:1849–1851. doi: 10.1126/science.1162253.
137. Luo S, Lu JY, Liu L, Yin Y, Chen C, Han X, *et al*. Divergent lncRNAs regulate gene expression and lineage differentiation in pluripotent cells. Cell Stem Cell 2016;18:637–652. doi: 10.1016/j.stem.2016.01.024.
138. Alexanian M, Maric D, Jenkinson SP, Mina M, Friedman CE, Ting CC, *et al*. A transcribed enhancer dictates mesendoderm specification in pluripotency. Nat Commun 2017;8:1806. doi: 10.1038/s41467-017-01804-w.
139. Ritter N, Ali T, Kopitchinski N, Schuster P, Beisaw A, Hendrix DA, *et al*. The lncRNA locus handsdown regulates cardiac gene programs and is essential for early mouse development. Dev Cell 2019;50:644–657.e8. doi: 10.1016/j.devcel.2019.07.013.
140. Marasco LE, Kornblihtt AR. The physiology of alternative splicing. Nat Rev Mol Cell Biol 2023;24:242–254. doi: 10.1038/s41580-022-00545-z.
141. Gao J, Zhang Y, Hu M, He T, Yin X, Hao T, *et al*. Alternative RNA splicing in cardiac diseases. Cardiovasc Innov Appli 2025; 10. doi: 10.15212/cvia.2024.0060.
142. Yang D, Wan X, Dennis AT, Bektik E, Wang Z, Costa MGS, *et al*. MicroRNA biophysically modulates cardiac action potential by direct binding to ion channel. Circulation 2021;143:1597–1613. doi: 10.1161/CIRCULATIONAHA.120.050098.
143. Fan J, Zhang X, Nie X, Li H, Yuan S, Dai B, *et al*. Nuclear miR-665 aggravates heart failure via suppressing phosphatase and tensin homolog transcription. Sci China Life Sci 2020;63:724–736. doi: 10.1007/s11427-018-9515-1.
144. Li H, Fan J, Zhao Y, Zhang X, Dai B, Zhan J, *et al*. Nuclear miR-320 mediates diabetes-induced cardiac dysfunction by activating transcription of fatty acid metabolic genes to cause lipotoxicity in the heart. Circ Res 2019;125:1106–1120. doi: 10.1161/CIRCRESAHA.119.314898.
145. Cordero J, Swaminathan G, Rogel-Ayala DG, Rubio K, Elsherbiny A, Mahmood S, *et al*. Nuclear microRNA 9 mediates G-quadruplex formation and 3D genome organization during TGF-beta-induced transcription. Nat Commun 2024;15:10711. doi: 10.1038/s41467-024-54740-x.
146. Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, *et al*. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. Nature 2008;456:980–984. doi: 10.1038/nature07511.
147. Besnier M, Shantikumar S, Anwar M, Dixit P, Chamorro-Jorganes A, Sweaad W, *et al*. miR-15a/-16 inhibit angiogenesis by targeting the Tie2 coding sequence: Therapeutic potential of a miR-15a/16 decoy system in limb ischemia. Mol Ther Nucleic Acids 2019;17:49–62. doi: 10.1016/j.omtn.2019.05.002.
148. Santovito D, Egea V, Bidzhekov K, Natarelli L, Mourao A, Blanchet X, *et al*. Autophagy unleashes noncanonical microRNA functions. Autophagy 2020;16:2294–2296. doi: 10.1080/15548627.2020.1830523.
149. Mohammad G, Kumar J, Kowluru RA. Mitochondrial genome-encoded long noncoding RNA cytochrome B and mitochondrial dysfunction in diabetic retinopathy. Antioxid Redox Signal 2023;39:817–828. doi: 10.1089/ars.2023.0303.
150. Sang L, Ju HQ, Yang Z, Ge Q, Zhang Z, Liu F, *et al*. Mitochondrial long non-coding RNA GAS5 tunes TCA metabolism in response to nutrient stress. Nat Metab 2021;3:90–106. doi: 10.1038/s42255-020-00325-z.
151. Zhao Y, Sun L, Wang RR, Hu JF, Cui J. The effects of mitochondria-associated long noncoding RNAs in cancer mitochondria: New players in an old arena. Crit Rev Oncol Hematol 2018;131:76–82. doi: 10.1016/j.critrevonc.2018.08.005.
152. Sun Y, Fan W, Xue R, Dong B, Liang Z, Chen C, *et al*. Transcribed ultraconserved regions, uc.323, ameliorates cardiac hypertrophy by regulating the transcription of CPT1b (carnitine palmitoyl transferase 1b). Hypertension 2020;75:79–90. doi: 10.1161/HYPERTENSIONAHA.119.13173.
153. Zhao Y, Riching AS, Knight WE, Chi C, Broadwell LJ, Du Y, *et al*. Cardiomyocyte-specific long noncoding RNA regulates alternative splicing of the triadin gene in the heart. Circulation 2022;146:699–714. doi: 10.1161/CIRCULATIONAHA.121.058017.
154. Du H, Zhao Y, Wen J, Dai B, Hu G, Zhou Y, *et al*. LncRNA DCRT protects against dilated cardiomyopathy by preventing NDUFS2 alternative splicing by binding to PTBP1. Circulation 2024;150:1030–1049. doi: 10.1161/CIRCULATIONAHA.123.067861.
155. Mo ZW, Peng YM, Zhang YX, Li Y, Kang BA, Chen YT, *et al*. High-density lipoprotein regulates angiogenesis by long non-coding RNA HDRACA. Signal Transduct Target Ther 2023;8:299. doi: 10.1038/s41392-023-01558-6.
156. Han C, Yang J, Sun J, Qin G. Extracellular vesicles in cardiovascular disease: Biological functions and therapeutic implications. Pharmacol Ther 2022;233:108025. doi: 10.1016/j.pharmthera.2021.108025.
157. Nie X, Fan J, Li H, Yin Z, Zhao Y, Dai B, *et al*. miR-217 promotes cardiac hypertrophy and dysfunction by targeting PTEN. Mol Ther Nucleic Acids 2018;12:254–266. doi: 10.1016/j.omtn.2018.05.013.
158. Kenneweg F, Bang C, Xiao K, Boulanger CM, Loyer X, Mazlan S, *et al*. Long noncoding RNA-enriched vesicles secreted by hypoxic cardiomyocytes drive cardiac fibrosis. Mol Ther Nucleic Acids 2019;18:363–374. doi: 10.1016/j.omtn.2019.09.003.
159. Bang C, Batkai S, Dangwal S, Gupta SK, Foinquinos A, Holzmann A, *et al*. Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. J Clin Invest 2014;124:2136–2146. doi: 10.1172/JCI70577.
160. Lei D, Li B, Isa Z, Ma X, Zhang B. Hypoxia-elicited cardiac microvascular endothelial cell-derived exosomal miR-210-3p alleviate hypoxia/reoxygenation-induced myocardial cell injury through inhibiting transferrin receptor 1-mediated ferroptosis. Tissue Cell 2022;79:101956. doi: 10.1016/j.tice.2022.101956.
161. Wang X, Huang W, Liu G, Cai W, Millard RW, Wang Y, *et al*. Cardiomyocytes mediate anti-angiogenesis in type 2 diabetic rats through the exosomal transfer of miR-320 into endothelial cells. J Mol Cell Cardiol 2014;74:139–150. doi: 10.1016/j.yjmcc.2014.05.001.
162. Lin S, Lin Y, Nery JR, Urich MA, Breschi A, Davis CA, *et al*. Comparison of the transcriptional landscapes between human and mouse tissues. Proc Natl Acad Sci U S A 2014;111:17224–17229. doi: 10.1073/pnas.1413624111.
163. Riehle C, Bauersachs J. Small animal models of heart failure. Cardiovasc Res 2019;115:1838–1849. doi: 10.1093/cvr/cvz161.
164. Ramanujam D, Schon AP, Beck C, Vaccarello P, Felician G, Dueck A, *et al*. MicroRNA-21-dependent macrophage-to-fibroblast signaling determines the cardiac response to pressure overload. Circulation 2021;143:1513–1525. doi: 10.1161/CIRCULATIONAHA.120.050682.
165. Nugrahani ASD, Widiarti W, Ramadhan RN, Dyah Kencono Wungu C, Susilo H, Mohd Amin I. Diagnostic and prognostic value of circulating microRNA-21 in heart failure: A systematic review and meta-analysis. Biomol Biomed 2025;26:912–923. doi: 10.17305/bb.2025.13164.
166. Reddy S, Hu DQ, Zhao M, Blay E, Jr., Sandeep N, Ong SG, *et al*. miR-21 is associated with fibrosis and right ventricular failure. JCI Insight 2017;2:e91625. doi: 10.1172/jci.insight.91625.
167. Patrick DM, Montgomery RL, Qi X, Obad S, Kauppinen S, Hill JA, *et al*. Stress-dependent cardiac remodeling occurs in the absence of microRNA-21 in mice. J Clin Invest 2010;120:3912–3916. doi: 10.1172/JCI43604.
168. Diener C, Keller A, Meese E. The miRNA-target interactions: An underestimated intricacy. Nucleic Acids Res 2024;52:1544–1557. doi: 10.1093/nar/gkad1142.
169. Bourque K, Jones-Tabah J, Petrin D, Martin RD, Tanny JC, Hebert TE. Comparing the signaling and transcriptome profiling landscapes of human iPSC-derived and primary rat neonatal cardiomyocytes. Sci Rep 2023;13:12248. doi: 10.1038/s41598-023-39525-4.

170. Ramanathan M, Porter DF, Khavari PA. Methods to study RNA-protein interactions. Nat Methods 2019;16:225–234. doi: 10.1038/s41592-019-0330-1.
171. Leisegang MS, Warwick T, Stotzel J, Brandes RP. RNA-DNA triplexes: Molecular mechanisms and functional relevance. Trends Biochem Sci 2024;49:532–544. doi: 10.1016/j.tibs.2024.03.009.
172. Mattick JS, Amaral PP, Carninci P, Carpenter S, Chang HY, Chen LL, *et al*. Long non-coding RNAs: Definitions, functions, challenges and recommendations. Nat Rev Mol Cell Biol 2023;24:430–447. doi: 10.1038/s41580-022-00566-8.
173. Bosson AD, Zamudio JR, Sharp PA. Endogenous miRNA and target concentrations determine susceptibility to potential ceRNA competition. Mol Cell 2014;56:347–359. doi: 10.1016/j.molcel.2014.09.018.
174. Nathanson L, Xia T, Deutscher MP. Nuclear protein synthesis: A re-evaluation. RNA 2003;9:9–13. doi: 10.1261/rna.2990203.
175. Carlevaro-Fita J, Johnson R. Global Positioning System: Understanding Long Noncoding RNAs through Subcellular Localization. Mol Cell 2019;73:869–883. doi: 10.1016/j.molcel.2019.02.008.
176. Gagnon KT, Li L, Janowski BA, Corey DR. Analysis of nuclear RNA interference in human cells by subcellular fractionation and argonaute loading. Nat Protoc 2014;9:2045–2060. doi: 10.1038/nprot.2014.135.
177. Udi Y, Zhang W, Stein ME, Ricardo-Lax I, Pasolli HA, Chait BT, *et al*. A general method for quantitative fractionation of mammalian cells. J Cell Biol 2023;222:e91625. doi: 10.1083/jcb.202209062.
178. Anunciado-Koza RVP, Guntur AR, Vary CP, Gartner CA, Nowak M, Koza RA. Purification of functional mouse skeletal muscle mitochondria using percoll density gradient centrifugation. BMC Res Notes 2023;16:243. doi: 10.1186/s13104-023-06519-4.
179. Liao PC, Bergamini C, Fato R, Pon LA, Pallotti F. Isolation of mitochondria from cells and tissues. Methods Cell Biol 2020;155:3–31. doi: 10.1016/bs.mcb.2019.10.002.
180. Stanway RR, Graewe S, Rennenberg A, Helm S, Heussler VT. Highly efficient subcloning of rodent malaria parasites by injection of single merosomes or detached cells. Nat Protoc 2009;4(:1433–1439. doi: 10.1038/nprot.2009.172.
181. Jusic A, Erpapazoglou Z, Dalgaard LT, Lakkisto P, de Gonzalo-Calvo D, Benczik B, *et al*. Guidelines for mitochondrial RNA analysis. Mol Ther Nucleic Acids 2024;35:102262. doi: 10.1016/j.omtn.2024.102262.
182. Young AP, Jackson DJ, Wyeth RC. A technical review and guide to RNA fluorescence in situ hybridization. PeerJ 2020;8:e8806. doi: 10.7717/peerj.8806.
183. Arora A, Goering R, Velez PT, Taliaferro JM. Visualization and quantification of subcellular RNA localization using single-molecule RNA fluorescence in situ hybridization. Methods Mol Biol 2022;2404:247–266. doi: 10.1007/978-1-0716-1851-6_13.
184. Srinivasan S, Yeri A, Cheah PS, Chung A, Danielson K, De Hoff P, *et al*. Small RNA sequencing across diverse biofluids identifies optimal methods for exRNA isolation. Cell 2019;177:446–462.e1. doi: 10.1016/j.cell.2019.03.024.
185. Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, *et al*. The microRNA spectrum in 12 body fluids. Clin Chem 2010;56:1733–1741. doi: 10.1373/clinchem.2010.147405.
186. Masson S, Batkai S, Beermann J, Bar C, Pfanne A, Thum S, *et al*. Circulating microRNA-132 levels improve risk prediction for heart failure hospitalization in patients with chronic heart failure. Eur J Heart Fail 2018;20:78–85. doi: 10.1002/ejhf.961.
187. Akat KM, Moore-McGriff D, Morozov P, Brown M, Gogakos T, Correa Da Rosa J, *et al*. Comparative RNA-sequencing analysis of myocardial and circulating small RNAs in human heart failure and their utility as biomarkers. Proc Natl Acad Sci U S A 2014;111:11151–11156. doi: 10.1073/pnas.1401724111.
188. Duong Van Huyen JP, Tible M, Gay A, Guillemain R, Aubert O, Varnous S, *et al*. MicroRNAs as non-invasive biomarkers of heart transplant rejection. Eur Heart J 2014;35:3194–3202. doi: 10.1093/eurheartj/ehu346.
189. Parvan R, Hosseinpour M, Moradi Y, Devaux Y, Cataliotti A, da Silva GJJ. Diagnostic performance of microRNAs in the detection of heart failure with reduced or preserved ejection fraction: A systematic review and meta-analysis. Eur J Heart Fail 2022;24:2212–222. doi: 10.1002/ejhf.2700.
190. Shah RV, Rong J, Larson MG, Yeri A, Ziegler O, Tanriverdi K, *et al*. Associations of circulating extracellular RNAs with myocardial remodeling and heart failure. JAMA Cardiol 2018;3:871–876. doi: 10.1001/jamacardio.2018.2371.
191. Perez-Carrillo L, Gimenez-Escamilla I, Gonzalez-Torrent I, Delgado-Arija M, Sanchez-Lazaro I, Garcia-Manzanares M, *et al*. Circulating long non-coding RNAs detection after heart transplantation and its accuracy in the diagnosis of acute cardiac rejection. Biomark Res 2024;12:49. doi: 10.1186/s40364-024-00590-0.
192. Zhang Y, Sun L, Xuan L, Pan Z, Li K, Liu S, *et al*. Reciprocal changes of circulating long non-coding RNAs ZFAS1 and CDR1AS predict acute myocardial infarction. Sci Rep 2016;6:22384. doi: 10.1038/srep22384.
193. Kumarswamy R, Bauters C, Volkmann I, Maury F, Fetisch J, Holzmann A, *et al*. Circulating long noncoding RNA, LIPCAR, predicts survival in patients with heart failure. Circ Res 2014;114:1569–1575. doi: 10.1161/CIRCRESAHA.114.303915.
194. Santer L, Lopez B, Ravassa S, Baer C, Riedel I, Chatterjee S, *et al*. Circulating long noncoding RNA LIPCAR predicts heart failure outcomes in patients without chronic kidney disease. Hypertension 2019;73:820–828. doi: 10.1161/HYPERTENSIONAHA.118.12261.
195. Meessen J, Bar C, di Dona FM, Staszewsky LI, Di Giulio P, Di Tano G, *et al*. LIPCAR Is Increased in Chronic Symptomatic HF Patients. A sub-study of the GISSI-HF Trial. Clin Chem 2021;67:1721–1731. doi: 10.1093/clinchem/hvab197.
196. Nemeth K, Bayraktar R, Ferracin M, Calin GA. Non-coding RNAs in disease: From mechanisms to therapeutics. Nat Rev Genet 2023;25:211–232. doi: 10.1038/s41576-023-00662-1.
197. Matsui M, Corey DR. Non-coding RNAs as drug targets. Nat Rev Drug Discov 2017;16:167–179. doi: 10.1038/nrd.2016.117.
198. Collotta D, Bertocchi I, Chiapello E, Collino M. Antisense oligonucleotides: A novel Frontier in pharmacological strategy. Front Pharmacol 2023;14:1304342. doi: 10.3389/fphar.2023.1304342.
199. Bennett CF. Therapeutic Antisense Oligonucleotides Are Coming of Age. Ann RevMed 2019;70:307–321. doi: 10.1146/annurev-med-041217-010829.
200. Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, *et al*. Silencing of microRNAs in vivo with 'antagomirs'. Nature 2005;438:685–689. doi: 10.1038/nature04303.
201. Watts JK, Corey DR. Silencing disease genes in the laboratory and the clinic. J Pathol 2012;226:365–379. doi: 10.1002/path.2993.
202. Hua Y, Vickers TA, Baker BF, Bennett CF, Krainer AR. Enhancement of SMN2 exon 7 inclusion by antisense oligonucleotides targeting the exon. PLoS Biol 2007;5:e73. doi: 10.1371/journal.pbio.0050073.
203. Hodges D, Crooke ST. Inhibition of splicing of wild-type and mutated luciferase-adenovirus pre-mRNAs by antisense oligonucleotides. Mol Pharmacol 1995;48:905–918.
204. Wilson RC, Doudna JA. Molecular mechanisms of RNA interference. Annu Rev Biophys 2013;42:217–239. doi: 10.1146/annurev-biophys-083012-130404.
205. Taubel J, Hauke W, Rump S, Viereck J, Batkai S, Poetzsch J, *et al*. Novel antisense therapy targeting microRNA-132 in patients with heart failure: Results of a first-in-human Phase 1b randomized, double-blind, placebo-controlled study. Eur Heart J 2021;42:178–188. doi: 10.1093/eurheartj/ehaa898.
206. Bauersachs J, Solomon SD, Anker SD, Antorrena-Miranda I, Batkai S, Viereck J, *et al*. Efficacy and safety of CDR132L in patients with reduced left ventricular ejection fraction after myocardial infarction: Rationale and design of the HF-REVERT trial. Eur J Heart Fail 2024;26:674–682. doi: 10.1002/ejhf.3139.
207. Nemecz M, Stefan DS, Comarita IK, Constantin A, Tanko G, Guja C, *et al*. Microvesicle-associated and circulating microRNAs in diabetic dyslipidemia: miR-218, miR-132, miR-143, and miR-21, miR-122, miR-155 have biomarker potential. Cardiovasc Diabetol 2023;22:260. doi: 10.1186/s12933-023-01988-0.
208. Koyama Y, Muguruma M, Horimoto Y, Narui K, Yamada A, Yamada K, *et al*. Hsa-miR-155-5p expression in primary breast tissue may have the potential for prediction of breast cancer brain recurrence: Results from the multi-institutional exploratory cohort study. Breast Cancer Res 2025;27:169. doi: 10.1186/s13058-025-02123-5.
209. Cerda A, Amaral AA, de Oliveira R, Moraes TI, Braga AA, Graciano-Saldarriaga ME, *et al*. Peripheral blood miRome identified miR-155 as potential biomarker of MetS and cardiometabolic risk in obese patients. Int J Mol Sci 2021;22:1468. doi: 10.3390/ijms22031468.
210. Shao C, Yang F, Qin Z, Jing X, Shu Y, Shen H. The value of miR-155 as a biomarker for the diagnosis and prognosis of lung cancer: A

systematic review with meta-analysis. BMC Cancer 2019;19:1103. doi: 10.1186/s12885-019-6297-6.
211. Jing L, Hou Y, Wu H, Miao Y, Li X, Cao J, *et al*. Transcriptome analysis of mRNA and miRNA in skeletal muscle indicates an important network for differential residual feed intake in pigs. Sci Rep 2015;5:11953. doi: 10.1038/srep11953.
212. Cui S, Sun B, Yin X, Guo X, Chao D, Zhang C, *et al*. Time-course responses of circulating microRNAs to three resistance training protocols in healthy young men. Sci Rep 2017;7:2203. doi: 10.1038/s41598-017-02294-y.
213. Chalchat E, Charlot K, Garcia-Vicencio S, Hertert P, Bauge S, Bourdon S, *et al*. Circulating microRNAs after a 24-h ultramarathon run in relation to muscle damage markers in elite athletes. Scand J Med Sci Sports 2021;31:1782–1795. doi: 10.1111/sms.14000.
214. Kumar MA, Baba SK, Sadida HQ, Marzooqi SA, Jerobin J, Altemani FH, *et al*. Extracellular vesicles as tools and targets in therapy for diseases. Signal Transduct Target Ther 2024;9:27. doi: 10.1038/s41392-024-01735-1.
215. Turchinovich A, Weiz L, Langheinz A, Burwinkel B. Characterization of extracellular circulating microRNA. Nucleic Acids Res 2011;39:7223–7233. doi: 10.1093/nar/gkr254.
216. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol 2011;13:423–433. doi: 10.1038/ncb2210.
217. McDonald JS, Milosevic D, Reddi HV, Grebe SK, Algeciras-Schimnich A. Analysis of circulating microRNA: Preanalytical and analytical challenges. Clin Chem 2011;57:833–840. doi: 10.1373/clinchem.2010.157198.
218. Mompeon A, Ortega-Paz L, Vidal-Gomez X, Costa TJ, Perez-Cremades D, Garcia-Blas S, *et al*. Disparate miRNA expression in serum and plasma of patients with acute myocardial infarction: A systematic and paired comparative analysis. Sci Rep 2020;10:5373. doi: 10.1038/s41598-020-61507-z.
219. Kim DJ, Linnstaedt S, Palma J, Park JC, Ntrivalas E, Kwak-Kim JY, *et al*. Plasma components affect accuracy of circulating cancer-related microRNA quantitation. J Mol Diagn 2012;14:71–80. doi: 10.1016/j.jmoldx.2011.09.002.
220. Kim SH, MacIntyre DA, Sykes L, Arianoglou M, Bennett PR, Terzidou V. Whole blood holding time prior to plasma processing alters microRNA expression profile. Front Genet 2021;12:818334. doi: 10.3389/fgene.2021.818334.
221. Kirschner MB, Edelman JJ, Kao SC, Vallely MP, van Zandwijk N, Reid G. The impact of hemolysis on cell-free microRNA biomarkers. Front Genet 2013;4:94. doi: 10.3389/fgene.2013.00094.
222. Benz F, Roderburg C, Vargas Cardenas D, Vucur M, Gautheron J, Koch A, *et al*. U6 is unsuitable for normalization of serum miRNA levels in patients with sepsis or liver fibrosis. Exp Mol Med 2013;45:e42. doi: 10.1038/emm.2013.81.
223. Pastor-Navarro B, Ramirez-Calvo M, Gil-Aldea I, Cortell-Granero I, Lopez-Guerrero JA. The Impact of tube type, centrifugation conditions, and hemolysis on plasma circulating microRNAs. Diagnostics (Basel) 2024;14:2369. doi: 10.3390/diagnostics14212369.
224. Viereck J, Thum T. Circulating noncoding RNAs as biomarkers of cardiovascular disease and injury. Circ Res 2017;120:381–399. doi: 10.1161/CIRCRESAHA.116.308434.
225. Schulte C, Barwari T, Joshi A, Zeller T, Mayr M. Noncoding RNAs versus protein biomarkers in cardiovascular disease. Trends Mol Med 2020;26:583–596. doi: 10.1016/j.molmed.2020.02.001.
226. Gil-Cabrerizo P, Simon-Yarza T, Garbayo E, Blanco-Prieto MJ. Navigating the landscape of RNA delivery systems in cardiovascular disease therapeutics. Adv Drug Deliv Rev 2024;208:115302. doi: 10.1016/j.addr.2024.115302.
227. Saber N, Senti ME, Schiffelers RM. Lipid nanoparticles for nucleic acid delivery beyond the Liver. Hum Gene Ther 2024;35:617–627. doi: 10.1089/hum.2024.106.
228. Kim Y, Landstrom AP, Shah SH, Wu JC, Seidman CE, American Heart A. Gene therapy in cardiovascular disease: recent advances and future directions in science: A science advisory from the American Heart Association. Circulation 2024;150:e471–e80. doi: 10.1161/CIR.0000000000001296.
229. Ma C, Yang Z, Wang J, She H, Tan L, Ye Q, *et al*. Exosomes miRNA-499a-5p targeted CD38 to alleviate anthraquinone induced cardiotoxicity: Experimental research. Int J Surg 2024;110:1992–2006. doi: 10.1097/JS9.0000000000001118.
230. Zhou L, Bi J, Chang S, Bai Z, Yu J, Wang R, *et al*. Self-assembled antibody-oligonucleotide conjugates for targeted delivery of complementary antisense oligonucleotides. Angew Chem Int Ed Engl 2025;64:e202415272. doi: 10.1002/anie.202415272.
231. Brillante S, Volpe M, Indrieri A. Advances in microRNA therapeutics: From preclinical to clinical studies. Hum Gene Ther 2024;35:628–648. doi: 10.1089/hum.2024.113.
232. Magill ST, Cambronne XA, Luikart BW, Lioy DT, Leighton BH, Westbrook GL, *et al*. microRNA-132 regulates dendritic growth and arborization of newborn neurons in the adult hippocampus. Proc Natl Acad Sci U S A 2010;107:20382–20387. doi: 10.1073/pnas.1015691107.
233. Ghafouri-Fard S, Abak A, Tavakkoli Avval S, Shoorei H, Taheri M, Samadian M. The impact of non-coding RNAs on macrophage polarization. Biomed Pharmacother 2021;142:112112. doi: 10.1016/j.biopha.2021.112112.
234. Kumarswamy R, Volkmann I, Beermann J, Napp LC, Jabs O, Bhayadia R, *et al*. Vascular importance of the miR-212/132 cluster. Eur Heart J 2014;35:3224–3231. doi: 10.1093/eurheartj/ehu344.
235. Bakshi N, Kanavalli M, Nowak KZ, Bandyra KJ. Human polynucleotide phosphorylase in mitochondrial RNA metabolism. Biosci Rep 2025;45:531–546. doi: 10.1042/BSR20240504.
236. Kura B, Kalocayova B, Devaux Y, Bartekova M. Potential clinical implications of miR-1 and miR-21 in heart disease and cardioprotection. Int J Mol Sci 2020;21:700. doi: 10.3390/ijms21030700.
237. Safieddine A, Coleno E, Lionneton F, Traboulsi AM, Salloum S, Lecellier CH, *et al*. HT-smFISH: A cost-effective and flexible workflow for high-throughput single-molecule RNA imaging. Nat Protoc 2023;18:157–187. doi: 10.1038/s41596-022-00750-2.
238. Xia C, Fan J, Emanuel G, Hao J, Zhuang X. Spatial transcriptome profiling by MERFISH reveals subcellular RNA compartmentalization and cell cycle-dependent gene expression. Proc Natl Acad Sci U S A 2019;116:19490–19499. doi: 10.1073/pnas.1912459116.
239. Allen R, Yokota T. Endosomal Escape and Nuclear Localization: Critical Barriers for Therapeutic Nucleic Acids. Molecules 2024;29:5997. doi: 10.3390/molecules29245997.
240. Kubo T, Zhelev Z, Bakalova R, Ohba H, Doi K, Fujii M. Controlled intracellular localization and enhanced antisense effect of oligonucleotides by chemical conjugation. Org Biomol Chem 2005;3:3257–3259. doi: 10.1039/b507691a.
241. Hill AC, Becker JP, Slominski D, Halloy F, Sondergaard C, Ravn J, *et al*. Peptide conjugates of a 2′-O-methoxyethyl phosphorothioate splice-switching oligonucleotide show increased entrapment in endosomes. ACS Omega 2023;8:40463–40481. doi: 10.1021/acsomega.3c05144.
242. Guzman-Villanueva D, Mendiola MR, Nguyen HX, Yambao F, Yu N, Weissig V. Conjugation of triphenylphosphonium cation to hydrophobic moieties to prepare mitochondria-targeting nanocarriers. Methods Mol Biol 2019;2000:183–189. doi: 10.1007/978-1-4939-9516-5_12.
243. Furukawa R, Yamada Y, Kawamura E, Harashima H. Mitochondrial delivery of antisense RNA by MITO-Porter results in mitochondrial RNA knockdown, and has a functional impact on mitochondria. Biomaterials 2015;57:107–115. doi: 10.1016/j.biomaterials.2015.04.022.
244. Wang Z, Guo W, Kuang X, Hou S, Liu H. Nanopreparations for mitochondria targeting drug delivery system: Current strategies and future prospective. Asian J Pharm Sci 2017;12:498–508. doi: 10.1016/j.ajps.2017.05.006.
245. Cao Y, Yang Y, Wang L, Li L, Zhang J, Gao X, *et al*. Analyses of long non-coding RNA and mRNA profiles in right ventricle myocardium of acute right heart failure in pulmonary arterial hypertension rats. Biomed Pharmacother 2018;106:1108–1115. doi: 10.1016/j.biopha.2018.07.057.
246. Zeng ZH, Wu WH, Peng Q, Sun YH, Liu JX. MicroRNA-132 mediates proliferation and migration of pulmonary smooth muscle cells via targeting PTEN. Mol Med Rep 2019;19:3823–3830. doi: 10.3892/mmr.2019.10053.
247. Culley MK, Chan SY. Mitochondrial metabolism in pulmonary hypertension: Beyond mountains there are mountains. J Clin Invest 2018;128:3704–3715. doi: 10.1172/JCI120847.
248. Tan Y, Zhang Z, Zheng C, Wintergerst KA, Keller BB, Cai L. Mechanisms of diabetic cardiomyopathy and potential therapeutic strategies: Preclinical and clinical evidence. Nat Rev Cardiol 2020;17:585–607. doi: 10.1038/s41569-020-0339-2.
249. Wang W, Jia YJ, Yang YL, Xue M, Zheng ZJ, Wang L, *et al*. LncRNA GAS5 exacerbates renal tubular epithelial fibrosis by acting as a competing endogenous RNA of miR-96-5p. Biomed Pharmacother 2020;121:109411. doi: 10.1016/j.biopha.2019.109411.

250. Tao H, Zhang JG, Qin RH, Dai C, Shi P, Yang JJ, *et al*. LncRNA GAS5 controls cardiac fibroblast activation and fibrosis by targeting miR-21 via PTEN/MMP-2 signaling pathway. Toxicology 2017;386:11–18. doi: 10.1016/j.tox.2017.05.007.
251. Baradan R, Hollander JM, Das S. Mitochondrial miRNAs in diabetes: Just the tip of the iceberg. Can J Physiol Pharmacol 2017;95:1156–1162. doi: 10.1139/cjpp-2016-0580.
252. Liu J, Zhang Y, Tian Y, Huang W, Tong N, Fu X. Integrative biology of extracellular vesicles in diabetes mellitus and diabetic complications. Theranostics 2022;12:1342–1372. doi: 10.7150/thno.65778.
253. Park YJ, Pang MG. Mitochondrial functionality in male fertility: From spermatogenesis to fertilization. Antioxidants (Basel) 2021;10:98. doi: 10.3390/antiox10010098.
254. Mai Z, Yang D, Wang D, Zhang J, Zhou Q, Han B, *et al*. A narrative review of mitochondrial dysfunction and male infertility. Transl Androl Urol 2024;13:2134–2145. doi: 10.21037/tau-24-262.
255. Yuan S, Schuster A, Tang C, Yu T, Ortogero N, Bao J, *et al*. Sperm-borne miRNAs and endo-siRNAs are important for fertilization and preimplantation embryonic development. Development 2016;143:635–647. doi: 10.1242/dev.131755.
256. Song R, Hu XQ, Zhang L. Mitochondrial MiRNA in cardiovascular function and disease. Cells 2019;8:1475. doi: 10.3390/cells8121475.
257. Liu X, Guo JW, Lin XC, Tuo YH, Peng WL, He SY, *et al*. Macrophage NFATc3 prevents foam cell formation and atherosclerosis: evidence and mechanisms. Eur Heart J 2021;42:4847–4861. doi: 10.1093/eurheartj/ehab660.
258. Di Mauro V, Crasto S, Colombo FS, Di Pasquale E, Catalucci D. Wnt signalling mediates miR-133a nuclear re-localization for the transcriptional control of Dnmt3b in cardiac cells. Sci Rep 2019;9:9320. doi: 10.1038/s41598-019-45818-4.

How to cite this article: Tang YY, Xie R, Cheng X, Wang XY, Bibli SI, Li HP, Chen C. microRNAs and long noncoding RNAs in heart failure: Biogenesis, subcellular functions, and clinical applications. Chin Med J 2026;XXX:1–31. doi: 10.1097/CM9.0000000000004177