BioMol Concepts, Vol. 3 (2012), pp. 213–223 • Copyright © by Walter de Gruyter • Berlin • Boston. DOI 10.1515/bmc-2011-0042

Review

Endothelial senescence and microRNA

Munekazu Yamakuchi

Department of Medicine, Aab Cardiovascular Research Institute, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642, USA

e-mail: Munekazu_Yamakuchi@urmc.rochester.edu

Abstract

Cellular senescence occurs when cells lose the ability to divide and proliferate. Endothelial cell senescence is associated with vascular diseases, such as atherosclerosis. In this review, I discuss the factors affecting endothelial cell senescence. Then I describe the role of microRNAs (miRNAs) in endothelial cell senescence. Understanding miRNA pathways in endothelial senescence may lead to new treatments for endothelial dysfunction and atherosclerosis.

Keywords: endothelial cells; microRNA; senescence; SIRT1.

Introduction

Fifty years ago, Hayflick and Moorhead discovered that primary cells cannot proliferate forever *in vitro*. They defined cellular senescence as the catastrophic loss of viability for primary cells at the end of their life span (1). Somatic cells have an intrinsically limited potential to replicate, the Hayflick limit. Why does this happen? One answer is that each mitosis shortens telomeres, and telomere shortening finally stops cell division. A telomere is a repetitive DNA sequence at the end of each chromosome. Telomeres limit deterioration of chromosomes and chromosome fusion. Blackburn and Greider identified a specific telomere terminal transferase activity in Tetrahymena extract, which was named telomerase, and they showed a model that telomere length could be extended in the 1980s (2). This discovery of telomerase has important implications for aging research.

Senescence is the process of aging, and it can be observed in cultured cells. Cellular senescence is the limited capacity of cells to divide in culture. In cultured human fibroblasts, cellular senescence was first observed as a process that stops proliferation, which is called replicative senescence. In contrast, extrinsic stimuli, such as DNA damage and mitogen signals, also cause cellular senescence (premature senescence). Generally, senescent cells have a characteristic enlarged and flattened cell shape (3) and express senescence-associated β galactosidase (SA-β-gal) (4). Senescent cells are resistant to mitogen-induced proliferation, show polyploidy, and altered gene expression (5). The tumor suppressors, p53, p16, and retinoblastoma protein (pRb) are increased by cellular senescence in normal somatic cells (6, 7).

Landmark studies of *Caenorhabditis elegans* and *Drosophila melanogaster* identified highly evolutionally conserved molecules that affect life span, dauer formation-16 (DAF-16) or the *Drosophila* DAF-16 (dFOXO). The insulin/insulin-like growth factor (IGF-1) pathway was the first pathway shown to be involved in aging in animals, and mutation within genes of the insulin/IGF-1 signaling pathway extend life span. In addition, yeast silent information regulator 2 (Sir2) was shown to regulate the aging process, and current aging studies have focused on its mammalian ortholog SIRT1. Aging also has been proposed to result from cumulative reactive oxygen-induced cellular damage (8).

MicroRNAs (miRNAs) are molecules that regulate a variety of physiological and pathological functions in almost all tissues and cells. miRNAs have emerged as important modulators of aging. This short review focuses on endothelial senescence and vascular aging. We summarize several key molecules that regulate endothelial senescence, especially emphasizing the role of miRNAs.

Vascular aging

Healthy blood vessels have normal vascular function, maintaining vascular dilation, limiting thrombosis, and suppressing inflammation. In contrast, aging vessels have abnormal vascular function, with impaired vasodilation, expression of proinflammatory markers, and increased vascular calcification (9, 11). Aging is the major risk factor for atherosclerosis (10). With aging, especially after 40 years, atherosclerosis and systolic hypertension increase. Features of aging arteries include endothelial cell dysfunction, vascular smooth muscle cell (VSMC) proliferation and migration, matrix fragmentation, collagenization, and glycation. Angiotensin II signaling is upregulated in aged arterial walls. Angiotensin II, angiotensin-converting enzyme (ACE), and angiotensin receptor AT1 are markedly increased with aging in arterial walls (12). Moreover, aging promotes the production of several molecules, such as monocyte chemotactic protein-1 (MCP-1), transforming growth factor-β1 (TGF-β1), and matrix metalloproteinase type II (MMP-2) (12). Aging alters the structure and functions of endothelial cells. Endothelial dysfunction in aged arteries and senescent-associated phenotypes in atherosclerotic coronary arteries are observed (13).

Endothelial senescence

Endothelial cells play an essential role in maintaining vascular homeostasis, but endothelial senescence impairs vascular angiogenesis, resulting in vascular dysfunction, which is a step in atherosclerotic plaque development (14, 13). Reduction of endothelial-dependent dilation was observed in aging endothelial cells, and flow-induced nitric oxide (NO)-mediated dilation of coronary arterioles is significantly diminished in aged rats (15, 16). There are multiple factors through which aging contributes to endothelial dysfunction, including continuous vascular stress and damage to endothelial cells (17). When endothelial cells age, a significant decrease in cell size is observed.

There are no specific biomarkers for endothelial aging. As described above, SA-β-gal, a lysosomal hydrolytic enzyme, is the most accepted marker (4, 18). Endothelial cells positive for SA-β-gal were detected in human atherosclerotic plaques because of abundant senescent cells in the plaques (19, 20). Another marker is senescence-associated heterochromatin foci (SAHF) formation (4, 21). This is a chromosomal condensed heterochromatin locus, which is critical for senescence induction. SAHF can inhibit the expression of genes that increase cellular proliferation. In endothelial cells, SAHF formation is not well studied. An inhibitor of cyclin-dependent kinase, p16INK4a is also used as a marker for endothelial cell senescence (22, 23).

Regulators of endothelial senescence

Telomere and telomerase

The telomere hypothesis is one concept that can explain replicative senescence (24). Endothelial cells in culture have a limited capacity to divide and finally reach replicative senescence after shortening of telomeric DNA. Telomeres at the ends of chromosomes contain the repeated DNA sequence (TTAGGG) to protect the end of chromosomes from excessive shortening during DNA replication. Telomeric DNA shortens by 25–100 bp with each round of cell division. Shortening of telomere causes severe DNA damage and stops cell cycle forever (25). Telomere length in endothelial cells of human abdominal aorta shortens with age (26, 27), and critical telomere shortening by endothelial cell division causes endothelial senescence (28). The length of telomeres in endothelial cells implicated in coronary atherosclerosis was shorter than that in normal endothelial cells (29). These observations suggest a strong correlation between telomeres and senescence in endothelial cells *in vitro* and *in vivo*.

Telomerase is a reverse transcriptase that synthesizes telomere DNA. This special enzyme can add telomere repeats to the 3′ end of the chromosomal DNA. More than 90% of human tumors strongly express telomerase (30), and telomere shortening in the absence of telomerase leads to replicative senescence and apoptosis. Telomerase activity in endothelial cells from aorta or umbilical vein is much lower than that in cancer cells, but suppression of telomerase activity in HUVEC by expressing a dominant negative of human telomerase (hTERT) catalytic subunit can reduce replicative capacity (31). Telomerase is associated with the survival and function of endothelial cells, and telomerase activity contributes to angiogenic properties and mitogenic activity in endothelial progenitor cells (EPC) (32, 33). The catalytic subunit of hTERT is a key molecule in the aging process, and PI3K-Akt signaling pathway plays an important role in regulating telomerase activity. Various therapeutic approaches have been tested to delay or stop endothelial cell senescence by controlling telomerase (20, 34, 35). For example, Moretti et al. demonstrated that VEGF increases hTERT expression and telomerase activity, and adenovirus-mediated transfer of hTERT promotes angiogenesis (36). Adenovirus-mediated hTERT transfer in ischemic rats induced the development of new capillaries and decreased apoptosis in vascular cells, suggesting that hTERT contributes to angiogenesis *in vivo*.

SIRT1

Yeast Sir2 family has been identified as regulators of life span as well as budding exhaustion in *Saccharomyces cerevisiae* model (37, 38). The ability of Sir2 to increase longevity was shown to be dependent on deacetylation and activation of DAF-16 and dFoxO proteins in *C. elegans* (39). Later, Sir2 was recognized as a histone deacetylase (40). In mammals, there are seven homologs of Sir2 (SIRT1–7). Among those proteins, SIRT1 is a nicotinamide adenine dinucleotide (NAD)-dependent deacetylase that has potential protective effect on vascular endothelial cells (41). Loss-of-function and gain-of-function of SIRT1 in mice studies demonstrated that SIRT1 plays a key role in the effect of caloric restriction upon metabolism (42–44). Calorie restriction and resveratrol, red wine polyphenol, could promote longevity. In mammals, however, there is no apparent evidence that SIRT1 can directly regulate life span in mammals yet.

SIRT1 controls a variety of transcriptional factor, such as p53, forkhead box O (FoxO), NF-κB, and peroxisome proliferator-activated receptor γ coactivator-1a (PGC-1a) (45–47). SIRT1 inhibits senescence by the deacetylation of FoxO3a, FoxO4, and p53. Insulin and IGF signaling affects life span in mammals (48). The FoxO family is a set of important molecules in insulin/IGF signaling, and FoxO proteins are regulated by posttranslational modifications, such as phosphorylation, acetylation, and ubiquitination. SIRT1 has been identified as a regulator of FoxO acetylation in response to oxidative stress. Sirt1 can regulate heart aging and resistance to oxidative stress through FoxO (49), although modulation of cardiac FoxO function by Sirt1 failed to increase life span (50). FoxO1 and FoxO3a are the most abundant FoxO isoforms in endothelial cells, and overexpression of FoxO1 or FoxO3a inhibits endothelial cell migration and tube formation and represses endothelial nitric oxide synthase (eNOS) expression (51). Akt negatively regulates the life span of endothelial cells, and Akt-induced senescence-like growth arrest in human endothelial cells was prevented by introduction of FoxO3a mutant that was resistant to Akt (52). Moreover, FoxO proteins upregulates antioxidant and

cytoprotective enzymes, such as catalase and MnSOD and induces a metabolic switch. Those evidences suggest that SIRT1 and FoxOs are crucial regulators of vascular homeostasis, which can affect cellular senescence.

Reactive oxygen species (ROS)

Cellular senescence is linked to ROS. ROS can damage DNA, leading to rapid-onset senescence (premature senescence), which is not completely related to telomere shortening. Harman proposed that free radicals are linked to aging, and subsequent studies have supported this idea (53). Alterations in intracellular ROS or changes in oxygen tension or expression of antioxidants can change senescence in culture cells. Also, radicals mediate oncogene induction of senescence in some cells. Limited evidence suggests that free radicals are also linked to aging of organisms. As atherosclerosis is accompanied by increased vascular oxidant stress, it is plausible that senescence plays a role in atherosclerosis (54–58).

Endothelial cells produce ROS, such as superoxide and hydrogen peroxide. ROS mediate a variety of biological responses as signaling molecules, which causes endothelial cell dysfunction. The major source of ROS in endothelial cells is NADPH oxidase (NOX). NOX is activated by cytokines, growth factors, hypoxia, and shear stress. NOX is composed of two groups of subunits: the catalytic components Nox1, Nox4, gp91phox; and the modulating components p22phox, p47phox, p67phox. Overexpression of NOX4 accelerates senescence in fibroblast (59). In endothelial cells, senescence is induced by oxidative stress. The expression of NOX p47phox is increased with aging in healthy men without changing the levels of the oxidant enzyme xanthine oxidase and antioxidant enzymes (60).

A balance of ROS-generating enzyme and antioxidant enzymes is important to regulate intracellular ROS levels. There are a variety of antioxidant enzymes including superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), heme oxygenase (HO), and thioredoxin-1 (Trx-1) system. The Trx-1 system has two oxidoreductases, thioredoxin reductase and Trx-1. Trx-1 is expressed in endothelial cells, and exerts antiapoptotic functions by scavenging intracellular hydrogen peroxide and by reducing sulfhydryl groups on proteins that have been oxidized. Trx-1 has been implicated in the regulation of senescence. Trx-1 and Trx reductase are decreased with age in rat kidney, but calorie restriction prevents these decreases (61). Trx-1 protein levels are also reduced in aged endothelial cells (62). Mitsui et al. has shown that transgenic mice overexpressing human Trx exhibited extended median and maximum life spans compared to wild-type mice (63).

Reducing oxidative stress prevents endothelial dysfunction with aging in human. Aging decreases peripheral conduit artery flow-mediated dilation, and ascorbic acid intravenous infusion restores this effect (64). Decreased endothelial cell-dependent vessel dilation is thought to contribute to age-associated development of cardiovascular disease. This endothelial cell senescence is associated with increased endothelial oxidative stress by upregulating NOX (60). These data suggest that ROS play a role in endothelial aging and dysfunction in humans.

Nitric oxide (NO)

NO is a free radical that regulates various functions in vascular cells. NO plays critical roles in regulation of endothelial function, vascular tone, and heart development (65–67). There are three isoforms of NO synthases (NOS) that catalyze the synthesis of NO; neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). In general, vascular endothelial cells express eNOS and, when inflamed, express iNOS. Endothelial cells relax the underlying VSMCs by releasing NO (67). This release of NO from endothelial cells is reduced in pathological conditions, such as diabetes or hypertension. Endothelium-dependent vasodilation and the basal release of NO are reduced during aging, as activity of eNOS is suppressed in senescent endothelial cells (68). Shear stress increases eNOS expression in young endothelial cells but not in aged endothelial cells (69). Akt protein level is reduced in senescent endothelial cells, causing low level of eNOS phosphorylation by Akt. *In vivo*, eNOS expression is downregulated in aorta of aged rats (70). Interestingly, NO has been shown to counteract senescence in vascular endothelial cells (69, 71, 72). One possible explanation for this is that NO can stimulate telomerase activity, which delays endothelial cell senescence (72). Ota et al. demonstrated that phosphodiesterase 3 (PDE3) inhibitor, cilostazol, promoted SIRT1 expression by increasing NO production in endothelial cells. This is another reason for the prevention of senescence by NO (73). Moreover, SIRT1 has been shown to deacetylate eNOS, which leads to more NO production and endothelial-dependent vasodilation (74). As SIRT1 is a positive regulator of cellular senescence, the function of SIRT1 to protect vascular aging may partially depend on the increase of NO by deacetylated eNOS.

Vascular miRNAs and endothelial senescence

What are miRNAs?

miRNAs are small noncoding RNAs that regulate gene expression by inhibiting translation or stability of their target mRNAs (75). miRNAs control a variety of cellular functions, such as proliferation, differentiation, apoptosis, and senescence (76–78). The biogenesis of miRNA is well studied. miRNA encoding genes are transcribed into primary transcript (pri-miRNA) by polymerase II and processed to smaller stem-loop structural miRNA precursor (pre-miRNA) by the microprocessor complex composed of RNase III enzyme, Drosha and diGeorge syndrome critical region gene 8 (DGCR8). Then, the pre-miRNA is exported to the cytoplasm by exportin-5 and diced into duplex mature miRNA by another RNase III, Dicer. This single-strand mature miRNA is incorporated into a special miRNA-protein complex (RISC), which binds to the 3′UTR of mRNAs when they have complementary binding sites to miRNA, leading either to translational suppression or degradation of target mRNAs. Therefore, two RNase III enzymes, Dicer and Drosha, are key enzymes to regulate miRNA biogenesis.

Some miRNAs are expressed in certain tissues or cell types (79). For example, miR-1 and miR-133 are considered to be muscle specific (80). Some miRNAs are highly expressed in endothelial cells (endothelial miRNAs) that modulate endothelial functions. We performed miRNA profiling in human aortic endothelial cells and found that miR-21, miR-126, miR-29 family, and let-7 family are relatively abundant in endothelial cells (Table 1). Although miR-21 and miR-29 are family-expressed ubiquitously, miR-126 is an endothelial-specific miRNA. Knockdown of miR-126 in zebrafish resulted in loss of vascular integrity and hemorrhage during embryonic development (81). Targeted deletion of miR-126 in mice causes leaky vessels and hemorrhaging because of a loss of vascular integrity and defects in endothelial cell proliferation, migration, and angiogenesis (82). Moreover, knockdown of miR-126 increased leukocyte adherence to endothelial cells by modulating vascular cell adhesion molecule 1 (VCAM-1) expression, suggesting that miR-126 controls vascular inflammation as well (83).

Drosha and Dicer affect senescence

How do Dicer and Drosha affect senescence? Global repression of miRNA maturation by knockdown of Dicer or Drosha or DGCR8 in mouse lung adenocarcinoma has been shown to promote cellular growth, transformation, and tumorigenesis (84). For primary cultured cells, a Dicer genetic knockout caused a rapid onset of cell senescence in mouse embryonic fibroblasts (MEFs) *in vitro* and *in vivo* (85). Dicer ablation increased the levels of p19Arf, p53, and phospho-p53. Those results suggested that loss of miRNA biogenesis induce premature cell senescence via p19Arf-p53 pathway. What is the role of Dicer and Drosha in cellular senescence? Srikantan et al. performed the experiments to touch this question. The expressions of Drosha and Dicer in senescent fibroblast cells are lower than those in young cells, indicating that aging makes less miRNA and more mRNA. Downregulation of Dicer or Drosha lowered miRNA levels, however, and decreased global translation (86). Dicer knockdown, but not Drosha, induced senescent cells. Those results suggest that the effect of Dicer and Drosha on senescence is partially independent of miRNA biogenesis, and those enzymes may have other functions on the regulation of senescence. Endothelial cell-specific knockout of Dicer and Drosha significantly reduced capillary sprouting and tube-forming activity and silencing of Dicer, not Drosha, reducing angiogenesis *in vivo* (87, 88). Those findings suggested that miRNAs play important roles in maintaining function in endothelial cells, but the role of Dicer or Drosha in endothelial cell senescence is still unknown.

Table 1 miRNA expression profiles in human primary endothelial cells.

HAEC		HUVEC	
1. miR-21	11. miR-30b	1. miR-21	11. miR-23a
2. miR-126	12. miR-34a	2. miR-126	12. miR-27a
3. miR-29a	13. miR-19b	3. miR-29a	13. miR-100
4. let-7a	14. miR-10b	4. miR-29b	14. miR-16
5. miR-29b	15. miR-23a	5. miR-424	15. let-7f
6. let-7g	16. miR-424	6. miR-217	16. miR-10a
7. miR-221	17. miR-16	7. miR-34a	17. miR-30a-5p
8. miR-15b	18. miR-216	8. miR-125b	18. miR-23a
9. miR-125b	19. miR-217	9. let-7a	19. miR-25
10. let-7f	20. miR-29c	10. let-7i	20. miR-15b

Total RNAs were extracted from HAECs and HUVECs and fractionated by size exclusion column chromatography and gel extraction. Small RNA (15–30 nt) were cloned into cloning vectors and sequenced. The number of miRNA was counted and ordered from the highest number of miRNAs. Data represent the top 20 miRNAs that are highly expressed in HAEC and HUVEC.

Longevity and miRNAs – profiling studies

Although several miRNAs have been identified as senescent-associated miRNAs, the relationship between aging and individual miRNAs is not well understood. The miRNA lin-4 and its target lin-14 have been shown to control the timing of larval development and life span in *C. elegans* through the insulin/IGF-1 pathway (89). The mammalian lin-4 miRNA homologs, the miR-125 family, might be able to regulate life span process, although it is not proved yet. Especially in cancer, a variety of functions of miRNAs have been discovered. A set of miRNAs, such as miR-20a and miR-34a, induce growth arrest and senescence by modulating p53 and Rb in cancer (90, 91). Those miRNAs are called senescence-associated miRNAs (SA-miRNAs) and play an important role in tumor suppression (92). There are more than 10 SA-miRNAs reported by now (Table 2). They listed possible target genes for senescence; however, the real targets of SA-miRNAs are still unclear because each miRNA has many potential target mRNAs. Lafferty-Whyte demonstrated several major pathways regulated by the SA-miRNAs. Those pathways are involved in (1) cytoskeletal remodeling process, (2) cell cycle, (3) regulation of transcription in the CREB pathway, and (4) TGF and WNT signaling (92).

For human primary culture cells, profiling of senescent miRNAs was performed. Maes et al. demonstrated the miRNA profile data of various conditions of WI-38 fibroblasts (93). They compared miRNAs of (1) young replicating fibroblasts, (2) fibroblasts in reversible growth arrest state, (3) fibroblasts in replicative senescence, and (4) fibroblasts in hydrogen peroxide-induced premature senescence. Their results represented that senescence increases miR-34a, miR-624, miR-638, and miR-377 and decreases miR-365 and miR-512-5p. Bonifacio et al. performed miRNA profiling of replicative senescent fibroblasts to find the effects of telomerase activity on miRNAs (94). More than 80 miRNAs, including miR-34a, miR-146a, miR-424-503 polycistron, miR-450, miR-542-3p, and miR-542-5p are significantly upregulated in senescent human foreskin (BJ) cells. They also compared miRNAs between early passage and senescent BJ cells and early and late passage immortalized fibroblasts (BJ-hTERT) that stably express the human telomerase reverse transcriptase subunit hTERT and discovered that a set of miRNAs, such as miR-21 and miR-19a, are possibly regulated by hTERT.

Table 2 miRNAs associated with endothelial senescence.

miRNA	Function	Target genes	References
miR-424	Cell cycle	MEK1, cyclin E1	Nakashima et al. (126)
miR-17	Cell cycle	p21/CDKN1A	Hackl et al. (127)
miR-20a	Cell cycle	p21/CDKN1A	Hackl et al. (127)
miR-106a	Cell cycle	p21/CDKN1A	Hackl et al. (127)
miR-34a	Cellular stress	SIRT1	Ito et al.; Zhao et al. (99, 100)
miR-217	Cellular stress	SIRT1	Menghini et al. (96)
miR-146a	Proliferation	NOX4	Vasa-Nicotera et al. (108)
miR-200c	Proliferation	ZEB1	Magenta et al. (105)

The miRNAs that regulate endothelial senescence and their potential target genes.

Overexpression of one senescence-upregulated miRNA, miR-143, induced growth arrest in young BJ cells but not in BJ-hTERT cells. Shin et al. identified SA-miRNAs in normal human keratinocytes (NHKs) (95). Most of altered miRNAs (>90%) were upregulated by replicative senescence, and they showed that ectopic expression of two SA-miRNAs, miR-137 or miR-668, induced senescence of NHKs.

miRNA profiling has been performed using senescent primary endothelial cells. According to the miRNA profiling data, several endothelial miRNAs including miR-17-5p, miR-21, miR-216, miR-217, miR-31b, and miR-181a/b were increased in human endothelial cells during aging (96). In contrast, some endothelial miRNAs, such as miR-146a, were downregulated in aging HUVEC. Among endothelial senescent-associated miRNAs, miR-217 expression was highly upregulated in aging HUVEC cells. Overexpression of miR-217 increases cellular senescence in endothelial cells, and SIRT1 is identified as a target of miR-217. Moreover, miR-217 is negatively correlated with SIRT1 expression in human atherosclerotic plaque, probably suggesting that miR-217 expression contributes to senescence in the development of atherosclerosis.

TERT1 and miRNAs

Bonifacio suggested that several miRNAs are controlled by TERT1 during aging (94). In cancer cells, the expressions of several miRNAs are altered by shorter telomeres (97). Human brain neuroepithelioma cell line, SK-N-MC with shortening telomeres, represents cellular senescence and increase miR-199a, miR-181a, miR-181b, miR-148a, and miR-143.

Do miRNAs regulate telomerase? miR-138 downregulates hTERT protein levels in thyroid carcinoma cells, and the expression of miR-138 is negatively correlated to hTERT expression, suggesting that miR-138 partially contributes to the development of thyroid carcinoma (98). As the expression of telomerase in endothelial cells is quite low, it would be interesting to find if knockdown of any endothelial miRNAs increases hTERT levels.

SIRT1 and miRNAs

As described above, miR-217 controls SIRT1 levels and induces senescence in endothelial cells. Many miRNAs have been identified to target SIRT1. miR-34a is the first miRNA found to regulate SIRT1. We have shown that miR-34a induces senescence and cell cycle arrest in endothelial cells. Overexpression of miR-34a inhibits SIRT1 expression, which is a key molecule for senescence in endothelial cells (99). miR-34a expressions in heart and spleen are higher in older mice than those in younger mice. Conversely, SIRT1 expression decreased with age. Our data suggested that miR-34a is one regulator of endothelial senescence. miR-34a also inhibits EPC-mediated angiogenesis by induction of senescence (100). In cardiomyocyte, miR-195 and miR-199a regulates SIRT1 expression (101, 102). miR-132 controls SIRT1 in adipose tissue (103). These miRNAs are also expressed in endothelial cells and affect endothelial cellular function, suggesting that they might play a role in endothelial senescence.

ROS and miRNAs

miRNAs modulate endothelial cell response to oxidative stress. miR-200 family is induced by hydrogen peroxide in endothelial cells (104). Hydrogen peroxide causes rapid pRb dephosphorylation by the activation of protein phosphatase 2A and upregulation of p53, followed by CDK inhibitor p21. Overexpression of miR-200c inhibits endothelial cell proliferation by increasing apoptosis and senescence through inhibition of Zinc finger E-box binding homeobox 1 protein (ZEB1 protein) expression. An increase in miR-200 family is observed in hindlimb ischemia model mice. Although the induction of miR-200c by a mouse hindlimb ischemia was attenuated in p66ShcA$^{-/-}$ mice, knockdown of miR-200 family partially rescues this effect of oxidative stress, suggesting miR-200 family is not sufficient to regulate endothelial senescence. Yang et al. provided that Trx1 upregulates miR-98, a member of the let-7 family in cardiomyocytes. Knockdown of miR-98 augmented angiotensin II-induced cardiac hypertrophy by modulating the expression of cyclin D2 (106). miR-98 inhibits the p53 pathway in lung adenocarcinoma epithelial cell line, suggesting that Trx1 might regulate senescence partially by miR-98 and p53 pathway in endothelial cells (105).

Dicer knockdown reduces ROS production by some stimuli, such as tumor necrosis factor-α (TNF-α) in human microvascular endothelial cells (107). Shilo et al. demonstrated one possible mechanism that the decrease of miRNA content upregulates transcription factor HBP-1, which downregulates

expression of p47phox protein, causing the ROS level to lower. As the expression of NOX4 is much higher than that of other NOX family in endothelial cells, the role of endothelial NOX4 is important to control ROS generation. In kidney, miR-25 is identified as a regulator of NOX4, a major catalytic subunit of NOX under hyperglycemia. In endothelial cells, miR-146a regulates NOX4 expression (108). The expression of miR-146a is decreasing in aging, and knockdown of miR-146a in HUVEC increased the number of senescent cells, providing one possible mechanism for the decrease in NOX-4-derived ROS production during endothelial senescence. Another group has shown that phorbol 12-myristate 13-acetate (PMA) induces miR-146a expression in human microvascular endothelial cells (109). PMA enhances angiogenesis through the activation of protein kinase C (PKC) in cultured endothelial cells and also upregulates NOX4 expression and ROS production in HUVEC-derived EA.hy 926 cells (110). These data suggest that miR-146a is one of the major regulators of ROS in endothelial cells and contribute to endothelial senescence.

eNOS and miRNA

NO alters the expression of miRNAs. NO induces the miR-200 family (miR-200a, miR-200b, miR-200c, and miR-429) in mouse ES cells (mES). miR-200 family targets Smad-interacting protein-1 (Sip1/ZEB2). Sip1/ZEB2 is a transcriptional repressor of TERT and controls early differentiation of mES cells. Therefore, NO affects differentiation of mES cells through the miR-200 family (111).

As vascular dysfunction with decreased NO availability is a hallmark of aging arteries, it is possible that miRNA regulates eNOS. Zhang et al. proposed that 27 nt small RNA that forms the intron 4 of eNOS suppresses eNOS expression by inhibiting eNOS promoter activity (112). What about miRNAs that might target eNOS? There is no convincing evidence of direct regulation of eNOS by specific miRNAs. However, eNOS is indirectly regulated by some miRNAs. Dicer knockdown increased activation of eNOS expression and activity in endothelial cells. The suppression of eNOS by Dicer knockdown is partially restored by overexpression of miR-221 and miR-222, suggesting that miR-221 and miR-222 indirectly control eNOS protein levels (113). miR-217 modulates eNOS expression by suppressing SIRT1 as shown above. miR-92a also regulates eNOS expression and activity. The transcription factor Kruppel-like factor 2 (KLF2) is a critical factor of endothelial cell development and vascular functions and is induced by shear stress (114). Shear stress downregulates miR-92a in endothelial cells, which increases KLF2 expression and enhancing eNOS levels and activity. Bonauer et al. provided the supporting data that miR-92a overexpression downregulates eNOS expression probably by inhibiting its target integrin subunits α5 (ITGA5) in endothelial cells (115). Shear stress increases miR-21 levels and inhibits its target phosphatase and tensin homolog (PTEN) in endothelial cells (116). Therefore, overexpression of miR-21 enhances Akt signaling, followed by increasing eNOS phosphorylation and NO production without changing eNOS expression.

p53 and miRNAs

How do miRNAs associated with aging regulate senescence? What are target genes of aging-associated miRNAs? We hypothesized that p53, tumor suppressor, is one possible candidate regulator for senescence-induced miR-34a. Activated p53 halts cell cycle progression, induces apoptosis, and accelerates cellular senescence in cancers. p53 functions not in cancer cells but in endothelial cells. The expression of p53 increases in endothelial cells of atherosclerotic lesions (117, 118). This elevation of p53 might limit neointimal thickening in atherosclerosis and angioplasty (119, 120). Knockdown of p53 pathway can cause senescence (121). Ectopic expression of p53 induces a senescent-like phenotype (122). We have shown that p53 induced miR-34a, and then, miR-34a increased senescence in endothelial cells, suggesting that endothelial cell senescence by p53 might be partially mediated by miR-34a, although more direct evidences are required.

Perspective

Manipulating miRNAs is a feasible therapeutic approach for human diseases (123). In mice, injection of antagomir, which is designed to knockdown a target miRNA, alters their phenotype. Systemic administration of antagomiR-92a enhanced blood vessel growth in mouse hindlimb ischemia model (115). In the same model, injection of antagomiR-126 reduced angiogenic response (124).

Can therapeutic miRNA modulate senescence? Many potential miRNAs can regulate endothelial senescence (Figure 1). Is it possible to slow the rate of aging by knocking down certain miRNAs? One challenge is that each miRNA has hundreds of target proteins, which makes a complicated miRNA-protein and miRNA-miRNA network. For example, p53 upregulates miR-34a expression, and miR-34a controls at least 100 gene expressions, including SIRT1. SIRT1 deacetylates and inactivates p53. When SIRT1 is downregulated by miR-34a, increased acetylated p53 makes more

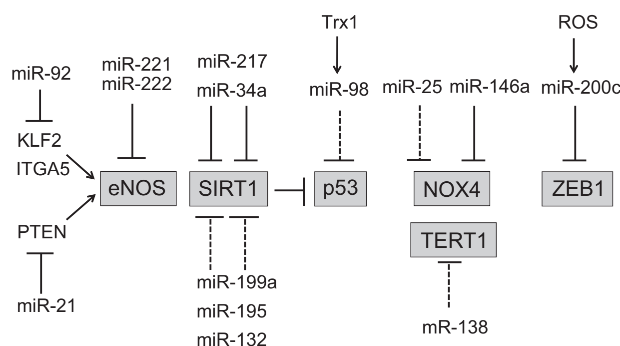


Figure 1 Regulation of endothelial senescence by miRNAs.
The major molecules in endothelial senescence are regulated by a variety of miRNAs. Dashed lines indicate miRNAs reported in non-endothelial cells.

miR-34a. This is one network of miRNA-p53 positive feedback model.

There are more questions that remain. How could we measure the stage of aging in endothelial cells? As endothelial cells are located at the interface between the blood and the vessel wall, a set of parameters in the blood, including NO, high-sensitivity C-reactive protein, asymmetric dimethylarginine (ADMA) has been used for biomarkers of endothelial cell function. miRNAs circulating in the blood are not only fascinating tools to diagnose diseases but are also therapeutic targets. miR-126 levels in blood were inversely correlated with age, heart failure, and type-2 diabetes (125). Little is known about circulating miRNAs in aging, and further research will define useful biomarkers for endothelial cell senescence.

Expert opinion

Sir William Osler once pointed out that a man is as old as his arteries. Since then, many aging studies have focused on the vasculature. Vascular endothelial cells play a pivotal role in the control of vascular homeostasis. With aging, the ability of endothelial cells to maintain vascular function and integrity is weakened, causing vascular inflammation, stiffness, and atherosclerosis. The biological mechanisms that underlie endothelial dysfunction during aging remain unknown. Recent remarkable progresses in studying miRNAs in the vasculature may help us to understand senescence in endothelial cells and may help us to develop novel therapies for aging.

Outlook

Which molecules alter miRNAs levels in endothelial cells? In cancer cells, p53 strongly increased the expression level of miR-34 family by modulating transcription or miRNA processing. This discovery revealed the further function of miR-34a and new p53 complicated networks in cancer. In the near future, the key molecules that control senescent-associated miRNAs in endothelial cell may be discovered, and the manipulation of miRNAs by these factors will attenuate age-related endothelial dysfunctions.

Highlights

- Cellular senescence is the limited capacity of cells to divide.
- A variety of factors, such as ROS and telomerase modulate cellular senescence.
- Endothelial senescence is associated with cardiovascular disease.
- SIRT1, p53, and eNOS may play critical roles in endothelial senescence.
- miRNAs that control endothelial senescence may play a key role in atherosclerosis or other diseases.
- miRNAs regulate cellular functions by modulating mRNA stability.
- A set of miRNAs including miR-34a and miR-217 controls endothelial senescence.

Acknowledgments

I thank Dr. Charles J. Lowenstein for helpful advice and discussions. This work was supported by Scientist Development Grant 835446N from the American Heart Association.

References

1. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res 1961; 25: 585–621.
2. Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell 1985; 43: 405–13.
3. Wagner M, Hampel B, Bernhard D, Hala M, Zwerschke W, Jansen-Durr P. Replicative senescence of human endothelial cells in vitro involves G1 arrest, polyploidization and senescence-associated apoptosis. Exp Gerontol 2001; 36: 1327–47.
4. Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, 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: 9363–7.
5. Yang D, McCrann DJ, Nguyen H, St Hilaire C, DePinho RA, Jones MR, Ravid K. Increased polyploidy in aortic vascular smooth muscle cells during aging is marked by cellular senescence. Aging Cell 2007; 6: 257–60.
6. Beausejour CM, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P, Campisi J. Reversal of human cellular senescence: roles of the p53 and p16 pathways. EMBO J 2003; 22: 4212–22.
7. Campisi J. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. Cell 2005; 120: 513–22.
8. Ashok BT, Ali R. The aging paradox: free radical theory of aging. Exp Gerontol 1999; 34: 293–303.
9. Kovacic JC, Moreno P, Nabel EG, Hachinski V, Fuster V. Cellular senescence, vascular disease, and aging: part 2 of a 2-part review: clinical vascular disease in the elderly. Circulation 2011; 123: 1900–10.
10. Virmani R, Avolio AP, Mergner WJ, Robinowitz M, Herderick EE, Cornhill JF, Guo SY, Liu TH, Ou DY, O'Rourke M. Effect of aging on aortic morphology in populations with high and low prevalence of hypertension and atherosclerosis. Comparison between occidental and Chinese communities. Am J Pathol 1991; 139: 1119–29.
11. Wang M, Zhang J, Jiang LQ, Spinetti G, Pintus G, Monticone R, Kolodgie FD, Virmani R, Lakatta EG. Proinflammatory profile within the grossly normal aged human aortic wall. Hypertension 2007; 50: 219–27.
12. Wang M, Monticone RE, Lakatta EG. Arterial aging: a journey into subclinical arterial disease. Curr Opin Nephrol Hypertens 2010; 19: 201–7.
13. Minamino T, Komuro I. Vascular aging: insights from studies on cellular senescence, stem cell aging, and progeroid syndromes. Nat Clin Pract Cardiovasc Med 2008; 5: 637–48.
14. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 2000; 101: 1899–906.

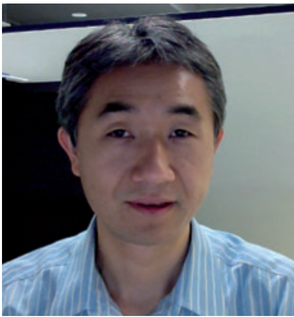
15. Csiszar A, Ungvari Z, Edwards JG, Kaminski P, Wolin MS, Koller A, Kaley G. Aging-induced phenotypic changes and oxidative stress impair coronary arteriolar function. Circ Res 2002; 90: 1159–66.
16. Gendron ME, Thorin-Trescases N, Villeneuve L, Thorin E. Aging associated with mild dyslipidemia reveals that COX-2 preserves dilation despite endothelial dysfunction. Am J Physiol Heart Circ Physiol 2007; 292: H451–8.
17. Schwartz SM, Gajdusek CM, Reidy MA, Selden SC 3rd, Haudenschild CC. Maintenance of integrity in aortic endothelium. Fed Proc 1980; 39: 2618–25.
18. Kurz DJ, Decary S, Hong Y, Erusalimsky JD. Senescence-associated (beta)-galactosidase reflects an increase in lysosomal mass during replicative ageing of human endothelial cells. J Cell Sci 2000; 113: 3613–22.
19. Vasile E, Tomita Y, Brown LF, Kocher O, Dvorak HF. Differential expression of thymosin beta-10 by early passage and senescent vascular endothelium is modulated by VPF/VEGF: evidence for senescent endothelial cells in vivo at sites of atherosclerosis. FASEB J 2001; 15: 458–66.
20. Minamino T, Miyauchi H, Yoshida T, Ishida Y, Yoshida H, Komuro I. Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction. Circulation 2002; 105: 1541–4.
21. Adams PD. Remodeling of chromatin structure in senescent cells and its potential impact on tumor suppression and aging. Gene 2007; 397: 84–93.
22. Finkel T, Serrano M, Blasco MA. The common biology of cancer and ageing. Nature 2007; 448: 767–74.
23. Yang DG, Liu L, Zheng XY. Cyclin-dependent kinase inhibitor p16(INK4a) and telomerase may co-modulate endothelial progenitor cells senescence. Ageing Res Rev 2008; 7: 137–46.
24. Greider CW. Telomeres and senescence: the history, the experiment, the future. Curr Biol 1998; 8: R178–81.
25. von Zglinicki T, Saretzki G, Ladhoff J, d'Adda di Fagagna F, Jackson SP. Human cell senescence as a DNA damage response. Mech Ageing Dev 2005; 126: 111–7.
26. Chang E, Harley CB. Telomere length and replicative aging in human vascular tissues. Proc Natl Acad Sci USA 1995; 92: 11190–4.
27. Aviv H, Khan MY, Skurnick J, Okuda K, Kimura M, Gardner J, Priolo L, Aviv A. Age dependent aneuploidy and telomere length of the human vascular endothelium. Atherosclerosis 2001; 159: 281–7.
28. Jakob S, Haendeler J. Molecular mechanisms involved in endothelial cell aging: role of telomerase reverse transcriptase. Z Gerontol Geriatr 2007; 40: 334–8.
29. Ogami M, Ikura Y, Ohsawa M, Matsuo T, Kayo S, Yoshimi N, Hai E, Shirai N, Ehara S, Komatsu R, Naruko T, Ueda M. Telomere shortening in human coronary artery diseases. Arterioscler Thromb Vasc Biol 2004; 24: 546–50.
30. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. Eur J Cancer 1997; 33: 787–91.
31. Erusalimsky JD, Skene C. Mechanisms of endothelial senescence. Exp Physiol 2009; 94: 299–304.
32. Murasawa S, Llevadot J, Silver M, Isner JM, Losordo DW, Asahara T. Constitutive human telomerase reverse transcriptase expression enhances regenerative properties of endothelial progenitor cells. Circulation 2002; 106: 1133–9.
33. Yang J, Nagavarapu U, Relloma K, Sjaastad MD, Moss WC, Passaniti A, Herron GS. Telomerized human microvasculature is functional in vivo. Nat Biotechnol 2001; 19: 219–24.
34. Bode-Boger SM, Martens-Lobenhoffer J, Tager M, Schroder H, Scalera F. Aspirin reduces endothelial cell senescence. Biochem Biophys Res Commun 2005; 334: 1226–32.
35. Haendeler J, Hoffmann J, Diehl JF, Vasa M, Spyridopoulos I, Zeiher AM, Dimmeler S. Antioxidants inhibit nuclear export of telomerase reverse transcriptase and delay replicative senescence of endothelial cells. Circ Res 2004; 94: 768–75.
36. Zaccagnini G, Gaetano C, Pietra LD, Nanni S, Grasselli A, Mangoni A, Benvenuto R, Fabrizi M, Truffa S, Germani A, Moretti F, Pontecorvi A, Sacchi A, Bacchetti S, Capogrossi MC, Farsetti A. Telomerase mediates vascular endothelial growth factor-dependent responsiveness in a rat model of hind limb ischemia. J Biol Chem 2005; 280: 14790–8.
37. Kennedy BK, Austriaco NR Jr, Zhang J, Guarente L. Mutation in the silencing gene SIR4 can delay aging in S. cerevisiae. Cell 1995; 80: 485–96.
38. Kaeberlein M, McVey M, Guarente L. The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. Genes Dev 1999; 13: 2570–80.
39. Guarente L, Picard F. Calorie restriction – the SIR2 connection. Cell 2005; 120: 473–82.
40. Imai S, Armstrong CM, Kaeberlein M, Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 2000; 403: 795–800.
41. Potente M, Dimmeler S. Emerging roles of SIRT1 in vascular endothelial homeostasis. Cell Cycle 2008; 7: 2117–22.
42. Haigis MC, Guarente LP. Mammalian sirtuins – emerging roles in physiology, aging, and calorie restriction. Genes Dev 2006; 20: 2913–21.
43. Banks AS, Kon N, Knight C, Matsumoto M, Gutierrez-Juarez R, Rossetti L, Gu W, Accili D. SirT1 gain of function increases energy efficiency and prevents diabetes in mice. Cell Metab 2008; 8: 333–41.
44. Feige JN, Lagouge M, Canto C, Strehle A, Houten SM, Milne JC, Lambert PD, Mataki C, Elliott PJ, Auwerx J. Specific SIRT1 activation mimics low energy levels and protects against diet-induced metabolic disorders by enhancing fat oxidation. Cell Metab 2008; 8: 347–58.
45. Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, Mayo MW. Modulation of NF-κB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J 2004; 23: 2369–80.
46. Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, Tran H, Ross SE, Mostoslavsky R, Cohen HY, Hu LS, Cheng HL, Jedrychowski MP, Gygi SP, Sinclair DA, Alt FW, Greenberg ME. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science 2004; 303: 2011–5.
47. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1α and SIRT1. Nature 2005; 434: 113–8.
48. Katic M, Kahn CR. The role of insulin and IGF-1 signaling in longevity. Cell Mol Life Sci 2005; 62: 320–43.
49. Alcendor RR, Gao S, Zhai P, Zablocki D, Holle E, Yu X, Tian B, Wagner T, Vatner SF, Sadoshima J. Sirt1 regulates aging and resistance to oxidative stress in the heart. Circ Res 2007; 100: 1512–21.
50. Sedding D, Haendeler J. Do we age on Sirt1 expression? Circ Res 2007; 100: 1396–8.
51. Potente M, Urbich C, Sasaki K, Hofmann WK, Heeschen C, Aicher A, Kollipara R, DePinho RA, Zeiher AM, Dimmeler S. Involvement of Foxo transcription factors in angiogenesis and postnatal neovascularization. J Clin Invest 2005; 115: 2382–92.

52. Miyauchi H, Minamino T, Tateno K, Kunieda T, Toko H, Komuro I. Akt negatively regulates the in vitro lifespan of human endothelial cells via a p53/p21-dependent pathway. EMBO J 2004; 23: 212–20.
53. Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol 1956; 11: 298–300.
54. Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. Arterioscler Thromb Vasc Biol 2005; 25: 29–38.
55. Labinskyy N, Csiszar A, Veress G, Stef G, Pacher P, Oroszi G, Wu J, Ungvari Z. Vascular dysfunction in aging: potential effects of resveratrol, an anti-inflammatory phytoestrogen. Curr Med Chem 2006; 13: 989–96.
56. Lakatta EG. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part III: cellular and molecular clues to heart and arterial aging. Circulation 2003; 107: 490–7.
57. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part II: the aging heart in health: links to heart disease. Circulation 2003; 107: 346–54.
58. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease. Circulation 2003; 107: 139–46.
59. Geiszt M, Kopp JB, Varnai P, Leto TL. Identification of renox, an NAD(P)H oxidase in kidney. Proc Natl Acad Sci USA 2000; 97: 8010–4.
60. Donato AJ, Eskurza I, Silver AE, Levy AS, Pierce GL, Gates PE, Seals DR. Direct evidence of endothelial oxidative stress with aging in humans: relation to impaired endothelium-dependent dilation and upregulation of nuclear factor-kappaB. Circ Res 2007; 100: 1659–66.
61. Cho CG, Kim HJ, Chung SW, Jung KJ, Shim KH, Yu BP, Yodoi J, Chung HY. Modulation of glutathione and thioredoxin systems by calorie restriction during the aging process. Exp Gerontol 2003; 38: 539–48.
62. Altschmied J, Haendeler J. Thioredoxin-1 and endothelial cell aging: role in cardiovascular diseases. Antioxid Redox Signal 2009; 11: 1733–40.
63. Mitsui A, Hamuro J, Nakamura H, Kondo N, Hirabayashi Y, Ishizaki-Koizumi S, Hirakawa T, Inoue T, Yodoi J. Overexpression of human thioredoxin in transgenic mice controls oxidative stress and life span. Antioxid Redox Signal 2002; 4: 693–6.
64. Eskurza I, Monahan KD, Robinson JA, Seals DR. Effect of acute and chronic ascorbic acid on flow-mediated dilatation with sedentary and physically active human ageing. J Physiol 2004; 556: 315–24.
65. Derakhshan B, Hao G, Gross SS. Balancing reactivity against selectivity: the evolution of protein S-nitrosylation as an effector of cell signaling by nitric oxide. Cardiovasc Res 2007; 75: 210–9.
66. Ignarro LJ. Nitric oxide: a unique endogenous signaling molecule in vascular biology. Biosci Rep 1999; 19: 51–71.
67. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987; 327: 524–6.
68. Gerhard M, Roddy MA, Creager SJ, Creager MA. Aging progressively impairs endothelium-dependent vasodilation in forearm resistance vessels of humans. Hypertension 1996; 27: 849–53.
69. Hoffmann J, Haendeler J, Aicher A, Rossig L, Vasa M, Zeiher AM, Dimmeler S. Aging enhances the sensitivity of endothelial cells toward apoptotic stimuli: important role of nitric oxide. Circ Res 2001; 89: 709–15.
70. Chou TC, Yen MH, Li CY, Ding YA. Alterations of nitric oxide synthase expression with aging and hypertension in rats. Hypertension 1998; 31: 643–8.
71. Hayashi T, Matsui-Hirai H, Miyazaki-Akita A, Fukatsu A, Funami J, Ding QF, Kamalanathan S, Hattori Y, Ignarro LJ, Iguchi A. Endothelial cellular senescence is inhibited by nitric oxide: implications in atherosclerosis associated with menopause and diabetes. Proc Natl Acad Sci USA 2006; 103: 17018–23.
72. Vasa M, Breitschopf K, Zeiher AM, Dimmeler S. Nitric oxide activates telomerase and delays endothelial cell senescence. Circ Res 2000; 87: 540–2.
73. Ota H, Eto M, Kano MR, Ogawa S, Iijima K, Akishita M, Ouchi Y. Cilostazol inhibits oxidative stress-induced premature senescence via upregulation of Sirt1 in human endothelial cells. Arterioscler Thromb Vasc Biol 2008; 28: 1634–9.
74. Mattagajasingh I, Kim CS, Naqvi A, Yamamori T, Hoffman TA, Jung SB, DeRicco J, Kasuno K, Irani K. SIRT1 promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase. Proc Natl Acad Sci USA 2007; 104: 14855–60.
75. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281–97.
76. Ambros V. The functions of animal microRNAs. Nature 2004; 431: 350–5.
77. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet 2004; 5: 522–31.
78. Croce CM. Oncogenes and cancer. N Engl J Med 2008; 358: 502–11.
79. Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T. Identification of tissue-specific microRNAs from mouse. Curr Biol 2002; 12: 735–9.
80. Sempere LF, Freemantle S, Pitha-Rowe I, Moss E, Dmitrovsky E, Ambros V. Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. Genome Biol 2004; 5: R13.
81. Fish JE, Santoro MM, Morton SU, Yu S, Yeh RF, Wythe JD, Ivey KN, Bruneau BG, Stainier DY, Srivastava D. miR-126 regulates angiogenic signaling and vascular integrity. Dev Cell 2008; 15: 272–84.
82. Wang S, Aurora AB, Johnson BA, Qi X, McAnally J, Hill JA, Richardson JA, Bassel-Duby R, Olson EN. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev Cell 2008; 15: 261–71.
83. Harris TA, Yamakuchi M, Ferlito M, Mendell JT, Lowenstein CJ. MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. Proc Natl Acad Sci USA 2008; 105: 1516–21.
84. Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T. Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nat Genet 2007; 39: 673–7.
85. Mudhasani R, Zhu Z, Hutvagner G, Eischen CM, Lyle S, Hall LL, Lawrence JB, Imbalzano AN, Jones SN. Loss of miRNA biogenesis induces p19Arf-p53 signaling and senescence in primary cells. J Cell Biol 2008; 181: 1055–63.
86. Srikantan S, Marasa BS, Becker KG, Gorospe M, Abdelmohsen K. Paradoxical microRNAs: individual gene repressors, global translation enhancers. Cell Cycle 2011; 10: 751–9.
87. Kuehbacher A, Urbich C, Zeiher AM, Dimmeler S. Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. Circ Res 2007; 101: 59–68.

88. Suarez Y, Fernandez-Hernando C, Yu J, Gerber SA, Harrison KD, Pober JS, Iruela-Arispe ML, Merkenschlager M, Sessa WC. Dicer-dependent endothelial microRNAs are necessary for postnatal angiogenesis. Proc Natl Acad Sci USA 2008; 105: 14082–7.
89. Boehm M, Slack F. A developmental timing microRNA and its target regulate life span in C. elegans. Science 2005; 310: 1954–7.
90. Tazawa H, Tsuchiya N, Izumiya M, Nakagama H. Tumor-suppressive miR-34a induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells. Proc Natl Acad Sci USA 2007; 104: 15472–7.
91. Poliseno L, Pitto L, Simili M, Mariani L, Riccardi L, Ciucci A, Rizzo M, Evangelista M, Mercatanti A, Pandolfi PP, Rainaldi G. The proto-oncogene LRF is under post-transcriptional control of MiR-20a: implications for senescence. PLoS One 2008; 3: e2542.
92. Lafferty-Whyte K, Cairney CJ, Jamieson NB, Oien KA, Keith WN. Pathway analysis of senescence-associated miRNA targets reveals common processes to different senescence induction mechanisms. Biochim Biophys Acta 2009; 1792: 341–52.
93. Maes OC, Sarojini H, Wang E. Stepwise up-regulation of microRNA expression levels from replicating to reversible and irreversible growth arrest states in WI-38 human fibroblasts. J Cell Physiol 2009; 221: 109–19.
94. Bonifacio LN, Jarstfer MB. MiRNA profile associated with replicative senescence, extended cell culture, and ectopic telomerase expression in human foreskin fibroblasts. PLoS One 2010; 5: e12519.
95. Shin KH, Pucar A, Kim RH, Bae SD, Chen W, Kang MK, Park NH. Identification of senescence-inducing microRNAs in normal human keratinocytes. Int J Oncol 2011; 39: 1205–11.
96. Menghini R, Casagrande V, Cardellini M, Martelli E, Terrinoni A, Amati F, Vasa-Nicotera M, Ippoliti A, Novelli G, Melino G, Lauro R, Federici M. MicroRNA 217 modulates endothelial cell senescence via silent information regulator 1. Circulation 2009; 120: 1524–32.
97. Uziel O, Beery E, Dronichev V, Samocha K, Gryaznov S, Weiss L, Slavin S, Kushnir M, Nordenberg Y, Rabinowitz C, Rinkevich B, Zehavi T, Lahav M. Telomere shortening sensitizes cancer cells to selected cytotoxic agents: in vitro and in vivo studies and putative mechanisms. PloS One 2010; 5: e9132.
98. Mitomo S, Maesawa C, Ogasawara S, Iwaya T, Shibazaki M, Yashima-Abo A, Kotani K, Oikawa H, Sakurai E, Izutsu N, Kato K, Komatsu H, Ikeda K, Wakabayashi G, Masuda T. Downregulation of miR-138 is associated with overexpression of human telomerase reverse transcriptase protein in human anaplastic thyroid carcinoma cell lines. Cancer Sci 2008; 99: 280–6.
99. Ito T, Yagi S, Yamakuchi M. MicroRNA-34a regulation of endothelial senescence. Biochem Biophys Res Commun 2010; 398: 735–40.
100. Zhao T, Li J, Chen AF. MicroRNA-34a induces endothelial progenitor cell senescence and impedes its angiogenesis via suppressing silent information regulator 1. Am J Physiol Endocrinol Metab 2010; 299: E110–6.
101. Zhu H, Yang Y, Wang Y, Li J, Schiller PW, Peng T. MicroRNA-195 promotes palmitate-induced apoptosis in cardiomyocytes by down-regulating Sirt1. Cardiovasc Res 2011; 92: 75–84.
102. Rane S, He M, Sayed D, Vashistha H, Malhotra A, Sadoshima J, Vatner DE, Vatner SF, Abdellatif M. Downregulation of miR-199a derepresses hypoxia-inducible factor-1α and Sirtuin 1 and recapitulates hypoxia preconditioning in cardiac myocytes. Circ Res 2009; 104: 879–86.
103. Strum JC, Johnson JH, Ward J, Xie H, Feild J, Hester A, Alford A, Waters KM. MicroRNA 132 regulates nutritional stress-induced chemokine production through repression of SirT1. Mol Endocrinol 2009; 23: 1876–84.
104. Magenta A, Cencioni C, Fasanaro P, Zaccagnini G, Greco S, Sarra-Ferraris G, Antonini A, Martelli F, Capogrossi MC. miR-200c is upregulated by oxidative stress and induces endothelial cell apoptosis and senescence via ZEB1 inhibition. Cell Death Differ 2011; 18: 1628–39.
105. Zhang S, Zhang C, Li Y, Wang P, Yue Z, Xie S. miR-98 regulates cisplatin-induced A549 cell death by inhibiting TP53 pathway. Biomed Pharmacother 2011; 65: 436–42.
106. Yang Y, Ago T, Zhai P, Abdellatif M, Sadoshima J. Thioredoxin 1 negatively regulates angiotensin II-induced cardiac hypertrophy through upregulation of miR-98/let-7. Circ Res 2011; 108: 305–13.
107. Shilo S, Roy S, Khanna S, Sen CK. Evidence for the involvement of miRNA in redox regulated angiogenic response of human microvascular endothelial cells. Arterioscler Thromb Vasc Biol 2008; 28: 471–7.
108. Vasa-Nicotera M, Chen H, Tucci P, Yang AL, Saintigny G, Menghini R, Mahè C, Agostini M, Knight RA, Melino G, Federici M. miR-146a is modulated in human endothelial cell with aging. Atherosclerosis 2011; 217: 326–30.
109. Hsieh CH, Rau CS, Jeng SF, Lin CJ, Chen YC, Wu CJ, Lu TH, Lu CH, Chang WN. Identification of the potential target genes of microRNA-146a induced by PMA treatment in human microvascular endothelial cells. Exp Cell Res 2010; 316: 1119–26.
110. Xu H, Goettsch C, Xia N, Horke S, Morawietz H, Forstermann U, Li H. Differential roles of PKCalpha and PKCepsilon in controlling the gene expression of Nox4 in human endothelial cells. Free Radic Biol Med 2008; 44: 1656–67.
111. Rosati J, Spallotta F, Nanni S, Grasselli A, Antonini A, Vincenti S, Presutti C, Colussi C, D'Angelo C, Biroccio A, Farsetti A, Capogrossi MC, Illi B, Gaetano C. Smad-interacting protein-1 and microRNA 200 family define a nitric oxide-dependent molecular circuitry involved in embryonic stem cell mesendoderm differentiation. Arterioscler Thromb Vasc Biol 2011; 31: 898–907.
112. Zhang MX, Ou H, Shen YH, Wang J, Coselli J, Wang XL. Regulation of endothelial nitric oxide synthase by small RNA. Proc Natl Acad Sci USA 2005; 102: 16967–72.
113. Suarez Y, Fernandez-Hernando C, Pober JS, Sessa WC. Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. Circ Res 2007; 100: 1164–73.
114. Wu W, Xiao H, Laguna-Fernandez A, Villarreal G Jr, Wang KC, Geary GG, Zhang Y, Wang WC, Huang HD, Zhou J, Li YS, Chien S, Garcia-Cardena G, Shyy JYJ. Flow-dependent regulation of Kruppel-like factor 2 is mediated by microRNA-92a. Circulation 2011; 124: 633–41.
115. Bonauer A, Carmona G, Iwasaki M, Mione M, Koyanagi M, Fischer A, Burchfield J, Fox H, Doebele C, Ohtani K, Chavakis E, Potente M, Tjwa M, Urbich C, Zeiher AM, Dimmeler S. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. Science 2009; 324: 1710–3.
116. Weber M, Baker MB, Moore JP, Searles CD. MiR-21 is induced in endothelial cells by shear stress and modulates apoptosis and eNOS activity. Biochem Biophys Res Commun 2010; 393: 643–8.

117. el-Deiry WS. Regulation of p53 downstream genes. Semin Cancer Biol 1998; 8: 345–57.
118. Ladislas R. Cellular and molecular mechanisms of aging and age related diseases. Pathol Oncol Res 2000; 6: 3–9.
119. Guevara NV, Kim HS, Antonova EI, Chan L. The absence of p53 accelerates atherosclerosis by increasing cell proliferation in vivo. Nat Med 1999; 5: 335–9.
120. Tanner FC, Yang ZY, Duckers E, Gordon D, Nabel GJ, Nabel EG. Expression of cyclin-dependent kinase inhibitors in vascular disease. Circ Res 1998; 82: 396–403.
121. Dirac AM, Bernards R. Reversal of senescence in mouse fibroblasts through lentiviral suppression of p53. J Biol Chem 2003; 278: 11731–4.
122. Itahana K, Campisi J, Dimri GP. Mechanisms of cellular senescence in human and mouse cells. Biogerontology 2004; 5: 1–10.
123. Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, Stoffel M. Silencing of microRNAs in vivo with'antagomirs'. Nature 2005; 438: 685–9.
124. Solingen CV, Seghers L, Bijkerk R, Duijs JMCG, Roeten MK, Oeveren-Rietdijk AMV, Baelde HJ, Monge M, Vos JB, Boer HCD, Quax PHA, Rabelink TJ, Zonneveld AJV. Antagomir-mediated silencing of endothelial cell specific microRNA-126 impairs ischemia-induced angiogenesis. J Cell Mol Med 2009; 13: 1577–85.
125. Zampetaki A, Kiechl S, Drozdov I, Willeit P, Mayr U, Prokopi M, Mayr A, Weger S, Oberhollenzer F, Bonora E, Shah A, Willeit J, Mayr M. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. Circ Res 2010; 107: 810–7.
126. Nakashima T, Jinnin M, Etoh T, Fukushima S, Masuguchi S, Maruo K, Inoue Y, Ishihara T, Ihn H. Down-regulation of mir-424 contributes to the abnormal angiogenesis via MEK1 and cyclin E1 in senile hemangioma: its implications to therapy. PloS one 2010; 5: e14334.
127. Hackl M, Brunner S, Fortschegger K, Schreiner C, Micutkova L, Muck C, Laschober GT, Lepperdinger G, Sampson N, Berger P, et al. miR-17, miR-19b, miR-20a, and miR-106a are down-regulated in human aging. Aging cell 2010; 9: 291–6.

Received July 20, 2011; accepted January 13, 2012



Munekazu Yamakuchi received his MD and PhD from Kagoshima University School of Medicine, Japan in 1998 and worked in Dr. Lowenstein's laboratory as a postdoctoral fellow at the Johns Hopkins University. He is currently a research assistant professor at the University of Rochester School of Medicine and Dentistry, Rochester, NY, USA. His major research area is vascular biology, but has recently become interested in microRNAs and the mechanism by which micro-RNAs regulate vascular inflammation.