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MicroRNA Changes in Human Arterial Endothelial Cells with Senescence: Relation to Apoptosis, eNOS and Inflammation

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Abstract

A senescent phenotype in endothelial cells is associated with increased apoptosis, reduced endothelial nitric oxide synthase (eNOS) and inflammation, which are implicated in arterial dysfunction and disease in humans. We tested the hypothesis that changes in microRNAs are associated with a senescent phenotype in human aortic endothelial cells (HAEC). Compared with early-passage HAEC, late-passage HAEC had a reduced proliferation rate and increased staining for senescence-associated beta-galactosidase and the tumor suppressor p16^{INK4a}. Late-passage senescent HAEC had reduced expression of proliferation-stimulating/apoptosis-suppressing miR-21, miR-214 and miR-92 and increased expression of tumor suppressors and apoptotic markers. eNOS-suppressing miR-221 and miR-222 were increased and eNOS protein and eNOS activation (phosphorylation at serine1177) were lower in senescent HAEC. Caveolin-1 inhibiting miR-133a was reduced and caveolin-1, a negative regulator of eNOS activity, was elevated in senescent HAEC. Inflammation-repressing miR-126 was reduced and inflammation-stimulating miR-125b was increased, whereas inflammatory proteins were greater in senescent HAEC. Development of a senescent arterial endothelial cell phenotype featuring reduced cell proliferation, enhanced apoptosis and inflammation and reduced eNOS is associated with changes in miRNAs linked to the regulation of these processes. Our results support the hypothesis that miRNAs could play a critical role in arterial endothelial cell senescence.

Keywords

vascular adhesion molecule; monocyte chemotactic protein-1; caspase; aging; nitric oxide

1. INTRODUCTION

Increasing age is an independent risk factor for cardiovascular diseases, including those linked to atherosclerosis (Castelli 1984). The vascular endothelium exerts an important influence on arterial wall function and health (Vita and Keaney 2002). Endothelial dysfunction, characterized by apoptosis (programmed cell death), diminished endothelial nitric oxide synthase (eNOS) production of nitric oxide (NO) and inflammation (Gimbrone and others 2000; Ungvari and others 2010), develops with age and is associated with the

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development and progression of atherosclerosis (Brandes and others 2005; Gimbrone and others 2000; Xu 2009).

During aging there is a gradual accumulation of senescent cells in mammalian tissues (Dimri and others 1995). Senescence is considered a protective mechanism against cancer, however, accumulation of senescent cells also may contribute to several age-related diseases (Rodier and Campisi 2011). Senescent cells have undergone irreversible cell cycle arrest due to either critically short telomeres (replicative senescence) or external stress induced by factors such as radiation, oxidative stress or altered DNA (stress-induced premature senescence) (Erusalimsky 2009). In atherosclerotic lesions, accumulation of senescent endothelial cells is considered an important factor contributing to the associated arterial dysfunction (Fenton and others 2001; Minamino and others 2002; Vasile and others 2001). Consistent with this, endothelial cells cultured to senescence *in vitro* show increased apoptosis (Hampel and others 2004), reduced eNOS (Yoon and others 2010) and elevated inflammatory mediators (Coppe and others 2010). However, the underlying molecular mechanisms contributing to endothelial cell senescence are incompletely understood.

MicroRNAs (miRNA) are small (~22 nucleotides) non-coding RNAs (Ghildiyal and Zamore 2009) that either degrade or translationally repress specific target mRNAs by imperfect pairing of the 5'-proximal "seed" region in the miRNA with the 3'-untranslated region of the mRNA (Bhattacharyya and others 2006). Each miRNA can regulate the expression of multiple mRNA targets, allowing miRNAs to orchestrate a wide variety of cellular responses. miRNAs are important regulators during development (Wienholds and Plasterk 2005), but more recently have been implicated in the control of lifespan (Ibanez-Ventoso and Driscoll 2009) and senescence (Hackl and others 2010; Li and others 2009a; Marasa and others 2010; Mudhasani and others 2008). It is unknown if alteration of miRNA expression is associated with the development of a senescent phenotype in arterial endothelial cells.

Here we test the novel hypothesis that a specific set of miRNAs previously reported to be expressed in endothelial cells and/or altered in models of cardiovascular disease (i.e., miR-21, -214, -92, -222/221, -133a, 125b and -126) (Chan and others 2009; Ji and others 2007; Poliseno and others 2006; Suarez and others 2007; Weber and others 2010), are changed in senescent arterial endothelial cells and are associated with altered apoptosis, eNOS protein expression/activation and atherosclerosis-linked inflammatory mediators. Specifically, we determined if, with senescence: 1) proliferation-stimulating/apoptosis-suppressing miR-21, -214 and -92 were reduced and associated with greater markers of tumor suppressors and apoptosis; 2) eNOS-suppressing miR-221 and -222 were increased and caveolin-1-suppressing miR-133a was reduced and associated with reduced eNOS and increased caveolin-1 protein; 3) inflammation-stimulating miR-125b and inflammation-repressing miR-126 were increased and reduced, respectively, and associated with increased inflammatory mediators.

2. METHODS

2.1. Cell culture

Human aortic endothelial cells (HAEC) were obtained from Lonza at a population doubling (PDL) of 19 and cultured in EBM-bullet kit media (Lonza, Walkersville, MD). PDLs were estimated at each passage using the following equation: $n = (\log_2 X - \log_2 Y)$ (with n = PDL, X = number of cells at the end of one passage, Y = number of cells that were seeded at the beginning of one passage). After 44 ± 1 population doublings, the late-passage cells had reached growth arrest and were considered senescent. Cells within PDL 24–30 (average: 27 ± 1) were used as early-passage (non-senescent) control cells. The growth rate of the cells was calculated in young and senescent cell cultures as the amount of PDLs performed per

day. The values for senescent cells were obtained from 6 repeated cultures of cells from early to late passage.

2.2. Senescence-associated beta-galactosidase (SA- β -gal) staining

To verify senescence, *in situ* staining for SA- β -gal was performed as described elsewhere (Dimri and others 1995). Briefly, cells were grown on 4-well cell culture slides, washed with PBS and fixed with 2% formaldehyde/0.2% glutaraldehyde in PBS for 5 min. Thereafter the cells were washed again with PBS and incubated with β -gal staining solution (150 mM NaCl, 2 mM MgCl₂, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 40 mM citric acid, 12 mM sodium phosphate, pH 6.0, containing 1 mg/ml 5-bromo-4-chloro-3-indolyl- β -D-galactoside (Sigma, St. Louis, MO) for 24 h at 37 °C. The slides were analyzed under a light microscope and pictures taken; 400–500 cells were counted and the percentage of blue staining senescent cells was analyzed.

2.3. Immunofluorescence

Early-passage control and senescent HAEC were seeded onto 4-well slides, fixed and washed as described above. After 1h of blocking with 5% donkey serum (Jackson ImmunoResearch, West Grove, PA), a primary antibody for p16^{INK4a} (p16 F-12, Santa Cruz, CA) was applied for 2h. The slide was washed in PBS and subsequently incubated with a secondary antibody, anti-mouse AF555 (Invitrogen, Carlsbad, CA) for 1h. To visualize cell nuclei, 4',6'-diamidino-2-phenylindole hydrochloride staining (DAPI) was added to the mounting medium. Slides were viewed using a fluorescence microscope (Eclipse 600; Nikon, Melville, N.Y., USA) and images captured by a Photometrics CoolSNAPfx digital camera (Roper Scientific Inc., Tucson, Ariz., USA). Images were then analyzed using Metamorph Software (Universal Imaging, Downingtown, PA) and the percentage of cells staining for nuclear p16^{INK4a} was determined in at least 300 cells. Separate slides were prepared as above but only exposed to DAPI for examination of the amount of apoptotic cells. At least 500 nuclei were counted from each slide. Cells with intact DAPI-staining nuclei were considered non-apoptotic, whereas cells with condensed DNA or completely disintegrated nuclei were considered apoptotic.

2.4. RT-PCR (miR)

RNA was prepared from early-passage control and senescent cells using mirVana miRNA isolation kit (Ambion, Austin, TX) according to the manufacturer's instructions. RT-PCR was performed using available primers and probes from Applied Biosystems for miR -21 (ID-397), -214 (ID-517), -92 (ID-430), -221 (ID-524), -222 (ID-525), -133a (ID-458), -125b (ID-449) and -126 (ID-450). Samples were amplified in duplicates and copy numbers were calculated according to their cycle of threshold values. Relative quantification was performed by comparing the ratios of the target cDNA copy numbers to those of the respective control RNU6B. Results were expressed as fold-change relative to the early-passage control cells.

2.5. Western blot analyses

Whole cell extracts were prepared from early-passage control and senescent cells. Briefly, cells were scraped off the plate in ice-cold RIPA lysis buffer containing protease and phosphatase inhibitors (Protease Inhibitor Cocktail Tablet, Roche, Indianapolis, IN) and 0.01% phosphatase inhibitor cocktail (Sigma, St. Louis, MO). 20 μ g of total protein was separated by 12% SDS-PAGE, and subjected to Western blot on to a nitrocellulose membrane. The following antibodies were used to quantify protein expression: anti-caveolin-1, -eNOS and -pENOS (BD Biosciences, San Jose, CA), anti-JNK1, -PTEN, -caspase-3, -p27^{KIP} and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Cell

Signaling, Danvers, MA). The blots were developed using the SuperSignal West Femto chemoluminescent substrate (Thermo Scientific, IL, U.S.A) and bands were visualized using a digital acquisition system (ChemiDoc-It; UVP, Upland, CA) and quantified using (ImageJ 1.42 software (NIH, Bethesda, MD). To account for differences in protein loading, expression was normalized to GAPDH.

2.6. Multiplex ELISA (inflammatory mediators)

The expression of the inflammatory mediators vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemotactic protein-1 (MCP-1) were assessed using enzyme-linked immunosorbent assay (ELISA, Searchlight Multiplex Immunoassay kit, Aushon Biosystems, Billerica, MA) according to manufacturer's instructions.

2.7. Statistics

Comparisons between early-passage control and senescent cells were made using independent t-tests. Data are presented as mean $\pm$ SEM. Significance was determined using $P < 0.05$.

3. RESULTS

3.1. Confirmation of senescence in late-passage HAEC

Compared with early-passage control cells, HAEC grown until a mean of 44 ± 1 PDLs exhibited an enlarged and flattened phenotype, lower proliferative capacity (0.1 ± 0.03 vs. 0.7 ± 0.1 PDLs/day) and an increased amount of cells staining for the senescence markers SA- β -gal and tumor suppressor p16^{INK4a} (all $P < 0.001$) (Figure 1). Thus, the late-passage HAEC were considered senescent.

3.2. miRNAs that stimulate proliferation and suppress apoptosis are reduced in senescent HAEC

Senescent HAEC had lower expression of proliferation-stimulating/apoptosis-suppressing miR-21 (0.4 ± 0.1 vs. 1.0 ± 0.2 fold-change) and miR-214 (0.3 ± 0.1 vs. 1.0 ± 0.3) compared with early-passage control cells ($P < 0.05$) (Figure 2A). The lower expression of miR-21 and -214 in senescent HAEC was associated with increases in protein expression of the anti-proliferative tumor suppressor PTEN and its downstream effector, the cell cycle repressor p27^{KIP} (Figure 2B). The reduction in miR-21 and -214 in senescent cells also was associated with an increase in % apoptotic cells and protein expression of the pro-apoptotic enzymes caspase-3 and c-Jun NH2-terminal kinase (JNK1) (Figure 2C and D). Proliferation stimulating miR-92a was decreased in senescent HAEC (0.4 ± 0.1 vs. 1.0 ± 0.2 , $P = 0.005$, Figure 2E).

3.3. eNOS-suppressing miRNAs are increased in senescent HAEC

Compared with early-passage control cells, eNOS-suppressing miR-221 (2.2 ± 0.2 vs. 1.0 ± 0.3) and miR-222 (1.7 ± 0.2 vs. 1.0 ± 0.2) were greater in senescent HAEC ($P < 0.05$, Figure 3A). This was associated with lower eNOS protein expression (0.5 ± 0.1 vs. 1.0 ± 0.1 , $P < 0.05$, Figure 3B). The decrease in miR-21 in senescent HAEC, a miRNA linked to phosphorylation of eNOS at serine 1177 and, thus, the activation state of the enzyme, was associated with a decrease in eNOS protein phosphorylated at that site (0.6 ± 0.1 vs. 1.0 ± 0.1 , $P < 0.05$, Figure 3B). Moreover, reduced expression of caveolin-1 regulating miR-133 (0.4 ± 0.1 vs. 1.0 ± 0.2) in senescent HAEC compared with control was associated with increased expression of caveolin-1 (1.8 ± 0.1 vs. 1.0 ± 0.1), a protein that inhibits the basal activity of eNOS (Figure 3C and D).

3.4. Senescence modulates miRNAs that influence vascular inflammation

miR-126, which regulates the cell adhesion molecule VCAM-1, was lower in senescent HAEC compared with early-passage control cells (0.2 ± 0.1 vs. 1.0 ± 0.1 , $P < 0.005$, Figure 4A) and was accompanied by a 3.6-fold increase in VCAM-1 expression ($P < 0.05$, Figure 4B). Compared with control cells, senescent HAEC also expressed elevated levels of miR-125b (3.0 ± 0.6 vs. 1.0 ± 0.2), a miRNA that indirectly upregulates MCP-1 (Figure 4C). The increase in miR-125b was associated with greater expression of MCP-1 protein (1.6 ± 0.1 vs. 1.0 ± 0.1 , $P < 0.005$, Figure 4D).

4. DISCUSSION

Here we report for the first time that changes in a specific set of miRNAs (miR-21, -214, -126, -92, -221, -222 and -125b) in human aortic endothelial cells with senescence are linked to reduced cell proliferation, increased apoptosis, decreased eNOS expression/activation and inflammation, and are associated with the development of a senescent phenotype.

4.1. Cell Proliferation and Apoptosis

The irreversible growth arrest occurring at senescence is due to induction of tumor suppressors such as p16^{INK4a} and p27^{Kip1} and p21^{Cip1} (Freedman and Folkman 2005; Wagner and others 2001). These proteins contribute to cell cycle arrest through inhibition of cyclin dependent kinases (CDK) and p21^{Cip1} plays a critical role by inhibiting CDK2 (Freedman and Folkman 2005). miRNAs are important regulators of cell proliferation and apoptosis. Genetic depletion of Dicer, a protein necessary for miRNA synthesis, induces senescence, suggesting that miRNAs are involved in regulating continuous cell cycle progression (Mudhasani and others 2008).

In the present study, we show that senescent HAEC demonstrate lower expression of miR-21 and miR-214 than early-passage HAEC, thus extending recent observations in T-cells and foreskin from aged individuals (Hackl and others 2010). These miRNAs are implicated in the regulation of cell proliferation through their common target, the tumor suppressor Phosphatase and Tensin Homolog (PTEN), inducing translational repression through binding the 3'-UTR of PTEN mRNA (Jindra and others 2010; Yang and others 2008; Zhang and others 2010). Indeed, we found an increased expression of PTEN, as well as its downstream effector p27^{Kip1} in senescent HAEC (Fig 2B). Upregulation of the PTEN pathway may contribute to the senescent phenotype, in concert with elevated levels of other tumor suppressors such as p16^{INK4a} and p21^{CIP}. MiR-92 also is implicated in proliferation and was reduced in senescent compared with early-passage control HAEC. MiR-92 is a part of the miR-17-92 cluster, also called Oncomir-1, which is upregulated in several proliferative tumor types (Jevnaker and others 2010). Thus, the reduction in miR-92 in senescent HAEC is consistent with the low proliferative capacity in these cells and is in agreement with what has been found recently in senescent fibroblasts and T-cells (Li and others 2009a). The amount of apoptotic cells increase with age and may contribute to organ dysfunction (Ungvari and others 2010). The present findings are consistent with previous results indicating that senescent endothelial cells show greater apoptosis (Wagner and others 2001). The present findings extend this prior work by providing new insight into the role of miRNAs in senescence-associated apoptosis in endothelial cells. The decrease in miR-21, a miRNA with anti-apoptotic effects acting through caspase-3 (Papagiannakopoulos and others 2008), likely contributed to the associated increases in apoptosis and caspase-3 observed in our senescent HAEC. Moreover, we found a decrease with senescence in miR-214, which also has been implicated in apoptosis (Cheng and others 2005; Yang and others 2009). miR-214 targets c-Jun NH2-terminal kinase (JNK1), a protein involved in activating apoptotic pathways (Yang and others 2009). Consistent with this, in the present

study we found increased expression of JNK1 in senescent compared with control HAEC. Thus, miR-21 and -214 may regulate several aspects of the senescent phenotype in HAEC including reductions in cell proliferation and increased rate of apoptosis (Figure 5).

Of note, the decrease in miR-21 in senescent HAEC observed in the present study is in contrast to findings of increases in this miRNA in senescent human umbilical vein endothelial cells (HUVEC)(Menghini and others 2009). This could be explained by the different type of endothelial cells used (HUVEC vs. HAEC) and/or by differences in the early-passage (control) cells, as up to 40% of the control cells stained for senescence in the previous study compared with only 5% in the current investigation.

4.2. eNOS

In a healthy endothelium, NO mediates important athero-protective functions by inhibiting monocyte activation/adhesion, proliferation of vascular smooth muscle cells and expression of proinflammatory cytokines. Aging causes endothelial dysfunction as a result of reduced NO bioavailability (Gimbrone and others 2000; Ungvari and others 2010). Senescent endothelial cells demonstrate reduced expression of eNOS (Sato and others 1993; Yoon and others 2010), the enzyme responsible for endothelial NO production. However, the underlying molecular mechanisms are incompletely understood.

In the present study, we provide new data supporting the possibility that the reductions in eNOS in senescent endothelial cells are mediated by changes in miRNA expression. We found that senescent HAEC had increased expression of miR -221 and -222. Both of these miRNAs are highly expressed in endothelial cells and are negatively associated with eNOS and related processes such as angiogenesis, cell migration and wound healing (Suarez and others 2007). The latter is mediated by reductions in the protein target of miR-221/222, the stem cell factor c-kit (Urbich and others 2008). However, because the eNOS mRNA does not express a target sequence for miR-221 and -222 (Suarez and others 2007), the regulation is thought to be indirect. The present results are in agreement with the previous observation that hyperglycemia, which can induce senescence with resulting decreases in eNOS (Chen and others 2007), is associated with increases in miR-221 in HUVEC (Li and others 2009b). miR-221 and -222 may be influenced differently by senescence in non-endothelial cells or tissues (Hackl and others 2010).

Another miRNA that may be involved in regulating eNOS is miR-21. MiR-21 expression is positively associated with phosphorylation of eNOS at serine 1177, a marker of activation of this enzyme (Weber and others 2010). We found that the reduction in miR-21 was accompanied by a 50% decrease in phosphorylated eNOS in senescent HAEC (Figure 3B). We also assessed caveolin-1, a major inhibitory protein for eNOS activation (Ju and others 1997). Recently, miR-133 was found to directly bind to the mRNA of caveolin-1 (Nohata and others 2011). Consistent with recent observations in non-endothelial cells (Hackl and others 2010), in the current study we observed a reduction in miR-133 in senescent HAEC, which was accompanied by an increase in protein expression of caveolin-1. In fibroblasts caveolin-1 promotes senescence through the p53 pathway (Bartholomew and others 2009). Our finding of an increase in caveolin-1 in senescent HAEC extends this observation by suggesting that miR-133 may induce senescence and reduce NO production in vascular endothelial cells. Overall, the changes in miR -221,-222, -21 and -133 in senescent HAEC observed here would lead to reduction in the amount and activity of eNOS, reducing the production of NO and contributing to a pro-atherosclerotic endothelial environment (Figure 5).

4.3. Inflammation

Senescence is associated with the production of inflammatory mediators (Rodier and Campisi 2011). This is thought to be a mechanism for the cell to signal that it should be removed by the immune system (Rodier and Campisi 2011). However, as senescent cells accumulate, this process may instead induce local inflammation that could worsen the state of dysfunction and disease.

During inflammation, VCAM-1 is expressed on the surface of endothelial cells to stimulate monocyte adhesion while the chemokine MCP-1 is released to mediate cell migration. MiR-126 is a miRNA that is highly expressed in endothelial cells, with inhibition of VCAM-1 as a primary target (Harris and others 2008). In the present study, we found that senescent HAEC had reduced expression of miR-126, which was accompanied by elevated VCAM-1 protein expression. Our results here are consistent with previous clinical observations that circulating miR-126 is decreased in patients with coronary artery disease (Fichtlscherer and others 2010). We also found increased expression of miR-125b in senescent HAEC, consistent with a recent observation in venous endothelial cells (Hackl and others 2010). MiR-125b is increased in vascular smooth muscle during diabetes, a state characterized by vascular inflammation (Villeneuve and others 2010). In that model, miR-125b was positively associated with MCP-1 protein expression. Interestingly, MCP-1 was identified as an indirect, positively regulated target of miR-125b via the transcription factor Suv39H1, a gene-silencing methyl-transferase (Villeneuve and others 2010) (Figure 5). In agreement with these observations, in the present study we found that levels of MCP-1 were elevated in senescent HAEC. Thus, it is possible that both miR-126 and -125b could contribute to the pro-inflammatory state associated with endothelial senescence.

4.4 Interpretation and Limitations

In the present study, all of the miRNAs that were assessed were altered in senescent HAECs. This is likely explained by the selection of miRNAs thought to be involved in senescence and expressed in vascular endothelial cells or other cardiovascular tissues. A recent study by Hackl and others 2010 examined the effects of senescence on the miRNA profile in different cell types and tissues, and found four miRNAs, not included in the present study, that were commonly down regulated. We also recognize that definitive cause and effect evidence of the relations between the senescence-associated changes in miRNAs reported here and their cellular effects will require manipulation of individual miRNAs with assessment of markers of the processes in question. Finally, the apoptosis rate in the early passage cells in the present study was low. This was most likely due to the method used, which detects only cells that have reached the terminal stage.

4.5. Conclusions

The results of the present study provide novel evidence that human arterial endothelial cells undergo changes in miRNA expression that can help to explain the anti-proliferative, pro-apoptotic, eNOS-inhibitory and pro-inflammatory phenotype associated with the development of senescence (Figure 5). As such, our findings may provide new insight into the molecular mechanisms underlying senescence-associated vascular dysfunction and disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

DAPI	4',6'-diamidino-2-phenylindole hydrochloride staining
eNOS	endothelial nitric oxide synthase
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
HAEC	human aortic endothelial cells
HUVEC	human umbilical vein endothelial cells
JNK1	c-Jun NH2-terminal kinase
miRNA	microRNA
MCP-1	monocyte chemotactic protein-1
NO	nitric oxide
peNOS	phosphorylated endothelial nitric oxide synthase
PTEN	Phosphatase and tensin homolog
PDL	population doubling
SA-β-gal	Senescence-associated beta-galactosidase
VCAM-1	vascular cell adhesion molecule-1

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Highlights

- Senescent endothelial cells show an altered expression of microRNAs
- miR-21, -214 and -92 were reduced and associated with low proliferation and increased apoptosis
- miR-221 -222 and -133 were altered and associated with reductions in eNOS
- miR-125b and -126 were altered and associated with inflammatory markers
- MicroRNAs could play an important role in arterial endothelial cell senescence

Figure 1. Proliferation rate and markers of senescence

Late-passage/senescent HAEC (44 ± 1 population doublings) have reduced proliferation rate (A) and an increased amount of cells staining for the senescence-associated markers p16^{INK4a} and beta-galactosidase (SA- β -gal) compared to early-passage cells (B).

Representative image of the p16^{INK4a} and SA- β -gal staining (C). Values are mean $\pm$ SEM, * $P < 0.05$.

Figure 2. miRNAs associated with proliferation and apoptosis

Reductions in PTEN-regulating miR-21 and miR-214 (A) and increases in PTEN and downstream tumor suppressor p27^{KIP} protein (B) and apoptosis and related proteins (C, D) in early passage (Early Pass, EP) and senescent HAEC. miR-92a, an oncomir upregulated in highly proliferating cells, was reduced in senescent HAEC (E). Representative western blot images (F). Values are mean±SEM, * P<0.05.

Figure 3. miRNAs associated with eNOS

eNOS suppressing miR-221 and -222 were increased in senescent HAEC (A) and associated with decreased eNOS protein and phosphorylation of eNOS at ser1177 (B). Caveolin-1 suppressing miR-133a was reduced (C) and caveolin-1 increased (D) in senescent compared to early passage HAEC. Representative western blot images (E). Values are mean $\pm$ SEM, * $P < 0.05$.

Figure 4. miRNAs associated with inflammation

Decreased endothelial-specific VCAM-1-regulating miR-126 and elevated protein expression of VCAM-1 in senescent HAEC (A and B). Increased miR-125b, a positive regulator of MCP-1, and MCP-1 protein expression in senescent HAEC (C and D). Protein expression was measured using multiplex ELISA. Values are mean $\pm$ SEM, * $P < 0.05$.

Figure 5. miRNAs involved in senescent endothelial phenotype

Schematic showing how miRNAs assessed in the present study may contribute to a senescent endothelial phenotype.