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Review article

Endothelial microRNAs and long noncoding RNAs in cardiovascular ageing

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ABSTRACT

Atherosclerosis and numerous other cardiovascular diseases develop in an age-dependent manner. The endothelial cells that line the vessel walls play an important role in the development of atherosclerosis. Non-coding RNA like microRNAs and long non-coding RNAs are known to play an important role in endothelial function and are implicated in the disease progression. Here, we summarize several microRNAs and long non-coding RNAs that are known to have an altered expression with endothelial aging and discuss their role in endothelial cell function and senescence. These processes contribute to aging-induced atherosclerosis development and by targeting the non-coding RNAs controlling endothelial cell function and senescence, atherosclerosis can potentially be attenuated.

1. Introduction

Ageing is the main independent risk factor for atherosclerosis development [1]. With the increase in lifespan globally, cardiovascular disease (CVD) in general, and atherosclerosis in particular will become an even more impactful disease than it currently already is [2,3]. It is therefore of importance to better understand the processes that contribute to age-induced atherosclerosis development. Next to well-known causative mechanisms in atherosclerosis like hyperlipidemia, hypertension and diabetes mellitus for example [4], the contribution of aging-induced mechanisms are less well described. Atherosclerosis involves many cell types in the artery wall, including endothelial cells, smooth muscle cells and leukocytes. Here we focus on the endothelial cells that line blood vessels and form the barrier between the blood and the underlying vessel wall. Since atherosclerosis development is initiated by endothelial pro-inflammatory activation that leads to the recruitment of leukocytes, endothelial cell function is one of the most-studied aspects of atherosclerosis.

Non-coding RNAs are, as the name already suggests, RNA molecules that do not code for proteins. These RNAs are by definition derived from the non-protein coding regions of the genome (also referred to as junk DNA in the past). Non-coding RNAs can roughly be divided in small non-coding RNAs and long non-coding RNAs [5]. Well known small non-coding RNAs are transfer RNAs (tRNAs) and microRNAs (miRNAs), whereas well known examples of long non-coding RNAs are ribosomal RNA (rRNAs) and long (intergenic) non-coding RNAs, usually abbreviated as lncRNA or lincRNA.

For miRNAs, it is relatively clear how these non-coding RNAs regulate cellular processes. MiRNAs mainly bind to 3′UTRs of messenger RNAs (mRNAs) and block translation into protein or promote the degradation of the targeted mRNA, thereby inhibiting gene expression. One miRNA can target several mRNAs, making these small molecules broad regulators of gene expression. LncRNAs on the other hand are a much more heterogeneous class of non-coding RNAs. Each lncRNA acts in a different manner via interaction with other RNAs, proteins or DNA, resulting in various molecular mechanisms. Several miRNAs and lncRNAs have been implicated to play a role in cardiovascular diseases like atherosclerosis, aneurysms, hypertension and heart failure. These non-coding RNAs are not always expressed or age-regulated in all cardiovascular cell types. In this review we focus on miRNAs and lncRNAs that are regulated by aging in endothelial cells.

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2. miRNAs

MiRNAs are a class of non-coding RNAs that are endogenously expressed and can regulate gene expression on a posttranscriptional level [6]. Since the first discovery of a miRNA (lin-4) in *Caenorhabditis elegans* in 1993 [7], miRNAs are shown to have tissue- and cell type-specific expression patterns [8] and play important roles in various vascular diseases [9–11]. They are involved in several key processes and mechanisms that lead to cardiovascular events, including vascular inflammation [12], diabetes [13] and also cardiovascular ageing [14]. This review provides an overview of miRNAs that are regulated by ageing and in ageing-associated CVDs and their endothelial cell (EC)-specific functions (Table 1 and Table 2).

miR-21 (hsa-miR-21-5p) is probably the best studied miRNA in the cardiovascular field. It is investigated in all major CVDs such as heart failure [22], abdominal aortic aneurysms (AAA) [23] and atherosclerosis [24]. miR-21 is upregulated in fibroblasts of failing hearts and regulates the ERK-MAP kinase signaling pathway [22]. Overexpression (OE) of miR-21 in a human prostate cancer cell line increased the expression of HIF-1a and VEGF and therefore induced tumor angiogenesis [15]. miR-21 OE was also shown to promote cardiac fibrosis via STAT3 by decreasing CADM1 expression, which leads to the assumption that miR-21 is also an important signaling molecule for cardiac remodeling [16]. miR-21 expression also has a vasculogenic effect by promoting proliferation and angiogenesis of endothelial progenitor cells [25]. There is also a described effect in vascular smooth muscle cells (VSMCs), where miR-21 was shown to modulate proliferation and apoptosis via PTEN and AKT in AAA [23], as well as REST in atherosclerosis [24]. miR-21 is also increased in senescent ECs, such as human umbilical vein ECs (HUVECs) [15,16]. Here, OE of miR-21 leads to growth arrest and cellular senescence in ECs, whereas knockdown (KD) increases the lifespan of the cells [16]. The expression of miR-21 can also be induced by oxLDL in senescent HUVECs [25]. A known target of miR-21 in ECs is DRP1, a regulator of mitochondrial fission [25]. miR-21 expression is regulated by NRF2, a basic leucine zipper that functions as a cellular antioxidant and protects against oxidative damage [15,20].

miR-34a (hsa-miR-34a-5p) is also a well-studied microRNA. It is investigated in the context of myocardial infarction, where it is found to be upregulated in ageing cardiomyocytes. One direct target of miR-34a is PNUTS and the key mechanism is therefore regulation of cardiac contractile function via induction of DNA damage responses and telomere attrition [26]. miR-34a is also found in ageing VSMCs [27] as well as ECs, where it is increased in ageing HUVECs with OE of miR-34a inducing senescence and suppression of proliferation *in vitro* [18]. Another target of miR-34a in ECs is SIRT1; cell cycle arrest and senescence are triggered via this axis, *in vitro* and in coronary artery disease (CAD) [27–29]. SIRT1 is an NAD-dependent deacetylase that is crucially involved in transcription regulation and coordination of several cellular processes, by deacetylating for example key regulators in CVDs such as NFKB, STAT3 and p53 [30]. miR-34a is regulated by NRF2 [28].

miR-92a (hsa-miR-92a-3p) is found in several vascular diseases, including atherosclerosis. It is enriched in atheroprone areas of mouse aortas and stimulated through oxLDL in ECs [18]. In the context of atherosclerosis, miR-92a is also found in extracellular vesicles of coronary artery patients. Via this pathway, ECs receive the miRNA and increase proliferation, migration and therefore angiogenesis via THBS1. THBS1 is an adhesive glycoprotein that has been shown to bind fibrinogen, laminin, collagens and integrins and therefore plays a role in platelet aggregation and angiogenesis. miR-92a is also upregulated in senescent HUVECs, which leads to inhibition of oxidative stress, apoptosis and inflammation via for example NRF2 [31]. It is also found upregulated in neo-intimal lesions following femoral injury models in mice [32]. Interestingly, the blockage of miR-92a was investigated in a first in-human study in the context of CVD and wound healing. Antisense oligonucleotides targeting miR-92a specifically were given to healthy individuals. Lower miR-92a levels could be observed in ECs and also in extracellular vesicles of the subjects, with target genes such as ITGA5 and CD93 increased [33].

Table 2
miRNAs in ageing ECs.

miRNAs increased in ageing ECs	miRNAs decreased in ageing ECs
Hsa-miR-21-5p [15,16]	Hsa-miR-126-3p/-5p [15,17]
Hsa-miR-34a-5p [18]	
Hsa-miR-92a-3p [19]	
Hsa-miR-100-5p [20]	
Hsa-miR-221/222-3p/5p [21]	

miR-100 (hsa-miR-100) is another microRNA found to be upregulated in atherosclerotic lesions and suppresses several endothelial adhesion molecules and stimulates EC autophagy. In humans it is shown to be inversely correlated with inflammatory cell content in the lesions. miR-100 OE results in impaired leucocyte-endothelial interaction. Pharmacological inhibition of miR-100 leads to enhanced plaque lesion formation in murine models, whereas a systemic miR-100 replacement therapy had protective effects and attenuated atherogenesis, resulting in overall decreased plaque area [19]. miR-100 is furthermore found upregulated in senescent HUVECs [20]. It is in involved in apoptosis, inflammation, and oxidative stress regulation in HUVECs *in vitro* [34]. A known regulator of miR-100 is NRF2 [28].

miR-221 and *miR-222* (hsa-miR-221/222-3p/5p) have been shown to play a role in promoting proliferation of different cancer cells. In the vascular context, miR-221 and miR-222 are found upregulated in VSMCs of atherosclerotic plaques and neointimal hyperplasia. Targets of miR-221/222 include p27 and p57, which control VSMC growth in this context [35]. But miR-221 and miR-222 are also upregulated in senescent ECs [21]. Here, both miRNAs suppress eNOS expression [17] and OE of miR-221 and miR-222 leads to inhibition of angiogenesis *in vitro* in HUVECs via targeting of c-Kit and other angiogenic factors, suppressing the formation of new capillaries [36]. C-Kit has previously been shown to play a protective role against atherosclerosis. One of its described functions is the preservation of the contractile VSMC phenotype [37]. miR-221/222 are also regulated by NRF2 [28].

miR-126 (both hsa-miR-126-3p and hsa-miR-126-5p) is the only miRNA in this review, that is described to be downregulated in senescent ECs [15,17]. This decrease in expression leads to impaired tube formation and delayed wound healing capacities, as well as decreased migration, proliferation and angiogenesis [17]. The effects of miR-126 can be summarized as athero-protective, by targeting of DLK1 and therefore reducing lesion formation in mice (only miR-126-5p, not

Table 1
miRNAs associated with CVDs and EC pathway regulation.

	Main ageing-associated CVDs	Regulated pathways in ECs
hsa-miR-21-5p	heart failure, AAA, atherosclerosis	Senescence, angiogenesis
hsa-miR-34a-5p	MI, arterial ageing	senescence, proliferation
hsa-miR-92a-3p	atherosclerosis, wound healing, arterial injury	proliferation, migration, angiogenesis, apoptosis, inflammation, oxidative stress
hsa-miR-100-5p	atherosclerosis	apoptosis, inflammation, oxidative stress, autophagy
hsa-miR-126-3p/5p	atherosclerosis	proliferation, inflammation, angiogenesis, cell adhesion
hsa-miR-221/222-3p/5p	atherosclerosis	angiogenesis

AAA: abdominal aortic aneurysms; MI: myocardial infarction.

miR-126-3p has this effect) [36]. OE of miR-126 limits leucocyte adherence to the endothelial cells and vascular inflammation [40] and results therefore in enhanced survival of ECs, also by rescuing EC proliferation capacities [36,38]. Targeted deletion of miR-126 in mice causes leaky vessels due to a loss of proper vascular integrity. Decreased vasculogenic effects including impaired proliferation, migration and angiogenic capacities also lead to dramatic cardiac phenotypes in these mice [39]. Experimentally validated targets of miR-126 include VCAM1 [40], DLK1 [36], and HIF1α [17] and NRF2 is a prominent regulator [20]. VCAM1 is a cell surface protein that mediates leucocyte adhesion to ECs and therefore plays a key role in the development of vascular diseases. DLK1 is a transmembrane protein that is involved in cell differentiation and HIF1α is a well-established master regulator of vascularization and remodeling. It plays important roles in oxygen homeostasis and also angiogenesis [41].

miRNAs can be targeted by therapeutic measures. Especially the miRNAs that are detrimental for vascular function could be targeted by loss-of-function measures (like antisense based therapeutics, antimiR oligonucleotides) to decrease their abundance and improve the fate of these cells [42,43]. Therapeutic approach should always be site-specific (local) to avoid accumulation in non-target cells and organs and to improve the overall outcome for animals and humans [39,41,44]. miR-126 is decreased in aged ECs. To improve functions of ECs via the hsa-miR-126-5p axis, another approach, namely gain-of-function by miRNA mimics or OE vectors for example should be chosen [42,45]. Again, a local site-specific approach should be used [46]. One caveat of this approach may be that in aged or diseased endothelial cells, the hsa-miR-126-5p targets may not be expressed in a comparable manner to young healthy cells, thereby limiting the potential of OE of hsa-miR-126-5p.

3. LncRNAs

LncRNAs are a heterogenic class of endogenously expressed non-coding RNAs that are larger than 200 nt. They are mostly transcribed by Pol II and many have 5′-end m7G caps and 3′-end poly(A) tails [47]. Remarkably, lncRNAs have a poor sequence conservation, but their structure and genomic region are often conserved. The molecular functions of lncRNAs are very diverse. In the nucleus they can bind to and thereby influence transcription factors, chromatin modifiers and pre-messenger RNAs or act as a scaffold in ribonucleoprotein complexes [5]. In the cytoplasm they can also mask binding sites of miRNAs or mRNAs and thereby alter the binding to their targets or alter the stability. LncRNAs are able to influence several important processes and mechanisms in the vascular system that can lead to cardiovascular events. Here we discuss lncRNAs which are known to be regulated with age in ECs and are associated with CVDs and/or implicated in endothelial function (Table 3 and Table 4).

Table 4
lncRNAs in ageing ECs.

lncRNAs increased in ageing ECs	lncRNAs decreased in ageing ECs
AERRIE	H19
ASncmtRNA-2	SNHG12
MALAT1	
MEG3	
MEG8	
NORAD	

3.1. AERRIE

LncRNA ‘Age and EndMT Regulated RNA In Endothelium’ (Aerrie or linc01013) has been shown to be upregulated with increased passage number in HUVECs [48]. Aerrie is also upregulated in plaques from symptomatic atherosclerotic patients compared to asymptotic patients, in Endothelial to mesenchymal transition (EndMT) and in the left ventricle of patients with ischemic heart disease. Aerrie interacts with YBX1 and is important in DNA damage signaling and repair.

3.2. ASncmtRNA-2

The mitochondrial lncRNA ASncmtRNA-2 is increased in ageing mice aortas and in late passage HUVECs [49]. ASncmtRNA-2 is expressed in ECs like human tonsil ECs and HUVECs [50], and OE leads to an accumulation of HUVECs in the G2/M phase [49]. ASncmtRNA-2 is thought to be stimulated by ROS and a reduction leads to a decrease in TGFβ1 and FN in human mesangial cells [51].

3.3. H19

In the embryonic stage the lncRNA H19 is highly expressed, but shortly after birth its expression decreases, except in skeletal muscle [52–57]. In the aging vasculature, H19 seems to be selectively downregulated in mouse intima compared to the media/adventitia [58]. In skin and human dermal fibroblasts H19 is also downregulated with age [59]. However, in atherosclerosis [54,60], arteriosclerosis obliterans [61], aortic aneurysms [62], CAD, ischemic stroke [63,64], diabetes [65] and calcific aortic valves [66] the expression of H19 is increased again. An increase of H19 has been found in rats after stroke and vascular injury [67–69], as well as in murine abdominal aortic aneurysm models [62], ApoE$^{-/-}$ mice [61] and in the diabetic mouse myocardium [70]. On the other hand, others have found a further repression of H19 in atherosclerotic plaques [58], no change in CAD in the peripheral blood or extracellular vesicles [71,72] or a reduction in ischemic mouse hearts [73,74]. An increased risk for CAD, ischemic stroke and hypertrophic cardiomyopathy has been described for genetic variations in H19 [69,75–77].

H19 is induced upon hypoxia, oxLDL treatment, TNFα stimulation and oxygen-glucose deprivation/reperfusion in ECs, and in the hind

Table 3
lncRNAs associated with CVDs and EC pathway regulation.

	Main ageing-associated CVDs	Regulated pathways in ECs
Aerrie	atherosclerosis, ischemic heart disease	DNA damage
ASncmtRNA-2		senescence
H19	atherosclerosis, AAA, CAD, stroke, diabetes, arterial injury, heart failure	senescence, proliferation, apoptosis, angiogenesis
MALAT1	atherosclerosis, hypertension, diabetes, congenital heart disease	proliferation, apoptosis, migration, angiogenesis, inflammation, autophagy
MEG3	CAD, hypertension, heart failure, hemorrhage, diabetes, metabolic syndrome, preeclampsia, MI	senescence, proliferation, apoptosis, migration, angiogenesis, autophagy, DNA damage
MEG8	ischemic heart disease	senescence, proliferation, apoptosis, angiogenesis
NORAD	atherosclerosis, CAD, MI	senescence, proliferation, apoptosis, migration, inflammation
SNHG12	atherosclerosis	senescence, apoptosis, angiogenesis, DNA damage

AAA: abdominal aortic aneurysms; CAD: coronary artery disease; MI: myocardial infarction.

limb ischemia mouse model [78–81].

Depletion of H19 in HUVECs leads to an accumulation of cells in the G0/G1 phase, increased β-galactosidase activity, increased apoptosis, reduced cell number, reduced sprouting, and increased expression of P16, P21, ICAM1 and VCAM1 [58,61,78]. OE on the other hand leads to increased proliferation, P38, P65 and N-cadherin, and suppressed apoptosis, E-cadherin, P16, P21 and STAT3 activity [58,60,61]. Oppositely, in oxLDL treated HUVECs H19 KD promotes cell viability and decreases apoptosis, ICAM1, VCAM1, IL1β, IL6, TNFα and ROS levels, possibly by interfering with let-7 bioavailability [80]. H19 expression leads to derepression of DICER and HMGA2 in HUVECs [82]. The exosomes of H19 silenced neurons undergoing oxygen-glucose deprivation/reperfusion increase the permeability of an astrocyte-EC co-culture via miR-18 and VEGF signaling [82].

In mice, H19 knockout increases leucocyte infiltration. These effects seem to be the result of increased activation of the IL6/STAT3 pathway [58]. OE of H19 in infarcted myocardium reduces the infarct size and improves the cardiac function. [77], while silencing H19 in aneurysm mouse models decreases aneurysm growth [62]. An extensive overview of H19 functions and mechanisms in cardiovascular disease has been published by Busscher et al. [83].

3.4. MALAT1

The lncRNA Metastasis Associated Lung Adenocarcinoma Transcript 1 (MALAT1) is also known as NEAT2. The expression of MALAT1 is increased in aging human aortic ECs [84], in (pulmonary artery) hypertension [85,86], blood samples of atherosclerotic [87], CAD and coronary heart disease patients [88,89], patients with unstable angina [90] and in diabetic patients and rats [91,92], while it is decreased in atherosclerotic plaques [93,94]. However, no change has been found in the extracellular vesicle MALAT1 expression in CAD patients [72]. Furthermore, genetic variations in the MALAT1 gene are associated with decreased risk for pulmonary arterial hypertension [95, 96], congenital heart disease [97], coronary atherosclerotic disease [98,96] and diabetic rethinopathy [99], but no correlation was found with ischemic stroke [77, 80] or myocardial infarction (MI) [100].

MALAT1 is induced in ECs by high glucose (HG) levels [101–103], oxygen-glucose deprivation (OGD) [104–107], hyperbaric oxygen [108], hypoxia and in the hind limb ischemia mouse model [78,109]. Silencing MALAT1 after OGD suppressed tube formation, proliferation and migration [104] and aggravates cell death and inflammation, middle carotid artery occlusion (MCAO)-induced brain infarction [105] and motor and learning ability in controlled cortical impact [104]. Hetero- and homozygous MALAT1-deficient ApoE$^{-/-}$ mice show increased atherosclerosis and inflammation [93,110]. However, in diabetic rats MALAT1 KD has positive effects and can rescue the pericyte loss, capillary degeneration, vascular leakage and retinal inflammation caused by the hyperglycemia [92].

There are several pathways discovered in which MALAT1 is involved, most of them involving MALAT1 binding to a miRNA. LPS-induced inflammation, ER stress and apoptosis in HUVECs can be reduced by MALAT1 silencing, acting via the miR-150/NF-κB axis [111]. MALAT1 has also shown to bind to miR-214 in HUVECs, thereby increasing X box-binding protein 1 expression [96]. [95]. As a result, proliferation and migration are inhibited. Furthermore, MALAT1 inhibition reduces both apoptosis and proliferation possibly by increasing the expression of hsa-miR-124-3p and hsa-miR-135a-5p and decreasing NR3C2 [86]. Another study shows that the MALAT1/miR-181b/TOX pathway is involved in oxLDL-induced inflammation and oxidative stress in HUVECs [87] and the MALAT1/miR-22-3p/CXCR2 pathway in oxLDL-induced endothelial injury [90]. In retinal microvascular ECs, knocking down MALAT1 leads to inhibition of EndMT, tube formation, proliferation and migration via the miR-205-5p/VEGF-A axis [103]. In this same cell type MALAT1 inhibition reduces permeability, tube formation, proliferation and migration via miR-125b/VE-cadherin/β-catenin [112]. In HUVECs, MALAT1 KD inhibits the inflammatory factors increased by HG by regulating the miR-361-3p/SOCS3 axis [104]. [101]. Furthermore, in EA. hy926 ECs the HG-induced pyroptosis was inhibited by MALAT1 knockdown partly via the miR-22/NLRP3 pathway [102]. In human coronary artery ECs hyperbaric oxygen-induced proliferation and tube formation was attenuated by MALAT1 silencing via the miR-92a/KLF2 pathway [108].

In mouse brain microvascular ECs MALAT1 regulated angiogenesis via the 15-LOX1/STAT3 pathway [106] and OGD/R-induced autophagy and survival in MALAT1-miR-26b-ULK2 regulatory axis [113].

Besides binding to miRNAs, MALAT1 has also been found to bind to different proteins. In mouse brain microvascular ECs and MCAO mouse tissue, MALAT1 was binding to Bim and E-selectin [105]. MALAT1 can also bind to EZH2 in CMVECs and thereby changes expression of EZH2 target genes like DAB2IP and Brachyury and OE of MALAT1 increases permeability [114].

3.5. MEG3

The lncRNA Maternally Expressed 3 (MEG3) has been found upregulated in aged mouse livers and in late passage HUVECs [115,116]. There is also a strong correlation between the expression of MEG3 and age in human cardiac atria samples [117]. Differential MEG3 expression is found in different diseases, like CAD [118]. There is also a strong correlation between the expression of MEG3 and age in human cardiac atria samples [115]. Differential MEG3 expression is found in different diseases, like CAD [119], pulmonary arterial hypertension [120], heart failure [118], intracerebral hemorrhage [121, 122], diabetes [91,123], metabolic syndrome [122] and preeclampsia [124]. MEG3 is shown to be increased in the heart after MI [118] and in the endothelium of obese mice or ApoE$^{-/-}$ mice fed with a high-fat diet [125,126]. The MEG3 increase in the latter was diminished by melatonin treatment [126]. Hypoxia and HG levels in HUVECs also increase MEG3 expression levels [109,127,128], although MEG3 levels were lower in diabetic mice and HG- or H_2O_2-induced retinal ECs [129]. MEG3 plays a role in angiogenesis, proliferation and apoptosis of ECs [115,119,127–133]. Knocking down MEG3 in HUVECs can inhibit VEGF-induced cell viability, migration, tube formation, angiogenesis and expression of CXCR4, SDF-1, pSmad2/3 and VEGFR2 [128,132]. It also reduces phagocytosed platelets and increases ROS production, while OE increases phagocytosed platelets, SOD activity, telomere length and membrane fluidity and reduced ROS production via miR-128 and Girdin [117]. MiR-9 inhibition by MEG3 is partly responsible for reduction of growth and capillary-like formations in HUVECs [133, 134], while miR-21 suppression by MEG3 leads to decreased proliferation and collage expression [118]. [119]. In HG-induced human retinal microvascular ECs MEG3 OE enhances cell viability and decreases apoptosis via the miR-19b/SOCS6-JAK2-STAT3 pathway [131]. In human aortic ECs, MEG3 is enhanced by oxLDL and increases pyroptosis probably via the MEG3/miR-223/NLRP3 axis [126].

Silencing MEG3 in HUVECs increases SA-β-gal activity and superoxide levels and impairs autophagy, mitochondrial structure and function, while MEG3 silencing in obese mice increases glucose intolerance and insulin resistance, which is diminished by knocking out P53 [125]. P53 signaling, DNA damage and apoptosis are induced by silencing of MEG3 and partly regulated by its binding to PTBP3 [132]. [130]. In diabetic mice MEG3 KD aggravates the retinal vascular leakage and increases the expression inflammatory markers, plus the knockdown increases cell viability by increasing PI3K phosphorylation in retinal ECs [129]. Silencing of Meg3 in mice can on the other hand improve perfusion after hind-limb ischemia [115, 117] and cardiac function after a MI [118] or transverse aortic constriction through the miR-361-5p/HDAC9 axis [135]. In Meg3-null embryos the cortical microvessel density is increased together with VEGF signaling pathway genes [134].

3.6. MEG8

The expression of lncRNA MEG8 is upregulated with increased passage number in HUVECs and iPSC derived cardiomyocytes [136]. MEG8 levels are also increased in the left ventricle of patients with ischemic heart disease compared to donors [137]. In addition, it has been found that the mouse homologue Rian is upregulated in aging mouse livers [116].

In HUVECs, MEG8 is induced upon hypoxia [78], and silencing of MEG8 leads to an increase of β-galactosidase and decrease in sprouting, proliferation and barrier function [136,137]. In human hemangioma ECs MEG8 silencing also inhibited proliferation as well as invasion and promoted apoptosis [138].

MEG8 binds to EZH2, a protein which is part of the PRC2 complex and induces H3K27 trimethylation, and thereby affects TFPI2 transcription [137]. This leads to changes in angiogenesis. In addition, MEG8 influences the expression of miRNA-370 and -494 by interacting with CIRPB and HADHB and this leads to changes in the endothelial barrier [136]. The regulation of miR-203 by MEG8 affects JAG1 and Notch1 expression as well as proliferation, invasion and apoptosis [138].

3.7. NORAD

Non-Coding RNA Activated By DNA Damage (NORAD), or linc00657, is a lncRNA which is highly expressed in ECs [109] and increased in aged passage HUVECs (p10) compared to young passage HUVECs (p3) [139]. NORAD expression is also increased in the serum of CAD patients [140], atherosclerosis patients. NORAD expression is also increased in the serum of CAD patients [141], atherosclerosis patients [142], in the pulmonary microvascular ECs of patients with acute lung injury [143], in the aortas of *ApoE*$^{-/-}$ mice fed with a high-fat diet, in the pulmonary microvascular ECs of patients with acute lung injury [140], in the aortas of *ApoE*$^{-/-}$ mice fed with a high-fat diet [141,144] and in the heart of MI mice [143].

NORAD expression in HUVECs is increased after 24h of hypoxia [109,143] and oxLDL treatment [139,141,142,145]. KD of NORAD in hypoxia-induced HUVECs resulted in decreased cell viability, migration and tube formation via miR-590-3p and its targets VEGFA, FGF1/2, while OE had the opposite effect [143]. Silencing of NORAD in oxLDL-treated HUVECs reversed the increase in apoptosis and EC injury marker levels via binding with HDAC6 to the VEGF promoter and regulating VEGF expression [141, 140].

Suppressing oxLDL-induced apoptosis, inflammatory response and EndMT via miR-30c-5p/Wnt7b/β-catenin is another discovered pathway followed by NORAD inhibition [142]. A third involved pathway reduces oxLDL-induced proliferation, migration, wound healing and tube formation after NORAD silencing via miR-590-3p/HIF1α [145]. However, in another study knocking down NORAD aggravated the decrease in cell viability and increase in apoptosis rate, senescence, oxidative stress levels and proinflammatory molecules [139]. The reason for this difference between studies could be that a six-fold higher dose of oxLDL was used in the latter.

LPS treatment *in vivo* in rat lung ECs or *in vitro* in human pulmonary microvascular ECs increased NORAD levels, while silencing NORAD *in vitro* protected the cells against a decrease in cell viability and increase in apoptosis and glucose intake via miR-30c-5p/LDHA axis [143]. LPS treatment *in vivo* in rat lung ECs or *in vitro* in human pulmonary microvascular ECs increased NORAD levels, while silencing NORAD *in vitro* protected the cells against a decrease in cell viability and increase in apoptosis and glucose intake via miR-30c-5p/LDHA axis [140].

In *ApoE*$^{-/-}$ mice silencing NORAD with lentivirus reduced apoptosis and EC injury markers. Additionally, it (partly) reversed the decrease in ejection fraction and fractional shortening and it reversed the increase in wall thickness of the aortic sinus and abdominal aorta, as well as the resistance index, lipid deposition and plaque area [140]. [141]. Another study found similar effects, showing NORAD KD with adeno-associated virus 9 in *ApoE*$^{-/-}$ mice suppresses plaque area, endothelial dysfunction, oxidative stress, inflammation, blood lipid levels, collagen fibers and CD68 levels via miR-495-3p/KLF5 [144]. However, the same study which found opposite effects after oxLDL treatment *in vitro*, found an aggravation of blood lipid levels, proinflammatory molecule expression and plaque area in *ApoE*$^{-/-}$ mice with NORAD KD with adenovirus [139]. This difference could be due to the dissimilarities in cholesterol levels reached in the different studies. In the latter study NORAD knockdown animals showed increased cholesterol levels compared to the control adenovirus injected animals [139], while in the other study the NORAD silenced mice showed decreased cholesterol levels compared to control siRNA treated mice [144].

3.8. SNHG12

The lncRNA small nucleolar host gene-12 (SNHG12) is decreased in expression in the aortic intima from aged C57Bl/6 mice [146]. This lncRNA also shows a reduced expression in human atherosclerotic arteries compared to control arteries which is more apparent in moderate or severe atherosclerosis compared to mild progression [146]. On the other hand, in peripheral artery disease patients with critical limb ischemia SNHG12 levels are increased in the gastrocnemius muscle [147].

In HUVECs the KD of SNHG12 leads to impaired wound healing, migration, proliferation and sprouting, while overexpression has the opposite effect [147]. These effects might take place due to the interaction of SNHG12 with IMP3, YBX1, DHX9, and/or DNA-PK [147].

High-fat diet in mice reduces the expression of Snhg12 in the aorta intima. Silencing Snhg12 induces the atherosclerotic lesion area, whereas the delivery of Snhg12 reduces plaque burden. SHGH12 interacts with DNA-PK mediating its binding to Ku70/80 and SNHG12 KD increases DNA damage and senescence markers both *in vitro* and *in vivo*, which is abrogated by nicotinamide riboside [146]. In addition, KD increases permeability and impaired efferocytosis.

Besides age and high-fat diet, SNHG12 is also downregulated after Ang-II induction in mice and HUVECs. Overexpressing SNHG12 leads to the inhibition of apoptosis and senescence markers *in vitro* and to the reduction of vascular endothelial injury in hypertensive mice [148]. This effect is mediated through the binding of SNHG12 to miR-25-3p, which targets SIRT6.

Furthermore, Snhg2 is also showed a trend towards reduced expression in ECs 4–7 days after femoral artery ligation in C57BL/6 mice, but increased expression after 11 days [147]. Knocking down Snhg12 leads to a slower blood flow recovery and decreased arterial diameter in C57BL/6, BALB/c and *db/db* mice.

However, in MCAO-reperfusion and OGD-exposed mouse brain ECs Snhg12 is increased [107,149]. KD of Snhg12 suppressed cell viability, migration and tube formation in OGD-injured cells, while Sngh12 OE had the opposite effect. Moreover, OE improved neurological function and reduced infarct volume after MCAO [149]. These effects are mediated by the binding of Snhg12 to miR-150, which influences the expression of its target VEGF.

4. Conclusion

Ageing and the associated CVDs are a healthcare burden and this will increase even more in the future [2]. Non-coding RNAs have been identified as key regulators in processes of several CVDs including atherosclerosis [93,144]. Both miRNAs and lncRNAs have been found to influence several pathways that are important for endothelial cell function, like senescence, proliferation, apoptosis, migration, inflammation, angiogenesis, autophagy and DNA damage. The miRNAs and lncRNAs discussed in this review have all been found to be increased or decreased in aged ECs and mutations or differences in expression have been found in different CVDs. Beside the non-coding RNAs, there are a

lot more miRNAs, lncRNAs but also circular RNAs expressed in ECs with a known functional role in cell function which might likewise play a role in endothelial aging [150–152]. Future studies should aim at identifying which non-coding RNAs can be used for diagnostics and/or treatment purposes in atherosclerosis. When studying which non-coding RNAs could be suitable for this, one should keep in mind that ECs have specific gene expression patterns in heterogeneous vascular beds and therefore responses to stimuli or therapies can differ between ECs from different parts of the vascular tree [153]. MiRNAs are well conserved between species, enabling rapid research *in vitro* and various species *in vivo* required to make a translation step. The primary sequences of lncRNAs on the other hand are much less conserved, making rapid translation more troublesome. Moreover, many lncRNAs seem to have evolved in primates only, making those even harder to study using animal models.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: LM is a scientific consultant and adviser for Novo Nordisk (Malov, Denmark), DrugFarm (Shanghai, China), and Angiolutions (Hannover, Germany), and received research funds from Roche Diagnostics (Rotkreuz, Switzerland).

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