Definition and review on a category of long non-coding RNA: Atherosclerosis-associated circulating lncRNA (ASCLncRNA)

Shanshan Lu[1], Qin Liang[1], Yanqing Huang[1], Fanming Meng[2] and Junwen Liu[1,3]

[1] Department of Histology and Embryology, School of Basic Medical Science, Central South University, Changsha, Hunan Province, China

[2] Department of Parasitology, School of Basic Medical Science, Central South University, Changsha, Hunan Province, China

[3] China-Africa Research Center of Infectious Diseases, School of Basic Medical Sciences, Central South University, Changsha, Hunan Province, China

ABSTRACT

Atherosclerosis (AS) is one of the most common cardiovascular system diseases which seriously affects public health in modern society. Finding potential biomarkers in the complicated pathological progression of AS is of great significance for the prevention and treatment of AS. Studies have shown that long noncoding RNAs (lncRNAs) can be widely involved in the regulation of many physiological processes, and have important roles in different stages of AS formation. LncRNAs can be secreted into the circulatory system through exosomes, microvesicles, and apoptotic bodies. Recently, increasing studies have been focused on the relationships between circulating lncRNAs and AS development. The lncRNAs in circulating blood are expected to be new non-invasive diagnostic markers for monitoring the progression of AS. We briefly reviewed the previously reported lncRNA transcripts which related to AS development and detectable in circulating blood, including *ANRIL*, *SENCR*, *CoroMarker*, *LIPCAR*, *HIF1α-AS1*, *LncRNA H19*, *APPAT*, *KCNQ1OT1*, *LncPPARδ*, *LincRNA-p21*, *MALAT1*, *MIAT*, and *UCA1*. Further researches and a definition of atherosclerosis-associated circulating lncRNA (ASCLncRNA) were also discussed.

Subjects Molecular Biology, Cardiology, Pathology

Keywords Atherosclerosis, Biomarker, Circulating lncRNAs, Non-invasive diagnosis

INTRODUCTION

Cardiovascular diseases (CVDs) seriously endanger human health around the world, taking the lives of around 17.9 million each year (*WHO, 2017*), which place a heavy financial burden on society and families. Atherosclerosis (AS) is the most common cause of CVDs (*Sanada et al., 2018*). Although it is an asymptomatic condition, the accumulation and rupture of atheromatous plaques in arteries can lead to serious consequences, such as coronary artery diseases (CADs), acute myocardial infarction (AMI), and heart failure (HF), etc (*Harada et al., 2014*; *Libby, Ridker & Hansson, 2011*). Plaque rupture is responsible for 75% of AMI with highest incidence occurs in male beyond age 45 and female beyond

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Corresponding authors
Fanming Meng, 22018112@csu.edu.cn
Junwen Liu, liujunwen@csu.edu.cn

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age 50 (*Pahwa & Jialal, 2019*). The AS progression involves dynamic changes in the vessel wall, such as endothelial dysfunction, macrophage activation, and phenotypic changes of vascular smooth muscle cells (VSMCs) (*Xi et al., 2013*).

Compared with other morbid states, AS is characterized by small lesions and insidious onset. At present, the diagnosis of AS is based on a combination of multiple detection methods, such as echocardiography, electrocardiogram, computed tomography scan, blood test, and angiography (*NHLBI, 2020*). However, the confirmed diagnosis of AS depends on surgery and pathological examination (like carotid endarterectomy), which can be traumatic and risky for patients. Considering the difficulty of detection on such subtle changes *in vivo*, identifying potential biomarkers associated with the complex pathological progress of AS is essential for the prevention and treatment of AS.

Long noncoding RNAs (lncRNAs) are defined as transcripts > 200 bp without protein-coding potential. It was previously considered to be the "noise" or "junk" of the genome and have no substantive function (*Palazzo & Lee, 2015*). Recent researches, however, found that lncRNAs functions in various kinds of cellular activities, which are related to many serious human diseases (*Wapinski & Chang, 2011*). LncRNAs can target microRNAs (miRNAs) to form the competing endogenous RNA (ceRNA) axes and then function in different stages of AS development (*Li, Zhu & Ge, 2016*; *Salmena et al., 2011*). They not only exist in cells but can also be detected in plasma or serum samples, which are called circulating lncRNAs (*Cao et al., 2019*; *Wang et al., 2017*). Circulating lncRNAs show great resistance to endogenous RNase, which makes them more stable in blood samples (*Tong et al., 2015*). All of these provide the necessary basis for finding circulating lncRNA as potential biomarkers in AS prevention and treatment.

In this review, we briefly discuss current advances of circulating lncRNAs in AS, which aim to facilitate our understanding of the relationships between them and promote the development of circulating lncRNAs as predicting AS biomarkers for clinical applications.

SURVEY METHODOLOGY

Our team put the focus on advances in the relationship between epigenetics and CVDs. We performed the literature search mainly using the PubMed database (https://www.ncbi.nlm.nih.gov/pubmed/), Web Of Science (http://www.webofknowledge.com), and Google scholar (scholar.google.cn). Based on the main keywords-"atherosclerosis", "coronary artery disease (or CAD)", "heart failure" and "stroke" combined with "lncRNA", "blood", "serum", or "plasma", "exosome", "microvesicle", "apoptotic body", relevant articles are extracted to classify and summarize the potential atherosclerosis-associated circulating lncRNAs. According to the search strategy, a total of 285 literatures were identified, including 55 reviews and 6 clinical trials. 282 of them have the full text. After reading the abstract and screening, 109 were then included which satisfied our goal. We did not refine factors such as journal, publishing date, or journal impact factors during our search. Ultimately, the time span of references in this review is from 1993 to 2020.

Circulating lncRNAs

Ideal biomarker refers to those molecules that can be used in non-invasive detection with relative stability, detection sensitivity, and specificity (*Qi, Zhou & Du, 2016*; *Shi & Yang, 2016*). But so far, lncRNAs used in scientific researches were usually derived from atherosclerotic plaques based on interventional methods (*Roth & Diederichs, 2016*). With the rapid improvements of detection methods, recent studies show that lncRNAs can be stably detected from body fluids (*Panzitt et al., 2007*; *Reis & Verjovski-Almeida, 2012*; *Tinzl et al., 2004*). LncRNAs can be secreted from tumor cells, then move into the circulatory system through exosomes, microvesicles, and apoptotic bodies, thus becoming a novel non-invasive diagnostic marker for monitoring the progression of cancers (*Wang et al., 2019a*). The variation of cellular components in blood-vessel cells during the development of AS make it possible for lncRNAs to migrate into the circulatory system (*Monteiro et al., 2019*).

Blood is the most widely distributed body fluid, carrying oxygen, nutrients, and signaling molecules to tissues and organs throughout the body. The circulating blood emphasizes a state of blood fluid distributing in the circulatory system of human body. Dynamic changes of particular substance in the blood may be closely related to specific disease states, such as cancers, CVDs, and nervous system diseases (*Chen et al., 2017*; *Fridman et al., 2017*; *Wang et al., 2018*). Such biomarkers can provide the following information: (1) identifying and classifying patient's condition; (2) diagnosing and monitoring disease states; and (3) guiding doctors to make appropriate therapeutic schedules and prognosis observations (*Heil & Tang, 2015*). The properties of lncRNAs determine their potential as biomarkers, such as lncRNA *PCA3* in prostate cancer, and lncRNAs *UCA1* in bladder cancer. Both of them can be easily detected in urine samples, and even have special discriminations for cancer types (*Hessels et al., 2003*; *Wang et al., 2006*). Later, lncRNAs with biomarker function in circulating blood were found in gastric cancer, lung cancer and breast cancer (*Dong et al., 2015*; *Liang et al., 2016*; *Zhang et al., 2016*).

AS is one of the most common diseases in the world. Studies on the correlation between circulating lncRNAs and AS development have been reported in recent years (*Chi et al., 2017*; *Pan et al., 2019*; *Wang et al., 2017*). If pathological changes of vessel can be found in the early stage based on the detection of circulating lncRNAs, it will be shade light on the initial intervention and treatment to reducing the morbidity and mortality of patients.

ASCLncRNA: atherosclerosis-associated circulating lncRNA

ANRIL (CDKN2B antisense RNA 1)

ANRIL, also known as *CDKN2B-AS1*, is located at chromosome 9p21 (*Pasmant et al., 2007*). The *ANRIL* gene contains 19 exons and can be transcribed into many spliceosomes with tissue specificity (*Folkersen et al., 2009*). Genome-wide association analysis (GWAS) revealed many disease-associated single nucleotide polymorphisms (SNPs) at the 9p21 locus (*Pasmant et al., 2011*). These SNPs are closely associated with the severity of coronary AS, cervical AS, peripheral arterial disease (*Congrains et al., 2012*; *Holdt et al., 2010*; *Holdt & Teupser, 2012*).

ANRIL exists in many AS-associated cells and tissues, such as VSMCs, endothelial cells (ECs), monocytes, macrophages, and carotid tissues. It plays a *trans*-regulatory role through binding to Alu elements in the promoter region of target genes, which is involved in the regulation of fatty acids, glucose metabolism, and inflammatory responses (*Bochenek et al., 2013*; *Holdt et al., 2013*).

In a sequencing analysis of whole blood samples from patients with AMI, researchers detected *ANRIL*'s significant reduction and selected it as a self-labeling molecule (*Vausort, Wagner & Devaux, 2014*). This is the first report on the dynamic expression level of *ANRIL* in circulating blood. Another research exhibits that *ANRIL* was up-regulated in peripheral venous blood from patients of type 2 diabetes mellitus complicated with AMI. Recently, circulating *ANRIL* was found to increase in the plasma of CAD patients compared with the controls and showed promise as a good diagnostic value for CAD because it could be related to the severity of inflammation, stenosis degree, and prognosis (*Hu & Hu, 2019*). The differences in research objects, such as arterial blood *vs*. venous blood, and the existence of comorbidity may be the core reason for the opposite results. Since the mechanism of *ANRIL*'s function in the development of AS is not very clear, its role as a biomarker in circulating blood remains to be explored.

SENCR (smooth muscle and endothelial cell-enriched migration/ differentiation-associated long noncoding RNA)

SENCR is a specifically cytoplasmic lncRNA enriched in vascular cells. It was firstly discovered in the high-throughput sequencing of human coronary artery SMCs (*Bell et al., 2014*). Bell and colleagues found *SENCR* seems to play a role in inhibiting phenotypic transformation and cell proliferation of VSMCs through downregulating the cell contraction-related gene (*Myocd*) and upregulating the promigration-associated genes (*Mdk/Ptn*) (*Bell et al., 2014*). It can inhibit the pathological migration of VSMCs to neointima during AS formation. Besides, *SENCR* could also bind CKAP4, a cytosolic protein in ECs, through a noncanonical RNA-binding domain. This interplay helps maintain the adherent junctions, membrane integrity, and permeability of ECs, thus protecting against AS (*Lyu et al., 2019*). The expression level of *SENCR*, which was isolated from the vessel wall ECs of patients with CVDs, was much lower than the control. It suggests that *SENCR* may be decreased in patients with endothelial dysfunction or AS (*Boulberdaa et al., 2016*). The decline of this atheroprotective lncRNA may provide a warning for the onset of AS.

By using amplification refractory mutation system-polymerase chain reaction, *SENCR* can be detected stably and sensitively in blood samples of patients with CAD (*Shahmoradi et al., 2017*). Besides, a large-scale investigation focused on the effect of pioglitazone on type 2 diabetes found that the expression of *SENCR* in the blood is related to the evaluation of diastolic function after drug treatment, and that its indication in drug treatment effect is better than other existing indicators (*De Gonzalo-Calvo et al., 2016b*).

CoroMarker (Aldo-keto reductase family 1 member B1 pseudogene 3)

Yang et al. screened 174 differentially expressed lncRNA transcripts in blood samples from CAD patients and healthy people through microarray analysis, then they further chose five candidate lncRNAs by improving screening criteria and verified them by quantitative

Polymerase Chain Reaction (qPCR) technology. Receiver operating characteristic (ROC) curve analysis indicated that lncRNA *AC100865.1* could meet the requirements of biomarkers and was named *CoroMarker*. Better sensitivity and predictive effects can be obtained when combined with other risk factors of CVDs (*Yang et al., 2015*). *CoroMarker* has now been demonstrated to be distributed in vesicles of circulating blood and monocytes. Knockdown of *CoroMarker* in THP-1 cells caused significant down-regulation of IL-1β, IL-6, and TNF-α, suggesting that *CoroMarker* may play an important role in the inflammatory response of AS (*Cai et al., 2016b*).

LIPCAR (mitochondrially encoded long non-coding cardiac associated RNA)

LIPCAR showed opposite expression trend before and after the AMI event; it was down-regulated at the early stage of AMI, but gradually increased in the subsequent period (*Kumarswamy et al., 2014*). Surprisingly, it was released from the mitochondrion in blood cells rather than cardiac cells, which may be the reason for the changes in *LIPCAR* expression in different stages of AMI (*Schulte et al., 2019*). Meng and colleagues investigated diagnostic value of 9 circulating lncRNAs in ST-segment elevation myocardial infarction and found that *LIPCAR* had better diagnostic accuracy than others (*Li et al., 2018a*).

In plasma, the expression of *LIPCAR* was inversely proportional to high-density lipoprotein cholesterol, suggesting it may lead to dyslipidemia, which is a key factor in the progression of AS (*Zhang et al., 2017*). Besides, overexpression of *LIPCAR* induced by the treatment of ox-LDL or platelet-derived growth factor BB could promote the proliferation, migration, and phenotypic transition of VSMCs, which proved the function of *LIPCAR* in the progression of AS (*Wang et al., 2019c*). The possibility of *LIPCAR* as biomarkers for CVDs was also confirmed by other studies (*De Gonzalo-Calvo et al., 2016a*; *Santer et al., 2019*). These may lay the theoretical basis for the use of *LIPCAR* as a biomarker for AS.

HIF1A-AS1 (HIF1A antisense RNA 1)

Brahma-related gene 1 (*BRG1*) is a central catalytic subunit in many chromatin-modifying enzymatic complexes and is involved in the regulation of gene expression through chromatin remodeling (*Zhou et al., 2009*). *HIF1A-AS1* was found to be correlated with *BRG1* in VSMCs detected by microarray in *BRG1* gain- and loss-of-function experiment. The interaction between them regulates the proliferation and apoptosis of VSMCs in vitro (*Wang et al., 2015c*). The function of *HIF1A-AS1* in VSMCs was confirmed by Xu et al. that its overexpression could inhibit cell proliferation (*Xu et al., 2019*). Silencing of *HIF1A-AS1* could promote the proliferation and reduce the hyperlipidemia-induced apoptosis of human umbilical vein ECs (*Wang et al., 2015b*). The potential role of *HIF1A-AS1* as a biomarker in circulating blood has already been verified in several cancers such as colorectal carcinoma and non-small cell lung cancer (*Gong et al., 2017*; *Tantai et al., 2015*). Both Zhao and Xu testified that expression of *HIF1A-AS1* was dramatically increased in the blood of patients with aneurysm (*Xu et al., 2019*; *Zhao et al., 2014*). *HIF1A-AS1* was also up-regulated in the exosomes extracted from the plasma sample of patients with AS, which was thought to be the result of the activation of VSMCs and ECs (*Wang et al., 2017*). These

studies laid a foundation for the clinical application of *HIF1A-AS1* in the AS diagnosis, but also ruled out its usage as a biomarker alone.

LncRNA H19 (H19 imprinted maternally expressed transcript)

LncRNA H19 is located near the insulin-like growth factor 2 (*IGF2*) gene in an imprinted region of chromosome 11 (*Giannoukakis et al., 1993*). The common polymorphisms of *lncRNA H19* were related to the risk and hazard level of CAD in a Chinese population (*Gao et al., 2015*). By using short hairpin RNA (shRNA) in ox- LDL-treated Raw264.7 cells, Han demonstrated that miR-130b is an important target gene of *lncRNA H19*. The axis could regulate inflammation response and lipid metabolism, which could be used as a targeting site of treating AS (*Han et al., 2018a*). Another experiment conducted by Zhang indicated that *lncRNA H19* acts as a ceRNA of miR-148b and thus modulates the WNT/β-catenin signaling pathway to promote proliferation and suppress apoptosis of ox-LDL-stimulated VSMCs (*Zhang et al., 2018*). Up to now, the expression level of H19 in the serum of patients with AS was all found to be up-regulated (*Cao et al., 2019*; *Han et al., 2018a*; *Pan, 2017*; *Zhang et al., 2018*).

APPAT (atherosclerotic plaque pathogenesis associated transcript)

The newly reported lncRNA *APPAT* also shows potential predictive function for AS progression. *APPAT* is a 669 bp intergenic long non-coding RNA containing four exons and localized in human chromosome 2. *APPAT* was found to be mainly distributed in the middle layer of the coronary vessel wall and located on the cytoplasmic region of VSMCs via immunofluorescence (*Meng et al., 2018*).

APPAT was discovered in blood samples. It declined inconspicuously in patients with angina pectoris (AP) through the method of case-control matching, whereas significantly down-regulated in AMI patients compared with the normal group. Further examination revealed a decrease in *APPAT* expression in tissues of severely stenotic coronary arteries. This detectable trend in blood, independent of disease factors such as diabetes and hypertension, has potential value for predicting and monitoring the development of AS (*Meng et al., 2018*).

KCNQ1OT1 (KCNQ1 opposite strand/antisense transcript 1)

Studies have shown that lncRNAs can affect the transcriptional activities of multiple genes by interacting with chromatin (*Saxena & Carninci, 2011*). The *Kcnq1* locus spans more than 1 Mb on the chromosome and is located on the short arm of human chromosome 11 (11p15.5), containing 8-10 protein-coding genes and lncRNA *KCNQ1OT1* (*Paulsen et al., 1998*). Through recruiting chromatin and DNA-modifying proteins, *KCNQ1OT1* interacts with chromatin to form a complex folding structure and then silences multiple target genes in this region (*Kanduri, 2011*).

The expression of *KCNQ1OT1* in atheromatous plaques was negatively correlated with the age of patients, laying the foundation for further revealing the association between age and atheromatous plaques (*Arslan et al., 2017*). Lately, it is also verified to be elevated in the peripheral blood monocytes of patients with CAD and blood samples of MI patients, which suggests its role in the diagnosis of AS (*Zhang et al., 2019*).

LncPPARδ (long noncoding peroxisome proliferator-activated receptor delta)

PPARδ belongs to the nuclear receptor superfamily (*Giordano Attianese & Desvergne, 2015*). Activation of *PPARδ* increases the cholesterol efflux of macrophages, inhibits the transmembrane migration activity of leukocytes or monocytes to the inner wall of arteries, and reduces the size of atheromatous plaques (*Ehrenborg & Skogsberg, 2013*). Cai and colleagues isolated a lncRNA transcript, *NONHSAT112178*, from the plasma of CAD patients and named it as *LncPPARδ*, which is located near the *PPARδ*. They further reported that the expression of *PPARδ* decreased significantly in THP-1 cells after the knock down *LncPPARδ*, indicating that *LncPPAR δ* may be involved in *PPARδ*-mediated inflammatory signaling pathway and play a role in the progression of AS and CAD. In the plasma test results, *LncPPARδ* expression is up-regulated in CAD patients and is shown to exist stably in the blood. When combined with factors such as gender and age, *LncPPAR δ* showed better predictive function for CAD patients (*Cai et al., 2016a*).

LincRNA-p21 (tumor protein p53 pathway corepressor 1)

LincRNA-p21 was firstly discovered in mice (*Huarte et al., 2010*). It interacts with heterogeneous nuclear ribonucleoprotein K (hnRNP-K), the repressive complex of *p53*, and then affects the expression of *p53*'s downstream target genes (*Barichievy et al., 2018*). Wu et al. found that it inhibits cell proliferation and helps bring about neointimal formation in damaged coronary arteries, thus affecting the progression of AS in mice. They also discovered that down-regulation of *LincRNA-p21* in human VSMCs can also promote cell proliferation and inhibit apoptosis (*Wu et al., 2014*). Hu et al. found that overexpression of *LincRNA-p21* shows the opposite trends in VSMCs (*Hu et al., 2019a*). These two consistent results suggested that *LincRNA-p21* may play a protective role against AS. *Cekin et al. (2018)* found that *LincRNA-p21* decreased about 7 fold in atherosclerotic coronary artery tissues compared with the normal arterials in the same individuals. The application of circulating *LincRNA-p21* exhibits a well predictive power on brain carotid tumors, chronic hepatitis (*Fayda et al., 2016*; *Yu et al., 2017*), and thoracic aortic aneurysms (*Hu et al., 2019a*). Although there is no direct evidence indicating the link between circulating *LincRNA-p21* with the progression of AS, it is reasonable to infer that circulating *LincRNA-p21* could also work as a marker for identifying AS given its effect on VSMCs when combined with other diagnostic indicators.

MALAT1 (metastasis associated lung adenocarcinoma transcript 1)

MALAT1, also known as *Neat2*, was firstly demonstrated to be related to the metastasis of non-small cell lung cancer (*Ji et al., 2003*). It binds with *polycomb 2* to regulate the proliferation of cells by relocating growth-control gene loci (*Yang et al., 2011*). *Michalik et al. (2014)* verified the significant function of *MALAT1* in the balancing of the phenotype of ECs, which affects vascular growth *in vivo*. It could act as ceRNAs with miRNAs (such as miR-216a-5p and miR-155) to inhibit inflammatory cytokine release and promote cell autophagy in ECs (*Li et al., 2018b*; *Wang et al., 2019b*).

MALAT1 was also found to be expressed highly in the macrophages of rats with diabetic AS (*Han et al., 2018b*), the plasma of patients with acute cerebral infarction (*Teng & Meng, 2019*), and serum of AS patients (*Wang et al., 2019b*). This can be partially explained

by the increase of *MALAT1* expression in exosomes secreted by ECs (*Gao et al., 2019*). Surprisingly, the expression is downregulated in both human and mouse atherosclerotic plaques (*Arslan et al., 2017*; *Cremer et al., 2019*). We hypothesize that the high expression of *MALAT1* in serum and exosomes originates from the cells surrounding pathological tissues and thus cause a decline in plaques. However, Li and colleagues found lower expression of *MALAT1* in exosomes of ox-LDL-treated ECs compared with the normal ECs (*Li et al., 2019*). This difference suggests that the role of *MALAT1* in AS remains unclear and deserves a multi-center investigation.

MIAT (myocardial infarction associated transcript)

MIAT was found in an SNP-rich region related to AMI. A small change in a single SNP locus can cause an up-regulation of *MIAT* expression levels (*Ishii et al., 2006*). Knockdown of *MIAT* in ECs can inhibit cell proliferation and migration in vitro (*Yan et al., 2015*). The expression of *MIAT* increased in blood samples of AS patients, while its target miR-181b was down-regulated. Further studies found that a ceRNA axis–*MIAT*-miR-181b-*STAT3* –in aortic SMCs may participate in cell proliferation and apoptosis and then affect the occurrence and development of AS (*Zhong et al., 2018*). The same expression trend was verified in the AS mice model, where *MIAT* inhibits efferocytosis through targeting the miR-149-5p/*CD47* to regulate plaque vulnerability (*Ye et al., 2019*). The *MIAT*-based ceRNA pattern provides a potential target for therapy of AS.

UCA1 (urothelial carcinoma associated 1)

UCA1 was originally identified as a highly specific and sensitive biomarker of bladder transitional cell carcinoma, which can be detected from urine (*Wang et al., 2006*). It could promote glucose metabolism, lactic acid production, cell proliferation, and inhibit apoptosis of bladder cancer cells (*Li et al., 2014*; *Wang et al., 2008*). The functions of UCA1 was later explored in other cells. For example, it could protect cardiomyocytes from H_2O_2-induced apoptosis by targeting miR-1(*Yan et al., 2016*). In cardiovascular diseases, Yin et al. found that the silencing of *UCA1* could induce apoptosis and repress the viability, migration, tube formation of human microvascular ECs (*Yin, Fu & Sun, 2018*). Hu et al. found that the knockdown of *UCA1* in THP-1 cells could repress the formation of foam cells and restrain the total cholesterol and triglyceride levels via sponging miR-206 (*Hu et al., 2019b*). Moreover, a stable presence of *UCA1* was also detected in circulating blood and serum exosomes (*Barbagallo et al., 2018*; *Wang et al., 2015a*; *Wang et al., 2006*). The expression of *UCA1* in plasma decreased in the early stage of AMI but gradually increased in the subsequent process (*Yan et al., 2016*). This phenomenon is very similar to the *LIPCAR* aforementioned (*Kumarswamy et al., 2014*). Given its good indicator role in cancer, we could expect the possibility of its clinical application in AS diagnosis and prognosis.

CONCLUSION

As a hotspot of non-coding RNAs, lncRNAs have attracted the attention of scholars because of their unique structural characteristics and functions. LncRNAs widely distribute in various organs, tissues, and cells, and have important roles in different types of CVDs,

such as CAD, AMI, and so on (*Huang, 2018*). At present, the researches on lncRNAs in the occurrence and development of AS mainly focus on their effects on the lipid metabolism, aberrant proteolysis and cell activities such as impaired function of ECs, modulation of VSMCs' phenotype, recruitment of inflammatory cells, the polarization of macrophages and formation of foam cell (*Fasolo et al., 2019*; *Li, Zhu & Ge, 2016*; *Zhou et al., 2016*). In the last several years, the advance of high throughput sequencing technology brought about abundant novel transcripts with or without function annotation. Researchers have classified lncRNAs into different types according to their sequence characteristics, locations on chromosomes, or functions. These classifications could provide brief information on their physical and chemical property. Besides, classification based on its role in biological or pathological processes could facilitate their further researches, especially for those correlated with a specific disease (*Jarroux, Morillon & Pinskaya, 2017*). For example, *SAL-RNAs was classified as Senescence-associated lncRNAs* (*Abdelmohsen et al., 2013*), *and PCA3/PCAT1 as Prostate cancer-associated transcripts* (*PCATs*) (*Mitobe et al., 2018*). *We suggest clustering the lncRNAs reviewed in the present paper into a category of Atherosclerosis-associated circulating lncRNA (ASCL* ncRNA). *Accordingly, the ASCL* ncRNA *should meet the following features: (1) human-sourced long non-coding RNA transcript, (2) detectable in circulating blood, (3) expression level changes with disease development.* This definition was limited in human beings because most lncRNAs sequence evolved rapidly and can't be detected as homologues in the different animal models (*Necsulea et al., 2014*). Further, lncRNA transcripts in this classification have a potentially predictive value for monitoring AS progression. Because of the high incidence and severity of AS in human beings, looking for potential non-invasive diagnostic methods and detectable markers for disease prevention, early diagnosis, and treatment, as well as providing a reliable reference for prognosis and follow-up observations has become an urgent task. Due to the chronic progression of AS, it is also necessary to screen out markers at different stages of disease progression. And the ASCLnRNA transcripts would be candidates. A summary on the characteristics of these ASCLnRNAs is collated in Table 1. Their function, potential mechanisms in human AS-related cells and dynamic changes in the circulating blood was provided here (Figs. 1 and 2).

The researches on ASCLnRNAs in circulating blood are limited because previous studies just describe the occurrence and variation characteristics of ASCLnRNAs in AS (*Chi et al., 2017*; *Zhang et al., 2019*). Before further application of lncRNAs as biomarkers, some questions should be explored and answered. First, current testing methods are still limited, laying the challenges for exploring the origin of lncRNAs in circulating blood. Second, research methods are lacking standardization, and the sample size is often small (*Moldovan et al., 2014*). Third, some ASCLnRNAs do not only exists in cells related to CVDs but also in some cancer cells, which reduces their potential as independent biomarkers. Last but not least, the expression level of lncRNAs is generally lower than protein-encoding genes, which poses a challenge to the large-scale screening for biomarkers with repeatability and reliability. A key factor for future research work is the standardization, including standardized sample extracting and processing methods. Through this way, we could easily compare the different researchers' data and draw reliable conclusions (*Kumar et al., 2019*). The advent of the latest third-generation full-length transcriptome sequencing technology

Table 1 Summary of atherosclerosis-associated circulating lncRNA (ASClncRNA).

ASCLncRNA	Official Full Name	Gene ID (NCBI/Ensembl)	Category	Location	Atherogenic/ atheroprotective	Disease type	sensitivity[a]	specificity[a]	PMID
ANRIL	CDmfKN2B antisense RNA 1	100048912/ ENSG00000240498	antisense	9p21.3	atherogenic	AMI/AS	81.6%-90.2%	59.7%-65.7%	30234067; 31411246; 23861667
SENCR	Smooth muscle and endothelial cell enriched migration/differentiation-associated lncRNA	100507392/ ENSG00000254703	antisense	11q24.3	atheroprotective	AS	–	–	30584103
CoroMarker	Aldo-keto reductase family 1 member B1 pseudogene 3	729347/ENSG00000213785	intergenic	11p15.2	atherogenic	CAD	76%	92.5%	26857419
LIPCAR	Mitochondrially encoded long non-coding cardiac associated RNA	–	antisense	–	atherogenic	CAD/HF	72.2%	62.3%	31603865; 28790415
HIF1A-AS1	HIF1A antisense RNA 1	–	antisense	14q23.2	unknown	AS	–		24875884
LncRNA H19	H19 imprinted maternally expressed transcript	283120/ ENSG00000130600	intergenic	11p15.5	atherogenic	AS	53.6%	73%	30778327; 28165553; 28790415
APPAT	–	ENST00000620272	intergenic		unknown	AMI	78.72%	93.02%	29372117
KCNQ1OT1	KCNQ1 opposite strand/antisense transcript 1	10984/ ENSG00000269821	antisense	11p15.5	unknown	AMI/CAD/AS	100%	60%	30941792
LncPPAR δ	–	–	intergenic	–	unknown	CAD	70%–82%	78%–94%	26871769
LincRNA-p21	Tumor protein p53 pathway corepressor 1	102800311	intergenic	6p21.2	atheroprotective	AS	–	–	25156994
MALAT1	Metastasis associated lung adenocarcinoma transcript 1	378938/ ENSG00000251562	intergenic	11q13.1	atheroprotective	AS	50%	63.6%	30586743; 31188931
MIAT	Myocardial infarction associated transcript	440823/ ENSG00000225783	intergenic	22q12.1	atherogenic	AS	0.955	0.727	31237148; 31188931
UCA1	Urothelial cancer associated 1	652995/ ENSG00000214049	intergenic	19p13.12	atherogenic	AMI	–	–	30633352

Notes.

[a]The data of specificity/sensitivity percentages were derived directly from the ROC analysis of the original text.

AMI, acute myocardial infarction; AS, atherosclerosis; CAD, coronary artery disease; HF, heart failure.

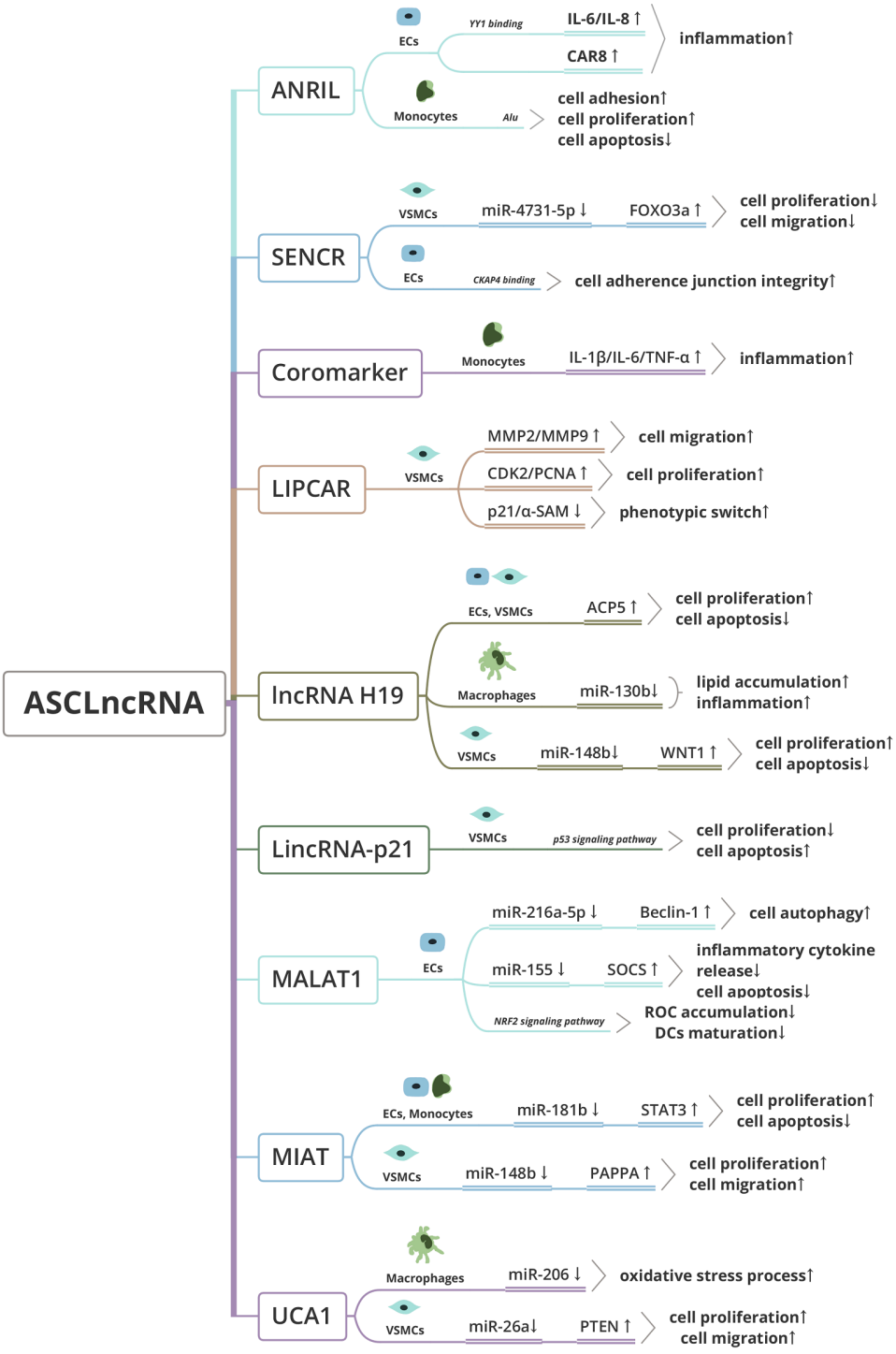

Figure 1 **Functional mechanisms of Atherosclerosis-associated circulating lncRNA (ASClncRNA).** ECs, endothelial cells; VSMCs, vascular smooth muscle cells.

Full-size DOI: 10.7717/peerj.10001/fig-1

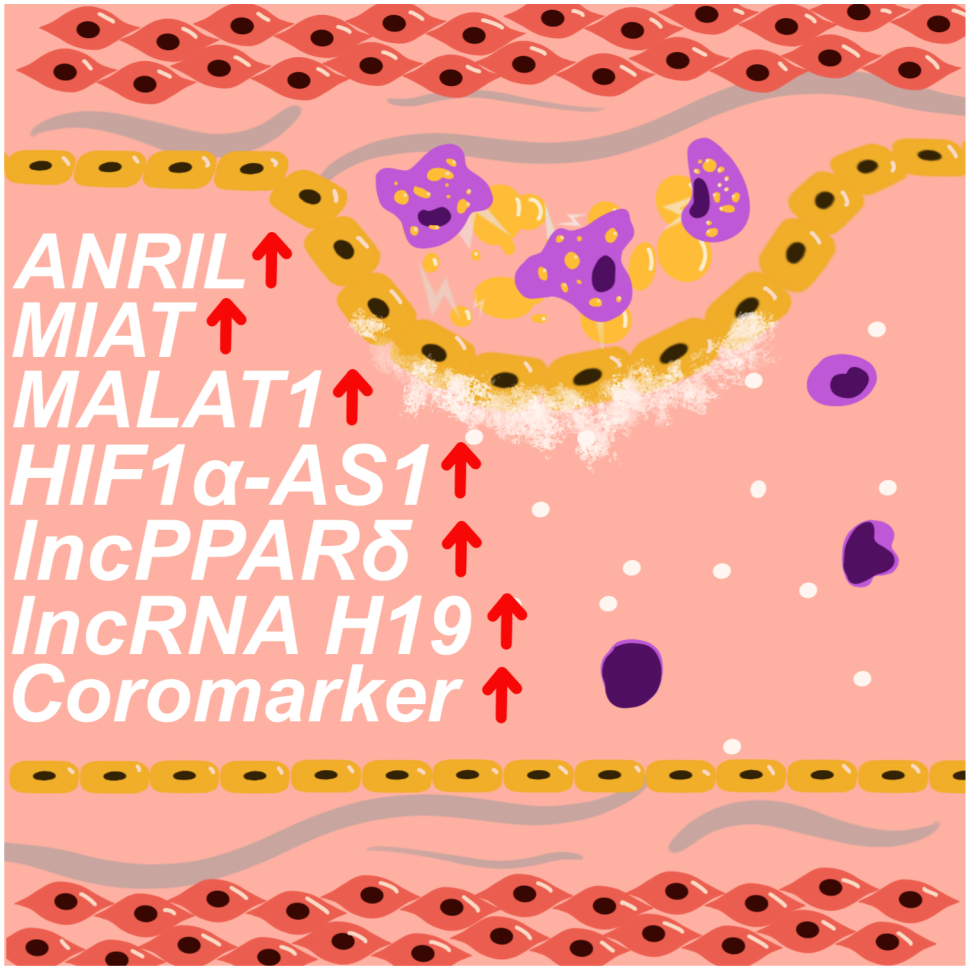

Figure 2 **The dynamic changes of Atherosclerosis-associated circulating lncRNA (ASCLncRNA) in blood.**

Full-size DOI: 10.7717/peerj.10001/fig-2

makes it possible to screen a large number of lncRNAs in different diseases (*Mercer et al., 2011*). Fortunately, we have seen similar work in cancer-related clinical practice. For example, *lncRNA-PCA3* has been widely recognized as a non-invasive diagnostic marker for prostate cancer (*Sanda et al., 2017*). Such work provides guidance and reference for finding AS-related biomarkers. The transcripts that meet the criterion of ASCLnRNA could form a candidate repository for further screening and validating researches on biomarkers for AS and could even become new therapeutic targets (*Skuratovskaia et al., 2019*).

Abbreviations

AMI	acute myocardial infarction
ANRIL	CDKN2B antisense RNA 1
APPAT	atherosclerotic plaque pathogenesis associated transcript
AS	atherosclerosis
ASCLncRNA	atherosclerosis-associated circulating lncRNA

BRG1	Brahma-related gene 1
CAD	coronary artery disease
ceRNA	competing endogenous RNA
ECs	endothelial cells
HF	heart failure
HIF1A-AS1	LncRNA HIF 1 alpha-antisense RNA 1
LncPPARδ	long noncoding Peroxisome proliferator-activated receptor delta
lncRNAs	long noncoding RNAs
MALAT1	Metastasis-associated lung adenocarcinoma transcript 1
MIAT	myocardial infarction associated transcript
miRNAs	microRNAs
qPCR	quantitative Polymerase Chain Reaction
SENCR	smooth muscle and endothelial cell-enriched migration/ differentiation-associated long noncoding RNA
SMCs	smooth muscle cells
SNPs	single nucleotide polymorphisms
UCA1	urothelial carcinoma associated 1
VSMCs	vascular smooth muscle cells

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Author Contributions

- Shanshan Lu performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Qin Liang and Yanqing Huang performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Fanming Meng and Junwen Liu conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

There is no raw data in this review.

REFERENCES

Abdelmohsen K, Panda A, Kang MJ, Xu J, Selimyan R, Yoon JH, Martindale JL, De S, Wood 3rd WH, Becker KG, Gorospe M. 2013. Senescence-associated lncRNAs: senescence-associated long noncoding RNAs. *Aging Cell* **12**:890–900 DOI 10.1111/acel.12115.

Arslan S, Berkan O, Lalem T, Ozbilum N, Goksel S, Korkmaz O, Cetin N, Devaux Y, Cardiolinc N. 2017. Long non-coding RNAs in the atherosclerotic plaque. *Atherosclerosis* **266**:176–181 DOI 10.1016/j.atherosclerosis.2017.10.012.

Barbagallo C, Brex D, Caponnetto A, Cirnigliaro M, Scalia M, Magnano A, Caltabiano R, Barbagallo D, Biondi A, Cappellani A, Basile F, Di Pietro C, Purrello M, Ragusa M. 2018. LncRNA UCA1, upregulated in CRC biopsies and downregulated in serum exosomes, controls mRNA expression by RNA-RNA interactions. *Molecular Therapy - Nucleic Acids* **12**:229–241 DOI 10.1016/j.omtn.2018.05.009.

Barichievy S, Naidoo J, Boulle M, Scholefield J, Parihar SP, Coussens AK, Brombacher F, Sigal A, Mhlanga MM. 2018. Viral apoptosis evasion via the MAPK pathway by use of a host long noncoding RNA. *Frontiers in Cellular Infection Microbiology* **8**:Article 263 DOI 10.3389/fcimb.2018.00263.

Bell RD, Long X, Lin M, Bergmann JH, Nanda V, Cowan SL, Zhou Q, Han Y, Spector DL, Zheng D, Miano JM. 2014. Identification and initial functional characterization of a human vascular cell-enriched long noncoding RNA. *Arteriosclerosis, Thrombosis, and Vascular Biology* **34**:1249–1259 DOI 10.1161/ATVBAHA.114.303240.

Bochenek G, Hasler R, El Mokhtari NE, Konig IR, Loos BG, Jepsen S, Rosenstiel P, Schreiber S, Schaefer AS. 2013. The large non-coding RNA ANRIL, which is associated with atherosclerosis, periodontitis and several forms of cancer, regulates ADIPOR1, VAMP3 and C11ORF10. *Human Molecular Genetics* **22**:4516–4527 DOI 10.1093/hmg/ddt299.

Boulberdaa M, Scott E, Ballantyne M, Garcia R, Descamps B, Angelini GD, Brittan M, Hunter A, McBride M, McClure J, Miano JM, Emanueli C, Mills NL, Mountford JC, Baker AH. 2016. A role for the long noncoding RNA SENCR in commitment and function of endothelial cells. *Molecular Therapy* **24**:978–990 DOI 10.1038/mt.2016.41.

Cai Y, Yang Y, Chen X, He D, Zhang X, Wen X, Hu J, Fu C, Qiu D, Jose PA, Zeng C, Zhou L. 2016a. Circulating "LncPPARdelta" from monocytes as a novel biomarker for coronary artery diseases. *Medicine* **95**:e2360 DOI 10.1097/MD.0000000000002360.

Cai Y, Yang Y, Chen X, Wu G, Zhang X, Liu Y, Yu J, Wang X, Fu J, Li C, Jose PA, Zeng C, Zhou L. 2016b. Circulating 'lncRNA OTTHUMT00000387022' from monocytes as a novel biomarker for coronary artery disease. *Cardiovascular Research* **112**:714–724 DOI 10.1093/cvr/cvw022.

Cao L, Zhang Z, Li Y, Zhao P, Chen Y. 2019. LncRNA H19/miR-let-7 axis participates in the regulation of ox-LDL-induced endothelial cell injury via targeting periostin. *International Immunopharmacology* **72**:496–503 DOI 10.1016/j.intimp.2019.04.042.

Cekin N, Ozcan A, Goksel S, Arslan S, Pinarbasi E, Berkan O. 2018. Decreased FENDRR and LincRNA-p21 expression in atherosclerotic plaque. *The Anatolian Journal of Cardiology* **19**:131–136 DOI 10.14744/AnatolJCardiol.2017.8081.

Chen C, Jin Y, Lo IL, Zhao H, Sun B, Zhao Q, Zheng J, Zhang XD. 2017. Complexity change in cardiovascular disease. *International Journal of Biological Sciences* **13**:1320–1328 DOI 10.7150/ijbs.19462.

Chi JS, Li JZ, Jia JJ, Zhang T, Liu XM, Yi L. 2017. Long non-coding RNA ANRIL in gene regulation and its duality in atherosclerosis. *Journal of Huazhong University of Science and Technology* **37**:816–822 DOI 10.1007/s11596-017-1812-y.

Congrains A, Kamide K, Oguro R, Yasuda O, Miyata K, Yamamoto E, Kawai T, Kusunoki H, Yamamoto H, Takeya Y, Yamamoto K, Onishi M, Sugimoto K, Katsuya T, Awata N, Ikebe K, Gondo Y, Oike Y, Ohishi M, Rakugi H. 2012. Genetic variants at the 9p21 locus contribute to atherosclerosis through modulation of ANRIL and CDKN2A/B. *Atherosclerosis* **220**:449–455 DOI 10.1016/j.atherosclerosis.2011.11.017.

Cremer S, Michalik KM, Fischer A, Pfisterer L, Jae N, Winter C, Boon RA, Muhly-Reinholz M, John D, Uchida S, Weber C, Poller W, Gunther S, Braun T, Li DY, Maegdefessel L, Perisic Matic L, Hedin U, Soehnlein O, Zeiher A, Dimmeler S. 2019. Hematopoietic deficiency of the long noncoding RNA MALAT1 promotes atherosclerosis and plaque inflammation. *Circulation* **139**:1320–1334 DOI 10.1161/CIRCULATIONAHA.117.029015.

De Gonzalo-Calvo D, Kenneweg F, Bang C, Toro R, Van der Meer RW, Rijzewijk LJ, Smit JW, Lamb HJ, Llorente-Cortes V, Thum T. 2016a. Circulating long-non coding RNAs as biomarkers of left ventricular diastolic function and remodelling in patients with well-controlled type 2 diabetes. *Scientific Reports* **6**:37354 DOI 10.1038/srep37354.

De Gonzalo-Calvo D, Kenneweg F, Bang C, Toro R, van der Meer RW, Rijzewijk LJ, Smit JW, Lamb HJ, Llorente-Cortes V, Thum T. 2016b. Circulating long noncoding RNAs in personalized medicine: response to pioglitazone therapy in type 2 diabetes. *Journal of the American College of Cardiology* **68**:2914–2916 DOI 10.1016/j.jacc.2016.10.014.

Dong L, Qi P, Xu MD, Ni SJ, Huang D, Xu QH, Weng WW, Tan C, Sheng WQ, Zhou XY, Du X. 2015. Circulating CUDR, LSINCT-5 and PTENP1 long noncoding RNAs in sera distinguish patients with gastric cancer from healthy controls. *International Journal of Cancer* **137**:1128–1135 DOI 10.1002/ijc.29484.

Ehrenborg E, Skogsberg J. 2013. Peroxisome proliferator-activated receptor delta and cardiovascular disease. *Atherosclerosis* **231**:95–106 DOI 10.1016/j.atherosclerosis.2013.08.027.

Fasolo F, Di Gregoli K, Maegdefessel L, Johnson JL. 2019. Non-coding RNAs in cardiovascular cell biology and atherosclerosis. *Cardiovascular Research* **115**:1732–1756 DOI 10.1093/cvr/cvz203.

Fayda M, Isin M, Tambas M, Guveli M, Meral R, Altun M, Sahin D, Ozkan G, Sanli Y, Isin H, Ozgur E, Gezer U. 2016. Do circulating long non-coding RNAs (lncRNAs) (LincRNA-p21, GAS 5, HOTAIR) predict the treatment response in patients with head and neck cancer treated with chemoradiotherapy? *Tumour Biology* **37**:3969–3978 DOI 10.1007/s13277-015-4189-1.

Folkersen L, Kyriakou T, Goel A, Peden J, Malarstig A, Paulsson-Berne G, Hamsten A, Hugh W, Franco-Cereceda A, Gabrielsen A, Eriksson P, consortia P. 2009. Relationship between CAD risk genotype in the chromosome 9p21 locus and gene expression. Identification of eight new ANRIL splice variants. *PLOS ONE* **4**:e7677 DOI 10.1371/journal.pone.0007677.

Fridman WH, Zitvogel L, Sautes-Fridman C, Kroemer G. 2017. The immune contexture in cancer prognosis and treatment. *Nature Reviews Clinical Oncology* **14**:717–734 DOI 10.1038/nrclinonc.2017.101.

Gao H, Wang X, Lin C, An Z, Yu J, Cao H, Fan Y, Liang X. 2019. Exosomal MALAT1 derived from ox-LDL-treated endothelial cells induce neutrophil extracellular traps to aggravate atherosclerosis. *Biological Chemistry* **401(3)**:367–376 DOI 10.1515/hsz-2019-0219.

Gao W, Zhu M, Wang H, Zhao S, Zhao D, Yang Y, Wang ZM, Wang F, Yang ZJ, Lu X, Wang LS. 2015. Association of polymorphisms in long non-coding RNA H19 with coronary artery disease risk in a Chinese population. *Mutation Research/DNA Repair* **772**:15–22 DOI 10.1016/j.mrfmmm.2014.12.009.

Giannoukakis N, Deal C, Paquette J, Goodyer CG, Polychronakos C. 1993. Parental genomic imprinting of the human IGF2 gene. *Nature Genetics* **4**:98–101 DOI 10.1038/ng0593-98.

Giordano Attianese GM, Desvergne B. 2015. Integrative and systemic approaches for evaluating PPARbeta/delta (PPARD) function. *Nucl Recept Signal* **13**:e001 DOI 10.1621/nrs.13001.

Gong W, Tian M, Qiu H, Yang Z. 2017. Elevated serum level of lncRNA-HIF1A-AS1 as a novel diagnostic predictor for worse prognosis in colorectal carcinoma. *Cancer Biomark* **20**:417–424 DOI 10.3233/CBM-170179.

Han Y, Ma J, Wang J, Wang L. 2018a. Silencing of H19 inhibits the adipogenesis and inflammation response in ox-LDL-treated Raw264.7 cells by up-regulating miR-130b. *Molecular Immunology* **93**:107–114 DOI 10.1016/j.molimm.2017.11.017.

Han Y, Qiu H, Pei X, Fan Y, Tian H, Geng J. 2018b. Low-dose sinapic acid abates the pyroptosis of macrophages by downregulation of lncRNA-MALAT1 in rats with diabetic atherosclerosis. *Journal of Cardiovascular Pharmacology* **71**:104–112 DOI 10.1097/FJC.0000000000000550.

Harada K, Harada K, Uetani T, Kataoka T, Takeshita M, Kunimura A, Takayama Y, Shinoda N, Kato B, Kato M, Marui N, Ishii H, Matsubara T, Amano T, Murohara

T. 2014. The different association of epicardial fat with coronary plaque in patients with acute coronary syndrome and patients with stable angina pectoris: analysis using integrated backscatter intravascular ultrasound. *Atherosclerosis* **236**:301–306 DOI 10.1016/j.atherosclerosis.2014.07.007.

Heil B, Tang WH. 2015. Biomarkers: their potential in the diagnosis and treatment of heart failure. *Cleveland Clinic Journal of Medicine* **82**:S28–S35 DOI 10.3949/ccjm.82.s2.05.

Hessels D, Klein Gunnewiek JM, Van Oort I, Karthaus HF, Van Leenders GJ, Van Balken B, Kiemeney LA, Witjes JA, Schalken JA. 2003. DD3(PCA3)-based molecular urine analysis for the diagnosis of prostate cancer. *European Urology* **44**:8–15 DOI 10.1016/s0302-2838(03)00201-x.

Holdt LM, Beutner F, Scholz M, Gielen S, Gabel G, Bergert H, Schuler G, Thiery J, Teupser D. 2010. ANRIL expression is associated with atherosclerosis risk at chromosome 9p21. *Arteriosclerosis, Thrombosis, and Vascular Biology* **30**:620–627 DOI 10.1161/ATVBAHA.109.196832.

Holdt LM, Hoffmann S, Sass K, Langenberger D, Scholz M, Krohn K, Finstermeier K, Stahringer A, Wilfert W, Beutner F, Gielen S, Schuler G, Gabel G, Bergert H, Bechmann I, Stadler PF, Thiery J, Teupser D. 2013. Alu elements in ANRIL non-coding RNA at chromosome 9p21 modulate atherogenic cell functions through trans-regulation of gene networks. *PLOS Genetics* **9**:e1003588 DOI 10.1371/journal.pgen.1003588.

Holdt LM, Teupser D. 2012. Recent studies of the human chromosome 9p21 locus, which is associated with atherosclerosis in human populations. *Arteriosclerosis, Thrombosis, and Vascular Biology* **32**:196–206 DOI 10.1161/ATVBAHA.111.232678.

Hu Y, Hu J. 2019. Diagnostic value of circulating lncRNA ANRIL and its correlation with coronary artery disease parameters. *Brazilian Journal of Medical and Biological Research* **52**:e8309 DOI 10.1590/1414-431X20198309.

Hu X, Ma R, Fu W, Zhang C, Du X. 2019b. LncRNA UCA1 sponges miR-206 to exacerbate oxidative stress and apoptosis induced by ox-LDL in human macrophages. *Journal of Cellular Physiology* **234**:14154–14160 DOI 10.1002/jcp.28109.

Hu W, Wang Z, Li Q, Wang J, Li L, Jiang G. 2019a. Upregulation of lincRNA-p21 in thoracic aortic aneurysms is involved in the regulation of proliferation and apoptosis of vascular smooth muscle cells by activating TGF-beta1 signaling pathway. *Journal of Cellular Biochemistry* **120**:4113–4120 DOI 10.1002/jcb.27696.

Huang Y. 2018. The novel regulatory role of lncRNA-miRNA-mRNA axis in cardiovascular diseases. *Journal of Cellular and Molecular Medicine* **22**:5768–5775 DOI 10.1111/jcmm.13866.

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. 2010. A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response. *Cell* **142**:409–419 DOI 10.1016/j.cell.2010.06.040.

Ishii N, Ozaki K, Sato H, Mizuno H, Saito S, Takahashi A, Miyamoto Y, Ikegawa S, Kamatani N, Hori M, Saito S, Nakamura Y, Tanaka T. 2006. Identification of a novel non-coding RNA, MIAT, that confers risk of myocardial infarction. *Journal of Human Genetics* **51**:1087–1099 DOI 10.1007/s10038-006-0070-9.

Jarroux J, Morillon A, Pinskaya M. 2017. History, discovery, and classification of lncRNAs. *Advances in Experimental Medicine and Biology* **1008**:1–46 DOI 10.1007/978-981-10-5203-3_1.

Ji P, Diederichs S, Wang W, Boing S, Metzger R, Schneider PM, Tidow N, Brandt B, Buerger H, Bulk E, Thomas M, Berdel WE, Serve H, Muller-Tidow C. 2003. MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer. *Oncogene* **22**:8031–8041 DOI 10.1038/sj.onc.1206928.

Kanduri C. 2011. Kcnq1ot1: a chromatin regulatory RNA. *Seminars in Cell & Developmental Biology* **22**:343–350 DOI 10.1016/j.semcdb.2011.02.020.

Kumar S, Williams D, Sur S, Wang JY, Jo H. 2019. Role of flow-sensitive microRNAs and long noncoding RNAs in vascular dysfunction and atherosclerosis. *Vascular Pharmacology* **114**:76–92 DOI 10.1016/j.vph.2018.10.001.

Kumarswamy R, Bauters C, Volkmann I, Maury F, Fetisch J, Holzmann A, Lemesle G, De Groote P, Pinet F, Thum T. 2014. Circulating long noncoding RNA, LIPCAR, predicts survival in patients with heart failure. *Circulation Research* **114**:1569–1575 DOI 10.1161/CIRCRESAHA.114.303915.

Li Z, Li X, Wu S, Xue M, Chen W. 2014. Long non-coding RNA UCA1 promotes glycolysis by upregulating hexokinase 2 through the mTOR-STAT3/microRNA143 pathway. *Cancer Science* **105**:951–955 DOI 10.1111/cas.12461.

Li S, Sun Y, Zhong L, Xiao Z, Yang M, Chen M, Wang C, Xie X, Chen X. 2018b. The suppression of ox-LDL-induced inflammatory cytokine release and apoptosis of HCAECs by long non-coding RNA-MALAT1 via regulating microRNA-155/SOCS1 pathway. *Nutrition Metabolism and Cardiovascular Diseases* **28**:1175–1187 DOI 10.1016/j.numecd.2018.06.017.

Li M, Wang YF, Yang XC, Xu L, Li WM, Xia K, Zhang DP, Wu RN, Gan T. 2018a. Circulating long noncoding RNA LIPCAR acts as a novel biomarker in patients with ST-segment elevation myocardial infarction. *Medical Science Monitor* **24**:5064–5070 DOI 10.12659/MSM.909348.

Li H, Zhu H, Ge J. 2016. Long noncoding RNA: recent updates in atherosclerosis. *International Journal of Biological Sciences* **12**:898–910 DOI 10.7150/ijbs.14430.

Li H, Zhu X, Hu L, Li Q, Ma J, Yan J. 2019. Loss of exosomal MALAT1 from ox-LDL-treated vascular endothelial cells induces maturation of dendritic cells in atherosclerosis development. *Cell Cycle* **18**:2255–2267 DOI 10.1080/15384101.2019.1642068.

Liang W, Lv T, Shi X, Liu H, Zhu Q, Zeng J, Yang W, Yin J, Song Y. 2016. Circulating long noncoding RNA GAS5 is a novel biomarker for the diagnosis of nonsmall cell lung cancer. *Medicine* **95**:e4608 DOI 10.1097/MD.0000000000004608.

Libby P, Ridker PM, Hansson GK. 2011. Progress and challenges in translating the biology of atherosclerosis. *Nature* **473**:317–325 DOI 10.1038/nature10146.

Lyu Q, Xu S, Lyu Y, Choi M, Christie CK, Slivano OJ, Rahman A, Jin ZG, Long X, Xu Y, Miano JM. 2019. SENCR stabilizes vascular endothelial cell adherens junctions through interaction with CKAP4. *Proceedings of the National Academy of Sciences of the United States of America* **116**:546–555 DOI 10.1073/pnas.1810729116.

Meng F, Yan J, Ma Q, Jiao Y, Han L, Xu J, Yang F, Liu J. 2018. Expression status and clinical significance of lncRNA APPAT in the progression of atherosclerosis. *PeerJ* **6**:e4246 DOI 10.7717/peerj.4246.

Mercer TR, Gerhardt DJ, Dinger ME, Crawford J, Trapnell C, Jeddeloh JA, Mattick JS, Rinn JL. 2011. Targeted RNA sequencing reveals the deep complexity of the human transcriptome. *Nature Biotechnology* **30**:99–104 DOI 10.1038/nbt.2024.

Michalik KM, You X, Manavski Y, Doddaballapur A, Zornig M, Braun T, John D, Ponomareva Y, Chen W, Uchida S, Boon RA, Dimmeler S. 2014. Long noncoding RNA MALAT1 regulates endothelial cell function and vessel growth. *Circulation Research* **114**:1389–1397 DOI 10.1161/CIRCRESAHA.114.303265.

Mitobe Y, Takayama KI, Horie-Inoue K, Inoue S. 2018. Prostate cancer-associated lncRNAs. *Cancer Letters* **418**:159–166 DOI 10.1016/j.canlet.2018.01.012.

Moldovan L, Batte KE, Trgovcich J, Wisler J, Marsh CB, Piper M. 2014. Methodological challenges in utilizing miRNAs as circulating biomarkers. *Journal of Cellular and Molecular Medicine* **18**:371–390 DOI 10.1111/jcmm.12236.

Monteiro JP, Bennett M, Rodor J, Caudrillier A, Ulitsky I, Baker AH. 2019. Endothelial function and dysfunction in the cardiovascular system: the long non-coding road. *Cardiovascular Research* **115**:1692–1704 DOI 10.1093/cvr/cvz154.

Necsulea A, Soumillon M, Warnefors M, Liechti A, Daish T, Zeller U, Baker JC, Grutzner F, Kaessmann H. 2014. The evolution of lncRNA repertoires and expression patterns in tetrapods. *Nature* **505**:635–640 DOI 10.1038/nature12943.

NHLBI. 2020. Atherosclerosis. *Available at https://www.nhlbi.nih.gov/health-topics/atherosclerosis*.

Pahwa R, Jialal I. 2019. StatPearls. In: *Atherosclerosis*. Treasure Island (FL): StatPearls Publishing.

Palazzo AF, Lee ES. 2015. Non-coding RNA: what is functional and what is junk? *Frontiers in Genetics* **6**:Article 2 DOI 10.3389/fgene.2015.00002.

Pan JX. 2017. LncRNA H19 promotes atherosclerosis by regulating MAPK and NF-kB signaling pathway. *European Review for Medical and Pharmacological Sciences* **21**:322–328.

Pan Z, Fan Z, Ma J, Liu H, Shen L, He B, Zhang M. 2019. Profiling and functional characterization of circulation LncRNAs that are associated with coronary atherosclerotic plaque stability. *American Journal of Translational Research* **11**:3801–3815.

Panzitt K, Tschernatsch MM, Guelly C, Moustafa T, Stradner M, Strohmaier HM, Buck CR, Denk H, Schroeder R, Trauner M, Zatloukal K. 2007. Characterization

of HULC, a novel gene with striking up-regulation in hepatocellular carcinoma, as noncoding RNA. *Gastroenterology* **132**:330–342 DOI 10.1053/j.gastro.2006.08.026.

Pasmant E, Laurendeau I, Heron D, Vidaud M, Vidaud D, Bieche I. 2007. Characterization of a germ-line deletion, including the entire INK4/ARF locus, in a melanoma-neural system tumor family: identification of ANRIL, an antisense noncoding RNA whose expression coclusters with ARF. *Cancer Research* **67**:3963–3969 DOI 10.1158/0008-5472.CAN-06-2004.

Pasmant E, Sabbagh A, Vidaud M, Bieche I. 2011. ANRIL, a long, noncoding RNA, is an unexpected major hotspot in GWAS. *FASEB Journal* **25**:444–448 DOI 10.1096/fj.10-172452.

Paulsen M, Davies KR, Bowden LM, Villar AJ, Franck O, Fuermann M, Dean WL, Moore TF, Rodrigues N, Davies KE, Hu RJ, Feinberg AP, Maher ER, Reik W, Walter J. 1998. Syntenic organization of the mouse distal chromosome 7 imprinting cluster and the Beckwith-Wiedemann syndrome region in chromosome 11p15.5. *Human Molecular Genetics* **7**:1149–1159 DOI 10.1093/hmg/7.7.1149.

Qi P, Zhou XY, Du X. 2016. Circulating long non-coding RNAs in cancer: current status and future perspectives. *Molecular Cancer* **15**:Article 39 DOI 10.1186/s12943-016-0524-4.

Reis EM, Verjovski-Almeida S. 2012. Perspectives of long non-coding RNAs in cancer diagnostics. *Frontiers in Genetics* **3**:32 DOI 10.3389/fgene.2012.00032.

Roth A, Diederichs S. 2016. Long noncoding RNAs in lung cancer. *Current Topics in Microbiology and Immunology* **394**:57–110 DOI 10.1007/82_2015_444.

Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. 2011. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell* **146**:353–358 DOI 10.1016/j.cell.2011.07.014.

Sanada F, Taniyama Y, Muratsu J, Otsu R, Shimizu H, Rakugi H, Morishita R. 2018. Gene-therapeutic strategies targeting angiogenesis in peripheral artery disease. *Medicine* **5(2)**:31 DOI 10.3390/medicines5020031.

Sanda MG, Feng Z, Howard DH, Tomlins SA, Sokoll LJ, Chan DW, Regan MM, Groskopf J, Chipman J, Patil DH, Salami SS, Scherr DS, Kagan J, Srivastava S, Thompson Jr IM, Siddiqui J, Fan J, Joon AY, Bantis LE, Rubin MA, Chinnayian AM, Wei JT, the E-PCASG, Bidair M, Kibel A, Lin DW, Lotan Y, Partin A, Taneja S. 2017. Association between combined TMPRSS2:ERG and PCA3 RNA urinary testing and detection of aggressive prostate cancer. *JAMA Oncology* **3**:1085–1093 DOI 10.1001/jamaoncol.2017.0177.

Santer L, Lopez B, Ravassa S, Baer C, Riedel I, Chatterjee S, Moreno MU, Gonzalez A, Querejeta R, Pinet F, Thum T, Diez J. 2019. Circulating long noncoding RNA LIPCAR predicts heart failure outcomes in patients without chronic kidney disease. *Hypertension* **73**:820–828 DOI 10.1161/HYPERTENSIONAHA.118.12261.

Saxena A, Carninci P. 2011. Long non-coding RNA modifies chromatin: epigenetic silencing by long non-coding RNAs. *Bioessays* **33**:830–839 DOI 10.1002/bies.201100084.

Schulte C, Barwari T, Joshi A, Theofilatos K, Zampetaki A, Barallobre-Barreiro J, Singh B, Sorensen NA, Neumann JT, Zeller T, Westermann D, Blankenberg S, Marber M, Liebetrau C, Mayr M. 2019. Comparative analysis of circulating noncoding RNAs versus protein biomarkers in the detection of myocardial injury. *Circulation Research* **125**:328–340 DOI 10.1161/CIRCRESAHA.119.314937.

Shahmoradi N, Nasiri M, Kamfiroozi H, Kheiry MA. 2017. Association of the rs555172 polymorphism in SENCR long non-coding RNA and atherosclerotic coronary artery disease. *Journal of Cardiovascular and Thoracic Research* **9**:170–174 DOI 10.15171/jcvtr.2017.29.

Shi Q, Yang X. 2016. Circulating microRNA and long noncoding RNA as biomarkers of cardiovascular diseases. *Journal of Cellular Physiology* **231**:751–755 DOI 10.1002/jcp.25174.

Skuratovskaia D, Vulf M, Komar A, Kirienkova E, Litvinova L. 2019. Promising directions in atherosclerosis treatment based on epigenetic regulation using microRNAs and long noncoding RNAs. *Biomolecules* **9**(**6**):226 DOI 10.3390/biom9060226.

Tantai J, Hu D, Yang Y, Geng J. 2015. Combined identification of long non-coding RNA XIST and HIF1A-AS1 in serum as an effective screening for non-small cell lung cancer. *International Journal of Clinical and Experimental Pathology* **8**:7887–7895.

Teng L, Meng R. 2019. Long non-coding RNA MALAT1 promotes acute cerebral infarction through miRNAs-mediated hs-CRP regulation. *Journal of Molecular Neuroscience* **69**:494–504 DOI 10.1007/s12031-019-01384-y.

Tinzl M, Marberger M, Horvath S, Chypre C. 2004. DD3PCA3 RNA analysis in urine–a new perspective for detecting prostate cancer. *European Urology* **46**:182–186 DOI 10.1016/j.eururo.2004.06.004.

Tong YS, Wang XW, Zhou XL, Liu ZH, Yang TX, Shi WH, Xie HW, Lv J, Wu QQ, Cao XF. 2015. Identification of the long non-coding RNA POU3F3 in plasma as a novel biomarker for diagnosis of esophageal squamous cell carcinoma. *Mol Cancer* **14**:Article 3 DOI 10.1186/1476-4598-14-3.

Vausort M, Wagner DR, Devaux Y. 2014. Long noncoding RNAs in patients with acute myocardial infarction. *Circulation Research* **115**:668–677 DOI 10.1161/CIRCRESAHA.115.303836.

Wang J, Chen L, Li H, Yang J, Gong Z, Wang B, Zhao X. 2015b. Clopidogrel reduces apoptosis and promotes proliferation of human vascular endothelial cells induced by palmitic acid via suppression of the long non-coding RNA HIF1A-AS1 in vitro. *Molecular and Cellular Biochemistry* **404**:203–210 DOI 10.1007/s11010-015-2379-1.

Wang X, Li D, Chen H, Wei X, Xu X. 2019c. Expression of long noncoding RNA LIPCAR promotes cell proliferation, cell migration, and change in phenotype of vascular smooth muscle cells. *Medical Science Monitor* **25**:7645–7651 DOI 10.12659/MSM.915681.

Wang F, Li X, Xie X, Zhao L, Chen W. 2008. UCA1, a non-protein-coding RNA up-regulated in bladder carcinoma and embryo, influencing cell growth and promoting invasion. *FEBS Letters* **582**:1919–1927 DOI 10.1016/j.febslet.2008.05.012.

Wang Y, Liang J, Xu J, Wang X, Zhang X, Wang W, Chen L, Yuan T. 2017. Circulating exosomes and exosomal lncRNA HIF1A-AS1 in atherosclerosis. *International Journal of Clinical and Experimental Pathology* **10**:8383–8388.

Wang HM, Lu JH, Chen WY, Gu AQ. 2015a. Upregulated lncRNA-UCA1 contributes to progression of lung cancer and is closely related to clinical diagnosis as a predictive biomarker in plasma. *International Journal of Clinical and Experimental Medicine* **8**:11824–11830.

Wang JJ, Wang X, Song YX, Zhao JH, Sun JX, Shi JX, Wu ZH, Wang ZN. 2019a. Circulating noncoding RNAs have a promising future acting as novel biomarkers for colorectal cancer. *Disease Markers* **2019**:Article 2587109 DOI 10.1155/2019/2587109.

Wang J, Wu X, Tian Y, Li X, Zhao X, Zhang M. 2018. Dynamic changes and diagnostic and prognostic significance of serum PCT, hs-CRP and s-100 protein in central nervous system infection. *Experimental and Therapeutic Medicine* **16**:5156–5160 DOI 10.3892/etm.2018.6866.

Wang K, Yang C, Shi J, Gao T. 2019b. Ox-LDL-induced lncRNA MALAT1 promotes autophagy in human umbilical vein endothelial cells by sponging miR-216a-5p and regulating Beclin-1 expression. *European Journal of Pharmacology* **858**:172338 DOI 10.1016/j.ejphar.2019.04.019.

Wang S, Zhang X, Yuan Y, Tan M, Zhang L, Xue X, Yan Y, Han L, Xu Z. 2015c. BRG1 expression is increased in thoracic aortic aneurysms and regulates proliferation and apoptosis of vascular smooth muscle cells through the long non-coding RNA HIF1A-AS1 in vitro. *European Journal of Cardio-Thoracic Surgery* **47**:439–446 DOI 10.1093/ejcts/ezu215.

Wang XS, Zhang Z, Wang HC, Cai JL, Xu QW, Li MQ, Chen YC, Qian XP, Lu TJ, Yu LZ, Zhang Y, Xin DQ, Na YQ, Chen WF. 2006. Rapid identification of UCA1 as a very sensitive and specific unique marker for human bladder carcinoma. *Clinical Cancer Research* **12**:4851–4858 DOI 10.1158/1078-0432.CCR-06-0134.

Wapinski O, Chang HY. 2011. Long noncoding RNAs and human disease. *Trends in Cell Biology* **21**:354–361 DOI 10.1016/j.tcb.2011.04.001.

WHO. 2017. Cardiovascular diseases. *Available at https://www.who.int/health-topics/cardiovascular-diseases/#tab=tab_1*.

Wu G, Cai J, Han Y, Chen J, Huang ZP, Chen C, Cai Y, Huang H, Yang Y, Liu Y, Xu Z, He D, Zhang X, Hu X, Pinello L, Zhong D, He F, Yuan GC, Wang DZ, Zeng C. 2014. LincRNA-p21 regulates neointima formation, vascular smooth muscle cell proliferation, apoptosis, and atherosclerosis by enhancing p53 activity. *Circulation* **130**:1452–1465 DOI 10.1161/CIRCULATIONAHA.114.011675.

Xi B, Shen Y, Reilly KH, Wang X, Mi J. 2013. Recapitulation of four hypertension susceptibility genes (CSK, CYP17A1, MTHFR, and FGF5) in East Asians. *Metabolism* **62**:196–203 DOI 10.1016/j.metabol.2012.07.008.

Xu J, Zhang Y, Chu L, Chen W, Du Y, Gu J. 2019. Long non-coding RNA HIF1A-AS1 is upregulated in intracranial aneurysms and participates in the regulation of proliferation of vascular smooth muscle cells by upregulating TGF-beta1. *Experimental and Therapeutic Medicine* **17**:1797–1801 DOI 10.3892/etm.2018.7144.

Yan B, Yao J, Liu JY, Li XM, Wang XQ, Li YJ, Tao ZF, Song YC, Chen Q, Jiang Q. 2015. lncRNA-MIAT regulates microvascular dysfunction by functioning as a competing endogenous RNA. *Circulation Research* **116**:1143–1156 DOI 10.1161/CIRCRESAHA.116.305510.

Yan Y, Zhang B, Liu N, Qi C, Xiao Y, Tian X, Li T, Liu B. 2016. Circulating long noncoding RNA UCA1 as a novel biomarker of acute myocardial infarction. *BioMed Research International* **2016**:Article 8079372 DOI 10.1155/2016/8079372.

Yang Y, Cai Y, Wu G, Chen X, Liu Y, Wang X, Yu J, Li C, Chen X, Jose PA, Zhou L, Zeng C. 2015. Plasma long non-coding RNA, CoroMarker, a novel biomarker for diagnosis of coronary artery disease. *Clinical Science* **129**:675–685 DOI 10.1042/CS20150121.

Yang L, Lin C, Liu W, Zhang J, Ohgi KA, Grinstein JD, Dorrestein PC, Rosenfeld MG. 2011. ncRNA- and Pc2 methylation-dependent gene relocation between nuclear structures mediates gene activation programs. *Cell* **147**:773–788 DOI 10.1016/j.cell.2011.08.054.

Ye ZM, Yang S, Xia YP, Hu RT, Chen S, Li BW, Chen SL, Luo XY, Mao L, Li Y, Jin H, Qin C, Hu B. 2019. LncRNA MIAT sponges miR-149-5p to inhibit efferocytosis in advanced atherosclerosis through CD47 upregulation. *Cell Death & Disease* **10**:Article 138 DOI 10.1038/s41419-019-1409-4.

Yin D, Fu C, Sun D. 2018. Silence of lncRNA UCA1 represses the growth and tube formation of human microvascular endothelial cells through miR-195. *Cellular Physiology and Biochemistry* **49**:1499–1511 DOI 10.1159/000493454.

Yu F, Zhou G, Huang K, Fan X, Li G, Chen B, Dong P, Zheng J. 2017. Serum lincRNA-p21 as a potential biomarker of liver fibrosis in chronic hepatitis B patients. *Journal of Viral Hepatitis* **24**:580–588 DOI 10.1111/jvh.12680.

Zhang L, Cheng H, Yue Y, Li S, Zhang D, He R. 2018. H19 knockdown suppresses proliferation and induces apoptosis by regulating miR-148b/WNT/beta-catenin in ox-LDL -stimulated vascular smooth muscle cells. *Journal of Biomedical Sciences* **25**:Article 311 DOI 10.1186/s12929-018-0418-4.

Zhang Z, Gao W, Long QQ, Zhang J, Li YF, Liu DC, Yan JJ, Yang ZJ, Wang LS. 2017. Increased plasma levels of lncRNA H19 and LIPCAR are associated with increased risk of coronary artery disease in a Chinese population. *Scientific Reports* **7**:7491 DOI 10.1038/s41598-017-07611-z.

Zhang K, Luo Z, Zhang Y, Zhang L, Wu L, Liu L, Yang J, Song X, Liu J. 2016. Circulating lncRNA H19 in plasma as a novel biomarker for breast cancer. *Cancer Biomark* **17**:187–194 DOI 10.3233/CBM-160630.

Zhang Y, Zhang L, Wang Y, Ding H, Xue S, Yu H, Hu L, Qi H, Wang Y, Zhu W, Liu D, Li P. 2019. KCNQ1OT1, HIF1A-AS2 and APOA1-AS are promising novel

biomarkers for diagnosis of coronary artery disease. *Clinical and Experimental Pharmacology and Physiology* **46**:635–642 DOI 10.1111/1440-1681.13094.

Zhao Y, Feng G, Wang Y, Yue Y, Zhao W. 2014. Regulation of apoptosis by long non-coding RNA HIF1A-AS1 in VSMCs: implications for TAA pathogenesis. *International Journal of Clinical and Experimental Pathology* **7**:7643–7652.

Zhong X, Ma X, Zhang L, Li Y, Li Y, He R. 2018. MIAT promotes proliferation and hinders apoptosis by modulating miR-181b/STAT3 axis in ox-LDL-induced atherosclerosis cell models. *Biomedicine and Pharmacotherapy* **97**:1078–1085 DOI 10.1016/j.biopha.2017.11.052.

Zhou T, Ding JW, Wang XA, Zheng XX. 2016. Long noncoding RNAs and atherosclerosis. *Atherosclerosis* **248**:51–61 DOI 10.1016/j.atherosclerosis.2016.02.025.

Zhou J, Zhang M, Fang H, El-Mounayri O, Rodenberg JM, Imbalzano AN, Herring BP. 2009. The SWI/SNF chromatin remodeling complex regulates myocardin-induced smooth muscle-specific gene expression. *Arteriosclerosis, Thrombosis, and Vascular Biology* **29**:921–928 DOI 10.1161/ATVBAHA.109.187229.