Endothelial Cell Aging: How miRNAs Contribute?

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Abstract: Endothelial cells (ECs) form monolayers and line the interior surfaces of blood vessels in the entire body. In most mammalian systems, the capacity of endothelial cells to divide is limited and endothelial cells are prone to be senescent. Aging of ECs and resultant endothelial dysfunction lead to a variety of vascular diseases such as atherosclerosis, diabetes mellitus, hypertension, and ischemic injury. However, the mechanism by which ECs get old and become senescent and the impact of endothelial senescence on the vascular function are not fully understood. Recent research has unveiled the crucial roles of miRNAs, which are small non-coding RNAs, in regulating endothelial cellular functions, including nitric oxide production, vascular inflammation, and anti-thromboformation. In this review, how senescent-related miRNAs are involved in controlling the functions of ECs will be discussed.

Keywords: microRNA; Endothelial cells; Senescence; SIRT1

1. Introduction

Aging is not a disease, but a series of physiological events that are usually inevitable [1,2]. In turn, aging becomes a risk factor for many diseases, such as stroke and heart failure, and accelerates age-related diseases [3]. Senescence is the biological aging of cells and represents the gradual deterioration of cellular function. Senescence of endothelial cells (ECs) impaired vascular functions, leading to aging of tissues and organ [4]. Several stimuli, including reactive oxygen species (ROS) [5,6], high glucose concentration [7], inflammatory cytokines [8], ionizing radiation [9], and telomere dysfunction [10], can induce senescence of ECs. There are several interesting signaling molecules associated with senescence in ECs [11]. For example, molecules contributing energy sensing pathways, mammalian target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK), are related to the process of senescence in ECs. Endothelial mitochondrial oxidative stress is implicated in senescent vascular events and AMPK plays a defensive role in this oxidative stress in aging ECs [12]. However, the exact mechanism by which these stimuli affect the signaling pathway for senescence of ECs has not been fully understood.

One of the major small non-coding RNAs, microRNAs (miRNAs) have been studied with focus on their function as well as their unique expression in tissues and organs for the last two decades [13,14]. Many miRNAs have been evaluated as biomarkers and therapeutic targets for cardiovascular diseases [15,16]. The expression of miR-21 was upregulated in aged hearts and the miR-21-induced fibroblastic phenotype in cardiac fibroblasts [17]. miR-22 enhanced senescence of cardiac fibroblasts, which accelerated cardiac hypertrophy and fibrosis [18]. Senescence of ECs is one of the major risk factors for atherosclerosis and cardiovascular diseases [19]. Recently, several miRNAs, such as miR-217 [20], miR-34a [21], and miR-146 [22], have been identified to control the senescent process of ECs. Therefore, the role of miRNAs in ECs could be more important to regulate the onset
and the process of cardiovascular diseases. We review the concept of vascular aging and senescence of ECs and endothelial miRNAs. Furthermore, the important miRNAs influencing endothelial senescence are lined up and their roles in controlling vascular aging are discussed.

2. Endothelial Cells: The Importance of this Silent Guardian of the Body

The structure and function of endothelial cells (ECs) are quite unique [23]. Every blood and lymphatic capillaries are covered by monolayer ECs, which possess properties of barriers to protect from invasions from the surrounding environment. ECs are spread through the whole body and the endothelium, monolayer ECs, in human can cover more than one thousand square meter of surface area throughout the body [24]. Endothelium separates systemic blood flow from underlying smooth muscle cells, fibroblasts, and pericytes and selectively connects with them by controlling the movement of fluids in blood and activated leukocytes extravasation at the place of inflammation. The function of ECs in each tissue and organ are not consistent. In arteries, the fast blood flow causes shear stress facilitates pro-atherosclerotic phenotypic change of arterial ECs [25]. In contrast, venous blood flow is relatively slow, therefore, ECs in veins are prone to augment procoagulant status [26].

Three major functions were basically classified, metabolic homeostasis, vascular hemodynamic control, and coagulation/trafficking regulation [27]. Vascular systems always need to be maintained by the balance between stimulators and inhibitors of a variety of signaling pathways. For example, pro-angiogenetic factors, such as the vascular endothelial growth factor (VEGF) inducesproliferation, migration, and activation of ECs, and anti-angiogenetic molecules, thrombospondins (TSP1 and TSP2), neutralize these effects when the level of VEGF signaling is overloaded [28]. Most ECs remain quiescent in blood flow and can proliferate in special situations. In vascular development, also called vasculogenesis, angioblast precursors differentiate into ECs and form a vascular network. Angiogenesis occurs from existing blood vessels when they get injured by inflammatory stimuli or are stimulated by tumor progression [29]. Lack of oxygen and nutrients for tumor proliferation stimulates proangiogenic signals, which mostly results in abnormal vasculatures.

3. Endothelial Senescence

Many stimuli, such as oxidative stress and inflammation induce and promote cellular senescence in ECs, which contribute to vascular dysfunctions. Endothelial senescence has been simply characterized by irreversible growth arrest and pro-inflammatory phenotypic change [11]. Classically, senescence has been categorized into two ways; replicative senescence [30]. Normal somatic cells cannot maintain the replicative capacity forever and go into growth arrest [31]. This has been termed by the Hayflick limit and this morphological change by the numerous cellular divisions us called replicative senescence [32,33]. In turn, exposure of oxidative stresses, hydrogen peroxide or hypoxia, induces cellular senescence, which is called stress-induced senescence [34,35]. There are some differences in messages between these two senescence states, even though they have such similar morphological alterations. In case of normal human diploid fibroblasts stimulated with hydrogen peroxide, many gene expressions have been upregulated or downregulated transcriptional factors, including heat shock protein 70 (HSP70), HSP27, cofflin 1 (COF1), and protein disulfide isomerase family A member 3 (PDIA3) [36].

ECs remain quiescent under physiological conditions in vascular systems. Under pathophysiological conditions such as wound healing, vascular inflammation, and tumorigenesis, ECs can replicate and proliferate, although the numbers of replication are limited. Therefore, aged ECs stop growing and senescent ECs impair their function and homeostasis in blood vessels, leading to the disruption of vascular integrity [37]. For example, senescent ECs decrease the ability of nitric oxide (NO) production. NO induces the relaxation of vascular smooth muscle cells, inhibits platelet aggregation, and blocks neutrophils/monocytes adhesion to ECs [38,39]. NO depletion leads to dysfunction of vascular homeostasis and development of hypertension, thrombosis, and atherosclerosis [40,41]. The molecular mechanisms underlying EC senescence has been intensively studied. Telomeres are repetitive DNA
sequences of TTAGGG located on the end of chromosomes to protect DNA from damage. One of the machinery to maintain the telomere length is to use telomerase, a telomeric repeats synthase. A forced increase of telomerase activity by the introduction of human catalytic component of telomerase (hTERT) in cultured ECs promotes cellular transformation [42]. Addition of NO to EC-rescued replicative reduction of telomerase activity [43,44]. Oncogenic proteins such as Ras and p53 are candidate molecules responsible for ECs senescence [45]. Foreman KE et al. identified altered gene expressions in senescent ECs [46]. Plasminogen activator inhibitor-1 (PAI-1) and p21 are increased; conversely, inhibitors of differentiation of DNA binding-1 (Id-1) and Cyclin A/B1 are decreased in senescent ECs [47,48].

4. Endothelial miRNAs

It has been speculated that more than 60% of genes that code for proteins are regulated by miRNAs in mammalian cells [49,50]. Therefore, miRNAs can control many proteins in ECs, leading to alteration of the function of ECs. The biogenesis of mature miRNAs is complex. The primary miRNAs, generated by polymerase II, are cut by two different endonucleases, Drosha and Dicer [51,52]. When Dicer is knocked down, the cells theoretically cannot produce mature miRNAs impairing the function of miRNAs. The experiments of Dicer downregulation in vitro and in vivo have been previously performed. Knockdown of Dicer in ECs suppressed expression of miRNAs that are crucial for biological functions in ECs, such as downregulation of nitric oxide synthase [53]. Moreover, mice with EC-specific deleted Dicer exhibited an impaired postnatal angiogenic response to VEGF [54]. In contrast, the silencing of Drosha inhibited capillary sprouting and tube-forming activity in vitro less than that of Dicer and Drosha knockdown and did not alter migrating activity in ECs [55]. These findings suggested that Dicer and Drosha possess an anti-angiogenic role in ECs, however, Drosha affects EC function less than Dicer. The authors demonstrated that the discrepancy of angiogenic activity in ECs between Dicer and Drosha was due to the difference of a subset of miRNAs processed by these enzymes.

Many studies have investigated the different patterns of miRNA expression by microarray analysis, quantitative RT-PCR, and deep RNA sequencing. In mice, tissue-specific miRNAs have been examined and this study revealed a huge difference of miRNA distribution between organs [56,57]. Tissues or Organs include a variety of cells and there is accumulating data for miRNA distribution in individual cell types. A series of endothelial miRNAs have been identified and described as regulators of important genes. Notably, ECs exist in almost all tissues throughout the body. Therefore, ECs in each tissue were not really of the same phenotype and differ in terms of flow, cell–cell junction, fenestration size, and glycocalyx [58]. Systematic bioinformatics analysis unveiled genes exhibiting an EC-restricted expression pattern. Most of the genes are abundantly expressed in different tissues; eighty-five messenger RNAs (mRNAs) were identified as EC-restricted genes using HUVEC (human umbilical vein EC), HAEC (human aortic EC), HCEC (human coronary EC), HPAEC (human pulmonary artery EC), and HMVEC (human microvascular ECs) [59]. Interestingly, miRNAs that are enriched in 3′ UTR of EC-restricted genes were extracted [59]. These miRNAs have potential to change ECs phenotypes by modulating these EC-restricted genes. McCall MN et al. investigated the profiling of miRNAs from HAEC, HCEC, HPAEC, HUVEC, HMVEC, and HBMVEC (human brain microvascular ECs) [59]. Interestingly, miRNAs that are enriched in 3′ UTR of EC-restricted genes were extracted [59]. These miRNAs have potential to change ECs phenotypes by modulating these EC-restricted genes. McCall MN et al. investigated the profiling of miRNAs from HAEC, HCEC, HPAEC, HUVEC, HMVEC, and HBMVEC (human brain microvascular ECs) and identified several miRNAs, including miR-99b, miR-20b, and let-7b, differed between these ECs [60]. Significant differences between the EC types were also shown by miRNA cluster groups; miR-17 cluster, miR-424 cluster, and miR-512 cluster, suggesting that these miRNA clusters are differentially regulated in each ECs type. For example, the expression of miR-424 cluster in microvascular ECs was higher than that in macrovascular ECs. The different expression of miR-424 in a variety of ECs might reflect the diversity of ECs functions depending on the size of blood vessels.

The methodological development of miRNA detection enabled researchers to detect miRNAs easily in any body fluid and tissue [61,62]. Accumulating evidence indicates that a variety of miRNAs are released from cells and circulate in the blood stream [63]. These cell-free miRNAs usually exist in extracellular vesicles, such as microparticles and exosomes. Some circulating miRNAs are...
incorporated into RNA-binding proteins, high-density lipoproteins (HDLs), nucleophosmin (NPM1), and Argonaute2 proteins (Ago2) [64–66]. Circulating miRNAs are also important biomarkers in patients with cardiovascular diseases [63]. Recently, aging adults have been studied for circulating miRNAs. Hooten NN et al. demonstrated that three serum miRNAs, miR-151a-3p, miR-181a-5p, and miR-1248, were decreased in old human individuals using deep RNA sequencing [67]. Senescent cells are characterized by permanent cell cycle arrest and acquisition of a senescence-associated secretory phenotype (SASP) [68]. This secretory vesicles propagate senescence to the surrounding cells and contribute to the inflammations that aid in aging [69]. Therefore, circulating miRNAs in senescent ECs are recognized as parts of the SASP.

5. Senescent miRNAs in ECs (Figure 1)

Several miRNAs related to the senescent process in ECs were individually discussed. Among them, the expressions of miR-126, miR-17-92, miR-23-27-24, and miR-221-222 are relatively restricted to ECs. Other miRNAs are expressed ubiquitously in many types of cells.

![Figure 1](image)

**Figure 1.** Schematic representation of endothelial miRNAs and their target in senescent ECs. The expression of miR-126, miR-17-92, miR23-27-24, and miR-221-222 are relatively abundant in ECs. In turn, miR-21, miR-181a, miR-146b, and miR-200 family, express ubiquitously in many types of cells, and influence the senescence of ECs. The increases of miR-34a and miR-217 suppress SIRT1 expression, leading to ECs aging.

5.1. SIRT1 and miRNAs—miR-217, miR-34a, and miR-21

Sir2, identified in *S. cerevisiae*, has been recognized as a key regulator of lifespan [70,71]. At least seven human orthologs of Sir2, SIRT1-SIRT7 were discovered and SIRT1 is now the most promising molecule to impact longevity [72]. SIRT1 is a nicotinamide adenine dinucleotide (NAD)-dependent deacetylase, which mainly regulates chromatin remodeling, stress responses, and DNA repair [73]. SIRT1 plays diverse roles in controlling cancer progression by deacetylating p53, forkhead box O1 (FoxO1), and peroxisome proliferating activated receptor gamma coactivator-1 alpha (PGC-1 alpha) [74]. Whether SIRT1 serves as a tumor promoter or suppressor is still controversial, however, the importance of SIRT1 on tumor biology no more remains dubious.

In ECs, SIRT1 is an important molecule to regulate their function. SIRT1 modulates NO synthesis by controlling endothelial NO synthase (eNOS) expression. Calorie restriction induced the expression of SIRT1, which was attenuated in eNOS knockout mice [75]. SIRT1 enhanced NO production by deacetylating eNOS [76]. These suggested that the endothelial SIRT1 maintains vascular homeostasis...
via NO production. Moreover, molecules regulated by SIRT1, such as FoxO1 and KLF2, also play crucial roles in ECs. Acetylation of FoxO1 was increased by loss of SIRT1 negatively controlled angiogenesis [77]. SIRT1 upregulated another transcriptional factor, Kruppel-like factor 2 (KLF2), inducing vasculo-protective gene expressions [78].

Several miRNAs associated with EC senescence were initially identified by Menghini R et al. in 2009 [20]. Comparison between young (population doubling levels; PDLs 8) and old (PDLs 44) HUVEC provided the list of senescence-upregulating miRNAs, including miR-217. The expression of miR-217 increased by treating HUVEC with hydrogen peroxide, which caused senescence of ECs. SIRT1 was identified as a target gene of miR-217. Since SIRT1 modulates deacetylation of FoxO1 and expression of eNOS in ECs, the increase of miR-217 might regulates the dysfunction of senescent ECs through SIRT1-FoxO1-eNOS pathway.

Overexpression of miR-34a downregulates SIRT1 expression [79]. The miR-34 family consists of miR-34a, -34b, and -34c that is located in different genes. Originally, miR-34a was reported as a tumor suppressor miRNA, and many cancers expressed a low level of miR-34a [80,81]. miR-34a attracted a great attention when p53 was proven to directly increase miR-34a. More than 50% of human cancers have p53 mutation, suggesting the importance of miR-34a in tumor progression. In fact, ectopic expression of miR-34a-induced apoptosis in many cancers [82–85]. Simultaneously, the role of SIRT1 in cancer progression has been studied, however, whether SIRT1 acts as a tumor suppressor or tumor progressor has not been elucidated yet [86]. The downstream signaling pathway of SIRT1 in each cancer might be different.

In the cardiovascular system, miR-34a was involved in apoptosis and senescence of vascular cells and heart [87,88]. Direct evidence of miR-34a in heart was provided using miR-34 knockout mice. The expression of miR-34a increased in aged mice hearts. Reduction of miR-34a inhibited myocardial infarction-induced cell death of cardiomyocytes and cardiac function in aged mice [89]. Protein phosphatase 1 regulatory subunit 10 (PPP1R10) was identified as an miR-34a target gene in this report. Delivery of antisense-miR-34a, which was knockdown of miR-34a, also improved cardiac remodeling after cardiac injury via coronary ligation [87]. In this experiment, several potential target genes of miR-34a, Bcl2, Cyclin D1, and SIRT1, were identified. These in vivo studies have clearly indicated the crucial role of miR-34a in acute myocardial infarction.

The role of miR-34a in ECs has been investigated. The expression of miR-34a increased in senescent HUVECs [21]. Overexpression of miR-34a-induced senescent phenotypes and decreased SIRT1 expression in young HUVECs. Delivery of miR-34a in endothelial progenitor cells (EPCs) increased senescence of EPCs and impaired angiogenesis via SIRT1 and FoxO1 pathway [90]. Taken together, the interaction of miR-34a and SIRT1 might be quite critical in regulating senescence in cardiovascular systems [91].

Kallistatin controls a variety of biological functions through two important domains, an active site and a heparin binding site [92]. The active site inhibits tissue kallikrein activity and increases expressions of eNOS and SIRT1. Since tissue kallikrein is one of the serine proteinase inhibitors to cleave low molecular weight kininogen, loss of Kallistatin results in hypertension and renal injury [93]. The heparin-binding site suppresses the signaling of VEGF, tumor necrosis factor alpha (TNF-alpha), and transforming growth factor beta (TGF-beta). Therefore, Kallistatin maintains homeostasis and protects from a series of pathological conditions [94]. Kallistatin exerts pleiotropic impacts on endothelial senescence. Kallistatin inhibited TNF-alpha induced senescence of endothelial progenitor cells by suppressing miR-21 and miR-34a [95]. miR-21 promotes fibroblastic alteration of ECs by TGF-beta, whose process is called ‘endothelial-to-mesenchymal transition (EndMT) [96]. In turn, miR-34a induced cellular senescence described above. In aorta of streptozotocin (STZ)-induced diabetic mice, Kallistatin improved vascular conditions during aging by decreasing superoxide production [95]. Kallistatin decreased the expressions of miR-21 and miR-34a decreased in aorta of this diabetic mice, which restored SIRT1 and eNOS levels. Moreover, the survival of Caenorhabditis elegans treated by
Kallistatin under heat and oxidative stress was significantly elongated. This enhancement of worm longevity might be the consequence of reduced oxidative stress and SIRT1 regulation by miRNAs.

5.2. miR-126

miR-126 is one of the key ‘molecules’ to control endothelial functions. miR-126 has been identified to be the only EC-specific miRNA in vertebrates [97]. miR-126 locates in an intron of EGF-like domain 7 (EGFL7) gene, which is produced and secreted by angiogenic stimuli [98]. Knockout of miR-126 in mice caused developmental defects of vasculature, leading to an increase in embryonic lethality by systemic hemorrhage [99–101]. In miR-126 knockout mice, two angiogenic proteins, Sprouty-related EVH1 domain-containing protein 1 (Sprd1) and a regulatory subunit of PI3K (p85 beta) were upregulated [99]. The survival of miR-126 knockout mice after myocardial infarction was reduced due to the decrease of angiogenic response [100]. In the experiment using zebrafish embryo, KL2F regulated miR-126-modulated vascular endothelial growth factor A (VEGF-A) signaling [102]. Moreover, miR-126 inhibited vascular inflammation through vascular cell adhesion molecule 1 (VCAM1) [97]. These suggested that miR-126 controls the physiological development of the vasculature, maintains homeostasis of cardiovascular system, and protects from vascular inflammation.

In general, miR-126 (known as miR-126-3p and its complement) and miR-126-3p are expressed in ECs. Recently, both miR-126-3p and miR-126-5p have emerged as potential biomarkers for atherosclerosis [103]. The level of miR-126-3p in plasma was downregulated in patients with diabetes mellitus (DM) because the level of endothelial miR-126-3p was decreased [104]. Similarly, plasma miR-126-5p was significantly downregulated in patients with severe coronary artery disease (CAD) [105]. This study has shown that aging, one of the factors associated with cardiovascular disease, was negatively associated with the decrease of plasma miR-126-5p. Another study revealed that the miR-126 (miR-126-3p) levels in circulating blood were positively associated with the age of healthy people, however, miR-126 in patients with type 2 diabetes mellitus (T2DM) did not significantly change with the age [106]. In vitro study showed that the level of miR-126 in HUVEC with high glucose was lower than that in HUVEC with normal glucose. Senescence-dependent increase of miR-126 might be a senescence-associated compensatory mechanism under non-diabetic condition.

5.3. miR-17-92 Cluster

The miR-17-92 cluster encodes six mature miRNAs; miR-17, miR-18a, miR-19a, miR-20a, miR-19b, and miR-92a. These miRNAs are well expressed in ECs and maintain vascular integrity [107]. Overexpression of miR-17, miR-18a, miR-19a, and miR-20b could suppress EC sprouting, and miR-92a-inhibited tube formation of ECs on Matrigel [108]. In case of ECs, the previous studies have mostly demonstrated that miR-17-92 negatively regulates angiogenesis [109]. However, there are controversies about the role of miR-17-92 in pro- or anti-angiogenic functions, since vascular features have not been investigated in miR-17-92 knockout mice yet.

Overexpression of the miR-17-92 cluster inhibited thrombospondin (TSP-1) and connective tissue growth factor (CTGF), suggesting that TSP-1 and CTGF are among of the targets of miR-17-92 cluster [110]. Expression of miRNAs in the miR-17-92 cluster decreased in aging mouse heart [111]. In mice models of aging-associated heart failure, CTGF and TSP-1 were increased, causing heart remodeling; miR-17-92 disrupted oncogenic ras-induced senescence in primary human fibroblasts (B) and WI38 cells) and p21 was a direct target of these miRNAs [112]. In ECs, cytokines promote senescence [113]; treatment of TNF alpha for 15 days altered the phenotype of HMVECs and one of miR-17-92, miR-20b, regulated retinoblastoma-like 1 (RBL1) [114]. Knockdown of miR-20b downregulated a cellular senescence marker, p16, indicating that miR-20b is more powerful in promoting EC senescence.
5.4. miR-23-27-24 Cluster

This unique cluster duplicates in two different locations; chromosome 9q22.32 and chromosome 19q13.13 (miR-23b, -27b, -24-1 and miR-23a, -27a, -24-2, respectively) [115]. The regulation of miR-23 and miR-27 is different from that of miR-24, at least, by special stimuli such as bone morphogenetic protein 2 (BMP2) [116]. Knockdown of miR-23 and miR-27 decreased VEGF induced angiogenesis, while knockdown of miR-24 in ECs increased vascularization and preserved cardiac function after myocardial infarction [117]. These three miRNAs are probably stimulated differently and work independently. Albeit the difference of only one nucleotide in the seed sequence, only miR-23b inhibited angiogenesis while miR-23a did not. Moreover, miR-23a reduced and miR-23b enhanced permeability of ECs. miR-23a and miR-23b target tight junction protein 2 (ZO-2) and junctional adhesion molecule C (JAM-C), respectively [118].

Dellago H et al. demonstrated that 12 miRNAs, including miR-23, miR-27, and miR-24, were upregulated in replicative senescent HUVECs [119]. The level of miR-23a is elevated in CAD patients and miR-23a regulates telomere shortening through telomeric repeat binding factor 2 (TRF2), suggesting that miR-23a might be able to modulate senescence [120]. miR-24 was involved in the process of senescence through targeting p16 [121], however, the role of miR-27 has not been elucidated yet. The impact of individual miRNAs from the miR-23-27-24 cluster on EC senescence should be studied in the future.

5.5. miR-222-221 Cluster

The cluster of miR-222-221 is highly expressed in ECs and has an antiangiogenic activity [54,122]. miR-222-221 inhibited proliferation and migration of ECs and induced apoptosis [123,124]. The expression of miR-222-221 was upregulated in balloon-injured rat carotid arteries [123]. In this model, knockdown of miR-222-221 decreased neointimal formation and increased re-endothelialization. Moreover, miR-222-221 was involved in the protective process against inflammation in ECs [125]. Target genes for miR-222 and miR-221 are quite different even though the seed sequences of both miRNAs are the same. Therefore, the functions of miR-221 and miR-222 often differ. For the function of ECs, miR-221 seems to be more important. By experiments in zebrafish, miR-221 was required for vascular development and linked to the VEGF signaling pathway [126].

The involvement of miR-222-221 in senescence of ECs has not been fully studied yet. Several genes, ETS1, cKIT, and ZEB2, were proven to be the targets of miR-222-221 in ECs [127]. Silencing Dicer increased eNOS expression in ECs and overexpression of miR-222-221 in Dicer knockdown EC-restored eNOS levels [54]. These data suggested that miR-222-221 might contribute to EC senescence.

5.6. miR-200 Family

The five miRNAs of the miR-200 family exit in two clusters. One has miR-200a, miR-200b, and miR-429 and the other has miR-200c and miR-141 in humans. Based on the observation that miR-200a level increased in the corpus cavernosum (CC) of aged rats with erectile dysfunction (ED) compared to young rats with ED, miR-200a in ECs was found to play a key role in the pathogenesis of ED in aged rats [128]. The expression of miR-200a was upregulated in cavernous endothelial cells (CECs) from aged ED rats, which influenced the downregulation of eNOS and cGMP expression. This suggested that miR-200a in senescent ECs was involved in the onset of EDs in the aged rat. Another group demonstrated that miR-200c, as well as miR-200a and miR-200b, has a key role in oxidative stress-induced apoptosis and senescence in HUVECs [129]. Introduction of miR-200c inhibited proliferation and induced senescence in HUVECs. ZEB1 was identified as one of the target genes of miR-200c. Downregulation of ZEB1 by miR-200c enhanced senescence in ECs. Moreover, the involvement of p53 in this pathway was addressed. Activation of p53 by oxidative stress induced miR-200c expression as well as miR-200a and miR-200b [130]. Taken together, the p53–miR-200 axis might regulate senescence of ECs.
5.7. miR-146a

Deng S et al. revealed that miR-146a was upregulated in lineage negative bone marrow cells in aged mice, which were enriched in endothelial progenitor cells (EPCs) [131]. They identified Polo-like kinase 2 (Plk2) as a target gene of miR-146a. Plk2 regulates the duplication of centrosomes and stress response by genotoxic damage [132, 133]. Overexpression of miR-146a enhanced senescence and augmented apoptosis, suggesting that miR-146a improves the capacity of vascular repair in EPCs.

Olivieri F et al. identified miRNAs specific for the senescent phenotype in different cultured ECs, including HUVECs, HAECs, and HCAECs [134]. The number of upregulated miRNAs in these senescent ECs was more than that of downregulated miRNAs. Highly upregulated miRNAs in aged ECs were miR-146a, miR-204, miR-367, and miR-9. The expression of miR-146a increased for 16 h after an hour treatment of hydrogen peroxide [134]. Stimulation of HUVECs with lipopolysaccharide (LPS) promoted the production of miR-146a in replicative senescent HUVECs [135]. Knockdown of miR-146a by antisense of miR-146a enhanced IRAK1 protein expression, the mediator of signaling pathway of inflammation [134]. Since pro-inflammatory conditions, such as chronic heart failure, accelerated senescence of ECs [136], the increase of miR-146a in ECs might represent senescence-associated pro-inflammatory conditions in the vasculature.

Senescence of ECs is highly associated with the increase of oxidant stress [137]. NADPH oxidase (NOX) plays important roles in regulating the production of ROS in ECs. There are many homologues of gp91phox, one of the integral membrane proteins of NOX. Among them, NOX4 is predominant in ECs [138]. The level of NOX4 was reduced by overexpression of miR-146a in HUVECs, leading to EC senescence [139]. Expression of miR-146a was decreased by the replicative senescence of ECs.

5.8. miR-181b

According to the profiling of replicative senescent HUVECs, the miR-181 family is quite important for senescence of ECs [134]. The level of miR-181a increased in aging HUVECs. Hori D et al. demonstrated that the expression of miR-181a in mice aorta did not change with age [140]. However, knockdown of miR-181a/b in EC significantly increased the production of nitric oxide (NO), even though the response of acetylcholine in the aortic ring of miR-181a/b knockout mice were not altered compared to wild-type mice [140]. In turn, miR-181b was one of the mechanosensitive miRNAs and upregulated in human aortic valve endothelial cells (HAVECs) sheared for 24 h under low-magnitude bidirectional shear stress (oscillatory shear stress; OS) [141]. Overexpression of miR-181b inhibited the tissue inhibitor of metalloproteinase 3 (TIMP3) in HAVECs under OS conditions suggesting that miR-181b induces ECM degradation by increased MMP activity. Although the role of miR-181b in ECs has not been studied yet, both miR-181a and miR-181b might contribute to senescence in ECs and protect from aged vascular events, such as atherosclerosis and aortic calcification.

6. Conclusion

Aging signs are a series of multifunctional events accompanied by structural alterations and also is usually associated with cardiovascular diseases. Endothelial senescence is directly connected to physiological longevity and the onset of diseases. EC-derived miRNAs or miRNAs affecting ECs have been studied, however, there are many questions left behind. The difference of miRNA localization or function in arteries and veins has been discussed, however, they have remained obscure. Moreover, the limitations of the in vitro studies come from the complexity of the culture of primary ECs. In vitro primary culture mostly alters the characteristics of endothelial cells. The functions and characters of ECs in culture differ from the tissues or organs where ECs are located. This review demonstrates a variety of miRNAs related to EC senescence. What is the real world of endothelial miRNAs? In theory, miRNAs function as fine tuners or buffers to gene fluctuations. However, there are still possibilities of important but unknown functions of miRNAs in ECs. Further studies are needed to unveil the role of miRNAs in regulating EC senescence.
Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bulterij, S.; Hull, R.S.; Bjork, V.C.; Roy, A.G. It is time to classify biological aging as a disease. *Front. Genet.* 2015, 6, 205. [CrossRef] [PubMed]

2. Gems, D. The aging-disease false dichotomy: Understanding senescence as pathology. *Front. Genet.* 2015, 6, 212. [CrossRef] [PubMed]

3. Niccoli, T.; Partridge, L. Ageing as a risk factor for disease. *Curr. Biol.* 2012, 22, R741–R752. [CrossRef] [PubMed]

4. Regina, C.; Panatta, E.; Candi, E.; Melino, G.; Amelio, I.; Balistreri, C.R.; Annicchiarico-Petruzzelli, M.; Di Daniele, N.; Ruvolo, G. Vascular ageing and endothelial cell senescence: Molecular mechanisms of physiology and diseases. *Mech. Aging Dev.* 2016, 159, 14–21. [CrossRef] [PubMed]

5. Liu, R.; Liu, H.; Ha, Y.; Tilton, R.G.; Zhang, W. Oxidative stress induces endothelial cell senescence via downregulation of Sirt6. *Biomed. Res. Int.* 2014, 2, 902842. [CrossRef] [PubMed]

6. Ota, H.; Eto, M.; Kano, M.R.; Ogawa, S.; Iijima, K.; Akishita, M.; Ouchiet, Y. Cilostazol inhibits oxidative stress-induced premature senescence via upregulation of Sirt1 in human endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 2008, 28, 1634–1639. [CrossRef] [PubMed]

7. Tang, Y.; Xu, J.; Qu, W.; Peng, X.; Xin, P.; Yang, X.; Ying, C.; Sun, X.; Hao, L. Resveratrol reduces vascular cell senescence through attenuation of oxidative stress by SIRT1/NADPH oxidase-dependent mechanisms. *J. Nutr. Biochem.* 2012, 23, 1410–1416. [CrossRef] [PubMed]

8. El Assar, M.; Angulo, J.; Rodriguez-Manas, L. Oxidative stress and vascular inflammation in aging. *Free Radic. Biol. Med.* 2013, 65, 380–401. [CrossRef] [PubMed]

9. Wang, Y.; Boerma, M.; Zhou, D. Ionizing Radiation-Induced Endothelial Cell Senescence and Cardiovascular Diseases. *Radiat. Res.* 2016, 186, 153–161. [CrossRef] [PubMed]

10. Liu, Y.; Bloom, S.I.; Donato, A.J. The Role of Senescence, Telomere Dysfunction and Shelterin in Vascular Aging. *Microcirculation* 2018. Available online: https://www.ncbi.nlm.nih.gov/pubmed/29924435 (accessed on 12 June 2018). [CrossRef] [PubMed]

11. Donato, A.J.; Morgan, R.G.; Walker, A.E.; Lesniewski, L.A. Cellular and molecular biology of aging endothelial cells. *J. Mol. Cell Cardiol.* 2015, 89, 122–135. [CrossRef] [PubMed]

12. Li, C.; Reif, M.M.; Craigie, S.M.; Kant, S.; Keaney, J.F., Jr. Endothelial AMPK activation induces mitochondrial biogenesis and stress adaptation via eNOS-dependent mTORC1 signaling. *Nitric. Oxide.* 2016, 55–56, 45–53. [CrossRef] [PubMed]

13. Ambros, V. The functions of animal microRNAs. *Nature* 2004, 431, 350–355. [CrossRef] [PubMed]

14. Berezikov, E. Evolution of microRNA diversity and regulation in animals. *Nat. Rev. Genet.* 2011, 12, 846–860. [CrossRef] [PubMed]

15. Dangwal, S.; Thum, T. MicroRNA therapeutics in cardiovascular disease models. *Annu. Rev. Pharmacol. Toxicol.* 2014, 54, 185–203. [CrossRef] [PubMed]

16. Condorelli, G.; Latronico, M.V.; Cavarretta, E. MicroRNAs in cardiovascular diseases: current knowledge and the road ahead. *J. Am. Coll. Cardiol.* 2014, 63, 2177–2187. [CrossRef] [PubMed]

17. Thum, T.; Gross, C.; Fiedler, J.; Fischer, T.; Kissler, S.; Bussen, M.; Galuppo, P.; Just, S.; Rottbauer, W.; Frantz, S. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature* 2008, 456, 980–984. [CrossRef] [PubMed]

18. Jazbutyte, V.; Fiedler, J.; Kneitz, S.; Galuppo, P.; Just, A.; Holzmann, A.; Bauersachs, J.; Thum, T. MicroRNA-22 increases senescence and activates cardiac fibroblasts in the aging heart. *Age* 2013, 5, 747–762. [CrossRef] [PubMed]

19. Voghel, G.; Thorin-Trescases, N.; Farhat, N.; Nguyen, A.; Villeneuve, L.; Mamabachi, A.M.; Fortier, A.; Perrault, L.P.; Carrier, M.; Thorin, E. Cellular senescence in endothelial cells from atherosclerotic patients is accelerated by oxidative stress associated with cardiovascular risk factors. *Mech. Ageing Dev.* 2007, 128, 662–671. [CrossRef] [PubMed]

20. Menghini, R.; Casagrande, V.; Cardellini, M.; Martelli, E.; Terrinoni, A.; Amati, F.; Vasa-Nicotera, M.; Ippoliti, A.; Novelli, G.; Melino, G. MicroRNA 217 modulates endothelial cell senescence via silent information regulator 1. *Circulation* 2009, 120, 1524–1532. [CrossRef] [PubMed]
21. Ito, T.; Yagi, S.; Yamakuchi, M. MicroRNA-34a regulation of endothelial senescence. *Biochem. Biophys Res. Commun.* 2010, 398, 735–740. [CrossRef] [PubMed]

22. Cheng, H.S.; Sivachandran, N.; Lau, A.; Boudreau, E.; Zhao, J.L.; Baltimore, D.; Delgado-Olguin, P.; Cybulsky, M.I.; Fish, J.E. MicroRNA-146 represses endothelial activation by inhibiting pro-inflammatory pathways. *EMBO Mol. Med.* 2013, 5, 1017–1034. [CrossRef] [PubMed]

23. Augustin, H.G.; Kozian, D.H.; Johnson, R.C. Differentiation of endothelial cells: Analysis of the constitutive and activated endothelial cell phenotypes. *Bioessays* 1994, 16, 901–906. [CrossRef] [PubMed]

24. Jaffe, E.A. Cell biology of endothelial cells. *Hum. Pathol.* 1987, 18, 234–239. [CrossRef]

25. Zhou, G.; Hamik, A.; Nayak, L.; Tian, H.; Shi, H.; Lu, Y.; Sharma, N.; Liao, X.; Hale, A.; Boerboom, L. Endothelial Kruppel-like factor 4 protects against atherothrombosis in mice. *J. Clin. Invest.* 2012, 122, 4727–4731. [CrossRef] [PubMed]

26. Yau, J.W.; Teoh, H.; Verma, S. Endothelial cell control of thrombosis. *BMC Cardiovasc. Disord.* 2015, 15, 130. [CrossRef] [PubMed]

27. Davidson, S.M. Endothelial mitochondria and heart disease. *Cardiovasc. Res.* 2010, 88, 58–66. [CrossRef] [PubMed]

28. Lawler, P.R.; Lawler, J. Molecular basis for the regulation of angiogenesis by thrombospondin-1 and-2. *Cold Spring Harb. Perspect. Med.* 2012, 2, a006627. [CrossRef] [PubMed]

29. Jain, R.K. Normalizing tumor microenvironment to treat cancer: Bench to bedside to biomarkers. *J. Clin. Oncol.* 2013, 31, 2205–2218. [CrossRef] [PubMed]

30. Kuilman, T.; Michaloglou, C.; Mooi, W.J.; Peeper, D.S. The essence of senescence. *Genes Dev.* 2010, 24, 2463–2479. [CrossRef] [PubMed]

31. Dimri, G.P.; Lee, X.; Basile, G.; Acosta, M.; Scott, G.; Roskelley, C.; Medrano, E.E.; Linskens, M.; Rubelj, I.; Pereira-Smith, O. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc. Natl. Acad. Sci. USA* 1995, 92, 9363–9367. [CrossRef] [PubMed]

32. Hayflick, L.; Moorhead, P.S. The serial cultivation of human diploid cell strains. *Exp. Cell Res.* 1961, 25, 585–621. [CrossRef]

33. Campisi, J. The biology of replicative senescence. *Eur. J. Cancer* 1997, 33, 703–709. [CrossRef]

34. Kong, Y.; Trabucco, S.E.; Zhang, H. Oxidative stress, mitochondrial dysfunction and the mitochondria theory of aging. *Interdiscip. Top. Gerontol.* 2014, 39, 86–107. [PubMed]

35. Barrientos, A. Complementary roles of mitochondrial respiration and ROS signaling on cellular aging and longevity. *Aging (Albany NY)* 2012, 4, 578–579. [CrossRef] [PubMed]

36. Aan, G.J.; Hairi, H.A.; Makpol, S.; Rahman, M.A.; Karsani, S.A. Differences in protein changes between stress-induced premature senescence and replicative senescence states. *Electrophoresis* 2013, 34, 2209–2217. [CrossRef] [PubMed]

37. Seals, D.R.; Jablonski, K.L.; Donato, A.J. Aging and vascular endothelial function in humans. *Clin. Sci.* 2011, 120, 357–375. [CrossRef] [PubMed]

38. Loscalzo, J. Nitric oxide insufficiency, platelet activation, and arterial thrombosis. *Circ. Res.* 2001, 88, 756–762. [CrossRef] [PubMed]

39. Hossain, M.; Qadri, S.M.; Liu, L. Inhibition of nitric oxide synthesis enhances leukocyte rolling and adhesion in human microvasculature. *J. Inflamm.* 2012, 9, 28. [CrossRef] [PubMed]

40. Tsikas, D.; Haufe, S.; Stichtenoth, D.O.; Jordan, J. Nitric oxide and hypertension. *J. Hypertens.* 2012, 30, 625–626. [CrossRef] [PubMed]

41. Walsh, T.; Donnelly, T.; Lyons, D. Impaired endothelial nitric oxide bioavailability: A common link between aging, hypertension, and atherogenesis? *J. Am. Geriatr. Soc.* 2009, 57, 140–145. [CrossRef] [PubMed]

42. Yang, J.; Chang, E.; Cherry, A.M.; Bangs, C.D.; Oei, Y.; Bodnar, A.; Bronstein, A.; Chiu, C.-P.; Scott Herron, G. Human endothelial cell life extension by telomerase expression. *J. Biol. Chem.* 1999, 274, 26141–26148. [CrossRef] [PubMed]

43. Vasa, M.; Breitschopf, K.; Zeiher, A.M.; Dimmeler, S. Nitric oxide activates telomerase and delays endothelial cell senescence. *Circ. Res.* 2000, 87, 540–542. [CrossRef] [PubMed]

44. Rossa, A.; Balsamo, A.; Gambino, R.; Dentelli, P.; Falcioni, R.; Cassader, M.; Pegoraro, L.; Pagano, G.; Brizzi, M.F. p53 Mediates the accelerated onset of senescence of endothelial progenitor cells in diabetes. *J. Biol. Chem.* 2006, 281, 4339–4347. [CrossRef] [PubMed]
45. Bajaj, A.; Zheng, Q.; Adam, A.; Vincent, P.; Pumiglia, K. Activation of endothelial ras signaling bypasses senescence and causes abnormal vascular morphogenesis. Cancer Res. 2010, 70, 3803–3812. [CrossRef] [PubMed]

46. Foreman, K.E.; Tang, J. Molecular mechanisms of replicative senescence in endothelial cells. Exp. Gerontol. 2003, 38, 1251–1257. [CrossRef] [PubMed]

47. Shelton, D.N.; Chang, E.; Whittier, P.S.; Choi, D.; Funk, W.D. Microarray analysis of replicative senescence. Curr. Biol. 1999, 9, 939–945. [CrossRef]

48. Tang, J.; Gordon, G.M.; Nickoloff, B.J.; Foreman, K.E. The helix-loop-helix protein id-1 delays onset of replicative senescence in human endothelial cells. Lab. Investig. 2002, 82, 1073–1079.

49. Friedman, R.C.; Farh, K.K.; Burge, C.B.; Bartel, D.P. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 2009, 19, 92–105. [CrossRef] [PubMed]

50. Bartel, D.P.; Chen, C.Z. Micromanagers of gene expression: The potentially widespread influence of metazoan microRNAs. Nat. Rev. Genet. 2004, 5, 396–400. [CrossRef] [PubMed]

51. Lee, Y.; Ahn, C.; Han, J.; Choi, H.; Yim, J.; Lee, J.; Provost, P.; Rådmark, O.; Kim, S.; et al. The nuclear RNome III Drosha initiates microRNA processing. Nature 2003, 425, 415–419. [CrossRef] [PubMed]

52. Ketting, R.F.; Fischer, S.E.; Bernstein, E.; Sijen, T.; Hannon, G.J.; Plasterk, R.H. Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in C. Elegans. Genes Dev. 2001, 15, 2654–2659. [CrossRef] [PubMed]

53. Suarez, Y.; Fernandez-Hernando, C.; Pober, J.S.; Sessa, W.C. Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. Circ. Res. 2007, 100, 1164–1173. [CrossRef] [PubMed]

54. Suarez, Y.; Fernandez-Hernando, C.; Yu, J.; Gerber, S.A.; Harrison, K.D.; Pober, J.S.; Luisa Iruela-Arispe, M.; Merkenschlager, M.; Sessa, W.C. Dicer-dependent endothelial microRNAs are necessary for postnatal angiogenesis. Proc. Natl. Acad. Sci. USA 2008, 105, 14082–14087. [CrossRef] [PubMed]

55. Kuehbacher, A.; Urbich, C.; Zeiher, A.M.; Dimmeler, S. Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. Circ. Res. 2007, 101, 59–68. [CrossRef] [PubMed]

56. Lagos-Quintana, M.; Rauhut, R.; Yalcin, A.; Meyer, J.; Lendeckel, W.; Tuschl, T. Identification of tissue-specific microRNAs from mouse. Curr. Biol. 2002, 12, 735–739. [CrossRef]

57. Fehlmann, T.; Ludwig, N.; Backes, C.; Meese, E.; Keller, A. Distribution of microRNA biomarker candidates in solid tissues and body fluids. RNA Biol. 2016, 13, 1084–1088. [CrossRef] [PubMed]

58. Aird, W.C. Phenotypic heterogeneity of the endothelium: II. Representative vascular beds. Circ. Res. 2007, 100, 174–190.

59. Bhasin, M.; Yuan, L.; Keskin, D.B.; Out, H.H.; Libermann, T.A.; Oettgen, P. Bioinformatic identification and characterization of human endothelial cell-restricted genes. BMC Genom. 2011, 12, 342. [CrossRef] [PubMed]

60. McCall, M.N.; Kent, O.A.; Yu, J.; Fox-Talbot, K.; Zaiman, A.L.; Halushka, M.K. MicroRNA profiling of diverse endothelial cell types. BMC Med. Genom. 2011, 4, 78. [CrossRef] [PubMed]

61. Rice, J.; Roberts, H.; Burton, J.; Pan, J.; States, V.; Rai, S.N.; Galandiu, S. Assay reproducibility in clinical studies of plasma miRNA. PLoS ONE 2015, 10, e0121948. [CrossRef] [PubMed]

62. Eriksen, A.H.; Andersen, R.F.; Pallisgaard, N.; Sorensen, F.B.; Jakobsen, A.; Hansen, T.F. MicroRNA Expression Profiling to Identify and Validate Reference Genes for the Relative Quantification of microRNA in Rectal Cancer. PLoS ONE 2016, 11, e0150593. [CrossRef] [PubMed]

63. Fichtlscherer, S.; Zeiher, A.M.; Dimmeler, S. Circulating microRNAs: biomarkers or mediators of cardiovascular diseases? Arterioscler. Thromb. Vasc. Biol. 2011, 31, 2383–2390. [CrossRef] [PubMed]

64. Wang, K.; Zhang, S.; Weber, J.; Baxter, D.; Galas, D.J. Export of microRNAs and microRNA-protective protein by mammalian cells. Nucleic Acids Res. 2010, 38, 7248–7259. [CrossRef] [PubMed]

65. Arroyo, J.D.; Chevillet, J.R.; Kroh, E.M.; Ruf, I.K.; Pritchard, C.C.; Gibson, D.F.; Mitchell, P.S.; Bennett, C.F.; Pogosova-Agadjanyan, E.L.; Stirewalt, D.L.; et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc. Natl. Acad. Sci. USA 2011, 108, 5003–5008. [CrossRef] [PubMed]

66. Vickers, K.C.; Palmisano, B.T.; Shocuri, B.M.; Shamburek, R.D.; Remaley, A.T. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat. Cell Biol. 2011, 13, 423–433. [CrossRef] [PubMed]
67. Noren Hooten, N.; Fitzpatrick, M.; Wood, W.H.; De, S.; Ejigu, N.; Zhang, Y.; Mattison, J.A.; Becker, K.G.; Zonderman, A.B.; Evans, M.K. Age-related changes in microRNA levels in serum. *Aging (Albany NY)* 2013, 5, 725–740. [CrossRef] [PubMed]

68. Childs, B.G.; Durik, M.; Baker, D.J.; van Deursen, J.M. Cellular senescence in aging and age-related disease: from mechanisms to therapy. *Nat. Med.* 2015, 21, 1424–1435. [CrossRef] [PubMed]

69. Kennedy, B.K.; Austriaco, N.R., Jr.; Zhang, J.; Guarente, L. Mutation in the silencing gene SIR4 can delay aging in *Saccharomyces cerevisiae*. *Cell* 1995, 80, 485–496. [CrossRef]

70. Tchkonia, T.; Zhu, Y.; van Deursen, J.; Campisi, J.; Kirkland, J.L. Cellular senescence and the senescent secretory phenotype: Therapeutic opportunities. *J. Clin. Investig.* 2013, 123, 966–972. [CrossRef] [PubMed]

71. Imai, S.; Armstrong, C.M.; Kaeberlein, M.; Guarente, L. Transcriptional silencing and longevity protein Sir2 in *Saccharomyces cerevisiae* by two different mechanisms. *Genes Dev.* 1999, 13, 2570–2580. [CrossRef] [PubMed]

72. Kaeberlein, M.; McVey, M.; Guarente, L. The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae*. *Cell* 2007, 129, 473–482. [CrossRef] [PubMed]

73. Imai, S.; Armstrong, C.M.; Kaeberlein, M.; Guarente, L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 2000, 403, 795–800. [CrossRef] [PubMed]

74. Salminen, A.; Kaarniranta, K. SIRT1: Regulation of longevity via autophagy. *Cell Signal.* 2009, 21, 1356–1360. [CrossRef] [PubMed]

75. Nisoli, E.; Tonello, C.; Cardile, A.; Cozzi, V.; Bracale, R.; Tedesco, L.; Falcone, S.; Valerio, A.; Cantoni, O.; Clementi, E.; et al. Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. *Science* 2005, 310, 314–317. [CrossRef] [PubMed]

76. Mattagajasingh, I.; Ki, C.S.; Naqvi, A.; Yamamori, T.; Hoffman, T.A.; Jung, S.B.; DeRicco, J.; Kasuno, K.; Irani, K. SIRT1 promotes endothelial-dependent vascular relaxation by activating endothelial nitric oxide synthase. *Proc. Natl. Acad. Sci. USA* 2007, 104, 14855–14860. [CrossRef] [PubMed]

77. Potente, M.; Ghaemi, L.; Baldessari, D.; Mostoslavsky, R.; Rossig, L.; Dequiedt, F.; Haendeler, J.; Mione, M.; Dejana, E.; Alt, F.W.; et al. SIRT1 controls endothelial angiogenic functions during vascular growth. *Genes Dev.* 2007, 21, 2644–2658. [CrossRef] [PubMed]

78. Gracia-Sancho, J.; Villarreal G., Jr.; Zhang, Y.; Garcia-Cardena, G. Activation of SIRT1 by resveratrol induces KLF2 expression conferring an endothelial vasoprotective phenotype. *Cardiovasc. Res.* 2010, 85, 514–519. [CrossRef] [PubMed]

79. Yamakuchi, M.; Ferlito, M.; Lowenstein, C.J. miR-34a repression of SIRT1 regulates apoptosis. *Proc. Natl. Acad. Sci. USA* 2008, 105, 13421–13426. [CrossRef] [PubMed]

80. Calin, G.A.; Sevignani, C.; Dumitru, C.D.; Hyslop, T.; Noch, E.; Yendamuri, S.; Shimizu, M.; Rattan, S.; Bullrich, F.; Negrini, M.; et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc. Natl. Acad. Sci. USA* 2004, 101, 2999–3004. [CrossRef] [PubMed]

81. Welch, C.; Chen, Y.; Stallings, R.L. MicroRNA-34a functions as a potential tumor suppressor by inducing apoptosis in neuroblastoma cells. *Oncogene* 2007, 26, 5017–5022. [CrossRef] [PubMed]

82. Bommer, G.T.; Gerin, I.; Feng, Y.; Kaczorowski, A.J.; Kuick, R.; Love, R.E. p53-mediated activation of miRNA34 candidate tumor-suppressor genes. *Curr. Biol.* 2007, 17, 1298–1307. [CrossRef] [PubMed]

83. Chang, T.C.; Wentzel, E.A.; Kent, O.A.; Ramachandran, K.; Mullendore, M.; Lee, K.H. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. *Mol. Cell* 2007, 26, 745–752. [CrossRef] [PubMed]

84. He, L.; He, X.; Lim, L.P.; de Stanchina, E.; Xuan, Z.; Liang, Y. A microRNA component of the p53 tumour suppressor network. *Nature* 2007, 447, 1130–1134. [CrossRef] [PubMed]

85. Tarasov, V.; Jung, P.; Verdoold, B.; Lodygin, D.; Epanchintsev, A.; Menssen, A. Differential regulation of microRNAs by p53 revealed by massively parallel sequencing: miR-34a is a p53 target that induces apoptosis and G1-arrest. *Cell Cycle* 2007, 6, 1586–1593. [CrossRef] [PubMed]

86. Lin, Z.; Fang, D. The Roles of SIRT1 in Cancer. *Genes Cancer* 2013, 4, 97–104. [CrossRef] [PubMed]

87. Yang, Y.; Cheng, H.W.; Qiu, Y.; Dupee, D.; Noonan, M.; Lin, Y.D. MicroRNA-34a Plays a Key Role in Cardiac Repair and Regeneration Following Myocardial Infarction. *Circ. Res.* 2015, 117, 450–459. [CrossRef] [PubMed]

88. Li, N.; Wang, K.; Li, P.F. MicroRNA-34 Family and Its Role in Cardiovascular Disease. *Crit. Rev. Eukaryot. Gene Expr.* 2015, 25, 293–297. [CrossRef] [PubMed]
89. Boon, R.A.; Iekushi, K.; Lechner, S.; Seeger, T.; Fischer, A.; Heydt, S. MicroRNA-34a regulates cardiac ageing and function. *Nature* 2013, 495, 107–110. [CrossRef] [PubMed]

90. Zhao, T.; Li, J.; Chen, A.F. MicroRNA-34a induces endothelial progenitor cell senescence and impedes its angiogenesis via suppressing silent information regulator 1. *Am. J. Physiol. Endocrinol. Metab.* 2010, 299, E110–E116. [CrossRef] [PubMed]

91. Yamakuchi, M.; Lowenstein, C.J. MiR-34, SIRT1 and p53: the feedback loop. *Cell Cycle* 2009, 8, 1516–1521. [CrossRef] [PubMed]

92. Chao, J.; Tillman, D.M.; Wang, M.Y.; Margolius, H.S.; Chao, L. Identification of a new tissue-kallikrein-binding protein. *Biochem. J.* 1986, 239, 325–331. [CrossRef] [PubMed]

93. Chao, J.; Shen, B.; Gao, L.; Xia, C.F.; Bledsoe, G.; Chao, L. Tissue kallikrein in cardiovascular, cerebrovascular and renal diseases and skin wound healing. *Biol. Chem.* 2010, 391, 345–355. [CrossRef] [PubMed]

94. Chao, J.; Bledsoe, G.; Chao, L. Protective Role of Kallistatin in Vascular and Organ Injury. *Hypertension* 2016, 68, 533–541. [CrossRef] [PubMed]

95. Guo, Y.; Li, P.; Gao, L.; Zhang, J.; Yang, Z.; Bledsoe, G. Kallistatin reduces vascular senescence and aging by regulating microRNA-34a-SIRT1 pathway. *Aging Cell* 2017, 16, 837–846. [CrossRef] [PubMed]

96. Kuhnert, F.; Mancuso, M.R.; Hampton, J.; Stankunas, K.; Asano, T.; Chen, C.Z. Attribution of vascular phenotypes of the murine Egfl7 locus to the microRNA miR-126. *Development* 2008, 135, 3989–3993. [CrossRef] [PubMed]

97. Nicoli, S.; Standley, C.; Walker, P.; Hurlstone, A.; Fogarty, K.E.; Lawson, N.D. MicroRNA-mediated integration of haemodynamics and Vegf signalling during angiogenesis. *Nature* 2010, 464, 1196–1200. [CrossRef] [PubMed]

98. Schober, A.; Nazari-Jahantigh, M.; Wei, Y.; Bidzhekov, K.; Gremse, F.; Grommes, J. MicroRNA-126-5p promotes endothelial proliferation and limits atherosclerosis by suppressing Dlk1. *Nat. Med.* 2014, 20, 368–376. [CrossRef] [PubMed]

99. Zampetaki, A.; Willeit, P.; Mayr, U.; Prokopi, M. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. *Circ. Res.* 2010, 107, 810–817. [CrossRef] [PubMed]

100. Li, H.Y.; Zhao, X.; Liu, Y.Z.; Meng, Z.; Wang, D.; Yang, F. Plasma MicroRNA-126-5p is Associated with the Complexity and Severity of Coronary Artery Disease in Patients with Stable Angina Pectoris. *Cell Physiol. Biochem.* 2016, 39, 837–846. [CrossRef] [PubMed]

101. Olivieri, F.; Bonafe, M.; Spazzafumo, L.; Gobbi, M.; Pratichizzo, F.; Recchioni, R. Age-and glycemia-related miR-126-3p levels in plasma and endothelial cells. *Aging (Albany NY)* 2014, 6, 771–787. [CrossRef] [PubMed]

102. Kuhnert, F.; Kuo, C.J. miR-17-92 angiogenesis micromanagement. *Blood* 2010, 115, 4631–4633. [CrossRef] [PubMed]

103. Doebel, C.; Bonauer, A.; Fischer, A.; Scholz, A.; Reiss, Y.; Urbich, C. Members of the microRNA-17-92 cluster exhibit a cell-intrinsic antiangiogenic function in endothelial cells. *Blood* 2010, 115, 4944–4950. [CrossRef] [PubMed]

104. Yin, R.; Wang, R.; Guo, L.; Zhang, W.; Lu, Y. MiR-17-3p inhibits angiogenesis by downregulating flk-1 in the cell growth signal pathway. *J. Vasc. Res.* 2013, 50, 157–166. [CrossRef] [PubMed]
110. Olive, V.; Jiang, I.; He, L. mir-17-92, a cluster of miRNAs in the midst of the cancer network. *Int. J. Biochem. Cell Biol.* 2010, 42, 1348–1354. [CrossRef] [PubMed]

111. Van Almen, G.C.; Verhesen, W.; van Leeuwen, R.E.; van de Vrie, M.; Eurlings, C.; Schellings, M.W. MicroRNA-18 and microRNA-19 regulate CTGF and TSP-1 expression in age-related heart failure. *Aging Cell* 2011, 10, 769–779. [CrossRef] [PubMed]

112. Hong, L.; Lai, M.; Chen, M.; Xie, C.; Liao, R.; Kang, Y.J. The miR-17-92 cluster of microRNAs confers tumorigenicity by inhibiting oncogene-induced senescence. *Cancer Res.* 2010, 70, 8547–8557. [CrossRef] [PubMed]

113. Zhang, Y.; Herbert, B.S.; Rajashekhar, G.; Ingram, D.A.; Yoder, M.C.; Clauss, M. Premature senescence of highly proliferative endothelial progenitor cells is induced by tumor necrosis factor-alpha via the p38 mitogen-activated protein kinase pathway. *FASEB J.* 2009, 23, 1358–1365. [CrossRef] [PubMed]

114. Wong, P.F.; Jamal, J.; Tong, K.L.; Khor, E.S.; Yeap, C.E.; Jong, H.L. Deregulation of has-miR-20b expression with erectile dysfunction and could attenuate endothelial function via SIRT1 inhibition. *Asian J. Androl.* 2010, 12, 74–79. [PubMed]

115. Li, J.; Zhao, Y.; Lu, Y.; Ritchie, W.; Grau, G.; Vadas, M. The Poly-cistronic miR-23-27-24 Complexes Target activity of the antiangiogenic homeobox gene GAX/MEOX2 by ZEB2 and microRNA-221. *Dev. Cell* 2012, 30, 208–214. [CrossRef] [PubMed]

116. Sun, F.; Wang, J.; Pan, Q.; Yu, Y.; Zhang, Y.; Wan, Y. Characterization of function and regulation of miR-24-1 and miR-31. *Biochem. Biophys. Res. Commun.* 2009, 380, 660–665. [CrossRef] [PubMed]

117. Li, J.; Zhao, Y.; Lu, Y.; Ritchie, W.; Grau, G.; Vadas, M. The Poly-cistronic miR-23-27-24 Complexes Target activity of the antiangiogenic homeobox gene GAX/MEOX2 by ZEB2 and microRNA-221. *Dev. Cell* 2012, 30, 208–214. [CrossRef] [PubMed]

118. Li, J.; Zhao, Y.; Lu, Y.; Ritchie, W.; Grau, G.; Vadas, M. The Poly-cistronic miR-23-27-24 Complexes Target activity of the antiangiogenic homeobox gene GAX/MEOX2 by ZEB2 and microRNA-221. *Dev. Cell* 2012, 30, 208–214. [CrossRef] [PubMed]

119. Dellago, H.; Preschitz-Kammerhofer, B.; Terlecki-Zaniewicz, L.; Schreiner, C.; Fortscheegger, K.; Chang, M.W. High levels of oncomiR-21 contribute to the senescence-induced growth arrest in normal human cells and its knock-down increases the replicative lifespan. *Aging Cell* 2013, 12, 446–458. [CrossRef] [PubMed]

120. Satoh, M.; Nasu, T.; Takahashi, Y.; Osaki, T.; Hitomi, S.; Morino, Y. Expression of miR-23a induces telomere shortening and is associated with poor clinical outcomes in patients with coronary artery disease. *Clin. Sci.* 2017, 131, 2007–2017. [CrossRef] [PubMed]

121. Lal, A.; Kim, H.H.; Abdelmohsen, K.; Kuwano, Y.; Pullmann, R., Jr; Srikanthan, S. p16(INK4a) translation suppressed by miR-24. *PLoS ONE* 2008, 3, e1864. [CrossRef] [PubMed]

122. Urbich, C.; Kuehbacher, A.; Dimmeler, S. Role of microRNAs in vascular diseases, inflammation, and angiogenesis. *Cardiovasc. Res.* 2008, 79, 581–588. [CrossRef] [PubMed]

123. Liu, X.; Cheng, Y.; Yang, J.; Xu, L.; Zhang, C. Cell-specific effects of miR-221/222 in vessels: Molecular mechanism and therapeutic application. *J. Mol. Cell. Cardiol.* 2012, 52, 245–255. [CrossRef] [PubMed]

124. Xue, Y.; Wei, Z.; Ding, H.; Wang, Q.; Zhou, Z.; Zheng, S. MicroRNA-19b/221/222 induces endothelial cell dysfunction via suppression of PGC-1alpha in the progression of atherosclerosis. *Atherosclerosis* 2015, 241, 671–681. [CrossRef] [PubMed]

125. Liu, C.W.; Sung, H.C.; Lin, S.R.; Wu, C.W.; Lee, C.W.; Lee, I.T. Resveratrol attenuates ICAM-1 expression and angiogenesis. *Cardiovasc. Res.* 2012, 93, 26–33. [CrossRef] [PubMed]

126. Nicoli, S.; Knypsyhausen, C.P.; Zhu, L.J.; Lakshmanan, A.; Lawson, N.D. miR-221 is required for endothelial tip cell behaviors during vascular development. *Dev. Cell* 2012, 22, 418–429. [CrossRef] [PubMed]

127. Chen, Y.; Banda, M.; Speyer, C.L.; Smith, J.S.; Rabson, A.B.; Gorski, D.H. Regulation of the expression and activity of the antiangiogenic homeobox gene GAX/MEOX2 by ZEB2 and microRNA-221. *Mol. Cell. Biol.* 2010, 30, 3902–3913. [CrossRef] [PubMed]

128. Pan, F.; Qiu, X.F.; Yu, W.; Zhang, Q.P.; Chen, Q.; Zhang, C.Y. MicroRNA-200a is up-regulated in aged rats with erectile dysfunction and could attenuate endothelial function via SIRT1 inhibition. *Asian J. Androl.* 2016, 18, 74–79. [PubMed]

129. Magenta, A.; Cencioni, C.; Fasanaro, P.; Zaccagnini, G.; Greco, S.; Sarra-Ferraris, G. miR-200c is upregulated by oxidative stress and induces endothelial cell apoptosis and senescence via ZEB1 inhibition. *Cell Death Differ.* 2011, 18, 1628–1639. [CrossRef] [PubMed]
130. Kim, T.; Veronese, A.; Pichiorri, F.; Lee, T.J.; Jeon, Y.J.; Volinia, S. p53 regulates epithelial-mesenchymal transition through microRNAs targeting ZEB1 and ZEB2. *J. Exp. Med.* 2011, 208, 875–883. [CrossRef] [PubMed]

131. Deng, S.; Wang, H.; Jia, C.; Zhu, S.; Chu, X.; Ma, Q. MicroRNA-146a Induces Lineage-Negative Bone Marrow Cell Apoptosis and Senescence by Targeting Polo-Like Kinase 2 Expression. *Arterioscler. Thromb. Vasc. Biol.* 2017, 37, 280–290. [CrossRef] [PubMed]

132. Haupt, S.; Haupt, Y. Mutant p53 subverts PLK2 function in a novel, reinforced loop of corruption. *Cell Cycle* 2012, 11, 217–218. [CrossRef] [PubMed]

133. Strebhardt, K. Multifaceted polo-like kinases: drug targets and antitargets for cancer therapy. *Nat. Rev. Drug Discov.* 2010, 9, 643–660. [CrossRef] [PubMed]

134. Olivieri, F.; Lazzarini, R.; Recchiioni, R.; Marcheselli, F.; Rippo, M.R.; Di Nuzzo, S. MiR-146a as marker of senescence-associated pro-inflammatory status in cells involved in vascular remodelling. *Age* 2013, 35, 1157–1172. [CrossRef] [PubMed]

135. Olivieri, F.; Lazzarini, R.; Babini, L.; Prattichizzo, F.; Rippo, M.R.; Tiano, L. Anti-inflammatory effect of ubiquinol-10 on young and senescent endothelial cells via miR-146a modulation. *Free Radic. Biol. Med.* 2013, 63, 410–420. [CrossRef] [PubMed]

136. Minamino, T.; Komuro, I. Vascular cell senescence: Contribution to atherosclerosis. *Circ. Res.* 2007, 100, 15–26. [CrossRef] [PubMed]

137. Forstermann, U. Oxidative stress in vascular disease: Causes, defense mechanisms and potential therapies. *Nat. Clin. Pract. Cardiovasc. Med.* 2008, 5, 338–349. [CrossRef] [PubMed]

138. Bedard, K.; Krause, K.H. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol. Rev.* 2007, 87, 245–313. [CrossRef] [PubMed]

139. Vasa-Nicotera, M.; Chen, H.; Tucci, P.; Yang, A.L.; Saintigny, G.; Menghini, R. miR-146a is modulated in human endothelial cell with aging. *Atherosclerosis* 2011, 217, 326–330. [CrossRef] [PubMed]

140. Hori, D.; Dunkerly-Eyring, B.; Nomura, Y.; Biswas, D.; Steppan, J.; Henao-Mejia, J. miR-181b regulates vascular stiffness age dependently in part by regulating TGF-beta signaling. *PLoS ONE* 2017, 12, e0174108. [CrossRef] [PubMed]

141. Heath, J.M.; Fernandez Esmerats, J.; Khambouneheuang, L.; Kumar, S.; Simmons, R.; Jo, H. Mechanosensitive microRNA-181b Regulates Aortic Valve Endothelial Matrix Degradation by Targeting TIMP3. *Cardiovasc. Eng. Technol.* 2018, 9, 141–150. [CrossRef] [PubMed]

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