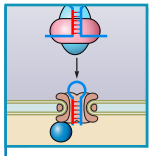
Physiol Rev 96: 1297–1325, 2016
Published August 17, 2016; doi:10.1152/physrev.00041.2015

NON-CODING RNAs IN DEVELOPMENT AND DISEASE: BACKGROUND, MECHANISMS, AND THERAPEUTIC APPROACHES

Julia Beermann, Maria-Teresa Piccoli, Janika Viereck, and Thomas Thum

Institute of Molecular and Translational Therapeutic Strategies, Hannover Medical School, Hannover, Germany; and National Heart and Lung Institute, Imperial College London, London, United Kingdom



Beermann J, Piccoli M-T, Viereck J, Thum T. Non-coding RNAs in Development and Disease: Background, Mechanisms, and Therapeutic Approaches. *Physiol Rev* 96: 1297–1325, 2016. Published August 17, 2016; doi:10.1152/physrev.00041.2015.—Advances in RNA-sequencing techniques have led to the discovery of thousands of non-coding transcripts with unknown function. There are several types of non-coding linear RNAs such as microRNAs (miRNA) and long non-coding RNAs (lncRNA), as well as circular RNAs (circRNA) consisting of a closed continuous loop. This review guides the reader through important aspects of non-coding RNA biology. This includes their biogenesis, mode of actions, physiological function, as well as their role in the disease context (such as in cancer or the cardiovascular system). We specifically focus on non-coding RNAs as potential therapeutic targets and diagnostic biomarkers.

I. INTRODUCTION

In the past years new technologies such as next generation deep sequencing have shown that most of the genome is transcribed into RNAs. However, only 1-2% of the human genome codes for proteins (68) dividing the RNA world in two halfs: *1*) RNAs that have coding potential and *2*) RNAs without coding potential, referred to as non-coding RNAs (ncRNAs). Although mRNAs were already studied extensively, ncRNAs account for the majority of RNAs. In the past, ncRNAs were considered as "evolutionary junk," but increasing evidence suggests a huge impact on several molecular mechanisms. Furthermore, the amount of ncRNAs in an organism correlates with its complexity. This suggests a tremendous influence of ncRNAs on the development and organization of higher structured animals.

NcRNAs are divided into two subclasses according to a relatively broad size threshold. NcRNAs smaller than 200 nucleotides (nt) are called small or short non-coding RNAs, whereas the ones longer than 200 nt are called long non-coding RNAs (lncRNAs). These two groups themselves are rather heterogeneous. Small ncRNAs can range from few to 200 nt, while lncRNAs have a size up to several kilobases. MicroRNAs (miRNAs) with a size of ~20 nt are the most known group of small ncRNAs and have been studied already extensively. Their main action is the negative regulation of gene expression by binding a target mRNA and induction of its degradation or inhibition of its translation. For detailed reviews on miRNA biology, see Ha and Narry Kim (98) or Castel and Martienssen (40).

Compared with miRNAs, the mechanistic characterization of lncRNAs is rather poor. This is partly due to the fact that lncRNAs can regulate gene expression at multiple levels in the cell through complex molecular mechanisms. Furthermore, unlike miRNAs, lncRNAs are relatively poorly conserved in terms of nucleotide sequence, even though they can be found in a broad range of species (96, 125, 220), including plants (256), yeast (111), prokaryotes (20), and viruses (227). This limits the availability of cellular and animal models for investigation of lncRNA functions, even though lncRNAs differing from each other in their nucleotide composition can still show the same three-dimensional structure, and therefore, the same molecular function (60, 217).

Increasing evidence is pointing towards lncRNAs as regulators of almost every cellular process, and expression of these non-coding molecules seems to be strictly regulated in physiological conditions as well as in several human diseases, including cancer (258, 286).

It is known that lncRNAs can be transcribed from almost every locus of the human genome and in different orientations compared with coding genes. Some lncRNAs are tran-

scribed from regions overlapping one or more exons of another coding transcript (sense lncRNAs), while others overlap coding genes on the antisense strand (antisense lncRNAs). Other lncRNAs arise from non-coding DNA sequences such as introns, or regulatory elements such as enhancers. Finally, some of them are transcribed from intergenic regions that do not overlap any other known coding gene, and have their own promoters and regulatory elements (261).

When considering the theoretical number of possible existing lncRNAs, it becomes evident that only a small amount of them have been already investigated. However, those that have been characterized showed the ability to regulate gene expression at a transcriptional and posttranscriptional level through interaction with nucleic acids and proteins in both a sequence-specific and a structure-specific manner (191, 290).

From a more general point of view, the classification and annotation of putative lncRNAs needs to be deeply assessed to exclude RNAs that are actually protein-coding. Indeed, it has been recently reported that, despite their classification as non-coding molecules, some lncRNAs can code for micropeptides (5). A skeletal muscle-specific RNA, which was assumed to be a lncRNA, encoded for a functional micropeptide, indicating that every newly investigated lncRNA needs to be validated as a non-coding transcript, before drawing conclusions on its regulatory role.

Recently, evidence showed that expression of ncRNAs is not limited to classical mechanisms. A backsplicing variant of expression gives rise to so-called circular RNAs (circRNA). CircRNAs are made of a covalently closed continuous loop and thus lack a 5' cap and 3' tail. This RNA species is not only conserved very well, it is also relatively stable and more tissue-specific (124).

The emerging links between non-coding RNAs and diseases have opened up a new field of therapeutic and diagnostic opportunities. Many miRNA have already successfully been shown to serve as biomarkers or therapeutic targets for many different diseases. There is also evidence that the same holds true for lncRNAs and circRNAs.

II. DEFINITION AND BIOGENESIS OF ncRNAs

It is evident that RNA molecules exhibit many more functions instead of being a simple template for protein synthesis. Considering the ability of RNA to form three-dimensional structures and to interact with DNA, proteins, and other RNA molecules, non-coding transcripts are assumed to be as versatile as proteins allowing them to orchestrate most cellular processes.

In addition, the number and categorized classes of such functional ncRNAs, ranging from ~20 to thousands of nucleotides, has been strongly expanded in the last decade. The length of ncRNAs is an important criterion to define two major classes of regulatory RNAs: small and long ncRNAs. In this review, we mainly focus on microRNAs and lncRNAs, including circRNAs and their role in developmental processes as well as in diseases such as cancer and mainly cardiovascular diseases.

A. MicroRNAs

MiRNAs are defined as single-stranded ncRNAs of ~20 nucleotides in length that are endogenously expressed and regulate gene expression on the posttranscriptional level. Since their first description in the worm *Caenorhabditis elegans* (155), thousands of miRNAs have been identified in flies, plants, and mammals (219). The conserved structure and biogenesis of this RNA species harbors the key to understand the modulation of gene expression by miRNAs.

Genes encoding for miRNAs are located throughout the genome, and a large proportion is found organized within clusters comprising multiple miRNAs. To some extent, miRNAs are encoded within or overlap to protein- or non-coding genes that relate their expression to the transcription and processing of such host genes. However, miRNAs can also arise from autonomous transcriptional units (214, 234). During biogenesis, miRNAs pass a multistep process including transcription, nuclear maturation, export, and cytoplasmatic processing before evolving into a functional RNA (see **FIGURE 1**).

A large proportion of miRNA genes are transcribed as polycistrons by RNA polymerase II (Pol II) (53), while a minor group depends on RNA Pol III. Transcription leads to large precursors (pri-miRNA) that exhibit mRNA-like modifications including 5' capping and 3' polyadenylation and that are characterized by a hairpin structure covering the mature miRNA sequence in the stem (11). This is followed by two processing events. The first is mediated by a nuclear complex, named microprocessor, comprising the DGCR8 protein (DiGeorge syndrome criticial region 8) that recognizes the pri-miRNA and directs the cleavage by RNAse III endonuclease Drosha (58, 156). The resulting hairpin precursor of ~60 nucleotides (pre-miRNA) translocates from the nucleus to the cytoplasm mediated by Exportin-5 and RAN-GTP (23, 176, 300). In a second step, the RNase III endonuclease Dicer interacting with TARBP (TAR RNA binding protein) cleaves the precursor close to the terminal loop releasing RNA duplexes of ~22 nucleotides (63). This duplex immediately associates with Ago proteins (Argonaute) assembling the miRNA-induced silencing complex (miRISC) (11). The mature miRNA (guide strand) retains in the complex, while the other strand of the duplex (passenger strand, miRNA*) is degraded. The se-

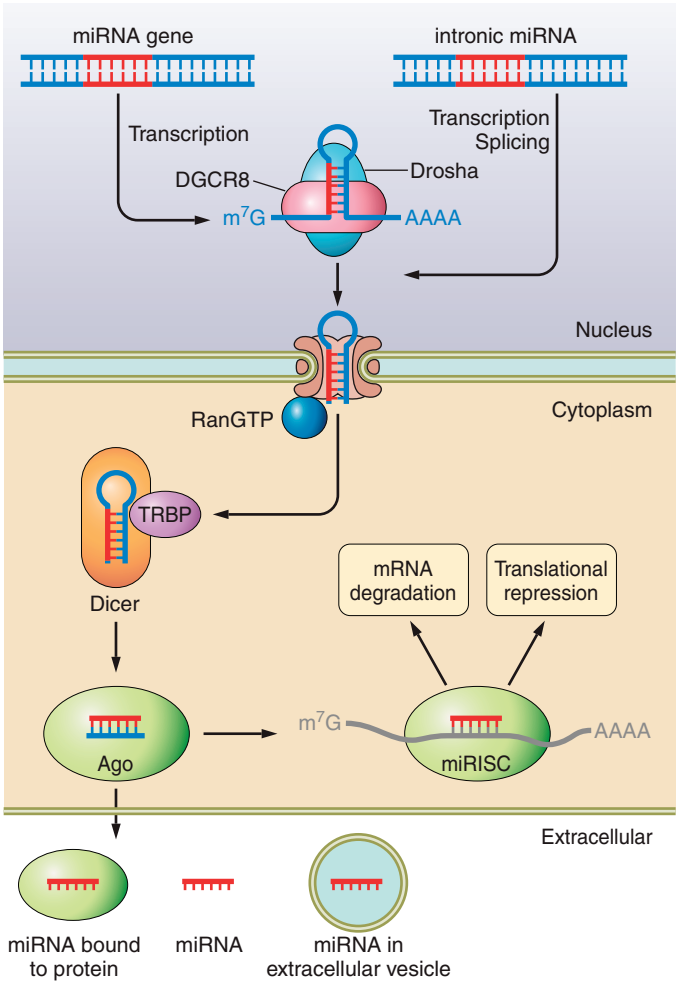


FIGURE 1. Biogenesis and function of microRNAs (miRNAs). miRNAs are transcribed as longer precursors or are derived from introns, and mature via endonucleolytic processing. Mature miRNAs are incorporated into the RNA-induced silencing complex (RISC) and regulate target transcript expression by degradation or translational repression. Non-coding RNAs (ncRNAs) can be secreted into the extracellular space, stabilized in vesicles or proteinous binding partners.

lection of the guide strand varies spatially as well as temporally and depends on properties of the precursor or further processing factors (82, 207).

Not all miRNAs known meet this classical or canonical route of biogenesis. In an alternative route (98), the noncanonical pathway, miRNAs are derived from short hairpin introns, named mirtrons (206). The first processing step by Drosha is substituted by the splicing machinery. This cleavage event generates an intermediate that is debranched before the hairpin structure is formed (98). The resulting pre-miRNAs are processed according to the canonical biogenesis pathway. Furthermore, pre-miRNAs can be derived from small nucleolar RNAs (snoRNAs) (69) or tRNA precursors (9).

B. LncRNAs

LncRNAs encompass the largest proportion of the non-coding transcriptome and are of emerging interest. However, they are the least studied ncRNA species, still a heterogeneous group and so far not well defined. New classes, characteristics, and functions are described and annotated each year. In general, the term lncRNA refers to transcripts that are longer than 200 nucleotides and do not harbor protein-coding signatures. This length threshold is a simple but convenient biophysical cut-off that separates lncRNAs from smaller ncRNA species such as miRNAs, siRNAs, and others (184, 197). Some lncRNAs, named macroRNAs, reach exceptional lengths, extending beyond 90 kb like the transcripts Airn and KCNQ1OT1 (141, 245). The criterion "non-coding" refers to the lack of open reading frames and/or conserved codons in transcripts, but remains controversial (8, 97, 118). Recent analysis provides evidence that lncRNAs can be engaged by ribosomes (16, 119), and some transcripts give rise to small peptides (119). However, the capability to encode a peptide does not necessarily exclude that a transcript serves as a functional RNA. Indeed, there is growing evidence of bifunctional transcripts serving both as protein templates and lncRNAs (64, 192).

Another type of classification of lncRNAs is based on their genomic organization or their relation to protein coding genes: *1*) sense lncRNAs overlap one or more exons of a coding gene; *2*) antisense transcripts exhibit partial or complete complementarity to transcripts on the opposite strand; *3*) intronic lncRNAs are produced from an intron of a gene; *4*) bidirectional transcripts share the same promoter with protein-coding genes, but are transcribed in the opposite direction; *5*) intergenic lncRNAs (lincRNAs) are located between protein-coding genes and are transcribed independently; *6*) enhancer RNAs (eRNAs) are produced from enhancer regions of protein-coding genes; and *7*) circRNAs originate from splicing events of protein coding genes and form covalently closed continuous loops.

Compared with protein-coding genes, which usually exhibit sequence similarities or identities among species, lncRNA transcripts in general appear less conserved, but more than introns or intergenic regions (94, 267). However, lncRNA can arise from ultraconserved regions (UCRs) (121), sequence stretches with near-perfect identity, and several transcripts exhibit conserved promoter regions and splice sites or are expressed from syntenic genomic loci relative to protein-coding genes (267), indicating that they might be functionally related regardless of their divergent sequences. Furthermore, conservation may be found in secondary structure motifs rather than in primary sequence stretches and/or due to functional interactions with DNA, RNA, or proteins (62). Differences in sequence conservation between coding and non-coding genes become evident comparing two well-studied lncRNAs. The X chromosome inactivating lncRNA Xist exhibits poor sequence similari-

ties, but well conserved functionality (78), while the sequence of the nuclear-enriched transcript Malat1 is well conserved among several mammals, but its role in murine and human cancer is divergent (62). Taken together, conservation of lncRNAs needs to be defined from a different angle considering sequence, structure, and function to entirely define the evolutionary relation between lncRNAs of different species.

1. LncRNA biogenesis and structure

The biogenesis of lncRNAs occurs in the nucleus and mirrors the synthesis of protein coding transcripts. lncRNA promoters are often epigenetically marked with histone modifications as well as bound and regulated by transcription factors favoring or hampering gene expression (96). As mRNAs, many lncRNAs are transcribed by Pol II, while other lncRNA promoters harbor structures that are more likely to be transcribed by Pol III. This regulation allows a precise fine-tuning of lncRNA expression to temporal or spatial events. In general, lncRNAs are present in lower amounts compared with mRNAs, but their expression is considerably more restricted to specific cell types (35, 60). Posttranscriptional processing is another feature that lncRNAs share with their protein-coding counterparts: a large proportion of lncRNAs is capped and polyadenylated (39, 131). RNA editing events are capable to alter non-coding transcripts as well (224). Nearly all lncRNAs exhibit canonical splice sites leading to at least two transcript isoforms that are mainly composed of two exons (60). In contrast to mRNAs, lncRNAs can be found in different compartments of the cell. After their biogenesis and processing, mRNAs are released into the cytoplasm that is true for several lncRNAs as well, while most of the non-coding transcripts retain in the nucleus and are recruited to the chromatin (60) (see **FIGURE 2**).

The final step in the lncRNA biogenesis and a crucial feature of lncRNAs is the ability to form thermodynamically stable structures. On the primary structure level, the functional activity of lncRNAs relies on Watson-Crick base pairing, facilitating direct interactions with other RNA molecules like mRNAs and miRNAs or DNA (204). Secondary structures including helices, hairpin loops, bulges, and pseudoknots are shaped by base-pairing as well or interactions of the ribose backbone that allow the formation of higher-order configurations (52, 192). In many cases, the structure determines the RNA function, rather than the primary sequence. For example, the function of HOTAIR as a tumor suppressor is based on conserved secondary structure elements that surround protein-binding motifs of this lncRNA (246). So far, three-dimensional RNA structures have been attempted more for ribozymes including components of the ribosome or spliceosome, rather than for lncRNAs (204). These findings and the fact that lncRNAs are capable to determine the macromolecular architectures like nuclear paraspeckles (48) suggest that non-coding transcripts are capable to form such higher-order structures as well. Although lncRNAs are suggested to be more structured than their protein-coding counterparts (178, 295), their structural versatility might play a critical role enabling lncRNAs to form binding sites for the interaction with proteins, DNA, and other RNA molecules. This suggests an important interplay between the lncRNA structures and their biological function. Nevertheless, the process of lncRNA folding including the discovery of auxiliary factors and the structure-to-function relationships of non-coding transcripts are still at the very beginning to be elucidated and will be an essential and interesting challenge for future research to uncover new regulatory mechanisms of lncRNAs.

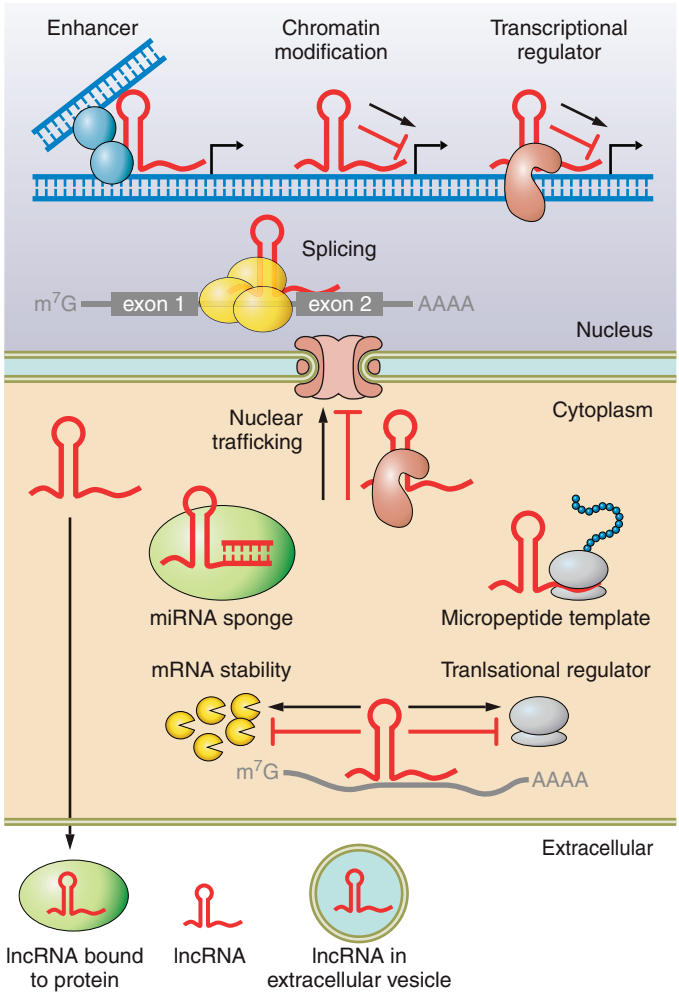


FIGURE 2. Biogenesis and function of long non-coding RNAs (lncRNAs). lncRNAs control the expression of genes in the nucleus by interacting with DNA, chromatin modifying complexes, and/or various transcriptional regulators. Cytoplasmatic lncRNAs act as sponges for other transcripts or proteins, serve as protein templates, or regulate mRNA degradation and translation. Like miRNAs, they also can be secreted and found in the extracellular space.

C. CircRNA Origin and Characteristics

The identification of circRNAs reshaped the perspective of the so far linear RNA world. Unlike known unidimensional

lncRNAs, the 3' and 5' ends of circRNAs normally present in a RNA molecule are covalently linked together forming single-stranded continuous loop structures. CircRNAs are found in all kingdoms of life; they are highly abundant in eukaryotes, evolutionary conserved, and can be specific for certain cell types or developmental stages (102, 186, 237, 303). Like linear lncRNAs, they are RNA species of a great variety. Corresponding genes are spread over the entire genome and located within or outside of protein coding genes. Their size ranges from 100 nt to over 4 kb and harbor single or multiple exons (237, 303). Specific characteristics of circRNAs are their extraordinary stability, due to the lack of exposed ends which are prone to nucleolytic degradation, and certain RNA folding determined by their structure (124).

CircRNAs usually arise from splicing events and circularization of exons or introns (237). Exonic circRNAs depend on spliceosomal splicing and can be generated by a process that is referred to as back splicing. Here, exons are spliced in reverse order by joining the upstream 3' splice site with a downstream 5' splice site leading to a circular product (50, 102, 186). In another mechanism exonic circRNAs result from exon skipping that forms a lariat structure harboring exons and introns (101, 124). This precursor undergoes self-splicing, whereas the intron is removed, and the lariat finally circularized (134) (see **FIGURE 3**).

Recent findings indicate that flanking intronic complementary sequences mediate exon circularization (302). The biogenesis of intronic circRNAs can be both dependent on and independent from the spliceosome. In spliceosomal splicing, circRNAs are derived from introns that are circularized at the 2'-5' branch point. This lariat harbors a 3' tail that is trimmed from the end to the branch point, but somehow escapes complete degradation resulting in a stabilized circRNA (303). Autonomously spliced intronic circRNAs evolve from autocatalytic ribozyme activities. Group I introns require an exogenous guanosine cofactor (202, 303), while group II introns undergo self-splicing (222, 303). Finally, a continuous loop is formed by a covalent 2'-5' phosphodiester bond. Following their biogenesis, circRNAs are released from the nucleus to the cytoplasm (102, 124, 186, 237), by a so far unknown transport mechanism, where they unfold their unique biological function described below.

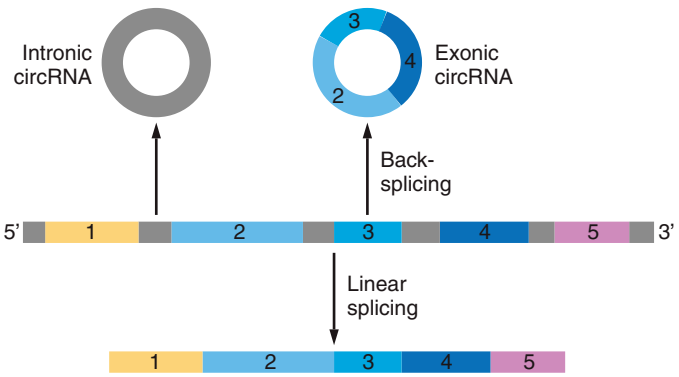


FIGURE 3. Biogenesis of circular RNAs. A model showing linear and back splicing for generating linear mRNA and exonic or intronic circRNA.

III. FUNCTION OF microRNAs, lncRNAs, AND circRNAs

A. Functions of microRNAs

The hallmark of the miRNA function is their ability to suppress gene expression by binding their target RNAs in a sequence-specific manner. Recognition is mainly mediated by Watson-Crick pairing of the miRNA seed region within matching nucleotides within the 3' untranslated region (3' UTR) of target RNA (11, 160). A small proportion of miRNAs is capable to regulate the expression of RNAs by recognizing and targeting other mRNA proportions (151). The strength of the binding between a target site and the miRNA depends on full or partial complementarity that mainly determines the nature of the target repression. The number, position, and accessibility of target sites, flanking regions, and secondary structures as well as sequences alterations by RNA are further determinants.

MiRNA-RNA targeting relies not only on the interaction between naked RNA molecules, but also on the association with effector proteins in the miRISC (11). When incorporated, miRNAs activate this complex and guide its activity to the target site. Finally, the interaction between miRNA and RISC leads to the target repression either by hampering the translational machinery or favoring nucleolytic mRNA degradation. Translation inhibition is mediated by Ago proteins that compete with factors that direct ribosomal subunits or make translation initiation accessible, or that lead to a dissociation of the translational machinery. Target degradation can be achieved by deadenylation from the 3' end or by decapping of the 5' end attracting endonucleolytic and exonucleolytic activities (71). More detailed functions about miRNAs can be found in the key reviews cited in **TABLE 1**.

Next to their conventional role in posttranscriptional gene regulation, there are several evidences that these ncRNAs appear also in unusual places and with surprising other functions. This includes findings of miRNAs to induce target translation by recruiting respective ribonucleoprotein complexes (273). Another recently discovered function of miRNA biology depends on their presence in the nucleus (164, 229). In contrast to the conventional assumptions of biogenesis and function in the cytoplasmatic compartment, certain miRNAs and adjacent Ago proteins are capable to accumulate in the nucleus where they influence the biogenesis of other miRNAs as well as other RNA species. So far, there is limited knowledge about the underlying mecha-

Table 1. *Key References summing up miRNA functions*

Authors	Reference Nos.
Bartel	12
Ha and Narry Kim	98
Lin and Gregory	168
Mendell and Olson	187
Sayed and Abdellatif	240
Winter et al.	291

miRNA, microRNA.

nisms. The functional relevance of nuclear miRNAs needs to be elucidated in more detail.

Another intriguing aspect of miRNA biology is the fact that miRNAs are not only restricted to processes within the cell, but that they are also present outside the cell in blood, plasma, breast milk, urine, and other body fluids (93). Circulating miRNAs are indeed highly stable and resistant to RNAse activity, since they are associated with proteinous carriers or incorporated into several forms of vesicles or microparticles. Here, miRNAs function as signaling molecules transferring genetic information between cells or tissues (276). Since miRNA expression patterns are strongly correlated with disease conditions including cardiovascular disease, circulating miRNAs are of great value for the use as diagnostic markers.

B. Functions of LncRNAs

Functions of lncRNAs are more complex and diverse than that of miRNAs. lncRNAs are generally expressed at a lower level but show more cell type-specific expression patterns than that of the protein-coding genes. Additionally, lncRNAs are less conserved than miRNAs (14, 96, 220) because they seem to be under more selective pressure especially in their promoter regions (60). These characteristics make it more challenging to establish functional motives for the action of lncRNA. The large number of transcripts [14,880 according to Derrien et al. (60), now already 118,770 human annotated lncRNAs according to lncipedia.org] is just in few parts investigated yet and many lncRNAs await functional characterization. Taking into consideration what has been published to date, different main groups of action for lncRNAs in accordance to Geisler and Coller (80) can be catalogued, where lncRNAs are grouped according to the level of acting on expression changes: lncRNAs acting as regulators of transcription; this takes place in the nucleus as well as in the cytoplasm by interacting of lncRNAs with transcription factors for instance. There are many examples of transcriptional regulation, either by enhancer RNAs (eRNAs) or *cis*- or *trans*-acting lncRNAs. For instance, IL1β-eRNA and IL1β-RBT46 eRNAs attenuate LPS-induced mRNA transcription in human innate immune responses (117); Xist regulates genomic imprinting in *cis* (153), whereas HOTAIR is a *trans*-acting regulator of the HOXD genes (228). lncRNAs can also act on the processing of mRNAs. Splicing is one mechanism that was shown to be influenced by lncRNAs. For instance, natural antisense transcripts (NATs) influence splicing patterns from the neuroblastoma MYC intron 1 splicing which is the sense encoded gene (17). Also MALAT1 is known to regulate Ser/Arg splicing factors via an unknown mechanism (264).

Another class of lncRNAs is involved in posttranscriptional control mechanisms; these lncRNAs can sequester miRNAs to keep them away from acting on their targets (like CDR1as, LINCMD1, or HULC). For instance, the cytoplasmic 1/2-sbsRNA promotes mRNA decay by base pairing with Alu-elements in the 3' UTR of their target mRNA (86). Another example is the lincRNA-p21, which suppresses target mRNA translation by recruitment of translation repressor proteins (301).

In the following, we want to guide the reader through the different so far known functions of lncRNAs. The classification given above facilitates us to state well-investigated examples of lncRNAs and go into more detail of their mode of action. This fascinating area of research revealed involvements in developmental regulation, chromatin modification, enhancer functions, genomic imprinting, DNA damage response, neural differentiation, and cell proliferation to name just a few of them.

1. Regulation of transcription

The level of lncRNA expression often correlates with that of antisense coding or neighboring genes but at lower levels (60). In contrast to proteins acting as chromatin modifiers, lncRNAs do not have to reenter the nucleus to exert their regulation. They can function in the same place where they are transcribed making them perfect candidates for a *cis*-regulatory way of action.

Xist is one of the earliest investigated lncRNAs. Discovered in 1991 (25, 27, 29, 30), Xist was shown to be involved in the complex regulation of X-chromosome inactivation (XCI), where it is also expressed. The process of XCI ensures that in female mammals the vast majority of genes from one X chromosome is silenced to correct for the double amount of X-chromosome genes (177). Inactivation of the X-chromosome is regulated spatially and temporarily due to the controlled transcription of the lncRNA Tsix and its antisense counterpart Xist, which is acting at the same time as a molecular signal and as a guide for the polycomb repression complex 2 (PRC2). PRC2 is a chromatin modifying complex that silences genes by methylation of histone 3 and, in early developmental stages, the loading of PRC2 onto the chromatin is prevented by Tsix (233, 253). After Xist transcription, PCR2 is recruited to the nucleation site

of inactive X-chromosome (305). Finally, Xist spreads through the entire X chromosome, which is turned into transcriptionally inactive heterochromatin (49, 180, 223). Additionally, Xist promotes this silencing by interaction with SHARP, a protein that recruits SMRT and HDAC3 to exclude Pol II from the inactive X (185).

This whole procedure shows how one single lncRNA can itself be regulated by a complex interplay of other *cis*-lncRNAs. Especially the sense/antisense regulatory pairing as seen here by Xist/Tsix seems to be a control mechanism repeatedly observed (72, 133).

Another example of antisense/sense pairing is the action of Airn on the paternal Igf2r cluster (150, 200, 245). Airn is a macroRNA, 108 kb in size, which is transcribed only from the paternal allele antisense to the Igf2r cluster (245). Like Xist, it functions in *cis* to silence Igf2r, Slc22a2, and Slc22a3 on the paternal allele. Similar to Xist, Airn regulates Slc22a3 expression by recruiting the histone methyltransferase EHMT2 to the genomic locus. Accumulated Airn at the promoter recruits EHMT2 and leads to target H3K9 methylation and allelic silencing (200). Interestingly, in case of Igf2r-repression, Airn silencing does not even require a stable RNA product. The lncRNA transcription itself is sufficient to interfere with Pol II recruiting to the target (150). The Airn sequence overlays the Igf2r promoter and this, by being continuously transcribed, prevents transcription initiation on the Igf2r promoter (150). This way of regulation fits the characteristics of macroRNAs.

Like Airn macroRNAs are rather long, not conserved, inefficiently spliced, and have a short half-life (241). It remains to be investigated whether there are more functional examples for this type of lncRNA.

Despite these well-known examples of *cis*-regulation (29, 140, 245), it has been shown so far that the vast majority of lncRNAs are not acting in a *cis*- but a *trans*-regulatory manner (95, 96). About 20% of the tested human lincRNAs are bound to PRC2 and others to other chromatin-modifying complexes. Moreover, a knockdown of HOTAIR and five other exemplary lincRNAs did not affect the expression levels of genes in vicinity, suggesting a *trans*-mechanistical regulation (135).

The human HOX loci harbor the sequences for over 200 lncRNAs (242). One of them is the *trans*-regulatory acting lncRNA HOTAIR (HOX Antisense Intergenic RNA) (228). It arises from the HOXC locus, but acts as a repressor of the HOXD locus which is not even on the same chromosome (228). HOTAIR physically associates not only with PRC2 but also with LSD1 (Lys-specific demethylase 1), both of them chromatin modifying proteins (265). HOTAIR acts as a scaffold by providing binding platforms to arrange the interaction of multiple enzymes, a function that has been described for many different lncRNAs.

LncRNAs can also arise from enhancer elements and may have enhancer-like functions. Enhancers are regions of DNA that can be bound by proteins to activate the transcription of their targeted genes (209). This is often, but not always, related to nearby located genes. Enhancer elements can activate genes independently of their genomic distance, orientation, and location (34, 210). Enhancer regions are one part of the complex machinery making it possible to adapt gene expression to specific events. It is likely that there are more than 1 million enhancer regions existing (213). A large number of enhancer regions produce RNA, the so-called eRNA or enhancer-associated lncRNA (elncRNA) (137). This class of long non-coding RNAs is detected in most cell types (199). Recent discoveries showed a role for eRNA in promoting gene expression through chromatin accessibility (198). It is not yet clear what part eRNAs play exactly in the regulation of enhancer activity. However, the production of eRNAs seems to be linked to enhancer activity. Loss-of-function studies showed that degradation of eRNA is enough to reduce expression of neighboring genes (162, 211, 282).

In the cytoplasm, non-coding RNAs can alter mRNA stability, protein trafficking, or stability (192). The regulation of the nuclear factor of activated T cells (NFAT) in the cytoplasm shows a complex organization of a lncRNA and several proteins. Using a shRNA collection to inhibit selected lncRNA candidates, a "dramatic activation" of NFAT was shown after targeting one specific lncRNA: NRON (non-coding repressor of NFAT) (289). A model was suggested where NRON complexes with proteins to regulate the trafficking of NFAT. The lncRNA serves as a platform of a high-molecular-weight complex; NRON stabilizes the scaffold protein IQGAP, resulting in a complex of NFAT and NFAT kinases in resting cells. The action of NFAT is regulated by its phosphorylation level. In the case of suiting stimuli of the cell, NFAT dissociates from the complex, is dephosphorylated and shuttled into the nucleus, where its leads to the expression of NFAT-regulated genes (243, 289).

2. Regulation of mRNA processing/posttranscriptional control

Since lncRNAs are very versatile regulators, they can exert their function in different compartments of the cell. Most of the in detail investigated lncRNAs so far are acting in the nucleus on the regulation of gene expression itself (e.g., H19, involved in imprinting; Anril, recruitment of PRC2 and PRC1; NEAT1, assembly of paraspeckles) (142, 179, 238, 298). However, there are also examples showing regulation at the mRNA level in the cytoplasm (see below for examples).

The lncRNA TINCR (terminal differentiation-induced ncRNA) was shown to be required for the control of human epidermal differentiation. It is a lncRNA 3.7 kb in size and is highly enriched in the cytoplasma of keratinocytes (144). TINCR is needed for normal induction of key protein mediators of epidermal differentiation, which was investigated by RNAi lack-of-function studies (144). Interestingly, a genome-wide interaction analysis revealed that TINCR interacts directly with a specific set of differentiation-associated mRNAs to stabilize them and keep up their abundance. The interaction is mediated via a so-called "TINCR box," a 25-nt motif enriched in target mRNAs as well as in TINCR itself which was detected by a de novo motif search. It was also shown that TINCR forms a complex with STAU1 to enable the interaction with mRNAs. STAU1 is a known RNA-binding protein, with no known involvement in epidermal differentiation so far (66, 136). TINCR seems to interact with STAU1 to form a complex necessary for mRNA stabilization (144), demonstrating a surprising mode of action of a non-coding RNA.

lncRNAs are often reported to be involved in the regulation of cell survival or apoptosis. Driven by the hypothesis that cell-cycle genes could harbor lncRNAs, Hung et al. (115) screened the genomic region of cyclins, cyclin-dependent kinases (CDK) and their inhibitors (CDKI) for potential lncRNA hits (115). This group discovered PANDA (P21 associated ncRNA DNA damage activated) to be a 5′-capped and polyadenylated nonspliced lncRNA that is transcribed antisense to the CDKI-coding gene CDKN1A. PANDA promotes cell survival by interacting with the transcription factor NF-YA and keeping it from occupying target gene promoters. PANDA and CDKN1A expression is both p53-dependent, but interestingly CDKN1A encodes a cell cycle inhibitor to mediate cell cycle arrest, whereas PANDA promotes cell survival by turning on the apoptotic gene expression program. The lncRNA acts as a RNA decoy by titrating away the transcription factor from its target. Intriguingly, the CDKN1A promoter not only codes for PANDA, but also four other lncRNAs. In 2010, the p53-induced lncRNA lincRNA-p21 (113) was identified which is located 15 kb upstream of CDKN1A and was reported to interfere with the cell's response to DNA damage. All these multiple transcripts encoded in the regulatory sequence upstream of CDKN1A acting on the same biological pathways seem to cooperate to regulate DNA damage response.

The above named examples illustrate the versatile and fascinating biology of lncRNAs and importance for the cell.

C. Functions of CircRNAs

CircRNAs were already described in the early 1990s, when Nigro et al. (203) performed a RNA expression assay and identified several abnormal spliced transcripts. These transcripts seemed to be somehow "scrambled" and joined at the ends. These "scrambled exons" showed for the first time a circular shape of RNA but seemed to be very low expressed (203). This assumption was contrasted when Capel et al. (38) published in 1993 a study about the circRNA Sry, which represents the most abundant RNA transcript in mouse testis.

First functional studies identified several modes of action for circRNAs (123). CircRNA expression is highly cell-type specific and well conserved among species (124, 236). In addition, circRNAs are more stable than associated linear mRNAs (124).

Certain circular RNA transcripts have been shown to arise from the exons of the Fmn gene, which is important for mouse limb development. Their high level of expression suggested an important biological role (43). The authors of this study proposed a function of the circRNA as a mRNA "trap"; the formation of circular RNA traps the transcript coming from the Fmn gene and regulates that way the level of the resulting protein (43).

In 2013, two groups simultaneously published reports about a highly stable circRNA expressed in human and mouse brain with competing endogenous RNA function: CiRS-7 (according to Hansen et al., Ref. 101) or CDR1as (according to Memczak et al., Ref. 186). This circRNA contains more than 70 conserved binding sites for miR-7 and can efficiently suppress its activity by acting as a molecular sponge (101, 186). CiRS-7/CDR1as interacts with AGO and is itself cleaved by miRNA-671. The expression level of the circRNA and its target is overlapping, thus showing a potentially high degree of interaction. In vivo studies revealed that a CiRS-7/CDR1as overexpression leads to a similar phenotype of impaired midbrain development than that of a miR-7 knockout mouse (186). Supporting this role of function for circRNAs, it was shown that also Sry serves as a miRNA sponge for miR-138 (101). More circRNAs have been reported to harbor multiple miRNA binding sites, which seems to be a typical feature of this class of RNA molecules.

IV. ROLE OF ncRNAs IN ANIMAL DEVELOPMENT

MiRNAs are conserved molecules with tightly regulated expression patterns that play an important role in development. As a matter of fact, mice lacking miRNAs because of Dicer deletion die at embryonic day 7.5 showing a depletion of multipotent stem cells (19).

To circumvent the lethality of Dicer deletion during embryonic life, several conditional knockout mice have been created, showing that, at least in mice, the lack of Dicer and,

likely, the lack of miRNAs as well, leads to impaired morphogenesis of the lung epithelium (104), the vertebrate limb (103), and the skin (6, 299). In the heart, conditional knockout of Dicer leads to progressive dilated cardiomyopathy and heart failure (44) as well as congenital cardiovascular abnormalities (239). The role of specific miRNAs in the development of different organs and systems has been reviewed in detail (240). In the following paragraph we summarize recent evidences for lncRNAs involved in animal development and specifically in cardiac development.

Developmentally complex organisms harbor an increasing number of lncRNA loci in their genomes and these ncRNAs appear to regulate a number of developmental processes, such as cell differentiation, organogenesis, genomic imprinting, and dosage compensation (73). Several lncRNAs are already known to play a role in neurodevelopment (47). The importance of lncRNAs in development is also demonstrated by the fact that RNA modifications such as N^6-methyl-adenosine (m6A), which are commonly found in mRNAs, are also present on lncRNAs during development. These lncRNAs are conserved, abundant, and necessary for proper differentation and exit from the pluripotency state of mouse and human embrionic stem cells (15).

One fundamental step of development is the transition from a pluripotent stage to a differentiated one, which is markedly accompanied by substantial epigenetic changes. Here, lncRNAs seem to play a crucial role in the maintenance as well as in the loss of pluripotency in embryonic stem cells. Guttman et al. (95) performed a comprehensive loss-of-function study on most lncRNAs expressed in mouse embryonic stem cells. This led to changes in gene expression that are comparable to the ones caused by the knockdown of known transcription factors involved in stem cell biology, including exit from the pluripotency state or upregulation of lineage commitment programs (95).

An important epigenetic event that is associated with the loss of pluripotency in embryonic stem cells is the inactivation of the X chromosome. This is a process in which the lncRNA Xist and its antisense counterpart Tsix were shown to play a key role (154). Reprogramming of adult differentiated cells into induced pluripotent stem cells (iPSCs) requires the reactivation of the X chromosome (XCR), which is directly linked to pluripotency. Payer et al. (221) have shown that the germline factor PRDM14 and the lncRNA Tsix are required for XCR and deficiencies in both factors can impair XCR and acquisition of pluripotency of iPSCs.

Another lncRNA that can control pluripotency as well as neural lineage commitment is the lncRNA called TUNA (Tcl1 upstream neuron-associated lincRNA). Through the generation of a shRNA library targeting lncRNAs in mouse embryonic stem cells Lin et al. (167) identified 20 lncRNAs involved in the maintenance of a pluripotency state. In particular, they found the lncRNA TUNA to form a complex with other RNA binding proteins to target the promoters of Nanog, Sox2, and Fgf4. In addition to showing an expression profile restricted to the central nervous system, TUNA is conserved in humans and zebrafish, where its knockdown caused impairment of the locomotor functions. Furthermore, expression of TUNA correlates significantly with the severity of Huntington's disease in humans (167).

The lncRNA linc-RoR was found to be a competing endogenous RNA that shares common miRNA-response elements with Oct4, Sox2, Nanog, and other key transcription factors of pluripotent stem cells. Therefore, linc-RoR may prevent the miRNA-mediated suppression of these transcription factors and maintain the self-renewing potential of embryonic stem cells (284).

Apart from having a key role in stem cell differentiation, lncRNAs are also involved in the establishment of pregnancy in animals. Neat1, in particular, is a lncRNA that can be found in animal cells as a component of nuclear bodies called paraspeckles. The physiological role of Neat1 is not well understood, even if it is clear that Neat1 is a regulator of gene expression through retention of hyperedited mRNAs and/or transcription factors (see **FIGURE 4**).

It has been shown that Neat1 is crucial for pregnancy in mice. In fact, Neat1 knockout mice show normal ovulation but fail to become pregnant due to a severe impairment of luteal tissue formation and consequent low progesterone (201).

Interestingly, Neat1 is also involved in the development of mammary gland and lactation. When Neat1 is genetically removed, the morphogenesis of mammary gland is severely impaired causing defects in lactation. These negative effects are due to the decreased ability of the mutant cells to undergo high rates of proliferation during lobular-alveolar development, linking for the first time the formation of Neat1-paraspeckles to a specific biological function in vivo (249).

Finally, another lncRNA involved in human pregnancy is Meg3. In particular, the lncRNA Meg3 is downregulated in placental samples from preeclamptic patients and gain- and loss-of-function experiments done in two different trophoblast cell lines showed that Meg3 can reduce apoptosis and promote migration of trophoblast cells, influencing in addition the expression of NF-κB, caspase-3, and Bax protein. Abnormal levels of Meg3 during pregnancy in trophoblast cells might be underlying the failure of uterine spiral artery remodeling contributing to preeclampsia (304).

A. LncRNAs and Cardiac Development

LncRNAs are not only involved in general developmental aspects but also for selected organ-specific developments.

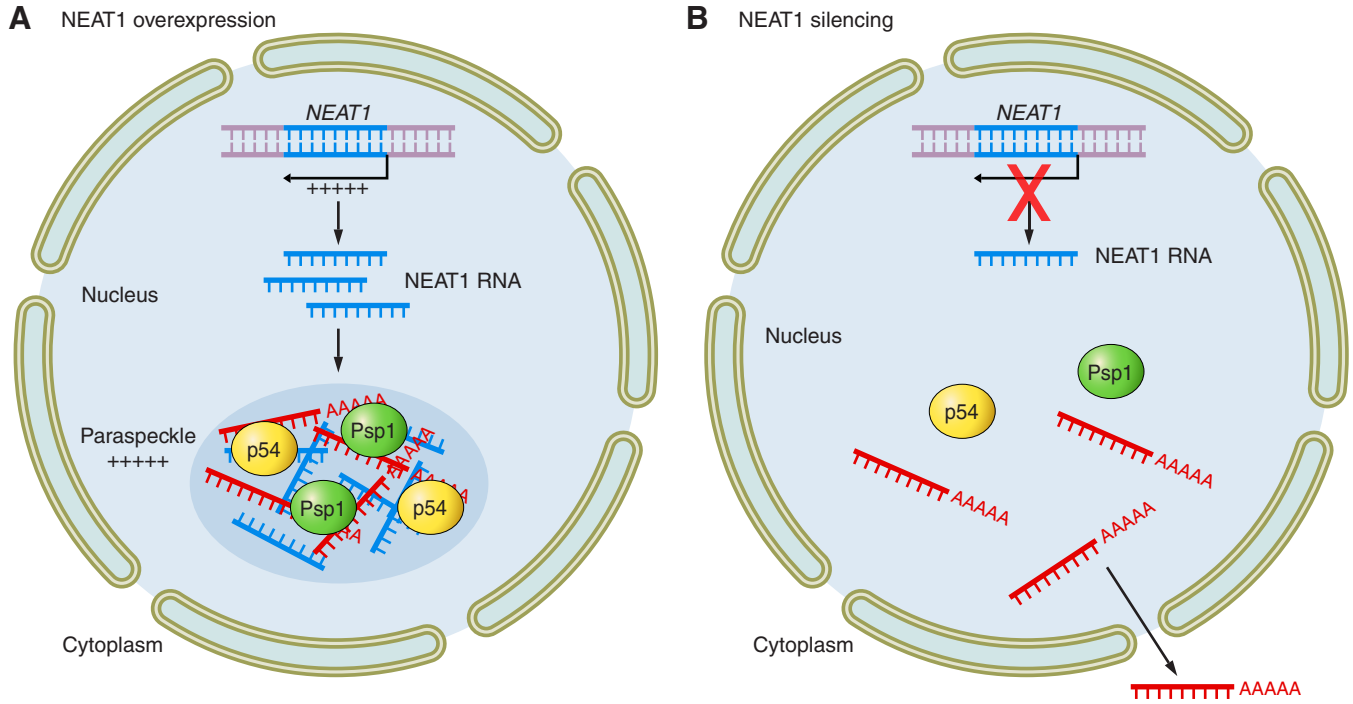


FIGURE 4. Structural role of NEAT1 in the formation of nuclear paraspeckles. NEAT1 (blue) is retained in nuclear structures, called paraspeckels, involved in nuclear retention of edited mRNAs (red). Overexpression of NEAT1 (*A*) increases paraspeckle number while silencing of NEAT1 via RNA interference (*B*) impairs their formation. Furthermore, NEAT1 directly binds to the mRNA editing proteins p54 and Psp1, allowing their incorporation into paraspeckels.

The first example of a lncRNA required for cardiomyocyte lineage commitment is the lncRNA Braveheart (Bvht) (138). The function of Bvht is to promote the expression of the protein mesoderm posterior 1 (MesP1), which is necessary for the development of cardiovascular progenitor cells. This function is probably carried out via the inhibition of PRC2-mediated silencing. However, the involvement of Bvht in heart development could only be described in mice, as this lncRNA is not conserved in humans, limiting translational implications of this study.

Epigenetic modulation of gene expression is crucial in developmental programs, and other lncRNAs seem to be involved in this process. The lncRNA Fendrr, for instance, was shown to be essential for proper heart development and probably exerts its functions at least in part by binding to chromatin remodeling complexes such as PRC2. As a matter of fact, mouse embryos lacking Fendrr show upregulation of transcription factors involved in lateral plate and cardiac mesoderm development (91). This upregulation correlates with a decreased occupancy of such factors by PRC2 complex as well as with epigenetic signatures of active expression. Since Fendrr can bind to epigenetic modifying complexes including both PRC2 and TrxG/MLL, it was suggested that the function of Fendrr could be to modulate the activity of such complexes to promote proper development of lateral mesoderm (91).

The regulatory networks involving lncRNAs in development are often complex. Examples are the lncRNAs SRA1 and linc-MD1, both involved in cell lineage differentiation, whose regulation must be tightly controlled in space and time. Linc-MD1 was first described as a sponge for miR-133 and miR-135, preventing these miRNAs from targeting Mef2c and Maml1, whose de-repression leads to muscle differentiation (41). More recently, a circuit of negative and positive feedbacks involving the ncRNAs linc-MD1, miR-133, and HuR protein during the early phases of muscle differentiation was described (157) (see **FIGURE 5**).

Specifically, the regulation of linc-MD1 expression can activate the progression towards later stages of muscle differentiation. Linc-MD1 forms a sponge for miR-133 and miR-135 and, interestingly, miR-133 is hosted by the linc-MD1 gene itself. Thus the transcription of one non-coding molecule excludes the biogenesis of the other. The factor controlling this switch between linc-MD1 and miR-133 is the HuR protein, which is targeted by miR-133 and normally triggers the accumulation of linc-MD1 by binding to the transcript and inhibiting Drosha cleavage (**FIGURE 5**). On the other hand, since HuR is a miR-133 target, the sponging activity of linc-MD1 reinforces HuR expression. Furthermore, HuR can cooperate with linc-MD1 in the cytoplasm, increasing the sponging ability of linc-MD1. The exit from this regulatory circuitry is triggered by an increase in miR-133, which is promoted by two unrelated miR-133 loci,

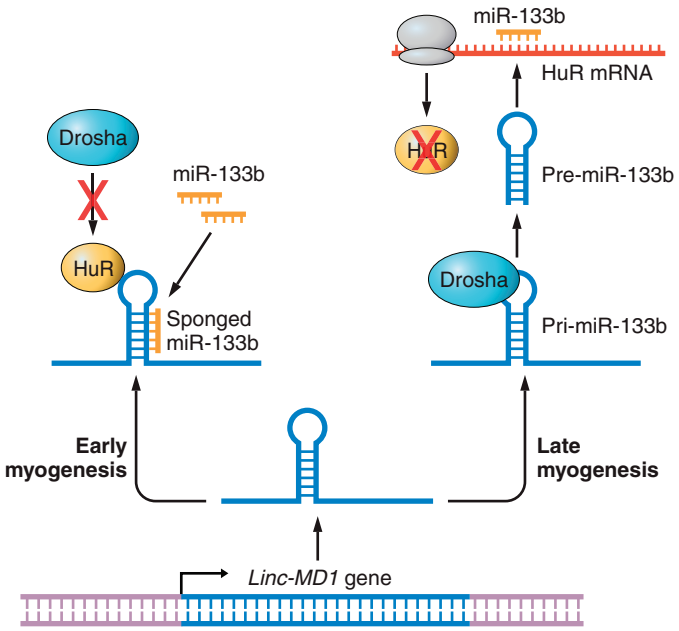


FIGURE 5. Feed-forward regulatory loop involving HuR protein and linc-MD1. In early phases of myogenesis, HuR protein consolidates the expression of linc-MD1, via inhibiting its Drosha-mediated cleavage. Therefore, the intact linc-MD1 transcript is free of sponging miR-133b, preventing the degradation of HuR. In late phases of myogenesis, an increase of miR-133b from unrelated loci leads to downregulation of HuR and consequent cleavage of linc-MD1 to generate further pre-miR-133b.

bringing to linc-MD1 downregulation and progression to later muscle differentiation stages.

Characteristics of the lncRNA SRA1 (steroid receptor RNA activator 1) include having a protein-coding counterpart arising from the same genomic location. SRA1 increases the activity of the transcription factor MyoD (myogenic differentiation 1), leading to myogenic differentiation. The SRA (steroid receptor RNA activator) gene has the property of functioning both as a ncRNA and a protein (SRAP) due to the alternative splicing of the first intron (114). Differently from SRA1, SRAP protein antagonizes muscle differentiation and the function of the lncRNA SRA1 by binding to its functional substructure STR7 **(FIGURE 6)**.

Additionally, many other lncRNAs have been described to be potentially important for muscular and/or heart development. However, clear insights into detailed functions and mechanisms are still missing. As an example, Matkovich et al. (182) identified 117 cardiac-enriched lncRNAs and compared expression profiles of these ncRNAs in normal embryonic, normal adult, and hypertrophic adult hearts. This group elegantly described a subset of lncRNAs that are characterized by a specific abundance in the embryonic heart tissue, which is slightly reflected in hypertrophic hearts. In some cases, these lncRNAs can directly regulate coding genes located in a 10-kb range of distance from their own locus or modulate NFkB- and CREB1-regulated genes, suggesting their importance for embryonic heart growth (182).

Further information on lncRNAs potentially important for heart development comes from the analysis of non-coding molecules that are deregulated in congenital heart diseases, which arise from abnormal heart development during embryogenesis. An insight into the role of lncRNA in the development of the cardiovascular system was provided by Song et al. (247) by finding an association between these non-coding molecules and ventricular septal defects (VSD). VSD are a common form of congenital heart disease and are tightly linked to a correct developmental program in the heart. The cause of such defects is currently not known, but the expression profile of lncRNAs is dramatically different in the heart tissue of VSD patients. In particular, the expression of two lncRNAs, ENST00000513542 and RP11-473L15.2, strongly correlated with the occurrence of VSD (247).

V. FUNCTIONS OF ncRNAs IN THE CARDIOVASCULAR SYSTEM

A. MiRNAs and LncRNAs in Cardiac Biology

Several excellent review articles are available about the importance of miRNAs in cardiac diseases (147, 208). Thus we here focus only on several miRNAs as examples for their potential use in directly affecting cardiomyocyte biology to develop therapeutic approaches. One of the most prohypertrophic miRNAs that was identified through a functional high-throughput library screen was miR-132. This miRNA

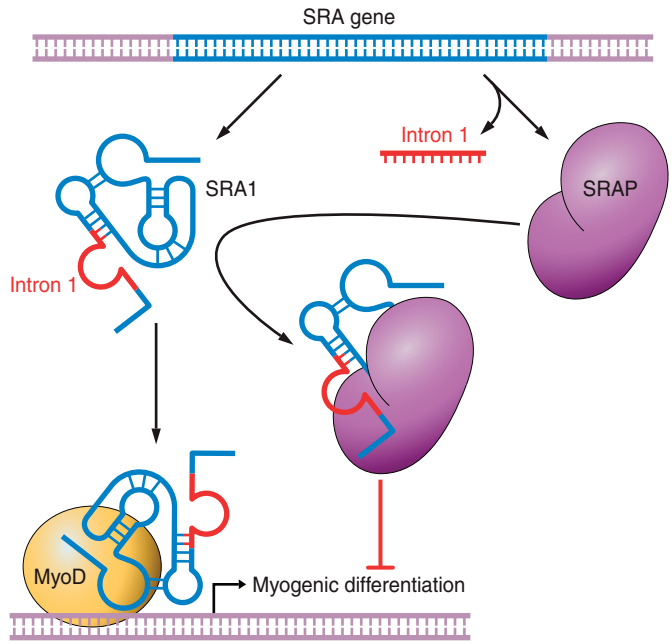


FIGURE 6. Regulation of myogenic differentiation by SRA ncRNA. The SRA gene can give rise to the ncRNA SRA1 or to the protein product SRAP according to retaining or splicing and degradation of the first intron. SRA1 is a coactivator of myogenic differentiation and cooperates with MyoD. On the other hand, SRAP can sequester SRA1, prevent its binding to MyoD, and inhibit myogenic differentiation.

is activated in both mouse models and humans with pathological cardiac remodeling (262, 266). Both in genetic and pharmacological deletion models, miR-132 silencing shows therapeutic efficacy to block cardiac remodeling by normalization of cardiac size and inhibition of fibrosis (266), paving the way for potential future clinical trials to block pathological cardiac remodeling in patients. Another miRNA with similar potential use is miR-25, and both miR-132 and miR-25 have been shown to target an important calcium handling protein in the cardiomyocyte, e.g., SERCA2 (280). However, there are conflicting results in the literature regarding miR-25 as a therapeutic target in cardiovascular disease. Another group has shown that in vivo inhibition of miR-25 by a specific miRNA inhibitor evoked spontaneous cardiac dysfunction and sensitized the murine myocardium to heart failure in a Hand2-dependent manner (65). There have been differences in the used chemistry and trial design in these two studies; for sure, more validation experiments need to be done to characterize the role of miR-25 better in cardiac diseases.

As miRNAs, also lncRNAs have been shown to regulate cardiomyocyte function (61, 261). An example is the lncRNA Chrf (cardiac hypertrophy-related factor). Chrf acts as a competing RNA and sequesters miR-489, thereby leading to cardiac hypertrophy (283). A more cardiac-specific antisense lncRNA, named myosin heavy-chain-associated RNA transcript (Mhrt), also exerts interesting cardiomyocyte functions; inhibition of Mhrt expression led to the induction of cardiomyopathy subsequent to pressure overload, whereas restoring Mhrt expression protected the heart from the development of hypertrophy and heart failure. Mhrt binds to the helicase domain of the transcription activator Brg1, which is essential for tethering Brg1 to its DNA targets, and prevents the expression of genes that are induced during stress through a Brg1-mediated chromatin remodeling mechanism (100).

Another recent publication identified the lncRNA Chast to promote cardiac remodeling and hypertrophy. The authors performed a global lncRNA expression profiling in mouse hearts exposed to 6 wk of transverse aortic constriction (TAC) or Sham. They found a set of 1,237 upregulated lncRNAs versus 1,623 downregulated lncRNAs. After stringent exclusion and selection of candidates, 22 lncRNAs were investigated. Silencing of Chast in vivo after TAC was beneficial in the mouse model, and the conservation of the lncRNA indicates the potential as a therapeutic target (277). These examples show the importance of lncRNAs in cardiomyocyte biology especially as therapeutic targets. Only a few lncRNAs have been functionally investigated in terms of cardiovascular biology, and it is more than likely that many exiting observations and potentially clinical useful targets will be discovered in the future.

B. MiRNAs and LncRNAs in Vascular Biology and Ischemia

NcRNAs have been shown to be crucial for the maintenance of vascular function. Certain miRNAs, that are activated during vascular stress such as miR-92a or miR-24, have been used as therapeutic targets, e.g., by injecting miRNA inhibitors in small and large animal models of myocardial infarction (MI) or kidney ischemia (24, 76, 108, 170). This shows the principal utility of miRNAs serving as powerful therpeutic targets to modulate vascularization in vivo. In contrast to ischemic organs, miR modulation can also be used to block vascularization in cancer; for instance, miR-92a delivery was shown to block endothelial proliferation in cancer models (7).

Currently much effort is undertaken to discover the role of lncRNAs in vascular biology. Mainly due to deep sequencing approaches, several endothelial expressed lncRNAs have been discovered such as MALAT1 or TUG1 (193). Indeed, the inhibition of MALAT1 impairs retina or hindlimb vascularization and thus may be an interesting therapeutic target despite its global expression throughout many cells in the body (193). Another recent report used next generation RNA sequencing to identify lncRNAs in human endothelial cells during hypoxia; several hypoxia-sensitive lncRNAs were characterized in more detail, e.g., LINC00323 and MIR503HG. Silencing of these lncRNA transcripts led to angiogenic defects, including repression of growth factor signaling and/or the key endothelial transcription factor GATA2. Their potential clinical importance for vascular structural integrity was demonstrated in an ex vivo model of human induced pluripotent stem cell-based engineered heart tissue showing great value of these lncRNAs to modulate tissue vascularization (74). The lncRNA p21 was shown to have a role in vascular endothelial apoptosis and cell cycle regulation (105). In a first study in the field, Boeckel et al. (22) have shown that circular RNAs identified by computational analysis of RNA generated from human endothelial cells with and without hypoxia are differentially regulated. A specific circRNA candidate, cZNF292, was shown to have proangiogenic activities in vitro showing that indeed also circRNAs in the vasculature have biological roles (22).

C. Role of MicroRNAs and LncRNAs in Fibrotic Diseases

Enhanced deposition of fibrous extracellular matrix occurs in the majority of human tissues as part of a physiological reparative response to acute tissue damage, leading to wound healing and scar formation (259). The term *fibrosis*, however, refers to a pathological and excessive tissue scarring which takes place during reactive responses to recurrent and persistent tissue injuries (77). Therefore, fibrosis is a common feature of many chronic diseases. Renal, hepatic,

pulmonary, and cardiac fibrosis are among the most common fibrotic diseases, and several reports described the role of different miRNA species in promoting or inhibiting fibrosis in these organs.

MiRNAs commonly involved in fibrotic diseases, whose expression can be regulated by a key profibrotic molecular signal such as transforming growth factor (TGF)-β, are miR-21, miR-29, and members of the miR-200 family.

MiR-21 and miR-29 have been shown to have, respectively, a pro- and anti-fibrotic role in well-established mouse models of kidney fibrosis, such as unilateral ureteral obstruction (UUO) or ischemic reperfusion injury (IRI). Under these conditions, expression of miR-21 increases, due to binding of R-Smads to the miR-21 promoter (306). Moreover, pharmacological inhibition of this miRNA attenuates fibrosis in the kidneys (306). On the contrary, expression of miR-29 decreases during kidney fibrosis (281), and its repression may be due to binding of Smad3 to a regulatory region located upstream from the miR-29b-2/29c cluster (225). Different from miR-21, restoration of miR-29 expression in vivo has a protective effect against renal fibrosis (225).

MiR-29 is also involved in liver fibrosis, as shown by Roderburg et al. (230). As a matter of fact, miR-29 downregulation in hepatic stellate cells is dependent on TGF-β1 and NF-κB signaling and leads to the upregulation of extracellular matrix genes (230).

Furthermore, miR-21 and miR-29 have also been described in lung fibrosis. miR-21 upregulation is induced by TGF-β in mice with bleomycin-induced lung fibrosis and promotes the activation of fibroblasts by targeting SMAD7 (169). MiR-29, on the contrary, is downregulated in the same model and appears to be repressed by TGF-β in human fetal lung fibroblast. Knockdown of miR-29 in these cells leads to derepression of extracellular matrix genes and fibrosis-related genes (54).

Additionally, miRNAs can regulate a process called epithelial-to-mesenchymal transition (EMT), which can be activated during tissue fibrosis and that consists in the loss of epithelial cell-cell and cell-basement membrane interactions followed by acquisition of mesenchymal properties (120). TGF-β can induce EMT through activation of the transcription factors ZEB1 and ZEB2. However, members of the miR-200 family can prevent EMT by repressing both factors (88). Furthermore, endothelial-to-mesenchymal transition (EndMT) has been shown to occur during fibrosis and to be dependent on the TGF-β-induced deregulation of miR-21 expression (149).

This antifibrotic role of miR-200 has been investigated in fibrotic kidneys (21423249), as well as in mouse models of lung fibrosis, where miR-200a, miR-200b, and miR-200c appear to be significantly downregulated (296).

Some recent reports have started to reveal the role of lncRNAs in fibrotic diseases as well, even though evidence is much more restricted compared with the miRNA field. Hou et al. (110) have recently shown that a lncRNA called linc-ROR can regulate EMT in immortalized human mammary epithelial cells by functioning as a competing endogenous RNA to miR-205 and by preventing the degradation of the miR-205 target ZEB2. Even though this finding is mainly related to the progression of breast cancer, a similar role for linc-ROR could be investigated in models of tissue fibrosis. In another study, Zhou et al. (307) used high-throughput RNA sequencing in wild-type and Smad3 knockout mouse models of UUO nephropathy and immunologically induced anti-glomerular basement membrane glomerulonephritis. They could identify and validate the deregulation of 21 novel lncRNAs whose expression is dependent on TGF-β/Smad3 activation. Understanding the function of these transcripts could provide new targets for the treatment of kidney diseases.

Further insights into the role of lncRNAs in fibrotic diseases come again from the nephrology field. As a matter of fact, under hyperglycemic conditions, miR-1207-5p and its host lncRNA plasmacytoma variant tanslocation 1 (PVT1) are expressed in human kidney cells and can increase the levels of plasminogen activator inhibitor 1 (PAI-1), TGF-β1, and fibronectin 1 (FN1) (3). Importantly, both regulation and functions of miR-1207-5p seem to be independent from the ones of PVT1 (3).

To identify lncRNAs that are deregulated in the course of lung fibrosis, Cao et al. (37) used a rat model of bleomycin-induced lung fibrosis in which they performed a microarray-based profiling of lncRNAs. Among the microarray hits, they could identify the lncRNAs AJ005396 and S69206, which were upregulated in the fibrotic lungs and localized in the cytoplasm of interstitial lung cells. The function of these ncRNAs, however, remains to be elucidated.

Furthermore, the conserved lncRNA maternally expressed gene 3 (Meg3), whose function has been so far mainly associated to repression of cancer growth, was recently discovered to be also an inhibitor of hepatic stellate cell activation and liver fibrogenesis (106). As a matter of fact, Meg3 is downregulated in a mouse model of liver fibrosis and in human fibrotic livers, likely due to hypermethylation of its promoter. Moreover, overexpression of this lncRNA in immortalized human hepatic stellate cells inhibited TGF-β1-induced proliferation and promoted apoptosis via activation of p53 (106). However, Meg3 effects in other fibrotic diseases are not known so far.

As anticipated, cardiac fibrosis is a hallmark of several cardiac pathologies implying a chronic myocardial stress. Fibrotic remodeling of the heart, however, is also a long-term consequence of myocardial infarction which leads to the replacement of necrotic tissue with a nonfunctional collagen-based scar as well as to the development of fibrosis remote from the infarct zone. The basic aspects of cardiac fibroblast biology and the involvement of ncRNAs in the regulation of cardiac fibroblast functions will be discussed separately in the following paragraphs.

1. MiRNAs, lncRNAs, and cardiac fibrosis

Fibroblasts are resident cells of connective tissues that generally have a mesenchymal origin. Key function of fibroblasts in all connective tissues across the human body is the production and homeostasis of the extracellular matrix (ECM). In spite of this functional feature, however, fibroblasts are more often identified on the basis of morphological characteristics, as spindle-shaped, flat cells showing multiple processes originating from the cell body. Above all, fibroblasts do not have a basement membrane, and this feature clearly separates cardiac fibroblasts (CFs) from all other permanent cell types of the heart (36).

CFs represent a major cardiac cell type which, in some organisms, can represent more than 50% of the total cardiac cell number (36). The function of CFs is not only to produce ECM components but also to preserve the biomechanical, electrical, and chemical properties of the heart. Additionally, through the production of autocrine and paracrine factors and via cell-cell interactions, CFs maintain the cardiac three-dimensional structure (36).

The notion that fibroblasts are a homogeneous cell type irrespective of their specific tissue location has been challenged by studies comparing the gene expression profiles of fibroblasts originating from different organs and showing that patterns of gene expression can highly vary between different fibroblast types (42). In particular, CFs remain poorly characterized in molecular terms and investigation in vivo of the mechanisms involved in cardiac fibroblast physiology and pathology is challenged by the relative lack of specific fibroblast markers (248).

Besides showing an increased proliferative activity during cardiomyopathy, CFs also tend to change their phenotype after stress and express specific markers of smooth muscle cells, especially the actin isoform α-smooth muscle actin (α-SMA). CFs showing these phenotypic and functional changes are called myofibroblasts and are particularly important during tissue repair and scarring after myocardial infarction (288). Myofibroblasts are indeed responsible for the organized deposition of matrix leading to the formation and subsequent remodeling of a scar at the site of infarction, where massive necrotic death of cardiomyocytes occurs. The origin of these differentiated cells remains unclear as well as the mechanisms leading to their persistence in the heart months and even years after infarction, causing fibrosis even in regions that are far from the site of injury. The mechanisms and functional importance of myofibroblast differentiation in cardiac pathologies other than myocardial infarction are still not well understood.

Of course, several growth factors and cytokines can influence the biology of CFs, and many of these molecules are also produced and secreted by fibroblasts themselves (276). The best characterized factor in this sense is TGF-β1. In the normal cardiac interstitium, TGF-β is present as a latent molecule unable to bind to its receptors (33). However, following cardiac injury, latent TGF-β is activated, and levels of TGF-β consistently increase in models of cardiac fibrosis as well as fibrotic human hearts. TGF-β is a potent inducer of myofibroblast differentiation and leads to increased expression of α-SMA and type I collagen (33). Additionally, TGF-β acts as a matrix-preserving molecule, by inducing the expression of protease inhibitors, and leads to increased CTGF levels, which is believed to cooperate with TGF-β to maintain a sustained fibrotic response (33).

TGF-β also regulates expression of pro-fibrotic and anti-fibrotic miRNAs in the heart, while its effects on the expression of regulatory lncRNAs that might have a function in CF biology are currently unknown.

NcRNAs, especially miRNAs, are deeply involved in myocardial fibrosis (259). As previously discussed, miR-21 and miR-29 have, respectively, a pro-fibrotic and an anti-fibrotic effect in several pathological conditions where tissue fibrosis is triggered. Similarly, these two miRNAs play a significant role in cardiac fibrosis.

MiR-21, in particular, is enriched in cardiac fibroblasts, regulating key pathways that are involved in CFs proliferation and activation. Moreover, miR-21 plays a role in the regulation of EndMT as well as EMT (28, 84).

Investigators have shown that the ERK-MAP kinase pathway is under control, in CFs, of miR-21 (263). Therefore, deregulation of miR-21 levels in CFs has a profound impact on ECM homeostasis. During heart failure, miR-21 expression is significantly upregulated in these cells, leading to an increased activation of the ERK-MAP kinase pathway. This occurs through the miR-21-mediated inhibition of sprouty homolog 1 (Spry1). Due to the action of miR-21, fibroblast survival and secretion of growth factors are affected, which correlates directly to the extent of fibrosis in the left ventricle and the hypertrophy of cardiomyocytes. Most importantly, the pharmacological silencing of miR-21 in vivo by using specific antisense chemistries is able to reduce the activation of the ERK-MAP kinase pathway and, consequently, the onset of fibrosis and hypertrophy following pressure overload (263).

Later on, other studies have shown that miR-21 has also an impact on expression of MMP2 and TGF-β receptor type III, thus acting on multiple pro-fibrotic pathways (163, 232).

As described earlier in this review, the biogenesis of miRNAs gives rise to a guide miRNA strand, which is normally responsible for gene silencing and regulation of cellular functions, and to a passenger strand, which was long believed to be simply degraded and to have no functional meaning. Recently, the biological functions of passenger strands (also called "star" strands or miR*) have started to be unraveled, including the ones of CFs-derived miR*. Interestingly, miR-21 has a key function in cardiac fibroblasts that is not only mediated by the guide strand, but also by the passenger strand. As a matter of fact, it has been recently shown that miR21* is produced by CFs, incorporated into exosomes, and secreted in the extracellular environment, inducing cardiomyocyte hypertrophy via its paracrine effect on these cells (10).

MiR-29 is also known to play a role in cardiac fibroblast biology. After myocardial infarction, miR-29 is downregulated in tissue areas that are close to the infarct zone (272). This downregulation is particularly relevant, due to the fact that miR-29, as described earlier in this review, targets a set of mRNAs coding for ECM components, including several collagen types, fibrillin and elastin. This means that the downregulation of miR-29 induces, in vivo, the derepression of ECM components leading to increased accumulation of matrix in the myocardium. Corresponding effects of miR-29 are observed in vitro when this miRNA is either artificially repressed or overexpressed (1). A potential role of miR-29 in other cell types such as cardiomyocytes remains to be investigated.

MiR-101 is additionally expressed in cardiac fibroblasts and silences the transcription factor c-*fos* leading to overexpression of its downstream target TGF-β1 and, consequently, to fibrosis development (216).

MiRNAs can contribute to the development of cardiac fibrosis even when their expression is typically observed in other cardiac and non-cardiac cell types. miR-1 and miR-133, for instance, are enriched in cardiomyocytes and can prevent cardiac fibrosis both directly and indirectly through regression of cardiac hypertrophy (132, 183). Furthermore, miRNAs might provoke a fibrotic response in the heart via immune cells such as macrophages, which express high levels of miR-155. The inhibition of this miRNA in mice reduces cardiac inflammation, hypertrophy, and dysfunction after pressure overload (107).

Evidence for lncRNAs to regulate cardiac fibroblast biology or to have a role in the development or progression of cardiac fibrosis is still very limited.

Expression profiles of lncRNAs have been analyzed by Jiang et al. (128) in angiotensin II (ANG II)-treated rat cardiac fibroblasts using a microarray-based approach. Alterations in lncRNA expression were accompanied by changes in mRNA levels indicating the activation of cell proliferation. The authors found that ANG II can dynamically regulate the expression of lncRNA-NR024118 and of the cell cycle inhibitor Cdkn1c, leading to downregulation of both (128). The angiotensin receptor blocker losartan, whose action is specific for the receptor tipe AT1, reversed the decrease of lncRNA-NR024118 and Cdkn1c while AT2 blockage did not lead to the same results, showing that the decrease of lncRNA-NR024118 and Cdkn1c induced by ANG II is mediated by AT1 receptor-dependent signaling (127).

However, the above-mentioned studies represent, so far, the only findings regarding lncRNAs in cardiac fibroblast biology and do not provide any functional information on NR024118 via gain- or loss-of-function experiments in vitro or in vivo, as well as any hint on the potential mechanism of action of this NR024118. Furthermore, there is no evidence, at present, about lncRNAs that can influence the development of cardiac fibrosis in vivo.

VI. TOOLS AND METHODS

A. Investigating miRNAs

MiRNA techniques have already been described in detail (see Refs. 55, 75, 270). Therefore, we will only give a very broad overview. The detection of miRNAs is usually done by two methods: the application of microarrays or detection by deep sequencing approaches. Deep sequencing is a more sensitive approach compared with microarray-based systems. It can also lead to the discovery of novel RNA sequences, while microarrays make use of a defined set of probes for detection. On the other hand, analyzing the output is more complex due to the massive amount of data, and high need of bioinformatic skills.

Validation of the screening results can be done with low effort and relatively low costs by quantitative real-time PCR (qRT-PCR). Former challenges harbored the design of the primer, to do reverse transcription because the transcript is so short. To date, there are many platforms available that offer optimized target-specific stem-loop reverse transcription primers. Other detection methods for defined miRNAs are Northern blotting and in situ hybridization (ISH).

MiRNAs often target not only one mRNA but multiple genes. Bioinformatic platforms are commonly used as a method to look for targets of a miRNA. Targetscan (160), miRanda(129), PicTar (143), or MicroCosm targets (89) are just some of numerous platforms available. **TABLE 2** gives an overview about several databases available.

Following bioinformatic-based predictions of targets of miRNAs, luciferase assays are often used to validate the predicted target. The 3' UTR of a putative target is cloned into a luciferase reporter downstream of the luciferase gene. A cell line is then cotransfected with the plasmid and the miRNA of interest. When the miRNA binds to the 3′ UTR of the potential target, the luciferase expression will be repressed. This way the luciferase activity goes down.

The expression level of a miRNA in vitro/in vivo can be altered by the application of miRNA mimics or anti-miRNAs, and it is discussed in more detail in the translational chapter.

Two excellent methodological reviews have been given by Eva van Rooij about the research of miRNA in general (270) and by Dangwal, Bang, and Thum with the focus on novel techniques (55).

B. Investigating lncRNAs

Next-generation sequencing is an efficient method to collect data sets with high accuracy and relatively low costs. To use the gathered data and analyze for lncRNAs, suitable bioinformatic workflows need to be applied. In addition, there are commercial arrays available to analyze the deregulation of a specific set of lncRNAs (e.g., Arraystar, Qiagen, Biocat, and many more). Another possibility to investigate the influence of lncRNAs is to make use of a genome-wide shRNA library to target a specific set of lncRNAs. Such a library can be applied and combined with other assays to measure the influence of lncRNA inhibition on signaling pathways or cell behavior. For instance, the lncRNA TUNA was identified by applying a shRNA library targeting 1,280 lincRNA in mouse embryonic stem cells containing Oct4-GFP (167). A review published in 2014 by Mohr et al. (195) summarizes nicely the assets and drawbacks of RNAi techniques.

To validate results obtained from a screen for lncRNAs, it is especially important to design primers exclusively detecting the ncRNA transcript. This design is crucial to distinguish coding from non-coding regions. Usually, the expression of a lncRNA is low. Also, the annotation of lncRNAs is still evolving and is not necessarily consistent throughout the different databases (like Refseq, UCSC, Ensembl). Often, lncRNAs are transcribed from pseudogenes, and corresponding primers not only detect the long non-coding transcript but also the original gene. Another difficulty occurs when lncRNAs are encoded sense or antisense to a known protein-coding gene.

To inhibit lncRNAs in vitro, different chemistries can be used. The so-called GapmeR technology will be discussed in translational section of this review. Cells can also be transduced with shRNAs targeting lncRNAs.

To improve our understanding on lncRNA mechanisms, it is important to evaluate the subcellular distribution of the molecule. This can already give a hint of the function, since lncRNAs involved in chromatin remodeling for instance are mainly detected in the nucleus. Plenty of lncRNAs were shown to interact with proteins; thus a pulldown of the lncRNA of interest can give evidence of potential interaction partners. This can be done by adding biotinylated lncRNA to a cell lysate like shown for Braveheart (138), Firre (99), or HOTAIR (181, 228). Pulldown of lncRNA/protein complexes harbors a lot of difficulties because it can give false-positive results. Recently, McHugh et al. (185) published a highly reproducible RNA antisense purification (RAP) approach to identify proteins that interact with a specific lncRNA directly

Table 2. *Softwares and databases used for miRNA research*

Tool	Purpose	Link	Reference Nos.
Targetscan	Target prediction	http://www.targetscan.org/	160
miRanda/microRNA.org	Target prediction	http://www.microrna.org/microrna/home.do	70, 129
PicTar	Target prediction	http://pictar.mdc-berlin.de/	143
MicroCosm Targets	Target prediction	http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/	70, 89, 90
miRDeep	miRNA identification from RNA sequencing data	http://www.australianprostatecentre.org/research/software/mirdeep-star	4
UEA sRNA workbench	miRNA identification from RNA sequencing data	http://srna-workbench.cmp.uea.ac.uk/	251
KEGG	Interactions/pathways	http://www.genome.jp/kegg/	130
Reactome	Interactions/pathways	http://www.reactome.org/	51
miR2Disease	Interactions/pathways	http://www.mir2disease.org/	126
RNAfold	Folding prediction	http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi	109
mfold	Folding prediction	http://mfold.rna.albany.edu/?q=mfold	308

in the cell. To do so, ultraviolet (UV) light is used to crosslink RNA and interacting proteins. Denaturing conditions are applied to crack noncovalent interactions. A mass spectrometry analysis is followed to identify binding proteins. To improve the sensitivity even more, cells can be cultured in SILAC medium to label the proteins and increase the accuracy of the mass spectrometry analysis. Applying this method to identify XIST-interacting proteins, the group was able to show interaction with 10 different proteins with high reproducibility. McHugh et al. (185) applied intense and multiple controls to narrow down the amount of false-positive detections. First, they compared the MS results from the XIST pulldown with the results from an abundantly expressed small nuclear RNA U1. Then, RAP was performed in cells not expressing XIST (uninduced cells) and in cells not crosslinked with UV prior to pulldown. Additionally, another nuclear enriched lncRNA was used for the pulldown. The advantage of this method is to first induce the lncRNA expression and then purify the lncRNA-protein complexes right from the cell without adding the lncRNA to a prepared cell lysate. This way many nonspecific interactions can be avoided. The addition of multiple, detailed controls increases the yield and reproducibility.

It is also important to validate an annotated sequence length of a newly discovered lncRNA. With the use of rapid amplification of cDNA ends (RACE), it is possible to amplify a lncRNA between a defined position inside the lncRNA and the 3' or 5' end of the sequence. This amplicon can then be cloned and sequenced to detect/validate the actual sequence.

To understand the role of a lncRNA in vivo, it is crucial to perform detailed loss- or gain-of-function studies. Making use of lentiviral overexpression to study the function of a lncRNA may however harbor some problems. Due to the random integration into the genome important genes might be disrupted. Also, there are limitations to influence the position of integration. However, for a lncRNA, its genomic position may be important because the molecule might influence genes in its vicinity. A very helpful guide about considerations before performing in vivo studies about lncRNA functions has been given by Bassett et al. (13).

Numerous lncRNA databases are available to search for lncRNAs and first hints of possible interactions and functions. A summary about some tools is given in the **TABLE 3**.

C. Investigating circRNAs

A completely new set of potential circRNAs can be predicted by screening existing RNA-sequencing data for circular RNAs (237). Long-read RNA-sequencing data can be used to screen for potential circRNAs. The algorithm needs to be specified for this special kind of molecules, since it might have been backsplicing to be produced. Two reports nicely show how a computational pipeline can be constructed to specifically detect new circRNAs (92, 186). Indeed, application of such new algorithms to RNA-sequencing data gives hints on potential existing circRNAs. The validation of these ncRNAs is in particular special, because obviously the gene where it is coming from has a different orientation. Exonic circRNAs need to be distinguished from other "backspliced" RNA molecules. These are molecules where the order of the exons is reversed compared with the annotated gene product (see Ref. 123).

To verify the predicted circRNAs, the expression can be accessed by using divergent primers in qPCRs. These primers do not amplify towards each other but are amplifying away from each other regarding the genomic region. That way it is possible to amplify the circle without amplifying the genomic regions (123, 124). Treatment of the sample with RNase R will further decrease the amount of linear RNAs (255). Separation of noncircular and circular RNA molecules in a Northern blot is also possible as circular RNAs have a faster migration. Performing another run on the same samples after digestion with RNase H will lead to

Table 3. *Softwares and databases used for lncRNA research*

Tool	Purpose	Link	Reference Nos.
NONCODE	ncRNA database	http://www.noncode.org/	31
CHIPBase	ChiP-Seq Data of ncRNA	http://deepbase.sysu.edu.cn/chipbase/	293
LNCipedia	Human lncRNA database	http://www.lncipedia.org/	278, 279
starBase v2.0	ncRNA interactions, interaction networks	http://starbase.sysu.edu.cn/	161, 294
lncRNome	lncRNA database	http://genome.igib.res.in/lncRNome/	21
DIANA-lncBase	Interaction prediction	http://diana.imis.athena-innovation.gr/DianaTools/index.php?r = site/index	218
HUGO	Standardized nomenclature for lncRNAs (and genes)	http://www.genenames.org/	87
PhyloCSF	Distinguishing between protein-coding and non-coding regions	https://github.com/mlin/PhyloCSF/wiki	166

two bands for a prior linear RNA molecule but only one band for a circRNA. Also there are different behaviors of noncircular versus circular RNA molecules in two-dimensional gel electrophoresis and Gel trap applications. circRNA will poorly migrate in two-dimensional gels and do not migrate at all in gel traps (123, 124, 186). It is important to distinguish circRNAs from other looped RNA structures.

First hints about the function of a potential circRNA can be accessed according to other RNA investigation tools. CircRNA bioinformatic platforms are just beginning to evolve. A brief overview is given in **TABLE 4**.

VII. ncRNAs IN THERAPY

With the discovery of the class of ncRNAs, a whole new field of mechanistic understanding and possible therapeutic interventions has evolved. Since microRNAs and also lncRNAs are involved in the regulation of a huge variety of diseases, it is a promising approach to convert new discoveries into therapies.

A. MiRNA Therapy

The group of miRNAs has been explored in some detail and several therapeutic interventions have been tested. In general, there are two different approaches to alter miRNA levels in disease settings. Their activity can either be beneficial or harmful. Thus its function can either be enhanced by mimicking miRNAs or they can be inhibited by targeting the miRNA sequence with antisense oligonucleotides (ASO).

1. Upregulation of miRNA for therapeutic approaches

To achieve an elevated expression level of beneficial miRNA, one can make use of adeno-associated viruses (AAV) or other viral gain-of-function approaches. Their injection leads to a transient introduction of gene copies into the host which drives a strong and long-lasting in vivo miRNA expression (139, 226). With AAV injection it is also possible to transduce specific tissues because each individual serotype shows a tropism towards different organs. For instance, the AAV subtypes 6, 8, and 9 are cardiotropic when given systemically (175, 215, 285). However, the distribution needs to be assessed individually because unintended upregulations in other organs are likely to occur. Novel AAV variants are necessary to target only specific organs or even specifc cell types by the use of cell-type specific promoters.

Another possibility is the use of miRNA mimicry to enhance the miRNA level. These miRNA "mimics" are small, synthetic, chemically modified double-stranded oligonucleotides. They are incorporated into the RISC complex and mimic endogenous miRNA functions. miRNA mimics are easy to synthesize, but their chemistry harbors some challenges in their delivery and design.

One example for an already developed miRNA therapy approach is MRX34 from Mirna Therapeutics to treat liver cancer (hepatocellular carcinoma; HCC). miR-34 is a naturally occurring tumor suppressor, and MRX34 mimics its function. MiR-34 delivery produced complete HCC regression in a mouse model (292). Currently it is in a multicenter, open-label phase 1 clinical trial (Ref. 26; ClinicalTrials.gov Identifier: NCT01829971).

In general, reagents that target miRNAs need to pass the cellular membrane. They should be prevented from degradation, and they need to be highly stable. This comes into play for the chemistry of miRNA mimics as well as for miRNA inhibitors.

2. Inhibitory approaches

Downregulation of miRNAs can be achieved by targeting them with antisense oligonucleotides (ASO). These antimiRNAs (or antagomirs) are single-stranded oligomers, complementary to the targeted miRNA. Several chemical modifications have been tested to enhance pharmacodynamics and pharmacokinetics of miRNA reagents and improve their function (260).

A modification on the parent phosphodiester backbone linkage creates an increased nuclease resistance and promotes cellular uptake. Furthermore, it improves binding to plasma proteins giving reduced urinary clearance (159). A commonly used variant is to convert the phosphodiester

Table 4. *Softwares and databases used for circRNA research*

Tool	Purpose	Link	Reference Nos.
CircRNABase	Predicts miRNA-circRNA interactions (embedded in starBase v2.0)	http://starbase.sysu.edu.cn/mirCircRNA.php	161, 294
circBase	circRNA database	http://www.circbase.org/	85
circ2Traits	Human circRNA database	http://gyanxet-beta.com/circdb/	83

into a phosphorothioate (PS), where a sulfur atom replaces one oxygen atom in the phosphate group. Additional modifications are possible when making use of non-ribose backbones like peptide nucleic acid oligos (PNA) or a phosphorodiamidate morpholino oligomer (PMO) (252, 271).

The conjugation of cholesterol to the 3' end of sequence facilitates cellular uptake and promotes in vivo stability. Antagomirs are synthetic cholesterol-conjugated 2-*O*-methyl RNA oligos of ~21–23 nucleotides which fully complement the miRNAs and effectively compete with miRNA target mRNAs. They contain phosphorothioate moieties to increase their stability. Antagomirs have a stronger binding to the miRNA-associated gene silencing complexes (miRNA-RISCs) (145).

The effect of modifications on the 2'-position of the sugar moiety is an increase in nuclease resistance and a higher affinity of Antagomirs oligonucleotides to their target miRNAs (56, 250). Such modifications can be 2'-*O*-methylation, 2'-*O*-methoxyethylation, or 2'-*O*-F modifications. The introduction of a 2'-*O*,4'-C-methylene bridge leads to a locked nucleic acid (LNA) chemistry.

LNA chemistry improves target affinity. They harbor a methylene bridge to lock the furanose ring. This leads to a higher efficiency and makes it possible to use shorter sequences to target the miRNA's seed sequence ("tiny LNA") (67, 205, 212). These tiny LNAs can be used to affect a whole family of miRNAs, because not the whole sequence is needed. Since a whole family of related miRNAs can be involved in a disease, tiny LNAs are a promising approach to target the whole group of disease-associated miRNAs without the need to address them individually. A study by Bernardo et al. (18) from 2012 compared the outcome of targeting only one member of the heart failure-related miR-34 family with the outcome after targeting the whole family with one tiny LNA. The level of the miR family members miR-34a, miR-34b and miR-34c are elevated in cardiac tissue in response to stress and thus they are a promising target for therapeutical approaches. Mice subjected to MI received a 8-mer LNA against the miR-34 familiy or a 15-mer LNA against miR-34a alone. The mice receiving LNA targeting miR-34a showed no benefit after MI. Interestingly, mice treated with the tiny LNA targeting multiple miR34 forms showed attenuated MI-induced morphological changes and improved functions (18). However, a greater specificity is only given when using anti-miRNAs complementary to the individual full length miR sequence. Also, there are sometimes sequences outside the miRNA seed sequence that are important for the function of a miRNA (148). Thus great care is needed when so-called tiny miRNA inhibitors are developed.

Another compound, *N*,*N*-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamine ("ZEN"), increases binding affinity and blocks nuclease degradation when positioned at or near each end of a single-stranded oligonucleotide (158).

This and many other oligonucleotide modifications may influence the inhibitory effects and need to be assessed individually.

Recently, a clinical translational approach was published, where a miRNA inhibition approach was used to treat patients with viral hepatitis C. The drug called "miravirsen" is a mixed locked nucleic acid (LNA)/DNA phosphorothioated sequence complementary to the 5' region of miR-122. This liver specific miRNA is needed in the life cycle of hepatitis C. Using LNA makes the reagent resistant to nuclease degradation and equips it with high affinity towards its target. It has recently been shown that miravirsen not only functions through the binding of mature miR-122, but also binds the stem-loop structure of pri- and pre-miR-122. In this way it inhibits the processing of the miR-122 precursors (79). There are currently several companies developing anti-hepatitis drugs by using anti-miRNA approaches.

B. LncRNA-Based Therapies

NcRNAs are appealing pharmacological and therapeutic targets because of their involvement in development and their general action in a cell type specific manner. Unfortunately, the modulation of lncRNAs in vivo is not very easy. Therefore, to date, there is no lncRNA drug brought into clinical trials. Nevertheless, plenty of promising approaches to regulate lncRNAs on a therapeutical level are in a preclinical phase.

Meng et al. (190) described in a recent publication the possibility of targeting the lncRNA Ube3a-ATS to treat the single gene disorder Angelman syndrome (AS) (190). AS is a complex neurodevelopmental disorder, characterized by mental retardation. The disorder is caused by the loss of the maternal allele of UBE3A in the brain, which encodes for an E3 ubiquitin ligase. This genomic region is regulated by an imprinting center leading to a silenced UBE3A expression on the paternal allele. The additional deficiency on the maternal chromosome leads to the loss of UBE3A expression and thus to AS (32, 188). The underlying silencing mechanism is provoked by the expression of the lncRNA Ube3a-ATS which lays antisense to the paternal gene and leads to its silencing (188, 231). In AS mouse model, it was possible to reduce Ube3a-ATS with ASO treatment and thus restore the level of paternal Ube3a, which in turn ameliorated some defects associated with the disease. This study reveals a promising therapy for a severe disease, especially because ASO therapy has been already tested in detail and is a perfect approach to silence RNAs including lncRNAs.

Targeting lncRNAs can also be done by the usage of so-called "GapmerRs." These DNA-LNA chimeric antisense

oligonucleotides are single-stranded and catalyze a degradation of target lncRNAs by recruiting RNase H (available from the company Exiqon). RNase H is present in the nucleus and cytoplasm and degrades RNA-DNA heteroduplexes (152). The therapeutic application of GapmerRs to target lncRNAs is still in its infancy.

One challenge of the investigation of lncRNAs is their poor conservation. Even if there can be major success in the therapeutic targeting of one lncRNA in a mouse model, it does not necessarily mean that these findings are transferable into humans. It will be thus especially important to investigate human lncRNAs for therapeutic approaches.

C. NcRNAs as Biomarkers

NcRNA expression patterns often correlate with a disease type and can provide specific diagnostic or even prognostic clinical information. Indeed, there is a great need for biomarkers that can define a subtype of disease to guide therapeutic interventions. Personalized medicine will help to treat patients more efficiently and thus also with lower costs for the health and economics systems.

1. Circulating miRNAs as biomarkers

A promising new field of research is the investigation of miRNAs as circulating biomarkers in body fluids. The process of miRNA secretion may be another way of intercellular communication between cells and/or organ systems. NcRNAs can be secreted in actively packed circulating particles like exosomes, microvesicles, or apoptotic bodies. In addition, they can be associated with HDL and other lipoprotein complexes (275). In contrast to this active secretion of ncRNAs, there is also a number of miRNAs entering the circulation system through passive leakage (45). Although once assumed to be unstable, it has been shown that circulating miRNAs are easy to detect and relatively stable. Indeed, inclusion of miRNAs in microvesicles, exosomes, apoptotic bodies, and complexes with proteins converts protection from RNase-dependent degradation (116, 244, 268). Thus miRNAs are very stable and their levels can be easily measured in body fluids like blood, serum, cerebrospinal fluid, or urine (173, 174, 194). They could have a great potential for an early noninvasive, specialized detection and indeed some examples have already been shown. See the review from Chen et al. (45) for more information about miRNA secretion.

Focusing on cardiovascular settings, microRNAs have been investigated as novel intercellular communicators. Bang et al. (10) were able to show a secretion of miR-21* from exosomes derived from fibroblast into cardiomyocytes. This unusual way of ncRNA exchange was shown to lead to cardiac hypertrophy (10). In another publication, it has been shown that the miRNA profile enables distinguishing heart failure patients with reduced from those with preserved ejection fraction (287). Other miRNAs in correlation with heart failure have been tested, among them miR-1, miR-133a, mir-208b, and miR-499. Meta studies are now performed to compare the findings of different groups and look for the best biomarker combination possible (46, 297). Especially the detection of multiple circulating miRNAs could be a more powerful diagnostic tool compared with the measurement of only one specific biomarker, as also shown by Derda et al. (59) for the differentiation of different forms of hypertrophic cardiomyopathies.

2. LncRNAs as biomarkers

Circulating lncRNAs show useful characteristics to serve as biomarkers, but it still needs to be studied how the extracellular release works for lncRNAs. So far, it has been shown that lncRNAs are packed in vesicles, but the detailed characterization is lacking (81, 257). An example for a circulating lncRNA is prostate cancer antigen 3 (PCA3). It is one of the most specific biomarkers for the diagnosis of prostate cancer. Recently a potential function for this biomarker-serving lncRNA was proposed. Downregulation of the tumor-suppressor gene PRUNE2 by PCA3 reveals this lncRNA to be a negative oncogenic regulator (235). A commercial kit for the detection of PCA3 called "Progensa PCA3 Assay" was launched by Gen-Probe Incorporated (San Diego, CA).

In line with this, more lncRNAs show promising features for diagnostic usage. The level of the circulating lncRNA TapSaki in plasma of patients with acute kidney injury is a predictor of mortality and is detectable in kidney biopsies as well as in plasma of these patients (171). LncRNAs were not only detectable in plasma, but also in urine of patients. For instance, it was possible to predict patients with ongoing kidney rejection after kidney transplantation by screening specific lncRNAs in urine (172).

The potential usage of cardiac lncRNA biomarkers has been investigated in plasma and whole blood of patients. The first proof of principle study of lncRNA detection in plasma was performed in patients with and without left ventricular remodeling after MI. It was possible to identify a mitochondrial-derived lncRNA named Lipcar, which marks patients with future cardiac remodeling and high risk of a death by heart failure. Vausort et al. (274) identified ANRIL and KCNQ1OT1 in full blood of patients. Measurements of these lncRNAs improved the prediction of left ventricular dysfunction (274). However, the potential of lncRNAs serving as biomarkers still needs to be investigated deeply. Further studies are needed to check the possibilities of body fluid-derived lncRNAs as biomarkers for various diseases. It is crucial to always examine whether ncRNA biomarkers are indeed better than the biomarkers in use. It might also help to make use of a combination of traditional biomarkers with novel ncRNA biomarkers to overcome

certain lacks of prognostic predictions. In addition to lncRNAs and miRNAs, circRNAs are promising new candidates for biomarker detection, since they are more abundant and stable in body fluids. But there are still only a few reports available.

VIII. CONCLUDING REMARKS

The emerging world of ncRNA is fascinating and shows a new level of the complexity of nature. The influence of ncRNAs on cellular biology is larger than initially expected and thus makes ncRNA research rather complex. However, despite the relatively short time period ncRNAs have been identified, there have been tremendous developments both in clinical applications and diagnostic approaches. It is likely that this field will develop into a new area of more specific and powerful drugs as well as personalized medicine approaches moving health care of patients to a new level. More and more ncRNA therapeutics will enter a formal drug development process. Additional information needs to be gathered, the pharmacokinetics and dynamics of potential ncRNA drugs need to be tested, and detailed toxic studies are necessary. More tools are essential to push the field forward. More clinical studies will enter into phase I/II studies, including the field of cardiovascular disease. There will be other, new molecules to inhibit small and long ncRNAs, forming a new armada of drugs to combat (much more mechanistically orientated) various diseases.

To keep up the pace and make this possible, a highly organized network of ncRNA research institutes is necessary. Knowledge needs to be exchanged fast and new techniques need to be implemented. Especially the assessment of biomarker potential could be scaled-up and performed in a cooperative manner. This will potentially also enable better prognostic options, for instance for certain cardiovascular disease subgroups.

In conclusion, the field of ncRNA research will undergo fast and important changes and gather significant and fascinating new knowledge.

ACKNOWLEDGMENTS

Address for reprint requests and other correspondence: T. Thum, Institute of Molecular and Translational Therapeutic Strategies, Hannover Medical School, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany (e-mail: Thum.Thomas@mh-hannover.de).

GRANTS

This study was supported by the IFB-Tx (BMBF 01E01302; to T. Thum), the REBIRTH Excellence Center (to T. Thum); Fondation Leducq (MIRVAD; to T. Thum), and the European Union funded ERC Consolidator Grant LongHeart (to T. Thum).

DISCLOSURES

T. Thum declared filing and licensing of several patents about non-coding RNAs in cardiovascular disease. T. Thum declares that he is cofounder of a miRNA company (Cardior Pharmaceuticals).

REFERENCES

1. Abonnenc M, Nabeebaccus AA, Mayr U, Barallobre-Barreiro J, Dong X, Cuello F, Sur S, Drozdov I, Langley SR, Lu R, Stathopoulou K, Didangelos A, Yin X, Zimmermann WH, Shah AM, Zampetaki A, Mayr M. Extracellular matrix secretion by cardiac fibroblasts: role of microRNA-29b and microRNA-30c. *Circ Res* 113: 1138–1147, 2013.

2. Ali SR, Ranjbarvaziri S, Talkhabi M, Zhao P, Subat A, Hojjat A, Kamran P, Muller AM, Volz KS, Tang Z, Red-Horse K, Ardehali R. Developmental heterogeneity of cardiac fibroblasts does not predict pathological proliferation and activation. *Circ Res* 115: 625–635, 2014.

3. Alvarez ML, Khosroheidari M, Eddy E, Kiefer J. Role of microRNA 1207-5P and its host gene, the long non-coding RNA Pvt1, as mediators of extracellular matrix accumulation in the kidney: implications for diabetic nephropathy. *PLoS One* 8: e77468, 2013.

4. An J, Lai J, Lehman ML, Nelson CC. miRDeep*: an integrated application tool for miRNA identification from RNA sequencing data. *Nucleic Acids Res* 41: 727–737, 2013.

5. Anderson DM, Anderson KM, Chang CL, Makarewich CA, Nelson BR, McAnally JR, Kasaragod P, Shelton JM, Liou J, Bassel-Duby R, Olson EN. A micropeptide encoded by a putative long non-coding RNA regulates muscle performance. *Cell* 160: 595–606, 2015.

6. Andl T, Murchison EP, Liu F, Zhang Y, Yunta-Gonzalez M, Tobias JW, Andl CD, Seykora JT, Hannon GJ, Millar SE. The miRNA-processing enzyme dicer is essential for the morphogenesis and maintenance of hair follicles. *Curr Biol* 16: 1041–1049, 2006.

7. Ando H, Okamoto A, Yokota M, Shimizu K, Asai T, Dewa T, Oku N. Development of a miR-92a delivery system for anti-angiogenesis-based cancer therapy. *J Gene Med* 15: 20–27, 2013.

8. Atianand MK, Fitzgerald KA. Long non-coding RNAs and control of gene expression in the immune system. *Trends Mol Med* 20: 623–631, 2014.

9. Babiarz JE, Ruby JG, Wang Y, Bartel DP, Blelloch R. Mouse ES cells express endogenous shRNAs, siRNAs, and other Microprocessor-independent, Dicer-dependent small RNAs. *Genes Dev* 22: 2773–2785, 2008.

10. 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, Schober A, Fiedler J, Thum T. Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. *J Clin Invest* 124: 2136–2146, 2014.

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

12. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, function. *Cell* 116: 281–297, 2004.

13. Bassett AR, Akhtar A, Barlow DP, Bird AP, Brockdorff N, Duboule D, Ephrussi A, Ferguson-Smith AC, Gingeras TR, Haerty W, Higgs DR, Miska EA, Ponting CP. Considerations when investigating lncRNA function in vivo. *Elife* 3: e03058, 2014.

14. Batista PJ, Chang HY. Long non-coding RNAs: cellular address codes in development and disease. *Cell* 152: 1298–1307, 2013.

15. Batista PJ, Molinie B, Wang J, Qu K, Zhang J, Li L, Bouley DM, Lujan E, Haddad B, Daneshvar K, Carter AC, Flynn RA, Zhou C, Lim KS, Dedon P, Wernig M, Mullen AC, Xing Y, Giallourakis CC, Chang HY. m(6)A RNA modification controls cell fate transition in mammalian embryonic stem cells. *Cell Stem Cell* 15: 707–719, 2014.

16. Bazzini AA, Johnstone TG, Christiano R, Mackowiak SD, Obermayer B, Fleming ES, Vejnar CE, Lee MT, Rajewsky N, Walther TC, Giraldez AJ. Identification of small

ORFs in vertebrates using ribosome footprinting and evolutionary conservation. *EMBO J* 33: 981–993, 2014.

17. Beltran M, Puig I, Pena C, Garcia JM, Alvarez AB, Pena R, Bonilla F, de Herreros AG. A natural antisense transcript regulates Zeb2/Sip1 gene expression during Snail1-induced epithelial-mesenchymal transition. *Genes Dev* 22: 756–769, 2008.

18. Bernardo BC, Gao XM, Winbanks CE, Boey EJ, Tham YK, Kiriazis H, Gregorevic P, Obad S, Kauppinen S, Du XJ, Lin RC, McMullen JR. Therapeutic inhibition of the miR-34 family attenuates pathological cardiac remodeling and improves heart function. *Proc Natl Acad Sci USA* 109: 17615–17620, 2012.

19. Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, Mills AA, Elledge SJ, Anderson KV, Hannon GJ. Dicer is essential for mouse development. *Nat Genet* 35: 215–217, 2003.

20. Bernstein HD, Zopf D, Freymann DM, Walter P. Functional substitution of the signal recognition particle 54-kDa subunit by its *Escherichia coli* homolog. *Proc Natl Acad Sci USA* 90: 5229–5233, 1993.

21. Bhartiya D, Pal K, Ghosh S, Kapoor S, Jalali S, Panwar B, Jain S, Sati S, Sengupta S, Sachidanandan C, Raghava GP, Sivasubbu S, Scaria V. lncRNome: a comprehensive knowledgebase of human long non-coding RNAs. *Database* 2013: bat034, 2013.

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

23. Bohnsack MT, Czaplinski K, Gorlich D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. *RNA* 10: 185–191, 2004.

24. Bonauer A, Carmona G, Iwasaki M, Mione M, Koyanagi M, Fischer A, Burchfield J, Fox H, Doebele C, Ohtani K, Chavakis E, Potente M, Tjwa M, Urbich C, Zeiher AM, Dimmeler S. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. *Science* 324: 1710–1713, 2009.

25. Borsani G, Tonlorenzi R, Simmler MC, Dandolo L, Arnaud D, Capra V, Grompe M, Pizzuti A, Muzny D, Lawrence C, Willard HF, Avner P, Ballabio A. Characterization of a murine gene expressed from the inactive X chromosome. *Nature* 351: 325–329, 1991.

26. Bouchie A. First microRNA mimic enters clinic. *Nat Biotechnol* 31: 577, 2013.

27. Brockdorff N, Ashworth A, Kay GF, McCabe VM, Norris DP, Cooper PJ, Swift S, Rastan S. The product of the mouse Xist gene is a 15 kb inactive X-specific transcript containing no conserved ORF and located in the nucleus. *Cell* 71: 515–526, 1992.

28. Bronnum H, Andersen DC, Schneider M, Sandberg MB, Eskildsen T, Nielsen SB, Kalluri R, Sheikh SP. miR-21 promotes fibrogenic epithelial-to-mesenchymal transition of epicardial mesothelial cells involving Programmed Cell Death 4 and Sprouty-1. *PLoS One* 8: e56280, 2013.

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

30. Brown CJ, Hendrich BD, Rupert JL, Lafreniere RG, Xing Y, Lawrence J, Willard HF. 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, 1992.

31. Bu D, Yu K, Sun S, Xie C, Skogerbo G, Miao R, Xiao H, Liao Q, Luo H, Zhao G, Zhao H, Liu Z, Liu C, Chen R, Zhao Y. NONCODE v3.0: integrative annotation of long non-coding RNAs. *Nucleic Acids Res* 40: D210–215, 2012.

32. Buiting K. Prader-Willi syndrome and Angelman syndrome. *Am J Med Genet C Semin Med Genet* 154C: 365–376, 2010.

33. Bujak M, Frangogiannis NG. The role of TGF-beta signaling in myocardial infarction and cardiac remodeling. *Cardiovasc Res* 74: 184–195, 2007.

34. Bulger M, Groudine M. Functional and mechanistic diversity of distal transcription enhancers. *Cell* 144: 327–339, 2011.

35. Cabili MN, Trapnell C, Goff L, Koziol M, Tazon-Vega B, Regev A, Rinn JL. Integrative annotation of human large intergenic non-coding RNAs reveals global properties and specific subclasses. *Genes Dev* 25: 1915–1927, 2011.

36. Camelliti P, Borg TK, Kohl P. Structural and functional characterisation of cardiac fibroblasts. *Cardiovasc Res* 65: 40–51, 2005.

37. Cao G, Zhang J, Wang M, Song X, Liu W, Mao C, Lv C. Differential expression of long non-coding RNAs in bleomycin-induced lung fibrosis. *Int J Mol Med* 32: 355–364, 2013.

38. Capel B, Swain A, Nicolis S, Hacker A, Walter M, Koopman P, Goodfellow P, Lovell-Badge R. Circular transcripts of the testis-determining gene Sry in adult mouse testis. *Cell* 73: 1019–1030, 1993.

39. Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, Oyama R, Ravasi T, Lenhard B, Wells C, Kodzius R, Shimokawa K, Bajic VB, Brenner SE, Batalov S, Forrest AR, Zavolan M, Davis MJ, Wilming LG, Aidinis V, Allen JE, Ambesi-Impiombato A, Apweiler R, Aturaliya RN, Bailey TL, Bansal M, Baxter L, Beisel KW, Bersano T, Bono H, Chalk AM, Chiu KP, Choudhary V, Christoffels A, Clutterbuck DR, Crowe ML, Dalla E, Dalrymple BP, de Bono B, Della Gatta G, di Bernardo D, Down T, Engstrom P, Fagiolini M, Faulkner G, Fletcher CF, Fukushima T, Furuno M, Futaki S, Gariboldi M, Georgii-Hemming P, Gingeras TR, Gojobori T, Green RE, Gustincich S, Harbers M, Hayashi Y, Hensch TK, Hirokawa N, Hill D, Huminiecki L, Iacono M, Ikeo K, Iwama A, Ishikawa T, Jakt M, Kanapin A, Katoh M, Kawasawa Y, Kelso J, Kitamura H, Kitano H, Kollias G, Krishnan SP, Kruger A, Kummerfeld SK, Kurochkin IV, Lareau LF, Lazarevic D, Lipovich L, Liu J, Liuni S, McWilliam S, Madan Babu M, Madera M, Marchionni L, Matsuda H, Matsuzawa S, Miki H, Mignone F, Miyake S, Morris K, Mottagui-Tabar S, Mulder N, Nakano N, Nakauchi H, Ng P, Nilsson R, Nishiguchi S, Nishikawa S, Nori F, Ohara O, Okazaki Y, Orlando V, Pang KC, Pavan WJ, Pavesi G, Pesole G, Petrovsky N, Piazza S, Reed J, Reid JF, Ring BZ, Ringwald M, Rost B, Ruan Y, Salzberg SL, Sandelin A, Schneider C, Schonbach C, Sekiguchi K, Semple CA, Seno S, Sessa L, Sheng Y, Shibata Y, Shimada H, Shimada K, Silva D, Sinclair B, Sperling S, Stupka E, Sugiura K, Sultana R, Takenaka Y, Taki K, Tammoja K, Tan SL, Tang S, Taylor MS, Tegner J, Teichmann SA, Ueda HR, van Nimwegen E, Verardo R, Wei CL, Yagi K, Yamanishi H, Zabarovsky E, Zhu S, Zimmer A, Hide W, Bult C, Grimmond SM, Teasdale RD, Liu ET, Brusic V, Quackenbush J, Wahlestedt C, Mattick JS, Hume DA, Kai C, Sasaki D, Tomaru Y, Fukuda S, Kanamori-Katayama M, Suzuki M, Aoki J, Arakawa T, Iida J, Imamura K, Itoh M, Kato T, Kawaji H, Kawagashira N, Kawashima T, Kojima M, Kondo S, Konno H, Nakano K, Ninomiya N, Nishio T, Okada M, Plessy C, Shibata K, Shiraki T, Suzuki S, Tagami M, Waki K, Watahiki A, Okamura-Oho Y, Suzuki H, Kawai J, Hayashizaki Y, FANTOM Consortium, RIKEN Genome Exploration Research Group and Genome Science Group (Genome Network Project Core Group). The transcriptional landscape of the mammalian genome. *Science* 309: 1559–1563, 2005.

40. Castel SE, Martienssen RA. RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond. *Nat Rev Genet* 14: 100–112, 2013.

41. Cesana M, Cacchiarelli D, Legnini I, Santini T, Sthandier O, Chinappi M, Tramontano A, Bozzoni I. A long non-coding RNA controls muscle differentiation by functioning as a competing endogenous RNA. *Cell* 147: 358–369, 2011.

42. Chang HY, Chi JT, Dudoit S, Bondre C, van de Rijn M, Botstein D, Brown PO. Diversity, topographic differentiation, and positional memory in human fibroblasts. *Proc Natl Acad Sci USA* 99: 12877–12882, 2002.

43. Chao CW, Chan DC, Kuo A, Leder P. The mouse formin (Fmn) gene: abundant circular RNA transcripts and gene-targeted deletion analysis. *Mol Med* 4: 614–628, 1998.

44. Chen JF, Murchison EP, Tang R, Callis TE, Tatsuguchi M, Deng Z, Rojas M, Hammond SM, Schneider MD, Selzman CH, Meissner G, Patterson C, Hannon GJ, Wang DZ. Targeted deletion of Dicer in the heart leads to dilated cardiomyopathy and heart failure. *Proc Natl Acad Sci USA* 105: 2111–2116, 2008.

45. Chen X, Liang H, Zhang J, Zen K, Zhang CY. Secreted microRNAs: a new form of intercellular communication. *Trends Cell Biol* 22: 125–132, 2012.

46. Cheng C, Wang Q, You W, Chen M, Xia J. MiRNAs as biomarkers of myocardial infarction: a meta-analysis. *PLoS One* 9: e88566, 2014.

47. Clark BS, Blackshaw S. Long non-coding RNA-dependent transcriptional regulation in neuronal development and disease. *Front Genet* 5: 164, 2014.

48. Clemson CM, Hutchinson JN, Sara SA, Ensminger AW, Fox AH, Chess A, Lawrence JB. An architectural role for a nuclear non-coding RNA: NEAT1 RNA is essential for the structure of paraspeckles. *Mol Cell* 33: 717–726, 2009.

49. Clemson CM, McNeil JA, Willard HF, Lawrence JB. XIST RNA paints the inactive X chromosome at interphase: evidence for a novel RNA involved in nuclear/chromosome structure. *J Cell Biol* 132: 259–275, 1996.

50. Cocquerelle C, Mascrez B, Hetuin D, Bailleul B. Mis-splicing yields circular RNA molecules. *FASEB J* 7: 155–160, 1993.

51. Croft D, Mundo AF, Haw R, Milacic M, Weiser J, Wu G, Caudy M, Garapati P, Gillespie M, Kamdar MR, Jassal B, Jupe S, Matthews L, May B, Palatnik S, Rothfels K, Shamovsky V, Song H, Williams M, Birney E, Hermjakob H, Stein L, D'Eustachio P. The Reactome pathway knowledgebase. *Nucleic Acids Res* 42: D472–477, 2014.

52. Cruz JA, Westhof E. The dynamic landscapes of RNA architecture. *Cell* 136: 604–609, 2009.

53. Cullen BR. Transcription and processing of human microRNA precursors. *Mol Cell* 16: 861–865, 2004.

54. Cushing L, Kuang PP, Qian J, Shao F, Wu J, Little F, Thannickal VJ, Cardoso WV, Lu J. miR-29 is a major regulator of genes associated with pulmonary fibrosis. *Am J Respir Cell Mol Biol* 45: 287–294, 2011.

55. Dangwal S, Bang C, Thum T. Novel techniques and targets in cardiovascular microRNA research. *Cardiovasc Res* 93: 545–554, 2012.

56. Davis S, Lollo B, Freier S, Esau C. Improved targeting of miRNA with antisense oligonucleotides. *Nucleic Acids Res* 34: 2294–2304, 2006.

57. De Lange FJ, Moorman AF, Anderson RH, Manner J, Soufan AT, de Gier-de Vries C, Schneider MD, Webb S, van den Hoff MJ, Christoffels VM. Lineage and morphogenetic analysis of the cardiac valves. *Circ Res* 95: 645–654, 2004.

58. Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ. Processing of primary microRNAs by the Microprocessor complex. *Nature* 432: 231–235, 2004.

59. Derda AA, Thum S, Lorenzen JM, Bavendiek U, Heineke J, Keyser B, Stuhrmann M, Givens RC, Kennel PJ, Schulze PC, Widder JD, Bauersachs J, Thum T. Blood-based microRNA signatures differentiate various forms of cardiac hypertrophy. *Int J Cardiol* 196: 115–122, 2015.

60. 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, Lassmann T, Carninci P, Brown JB, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Shiekhattar R, Gingeras TR, Hubbard TJ, Notredame C, Harrow J, Guigo R. The GENCODE v7 catalog of human long non-coding RNAs: analysis of their gene structure, evolution, and expression. *Genome Res* 22: 1775–1789, 2012.

61. Devaux Y, Zangrando J, Schroen B, Creemers EE, Pedrazzini T, Chang CP, Dorn GW 2nd, Thum T, Heymans S, Cardiolinc Network. Long non-coding RNAs in cardiac development and ageing. *Nat Rev Cardiol* 12: 415–425, 2015.

62. Diederichs S. The four dimensions of non-coding RNA conservation. *Trends Genet* 30: 121–123, 2014.

63. Diederichs S, Haber DA. Dual role for argonautes in microRNA processing and posttranscriptional regulation of microRNA expression. *Cell* 131: 1097–1108, 2007.

64. Dinger ME, Gascoigne DK, Mattick JS. The evolution of RNAs with multiple functions. *Biochimie* 93: 2013–2018, 2011.

65. Dirkx E, Gladka MM, Philippen LE, Armand AS, Kinet V, Leptidis S, El Azzouzi H, Salic K, Bourajjaj M, da Silva GJ, Olieslagers S, van der Nagel R, de Weger R, Bitsch N, Kisters N, Seyen S, Morikawa Y, Chanoine C, Heymans S, Volders PG, Thum T, Dimmeler S, Cserjesi P, Eschenhagen T, da Costa Martins PA, De Windt LJ. Nfat and miR-25 cooperate to reactivate the transcription factor Hand2 in heart failure. *Nat Cell Biol* 15: 11: 1282–1293, 2013.

66. Dugre-Brisson S, Elvira G, Boulay K, Chatel-Chaix L, Mouland AJ, DesGroseillers L. Interaction of Staufen1 with the 5' end of mRNA facilitates translation of these RNAs. *Nucleic Acids Res* 33: 15: 4797–4812, 2005.

67. Elmen J, Lindow M, Schutz S, Lawrence M, Petri A, Obad S, Lindholm M, Hedtjarn M, Hansen HF, Berger U, Gullans S, Kearney P, Sarnow P, Straarup EM, Kauppinen S. LNA-mediated microRNA silencing in non-human primates. *Nature* 452: 896–899, 2008.

68. ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature* 489: 57–74, 2012.

69. Ender C, Krek A, Friedlander MR, Beitzinger M, Weinmann L, Chen W, Pfeffer S, Rajewsky N, Meister G. A human snoRNA with microRNA-like functions. *Mol Cell* 32: 519–528, 2008.

70. Enright AJ, John B, Gaul U, Tuschl T, Sander C, Marks DS. MicroRNA targets in *Drosophila*. *Genome Biol* 5: R1, 2003.

71. Eulalio A, Huntzinger E, Izaurralde E. Getting to the root of miRNA-mediated gene silencing. *Cell* 132: 9–14, 2008.

72. Fahey ME, Moore TF, Higgins DG. Overlapping antisense transcription in the human genome. *Comp Funct Genomics* 3: 244–253, 2002.

73. Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. *Nat Rev Genet* 15: 7–21, 2014.

74. Fiedler J, Breckwoldt K, Remmele CW, Hartmann D, Dittrich M, Pfanne A, Just A, Xiao K, Kunz M, Muller T, Hansen A, Geffers R, Dandekar T, Eschenhagen T, Thum T. Development of long non-coding RNA-based strategies to modulate tissue vascularization. *J Am Coll Cardiol* 66: 2005–2015, 2015.

75. Fiedler J, Gupta SK, Thum T. Identification of cardiovascular microRNA targetomes. *J Mol Cell Cardiol* 51: 674–681, 2011.

76. Fiedler J, Jazbutyte V, Kirchmaier BC, Gupta SK, Lorenzen J, Hartmann D, Galuppo P, Kneitz S, Pena JT, Sohn-Lee C, Loyer X, Soutschek J, Brand T, Tuschl T, Heineke J, Martin U, Schulte-Merker S, Ertl G, Engelhardt S, Bauersachs J, Thum T. MicroRNA-24 regulates vascularity after myocardial infarction. *Circulation* 124: 720–730, 2011.

77. Friedman SL, Sheppard D, Duffield JS, Violette S. Therapy for fibrotic diseases: nearing the starting line. *Sci Transl Med* 167: 167sr1, 2013.

78. Galupa R, Heard E. X-chromosome inactivation: new insights into *cis* and *trans* regulation. *Curr Opin Genet Dev* 31: 57–66, 2015.

79. Gebert LF, Rebhan MA, Crivelli SE, Denzler R, Stoffel M, Hall J. Miravirsen (SPC3649) can inhibit the biogenesis of miR-122. *Nucleic Acids Res* 42: 609–621, 2014.

80. Geisler S, Coller J. RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. *Nat Rev Mol Cell Biol* 14: 699–712, 2013.

81. Gezer U, Ozgur E, Cetinkaya M, Isin M, Dalay N. Long non-coding RNAs with low expression levels in cells are enriched in secreted exosomes. *Cell Biol Int* 38: 1076–1079, 2014.

82. Ghildiyal M, Xu J, Seitz H, Weng Z, Zamore PD. Sorting of *Drosophila* small silencing RNAs partitions microRNA* strands into the RNA interference pathway. *RNA* 16: 43–56, 2010.

83. Ghosal S, Das S, Sen R, Basak P, Chakrabarti J. Circ2Traits: a comprehensive database for circular RNA potentially associated with disease and traits. *Front Genet* 4: 283, 2013.

84. Ghosh AK, Nagpal V, Covington JW, Michaels MA, Vaughan DE. Molecular basis of cardiac endothelial-to-mesenchymal transition (EndMT): differential expression of microRNAs during EndMT. *Cell Signal* 24: 1031–1036, 2012.

85. Glazar P, Papavasileiou P, Rajewsky N. circBase: a database for circular RNAs. *RNA* 20: 1666–1670, 2014.

86. Gong C, Maquat LE. lncRNAs transactivate STAU1-mediated mRNA decay by duplexing with 3' UTRs via Alu elements. *Nature* 470: 284–288, 2011.

87. Gray KA, Yates B, Seal RL, Wright MW, Bruford Genenames EA. org: the HGNC resources in 2015. *Nucleic Acids Res* 43: D1079–1085, 2015.

88. Gregory PA, Bracken CP, Smith E, Bert AG, Wright JA, Roslan S, Morris M, Wyatt L, Farshid G, Lim YY, Lindeman GJ, Shannon MF, Drew PA, Khew-Goodall Y, Goodall GJ. An autocrine TGF-beta/ZEB/miR-200 signaling network regulates establishment and maintenance of epithelial-mesenchymal transition. *Mol Biol Cell* 22: 1686–1698, 2011.

89. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res* 34: D140–144, 2006.

90. Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ. miRBase: tools for microRNA genomics. *Nucleic Acids Res* 36: D154–158, 2008.

91. Grote P, Wittler L, Hendrix D, Koch F, Wahrisch S, Beisaw A, Macura K, Blass G, Kellis M, Werber M, Herrmann BG. The tissue-specific lncRNA Fendrr is an essential regulator of heart and body wall development in the mouse. *Dev Cell* 24: 206–214, 2013.

92. Guo JU, Agarwal V, Guo H, Bartel DP. Expanded identification and characterization of mammalian circular RNAs. *Genome Biol* 15: 409-014-0409-z, 2014.

93. Gupta SK, Bang C, Thum T. Circulating microRNAs as biomarkers and potential paracrine mediators of cardiovascular disease. *Circ Cardiovasc Genet* 3: 484–488, 2010.

94. Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP, Cabili MN, Jaenisch R, Mikkelsen TS, Jacks T, Hacohen N, Bernstein BE, Kellis M, Regev A, Rinn JL, Lander ES. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. *Nature* 458: 223–227, 2009.

95. Guttman M, Donaghey J, Carey BW, Garber M, Grenier JK, Munson G, Young G, Lucas AB, Ach R, Bruhn L, Yang X, Amit I, Meissner A, Regev A, Rinn JL, Root DE, Lander ES. lincRNAs act in the circuitry controlling pluripotency and differentiation. *Nature* 477: 295–300, 2011.

96. Guttman M, Rinn JL. Modular regulatory principles of large non-coding RNAs. *Nature* 482: 339–346, 2012.

97. Guttman M, Russell P, Ingolia NT, Weissman JS, Lander ES. Ribosome profiling provides evidence that large non-coding RNAs do not encode proteins. *Cell* 154: 240–251, 2013.

98. Ha M, Kim VN. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol* 15: 509–524, 2014.

99. Hacisuleyman E, Goff LA, Trapnell C, Williams A, Henao-Mejia J, Sun L, McClanahan P, Hendrickson DG, Sauvageau M, Kelley DR, Morse M, Engreitz J, Lander ES, Guttman M, Lodish HF, Flavell R, Raj A, Rinn JL. Topological organization of multichromosomal regions by the long intergenic non-coding RNA Firre. *Nat Struct Mol Biol* 21: 198–206, 2014.

100. Han P, Li W, Lin CH, Yang J, Shang C, Nurnberg ST, Jin KK, Xu W, Lin CY, Lin CJ, Xiong Y, Chien HC, Zhou B, Ashley E, Bernstein D, Chen PS, Chen HS, Quertermous T, Chang CP. A long non-coding RNA protects the heart from pathological hypertrophy. *Nature* 514: 102–106, 2014.

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

102. Hansen TB, Wiklund ED, Bramsen JB, Villadsen SB, Statham AL, Clark SJ, Kjems J. miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA. *EMBO J* 30: 4414–4422, 2011.

103. Harfe BD, McManus MT, Mansfield JH, Hornstein E, Tabin CJ. The RNaseIII enzyme Dicer is required for morphogenesis but not patterning of the vertebrate limb. *Proc Natl Acad Sci USA* 102: 10898–10903, 2005.

104. Harris KS, Zhang Z, McManus MT, Harfe BD, Sun X. Dicer function is essential for lung epithelium morphogenesis. *Proc Natl Acad Sci USA* 103: 2208–2213, 2006.

105. He C, Ding JW, Li S, Wu H, Jiang YR, Yang W, Teng L, Yang J, Yang J. The role of long intergenic Non-coding RNA p21 in vascular endothelial cells. *DNA Cell Biol* 34: 677–683, 2015.

106. He Y, Wu YT, Huang C, Meng XM, Ma TT, Wu BM, Xu FY, Zhang L, Lv XW, Li J. Inhibitory effects of long non-coding RNA MEG3 on hepatic stellate cells activation and liver fibrogenesis. *Biochim Biophys Acta* 1842: 11: 2204–2215, 2014.

107. Heymans S, Corsten MF, Verhesen W, Carai P, van Leeuwen RE, Custers K, Peters T, Hazebroek M, Stoger L, Wijnands E, Janssen BJ, Creemers EE, Pinto YM, Grimm D, Schurmann N, Vigorito E, Thum T, Stassen F, Yin X, Mayr M, de Windt LJ, Lutgens E, Wouters K, de Winther MP, Zacchigna S, Giacca M, van Bilsen M, Papageorgiou AP, Schroen B. Macrophage microRNA-155 promotes cardiac hypertrophy and failure. *Circulation* 128: 1420–1432, 2013.

108. Hinkel R, Penzkofer D, Zuhlke S, Fischer A, Husada W, Xu QF, Baloch E, van Rooij E, Zeiher AM, Kupatt C, Dimmeler S. Inhibition of microRNA-92a protects against ischemia/reperfusion injury in a large-animal model. *Circulation* 128: 1066–1075, 2013.

109. Hofacker IL. Vienna RNA secondary structure server. *Nucleic Acids Res* 31: 3429–3431, 2003.

110. Hou P, Zhao Y, Li Z, Yao R, Ma M, Gao Y, Zhao L, Zhang Y, Huang B, Lu J. LincRNA-ROR induces epithelial-to-mesenchymal transition and contributes to breast cancer tumorigenesis and metastasis. *Cell Death Dis* 5: e1287, 2014.

111. Houseley J, Rubbi L, Grunstein M, Tollervey D, Vogelauer M. A ncRNA modulates histone modification and mRNA induction in the yeast GAL gene cluster. *Mol Cell* 32: 685–695, 2008.

112. Huang HS, Allen JA, Mabb AM, King IF, Miriyala J, Taylor-Blake B, Sciaky N, Dutton JW, Jr Lee HM, Chen X, Jin J, Bridges AS, Zylka MJ, Roth BL, Philpot BD. Topoisomerase inhibitors unsilence the dormant allele of Ube3a in neurons. *Nature* 481: 185–189, 2011.

113. Huarte M, Guttman M, Feldser D, Garber M, Koziol MJ, Kenzelmann-Broz D, Khalil AM, Zuk O, Amit I, Rabani M, Attardi LD, Regev A, Lander ES, Jacks T, Rinn JL. A large intergenic non-coding RNA induced by p53 mediates global gene repression in the p53 response. *Cell* 142: 409–419, 2010.

114. Hube F, Velasco G, Rollin J, Furling D, Francastel C. Steroid receptor RNA activator protein binds to and counteracts SRA RNA-mediated activation of MyoD and muscle differentiation. *Nucleic Acids Res* 39: 513–525, 2011.

115. Hung T, Wang Y, Lin MF, Koegel AK, Kotake Y, Grant GD, Horlings HM, Shah N, Umbricht C, Wang P, Wang Y, Kong B, Langerod A, Borresen-Dale AL, Kim SK, van de Vijver M, Sukumar S, Whitfield ML, Kellis M, Xiong Y, Wong DJ, Chang HY. Extensive and coordinated transcription of non-coding RNAs within cell-cycle promoters. *Nat Genet* 43: 621–629, 2011.

116. Hunter MP, Ismail N, Zhang X, Aguda BD, Lee EJ, Yu L, Xiao T, Schafer J, Lee ML, Schmittgen TD, Nana-Sinkam SP, Jarjoura D, Marsh CB. Detection of microRNA expression in human peripheral blood microvesicles. *PLoS One* 3: e3694, 2008.

117. Ilott NE, Heward JA, Roux B, Tsitsiou E, Fenwick PS, Lenzi L, Goodhead I, Hertz-Fowler C, Heger A, Hall N, Donnelly LE, Sims D, Lindsay MA. Long non-coding RNAs and enhancer RNAs regulate the lipopolysaccharide-induced inflammatory response in human monocytes. *Nat Commun* 5: 3979, 2014.

118. Ingolia NT, Brar GA, Stern-Ginossar N, Harris MS, Talhouarne GJ, Jackson SE, Wills MR, Weissman JS. Ribosome profiling reveals pervasive translation outside of annotated protein-coding genes. *Cell Rep* 8: 1365–1379, 2014.

119. Ingolia NT, Lareau LF, Weissman JS. Ribosome profiling of mouse embryonic stem cells reveals the complexity and dynamics of mammalian proteomes. *Cell* 147: 789–802, 2011.

120. Iwano M, Plieth D, Danoff TM, Xue C, Okada H, Neilson EG. Evidence that fibroblasts derive from epithelium during tissue fibrosis. *J Clin Invest* 110: 341–350, 2002.

121. Iyer MK, Niknafs YS, Malik R, Singhal U, Sahu A, Hosono Y, Barrette TR, Prensner JR, Evans JR, Zhao S, Poliakov A, Cao X, Dhanasekaran SM, Wu YM, Robinson DR, Beer DG, Feng FY, Iyer HK, Chinnaiyan AM. The landscape of long non-coding RNAs in the human transcriptome. *Nat Genet* 47: 199–208, 2015.

122. Janssen HL, 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. Treatment of HCV infection by targeting microRNA. *N Engl J Med* 368: 1685–1694, 2013.

123. Jeck WR, Sharpless NE. Detecting and characterizing circular RNAs. *Nat Biotechnol* 32: 453–461, 2014.

124. Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, Marzluff WF, Sharpless NE. Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA* 19: 141–157, 2013.

125. Jia H, Osak M, Bogu GK, Stanton LW, Johnson R, Lipovich L. Genome-wide computational identification and manual annotation of human long non-coding RNA genes. *RNA* 16: 1478–1487, 2010.

126. Jiang Q, Wang Y, Hao Y, Juan L, Teng M, Zhang X, Li M, Wang G, Liu Y. miR2Disease: a manually curated database for microRNA deregulation in human disease. *Nucleic Acids Res* 37: D98–104, 2009.

127. Jiang X, Zhang F, Ning Q. Losartan reverses the down-expression of long non-coding RNA-NR024118 and Cdkn1c induced by angiotensin II in adult rat cardiac fibroblasts. *Pathol Biol* 63: 122–125, 2015.

128. Jiang XY, Ning QL. Expression profiling of long non-coding RNAs and the dynamic changes of lncRNA-NR024118 and Cdkn1c in angiotensin II-treated cardiac fibroblasts. *Int J Clin Exp Pathol* 7: 1325–1336, 2014.

129. John B, Enright AJ, Aravin A, Tuschl T, Sander C, Marks DS. Human MicroRNA targets. *PLoS Biol* 2: e363, 2004.

130. Kanehisa M, Goto S, Furumichi M, Tanabe M, Hirakawa M. KEGG for representation and analysis of molecular networks involving diseases and drugs. *Nucleic Acids Res* 38: D355–360, 2010.

131. Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Willingham AT, Stadler PF, Hertel J, Hackermuller J, Hofacker IL, Bell I, Cheung E, Drenkow J, Dumais E, Patel S, Helt G, Ganesh M, Ghosh S, Piccolboni A, Sementchenko V, Tammana H, Gingeras TR. RNA maps reveal new RNA classes and a possible function for pervasive transcription. *Science* 316: 1484–1488, 2007.

132. Karakikes I, Chaanine AH, Kang S, Mukete BN, Jeong D, Zhang S, Hajjar RJ, Lebeche D. Therapeutic cardiac-targeted delivery of miR-1 reverses pressure overload-induced cardiac hypertrophy and attenuates pathological remodeling. *J Am Heart Assoc* 2: e000078, 2013.

133. Katayama S, Tomaru Y, Kasukawa T, Waki K, Nakanishi M, Nakamura M, Nishida H, Yap CC, Suzuki M, Kawai J, Suzuki H, Carninci P, Hayashizaki Y, Wells C, Frith M, Ravasi T, Pang KC, Hallinan J, Mattick J, Hume DA, Lipovich L, Batalov S, Engstrom PG, Mizuno Y, Faghihi MA, Sandelin A, Chalk AM, Mottagui-Tabar S, Liang Z, Lenhard B, Wahlestedt C, RIKEN Genome Exploration Research Group, Genome Science Group (Genome Network Project Core Group), FANTOM Consortium. Antisense transcription in the mammalian transcriptome. *Science* 309: 1564–1566, 2005.

134. Kelly S, Greenman C, Cook PR, Papantonis A. Exon skipping is correlated with exon circularization. *J Mol Biol* 427: 2414–2417, 2015.

135. Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, Thomas K, Presser A, Bernstein BE, van Oudenaarden A, Regev A, Lander ES, Rinn JL. Many human large intergenic non-coding RNAs associate with chromatin-modifying complexes and affect gene expression. *Proc Natl Acad Sci USA* 106: 11667–11672, 2009.

136. Kiebler MA, Hemraj I, Verkade P, Kohrmann M, Fortes P, Marion RM, Ortin J, Dotti CG. The mammalian staufen protein localizes to the somatodendritic domain of cultured hippocampal neurons: implications for its involvement in mRNA transport. *J Neurosci* 19: 288–297, 1999.

137. Kim TK, 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. Widespread transcription at neuronal activity-regulated enhancers. *Nature* 465: 182–187, 2010.

138. Klattenhoff CA, Scheuermann JC, Surface LE, Bradley RK, Fields PA, Steinhauser ML, Ding H, Butty VL, Torrey L, Haas S, Abo R, Tabebordbar M, Lee RT, Burge CB, Boyer LA. Braveheart, a long non-coding RNA required for cardiovascular lineage commitment. *Cell* 152: 570–583, 2013.

139. Knabel MK, Ramachandran K, Karhadkar S, Hwang HW, Creamer TJ, Chivukula RR, Sheikh F, Clark KR, Torbenson M, Montgomery RA, Cameron AM, Mendell JT, Warren DS. Systemic delivery of scAAV8-encoded MiR-29a ameliorates hepatic fibrosis in carbon tetrachloride-treated mice. *PLoS One* 10: e0124411, 2015.

140. Koerner MV, Pauler FM, Huang R, Barlow DP. The function of non-coding RNAs in genomic imprinting. *Development* 136: 1771–1783, 2009.

141. Korostowski L, Raval A, Breuer G, Engel N. Enhancer-driven chromatin interactions during development promote escape from silencing by a long non-coding RNA. *Epigenetics Chromatin* 4: 21–8935-4-21, 2011.

142. Kotake Y, Nakagawa T, Kitagawa K, Suzuki S, Liu N, Kitagawa M, Xiong Y. Long non-coding RNA ANRIL is required for the PRC2 recruitment to and silencing of p15(INK4B) tumor suppressor gene. *Oncogene* 30: 1956–1962, 2011.

143. Krek A, Grun D, Poy MN, Wolf R, Rosenberg L, Epstein EJ, MacMenamin P, da Piedade I, Gunsalus KC, Stoffel M, Rajewsky N. Combinatorial microRNA target predictions. *Nat Genet* 37: 495–500, 2005.

144. Kretz M, Siprashvili Z, Chu C, Webster DE, Zehnder A, Qu K, Lee CS, Flockhart RJ, Groff AF, Chow J, Johnston D, Kim GE, Spitale RC, Flynn RA, Zheng GX, Aiyer S, Raj A, Rinn JL, Chang HY, Khavari PA. Control of somatic tissue differentiation by the long non-coding RNA TINCR. *Nature* 493: 231–235, 2013.

145. Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, Stoffel M. Silencing of microRNAs in vivo with "antagomirs." *Nature* 438: 685–689, 2005.

146. Kumarswamy R, Bauters C, Volkmann I, Maury F, Fetisch J, Holzmann A, Lemesle G, de Groote P, Pinet F, Thum T. Circulating long non-coding RNA, LIPCAR, predicts survival in patients with heart failure. *Circ Res* 114: 1569–1575, 2014.

147. Kumarswamy R, Thum T. Non-coding RNAs in cardiac remodeling and heart failure. *Circ Res* 113: 676–689, 2013.

148. Kumarswamy R, Volkmann I, Beermann J, Napp LC, Jabs O, Bhayadia R, Melk A, Ucar A, Chowdhury K, Lorenzen JM, Gupta SK, Batkai S, Thum T. Vascular importance of the miR-212/132 cluster. *Eur Heart J* 35: 3224–3231, 2014.

149. Kumarswamy R, Volkmann I, Jazbutyte V, Dangwal S, Park DH, Thum T. Transforming growth factor-beta-induced endothelial-to-mesenchymal transition is partly mediated by microRNA-21. *Arterioscler Thromb Vasc Biol* 32: 361–369, 2012.

150. Latos PA, Pauler FM, Koerner MV, Senergin HB, Hudson QJ, Stocsits RR, Allhoff W, Stricker SH, Klement RM, Warczok KE, Aumayr K, Pasierbek P, Barlow DP. Airn transcriptional overlap, but not its lncRNA products, induces imprinted Igf2r silencing. *Science* 338: 1469–1472, 2012.

151. Lee I, Ajay SS, Yook JI, Kim HS, Hong SH, Kim NH, Dhanasekaran SM, Chinnaiyan AM, Athey BD. New class of microRNA targets containing simultaneous 5'-UTR and 3'-UTR interaction sites. *Genome Res* 19: 1175–1183, 2009.

152. Lee JE, Bennett CF, Cooper TA. RNase H-mediated degradation of toxic RNA in myotonic dystrophy type 1. *Proc Natl Acad Sci USA* 109: 4221–4226, 2012.

153. Lee JT, Bartolomei MS. X-inactivation, imprinting, and long non-coding RNAs in health and disease. *Cell* 152: 1308–1323, 2013.

154. Lee JT, Davidow LS, Warshawsky D. Tsix, a gene antisense to Xist at the X-inactivation centre. *Nat Genet* 21: 400–404, 1999.

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

156. Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Radmark O, Kim S, Kim VN. The nuclear RNase III Drosha initiates microRNA processing. *Nature* 425: 415–419, 2003.

157. Legnini I, Morlando M, Mangiavacchi A, Fatica A, Bozzoni I. A feedforward regulatory loop between HuR and the long non-coding RNA linc-MD1 controls early phases of myogenesis. *Mol Cell* 53: 506–514, 2014.

158. Lennox KA, Owczarzy R, Thomas DM, Walder JA, Behlke MA. Improved performance of anti-miRNA oligonucleotides using a novel non-nucleotide modifier. *Mol Ther Nucleic Acids* 2: e117, 2013.

159. Levin AA. A review of the issues in the pharmacokinetics and toxicology of phosphorothioate antisense oligonucleotides. *Biochim Biophys Acta* 1489: 69–84, 1999.

160. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 120: 15–20, 2005.

161. Li JH, Liu S, Zhou H, Qu LH, Yang JH. StarBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Res* 42: D92–97, 2014.

162. Li W, Notani D, Ma Q, Tanasa B, Nunez E, Chen AY, Merkurjev D, Zhang J, Ohgi K, Song X, Oh S, Kim HS, Glass CK, Rosenfeld MG. Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature* 498: 516–520, 2013.

163. Liang H, Zhang C, Ban T, Liu Y, Mei L, Piao X, Zhao D, Lu Y, Chu W, Yang B. A novel reciprocal loop between microRNA-21 and TGFbetaRIII is involved in cardiac fibrosis. *Int J Biochem Cell Biol* 44: 2152–2160, 2012.

164. Liang H, Zhang J, Zen K, Zhang CY, Chen X. Nuclear microRNAs and their unconventional role in regulating non-coding RNAs. *Protein Cell* 4: 325–330, 2013.

165. Lie-Venema H, van den Akker NM, Bax NA, Winter EM, Maas S, Kekarainen T, Hoeben RC, deRuiter MC, Poelmann RE, Gittenberger-de Groot AC. Origin, fate, and function of epicardium-derived cells (EPDCs) in normal and abnormal cardiac development. *Scientific World J* 7: 1777–1798, 2007.

166. Lin MF, Jungreis I, Kellis M. PhyloCSF: a comparative genomics method to distinguish protein coding and non-coding regions. *Bioinformatics* 27: i275–282, 2011.

167. Lin N, Chang KY, Li Z, Gates K, Rana ZA, Dang J, Zhang D, Han T, Yang CS, Cunningham TJ, Head SR, Duester G, Dong PD, Rana TM. An evolutionarily conserved long non-coding RNA TUNA controls pluripotency and neural lineage commitment. *Mol Cell* 53: 1005–1019, 2014.

168. Lin S, Gregory RI. MicroRNA biogenesis pathways in cancer. *Nat Rev Cancer* 15: 321–333, 2015.

169. Liu G, Friggeri A, Yang Y, Milosevic J, Ding Q, Thannickal VJ, Kaminski N, Abraham E. miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. *J Exp Med* 207: 1589–1597, 2010.

170. Lorenzen JM, Kaucsar T, Schauerte C, Schmitt R, Rong S, Hubner A, Scherf K, Fiedler J, Martino F, Kumarswamy R, Kolling M, Sorensen I, Hinz H, Heineke J, van Rooij E, Haller H, Thum T. MicroRNA-24 antagonism prevents renal ischemia reperfusion injury. *J Am Soc Nephrol* 25: 2717–2729, 2014.

171. Lorenzen JM, Schauerte C, Kielstein JT, Hubner A, Martino F, Fiedler J, Gupta SK, Faulhaber-Walter R, Kumarswamy R, Hafer C, Haller H, Fliser D, Thum T. Circulating long non-coding RNATapSaki is a predictor of mortality in critically ill patients with acute kidney injury. *Clin Chem* 61: 191–201, 2015.

172. Lorenzen JM, Schauerte C, Kolling M, Hubner A, Knapp M, Haller H, Thum T. Long non-coding RNAs in urine are detectable and may enable early detection of acute T cell-mediated rejection of renal allografts. *Clin Chem* 61: 1505–1514, 2015.

173. Lorenzen JM, Thum T. Circulating and urinary microRNAs in kidney disease. *Clin J Am Soc Nephrol* 7: 1528–1533, 2012.

174. Lorenzen JM, Volkmann I, Fiedler J, Schmidt M, Scheffner I, Haller H, Gwinner W, Thum T. Urinary miR-210 as a mediator of acute T-cell mediated rejection in renal allograft recipients. *Am J Transplant* 11: 2221–2227, 2011.

175. Lovric J, Mano M, Zentilin L, Eulalio A, Zacchigna S, Giacca M. Terminal differentiation of cardiac and skeletal myocytes induces permissivity to AAV transduction by relieving inhibition imposed by DNA damage response proteins. *Mol Ther* 20: 2087–2097, 2012.

176. Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. *Science* 303: 95–98, 2004.

177. Lyon MF. Gene action in the X-chromosome of the mouse (*Mus musculus L.*). *Nature* 190: 372–373, 1961.

178. Managadze D, Rogozin IB, Chernikova D, Shabalina SA, Koonin EV. Negative correlation between expression level and evolutionary rate of long intergenic non-coding RNAs. *Genome Biol Evol* 3: 1390–1404, 2011.

179. Mao YS, Sunwoo H, Zhang B, Spector DL. Direct visualization of the co-transcriptional assembly of a nuclear body by non-coding RNAs. *Nat Cell Biol* 13: 95–101, 2011.

180. Marahrens Y, Panning B, Dausman J, Strauss W, Jaenisch R. Xist-deficient mice are defective in dosage compensation but not spermatogenesis. *Genes Dev* 11: 156–166, 1997.

181. Marin-Bejar O, Huarte M. RNA pulldown protocol for in vitro detection and identification of RNA-associated proteins. *Methods Mol Biol* 1206: 87–95, 2015.

182. Matkovich SJ, Edwards JR, Grossenheider TC, de Guzman Strong C, Dorn GW 2nd. Epigenetic coordination of embryonic heart transcription by dynamically regulated long non-coding RNAs. *Proc Natl Acad Sci USA* 111: 12264–12269, 2014.

183. Matkovich SJ, Wang W, Tu Y, Eschenbacher WH, Dorn LE, Condorelli G, Diwan A, Nerbonne JM, Dorn GW 2nd. MicroRNA-133a protects against myocardial fibrosis and modulates electrical repolarization without affecting hypertrophy in pressure-overloaded adult hearts. *Circ Res* 106: 166–175, 2010.

184. Mattick JS, Rinn JL. Discovery and annotation of long non-coding RNAs. *Nat Struct Mol Biol* 22: 5–7, 2015.

185. McHugh CA, Chen CK, 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, Guttman M. The Xist lncRNA interacts directly with SHARP to silence transcription through HDAC3. *Nature* 521: 232–236, 2015.

186. 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. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 495: 333–338, 2013.

187. Mendell JT, Olson EN. MicroRNAs in stress signaling and human disease. *Cell* 148: 1172–1187, 2012.

188. Meng L, Person RE, Beaudet AL. Ube3a-ATS is an atypical RNA polymerase II transcript that represses the paternal expression of Ube3a. *Hum Mol Genet* 21: 3001–3012, 2012.

189. Meng L, Person RE, Huang W, Zhu PJ, Costa-Mattioli M, Beaudet AL. Truncation of Ube3a-ATS unsilences paternal Ube3a and ameliorates behavioral defects in the Angelman syndrome mouse model. *PLoS Genet* 9: e1004039, 2013.

190. Meng L, Ward AJ, Chun S, Bennett CF, Beaudet AL, Rigo F. Towards a therapy for Angelman syndrome by targeting a long non-coding RNA. *Nature* 518: 409–412, 2015.

191. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet* 10: 155–159, 2009.

192. Mercer TR, Mattick JS. Structure and function of long non-coding RNAs in epigenetic regulation. *Nat Struct Mol Biol* 20: 300–307, 2013.

193. Michalik KM, You X, Manavski Y, Doddaballapur A, Zornig M, Braun T, John D, Ponomareva Y, Chen W, Uchida S, Boon RA, Dimmeler S. Long non-coding RNA MALAT1 regulates endothelial cell function and vessel growth. *Circ Res* 114: 1389–1397, 2014.

194. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* 105: 10513–10518, 2008.

195. Mohr SE, Smith JA, Shamu CE, Neumuller RA, Perrimon N. RNAi screening comes of age: improved techniques and complementary approaches. *Nat Rev Mol Cell Biol* 15: 591–600, 2014.

196. Moore-Morris T, Guimaraes-Camboa N, Banerjee I, Zambon AC, Kisseleva T, Velayoudon A, Stallcup WB, Gu Y, Dalton ND, Cedenilla M, Gomez-Amaro R, Zhou B, Brenner DA, Peterson KL, Chen J, Evans SM. Resident fibroblast lineages mediate pressure overload-induced cardiac fibrosis. *J Clin Invest* 124: 2921–2934, 2014.

197. Morris KV, Mattick JS. The rise of regulatory RNA. *Nat Rev Genet* 15: 423–437, 2014.

198. Mousavi K, Zare H, Dell'orso S, Grontved L, Gutierrez-Cruz G, Derfoul A, Hager GL, Sartorelli V. eRNAs promote transcription by establishing chromatin accessibility at defined genomic loci. *Mol Cell* 51: 606–617, 2013.

199. Mousavi K, Zare H, Koulnis M, Sartorelli V. The emerging roles of eRNAs in transcriptional regulatory networks. *RNA Biol* 11: 106–110, 2014.

200. Nagano T, Mitchell JA, Sanz LA, Pauler FM, Ferguson-Smith AC, Feil R, Fraser P. The Air non-coding RNA epigenetically silences transcription by targeting G9a to chromatin. *Science* 322: 1717–1720, 2008.

201. Nakagawa S, Shimada M, Yanaka K, Mito M, Arai T, Takahashi E, Fujita Y, Fujimori T, Standaert L, Marine JC, Hirose T. The lncRNA Neat1 is required for corpus luteum formation and the establishment of pregnancy in a subpopulation of mice. *Development* 141: 4618–4627, 2014.

202. Nielsen H, Fiskaa T, Birgisdottir AB, Haugen P, Einvik C, Johansen S. The ability to form full-length intron RNA circles is a general property of nuclear group I introns. *RNA* 9: 1464–1475, 2003.

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

204. Novikova IV, Hennelly SP, Tung CS, Sanbonmatsu KY. Rise of the RNA machines: exploring the structure of long non-coding RNAs. *J Mol Biol* 425: 3731–3746, 2013.

205. Obad S, dos Santos CO, Petri A, Heidenblad M, Broom O, Ruse C, Fu C, Lindow M, Stenvang J, Straarup EM, Hansen HF, Koch T, Pappin D, Hannon GJ, Kauppinen S. Silencing of microRNA families by seed-targeting tiny LNAs. *Nat Genet* 43: 371–378, 2011.

206. Okamura K, Hagen JW, Duan H, Tyler DM, Lai EC. The mirtron pathway generates microRNA-class regulatory RNAs in *Drosophila*. *Cell* 130: 89–100, 2007.

207. Okamura K, Liu N, Lai EC. Distinct mechanisms for microRNA strand selection by *Drosophila* Argonautes. *Mol Cell* 36: 431–444, 2009.

208. Olson EN. MicroRNAs as therapeutic targets and biomarkers of cardiovascular disease. *Sci Transl Med* 6: 239ps3, 2014.

209. Ong CT, Corces VG. Enhancers: emerging roles in cell fate specification. *EMBO Rep* 13: 423–430, 2012.

210. Ong CT, Corces VG. Enhancer function: new insights into the regulation of tissue-specific gene expression. *Nat Rev Genet* 12: 283–293, 2011.

211. Orom UA, Derrien T, Beringer M, Gumireddy K, Gardini A, Bussotti G, Lai F, Zytnicki M, Notredame C, Huang Q, Guigo R, Shiekhattar R. Long non-coding RNAs with enhancer-like function in human cells. *Cell* 143: 46–58, 2010.

212. Orom UA, Kauppinen S, Lund AH. LNA-modified oligonucleotides mediate specific inhibition of microRNA function. *Gene* 372: 137–141, 2006.

213. Ounzain S, Micheletti R, Beckmann T, Schroen B, Alexanian M, Pezzuto I, Crippa S, Nemir M, Sarre A, Johnson R, Dauvillier J, Burdet F, Ibberson M, Guigo R, Xenarios I, Heymans S, Pedrazzini T. Genome-wide profiling of the cardiac transcriptome after myocardial infarction identifies novel heart-specific long non-coding RNAs. *Eur Heart J* 36: 353–368, 2015.

214. Ozsolak F, Poling LL, Wang Z, Liu H, Liu XS, Roeder RG, Zhang X, Song JS, Fisher DE. Chromatin structure analyses identify miRNA promoters. *Genes Dev* 22: 22: 3172–3183, 2008.

215. Pan X, Yue Y, Zhang K, Hakim CH, Kodippili K, McDonald T, Duan D. AAV-8 is more efficient than AAV-9 in transducing neonatal dog heart. *Hum Gene Ther Methods* 26: 54–61, 2015.

216. Pan Z, Sun X, Shan H, Wang N, Wang J, Ren J, Feng S, Xie L, Lu C, Yuan Y, Zhang Y, Wang Y, Lu Y, Yang B. MicroRNA-101 inhibited postinfarct cardiac fibrosis and improved left ventricular compliance via the FBJ osteosarcoma oncogene/transforming growth factor-beta1 pathway. *Circulation* 126: 840–850, 2012.

217. Pang KC, Frith MC, Mattick JS. Rapid evolution of non-coding RNAs: lack of conservation does not mean lack of function. *Trends Genet* 22: 1–5, 2006.

218. Paraskevopoulou MD, Georgakilas G, Kostoulas N, Reczko M, Maragkakis M, Dalamagas TM, Hatzigeorgiou AG. DIANA-LncBase: experimentally verified and computationally predicted microRNA targets on long non-coding RNAs. *Nucleic Acids Res* 41: D239–245, 2013.

219. Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, Ball EE, Degnan B, Muller P, Spring J, Srinivasan A, Fishman M, Finnerty J, Corbo J, Levine M, Leahy P, Davidson E, Ruvkun G. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature* 408: 86–89, 2000.

220. Pauli A, Valen E, Lin MF, Garber M, Vastenhouw NL, Levin JZ, Fan L, Sandelin A, Rinn JL, Regev A, Schier AF. Systematic identification of long non-coding RNAs expressed during zebrafish embryogenesis. *Genome Res* 22: 577–591, 2012.

221. Payer B, Rosenberg M, Yamaji M, Yabuta Y, Koyanagi-Aoi M, Hayashi K, Yamanaka S, Saitou M, Lee JT. Tsix RNA and the germline factor, PRDM14, link X reactivation and stem cell reprogramming. *Mol Cell* 52: 805–818, 2013.

222. Peebles CL, Perlman PS, Mecklenburg KL, Petrillo ML, Tabor JH, Jarrell KA, Cheng HL. A self-splicing RNA excises an intron lariat. *Cell* 44: 213–223, 1986.

223. Penny GD, Kay GF, Sheardown SA, Rastan S, Brockdorff N. Requirement for Xist in X chromosome inactivation. *Nature* 379: 131–137, 1996.

224. Picardi E, D'Erchia AM, Gallo A, Montalvo A, Pesole G. Uncovering RNA editing sites in long non-coding RNAs. *Front Bioeng Biotechnol* 2: 64, 2014.

225. Qin W, Chung AC, Huang XR, Meng XM, Hui DS, Yu CM, Sung JJ, Lan HY. TGF-beta/Smad3 signaling promotes renal fibrosis by inhibiting miR-29. *J Am Soc Nephrol* 22: 1462–1474, 2011.

226. Quattrocelli M, Crippa S, Montecchiani C, Camps J, Cornaglia AI, Boldrin L, Morgan J, Calligaro A, Casasco A, Orlacchio A, Gijsbers R, D'Hooge J, Toelen J, Janssens S, Sampaolesi M. Long-term miR-669a therapy alleviates chronic dilated cardiomyopathy in dystrophic mice. *J Am Heart Assoc* 2: e000284, 2013.

227. Reeves MB, Davies AA, McSharry BP, Wilkinson GW, Sinclair JH. Complex I binding by a virally encoded RNA regulates mitochondria-induced cell death. *Science* 316: 1345–1348, 2007.

228. Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E, Chang HY. Functional demarcation of active and silent chromatin domains in human HOX loci by non-coding RNAs. *Cell* 129: 1311–1323, 2007.

229. Roberts TC. The microRNA biology of the mammalian nucleus. *Mol Ther Nucleic Acids* 3: e188, 2014.

230. Roderburg C, Urban GW, Bettermann K, Vucur M, Zimmermann H, Schmidt S, Janssen J, Koppe C, Knolle P, Castoldi M, Tacke F, Trautwein C, Luedde T. MicroRNA profiling reveals a role for miR-29 in human and murine liver fibrosis. *Hepatology* 53: 209–218, 2011.

231. Rougeulle C, Cardoso C, Fontes M, Colleaux L, Lalande M. An imprinted antisense RNA overlaps UBE3A and a second maternally expressed transcript. *Nat Genet* 19: 15–16, 1998.

232. Roy S, Khanna S, Hussain SR, Biswas S, Azad A, Rink C, Gnyawali S, Shilo S, Nuovo GJ, Sen CK. MicroRNA expression in response to murine myocardial infarction: miR-21 regulates fibroblast metalloprotease-2 via phosphatase and tensin homologue. *Cardiovasc Res* 82: 21–29, 2009.

233. Sado T, Hoki Y, Sasaki H. Tsix silences Xist through modification of chromatin structure. *Dev Cell* 9: 159–165, 2005.

234. Saini HK, Griffiths-Jones S, Enright AJ. Genomic analysis of human microRNA transcripts. *Proc Natl Acad Sci USA* 104: 17719–17724, 2007.

235. Salameh A, Lee AK, Cardo-Vila M, Nunes DN, Efstathiou E, Staquicini FI, Dobroff AS, Marchio S, Navone NM, Hosoya H, Lauer RC, Wen S, Salmeron CC, Hoang A, Newsham I, Lima LA, Carraro DM, Oliviero S, Kolonin MG, Sidman RL, Do KA, Troncoso P, Logothetis CJ, Brentani RR, Calin GA, Cavenee WK, Dias-Neto E, Pasqualini R, Arap W. PRUNE2 is a human prostate cancer suppressor regulated by the intronic long non-coding RNA PCA3. *Proc Natl Acad Sci USA* 112: 8403–8408, 2015.

236. Salzman J, Chen RE, Olsen MN, Wang PL, Brown PO. Cell-type specific features of circular RNA expression. *PLoS Genet* 9: e1003777, 2013.

237. Salzman J, Gawad C, Wang PL, Lacayo N, Brown PO. Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. *PLoS One* 7: e30733, 2012.

238. Sasaki YT, Ideue T, Sano M, Mituyama T, Hirose T. MENepsilon/beta non-coding RNAs are essential for structural integrity of nuclear paraspeckles. *Proc Natl Acad Sci USA* 106: 2525–2530, 2009.

239. Saxena A, Tabin CJ. miRNA-processing enzyme Dicer is necessary for cardiac outflow tract alignment and chamber septation. *Proc Natl Acad Sci USA* 107: 87–91, 2010.

240. Sayed D, Abdellatif M. MicroRNAs in development and disease. *Physiol Rev* 91: 827–887, 2011.

241. Seidl CI, Stricker SH, Barlow DP. The imprinted Air ncRNA is an atypical RNAPII transcript that evades splicing and escapes nuclear export. *EMBO J* 25: 3565–3575, 2006.

242. Sessa L, Breiling A, Lavorgna G, Silvestri L, Casari G, Orlando V. Non-coding RNA synthesis and loss of Polycomb group repression accompanies the colinear activation of the human HOXA cluster. *RNA* 13: 223–239, 2007.

243. Sharma S, Findlay GM, Bandukwala HS, Oberdoerffer S, Baust B, Li Z, Schmidt V, Hogan PG, Sacks DB, Rao A. Dephosphorylation of the nuclear factor of activated T cells (NFAT) transcription factor is regulated by an RNA-protein scaffold complex. *Proc Natl Acad Sci USA* 108: 11381–11386, 2011.

244. Skog J, Wurdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT, Jr Carter BS, Krichevsky AM, Breakefield XO. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol* 10: 1470–1476, 2008.

245. Sleutels F, Zwart R, Barlow DP. The non-coding Air RNA is required for silencing autosomal imprinted genes. *Nature* 415: 810–813, 2002.

246. Somarowthu S, Legiewicz M, Chillon I, Marcia M, Liu F, Pyle AM. HOTAIR forms an intricate and modular secondary structure. *Mol Cell* 58: 353–361, 2015.

247. Song G, Shen Y, Zhu J, Liu H, Liu M, Shen YQ, Zhu S, Kong X, Yu Z, Qian L. Integrated analysis of dysregulated lncRNA expression in fetal cardiac tissues with ventricular septal defect. *PLoS One* 8: e77492, 2013.

248. Souders CA, Bowers SL, Baudino TA. Cardiac fibroblast: the renaissance cell. *Circ Res* 105: 1164–1176, 2009.

249. Standaert L, Adriaens C, Radaelli E, Van Keymeulen A, Blanpain C, Hirose T, Nakagawa S, Marine JC. The long non-coding RNA Neat1 is required for mammary gland development and lactation. *RNA* 20: 1844–1849, 2014.

250. Stenvang J, Petri A, Lindow M, Obad S, Kauppinen S. Inhibition of microRNA function by antimiR oligonucleotides. *Silence* 3: 1-907X-3-1, 2012.

251. Stocks MB, Moxon S, Mapleson D, Woolfenden HC, Mohorianu I, Folkes L, Schwach F, Dalmay T, Moulton V. The UEA sRNA workbench: a suite of tools for analysing and visualizing next generation sequencing microRNA and small RNA datasets. *Bioinformatics* 28: 2059–2061, 2012.

252. Summerton J, Weller D. Morpholino antisense oligomers: design, preparation, and properties. *Antisense Nucleic Acid Drug Dev* 7: 187–195, 1997.

253. Sun BK, Deaton AM, Lee JT. A transient heterochromatic state in Xist preempts X inactivation choice without RNA stabilization. *Mol Cell* 21: 617–628, 2006.

254. Sundberg C, Ivarsson M, Gerdin B, Rubin K. Pericytes as collagen-producing cells in excessive dermal scarring. *Lab Invest* 74: 452–466, 1996.

255. Suzuki H, Zuo Y, Wang J, Zhang MQ, Malhotra A, Mayeda A. Characterization of RNase R-digested cellular RNA source that consists of lariat and circular RNAs from pre-mRNA splicing. *Nucleic Acids Res* 34: e63, 2006.

256. Swiezewski S, Liu F, Magusin A, Dean C. Cold-induced silencing by long antisense transcripts of an *Arabidopsis* Polycomb target. *Nature* 462: 799–802, 2009.

257. Takahashi K, Yan IK, Haga H, Patel T. Modulation of hypoxia-signaling pathways by extracellular linc-RoR. *J Cell Sci* 127: 1585–1594, 2014.

258. Tano K, Akimitsu N. Long non-coding RNAs in cancer progression. *Front Genet* 3: 219, 2012.

259. Thum T. Non-coding RNAs and myocardial fibrosis. *Nat Rev Cardiol* 11: 655–663, 2014.

260. Thum T. MicroRNA therapeutics in cardiovascular medicine. *EMBO Mol Med* 4: 3–14, 2012.

261. Thum T, Condorelli G. Long non-coding RNAs and microRNAs in cardiovascular pathophysiology. *Circ Res* 116: 751–762, 2015.

262. Thum T, Galuppo P, Wolf C, Fiedler J, Kneitz S, van Laake LW, Doevendans PA, Mummery CL, Borlak J, Haverich A, Gross C, Engelhardt S, Ertl G, Bauersachs J. MicroRNAs in the human heart: a clue to fetal gene reprogramming in heart failure. *Circulation* 116: 258–267, 2007.

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

264. Tripathi V, Ellis JD, Shen Z, Song DY, Pan Q, Watt AT, Freier SM, Bennett CF, Sharma A, Bubulya PA, Blencowe BJ, Prasanth SG, Prasanth KV. The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. *Mol Cell* 39: 925–938, 2010.

265. Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, Shi Y, Segal E, Chang HY. Long non-coding RNA as modular scaffold of histone modification complexes. *Science* 329: 689–693, 2010.

266. 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. The miRNA-212/132 family regulates both cardiac hypertrophy and cardiomyocyte autophagy. *Nat Commun* 3: 1078, 2012.

267. Ulitsky I, Shkumatava A, Jan CH, Sive H, Bartel DP. Conserved function of lincRNAs in vertebrate embryonic development despite rapid sequence evolution. *Cell* 147: 1537–1550, 2011.

268. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 9: 654–659, 2007.

269. Van Amerongen MJ, Bou-Gharios G, Popa E, van Ark J, Petersen AH, van Dam GM, van Luyn MJ, Harmsen MC. Bone marrow-derived myofibroblasts contribute functionally to scar formation after myocardial infarction. *J Pathol* 214: 377–386, 2008.

270. Van Rooij E. The art of microRNA research. *Circ Res* 108: 219–234, 2011.

271. Van Rooij E, Purcell AL, Levin AA. Developing microRNA therapeutics. *Circ Res* 110: 496–507, 2012.

272. Van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, Hill JA, Olson EN. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. *Proc Natl Acad Sci USA* 105: 13027–13032, 2008.

273. Vasudevan S, Tong Y, Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. *Science* 318: 1931–1934, 2007.

274. Vausort M, Wagner DR, Devaux Y. Long non-coding RNAs in patients with acute myocardial infarction. *Circ Res* 115: 668–677, 2014.

275. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nat Cell Biol* 13: 423–433, 2011.

276. Viereck J, Bang C, Foinquinos A, Thum T. Regulatory RNAs and paracrine networks in the heart. *Cardiovasc Res* 102: 290–301, 2014.

277. 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. Long noncoding RNA Chast promotes cardiac remodeling. *Sci Transl Med* 8: 326ra22, 2016.

278. Volders PJ, Helsens K, Wang X, Menten B, Martens L, Gevaert K, Vandesompele J, Mestdagh P. LNCipedia: a database for annotated human lncRNA transcript sequences and structures. *Nucleic Acids Res* 41: D246–251, 2013.

279. Volders PJ, Verheggen K, Menschaert G, Vandepoele K, Martens L, Vandesompele J, Mestdagh P. An update on LNCipedia: a database for annotated human lncRNA sequences. *Nucleic Acids Res* 43: D174–180, 2015.

280. Wahlquist C, Jeong D, Rojas-Munoz A, Kho C, Lee A, Mitsuyama S, van Mil A, Park WJ, Sluijter JP, Doevendans PA, Hajjar RJ, Mercola M. Inhibition of miR-25 improves cardiac contractility in the failing heart. *Nature* 508: 531–535, 2014.

281. Wang B, Komers R, Carew R, Winbanks CE, Xu B, Herman-Edelstein M, Koh P, Thomas M, Jandeleit-Dahm K, Gregorevic P, Cooper ME, Kantharidis P. Suppression of microRNA-29 expression by TGF-beta1 promotes collagen expression and renal fibrosis. *J Am Soc Nephrol* 23: 252–265, 2012.

282. Wang D, Garcia-Bassets I, Benner C, Li W, Su X, Zhou Y, Qiu J, Liu W, Kaikkonen MU, Ohgi KA, Glass CK, Rosenfeld MG, Fu XD. Reprogramming transcription by distinct classes of enhancers functionally defined by eRNA. *Nature* 474: 390–394, 2011.

283. Wang K, Liu F, Zhou LY, Long B, Yuan SM, Wang Y, Liu CY, Sun T, Zhang XJ, Li PF. The long non-coding RNA CHRF regulates cardiac hypertrophy by targeting miR-489. *Circ Res* 114: 1377–1388, 2014.

284. Wang Y, Xu Z, Jiang J, Xu C, Kang J, Xiao L, Wu M, Xiong J, Guo X, Liu H. Endogenous miRNA sponge lincRNA-RoR regulates Oct4, Nanog, and Sox2 in human embryonic stem cell self-renewal. *Dev Cell* 25: 69–80, 2013.

285. Wang Z, Zhu T, Qiao C, Zhou L, Wang B, Zhang J, Chen C, Li J, Xiao X. Adeno-associated virus serotype 8 efficiently delivers genes to muscle and heart. *Nat Biotechnol* 23: 321–328, 2005.

286. Wapinski O, Chang HY. Long non-coding RNAs and human disease. *Trends Cell Biol* 21: 354–361, 2011.

287. Watson CJ, Gupta SK, O'Connell E, Thum S, Glezeva N, Fendrich J, Gallagher J, Ledwidge M, Grote-Levi L, McDonald K, Thum T. MicroRNA signatures differentiate preserved from reduced ejection fraction heart failure. *Eur J Heart Fail* 17: 405–415, 2015.

288. Weber KT, Sun Y, Bhattacharya SK, Ahokas RA, Gerling IC. Myofibroblast-mediated mechanisms of pathological remodelling of the heart. *Nat Rev Cardiol* 10: 15–26, 2013.

289. Willingham AT, Orth AP, Batalov S, Peters EC, Wen BG, Aza-Blanc P, Hogenesch JB, Schultz PG. A strategy for probing the function of non-coding RNAs finds a repressor of NFAT. *Science* 309: 1570–1573, 2005.

290. Wilusz JE, Sunwoo H, Spector DL. Long non-coding RNAs: functional surprises from the RNA world. *Genes Dev* 23: 1494–1504, 2009.

291. Winter J, Jung S, Keller S, Gregory RI, Diederichs S. Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat Cell Biol* 11: 228–234, 2009.

292. Xu Y, Liu L, Liu J, Zhang Y, Zhu J, Chen J, Liu S, Liu Z, Shi H, Shen H, Hu Z. A potentially functional polymorphism in the promoter region of miR-34b/c is associated with an increased risk for primary hepatocellular carcinoma. *Int J Cancer* 128: 412–417, 2011.

293. Yang JH, Li JH, Jiang S, Zhou H, Qu LH. ChIPBase: a database for decoding the transcriptional regulation of long non-coding RNA and microRNA genes from ChIP-Seq data. *Nucleic Acids Res* 41: D177–187, 2013.

294. Yang JH, Li JH, Shao P, Zhou H, Chen YQ, Qu LH. starBase: a database for exploring microRNA-mRNA interaction maps from Argonaute CLIP-Seq and Degradome-Seq data. *Nucleic Acids Res* 39: D202–209, 2011.

295. Yang JR, Zhang J. Human long non-coding RNAs are substantially less folded than messenger RNAs. *Mol Biol Evol* 32: 970–977, 2015.

296. Yang S, Banerjee S, de Freitas A, Sanders YY, Ding Q, Matalon S, Thannickal VJ, Abraham E, Liu G. Participation of miR-200 in pulmonary fibrosis. *Am J Pathol* 180: 484–493, 2012.

297. Yang W, Shao J, Bai X, Zhang G. Expression of Plasma microRNA-1/21/ 208a/499 in myocardial ischemic reperfusion injury. *Cardiology* 130: 237–241, 2015.

298. Yap KL, Li S, Munoz-Cabello AM, Raguz S, Zeng L, Mujtaba S, Gil J, Walsh MJ, Zhou MM. Molecular interplay of the non-coding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. *Mol Cell* 38: 662–674, 2010.

299. Yi R, O'Carroll D, Pasolli HA, Zhang Z, Dietrich FS, Tarakhovsky A, Fuchs E. Morphogenesis in skin is governed by discrete sets of differentially expressed microRNAs. *Nat Genet* 38: 356–362, 2006.

300. Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev* 17: 3011–3016, 2003.

301. Yoon JH, Abdelmohsen K, Srikantan S, Yang X, Martindale JL, De S, Huarte M, Zhan M, Becker KG, Gorospe M. LincRNA-p21 suppresses target mRNA translation. *Mol Cell* 47: 648–655, 2012.

302. Zhang XO, Wang HB, Zhang Y, Lu X, Chen LL, Yang L. Complementary sequence-mediated exon circularization. *Cell* 159: 134–147, 2014.

303. Zhang Y, Zhang XO, Chen T, Xiang JF, Yin QF, Xing YH, Zhu S, Yang L, Chen LL. Circular intronic long non-coding RNAs. *Mol Cell* 51: 792–806, 2013.

304. Zhang Y, Zou Y, Wang W, Zuo Q, Jiang Z, Sun M, De W, Sun L. Down-regulated long non-coding RNA MEG3 and its effect on promoting apoptosis and suppressing migration of trophoblast cells. *J Cell Biochem* 116: 542–550, 2015.

305. Zhao J, Sun BK, Erwin JA, Song JJ, Lee JT. Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. *Science* 322: 750–756, 2008.

306. Zhong X, Chung AC, Chen HY, Meng XM, Lan HY. Smad3-mediated upregulation of miR-21 promotes renal fibrosis. *J Am Soc Nephrol* 22: 1668–1681, 2011.

307. Zhou Q, Chung AC, Huang XR, Dong Y, Yu X, Lan HY. Identification of novel long non-coding RNAs associated with TGF-beta/Smad3-mediated renal inflammation and fibrosis by RNA sequencing. *Am J Pathol* 184: 409–417, 2014.

308. Zuker M. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res* 31: 3406–3415, 2003.