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Non-coding RNAs and atherosclerosis

Binod Aryal, MS[#], **Noemi Rotllan, Ph.D**[#], and **Carlos Fernández-Hernando, Ph.D**[#]
Vascular Biology and Therapeutics Program, Yale University School of Medicine, New Haven, Connecticut, USA. Integrative Cell Signaling and Neurobiology of Metabolism Program, Section of Comparative Medicine, Yale University School of Medicine, New Haven, Connecticut, USA.

[#] These authors contributed equally to this work.

Abstract

Non-coding RNAs (ncRNAs) represent a class of RNA molecules that typically do not code for proteins. Emerging data suggest that ncRNAs play an important role in several physiological and pathological conditions such as cancer and cardiovascular diseases (CVDs) including atherosclerosis. The best-characterized ncRNAs are the microRNAs (miRNAs), which are small, ~22 nucleotide (nt) sequences of RNA that regulate gene expression at the posttranscriptional level through transcript degradation or translational repression. MiRNAs control several aspects of atherosclerosis including endothelial cell, vascular smooth cell, and macrophage functions as well as lipoprotein metabolism. Apart from miRNAs, recently ncRNAs, especially long ncRNAs (lncRNAs), have emerged as important potential regulators of the progression of atherosclerosis. However, the molecular mechanism of their regulation and function as well as significance of other ncRNAs such as small nucleolar RNAs (snoRNAs) during atherogenesis is largely unknown. In this review, we summarize the recent findings in the field, highlighting the importance of ncRNAs in atherosclerosis and discuss their potential use as therapeutic targets in CVDs.

Keywords

microRNA; LncRNA; atherosclerosis; lipid metabolism

INTRODUCTION

In recent years, there has been a surge of interest in understanding RNA biology and its implication in human health and disease processes. High-throughput transcriptome analyses have identified a large number of non-coding RNAs (ncRNAs) in mammalian genome. While only 1.5% of human genome is responsible for protein coding genes, a vast majority of non-coding regulatory elements are transcribed into ncRNAs (1, 2). For many years the

[#]**Corresponding author**: Carlos Fernández-Hernando, PhD 10 Amistad Street, Amistad Research Building, Room 337C, New Haven, CT 06510 Yale University School of Medicine Tel: (203) 737-4615 Fax: (203) 737-2290 carlos.fernandez@yale.edu.

CONFLICTS OF INTEREST

Binod Aryal and Noemi Rotllan declare that they have no conflict of interest. Carlos Fernandez-Hernando has patents on the use of microRNA-33 inhibitors.

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function of these RNA molecules remained unknown and thus were regarded as the “Dark Matter” of biology. Recent findings from several studies have shown that the non-coding portion of the genome is of crucial importance for diseases and for the regulation of biological processes including differentiation, development, post-transcriptional regulation of gene expression and epigenetic regulation (3-5). MicroRNAs, ~22 nt long non-coding sequences involved in the post-transcriptional regulation of gene expression, are by far the most extensively studied ncRNAs. Initially discovered in *Caenorhabditis elegans* as developmental regulators (6), miRNAs are now known to control gene expression in most animals and are involved in a wide spectrum of biological processes including development, cell proliferation, lipid metabolism, angiogenesis, and tumorigenesis among others (7-9). Apart from miRNAs, roles of several other noncoding RNA species including long non-coding RNAs (lncRNAs), PIWI-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), transcribed ultraconserved regions (t-UCRs), and others have been surfacing for these processes.

Cardiovascular diseases represent a major health problem and the leading cause of death in the Western societies (10). Atherosclerosis, the major form of cardiovascular disease, is a chronic inflammatory process characterized by the deposition of inflammatory plaques within the arterial wall and involves the complex interaction of several different cell types and modified lipoproteins (11). Several studies have shown that altered ncRNA expression and function have been implicated in the atherosclerotic process.

In this review article, we summarize the recent findings in the field and discuss the potential therapeutic application of targeting ncRNAs for treating cardiometabolic disorders.

Non-coding RNAs

Regulatory ncRNAs can be classified into three different classes based on their transcript-size; 1) short ncRNAs, 2) medium ncRNAs and 3) long ncRNAs (lncRNAs) (**Table 1**). Short ncRNAs represent a class of ncRNAs smaller than 30 nt in length and include very well documented microRNAs (miRNAs), piRNAs and tiRNAs. Medium ncRNAs, typically 20-300 nucleotides in length, include PROMPTs, snRNAs, and TSSa-RNAs. Long ncRNAs (lncRNAs) are usually longer than 200 nucleotides and comprise a wide variety of RNAs including lincRNAs, eRNAs, T-UCRs and NATs among others. The regulation and processing of these ncRNAs are described elsewhere (4, 12, 13). Here, we will focus mainly on miRNAs and lncRNAs that have been established as important regulators of several aspects of atherosclerosis.

Small ncRNA (miRNA)—The most widely studied class of ncRNAs are miRNAs, which are small ncRNAs (~22 nt), that in animals, mediate post-transcriptional gene repression by controlling the translation of mRNA into protein (14, 15). miRNAs are estimated to regulate the translation of more than 60% of protein-coding genes (16). As a consequence, these small endogenous silencers have emerged as critical regulators of a diverse range of biological processes, including proliferation, differentiation, apoptosis and development. Interestingly, while some miRNAs regulate individual target genes, others can function as a master regulator of biological processes by controlling the expression of numerous genes

involved in the same physiological pathway. In most cases, miRNAs function to modulate or fine-tune cellular phenotypes rather than working as regulatory on/off switches. Biogenesis of miRNAs takes places through a multi-step process that involves the RNase III enzyme DROSHA and DICER and finally results in the production of mature miRNAs. The guide strand (5p), is loaded into the RNA-induced silencing complex (RISC) targeting the 3' untranslated region (3'UTR) of mRNA transcripts (17, 18). After binding to the 3'UTR of their target genes, miRNAs regulate protein expression through mRNA destabilization and/or by inhibition of translation. In order to repress the transcript, it is crucial that the nucleotides in position 2-8 of the miRNA, the seed sequence, are almost perfectly complimentary to regions at the 3'UTR of their target genes (19).

Long ncRNAs (lncRNAs)—lncRNAs are heterogenous groups of transcribed RNA molecules ranging from 200 to 100,000 nt in length. Based on their genomic location relative to well-established markers such as protein-coding genes, lncRNAs can be classified into several sub groups (20); 1) long intergenic ncRNAs, 2) natural antisense transcripts, 3) enhancer-like ncRNAs, and 4) transcribed ultra-conserved regions. Long intergenic ncRNAs (lincRNAs) are distinct transcriptional units located in sequence spaces that do not overlap protein-coding genes. Examples include HOTAIR and MALAT1. Natural antisense transcripts (NATs) are RNA molecules transcribed opposite to the sense DNA strand of annotated transcription units while enhancer-like ncRNAs (eRNAs) are short bidirectional products from enhancers that are not processed. Transcribed ultra-conserved regions (T-UCRs) are transcripts from genomic regions evolutionarily conserved among mammalian species. Moreover, lncRNAs also include pseudogenes, gene transcripts that have lost their coding potential due to nonsense, frameshift, and other mutations (20, 21). lncRNAs make up a large proportion of the mammalian transcriptome. There are 73,370 lncRNA entries from 1,239 organisms according to NONCODE v3.050; however, less than 200 of these lncRNAs have been functionally annotated (21). LncRNAs can regulate gene expression through a variety of mechanisms, including epigenetic modification of DNA, alternative splicing, post-transcriptional gene regulation and mRNA stability and translation (22, 23). They have been reported to control every level of the gene expression program in various physiological processes including development, proliferation, differentiation, apoptosis, and metabolism (24-26). lncRNAs can regulate gene expression by executing as signals, decoys, guides and scaffolds and acting as repressors or activators to modulate the process of gene transcription and translation (27). For instance, PANDA, a lncRNA induced by DNA damage from the CDKN1A promoter, acts as a decoy molecule. Specifically, PANDA limits the expression of proapoptotic genes to favor cell cycle arrest following DNA damage through the binding and sequestration of NF-YA, a nuclear transcription factor that activates the apoptotic program upon DNA damage, thereby promoting cell survival (28). Similarly, lncRNAs can also act as scaffolds bringing together multiple proteins to form ribonucleoprotein complexes. For example, the lncRNA HOTAIR (HOX Antisense Intergenic RNA) acts as a scaffold for the polycomb repressive complex 2 (PRC2) and LSD1/CoREST/REST complex and coordinates targeting of PRC2 and LSD1 to chromatin resulting in histone modification and transcriptional repression (29).

Small ncRNA (MiRNAs) and atherosclerosis

MiRNAs were primarily described in cancer but recent exciting findings have also demonstrated a key role in CVD, including atherosclerosis. The progression of atherosclerosis is characterized by very well established steps including macrophage foam cell formation, fatty streak accumulation, migration and proliferation of vascular smooth muscle cell (VSMC) and fibrous cap formation. This multifactorial disease is caused by the interaction of many key components, including lipoproteins, macrophages, T cells and arterial wall components such as endothelial cells (EC) and VSMCs. A growing body of studies have shown that miRNAs regulates ECs, VSMC, and macrophage functions, as well lipid metabolism, thereby controlling the progression of atherosclerosis.

Lipoprotein metabolism and miRNAs—Since the early discovery in the mammalian genome, miRNAs have become essential regulators of lipid and glucose metabolism (30). MicroRNAs such as miR-122, miR-33, miR-144, miR-758 and miR-106 have been described to play important roles in regulating lipid homeostasis by targeting crucial genes of cholesterol metabolism and fatty acid oxidation (31-36). miR-33a and miR-33b are intronic miRNAs encoded within the sterol response element binding protein genes, SREBP2 and SREBP1 respectively. Both miRNAs repress the expression of genes involved in cholesterol efflux, fatty acid oxidation and glucose production including adenosine tri-phosphate binding cassette A1 (ABCA1), carnitine O-octanyl transferase (CROT), carnitine palmitoyltransferase 1A (CPT1a), hydroxyacyl-CoA dehydrogenase-3-ketoacyl-CoA (HADHB), AMP-activated protein kinase (AMPK), phosphoenolpyruvate carboxykinase (PCK1) and glucose-6-phosphatase (G6PC) (33, 37). Recent reports in different animal models have shown that the therapeutic inhibition of miR-33 increases circulating high-density lipoprotein cholesterol (HDL-C) (38-40). Similar effects were observed in mice lacking miR-33. As expected by its significant effect on raising plasma HDL-C levels, antagonism of miR-33 attenuates the progression and enhances the regression of atherosclerosis in mice. Similarly, genetic ablation of miR-33 markedly reduces atherogenesis in ApoE deficient mice. In addition to miR-33, other miRNAs including miR-144 and miR-748 also regulate ABCA1 expression. Interestingly, silencing miR-144 increases plasma HDL-C in mice suggesting that miR-144 inhibition might be useful for treating atherosclerosis.

High-levels of circulating low-density lipoprotein (LDL) is the major risk factor that predisposes an individual to suffer coronary heart disease (CHD). In this regard, hepatic miR-122 and miR-30c expression have been shown to control plasma LDL cholesterol levels. Two classic studies demonstrated that antagonism miR-122 results in a significant reduction of circulating LDL cholesterol in mice and non-human primates. miR-122 is the most abundant miRNA in the liver and its absence decreases the hepatic expression of genes involved in cholesterol synthesis and lipoprotein secretion. One recent study has also shown that miR-30c expression also controls LDL cholesterol levels by regulating the secretion of lipoproteins in the liver. miR-30c regulates the expression of microsomal triglyceride transfer protein (MTP), which is essential for the assembly of lipoproteins in the liver. Intriguingly, hepatic miR-30c overexpression results in a marked reduction of plasma cholesterol levels, thereby ameliorating the progression of atherosclerosis in mice (41).

Endothelial dysfunction and miRNAs—The initial step in the development of atherosclerotic lesions is mediated by the endothelium. Many stimuli such as oxidized low-density lipoproteins (ox-LDLs), cholesterol, diabetes mellitus, hypertension and others impair endothelial function, increasing permeability and expression of leucocytes adhesion proteins, such as E-selectin and vascular cell adhesion protein 1 (VCAM-1), thereby allowing leukocytes to migrate into the vessel wall. The first evidence of the important role of miRNAs and endothelial dysfunction was provided by the observation that silencing DICER in ECs impairs angiogenesis *in vitro.* Several studies have recently shown the importance of some specific miRNAs including miR-126, miR-31, miR-181b and miR-92a in regulating endothelial cell functions. Mir-126 is highly expressed in ECs and regulates monocyte adhesion by directly targeting VCAM-1 (42). Interestingly, administration of miR-126 in a mouse model of atherosclerosis may reduce macrophage and apoptotic cell content, thereby limiting the size of the plaque and conferring a milder inflammatory reaction (43). In contrast, a recent paper has shown the contribution of miR-126 in neointimal hyperplasia and the atheroprone role of this miRNA in SMCs (44). In addition to miR-126, miR-31 and miR-17-3p also regulate vascular inflammation by controlling the expression of VCAM-1, I-CAM1 and E-SEL(45). VCAM-1 levels in ECs are also regulated by miR-21 but its contribution during the progression of atherosclerosis is somehow controversial. While miR-21 is upregulated in human atherosclerotic plaques (46) and aberrant expression of this miRNA was found in vascular neointimal lesion formation (47), miR-21 inhibits proliferator-activated receptor-alpha (PPAR), leading to enhanced expression of VCAM-1 (48). Whether an increase in miR-21 in ECs is beneficial or deleterious remains to be clarified. In a very recent study, Di Bernardini and colleagues, suggest that in induced pluripotent stem cells (iPSCs) pre-differentiated with VEGF, miR-21 targets PTEN/Akt and induces TGF- 2, therefore mediating endothelial differentiation, which might provide the basic information for stem cell therapy of vascular diseases (49). Other miRNAs are involved in the modulation of pro-inflammatory genes in ECs, such as miR-10a, which inhibits VCAM-1 and E-SEL or the NF-□ (50). This signaling pathway is also modulated by miR-181-b, which directly targets the importin subunit alpha-4 (KPNA4), a protein required for NF-□ (51). Recently, the same group has described that systemic delivery of miR-181b in apolipoprotein E (apoE) deficient mice inhibits NF-the inhibition of importin-a3 (52). MiR-146a and miR-146b have to be added to this microRNA-mediated NF-□B regulatory network in ECs. Both miRNAs control the activation of the EGR and AP-1 pathways and directly targets HuR, which promotes endothelial activation by antagonizing the endothelial nitric oxide synthase (eNOS) expression (53). miR-155 and miR-221/222 also play a role in modulating endothelial inflammation by down-regulating eNOS expression (54, 55), ETS-1 and its downstream inflammatory molecules in ECs (56). Neovessel formation can also be regulated by miR-222, which silences the signal transducer and activator of transcription 5A (STAT5A) (57). In fact, there is a negative correlation between miR-222 and STAT5A in ECs from advanced neovascularized plaques (57). A recent microRNA that regulates endothelial inflammation by targeting TIMP3 is miR-712, an atypical mechanosensitive miRNA originating from the RN45S gene (58). Moreover, miR-205 was identified as a human homologue of the murine miR-712 and shown to share the same seed sequence (58).

Angiogenesis is also regulated by the miR-17-92 cluster. Vascular endothelial growth factor (VEGF) regulates the expression of miR-17-92 and targets thrombospondin (TSP1), an anti-angiogenic molecule (59). However, the role of every member of the miR-17-92 cluster in regulating angiogenesis is not totally clear and appears to be complex as it has shown contradicting roles in different reports (59). Shear stress also regulates endothelial cell activation through miRNAs. In this regard, atheroprotective laminar shear flow downregulates miR-92a, a member of the miR-17-92 cluster, and increases the expression of some targets such as Krupple-like factor 2 (KLF2) or KLF4 (60). A recent paper published by Loyer *et al* reported for the first time the pro-atherogenic role of miR-92a, which specifically promotes endothelial dysfunction and inflammation in a low-dependent manner. They identified SOCS5 as a new target for miR-92-a and STAT3 as a transcription factor that regulates its expression (61). Although the role of miR-27 during atherosclerosis has not been elucidated, there is a good reason to speculate that miR-27 may serve as a potential indicator for atherosclerosis. Most evidence suggests that the miR-27 family may be a genuine proatherogenic-gene and that it may play an important role in the regulation of angiogenesis, adipogenesis, inflammation, lipid metabolism, oxidative stress and insulin signaling (62). Genetic silencing of DICER and DROSHA led to significantly reduced miR-27 expression in ECs (63). Akhtar *et al* have shown that increased expression of metalloproteinase-13 (MMP-13) correlated with down-regulation of miR-27b (64), indicating that miR-27 may contribute to plaque formation in atherosclerosis through regulating the expression of MMP-13 miR-27a/b also targets SEMA6A, thereby regulating EC adhesion and angiogenesis (65, 66)..

Aging is another risk factor for the development of atherosclerosis. Since EC senescence contributes to age related impairment of angiogenesis and vascular relaxation, miRNAs involved in the senescence process may be also related to plaque formation. In this regard, it has been recently described that miR-217 exerts negative effects on the vascular endothelium by enhancing endothelial senescence (67), as well as miR-34a (68). In contrast, miR-146a has been shown to delay EC senescence (69).

Vascular smooth muscle cells regulation and miRNAs—VSMC migration and proliferation are crucial in atherosclerosis progression. The switch of VSMC phenotype contributes to neointima and plaque formation. miRNAs are essential for VSMC development and function by regulating both differentiation and proliferation. In addition, some endothelial miRNAs can be exported via exosome and affect VSMC functions. Importantly, alterations in EC-VSMC communication have been implicated in atherosclerosis. Increased miR-21 regulates VSMC function via targeting tropomyosin-1 (TPM1) (70), programmed cell death protein-4 (PDCD4) (47,71), sprouty-2 (SPRY2), and PPARA in atherosclerosis (72). Another well-know miRNA that has been shown to regulate VSMC phenotype and function is miR-143/145. Several studies have demonstrated that miR-143/145 play a key role in SMC phenotypic switching in response to vascular injury. The effect of miR-143/145 in controlling SMC phenotypic switching is likely mediated through the targeting of multiple transcription factors, such as KLF4, KLF5, and a member of the ETS oncogene family (ELK-1). The activity of these transcription factors are linked to the repression of SMC differentiation genes (73-76). Moreover, loss of miR-145 results in

the formation of podosomes, which are actin-rich membrane protrusions that are involved in VSMC migration (77). In addition to the miR-143/145 cluster, miR146a and miR1/133 can also modulate KLF4 expression in VSMCs (78-81).

In a series of elegant, yet independent studies, it has recently been shown that miR-29 regulates vascular integrity by controlling multiple genes encoded for extracellular matrix proteins including multiple collagens, fibrilins, and elastin (82). miR-29 expression is upregulated in the arteries isolated from mouse model of aneuryms and in biopsies of human thoracic aneuryms. Importantly, inhibition of miR-29 enhances elastin levels in cells haploinsufficient for elastin and improves bioengineered vessels by increasing elastin expression (83). miR-29 expression levels are regulated during aging in the arterial wall. Arteries isolated from old mice expressed significantly higher levels of miR-29 compared with young mice. Moreover, miR-29 expression is also upregulated in aortas isolated from mice fed a high-fat diet. Together, these observations suggest a potential implication of this miRNA during the progression of atherosclerosis where aging and dyslipidemia are major risk factors.

Several miRNAs has been shown to play important roles in regulating VSMC proliferation including miR-221/222, miR-208 and miR-132. miR-221/222 expression is upregulated and localized in the VSMC of injured vascular walls, resulting in VSMC proliferation and neointimal hyperplasia (84, 85). Zhang *et al* demonstrated that insulin upregulated miR-208 expression and increased VSCM proliferation by targeting p21 (86). Finally, miR-132 has been reported as a novel regulator of VSCM proliferation that represses neointimal formation by inhibiting leucine-rich repeat (in Flightless 1) interacting protein-1 (LRRFIP1) expression (87).

Although let-7 is important during cancer development, it has been recently identified as a key player in cardiovascular disease. Let-7 has nine members in humans. Yu *et al* have described that let-7d inhibits VSMC proliferation through targeting KRAS (88). Moreover, ox-LDL inhibited let-7g expression via the LOX-1/ROS/ERK/AP-1 pathway and, inversely, let-7g was shown to directly target LOX-1 to compromise a negative feedback regulation that was involved in VSMC proliferation and migration (89). Recently, let-7g overexpression has been described to negatively regulate apoptosis induced by ox-LDL in vascular endothelial cells by targeting capase-3 (CASP3) expression (90). Liao *et al* in another recent study, have shown that the decreased let-7g levels impair endothelial function and increase the risk of cardiovascular disease through targeting TGF-β and sirtuin-1 (SIRT-1) signaling (91).

Arterial calcification is a key pathologic component of atherosclerosis and a hallmark of it is the phenotypic transition of VSMCs to osteoblast-like cells. Several studies have demonstrated that microRNAs also regulate osteoblast differentiation. In a recent study, the role of miR-133 in regulating VSMC-mediated arterial calcification via direct suppression of runt-related transcription factor 2 (RUNX2), a transcription factor involved in osteogenesis, has been characterized(92). Additionally, miR-181a protects against angiotensin II-induced osteopontin expression in VSMC (93).

Altogether, these studies strongly suggest that miRNAs regulate multiple aspects of VSMC biology including phenotypic differentiation, migration and proliferation.

Macrophage activation and miRNAs—One of the pathological hallmarks of atherosclerosis is the accumulation of cholesterol by macrophages (11). The lipid-laden macrophages are named foam cells, and they contribute significantly to the formation of atherosclerotic lesions. Moreover, macrophages are cells involved in the innate immune system and can also release inflammatory substances that influence atherosclerosis. Recently, some studies have reported that miRNAs play an important role in foam cell formation and the inflammation process in atherosclerosis. A recent paper determined that miR-9 could functionally decrease the formation of foam cells from THP-1-derived macrophages by reducing acetyl-CoA acetyltransferase 1 (ACAT1) protein following the enzymatic activity (94). However, further studies are needed to rule out if miR-9 affects lipid-uptake, binding or the cholesterol efflux from ox-LDL. Moreover, miR-9 regulates the expression of PPAR in human monocytes during the inflammatory response (95). Mir-125a-5p is not only important in endothelial dysfunction but also during foam cell formation. It has been shown that miR-125a-5p inhibits lipid uptake and the inflammatory responses in ox-LDL-stimulated monocytes/macrophages through modulation of oxysterol binding protein-like 9 (ORP9) expression (96). Silencing of the endogenous miR-155 in macrophages significantly enhances lipid uptake, upregulates the expression of scavenger receptors, and promotes the release of several pro-inflammatory cytokines including IL-6, IL-□ (97). Moreover, miR-155 promoted the expression of chemokine (C-C motif) ligand 2 (CCL2) and suppressed B cell leukemia/lymphoma 6 (Bcl-6), a transcription factor that inhibits NF-□B (98). However, another study has opposite results showing that hematopoietic deficiency of this miRNA increased the size and the instability of the plaque, possibly by the inhibition of lipid uptake and the inflammatory responses in monocytes (99). Thus, more studies need to be done in order to elucidate the role of miR-155 during the progression of atherosclerosis.

Two main risk factors, hyperlipidemia and infectious disease, point towards the innate immunity mechanism as a potential contributor to pro-atherogenic inflammation. The Toll-like receptors (TLRs) are the pro-inflammatory sensors of pathogens and oxidized lipids that control inflammation in infection diseases and atherosclerosis. In addition to regulating inflammation by controlling NFKB activation, TLR ligands also stimulate the expression of several miRNAs including miR-155, miR-146 and miR-147 associated with inflammation and the progression of atherosclerosis. mir-146a inhibits accumulation of oxLDL-induced lipid and the inflammatory response via targeting TLR4 (100). miR-147 has also been described as a negative regulator of the TLR-associated signaling events in macrophages (101).

lncRNAs and atherosclerosis

Although lncRNAs are reported in a wide range of physiological functions, their role in the progression of cardiovascular disease and atherosclerosis is poorly known and is limited to few examples. MIAT (myocardial infarction-associated transcript) was one of the earliest lncRNAs identified as a risk factor for cardiovascular disease. Particularly, through a large-

scale case-control association study, it was shown that altered expression of MIAT by one particular SNP confers susceptibility to myocardial infarction (102). The molecular mechanism underlying this correlation, however, is still unknown. Several later studies found that the chromosome 9p21 locus is the strongest genetic risk factor for coronary artery disease (103-105). This region contains a lincRNA designated antisense noncoding RNA in the INK4 locus (CDKN2B-AS also kwon as ANRIL). ANRIL is transcribed in an anti-sense direction with respect to the primary INK4 and ARF transcripts. It is known to be involved in tumor suppression. Specifically, it binds to components of the polycomb repression complexes 1 and 2 (PRC1 and PRC2) and mediates transcriptional repression of the INK4 locus by H3K27 trimethylation in cis (106,107). In addition, there are ample of evidence demonstrating a direct role of ANRIL in cardiovascular diseases. ANRIL is expressed in many cells and tissues involved in the atherogenic processes including smooth muscle cells, vascular endothelial cells, human monocyte-derived macrophage cells, and RNA extracted from carotid and arterectomy (108). A recent study has shown that increased expression of ANRIL transcripts is directly correlated with the severity of atherosclerosis. ANRIL regulates target genes in trans leading to increased cell proliferation, increased cell adhesion and decreased apoptosis, all processes essential for atherosclerosis. This trans regulation is dependent on Alu motifs, which are present on the promoters of ANRIL target genes and mirrored in ANRIL RNA transcripts (108). Moreover, another study shows that ANRIL regulates the expression of ADIPOR1, VAMP3 and C11ORF10 in a time-dependent manner. These genes demonstrate a well-described role in fatty acid and glucose metabolism as well as inflammation. Furthermore, the study found that the VAMP3 gene locus is a risk factor for coronary artery disease and C11ORF10 carries a risk variant for metabolic disorders (109). All these data present strong evidence for the role of ANRIL in cardiovascular diseases.

There have been several studies aimed at identifying and underlining the specific roles of lncRNAs in a tissue-specific manner in vascular biology. For example, Lnc-Ang362 was identified as a differentially expressed noncoding RNA in rat VSMCs in response to Angiotensin II (AngII) using transcriptome and epigenomic profiling. The study found that microRNAs miR-221 and miR-222, which are associated with smooth muscle cell proliferation and neointimal hyperplasia in response to vascular injury, are co-transcribed with Lnc-Ang362. Interestingly, knockdown of Lnc-Ang362 reduces the expression of these microRNAs and VSMC proliferation (25). Apart from VSMCs, endothelial cell integrity is an important component of normal vasculature, which can be deregulated during atherosclerosis. In particular, endothelial nitric-oxide synthase (NOS3) is an important enzyme that maintains this integrity by producing nitric oxide. Natural antisense transcript (NAT) to NOS3, NOS3 antisense (NOS3-AS) is induced by hypoxia in endothelial cells and regulates NOS3 expression in a post-transcriptional manner under normoxic and hypoxic conditions. Overexpression of NOS3-AS reduces NOS3 expression while inhibition shows an opposite effect (110, 111). Similarly, tie-1, a cell-surface tyrosine kinase receptor for angiopoietin ligands important for vascular development in vertebrates, is regulated *in vitro* and *in vivo* by its lnc anti-sense transcript tie-1AS lncRNA. Tie-1AS lncRNA binds to tie-1 mRNA resulting in downregulation of tie-1 protein and results in defects in endothelial cell contacts. Overexpression of tie-1AS lncRNA resulted in defects in EC junctions and tube

formation (112). A very recent study has shown that another NAT APOA1-AS acts as a negative transcriptional regulator of APOA1, a major component of HDL particles, both *in vitro* and *in vivo* (113). APOA1 is the major protein component of high-density lipoprotein (HDL), an important lipoprotein that is involved in reverse cholesterol transport and is associated with reduced atherosclerosis (114). This study found that APOA1-AS can modulate distinct histone methylation patterns and thereby expression of APOA1 as well as two neighboring genes through the recruitment of histone-modifying enzymes (113). Moreover, lncRNAs are implicated in inflammation and the innate immune response as well. Recently, lncCox2, a lncRNA proximal to Cox2 gene, was identified using whole transcriptome analysis of mouse bone marrow derived macrophages (BMDMs) stimulated with TLR ligands. This study shows that lncCox2 can mediate both activation and repression of distinct classes of immune genes (115).

Taken together, these observations suggest that lncRNAs are emerging players in cardiovascular diseases and atherosclerosis. LncRNAs can affect several processes associated with atherosclerosis including VSMC proliferation, endothelial function, lipid metabolism and inflammation. However, our knowledge on lncRNAs, their regulation and functions is still in infancy and as such, requires further investigation to identify and characterize myriads of other lncRNAs and explore their potential therapeutic application.

Concluding remarks

Non-coding RNAs have emerged as critical regulators of several disease processes including atherosclerosis. miRNAs, in particular, are well documented in terms of dysregulation of several aspects of atherosclerosis including lipid metabolism, cholesterol homeostasis, endothelial dysfunction and inflammation. Their conservation between species suggests that the biological pathways where miRNAs play a role may have been conserved. For that reason, miRNAs have a therapeutic potential and different approaches have been undertaken to examine it. For example, antagonists of miR-33 in vivo increase plasma HDL levels and the progression of atherosclerosis in mice and non-human primates (39, 40). Moreover, signaling pathways that are regulated by miRNAs could be potential therapeutic targets as well. On the other hand, although several thousands of lncRNAs have been identified in mammals, only few of them are functionally annotated, especially in association with cardiovascular diseases. However, given the rapid advancement in the field and immense attention garnered by lncRNAs in recent times, we anticipate that the field will witness major discoveries on the role of lncRNAs in many cellular and molecular processes. The knowledge on these processes will be of substantial value to identify novel genes and molecular pathways for therapeutic interventions against diseases including atherosclerosis.

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Table. 1

Non-coding RNA and their biological functions

Name	Size	Function
Short ncRNAs		
Micro RNAs (miRNAs)	19-24 bp	mRNA degradation, translational inhibition
PIWI-interacting RNAs (piRNAs)	26-31 bp	Transposon repression, epigenetic modification
Transcription initiation RNAs (tiRNAs)	17-18 bp	RNAPII backtracking, transcriptional regulation
Medium ncRNAs		
Promoter upstream targets (PROMPTs)	<200 bp	Transcription activation
Small nucleolar RNAs (snoRNAs)	60-300 bp	RNA modification, rRNA processing
TSS-associated RNAs (TSSa-RNAs)	20-90 bp	Transcription maintenance
Long ncRNAs		
Long intergenic ncRNAs (lincRNAs)	>200 bp	Epigenetic regulators of transcription
Natural antisense transcripts (NATs)	>200 bp	mRNA stability, transcriptional regulation
Enhancer-like ncRNA (eRNAs)	50-2000 bp	Transcriptional gene activation
Transcribed ultraconserved regions (T-UCRs)	>200 bp	Regulation of miRNA and mRNA levels
Other long ncRNAs	>200 bp	Various

Table 2

miRNA regulation of lipid metabolism and macrophages, ECs and VSMCs functions

Cell type/processes	ncRNA	mRNA target	Function	References
Lipid Metabolism	miR-33a/b	ABCA1, ABCG1, CPT1A, CROT, HADHB	Cholesterol efflux, fatty acid β-oxidation	(*33*, *37*)
	miR-144, miR-758, miR-106	ABCA1	Cholesterol efflux	(*34-36*)
	miR-30c	MTP, LPGAT1	Cholesterol synthesis, lipoprotein secretion	(*41*)
	lnc APOA1-AS	APOA1 gene cluster	Epigenetic modulation of APOA1 expression	(*113*)
Macrophages	miR-155	LX1, CD36 CD68, MyD88, BCL6	Lipid uptake and inflammation	(*97-99*)
	miR-125a-5p	ORP9	Lipid uptake and inflammation	(*96*)
	miR-146a	TLR4	TH1 response	(*100*)
	miR-147		Inflammation	(*101*)
	miR-9	PPARδ	Inflammation	(*95*)
	miR-342-5p	AKT1	Inflammation	
	lncCox2	??	Inflammation	(*115*)
VSMCs	miR-21	TPM1, PDCD4, PPARα	Proliferation, migration and apoptosis	(*70-72*)
	miR-143/145	KLF4, KLF5, ELK-1	Phenotype switching, podosome formation	(*73-76*)
	miR-146a			
	miR-1/33	KLF4, Sp-1	Proliferation	(*79-81*)
	miR-221/222	p27, p57, c-kit,	Proliferation, migration and apoptosis	(*84*, *85*)
	miR-29	ELN	Elastin formation	(*82*, *83*)
	miR-208	p21	Proliferation	(*86*)
	let-7d	KRAS	Proliferation	(*88*)
	let-7g	LOX-1	Proliferation and migration	(*89*, (*90*)
	miR-132	LRRFIP1	Proliferation	(*87*)
	miR-133a	RUNX2	Osteogenic differentiation	(*92*)
	miR-181a	OPN	Adhesion and oseopontin formation	(*93*)
	lnc ANRIL	??	Strong risk factor for athero, proliferation, adhesion, apoptosis	(*103-105*), (*108*)
	lnc-Ang362	miR-221/222	Proliferation	(*25*)
ECs	miR-126	SPRED1, VCAM-1	Monocyte adhesion	(*42*)
	miR-19-92 miR-17-3p, miR-31	ICAM-1, SELE	Inflammation	(*45*)
	miR-92a	eNOS, KLF2, KLF4, SOCS5	vasodilation, inflammation	(*60*), (*61*)
	miR-155, miR-221/222	eNOS, ETS-1	Inflammation	(*54*, *55*)
	miR-712	TIMP3	Inflammation	(*58*)
	miR-10	VCAM-1, SELE	Inflammation	(*50*)
	miR-181b	KPNA4 (importin-a3)	Inflammation	(*42*)
	miR-27	SEMA6A	EC adhesion, angiogenesis	(*65*, *66*)
	miR-34a, miR-217	SIRT-1	Senescence	(*67*), (*68*)
	miR-146	HuR, NOX4	EC activation, aging	(*53*), (*69*)

Cell type/processes	ncRNA	mRNA target	Function	References
	let-7g	CASP-3, SIRT-1, TGF-β	Apoptosis, inflammation, angiogenesis	(*90*), (*91*)
	NAT sONE	eNOS	Reduces eNOS expression	(*110*, *111*)
	lnc Tie-As	TIE-1	Vascular integrity, inflammation	(*112*)