Abstract  Molecular mechanisms underlying heart failure (HF) are only partly understood. Non-coding RNAs (ncRNAs) have been reported to control function and signalling routes in the...
myocardium. As ncRNAs such as microRNAs (miRNAs), long non-coding RNAs (lncRNAs) or circular RNAs (circRNAs) can be selectively targeted via pharmacological approaches, this opens new avenues for diagnostic and therapeutic approaches. Here, we review the main ncRNA classes and how they influence cardiac biology. In addition we provide insight into the role of ncRNAs in chemotherapy-induced cardiac dysfunction. To provide a better understanding of ncRNAs in cardiovascular biology we present an outlook on specialized functions such as chromatin remodelling, biomarker potential and the recently discovered ncRNA-derived micropeptides.

**Introduction**

For a long time, one of the central dogmata of biology was simply ‘DNA makes RNA makes protein’, but in the last several years it has become clear that this hypothesis may need to be revised. As indicated in Fig. 1A about three-quarters of the genome is actively transcribed, and not-transcribed areas of the genome mainly locate to the telomere ends and the centromere regions of the chromosomes (greyed out chromosome regions) (Frith et al. 2005; Djebari et al. 2012). The actively transcribed part spans both repeated (grey) and unique sequences. Since sequencing technologies have significantly advanced, it became clear that only 2–3% of the genome encodes proteins containing exons (E, orange). Instead, other unique genomic regions containing regulatory sequences such as promoter and enhancer regions (blue) as well as intronic regions (purple) present the overwhelming portion of arising transcripts. No longer being just an intermediate step between DNA and proteins these non-coding RNAs (ncRNAs), that may contain exons of protein-coding genes, span a wide range, arbitrarily classified by their size (below or above 200 nt, denoted “short” and “long” in Fig. 1B) and basic molecular features. In this review we summarize key findings on ncRNAs and their diagnostic and therapeutic application in the context of heart failure as presented recently by Prof. Thomas Thum at the Gordon Research Conference on Cardiac Regulatory Mechanisms, June 3–8, 2018.

In 1993, Lee and colleagues identified the first short non-coding transcript which interacted with a messenger RNA (mRNA) (Lee et al. 1993). By binding to complementary sequences in the 3′-untranslated region (UTR) of mRNAs, these microRNAs (miRNAs, also miR-#), short and highly conserved oligonucleotides of ~22 nt, led to mRNA degradation or translational repression by directing a silencing complex (Bartel, 2009). The *modus operandi* is directly dependent on sequence complementarity – and as miRNAs have a rather short binding site of just 6–8 bp, they usually exhibit rather broad specificity. As such, >60% of all mRNAs possess such binding sites, potentially being targets of miRNA regulation (Friedman et al. 2009). As of now 1917 human miRNA sequences are known (included in the “short ncRNA” category, Fig. 1B), allowing for a high degree of tissue- or disease-specific regulation despite their broad specificity (Griffiths-Jones, 2004; Kozomara & Griffiths-Jones, 2014). Some miRNAs, e.g. miR-146a or miR-21∗ (asterisk indicates the usually degraded miRNA passenger strand), can even act on other cell types in a paracrine fashion through exosome transport (Halkein et al. 2013; Bang et al. 2014).

In contrast, long non-coding RNAs (lncRNAs) are single-stranded RNA molecules of >200 nt, often up to several kilobases (included in the “long ncRNA” category, Fig. 1B). As lncRNAs described a very heterogeneous class of molecules, they are often further subdivided by their genomic location. As such, lncRNAs can span several exonic and intronic regions of protein-coding genes (sense), solely reside within introns (intronic), be transcribed from the opposite strand (antisense and bidirectional) or be located within the former “junk DNA” in-between protein-coding genes (intergenic). As lncRNAs contain palindromic stretches within their sequence they can form simple or complex loops and hairpin structures. By arranging the single loops into three-dimensional structures, lncRNAs can bridge the gap between nucleic acids and proteins, being an integral scaffold for protein complexes in transcription and translation, e.g. the lncRNA NEAT1 maintaining...
ncRNAs in cardiac disease

In the last years several reports have underlined that inhibition of miRNAs has been associated with therapeutic effects during heart failure (HF) or ischaemic injury.
In line with this, Ucar et al. identified the miR-132/212 cluster consisting of two miRNAs driving cardiomyocyte hypertrophy. Whereas cardiomyocyte-specific overexpression led to cardiomyocyte hypertrophy, heart failure and death in mice, the silencing of miR-132 protected against heart failure induced by pressure overload (Ucar et al. 2012). Another factor promoting cardiomyocyte hypertrophy is the IncRNA CHAST, which was found to be upregulated in cardiomyocytes of transverse aortic constriction (TAC)-operated mice and hypertrophic heart tissue from aortic stenosis patients. Therapeutic gain-of-function led to induction of cardiomyocyte hypertrophy indicating a pivotal role for cardiac performance (Viereck et al. 2016). While these ncRNAs actively promote hypertrophy, negative regulation of hypertrophy by ncRNAs has also been described. An outstanding example of such a preventive IncRNA is Mhrt (or Myheart), which prevents the helicase domain of the Brg1-Hdac-Parp chromatin repressor complex from recognizing its target sequence by competitive binding and thereby protecting the heart from cardiomyocyte hypertrophy (Han et al. 2014). In contrast to those heart failure-associated ncRNAs Eulalio and colleagues deciphered the role of miRNAs in early cardiac development and showed that administration of miR-199a and miR-590 in neonatal rodents could enhance cardiomyocyte proliferation as another layer of cardiac therapeutics (Eulalio et al. 2012).

While these ncRNAs are acting on cardiomyocytes, the first proof-of-concept of treating cardiac disease with miRNA-antagonists focused on miR-21, a microRNA selectively upregulated in fibroblasts of the failing heart. Silencing of miR-21 induced repression of ERK1/2-dependent growth factor signalling leading to regression of interstitial cardiac hypertrophy and fibrosis in a murine pressure overload model (Thum et al. 2008). Similarly, inhibition of the IncRNA MEG3 decreased cardiac fibrosis and improved cardiac parameters by targeting the production of matrix metalloproteinase-2 (MMP-2) (Piccoli et al. 2017).

A driving force of the controlled revascularization response after cardiac ischaemia is GATA2, a major transcription factor highly associated to miRNA biology. As such, GATA2 is both controlled by miR-24, but also induces miR-126 and miR-221 expression (Fiedler et al. 2011; Hartmann et al. 2016). Moreover, GATA2 was also repressed after silencing of the IncRNA MIR503HG, highlighting the importance of IncRNAs towards maintaining balanced gene expression as well (Fiedler et al. 2015). Interestingly, upregulation of miR-503 (the miRNA transcribed from the same locus as MIR503HG) was shown to inhibit angiogenesis independent of GATA2 (Zhou et al. 2013; Wen et al. 2018). Aside from interacting with transcription factors, members of the miR-17–92 cluster regulate vascular signalling in endothelial cells by targeting the mRNAs of several proangiogenic genes (Doebbe et al. 2010). Another central regulator of endothelial cell biology is miR-146a, a microRNA induced upon peripartum cardiomyopathy in patients or in pressure overload models. Interestingly, miR-146a is released from those endothelial cells via exosomes and then acts on cardiomyocytes where it promotes cardiac hypertrophy and left ventricular dysfunction (Halkein et al. 2013; Heggermont et al. 2017). In the context of atherosclerosis, miR-146a was described to restrict pro-inflammatory signalling in endothelial cells, highlighting an atheroprotective role of this microRNA (Cheng et al. 2017).

Taking research from linearized to the previously mentioned circularized RNA structures, Boeckel et al. reported the induction of a circRNA, cZNF292, in a model of endothelial hypoxia. Specific silencing of the circRNA, but not the host gene, significantly inhibited sprouting and tube formation (Boeckel et al. 2015). Interestingly, cZNF292 does not contain miRNA binding sites, strongly implying a miRNA-independent mechanism for circRNA-dependent regulation. The diverse roles of ncRNAs in the cardiac phenotype (hypertrophy, vascularization, protein signalling and potential use as biomarker) are summarized in the Abstract figure.

Non-coding RNAs in chemotherapy-induced cardiac dysfunction

State-of-the-art for the treatment of cancer is mainly based on the application of chemotherapeutics. While targeted delivery approaches have increased over the past years, these compounds, mostly potent cytostatica or cytotoxins, still also reach healthy tissue. Depending on mechanism of action and condition of the patient, the high doses needed for successful treatment of the cancer (to manage the risk of secondary malignancies) often also result in secondary damage to whole organ systems. This is especially true for cardiac tissue with its extraordinary situation of constant, high workload and low regenerative capabilities, eventually leading to chemotherapy-related cardiac dysfunctions (CRCDs). Efforts were made to distinguish between permanent myocardial damage (type I CRCD) and reversible dysfunction (type II CRCD) based on mechanism and dose dependency (Ewer & Lippman, 2005). Over several decades studies reported type I CRCD after treatment with anthracyclines such as doxorubicin (Doxo), visibly e.g. in the high rate of human cancer patients developing congestive heart failure (Hoff et al. 1979; Lipshultz et al. 1991, 2008; Seidman et al. 2002; Swain et al. 2003). This is contrasted to type II CRCD generally reported by upcoming targeted therapies such as the antibody Trastuzumab (Ewer & Lippman, 2005; Ewer & Ewer, 2010).
In this setting, Gupta and colleagues recently highlighted the importance of circRNA formation during chemotherapy-induced cardiotoxicity (Gupta et al. 2018). In murine myocardium, Doxo treatment strongly repressed expression of the RNA-binding protein Qki5 (Quaking). In line with this, therapeutic Qki5 over-expression was successful to counteract detrimental cardiac effects of Doxo treatment in mice. Interestingly, repression of cardiac Qki5 efficiently lowered expression of circRNAs derived from important cardiomyocyte genes, e.g. Ttn (Titin), thereby implying circRNAs play an important anti-apoptotic role in cardiac muscle (Gupta et al. 2018). The effects of Doxo-based chemotherapy are not entirely understood, as several recent long-term follow-up studies of Doxo treatment could not confirm worsening of cardiac function (Ganz et al. 2017) or identified valvular abnormalities, but not congestive heart failure (Materazzo et al. 2017). Nevertheless, the study of Gupta and colleagues linked circRNA biogenesis and function with a promising new route for potential adjuvant therapy of (circular) ncRNAs during chemotherapy. Importantly, most data on potential ncRNA therapies are derived from in vitro studies or animal models with restricted translatability, given the limited conservation of lncRNAs across species (compare ‘Introduction’). Therefore current clinical trials of non-coding RNA-based therapies focus on miRNAs: antifibrotic properties of anti-miR-21 intervention are currently being studied in a phase II trial for the treatment of Alport syndrome (NCT02855268) and Miravirsen represents a miR-122 antagonist against hepatitis C virus infection (Lindow & Kauppinen, 2012; Janssen et al. 2013; Ottosen et al. 2015). Although these examples are not about cardiac diseases, ncRNA-based therapies are already in clinical trials and will explore the general potential and safety of this molecule class. Nevertheless, more research is needed to determine the optimal formulation and delivery of ncRNAs, to investigate their pharmacokinetics, and to standardize ncRNA therapy. To achieve this goal, intensive research on delivery routes will initiate a path towards therapeutic application of miRNAs, lncRNAs or circRNAs in cardiac disease.

**Specialized ncRNA function**

The functions and mechanisms described above are now established, leading to the development of ncRNA-based drugs. In addition, we want to offer insight into recent developments which we believe will become relevant over the coming years.

One subclass of intergenic ncRNAs are enhancer-derived lncRNAs (eRNAs, included in the “long ncRNA” category, Fig. 1B), which are synthesized specifically at enhancers, thereby regulating mRNA transcription (by this means being in close proximity with the transcription complex) (Kim et al. 2010). Another important subclass, originally termed ncRNA-a, which are characterized by another set of histone marks, describes ncRNAs able to activate the expression of neighbouring genes, both in cis and in trans (Ørom et al. 2010). Whether or not these reports describe truly distinct classes of ncRNAs, both variants establish chromatin accessibility by recruiting chromatin remodelling to enable polymerase II assembly, e.g. by directing chromatin looping through interaction with the Mediator complex (Lai et al. 2013), the Long intervening/intergenic noncoding RNA (linc RNA) Yam-1 enabling transcription of miR-715 in myoblasts (Lu et al. 2013), or eRNAs enabling MyoD access (Mousavi et al. 2013). Ounzain and colleagues identified CARMEN, a lncRNA derived from a super enhancer (SE). By directing components of polycomb repressive complex 2 (PRC2), CARMEN regulates cardiomyocyte differentiation and maintains this functional state (Ounzain et al. 2015). Similarly, IncRNA Wisper is SE-derived, but regulates cardiac fibroblast proliferation, migration and survival. Pharmacological intervention effectively reduced fibrotic scar formation supporting cardiac healing (Micheletti et al. 2017).

The complexity of IncRNAs also offers possible interactions with ribosomes suggesting that a certain set of IncRNAs is able to encode for short peptide sequences, so-called micropeptides. By way of example, Anderson et al. identified myoregulin, a 46 amino acid micropeptide constraining Ca^{2+} handling in mice (Anderson et al. 2015). Recently, the same group identified DWORF, a 34 amino acid micropeptide activating the Ca^{2+} pump SERCA (Nelson et al. 2016). It is under debate whether these micropeptides are actually functional, e.g. as inter- or intracellular messengers, or only derive due to pioneering rounds of ribosomes before nonsense-mediated decay can take place, as experimental identification and validation is challenging and calls for integration of carefully conducted proteome-based approaches. Similarly, it is unclear how to correctly distinguish between true ncRNAs encoding micropeptides and misannotations of protein-coding genes, which harbour short open reading frames (Bánfi et al. 2012).

Given the sheer number of different ncRNAs, tissue-specific expression could open their potential use as biomarkers. One candidate microRNA could be miR-122, a miRNA highly liver specific and associated with risk of developing metabolic syndrome (Willeit et al. 2016, 2017). Also type 2 diabetes has been associated with a certain miRNA expression pattern, especially loss of miR-126 in endothelial cells, thereby linking diabetes and impaired angiogenesis (Zampetaki et al. 2010). Zampetaki et al. further investigated this pattern and found platelets as a major miRNA reservoir (Zampetaki et al. 2012). Also lncRNAs have been found to be possible biomarkers, e.g. Kumarswamy et al. identified the mitochondrial lncRNA...
LIPCAR to be a predictive factor in plasma of myocardial infarction patients (Kumarswamy et al. 2014).

Based on their resistant structure, circRNAs naturally lend themselves as biomarker candidates. Indeed, Memczak et al. could identify >4000 distinct circRNAs in human blood (Memczak et al. 2015). Which ncRNA subtype is successful to become a superior biomarker compared to troponin-based evaluation needs to be determined in future research.

Outlook

In the last 20–25 years, it has become increasingly obvious that central concepts of biology are insufficient to describe the larger picture, e.g. gene number and genome size are not predictive of an organism’s perceived complexity (Gregory 2004).

Cook and colleagues noticed the enrichment of active RNA polymerase II in discrete foci of the nucleus, which they termed “transcription factories” (Iborra et al. 1996). These foci are able to enrich and concentrate proteins, and possibly other components as well (Eskiw et al. 2008). Although the precise components are unknown, the foci seem to be held together by some kind of underlying scaffold which counteracts diffusion and aids forming new foci after mitosis. Given the multi-layered model of ncRNA interactions, it would be no surprise if ncRNAs would also be part of these foci, possibly as part of the scaffold directing chromatin loops and transcriptional compartments into proximity (similar to the function of IncRNA NEAT1 in paraspeckles, Sasaki et al. 2009). The direct involvement of IncRNAs in the transcriptional machinery is providing first evidence that the assembly and regulation of multiple-molecule complexes are not the sole domain of proteins, but rather proteins binding to DNA and RNA can profit from the guidance of stretches from IncRNA molecules. Next to these speculative intracellular functions of IncRNAs in particular, validation of miR-based therapeutic strategies to improve cardiac output will be in demand as a field of cardiac drug research in the near future. This also implies that multiple efforts have to be taken in identifying true target structures to promote pharmacological evaluation at the pre-clinical and clinical level.

References

Anderson DM, Anderson KM, Chang C-L, Makarewich CA, Nelson BR, McAnally JR, Kasaragod P, Shelton JM, Liou J, Bassel-Duby R & Olson EN (2015). A micropeptide encoded by a putative long noncoding RNA regulates muscle performance. Cell 160, 595–606.

Bánffai B, Jia H, Khatun J, Wood E, Risk B, Gundling WE, Kundaje A, Gunawardena HP, Yu Y, Xie L, Krajewski K, Strahl BD, Chen X, Bickel P, Giddings MC, Brown JB & Lipovich L (2012). Long noncoding RNAs are rarely translated in two human cell lines. Genome Res 22, 1646–1657.

Bang C, Batkai S, Dangwal S, Gupta SK, Foinquinos A, Holzmann A, Just A, Remke J, Zimmer K, Zeug A, Ponimaskin E, Schmiedl A, Yin X, Mayr M, Halder R, Fischer A, Engelhardt S, Wei Y, Scholer A, Fiedler J & Thum T (2014). Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. J Clin Invest 124, 2136–2146.

Bartel DP (2009). MicroRNAs: target recognition and regulatory functions. Cell 136, 215–233.

Beermann J, Piccoli MT, Vieriek J & Thum T (2016). Non-coding RNAs in development and disease: background, mechanisms, and therapeutic approaches. Physiol Rev 96, 1297–1325.

Boeckel JN, Jae N, Heumuller AW, Chen W, Boon RA, Stellos K, Zeiher AM, John D, Uchida S & Dimmeler S (2015). Identification and characterization of hypoxia-regulated endothelial circular RNA. Circ Res 117, 884–890.

Brown CJ, Ballabio A, Rupert JL, Lafreniere RG, Grompe M, Tonlorenzi R & Willard HF (1991). A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. Nature 349, 38–44.

Brown CJ, Hendrich BD, Rupert JL, Lafreniere RG, Xing Y, Lawrence J & Willard HF (1992). The human XIST gene: Analysis of a 17 kb inactive X-specific RNA that contains conserved repeats and is highly localized within the nucleus. Cell 71, 527–542.

Cai X & Cullen BR (2007). The imprinted H19 noncoding RNA is a primary microRNA precursor. RNA 13, 313–316.

Cheng HS, Besla R, Li A, Chen Z, Shikatani EA, Nazari-Jahantigh M, Hammoutene A, Nguyen MA, Geoffrion M, Cai L, Khyzha N, Li T, MacParland SA, Husain M, Cybulsky MI, Boulanger CM, Temel RE, Scholer A, Rayner KJ, Robbins CS & Fish JE (2017). Paradoxical suppression of atherosclerosis in the absence of microRNA-146a. Circ Res 121, 354–367.

Cocqueree C, Mascarez B, Héutin D & Bailleul B (1993). Mis-splicing yields circular RNA molecules. FASEB J 7, 155–160.

Derrien T, Johnson R, Bussotti G, Tanzer A, Kola I, Gisselbrecht S, Besse C, Sfeir A, Pasquinelli AE, Karlsson E, Norgaard-K逼ock T, Panopoulos A, Beyenbach K, Boeckel JN, Jaen H, Heumuller A, Chen W, Boon RA, Stellos K, Zeiher AM, John D, Uchida S & Dimmeler S (2012). FASEB J 26, 3540–3550. https://doi.org/10.1096/fj.12-217581.

Eskiw C, Dean G, Barce-Pick SG, Beacham J, Cook AP, Turner CC, Boettiger A, Albert V, Rice TP, Brown CJ, Ballabio A, Rupert JL, Lafreniere RG, Grompe M, Tonlorenzi R & Willard HF (2014). Non-coding RNAs in development and disease: background, mechanisms, and therapeutic approaches. Physiol Rev 96, 1297–1325.

Fischer A, Engelhardt S, Wei Y, Scholer A, Fiedler J & Thum T (2014). Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. J Clin Invest 124, 2136–2146.

Bartel DP (2009). MicroRNAs: target recognition and regulatory functions. Cell 136, 215–233.

Beermann J, Piccoli MT, Vieriek J & Thum T (2016). Non-coding RNAs in development and disease: background, mechanisms, and therapeutic approaches. Physiol Rev 96, 1297–1325.

Boeckel JN, Jae N, Heumuller AW, Chen W, Boon RA, Stellos K, Zeiher AM, John D, Uchida S & Dimmeler S (2015). Identification and characterization of hypoxia-regulated endothelial circular RNA. Circ Res 117, 884–890.

Brown CJ, Ballabio A, Rupert JL, Lafreniere RG, Xing Y, Lawrence J & Willard HF (1992). The human XIST gene: Analysis of a 17 kb inactive X-specific RNA that contains conserved repeats and is highly localized within the nucleus. Cell 71, 527–542.

Cai X & Cullen BR (2007). The imprinted H19 noncoding RNA is a primary microRNA precursor. RNA 13, 313–316.

Cheng HS, Besla R, Li A, Chen Z, Shikatani EA, Nazari-Jahantigh M, Hammoutene A, Nguyen MA, Geoffrion M, Cai L, Khyzha N, Li T, MacParland SA, Husain M, Cybulsky MI, Boulanger CM, Temel RE, Scholer A, Rayner KJ, Robbins CS & Fish JE (2017). Paradoxical suppression of atherosclerosis in the absence of microRNA-146a. Circ Res 121, 354–367.

Cocqueree C, Mascarez B, Héutin D & Bailleul B (1993). Mis-splicing yields circular RNA molecules. FASEB J 7, 155–160.

Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, Lagarde J, Veeravalli L, Ruan X, Ruan Y, Lasman T, Carninci P, Brown JB, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Shiekhattar R, Gingeras TR, Hubbard TJ, Notredame C, Harrow J & Guigó R (2012). The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome research 22, 1775–1789. https://doi.org/10.1101/gr.132159.111.
Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, Tanzer A, Lagarde J, Lin W, Schlesinger F, Xue C, Marinov GK, Khatun J, Williams BA, Zaleski C, Rozowsky J, Röder M, Kukok M, Abdelhamid RF, Alioto T, Antoshechkin I, Baer MT, Bar NS, Batut P, Bell K, Bell I, Chakrabortty S, Chen X, Chrast J, Curado J, Durrien T, Drenkow J, Dumais E, Dumais J, Duttagupta R, Falconnet E, Fastuca M, Fejes-Toth K, Ferreira P, Foisac S, Fullwood MJ, Gao H, Gonzalez D, Gordon A, Gunawardena H, Howald C, Jha S, Johnson R, Kapranov P, King B, Kingswood C, Luo OJ, Park E, Persaud K, Preall JB, Ribeca P, Risk B, Roër Y, Sammeth M, Schaffer L, See L-H, Shahab A, Skancke J, Suzuki AM, Takahashi H, Tilgner H, Trout D, Walters N, Wang H, Wrobel J, Yu Y, Ruan X, Hayashizaki Y, Harrow J, Gerstein M, Hubbard T, Reymond A, Antonarakis SE, Hannon G, Giddings MC, Ruan Y, Wold B, Carninci P, Guigó R & Gingeras TR (2012). Landscape of transcription in human cells. *Nature* **489**, 101–108.

Doebele C, Bonauer A, Fischer A, Scholz A, Reiss Y, Urbich C, Jebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, Tanzer A, Lagarde J, Lin W, Schlesinger F, Xue C, Marinov GK, Khatun J, Williams BA, Zaleski C, Rozowsky J, Röder M, Kukok M, Abdelhamid RF, Alioto T, Antoshechkin I, Baer MT, Bar NS, Batut P, Bell K, Bell I, Chakrabortty S, Chen X, Chrast J, Curado J, Durrien T, Drenkow J, Dumais E, Dumais J, Duttagupta R, Falconnet E, Fastuca M, Fejes-Toth K, Ferreira P, Foisac S, Fullwood MJ, Gao H, Gonzalez D, Gordon A, Gunawardena H, Howald C, Jha S, Johnson R, Kapranov P, King B, Kingswood C, Luo OJ, Park E, Persaud K, Preall JB, Ribeca P, Risk B, Roër Y, Sammeth M, Schaffer L, See L-H, Shahab A, Skancke J, Suzuki AM, Takahashi H, Tilgner H, Trout D, Walters N, Wang H, Wrobel J, Yu Y, Ruan X, Hayashizaki Y, Harrow J, Gerstein M, Hubbard T, Reymond A, Antonarakis SE, Hannon G, Giddings MC, Ruan Y, Wold B, Carninci P, Guigó R & Gingeras TR (2012). Landscape of transcription in human cells. *Nature* **489**, 101–108.

Ewer MS & Ewer SM (2010). Cardiotoxicity of anticancer treatments: what the cardiologist needs to know. *J Clin Invest* **124**, 2143–2154.

Halseen K, Tabruyn SP, Ricke-Hoch M, Haghikia A, Nguyen NQ, Scherr M, Castermans K, Malvaux L, Lambert V, Thiry M, Sliwa K, Noel A, Martial JA, Hilfiker-Kleiner D & Struman I (2013). MicroRNA-146a is a therapeutic target and biomarker for peripartum cardiomyopathy. *J Clin Invest* **123**, 2143–2154.

Han P, Li W, Lin C-H, Yang J, Shang G, Nuernberg ST, Jin KK, Xu W, Lin C-Y, Lin C-J, Xiong Y, Chien H, Zhou B, Ashley E, Bernstein D, Chen P-S, Chen H-SV, Quertermous T & Chang C-P (2014). A long noncoding RNA protects the heart from pathological hypertrophy. *Nature* **514**, 102–106.

Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK & Kjems J (2013). Natural RNA circles function as efficient microRNA sponges. *Nature* **495**, 384–388.

Hartmann D, Fiedler J, Sonnenschein K, Just A, Pfanne A, Zimmer K, Remke J, Foinquinos A, Butzlafl M, Schimmel K, Maegdefessel L, Hilfiker-Kleiner D, Lachmann N, Schober A, Froese N, Heinke J, Bauersachs J, Batkai S & Thum T (2016). MicroRNA-based therapy of GATA2-deficient vascular disease. *Circulation* **134**, 1973–1990.

Heggermont WA, Papageorgiou AP, Quaegebeur A, Deckx S, Carai P, Verhesen W, Eelen G, Schoors S, van Leeuwen R, Alekseev S, Elzenaar I, Vinckier S, Pokreisz P, Walravens AS, Gijsbers R, van den Haute C, Nickel A, Schroen B, van Bilzen M, Janssens S, Maack C, Pinto Y, Carmeliet P & Heymans S (2017). Inhibition of microRNA-146a and overexpression of its target dihydrolipoyl succinyltransferase protect against pressure overload-induced cardiac hypertrophy and dysfunction. *Circulation* **136**, 747–761.

Hullinger TG, Montgomery RL, Seto AG, Dickinson BA, Semus HM, Lynch JM, Dalby CM, Robinson K, Stack C, Latimer PA, Hare JM, Olson EN & van Rooij E (2012). Inhibition of miR-15 protects against cardiac ischemic injury. *Circ Res* **110**, 71–81.
McHugh CA, Pombo A, Jackson DA & Cook PR (1996). Active RNA polymerases are localized within discrete transcription 'factories' in human nuclei. J Cell Sci 109, 1427–1436.

Jakobi T, Czaja-Hasse LF, Reinhardt R & Dieterich C (2016). Profiling and validation of the circular RNA repertoire in adult murine hearts. Genomics Proteomics Bioinformatics 14, 216–223.

Janssen HLA, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, Patel K, van der Meer AJ, Patick AK, Chen A, Zhou Y, Persson R, King BD, Kauppinen S, Levin AA & Hodges MR (2013). Treatment of HCV infection by targeting microRNA. N Engl J Med 368, 1685–1694.

Kim T-K, Hemberg M, Gray JM, Costa AM, Bear DM, Wu J, Harmin DA, Laptewicz M, Barbara-Haley K, Kuersten S, Markenscoff-Papadimitriou E, Kuhl D, Bito H, Worley PF, Kreiman G & Greenberg ME (2010). Widespread transcription at neuronal activity-regulated enhancers. Nature 463, 182–187.

Kozomara A, Birgaoanu M & Griffiths-Jones S (2019). miRBase: from microRNA sequences to function. Nucleic Acids Res 47, D155–D162.

Kozomara A & Griffiths-Jones S (2014). miRBase: annotating high confidence microRNAs using deep sequencing. Nucleic Acids Res 42, D68–D73.

Kumarswamy R, Bauters C, Volkmann I, Maury F, Fetisch J, Kozomara A, Bauters C, Volkmann I, Maury F, Fetisch J, Kozomara A & Griffiths-Jones S (2014). miRBase: annotating high confidence microRNAs using deep sequencing. Nucleic Acids Res 42, D68–D73.

Lai F, Orom UA, Cesaroni M, Beringer M, Taatjes DJ, Blobel GA & Shekhattar R (2013). Activating RNAs associate with Mediator to enhance chromatin architecture and transcription. Nature 494, 497–501.

Lee RC, Feinbaum RL & Ambros V (1993). The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75, 843–854.

Lindow M & Kauppinen S (2012). Discovering the first microRNA-targeted drug. J Cell Biol 199, 407–412.

Lipshultz SE, Alvareza JA & Scully RE (2008). Anthracycline associated cardiotoxicity in survivors of childhood cancer. Heart 94, 525–533.

Lipshultz SE, Colan SD, Gelber RD, Perez-Atayde AR, Sallan SE & Sanders SP (1991). Late cardiac effects of doxorubicin therapy for acute lymphoblastic leukemia in childhood. N Engl J Med 324, 808–815.

Lu L, Sun K, Chen X, Zhao Y, Wang L, Zhou L, Sun H & Wang H (2013). Genome-wide survey by ChIP-seq reveals YY1 regulation of lncRNAs in skeletal myogenesis. EMBO J 32, 2575–2588.

McHugh CA, Chen C-K, Chow A, Surka CF, Tran C, McDonel P, Pandya-Jones A, Blanco M, Burghard C, Moradian A, Sweredoski MJ, Shishkin AA, Su J, Lander ES, Hess S, Plath K & Guttmann M (2015). The Xist IncRNA interacts directly with SHARP to silence transcription through HDAC3. Nature 521, 232–236.

Materazzo C, Massimino M, Schiavello E, Podda M, Gandola L, Cefalo G, Catania S, Meaza C, Moschetti I & Terzeniani M (2017). Clinical and subclinical cardiac late effects in pediatric Hodgkin’s lymphoma survivors. Tumori 103, 566–571.

Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M, Loewer A, Ziebold U, Landthaler M, Kocks C, Le Noble F & Rajewsky N (2013). Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 495, 333–338.

Memczak S, Papavasileiou P, Peters O & Rajewsky N (2015). Identification and characterization of circular RNAs as a new class of putative biomarkers in human blood. PLoS One 10, e0141214.

Micheletti R, Plaisance I, Abraham BJ, Sarre A, Ting C-C, Alexanian M, Maric D, Maison D, Nemir M, Young RA, Schroen B, Gonzalez A, Ounzain S & Pedrazzini T (2017). The long noncoding RNA Wisp4 controls cardiac fibrosis and remodeling. Sci Transl Med 9, eaa9118.

Montgomery RL, Hullinger TG, Semus HN, Dickinson BA, Seto AG, Lynch JM, Stack C, Latimer PA, Olson EN & van Rooij E (2011). Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure. Circulation 124, 1537–1547.

Mousavi K, Zare H, Dell’Orso S, Grontved L, Gutierrez-Cruz G, Dorfau A, Hager GL & Sartorelli V (2013). eRNAs promote transcription by establishing chromatin accessibility at defined genomic loci. Mol Cell 51, 606–617.

Nelson BR, Makarewich CA, Anderson DM, Winders BR, Troupes CD, Wu F, Reese AL, McAnally JR, Chen X, Kavalali ET, Cannon SC, Houser SR, Bassel-Duby R & Olson EN (2016). A peptide encoded by a transcript annotated as long noncoding RNA enhances SERCA activity in muscle. Science 351, 271–275.

Nigro JM, Cho KR, Fearon ER, Kern SE, Ruppert JM, Oliner JD, Kinzler KW & Vogelstein B (1991). Scrambled exons. Cell 64, 607–613.

Orom UA, Derrien T, Beringer M, Gumireddy K, Gardini A, Bussotti G, Lai F, Zytnicki M, Notredame C, Huang Q, Guigo R & Shekhattar R (2010). Long noncoding RNAs with enhancer-like function in human cells. Cell 143, 46–58.

Ottoson S, Parsley TB, Yang L, Zeh K, van Doorn L-J, van der Veer E, Raney AK, Hodges MR & Patrick AK (2015). In vitro antiviral activity and preclinical and clinical resistance profile of miraviren, a novel anti-hepatitis C virus therapeutic targeting the human factor miR-122. Antimicrob Agents Chemother 59, 599–608.

Ounzain S, Micheletti R, Arnan C, Plaisance I, Cecchi D, Schroen B, Reverter F, Alexanian M, Gonzales C, Ng SY, Bussotti G, Pizzuto I, Notredame C, Heymans S, Guigo R, Johnson R & Pedrazzini T (2015). CARMEN, a human super enhancer-associated long noncoding RNA controlling cardiac specification, differentiation and homeostasis. J Mol Cell Cardiol 89, 98–112.

Piccoli MT, Gupta SK, Vierbeck J, Foinquinos A, Samolovac S, Kramer FL, Garg A, Remke J, Zimmer K, Batkai S & Thum T (2017). Inhibition of the cardiac fibroblast-enriched lncRNA Meg3 prevents cardiac fibrosis and diastolic dysfunction. Circ Res 121, 575–583.

Sasaki YTF, Ideue T, Sano M, Mituyama T & Hirose T (2009). The long noncoding RNA Meg3 prevents cardiac fibrosis and diastolic dysfunction. Circ Res 104, D155–D162.
Seidman A, Hudis C, Pierrri MK, Shak S, Paton V, Ashby M, Murphy M, Stewart SJ & Keefe D (2002). Cardiac dysfunction in the trastuzumab clinical trials experience. *J Clin Oncol* **20**, 1215–1221.

Swain SM, Whaley FS & Ewer MS (2003). Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. *Cancer* **97**, 1215–1221.

Swain SM, Whaley FS & Ewer MS (2003). Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. *Cancer* **97**, 1215–1221.

Thum T, Gross C, Fiedler J, Fischer T, Kessler S, Bussen M, Galuppo P, Just S, Rottbauer W, Frantz S, Castoldi M, Soutschek J, Koteliantsky V, Rosenwald A, Basson MA, Licht JD, Pena JTR, Rouhanifard SH, Muckenthaler MU, Tuschl T, Martin GR, Bauersachs J & Engelhardt S (2008). MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature* **456**, 980–984.

Ucar A, Gupta SK, Fiedler J, Erikci E, Kardasinski M, Batkai S, Dangwal S, Kumarswamy R, Bang C, Holzmann A, Remke J, Caprio M, Jentzsch C, Engelhardt S, Geisendorf S, Glas C, Hofmann TG, Nessling M, Richter K, Schiffer M, Carrier L, Napp LC, Bauersachs J, Chowdhury K & Thum T (2012). The miRNA-212/132 family regulates both cardiac hypertrophy and cardiomyocyte autophagy. *Nat Commun* **3**, 1078.

Viereck J, Kumarswamy R, Foinquinos A, Xiao K, Avramopoulos P, Kunz M, Dittrich M, Maetzig T, Zimmer K, Remke J, Just A, Fendrich J, Scherf K, Bolesani E, Schambach A, Weidemann F, Zweigerdt R, de Windt LJ, Engelhardt S, Dandekar T, Batkai S & Thum T (2016). Long noncoding RNA Chast promotes cardiac remodeling. *Sci Transl Med* **8**, 326ra22.

von Hoff DD, Layard MW, Basa P, Davis HL, von Hoff AL, Rozencweig M & Muggia FM (1979). Risk factors for doxorubicin-induced congestive heart failure. *Ann Intern Med* **91**, 710–717.

Wen Y, Chen R, Zhu C, Qiao H, Liu Y, Ji H, Miao J, Chen L, Liu X & Yang Y (2018). MiR-503 suppresses hypoxia-induced proliferation, migration and angiogenesis of endothelial progenitor cells by targeting Apelin. *Peptides* **105**, 58–65.

Willeit P, Skroblin P, Kiechl S, Fernandez-Hernando C & Mayr M (2016). Liver microRNAs: potential mediators and biomarkers for metabolic and cardiovascular disease? *Eur Heart J* **37**, 3260–3266.

Willeit P, Skroblin P, Moschen AR, Yin X, Kaudewitz D, Zampetaki A, Barwari T, Whitehead M, Ramirez CM, Goedeke L, Rotllan N, Bonora E, Hughes AD, Santer P, Fernandez-Hernando C, Tilg H, Willeit J, Kiechl S & Mayr M (2017). Circulating microRNA-122 is associated with the risk of new-onset metabolic syndrome and type 2 diabetes. *Diabetes* **66**, 347–357.

Zampetaki A, Kiechl S, Drozdev I, Willeit P, Mayr U, Prokopi M, Mayr A, Weger S, Oberhollenzer F, Bonora E, Shah A, Willeit J & Mayr M (2010). Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. *Circ Res* **107**, 810–817.

Zampetaki A, Willeit P, Tilling L, Drozdev I, Prokopi M, Renard JM, Mayr A, Weger S, Schett G, Shah A, Boulanger CM, Willeit J, Chowiencyzk PJ, Kiechl S & Mayr M (2012). Prospective study on circulating microRNAs and risk of myocardial infarction. *J Am Coll Cardiol* **60**, 290–299.

Zhou B, Ma R, Si W, Li S, Xu Y, Tu X & Wang Q (2013). MicroRNA-503 targets FGF2 and VEGFA and inhibits tumor angiogenesis and growth. *Cancer Lett* **333**, 159–169.

**Additional information**

**Competing interests**

T.T. and J.F. have filed several patents about ncRNAs. T.T. is co-founder of Cardior Pharmaceuticals. F.P.K. declares no competing interest.

**Author contributions**

T.T. held the presentation at GRC related to the manuscript. F.P.K. and J.F. wrote the initial draft of the manuscript, all authors revised later versions. F.P.K. designed the initial drafts of the figures, F.P.K. and J.F. revised the figures. All authors approved the final version of the figures and the manuscript, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

**Funding**

The study was supported by the ERC Grant LONGHEART, and the ERA Net Grant Expert (to TT).

**Acknowledgements**

F.P.K. is enrolled in the Molecular Medicine programme of the Hannover Biomedical Research School (HBR S). The image “Heart” of the Abstract figure by Servier is licensed under CC BY 3.0.