Vascular Pharmacology 114 (2019) 13–22

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Review

Long non-coding RNAs in vascular biology and disease

Nicolas Jaé, Andreas W. Heumüller, Youssef Fouani, Stefanie Dimmeler*

Institute for Cardiovascular Regeneration, Goethe University, Frankfurt am Main 60590, Germany

A B S T R A C T

The advent of deep sequencing technologies recently unraveled the complexity of the human genome: Although almost entirely transcribed, only a very minor part of our genome actually accounts for protein coding exons and most is considered non-coding. Among the non-coding transcripts, long non-coding RNAs (lncRNAs) constitute a rather heterogeneous group of linear as well as circular RNAs (circRNAs). LncRNAs act *via* multiple mechanisms and several lncRNAs were shown to be involved in vascular development, growth and remodeling. For example, the lncRNAs PUNISHER, MALAT1, MEG3, and GATA6-AS regulate vessel formation *in vivo*, whereas lincRNA-p21 controls smooth muscle cell function and neointima formation. For several other lncRNAs (*e.g.* SENCR, SMILR, and HypERlnc) functional roles in smooth muscle cells/pericytes have been described *in vitro*. Less information is available with respect to the function of circRNAs. Here most studies report on expression profiles but some circRNAs (*e.g.* cANRIL or cZNF292) may also play critical roles in smooth muscle or endothelial cells *in vitro*. This review summarizes the current knowledge of lncRNA and circRNA functions in vascular biology and disease and discusses their potential use as biomarkers.

1. Introduction

In the early days of molecular biology it was recognized that genome sizes of different organisms and their presumed number of genes, reflected by species complexity, do not necessarily correlate, an observation described as c-value paradox [1]. Consequentially, it was deduced that genomes harbor huge amounts of so-called “junk DNA”, a term implying total non-functionality. However, this assumption was challenged by early RNA-DNA hybridization reactions, which revealed that so-called heterogeneous nuclear RNAs cover a larger genomic portion than messenger RNAs (mRNA) [2]. This obvious imbalance was to some extent solved by the stepwise discovery of intronic sequences and distinct functional non-coding RNAs (ncRNAs). An anticipated milestone in this regard, the completion of the human genome project, still held a big surprise: Although the number of human genes has been constantly corrected downwards, the first draft of the human genome in 2001 still estimated 30,000 to 40,000 genes to be protein-coding. This count, however, was subsequently reduced to only ~20,000 protein-coding genes [3], lagging far behind initial estimates. To put this another way: Only about 2% of the human genome is actually transcribed into mRNA [4,5]; a number surprisingly small. However, with the development of high-throughput sequencing technologies it became evident that the vast majority of the human genome is actively transcribed [6]. These observations unveiled that our transcriptome is predominantly non-coding, and at the same time brought up the question for the biological implications of the tens of thousands of newly identified ncRNAs, initiating a new era in RNA biology. The steadily growing importance of this research field is also reflected by our view on the RNA landscape. Whereas for a long time the main RNA participants in gene expression, mRNAs, ribosomal RNAs (rRNAs) and transfer RNAs were at the center of RNA research, this role was gradually taken over by novel classes of functional RNAs (Fig. 1). This process was initiated by the identification of small nuclear RNAs as central building blocks of the spliceosome and heavily attracted attention with the discovery of gene regulatory microRNAs (miRNAs) in 2001 [7]. Today, this spectrum is complemented by lncRNAs and derivatives, as well as by circRNAs. Importantly, our current knowledge of linear and circular lncRNAs is primarily descriptive, based on massive data generation by RNA deep sequencing and complicated by the analysis of distinct disease states, cell lines, tissues and the observation that some putative ncRNAs can encode micropeptides [8,9]. Nevertheless, first studies have begun to decipher the molecular functions of lncRNAs and circRNAs (Fig. 2). This review aims to summarize the current scientific knowledge of these transcripts in vascular biology (Table 1).

* Corresponding author at: Institute of Cardiovascular Regeneration, Centre for Molecular Medicine, Goethe University Frankfurt am Main, Theodor-Stern-Kai 7, D-60590 Frankfurt, Germany.

E-mail address: Dimmeler@em.uni-frankfurt.de (S. Dimmeler).

https://doi.org/10.1016/j.vph.2018.03.003

Received 24 December 2017; Received in revised form 28 March 2018; Accepted 28 March 2018

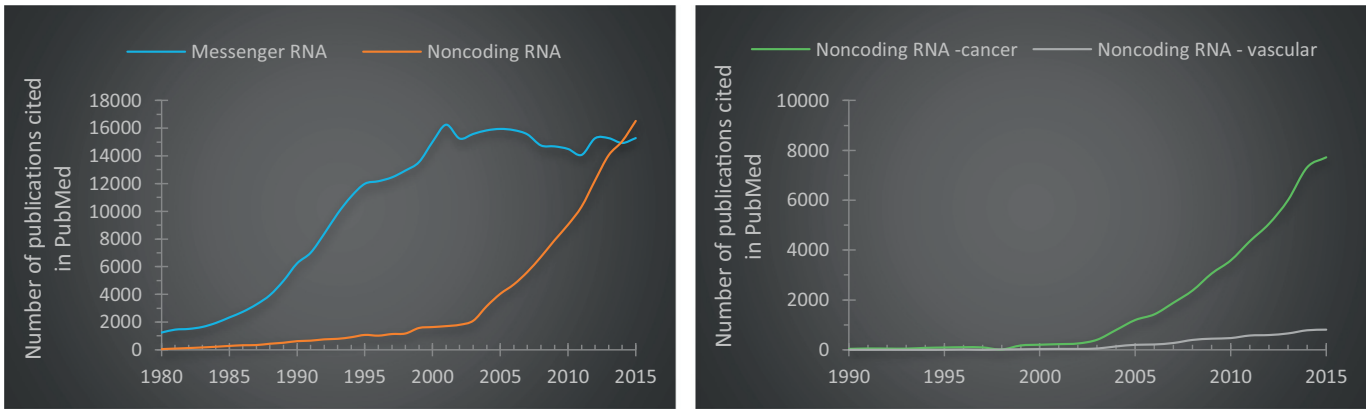

Fig. 1. Number of PubMed-listed publications from 1980/1990 to 2015, using *messenger RNA* (blue) *or noncoding RNA* (orange) and *noncoding RNA and cancer* (green), or *noncoding RNA and vascular* (grey) as keywords. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

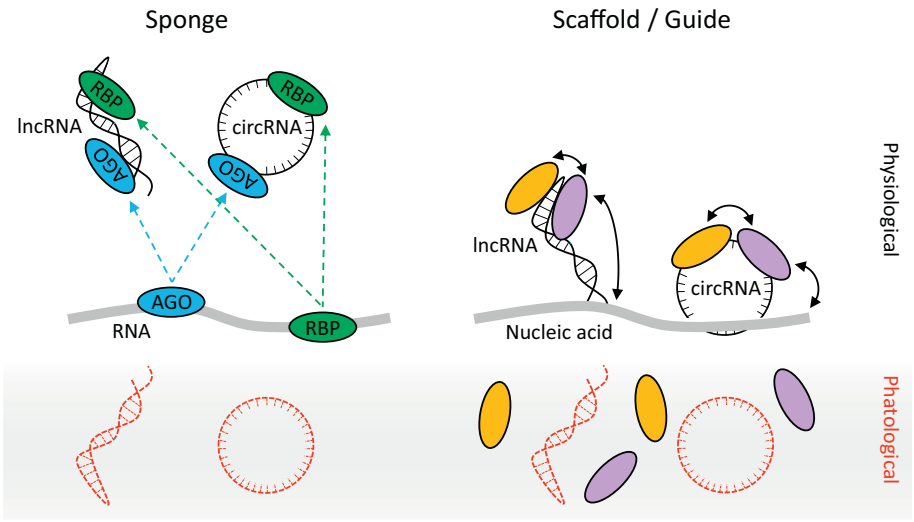

Fig. 2. Molecular functions of long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs). Left: lncRNAs and circRNAs can compete with other RNA species for the interaction with RNA binding proteins (RBPs) or miRNA-loaded RISC complexes (AGO), thereby interfering with normal protein targeting. Right: lncRNAs and circRNAs can act as scaffolds or guides, enabling various interactions between proteins and nucleic acids. Bottom: Under pathological conditions, changed lncRNA and circRNA expression levels (e.g. reduced levels as shown) can have a negative impact on the above mentioned functions.

2. Long non-coding RNAs

LncRNAs comprise a very heterogeneous group of non-protein-coding RNAs, lacking a clear classification and nomenclature. However, in order to distinguish them from small ncRNAs, a commonly accepted feature is a length > 200 nucleotides. Frequently, lncRNAs share several aspects with protein-coding mRNAs. In this regard, the majority of them seem to be 5′ capped, spliced, and 3′ polyadenylated, but interestingly, some of the most abundant lncRNAs seem to lack processing or are processed in non-canonical ways [10]. Based on their origin of transcription, with respect to their neighboring genes and genomic elements, lncRNAs can be sub-categorized into sense, natural antisense, intronic, intergenic, bidirectional promoter lncRNAs, and enhancer lncRNAs [11,12] (Fig. 3). Different studies have shown that lncRNAs display spatiotemporal expression patterns [13,14] and their aberrant expression and dysfunction is linked to several pathologies [15,16]. The molecular functions of lncRNAs are very diverse; the primary sequence of lncRNAs is only poorly conserved, however, some transcripts show positional conservation [17] and functionality is often maintained by a higher degree of structural conservation [18,19]. Of note, the molecular mechanisms of lncRNAs are closely linked to their cellular function. Many chromatin states modifying lncRNAs act in *cis* and recruit chromatin modifying enzymes to their site of transcription, thus regulating the expression of neighboring genes. In contrast, *trans*-regulatory lncRNAs recruit their binding partners to distal chromosomal sites. As a third possibility, lncRNAs can be tethered to their site of transcription, however, act on distal genomic sites, based on the three-dimensionality of the chromatin [20]. Besides their well-known role in epigenetic gene regulation, other nuclear lncRNAs were described to influence gene expression by acting as guides, scaffolds, or decoys for transcription factors, whereas a different subset focuses on the regulation of pre-mRNA splicing *via* association with RNA binding proteins. In contrast to nuclear lncRNAs, cytoplasmic lncRNAs were shown to post-transcriptionally regulate gene expression by acting as an endogenous sponge for miRNAs, thereby fine tuning the availability of these small ncRNAs at their site of action, or through modulating translation and mRNA stability [21,22].

2.1. LncRNAs in endothelial cells

Increasing evidence suggests that lncRNAs play crucial roles during vascular development and angiogenesis, two processes relying on carefully regulated gene expression. However, our insight into the contribution of lncRNAs is still at early stages. The first lncRNA, which was found to play a regulatory role in vascular development, is tie-1AS [23]. This lncRNA was identified in zebrafish and is expressed temporally and spatially with its native target, the tie-1 coding transcript, which plays a critical role in angiogenesis and blood vessel stability. Mechanistically, tie-1AS regulates tie-1 expression levels by binding to the 3′ region of the tie-1 mRNA [23].

Table 1
Selected lncRNAs with proposed *in vivo* functions.

	Function	Model	Subcellular localization	Mechanism of action	Reference
LncRNA					
ANRIL	Possesses atherogenic functions and harbors atherosclerosis-associated SNPs	Human	Nuclear	Induces cell adhesion/proliferation and suppresses apoptosis. Regulates gene expression through recruiting polycomb repressive complexes 1 and 2 *via Alu*-repeats	[54,60]
GATA6-AS	Hypoxia-induced lncRNA that controls angiogenic functions of endothelial cells as well as endothelial-to-mesenchymal transition	*In vivo* and xenograft model	Nuclear	Interacts with LOXL2 to modulate H3K4me3 levels at promoters of angiogenesis-associated genes	[45]
H19	Re-expressed in atherosclerotic plaques to recapitulate earlier developmental stages	Human	Nuclear, cytoplasmic	Sponges members of the let-7 microRNA family to regulate endothelial and smooth muscle cell functions	[61,66]
lincRNA-p21	Regulates vascular smooth muscle cell proliferation and apoptosis	Mice	Nuclear	Enhances p53 transcriptional activity *via* binding to E3 ubiquitin-protein ligase	[68]
MALAT1	Controls hind limb ischemia-induced angiogenesis and vascularization of the neonatal retina	Mice	Nuclear	Regulates endothelial proliferation and apoptosis under stress or pro-inflammatory conditions	[27,30]
MIAT	Induced in diabetic retinas and under high-glucose stressed cells. Modulates angiogenic functions of endothelial cells	Rat	Nuclear	Sponges endogenous miR-150-5p to regulate VEGF expression	[36,37]
MEG3	Attenuates angiogenesis during aging and impairs perfusion following hind limb ischemia. MEG3 downregulation was observed in patients with pulmonary arterial hypertension	Mice, human	Not determined	Regulates angiogenesis probably through modulating VEGF expression. Pro-proliferative and pro-migratory function in smooth muscle cells is presumably accomplished through p53 pathway regulation	[38,50]
PUNISHER	Involved in endothelial cell function as well as vascular and cardiac development	Zebrafish, mice	Nuclear	Regulates histone H3 phosphorylation and acetyl-low-density lipoprotein uptake	[24]
Tie-1 AS	Regulates endothelial cell junctions and vascular integrity	Zebrafish, mice	Cytoplasmic	Targets the 3′-UTR of tie-1 to regulate its expression	[23]
circRNA					
circANRIL	Regulates apoptosis and cell proliferation *in vitro*. Associated with atheroprotective functions.	Human	Not determined	Impairs rRNA maturation by interaction with the pre-ribosomal factor PES1 leading to an increase in nucleolar stress and p53 stabilization.	[112]

The effect of lncRNAs on endothelial differentiation and vascular development was further investigated by Kurian and co-workers. Their study investigated the role of differentially regulated lncRNAs during endothelial differentiation of human stem cells, leading to the identification of three novel lncRNAs which are regulated during the process of endothelial commitment [24]. A stage specific analysis revealed that these uncharacterized lncRNAs, named TERMINATOR, ALIEN, and PUNISHER, were specifically expressed in undifferentiated pluripotent stem cells, cardiovascular progenitors, and differentiated endothelial cells, respectively. The characterization of these lncRNAs confirmed a specific function of each transcript for a developmental stage [24]. In this context, PUNISHER was explicitly expressed in terminally differentiated endothelial cells and its expression positively correlated with transcripts participating in vascular development, and inversely with cell cycle, DNA damage response genes, and chromatin modifiers. Interestingly, silencing of PUNISHER *in vivo* resulted in severe vascular defects and compromised endothelial cell function [24]. SENCR is another lncRNA that was reported to be regulated upon differentiation of pluripotent cells to the endothelial lineage [25]. Although initially described to primarily function as a smooth muscle lncRNA (see Section 2.2.), SENCR also affects endothelial cell differentiation and function [25]. Forced expression of SENCR drove mesodermal and endothelial commitment by inhibiting the expression of pluripotency markers as well as ectodermal and endodermal genes. In addition, loss and gain-of-function assays suggested that SENCR acts as a pro-angiogenic and pro-migratory lncRNA, which regulates the expression of genes involved in both angiogenesis and migration of endothelial cells [25].

The lncRNA MALAT1 was first identified as highly abundant lung cancer metastasis biomarker and its aberrant expression was found to be associated with several cancers [26]. Its distinct role in endothelial biology was later on defined by Michalik et al. [27]: In endothelial cells, MALAT1 expression was induced by hypoxia, and siRNA-mediated silencing of MALAT1 reduced functional sprouting angiogenesis and endothelial cell proliferation. Transcriptome profiling upon MALAT1 depletion demonstrated that S-phase cyclins (CCNA2, CCNB1, and CCNB2), as well as cell cycle inhibitors were among the significantly regulated genes. Whereas mechanistic studies showed that MALAT1 controls epigenetic mechanisms and RNA splicing in cancer cells [28] [29], the precise molecular targets in endothelial cells are still unclear. *In vivo*, *Malat1*−/− mice showed a significant reduction in vascularization of the neonatal retina caused by impaired endothelial cell proliferation. Furthermore, Malat1 targeting using LNA GapmeRs *in vivo* impaired hind limb ischemia-induced angiogenesis [27]. The pro-proliferative and pro-angiogenic function of *MALAT1* was supported by additional studies showing that silencing of Malat1 may ameliorate diabetic retinopathy [30,31]. Furthermore, high glucose levels were found to induce Malat1 expression and to induce an inflammatory response in endothelial cells [31]. In 2017, Zhang et al. extended the functional spectrum of Malat1 by analyzing its role in cerebrovascular pathogenesis of ischemic stroke [32]. Malat1 was regulated by oxygen and glucose deprivation-induced cell death and was identified to play anti-apoptotic and anti-inflammatory roles *in vitro* and *in vivo*, most likely by direct interaction with the pro-apoptotic factor Bim and the pro-inflammatory *E*-selectin. Of note, it is unclear whether the profound effects of MALAT1 in the different models are solely due to an effect on vascular cells. MALAT1 is highly expressed in hematopoietic cells and the regulation of inflammation may well contribute to some of the effects described above. For example, knockdown of MALAT1 increased LPS-induced expression of inflammatory cytokines in macrophages [33]. Interestingly, the functions of MALAT1 on the immune system may be mediated *via* the tRNA-like processed mascRNA, which is part of the primary RNA transcript of MALAT1 [34]. Moreover, immune regulatory functions of MALAT1 were particularly observed under stress conditions [34]. This is consistent with the findings that *Malat1*-deficiency does not affect vascular homeostasis (*Malat1* deficient mice have no vascular dysfunction under baseline [27]) and suggests that

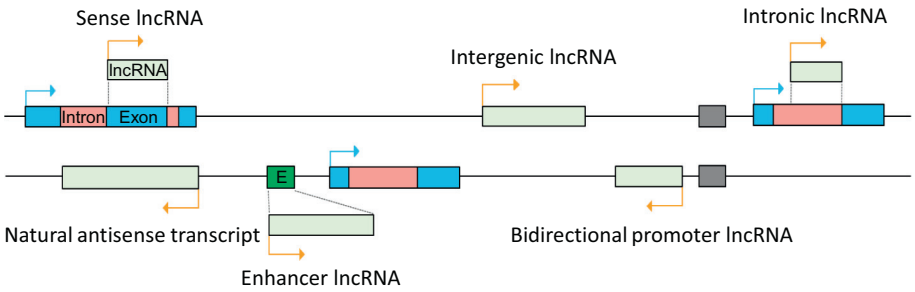

Fig. 3. Schematic representation of the various types of lncRNAs. LncRNAs are categorized depending on the genomic loci where they are transcribed from with respect to coding genes and other genomic elements. Exonic and intronic sequences of protein-coding genes are represented by blue and red boxes, and enhancer sequences and promoter sequences by dark green and grey boxes, respectively. lncRNAs are shown in light green and transcription start sites are indicated by arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

this lncRNA may have a critical role in adaptation under stress or pro-inflammatory conditions.

MIAT (also known as RNCR2, AK028326, or GOMAFU) was first described to harbor a single nucleotide polymorphism (SNP) (rs2301523) that is significantly enriched in myocardial infarction patients [35]. Yan and co-authors investigated the role of this well-conserved lncRNA in diabetes mellitus-induced microvascular dysfunction [36]. In their study, they demonstrated that the nuclear lncRNA MIAT is expressed in several retinal layers in humans and rats. Moreover, MIAT expression was upregulated in diabetic retinas and endothelial cells exposed to high glucose stress [36]. Depletion of MIAT enhanced visual function and improved diabetes mellitus-induced vascular leakage in diabetic rats. MIAT also demonstrated a pro-angiogenic function *in vitro*, as siRNA-mediated silencing in endothelial cells significantly decreased the number of migrating cells as well as TNF-α- or VEGF-induced angiogenesis. Using *in silico* predictions, Yan et al. postulated that MIAT might be targeted by several miRNAs, including miR-29 family members and miR-150-5p but also showed that MIAT may act as sponge for miR-150-5p to regulate VEGF and thereby angiogenesis [36]. How this complex interplay between MIAT and the miRNAs is regulated (degradation *versus* sponging) remains to be clarified [37].

The lncRNA MEG3 was initially demonstrated to act as a tumor suppressor and was found to be downregulated in several cancers [26]. Further investigation revealed that MEG3 was significantly upregulated during aging of endothelial cells. GapmeR-mediated silencing of this lncRNA induced sprouting angiogenesis *in vitro,* augmented perfusion after hind limb ischemia in old mice and, thus, rescued aging impaired angiogenesis [38]. In addition, Meg3-deficient mice showed augmented expression of angiogenic genes such as VEGF [39]. However, the Meg3 locus also includes the miRNAs from the 14q32 cluster, which are encoded by the same primary transcript, and several members of this cluster have known angiogenic properties [40]. Thus, it remains to be determined whether the effects observed in Meg3−/− mice are indeed mediated by the lncRNA or might be secondary to the deletion of the microRNA cluster. Interestingly, Meg3 inhibition also affected fibroblast function *in vitro* and prevented cardiac fibrosis as well as diastolic dysfunction *in vivo* [41]. It would be of interest to determine to what extent the endothelial protective and neovascularization inducing effects of MEG3 inhibition might have contributed to these therapeutic benefits.

Leisengang and co-authors identified an intronic lncRNA named MANTIS, which is transcribed antisense of Annexin A. It was demonstrated that MANTIS contributes to endothelial cell integrity through association with the ATP-dependent helicase BRG1, a component of the chromatin-remodeling complex SWI/SNF, in order to stabilize its interaction with the ATPase function stimulator BAF155. Moreover, this MANTIS-BRG1 association was found to regulate the expression of angiogenic genes, such as *SOX18*, *SMAD6*, and *COUP TFII*, in *trans* by facilitating chromatin remodeling into a more accessible structure for RNA polymerase II binding. These results were in line with MANTIS loss-of-function assays in endothelial cells, where MANTIS depletion attenuated the angiogenic and migratory capacity of these cells [42].

Preliminary evidence for a regulatory function in the vascular system was also provided for the lncRNAs HOTTIP [43] and LINC00341 [44]. HOTTIP levels were found to be elevated in coronary artery disease (CAD) samples and ectopic expression of HOTTIP increased endothelial cell proliferation and migration. Albeit the underlying mechanism is not clarified, activation of the Wnt/β-catenin pathway might play a role [43]. For LINC00341, expression levels were found to be elevated under atheroprotective flow and overexpression of this lncRNA in endothelial cells resulted in an anti-inflammatory response, which the authors ascribe to LINC00341-mediated recruitment of the repressive PRC2 complex to the *VCAM1* promoter [44].

Finally, a recent study by Neumann et al. defined the role of the long non-coding antisense transcript of GATA6 (GATA6-AS) in endothelial biology [45].This nuclear lncRNA was found to be hypoxia-induced and a functional characterization *in vitro* revealed its implication in the cardio-vascular disease-associated process of endothelial-to-mesenchymal transition, and to a lesser extent, in endothelial cell sprouting and migration. GATA6-AS silencing further promoted vessel formation in a human endothelial cell based xenograft model *in vivo*. Mechanistically, the authors identified the histone H3 lysine 4 deaminase LOXL2 as a GATA6-AS-associated protein and uncovered a set of angiogenesis-related genes that are inversely regulated by LOXL2 and GATA6-AS silencing. In particular, GATA6-AS silencing reduced H3K4me3 marks at the promoters of periostin and cyclooxygenase-2, leading to the conclusion that GATA6-AS acts as negative regulator of nuclear LOXL2 function.

A role of a lncRNA as microRNA host gene was discovered by Leung et al. who analyzed angiotensin II-regulated genes in rat vascular smooth muscle cells [46]. By combining RNA sequencing and ChIP sequencing analysis, the authors identified several lncRNAs to be regulated, among them Lnc-Ang362, which was found to be induced by angiotensin II. Interestingly, Lnc-Ang362 was identified as host transcript for two microRNAs, miR-221 and miR-222, and interference with Lnc-Ang362 expression was accompanied by a reduced expression of both microRNAs as well as by reduced cell proliferation.

2.2. LncRNAs in smooth muscle cells

SENCR was among the first lncRNAs identified to be enriched in smooth muscle cells and was found to inhibit migration and expression of contractile genes (*e.g. Myocardin*) [47]. Cytoplasm-enriched SENCR failed to exhibit *cis*-regulatory function and did not regulate the expression of its antisense coding transcript FLI1 in endothelial and smooth muscle cells. The smooth muscle enriched lncRNA SMILR is another regulator of smooth muscle cell function that drives smooth muscle cell proliferation [48]. Of note, the expression of SMILR and genes proximal to SMILR was also altered by interleukin-1α/platelet-derived growth factor treatment. The authors further suggest that SMILR controls the expression of nearby coding genes such as *HAS2*, which encodes an enzyme that synthesizes hyaluronic acid, which typically accumulates in atherosclerotic lesions.

In line with its biological function in endothelial cells, MALAT1 was also shown to promote smooth muscle cell proliferation and migration by regulating cell cycle genes [49]. Of note, Brock et al. provided

evidence that hypoxia is also up-regulating MALAT1 expression in smooth muscle cells in a HIF1α dependent manner [49].

Recently, the role of MEG3 in patients with pulmonary arterial hypertension was examined which revealed significantly reduced MEG3 expression levels in patients compared to healthy controls [50]. *In vitro*, exposure of pulmonary artery smooth muscle cells to hypoxic conditions mimicked the observed downregulation of MEG3. Moreover, siRNA-mediated silencing of MEG3 promoted cell proliferation and migration along with an increased expression of cell cycle regulating proteins, effects the authors finally linked back to MEG3 regulating the p53 pathway in pulmonary artery smooth muscle cells [50].

A recent study by Bischoff et al. addressed the role of the lncRNA HypERlnc in pericytes as well as its regulation in human heart failure and idiopathic pulmonary arterial hypertension [51]. HypERlnc was identified to be hypoxia-induced in human primary pericytes and silencing of HypERlnc strongly reduced cell viability and proliferation along with pericyte dedifferentiation, increased endothelial permeability and reduced endothelial-pericyte recruitment *in vitro*. In turn, forced expression of HypERlnc was accompanied with the upregulation of pericyte marker genes. By using transcription factor arrays, the authors showed an activation of endoplasmic reticulum stress-related transcription factors upon HypERlnc knockdown and reported a coinciding upregulation of the endoplasmic reticulum stress markers IRE1α, ATF6, and soluble BiP. Gene ontology analysis of HypERlnc-regulated genes suggested a role of this lncRNA in cardiovascular disease states, a notion which was supported by significantly reduced HypERlnc levels in human cardiac tissue from patients with heart failure and a significant correlation of HypERlnc expression with pericyte markers in lungs from patients diagnosed with idiopathic pulmonary arterial hypertension.

2.3. LncRNAs in atherosclerosis

Although several lncRNAs have been demonstrated to control endothelial or smooth muscle cell functions, little is known with respect to the *in vivo* roles of most lncRNAs and particularly regarding their role in atherosclerosis. Atherosclerosis is one of the most prevalent chronic vascular diseases, which develops predominantly at branch points or curvatures of large arteries, where the flow is disturbed. Atherosclerosis is initiated by endothelial activation and dysfunction, followed by inflammatory response and subsequent accumulation of malfunctioning smooth muscle cell, extracellular lipid disposition, and fibrous tissue formation [52]. First insights suggesting that lncRNAs may be important regulators of atherosclerosis came from GWAS studies, showing a strong association of SNPs in non-coding genes with atherosclerosis. The antisense ncRNA in the *INK4* locus, ANRIL, represents an example of a lncRNA gene harboring a hotspot of atherosclerosis-associated SNPs [53]. These mutations were found to disrupt ANRIL expression [54,55], splicing [56], as well as its molecular function. ANRIL is transcribed antisense to the cyclin-dependent kinase inhibitors CDKN2A/B. Initial studies focused on the *cis*-regulatory function of ANRIL [57] and its association with cancer [58]. However, in 2010 Holdt et al. assessed the correlation between ANRIL expression and atherosclerosis in a cohort of patients with CAD. Four SNPs (rs10757274, rs2383206, rs2383207, and rs10757278) in the Chr9p21 locus were found to correlate with several atherosclerosis phenotypes. Moreover, two annotated isoforms of ANRIL, EU741058 and NR_003529 were found to be upregulated in atherosclerotic plaques [54]. In a later work, Holdt et al. investigated the mechanism by which a subset of ANRIL isoforms carries out their atherogenic function. Overexpression of the ANRIL isoforms 1–4 modulated gene expression in *trans* and subsequently induced cell adhesion and proliferation but reduced apoptosis. These dysregulated cellular functions represent hallmarks of atherogenesis. In line with the reports on its *cis*-regulatory role [59], it was demonstrated that ANRIL regulates gene expression epigenetically through recruiting repressive components of the polycomb complexes 1 and 2 to ANRIL-target gene promoters *via Alu*-repeats [60].

Besides ANRIL, the lncRNA H19 was identified to be associated with atherosclerosis [61,62]. This developmentally regulated lncRNA belongs to an imprinted gene cluster, which was initially identified to play an important role in development and growth control [63] as well as in tumor biology [64,65]. Already in 1996, a first study suggested that H19 is re-expressed in human atherosclerotic plaques [61]. It was demonstrated that H19 is regulated *via* the insulin growth factor family and recapitulates earlier developmental states. Mechanistically, H19 harbors canonical and non-canonical binding sites for the let-7 microRNA family and therefore is considered to act as molecular microRNA sponge [66]. Let-7 family members are well-known to regulate endothelial and smooth muscle cell functions [67]. Therefore, one may speculate that sponging of let-7 family members may mediate the biological function of H19. However, the impact of this sponging function on vascular biology is still not well understood and it needs to be taken into consideration, how the lowly expressed lncRNA H19 can interfere with the numerous members of the let-7 family, which are all highly expressed in vascular cells.

One of the few lncRNAs with documented *in vivo* effects is lincRNA-p21 [68]. The expression of lincRNA-p21 was downregulated in murine atherosclerotic plaques and in patients with CAD and silencing of lincRNA-p21 was shown to increase cell proliferation and to inhibit apoptosis in vascular smooth muscle cells and macrophages *in vitro* [68]. Moreover, inhibition of lincRNA-p21 *in vivo* resulted in neointimal hyperplasia in a carotid artery injury model. Mechanistically, lincRNA-p21 enhanced p53 transcriptional activity, at least in part, *via* binding to the E3 ubiquitin-protein ligase MDM2.

Oxidized low-density lipoprotein cholesterol (oxLDL) is known to promote atherosclerosis in multiple ways. In order to analyze the involvement of lncRNAs in the pathogenesis of atherosclerosis, two studies evaluated the expression of mRNAs and lncRNAs in smooth muscle and endothelial cells upon stimulation with oxLDL [69,70]. Singh et al. identified CLDN10-AS1, which is found in antisense orientation to *CLDN10*, and *CTC-459I6.1*, which shares a small overlap with *RASGRF2*, as top-regulated transcripts [69]. Even though this study does not deal with the functional roles of the identified lncRNAs, it provides a comprehensive summary of potential candidate genes involved in atherogenesis. In the second study by Shan et al., oxLDL was shown to induce the lncRNA RNCR3 in endothelial and smooth muscle cells [70]. RNCR3 expression was also increased in atherosclerotic lesions and silencing of RNCR3 inhibited endothelial cell proliferation next to regulating smooth muscle cell migration and proliferation *in vitro*. Shan et al. claim that RNCR3 may act as sponge for miR-185-5p [70]. *TGFB2* overlapping transcript 1 (TGFB2-OT1) is another oxLDL-induced lncRNA, which regulates vascular inflammation and autophagy [71]. Although the study does not address the role in atherosclerosis, *in vitro* studies suggest that TGFB2-OT1 may act as microRNA sponge to control autophagy in endothelial cells.

2.4. LncRNAs as biomarkers

Finally, it has also been investigated whether lncRNAs detected in plasma samples or whole blood in humans might be useful as disease biomarkers. First studies suggest that extracellular circulating lncRNAs are dysregulated in patients with cardiovascular diseases. For example, H19 was shown to be increased in plasma or serum samples of humans with atherosclerotic disease [62,72]. Also LIPCAR, a lncRNA previously reported to correlate with mortality in patients with heart failure [73], was significantly increased in patients with CAD [72]. The smooth muscle regulatory lncRNA SMILR was also highly expressed in unstable atherosclerotic plaques and was augmented in plasma from patients with high levels of plasma C-reactive protein [48].

3. Circular RNAs

Circular RNAs are referred to as a novel type of non-coding RNA, which differ from linear RNAs as they are covalently closed, do not

possess strand polarities and are generated in a process termed back-splicing in which a downstream sequence is spliced to an upstream one [74]. Given that back-splicing is less favorable compared to linear splicing [75], most circRNAs are expressed at low rates, although numerous exceptions have been identified [74]. Initially, RNA circles were discovered in plant viroids [76] and the hepatitis delta virus [77]. In eukaryotes, first evidence of a circular transcript has been shown by electron microscopy as early as 1979 [78] and various individual circRNAs have been identified in the 1990s [79–86]. However, given the rather low expression rates of circRNAs, their existence was mostly attributed to mis-splicing events and they were therefore regarded as non-functional [87]. However, in 2010 Burd et al. reported multiple circRNAs generated from the lncRNA *ANRIL* gene locus and associated them with an atherosclerotic vascular disease genotype [56]. In a first RNA-sequencing data-based study in 2012, Salzmann et al. showed that exon scrambling occurs for hundreds of human genes, and that transcripts with such are most likely to be circular RNAs [74]. Subsequently, using improved computational approaches, Memzack and Salzmann showed that circRNAs are expressed cell-type-specific in humans and other animals [88,89]. Memzack et al. further presented evidence for the circRNA CDR1as (also known as ciRS-7) to act as an efficient miR-7 sponge, thereby influencing gene levels post-transcriptionally [88]. Today, we know that circRNA expression occurs among a variety of species such as rats [90–92], mice [89,90,93], flies [89,94,95] and nematodes [88,96], and circRNAs are regulated during development, aging [97,98] and in disease [99]. Although thousands of circRNAs have meanwhile been identified, the overlap between the identified circRNAs of different studies is relatively small. In this regard, Hansen et al. reported that the overlap of commonly identified circRNAs between five circRNA identification tools only attributes to 16.8% [100]. In principal, the identification of a circRNA is tied to a sequencing read covering the back-spliced exons. During circRNA biogenesis, a downstream splice donor is reversely joined with an upstream splice acceptor, which is referred to as back-splicing and thus generates a covalently closed RNA circle and the back-splice site sequence [74,89] (Fig. 4). There is strong evidence that back-splicing is carried out by the canonical spliceosomal machinery [101] and circRNA biogenesis therefore frequently competes with linear splicing [102]. Given that for most transcripts splicing occurs co-transcriptionally, the rate of circRNA formation depends on either unfavorable linear splicing [74,102,103] or is tied to exon-skipping events

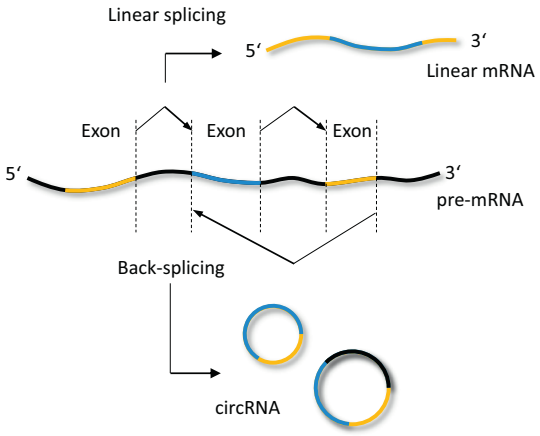

Fig. 4. Schematic comparison of linear and circular splicing reactions. Top: For linear splicing, an upstream splice donor site is joined with a downstream splice acceptor site in a sequential order. Bottom: Incontrast, circularization is achieved by linking a downstream splice donor site to an upstream splice acceptor site. Generated circRNAs may consist of exons only or retain intronic sequences. Exons are depicted in yellow or blue and intronic sequences in black. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and lariat formation [74,89,103]. This may be partly achieved as introns adjacent to the back-spliced exons are longer and less often take part in alternative splicing [74,103]. Furthermore, evidence has accumulated that circularization is strongly enhanced when the respective splice junctions are in close proximity to each other, which can be influenced by both, *cis*-elements [95,96,103–105] and *trans*-acting protein factors [95,102,106]. *cis*-Elements mainly refer to reverse complementary sequences in the neighboring introns such as *Alu* repeats in humans [103,105] or inverted repeats in mice and flies [95,104,107], which lead to the formation of hairpin-like structures. Ivanov et al. further showed that the RNA editing factor ADAR edits *Alu* repeats and thereby influences circRNA splicing by either disrupting or enhancing hairpin formation [96]. Aside from nucleic acid interactions, proteins have been shown to influence circRNA splicing as well. Conn et al. showed that the RNA-binding protein Quaking (QKI) enhances circularization by binding to the flanking regions of circRNAs during epithelial-to-mesenchymal transition [106]. Moreover, the splicing factor muscleblind (MBL) strongly regulates circRNA formation from its own gene in humans and flies [102]. The group of Wilusz further reported that multiple SR and hnRNP proteins support circRNA production in flies [95]. These studies indicate that circRNA formation is a highly regulated and dynamic process, arguing towards a biological function. Ever since the first RNA-sequencing studies on circRNAs, the functionality of circRNAs has been extensively discussed. Given their lack of strand ends, circRNAs are not targeted by exonuclease-mediated decay and therefore are more stable than most linear RNAs, which may partly compensate for the low expression levels of circRNAs. To date, the miRNA-sponge function is the best-studied mechanism by which circRNAs influence cellular homeostasis [88,108]. The most prominent example is the circRNA ciRS-7 which harbors > 63 conserved miR-7 target sites and has been reported to regulate miR-7 and its downstream targets in a wide range of cell types [108–110]. However, based on bioinformatics analyses Guo et al. proposed that only a minority of circRNAs contain multiple miRNA target sites, arguing against a general function of circRNAs as competing endogenous RNAs [93]. Similar results were obtained when bioinformatically assessing microRNA binding sites in endothelial circRNAs [111]. Other studies explored the possibility of circRNAs interacting with proteins and showed evidence that circRNAs can act as sponge or scaffold for RNA binding proteins [102,112–114]. Although circRNAs mainly localize to the cytoplasm, a nuclear subset mainly consisting of circRNAs including intronic sequences has been reported to influence the transcription and splicing of their respective host genes [115]. Recently, cap-independent translation was observed to occur from circRNAs, although the generated peptides are yet to be associated with distinct functions [116,117].

3.1. CircRNAs in endothelial cells

Although we are just beginning to unravel the molecular mechanisms of circRNAs, several studies have addressed the expression and regulation of circRNAs in the circulatory system. In regard to the vasculature, Boeckel et al. identified > 7000 circRNAs in human umbilical vein endothelial cells (HUVECs), and showed that circRNA levels are altered under hypoxic conditions. Furthermore, they provide evidence that the hypoxia regulated circRNA cZNF292 influences angiogenesis and cell proliferation in endothelial cells by circRNA silencing experiments [111]. This work is supported by Dang et al. who provide a microarray-based analysis of hypoxia regulated circRNAs in HUVECs [118]. In preliminary data, the authors identified the circRNA hsa_circ_0010729 as putative regulator of endothelial cell proliferation and apoptosis based on loss-of-function experiments. They further link these results to an interaction network between hsa_circ_0010729, miR-186 and HIF-1α. Similar results are provided by Zheng and colleagues who reported the hypoxic regulation of the circRNA hsa_circ_000595 in human aortic smooth muscle cells and a putative interaction between this circRNA and miR-19 thereby regulating apoptosis [119].

3.2. CircRNA in atherosclerosis

Circular transcripts of the lncRNA gene locus *ANRIL* (circANRIL) have been among the first identified circRNAs [56]. Interestingly, this locus was previously already associated with atherosclerotic disease, based on GWAS studies [120]. Burd et al.could correlate the expression of the circular ANRIL transcripts with these SNPs [56]. Interestingly, Holdt and colleagues further confirmed that linear ANRIL is positively correlated with increasing atherosclerotic burden, but showed that circANRIL has the opposite effect and high circANRIL levels were associated with atheroprotection [112]. Overexpression and silencing experiments in HEK cells revealed that circANRIL controls apoptosis and cell proliferation *in vitro*. Mechanistically, circANRIL was demonstrated to interact with the pre-ribosomal assembly factor PES1 thereby interfering with rRNA maturation. CircANRIL-mediated impaired rRNA processing was further linked to induction of nucleolar stress and p53 stabilization thereby affecting cell proliferation and apoptosis. It is worth mentioning, that a different study by Song et al. assessed the influence of a circular ANRIL transcript (referred to as cANRIL) in a high-fat diet and vitamin D_3-dependent atherosclerosis model in rats [121]. However, the authors state that overexpression of human cANRIL in rats leads to an increase in coronary endothelial apoptosis and an increase in inflammatory molecules in rat blood sera, suggesting an atherosclerosis promoting function instead.

One of the major risk factors in the onset of atherosclerosis is the increase in overall low density lipoproteins (LDL). Therefore, Li and colleagues investigated the expression profile of endothelial cells treated with oxLDL [122]. Microarray results revealed that > 900 circRNAs were differentially expressed of which hsa_circ_0003575 was the most strongly upregulated circRNA. The authors further showed that hsa_circ_0003575 silencing resulted in enhanced cell migration and angiogenic capacity in HUVECs. Mechanistically, Li et al. predicted putative miRNA-binding sites within hsa_circ_0003575 and suggest that hsa_circ_0003575 may influence cellular functions by acting as miRNA sponge.

3.3. CircRNAs as biomarkers

Since circRNAs have moved into the focus of research, a putative function of circRNAs as biomarker has been extensively discussed [123]. This is primarily due to the high stability of circRNAs owing to their resistance towards exonuclease degradation. Especially in the blood, where the level of RNases is comparably high, circRNAs may be more reliably measurable. Furthermore, other features such as their principal abundance [88,89], and their cell-type specific and disease-dependent regulation underline the capacity of circRNAs as putative biomarkers. In principal, circRNAs are detectable in human whole blood [124], plasma [125], saliva [126] and exosomes [127], all of which are collectable using non-invasive methods. Many circRNAs have been proposed as biomarkers in cancer [128], however, only few studies so far addressed circRNAs as putative biomarkers in cardiovascular diseases. Salgado-Somoza and colleagues analyzed circRNA levels in patients following acute myocardial infarction. They report a circRNA referred to as MICRA to be associated with heart failure development in a cohort of 472 patients. Furthermore, additionally including measurements of MICRA levels in a multivariate analysis with known predictive markers significantly improved the prediction of heart failure [129]. Zhao et al. addressed the circRNA expression pattern in peripheral blood of healthy volunteers and such suffering from CAD [130]. Using microarrays, they identified 22 differentially expressed circRNAs. Among these they identified the circRNA hsa_circ_0124644 as putative diagnostic biomarker of CAD. Their results showed that including hsa_circ_0124644 into an established set of CAD biomarkers increased the diagnostic value in an independent cohort of 115 control and 137 CAD patient samples.

4. Summary and outlook

Evidence is increasing that at least a part of the huge number of non-coding transcripts and circRNAs affect vascular biology and disease. However, the field is still in its infancies and most studies are still preliminary and limited to *in vitro* studies. Although first genetic knock out mouse models confirm the functional importance of both lncRNAs and circRNAs, one has to consider that deleting a lncRNA locus may not necessarily only affect the expression of a non-coding transcript, which acts as regulatory RNA, but the transcription of the RNA itself may establish a permissive chromatin environment [131]. Particularly for circRNAs only one paper so far demonstrated the *in vivo* function of the circRNA cdr1AS [132], but also in this case the entire locus was deleted. Since the linear mRNA was admittedly little expressed, the authors suggest that the impaired brain function is likely due to the loss of the circRNA [132]. The functional validation of lncRNAs is even further complicated by the poor conservation of the transcripts, which are often only primate-specific. Thus, the use of conventional mouse models may not necessarily decipher the full activity and the path to a potential translation of the findings may require humanized or non-human primate models.

But before even considering the clinical translation of the studies, it will also be essential to better understand the diverse mechanisms of actions of these lncRNAs. Only very few studies so far systematically addressed the interaction of lncRNAs or circRNAs with proteins, RNA or DNA and most studies focus on anticipated functions, which are easily measurable (*e.g.* microRNA sponging, *cis*-regulatory effects). Overall, there is ample room to identify so far uncharacterized lncRNAs with important biological functions and potentially also novel mechanistic circuits that control vascular homeostasis and disease.

Disclosure

N.J. and S.D. applied for a patent on hypoxia-regulated lncRNAs and circRNAs.

Acknowledgements

The authors would like to thank the DFG (SFB902) and the European Research Council for support (ERC grant Angiolnc).

References

[1] C.A. Thomas, The genetic organization of chromosomes, Annu. Rev. Genet. 5 (1971) 237–256.
[2] B.R. Hough, M.J. Smith, R.J. Britten, E.H. Davidson, Sequence complexity of heterogeneous nuclear RNA in sea urchin embryos, Cell 5 (1975) 291–299.
[3] C. Southan, Last rolls of the yoyo: Assessing the human canonical protein count, F1000Research 6 (2017) 448.
[4] E.S. Lander, L.M. Linton, B. Birren, C. Nusbaum, M.C. Zody, J. Baldwin, K. Devon, K. Dewar, M. Doyle, W. FitzHugh, R. Funke, D. Gage, K. Harris, A. Heaford, J. Howland, L. Kann, J. Lehoczky, R. LeVine, P. McEwan, K. McKernan, J. Meldrim, J.P. Mesirov, C. Miranda, W. Morris, J. Naylor, C. Raymond, M. Rosetti, R. Santos, A. Sheridan, C. Sougnez, N. Stange-Thomann, N. Stojanovic, A. Subramanian, D. Wyman, J. Rogers, J. Sulston, R. Ainscough, S. Beck, D. Bentley, J. Burton, C. Clee, N. Carter, A. Coulson, R. Deadman, P. Deloukas, A. Dunham, I. Dunham, R. Durbin, L. French, D. Grafham, S. Gregory, T. Hubbard, S. Humphray, A. Hunt, M. Jones, C. Lloyd, A. McMurray, L. Matthews, S. Mercer, S. Milne, J.C. Mullikin, A. Mungall, R. Plumb, M. Ross, R. Shownkeen, S. Sims, R.H. Waterston, R.K. Wilson, L.W. Hillier, J.D. McPherson, M.A. Marra, E.R. Mardis, L.A. Fulton, A.T. Chinwalla, K.H. Pepin, W.R. Gish, S.L. Chissoe, M.C. Wendl, K.D. Delehaunty, T.L. Miner, A. Delehaunty, J.B. Kramer, L.L. Cook, R.S. Fulton, D.L. Johnson, P.J. Minx, S.W. Clifton, T. Hawkins, E. Branscomb, P. Predki, P. Richardson, S. Wenning, T. Slezak, N. Doggett, J.F. Cheng, A. Olsen, S. Lucas, C. Elkin, E. Uberbacher, M. Frazier, R.A. Gibbs, D.M. Muzny, S.E. Scherer, J.B. Bouck, E.J. Sodergren, K.C. Worley, C.M. Rives, J.H. Gorrell, M.L. Metzker, S.L. Naylor, R.S. Kucherlapati, D.L. Nelson, G.M. Weinstock, Y. Sakaki, A. Fujiyama, M. Hattori, T. Yada, A. Toyoda, T. Itoh, C. Kawagoe, H. Watanabe, Y. Totoki, T. Taylor, J. Weissenbach, R. Heilig, W. Saurin, F. Artiguenave, P. Brottier, T. Bruls, E. Pelletier, C. Robert, P. Wincker, D.R. Smith, L. Doucette-Stamm, M. Rubenfield, K. Weinstock, H.M. Lee, J. Dubois, A. Rosenthal, M. Platzer, G. Nyakatura, S. Taudien, A. Rump, H. Yang, J. Yu, J. Wang, G. Huang,

J. Gu, L. Hood, L. Rowen, A. Madan, S. Qin, R.W. Davis, N.A. Federspiel, A.P. Abola, M.J. Proctor, R.M. Myers, J. Schmutz, M. Dickson, J. Grimwood, D.R. Cox, M.V. Olson, R. Kaul, N. Shimizu, K. Kawasaki, S. Minoshima, G.A. Evans, M. Athanasiou, R. Schultz, B.A. Roe, F. Chen, H. Pan, J. Ramser, H. Lehrach, R. Reinhardt, W.R. McCombie, M. de la Bastide, N. Dedhia, H. Blöcker, K. Hornischer, G. Nordsiek, R. Agarwala, L. Aravind, J.A. Bailey, A. Bateman, S. Batzoglou, E. Birney, P. Bork, D.G. Brown, C.B. Burge, L. Cerutti, H.C. Chen, D. Church, M. Clamp, R.R. Copley, T. Doerks, S.R. Eddy, E.E. Eichler, T.S. Furey, J. Galagan, J.G. Gilbert, C. Harmon, Y. Hayashizaki, D. Haussler, H. Hermjakob, K. Hokamp, W. Jang, L.S. Johnson, T.A. Jones, S. Kasif, A. Kaspryzk, S. Kennedy, W.J. Kent, P. Kitts, E.V. Koonin, I. Korf, D. Kulp, D. Lancet, T.M. Lowe, A. McLysaght, T. Mikkelsen, J.V. Moran, N. Mulder, V.J. Pollara, C.P. Ponting, G. Schuler, J. Schultz, G. Slater, A.F. Smit, E. Stupka, J. Szustakowski, D. Thierry-Mieg, J. Thierry-Mieg, L. Wagner, J. Wallis, R. Wheeler, A. Williams, Y.I. Wolf, K.H. Wolfe, S.P. Yang, R.F. Yeh, F. Collins, M.S. Guyer, J. Peterson, A. Felsenfeld, K.A. Wetterstrand, A. Patrinos, M.J. Morgan, P. de Jong, J.J. Catanese, K. Osoegawa, H. Shizuya, S. Choi, Y.J. Chen, J. Szustakowki, Initial sequencing and analysis of the human genome, Nature 409 (2001) 860–921.

[5] J.C. Venter, M.D. Adams, E.W. Myers, P.W. Li, R.J. Mural, G.G. Sutton, H.O. Smith, M. Yandell, C.A. Evans, R.A. Holt, J.D. Gocayne, P. Amanatides, R.M. Ballew, D.H. Huson, J.R. Wortman, Q. Zhang, C.D. Kodira, X.H. Zheng, L. Chen, M. Skupski, G. Subramanian, P.D. Thomas, J. Zhang, G.L. Gabor Miklos, C. Nelson, S. Broder, A.G. Clark, J. Nadeau, V.A. McKusick, N. Zinder, A.J. Levine, R.J. Roberts, M. Simon, C. Slayman, M. Hunkapiller, R. Bolanos, A. Delcher, I. Dew, D. Fasulo, M. Flanigan, L. Florea, A. Halpern, S. Hannenhalli, S. Kravitz, S. Levy, C. Mobarry, K. Reinert, K. Remington, J. Abu-Threideh, E. Beasley, K. Biddick, V. Bonazzi, R. Brandon, M. Cargill, I. Chandramouliswaran, R. Charlab, K. Chaturvedi, Z. Deng, V. Di Francesco, P. Dunn, K. Eilbeck, C. Evangelista, A.E. Gabrielian, W. Gan, W. Ge, F. Gong, Z. Gu, P. Guan, T.J. Heiman, M.E. Higgins, R.R. Ji, Z. Ke, K.A. Ketchum, Z. Lai, Y. Lei, Z. Li, J. Li, Y. Liang, X. Lin, F. Lu, G.V. Merkulov, N. Milshina, H.M. Moore, A.K. Naik, V.A. Narayan, B. Neelam, D. Nusskern, D.B. Rusch, S. Salzberg, W. Shao, B. Shue, J. Sun, Z. Wang, A. Wang, X. Wang, J. Wang, M. Wei, R. Wides, C. Xiao, C. Yan, A. Yao, J. Ye, M. Zhan, W. Zhang, H. Zhang, Q. Zhao, L. Zheng, F. Zhong, W. Zhong, S. Zhu, S. Zhao, D. Gilbert, S. Baumhueter, G. Spier, C. Carter, A. Cravchik, T. Woodage, F. Ali, H. An, A. Awe, D. Baldwin, H. Baden, M. Barnstead, I. Barrow, K. Beeson, D. Busam, A. Carver, A. Center, M.L. Cheng, L. Curry, S. Danaher, L. Davenport, R. Desilets, S. Dietz, K. Dodson, L. Doup, S. Ferriera, N. Garg, A. Gluecksmann, B. Hart, J. Haynes, C. Haynes, C. Heiner, S. Hladun, D. Hostin, J. Houck, T. Howland, C. Ibegwam, J. Johnson, F. Kalush, L. Kline, S. Koduru, A. Love, F. Mann, D. May, S. McCawley, T. McIntosh, I. McMullen, M. Moy, L. Moy, B. Murphy, K. Nelson, C. Pfannkoch, E. Pratts, V. Puri, H. Qureshi, M. Reardon, R. Rodriguez, Y.H. Rogers, D. Romblad, B. Ruhfel, R. Scott, C. Sitter, M. Smallwood, E. Stewart, R. Strong, E. Suh, R. Thomas, N.N. Tint, S. Tse, C. Vech, G. Wang, J. Wetter, S. Williams, M. Williams, S. Windsor, E. Winn-Deen, K. Wolfe, J. Zaveri, K. Zaveri, J.F. Abril, R. Guigó, M.J. Campbell, K.V. Sjolander, B. Karlak, A. Kejariwal, H. Mi, B. Lazareva, T. Hatton, A. Narechania, K. Diemer, A. Muruganujan, N. Guo, S. Sato, V. Bafna, S. Istrail, R. Lippert, R. Schwartz, B. Walenz, S. Yooseph, D. Allen, A. Basu, J. Baxendale, L. Blick, M. Caminha, J. Carnes-Stine, P. Caulk, Y.H. Chiang, M. Coyne, C. Dahlke, A. Mays, M. Dombroski, M. Donnelly, D. Ely, S. Esparham, C. Fosler, H. Gire, S. Glanowski, K. Glasser, A. Glodek, M. Gorokhov, K. Graham, B. Gropman, M. Harris, J. Heil, S. Henderson, J. Hoover, D. Jennings, C. Jordan, J. Jordan, J. Kasha, L. Kagan, C. Kraft, A. Levitsky, M. Lewis, X. Liu, J. Lopez, D. Ma, W. Majoros, J. McDaniel, S. Murphy, M. Newman, T. Nguyen, N. Nguyen, M. Nodell, S. Pan, J. Peck, M. Peterson, W. Rowe, R. Sanders, J. Scott, M. Simpson, T. Smith, A. Sprague, T. Stockwell, R. Turner, E. Venter, M. Wang, M. Wen, D. Wu, M. Wu, A. Xia, A. Zandieh, X. Zhu, The sequence of the human genome, Science 291 (2001) 1304–1351.

[6] T. Jensen, A. Jacquier, D. Libri, Dealing with pervasive transcription, Mol. Cell 52 (2013) 473–484.

[7] T.R. Cech, J.A. Steitz, The noncoding RNA revolution - trashing old rules to forge new ones, Cell 157 (2014) 77–94.

[8] B.R. Nelson, C.A. Makarewich, D.M. Anderson, B.R. Winders, C.D. Troupes, F. Wu, A.L. Reese, J.R. McAnally, X. Chen, E.T. Kavalali, S.C. Cannon, S.R. Houser, R. Bassel-Duby, E.N. Olson, A peptide encoded by a transcript annotated as long noncoding RNA enhances SERCA activity in muscle, Science 351 (2016) 271–275.

[9] D.M. Anderson, K.M. Anderson, C.-L. Chang, C.A. Makarewich, B.R. Nelson, J.R. McAnally, P. Kasaragod, J.M. Shelton, J. Liou, R. Bassel-Duby, E.N. Olson, A micropeptide encoded by a putative long noncoding RNA regulates muscle performance, Cell 160 (2015) 595–606.

[10] J.E. Wilusz, Long noncoding RNAs: re-writing dogmas of RNA processing and stability, Biochim. Biophys. Acta Gene Regul. Mech. 1859 (2016) 128–138.

[11] S. Uchida, S. Dimmeler, Long noncoding RNAs in cardiovascular diseases, Circ. Res. 116 (2015) 737–750.

[12] L. Ma, V.B. Bajic, Z. Zhang, On the classification of long non-coding RNAs, RNA Biol. 10 (2013) 925–933.

[13] A. Pauli, E. Valen, M.F. Lin, M. Garber, N.L. Vastenhouw, J.Z. Levin, L. Fan, A. Sandelin, J.L. Rinn, A. Regev, A.F. Schier, Systematic identification of long noncoding RNAs expressed during zebrafish embryogenesis, Genome Res. 22 (2012) 577–591.

[14] L.A. Goff, A.F. Groff, M. Sauvageau, Z. Trayes-Gibson, D.B. Sanchez-Gomez, M. Morse, R.D. Martin, L.E. Elcavage, S.C. Liapis, M. Gonzalez-Celeiro, O. Plana, E. Li, C. Gerhardinger, G.S. Tomassy, P. Arlotta, J.L. Rinn, Spatiotemporal expression and transcriptional perturbations by long noncoding RNAs in the mouse brain, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 6855–6862.

[15] M.-T. Melissari, P. Grote, Roles for long non-coding RNAs in physiology and disease, Pflügers Arch. 468 (2016) 945–958.

[16] J. Li, Z. Xuan, C. Liu, Long non-coding RNAs and complex human diseases, Int. J. Mol. Sci. 14 (2013) 18790–18808.

[17] P.G. Engström, H. Suzuki, N. Ninomiya, A. Akalin, L. Sessa, G. Lavorgna, A. Brozzi, L. Luzi, S.L. Tan, L. Yang, G. Kunarso, E.L.-C. Ng, S. Batalov, C. Wahlestedt, C. Kai, J. Kawai, P. Carninci, Y. Hayashizaki, C. Wells, V.B. Bajic, V. Orlando, J.F. Reid, B. Lenhard, L. Lipovich, Complex loci in human and mouse genomes, PLoS Genet. 2 (2006) e47.

[18] P. Johnsson, L. Lipovich, D. Grandér, K.V. Morris, Evolutionary conservation of long non-coding RNAs; sequence, structure, function, Biochim. Biophys. Acta 1840 (2014) 1063–1071.

[19] K.C. Pang, M.C. Frith, J.S. Mattick, Rapid evolution of noncoding RNAs: lack of conservation does not mean lack of function, Trends Genet. 22 (2006) 1–5.

[20] R. Bonasio, R. Shiekhattar, Regulation of transcription by long noncoding RNAs, Annu. Rev. Genet. 48 (2014) 433–455.

[21] T.R. Mercer, M.E. Dinger, J.S. Mattick, Long non-coding RNAs: insights into functions, Nat. Rev. Genet. 10 (2009) 155–159.

[22] F.P. Marchese, I. Raimondi, M. Huarte, The multidimensional mechanisms of long noncoding RNA function, Genome Biol. 18 (2017) 206.

[23] K. Li, Y. Blum, A. Verma, Z. Liu, K. Pramanik, N.R. Leigh, C.Z. Chun, G.V. Samant, B. Zhao, M.K. Garnaas, M.A. Horswill, S.A. Stanhope, P.E. North, R.Q. Miao, G.A. Wilkinson, M. Affolter, R. Ramchandran, A noncoding antisense RNA in tie-1 locus regulates tie-1 function in vivo, Blood 115 (2010) 133–139.

[24] L. Kurian, A. Aguirre, I. Sancho-Martinez, C. Benner, T. Hishida, T.B. Nguyen, P. Reddy, E. Nivet, M.N. Krause, D.A. Nelles, C. Rodriguez Esteban, J.M. Campistol, G.W. Yeo, J.C. Izpisua Belmonte, Identification of novel long noncoding RNAs underlying vertebrate cardiovascular development, Circulation 131 (2015) 1278–1290.

[25] M. Boulberdaa, E. Scott, M. Ballantyne, R. Garcia, B. Descamps, G.D. Angelini, M. Brittan, A. Hunter, M. McBride, J. McClure, J.M. Miano, C. Emanueli, N.L. Mills, J.C. Mountford, A.H. Baker, A role for the long noncoding RNA SENCR in commitment and function of endothelial cells, Mol. Ther. 24 (2016) 978–990.

[26] S. Dhamija, S. Diederichs, From junk to master regulators of invasion: LncRNA functions in migration, EMT and metastasis, Int. J. Cancer 139 (2016) 269–280.

[27] K.M. Michalik, X. You, Y. Manavski, A. Doddaballapur, M. Zörnig, T. Braun, D. John, Y. Ponomareva, W. Chen, S. Uchida, R.A. Boon, S. Dimmeler, Long noncoding RNA MALAT1 regulates endothelial cell function and vessel growth, Circ. Res. 114 (2014) 1389–1397.

[28] L. Yang, C. Lin, W. Liu, J. Zhang, K.A. Ohgi, J.D. Grinstein, P.C. Dorrestein, M.G. Rosenfeld, NcRNA- and Pc2 methylation-dependent gene relocation between nuclear structures mediates gene activation programs, Cell 147 (2011) 773–788.

[29] V. Tripathi, J.D. Ellis, Z. Shen, D.Y. Song, Q. Pan, A.T. Watt, S.M. Freier, C.F. Bennett, A. Sharma, P.A. Bubulya, B.J. Blencowe, S.G. Prasanth, K.V. Prasanth, The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation, Mol. Cell 39 (2010) 925–938.

[30] J.Y. Liu, J. Yao, X.M. Li, Y.C. Song, X.Q. Wang, Y.J. Li, B. Yan, Q. Jiang, Pathogenic role of lncRNA-MALAT1 in endothelial cell dysfunction in diabetes mellitus, Cell Death Dis. 5 (2014) e1506, , http://dx.doi.org/10.1038/cddis.2014.466.

[31] P. Puthanveetil, S. Chen, B. Feng, A. Gautam, S. Chakrabarti, Long non-coding RNA MALAT1 regulates hyperglycaemia induced inflammatory process in the endothelial cells, J. Cell. Mol. Med. 19 (2015) 1418–1425.

[32] X. Zhang, X. Tang, K. Liu, M.H. Hamblin, K.-J. Yin, Long noncoding RNA Malat1 regulates cerebrovascular pathologies in ischemic stroke, J. Neurosci. 37 (2017) 1797–1806.

[33] G. Zhao, Z. Su, D. Song, Y. Mao, X. Mao, The long noncoding RNA MALAT1 regulates the lipopolysaccharide-induced inflammatory response through its interaction with NF-κB, FEBS Lett. (2016) 2884–2895.

[34] M. Gast, B. Schroen, A. Voigt, J. Haas, U. Kuehl, D. Lassner, C. Skurk, F. Escher, X. Wang, A. Kratzer, K. Michalik, A. Papageorgiou, T. Peters, M. Loebel, S. Wilk, N. Althof, K.V. Prasanth, H. Katus, B. Meder, S. Nakagawa, C. Scheibenbogen, H.P. Schultheiss, U. Landmesser, S. Dimmeler, S. Heymans, W. Poller, Long non-coding RNA MALAT1-derived mascRNA is involved in cardiovascular innate immunity, J. Mol. Cell Biol. 8 (2016) 178–181.

[35] Y. Ohnishi, T. Tanaka, R. Yamada, K. Suematsu, M. Minami, K. Fujii, N. Hoki, K. Kodama, S. Nagata, T. Hayashi, N. Kinoshita, H. Sato, H. Sato, T. Kuzuya, H. Takeda, M. Hori, Y. Nakamura, Identification of 187 single nucleotide polymorphisms (SNPs) among 41 candidate genes for ischemic heart disease in the Japanese population, Hum. Genet. 106 (2000) 288–292.

[36] B. Yan, J. Yao, J.-Y. Liu, X.-M. Li, X.-Q. Wang, Y.-J. Li, Z.-F. Tao, Y.-C. Song, Q. Chen, Q. Jiang, lncRNA-MIAT regulates microvascular dysfunction by functioning as a competing endogenous RNA, Circ. Res. 116 (2015) 1143–1156.

[37] N. Jaé, S. Dimmeler, Long noncoding RNAs in diabetic retinopathy, Circ. Res. 116 (2015) 1104–1106.

[38] R.A. Boon, P. Hofmann, K.M. Michalik, N. Lozano-Vidal, D. Berghäuser, A. Fischer, A. Knau, N. Jaé, C. Schürmann, S. Dimmeler, Long noncoding RNA Meg3 controls endothelial cell aging and function: implications for regenerative angiogenesis, J. Am. Coll. Cardiol. 68 (2016) 2589–2591.

[39] F.E. Gordon, C.L. Nutt, P. Cheunsuchon, Y. Nakayama, K.A. Provencher, K.A. Rice, Y. Zhou, X. Zhang, A. Klibanski, Increased expression of angiogenic genes in the brains of mouse Meg3-null embryos, Endocrinology 151 (2010) 2443–2452.

[40] S. Dimmeler, S. Yiä-Herttuala, 14q32 miRNA cluster takes center stage in neovascularization, Circ. Res. 115 (2014) 680–682.

[41] M.T. Piccoli, S.K. Gupta, J. Viereck, A. Foinquinos, S. Samolovac, F.L. Kramer,

A. Garg, J. Remke, K. Zimmer, S. Batkai, T. Thum, Inhibition of the cardiac fibroblast-enriched lncRNA Meg3 prevents cardiac fibrosis and diastolic dysfunction, Circ. Res. 121 (2017) 575–583, http://dx.doi.org/10.1161/CIRCRESAHA.117.310624.

[42] M.S. Leisegang, C. Fork, I. Josipovic, F.M. Richter, J. Preussner, J. Hu, M.J. Miller, J. Epah, P. Hofmann, S. Günther, F. Moll, C. Valasarajan, J. Heidler, Y. Ponomareva, T.M. Freiman, L. Maegdefessel, K.H. Plate, M. Mittelbronn, S. Uchida, C. Künne, K. Stellos, R.T. Schermuly, N. Weissmann, K. Devraj, I. Wittig, R.A. Boon, S. Dimmeler, S.S. Pullamsetti, M. Looso, F.J. Miller, R.P. Brandes, Long noncoding RNA MANTIS facilitates endothelial angiogenic function, Circulation 136 (2017) 65–79.

[43] B. Liao, R. Chen, F. Lin, A. Mai, J. Chen, H. Li, Z. Xu, S. Dong, Long noncoding RNA HOTTIP promotes endothelial cell proliferation and migration via activation of the Wnt/β-catenin pathway, J. Cell. Biochem. 119 (3) (2018 Mar) 2797–2805.

[44] T.-S. Huang, K.-C. Wang, S. Quon, P. Nguyen, T.-Y. Chang, Z. Chen, Y.-S. Li, S. Subramaniam, J. Shyy, S. Chien, LINC00341 exerts an anti-inflammatory effect on endothelial cells by repressing VCAM1, Physiol. Genomics 49 (2017) 339–345.

[45] P. Neumann, N. Jaé, A. Knau, S.F. Glaser, Y. Fouani, O. Rossbach, M. Krüger, D. John, A. Bindereif, P. Grote, R.A. Boon, S. Dimmeler, The lncRNA GATA6-AS epigenetically regulates endothelial gene expression via interaction with LOXL2, Nat. Commun. 9 (2018) 237.

[46] A. Leung, C. Trac, W. Jin, L. Lanting, A. Akbany, P. Saetrom, D.E. Schones, R. Natarajan, Novel long noncoding RNAs are regulated by angiotensin II in vascular smooth muscle cells, Circ. Res. 113 (2013) 266–278.

[47] R.D. Bell, X. Long, M. Lin, J.H. Bergmann, V. Nanda, S.L. Cowan, Q. Zhou, Y. Han, D.L. Spector, D. Zheng, J.M. Miano, Identification and initial functional characterization of a human vascular cell-enriched long noncoding RNA, Arterioscler. Thromb. Vasc. Biol. 34 (2014) 1249–1259.

[48] M.D. Ballantyne, K. Pinel, R. Dakin, A.T. Vesey, L. Diver, R. Mackenzie, R. Garcia, P. Welsh, N. Sattar, G. Hamilton, N. Joshi, M.R. Dweck, J.M. Miano, M.W. McBride, D.E. Newby, R.A. McDonald, A.H. Baker, Smooth muscle enriched long noncoding RNA (SMILR) regulates cell proliferation, Circulation 133 (2016) 2050–2065.

[49] M. Brock, C. Schuoler, C. Leuenberger, C. Bühlmann, T.J. Haider, J. Vogel, S. Ulrich, M. Gassmann, M. Kohler, L.C. Huber, Analysis of hypoxia-induced noncoding RNAs reveals metastasis-associated lung adenocarcinoma transcript 1 as an important regulator of vascular smooth muscle cell proliferation, Exp. Biol. Med. 242 (2017) 487–496.

[50] Z. Sun, X. Nie, S. Sun, S. Dong, C. Yuan, Y. Li, B. Xiao, D. Jie, Y. Liu, Long noncoding RNA MEG3 downregulation triggers human pulmonary artery smooth muscle cell proliferation and migration via the p53 signaling pathway, Cell. Physiol. Biochem. 42 (2017) 2569–2581.

[51] F.C. Bischoff, A. Werner, D. John, J.-N. Boeckel, M.-T. Melissari, P. Grote, S.F. Glaser, S. Demolli, S. Uchida, K.M. Michalik, B. Meder, H.A. Katus, J. Haas, W. Chen, S.S. Pullamsetti, W. Seeger, A.M. Zeiher, S. Dimmeler, C.M. Zehendner, Identification and functional characterization of hypoxia-induced endoplasmic reticulum stress regulating lncRNA (HypERlnc) in pericytes, Circ. Res. 121 (2017) 368–375.

[52] C. Weber, L. Badimon, F. Mach, E.P.C. van Der Vorst, Therapeutic strategies for atherosclerosis and atherothrombosis: past, present and future, Thromb. Haemost. 117 (2017) 1258–1264.

[53] M.S. Cunnington, M.S. Koref, B.M. Mayosi, J. Burn, B. Keavney, Chromosome 9p21 SNPs associated with multiple disease phenotypes correlate with ANRIL expression, PLoS Genet. 6 (2010).

[54] L.M. Holdt, F. Beutner, M. Scholz, S. Gielen, G. Gäbel, H. Bergert, G. Schuler, J. Thiery, D. Teupser, ANRIL expression is associated with atherosclerosis risk at chromosome 9p21, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 620–627.

[55] A. Congrains, K. Kamide, R. Oguro, O. Yasuda, K. Miyata, E. Yamamoto, T. Kawai, H. Kusunoki, H. Yamamoto, Y. Takeya, K. Yamamoto, M. Onishi, K. Sugimoto, T. Katsuya, N. Awata, K. Ikebe, Y. Gondo, Y. Oike, M. Ohishi, H. Rakugi, Genetic variants at the 9p21 locus contribute to atherosclerosis through modulation of ANRIL and CDKN2A/B, Atherosclerosis 220 (2012) 449–455.

[56] C.E. Burd, W.R. Jeck, Y. Liu, H.K. Sanoff, Z. Wang, N.E. Sharpless, Expression of linear and novel circular forms of an INK4/ARF-associated non-coding RNA correlates with atherosclerosis risk, PLoS Genet. 6 (2010) e1001233.

[57] Y. Kotake, T. Nakagawa, K. Kitagawa, S. Suzuki, N. Liu, M. Kitagawa, Y. Xiong, Long non-coding RNA ANRIL is required for the PRC2 recruitment to and silencing of p15INK4B tumor suppressor gene, Oncogene 30 (2011) 1956–1962.

[58] K.L. Yap, S. Li, A.M. Muñoz-Cabello, S. Raguz, L. Zeng, S. Mujtaba, J. Gil, M.J. Walsh, M.-M. Zhou, Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a, Mol. Cell 38 (2010) 662–674.

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

[60] L.M. Holdt, S. Hoffmann, K. Sass, D. Langenberger, M. Scholz, K. Krohn, K. Finstermeier, A. Stahringer, W. Wilfert, F. Beutner, S. Gielen, G. Schuler, G. Gäbel, H. Bergert, I. Bechmann, P.F. Stadler, J. Thiery, D. Teupser, Alu elements in ANRIL non-coding RNA at chromosome 9p21 modulate atherogenic cell functions through trans-regulation of gene networks, PLoS Genet. 9 (2013) e1003588.

[61] D.K.M. Han, Z.Z. Khaing, R.A. Pollock, C.C. Haudenschild, G. Liau, H19, a marker of developmental transition, is reexpressed in human atherosclerotic plaques and is regulated by the insulin family of growth factors in cultured rabbit smooth muscle cells, J. Clin. Invest. 97 (1996) 1276–1285.

[62] J.X. Pan, LncRNA H19 promotes atherosclerosis by regulating MAPK and NF-kB signaling pathway, Eur. Rev. Med. Pharmacol. Sci. 21 (2017) 322–328.

[63] A. Gabory, H. Jammes, L. Dandolo, The H19 locus: role of an imprinted non-coding RNA in growth and development, BioEssays 32 (2010) 473–480.

[64] I.J. Matouk, N. DeGroot, S. Mezan, S. Ayesh, R. Abu-Lail, A. Hochberg, E. Galun, The H19 non-coding RNA is essential for human tumor growth, PLoS One (2007) 2.

[65] T. Yoshimizu, A. Miroglio, M.-A. Ripoche, A. Gabory, M. Vernucci, A. Riccio, S. Colnot, C. Godard, B. Terris, H. Jammes, L. Dandolo, The H19 locus acts in vivo as a tumor suppressor, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 12417–12422.

[66] A.N. Kallen, X.-B. Zhou, J. Xu, C. Qiao, J. Ma, L. Yan, L. Lu, C. Liu, J.-S. Yi, H. Zhang, W. Min, A.M. Bennett, R.I. Gregory, Y. Ding, Y. Huang, The imprinted H19 lncRNA antagonizes let-7 microRNAs, Mol. Cell 52 (2013) 101–112.

[67] M.-H. Bao, X. Feng, Y.-W. Zhang, X.-Y. Lou, Y. Cheng, H.-H. Zhou, Let-7 in cardiovascular diseases, heart development and cardiovascular differentiation from stem cells, Int. J. Mol. Sci. 14 (2013) 23086–23102.

[68] G. Wu, J. Cai, Y. Han, J. Chen, Z.-P. Huang, C. Chen, Y. Cai, H. Huang, Y. Yang, Y. Liu, Z. Xu, D. He, X. Zhang, X. Hu, L. Pinello, D. Zhong, F. He, G.-C. Yuan, D.-Z. Wang, C. Zeng, LincRNA-p21 regulates neointima formation, vascular smooth muscle cell proliferation, apoptosis, and atherosclerosis by enhancing p53 activity, Circulation 130 (2014) 1452–1465.

[69] K.K. Singh, P.N. Matkar, Y. Pan, A. Quan, V. Gupta, H. Teoh, M. Al-Omran, S. Verma, Endothelial long non-coding RNAs regulated by oxidized LDL, Mol. Cell. Biochem. 431 (2017) 139–149.

[70] K. Shan, Q. Jiang, X.Q. Wang, Y.N.Z. Wang, H. Yang, M.D. Yao, C. Liu, X.M. Li, J. Yao, B. Liu, Y.Y. Zhang, J. Yong, B. Yan, Role of long non-coding RNA-RNCR3 in atherosclerosis-related vascular dysfunction, Cell Death Dis. 7 (2016).

[71] S.Y. Huang, W. Lu, D. Ge, N. Meng, Y. Li, L. Su, S.L. Zhang, Y. Zhang, B.X. Zhao, J.Y. Miao, A new microRNA signal pathway regulated by long noncoding RNA TGFB2-OT1 in autophagy and inflammation of vascular endothelial cells, Autophagy 11 (2015) 2172–2183.

[72] Z. Zhang, W. Gao, Q.-Q. Long, J. Zhang, Y.-F. Li, D.-C. Liu, J.-J. Yan, Z.-J. Yang, L.-S. Wang, Increased plasma levels of lncRNA H19 and LIPCAR are associated with increased risk of coronary artery disease in a Chinese population, Sci. Rep. 7 (2017) 7491.

[73] R. Kumarswamy, C. Bauters, I. Volkmann, F. Maury, J. Fetisch, A. Holzmann, G. Lemesle, P. de Groote, F. Pinet, T. Thum, Circulating long noncoding RNA, LIPCAR, predicts survival in patients with heart failure, Circ. Res. 114 (2014) 1569–1575.

[74] J. Salzman, C. Gawad, P.L. Wang, N. Lacayo, P.O. Brown, Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types, PLoS One 7 (2012).

[75] Y. Zhang, W. Xue, X. Li, J. Zhang, S. Chen, J.-L. Zhang, L. Yang, L.-L. Chen, The biogenesis of nascent circular RNAs, Cell Rep. 15 (2016) 611–624.

[76] H.L. Sanger, G. Klotz, D. Riesner, H.J. Gross, A.K. Kleinschmidt, Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures, Proc. Natl. Acad. Sci. U. S. A. 73 (1976) 3852–3856.

[77] A. Kos, R. Dijkema, A.C. Arnberg, P.H. Van Der Meide, H. Schellekens, The hepatitis delta (δ) virus possesses a circular RNA, Nature 323 (1986) 558–560.

[78] M.T. Hsu, M. Coca-Prados, Electron microscopic evidence for the circular form of RNA in the cytoplasm of eukaryotic cells, Nature 280 (1979) 339–340.

[79] J.M. Nigro, K.R. Cho, E.R. Fearon, S.E. Kern, J.M. Ruppert, J.D. Oliner, K.W. Kinzler, B. Vogelstein, Scrambled exons, Cell 64 (1991) 607–613.

[80] C. Cocquerelle, P. Daubersies, M. a Majérus, J.P. Kerckaert, B. Bailleul, Splicing with inverted order of exons occurs proximal to large introns, EMBO J. 11 (1992) 1095–1098.

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

[82] P.G. Zaphiropoulos, Circular RNAs from transcripts of the rat cytochrome P450 2C24 gene: correlation with exon skipping, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 6536–6541.

[83] C. Caldas, C.W. So, A. MacGregor, A.M. Ford, B. McDonald, L.C. Chan, L.M. Wiedemann, Exon scrambling of MLL transcripts occur commonly and mimic partial genomic duplication of the gene, Gene 208 (1998) 167–176.

[84] C.W. Chao, D.C. Chan, A. Kuo, P. Leder, The mouse formin (Fmn) gene: abundant circular RNA transcripts and gene-targeted deletion analysis, Mol. Med. 4 (1998) 614–628.

[85] X.F. Li, J. Lytton, A circularized sodium-calcium exchanger exon 2 transcript, J. Biol. Chem. 274 (1999) 8153–8160.

[86] Y. Takeshima a Surono, T. Wibawa, M. Ikezawa, I. Nonaka, M. Matsuo, Circular dystrophin RNAs consisting of exons that were skipped by alternative splicing, Hum. Mol. Genet. 8 (1999) 493–500.

[87] C. Cocquerelle, B. Mascrez, D. Hétuin, B. Bailleul, Mis-splicing yields circular RNA molecules, FASEB J. 7 (1993) 155–160.

[88] S. Memczak, M. Jens, A. Elefsinioti, F. Torti, J. Krueger, A. Rybak, L. Maier, S.D. Mackowiak, L.H. Gregersen, M. Munschauer, A. Loewer, U. Ziebold, M. Landthaler, C. Kocks, F. le Noble, N. Rajewsky, Circular RNAs are a large class of animal RNAs with regulatory potency, Nature 495 (2013) 333–338, http://dx.doi.org/10.1038/nature11928.

[89] J. Salzman, R.E. Chen, M.N. Olsen, P.L. Wang, P.O. Brown, Cell-type specific features of circular RNA expression, PLoS Genet. 9 (2013).

[90] S. Werfel, S. Nothjunge, T. Schwarzmayr, T.-M. Strom, T. Meitinger, S. Engelhardt, Characterization of circular RNAs in human, mouse and rat hearts, J. Mol. Cell. Cardiol. 98 (2016) 103–107.

[91] X. Cheng, B. Joe, Circular RNAs in rat models of cardiovascular and renal diseases, Physiol. Genomics 49 (2017) 484–490.

[92] L. Li, J. Guo, Y. Chen, C. Chang, C. Xu, Comprehensive CircRNA expression profile

and selection of key CircRNAs during priming phase of rat liver regeneration, BMC Genomics 18 (2017) 80.
[93] J.U. Guo, V. Agarwal, H. Guo, D.P. Bartel, Expanded identification and characterization of mammalian circular RNAs, Genome Biol. 15 (2014) 409.
[94] J.O. Westholm, P. Miura, S. Olson, S. Shenker, B. Joseph, P. Sanfilippo, S.E. Celniker, B.R. Graveley, E.C. Lai, Genome-wide analysis of Drosophila circular RNAs reveals their structural and sequence properties and age-dependent neural accumulation, Cell Rep. 9 (2014) 1966–1981.
[95] M.C. Kramer, D. Liang, D.C. Tatomer, B. Gold, Z.M. March, S. Cherry, J.E. Wilusz, Combinatorial control of Drosophila circular RNA expression by intronic repeats, hnRNPs, and SR proteins, Genes Dev. 29 (2015) 2168–2182.
[96] A. Ivanov, S. Memczak, E. Wyler, F. Torti, H.T. Porath, M.R. Orejuela, M. Piechotta, E.Y. Levanon, M. Landthaler, C. Dieterich, N. Rajewsky, Analysis of intron sequences reveals hallmarks of circular RNA biogenesis in animals, Cell Rep. 10 (2015) 170–177.
[97] H. Gruner, M. Cortés-López, D.A. Cooper, M. Bauer, P. Miura, CircRNA accumulation in the aging mouse brain, Sci. Rep. 6 (2016).
[98] X. You, I. Vlatkovic, A. Babic, T. Will, I. Epstein, G. Tushev, G. Akbalik, M. Wang, C. Glock, C. Quedenau, X. Wang, J. Hou, H. Liu, W. Sun, S. Sambandan, T. Chen, E.M. Schuman, W. Chen, Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity, Nat. Neurosci. 18 (2015) 603–610.
[99] J. Greene, A.-M. Baird, L. Brady, M. Lim, S.G. Gray, R. McDermott, S.P. Finn, Circular RNAs: biogenesis, function and role in human diseases, Front. Mol. Biosci. 4 (2017).
[100] T.B. Hansen, M.T. Venø, C.K. Damgaard, J. Kjems, Comparison of circular RNA prediction tools, Nucleic Acids Res. (2015) 44.
[101] S. Starke, I. Jost, O. Rossbach, T. Schneider, S. Schreiner, L.H. Hung, A. Bindereif, Exon circularization requires canonical splice signals, Cell Rep. 10 (2015) 103–111.
[102] R. Ashwal-Fluss, M. Meyer, N.R. Pamudurti, A. Ivanov, O. Bartok, M. Hanan, N. Evantal, S. Memczak, N. Rajewsky, S. Kadener, CircRNA biogenesis competes with pre-mRNA splicing, Mol. Cell 56 (2014) 55–66.
[103] W.R. Jeck, J.A. Sorrentino, K. Wang, M.K. Slevin, C.E. Burd, J. Liu, W.F. Marzluff, N.E. Sharpless, Circular RNAs are abundant, conserved, and associated with ALU repeats, RNA 19 (2012) 141–157.
[104] X.O. Zhang, H. Bin Wang, Y. Zhang, X. Lu, L.L. Chen, L. Yang, Complementary sequence-mediated exon circularization, Cell 159 (2014) 134–147.
[105] D. Liang, J.E. Wilusz, Short intronic repeat sequences facilitate circular RNA production, Genes Dev. 28 (2014) 2233–2247.
[106] S.J. Conn, K.A. Pillman, J. Toubia, V.M. Conn, M. Salmanidis, C.A. Phillips, S. Roslan, A.W. Schreiber, P.A. Gregory, G.J. Goodall, The RNA binding protein quaking regulates formation of circRNAs, Cell 160 (2015) 1125–1134.
[107] R.A. Dubin, M.A. Kazmi, H. Ostrer, Inverted repeats are necessary for circularization of the mouse testis Sry transcript, Gene 167 (1995) 245–248.
[108] T.B. Hansen, T.I. Jensen, B.H. Clausen, J.B. Bramsen, B. Finsen, C.K. Damgaard, J. Kjems, Natural RNA circles function as efficient microRNA sponges, Nature 495 (2013) 384–388.
[109] H. Xu, S. Guo, W. Li, P. Yu, The circular RNA Cdr1as, via miR-7 and its targets, regulates insulin transcription and secretion in islet cells, Sci. Rep. 5 (2015).
[110] L. Yu, X. Gong, L. Sun, Q. Zhou, B. Lu, L. Zhu, The circular RNA Cdr1as act as an oncogene in hepatocellular carcinoma through targeting miR-7 expression, PLoS One 11 (2016).
[111] J.-N. Boeckel, N. Jaé, A.W. Heumüller, W. Chen, R.A. Boon, K. Stellos, A.M. Zeiher, D. John, S. Uchida, S. Dimmeler, Identification and characterization of hypoxia-regulated endothelial circular RNA, Circ. Res. 117 (2015) 884–890.
[112] L.M. Holdt, A. Stahringer, K. Sass, G. Pichler, N.A. Kulak, W. Wilfert, A. Kohlmaier, A. Herbst, B.H. Northoff, A. Nicolaou, G. Gäbel, F. Beutner, M. Scholz, J. Thiery, K. Musunuru, K. Krohn, M. Mann, D. Teupser, Circular non-coding RNA ANRIL modulates ribosomal RNA maturation and atherosclerosis in humans, Nat. Commun. 7 (2016).
[113] W.W. Du, W. Yang, E. Liu, Z. Yang, P. Dhaliwal, B.B. Yang, Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2, Nucleic Acids Res. 44 (2016) 2846–2858.
[114] W.W. Du, W. Yang, Y. Chen, Z.K. Wu, F.S. Foster, Z. Yang, X. Li, B.B. Yang, Foxo3 circular RNApromotes cardiac senescence by modulating multiple factors associated with stress and senescence responses, Eur. Heart J. 38 (2017) 1402–1412.
[115] Z. Li, C. Huang, C. Bao, L. Chen, M. Lin, X. Wang, G. Zhong, B. Yu, W. Hu, L. Dai, P. Zhu, Z. Chang, Q. Wu, Y. Zhao, Y. Jia, P. Xu, H. Liu, G. Shan, Exon-intron circular RNAs regulate transcription in the nucleus, Nat. Struct. Mol. Biol. 22 (2015) 256–264.
[116] N.R. Pamudurti, O. Bartok, M. Jens, R. Ashwal-Fluss, C. Stottmeister, L. Ruhe, M. Hanan, E. Wyler, D. Perez-Hernandez, E. Ramberger, S. Shenzis, M. Samson, G. Dittmar, M. Landthaler, M. Chekulaeva, N. Rajewsky, S. Kadener, Translation of CircRNAs, Mol. Cell 66 (2017) 9–21.e7.
[117] Y.Y. Yang, X. Fan, M. Mao, X. Song, P. Wu, Y. Zhang, Y. Jin, Y.Y. Yang, L.L. Chen, Y. Wang, C.C.L. Wong, X. Xiao, Z. Wang, Extensive translation of circular RNAs driven by N 6 -methyladenosine, Cell Res. 27 (2017) 626–641.
[118] R.Y. Dang, F.L. Liu, Y. Li, Circular RNA hsa_circ_0010729 regulates vascular endothelial cell proliferation and apoptosis by targeting the miR-186/HIF-1α axis, Biochem. Biophys. Res. Commun. 490 (2017) 104–110.
[119] C. Zheng, H. Niu, M. Li, H. Zhang, Z. Yang, L. Tian, Z. Wu, D. Li, X. Chen, Cyclic RNA hsa-circ-000595 regulates apoptosis of aortic smooth muscle cells, Mol. Med. Rep. 12 (2015) 6656–6662.
[120] M.J. Bown, P.S. Braund, J. Thompson, N.J.M. London, N.J. Samani, R.D. Sayers, Association between the coronary artery disease risk locus on chromosome 9p21.3 and abdominal aortic aneurysm, Circ. Genet. 1 (2008) 39–42.
[121] C.-L. Song, J.-P. Wang, X. Xue, N. Liu, X.-H. Zhang, Z. Zhao, J.-G. Liu, C.-P. Zhang, Z.-H. Piao, Y. Liu, Y.-B. Yang, Effect of circular ANRIL on the inflammatory response of vascular endothelial cells in a rat model of coronary atherosclerosis, Cell. Physiol. Biochem. (2017) 1202–1212.
[122] C.Y. Li, L. Ma, B. Yu, Circular RNA hsa_circ_0003575 regulates oxLDL induced vascular endothelial cells proliferation and angiogenesis, Biomed Pharmacother 95 (2017) 1514–1519.
[123] Y. Devaux, E.E. Creemers, R.A. Boon, S. Werfel, T. Thum, S. Engelhardt, S. Dimmeler, I. Squire, Circular RNAs in heart failure, Eur. J. Heart Fail. 19 (2017) 701–709.
[124] S. Memczak, P. Papavasileiou, O. Peters, N. Rajewsky, Identification and characterization of circular RNAs as a new class of putative biomarkers in human blood, PLoS One 10 (2015) e0141214.
[125] W. Koh, W. Pan, C. Gawad, H.C. Fan, G.A. Kerchner, T. Wyss-Coray, Y.J. Blumenfeld, Y.Y. El-Sayed, S.R. Quake, Noninvasive in vivo monitoring of tissue-specific global gene expression in humans, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 7361–7366.
[126] J.H. Bahn, Q. Zhang, F. Li, T.M. Chan, X. Lin, Y. Kim, D.T.W. Wong, X. Xiao, The landscape of MicroRNA, piwi-interacting RNA, and circular RNA in human saliva, Clin. Chem. 61 (2015) 221–230.
[127] Y. Li, Q. Zheng, C. Bao, S. Li, W. Guo, J. Zhao, D. Chen, J. Gu, X. He, S. Huang, Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis, Cell Res. 25 (2015) 981–984.
[128] S. Meng, H. Zhou, Z. Feng, Z. Xu, Y. Tang, P. Li, M. Wu, CircRNA: functions and properties of a novel potential biomarker for cancer, Mol. Cancer 16 (2017) 94.
[129] A. Salgado-Somoza, L. Zhang, M. Vausort, Y. Devaux, The circular RNA MICRA for risk stratification after myocardial infarction, IJC Hear. Vasc. 17 (2017) 33–36.
[130] Z. Zhao, X. Li, C. Gao, D. Jian, P. Hao, L. Rao, M. Li, Peripheral blood circular RNA hsa_circ_0124644 can be used as a diagnostic biomarker of coronary artery disease, Sci. Rep. 7 (2017) 39918.
[131] K.M. Anderson, D.M. Anderson, J.R. McAnally, J.M. Shelton, R. Bassel-Duby, E.N. Olson, Transcription of the non-coding RNA upperhand controls Hand2 expression and heart development, Nature 539 (2016) 433–436.
[132] M. Piwecka, P. Glažar, L.R. Hernandez-Miranda, S. Memczak, S.A. Wolf, A. Rybak-Wolf, A. Filipchyk, F. Klironomos, C.A. Cerda Jara, P. Fenske, T. Trimbuch, V. Zywitza, M. Plass, L. Schreyer, S. Ayoub, C. Kocks, R. Kühn, C. Rosenmund, C. Birchmeier, N. Rajewsky, Loss of a mammalian circular RNA locus causes miRNA deregulation and affects brain function, Science 357 (2017) eaam8526.