Gene expression reprogramming is the base of pathological cardiac hypertrophy and heart failure (HF). Mechanisms similar to those that govern gene expression in heart development are at the root of those that occur in hypertrophy and HF.1 For example, pathological cardiac hypertrophy is associated with upregulation of certain fetal genes on the one hand and downregulation of several adult genes on the other, causing the often inevitable deterioration in heart function.2,3 Historically, proteins were thought to be the main players in gene expression regulation, but with the development of new, high-resolution technologies for the analysis of the transcriptome, this paradigm has fallen. In fact, it has now been determined that although about three-quarters of the mammalian genome is transcribed, <2% is ultimately translated into protein.4 These non–protein-coding transcripts form a vast non–protein-coding RNA (ncRNA) world that we have only recently started to study (Figure 1). The fundamental importance of this ncRNA world is inferred from the fact that the degree of complexity of a species correlates better with the amount of DNA that is transcribed into ncRNA than with the number of protein-coding genes.5 Many ncRNAs with a gene regulatory function have been identified to date and, similarly to proteins, found to act at various steps along the protein biosynthetic process, including transcription, RNA maturation, translation, and protein degradation. This discovery is fueling a new era in pathophysiology.

Abstract: RNAs not encoding proteins have gained prominence over the last couple of decades as fundamental regulators of cellular function. Not surprisingly, their dysregulation is increasingly being linked to pathology. Here, we review recent reports investigating the pathophysiological relevance of this species of RNA for the cardiovascular system, concentrating mainly on recent findings on long noncoding RNAs and microRNAs in cardiac hypertrophy and failure. (Circ Res. 2015;116:751-762. DOI: 10.1161/CIRCRESAHA.116.303549.)

Key Words: cardiac hypertrophy ■ cardiac remodelling ■ heart failure ■ lncRNAs ■ microRNAs ■ myocardial infarction

Original received August 1, 2014; revision received September 5, 2014; accepted September 23, 2014. In December 2014, the average time from submission to first decision for all original research papers submitted to Circulation Research was 14.47 days.

From the Institute of Molecular and Translational Therapeutic Strategies (IMTTS), Integrated Research and Treatment Center Transplantation, and REBIRTH Excellence Cluster, Hannover Medical School, Hannover, Germany (T.T.); National Heart and Lung Institute, Imperial College London, London, United Kingdom (T.T.); Humanitas Clinical and Research Center, Rozzano, Milan, Italy (G.C.); Institute of Genetics and Biomedical Research, National Research Country of Italy, Milan, Italy (G.C.); University of Milan, Milan, Italy (G.C.); and Department of Cardiovascular Sciences, University of Leicester, Leicester, United Kingdom (G.C.).

Correspondence to Thomas Thum, MD, PhD, Institute of Molecular and Translational Therapeutic Strategies (IMTTS), Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany. E-mail thum.thomas@mh-hannover.de; or Gianluigi Condorelli, MD, PhD, Humanitas Clinical and Research Center, Via Manzoni 56, Rozzano (MI) 20089, Italy. E-mail gianluigi-condorelli@humanitasresearch.it

© 2015 American Heart Association, Inc.

Circulation Research is available at http://circres.ahajournals.org DOI: 10.1161/CIRCRESAHA.116.303549

751
MicroRNA Function and Therapeutic Utility in Important Cardiovascular Pathophysiologic Conditions

There are excellent reviews available on the mechanistic role of microRNAs and their potential use as therapeutic targets. We thus only briefly report on novel aspects of microRNAs in pathophysiologic conditions. We will consider the following cardiovascular pathophysiologies: (1) adverse cardiac hypertrophy, (2) cardiac fibrosis, (3) cardiac vessel rarefication/density, and (4) cardiac metabolism.

MicroRNAs in Left Ventricular Hypertrophy

Pathological stress can activate signaling pathways that trigger cardiomyocyte hypertrophy. An important example is the activation of the calcineurin–nuclear factor of activated T-cells (NFAT) pathway, whereby the calcium-dependent phosphatase calcineurin dephosphorylates the transcription factor NFAT3, enabling it to translocate to the nucleus to activate multiple hypertrophy-related genes. One of the first studies that mechanistically investigated the importance of a microRNA in cardiac hypertrophy was from the Olson laboratory, showing miR-208 to be crucially involved in hypertrophic signaling. Another early report demonstrated that pressure-overloaded mice had low cardiac miR-133 levels; coherently, inhibition of miR-133 with an anti-miR-133 oligonucleotide in overloaded mice had low cardiac miR-133 levels; coherently, inhibition of miR-133 with an anti-miR-133 oligonucleotide generated cardiac hypertrophy in vivo.

miR-1 and miR-133—which are encoded as part of the same bicistronic unit—are both inversely related to cardiac hypertrophy. In particular, miR-1 modulates the insulin-like growth factor-1 pathway either directly, inhibiting insulin-like growth factor-1 and its receptor, or by downregulating secreted targets related to this pathway. miR-1 is able to attenuate cardiomyocyte hypertrophy in cultured cardiomyocytes and in the intact adult heart by regulation of cardiomyocyte growth responses through modulation of calcium signaling components such as calmodulin. miR-133 modulates inotropism by regulating the expression of multiple components of the β1-adrenergic pathway, including the receptor itself.

In addition to relatively abundant cardiac microRNAs, many microRNAs are expressed at relatively low levels under basal conditions and during pathological stress are strongly upregulated, becoming, thus, functionally important during disease. An example is the brain-enriched microRNA miR-212/132 family, which becomes activated during HF in humans and animal models. This microRNA family regulates cardiac hypertrophy and autophagy in cardiomyocytes by targeting the antihypertrophic and proautophagic transcription factor forkhead box O3, leading to hyperactivation of the prohypertrophic calcineurin/NFAT signaling pathway. Both genetic and pharmacological inhibition of miR-132 rescued cardiac hypertrophy and HF in mice, offering a possible therapeutic approach for this pathology.

In addition, microRNAs regulate intracellular calcium homeostasis, directly contributing to cardiac contractility. For instance, impaired myocardial sarcoplasmic reticulum calcium ATPase 2a activity is a hallmark of failing hearts, and clinical studies using genetic sarcoplasmic reticulum calcium ATPase 2 overexpression constructs led to beneficial effects in patients with HF. In studies with rats, it was shown that sarcoplasmic reticulum calcium ATPase 2 therapy after myocardial infarction...
(MI) restored miR-1 levels, leading to normalized expression levels of the sodium–calcium exchanger. miR-214 also has a cardioprotective role during IR injury by repression of Ncx1, a key regulator of Ca\(^{2+}\) influx. Recently, a report showed in vivo inhibition of miR-25 by a specific antagonist to evoke spontaneous cardiac dysfunction and HF, whereas others reported inhibition of miR-25 to improve cardiac contractility by interacting with the sarcoplasmic reticulum calcium uptake pump sarcoplasmic reticulum calcium ATPase 2. Thus, the exact mechanism and function of miR-25 in the heart remains controversial, and future studies are needed to resolve this issue. Other microRNAs involved in cardiac calcium handling have been recently summarized elsewhere.

### MicroRNAs in Cardiac Fibrosis

Cardiac fibrosis is the exaggerated activation of (mostly) resident cardiac fibroblasts, leading to increased secretion of extracellular matrix proteins, growth factors, cytokines, and genetic material. There are several microRNAs enriched in cardiac fibroblasts that have been shown to serve as powerful regulators of various cellular processes, including proliferation and growth factor secretion. For instance, miR-133 and miR-30, which control expression of connective tissue growth factor—a protein tightly linked to fibrosis development—are also usually downregulated under cardiac stress. miR-21 was shown to silence the ERK–MAP kinase inhibitor sprouty-1, which is mechanistically involved in fibrosis development. As microRNAs usually have dozens of targets, it is interesting that miR-21 has also been found to promote cardiac fibrosis by regulating other targets, such as transforming growth factor-\(\beta\) receptor III and matrix metalloprotease-2. Surprisingly, miR-21 knockout mice developed cardiac fibrosis under cardiac stress, whereas pharmacological inhibition of miR-21 inhibited fibrosis in the heart and other organs, such as lung and kidney. In contrast to miR-21, which is activated during cardiac stress, miR-29 is downregulated and leads to a derepression of many fibrosis-related genes, such as collagen, elastin, and fibrillin. Both miR-21 and miR-29 have been shown to serve as potential therapeutic targets for cardiac fibrosis. Interestingly, circulating levels of miR-29 were found to directly correlate with hypertrophy and fibrosis in patients with hypertrophic cardiomyopathy, whereas other circulating miRNA pattern may discern between patients with heart failure with reduced ejection fractions (HFrEF) and patients with heart failure with preserved ejections fraction (HfPEF). Thus, monitoring the level of this microRNA could be useful for prognostic/therapeutic purposes in various clinical settings.

### MicroRNAs Regulating Cardiac Vessel Density

Each cardiomyocyte in the heart is usually surrounded by \(\approx 4\) capillaries so as to provide sufficient energy and oxygen transport. This balance is derailed under cardiac stress, such as with MI, cardiac fibrosis, or HF. There is a growing interest in strategies to improve vessel density leading to smaller scar formation after MI and for treatment of HfPEF. Endothelial knockout of Dicer—a key protein involved in microRNA maturation—led to endothelial dysfunction, demonstrating the key role of microRNAs in endothelial biology. One of these, the endothelial cell-restricted microRNA miR-126, was found to mediate developmental angiogenesis in vivo. Indeed, deletion of miR-126 led to loss of vascular integrity and defects in endothelial cell proliferation, migration, and angiogenesis. Thus, approaches to deliver miR-126 are likely to be beneficial to endothelial cells and the improvement of vascular integrity and repair. A further microRNA involved in cardiac angiogenesis is miR-92a: this microRNA is highly, but not exclusively, expressed in human endothelial cells and controls the growth of new blood vessels. By using mouse models of limb ischemia and MI, it was shown that systemic blockade of miR-92a led to enhanced blood vessel growth and functional recovery of damaged tissue.

Another microRNA involved in the regulation of cardiac vascularization is miR-24: this microRNA is one of the most highly expressed in cardiac endothelial cells and is further up-regulated after ischemia in various organs. High levels of miR-24 induced endothelial cell apoptosis and abolished endothelial capillary network formation, partly through targeting the endothelial transcription factor GATA2 and the p21-activated kinase PAK4. Importantly, blockade of endothelial miR-24 limited MI size in mice, via enhancement of vascularity, eventually leading to preserved cardiac function and survival. In addition, miR-24 regulates smooth muscle cell functions, and miR-24 overexpression inhibited development of the vasculature in a model of engineered heart tissue.

### MicroRNAs Regulating Cardiac Metabolism

A relatively new aspect of the role of microRNAs in the heart is the control of energy homeostasis and metabolism not only in organs such as the liver but also in the cardiovascular system. The idea that the heart may be considered an engine dependent on fuel and that the failing heart is an engine out of fuel is relatively old. A main system in the heart to create energy is the creatine kinase system, and there are several nuclear-receptor transcription factors, such as nuclear receptors of the peroxisome proliferator–activated receptor (PPAR) family, which partly regulate this system and are also responsible for substrate utilization from fatty acids to glucose. Interestingly, the miR-199a/214 cluster shares peroxisome proliferator–activated receptor \(\delta\) as a common target. Silencing of both miR-199a and miR-214 in mice undergoing pressure overload improved cardiac function and restored mitochondrial fatty acid oxidation. Thus, microRNA manipulation can facilitate a metabolic shift from predominant reliance on fatty acid utilization in the healthy myocardium toward increased reliance on glucose metabolism at the onset of HF. This may open up new microRNA-based therapeutic concepts.

Another important regulator of energy homeostasis is the peroxisome proliferator–activated receptor \(\gamma\) coactivator 1\(\beta\), a transcriptional coactivator that regulates metabolism and mitochondrial biogenesis through stimulation of nuclear hormone receptors. The peroxisome proliferator–activated receptor \(\gamma\) coactivator 1\(\beta\) gene harbors the genes for miR-378 and miR-378*, which counterbalance the metabolic actions of peroxisome proliferator–activated receptor \(\gamma\) coactivator 1\(\beta\): indeed, mice genetically lacking miR-378 and miR-378* were resistant to high-fat diet–induced obesity. Other targets of miR-378 and miR-378* are carnitine \(O\)-acyltransferase, a mitochondrial enzyme involved in fatty acid metabolism, and mediator...
Long Noncoding RNAs

lncRNAs are transcripts >200 nucleotides long that have no known protein-coding function. However, this definition may be preliminary; in fact, many lncRNAs have been recently reported to encode short peptides in human tissues. Nevertheless, lncRNAs can be classified as: (1) sense lncRNAs, when they overlap one or more exons of another transcript on the same strand; (2) antisense lncRNAs, when they overlap one or more exons of another transcript on the opposite strand; (3) bidirectional lncRNAs, when their expression and that of a neighboring coding transcript on the opposite strand are initiated in close genomic proximity; (4) intronic lncRNAs, when they are derived from an intron of another transcript; and (5) intergenic lncRNAs, when found as an independent unit within the genomic interval between 2 genes (Figure 2).

The precise mechanisms of the various functions of lncRNA are the subject of intense investigation. lncRNAs can interact with the whole gamut of macromolecules present within the cell, including other RNA species, proteins, and DNA. In fact, complementary sites on the lncRNA allow them to recognize and bind to miRNAs, microRNAs, or even other lncRNAs and act as highly specific sensors for their regulation. Protein-binding sites allow them to interact with proteins, generating ribonucleoprotein particles with different functions. Moreover, the ability for conformational switching, whereby the activity of the lncRNA is activated or suppressed by an external signal, renders them as exquisite regulatory devices. The formation of binding sites and the ability to undergo allosteric transition are possible because of the capacity of these ncRNAs to fold into a variety of thermodynamically stable secondary structures, such as double helices, hairpin loops, bulges, and pseudoknots, which in turn can develop into higher-order tertiary interactions, generating a complex structural landscape. Not surprisingly, lncRNAs have been found to have high folding energy, which distinguishes them from mRNAs.

In general, lncRNAs can be divided into nuclear lncRNAs and cytoplasmic lncRNAs: the former guide chromatin modifiers such as DNA or histone methyltransferases and polycomb repressive complex (PRC) 2 to specific genomic loci. The result is prompting of a repressive heterochromatin state and, hence, downregulation of transcription; in contrast, transcriptional activation has been observed with recruitment of mixed lineage leukemia 1—a histone methyltransferase—and by lncRNAs acting similarly to enhancers. Cytoplasmic lncRNAs modulate gene expression either positively or negatively at the translational level by binding to targeted mRNAs or as binding sponging up transcription factors and chromatin modifiers, preventing them from effecting translational repression of their targets.

Wang and Chang classified lncRNAs into 4 main categories based on the molecular mechanism (Figure 3): (1) Signal lncRNAs serve as molecular signals due to temporal and spatial restriction of their transcription to interpret cellular context or as a response to specific stimuli. Air (antisense Igf2r RNA) and Kcnq1ot1 (potassium voltage-gated channel subfamily Q member 1 overlapping transcript) are examples: they mediate transcriptional silencing of multiple genes by interacting with chromatin and recruiting the chromatin-modifying machinery; (2) Decoy lncRNAs bind and titrate away protein, in effect sponging up transcription factors and chromatin modifiers to produce broad changes in the transcriptome. NEAT2 binds to and sequesters several splicing factors to nuclear speckles; depletion of NEAT2 alters splicing-factor localization and activity,
leading to an altered pattern of alternative splicing for a set of pre-mRNAs; (3) Guide IncRNAs act as molecular chaperons, localizing ribonucleoproteins to specific chromatin targets. This activity can cause changes in the gene expression of neighboring (cis) or distantly located (trans) genes that cannot be easily predicted by just the IncRNA sequence itself. An example is HOTAIR (Hox antisense intergenic RNA), which is involved in protein degradation promoting ubiquitin-mediated proteolysis through binding E3 ubiquitin ligase; and (4) Scaffold IncRNAs have multiple domains so that they bind distinct proteins to form complexes with functions such as transcriptional activation or repression. Thus, the IncRNA serves as an adaptor to form the functional protein complex. TERRA (telomeric repeat-containing RNA), which is part of telomeric heterochromatin in addition to being present in the nucleoplasm, is a classic example of an RNA scaffold and is essential for telomerase function.

**Biological Role of IncRNAs**

Thousands of eukaryotic IncRNAs have been identified, with many found to be species specific but less conserved than protein-coding genes. Moreover, the expression profile of IncRNAs seems to be more cell-type specific than that of protein-coding genes and changes with differentiation and the developmental stage of an organism. IncRNAs are being implicated in many biological processes. Below we summarize the main recent findings on these functions.

**Cell Fate Specification**

IncRNAs provide an additional layer of regulation, in addition to that of proteins, for the specification of cellular identity. For example, the muscle-specific IncRNA linc-MD1 is involved in muscle cell differentiation, acting as a competing endogenous RNA in mouse and human myoblasts: in fact, downregulation and overexpression of linc-MD1 (long intergenic noncoding RNA muscle differentiation 1) correlated respectively with retardation and anticipation of the muscle differentiation program.

**Chromatin Modification**

IncRNAs may regulate transcription by recruitment of chromatin-remodeling complexes that generate epigenetic changes. For example, Kcnq1ot1, Air, and HOTAIR promote the formation of repressive chromatin across large areas of the genome by recruiting epigenetic enzymes, such as PRC1/2, which mediate mono-ubiquitylation of lysine 119 on histone 2A and trimethylation of lysine 27 on histone H3, respectively.

**Enhancer Functions**

IncRNAs have also been discovered to have enhancer-like functions. Enhancers are cis-acting regulatory sequences localized in intragenic and intergenic regions and play an important role in transcription regulation; these genetic elements contribute to defining the transcription program in cell differentiation and development, and their activity has been found altered in several human diseases, including cardiac hypertrophy. Knockdown of some IncRNAs was found to cause the downregulation of neighboring protein-coding genes, and in vitro luciferase assays revealed that they acted in cis in an orientation-independent manner. Among these, ncRNA-a7 was found to induce transcriptional activation of snail family zinc finger 1 (Snail), a gene encoding a transcription factor important for mesoderm formation during embryonic development. Another example is HOTTIP, an IncRNA that promotes the transcription of homeotic genes through the binding of WDR5 (WD repeat domain 5)/mixed-lineage leukemia, a protein complex that methylates lysine 4 of histone H3, a histone marker associated with transcription activation.

**RNA Splicing**

Alternative splicing of pre-mRNAs increases the diversity of the proteome by generating diverse proteins from a single mRNA. NEAT2 (nuclear-enriched noncoding transcript 2)—previously known as MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) because it was specifically upregulated in metastatic non–small-cell lung cancer cells—is an IncRNA that accumulates in the nucleus and regulates alternative splicing through interaction with serine/arginine-rich nuclear phosphoproteins of the splicing machinery.

**Nuclear Architecture and Compartmentalization**

IncRNAs also contribute to the building of cellular architecture. For example, upregulation of NEAT1 (nuclear paraspeckle...
assembly transcript 1) has been attributed to enlargement of paraspeckles, such as that occurring during myotube differentiation. Paraspeckles are ribonucleoprotein bodies found in the interchromatin space of mammalian cell nuclei and control transcription initiation, coactivation and corepression, RNA splicing, and transcription termination by retaining RNA in the nucleus. Given the large number of long non-coding transcripts currently being discovered through whole-transcriptome analysis, paraspeckles may be a paradigm for a class of subnuclear bodies formed around lncRNAs.

**X Chromosome Inactivation**

X chromosome inactivation is a process occurring in female somatic cells whereby genes on the inactive X chromosome are inactivated by the formation of a chromatin structure associated with transcription repression. The lncRNA *Xist* (X-inactive specific transcript) is required for this silencing, although the exact mechanism of *Xist*-mediated X chromosome inactivation is yet to be fully elucidated.

**Genomic Imprinting**

Inactivation of either the maternally or the paternally inherited allele—known as genomic imprinting—plays a crucial role in mammalian development. In addition to mRNAs, many imprinted gene loci express also a significant number of lncRNAs (eg, *Air*, *Kcnq1ot1*, and *H19*). These seem to have important roles in regulating the expression of neighboring imprinted protein-coding genes in cis.

**Other Functions**

Many other functions probably await discovery: for example, *NRON* (noncoding repressor of NFAT) regulates nuclear trafficking of NFAT, and because lncRNAs are also located in the cytoplasm, they may have other roles in cell biology.

**lncRNAs and Heart Development**

The expression of lncRNAs involved in the control of pluripotency and lineage specification is modulated during development. For instance, *Xist* becomes repressed while its antisense partner, *Tsix*, becomes upregulated at various stages of embryonic development. Knockdown of several IncRNAs has demonstrated that they are important to in the circuitry controlling the embryonic stem cell state, as it causes either exit from the pluripotent state or upregulation of lineage commitment programs. Comprehensive quantitative RNA sequencing of mouse heart has revealed the presence of ≈321 lncRNAs in the myocardium; of these, 117 were cardiac-enriched. It was determined that the lncRNA profile changed significantly during development, in particular during embryonic maturation.

The study of cardiac lncRNAs is less advanced than cardiac microRNAs. To date, only 2 lncRNAs—*Bvht* (Braveheart) and *Fendrr* (Foxf1 adjacent noncoding developmental regulatory RNA)—have been demonstrated particularly important in this field.

*Bvht* acts upstream of *MesP1* (mesoderm posterior 1)—a master gene of cardiovascular lineage commitment—and is required for cardiomyocyte differentiation and maintenance of cardiomyocyte phenotype by regulating the expression of core gene regulatory networks involved in defining cardiovascular cell fate. It regulates cardiomyocyte differentiation by modulating the epigenetic profile of cells through interaction with the PRC2 component SUZ12 (suppressor of zeste 12 homolog). However, *Bvht* is specific to the mouse, so any pertinence of the mechanism to humans remains to be revealed.

By contrast, orthologous transcripts of *Fendrr* are found in mice, rats, and humans. *Fendrr*-deficient mice present with thin ventricular walls due to hypoplasia linked to altered cardiomyocyte proliferation and die at the embryonic stage. *Fendrr* regulates the expression of important cardiac transcription factors, such GATA-6, FOXF1, IRX3, NKX2-5, PITX2, and TBX3, by controlling the epigenetic profile of their gene promoters. In fact, *Fendrr* can bind either PRC2 or trithorax group/mixed-lineage leukemia protein complexes to induce respectively trimethylation of H3 at lysine 27 and lysine 4 (Figure 4). The interaction of the lncRNA with the protein targets the latter to specific gene promoters.

---

**Figure 4.** Schematic of the mechanism of *Fendrr* as an example of a long noncoding RNA involved in heart development. *Fendrr* can recruit either polycomb repressive complex 2 (PRC2) or mixed lineage leukemia 1 (MLL1) and tether them to the promoters of cardiac transcription factor (TF) genes. In the former case, repressive histone marks (H3K4me3) are then laid, closing the chromatin structure and downregulating genes in trans; in the latter case, activating marks are laid (H3K27me3), opening chromatin structure and upregulating gene transcription (Illustration Credit: Ben Smith).
IncRNAs and Cardiac Pathology

Studies have started to parse the role of IncRNAs also in the pathogenesis of cardiac diseases. Deep sequencing revealed that the profile of myocardial IncRNAs is altered upon HF in humans and that left ventricular assist devices normalized a subset of differentially expressed IncRNAs; moreover, the IncRNA expression signature was more effective at distinguishing nonischemic from ischemic failing myocardium than were the microRNA or mRNA profiles. However, in contrast to the dynamic IncRNA profile found during embryonic development of the mouse heart, stress—such as pressure overload—during adult life was associated in changes with only a small fraction of myocardial IncRNAs, indicating differential regulation between IncRNAs and conventional genes.99

In mice, the IncRNA Chrf (cardiac hypertrophy–related factor) was found to act as a competing endogenous RNA sequestering miR-489, a microRNA targeting the mRNA of myeloid differentiation primary response gene 88 (Myd88), the upregulation of which is known to induce hypertrophy.103 A genome-wide association study identified 6 single-nucleotide polymorphisms in the IncRNA MIAT (myocardial infarction associated transcript) associated with MI; one single-nucleotide polymorphism (A11741G) caused a 1.3-fold increase in MIAT transcription in vitro.104 Another study revealed 15 IncRNAs modulated in pressure-overloaded mouse heart.105 A genome-wide association study on 23,000 participants identified a genomic locus containing ANRIL (antisense noncoding RNA in the INK4 locus) that harbored several single-nucleotide polymorphisms associated with increased susceptibility to coronary artery disease and several other diseases, including cancer.106 ANRIL is an antisense ncRNA transcribed with the INK4b–ARF–INK4a gene cluster, which it regulates through the recruitment of PRC1 and PRC2 to promote a repressive chromatin structure.107 Moreover, mice expressing a truncated Kcnq1ot1 had upregulation of the potassium channel gene Kcnq1. This IncRNA is an ≈60 kb unspliced RNA whose transcription starts antisense to intron 10 of Kcnq1, its host gene.108 Misexpression of these IncRNAs was hypothesized to cause disease through dysregulation of key cardiac proteins. In the vascular system, MALAT1 is enriched in endothelial cells and regulates the angiogenic features of vascular cells.109

Recently, the locus of the cardiac-specific gene myosin heavy chain 7 (Myh7) was demonstrated to harbor a cluster of antisense IncRNAs, which were named myosin heavy-chain-associated RNA transcripts (Mhrt); inhibition of Mhrt expression was found to be an essential step for the induction of cardiomyopathy subsequent to pressure overload.110 In fact, restoring Mhrt expression levels protected the heart from the development of hypertrophy and HF. Mechanistically, Mhrt antagonized the function of Brg1, a stress-activated, ATP-dependent chromatin-remodeling factor previously found involved in the regulation of pathological gene expression.111 Mhrt binds to the helicase domain of Brg1, which is essential for tethering Brg1 to its DNA targets, and prevents the expression of genes that are induced during stress through a Brg1-mediated chromatin remodeling mechanism (Figure 5).

Another IncRNA that has been recently linked to cardiac pathology is Carl (cardiac apoptosis–related IncRNA).112 In mouse cardiomyocytes, Carl bound to and sequestered miR-539, a microRNA found to target the mRNA of the PHB2 subunit of prohibitin, a protein localized to the inner mitochondrial membrane, where it has a role in mitochondrial homeostasis. Downregulation of PHB2 during pathological insults, such as anoxia and ischemia/reperfusion, was found to be dependent on upregulation of miR-539, promoting mitochondrial fission and cardiomyocyte apoptosis. Carl acted as the endogenous sponge for this microRNA, regulating mitochondrial morphology and cell death under normal conditions.

ncRNAs and Paracrine Signaling

There is recent evidence that multiple cell types in the cardiovascular system are able to generate various kinds of vesicles that vehiculate paracrine signals. This has been reviewed recently,113,114 and thus, here we will only focus on the importance of vesicle-enriched ncRNAs in cardiovascular pathophysiology.

Figure 5. Schematic of the mechanism of Mhrt as an example of a long noncoding RNA involved in heart pathology. Under physiological conditions, Mhrt is expressed along with its host gene, Myh7. Mhrt then binds to BRG1 complexes, effectively sponging them up so as to blockade attachment to BRG1-responsive genes. Downregulation of Mhrt as a consequence of cardiac stress releases Mhrt-mediated BRG1 complex inhibition, allowing the opening up of chromatin and the transcription of hypertrophy-related genes (Illustration Credit: Ben Smith).
Different kinds of vesicles are produced by cardiovascular cells, including—from the largest to the smallest in size—apoptotic bodies, microvesicles, and exosomes. They all contain specific cargoes of protein, microRNA, and mRNA. It was recently shown for exosomes that they are particularly enriched in small RNAs, such as microRNAs. Exosomes are membrane vesicles with a diameter of 30 to 100 nm and, thus, can only be visualized by electron microscopy or fluorescent-labeling strategies. Exosomes are formed by fusion of multivesicular bodies with the plasma membrane, leading to a release of exosomes into the extracellular space. As the microRNA and mRNA content of exosomes is highly specific and does not simply reflect the composition in the cytoplasm, there must be a specific transport and shuttling system that specifically conveys only selected RNAs to the exosomes. Potential mechanisms include chaperones, such as HSC70 (heat shock cognate 70), Rab GTPases (guanine triphosphatases), as well as the autophagy pathway. Indeed, it is likely that activation or inhibition of cell-type specific autophagy processes may affect multivesicular body formation and composition, leading to altered exosomal microRNA content due to multivesicular bodies associating with components of microRNA effector complexes.

Of potential interest for the clinician, secreted exosomes can be isolated from biological fluids and their microRNA profile assessed, enabling them to serve as potential biomarkers for certain diseases. This has recently been suggested also for extracellular lncRNAs, which can be detected in the plasma of patients with MI. Of considerable importance are recent findings that demonstrate that secreted ncRNAs have a paracrine potential, for example, through uptake by recipient cells and subsequent binding to and regulation of the recipient cells’ mRNAs.

In addition, apoptotic bodies enriched in miR-126 have been reported to limit atherosclerosis development. Further evidence for the crucial role of miR-126 as an important protector of the endothelial system was recently obtained with miR-126 knockout mice, which have impaired endothelial proliferation. A further example of atheroprotective actions based on microRNA trafficking was shown for shear stress–stimulated human endothelial cells that produced vesicles enriched in miR-143/145 that led to reduced atherosclerotic lesion formation in aortae of ApoE knockout mice. Likewise, circulating proangiogenic cells were able to secrete exosomes enriched in miR-15a/16, leading to modulation of vascular density in critical limb ischemia. Human cardiac progenitor cells secreted vesicles enriched in miR-210, miR-132, and miR-146-3p, which protected cardiomycocytes during MI. Likewise, GATA4–gene–engineered mesenchymal stem cells secreted miR-221–enriched microvesicles that also led to cardiomycocyte protection on vesicle uptake. Other cell types with evidence of an ability to secrete microRNAs are cardiac fibroblasts and inflammatory cells, both involved in the cardiac fibrosis process. Cardiac fibroblast–secreted exosomes were enriched in passenger strand microRNAs, such as miR-219*, which could be taken up by recipient cardiomycocytes, provoking hypertrophy. Macrophages were also recently shown to produce microvesicles transferring miR-223 to monocytes, endothelial cells, and fibroblasts.

In addition to being cargoed within vesicles, microRNAs can also associate with lipoprotein-based carriers in biological fluids, such as high-density lipoproteins. For example, it was reported that endothelial ICAM-1 (intercellular adhesion molecule 1) expression could be regulated through transfer of miR-223, suggesting a new mechanism for the anti-inflammatory and antiatherosclerotic properties of high-density lipoproteins.

As for microRNAs, lncRNAs are also highly stable in biological fluids, and the circulating lncRNA profile has been found altered upon HF in mice. In humans, levels of aHIF, KCNQ1OT1, and MALAT1 were found increased, and that of ANRIL decreased, in peripheral blood after MI; moreover, levels of ANRIL, KCNQ1OT1, MIAT, and MALAT1 were higher in patients with ST-segment–elevation myocardial infarction than with non–ST-segment–elevation myocardial infarction and were found to be significant univariable predictors of left ventricular dysfunction, indicating that the measurement of circulating lncRNAs may be used for prognosis. Finally, the lncRNA LIPCAR (long intergenic noncoding RNA predicting cardiac remodeling), reported detectable in plasma of post-MI patients, was determined to have a potential to predict onset of future cardiac remodeling processes and survival.

In conclusion, there is cumulative evidence that cardiovascular cells communicate with each other via the transfer of ncRNAs. The finding that vesicles contain unique ncRNA compositions, including enrichment with passenger strand microRNAs, clearly needs further mechanistically orientated studies. Their use as diagnostic markers is intriguing and may open a new avenue for cardiovascular biomarker research. Finally, circulating vesicles containing ncRNAs may serve as new treatment targets through manipulation of derailed intercellular communication systems.

Therapeutic Prospective for ncRNAs

The above has highlighted how inappropriately high or low ncRNA expression can lead to cardiovascular disease. Therefore, manipulating their levels, through either gene therapy to increase the expression of disease-downregulated ncRNAs or inhibition of disease-upregulated ones, could represent new therapeutic strategies. Regarding the former, exogenous administration of microRNAs has been shown to restore left ventricular mass and promote functional recovery after MI in mice. However, it is difficult to foresee the application of viral vectors to increase microRNA expression in the clinic, at least for the near future. Regarding the latter strategy, inhibition of inappropriately upregulated microRNAs can be achieved through antisense oligonucleotides. These antisense sequences must be chemically modified to improve stability and tissue distribution of the underlying nucleotide structure, for example, through the use of phosphorothioate backbone linkages (which enhance binding affinity and confer nucleae resistance), and conjugation with 2′-O-methyl-cholesterol (which facilitates cellular uptake). Anti-microRNAs can have different lengths: antagomiRs are antisense molecules of the whole microRNA sequence that have a phosphorothioate-modified backbone and are conjugated with 2′-O-methoxycholesterol; locked nucleic acids are 8- or 15-base antisense molecules with 2′ sugar modifications (2′-fluoro and 2′-O-methoxyethyl groups).
creating high affinity for the target. There are also locked nucleic acid–based chemistries allowing targeting of lncRNAs in vitro and in vivo. So-called long RNA gapmeRs are antisense oligonucleotides containing a central stretch (gap) of DNA monomers flanked by blocks of locked nucleic acid–modified nucleotides; these produce increased target affinity and nucleo-

To date, use of the antisense approach in the cardiovascular field has been highly successful experimentally. However, the efficacy of the drugs must be titrated against their off-target effects—such as interference with the complement cascade and activation of innate immunity—and toxicity.136 Up to now, only the antagoniR anti-miR-122 has made it through to a phase-II clinical trial (for the treatment of hepatitis C virus infection).137 The use of anti-microRNA in the cardiovascular arena is still in the preclinical phase.

Acknowledgments
We thank members of our laboratories for their support and critically reading of this review. In particular, Dr Condorelli thanks Michael V.G. Latronico, Roberto Papait, and Giuliano Giuseppe Stirparo. We apologize for not having included many significant reports due to space constraints.

Sources of Funding
The support of the European Commission–supported project FIBROTARGET, the Integrated Research and Treatment Center Transplantation (IFB-Tx; grant number 01EO1302), and the Foundation Leducq (Project MIRV AD) to Dr Thum is kindly acknowl-

Disclosures
None.

References
2. Lompre AM, Schwartz K, d’Albis A, Lacombe G, Van Thiern M, Swynghedauw B. Myosin isoenzyme redistribution in chronic heart over-

9. Saxena A, Carninci P. Long non-coding RNA modifies chromatin: epi-

10. Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annota-

11. Liang Y, Ridzon D, Wong L, Chen C. Characterization of microRNA expres-

21. von Roussi E, Sutherland LB, Qi X, Richardson JA, Hill J, Olson EN. Control of stress-dependent cardiac growth and gene expression by a microR-

26. Castaldo A, Zaglia T, Di Mauro V, et al. MicroRNA-133 modulates the β1-


28. Eisinger JR, Pheasant M, Mattick JS. The relationship between non-protein-


Long Noncoding RNAs and MicroRNAs in Cardiovascular Pathophysiology
Thomas Thum and Gianluigi Condorelli

Circ Res. 2015;116:751-762
doi: 10.1161/CIRCRESAHA.116.303549
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/116/4/751

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/