



Regulatory RNAs in Heart Failure

ABSTRACT: Cardiovascular disease is an enormous socioeconomic burden worldwide and remains a leading cause of mortality and disability despite significant efforts to improve treatments and personalize healthcare. Heart failure is the main manifestation of cardiovascular disease and has reached epidemic proportions. Heart failure follows a loss of cardiac homeostasis, which relies on a tight regulation of gene expression. This regulation is under the control of multiple types of RNA molecules, some encoding proteins (the so-called messenger RNAs) and others lacking protein-coding potential, named noncoding RNAs. In this review article, we aim to revisit the notion of regulatory RNA, which has been thus far mainly confined to noncoding RNA. Regulatory RNA, which we propose to abbreviate as regRNA, can include both protein-coding RNAs and noncoding RNAs, as long as they contribute, directly or indirectly, to the regulation of gene expression. We will address the regulation and functional role of messenger RNAs, microRNAs, long noncoding RNAs, and circular RNAs (ie, regRNAs) in heart failure. We will debate the utility of regRNAs to diagnose, prognosticate, and treat heart failure, and we will provide directions for future work.

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INTRODUCTION: THE EVER-GROWING BURDEN OF HEART FAILURE

Heart failure is an ever-growing problem for which new therapeutic interventions are still required. This is despite remarkable advances made in our understanding of heart failure and in our management of the condition. So what is the problem with heart failure? There are two major issues: (1) vast numbers of patients have the condition; and (2) despite treatment advances, the prognosis remains poor. The estimated prevalence of heart failure in industrialized societies is 1% to 2% of all adults, rising to >10% of those >70 years of age. In Europe, the lifetime risk for an individual 55 years of age is estimated at 33% for men and 28% for women,¹ and in the United States the lifetime risk is 20% for those 40 years of age or more.² In the United States, more than 650 000 incident cases are identified annually. Although this figure has remained relatively stable, improvements in management and subsequent survival have led to the increasing prevalence of heart failure in the US population³ and in most other industrialized countries;⁴ developing countries face an epidemic yet to materialize fully.

In this article, we describe the place of RNAs in aspects of heart failure and their potential as novel clinical and therapeutic biomarkers. We introduce the concept

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analysis

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of regulatory RNAs (regRNAs) and gather the current knowledge of the role of RNA molecules with regulatory functions (ie, regRNAs) in several pathophysiological pathways associated with heart failure, namely cardiac hypertrophy, inflammation, regeneration, and angiogenesis. We emphasize the translational potential of regRNAs and propose directions for future work. Because of the large amount of available data in the field and space limitations for this article, we unfortunately could not cite several interesting works.

THE RNA FAMILY: REGULATORY RNAS REVISITED

For many years, the scientific community has mostly dedicated attention to a mere $\approx 2\%$ of the human genome corresponding to protein-coding exons of messenger RNAs (mRNAs). Ribosomal RNAs, transfer RNAs, small nuclear and nucleolar RNAs, although known for many years, displayed mainly housekeeping functions dedicated to mRNA maturation and protein synthesis. From the beginning of this century, however, high-throughput RNA-sequencing analysis of the genomic functional elements has clearly shown that more than 70% of the human genome is transcribed. It is now clear that the genome portions previously indicated as “junk DNA” are transcribed, giving origin to a large and heterogeneous family of RNA molecules with a variety of functions. Indeed, RNAs have surfaced as a promising tool in the field of diagnostics, therapeutics, and personalized medicine. Figure 1 displays the categories of RNAs known so far and constituting the “RNA family.” Several categories possess regulatory roles and can be grouped into a subfamily that we propose to name regRNAs.

Regulatory RNAs are composed mostly of noncoding RNAs (ncRNAs). MicroRNAs (miRNAs), long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs) have gained increased interest in the cardiovascular community for their ability to modulate cellular responses. These should be intended as operative categories, given that the distinction between coding and noncoding RNAs is at times blurred, with several cases of RNA species displaying dual roles encoding microproteins (less than 100 amino acids). Accordingly, the genomewide analysis of heart transcriptomes, characterized using ribosome profiling, identified hundreds of microproteins, expressed from both lncRNAs and circRNAs.⁵ Some of these microproteins are expressed from functionally characterized lncRNAs, suggesting their contribution to biological functions previously assigned to the lncRNA. On the other hand, protein-coding sequences display evidence of other embedded functions, as indicated by constraints on synonymous codon usage.

Regulatory RNAs can be part of complex structures or interact with RNA-binding proteins, DNA, and other RNA types, regulating their activity. They display distinctive temporal and spatial patterns of expression, indicating a precise regulation of their expression. They can be subjected to methylation, the most important being N6-methyladenosine. Furthermore, given that certain regRNAs demonstrate increased stability in the blood and that dysregulated numbers represent different disease states, they may function as important disease biomarkers. Noncoding RNAs are classified for practical reasons according to their size, less or more than 200 nucleotides long, into short- and long-ncRNAs, respectively (Figure 1). The main mechanisms of action of ncRNAs are summarized in Figure 2.

Short Noncoding RNAs

Short ncRNAs include different RNA categories, such as small interfering RNAs, piwi-interacting RNAs, and miRNAs, that are the most widely studied.

In most circumstances, miRNAs are transcribed as long precursors in which miRNA-containing hairpins are cleaved by the sequential actions of DROSHA-DGCR8 (Drosha Ribonuclease III; DiGeorge Syndrome Critical Region Gene 8) microprocessor in the nucleus and of DICER-TRBP (Dicer 1, Ribonuclease III; transactivation response element RNA-binding protein) complex in the cytoplasm. The product is a ≈ 22 -nt mature miRNA that binds to Argonaute proteins to form the RNA-induced silencing complex, which mediates target-gene silencing by mRNA degradation or translation inhibition. MiRNA binding sites have been identified in 3' and 5' untranslated regions, introns as well as exonic regions of protein-coding mRNAs and lncRNAs. Moreover, miRNAs targeting of the 5'-untranslated region can even induce gene expression, and a role in both negative and positive gene transcriptional regulation by intranuclear miRNAs is also emerging.⁶

Regardless of their mechanism of action, each miRNA can regulate hundreds of mRNAs, often targeting whole pathways, and each transcript can be targeted by more than 1 miRNA, in a combinatorial way. In addition, miRNA families have identical seed-sequences, likely sharing most targets. The result is a complex network, often displaying a high degree of redundancy, that may be necessary to stabilize central pathways.

Long Noncoding RNAs

The classification of lncRNAs is not standardized, and their nomenclature is often related to experimental features, such as tissue specificity, mechanism of action, or molecular function. One possible classification is according to their genomic location related to protein-coding genes. For example, natural antisense lncRNAs

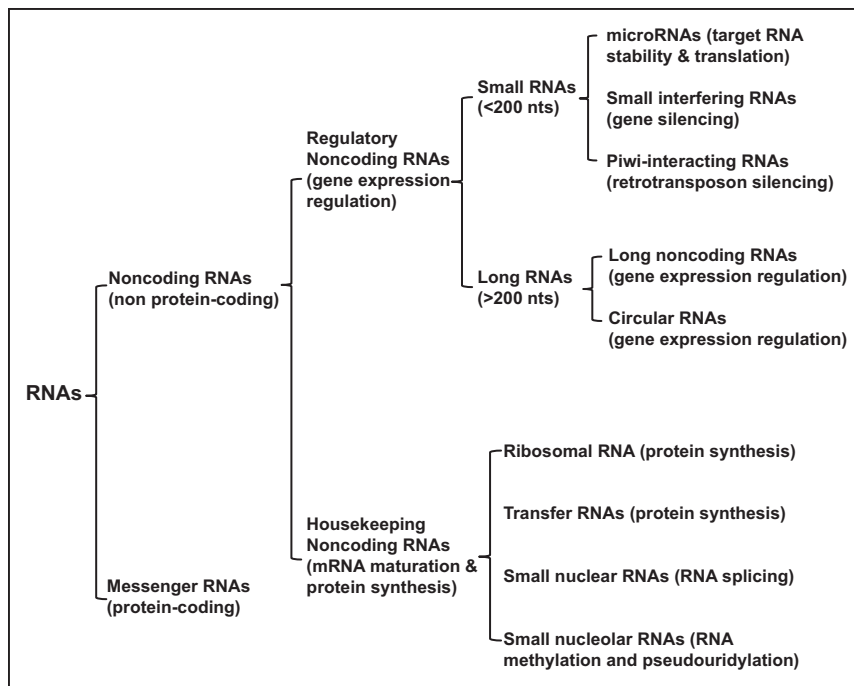


Figure 1. The RNA family and regulatory RNAs.

Nts indicates nucleotides.

are expressed from the opposite DNA-strand of mRNAs, whereas long intergenic noncoding RNA (lincRNAs) are localized in intergenic regions.

Although there are a few examples of lincRNAs displaying a conserved primary sequence, conservation is generally weak. However, different computational methods indicate that the secondary structure of lincRNAs is often conserved, suggesting that structural constraints may be more relevant than the nucleotide sequence in maintaining function.

Indeed, while many lincRNAs act in trans, leaving the site of transcription and executing a variety of cellular functions throughout the cell, others work in cis, affecting the expression and/or chromatin state of

nearby genes. Among cis-acting lincRNAs, some regulate the expression of neighboring genes through recruitment of regulatory factors to the originating loci, thereby modulating their function. A prototype of this class of lincRNAs is represented by XIST (X-inactive specific transcript). For other lincRNAs such as Airn or Blutr, the process of transcription or splicing of the lincRNA and not the transcript itself elicits the regulation of gene expression. For yet other lincRNAs such as lincRNA-p21, the functional elements are only the DNA sequences within the promoter, regardless of the encoded RNA or its generation.

Despite these specificities, mechanisms of action and functions of an ever-increasing number of

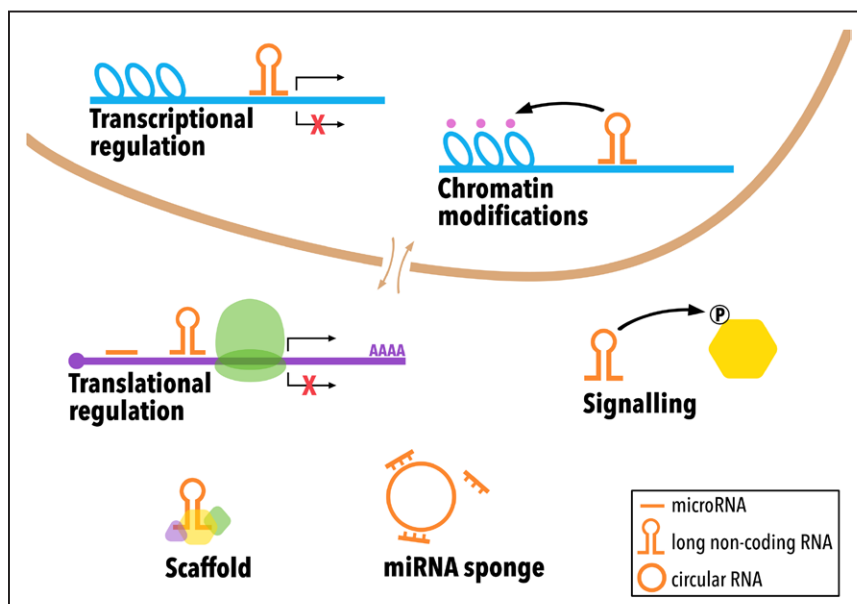


Figure 2. Main mechanisms of action of ncRNAs.

Noncoding RNAs (microRNAs, long non-coding RNAs, and circular RNAs), represented in orange, act in different ways to regulate gene expression, such as chromatin modification, transcriptional and translational activation and repression, protein signalling, scaffold, and miRNA sponge. miRNA indicates microRNA.

lncRNAs have started to be elucidated, notably in the cardiovascular field.⁷ One of the best-studied features of lncRNA is their role in the epigenetic regulation of gene expression. Some nuclear lncRNAs, such as XIST, work as scaffolds for chromatin modification enzymes, modulating positively or negatively, reversibly or irreversibly, the expression of individual genes and whole chromosomal regions. lncRNAs can bring together transcriptional repressors and activators, modulating the rates of RNA Polymerase II initiation and elongation, interacting with a variety of complexes, such as Polycomb Repressive Complex 1 or 2. Some nuclear lncRNAs have been implicated in maintaining nuclear bodies, such as nuclear speckles, built on the architectural lncRNA, NEAT1.⁸ Other lncRNAs regulate gene expression posttranscriptionally, modulating mRNA maturation and stability through different mechanisms. For example, some antisense lncRNAs can base-pair with sense-mRNAs, thereby altering splice-site recognition and spliceosome recruitment.⁹

Other lncRNAs, such as MALAT1 (metastasis-associated lung adenocarcinoma transcript 1), do not hybridize with the target mRNA but can affect mRNA splicing through cooperation with the splicing machinery.¹⁰ lncRNAs can induce both stabilization and destabilization of target mRNA transcripts. Some lncRNAs act as a molecular scaffold, such as H19, which favors mRNA degradation mediated by the protein KSRP of myogenin.¹¹ Others bind their complementary sense transcripts and mask miRNA binding sites, such as BACE1 antisense that protects BACE1 from miR-485-5p inhibition.¹² Several lncRNAs act as competing endogenous RNAs (ceRNAs), sponging miRNAs and possibly other regulatory factors. As an example, linc-MD1 is encoded by the genomic locus containing the miR-206 and miR-133b and works as ceRNA for miR-133 and miR-135.¹³ lncRNAs can also affect gene expression at the posttranslational level, inducing ubiquitin-mediated proteolysis. For example, HOTAIR, in addition to its epigenetic function, can bind to E3 ubiquitin-ligases bearing RNA-binding domains, along with their respective ubiquitination substrates, facilitating their ubiquitination and degradation.¹⁴

A particular class of lncRNAs is represented by circRNAs, that are the product of back-splicing events and that can accumulate at relatively high levels, at least in part, because of their resistance to exonucleases.¹⁵ One prominent function of circRNAs is to act as ceRNA (miRNA sponges), but they can also modulate RNA transcription, splicing, turnover, translation, and may even have protein coding potential.¹⁵ Regardless of the specific ceRNA nature, miRNA/ceRNA stoichiometry is essential for effective miRNA sequestration. Indeed, although virtually all ncRNAs display at least 1 putative miRNA binding-site, only a few are expressed at levels sufficiently high to operate as ceRNAs.

Overall, regRNAs constitute a diverse class of RNA molecules that significantly affect gene expression and are of paramount importance for the maintenance of cardiac homeostasis. Multiple regRNAs are dysregulated in the failing heart, and many of them functionally contribute to the development and progression of heart failure.

Epitranscriptome

RNAs are subjected to diverse types of chemical modifications, which affect their fate and impact cellular responses, a field called epitranscriptomics. Although chemical modifications on mRNA are known for decades, studies show that ncRNAs also undergo different modifications (Figure 3). However, investigations on regRNA modifications and their effects are restricted because of technical limitations. It has been suggested that the epitranscriptome in regRNAs affects their regulatory activity, stability, protection from degradation, and recruitment of proteins to specific sites of the regRNA.¹⁶ The epitranscriptome provides another layer of complexity to the transcriptome.

REGULATORY RNAS IN THE CIRCULATION

Although RNA molecules are usually considered unstable, particularly in the circulation because of the abundance of exonucleases, different types of RNA can be found intact. Both coding and ncRNAs are protected from degradation through different means, such as packed in lipid vesicles (eg, exosomes and microvesicles), bound to high-density lipoproteins or proteins, especially Argonaute 1 and 2 (which is the case of miRNAs), and by their circular structure. CircRNAs are significantly more stable than linear RNAs because of their lack of free ends, poly(A) tails, and 5' caps. The stability of RNAs in the circulation reinforces their intercellular communication and regulatory roles. Likewise, their presence in extracellular vesicles further supports such a biological function. There is increasing evidence that cells package and export different molecules, including regRNAs, in extracellular vesicles. These are taken up by recipient cells, where their cargo can induce expression and functional changes.

The spectrum of regRNAs in the circulation is highly dynamic, according to the health status of an individual. Numerous regRNAs have been described to have distinct profiles associated with specific pathophysiological states. There are many reports of miRNAs, lncRNAs, and circRNAs for which altered levels correlate with different cardiovascular diseases such as heart failure. Circulating regRNA profiles have also been reported to change in response to environmental and lifestyle factors, such as exercise, diet, and circadian rhythm.¹⁷

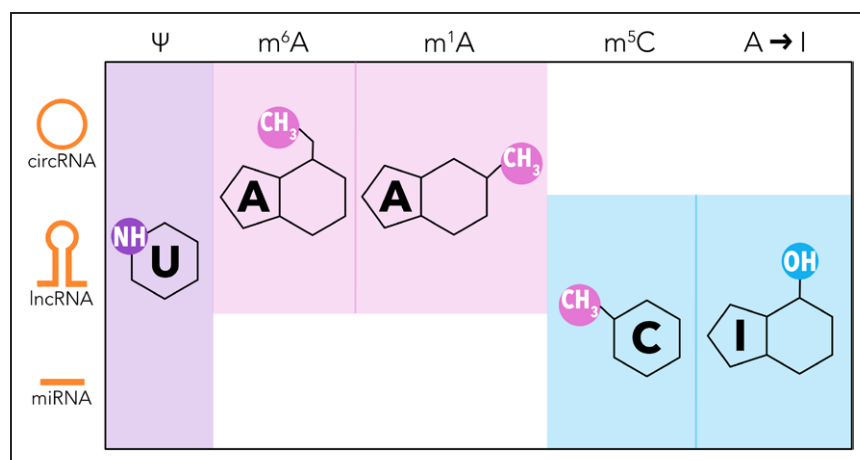


Figure 3. Most frequent posttranscriptional modifications in regRNAs.

Simplified chemical structure of modified RNA nitrogenous bases: purple, pseudouridine [y], reported in the three ncRNAs; pink, N6-methyladenosine [m6A] and N1-methyladenosine [m1A], observed in circRNA and lncRNA; blue, 5-methylcytosine [m5C] and adenosine to inosine editing [A to I], occurring in lncRNA and miRNA. circRNA indicates circular RNA; lncRNA, long non-coding RNA; miRNA, microRNA; and regRNA, regulatory RNA.

RegRNAs, particularly miRNAs, have characteristics that make them prominent clinical biomarkers: stability, ease of access, the possibility to be measured in a routine lab, and specificity to pathophysiological states. Indeed, different miRNAs or profiles of miRNAs have been described as potential biomarkers for virtually all cardiovascular diseases, as well as lncRNAs and circRNAs, although less extensively. Circulating regRNAs could serve not only as prognostic and diagnostic biomarkers for disease but also for treatment selection and monitoring, and as therapeutic targets.¹⁸

REGULATORY RNAs IN CARDIAC HYPERTROPHY

MiRNAs

Cardiac hypertrophy is a hallmark of heart failure. Numerous miRNA-mediated mechanisms underlying cardiac hypertrophy have been identified (Table 1).

MiRNA-148a was shown to control the transition from concentric hypertrophy toward dilation upon pressure overload in mice.¹⁹ Adeno-associated viral delivery of miR-148a protected the mouse heart from pressure overload-induced systolic dysfunction, whereas antagomir-mediated silencing of miR-148a caused cardiac dilation and failure. MiR-146a induced cardiac dysfunction during maladaptive hypertrophy through the suppression of cardiac SUMO1 expression.²⁰ Overexpression of miR-146a using AAV9 (adeno associated virus 9) vectors reduced SUMO1 expression, SERCA2a SUMOylation, and cardiac contractility both in vitro and in vivo. It is interesting that transdifferentiation of fibroblasts triggers miR-146a upregulation and secretion through extracellular vesicles. This transfer was identified as the causative mechanism of miR-146a upregulation in failing cardiomyocytes. MiR-29 promotes pathologic hypertrophy of cardiac myocytes and overall cardiac dysfunction.²¹ In a mouse model of cardiac pressure overload, both global genetic deletion

of miR-29 and anti-miR-29 infusion prevented cardiac hypertrophy and fibrosis and improved cardiac function. Targeted deletion of miR-29 in cardiomyocytes in vivo also prevented cardiac hypertrophy and fibrosis, implying that the role of miR-29 in cardiomyocytes dominates over that in nonmyocyte cell types. The effects of miR-29 on cardiomyocyte hypertrophy were at least partly mediated by several targets involved in Wnt signaling.

Overexpression of miR-135b may attenuate pathological cardiac hypertrophy by targeting CACNA1C.²² MiR-19a/b-3p was found to be antihypertrophic in a model of angiotensin II-induced hypertrophy, an effect involving the phosphodiesterase PDE5A, a leading factor contributing to cGMP signaling and cardiac hypertrophy.²³ The miR-99 family fine-tunes Egr-1/Akt1 signaling and thereby balance cardiomyocyte physiological and pathological hypertrophic responses.²⁴ MiR-672-5p, which is downregulated in hypertrophic cardiomyocytes, reduces cardiomyocyte hypertrophy in vitro by inhibiting JUN expression.²⁵ Furthermore, cardiac hypertrophy was shown to be attenuated in angiotensin II-infused mice that received tail vein injection of miR-92b-3p mimic.²⁶ Myocyte-specific enhancer factor 2D was identified as a potential target gene mediating this effect. Cardiac-specific overexpression of miR-18 via AAV2 protected against cardiac dilatation during hypertension-induced heart failure.²⁷ Here, the p53-miR-18-HSF2-IGF-IIR axis was claimed to be involved. In a mouse model of cardiac hypertrophy induced by phenylephrine, the administration of miR-485-5p agomir significantly decreased phenylephrine-induced SUMO E3 ligase, mitochondrial anchored protein ligase, and hypertrophic markers atrial natriuretic peptide and β -myosin heavy chain, and protected against cardiac dysfunction.²⁸ MiR-297 promotes cardiomyocyte hypertrophy by inhibiting the expression of Sigma-1 receptor, a ligand-regulated endoplasmic reticulum chaperone involved in cardiac hypertrophy, and activation of endoplasmic reticulum stress signaling.²⁹

Table 1. Regulatory RNAs and RNA Binding Proteins Associated With Cardiac Hypertrophy

Regulatory RNAs	Experimental Model	Effect on Hypertrophy	Mechanism of Action	References
miRNAs				
miR-148a	TAC in mouse	Prevents transition of pressure-overload induced concentric hypertrophic remodeling toward eccentric hypertrophy	STAT3 signaling	19
miR-146a	TAC in mouse; AAV9-miR-146a in mouse	Induction	SUMO1/SERCA2a signaling	20
miR-29	TAC in mouse	MiR-29 inhibition prevents hypertrophy	Wnt signaling	21
miR-135b	TAC in mice; nMCM+AngII	Attenuation	CACNA1C	22
miR-19a/b-3p	AngII in mice	Reduction	Phosphodiesterase 5A	23
miR-99	Swimming or isoproterenol in rats; H9c2+ α -2M or isoprenaline	Balance physiological/ pathological hypertrophy	Akt-1 signaling	24
miR-672-5p	nRCM+AngII	Downregulation allows hypertrophy	JUN	25
miR-92b-3p	AngII in mouse	Attenuation	MEF2D	26
miR-18	AngII in mouse and nRCM	Downregulation allows hypertrophy	IGF-IIR signaling	27
miR-485-5p	Phenylephrin in mouse	Reduction	Mitochondrial fission	28
miR-297	TAC in mouse; nRCM+AngII	Promotes	Endoplasmic reticulum stress	29
lncRNAs				
Mhrt	TAC in mouse	Downregulation allows hypertrophy	Chromatin remodeling	30
Chaer	TAC in mouse; nRCM+ Phenylephrin	Necessary for hypertrophy	Epigenetic reprogramming and induction of hypertrophic genes	31
TINCR	TAC in mouse	Attenuation	Epigenetic silencing CaMKII	32
Chast	TAC in mouse; RCM+ Phenylephrin	Induction	Regulation of autophagy and hypertrophy via Pleckstrin homology domain-containing protein family M member 1	33
XIST	nMCM+Phenylephrin	Attenuation	MiR-330-3p/S100B	34
CHRF	nMCM+AngII; nMCM+ Isoproterenol	Induction	CeRNA; inhibits antihypertrophic miR-489; ceRNA of miR-93 to sequester miR-93 from Akt3	35
Plscr4	TAC in mouse; nMCM+AngII	Attenuation	CeRNA; inhibits prohypertrophic miR-214	36
MIAT	H9c2+AngII	Enhancement	CeRNA; inhibits antihypertrophic miR-150	37
H19	nMCM+Phenylephrin	Inhibition	Through miR-675 function, partly on CAMKII δ	38
HOTAIR	nMCM+AngII	Inhibition	CeRNA for miR-19/PTEN	39
lncRNA-ROR	nRCM	Knockdown reduces BNP and ANP levels	Possibly through interaction with miR-133	40
RNA binding proteins				
Rbm24	AAV9-Rbm24 in mouse; zebrafish	Sarcomere assembly and cardiomyocyte contractility)	—	41,42
HuR	nRCM+Phenylephrin	Promotion	Modulation of nuclear factor of activated T-cells activity	43
PCBP2	nRCM+AngII	Inhibition	Downregulation increases stability of prohypertrophic GPR56	44

α -2M indicates alpha-2-Macroglobulin; AngII, angiotensin II; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; ceRNA, competing endogenous RNA; circRNA, circular RNA; IGF-IIR, insulin-like growth factor II receptor; lncRNA, long noncoding RNA; MEF2D, myocyte-specific enhancer factor 2D; miRNA, microRNA; nMCM, neonatal mouse cardiomyocyte; nRCM, neonatal rat cardiomyocyte; and TAC, transverse aortic constriction.

MicroRNA-Associated Feed-Forward Loops Associated With Cardiomyocyte Hypertrophy

MicroRNA-associated feed-forward loops (miR-FFL) are 3-gene modules. Mining published expression data led to the identification of 22 miR-FFL motifs dysregulated

in cardiac hypertrophy.⁴⁵ These modules included 17 miRNAs, including miR-20a and -b, miR-21, let-7b, miR-16, miR-9, miR-106a, miR-15, miR-93, miR-125b, miR-34b, miR-1, miR-30a, miR-130b, miR-92b, miR-335, and miR-425. Most of these miRNAs are known

to be associated with cardiomyocyte hypertrophy and cardiac dysfunction.

Long Noncoding RNAs

Long Noncoding RNAs Functioning Through (Epi) Genetic Mechanism

Whereas more than a decade of research into the roles of miRNAs in cardiomyocyte hypertrophy has resulted in a vast amount of data, the implications of lncRNAs in cardiomyocyte hypertrophy are still limited (Table 1).

The first study implicating a lncRNA in cardiac hypertrophy identified a new antisense transcript of myosin heavy chain 7 formed by alternative splicing, called *MHRT* (Myosin Heavy Chain Associated RNA Transcript).³⁰ *MHRT* is highly expressed in the mouse and human adult heart and functions through the modification of Brg1 (brahma-related gene 1)-mediated chromatin remodeling. The *MHRT*-Brg1 feedback circuit appears crucial for cardiac function and is conserved in humans. More recently, it was suggested that *MHRT* acts together with myocardin in a regulatory feedback mechanism in the regulation of cardiac hypertrophy.⁴⁶ *MHRT* was found to affect the acetylation of myocardin by HDAC5 and proposed to inhibit thereby cardiac hypertrophy induced by myocardin. Moreover, myocardin also directly activated *MHRT* transcription through binding to the CarG box.

Another identified lncRNA with a function in epigenetic reprogramming of hypertrophic cardiomyocytes is *Chaer*.³¹ *Chaer* modulates the chromatin remodeling function of polycomb repressor complex 2 through direct interaction with its catalytic subunit, and in this way affects histone methylation and the expression of cardiac hypertrophic genes. *TINCR* (Terminal differentiation-induced ncRNA) has been linked to the epigenetic silencing of CaMKII via the direct targeting of the methyltransferase enhancer of zeste 2 that associates with the CaMKII δ promoter.³² Enforced expression of *TINCR* attenuated cardiac hypertrophy in pressure-overloaded mice. Both *Chaer* and *TINCR* are annotated in human, but whether the epigenetic mechanisms described in mice are conserved in humans remains to be demonstrated.

The lncRNA *Chast* (cardiac hypertrophy-associated transcript), on the other hand, functions through a cis-regulatory action directly on the gene located on the opposite strand: *pleckstrin homology domain-containing protein family M member 1*, an autophagy regulator. Through a yet unknown mechanism, *Chast* induced cardiomyocyte hypertrophy in vitro and in vivo, whereas silencing of *Chast* attenuated cardiac remodeling after pressure overload.³³

An important subclass of lncRNAs with epigenetic functions is that of the enhancer lncRNAs, derived from the enhancer regions of the genome that among

other processes regulate gene expression reprogramming during cardiomyocyte hypertrophy.⁴⁷

Long Noncoding RNAs With Proposed Scaffold-Based Mechanisms

Searching for competitive endogenous RNA interactions between lncRNAs and miRNAs indirectly affecting mRNAs has become an exciting area of research attesting the complexity of the cross-talks between different regRNA species.

The lncRNA *XIST* was upregulated in hypertrophic murine hearts and phenylephrine-treated cardiomyocytes.³⁴ Inhibition of *XIST* induced cardiomyocyte hypertrophy while overexpression of *XIST* attenuated cardiomyocyte hypertrophy induced by phenylephrine in vitro. These effects may involve a sponging effect of *XIST* for miR-330-3p, leading to enhanced S100B expression and cardiomyocyte hypertrophy.

The lncRNA *CHRF* (cardiac hypertrophy related factor) is also upregulated in cardiomyocyte hypertrophy where it scavenges miR-489, thereby indirectly regulating the expression levels of the *myeloid differentiation primary response gene 88*.³⁵ The lncRNA *Plscr4* may down-regulate cardiac hypertrophy through the miR-214-Mfn2 axis.³⁶ Also, the *MIAT* (myocardial infarction associated transcript) was found to contribute to cardiomyocyte hypertrophy in vitro through a miR-150-5p/P300 axis.³⁷ The lncRNA *H19* was shown to inhibit cardiomyocyte hypertrophy in vitro, and these effects were completely dependent on its encoded miR-675 and may partly involve the downstream target CAMKII δ .³⁸ lncRNA *HOTAIR* may function as a miR-19-sponge to modulate PTEN levels.³⁹ *Human large intergenic non-coding RNA ROR* (lncRNA-ROR) was shown to reduce ANP and BNP protein levels in cardiomyocytes in vitro and may do so via its interaction with miR-133.⁴⁰

RNA Binding Proteins

Dedicated RNA binding proteins (RBPs) are pivotal to the functioning of regRNAs. It is important to note that knowledge emerges on an intricate RBP-dependent interplay between miRNA-based gene silencing of mRNAs and alternative splicing of pre-mRNAs, a crucial posttranscriptional mechanism implicated in the cellular stress response. For example, the RBP family Rbfox regulates both tissue-specific pre-mRNA splicing and miRNA processing, and both Rbfox1 and -2 were recently linked to heart failure, the latter through regulation of miR-34a.⁴⁸ Moreover, the recognition of the importance of tightly controlled alternative pre-mRNA splicing in cardiac homeostasis is emerging. RNA binding proteins play a major role in RNA splicing. The first identified RBP with a role in alternative splicing during heart failure was alternative splicing factor 1 (ASF/SF2), regulating the remodeling-induced splicing switch of

CaMKII δ .⁴⁹ The RNA binding protein RBM24 is a pivotal cardiac splice factor, which governs sarcomerogenesis in the heart by controlling the expression of alternative protein isoforms.⁴¹ Although a role in hypertrophy is unclear, AAV9-mediated cardiomyocyte Rbm24 overexpression in mice was recently shown to slightly increase heart weights, but this may have been a result of increased fibrosis in these mice.⁴² RBM38, a homolog of RBM24, has been implicated in RNA splicing, RNA stability, and RNA translation. Yet, Rbm38 deficiency did not affect cardiac hypertrophy *in vivo* and was found to be dispensable during pressure overload-induced cardiac remodeling in mice.⁵⁰

The RNA binding protein HuR (human antigen R) interacts with specific AU-rich domains in target mRNAs and is highly expressed in many cell types, including cardiomyocytes. Upon hypertrophic stimulation, HuR undergoes cytoplasmic translocation, indicative of its activation, in cultured cardiac myocytes.⁴³ HuR activation was necessary for Gq-mediated hypertrophic growth of neonatal rat ventricular myocytes and may function through the activation of the transcription factor nuclear factor of activated T cells.

The RNA-binding protein PCBP2 (Poly(C)-binding protein 2) is downregulated in failing human hearts and mouse hypertrophied hearts.⁴⁴ PCBP2 knockdown promoted angiotensin II-induced hypertrophy of neonatal cardiomyocytes and H9C2 cells, whereas PCBP2 overexpression promoted opposite effects. Furthermore, PCBP2 inhibited prohypertrophic GPR56 expression by promoting its mRNA degeneration in cardiomyocytes.

REGULATORY RNAs IN CARDIAC INFLAMMATION

Cardiac and systemic inflammation is associated with chronic heart failure as well as being a contributing factor to metabolic syndrome, type 2 diabetes, atherosclerosis, aortic aneurysm, and aging. Several regRNAs are modulated and participate in the regulation of cardiac inflammation (Table 2).

MiRNAs

Translational miRNA expression studies sustain causal links between miRNA dysregulation and cardiac inflammation and have identified potential miRNA therapeutic targets. In human myocarditis samples, a microarray screen identified 107 miRNAs differentially expressed as compared with control hearts, of which 21 were upregulated and 37 were down-regulated.⁵¹ MiR-155, miR-146b, and miR-21 were upregulated, and inhibition of miR-155,⁵² miR-21, and miR-146b,⁵⁶ reduced myocardial inflammation and necrosis in coxsackievirus-B3 or autoimmune myocarditis in mice. Likewise, miR-155

inhibits PU.1 and SOCS1 in the heart and *per se* derepresses the proinflammatory cytokines, facilitating activation of T-cells and monocytes.⁶⁸ In addition, miR-155 amplifies nitric oxide/cGMP signaling in the heart and increases cardiac inflammation upon a septic shock in mice and human hearts.⁵³ Combined inhibition of miR-21 and miR-146a reduced cardiac dysfunction after myocardial infarction in a model of viral myocarditis and decreased the expression levels of T-helper cell 17 and RAR-related orphan receptor gamma ROR γ t in mice.⁵⁶

MicroRNA-21 is particularly increased in the border and infarct zones during the early inflammatory phase of remodeling following myocardial infarction in mice and prevents excessive inflammation and cardiac dysfunction.⁵⁴ Indeed, miR-21 knockout mice have elevated baseline levels of inflammatory cytokines, CD11b⁺ monocytes and macrophages in the heart and, after myocardial infarction, they exhibit higher mortality rates, worse cardiac dysfunction, and increased infarct and scar areas as compared with wild-type mice.⁵⁴

Systemic inhibition of the miR-221/-222 cluster in mice infected by coxsackievirus-B3 increased the cardiac viral load and aggravated cardiac inflammation and injury.⁵⁷ Regulation of inflammation by miR-221/-222 involves the target genes ETS1/2, IRF2, BCL2L11, TOX, BMF, and CXCL12. It is interesting to note that the miR-221/-222 cluster regulates both inflammation and cardiac virulence.

Long Noncoding RNAs

Besides miRNAs, lncRNAs are also implicated in immune modulation in the heart, and some regulatory networks involving miRNAs and lncRNAs have been linked to cardiac inflammation.

A translational study exploring the involvement of lncRNAs in antiviral capacity in patients with coxsackievirus-B3 cardiomyopathy conferred beneficial immunoregulatory functions to the lncRNA MALAT1 and its enzymatic processing product MALAT1-associated small cytoplasmic RNA.⁵⁸ MALAT1-associated small cytoplasmic RNA overexpression in cardiomyocytes induced antiviral immunity genes and rendered cells resistant to virus infection. MALAT1-associated small cytoplasmic RNA ablation in monocytes significantly increased the expression of proinflammatory proteins, such as FASLG, FAS, TNF- α , and IL6. In autoimmune myocarditis in mice, MALAT1 induces tolerogenic dendritic cells and regulatory T cells, via miR-155/dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin/IL10 axis.⁶³ MALAT1 also inhibits immune activation and atherosclerosis development in ApoE-deficient mice.⁶¹

The lncRNA HOTAIR is intergenic and antisense to the HOXC gene. Inhibiting HOTAIR improved cardiac function during lipopolysaccharide-induced sepsis in mice, presumably via inhibition of TNF- α and NF κ B/

Table 2. Regulatory RNAs Associated With Cardiac Inflammation

Regulatory RNA	Experimental Model	Effect on Inflammation	Mechanism of Action	References
miRNAs				
miR-155	Viral myocarditis	Activating	MiR-155 inhibition activates PU.1, an ETS-domain containing transcription factor that activates gene expression during myeloid and B-lymphoid cell development	51
	Viral myocarditis in miR-155 ^{-/-} mice	Activating	MiR-155 ^{-/-} mice show elevated levels of M2 macrophages and decreased levels of M1 macrophages	52
	Sepsis	Activating	MiR-155 targets the angiotensin type 1 receptor, impairing Ang II response to septic stress and amplified NO-cGMP-PKG signaling by targeting CD47	53
miR-21	Myocardial infarction	Protective	MiR-21 overexpression decreases KBTBD7 expression in the infarct border zone, reducing activation of MAP kinase kinases 3/6, which activate the NF-κB pathway	54
	Viral myocarditis	Activating	MiR-21 targets YOD1, hence enhancing the K48-linked ubiquitination and degradation of desmin, inducing the disruption of desmosome structures in cardiomyocytes	55
miR-146b miR-21	Viral myocarditis	Activating	Inhibition of miR-21 and miR-146b decreases Th17 and RORγt	56
miR-221/222	Viral myocarditis	Protective	MiR-221/222 family targets <i>STAT1</i> , <i>STAT3</i> , <i>ETS1</i> , <i>ETS2</i> , <i>IRF2</i> , <i>CXCL12</i> , <i>BCL2L11</i> , <i>BMF</i> , <i>TOX</i> , and <i>CD4</i> ; overexpression of miR-221/222 blunts response to CVB3 infection and IL-6, TNF-α, and IFN-γ pathways	57
lncRNAs				
MascRNA	Viral myocarditis	Protective	Role in cardiovascular innate immunity	58
NEAT1	SiRNA knockdown in THP1 monocytes	Activating	Repression of <i>NEAT1</i> or its target <i>miR-342-3p</i> reduces apoptosis and inflammation in response to oxLDLs	59
	<i>Neat1</i> ^{-/-} mice	Protective	<i>Neat1</i> ^{-/-} mice exhibits elevated aortic wall CD68+ cell infiltration and myocardial inflammation	60
MALAT1	<i>Malat1</i> ^{-/-} mice	Protective	<i>Malat1</i> deficiency impairs macrophage phagocytosis	61
	Sepsis	Activating	Inhibition of <i>Malat1</i> in cardiomyocytes prevents upregulation of TNF-α	62
	EAM	Protective	<i>Malat1</i> improves immune tolerance in EAM by sponging miR-155, supporting homeostasis of tolerogenic dendritic cells, promoting IL-10 and suppressing IL-6 and IL-12 production	63
THRIL	Sepsis (in vitro)	Unclear	<i>THRIL</i> is required for hnRNPL-associated TNF-α activation	64
HOTAIR	Sepsis	Activating	<i>HOTAIR</i> promotes TNF-α production through NF-κB activation	65
ANRIL/CDKN2BAS	Ischemia-reperfusion injury	Activating	<i>ANRIL</i> and its target <i>miR-181b</i> are antithetically regulated, activating NF-κB signaling	66
circRNAs				
cANRIL	Coronary atherosclerosis in rat	Activating	<i>cANRIL</i> affects ribosomal RNA biogenesis and inflammation in vascular endothelial cells and macrophages	67

cANRIL indicates circular ANRIL; circRNA, circular RNA; EAM, experimental autoimmune myocarditis; lncRNA, long noncoding RNA; mascRNA, MALAT1-associated small cytoplasmic RNA; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; miRNA, microRNA; and oxLDL, oxidized low-density lipoprotein.

p65 phosphorylation.⁶⁵ The lncRNA ANRIL/INK4/CDKN2B-AS1A is encoded by a risk haplotype at the locus 9p21.3 identified in genome-wide association studies as conveying risk for coronary artery disease and myocardial infarction as well as other inflammatory conditions. ANRIL has a circRNA counterpart (cANRIL) thought to modulate proinflammatory gene expression in vascular endothelial cells (ECs), inciting coronary atherosclerosis.⁶⁹

Other lncRNAs, which may benefit from further investigation in cardiac inflammatory disease, include

CARL (cardiac and apoptosis-related lncRNA), and THRIL (TNFα and hnRNPL related immunoregulatory lincRNA). The first is involved in macrophage activation and NF-κB signaling and inhibits mitochondrial fission and apoptosis in cardiomyocytes.⁷⁰ The second regulates expression of key immune response genes, including TNF-α.⁶⁴

Circular RNAs have also been associated with inflammation. Expression levels of the circRNA MICRA in inflammatory blood cells of patients after acute myocardial infarction are associated with the development of heart failure.⁷¹ *cANRIL* modulates the maturation of

ribosomal RNAs and confers protection from atherogenesis, providing evidence that circularization of lncRNAs (ANRIL in this particular case), can affect RNA processing and disease development.⁶⁷

REGULATORY RNAs IN CARDIAC REGENERATION

Regeneration pathways are highly preserved between species. However, because of early postnatal cell cycle exit of cardiomyocytes and failure to activate traditional regeneration programs upon injury, the adult mammalian heart exhibits limited regenerative capacities. Common mechanisms of successful cardiac regeneration seen in lower vertebrates and neonatal mice include controlled inflammation and matrix remodeling centering around cardiomyocyte dedifferentiation and proliferation. In contrast, new cardiomyocyte formation resulting from endogenous differentiation of progenitor cells after injury has lately been put into question. Therefore, strategies to induce cardiomyocyte proliferation and to facilitate cardiomyogenic differentiation of stem/progenitor cells for therapeutic application or to directly reprogram nonmyocytes into cardiomyocytes are currently under intense investigation. All

these processes require sophisticated gene regulation involving regRNAs (Table 3).

MiRNAs

Screening for miRNAs that regulate postnatal cardiomyocyte proliferation revealed that miR-590 and miR-199a induce cell cycle reentry and proliferation of adult cardiomyocytes, thereby improving outcome after myocardial infarction.⁷² Similarly, miR-302–367 cluster promotes cardiomyocyte proliferation, and its transient activation was associated with reduced scarring and improved function after myocardial infarction in mice.⁷³ In contrast, the miR-15 family induces postnatal cell cycle exit, and its inhibition from the early postnatal stage onwards allows for retained regeneration of the adult mouse heart.⁷⁴ Dedifferentiation and proliferation of cardiomyocytes with consecutive regeneration after myocardial infarction could also be induced in adult mice upon miR-99/100 and let-7a/c inhibition.⁷⁵ These data support the therapeutic targeting of miRNAs to activate preserved regeneration programs in the adult mammalian heart.

RegRNAs play essential roles during cardiac development, which could open new avenues for cell therapy and reprogramming. The muscle-specific myomiR

Table 3. Regulatory RNAs Associated With Cardiac Regeneration

Regulatory RNA	Experimental Model	Effect on Regeneration	Mechanism of Action	References
mRNAs				
miR-590, miR-199a	Myocardial infarction, mice, overexpression	Improvement	Cell cycle reentry, c cardiomyocyte proliferation	72
miR-302–367 cluster	Myocardial infarction, mice, transient mimics	Improvement	Cell cycle reentry, cardiomyocyte proliferation	73
miR-15 family	Myocardial infarction, mice, downregulation	Improvement	Cardiomyocyte proliferation	74
miR-99/100, let-7a/c	Myocardial infarction, mice, downregulation	Improvement	Cardiomyocyte dedifferentiation and proliferation	75
miR-17-92	Myocardial infarction, mice, overexpression	Improvement	Cardiomyocyte proliferation	76
miR-1, miRs-133 (208, 499)	Myocardial infarction, mice, lentiviral delivery	?	Reprogramming of cardiac fibroblasts to cardiomyocytes	77
lncRNAs				
<i>Braveheart</i>	Mouse embryonic stem cells, overexpression	?	Cardiac fate commitment	78
<i>Fendrr</i>	Mouse embryos, null mutants	?	Impaired cardiac development	79
<i>TERMINATOR</i>	Human embryonic stem cells, downregulation	?	Loss of pluripotency, apoptosis	80
<i>ALIEN</i>	Zebrafish embryos, human vascular progenitor cells, downregulation	?	Impaired cardiovascular development	80
<i>CARMEN</i>	Mouse embryonic stem cells, human fetal cardiac progenitor cells, downregulation	?	Impaired cardiac differentiation	81
<i>METEOR/linc1405</i>	Mouse embryonic stem cells, (human induced pluripotent stem cells), abrogation	?	Impaired cardiac differentiation	82
circRNAs				
To be defined				

? indicates unknown; circRNA, circular RNA; lncRNA, long noncoding RNA; and miRNA, microRNA.

families miR-1 and miR-133 are highly preserved between species and their tight regulation is required for balancing proliferation and differentiation during cardiogenesis, supporting a role for miRNAs in gene dosage during development. Both miR-1 and miR-133 have been used for reprogramming of cardiac fibroblasts to cardiomyocytes.⁷⁷ As another example of miRNAs exerting multiple functions, the miR-17/92 cluster induces cardiomyocyte proliferation in the adult mouse.⁷⁶

Long Noncoding RNAs

Of interest to cardiac regeneration are also lncRNAs. Among the first identified was *Braveheart*, which regulates embryonic cardiovascular progenitor cell differentiation in the mouse via epigenetic modulation of cardiac transcription factor expression.⁷⁸ Similar functions were shown for *Fendrr*, which is indispensable for normal cardiogenesis in mice.⁷⁹ lncRNAs are less preserved than miRNAs, and whereas a human homolog for *Braveheart* is missing, *TERMINATOR*, *ALIEN*,⁸⁰ *CARMEN*,⁸¹ and *METEOR/linc1405*⁸² have been implicated in human stem and progenitor cell differentiation. lncRNAs also regulate cardiomyocyte proliferation. Cardiomyocyte dedifferentiation and cell cycle re-entry occur in adult mouse and human cardiomyocytes as part of a myocardial stress response guided by lncRNAs.⁸³ Although circRNAs are dynamically regulated during human cardiac development, their identities, regulation, and roles have still to be revealed.

Recent initiatives that synthesize publicly available gene expression data in cardiac regeneration provide valuable resources for further research in the field (eg, <https://regendbase.org>).

REGULATORY RNAS IN THE VASCULAR SYSTEM

Changes in both the coronary and peripheral vasculature are key to the development of heart failure. Coronary vessel remodeling contributes to impaired myocardial contractility, while peripheral vascular remodeling results in decreased compliance, increased resistance and decreased capacitance in the arterial circulation, thus increasing the afterload and exacerbating the heart failure. Dysfunction of ECs and consequent alterations in vascular smooth muscle cells are central to this altered vascular structure and function.

Prevention or reversal of this remodeling may help to restore homeostasis and protect against heart failure, as would harnessing the mechanisms of angiogenesis to promote neovascularization. The role of regRNAs in these phenotypic changes in vascular cells is increasingly recognized (Table 4).

Messenger RNAs

A global change in the localization of mRNA molecules in ECs has been shown to regulate blood vessel sprouting and EC migration during angiogenesis, and mRNA transcript allocation controls tissue morphogenesis.⁹⁹

MiRNAs

Angiogenic early outgrowth cells from chronic heart failure patients show reduced miR-126 and miR-130a, which impairs their ability to improve cardiac function,⁸⁴ while levels of miR-126 and miR-508-5p in endothelial progenitor cells are prognostic for chronic heart failure in ischemic and nonischemic cardiomyopathy patients respectively.⁸⁵ Mir-214⁸⁷ and miR-665⁸⁸ are both increased in failing human hearts and in the circulation. Inhibition of each of these miRNAs improved cardiac function and angiogenesis in mouse models of hypertrophy.^{87,88} Recently, it was postulated that the K_{ATP} channel openers iptakalim and natakalin may exert protective effects on the endothelium via a miR-1-3p/ET-1 pathway, although the evidence at this stage is mostly in vitro.⁸⁹

More broadly, numerous miRNAs (eg, miR-126, miR-17-92a cluster, miR-143/145, miR-210, miR-24, miR-15a/16, and miR-221/222) have been shown to have important roles in angiogenesis and are thus potential therapeutic targets and biomarkers of heart failure.

Long Noncoding RNAs

Long noncoding RNAs have also come into view as regulators of numerous pathways involved in the pathophysiology of the vascular system associated with heart failure. In vitro silencing of MALAT1 switched ECs from a proliferative to a promigratory phenotype, and its inhibition in vivo reduced vascular growth.⁹⁰ HIF 1 antisense RNA¹⁹² and lincRNA-p21⁹³ are implicated in vascular smooth muscle cell regulation while TGF β 2-OT1⁹¹ or mitochondrial ASncmtRNA-2⁹⁴ play roles in inflammation or senescence of vascular ECs, respectively. MANTIS controls transcription factor expression in ECs via interaction with BRG-1, part of the SWI/SNF complex, to promote angiogenic functions.⁹⁵ Hyperlnc is expressed in pericytes and is downregulated in hearts of patients with heart failure.⁹⁶ Silencing of Hyperlnc in pericytes in vitro resulted in dedifferentiation, attributed to increased endoplasmic reticulum stress.

Circular RNAs are expressed and regulated in the vascular system, although their role in heart failure development is still poorly known. CircWDR77 targets FGF-2 to modulate vascular smooth muscle cell proliferation and migration by sponging miR-124.⁹⁷ In another study, circRNA-MYLK acted as a ceRNA for miR-29a, contributing to endothelial-mesenchymal transition by activating VEGFA/VEGFR2 pathway.⁹⁸ It is also worth mentioning that endothelial lncRNAs and circRNAs are

Table 4. Regulatory RNAs in the Vascular System

Regulatory RNA	Experimental Model	Effect in Vascular System	Mechanism of Action	References
mRNAs				
miR-126-3p	Human early outgrowth cells in myocardial infarction mouse	Proangiogenic	Downregulated in early outgrowth cells in heart failure	84
	Human endothelial progenitor cells	Prognostic for chronic heart failure secondary to ischemic cardiomyopathy	Unknown	85
	Human and mouse endothelial cells	Proangiogenic, maintains vascular homeostasis	Multiple, including regulation of VEGF, NOS3, SIRT1	For review see ⁸⁶
miR-130a	Human early outgrowth cells in myocardial infarction mouse	Proangiogenic	Downregulated in early outgrowth cells in heart failure	84
miR-508-5p	Human endothelial progenitor cells	Prognostic for chronic heart failure secondary to non-ischemic cardiomyopathy	Unknown	85
miR-214	Human failing heart, isoprenaline-treated mouse heart, HUVECs	Anti-angiogenic	Upregulated in heart failure; silencing improves angiogenesis and cardiac function in isoprenaline-treated mice; targets XBP1 in vitro	87
miR-665	Human failing heart, HUVECs, TAC mouse heart	Anti-angiogenic	Upregulated in heart failure; affects endothelial cell function; targets CD34 expression; silencing improves cardiac function in TAC mice	88
miR-1-3p	Rat primary cardiac microvascular endothelial cells, isoprenaline-treated rat heart	May mediate protective effect of iptakalim/natakalm treatment	Heart expression restored by iptakalim/natakalm; targets endothelin-1 expression in vitro	89
lncRNAs				
MALAT1	HUVECs, mouse hindlimb ischemia	Silencing is promigratory, antiproliferative	Modulates cell cycle regulators	90
TGFB2-OT1	HUVECs	Autophagy	ceRNA; binds miR-3960, miR-4488, miR-4559	91
HIF1A-AS1	Human aortic smooth muscle cells	Apoptosis and proliferation	Unknown	92
LincRNA-p21	Human smooth muscle cells, mouse carotid artery injury	Apoptosis and proliferation	Modulates p53	93
ASncmtRNA-2	HUVECs	Senescence	Possible precursor of miR-4485, miR-1973	94
MANTIS	HUVECs	Proangiogenic	Interacts with BRG-1 (SWI/SNF)	95
HypERlnc	Human pericytes	Maintains pericyte differentiation	Protects against endoplasmic reticulum stress	96
circRNAs				
circWDR77	Human vascular smooth muscle cells	Proliferation and migration	Sponges miR-124 to regulate FGF2	97
circRNA-MYLK	HUVECs	Proliferation and migration, endothelial-mesenchyme transition	ceRNA for miR-29a, targeting VEGFA and Ras/ERK	98

circRNA indicates circular RNA; HUVEC, human umbilical vein endothelial cell; lncRNA, long noncoding RNA; miRNA, microRNA; and TAC, transverse aortic constriction.

regulated by hypoxia and may contribute to regulating fibrosis.

TRANSLATIONAL RELEVANCE OF REGULATORY RNAs

The discovery of novel regRNAs implicated in heart failure has progressed extremely rapidly over the past few years, yet has not come to an end. The question is how to translate research findings into biologically and clinically relevant information.

Given their relative cell-type specific expression and tight temporal and spatial regulation, regRNAs make attractive therapeutic targets and biomarkers for heart failure. The potential clinical applications of regRNAs are diverse, particularly as tools for personalized medicine. The identification of regRNA profiles in heart failure may lead to new preventive measures, diagnostics, and therapies. It may also have an added value to existing tools and biomarkers, giving molecular snapshots of specific moments throughout the evolution of a disease, and, thus, providing additional information

to guide clinical decisions. Understanding the interactions between the different types of RNA and how they affect gene expression will help fill critical gaps in our knowledge, enabling such translational applications.

Although there is compelling evidence for the potential of regRNAs in clinical applications, our mechanistic insights into the molecular pathophysiology underlying heart failure have not yet emerged into new therapeutic or diagnostic options based on regRNAs. This is partly because of technical and nontechnical hindrances. For example, comparing and reproducing results from different studies is challenging. Translational transcriptomics studies in the cardiovascular field, as well as other fields, must rely on a consensus of best practices and experimental standards for all steps of the process, such as study design, sample type and size, methods of normalization and analysis, specificity of the detection and targeting of regRNAs.¹⁰⁰ Extensive progresses in these technical aspects are paramount for the use of RNA-based diagnostic and therapeutic approaches.

CONCLUSION AND FUTURE DIRECTIONS

It has become increasingly evident that RNAs possess a plethora of regulatory roles, some of which remain to be unraveled. A better knowledge of the role of regRNAs may ultimately foster the development of novel RNA-based therapeutic approaches (Figure 4).

While the role of miRNAs in heart failure development and progression has been extensively studied, the knowledge on lncRNAs is far scarcer. Although deep RNA-sequencing experiments revealed an extensive regulation of lncRNAs in the failing heart, only a few lncRNAs have been functionally involved in heart failure processes. Even less well known, circRNAs also appear to be regulated during heart failure, while their role in the heart has still to be fully uncovered.

A few reports highlight the importance of RNA binding proteins that are necessary to maintain cardiac homeostasis through tight control of pre-mRNA splicing. Dysregulation of these RNA binding proteins has been observed in the failing heart, and further research is needed to systematically characterize their relevance in heart failure and evaluate their therapeutic and diagnostic potential. Since these proteins act at a very early stage of gene expression (pre-mRNA splicing), it is plausible that the study of their regulation may provide early indicators of subtle changes in gene regulatory networks that contribute to heart failure development.

Secondary analyzes of publicly-available high throughput datasets (eg, RNA-sequencing) with constantly improved bioinformatics tools have the potential to discover interesting novel targets through data sharing and collaborative work. Any new target requires extensive characterization and validation using

1. Improve our knowledge of the role of regRNAs in heart failure, particularly lncRNAs and circRNAs
2. Study the role of RNA binding proteins and miRNA-associated feed-forward loops in regRNA networks
3. Optimize bioinformatics tools to study regRNA networks
4. Perform secondary analyses of publicly available high throughput datasets
5. Identify technical gaps and define methodological standards
6. Use standardized methods for better reproducibility of findings
7. Improve the specificity of detecting and targeting regRNAs
8. Share data and expertise through collaborative work
9. Work translationally for the patient's benefit.

Figure 4. Future directions.

circRNA indicates circular RNA; lncRNA, long non-coding RNA; miRNA, microRNA; and regRNA, regulatory RNA.

independent methods before being considered for further development as a drug or a biomarker.

Now that also more direct mRNA targets of miRNAs become validated, pathway analysis possibilities based on systems biology concepts emerge. As such, the identification of miR-FFLs represents a new era in disease research and may help shed light on the diversity of miRNA-mediated pathways identified so far.

The development of bioinformatics has greatly contributed to a better knowledge of the role of regRNAs in heart failure. Intriguingly, most of the lncRNA studies described scaffold-based mechanisms ("sponge effect"), in which they prevent miRNAs from exerting their roles on mRNAs. It is unclear whether this tripartite mechanism is indeed widely effective in cardiac cells. Deciphering the functions of lncRNAs is not trivial. The identification of putative miRNA binding sites is relatively easy using current *in silico* tools, as it is based on sequence complementarity. Identifying interactions of lncRNAs with proteins or DNA, on the other hand, requires more complicated and time-consuming biochemical assays, especially when performed in an unbiased manner. Thus, more efforts are required to deepen our understanding of the role of regRNA networks in heart failure.

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Disclosures

None.

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