



Published in final edited form as:

Nat Rev Cardiol. 2024 May ; 21(5): 326–345. doi:10.1038/s41569-023-00952-5.

Long non-coding RNAs in cardiac hypertrophy and heart failure: functions, mechanisms and clinical prospects

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Abstract

The surge in reports describing non-coding RNAs (ncRNAs) has focused attention on their possible biological roles and effect on development and disease. ncRNAs have been touted as previously uncharacterized regulators of gene expression and cellular processes, possibly working to fine-tune these functions. The sheer number of ncRNAs identified has outpaced the capacity to characterize each molecule thoroughly and to reliably establish its clinical relevance; it has, nonetheless, created excitement about their potential as molecular targets for novel therapeutic approaches to treat human disease. In this Review, we focus on one category of ncRNAs — long non-coding RNAs — and their expression, functions and molecular mechanisms in cardiac hypertrophy and heart failure. We further discuss the prospects for this specific class of ncRNAs as novel targets for the diagnosis and treatment of these conditions.

ToC

In this Review, Mably and Wang summarize the expression, functions and molecular mechanisms of long non-coding RNAs (lncRNAs) in the cardiac hypertrophy and failure. The authors also discuss lncRNAs as novel targets for the diagnosis and treatment of these conditions.

Introduction

Although only 1% of the human genome is estimated to be composed of protein-coding genes, nearly 80% is transcribed and functional; therefore, only ~2% of transcripts encode proteins, meaning that the vast majority of transcription products are categorized as being non-coding^{1,2}. As a result of this observation, scientists are faced with a conundrum: either the process of transcription is highly non-specific and inefficient, resulting in a huge amount of material that serves no biological purpose, or these non-coding transcripts do indeed have a function that remains to be fully defined. Since the first description of microRNAs (miRNAs) in 1993^{3,4}, evidence has continued to accumulate that supports

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Author contributions

Both authors contributed substantially to all aspects of the article.

Competing interests

The authors declare no competing interests.

the latter of the two possibilities. Additionally, these studies suggest that miRNAs and other non-coding RNAs (ncRNAs) could have important roles in the regulation of gene expression, including in cardiac disease states⁵. Multiple studies and biotech start-ups have been initiated to examine the potential use of ncRNAs as therapeutic targets or agents; therefore, a comprehensive understanding of their biology and assessment of their clinical potential are necessary as the field progresses.

Long non-coding RNAs (lncRNAs) are a subgroup of ncRNAs that are broadly defined as being greater than 200 nucleotides in length but they can be as long as 100 kilobases^{6,7}. They are less conserved across species and expressed at lower levels than their coding (mRNA) counterparts. Depending on their genomic environment, synthesis, structure and function, lncRNAs can be further subclassified as intergenic (lincRNA), enhancer (elncRNA), promoter (plncRNA) or antisense (lncRNA-as); some lncRNAs can form a circular structure and form part of the broader circular RNA (circRNA) category. The function and molecular mechanisms of lncRNA action show wide heterogeneity, depending on their subcellular location and interaction partners (Fig. 1). Based on their mechanism of action, they can be categorized into four broad groups: signal lncRNAs, which influence the actions of transcription factors or signalling pathways; decoy lncRNAs, which sequester transcription factors, other proteins or miRNAs to prevent their normal function; guide lncRNAs, which can recruit proteins to target nearby (*cis*) or distant (*trans*) target genes; and scaffold lncRNAs, which bring together and/or stabilize multiple proteins to form larger complexes⁸. They can be further characterized on the basis of their genomic location and context: sense lncRNAs are derived from the sense strand of a protein-coding gene, can include intronic regions and can also overlap with a substantial portion of the protein-coding sequence; antisense lncRNAs are transcribed from the antisense strand of a protein-coding gene and can also overlap with the protein-coding sequence and include intronic sequence; intergenic lncRNAs are transcribed from both strands of DNA between genes; and intronic lncRNAs are transcribed from introns between the exons of protein-coding genes⁹. These lncRNAs can also function bidirectionally in *cis* to influence the expression of genes at either their 5' or 3' ends (Fig. 1a)⁸. In the nucleus, lncRNAs participate in chromatin organization, gene transcription, and the modification and splicing of nascent RNAs (Fig. 1b)¹⁰. In the cytoplasm, lncRNAs can modulate mRNA turnover, storage and protein translation (Fig. 1c–e)¹¹. Given the effect of lncRNAs on these essential cellular processes, their influence inevitably extends to broader biological processes and physiological functions.

Two of the aforementioned properties of lncRNAs have created challenges to establishing their functions and mechanisms; their poor sequence conservation makes animal modelling difficult and their characteristically low expression levels is a challenge to assessing their endogenous *in vivo* functions. Some studies have shown that many lncRNAs have non-essential roles during heart development and in cardiac physiology, and multiple others have demonstrated that the expression of many lncRNAs is altered under pathological cardiac conditions; therefore, lncRNAs have a key role in cardiac remodelling in response to stress. In this Review, we first summarize the expression of lncRNAs and the functional involvement of lncRNAs in cardiac hypertrophy, based on studies using *in vitro* systems and/or *in vivo* animal models (see Box 1 for more information about these models).

We discuss well-characterized lncRNAs with in vivo cardiac phenotypes and highlight commonalities among these studies. However, we have restricted this Review to a discussion of linear lncRNAs to avoid overlap with other reviews focused on the roles of circular lncRNAs in cardiac hypertrophy^{12,13}. Next, we provide an overview of the mechanisms by which lncRNAs mediate their effects and speculate about their clinical potential. lncRNAs could represent a novel class of molecules that might function as biomarkers and/or as therapeutic targets in the search for new approaches in the treatment of cardiovascular disease.

lncRNA expression and association with cardiac hypertrophy and heart disease

Multidimensional comparative transcriptomics analyses across various developmental time points, organs, and species have demonstrated that lncRNAs are preferentially expressed in adult tissues¹⁴. Additional integrated transcriptomics and epigenomics studies in developmental models, as well as adult mouse and human hearts, have identified additional novel lncRNAs that are associated with cardiovascular disease¹⁵. lncRNAs are expressed at low levels across cell and tissue types; however, lncRNAs that show tissue-restricted and higher expression patterns have been identified. Many of these more tissue-restricted and robustly expressed lncRNAs show greater evolutionary conservation, in particular around their promoter regions^{16,17}, which suggests a paradigm in which tissue specificity and expression level correlate with preserved function across species.

An analysis of the cardiomyocyte transcriptomes of mouse and human hearts revealed that lincRNAs were key nodal regulators of subpopulations of cardiomyocytes¹⁸. In this report from 2017, investigators used single-nucleus RNA sequencing to profile the transcriptome from the hearts of adult mice and humans, in both failing and non-failing conditions¹⁸. They assessed the function of these lincRNAs by knocking down their levels in mouse ventricular cardiomyocytes and demonstrated changes in the expression patterns of genes involved in dedifferentiation and the cell cycle. These results led the researchers to ascribe possible roles for these lincRNAs in the regulation of regeneration of adult cardiomyocyte subpopulations¹⁸. Other annotated lncRNAs have the potential to encode micropeptides, thereby directly contributing to the expressed transcriptome of cardiac cell types^{19,20}. This insight suggests another, possibly complementary, mechanism by which lncRNAs might contribute to the pathophysiology of cardiac hypertrophy and substantially increases the complexity of lncRNA biology^{19,20}.

Mouse models of myocardial infarction (MI) are extensively used to study cardiac damage caused by ischaemia–reperfusion. Investigators studying the heart transcriptome in one of these mouse models used traditional RNA sequencing to determine that the expression of certain lncRNAs was strongly correlated with specific parameters of cardiac function and dimensions²¹. These data suggest that specific lncRNAs are associated with cardiac-specific genomic enhancers and thereby influence the transcription of genes involved in cardiogenesis and pathological remodelling²¹.

A systematic analysis of the lncRNA expression profiles between normal embryonic and adult cardiac tissue identified 157 lncRNAs with differential expression²². However, when the expression profiles of adult normal and hypertrophic cardiac tissue were compared, only 17 differentially expressed lncRNAs were observed after transverse aortic constriction (TAC) surgery (analysis performed 1 week and 4 weeks after TAC)²². This finding is intriguing because it shows a lncRNA response that is different from the dogma for the expressed transcriptome in the heart (that many fetal, protein-coding mRNAs (and their products) that are highly expressed in the developing and fetal heart are also induced in the hypertrophic heart^{23,24}). The observation that fetal lncRNAs are not re-expressed in the hypertrophic heart to a high degree suggests an important role during development that is more subtle during the response of the heart to haemodynamic stress²².

In an attempt to characterize the transcriptome changes in diseased hearts, researchers performed next-generation sequencing using tissue collected from human left ventricles; samples collected from non-failing hearts were analysed and compared with samples from paired non-ischaemic and ischaemic failing left ventricular (LV) tissue collected before or after LV assist device implantation²⁵. The investigators found that the expression profiles of lncRNAs, but not those of mRNAs or miRNAs, could discriminate between failing hearts with different pathologies. In particular, lncRNAs were markedly altered in response to LV assist device support, suggesting they could be sensitive markers of heart failure (HF) and might have important roles in the pathogenesis of HF and in the reverse remodelling observed in response to mechanical support^{25,26}. Another interesting finding from this study was the detection of a high abundance of lncRNAs of mitochondrial origin (71%)²⁵. Given that the mitochondrial number and function are dynamically regulated and altered in the heart during various pathophysiological conditions, these mitochondrially encoded lncRNAs might have previously unrecognized roles in the response of the heart to stress. However, due to the small sample size of this analysis (eight in each group), these data need to be validated by further studies to test their reproducibility and whether other tissues and organs respond similarly. The presence of mitochondrial lncRNAs at these elevated levels would introduce another mechanism by which this organelle could actively influence cardiac function and disease. Further investigation to establish the function of these lncRNAs in the heart, as well as their mechanisms of action, will not only contribute to a better understanding of lncRNA biology but will also provide important insights into their roles in cardiovascular disease.

lncRNA mechanisms of action in the heart

lncRNAs are detectable in most cells and tissues, with many showing a tissue-specific or cell-type-specific pattern of expression²⁷. lncRNA transcripts can be found in the nucleus or cytoplasm, and their subcellular localization can provide insights into their function (Fig. 1)^{28–30}. In the nucleus, these functions can involve direct interactions with DNA, RNA and proteins, allowing lncRNAs to modulate chromatin function, regulate the assembly and function of nuclear bodies, and mediate the association between DNA and epigenetic factors, transcription factors and even RNA polymerase (Fig. 1a)³¹. Alternatively, lncRNAs have been shown to alter the stability and translation of cytoplasmic mRNAs and thereby interfere with signalling pathways (Fig. 1b–e)^{11,32}. lncRNAs might also function as sponges

for miRNAs and other RNAs in the cytoplasm (Fig. 1d)^{33,34}. In addition, emerging evidence suggests that many lncRNAs function in a *cis*-regulatory manner, which can confine their effect on gene expression to specific transcripts, depending on their own genomic context (Fig. 1a)^{35,36}.

***Cis*-regulatory or *trans*-regulatory functions**

Multiple mechanisms of action have now been described for what has become the broad category of *cis*-regulatory lncRNAs^{35,36}. However, many of these mechanisms are shared with lncRNAs that do not demonstrate clear *cis*-regulatory roles. Additionally, many lncRNAs are likely to possess both *cis*-regulatory and *trans*-regulatory capacities³⁷ (Fig. 1a). Therefore, in this section, we discuss several lncRNAs with defined mechanisms of action and note for each whether these actions are in *cis* or *trans*, if known.

Enhancer interaction.—The lncRNA Uph (upperhand) has been shown to be crucial to heart development, given that blocking its transcription in mice results in right ventricular hypoplasia and embryonic lethality³⁸. However, the process of Uph transcription itself, rather than the mature transcript, was shown to be required for proper *Hand2* expression³⁸. This analysis revealed that Uph has a crucial role in maintaining the super-enhancer signature at the *Hand2* enhancer locus, thereby promoting RNA polymerase II elongation and ultimately controlling *Hand2* gene transcription³⁸.

Antisense lncRNAs.—AIRN (also called AIR) is a lncRNA transcribed in the antisense orientation to the *IGF2R* gene^{39,40}. Studies in mice and in human cells have shown that AIRN regulates the expression of the nearby imprinted protein-coding genes *IGF2R* (maternally derived), *SLC22A2* and *SLC22A3* by a *cis*-regulatory mechanism^{41,42}. AIRN is highly expressed in the heart⁴³; in a mouse model of MI, its expression is downregulated in non-infarcted regions of the heart, and silencing of Airn induces cell death in cardiomyocytes⁴³. The regulatory activity of Airn is facilitated by its interaction with the product of the nearby gene *Igf2bp2*, which encodes an RNA-binding protein that regulates the translation of target genes, including that of *Igf2bp2* itself⁴³. These findings are intriguing but were discovered using cell-based assays, without confirmation using in vivo models. Therefore, the role of AIRN in heart development and disease remains to be confirmed. Further studies to determine whether reduced levels of AIRN or its absence is associated with heart defects either in mouse models or in cardiovascular disease in humans would be valuable to assess its contribution to cardiac function.

Alternative splicing.—TRDN-AS is transcribed as an antisense transcript from the *TRDN* locus encoding the protein triadin (TRDN)^{44,45}, establishing alternative splicing as another process that can be regulated by lncRNAs to influence cardiac function. TRDN is a sarcoplasmic reticulum membrane protein and it is known to have an important role in Ca²⁺ homeostasis and cardiomyocyte function; mutations in the *TRDN* gene have been linked to cardiac dysfunction and arrhythmias^{46,47}. TRDN-AS is expressed in cardiomyocytes and co-localizes and interacts with serine/arginine splicing factors in the nucleus, where it efficiently recruits them to the triadin precursor mRNA^{44,45}. Loss of Trdn-as in the heart has been shown to sensitize mice to cardiac arrhythmias in response to catecholamine challenge,

at least in part due to a reduction in cardiac triadin transcript and protein levels⁴⁵. Of note, in contrast to the levels of TRDN, the transcript levels of TRDN-AS are increased in the hearts of patients with HF⁴⁴, consistent with studies on the influence of Trdn-as deletion on Trdn protein levels in mouse⁴⁵. These data suggest that this conserved lncRNA has the potential to be a novel therapeutic target for the treatment of HF and arrhythmias. Although analysis of TRDN-AS convincingly shows that it functions as a *trans*-regulatory factor that modulates the alternative splicing of the TRDN-coding transcripts, further studies are required to determine whether it also functions as a *cis*-regulatory element to modulate triadin expression.

Protein interactions.—The function of the lncRNA CCRR (cardiac conduction regulatory RNA) has been linked to cardiac arrhythmias⁴⁸. The expression of *Ccrr* is downregulated in a mouse model of HF and is associated with slow cardiac conduction and increased arrhythmogenicity⁴⁸. Furthermore, silencing *Ccrr* in the heart induces arrhythmias in mice⁴⁸. Functional analysis of *Ccrr* determined that its inhibition affects the structure and function of intercalated discs and gap junctions, thereby slowing longitudinal cardiac conduction⁴⁸. Mechanistically, *Ccrr* directly binds to the connexin 43 (Cx43)-interacting protein, CIP85, to block endocytic trafficking of Cx43 and prevent its degradation. This lncRNA is conserved in humans, and reduced CCRR expression was found in patients with HF⁴⁸. Therefore, CCRR would be an interesting target for the design of new approaches to the treatment of pathological arrhythmias.

Caren (cardiomyocyte-enriched non-coding transcript) is a lncRNA that is enriched in cardiomyocytes and located in the cytoplasm⁴⁹. Given that Caren does not influence the expression levels of neighbouring genes, a *cis*-regulatory mechanism is unlikely. Instead, Caren transcripts decrease the translation of its downstream target *Hint1* (encoding histidine triad nucleotide-binding protein 1)⁴⁹. In the heart, Caren has a protective role by inactivating the serine-protein kinase ATM-mediated DNA damage response pathway and activating mitochondrial bioenergetics⁴⁹. This cardioprotective role of Caren suggests that it has the potential to be a novel therapeutic target for the treatment of heart disease.

The lncRNA CARDINAL (myocardin-adjacent lncRNA) is encoded by a genomic locus adjacent to the *MYOCD* gene, encoding myocardin (a potent transcriptional coactivator of serum response factor (SRF))⁵⁰. Cardinal and *Myocd* also share overlapping but distinct cardiac-muscle-specific, smooth-muscle-specific and embryonic expression patterns⁵⁰. The Cardinal transcript is present in both the nucleus and the cytoplasm of cardiomyocytes⁵⁰. Nuclear Cardinal forms a complex with SRF to repress the transcription of the pro-mitogenic gene *Fos*, encoding protein c-Fos⁵⁰. Cardinal-deficient mice have aberrant expression of TCF–SRF-dependent mitogenic genes and reduced cardiac contractility⁵⁰; these data suggest that nuclear CARDINAL might function as an RNA cofactor for SRF to regulate the transcription of target genes. However, it remains unclear whether CARDINAL has a *cis*-regulatory function to regulate the expression of nearby genes, such as myocardin, or how cytosolic CARDINAL functions. Furthermore, it is unknown whether CARDINAL is involved in cardiac remodelling and dysfunction in response to pathophysiological stress.

In a related study, the tissue-specific lncRNA CARMN (or CARMEN, cardiac mesoderm enhancer-associated non-coding RNA) was found to interact directly with MYOCD to modulate its ability to influence cardiac and smooth muscle gene expression^{51,52}. CARMN is encoded by the genomic region that includes the miRNA-143–miRNA-145 cluster; however, the aforementioned function of CARMN seems to be independent of the activity of these miRNAs^{51,52}. Another study reported that CARMN interacts directly with SRF to regulate gene expression and its role in vascular smooth muscle⁵³.

The lncRNA Cpmer (cytoplasmic mesoderm regulator) uses an RNA–RNA pairing mechanism to specifically recognize *Eomes* mRNA (encoding the protein eomesodermin, a transcription factor involved in early mesoderm development)⁵⁴. This interaction facilitates binding of the *Eomes* transcript to the translational elongation factor eEF1A2 and subsequent translation of the eomesodermin protein⁵⁴. The ability of CPMER to regulate eomesodermin translation is conserved between mice and humans; in addition, Cpmer has been shown to promote the differentiation of both mouse and human embryonic stem cells into cardiomyocytes⁵⁴.

Molecular sponging.—Overexpression of the lncRNA Plscr4 (phospholipid scramblase 4) in mice can reduce angiotensin II-induced cardiomyocyte hypertrophy, whereas depletion of Plscr4 induces hypertrophy⁵⁵. Plscr4 overexpression can also attenuate TAC-induced cardiac hypertrophy in mice⁵⁵. Mechanistically, evidence supports a role for Plscr4 as a natural sequestering agent for miRNA-214, thereby acting as an miRNA-214 sponge. In support of this hypothesis, Plscr4 overexpression in angiotensin II-treated and normal cardiomyocytes decreases the levels of miRNA-214⁵⁵. This finding was validated in mouse hearts and was proposed as an explanation for the observed reduction in cardiomyocyte hypertrophy⁵⁵. By contrast, Plscr4 knockdown increases miRNA-214 levels, thereby promoting cardiomyocyte hypertrophy⁵⁵.

lncRNAs that regulate cardiac hypertrophy

Many lncRNAs expressed in cardiac tissue have been investigated for their influence on gene expression in the heart. As more detailed studies are published, we will gain a better understanding of the roles of these molecules on cardiac function, and certain lncRNAs will inevitably gain prominence because of their effect on processes such as cardiac hypertrophy and remodelling. This trend would be similar to what was observed during research into the roles of miRNAs in the heart, when specific cardiac miRNAs (such as miRNA-1 and miRNA-133) garnered greater attention because of their important roles in cardiac tissue^{56–60}. Studies on some lncRNAs have already provided interesting information into their mechanisms of action, as well as possible links to cardiac disease processes. We highlight some of these in the following section (a more comprehensive list is provided in Box 2, with details provided in Tables 1,2).

H19

H19 is a lncRNA that is expressed at high levels in both cardiac and skeletal muscle⁶¹. The function of H19 has also been extensively characterized, with published studies linking it with the genomic imprinting disorders Silver–Russell syndrome and Beckwith–Wiedemann

syndrome^{62,63} and it has also emerged as a very promising therapeutic target for cancer treatment⁶⁴. Several studies have examined the function of *H19* in cardiac hypertrophy and HF^{61,65–68}. These studies revealed that H19 expression is induced during the early stages of cardiac hypertrophy, then downregulated during decompensation. In response to adeno-associated virus 9 (AAV9)-mediated delivery of *H19* to mouse hearts, it was possible to attenuate the development of cardiac hypertrophy, in part by suppression of nuclear factor of activated T cells (NFAT) expression⁶¹, a transcription factor associated with the induction of the hypertrophic gene expression programme in the heart. The suppression of NFAT activity was proposed to be mediated by direct interaction between H19 and the polycomb repressive complex 2 (PRC2), which led to a reduction in tescalcin levels and function, resulting in a reduction in NFAT expression⁶¹ (Fig. 2).

H19 overexpression has been reported to reduce the size of cardiomyocytes both at baseline and in response to the hypertrophic agonist phenylephrine in vitro; conversely, knockdown of H19 induced cardiomyocyte hypertrophy⁶⁸. Apart from its role in cardiac hypertrophy, H19 has also been linked to cardiomyocyte proliferation, metabolism and cardiac regeneration through its association with protein lin-28 homologue A (LIN28a)⁶⁹. The ability of LIN28a (a multifunctional RNA-binding protein) to induce cardiomyocyte metabolism and cell cycle activity were blunted when H19 was inhibited in neonatal rat ventricular cardiomyocytes⁶⁹. However, the mechanism by which H19 controls these biological processes has not been fully characterized.

The mechanism of action of this lncRNA is further complicated by the fact that another regulatory molecule, miRNA-675, is embedded in the first exon of *H19*⁷⁰. Whether H19 regulates cardiomyocyte hypertrophy independently of miRNA-675 or whether the two molecules function together remains unclear. Moreover, the excision of miRNA-675 from H19 is further regulated by the stress-response RNA-binding protein HuR, establishing a multi-tiered regulatory node in the regulation of cardiac hypertrophy⁷⁰. Evidence suggests that miRNA-675 helps to mediate the function of H19 in regulating hypertrophy, given that an H19 fragment without the pre-miRNA-675 (or with mutant) sequences does not inhibit cardiomyocyte hypertrophy⁶⁸.

In another study into H19 regulation of the dystrophin protein, a novel mechanism of lncRNA action was discovered. H19 was found to bind to the tail end of the dystrophin cysteine-rich domain, which protected a lysine residue from ubiquitination, thereby stabilizing the dystrophin protein^{71,72}. Moreover, in a mouse model of muscular dystrophy, agrin-conjugated H19 mimics were shown to retain the ability to stabilize dystrophin, resulting in improved muscle function and alleviation of the muscular dystrophy-associated cardiomyopathy⁷¹.

Despite these findings, some controversy remains about the role of H19 in the heart, given that some studies suggest it is cardioprotective whereas others suggest it promotes heart disease⁶¹. Furthermore, although H19 is more abundant in cardiomyocytes than in cardiac fibroblasts, it is even more highly expressed in endothelial cells than either of those cell types, which suggests possible disparate functions in different cell lineages⁶¹. Although this observation alone does not preclude an important role for H19 in the heart, it does suggest

that its mechanism of action could be more complicated; perhaps the influence of H19 on cardiomyocyte function is a result of H19 expression and function in an adjacent cell type. Further study will be required to resolve these questions before consideration of H19 as therapeutic agent would be reasonable.

CHRF

CHRF (cardiac hypertrophy related factor) was among the first-characterized lncRNAs determined to have a role in heart tissue; it was initially identified as a target of miRNA-489⁷³. By binding directly to miRNA-489, CHRF acts as a sponge to prevent miRNA-489 from binding to myeloid differentiation primary response protein 88 (MYD88), its regulatory target; in so doing, CHRF removes the repressive action of miRNA-489 on MYD88 and induces cardiac hypertrophy⁷³ (Fig. 3). This study also demonstrated that exogenous expression of *Chrf* led to increased apoptosis of cardiomyocytes in mouse hearts⁷³; however, knockdown of *Chrf* using small interfering RNA (siRNA) also restored atrial natriuretic factor (Anf) and β -myosin heavy chain (β -Mhc) levels in the heart to normal⁷³. Although some aspects of this study indicate that CHRF is a promising lncRNA for regulating cardiac hypertrophy and function by acting as a miRNA sponge, follow-up studies to characterize this function further have not yet been conducted.

ZFAS1

ZFAS1 (ZNF1 antisense RNA 1) is another lncRNA that seems to participate in the crucial biological process of cardiomyocyte contractility⁷⁴. ZFAS1 is an antisense lncRNA transcribed from the 5' end region of the protein-coding gene *ZNF1*, and circulating levels of ZFAS1 were measured by quantitative polymerase chain reaction and found to be decreased in whole-blood samples from patients with acute MI⁷⁵. Knockdown of endogenous *Zfas1* in mice partially protects the heart against ischaemia-induced contractile dysfunction, whereas AAV-based overexpression of *Zfas1* in the heart impairs contractile function of cardiomyocytes⁷⁴. Preliminary studies suggest that *Zfas1* overexpression results in intracellular Ca^{2+} overload in cardiomyocytes, providing insights into its mechanism of action⁷⁴. ZFAS1 is located in the cytoplasm and is associated with the sarcoplasmic reticulum, where it directly binds to the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a) protein to limit its activity and reduce cardiomyocyte contraction⁷⁴ (Fig. 2).

This observation raises intriguing possibilities when considered in the light of a previous study that reported the identification of a dwarf open reading frame (DWORF) in an annotated lncRNA gene⁷⁶. DWORF encodes a micropeptide localized to the sarcoplasmic reticulum membrane and increases SERCA2a activity by displacing inhibitors of the SERCA2a pump, including phospholamban, sarcolipin and myoregulin⁷⁶. Further investigation to determine whether ZFAS1 and DWORF interact physically and/or functionally to regulate the activity of SERCA2a in cardiomyocytes is ongoing. However, given that the *ZFAS1* gene is conserved across species, it might function as a novel biomarker and/or therapeutic target for ischaemic cardiac disease. At this time, studies in mouse and rat have determined that inhibition of endogenous *Zfas1* improves cardiac function in response to MI^{74,77}; however, whether ZFAS1 is involved in MI-induced myocardial death and/or cardiac regeneration is uncertain. Furthermore, caution will need

to be exercised when considering applications in humans because increased expression of ZFAS1 has been linked with cancer and other diseases^{78–80}.

CHAST

CHAST (cardiac hypertrophy-associated transcript) was originally identified and reported as a lncRNA that was dysregulated during cardiac hypertrophy^{61,81}. Not surprisingly its transcription is regulated by the NFAT signalling pathway, an important component of the cellular processes that govern cardiac hypertrophy and pathological remodelling⁷⁴. Through a series of gain-of-function and loss-of function studies in mice, Chast overexpression was shown to be sufficient to induce cardiomyocyte hypertrophy both in vitro using isolated cardiomyocytes and in vivo using mouse models⁸¹. In an attempt to define a therapeutic approach, GapmeRs (a form of antisense oligonucleotide) were used to inhibit Chast activity and were able to prevent the development of hypertrophy when applied early after TAC⁸¹. If treatment was delayed, the use of GapmeRs could still attenuate TAC-induced pathological cardiac remodelling⁸¹.

The genetic sequence of Chast corresponds to the antisense strand of two adjacent genes, *Arhgap27* and *Plekhm1*⁸¹. Chast has been proposed to act as a *cis* regulatory factor for *Plekhm1*⁸¹, but how these two interact to modulate cardiac hypertrophy has not been determined. Chast is highly conserved across mammalian species, and expression of the CHAST homologue in humans was also substantially upregulated in the hypertrophic hearts of patients with aortic stenosis⁸¹. These findings support further research into the possible value of CHAST as a target for therapeutic approaches to treat cardiac hypertrophy, but there is still little independent evidence to support the role of CHAST in cardiac remodelling. Further studies to elucidate the molecular mechanisms underlying the observed function would help to justify follow-up studies to establish the therapeutic potential of this lncRNA for the treatment of cardiac disease.

CHAER

Chaer (cardiac-hypertrophy-associated epigenetic regulator) is another lncRNA that has an important role in cardiac hypertrophy and remodelling^{82–84}. Inhibition of Chaer expression in mouse hearts substantially attenuated pressure overload-induced cardiac hypertrophy and heart dysfunction before the onset of remodelling⁸²; however, inhibition of Chaer was not sufficient to correct the cardiac maladaptive remodelling after the onset of pressure overload^{82,83}. These data support a role for Chaer as an early mediator in the events leading to cardiac hypertrophy and HF. Chaer has been shown to interact directly with PRC2, a key epigenetic regulator that functions to maintain transcriptional repression⁸². This activity is transient and stress-dependent and modulates the transcription and function of hypertrophy-related genes⁸² (Fig. 4). This study provides an important piece of evidence as to the mechanism of action of CHAER, but whether it has additional roles in other modes of gene transcription and/or in post-translational modification and protein translation remains to be determined. Studies on possible clinical applications of CHAER have not been conducted.

MALAT1

MALAT1 (metastasis-associated lung adenocarcinoma transcript 1), also known also NEAT2 (nuclear enriched abundant transcript 2), is a nuclear lncRNA that is widely expressed in multiple tissues, including the heart⁸⁵. Algorithmic analysis indicates that the MALAT1 transcript has low protein-coding potential⁸⁶. MALAT1 was also one of the first lncRNAs to be extensively studied in multiple biological systems because its dysregulation has been associated with several disease conditions⁸⁷. The ~7 kb MALAT1 primary transcript localizes to the nucleus, whereas MALAT1-associated small cytoplasmic RNA (mascRNA) is found exclusively in the cytoplasm⁸⁸. MALAT1 regulates vascular endothelial cell proliferation and angiogenesis^{89,90}, suggesting that this lncRNA might have a role in cardiac remodelling. However, studies using a loss-of-function genetic mouse model indicated that Malat1 had no significant effect on pathological cardiac hypertrophy, although it remains a topic of active research⁹¹.

MIAT

MIAT (myocardial infarction-associated transcript) was originally identified from the mouse nervous system as an mRNA-like non-coding gene called Gomafu⁹². Further analysis revealed that MIAT is a highly conserved intergenic lncRNA; mutations or single-nucleotide polymorphisms of this lncRNA are associated with increased risks of ischaemic heart disease and/or MI^{93,94}. Furthermore, patients with chronic Chagas disease and dilated cardiomyopathy have increased MIAT expression in heart tissue⁹⁵, and patients with type 2 diabetes mellitus and maladaptive cardiac remodelling demonstrate similar increases in serum MIAT levels⁹⁶. Studies in mice show that genetic deletion of *Miat* attenuates pathological hypertrophy and HF induced by pressure overload or angiotensin II infusion⁹⁷. Although the molecular mechanism remains unclear, it was observed that the expression levels of SERCA2a and ryanodine receptor 2 in the hearts of these *Miat*-null mice exposed to pressure overload or angiotensin II were restored to normal levels; restored expression of these, and possibly other, proteins from the reduced levels typically associated with cardiac hypertrophy might contribute to the observed cardiac protection.

A similar finding was reported when small hairpin (shRNA)-mediated inhibition of *Miat* was used to protect mouse hearts against MI-induced cardiac injury⁹⁸. The investigators found that *Miat* directly interacted with translocator protein homologue (TSPO)⁹⁸, which is primarily found on the outer mitochondrial membrane, suggesting that MIAT might act as a pro-apoptotic lncRNA. The researchers propose that during stress, mitochondria are damaged and the mitochondrial death pathway is triggered by MIAT through TSPO. Another independent study, using both gain-of-function and loss-of-function approaches, found that *Miat* overexpression worsens cardiac remodelling and that genetic deletion of *Miat* protects hearts against MI-mediated injury⁹⁹, consistent with previous findings. The researchers suggest that MIAT functions as a sponge for miRNA-150, consistent with the finding that overexpression of miRNA-150 attenuates the detrimental effects of MIAT in response to MI⁹⁹.

In addition to its role in cardiomyocytes and cardiomyopathy, the function of MIAT has been linked to smooth muscle cells and atherosclerosis¹⁰⁰. Serum levels of MIAT were found to

be markedly upregulated in patients with advanced carotid artery atherosclerotic lesions¹⁰¹. In human cultured carotid smooth muscle cells, nuclear MIAT binds to the promoter region of the *KLF4* gene to increase its transcription¹⁰⁰. GapmeR-based MIAT knockdown led to decreased proliferation and migration of human carotid artery smooth muscle cells in culture but also increased apoptosis¹⁰⁰. This finding is in sharp contrast to those in cardiomyocytes, in which MIAT knockdown seems to be beneficial and reduces cardiomyocyte apoptosis in response to stress⁹⁸. These conflicting tissue-specific functions will require consideration if MIAT is further investigated as a therapeutic target for cardiovascular disease.

XIST

XIST (*X*-inactive specific transcript) was one of the first lncRNAs to be linked to X chromosome inactivation via an epigenetic mechanism¹⁰². Other studies have reported that XIST is expressed in the heart and has a role in the regulation of cardiomyocyte hypertrophy¹⁰³. Initial studies described high levels of XIST expression in the hearts of female patients with idiopathic dilated cardiomyopathy and the onset of HF¹⁰³. Although this study did not provide sufficient evidence to establish a firm role for XIST in cardiomyopathy, it does support a role for this lncRNA in the regulation of cardiac function. Multiple reports suggest that XIST might regulate cardiomyocyte hypertrophy by targeting miRNAs^{104,105} (Fig. 3). These studies used only in vitro models and must, therefore, be interpreted with caution, but the observation that the loss of *Xist* in the *mdx* mouse (a model of muscular dystrophy) leads to a delay in the onset of HF supports a role for XIST in the regulation of cardiac function, either directly or indirectly^{106,107}.

HOTAIR

HOTAIR (HOX antisense intergenic RNA) has been well studied in many biological systems and disease states, including cancer¹⁰⁸, but has also been linked to cardiomyocyte function and cardiac hypertrophy^{109–111}. HOTAIR can suppress the expression of the calcium channel, Ca_v1.2 (Fig. 2), whereas knockdown of HOTAIR promotes the expression of Ca_v1.2 in human cardiomyocytes in vitro¹⁰⁹. Other studies have expanded on this work to demonstrate that *Hotair* is downregulated in heart tissue from TAC-operated mice as well as in cultured cardiomyocytes treated with angiotensin II to induce a cardiac hypertrophy phenotype in vitro¹¹⁰. Overexpression of *Hotair* in these systems suppresses the cardiac hypertrophy molecular profile¹¹⁰. A role for HOTAIR in cardiac regeneration has also been suggested by a study examining its expression during mouse neonatal development¹¹¹.

lncRNAs expressed in striated muscle: roles in myogenesis, cardiac function and disease

Cardiac striated muscle and skeletal striated muscle share numerous structural and physiological characteristics, including the sarcomere, the basic contractile unit of the myocyte. In cardiomyocytes, the genes *MYH6* and *MYH7* encode the myosin heavy chain- α (MHC α) and MHC β proteins, respectively. In the adult ventricular tissue of large mammals such as humans, MYH β is the more abundant form, whereas in small mammals such as mice, MYH α is more prevalent¹¹². This difference in the expression patterns of *MYH6* and *MYH7* in large versus small mammals has been well studied and correlates

with the requirement for the higher actin-activated ATPase of MYH α to achieve the higher cardiac contractility rates in small animals. *MYH6* is also predominantly expressed in the atria of both large and small mammals¹¹³. Subsequent studies of the genomic regions in mice revealed that two microRNAs (miRNA-208a and miRNA-208b) are encoded in the introns of *Myh6* and *Myh7*, respectively^{114–116}. These microRNAs are functional and expressed in a cardiac-specific manner. A cluster of lncRNA transcripts have also been reported as antisense transcripts from the *Myh7* locus¹¹⁷. We discuss these below, and others are summarized in Table 3.

MHRT

MHRT (or MYHEART, myosin heavy-chain-associated RNA transcripts) was first identified as a cluster of cardiac-specific lncRNAs that were induced during pathophysiological hypertrophy¹¹⁷. Through a series of experiments conducted both in vitro and in vivo, including the use of mouse models of cardiac hypertrophy, it was determined that repression of *Mhrt* expression under conditions of stress was essential for the development of cardiomyopathy, whereas restoration of *Mhrt* to its pre-stress levels could protect the heart against hypertrophy and failure¹¹⁷. The *Mhrt*–transcription activator BRG1 feedback circuit has been implicated in this process; under pathological stress, the BRG1–HDAC–PARP chromatin-repressing signal is activated, which inhibits *Mhrt* transcription in the heart¹¹⁷. Conversely, *Mhrt* binding to BRG1 prevents BRG1 from recognizing its genomic DNA targets, thereby inhibiting chromatin targeting and gene regulation by BRG1. This finding suggests that certain lncRNAs are embedded in protein-coding genomic loci and can regulate cardiac function and disease in response to stress, thereby participating in feedback and/or feedforward regulatory cascades in the cardiac system^{118,119}.

Mhrt779 is a member of the *Mhrt* lncRNA cluster and an antisense RNA of *Myh7*¹²⁰. Mechanistically, *Mhrt779* seems to participate in epigenetic regulation by directly associating with BRG1. This association might be important to its role in conferring antihypertrophic memory, which refers to a cellular adaptation mechanism in which previous exposure to certain stimuli or stress, such as exercise-induced physiological cardiac hypertrophy, can produce a protective effect on the heart against pathological hypertrophic stressors, such as pressure overload. Given that physiological cardiac hypertrophy produced by exercise hypertrophic preconditioning leads to an increase in the levels of *Mhrt779*, this lncRNA is thought to be a component in the process to protect the heart from maladaptive remodelling in response to pathological cardiac stress resulting from pressure overload¹²⁰. These data, which reveal the importance of elevated levels of *Mhrt779*, partially explains why healthy exercise is good for the heart. However, the finding also raises new questions about whether less-intense exercise can also induce *Mhrt779* expression and, therefore, protect the heart. What other factors, in addition to *Mhrt779*, contribute to cardiac protection remains to be determined^{121,122}.

lncMYH7b (lnc myosin heavy chain 7b) is produced from the *MYH7b* locus, which encodes a unique member of the myosin heavy chain family that is expressed in striated muscles but does not seem to produce a full-length protein¹²³. Instead, a post-transcriptional exon-skipping mechanism occurs in mammalian cardiac muscle to produce an abridged

transcript¹²³. This locus also produces an intronic microRNA, miRNA-499, which has a role in regulating muscle fibre identity by activating slow myofibre and repressing fast myofibre gene programmes^{115,124}. This function is redundant with that of another miRNA, miRNA-208b, which originates from the *MYH7* locus^{115,124}. Previous studies suggested that the muscle fibre type-specific function was modulated by miRNA-499 through the regulation of mitochondrial oxidative metabolism¹²⁵. However, a recent study revealed a new miRNA-499-independent mechanism for the non-coding exon-skipped RNA from the *MYH7b* locus (lncMYH7b). Mechanistically, lncMYH7b has been suggested to control the activity of a member of the TEA domain transcription factor family (TEAD3), thereby regulating the ratio of MHC β to MHC α in cardiomyocytes and, in turn, cardiomyocyte function¹²⁶. Further studies are needed to establish the functional correlation between the protein-coding, miRNA and lncRNA components of this genomic locus. Furthermore, there might be additional, uncharacterized non-coding components in other contractile protein loci that have traditionally been thought to encode only protein-coding transcripts.

CHARME

CHARME (chromatin architect of muscle expression) was originally designated as lnc-31 in mouse because it was derived from the same genomic locus as miRNA-31^{127,128}. CHARME has been characterized as a chromatin-associated muscle-specific RNA that is expressed in striated muscles¹²⁹. CHARME is conserved between humans and mice, and its expression increases during myogenic differentiation¹²⁹. CHARME transcripts were detected in the nucleus of myocytes, and its inhibition has been shown to reduce the expression of genes involved in myogenic differentiation and the myogenic programme¹²⁹. Genetic deletion of Charme results in a reduction in skeletal muscle morphogenesis, as well as a cardiac remodelling phenotype characterized by changes in cardiomyocyte size and structure; these latter changes influence the sculpting of the heart¹²⁹. Charme is targeted to specific chromosomal sites and is necessary for the disassembly of chromosomal domains and the expression of myogenic genes in skeletal muscle myocytes¹²⁹. Additionally, Charme has been shown to interact physically with several proteins involved in splicing regulation, including Ptb1 and Matr3, to modulate cardiac gene expression and function¹³⁰. Interestingly, several genes regulated by CHARME are associated with human cardiomyopathies¹³⁰, suggesting an important and evolutionarily conserved role in muscle function and disease.

TRDN-AS

TRDN-AS, as discussed in the previous section, is transcribed as an antisense transcript from the locus encoding TRDN^{44,45}. In addition to its function in cardiac tissue discussed above, TRDN-AS was originally discovered to directly regulate the balance between the alternative spliced forms of the triadin gene in cardiac and skeletal muscle⁴⁵. This lncRNA could, therefore, have important roles and potential therapeutic value for the treatment of diseases affecting both cardiac and skeletal muscle.

lncRNAs expressed in non-cardiomyocyte cells that influence cardiac hypertrophy, remodelling and disease

In addition to roles for cardiomyocyte-expressed lncRNAs in the regulation of cardiac hypertrophy and HF, important insights have been made into the functions of lncRNAs expressed by non-cardiomyocyte cells in the remodelling of the heart in response to stress. In particular, lncRNAs that regulate the expression of genes associated with fibrosis during cardiac remodelling have been found to be crucial to cardiac function during disease progression^{131,132}. Given the severe detrimental effects of cardiac fibrosis on cardiac function, the importance of understanding the mechanisms by which lncRNAs influence this process is paramount.

WISPER

WISPER (WISP2 super-enhancer-associated RNA) is a conserved lncRNA that is enriched in cardiac fibroblasts, and study of WISPER has provided important insights into the important roles of lncRNAs in other cardiac cell types. Examination of Wisper expression under stress conditions using a mouse model of renovascular hypertension¹³³ confirmed that it was elevated in response to volume overload-induced cardiac hypertrophy and fibrosis¹³⁴. In addition, Wisper silencing reduced the expression of cardiac stress markers¹³⁴, suggesting a possible approach to reverse the cardiac hypertrophy. Knockdown of Wisper significantly reduces fibroblast proliferation and migration and the expression of cardiac fibroblast gene programmes that are crucial for cell identity, extracellular matrix deposition, proliferation and survival¹³⁴. Furthermore, Wisper inhibition protected the mouse heart against MI-induced fibrosis and cardiac dysfunction¹³⁴, suggesting that it might be a therapeutic target for treating heart disease.

MEG3

MEG3 (maternally expressed gene 3) is a genomically imprinted gene that is transcribed in cardiac fibroblasts and has an important role in regulating cardiac remodelling in response to stress^{135,136}. Using gain-of-function and loss-of-function assays both in vitro and in vivo, investigators demonstrated that Meg3 inhibition reduces cardiac hypertrophy and fibrosis in the heart in response to TAC-induced pressure overload¹³⁶. The mechanism of Meg3 has been studied in the brain as well because of its role in motor neuron development and autophagy after cerebral ischaemia–reperfusion injury^{137,138}. Nuclear MEG3 interacts with the transcription factor p53 through conserved pseudoknot structures (or kissing loops) that facilitate its regulation of p53¹³⁹. This change in p53 activity influences the expression of a variety of genes involved in cardiac remodelling and fibrosis, including *MMP2*¹³⁶.

PFL

Pfl (pro-fibrotic lncRNA) is upregulated in mouse hearts in response to MI¹⁴⁰. Further study revealed that Pfl was enriched in cardiac fibroblasts, and its expression increased during cardiac remodelling involving fibrosis¹⁴⁰. Adenovirus-mediated shRNA knockdown of Pfl attenuated cardiac interstitial fibrosis and improved cardiac function in response to MI induced in mice by coronary artery ligation¹⁴⁰. Overexpression of Pfl in vitro

promotes fibroblast proliferation and the fibroblast–myofibroblast transition in mouse cardiac fibroblasts¹⁴⁰. Conversely, inhibition of Pfl diminishes transforming growth factor- β 1-induced myofibroblast generation and fibrogenesis¹⁴⁰. Mechanistically, Pfl was shown to act as a competitive endogenous RNA of let-7d; forced expression of Pfl reduced let-7d expression and activity^{140,141}.

Micropeptides expressed in the heart that regulate cardiac function

lncRNAs are typically defined as RNA transcripts >200 nucleotides in size without protein-coding potential; however, this definition has proven to be inaccurate given that some annotated lncRNAs actually encode micropeptides. As their name suggests, micropeptides typically encode translational products of ~100 amino acids and are derived from short open reading frames (sORFs) from larger transcripts¹⁴². Evidence for the existence of micropeptides in mammalian cells is ever increasing, but their functions remain largely unknown. However, several micropeptides have been identified in striated muscles (cardiac and skeletal muscle) with roles in the regulation of development and function of those tissues^{19,143}. Myomixer, a micropeptide that is enriched in developing and regenerating skeletal muscle, was reported to control the crucial step in myofibre formation during muscle development¹⁴⁴. Another muscle-enriched micropeptide, DWORF, was found to be located in the sarcoplasmic reticulum of striated muscles and could activate SERCA to prevent pathological remodelling and Ca²⁺ dysregulation in a mouse model of HF^{76,145}.

Investigators have attempted to systematically identify micropeptide-coding genes in hypertrophic cardiomyocytes¹⁹. Overall, >10,000 open reading frames were detected from the deep sequencing of ribosome-protected fragments¹⁹. The investigators identified >100 uncharacterized sORFs in genes that were originally annotated as encoding lncRNAs but were found to encode micropeptides¹⁹. Among 15 candidates that were experimentally tested, the investigators verified the coding potential of 11 sORFs. They demonstrated that these micropeptides participate in the regulation of cardiomyocyte hypertrophy by divergent mechanisms, such as modulation of oxidative phosphorylation, the calcium signalling pathway and the mitogen-activated protein kinase pathway¹⁹. These microproteins are located in various cell compartments; intriguingly, many are found localized to the mitochondria, possibly related to roles in responding to stress²⁰. Further studies might reveal previously uncharacterized micropeptides that have important roles in cardiomyocyte biology and heart disease.

Clinical implications

The COVID-19 pandemic marked the emergence of RNA-based technologies as a powerful set of tools for the development of new medicines. Although the application was a more traditional use of RNA in its protein-coding capacity, it proved to be a rapid and successful approach for vaccine generation. The interest in RNA-based methodologies that it created will continue to spawn new strategies for the prevention and treatment of disease. The value of lncRNAs as clinical reagents has only just begun to be investigated but their clinical utility is already becoming apparent. One study identified a panel of 2,906 lncRNAs that were either cardiac-enriched or differentially expressed between failing and non-failing

hearts, and researchers could differentiate between patients with myocarditis and those with acute MI based on the levels of lncRNAs in blood samples¹⁴⁶. Other preliminary studies on lncRNA function also report the potential utility of these RNAs as either biomarkers or potential targets for therapeutic study. One study examining the value of lncRNAs as biomarkers for myocardial injury suggested that measuring the circulating levels of these molecules for the detection of cardiac disease or damage could be valuable¹⁴⁷; however, the field is too immature to make any clear predictions about their usefulness. Studies characterizing lncRNAs for use as biomarkers of cardiac hypertrophy and disease are summarized in Table 4.

Future studies will continue to characterize novel lncRNAs and the molecular mechanisms they use to influence gene and protein expression and function. The definition of these mechanisms alone might lead to the development of new therapeutic strategies, as well as identifying new potential molecular targets. Approaches to exploit lncRNAs for clinical use will also need to be developed, although some tools are already available. The use of AAVs has been labelled as a disruptive innovation in the pharmaceutical industry for gene delivery. In addition to their traditional use for the expression of mRNAs to restore or augment protein function, they could be adopted for use in expression of ncRNAs, such as the lncRNAs described in this Review, if the efficacy of lncRNAs to improve cardiac function is validated in clinical studies. There are already several pharmaceutical companies heavily invested in the use of antisense oligonucleotides for therapeutic strategies, and this approach could similarly be used to reduce the level of a lncRNA (a GapmeR approach) if its expression is detrimental to heart function.

One approach that warrants a much more substantial discussion than is possible here is use of the CRISPR/Cas9 genome editing system to target lncRNAs in patients with cardiovascular disease¹⁴⁸. In addition to the use of CRISPR/Cas9 genome editing to delete part or all of a locus, more sophisticated CRISPR/Cas9 techniques are available and are being refined that would allow more precise targeting and intervention. Using such an approach to target a promoter region could allow the expression of a lncRNA to be fine-tuned to either increase or decrease its activity or possibly even alter its function. Whereas the use of a reagent such as a GapmeR would be transient, a CRISPR/Cas9 intervention would be longer lasting (although this durability might not be desirable under all circumstances). In addition, the potential off-target DNA changes associated with the use of CRISPR/Cas9 also have to be considered¹⁴⁹. Gene editing is also not trivial when targeting non-coding genes because they lack an open reading frame¹⁴⁸.

The results of the studies we have described examining the effects of increasing or decreasing the levels of lncRNAs in animal models are very encouraging. However, one caveat of this body of work is that the poorly conserved nature of ncRNAs across species means that the interpretation of animal studies in the context of human disease must be carefully considered. The gap between these preclinical studies and use in humans is large and will require questions about the specificity and sensitivity of lncRNAs and standard criteria required for any small-molecule therapy to be thoroughly addressed. As with most drugs, the ability to target a specific organ or tissue is often the greatest obstacle; therefore,

lncRNAs that are enriched in cardiac tissue might prove the most amenable to clinical applications.

Conclusions

Many early studies suggested that lncRNAs might be biologically unimportant because they did not have profound effects on heart development. However, subsequent studies suggested that lncRNAs have important effects on heart function during cardiac stress. This realization is a reason for optimism for the clinical potential of lncRNAs, as they could emerge as major regulators during cell stress responses and in pathophysiological heart conditions.

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Key points

- Long non-coding RNAs (lncRNAs) are a class of non-coding RNAs (ncRNAs) that can regulate gene expression at multiple levels, including transcription, RNA splicing, and protein translation.
- There are a variety of lncRNA classes, based on their structures and/or mechanisms of action but their study has been limited by their low levels of expression and relatively poor conservation between species.
- LncRNAs are critical regulators of cell differentiation, development, and disease; in the heart, their expression is often associated with stress conditions and they participate in the pathophysiological remodelling associated with cardiac hypertrophy and heart failure.
- Studies have shown that dysregulation of lncRNAs is associated with a variety of cardiac diseases, including coronary artery disease (CAD), myocardial infarction (MI), heart failure (HF), and arrhythmias.
- Indications from studies in animal models and humans reveal that lncRNAs have great potential for disease diagnosis as biomarkers and as novel targets for the treatment of cardiac disease.

Box 1 |**Definition and mouse models of cardiac hypertrophy**

Cardiac hypertrophy is an adaptive response by the heart to changes in pressure or volume. This process can be beneficial, such as the hypertrophy associated with exercise observed in athletes. However, multiple disease processes, including genetic mutations in sarcomeric (or other) proteins or loss of cardiac muscle mass as a result of previous damage, can induce pathological hypertrophy. This type of hypertrophy is observed in patients with ischaemic heart disease, hypertension and/or valvular defects. Given that pathological cardiac hypertrophy impairs heart function, an inability to reverse it results in continuing deterioration, that can lead to heart failure and death.

Mice are the most widely used animal models for in vivo studies of roles for long non-coding RNAs (and other molecules) on heart function. One of the most widely used surgical models in mouse is the transverse aortic constriction technique to study pressure overload-induced cardiac hypertrophy and heart failure²⁰⁰. During the early stages, transverse aortic constriction surgery often leads to a compensatory hypertrophy associated with a transient increase in cardiac contractility. However, the sustained haemodynamic overload eventually results in a maladaptive response, leading to cardiac dilatation and heart failure. A mouse model of myocardial infarction is also commonly used and involves the surgical ligation of the left anterior descending coronary artery²⁰¹. The artery ligation can be either permanent or transient (a model of ischaemia–reperfusion injury). As would be expected, permanent ligation results in substantially more damage to the heart tissue, leading to an elevation in apoptosis and scarring. Transient ligation results in less initial damage and is associated with a smaller amount of scarring but is accompanied by necrotic damage associated with reperfusion after removal of the occlusion. Both models are relevant to the pathological processes observed in patients with cardiac disease.

Box 2 |**lncRNAs associated with cardiac hypertrophy**

- Ahit (antihypertrophic interrelated transcript)¹⁶³
- ANRIL (antisense non-coding RNA in the *INK4* locus)¹⁵⁰
- APF (autophagy promoting factor)¹⁷⁴
- AZIN2-sv (antizyme inhibitor 2-splice variant)^{172,173}
- CAIF (cardiac autophagy inhibitory factor)¹⁷⁵
- Caren (cardiomyocyte-enriched non-coding transcript)⁴⁹
- Carmen or Carmn (cardiac mesoderm enhancer-associated non-coding RNA)¹⁷⁶
- CCRR (cardiac conduction regulatory RNA) (also known as AK045950)⁴⁸
- Chaer (cardiac-hypertrophy-associated epigenetic regulator)⁸²
- CHAIR (cardiomyocyte hypertrophic associated inhibitory RNA) (also known as 4632428C04Rik)¹⁵¹
- CHAR (cardiac hypertrophy-associated regulator)¹⁶⁴
- Chast (cardiac hypertrophy-associated transcript)⁸¹
- CHRf (cardiac hypertrophy related factor)^{73,152}
- CPhar (cardiac physiological hypertrophy-associated regulator)^{165,202}
- CRRL (cardiomyocyte regeneration-related lncRNA)¹⁷⁷
- ECRAR (endogenous cardiac regeneration-associated regulator)¹⁷⁸
- Fendrr (FOXF1 adjacent non-coding developmental regulatory RNA)¹⁵³
- GAS5 (growth arrest-specific 5)¹⁶⁶
- Gm15834¹⁵⁴
- H19^{68,71,72,167}
- Hyperlnc (hypoxia-induced endoplasmic reticulum stress regulating long non-coding RNAs)^{155,156}
- lncCytB (lncRNA CytB)¹⁶⁸
- lncDACH1 (long non-coding RNA dachshund homologue 1)¹⁵⁷
- lncExACT1 (long non-coding exercise associated transcript 1)^{158,159}
- lncMYH7b (long non-coding myosin heavy chain 7b)¹²⁶
- Meg3 (maternally expressed gene 3)^{135,136,160,179}

- Mhrt or Myheart (myosin heavy-chain-associated RNA transcripts)^{117–119,169,203}
- Mhrt779^{120–122}
- MIAT (myocardial infarction-associated transcript)^{97,99}
- NRON (non-coding repressor of nuclear factor of activated T cells)¹⁶¹
- OIP5-as1 (Opa-interacting protein 5-antisense 1) (also known as 1700020I14Rik or Cyrano)¹⁷⁰
- Sirt1-as (silent information regulator 1-antisense)¹⁶²
- Trdn-as (triadin-as)⁴⁵
- Wisper (WISP2 super-enhancer-associated RNA)¹³⁴
- ZNF593-as (zinc finger protein 593-antisense) (also known as RP11-96L14.7 or ENST00000448923.2)¹⁷¹

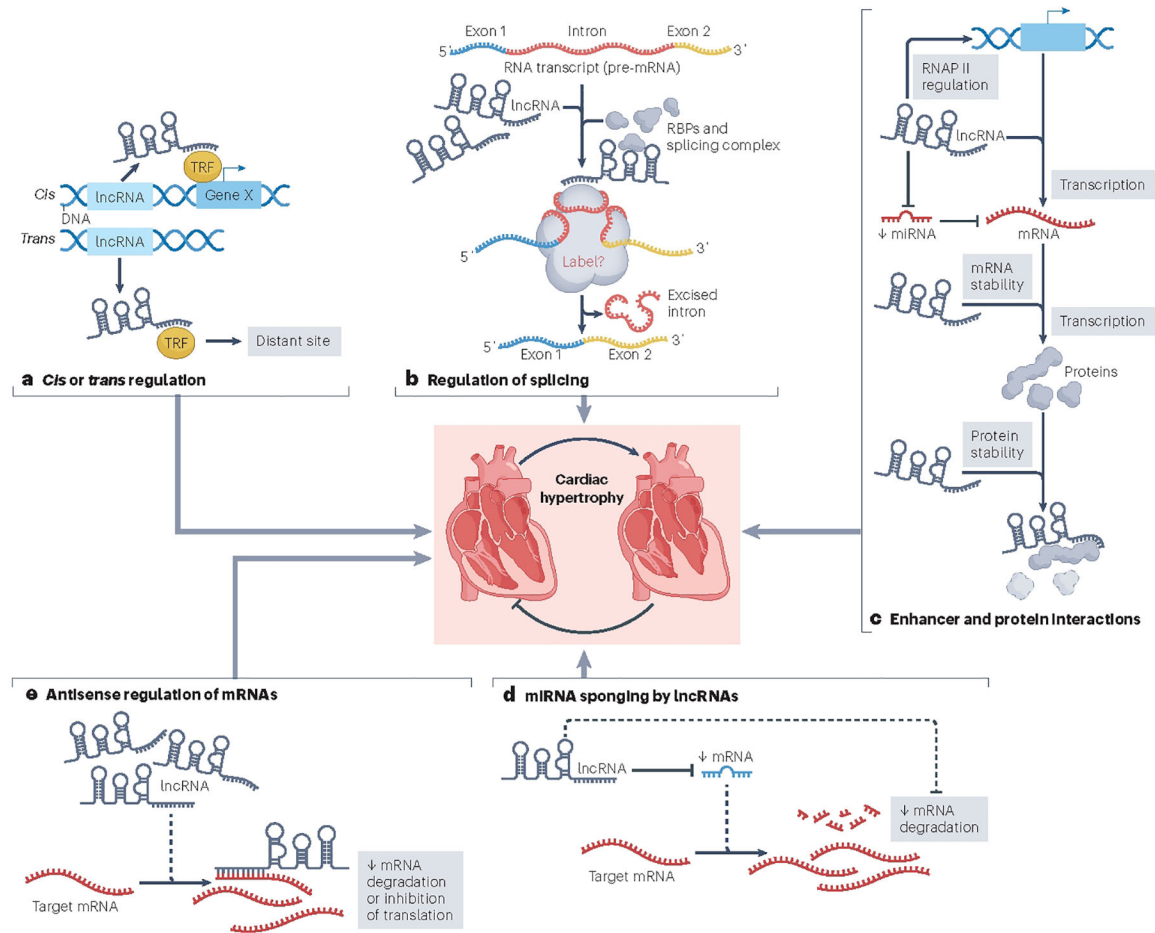


Fig. 1 | lncRNA mechanisms of action.

The figure shows mechanism by which long non-coding RNAs (lncRNAs) have been shown to exert their function in the heart and lead to cardiac hypertrophy. **a**, *Cis* or *trans* regulation. lncRNAs can interact with transcription factors (TFs) to facilitate their activity and promote transcription in *cis* of a nearby gene (gene X). This lncRNA–TF interaction can also facilitate transcription in *trans* of a gene at a distant site (gene Y). Alternatively, lncRNA–TF interactions can limit the activity of the TF or the lncRNA could participate in the stabilization of a transcriptional repressor complex, thereby reducing transcription. **b**, Regulation of alternative splicing. lncRNAs can interact with RNA-binding proteins (RBPs) to facilitate the formation of alternative splice variants. **c**, Protein interactions. lncRNAs can have a direct influence on the stabilization of the RNA polymerase II (Pol II) complex to promote transcription. Some lncRNAs can also stabilize proteins to facilitate or prolong their activity. **d**, microRNA (miRNA) sponging by lncRNAs. lncRNAs are also able to interact with and limit the activity of miRNAs. In one mechanism, lncRNAs directly sequester and prevent miRNAs from degrading target mRNAs. In a second mechanism, lncRNAs interact with and suppress the ability of miRNAs to inhibit translation. **e**, Antisense regulation of mRNAs. lncRNAs can influence the stability of an mRNA through direct interaction and by preventing its degradation. Other mechanisms might exist but remain to be validated in the heart.

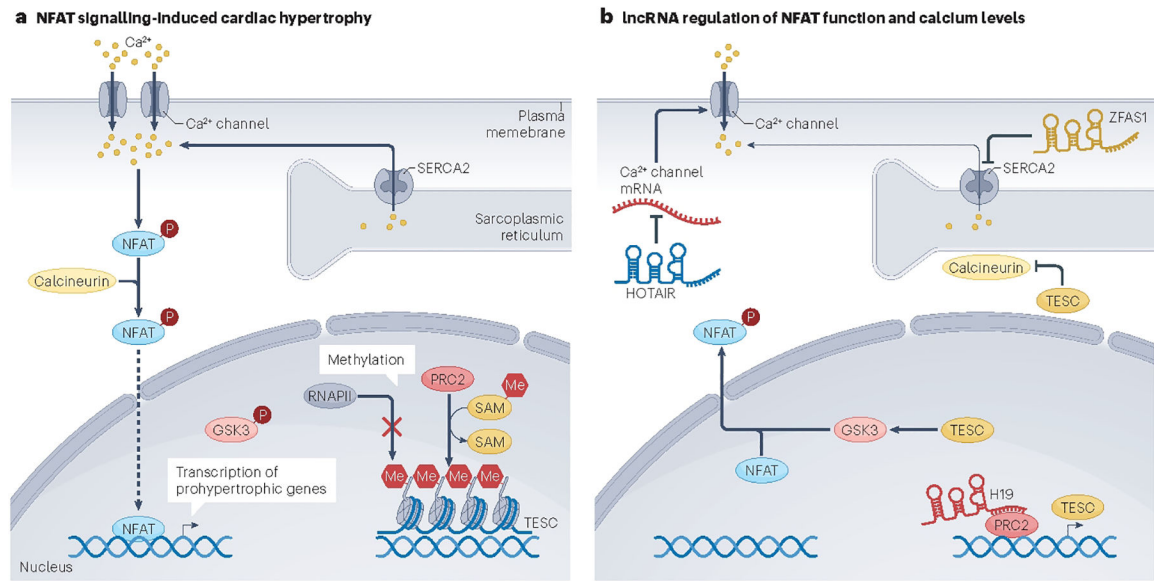


Fig. 2 | lncRNAs regulation of cardiac hypertrophy induced by calcium and NFAT.

a, Calcium is a key mediator of cardiac hypertrophy. Calcium enters the cell via calcium channels and is sequestered into the sarcoplasmic reticulum via the sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (SERCA2). The long non-coding RNA (lncRNA) ZFAS1 (ZNF1 antisense RNA 1) binds to and limits the activity of SERCA2 protein, resulting in elevated intracellular calcium levels. In the presence of calcium, calcineurin dephosphorylates nuclear factor of activated T cells (NFAT), which translocates to the nucleus and activates the transcription of prohypertrophic genes, including the lncRNA ZFAS1. **b**, The lncRNA HOTAIR (HOX antisense intergenic RNA) is proposed to alter calcium homeostasis by decreasing Ca^{2+} channel activity. Another lncRNA, H19, binds to the protein polycomb repressive complex 2 (PRC2) and thereby promotes the transcription of the *TESC* gene encoding tescalcin (TESC). The TESC protein inhibits the phosphorylation of glycogen synthase kinase 3 (GSK3), thereby enabling GSK3 to phosphorylate and prevent the DNA-binding function of NFAT. Similarly, TESC suppresses the phosphatase activity of calcineurin A¹⁹⁹. This pathway results in suppression of the hypertrophic gene response initiated by NFAT activation. Me, methyl group; P, phosphate group; Pol II, RNA polymerase II; SAM, *S*-adenosyl methionine.

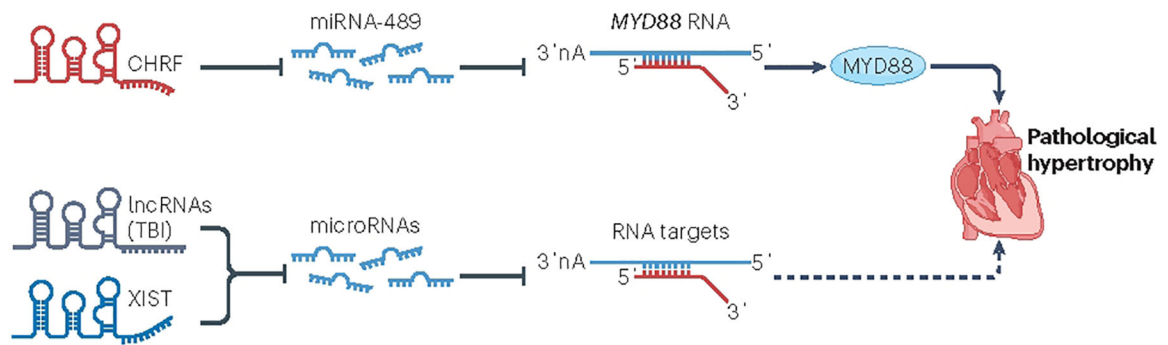


Fig. 3 |. lncRNA regulation of miRNA-dependent cardiac hypertrophy.

Long non-coding RNAs (lncRNAs), such as CHRF (cardiac hypertrophy related factor) and XIST (X-inactive specific transcript), sequester microRNAs (miRNAs) and prevent their binding to target mRNAs. Myeloid differentiation primary response 88 (MYD88) has been shown to promote inflammatory signalling and lead to cardiac hypertrophy after myocardial infarction. miR-489 suppresses MYD88 activity. Given that CHRF acts as an endogenous sponge of miR-489 and downregulates miR-489 expression levels, CHRF releases MYD88 from the inhibitory influence of this miRNA, enabling the downstream cardiac hypertrophic response. Similarly, the Toll-like receptor 2 (TLR2) is essential for activating the IGF1–PI3K–AKT pathway and promoting cardiac hypertrophy. miR-101 directly targets TLR2 to repress its activity. By suppressing the action of miR-101, the lncRNA XIST activates the TLR2-dependent cardiac hypertrophic response.

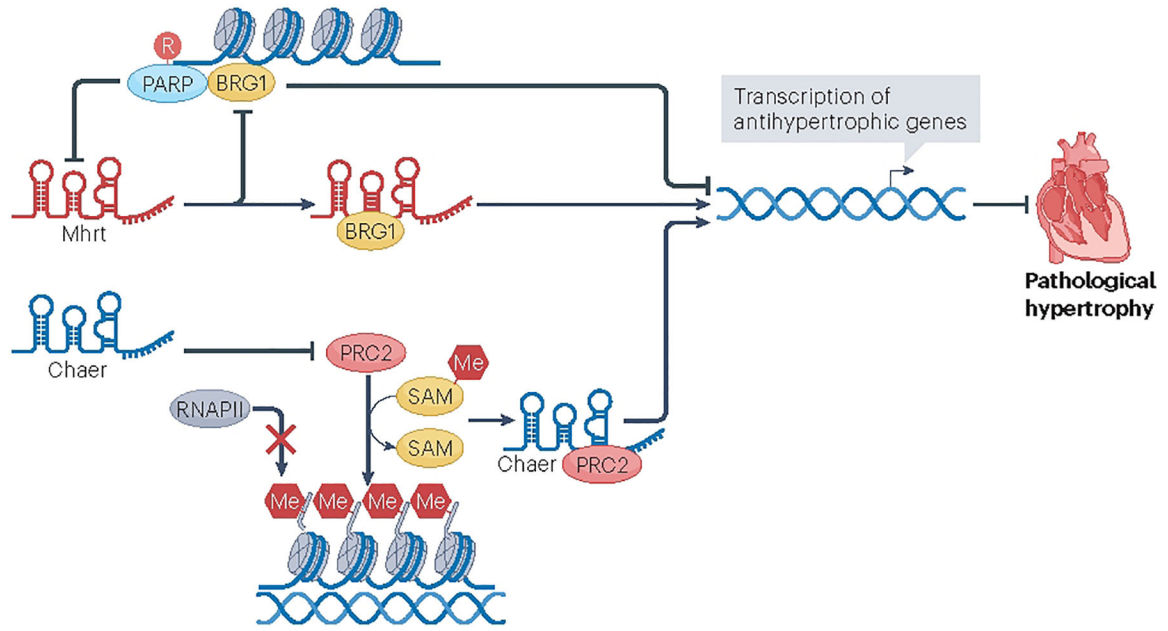


Fig. 4 |. lncRNA regulation of chromatin-mediated cardiac hypertrophy.

Long non-coding RNAs (lncRNAs) such as Mhrt (myosin heavy-chain-associated RNA transcripts) and Chaer (cardiac-hypertrophy-associated epigenetic regulator) have been shown to regulate the hypertrophic response through their interaction with chromatin-regulatory complexes. Mhrt binds the transcription activator BRG1 and prevents it from recognizing its genomic DNA targets. This releases the chromatin from its inhibitory conformation and facilitates the transcription of an antihypertrophic gene profile. Similarly, Chaer directly binds to polycomb repressive complex 2 (PRC2), releasing chromatin from a repressed state; however, this lncRNA enables the hypertrophic response, suggesting the activation of a hypertrophic gene profile. Me, methyl group; PARP, poly (ADP-ribose) polymerase; Pol II, RNA polymerase II; R, ribosyl group; SAM, *S*-adenosyl methionine.

Table 1 |

lncRNAs that induce cardiac hypertrophy and/or heart failure

lncRNA	Models	Functions	Interactions and effects	Ref.
ANRIL (antisense non-coding RNA in the <i>INK4</i> locus)	Rat (diabetic model)	Downregulated ANRIL improved cardiac function index and decreased expression of inflammatory factors, resulting in decreased myocardial collagen deposition area and cardiomyocyte apoptosis and reduced levels of oxidative stress in myocardial tissue	Not determined	150
Chaer (cardiac hypertrophy-associated epigenetic regulator)	Mouse	Associated with the development of cardiac hypertrophy	Interacts with the catalytic subunit of PRC2	82
CHAIR (cardiomyocyte hypertrophic associated inhibitory RNA; 4632428C04Rik)	Human and mouse	Loss of CHAIR has no effect on normal hearts; however, in response to stress, it accelerates heart functional decline, increases hypertrophy and exacerbates heart failure	Interacts with DNMT3A to inhibit its DNA-binding activity	151
Chast (cardiac hypertrophy-associated transcript)	Human and mouse	Virus-based overexpression of Chast is sufficient to induce cardiomyocyte hypertrophy in vitro and in vivo; GapmeR-mediated silencing of Chast both prevented and attenuated TAC-induced pathological cardiac remodelling, with no toxicological signs or adverse effects	Negatively regulates PLEKHM1 (located on the opposite strand to Chast), which impedes cardiomyocyte autophagy and drives hypertrophy	81
CHRF (cardiac hypertrophy related factor) ^{73,152}	Mouse and in vitro model	Small interfering RNA-mediated knockdown of CHRF attenuates ANF and MHC β levels in the heart	Binds to miRNA-489 to reduce its levels (microRNA sponge)	73
Fendrr (FOXF1 adjacent non-coding developmental regulatory RNA)	Mouse and in vitro model	Fendrr loss of function reduces cardiac fibrosis induced by TAC	Binds to miRNA-106b	153
Gm15834	Mouse (TAC and angiotensin II infusion models)	Forced expression of Gm15834 increases cardiomyocyte autophagy and promotes myocardial hypertrophy; silencing of Gm15834 attenuates autophagy-induced myocardial hypertrophy	Binds to miRNA-30b-3p to function as an endogenous sponge	154
HypERlnc (hypoxia-induced endoplasmic reticulum stress regulating long non-coding RNAs) ^{155,156}	In vitro	Silencing of HypERlnc decreases cell viability and proliferation and results in pericyte dedifferentiation; associated with increased endothelial permeability in co-cultures consisting of human primary pericyte and human coronary microvascular endothelial cells	Endoplasmic reticulum stress-related transcription factors were prominently activated by HypERlnc knockdown	155
LncDACH1 (long non-coding RNA-Dachshund homologue 1; DACH1)	Mouse	Transgenic overexpression of LncDACH1 in cardiomyocytes leads to impaired cardiac function, reduced calcium transient and cell shortening, and decreased SERCA2a protein expression; by contrast, conditional knockout of LncDACH1 in TAC-treated mouse cardiomyocytes results in increased calcium transients, cell shortening and SERCA2a protein expression and improved cardiac function	Binds to SERCA2a	157
lncExACT1 (long non-coding exercise associated transcript 1)	Mouse (exercise model)	lncExACT1 inhibition induced physiological hypertrophy and cardiomyogenesis	Interacts with DCHS2	158,159
MIAT (myocardial infarction-associated transcript)	Mouse (myocardial infarction model)	Inhibition of MIAT protects the heart against myocardial infarction	Interacts with miRNA-150 and HOXA4	99
	Mouse (TAC and angiotensin II infusion models)	Genetic ablation of MIAT attenuates pathological hypertrophy and heart failure	Not available	97

lncRNA	Models	Functions	Interactions and effects	Ref.
Meg3 (maternally expressed gene 3) ^{135,136}	Human (induced pluripotent stem cells) and mouse	Inhibition of Meg3 in vivo after TAC prevented cardiac MMP2 induction, leading to decreased cardiac fibrosis and improved diastolic performance; mostly expressed by cardiac fibroblasts; undergoes transcriptional downregulation during late cardiac remodelling	Interacts with p53 to increase its binding and activity, inducing expression of profibrotic <i>MMP2</i> gene	136
	In vitro	Silenced Meg3 inhibited cardiomyocyte hypertrophy and reversed other hypertrophic responses	Might regulate miRNA-361–5p and HDAC9 by acting as a competing endogenous RNA; upregulated by the transcription factor STAT3	160
NRON (non-coding repressor of nuclear factor of activated T cells)	Mouse (TAC model)	In a gain-of-function mouse model, hypertrophic cardiomyopathy is worsened; by contrast, loss of function attenuates symptoms	Influences the transcription programme for hypertrophic cardiomyopathy; nuclear localization	161
Sirt1-as (silent information regulator 1-antisense)	Mouse	Overexpression of Sirt1-as increases cardiomyocyte proliferation, attenuates cardiomyocyte apoptosis, improves cardiac function and decreases mortality after myocardial infarction	Binds to the <i>Sirt1</i> 3'-UTR to increase the stability of <i>Sirt1</i> mRNA and increase abundance at both the mRNA and protein levels	162

Multiple long non-coding RNAs (lncRNAs) have been described with roles in heart development and cardiac cell growth. lncRNAs with defined roles in cardiac hypertrophy and/or heart failure are described here, but this list is rapidly expanding. Some lncRNAs are presented on two rows due to substantial differences in the experimental research design and/or conclusions. miRNA, microRNA; TAC, transverse aortic constriction; UTR, untranslated region.

Table 2 |

lncRNAs that suppress cardiac hypertrophy and ameliorate cardiac function

lncRNA	Models	Functions	Interactions and effects	Ref.
Ahit (antihypertrophic interrelated transcript)	Mouse and in vitro (rat cardiomyocytes)	Inhibition of Ahit induces cardiac hypertrophy, both in vitro and in vivo	Interacts with SUZ12	163
Caren (cardiomyocyte-enriched non-coding transcript)	Mouse	Cardioprotective effects by regulating the translation of a distant gene and maintaining cardiomyocyte homeostasis	Interacts with HINT1	49
CHAR (cardiac hypertrophy-associated regulator)	Mouse and in vitro (cardiomyocytes)	CHAR downregulation is sufficient to induce hypertrophic phenotypes in healthy mice; overexpression of CHAR reduced the hypertrophic response	Interacts with miRNA-20b (downregulation)	164
CPhar (cardiac physiological hypertrophy-associated regulator)	Mouse (exercise model)	Overexpression of CPhar prevents myocardial ischaemia-reperfusion injury and cardiac dysfunction in vivo	CPhar works with DDX17 as a binding partner to sequester C/EBP β , leading to decreased levels of ATF7	165
GAS5 (growth arrest-specific 5)	Mouse (<i>Srsf4</i> knock-out)	Represses cardiac hypertrophy in <i>Srsf4</i> knock-out mice	GAS5 is a repressor of the glucocorticoid receptor	166
H19	Human and mouse	H19 inhibits E3 ligase-dependent polyubiquitination at Lys3584 of dystrophin (referred to as Ub-DMD), impeding dystrophin protein degradation	Interacts with dystrophin	71,72
	In vitro (cardiomyocytes)	H19-miRNR-675 axis acts as a negative regulator of cardiac hypertrophy by targeting CaMKII δ	Inhibition of miRNA-675 reversed the increase in cardiomyocyte size associated with H19 overexpression; implicates miRNA-675 derived from H19 in cardiac hypertrophy	68,167
lncctyb (lncRNA cytb)	Mouse	Cytosolic lncRNA; function consistent with microRNA sponge	Interacts with miRNA-103-3p	168
lncMYH7b	In vitro (induced pluripotent stem cell-derived cardiomyocytes)	lncMYH7b regulates the ratio of MHC β to MHC α , thereby influencing cardiac rhythm	Interacts with TEAD3	126
Mhrt or Myheart (myosin heavy-chain-associated RNA transcripts)	Mouse	Highly expressed in adult heart; stress represses Mhrt expression; overexpression of Mhrt protects the heart against cardiac hypertrophy and heart failure in response to stress	Mhrt antagonizes the function of the chromatin remodelling enzyme BRG1	117
Mhrt (lncRNA-Mhrt)	Mouse and in vitro (cardiomyocytes)	lncRNA-Mhrt inhibits cardiac hypertrophy by inhibiting myocardin	Interacts with myocardin through miRNA-145a-5p	169
Mhrt779	Mouse (exercise and TAC models)	Mhrt779 increases antihypertrophic effects associated with other interventions	Interacts with BRG1-HDAC2	120-122
OIP5-AS1 (Opa interacting protein 5-antisense RNA 1; 1700020I14Rik; Cyran)	Mouse (knock-out model)	Heart failure associated with TAC-induced pressure overload is exacerbated in female (but not male) knock-out mice	Interacts with genes involved with mitochondrial function	170
Trdn-as (triadin-antisense)	Mouse and in vitro	Role in cardiac conduction through splicing of target genes	Interacts with triadin	45
ZNF593-as (RP11-96L14.7; ENST00000448923.2)	Mouse	Localized in the cytoplasm of cardiomyocytes; levels are decreased in the failing hearts of patients with dilated cardiomyopathy; improves TAC-induced cardiac dysfunction	ZNF593-as acts as a guide or scaffold for HNRNPC with <i>RYR2</i> mRNA, thereby stabilizing <i>RYR2</i> mRNA	171

lncRNA, long non-coding RNA; miRNA, microRNA; TAC, transverse aortic constriction.

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Table 3 |

lncRNAs involved in cardiomyocyte proliferation, cardiac regeneration or cardiac conduction

lncRNA	Models	Functions	Interactions and effects	Ref.
AZIN2-sv (AZIN2-splice variant) ^{172,173}	Rat	AZIN2-sv suppresses endogenous cardiac regeneration by targeting the PTEN-AKT pathway; knock-down of AZIN2-sv attenuates ventricular remodelling and improves cardiac function after myocardial infarction	Stabilizes PTEN through binding; acts as a sponge of miRNA-214 to release PTEN	172
APF (autophagy promoting factor)	Mouse	Inhibition of APF reduces ischaemia-reperfusion injury after myocardial infarction in vivo	Binds to and regulates miRNA-188-3p	174
CAIF (cardiac autophagy inhibitory factor)	In vitro (cardiomyocytes)	CAIF suppresses cardiac autophagy and attenuates damage from myocardial infarction	Binds to p53 protein and blocks p53-mediated myocardial transcription	175
CARMEN (cardiac mesoderm enhancer-associated non-coding RNA)	Human and mouse	Expression of CARMEN is activated during pathological remodelling in mouse and human hearts; necessary for maintaining cardiac identity in differentiated cardiomyocytes; knock-down inhibits specification and differentiation in cardiac precursor cells	Interacts with SUZ12 and EZH2	176
CCRR (cardiac conduction regulatory RNA; AK045950)	Human and mouse	CCRR is downregulated in humans with heart failure and a mouse model of heart failure; inhibition of CCRR induces arrhythmias in healthy mice (eliminated by CCRR overexpression); heart failure or CCRR knock-down damages intercalated discs and gap junctions to slow longitudinal cardiac conduction	Binds to CIP85	48
CRRL (cardiomyocyte regeneration-related lncRNA)	Mouse	Loss of CRRL attenuates remodelling after myocardial infarction and preserves cardiac function in adult rats; CRRL promotes cardiomyocyte proliferation	CRRL acts as a competing endogenous RNA by binding to miRNA-199a-3p, which results in increased expression of HOPX	177
ECRAR (endogenous cardiac regeneration-associated regulator)	Human and rat	Promotes DNA synthesis, mitosis and cytokinesis in postnatal day 7 and adult rat cardiomyocytes; overexpression stimulates myocardial regeneration after myocardial infarction; knock-down of ECRAR inhibited postnatal day 1 cardiomyocyte proliferation and prevented recovery after myocardial infarction	ECRAR directly binds and promotes phosphorylation of ERK1/2, resulting in activation of cyclin D1 and cyclin E1, which, in turn, activate E2F1	178
Meg3 (maternally expressed gene 3)	Human	Meg3 was increased in samples from patients with heart failure; Meg3 has been shown to have pro-apoptotic properties; mice with Meg3 knock-down show improvement in cardiac function after myocardial infarction	Interacts with the RNA-binding protein FUS	179
Wisper (WISP2 super-enhancer-associated RNA)	Mouse	Enriched in cardiac fibroblasts; antisense oligonucleotide-mediated silencing of Wisper in vivo attenuates myocardial infarction-induced fibrosis and cardiac dysfunction	Associates with TIAL1 to control expression of a profibrotic form of PLOD2 (which is involved in collagen crosslinking and stabilization of the extracellular matrix)	134

Long non-coding RNAs (lncRNAs) with roles in other cardiac growth processes and functions have also been defined. This list of lncRNAs overlaps with those that affect cardiac hypertrophy. miRNA, microRNA.

Table 4 |

lncRNAs that could function as biomarkers for cardiac hypertrophy and cardiac disease

lncRNAs	Models	Observed change / study findings	Study population parameters	Ref.
aHIF, ANRIL, KCNQ1OT1, MALAT1, MIAT	Human	Whole-blood levels of aHIF, KCNQ1OT1 and MALAT1 were higher in patients with MI, whereas the level of ANRIL was lower; the level of MIAT was similar in both groups	Patients with STEMI: $n = 274$, male/female = 199/75, median age 61 years, range 35–89 years; patients with NSTEMI: $n = 140$, male/female = 102/38, median age 62 years, range 30–89 years; controls: $n = 86$, male/female = 70/16, median age 61 years, range 25–82 years	180
Ahit	Human	Upregulated in serum samples from patients with hypertensive heart disease (compared with individuals with non-hypertrophic hearts)	Patients with cardiac hypertrophy: male/female = 6/5; controls: male/female = 8/7	163
BACE1, BACE1-as	Human and mouse	Levels of both transcripts increased in tissue biopsied from left ventricle of patients with HF; transcript levels also increased in a mouse model of ischaemic HF	Patients with HF: $n = 18$, male/female = 17/1; aged 65.0 ± 0.6 years; controls: $n = 17$, male/female = 10/6 (noted discrepancy in numbers from report); aged 58.3 ± 3.4 years	181
Carmen, Fendrr, Mhrt	Human	Left ventricular mass index showed a negative correlation with levels of Mhrt and Fendrr and a positive correlation with levels of Carmen; analysis performed using peripheral blood mononuclear cells from patients with essential hypertension associated with left ventricular hypertrophy	Patients with hypertension: $n = 80$, male/female = 32/48, aged 67 ± 8 years; controls: $n = 25$, male/female = 8/17, aged 64 ± 5 years	182
H19	Human	Single-nucleotide polymorphisms identified that affect the secondary structure of H19	Patients with DCM: $n = 96$, male/female = 70/26, mean age 53 years, range 22–80 years; controls: $n = 259$, male/female = 129/130, from the 1000 Genomes Project); variants confirmed with cohort of 1,084 patients with DCM and 751 disease-free controls (overall mean age 43 (range 0–83) years, 31% female)	183
	Human, mouse and rat	Dynamically expressed in pathological hearts (changes observed in patient plasma samples, as well as in TAC mouse model of cardiac hypertrophy and rat model of right ventricular failure (monocrotaline treated and pulmonary artery banded))	Aortic stenosis: $n = 24$, male/female = 16/8, aged 70 ± 17 years; HCM: $n = 12$, male/female = 6/6; aged 17 ± 20 years; control hearts: $n = 24$, male/female = 14/10, aged 39 ± 13 years. Failing hearts: $n = 12$, male/female = 7/5, aged 72 ± 4 years, fetal hearts: $n = 4$, aged 12–14 weeks; control (non-failing) hearts: $n = 4$, male/female = 2/2, aged 48 ± 4 years. LVAD hearts: $n = 12$, male/female = 9/3, aged 54 ± 6 years; LVAD control hearts: $n = 9$, male/female = 1/8, aged 40 ± 13 years. ^{61,67} Patients with IPAH: $n = 52$, male/female = 21/31, aged 61 ± 16 years; patients with CTD-IPAH: $n = 21$, male/female = 5/16, aged 69 ± 8 years; controls: $n = 57$, male/female = 40/17, aged 46 ± 19 years ^{61,67}	61,67
	Human	H19 variants associated with elevated risk of developing HCM; genotyped two H19 SNPs in 405 patients with HCM and 550 controls; sequence determined in 100 patients; the incidence of the H19 rs2107425 CC genotype (C homozygous) was higher in patients without sarcomere mutations	Patients with sarcomere-negative HCM: $n = 225$, male/female = 140/85, aged 56 ± 14 years; patients with sarcomere-positive HCM: $n = 180$, male/female = 117/63, aged 46 ± 12 years; controls: $n = 550$, aged 60–85 years	184
H19 and LIPCAR	Human	Increased plasma levels associated with increased risk of coronary artery disease in a Chinese population	Patients with coronary artery disease: $n = 300$, male/female = 188/112, aged 64 ± 11 years; controls: $n = 180$, male/female = 108/72, aged 63 ± 10 years	185
H19, HOTAIR, RMRP	Human and mouse	Levels increased in tissue biopsied from left ventricle of patients with ischaemic DCM; also increased in TAC mouse model of cardiac hypertrophy	Patients with ischaemic DCM or HF: $n = 18$, male/female = 17/1, aged 65 ± 1 years; controls: $n = 16$, male/female = 10/6, aged 58 ± 3 years	186

lncRNAs	Models	Observed change / study findings	Study population parameters	Ref.
Heat2	Human	Levels elevated in the blood of patients with HF; enriched in circulating immune cells	Next-generation sequencing cohort (cohort 1): patients with HF: $n = 4$, male/female = 4/0, aged 62 ± 14.5 years; controls: $n = 4$, male/female = 4/0, aged 29 ± 2.8 years. First validation cohort (cohort 2): patients with HF: $n = 16$, male/female = 3/13, aged 65 ± 11.1 years; controls: $n = 8$, male/female = 2/6, aged 64 ± 5.2 years. Second validation cohort (cohort 3): patients with HF: $n = 69$, male/female = 58/11, aged 65 ± 10.5 years; controls: $n = 38$, male/female = 27/11, aged 63.5 ± 12.1 years	187
LIPCAR ^{188,189}	Human	Plasma levels increased in patients with HF and higher NYHA class	LIPCAR subcohort: patients with HF: $n = 967$, male/female = 775/192, aged 70 ± 10.8 years	190
	Human	Increased plasma levels in patients with HF after acute MI	Patients with HF after acute MI: $n = 59$, male/female = 33/26, aged 64 ± 7.2 years; patients without HF after acute MI: $n = 68$, male/female = 39/29, aged 63 ± 10.5 years	191
	Human	Plasma levels elevated in patients with HF with reduced ejection fraction and associated with left ventricular remodelling and poor outcomes	Patients with CKD: $n = 100$, male/female = 41/59, aged 77 ± 8.7 years; patients without CKD: $n = 134$, male/female = 117/17, aged 72 ± 8.9 years	192
	Human	Initially downregulated after MI but upregulated during later stages (plasma RNA isolated from three independent cohorts (788 patients) who developed HF with abnormal cardiac remodelling)	Cohort with left ventricular remodelling: patients with acute MI: $n = 246$, male/female = 200/46, aged 57 ± 14 years. First cohort with HF: patients with ischaemic HF: $n = 164$, male/female = 136/28; aged 56 ± 11 years; patients with non-ischaemic HF: $n = 180$, male/female = 139/41, aged 53 ± 11 years. Second cohort with HF: patients with HF and subsequent cardiovascular death: $n = 99$, male/female = 91/8, aged 59 ± 11 years; patients with HF and no subsequent cardiovascular death: $n = 99$, male/female = 91/8, aged 59 ± 11 years	188
lncCytB	Human and mouse (TAC model)	Plasma levels decreased in patients with HF; cytosolic lncRNA	Patients with HF: $n = 12$, male/female = 6/6, aged 60 ± 2.8 years; controls: $n = 11$, male/female = 5/6, aged 54 ± 2.4 years	168
lncExACT1	Human	Increased plasma levels in patients with HF	Patients with HF with reduced ejection fraction: $n = 18$; patients with HF with preserved ejection fraction: $n = 16$; controls or patients with supraventricular tachycardia but no HF: $n = 8$	158
lncHrt	Human	Levels decreased in the hearts of patients with DCM	Patients with DCM: $n = 6$, aged 49–68 years; controls: $n = 2$, aged 15–30 years	193
lncRNA ENST00000507296 and ENST00000532365	Human	Plasma levels of lncRNAs ENST00000507296 and ENST00000532365 correlated with cardiac function in patients with DCM	Screening cohort: patients with DCM: $n = 10$, male/female = 9/1, aged 63–82 years; controls: $n = 10$, male/female = 10/0, aged 33–56 years. Validation cohort: patients with DCM: $n = 64$, male/female = 42/22, aged 56 ± 14.6 years; controls: $n = 64$, male/female = 36/28, aged 56 ± 10.7 years. Replication cohort: patients with DCM: $n = 198$, male/female = 144/54, aged 55 ± 0.9 years; controls: $n = 198$, male/female = 133/65, aged 54 ± 0.7 years. Follow-up cohort: patients with DCM (with exclusion of patients with other severe systemic diseases (such as renal failure or hepatic diseases) and malignant tumours or congenital heart diseases or clinically significant valvular diseases): $n = 552$, male/female = 392/160, aged 55 ± 13 years	194
Mhrt	Human	Plasma levels of Mhrt were downregulated in patients with chronic HF (compared with healthy controls); potential use of circulating MHRT levels as a diagnostic and prognostic marker for chronic HF	Patients with chronic HF: $n = 88$, male/female = 50/38, aged 55 ± 6.6 years; controls: $n = 65$, male/female = 40/25, aged 54 ± 5.9 years	195
Mhrt, NRON	Human	Plasma levels of both lncRNAs higher in patients with HF than in	Patients with HF: $n = 72$, male/female = 47/25, aged 59 ± 11.2 years; controls: $n = 60$, male/female = 37/23, aged 60 ± 12 years	196

lncRNAs	Models	Observed change / study findings	Study population parameters	Ref.
		controls (analysis conducted using real-time RT-PCR)		
NEAT1	Human	Increased serum levels of NEAT1 associated with decreased microRNA-129-5p expression in patients with chronic HF	Patients with chronic HF: $n = 70$, male/female = 37/33, aged 67 ± 1.6 years; controls: $n = 62$, male/female = 34/28, aged 67 ± 1.7 years	197
NRF	Human	Potential marker to determine the risk of HF after acute MI	Patients with HF after acute MI: $n = 76$; patients without HF after acute MI: $n = 58$	198

CKD, chronic kidney disease; CTD, connective tissue disease-associated; DCM, dilated cardiomyopathy; HF, heart failure; HCM, hypertrophic cardiomyopathy; IPAH, idiopathic pulmonary arterial hypertension; lncRNA, long non-coding RNA; LVAD, left ventricular assist device; MI, myocardial infarction; NSTEMI, non-ST-segment elevation myocardial infarction; TAC, transverse aortic constriction; RT-PCR, reverse transcription polymerase chain reaction; STEMI, ST-segment elevation myocardial infarction;.

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